Vitiligo

Mauro Picardo
Alain Taïeb
Editors

Second Edition

Springer

Vitiligo

Mauro Picardo • Alain Taïeb
Editors

Vitiligo

Second Edition 2019

Springer

Editors
Mauro Picardo
Cutaneous Physiopathology & CIRM
San Gallicano Dermatological Institute
Rome
Italy

Alain Taïeb
Hôpital Saint André, Service de
Dermatologie Adulte et Pédiatrique
INSERM U 1035, Université de Bordeaux
Bordeaux
France

ISBN 978-3-319-62958-2 ISBN 978-3-319-62960-5 (eBook)
https://doi.org/10.1007/978-3-319-62960-5

Preface to the First Edition

Vitiligo has been, until recently, a rather neglected area in dermatology and medicine. Patients complain about this situation, which has offered avenues to quacks, and has led to the near orphan status of the disease. The apparently, simple and poorly symptomatic presentation of the disease has been a strong disadvantage to its study, as compared to other common chronic skin disorders such as psoriasis and atopic dermatitis. Vitiligo is still considered by doctors as a non disease, a simple aesthetic problem. A good skin-based angle of attack is also lacking because generalized vitiligo is clearly epitomizing the view of skin diseases as simple targets of a systemic unknown dysregulation (diathesis), reflecting the Hippocratic doctrine. This view has mostly restricted vitiligo to the manifestation of an auto-immune diathesis in the past 30 years. Thus, skin events, which are easily detected using skin biospies in most other situations, have not been precisely recorded, with the argument that a clinical diagnosis was sufficient for the management (or most commonly absence of management) of the patient.

This book is an international effort to summarize the information gathered about this disorder at the clinical, pathophysiological and therapeutic levels. Its primary aim is to bridge current knowledge at the clinical and investigative level, to point to the many unsolved issues, and to delineate future priorities for research. Its impetus was also to provide the best guidelines for integrated patient care, which is currently possible at a very limited number of places around the world, especially for surgical procedures.

A striking feature in the vitiligo field was, until recently, the absence of consensus on definitions, nomenclature, and outcome measures. With a group of European dermatologists, who had a strong interest in vitiligo and pigment cell research, we had launched some years ago, the Vitiligo European Task Force (VETF). The VETF has addressed those issues as a priority. This group, joined by other colleagues from the rest of the world also involved in the vitiligo research community, has communicated its experience in this book. We have tried to pilot the editing of the book according to consistent principles based on discussions held at VETF meetings and international IPCC (international pigment cell conference) workshops. However, some areas remain controversial and we have highlighted the existing conflicting issues and uncertainties.

The correction to this book is available at https://doi.org/10.1007/978-3-319-62960-5_47.

After reviewing the field, much needs to be done. In particular, besides basic research based on the many hypotheses raised, new unbiased epidemiological, clinical, histopathological, natural history, and therapeutic data are clearly needed. They should be confronted by genetics and other investigative variables to better define the disease and its subsets. We hope that the combined efforts of all participating authors will prove useful to bring more attention to this field, and we are confident that both the research community (the mystery of melanocyte loss in vitiligo is a true scientific challenge) and the drug industry (the potential market is large) will be stimulated to bring in new treatment strategies to this large number of patients with unmet needs.

Bordeaux, France — Alain Taïeb
Rome, Italy — Mauro Picardo

Preface to the Second Edition

Nearly 10 years after the first edition, it was necessary to update but also to reassess the field more globally. What has changed in our vision of vitiligo in about a decade?

Developments in the field have included classification, identification of new identities, pathogenic mechanisms, investigation of normal appearing skin, and new approaches for diagnosis, treatment, and maintenance of treatment, just to mention a few. To reflect the expansion of the field, we have benefited of the expertise of a larger community of scientists and physicians, but in parallel we also tried to improve the book plan with the underlying challenging endeavor to simplify and limit redundancies.

For disease definition, clinical aspects have been better delineated, and international efforts conducted under the auspices of the Vitiligo Global Issues Consensus Conferences have simplified nomenclature and classification as well as scoring and paved the way to more uniform outcome research tools. Considering disease expression, the epidemic of vitiligoid depigmentation caused by immunotherapies has questioned the immune mechanisms of melanocyte loss in common vitiligo, a central part of disease pathophysiology.

The basic understanding of the disease for its immune/inflammatory component has undergone significant progresses and seems at work in all subsets of the disease, with immediate practical consequences but also more durable translational perspectives for therapy. Melanocyte stability, melanocyte regeneration, and epigenetics are relatively new fields in terms of basic research explored in this new edition with also important potential added value for therapy.

For therapy, our perspective has clearly changed over the last decade for segmental vitiligo which was considered previously as poorly or not at all responsive to medical treatment. Given the efficacy of early medical intervention now reported by several investigators, vitiligo of any subtype should better be considered as a therapeutic emergency to avoid irreversible immune/inflammatory losses in pigment cells. The advantage of vitiligo over type 1 diabetes is the visibility and early detection of tissue damage in most patients, allowing more reactivity. However, patients do still complain, and this has not sufficiently changed over the last decade, of the absence of interest of doctors for their disease. Patients' voices are probably better heard with the more coordinated action of patients' advocacies, but much more education is needed for nonspecialist and specialist physicians to use the current medical

and surgical armamentarium for vitiligo. Fortunately, things should change for the availability of registered treatments, since the next decade should clearly be the era of drug development for vitiligo, following for dermatology significant progresses already accomplished for other major skin disorders such as psoriasis, atopic dermatitis, and alopecia areata.

Finally, we want to thank warmly the large group of international colleagues who accepted to participate in this second edition of the book.

Bordeaux, France — Alain Taïeb
Rome, Italy — Mauro Picardo

Contents

Contributors

Setsuya Aiba Department of Dermatology, Tohoku University Graduate School of Medicine, Sendai, Japan

Aleissa Ahmed The National Center of Vitiligo and Psoriasis, Riyadh, Kingdom of Saudi Arabia

Abdulrahman Aljamal The National Center of Vitiligo and Psoriasis, Riyadh, Kingdom of Saudi Arabia

Mohammed Aljamal The National Center of Vitiligo and Psoriasis, Riyadh, Kingdom of Saudi Arabia

Tag S. Anbar (in memory) Department of Dermatology and Andrology, Faculty of Medicine, Al-Minya University, Al-Minya, Egypt

Marco Ardigò San Gallicano Dermatological Institute, IRCCS, Rome, Italy

Ratnam Attili Visakha Institute of Skin and Allergy, Visakhapatnam, India

Markus Böhm Department of Dermatology, University of Münster, Münster, Germany

Laïla Benzekri Department of Dermatology, CHU Ibn Sina, Mohammed V University, Rabat, Morocco

Anuradha Bishnoi Department of Dermatology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Katia Boniface INSERM U 1035, University of Bordeaux, France

INSERM U1035, BMGIC, Immunodermatology team, ATIP-AVENIR, Université de Bordeaux, Bordeaux, France

Muriel Cario-André INSERM U 1035, Centre de référence des maladies rares de la peau, Université de Bordeaux, Bordeaux, France

Sai Yee Chuah National Skin Centre, Singapore, Singapore

Carlo Cota Dermatopathology Unit, San Gallicano Dermatologic Institute (IRCCS), Rome, Italy

Dipankar De Department of Dermatology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Maria Lucia Dell'Anna Cutaneous Physiopathology, San Gallicano Dermatological Institute, IFO, Rome, Italy

Véronique Delmas INSERM U1021, Normal and Pathological Development of Melanocytes, Institut Curie, PSL Research University, Orsay, France

Alida DePase Associazione Ricerca Informazione per la Vitiligine (ARIV), Cernusco Lombardone, LC, Italy

Mari Dezawa Department of Stem Cell Biology and Histology, Tohoku University Graduate School of Medicine, Sendai, Japan

Amira A. Eid Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Gisela F. Erf Division of Agriculture, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR, USA

Khaled Ezzedine Service de dermatologie, Hôpital Henri Mondor, France

Emily Yiping Gan National Skin Centre, Singapore, Singapore

Yvon Gauthier Former Consultant, Department of Dermatology, CHU de Bordeaux, France

Boon Kee Goh Skin Physicians Pte Ltd, Mount Elizabeth Medical, Singapore, Singapore

Pearl E. Grimes Vitiligo & Pigmentation Institute of Southern California, Los Angeles, CA, USA

Seung-Kyung Hann Korea Institute of Vitiligo Research, Drs. Woo & Hann's skin clinic, Seoul, South Korea

Candrice Heath Vitiligo & Pigmentation Institute of Southern California, Los Angeles, CA, USA

Rehab A. Hegazy Department of Dermatology, Faculty of Medicine, Cairo University, Cairo, Egypt

Steven W. Henning Oncology Research Institute, Loyola University Chicago, Maywood, IL, USA

Thomas Jouary Service de Dermatologie, Hôpital Saint André, CHU de Bordeaux, Bordeaux, France

Hee Young Kang Department of Dermatology, Ajou University School of Medicine, Suwon, Korea

Panagiota Kostopoulou Service de Dermatologie, Hôpital Saint André, Bordeaux, France

Daniela Kovacs Cutaneous Physiopathology and Integrated Center of Metabolomics Research, San Gallicano Dermatologic Institute (IRCCS), Rome, Italy

Prasad Kumarasinghe Department of Dermatology, Royal Perth Hospital, Perth, WA, Australia

Cheng-Che Eric Lan Department of Dermatology, Kaohsiung Medical University, Kaohsiung, Taiwan

Lionel Larue INSERM U1021, Normal and Pathological Development of Melanocytes, Institut Curie, PSL Research University, Orsay, France

I. Caroline Le Poole Professor of Dermatology, Microbiology and Immunology Northwestern University at Chicago, IL, USA

Giovanni Leone San Gallicano Dermatological Institute IRCCS, Rome, Italy

Henry W. Lim Henry Ford Immunology Program, Department of Dermatology, Henry Ford Hospital, Detroit, MI, USA

Jean-Marie Meurant Association Française du Vitiligo, Paris, France

Qing-Sheng Mi Henry Ford Immunology Program, Department of Dermatology, Henry Ford Hospital, Detroit, MI, USA

Silvia Moretti Division of Clinical Preventive and Oncologic Dermatology, University of Florence, Florence, Italy

Fanny Morice-Picard Department of Dermatology, Hôpital Pellegrin-Enfants, Bordeaux, France

Sanjeev Mulekar The National Center of Vitiligo and Psoriasis, Riyadh, Kingdom of Saudi Arabia

Onyeka Obioha Vitiligo and Pigmentation Institute of Southern California, Los Angeles, CA, USA

Jean-Paul Ortonne Department of Dermatology, Archet-2 hospital, Nice, France

Alessia Pacifico San Gallicano Dermatological Isitute, IRCCS, Rome, Italy

Davinder Parsad Department of Dermatology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Thierry Passeron Department of Dermatology, University Hospital of Nice, Nice Cedex 3, France

Mauro Picardo Cutaneous Physiopathology and CIRM, San Gallicano Dermatological Institute, IRCCS, Rome, Italy

M. Ramam Department of Dermatology and Venereology, All India Institute of Medical Sciences, New Delhi, India

Julien Seneschal Service de dermatologie, Hôpital St André, CHU de Bordeaux, Bordeaux, France

Suzan Shalaby Department of Dermatology, Faculty of Medicine, Cairo University, Cairo, Egypt

Reinhart Speeckaert Department of Dermatology, Ghent University Hospital, Ghent, Belgium

Richard A. Spritz Human Medical Genetics and Genomics Program, University of Colorado School of Medicine, Aurora, CO, USA

Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO, USA

Alain Taïeb Service de Dermatologie, Hôpital St André, CHU de Bordeaux, Bordeaux, France

Department of Dermatology and Pediatric Dermatology, Bordeaux University Hospitals, and INSERM U 1035, University of Bordeaux, Bordeaux, France

Steven Thng National Skin Centre, Singapore, Singapore

Kenichiro Tsuchiyama Department of Dermatology, Tohoku University Graduate School of Medicine, Sendai, Japan

Wietze Van der Veen Department of Dermatology, Institute for Pigment Cell Disorders, AMC, Amsterdam, The Netherlands

Nanja van Geel Department of Dermatology, Ghent University Hospital, Ghent, Belgium

Carole Van Haverbeke Department of Dermatology, Ghent University Hospital, Ghent, Belgium

Charlotte Vrijman Department of Dermatology, Ziekenhuisgroep Twente, Hengelo, The Netherlands

Kirsten C. Webb Division of Dermatology, Department of Medicine, Loyola University Chicago, Maywood, IL, USA

Ching-Shuang Wu Faculty of Biomedical Laboratory Science, Kaohsiung Medical University, Kaohsiung, Taiwan

Savita Yadav Department of Dermatology and Venereology, All India Institute of Medical Sciences, New Delhi, India

Kenshi Yamazaki Department of Dermatology, Tohoku University Graduate School of Medicine, Sendai, Japan

Hsin-Su Yu Department of Dermatology, Kaohsiung Medical University, Kaohsiung, Taiwan

Li Zhou Henry Ford Immunology Program, Department of Dermatology, Henry Ford Hospital, Detroit, MI, USA

Part I

Defining the Disease

Historical Aspects of Vitiligo

1

Yvon Gauthier and Laila Benzekri

Contents

Abstract

Vitiligo was recognized long ago under different names. Firstly, the *Ebers Papyrus* (circa 1500 BC) mentions two types of diseases affecting the colour of the skin, one being probably leprosy and the other vitiligo. In the ancient Vedic scripture of India (circa 1400 BC), Sanskrit words "kilas" and "svitra" (white patches on the skin) can be found. The early classics of the Far East (1200 BC) mention "shirabito" (white man). The Hebrew word "Zoorat" in the bible corresponds to a group of achromic diseases including vitiligo. "Baras and alabras" were the Arabic names used to describe vitiligo. The term "vitiligo" itself was introduced in the first century of our era. The confusion of leprosy with vitiligo in the Old Testament under "Zoorat" is an important cause for the social stigma attached to white spots on the skin. Detailed and effective treatments for vitiligo are found in different sacred books. The modern photochemotherapy is an improvement of a photochemotherapy practised in the ancient world with herbals containing furocoumarins (mainly *Ammi majus Linnaeus*, *Psoralea corylifolia*) and sun. In the mid-1960s the synthetic furocoumarin (trioxsalen and trimethylpsoralen) were developed. The effectiveness of PUVA (psoralen + UVA) for the treatment of some patients

Y. Gauthier (✉)
Former Consultant, Department of Dermatology, CHU de Bordeaux, Bordeaux, France

L. Benzekri
Department of Dermatology, CHU Ibn Sina, Mohammed V University, Rabat, Morocco

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_1

with vitiligo was confirmed during 1974–1982. More recently, narrowband UVB therapy, local microphototherapy, excimer laser, topical treatments with corticosteroids and calcineurin inhibitors and melanocyte transplantation have been successively introduced.

Key Points

- The term vitiligo was introduced in the first century of our era.
- The mistaking of leprosy for vitiligo in the Old Testament under Zoorat or Zaraath is an important cause for the social stigma attached to white spots on the skin.
- Modern photochemotherapy (PUVA) was an improvement of photochemotherapy practised in the ancient world with herbals containing furocoumarins.
- The stigma historically associated with the disease has not disappeared, and information and educational programmes should be implemented to fight this problem, especially the fear of contagion.

The physician must know what his predecessors have known, if he does not want to deceive both himself and others.

—Hippocrates

1.1 Before Vitiligo: Understanding Old Terms Meaning White Skin Spots

Even though the term vitiligo appeared in the first century of our era, descriptions of the disease now known as vitiligo can be found in the ancient medical classics of the second millennium BC [1, 2]. The earliest reference to the disease was found in 2200 BC in the period of Aushooryan according to the ancient literature of Iran "Tarkh-e-Tibble". In the *Ebers Papyrus* (circa 1500 BC) [3], there is a mention of two types of diseases affecting the colour of the skin. One of them which is associated with swellings and recommended to be left alone "Thou shall anything to-do it" is probably leprosy. The other, manifested with only changes of colour, is likely to be vitiligo, as this is said to have been treated (Fig. 1.1).

Hundreds of years before Christ, vitiligo was present in ancient Indian sacred books such as the Atharva Veda (1400 BC) and the Buddhist sacred book Vinay Pitak (224–544 BC). Atharva Veda gives a description of vitiligo but also of many achromic or hypochromic diseases under different names "Kilasa", "SvetaKhista" and "Charak". The Sanskrit word "kilas" is derived from kil meaning white. So "kilas" means "which throw away colour"; the term "SvetaKhista" was used meaning white leprosy, and vitiligo was probably confused with macular leprosy. The term "Charak" used by villagers means "which spreads or is secret". In the Buddhist "Vinay Pitak", the term "kilas" was also used to describe the white spots on the skin [4].

Descriptions of vitiligo and other leukodermas can also be found in other ancient Indian writings such as the *Charaka Samhita* (800 BC), Manusmriti (200 BC) and *Amorkasha* (600 AC). There are also references to disease with a whitening of the skin in the early classics of the Far East. In "Makataminoharai", a collection of Shinto prayers (dating back to about 1200 BC), there is mention of a disease "shirabito" which literally means white man. This also could have been vitiligo.

Though vitiligo, the disease with white spots, was recognized in the ancient times, it was frequently confused with leprosy. Even Hippocrates (460–355 BC) did not differentiate vitiligo and leprosy and included lichen, leprosy, psoriasis and vitiligo under the same category. In the Old Testament of the Holy Bible, the Hebrew word "Zoorat" is referring to a group of skin diseases that where classified into five categories [1]:

1. White spots per se interpreted as vitiligo or post-inflammatory leukoderma
2. White spots associated with inflammation
3. White spots associated with scaling
4. White spots associated with atrophy
5. White spots associated with the regrowth of white hairs

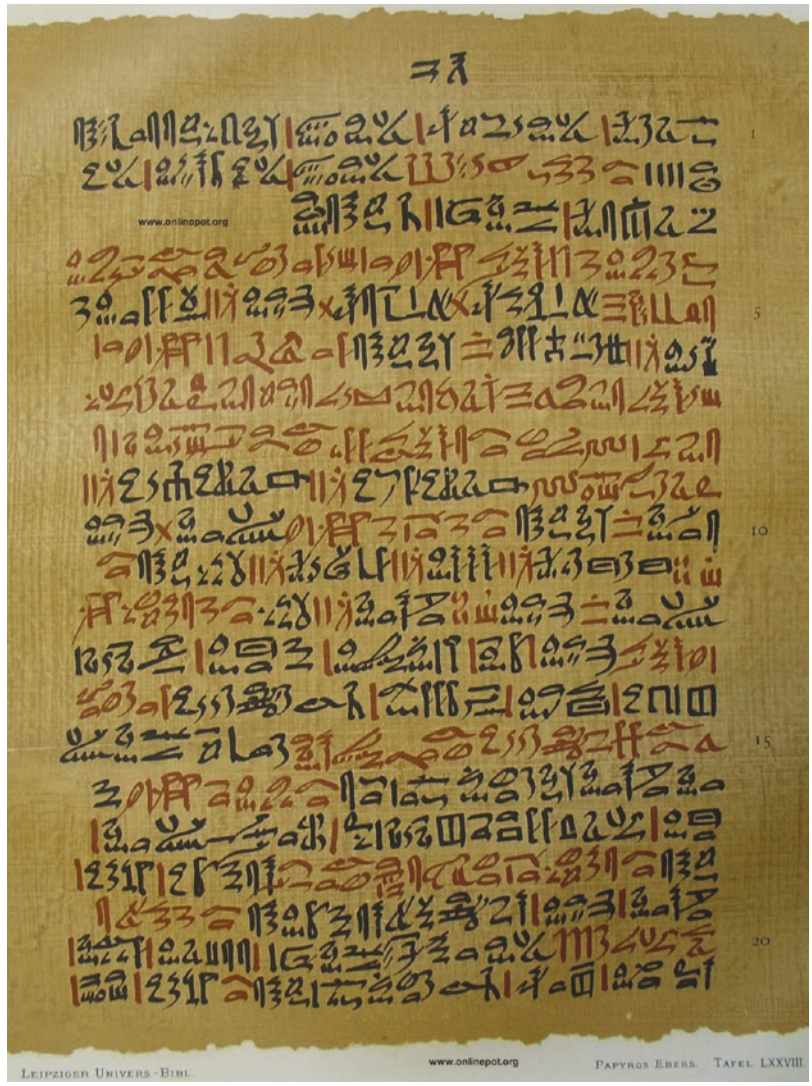


Fig. 1.1 *Papyrus* (Published under Creative Common Licence CCO 1.0, Leipzig, University Library, Papyrus Ebers, Table XXIII, Column LXXVII-LXXIX, https://papyri.uni-leipzig.de/receive/UBLPapyri_text_00038150)

Ptolemy II (250 BC) demanded the translation of the Hebrew Bible into Greek so that more people could understand the Bible. The word "Zoorat'" was unfortunately translated in Greek versions of the Bible as "white leprosy" (Leviticus Chap. XIII). According to modern dermatologists and theologians, biblical leprosy represented not a specific illness but psoriasis or leukoderma and other disorders perceived to be associated with a spiritual uncleanliness. Consequently, persons with white spots, independent of the cause, were isolated from the healthy ones [1, 2, 5, 6].

"Bohak", "baras" and "alabras" are the Arabic names used to describe vitiligo. "Baras" means "white skin". In the Koran (Surah the family of Imran chapter 3, verse 48, and Surah the table spread chapter 5 verse 109), we can read (Fig. 1.2):

> *In accord with God' will Jesus was able to cure patients with vitiligo*

"Alabras" was translated as leprosy in many languages. However, Tahar Ben Achour [7] in 1973 explained that "alabras" meant vitiligo. For this theologian, the aetiology of vitiligo in the Koran is unknown, but it is inherited and is not contagious.

The name vitiligo was first used by the famous Roman physician Celsus at the second century AC

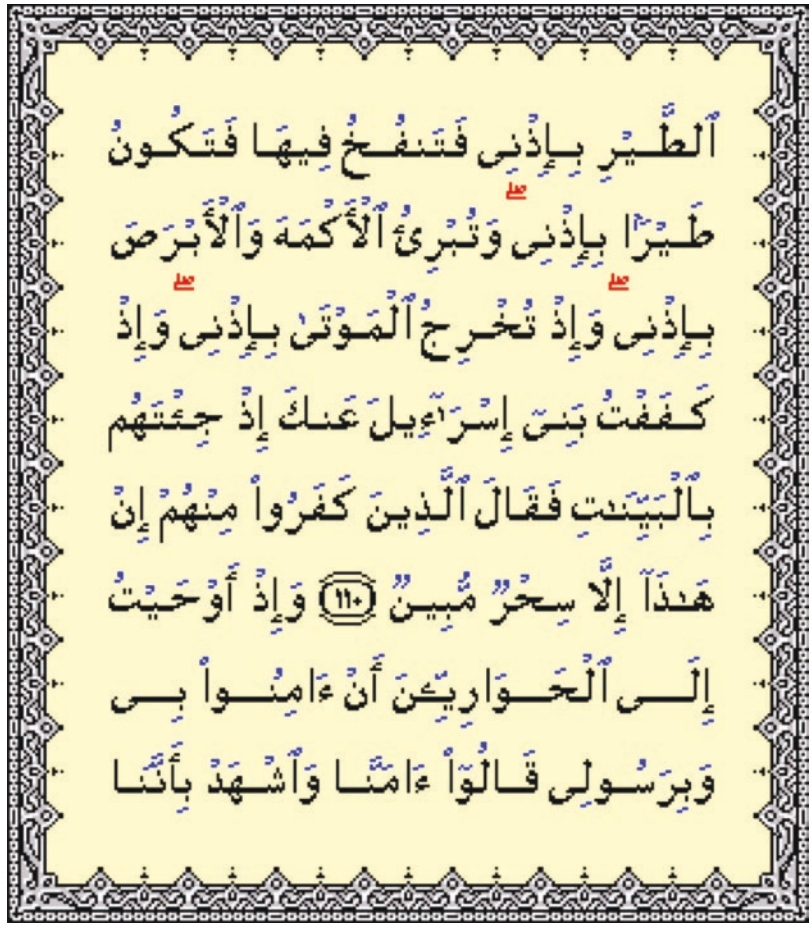

ٱلطَّيۡرِ بِإِذۡنِي فَتَنفُخُ فِيهَا فَتَكُونُ
طَيۡرَۢا بِإِذۡنِي وَتُبۡرِئُ ٱلۡأَكۡمَهَ وَٱلۡأَبۡرَصَ
بِإِذۡنِي وَإِذۡ تُخۡرِجُ ٱلۡمَوۡتَىٰ بِإِذۡنِي وَإِذۡ
كَفَفۡتُ بَنِيٓ إِسۡرَٰٓءِيلَ عَنكَ إِذۡ جِئۡتَهُمۡ
بِٱلۡبَيِّنَٰتِ فَقَالَ ٱلَّذِينَ كَفَرُواْ مِنۡهُمۡ إِنۡ
هَٰذَآ إِلَّا سِحۡرٞ مُّبِينٞ ﴿١١٠﴾ وَإِذۡ أَوۡحَيۡتُ
إِلَى ٱلۡحَوَارِيِّـۧنَ أَنۡ ءَامِنُواْ بِي
وَبِرَسُولِي قَالُوٓاْ ءَامَنَّا وَٱشۡهَدۡ بِأَنَّنَا

Fig. 1.2 From the Koran, Surah "The Family of Imran", Chapter 3 verse 48

in his medical classic *De Medicina*. The word vitiligo has often been said to have derived from "vitium" (defect or blemish) rather than "vitellus" meaning calf [2].

1.2 From Celsus to the Modern Period

Over the last century and up to now, the alternative use of the terms of "vitiligo" and "leukoderma" has perpetuated confusion in the medical literature. Pearson, just after the end of the nineteenth century, described under the name leukoderma a disease which seems to be vitiligo. In the late twentieth century, the elusive nature of vitiligo has led to prudent definitions excluding disorders of established aetiology. "Vitiligo can be described as an acquired primary, usually progressive, melanocyte loss of still mysterious aetiology, clinically characterized by circumscribed achromic macules often associated with leukotrichia and progressive disappearance of melanocytes in the involved skin". However, controversies still remain about achromic macules occurring during the evolution of malignant melanoma and lymphomas. Are they "vitiligoid lesions" or true vitiligo? (see Chap. 16).

1.3 Social Status of Vitiligo Patients Across the Ages

The history of vitiligo reveals much information regarding the social stigma of patients suffering from this disease. In the Buddhist literature (624–544 BC), we can read "men and women suffering from the disease named Kilasa were not eligible to

get ordainment". The social implication of this disease is also well documented in *Rgveda* (Indian book) "persons suffering from switra and their progeny are disqualified from marrying others" [4].

Herodotus (484–425 BC) in his book *Clio* (1138) has written "if a Persian has leprosy or white sickness he is not allowed to enter into a city or to have dealings with other Persians, he must have sinned against the sun. Foreigners suffering from this disorder are forced to leave the country; even white pigeons are often driven away, as guilty of the same offence".

In Leviticus XIII, 34, white spot diseases are considered as a punishment sent by God: "Anyone with these skin affections must wear torn clothes and have his hair dishevelled, he must conceal his upper lip, and call out "unclean, unclean". So long the disease persists, he is to be considered virtually unclean and live alone outside the camp". During many centuries the stigma of leprosy was strengthened by old edicts and cruel laws [1].

In 1943, according to the initiative of Pope Pius XII and the American Catholic office, the church added the following note with reference to Leviticus XIII "Various kinds of skin blemishes are treated here which were not contagious but simply disqualified their subjects from associations with others, until they were declared ritually clean. The Hebrew term used does not refer to Hansen's disease, currently called leprosy" [1, 2].

Islamic theologians consider that baras is a defect in the couple. Thus, the husband or the wife has the choice to divorce. Arabic kings talked to vitiligo patients only behind a screen as reported by the poet Harith bin Hilliza [7].

Therefore the patient and their parents should be reassured about the non-infectious origin of vitiligo. Unfortunately, patients with vitiligo are still regarded as social outcasts in some countries.

1.4 Early History of the Medical Treatment of Vitiligo

The *Ebers Papyrus* (circa 1500 BC), the Atharva Veda (circa 1400 BC), other Indian and Buddhist medical literature (circa 200 AC) and Chinese manuscript from the Sung period (circa 700 AC) make reference to the treatment of vitiligo with black seeds from the plant Bawachee or Vasuchika which is now called *Psoralea corylifolia*.

Photochemotherapy was practised in the ancient world by physicians and herbalists who used boiled extracts of leaves, seeds or the roots of *Ammi majus Linnaeus* in Egypt (Fig. 1.3) or *Psoralea corylifolia* in India (Fig. 1.4) which contain psoralens and

Fig. 1.3 *Ammi majus Linnaeus* (AML) a weed which grows throughout the Nile Valley. The seeds of AML were used in ancient Egypt as a therapy for leucoderma (Published under Creative Commons Attribution ShareAlike 3.0 License, https://commons.wikimedia.org/wiki/File:Ammi_majus_001.JPG)

Fig. 1.4 *Psoralea corylifolia* grows in India. Preparations for the treatment of vitiligo were made from seeds of this plant (Photo by Biswarup Ganguly, published under Creative Commons Attribution ShareAlike 3.0 License, https://commons.wikimedia.org/w/index.php?curid=23839722)

several furocoumarins [6]. These preparations made from seeds obtained from herbal stores were either applied to the skin or ingested before solar exposure. In the ancient "Ayurvedic system of medicine", the use of *Psoralea corylifolia* (Leguminosae family) for inducing the repigmentation of vitiligo is carefully recorded. In *Charaka Samhita* both topical and systemic uses of figs are also recommended before sun exposure. Figs are known to contain both psoralens and glucosides of psoralens. In Atharva Veda (2000–1400 BC) and many other writings of this period, the treatment of vitiligo with "Bawachee" (*Psoralea corylifolia*) is described to be associated with exposure to solar radiation and worshipping prayer. This is illustrated in Atharva Veda by the following poem to one of the plant used.

Born by night art thou, O plant
Dark, black, sable, do thou
That art rich in colour, stain
This leprosy and white grey spots.
Even colour is the name of thy mother,
Even colour is the name of thy father.
Thou, O plant produced even colour,
Render this spot to even colour

According to Indian medical books, the two most commonly applied and effective herbs were " Malapu" (*Ficus hispida*) and "Bawachee" (*Psoralea corylifolia*). These herbs were administered orally or topically followed by exposure to sunlight until sweating was observed. Blisters were then produced and after their rupture, repigmentation occurred. In Ayurvedic, the association of internal treatment consisting of dried ginger, black pepper, pippali and leadwort root fermented in cow's urine and local ointment with a paste made of several medicinal herbs including *Psoralea corylifolia* was proposed to induce the repigmentation of vitiligo [4].

Another important plant *Ammi majus Linnaeus* [4, 8, 9], which grows throughout the Nile Valley as a weed, has been used many centuries BC as a treatment for leukoderma. Ibn el Bitar in his thirteenth-century book *Mafradat Al Adwiya* described the treatment of vitiligo "baras" with the seeds of aatrillal (*Ammi majus*) and sunlight [10]. Aatrillal seemed to be a common therapy for leukoderma in "Ben Shoerb", a Berberian tribe living in the north-western African desert. Egyptian herbalists used these herbs in the form of a powder [11]. Through the ages, in Egypt, topical or oral use of seeds from the plant *Ammi majus* followed by sunlight exposure as mentioned earlier in India was the mainstay of treatments.

Natural psoralens have been found in more than 30 plants, including lime, lemon, bergamot, celery, fig and clove [12].

1.5 Modern History of the Medical Treatment of Vitiligo

In 1938 Kuske investigated phytophotodermatitis occurring on areas of the skin which have been in contact with certain plants and also

exposed to sunlight [13]. Extensive research began in 1941 in Cairo, Egypt, in the laboratory of Fahmi and his group. Three crystalline compounds were obtained from *Ammi majus* L. and named ammoidin, ammidin and majudin [11]. El Mofty [14] was the first, in the early 1940s, to use crystalline methoxsalen followed by exposure to sunlight for the treatment of vitiligo. The results were found good, and shortly afterwards two of the compounds, 8-methoxypsoralen and 8-isoamyleneoxypsoralen, were produced by an Egyptian firm in Cairo. In 1957 Harris and Lerner isolated a melanocyte-stimulating hormone or MSH which they obtained from the pituitary glands of pigs and studied its skin-darkening effects in hypopituitarism skin and vitiligo skin [10]. No changes were seen in the vitiliginous areas after alpha-MSH treatment. Furthermore microscopic examination of DOPA-stained skin sheets from the injected sites and from normal skin did not reveal any significant increase in the number of melanocytes [15]. However more recently, afamelanotide, an analogue of alpha-melanocyte-stimulating hormone, was shown to promote melanoblast differentiation and proliferation of melanocytes in vitiligo skin [16]. In 1974 a new high-intensity UVA light source was initially used in combination with either oral 8 MOP or 4,5,8-trimethylpsoralen for the treatment of vitiligo. It was the beginning of PUVA therapy which permitted to induce a good repigmentation on hairy skin using the melanocytes from the follicular reservoir. The period from 1974 to 1988 was the period of photomedicine that established the therapeutic effectiveness of psoralens in combination with UVA in the treatment of vitiligo, psoriasis and various other skin diseases [17].

More recently several strategies were used [18] including topical and systemic corticosteroids, topical calcineurin inhibitors, photochemotherapy (oral and topical) such as psoralen plus UVA (PUVA), psoralen plus sunlight (PUVA sol), broad- and narrowband UVB phototherapy, 308 nm microphototherapy [2], excimer light and combination phototherapy (see Chap. 38).

1.6 Modern History of Surgical Treatment of Vitiligo

Many investigators have tried to induce repigmentation in patients with vitiligo not responding to medical therapies with grafting and transplantation techniques. Successively the dermoepidermal grafts were introduced by Behl in 1964 [19]. Epidermal grafting with suction blisters was successfully used for the first time in 1971 by Falabella [20] on segmental vitiligo and post-burn depigmentation. Minigrafting was proposed in 1983 by Falabella in three patients with segmental vitiligo [21]. In 1989 epidermis with melanocytes was grown for the first time for repigmentation purposes by Falabella et al. [22]. The use of epidermal suspensions for repigmentation of stable vitiligo macules was initially described in 1992 by Gauthier [23]. This method was improved in 1998 by Olsson and Juhlin [8]. In 1992 pure melanocyte suspensions were cultured and implanted in patients with vitiligo by Olsson and Juhlin [24]. A new approach to simplify the delivery of cultured melanocytes to patients via a cell delivery carrier which could also be used for transport of cells over considerable distances has been proposed by Mac Neil and Eves [25]. Because melanocyte stem cells reside in the outer root sheath of hair follicles, suspension of outer root sheath cells has been used as a source of melanocytes when transplanted into patients with vitiligo [26, 27].

1.7 Conclusions

Vitiligo, under different names, was recognized and feared over several centuries. Even though modern phototherapies and photochemotherapies represent an improvement of the herbal and sun ancient world techniques, our current therapies, including surgical techniques, fail in a large number of cases. The stigma historically associated with the disease has not disappeared, and information and educational programmes should be implemented to fight this problem, especially the fear of contagion.

References

1. Goldman L, Richard S, Moraites R. White spots in Biblical Times. Arch Dermatol. 1966;93:744–53.
2. Ortonne JP, Mosher DP, Fitzpatrick TB. Vitiligo and other hypomelanoses of hair and skin. New York, NY: Plenum medical school; 1983. p. 129–32.
3. Ebbel B. The papyrus Ebers. Copenhagen: Levin and Munksgaard; 1963.
4. Donata SR, Kesavan M, Austin SR. Clinical trial of certain Ayurveda medicines indicated in vitiligo. Ancient Sci Life. 1990;4:202–6.
5. Nair BK. Vitiligo: a retrospect. Int J Dermatol. 1978;17:755–7.
6. Panda AK. The medicohistorical perspective of vitiligo. Bull Ind Hist Med. 2005;25:41–6.
7. Ben Achour T. TafsirAt Tahir Wat-Tanwir. Edn Dar SahouneHannadi, city, 1973.
8. Olsson M, Juhlin M. Leucoderma treated by transplantation of a basal cell layer enriched suspension. Br J Dermatol. 1998;138:644–8.
9. Prasad PV, Bhatnagar VK. Medico-historical study of "Kilasa" (vitiligo/leucoderma) a common skin disorder. Bull Ind Inst Hist Med. 2003;33:113–27.
10. Harris JL, Lerner AB. Amino acid sequence of alpha-melanocyte stimulating hormone. Nature. 1957;179:1346–7.
11. Fahmy IR, Abn-Shady H. Pharmacological study and isolation of crystalline constituents: Amoïdin. Quart J Pharm and Pharmacol. 1947;20:281–6.
12. Pathak M, Daniels F, Fitzpatrick TB. The presently known distribution of furocoumarins (psoralens) in plants. J Invest Dermatol. 1962;39:225–39.
13. Kuske H. Experimentelle untershungersur Photosensibiliste der Haut durch pflanzliche Wisrktoffe. Arch F Dermatol et Syphil. 1938;178:112–4.
14. El Mofty AM. A preliminary clinical report on the treatment of leukodermias with Ammi Majus Linn. J Roy Egypt. 1948;31:651–6.
15. Lerner AB, McGuire JS. Vitiligo and sympathectomy: the effect of sympathectomy and alpha-melanocyte stimulating hormone. Arch Dermatol. 1966;94:269–78.
16. Lim HW, Grimes PE, Agbai O, et al. Afamelanotide and narrowband UVB phototherapy for the treatment of vitiligo: a randomized multicenter trial. JAMA Dermatol. 2015;151(1):42–50.
17. Pathak M, Fitzpatrick TB. The evolution of photochemotherapy with psoralens and UVA (PUVA) 2000BC to 1992 AD. J Photochem Photobiol. 1992;14:3–22.
18. Lotti TM, Menchini G, Andreassi L. UV-B radiation microphototherapy. An elective treatment for segmental vitiligo. J Eur Acad Dermatol Venereol. 1999;12:102–8.
19. Behl PN. Treatment of vitiligo with homologous thin Thiersch skin grafts. Curr Med Pract. 1964;8:218–21.
20. Falabella R. Epidermal grafting: an original technique and its application in achromic areas. Arch Dermatol. 1971;104:592–600.
21. Falabella R. Repigmentation of leucoderma by autologous epidermal grafting. J Dermatol Surg Oncol. 1984;10:136–44.
22. Falabella R, Escobar C, Borrero L. Treatment of refractory and stable vitiligo by transplantation of in vitro cultured epidermal autografts bearing melanocytes. J Am Acad Dermatol. 1992;262(1):230–6.
23. Gauthier Y, Surleve-Bazeille JE. Autologous grafting with non cultured melanocytes: a simplified method for treatment of depigmented lesions. J Am Acad Dermatol. 1992;26:191–4.
24. Olsson M, Juhlin M. Melanocyte transplantation in vitiligo. Lancet. 1992;34(8825):981.
25. Mac Neil S, Eves P. Simplifying the delivery of cultured melanocytes. In: Gupta S, Olsson MJ, Kanwar A, editors. Surgical management of vitiligo. Oxford: Blackwell; 2007. p. 191–201.
26. Singh C, Parsad D, Kanwar AJ, Dogras AS, Kumar R. Comparison between autologous non cultured extracted hair follicle outer root sheath cell suspension and autologous non cultured epidermal cell suspension in the treatment of stable vitiligo: a randomized study. Br J Dermatol. 2013;169(2):287–93.
27. Vanscheidt W, Hunziker T. Repigmentation by outer-root sheath-derived melanocytes: proof of concept in vitiligo and leucoderma. Dermatology. 2009;218(4):342–3.

Definitions and Classification

2

Alain Taïeb and Mauro Picardo

Contents

Abstract

Since the first edition of this textbook, the nomenclature has been revised after an extensive international work within the Vitiligo Global Issues Consensus Conference (VGICC). Vitiligo/nonsegmental vitiligo (NSV) is a consensus umbrella term for all forms of generalized vitiligo. It is an acquired chronic pigmentation disorder characterized by white patches, often symmetrical, which usually increases in size with time, corresponding to a substantial loss of functioning epidermal and sometimes hair follicle melanocytes. The two other subsets of vitiligo defined by the international consensus are segmental vitiligo and unclassified/undetermined vitiligo which correspond to focal disease and rare variants. A series of hypopigmented disorders may masquerade as vitiligo, and some of them need to be ruled out by specific procedures including a skin biopsy.

A. Taïeb (✉)
Department of Dermatology and Pediatric Dermatology, Bordeaux University Hospitals, and INSERM U 1035, University of Bordeaux, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

M. Picardo
Cutaneous Physiopathology and CIRM, San Gallicano Dermatological Institute, IRCCS, Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_2

Key Points

- An international consensus has been reached for the classification of vitiligo in three major categories, namely, vitiligo or nonsegmental vitiligo, segmental vitiligo, and unclassified/undetermined vitiligo.
- Vitiligo/NSV is an acquired chronic pigmentation disorder characterized by white patches, often symmetrical, which usually increases in size with time, corresponding to a substantial loss of functioning epidermal and sometimes hair follicle melanocytes.
- A series of hypopigmented disorders may masquerade as vitiligo, and some of them need to be ruled out by specific procedures including a skin biopsy.

2.1 VETF Position Paper Definition and Classification

Although vitiligo is clinically identified without much difficulty in most instances, difficulties arise when searching for the vitiligo literature due to a lack of consensus in definitions. In order to address this problem, the Vitiligo European Task Force (VETF) proposed after several meetings and workshops a set of consensus statements in 2007, including descriptive definitions, classification, and scoring [1]. For common generalized vitiligo, the definition was as follows: "Vitiligo (nonsegmental or NSV) is an acquired chronic pigmentation disorder characterized by white patches, often symmetrical, which usually increases in size with time, corresponding to a substantial loss of functioning epidermal and sometimes hair follicle melanocytes." As it, the VETF definition (1) was not specific enough and needed to be completed by a list of disorders which may clinically resemble vitiligo but which are clearly attributable to known etiologic factors; (2) it intentionally made no reference to either a single disease or to a syndrome of various causes.

Concerning classification, the simplest one, proposed by Koga [2], distinguishing segmental (SV) and nonsegmental (NSV) forms of disease, served as a pragmatic framework in the 2007 VETF classification, which in addition introduced the recently identified subset mixed vitiligo (MV) [3, 4].

In 2011, the Vitiligo Global Issues Consensus Conference (VGICC) proposed a novel classification and nomenclature, based on a revision of the 2007 VETF classification [Table 2.1]. For the sake of consistency, the definitions used throughout the book are those proposed by the VGICC and reviewed thereafter.

Table 2.1 Bordeaux VGICC classification of vitiligo (2011)

Type of vitiligo	Subtypes	*Consensus statements* and other remarks
Vitiligo (V)/ nonsegmental vitiligo (NSV)	Acrofacial Mucosal (more than one mucosal site) Generalized Universal Mixed (associated with SV) Rare variants	*The term "vitiligo" is the recommended umbrella term for all nonsegmental forms of vitiligo. As a transition, vitiligo/NSV can be used* *Mixed vitiligo, being the coexistence of SV + V, is a subgroup of V* Subtyping may not reflect a distinct nature but is a useful information for epidemiologic studies
Segmental vitiligo (SV)	Uni-, bi-, or plurisegmental	*Segmental vitiligo refers to a clinically unambiguous segmental distribution of depigmented lesions, typically associated with rapid onset and with leukotrichia. There is no consensus concerning the mechanism underlying lesion distribution in SV*
Undetermined/ unclassified vitiligo (UnV)	Focal Mucosal (one site in isolation)	*Focal vitiligo, a term that applies to localized macules characterized by loss of melanocytes, is assigned to the category UnV until more definitive classification can be made on clinical grounds (generally after 1–2 years of follow-up). Cases with long-lasting focal lesions or of pure mucosal vitiligo, if not classified as SV, may remain "unclassifiable"*

2.2 International Consensus on Nomenclature

The VETF, which includes medical experts, scientists, and representatives of patient support groups, drafted a preliminary list of issues of global importance for clinical research and patient management which was sent to even regional working groups were established: North and South Africa, North and South/Central America, Europe, Middle East, Continental Asia/Singapore, Japan/Taiwan, and the Pacific. Classification and nomenclature were the first items selected besides definition of stable disease, definition of Koebner's phenomenon (KP), and significance/individualization of "autoimmune vitiligo."

In January 2011, each group submitted a preliminary contribution addressing the four selected topics. A first round of discussion of the VGICC was held in Seoul, Korea, in May 2011, prior to the 22nd World Congress of Dermatology attended by representatives from all seven regional groups. Following this first meeting, a draft consensus document was prepared and circulated for further comments. The final round of discussion was held in Bordeaux, France, in September 2011 at the 21st IPCC, the draft revised accordingly and published in 2012 [5].

2.3 Key Points of Consensus and Definitions/Glossary

2.3.1 Vitiligo/NSV, A Consensus Umbrella Term for All Forms of Generalized Vitiligo

Although not fully satisfactory, the term "nonsegmental vitiligo" is currently used to describe different clinical subtypes of vitiligo that are all clearly distinct from SV including acrofacial, generalized, mucosal (multifocal), and universal vitiligo. The terms "bilateral" and "unilateral" vitiligo have been used as alternative approaches to classification but raised possible confusion with mixed vitiligo and multisegmental vitiligo (see respective definitions in next sections). The VGICC participants agreed that the term "vulgaris," as synonymous with "common," conveys a pejorative connotation to patients and the general public and should not be used. The validation of the term vitiligo (alone) as a synonym of nonsegmental vitiligo was agreed upon, because it is the most common presentation and that for nonspecialists, it is difficult to understand the negative naming of the disease; however, a test period with the double name vitiligo/NSV was suggested to avoid confusion.

2.3.2 Definitions

The following descriptive consensus statements were proposed in the accompanying glossary of the VGICC consensus [6]:

2.3.2.1 Vitiligo/Nonsegmental Vitiligo Subsets

- **Generalized common vitiligo (formerly referred to as vitiligo vulgaris)**: This most common form of vitiligo is characterized by asymptomatic, well-circumscribed, milky-white macules involving multiple parts of the body, usually in a symmetrical pattern. The disease can start at any site of the body, but the fingers, hands, and face are frequently the initial sites.
- **Acrofacial vitiligo**: In acrofacial vitiligo, the involved sites are usually limited to the face, head, hands, and feet. A distinctive feature is depigmentation of the distal fingers and facial orifices, and a common genital involvement. It may later include other body sites, resulting in typical generalized vitiligo.
- **Vitiligo universalis**: Vitiligo universalis is the most extensive form of the disease and generally occurs in adulthood. "Universalis" is generally used when depigmentation is virtually universal (80–90% of the body surface), but some pigmentation may be still present. Hairs may also be partially spared. Whereas this diagnosis is easy in dark-skinned individuals, it may be difficult in very fair-skinned individuals. Usually, vitiligo universalis is preceded by generalized vitiligo that gradually evolves to complete or near-complete depigmentation of the skin and hair.

2.3.2.2 Segmental Vitiligo

Mono-segmental vitiligo is the most common form of SV, referring to the presence of one or more white depigmented macules distributed on one side of the body, usually respecting the midline (although some lesions may partly cross the midline), early follicular involvement (leukotrichia), and rapid development over a few weeks or months and overall protracted course. Rarely, segmental vitiligo may refer to multiple segmental lesions distributed either unilaterally or bilaterally. The onset may be simultaneous or not. A clear segmental distribution of the lesions with midline demarcation, together with the associated features described in mono-segmental cases (leukotrichia, protracted course), distinguishes this subset versus vitiligo/NSV in bilateral cases.

2.4 Pending Classification Issues/Rare Variants (see Chap. 1.5.4)

The Bordeaux VGICC classification suggests to leave focal cutaneous or mucosal vitiligo within the category undetermined/unclassified vitiligo (UnV) and discusses some rare variants:

- **Focal vitiligo**: Focal vitiligo refers to a small isolated patch that does not fit a segmental distribution and which has not evolved into vitiligo/NSV after a period of at least 2 years. This form of vitiligo may evolve into either SV or vitiligo/NSV.
- **Vitiligo punctata**: Lesions present as sharply demarcated depigmented punctiform 1–1.5 mm macules involving any area of the body, different from hypomelanosis guttata and the hereditary dyschromatoses.
- **Vitiligo minor/hypochromic vitiligo**: This form of NSV is rarely reported. The disease seems to be limited to dark-skinned individuals. The term "minor" does not strictly refer to the limitation of the disease to a restricted surface area but rather to the partial defect in pigmentation. The relation to true vitiligo comes from pathology and coexistence with conventional vitiligo macules. The differential diagnosis from early-stage cutaneous lymphoma is of primary importance, and repeated biopsies with molecular studies of clonality may be needed. A recent case series has been reported under the name "hypochromic vitiligo" [7].
- **Follicular vitiligo**: This refers to a form of generalized vitiligo seen in a young black patient that primarily involved the follicular reservoir with limited skin involvement, contrasting with marked generalized hair whitening, and melanocyte loss in hair follicles [5]. A recent case series has been published which documents in all patients' significant whitening of their body and, in some, scalp hairs before cutaneous depigmentation. Examination revealed classic generalized depigmented lesions of vitiligo and an impressive presence of leukotrichia, not only in vitiliginous areas but also in areas with clinically normal-appearing skin. Punch biopsy specimen of the leukotrichia and vitiligo lesions demonstrated loss of melanocytes and precursors in the basal epidermis and hair follicles [8].

2.5 Differential Diagnosis

2.5.1 Conditions to Exclude from the Definition of Vitiligo/NSV

2.5.1.1 Inherited or Genetically Induced Hypomelanoses

Usually, contrary to vitiligo, hypopigmented patches are present at birth, but in patients of low phototype, hypopigmented patches are usually discovered after the first sun exposure, sometimes in the second or third year of life. The group of genetic disorders to exclude is detailed in Table 2.2 and Figs. 2.1, 2.2, 2.3, and 2.4. Piebaldism may be mistaken for vitiligo when the patient without informative family history comes with symmetrical limb patches without

Table 2.2 Monogenic hypomelanoses

Disorder/OMIM number	Clinical presentation	Transmission/diagnosis
Piebaldism/172800	White forelock, anterior body midline depigmentation, bilateral shin depigmentation	Autosomal dominant. Skin biopsy: usually, the absence of c-Kit protein immunostaining in melanocytes Molecular analysis of *c-KIT* gene or *SNA1* gene
Tuberous sclerosis/191100	Small or larger (ash-leaf) white spots, seizures; other usually later cutaneous symptoms (shagreen patches, angiofibromas, etc.)	Autosomal dominant, brain imaging, cardiac and renal imaging Molecular analysis of TSC1 and 2
Ito's hypomelanosis/300337	Blaschkolinear distribution, uni- or bilateral of hypopigmented streaks	Sporadic Chromosomal or genetic mosaicism (blood or skin cells)
Waardenburg's syndrome/193500 (type I)	White forelock, hypertelorism, deafness (variable according to genotype) Possible association to congenital megacolon (Hirschsprung's disease)	Autosomal dominant Genetic testing according to phenotype (several clinical variants and six possible causative genes mutated)
Hermansky-Pudlak syndrome/203300 (type 1)	Diffuse depigmentation pattern, eye pigment dilution, hemorrhagic diathesis Specific ethnic background	Autosomal dominant, genetic heterogeneity (eight possible causative genes) Molecular testing possible according to ethnic background and phenotype
Menkès syndrome/300011	Hair and body diffuse pigment dilution, pili torti, neurodegenerative changes	X-linked recessive ATP7A gene mutations; affects cupper-dependent enzymes Molecular testing possible
Ziprkowski-Margolis syndrome/300700	Iris heterochromia, depigmentation (diffuse) + hyperpigmented macules can remain, neurosensorial deafness	Described in Israel in one family X-linked recessive Mapped to Xq26.3-q27.1
Griscelli's syndrome 214450 (type 1)	Silvery hairs usually diffuse liver enlargement and symptoms related to immunodeficiency	Autosomal recessive, three types corresponding to three different causative genes Molecular testing possible

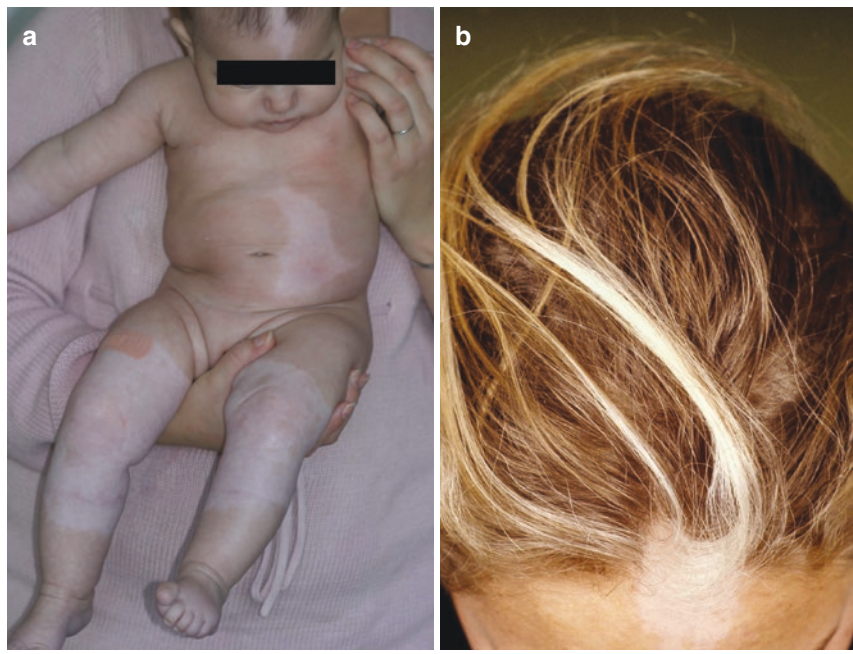


Fig. 2.1 Piebaldism: diffuse form without spontaneous repigmentation (**a**); limited form (white forelock) in the mother (**b**); typical spontaneous repigmentation pattern in an adult (courtesy of Dr Y Gauthier) (**c**); repigmentation the two first years of life (**d**–**f**), becoming clearly trichrome in (**f**)

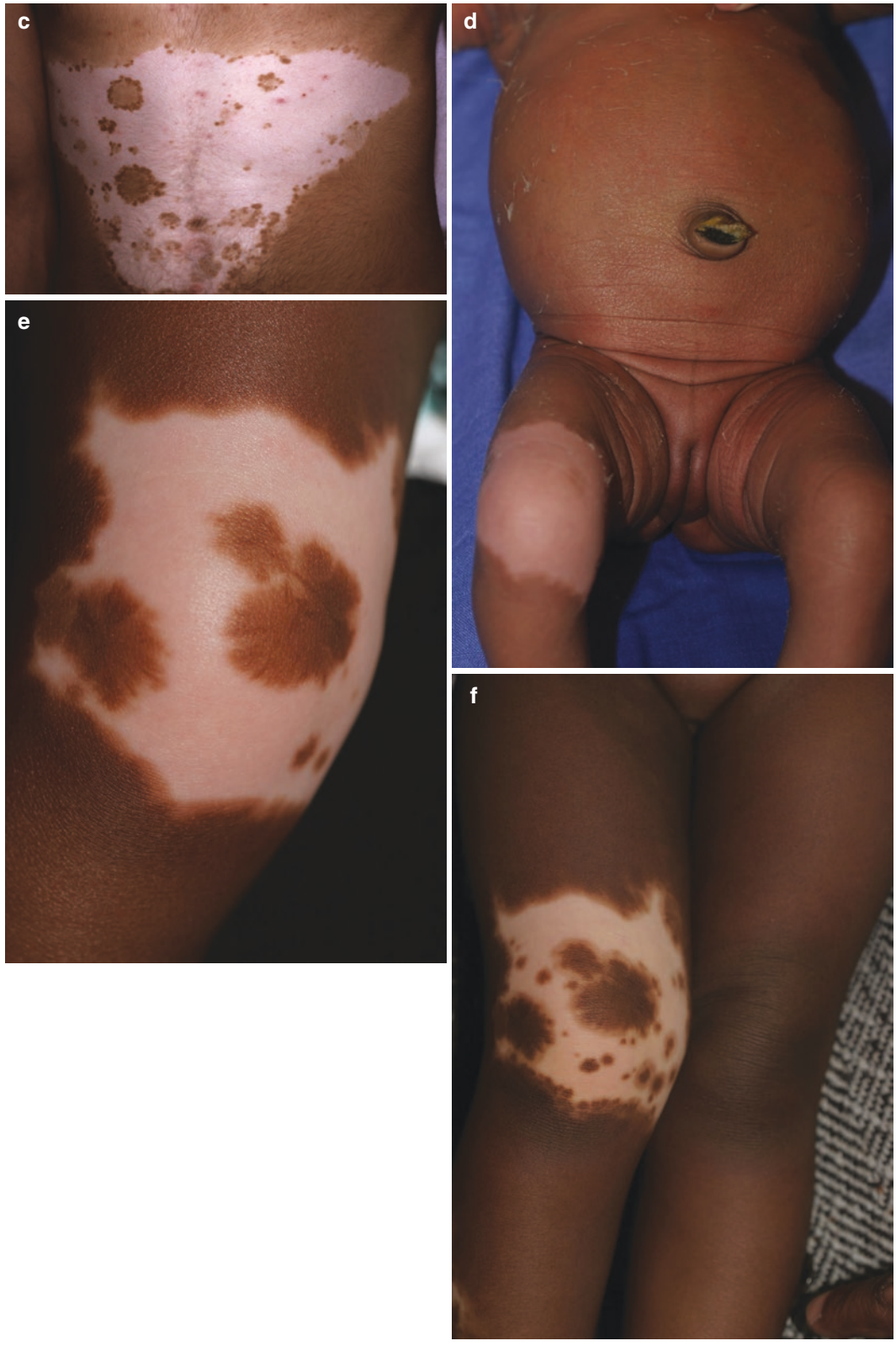


Fig. 2.1 (continued)

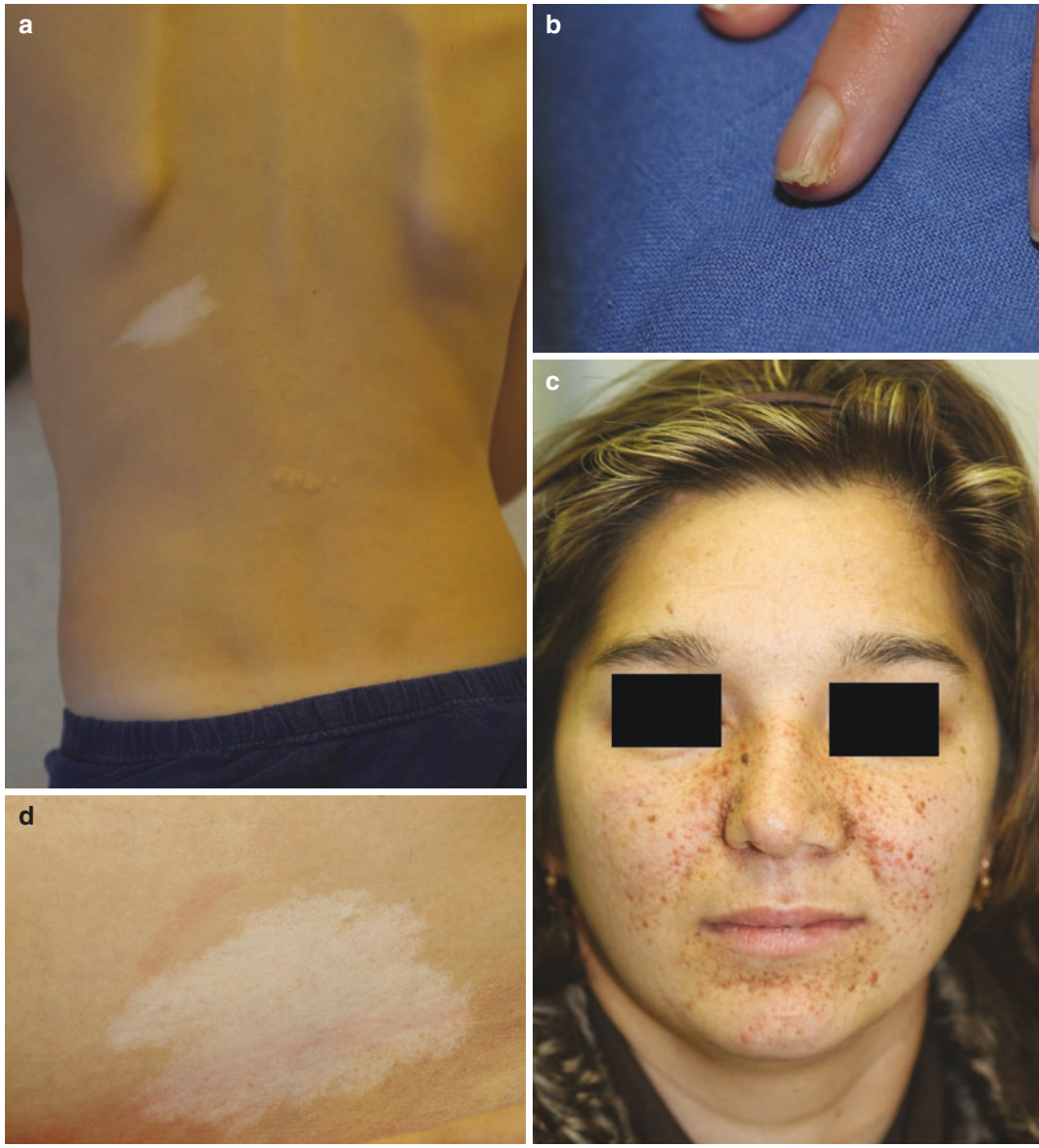


Fig. 2.2 Tuberous sclerosis (**a**) ash-leaf macules and shagreen patch on the dorsum of a young patient; Koenen's periungual tumor in the same patient (**b**); facial angiofibromas (**c**) and large vitiligoid patch (**d**) of the abdominal belt in a 25-year-old female patient

midline anomalies. The hyperpigmented rim at the interface of depigmented and normally pigmented skin is characteristic of vitiligo after sun exposure, but may be also seen in piebaldism, in addition with perifollicular central hyperpigmented patches. Piebaldism shows however a ventral distribution of lesions and early onset. As usual for monogenetic disorders, information about specific ethnic background/consanguinity and a detailed family tree are mandatory.

On the opposite, vitiligo universalis is sometimes misdiagnosed as albinism when the history cannot be obtained properly (Chap. 1.5.1). Classic oculocutaneous albinism with hair depigmentation and nystagmus at birth is not a consideration, but milder syndromic albinisms should be mentioned (Table 2.2). Some rare vitiligo-like or true vitiligo conditions ("syndromic vitiligo") are seen in the context of monogenic disorders. They are reviewed separately in Chap. 1.5.8.

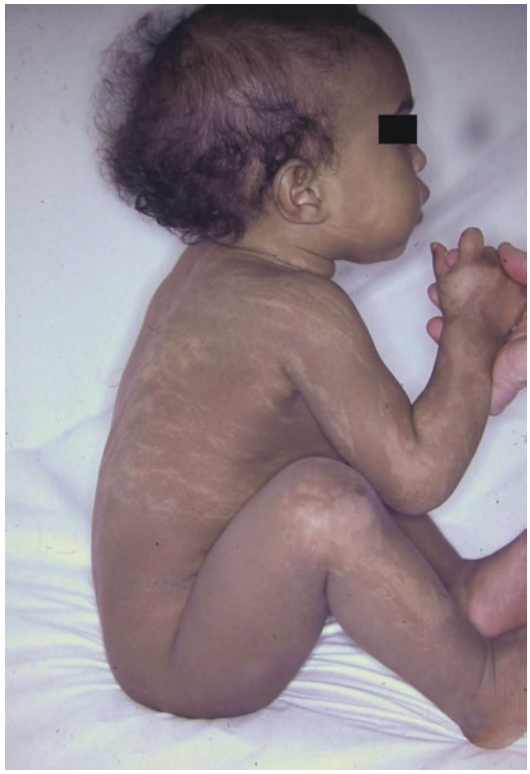


Fig. 2.3 Hypomelanosis of Ito (courtesy of Dr O Enjolras, France). Note the striking blaschkolinear pattern (thin bands of depigmentation following the dorsoventral pattern of skin development)

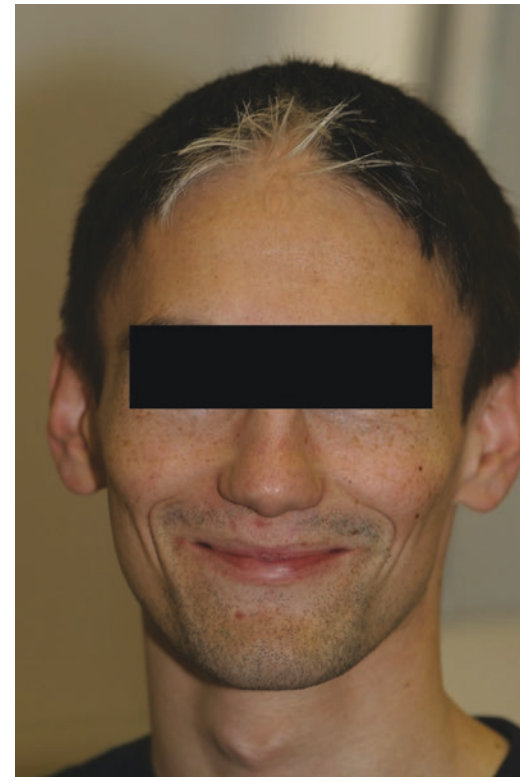


Fig. 2.4 Waardenburg's syndrome

2.5.1.2 Post-inflammatory Hypomelanoses (Fig. 2.5)

There are two main processes for melanin pigment to be eliminated following inflammation. First, in inflammatory disorders accompanied by an increased epidermal turnover (e.g., psoriasis, atopic dermatitis), pigment is lost upwards within eliminated cells, resulting in focal pigment dilution until cessation of inflammation and recovery of melanin production/distribution within the epidermis. Second, when an acute lichenoid/cytotoxic infiltrate attacks the epidermal basal layer (e.g., lichen planus, toxic drug reactions), there is a leakage of epidermal pigment into the superficial dermis ("pigment incontinence"), which is removed when the inflammation has stopped by macrophages ("melanophages"), a process which takes months or years. In vitiligo/NSV there is limited evidence of pigment incontinence, which may occur during the acceleration phase of depigmentation, in the case of mild subclinical lichenoid inflammation (Chap. 1.4). In the majority of cases, a progressive pigment dilution accompanies the disappearance of pigment cells. Distinguishing NSV from post-inflammatory hypomelanoses is usually made on clinical grounds rather than on histopathology, when the primary skin inflammatory disease can be diagnosed clinically (e.g., scalp or plaque psoriasis, flexural dermatitis for atopic dermatitis, scleroderma plaques, lichen sclerosus "white spot disease," etc.). However, true association may occur [9] and sometimes in similar locations, suggesting pathophysiological links, such as koebnerization in the case of pruritic dermatoses followed by vitiliginous patches (see Chap. 1.5.7). In difficult cases, a biopsy can be useful to make an accurate diagnosis, e.g., showing spongiosis (eczema) or psoriasiform changes with neutrophils in the stratum corneum (psoriasis).

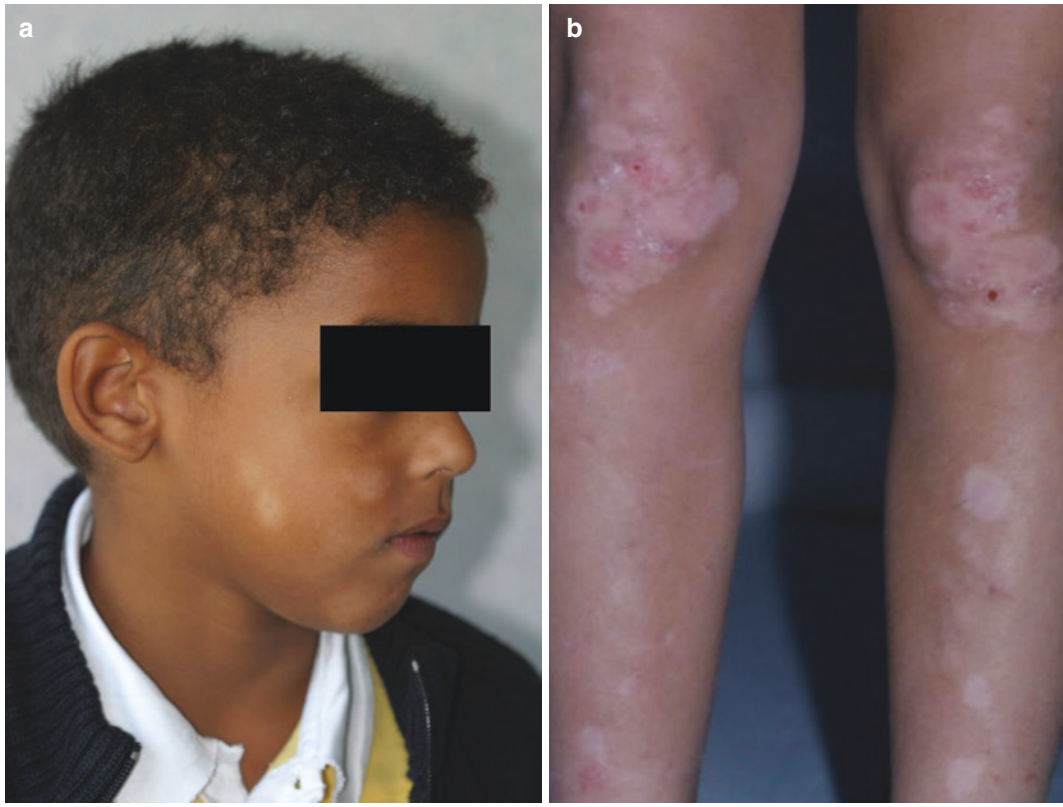


Fig. 2.5 Dermatitis with post-inflammatory hypopigmentation: pityriasis alba in a child with mild atopic dermatitis (**a**); psoriasis (**b**)

2.5.1.3 Para-malignant Hypomelanoses/Mycosis Fungoides (Fig. 2.6)

In dark-skinned patients, skin whitening may correspond to an early stage of epidermotropic T cell lymphoma. When signs of inflammation and skin infiltration are lacking, this presentation can be misleading [10]. A biopsy is able to show diagnostic changes (large size and atypia of epidermotropic lymphocytes especially in the basal layer, Chap. 1.4). The mechanism of pigment dilution in mycosis fungoides has been studied, and a reduced expression of c-Kit has been shown. The loss of c-Kit, and subsequent downstream effects on melanocyte survival, might be initiated by cytotoxic effects of melanosomal-antigen-specific CD8-positive neoplastic T lymphocytes [10].

2.5.1.4 Para-malignant Hypomelanoses/Melanoma-Associated Depigmentation (Fig. 2.7)

The vitiligoid changes associated with melanoma may result from a halo of depigmentation around a cutaneous melanoma (malignant Sutton's phenomenon) to more widespread vitiligoid changes. The margins of such vitiligoid lesions under Wood's lamp are usually less distinct than those of common vitiligo, and depigmentation is usually incomplete [11]. Koebner's phenomenon is usually absent. The prognostic value of depigmentation in the context of melanoma treated with interferon has been established [12]. Chapter 16 discusses the vitiligoid changes after immune checkpoint inhibitors, a frequent side effect since the introduction of these molecules in metastatic melanoma.

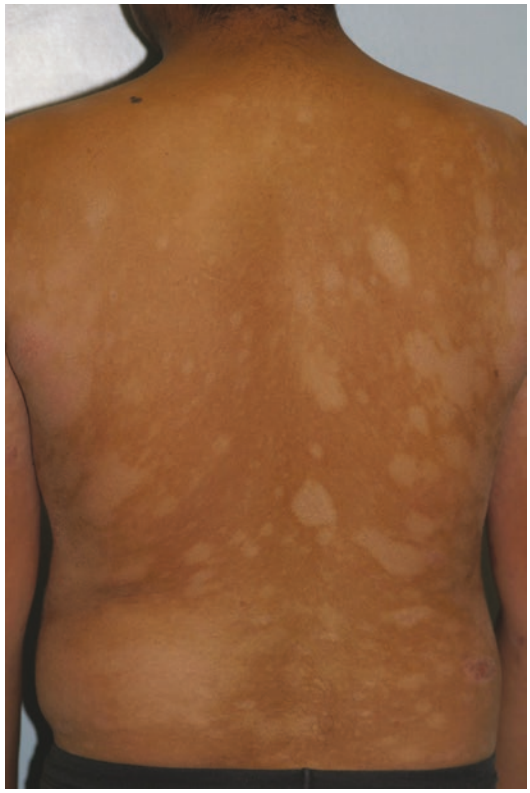


Fig. 2.6 Mycosis fungoides can present as hypopigmented patches in dark-skinned individuals. Histopathology is diagnostic

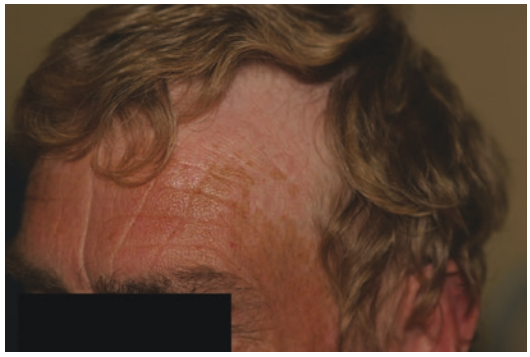


Fig.2.7 Melanoma-associated depigmentation: Melanoma-associated leukoderma is associated with spontaneous or vaccine-induced regression of a primary melanoma

2.5.1.5 Para-infectious Hypopigmentation

Tinea versicolor can cause vitiligoid changes, generally after treatment in the absence of re-exposure to UV light. However, distribution, shape of the lesion, and some scaling and green fluorescence of

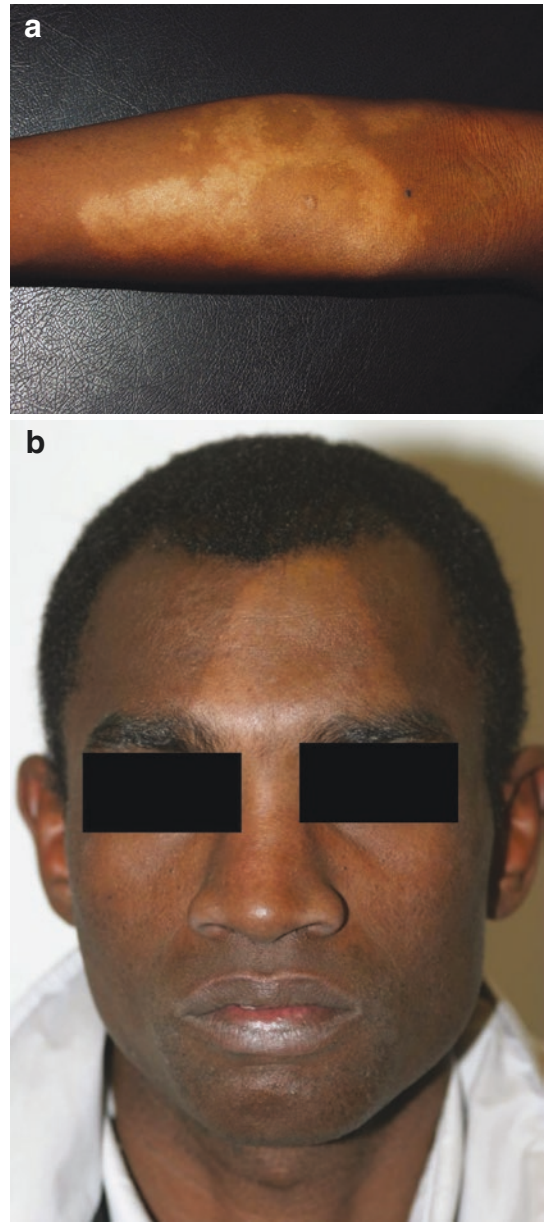


Fig. 2.8 Indeterminate leprosy (**a** elbow, **b** forehead) should be suspected in individuals living in endemic areas. Loss of sensitivity is usually associated

untreated lesions allow a definite diagnosis. Indeterminate leprosy (Fig. 2.8) is manifested by hypochromic patches which are hypoesthetic under light touch. In both cases, the infectious process can inhibit melanogenesis through largely unknown mechanisms. For tinea versicolor, toxic effects on pigment synthesis by fungal metabolites, especially tryptophan-derived metabolites of *M. furfur*,

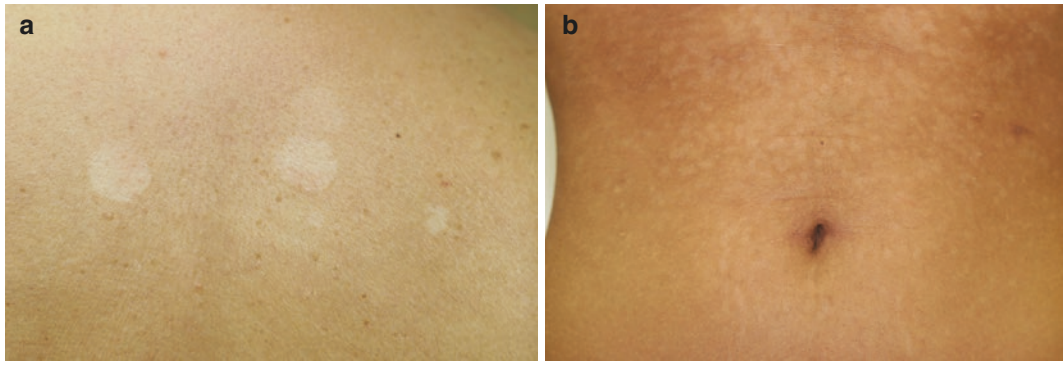


Fig. 2.9 Hypopigmented vitiligoid sequel of pityriasis versicolor (**a**) (upper back) and acquired macular hypomelanosis (**b**) (abdomen) (Guillet-Westerhof syndrome)

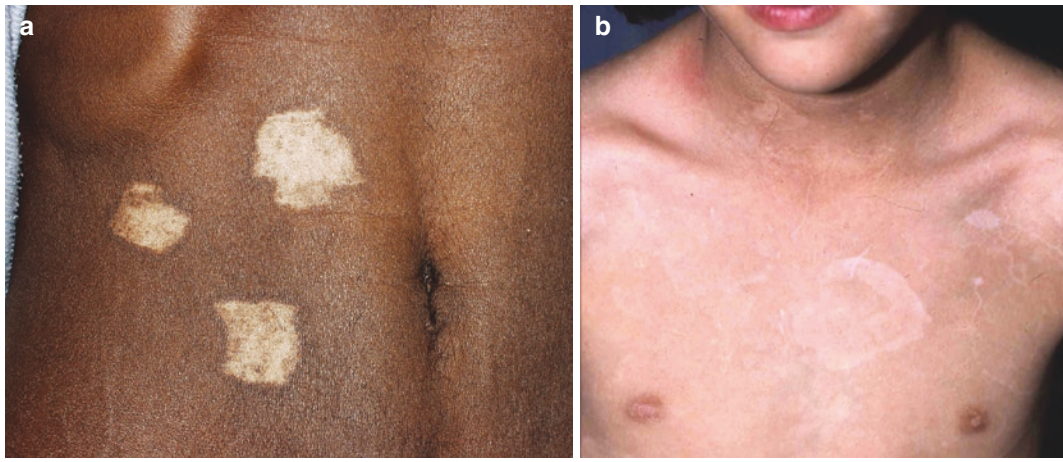


Fig. 2.10 Posttraumatic depigmentation (**a**): note the unusual square limits; (**b**) definitive depigmentation following toxic epidermal necrolysis

have been discussed [13] (Fig. 2.9a). Acquired macular hypomelanosis (Guillet-Westerhof disease) (Fig. 2.9b) is seen in young adults and frequently referred to as a "recalcitrant pityriasis versicolor" [14–16]. The white macules are present on the trunk and more marked on the lower back and axillae. The role of *Propionibacterium acnes* has been based on red spots centering the macules under Wood's lamp, but this finding is not constant. This disease remains largely unknown by dermatologists and is usually not diagnosed but responds well to UVB therapy.

2.5.1.6 Posttraumatic Leukoderma

When the melanocyte reservoir is depleted, as after deep burns or scars, which remove the hair follicles entirely or when the bulge area which contains melanocyte precursors is destroyed, the resulting wound healing process will not recapitulate pigmentation from the center, and marginal repigmentation fails to compensate the loss. It may sometimes be difficult to distinguish some aspects from true vitiligo, when scarring is not obvious. This is the case in depigmenting sequels of toxic epidermal necrolysis (Fig. 2.10) [17].

2.5.1.7 Melasma

This common hypermelanotic disorder is surprisingly a not so rare diagnostic pitfall in the vitiligo clinic when the hyperpigmented facial lesions surround normal but hypochromic-looking skin (Fig. 2.11). Usually the pattern is different from vitiligo, and the examination of other body sites allows a definitive diagnosis.

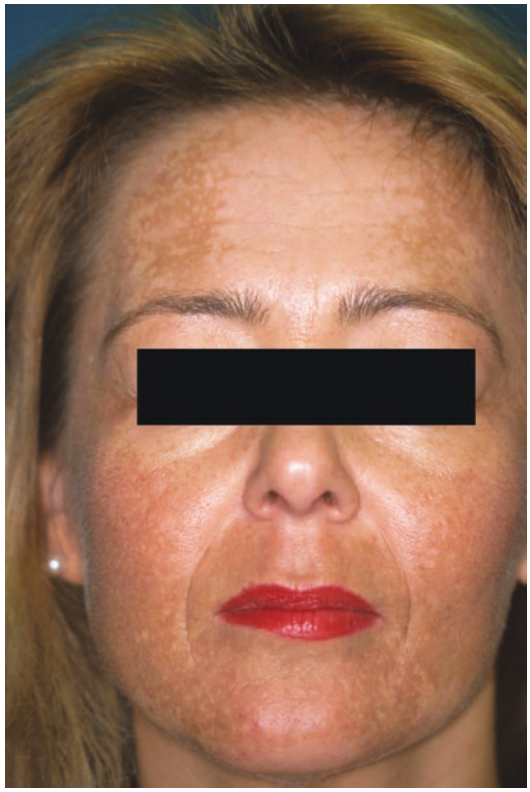


Fig. 2.11 Melasma: a complete skin examination helped by Wood's lamp examination is mandatory in this setting

2.5.1.8 Occupational and Drug-Induced Depigmentation (Chap. 1.5.7)

The so-called occupational vitiligo is a subset of vitiligo triggered by an occupational exposure, which evolves from contact depigmentation caused generally by a phenolic-catecholic derivative to a generalized phenomenon. When depigmentation is widespread, the limits between drug-induced depigmentation and true vitiligo are not easy to delineate. An occupational medicine advice can be helpful. For systemic drugs, except the depigmentation occurring after toxic epidermal necrolysis, which is related to a kind of superficial burn-like injury but also a rare type of universal depigmentation [18], other drugs may rarely cause vitiligoid depigmentation (chloroquine, fluphenazine, physostigmine) [19]. More recently vitiligoid depigmentations following imatinib treatment for chronic myeloid leukemia have been reported and are thought to be mediated via c-Kit signaling downregulation [20], as well as topically induced vitiligoid lesions following imiquimod therapy [21]. The long-term use of potent topical corticosteroids may cause vitiligoid depigmentation (Fig. 2.12).

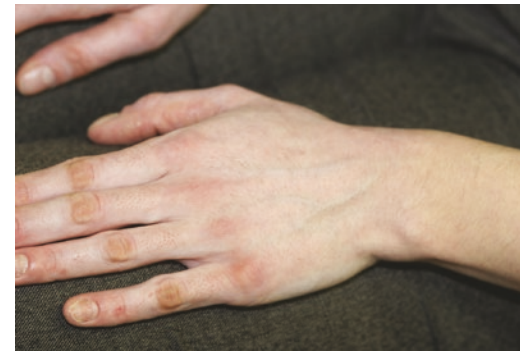


Fig. 2.12 Vitiligoid depigmentation of dorsum of hands in a patient with hand eczema. Note the persistence of pigment on knuckle pads

2.5.2 Conditions to Exclude from the Definition of SV

Although several disorders may pose problems, congenital hypomelanoses of segmental distribution named collectively naevus depigmentosus or achromic nevus [22, 23], which might correspond to somatic mosaic defects of cutaneous pigmentary genes, are the most common differential diagnoses. Naevus depigmentosus is usually congenital or detectable in the first year of life and stable in size in proportion to the child's growth (Fig. 2.13). The lesion usually contains a normal or subnormal number of melanocytes compared with control perilesional skin, but the production of melanin pigment is reduced. Sun exposure may attenuate the difference in pigmentation from the normal skin. In difficult cases, a biopsy is needed to differentiate naevus depigmentosus from SV. Segmental or hemicorporeal hypomelanosis of Ito (Fig. 2.3), which corresponds to another type of cutaneous mosaicism made of narrow depigmented streaks following Blaschko's lines, is not in practice a real differential diagnosis problem for multisegmental vitiligo (Table 2.2).

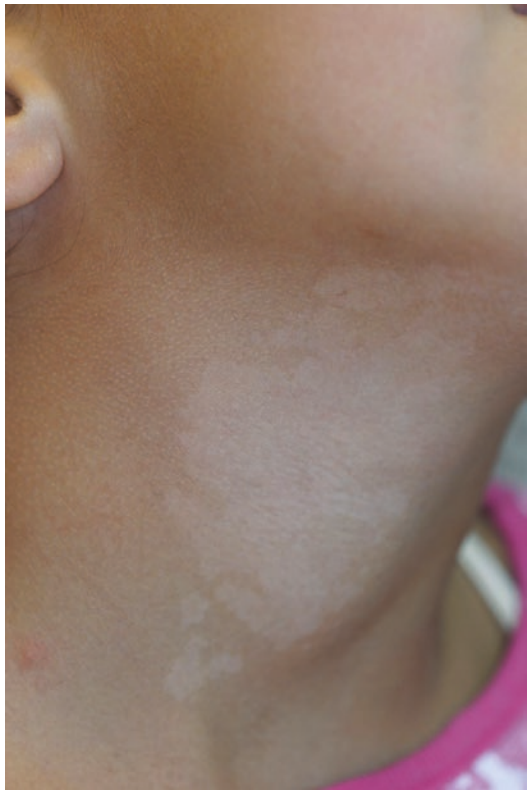


Fig. 2.13 Nevus depigmentosus, a common pitfall for the diagnosis of segmental vitiligo (see text)

References

1. Taïeb A, Picardo M, VETF Members. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell Res. 2007;20:27–35.
2. Koga M. Vitiligo: a new classification and therapy. Br J Dermatol. 1977;97:255–61.
3. Gauthier Y, Cario-Andre M, Taieb A. A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? Pigment Cell Res. 2003;16:322–32.
4. Mulekar SV, Al Issa A, Asaad M, et al. Mixed vitiligo. J Cutan Med Surg. 2006;10:104–7.
5. Ezzedine K, Amazan E, Séneschal J, Cario-André M, Léauté-Labrèze C, Vergier B, Boralevi F, Taieb A. Follicular vitiligo: a new form of vitiligo. Pigment Cell Melanoma Res. 2012;25:527–9.
6. Ezzedine K, Lim HW, Suzuki T, Katayama I, Hamzavi I, Lan CC, Goh BK, Anbar T, Silva de Castro C, Lee AY, Parsad D, van Geel N, Le Poole IC, Oiso N, Benzekri L, Spritz R, Gauthier Y, Hann SK, Picardo M, Taieb A. Vitiligo Global Issue Consensus Conference Panelists. Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. Pigment Cell Melanoma Res. 2012;25:E1–13.
7. Ezzedine K, Mahé A, van Geel N, Cardot-Leccia N, Gauthier Y, Descamps V, Al Issa A, Ly F, Chosidow O, Taïeb A, Passeron T. Hypochromic vitiligo: delineation of a new entity. Br J Dermatol. 2015;172:716–21.
8. Gan EY, Cario-André M, Pain C, Goussot JF, Taïeb A, Seneschal J, Ezzedine K. Follicular vitiligo: a report of 8 cases. J Am Acad Dermatol. 2016;74:1178–84.
9. Berger TG, Kiesewetter F, Maczek C, et al. Psoriasis confined strictly to vitiligo areas--a Koebner-like phenomenon? J Eur Acad Dermatol Venereol. 2006;20:178–83.
10. Singh ZN, Tretiakova MS, Shea CR, Petronic-Rosic VM. Decreased CD117 expression in hypopigmented mycosis fungoides correlates with hypomelanosis: lessons learned from vitiligo. Mod Pathol. 2006;19:1255–60.
11. Hartmann A, Bedenk C, Keikavoussi P, et al. Vitiligo and melanoma-associated hypopigmentation (MAH):shared and discriminative features. J Dtsch Dermatol Ges. 2008;6:1053–9.
12. Gogas H, Ioannovich J, Dafni U, et al. Prognostic significance of autoimmunity during treatment of melanoma with interferon. N Engl J Med. 2006;354:709–18.
13. Thoma W, Krämer HJ, Mayser P. Pityriasis versicolor alba. J Eur Acad Dermatol Venereol. 2005;19:147–52.
14. Guillet G, Helenon R, Gauthier Y, et al. Progressive macular hypomelanosis of the trunk: primary acquired hypopigmentation. J Cutan Pathol. 1988;15:286–9.
15. Relyveld GN, Dingemans KP, Menke HE, et al. Ultrastructural findings in progressive macular hypomelanosis indicate decreased melanin production. J Eur Acad Dermatol Venereol. 2008;22:568–74.
16. Westerhof W, Relyveld GN, Kingswijk MM, et al. Propionibacterium acnes and the pathogenesis of progressive macular hypomelanosis. Arch Dermatol. 2004;140:210–4.
17. Oplatek A, Brown K, Sen S, et al. Long-term follow-up of patients treated for toxic epidermal necrolysis. J Burn Care Res. 2006;27:26–33.
18. Smith DA, Burgdorf WH. Universal cutaneous depigmentation following phenytoin-induced toxic epidermal necrolysis. J Am Acad Dermatol. 1984;10:106–9.
19. Boissy RE, Manga P. On the etiology of contact/occupational vitiligo. Pigment Cell Res. 2004;17:208–14.
20. Cario-André M, Ardilouze L, Pain C, et al. Imatinib mesilate inhibits melanogenesis in vitro. Br J Dermatol. 2006;155:493–4.
21. Mashiah J, Brenner S. Possible mechanisms in the induction of vitiligo-like hypopigmentation by topical imiquimod. Clin Exp Dermatol. 2008;33:74–6.
22. Hann SK, Lee HJ. Segmental vitiligo: clinical findings in 208 patients. J Am Acad Dermatol. 1996;35:671–4.
23. Lee HS, Chun YS, Hann SK. Nevus depigmentosus: clinical features and histopathologic characteristics in 67 patients. J Am Acad Dermatol. 1999;40:21–6.

Vitiligo: Histopathology, Including Electron Microscopy

3

Carlo Cota and Daniela Kovacs

Contents

Abstract

The diagnosis of vitiligo is mainly based on clinical features; thus the role of the histological evaluation is generally poorly considered. However, the increasing interest in predicting the stability of the disease has reawakened awareness of the histopathological features of vitiligo. Histopathologically, both epidermal and dermal changes characterize vitiligo, mainly in relation to the activity and duration of the disease. Microscopical alterations of vitiligo may be subtle and overlooked in routine practice; thus a clinicopathological correlation is crucial for a proper diagnosis.

C. Cota (✉)
Dermatopathology Unit, San Gallicano Dermatologic Institute (IRCCS), Rome, Italy
e-mail: carlo.cota@ifo.gov.it

D. Kovacs
Laboratory of Cutaneous Physiopathology and Integrated Center of Metabolomics Research, San Gallicano Dermatologic Institute (IRCCS), Rome, Italy

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_3

Key Points

- Lack of melanocytes and reduction/absence of melanin in long-lasting lesions of stable disease
- Clear cells in semi-thin sections in long-lasting disease (25 years), associated with positive DOPA reaction (residual melanocytes in depigmented lesional skin)
- Progressive reduction of melanocytes along the lesional borders, with residual melanocytes at the edges of the lesions
- Focal spongiosis at the basal layer or the Malpighian layers of the epidermis, along with epidermal mononuclear cell infiltration and dermal perivascular lymphocytic infiltrates, in advancing edge of actively spreading or inflammatory vitiligo
- Increased number/activity of melanocytes and reduction of degenerative features in both melanocytes and keratinocytes after phototherapy
- "Microdepigmentation" (loss of melanocytes and T cell epidermal infiltration) in areas of macroscopically uninvolved skin of active vitiligo patients
- Perilesional melanocytes well connected to the neighboring cells by long dendrites in stable disease vs. shorter dendrites and weak connection to surrounding keratinocytes in active form (electron microscopy)
- Keratinocytes with focal desmosome interruptions, few melanosomes and extracellular granular material, and swollen mitochondria in the active disease (electron microscopy)

3.1 Introduction

The diagnosis of vitiligo is mainly based on clinical features; thus the role of the histological evaluation is generally discussed. In some instances, a skin biopsy is useful to differentiate a vitiliginous lesion from other hypopigmented disorders, especially in its early spreading stage [1]. The increasing interest in predicting the stability of the disease has reawakened awareness of the histopathological features of vitiligo. Several morphological abnormalities of lesional and perilesional areas have been highlighted by routine histology, histochemical/immunohistochemical stainings, and electron microscopy. In addition, the presence of widespread cutaneous alterations has also been described in normally pigmented areas of vitiligo patients, focusing interest on normally appearing skin.

Histopathologically, both epidermal and dermal changes characterize vitiligo, mainly in relation to the activity and duration of the disease. Indeed, early-stage/unstable progressive vitiligo is expected to show more prominent morphological changes than the stable disease [2]. In this context, biopsy site selection is pivotal. If the disease is stable, the biopsy should be performed on the margin of a representative macula, including adjacent normal-appearing skin in order to compare the two areas histologically. In the case of unstable spreading vitiligo, a biopsy including the edges of a newly appeared macula or of a lesion with a recently increased size will be preferred [2, 3].

One should remember that microscopical alterations of vitiligo may be subtle and overlooked in routine practice; thus a clinico-pathological correlation is crucial for a proper diagnosis.

3.2 Histopathology of Lesional, Marginal, and Perilesional Skin

The loss of melanocytes in the basal layer of the lesional epidermis is the hallmark of vitiligo, varying on the life of the lesion [3].

Long-lasting lesions of stable disease commonly show a lack of melanocytes associated with a reduction or absence of melanin (Fig. 3.1). If basal melanocytes of normally pigmented epidermis typically appear as clear cells due to fixation by routine histology, depigmented vitiliginous lesions display a deficit of clear cells and an absence of DOPA reaction, which allows for the detection of functional melanin-producing melanocytes (Table 3.1). Fontana-Masson silver staining

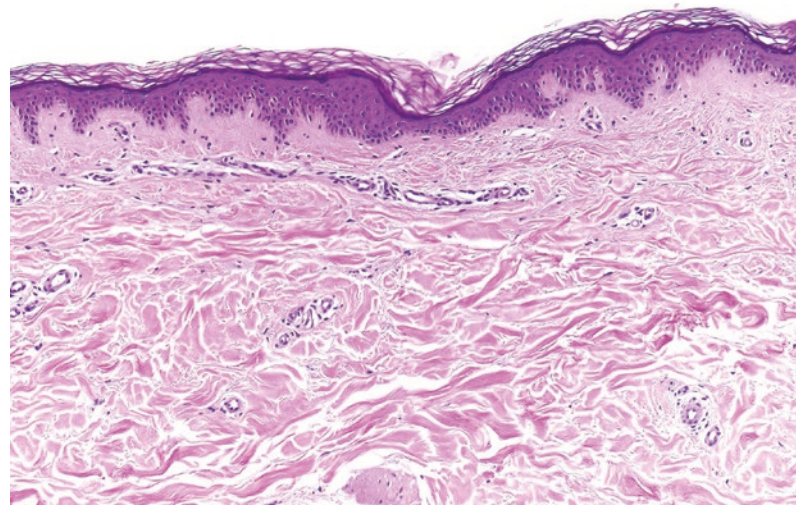


Fig. 3.1 Histopathological examination of stable lesional vitiligo shows the lack of melanocytes and melanin in the epidermis and the absence of dermal inflammatory infiltrate (HE 100×)

Table 3.1 Special stains and antibodies to detect melanocytes

Special stains	Staining target	Staining pattern	References
Fontana-Masson	Melanin	Black silver precipitates	[10]
DOPA reaction	Melanocytes (enzymatically active)	Brown black, cytoplasmic	[47]
Antibodies			
Microphtalmia transcription factor (MITF)	Melanocytes: it may be expressed by macrophages, lymphocytes, fibroblasts, Schwann cells, and smooth muscle cells	Nuclear	[48, 49]
Tyrosinase (T311, MAT-1)	Melanocytes, melanocytic differentiation marker	Cytoplasmic	[50, 51]
MART1/melanA Melanoma antigen recognized by T cells-1 (A103, M2-7C10)	Melanocytes, melanosomal component	Cytoplasmic	[52, 53]
S100 protein	Melanocytes, family of calcium-binding proteins Low specificity: Langerhans cells, histiocytes, Schwann cells, and sweat glands	Nuclear and cytoplasmic	[47]
c-Kit, stem cell factor receptor	Melanocytes. Not specific, it recognizes also germ cells, epithelial cells (breast and kidney), mast cells, and several other hematopoetic cell types	Cell surface	[54]
Tyrosinase-related protein 1 (TRP1, TYRP1)	Melanocytes Melanocytic differentiation marker		[50]
Dopachrome tautomerase-/ tyrosinase-related protein 2 (DCT, TRP2)	Melanocytes and melanoblasts		[50, 55]
HMB-45 glycoprotein 100 (gp100)	Fetal, neonatal, and stimulated adult melanocytes, associated to immature, pre-melanosomes It may react with cells of angiomyolipoma and lesions of the PEComa group of neoplasms	Cytoplasmic	[56, 57]
NKI-beteb PMEL17/gp100 melanosome-associated antigen	Melanocytes	Cytoplasmic	[58]

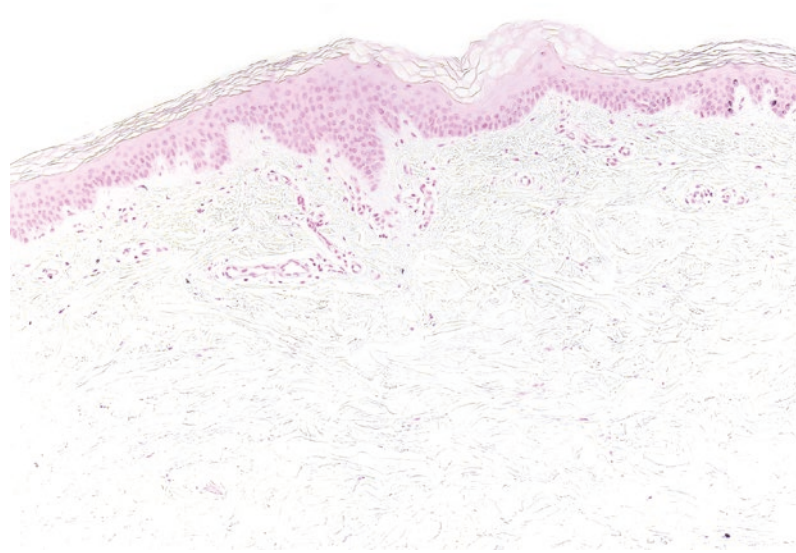


Fig. 3.2 Cutaneous biopsy of lesional skin shows the absence of melanin in the basal layer of the epidermis, as assessed by Fontana-Masson stain (FM 100×)

corroborates the loss of melanin in the epidermal basal layer of these patients (Fig. 3.2) (Table 3.1). However, the identification of clear cells in semi-thin sections of biopsies from patients with even long-lasting disease (25 years) associated with positive DOPA reaction has suggested the presence of residual melanocytes in depigmented lesional skin, although no reactivity for melanocyte markers has been detected [4]. In line with these observations, Anbar et al. found scanty melanocytes by H&E in the center of vitiligous areas in 3 out of 30 biopsies evaluated [5]. These cells did not stain with DOPA or HMB45 marker, suggesting the inactivity of these cells. In the same work, most of marginal melanocytes, stained with DOPA or HMB45, show retracted dendrites, whereas perilesional ones display normal dendrites [5]. By studying 16 patients with progressive and stable disease, Kubanov et al. demonstrated melanocytes and melanin granules in lesional skin, and Kim et al. found melanocytes in lesions lasting 5 or more years [6, 7]. If the follicular melanocytes are not usually involved in the early stages of the disease, they appear to be the first target in follicular vitiligo, reinforcing the hypothesis that follicular and epidermal melanocytes probably exhibit different antigenic profiles [8]. The progressive reduction in the number of melanocytes has been observed along the lesional borders [9], with residual melanocytes at the edges of the lesions (Fig. 3.3). The latter are described as larger, with low pigmentation and with longer, pronouncedly arborized, dendrites [10]. Degenerative changes and fragmentation of these cells in marginal skin have also been reported by Abdel-Naser et al. and Van den Wijngaard et al. [9, 11]. Additional epidermal changes appear scant in established lesions, whereas they are most frequently correlated with an unstable, clinically dynamic, disease, being more evident in the active margin of the lesions.

Sparse to mild lymphocytic infiltration may be seen in the papillary dermis, sometimes with a lichenoid interface dermatitis pattern. Lymphocytes at the basal layer of the epidermis may be arranged as "small lymphocytic nests" or "focal lichenoid infiltrates," and, in many cases, they surround or are in close vicinity to melanocytes [12]. Epidermal-clustered lymphocytes may also show epidermotropism and mimic small Pautrier microabscess observed in mycosis fungoides, thus prompting a misdiagnosis.

Focal spongiosis involving the basal layer or the Malpighian layers of the epidermis, along with epidermal mononuclear cell infiltration and dermal perivascular lymphocytic infiltrates, may be observed in biopsy specimens taken from the advancing edge of actively spreading or inflammatory vitiligo (stage I lesions—onset

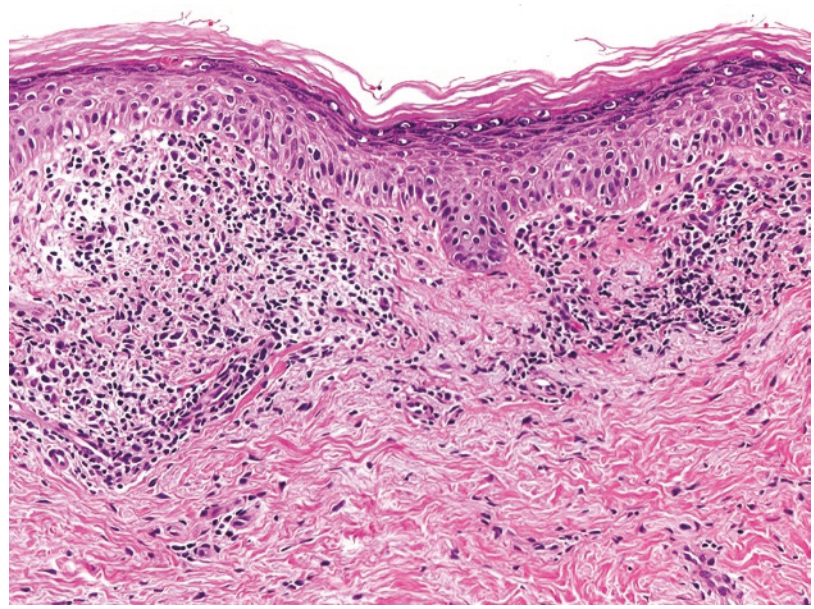


Fig. 3.3 Skin biopsy of lesional/marginal and perilesional skin shows the progressive reduction in the number of melanocytes with residual melanocytic cells at the edges of the lesions, as demonstrated by immunohistochemical reactivity for Melan-A (Melan-A 25×). Courtesy of Prof. L. Cerroni, "Dermatopathology Unit, Department of Dermatology, Medical University of Graz" Graz, Austria

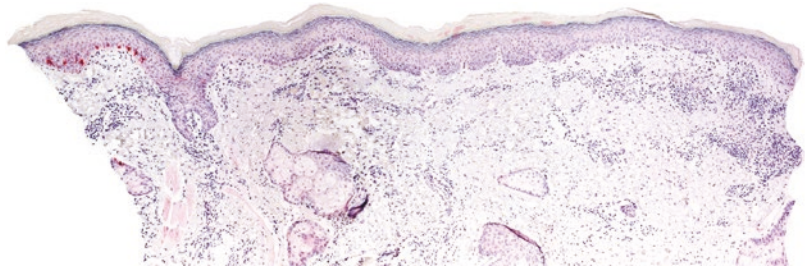


Fig. 3.4 Cutaneous biopsy of the advancing edge of actively spreading or inflammatory vitiligo shows focal spongiosis of the basal layer of the epidermis, as well as dermal, mainly perivascular, lymphocytic infiltrates. Exocytosis of mononuclear cells may also be seen (HE 200×). Courtesy of Prof. L. Cerroni, "Dermatopathology Unit, Department of Dermatology, Medical University of Graz" Graz, Austria

3–13 weeks) (Fig. 3.4). This has been demonstrated by evaluating epon-embedded sections from 25 patients [13]. The inflammatory pattern can be interpreted as non-specific, with no proper clinical information, and easily misdiagnosed as spongiotic or lichenoid disorders.

In thinner sections, a focal but severe vacuolar degeneration of basal keratinocytes mainly observed around dermal papillae as well as a thickening of the basement membrane have been described [14, 15]. Thickening of the stratum corneum and underlying epidermis associated with an elongation of the basement membrane (defined as the result of an increased number and/or length of epidermal rete ridges) has been reported in the central area of vitiliginous patches as opposed to the periphery, as a possible reparative/protective phenomenon in a study of 33 Greek patients [16]. A recent study has demonstrated an increased thickening of the stratum corneum of viable epidermis (defined as the thickness measured from the stratum granulosum to the basal layer) and of the total epidermis in vitiliginous lesions compared to adjacent normal-appearing skin [17]. These features were statistically significant in sun-exposed areas, suggesting a protective role against recurrent UV exposure [17]. Although we usually observe an increased number of Langerhans cells in the lesional area of vitiligo, conflicting observations regarding the

presence of these dendritic cells have been reported. A reduced [18], an increased [17], or an unmodified [19, 20] amount have been observed in different studies. A tendency to a higher number of Langerhans cells along the basement membrane has been confirmed by Le Poole et al. [9], and the hypothesis that these cells may replace lacking basal epidermal melanocytes has been put forth [21].

As concerns the dermal compartment, perivascular lymphocytic infiltrate in the upper dermis has been described in recently spreading lesions, whereas no inflammatory infiltrate is seldom noticed in established depigmented skin areas [13]. Melanophages as well as inflammatory and degenerative changes in sweat glands, dermal nerves, and nerve endings have also been observed [1, 22]. An increased number of dermal blood vessels with angioneogenesis accompanied the epidermal hyperplasia as a possible reparative/protective phenomenon in a study of 33 Greek patients [16].

In evaluating the histopathological features of 66 patients with stable and unstable vitiligo, Yadav et al. recently showed that five parameters—spongiosis, basal layer vacuolization, epidermal/dermal lymphocytes, and melanophages—were more recurrent in the unstable disease compared to the stable disease, suggesting these variables to be potential values of the disease activity [2]. In an attempt to correlate the clinical aspect to the histological features of vitiligo, Benzekri et al. distinguished hypomelanotic lesions with poorly defined borders and amelanotyc lesions with sharply demarcated borders [23]. The former were the expression of active spreading vitiligo characterized histopathologically by a progressive loss of melanocytes, epidermal spongiosis, vacuolar degeneration of basal cells, clustered lymphocytic infiltration, and dermal melanophages. On the contrary, amelanotic lesions with sharply demarcated borders were the clinical picture of a stable disease, featured by the normal epidermis, in the majority of cases, with a well-defined depigmentation and scant inflammatory infiltrates.

The histopathological evaluation of skin biopsies following therapies, such as phototherapy, shows an increase in the number and activity of melanocytes as well as a reduction of degenerative features in both melanocytes and keratinocytes [5]. In our experience, the repigmentation observed after punch grafts is due to the horizontal migration of activated melanocytes from donor sites through the enlargement of the intercellular spaces and the release of pro-melanogenic mediators. The regular function of these melanocytes has been proven by the reactivity for MiTF, c-Kit, MART1, and TRP1 as well as by the presence of melanin in the neighboring keratinocytes [24].

3.3 Histopathology of Normal-Appearing Skin

In our experience, we have not observed significant differences in the amount and distribution of melanocytes between normal-appearing vitiligo skin and healthy control, in agreement with other studies [9, 25]. However, "microdepigmentation," consisting of the loss of melanocytes in the presence of a parallel T cell epidermal infiltration, has been identified in areas of macroscopically uninvolved skin of active vitiligo patients [26]. Analyzing histological changes in skin distant from depigmented areas, Aslanian and coworkers also showed a decreased pigmentation of the epidermal basal layer as well as dermal pigment incontinence with the presence of melanophages [27].

The presence of melanocytes located suprabasally is occasionally detected in uninvolved skin areas (Fig. 3.5). This observation supports the melanocytorrhagy theory, which has been proposed as one of the mechanisms responsible for melanocyte loss in vitiligo. Based on this hypothesis, melanocyte disappearance is caused by chronic detachment and transepidermal loss due to abnormal responses to mechanical stresses, such as friction or other traumas [28]. Besides the presence of alteration related to melanocytes and pigmentation, keratinocytes may be involved. In semi-thin sections, mostly at the basal layer, keratinocytes showed degenerative changes in areas distant from depigmented skin [14]. On the other hand, Gokhale and Mehta did not observe

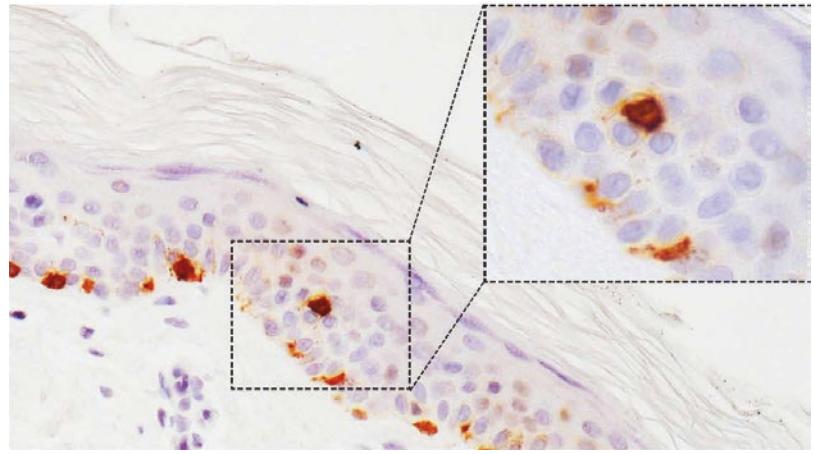


Fig. 3.5 Normal-appearing skin shows the presence of melanocytes located suprabasally, as observed with Melan-A immunostaining (Melan-A 200×, enlarged view 400×). Observation from Dermatopatology Laboratory, San Gallicano Dermatological Institute, Rome

any degenerative or inflammatory modifications or the presence of suprabasal clear cells in normal-appearing vitiligo skin [22].

3.4 Special Stains and Immunohistochemistry in Vitiligo

The detection of melanocytes and the nature of inflammatory infiltrates have been studied by immunohistochemistry.

Several authors performed immunohistochemical analyses using antibodies directed at different target antigens, with the aim of identifying and possibly characterizing melanocytes of vitiligo skin. Employing a large panel of antibodies directed at distinct melanocyte-related antigens (one polyclonal and 17 monoclonal antibodies), Le Poole and co-workers showed a loss of melanocytes in lesional areas and no significant differences in the number of cells between non-lesional and control skin [25]. Norris et al. showed an absence of melanocytes positively stained for the stem cell factor receptor c-KIT in vitiliginous skin [29]. Other studies show that melanocytes may be sporadically present in some depigmented skin, even in stable, long-lasting lesions, as reported by Tobin et al. who found rare clear cells and positive DOPA reaction in the epidermis of vitiligo patients with stable disease lasting 25 years. However, no reactivity for the melanocyte markers NKI-beteb or HMB-45 was detected [4]. Kubanov et al. observed Melan-A-positive cells and melanin granules in depigmented skin of patients with a disease duration ranging from 21 months to 40 years [6]. The hypothesis that these residual melanocytes may be functional has also been proposed [7]. Anbar et al. detected a few melanocytes in lesional areas of 3 out of 30 biopsies analyzed by routine E&E staining and electron microscopy [5]. However, these cells did not react with DOPA or HMB-45 antibody by immunohistochemistry, thus suggesting their inactivity. Melanocytes positive for DOPA and HMB-45 are instead detected in marginal and perilesional areas [5]. A decrease of positive melanocytes was also observed in perilesional normal skin compared to that of healthy controls, when using the Melan-A antibody [6]. Melanocyte-specific markers have also been employed to analyze the number and distribution of melanocytes in normal-appearing skin. Van den Wijngaard and co-workers demonstrated a similar amount and distribution of melanocytes in non-lesional vitiligo skin compared to normal skin, using NKI-beteb antibody by immunohistochemistry [9]. Similarly, Wagner et al. did not observe any differences in the overall number of melanocytes in vitiligo normal-appearing skin compared to the normal control utilizing the Trp2 melanocyte marker [30]. However, the authors report the presence of melanocytes located in the upper epidermal layers and demonstrate more basal keratinocytes between two melanocytes, pointing to an

irregular ratio in the number of cells forming the epidermal melanin unit [30].

The inflammatory infiltrate, as shown by histopathological examination, depends on the life of the lesions. Immunohistochemical analysis of the lymphocytic infiltrate by Al Badri et al. showed, in keeping with the histology, a greater number of epidermal and dermal CD3+, CD4+, and CD8+ cells at the margins of depigmented skin [31]. Later studies have focused on the differences between stable and unstable diseases. Ahn et al. demonstrated the higher expression of ICAM1 and CD4 in the epidermis of actively spreading vitiligo compared to the stable disease [32]. No differences were found regarding CD8 and HLA-DR. However, several studies have shown an increase in the CD8 T lymphocytes in active perilesional skin of unstable disease [33, 34]. These cells are located in the epidermis/papillary dermis, often juxtaposed to the remaining melanocytes [33]. Clustered CLA+ lymphocytes at the epidermal/dermal junction may express cytotoxic markers, such as perforin and granzyme-B at the site of melanocyte destruction [9]. In addition, Le Poole et al. also found a high number of CD68+ macrophages in the papillary dermis of perilesional and lesional skin [9].

Finally, Kim et al. measured the dermal inflammation, by quantitative analysis, observing that CD3+ cells accounted for 65.38% of the inflammatory cells, whereas CD20+, CD68+, and CD1a+ cells accounted for 5.67%, 13.5%, and 1.89%, respectively [7].

3.5 Electron Microscopy: Lesional, Marginal, and Perilesional Skin

As for light microscopy, ultrastructural features of vitiligo skin may differ according to the active progressing or stable stage of the disease.

3.6 Melanocytes

Divergent conclusions have been reported regarding the presence of melanocytes in depigmented areas. Several studies have described the loss of these cells in the central region of lesional skin [35–39]. However, Tobin et al. hypothesized the presence of residual altered functioning melanocytes as the source of pigment granules detected in basal and suprabasal keratinocytes of lesional areas [4]. Residual melanocytes with poorly melanized melanosomes, vacuolization, and dilated rough endoplasmic reticulum have also been demonstrated by Kim et al. [7]. In early active spreading vitiligo, residual melanocytes in the center and in the edges of the macular lesions may show cytoplasm vacuolization, fatty degeneration, pyknosis, and atypical/small melanosomes. Sometimes, apparently normal melanocytes have been observed at the margins of the lesions [36]. Breathnach et al. have described many melanocytes on the pigmented side of the margin with minimal melanogenic activity and more evident filamentous structures in the cytoplasm [35]. In addition, melanocytes located in the perilesional skin have been reported to show electron-lucent vacuoles [39, 40] (Fig. 3.6).

In contrast to stable disease, where perilesional melanocytes appeared well connected to the neighboring cells by long dendrites, melanocytes from active spreading disease showed shorter dendrites and a weak connection to surrounding keratinocytes, as demonstrated by Ding and co-workers. Mitochondria appeared swollen with obscure cristae, irregular and vacuolated, mainly in active lesions [41]. While Prignano et al. observed well-preserved perilesional melanocytes (with abundant organelles, well-preserved melanosomes mainly at the second, third, and fourth stages of differentiation but enlarged mitochondria), Anbar et al. reported degenerative changes in melanocytes and keratinocytes located in marginal and perilesional vitiliginous areas. These cells showed cytoplasmic vacuolization, pyknotic nuclei, and peripheral margination of nuclear chromatin [5, 37].

3.7 Keratinocytes

Regarding the ultrastructural features of lesional keratinocytes, Galadari et al. did not find any alterations in the center of the lesions [36]. In line

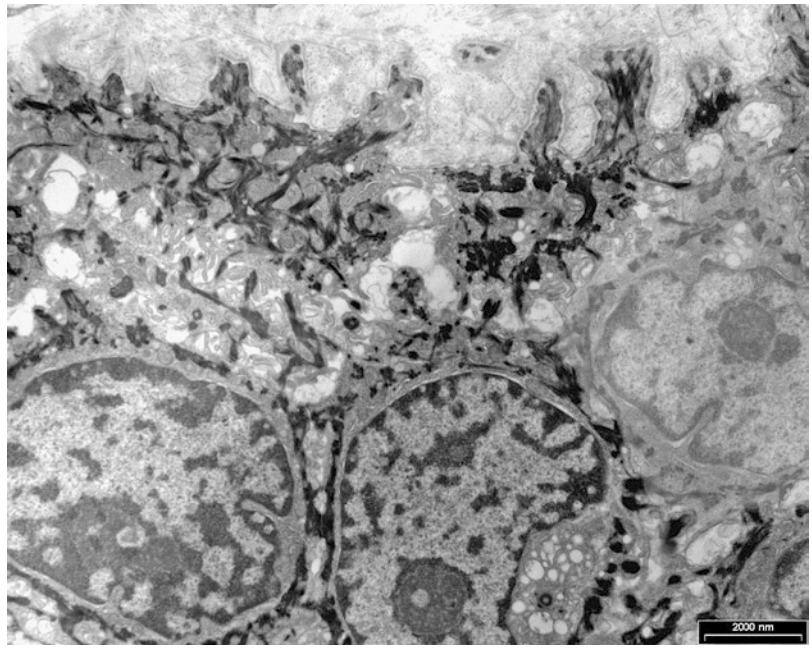


Fig. 3.6 Ultrastructural analysis of lesional vitiligo skin showing the absence of melanosomes inside keratinocytes and mild spongiosis. Courtesy of Prof. M.R. Torrisi and Dr. S. Raffa, Cellular Diagnostics Unit, Sant'Andrea Hospital; Dept. of Clinical and Molecular Medicine, Sapienza University of Rome, Italy

with these observations, Prignano et al. described lesional keratinocytes with no cytoskeletal alterations or organelle rearrangement, except for some lighter areas possibly representing vacuoles [37]. On the contrary, other authors noticed a varied level of degeneration in lesional keratinocytes, including irregular nuclei, alterations in the cytoplasm consisting of dilated Golgi complex and/or endoplasmic reticulum, vacuolar degeneration, and fragmented tonofilaments [7, 42]. Ding et al. also described keratinocytes with focal desmosome interruptions, few melanosomes associated with the presence of extracellular granular material and swollen mitochondria, mainly in the active disease [41] (Fig. 3.7).

As demonstrated by Anbar et al., lesional, marginal, and perilesional keratinocytes exhibited dilatation of the rough endoplasmic reticulum, cytoplasmic vacuoles, peripheral margination of the fragmented tonofilaments, pyknosis, peripheral margination of the nuclear chromatin, and an absence of nucleoli. Extracellular granular material (EGM) located in the dilated intercellular space between keratinocytes has also been observed [5]. Moellmann et al., who observed these abnormalities more abundantly in rapidly progressing or stable disease compared to treated and repigmenting patients, had already described

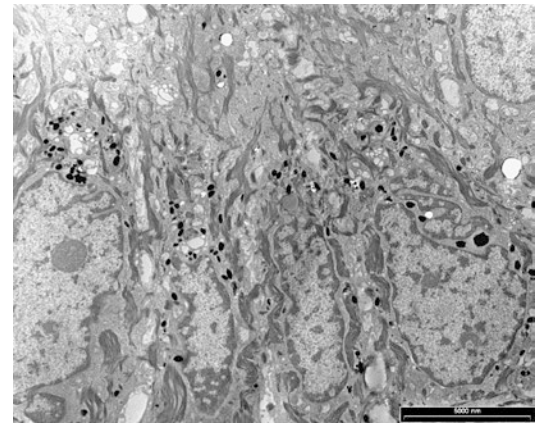


Fig. 3.7 Ultrastructural analysis of normal-appearing vitiligo skin. The presence of melanosomes inside keratinocytes is evident. Courtesy of Prof. M.R. Torrisi and Dr. S. Raffa, Cellular Diagnostics Unit, Sant'Andrea Hospital; Dept. of Clinical and Molecular Medicine, Sapienza University Rome, Italy

the presence of EGM deposits, associated with focal vacuolar degeneration of keratinocytes [14]. Fawzy and co-workers confirmed the existence of vacuolar degeneration in the basal and suprabasal lesional cells, extensive intercellular areas showing extracellular granular material [39]. The authors have highlighted two types of keratinocyte modifications: (1) electron-lucent degeneration

with cytoplasmic vacuolizations with both large indented and small electron-dense nuclei and (2) dark degeneration with electron-dense cytoplasm, margination of clumped tonofilaments, electron-dense melanosomes, and electron-dense nuclei with dispersed chromatin and distinct nucleoli. Perilesional keratinocytes show vacuoles, focally interrupted desmosomes, swollen mitochondria, and rearranged cristae, as well as granular material in the cytoplasm and in the extracellular spaces, especially in the active disease [37, 38]. Bartosik et al. have described the presence of melanosomes and, more recurrently, melanosome complexes in basal keratinocytes located in the center of lesions of long-lasting stable vitiligo, suggesting the possible persistence of melanin in keratinocytes some time after the appearance of the disease [43].

3.8 Langerhans Cells

Langerhans cells have been observed to apparently replace melanocytes in central regions of vitiligo lesions [35]. These cells are characterized by the presence of many Birbeck granules, intracytoplasmatic vacuoles, dilated Golgi complexes, and sometimes, irregular nuclei and dilated endoplasmic reticulum, damaged mitochondria, and small indentations in the cytoplasm of the adjacent keratinocytes [7, 42]. Prignano et al. have observed numerous Langerhans cells in both basal and suprabasal layers of lesional skin. They present an indented nucleus, rough endoplasmic reticulum and Golgi apparatus in the cytoplasm, typical Birbeck granules, and membrane alterations in the mitochondria. The authors have also described an additional group of clear cells, resembling less differentiated Langerhans cells, characterized by the absence of typical Birbeck granules but abounding in structures with a similar electron density [37, 38].

The basal layer of pigmented marginal areas presents more abundant Langerhans cells compared to normal control skin, as demonstrated by Breathnach [35]. Prignano et al. have described an inclination of these cells to be localized in the suprabasal layers in perilesional skin [37, 38]. They show rarefied chromatin in the nuclei, mitochondria alterations with rarefaction, or destructions of their membranes [38].

3.9 Basal Membrane

The basal membrane has been reported to be normal in many cases of vitiligo. However, both a thicker lamina densa and a focal disappearance of the basement membrane with a passage of granular material and vesicles from the epidermis into the dermis have been described in lesional skin [39, 42]. Prignano et al. also observed interruptions in the basal membrane of perilesional areas, with no significant alterations in the hypopigmented vitiliginous skin [37]. On the other hand, early reports described multiple focal replication or "layering" of the basement membrane beneath melanocytes in the perilesional pigmented margin [35].

3.10 Dermis

The cellularity of lesional and marginal areas appears greater than normal with an increase of small vessels, Schwann cells, and in some cases, mast cells. Lymphocytes and active fibroblasts are more copious at the dermo-epidermal junction compared to the control [35]. The presence of lymphocytes with cytoplasmic protrusions both in the dermis and in contact with degenerated keratinocytes has been observed in the central areas of vitiligous lesions [42]. Nerve alterations, including axon swelling; axon membrane discontinuity; reduplication or multiplicity of Schwann cell basement membrane; increase of Schwann cell cytoplasmic RNP granules, endoplasmic reticulum, and mitochondria; extrusion of degenerate axons from Schwann cytoplasm; and regenerating axons, have been observed in central and marginal areas of vitiligo lesions [35]. Changes consisting of regeneration and degeneration of dermal nerves have also been reported by Al'Abadie et al., who observed an increased thickening, often with reduplication, of

the basement membrane of Schwann cells surrounding the nerve fibers in both lesional and marginal areas [44].

3.11 Normally Pigmented Skin

Ultrastructurally, several studies were unable to detect significant differences in healthy skin of vitiligo patients compared to normal skin. Normal-appearing melanocytes in size and number, with melanosomes at all stages of differentiation, structured rough endoplasmic reticulum, and large but well-preserved mitochondria were detected. Occasionally, some melanocytes showed intracellular edema and vacuoles. Keratinocytes appeared as normal cells with only two cases showing reduced distribution of the cytoskeleton. Langerhans cells show the typical indented nucleus, rough endoplasmic reticulum, mitochondria, and Golgi complex [38]. The basal membrane looked well developed without degenerative signs [37, 38]. These findings differ from the observations of Moellmann et al. who observed that the accumulation of extracellular granular material (EGM) and foci of vacuolar degeneration of basal keratinocytes (consisting of swollen mitochondria, dilated endoplasmic reticulum, cytoplasmic vacuoles, loss of desmosomes and hemidesmosomes) were more evident in normally pigmented skin of patients with rapidly progressing or stable disease [14].

3.12 Differential Diagnosis

Diagnosis of vitiligo may be challenging, especially in the early spreading inflammatory phase. Although in some cases, an accurate medical history associated with a careful clinical inspection leads to the correct diagnosis. A histological examination is useful to rule out other hypopigmented disorders that may be present in a differential diagnosis [3]. In most cases, an analysis of melanocytes and melanin distribution as well as the inflammatory infiltrate features represent clues that allow for a histopathological confirmation of the diagnosis of vitiligo.

Differential diagnosis of vitiligo mainly includes nevus depigmentosus, hypopigmented mycosis fungoides, lichen sclerosus, and pityriasis alba [45].

Morphologic abnormalities of melanosomes and functional defects of melanocytes that appeared normal in number, compared to the normal epidermis, featured nevus depigmentosus, a congenital hypopigmented nonprogressive isolated macule. In a study, melanocyte counts were significantly decreased in nevus depigmentosus with GP-100 and MART-1 stain for melanocytes, whereas no significant differences in the number of these cells were identified with S100 [46]. Compared to nevus depigmentosus, vitiligo shows a significant decrease in the number of melanocytes and mild inflammatory dermal infiltrate.

Hypopigmented mycosis fungoides may mimic both clinical and histopathological inflammatory vitiligo. Epidermotropism, hydropic degeneration of basal cells, partial loss of pigment, preservation of some basal melanocytes, increased density of dermal lymphocytic infiltrate, and wiry fibrosis of the papillary dermis are all clues significantly associated with the histopathological diagnosis of hypopigmented mycosis fungoides [15]. On the other hand, focal thickening of the basal membrane, a complete loss of melanocytes, an absence of melanin, and an absence or sparseness of a mild lymphocytic infiltrate in the dermal papillae characterized vitiligo and differentiated it from mycosis fungoides [15].

Microscopical features allow for an easy differentiation between vitiligo and lichen sclerosus, which is histopathologically characterized by vacuolar interface changes, lichenoid inflammatory infiltrate, and papillary dermal sclerosis.

Pityriasis alba is a common benign dermatoses with a predilection for the upper part of the body in atopic preadolescent and adolescent individuals. Notwithstanding the paucity of morphologic studies in literature, the latter seems to be characterized by an irregular pigmentation of the basal layer with a normal melanocytic number associated with additional skin changes, such as follicular spongiosis, mild acanthosis of epidermis, and

sebaceous gland atrophy. However, the clinical presentation is quite characteristic and allows it to be differentiated from vitiliginous lesions [45].

References

1. Montes LF, Abulafia J, Wilborn WH, et al. Value of histopathology in vitiligo. Int J Dermatol. 2003;42(1):57–61.
2. Yadav AK, Singh P, Khunger N. Clinicopathologic analysis of stable and unstable vitiligo: a study of 66 cases. Am J Dermatopathol. 2016;38(8):608–13. https://doi.org/10.1097/DAD.0000000000000539.
3. Bolognia JL, Jorizzo JL, Schaffer JV. Dermatology. 3rd ed. London: Elsevier; 2012.
4. Tobin DJ, Swanson NN, Pittelkow MR, et al. Melanocytes are not absent in lesional skin of long duration vitiligo. J Pathol. 2000;191:407–16.
5. Anbar TS, El-Sawy AE, Attia SK, et al. Effect of PUVA therapy on melanocytes and keratinocytes in non-segmental vitiligo: histopathological, immuno-histochemical and ultrastructural study. Photodermatol Photoimmunol Photomed. 2012;28(1):17–25. https://doi.org/10.1111/j.1600-0781.2011.00631.x.
6. Kubanov A, Proshutinskaia D, Volnukhin V, et al. Immunohistochemical analysis of melanocyte content in different zones of vitiligo lesions using the Melan-A marker. Acta Dermatovenerol Alp Pannonica Adriat. 2016;25(1):5–9.
7. Kim YC, Kim YJ, Kang HY, et al. Histopathologic features in vitiligo. Am J Dermatopathol. 2008;30(2):112–6. https://doi.org/10.1097/DAD.0b013e3181651511.
8. Gan EY, Cario-André M, Pain C, et al. Follicular vitiligo: a report of 8 cases. J Am Acad Dermatol. 2016;74:1178–84.
9. van den Wijngaard R, Wankowicz-Kalinska A, Le Poole C, et al. Local immune response in skin of generalized vitiligo patients. Destruction of melanocytes is associated with the prominent presence of CLA+ T cells at the perilesional site. Lab Invest. 2000;80(8):1299–309.
10. Weedon D. Weedon's skin pathology. 3rd ed. London: Elsevier; 2010.
11. Abdel-Naser MB, Krüger-Krasagakes S, Krasagakis K, et al. Further evidence for involvement of both cell mediated and humoral immunity in generalized vitiligo. Pigment Cell Res. 1994;7(1):1–8.
12. Attili VR, Attili SK. Lichenoid inflammation in vitiligo – a clinical and histopathologic review of 210 cases. Int J Dermatol. 2008;47:663–9.
13. Sharquie KE, Mehenna SH, Naji AA, et al. Inflammatory changes in vitiligo: stage I and II depigmentation. Am J Dermatopathol. 2004;26(2):108–12.
14. Moellmann G, Klein-Angerer S, Scollay DA, et al. Extracellular granular material and degeneration of keratinocytes in the normally pigmented epidermis of patients with vitiligo. J Invest Dermatol. 1982;79(5):321–30.
15. El-Darouti MA, Marzouk SA, Azzam O, et al. Vitiligo vs. hypopigmented mycosis fungoides (histopathological and immunohistochemical study, univariate analysis). Eur J Dermatol. 2006;16(1):17–22.
16. Stylianos V, Eleftherios I, Nikolaos K, et al. Correlating epidermal thickness and basement membrane length to angiogenesis in the centre and the periphery of vitiligo lesion. Indian J Dermatol Venereol Leprol. 2012;78(3):368–71. https://doi.org/10.4103/0378-6323.95462.
17. Jung SE, Kang HY, Lee ES, et al. Changes of epidermal thickness in vitiligo. Am J Dermatopathol. 2015;37(4):289–92.
18. Kao CH, Yu HS. Depletion and repopulation of Langerhans cells in nonsegmental type vitiligo. J Dermatol. 1990;17(5):287–96.
19. Brown J, Winklemann RK, Wolff K. Langerhans cells in vitiligo: a qualitative study. J Invest Dermatol. 1967;49(4):386–90.
20. Claudy AL, Rouchouse B. Langerhans' cell and vitiligo: quantitative study of T6 and HLA-DR antigen-expressing cells. Acta Derm Venereol. 1984;64(4):334–6.
21. Meheregan AH, Hashimoto K. Pinkus' guide to dermatopathology. Norwalk, CT: Appleton & Lange; 1991.
22. Gokhale BB, Mehta LN. Histopathology of vitiliginous skin. Int J Dermatol. 1983;22(8):477–80.
23. Benzekri L, Gauthier Y, Hamada S, et al. Clinical features and histological findings are potential indicators of activity in lesions of common vitiligo. Br J Dermatol. 2013;168(2):265–71. https://doi.org/10.1111/bjd.12034.
24. Kovacs D, Abdel-Raouf H, Al-Khayyat M, et al. Vitiligo: characterized of melanocytes in repigmented skin after punch grafting. J Eur Acad Dermatol Venereol. 2015;29:581–90. https://doi.org/10.1111/jdv.12647.
25. Le Poole IC, van den Wijngaard RM, Westerhof W, et al. Presence or absence of melanocytes in vitiligo lesions: an immunohistochemical investigation. J Invest Dermatol. 1993;100(6):816–22.
26. Wańkowicz-Kalińska A, van den Wijngaard RM, Tigges BJ, et al. Immunopolarization of CD4+ and CD8+ T cells to Type-1-like is associated with melanocyte loss in human vitiligo. Lab Invest. 2003;83(5):683–95.
27. Pretti Aslanian FM, Noé RA, Cuzzi T, et al. Abnormal histological findings in active vitiligo include the normal-appearing skin. Pigment Cell Res. 2007;20(2):144–5.
28. Gauthier Y, Cario Andre M, Taïeb A. A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? Pigment Cell Res. 2003;16:322–32.
29. Norris A, Todd C, Graham A, et al. The expression of the c-kit receptor by epidermal melano-

cytes may be reduced in vitiligo. Br J Dermatol. 1996;134(2):299–306.
30. Wagner RY, Luciani F, Cario-André M, et al. Altered E-cadherin levels and distribution in melanocytes precede clinical manifestations of vitiligo. J Invest Dermatol. 2015;135(7):1810–9. https://doi.org/10.1038/jid.2015.25.
31. Al Badri AMT, Tood PM, Garioch JJ, et al. An immunohistochemical study of cutaneous lymphocytes in vitiligo. J Pathol. 1993;170:149–55.
32. Ahn SK, Choi EH, Lee SH, et al. Immunohistochemical studies from vitiligo – comparison between active and inactive lesions. Yonsei Med J. 1994;35:404–10.
33. Le Poole IC, Van den Wijngaard RM, Westerhof W, et al. Presence of T-cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. Am J Pathol. 1996;148:1219–28.
34. Pretti Aslanian FM, Noè RAM, Antelo DP, et al. Immunohistochemical findings in active vitiligo including depigmenting lesions and non-lesional skin. Open Dermatol J. 2008;2:105–10.
35. Breathnach AS, Bor S, Wyllie LM. Electron microscopy of peripheral nerve terminals and marginal melanocytes in vitiligo. J Invest Dermatol. 1966;47(2):125–40.
36. Galadari E, Mehregan AH, Hashimoto K. Ultrastructural study of vitiligo. Int J Dermatol. 1983;32(4):269–71.
37. Prignano F, Pescitelli L, Becatti M, et al. Ultrastructural and functional alterations of mitochondria in perilesional vitiligo skin. J Dermatol Sci. 2009;54(3):157–67. https://doi.org/10.1016/j.jdermsci.2009.02.004.
38. Prignano F, Ricceri F, Bianchi B, et al. Dendritic cells: ultrastructural and immunophenotypical changes upon nb-UVB in vitiligo skin. Arch Dermatol Res. 2011;303(4):231–8. https://doi.org/10.1007/s00403-010-1109-5.
39. Fawzy MM, El Maadawi ZM, Hegazy RA, et al. Vitiligo - the story from within: a transmission electron microscopic study before and after narrow-band ultraviolet B. Ultrastruct Pathol. 2016;40(5):265–75. https://doi.org/10.1080/01913123.2016.1218987.
40. Hann SK, Park YK, Lee KG, et al. Epidermal changes in active vitiligo. J Dermatol. 1992;19(4):217–22.
41. Ding GZ, Zhao WE, Li X, et al. A comparative study of mitochondrial ultrastructure in melanocytes from perilesional vitiligo skin and perilesional halo nevi skin. Arch Dermatol Res. 2015;307(3):281–9. https://doi.org/10.1007/s00403-015-1544-4.
42. Panuncio AL, Vignale R. Ultrastructural studies in stable vitiligo. Am J Dermatopathol. 2003;25(1):16–20.
43. Bartosik J, Wulf HC, Kobayasi T. Melanin and melanosome complexes in long standing stable vitiligo—an ultrastructural study. Eur J Dermatol. 1998;8(2):95–7.
44. Al'Abadie MS, Warren MA, Bleehen SS, et al. Morphologic observations on the dermal nerves in vitiligo: an ultrastructural study. Int J Dermatol. 1995;34(12):837–40.
45. Iannella G, Greco A, Didona D, et al. Vitiligo: pathogenesis, clinical variants and treatment approaches. Autoimmun Rev. 2016;15:335–43.
46. Kim SK, Kang HY, Lee ES, et al. Clinical and histopathologic characteristics of nevus depigmentosus. J Am Acad Dermatol. 2006;55:423–8.
47. Dean NR, Brennan J, Haynes J, et al. Immunohistochemical labeling of normal melanocytes. Appl Immunohistochem Mol Morphol. 2002;10(3):199–204.
48. King R, Weilbaecher KN, McGill G, et al. Microphthalmia transcription factor. A sensitive and specific melanocyte marker for Melanoma Diagnosis. Am J Pathol. 1999;155(3):731–8.
49. Prieto VG, Shea CR. Immunohistochemistry of melanocytic proliferations. Arch Pathol Lab Med. 2011;135(7):853–9. https://doi.org/10.1043/2009-0717-RAR.1.
50. Passeron T, Coelho SG, Miyamura Y, et al. Immunohistochemistry and in situ hybridization in the study of human skin melanocytes. Exp Dermatol. 2007;16(3):162–70.
51. Clarkson KS, Sturdgess IC, Molyneux AJ. The usefulness of tyrosinase in the immunohistochemical assessment of melanocytic lesions: a comparison of the novel T311 antibody (anti-tyrosinase) with S-100, HMB45, and A103 (anti-melan-A). J Clin Pathol. 2001;54(3):196–200.
52. Kawakami Y, Eliyahu S, Delgado CH, et al. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci U S A. 1994;91(9):3515–9.
53. Coulie PG, Brichard V, Van Pel A, et al. A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med. 1994;180(1):35–42.
54. Grabbe J, Welker P, Dippel E, et al. Stem cell factor, a novel cutaneous growth factor for mast cells and melanocytes. Arch Dermatol Res. 1994;287(1):78–84.
55. Steel KP, Davidson DR, Jackson IJ. TRP-2/DT, a new early melanoblast marker, shows that steel growth factor (c-kit ligand) is a survival factor. Development. 1992;115(4):1111–9.
56. Gown AM, Vogel AM, Hoak D, et al. Monoclonal antibodies specific for melanocytic tumors distinguish subpopulations of melanocytes. Am J Pathol. 1986;123(2):195–203.
57. Smoller BR, Hsu A, Krueger J. HMB-45 monoclonal antibody recognizes an inducible and reversible melanocyte cytoplasmic protein. J Cutan Pathol. 1991;18(5):315–22.
58. Vennegoor C, Hageman P, Van Nouhuijs H, et al. A monoclonal antibody specific for cells of the melanocyte lineage. Am J Pathol. 1988;130(1):179–92.

Introduction to Clinical Aspects Chapters

4

Alain Taïeb and Mauro Picardo

Abstract

The following chapters cover the entities delineated in the current nosology of vitiligo (vitiligo/NSV and its variants Chap. 5), segmental vitiligo Chap. 6, mixed vitiligo Chap. 7, and rare variants Chap. 8, in accordance with the recent VGICC international consensus summarized in Chap. 2. The clinical aspects and natural history are treated in more detail and illustrated.

In addition, specific clinical aspects are highlighted such as subtypes of mucocutaneous pigment cell involved by vitiligo: mucosal, hair follicle, and nevus cells Chap. 9; involvement of extracutaneous melanocytes Chap. 10; environmental triggers such as Koebner's phenomenon and occupational vitiligo Chaps. 11 and 12 associated disorders and comorbidities including autoimmune/inflammatory disorders, immunodeficiencies, and rare monogenic diseases Chap. 13; age and vitiligo, childhood, pregnancy, and late-onset disease Chap. 14; skin colour Chap. 15; and vitiligoid changes occurring during immunotherapies of melanoma Chap. 16.

A. Taïeb (✉)
Service de Dermatologie, Hôpital St André, CHU de Bordeaux, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

M. Picardo
Cutaneous Physiopathology and CIRM, San Gallicano Dermatological Institute, IRCSS, Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_4

5 Vitiligo/nonsegmental Vitiligo Including Acrofacial and Universalis

Thierry Passeron, Jean-Paul Ortonne, Prasad Kumarasinghe, and Alain Taïeb

Contents

T. Passeron
Department of Dermatology, University Hospital of Nice, Nice, France
e-mail: passeron@unice.fr

J.-P. Ortonne
Department of Dermatology, Archet-2 hospital, Nice, France

P. Kumarasinghe
Department of Dermatology, Royal Perth Hospital, Perth, WA, Australia

A. Taïeb (✉)
Service de Dermatologie, Hôpital St André, CHU de Bordeaux, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

Abstract

Based on the VGICC classification, vitiligo/NSV, a consensus umbrella term for all forms of generalized vitiligo, is meant to describe different clinical subtypes of vitiligo that are all clearly distinct from SV including acrofacial, generalized, mucosal (multifocal), and universal. According to the VETF definition, generalized vitiligo is characterized by asymptomatic well-circumscribed milky-white macules involving both sides of the body with usually a symmetrical pattern. In acrofacial vitiligo (AFV), the involved sites are by

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_5

definition limited to the face, head, hands, and feet with typically depigmentation of the distal fingers and facial orifices. Vitiligo universalisis a rapidly progressive form of the disease, which needs a more in-depth investigation especially for associated autoimmunity.

Key Points

- The existence of various phenotypes among vitiligo/NSV (e.g. acrofacial or vulgaris) is suggested by clinical observation and possibly by genetic associations.
- Half of initially "focal" vitiligo patients evolve into vitiligo/NSV.
- The fingers, hands, and face are frequently reported to be the initial sites by the patients.
- Palms and soles are frequently involved if a systematic Wood's lamp examination is performed.
- Most of the 'spontaneous' repigmentation reported by the patients is correlated to sun exposure.
- Multichrome vitiligo refers to various degrees of depigmentation within a vitiligo macule, a phenomenon noted in dark skin.
- Vitiligo universalis is the most uncommon form of vitiligo and is probably the extreme severity end of the spectrum of vitiligo/NSV.
- For vitiligo universalis, patients have to be informed that associated autoimmune diseases may manifest months or years after being diagnosed with vitiligo.
- For vitiligo universalis, sun protection is important to avoid sunburns and other sequelae.
- For vitiligo universalis, depigmenting creams such as monobenzyl ether of hydroquinone or laser depigmentation may be used to depigment small areas of breakthrough pigmentation.

5.1 Introduction

Based on the VGICC classification [1], vitiligo/NSV, a consensus umbrella term for all forms of generalized vitiligo, is meant to describe different clinical subtypes of vitiligo that are all clearly distinct from SV including acrofacial, generalized, mucosal (multifocal), and universal (Chap. 1.2). Mixed vitiligo and mucosal vitiligo (more than one mucosal site) are also included under the vitiligo/NSV umbrella but discussed separately (respectively, Chaps. 1.5.3 and 1.5.5). Generalized vitiligo (*formerly vulgaris*) is the most common form of vitiligo/NSV corresponding to several achromic patches symmetrically distributed on the body [2]. In the largest series of patients (from China), it accounted for 41% of cases [3].

Although large clinicogenetic databases are currently lacking, different phenotypes of vitiligo may have different genetic backgrounds [4].

5.2 Generalized Vitiligo

Generalized vitiligo is characterized by asymptomatic well-circumscribed milky-white macules involving both sides of the body with usually a symmetrical pattern [5]. The shape of individual macules is round to oval with slightly brushed to fairly distinct margins (Fig. 5.1a). Initial spot size varies from a few to several centimetres in diameter. The depigmentation is macular, and the epidermis shows no sign of atrophy, telangiectasias, or any other signs. The presence of epidermal atrophy should suggest some other disorders, especially lichen sclerosus (Chap. 1.2). A transient erythema, which can be clinically misleading, is frequently observed after ultraviolet (UV) exposure, but the history is usually contributive (Fig. 5.1b). Hyperpigmented lesional borders are not uncommon, especially in dark skin patients and after UV exposure. Hairs are usually spared and remain pigmented, but in some cases hair depigmentation may also occur simultaneously. In the scalp, vitiligo usually leads to localized patches of grey or white hairs,

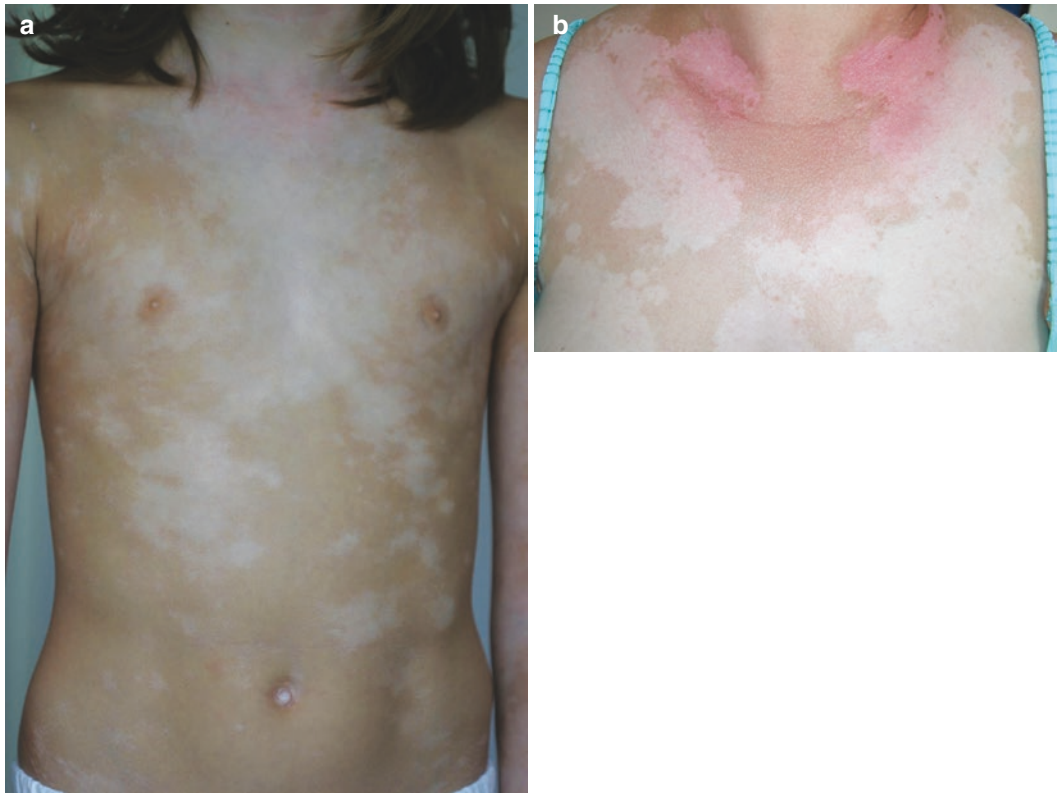


Fig. 5.1 Extensive generalized vitiligo with mostly symmetrical lesions in a child with fuzzy borders indicative of progressive disease (**a**) and erythema in sun-exposed areas in an adult (**b**)

but total depigmentation of the scalp hair may occur. Depigmented body hairs within vitiligo macules are considered as markers of poor repigmentation prognosis [6]. Follicular vitiligo has been recently delineated as a variant with initial and prominent hair depigmentation [7].

Vitiligo patches are easy to recognize on darker phototypes, but depigmentation is sometimes difficult to detect in patients with very fair skin. Wood's lamp or 365 nm monochromatic lamp examination is very helpful to delineate the areas involved in light-coloured individuals and also to assess the remaining reservoir of melanocytes. Even in darker skin individuals, palms and soles are light coloured and need to be examined with Wood's lamp. Systematic examination of palms and soles suggest that palmoplantar vitiligo is not uncommon, like in psoriasis. Wood's lamp examination can also show the earliest signs of repigmentation at the border of the lesion or in perifollicular areas (Fig. 5.2).

5.2.1 Distribution

Generalized vitiligo can start at any site of the body, but the fingers, hands, and face are frequently reported to be the initial sites by the patients. It has been reported that when the hands are the initial site, vitiligo most commonly progresses to the face, explaining the frequency of acrofacial vitiligo in those patients [8]. The same study suggests that when the posterior trunk, hands, or feet are the initial sites, vitiligo tends to have a more widespread progression. The extensor surfaces are commonly affected including interphalangeal joints, metacarpal/metatarsal interphalangeal joints, elbows, and knees. Other surfaces involved include volar wrists, malleoli, umbilicus, lumbosacral area, anterior tibia, and axillae. The role of the Koebner phenomenon in the distribution pattern is discussed in Chap. 1.5.7. Most of the time, the distribution of the lesions is clearly symmetri-

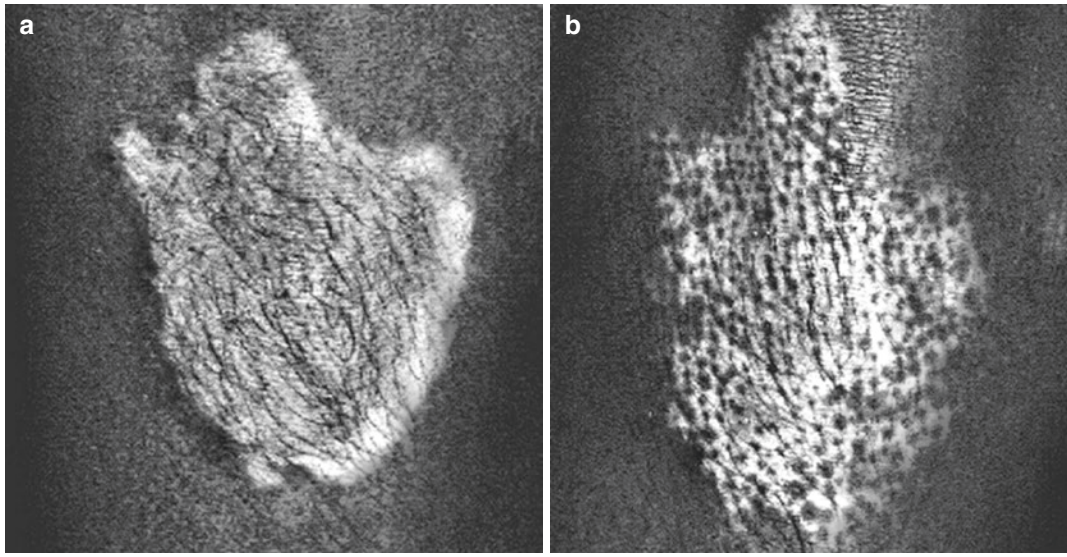


Fig. 5.2 Aspect of vitiligo lesion of the knee in Wood's lamp examination (**a**). Note the persistence of a grey pattern in the centre of the lesion, suggesting of a remaining melanocyte reservoir; (**b**) same lesion during after phototherapy. Note the perifollicular repigmentation

cal. Vitiligo macules may also be periorificial and involve the skin around the eyes, nose, ears, mouth, and anus. Periungual involvement may occur alone or with certain cutaneous or mucosal surfaces (lip-tip vitiligo-, distal penis, nipples). Vitiligo can affect genital areas and in some cases almost exclusively. This location should be discussed with the patients because it causes a great concern.

5.2.2 Natural Course

The evolution of generalized vitiligo is unpredictable. Focal vitiligo, although stable for a time, may be a precursor of generalized vitiligo. In a large series of vitiligo patients, about three-quarters of the patients occurred as focal vitiligo, but more than half of those patients evolved to a generalized form [3]. The natural course of common vitiligo is often one of abrupt onset, followed by progression for a time; then a period of stability follows and may last for some time, even decades. This may be followed later by another period of more rapid evolution. Periods of rapid progression last often less than a year, after which there is little extension or regression. The evolution to vitiligo universalis is rare (Sect. 5.4). The most common course is one of gradual extension of existing macules and periodic development of new ones. Vitiligo can repigment spontaneously, but this phenomenon is rare and mostly localized to some lesions. Most of the 'spontaneous' repigmentation reported by the patients is correlated to sun exposure.

5.2.3 Clinical Variants

5.2.3.1 Multichrome Vitiligo

This form of vitiligo is mostly seen in darker phototypes. Within a vitiligo lesion, areas of depigmentation coexist with hypopigmented areas and with normal colour as in the surrounding skin (Fig. 5.3). In the hypopigmented area, a partial loss of melanocyte is observed. Trichrome vitiligo is commonly used to describe this pattern, but

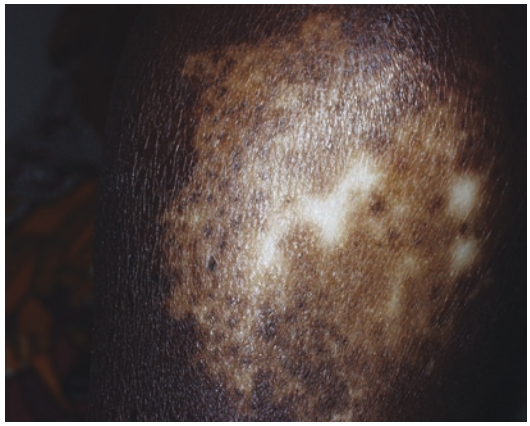


Fig. 5.3 Multichrome vitiligo of the leg

various degrees of hypopigmentation can be observed leading to trichrome, quadrichrome, or pentachrome vitiligo [9, 10]. Thus, the term "multichrome vitiligo" should be preferred.

5.2.3.2 Blue Vitiligo

Postinflammatory hyperpigmentation leading to a blue discolouration of vitiligo patches has been reported after inflammatory vitiligo [11].

5.3 Acrofacial Vitiligo

In acrofacial vitiligo (AFV), the involved sites are by definition (Chap. 2) limited to the face, head, hands, and feet with typically depigmentation of the distal fingers and facial orifices. Since it may later include other body sites, resulting in typical generalized vitiligo, substantial variation in estimates of this subtype exists in the literature, according to stage and more or less strict application of the definition, in particular involvement of only acral sites without periorificial involvement, or mixed forms with coexisting non-acrofacial lesions. According to recent large series, 6.8% of Chinese Han and non-Han [12], 5.8% of French [1], 25.6% of Brazilian [13], and 44% of Indian [14] vitiligo patients received a diagnosis of AFV. There are no series looking specifically at the natural history of AFV. The association with autoimmune thyroid disease seems less strong when compared with generalized vitiligo [13]. The Northeastern Chinese series showed an association with mucosal vitiligo [12]. This association is also strongly suggested by the study of Attili, which indicates that lichen sclerosus needs in addition to be also actively researched in this context and could be underestimated because a thorough examination of genital areas is frequently omitted [15]. Of 217 cases of AFV recorded over a period of 12 years by the authors, 116 (53%) had associated oral/genital lesions. Among these, 15 patients demonstrated typical clinical as well as histological features of lichen sclerosus. Interestingly one female patient had cutaneous lichen sclerosus guttata among acral lesions of vitiligo [15].

5.4 Vitiligo Universalis

Acquired complete depigmentation or nearly complete depigmentation of the skin (and sometime hairs) is termed vitiligo universalis (VU), which is the most extensive form of vitiligo/NSV. This form can start as common nonsegmental vitiligo (NSV) and advance to complete depigmentation of the skin and hair. True segmental vitiligo (SV) does not progress to vitiligo universalis. Within the group of vitiligo/NSV, it is not clear what factors trigger the cascade of complete or near-complete depigmentation of the integument in VU.

There appears to be some minor differences in the prevalence of vitiligo among different populations, but no specific data is available on VU [16]. VU is certainly the most uncommon clinical manifestation of vitiligo and appears to occur worldwide. It is not clear whether VU has the same prevalence among fair-skinned Caucasians as compared to Asians or Africans, where it is easily recognizable. Although vitiligo may occur at any age, VU is uncommon in

childhood. The population prevalence of VU is not known. In a study by Song et al. [17], it has been reported that there was only one case of VU among 1315 vitiligo patients. In a retrospective study we carried out at the National Skin Centre, Singapore [18], 1.4% (4 patients out of 282 vitiligo patients) had vitiligo universalis. Male to female ratio of vitiligo universalis appears to be the same. More data are needed to confirm this observation.

5.4.1 Clinical Features

In VU, nearly all the skin becomes completely depigmented. However, in sun-exposed areas there can be minor perifollicular, discrete, or coalescent pigmentation (Figs. 5.4, 5.5, 5.6, and 5.7). Areolae and genitalia may depigment either early in the disease process or later. Some VU patients may show areas of partial depigmentation, appearing clinically as trichrome vitiligo. However, these apparently resisting areas may also lose colour and become uniformly depigmented.

Some patients retain the dark hairs on the scalp at least in the early course of disease. Pubic and axillary hairs may or may not be depigmented. Body hairs often get depigmented in affected areas. Generally, in established VU, the hairs in the depigmented skin also get depigmented.

Iris pigmentation is usually unaffected, but exceptions exist. In some VU patients, associated uveitis may even lead to blindness. Mucosae, particularly the lips, gums, and genitalia too, may be partially or completely depigmented [19].

According to a Turkish study [20], minor hearing defects are not uncommon in NSV patients. These authors have reported that 14% of NSV patients have at least mild hyperacusis. Eye and ear symptoms in extensive vitiligo patients are likely to be due to the loss of melanocytes in these organs. This is a prominent feature in Vogt-Koyanagi-Harada syndrome (Chaps. 8 and 10).

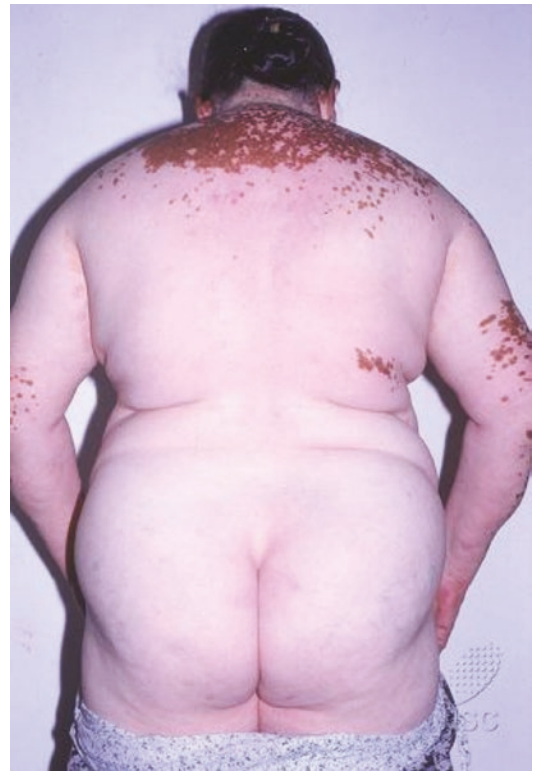


Fig. 5.4 A Singaporean woman of Indian origin with vitiligo universalis and breakthrough repigmentation on exposed areas (Photograph, courtesy, National Skin Centre, Singapore)

5.4.2 Precipitating Factors and Progression

It is uncertain what factors (genetic or environmental) precipitate common NSV to progress to VU. Often the progression is symmetrical. There is no evidence that vitiligo universalis increases the risk of another vitiligo universalis case among family members. No sufficient genetic data is available which would help determining a risk of vitiligo universalis in a new case of NSV in a vitiligo family.

Extensive NSV can evolve into VU. Sometimes this evolution can be rather rapid after a period of apparent stability (personal observation). Depigmentation may spread from the edges of existing lesion or by occurrence of new macules.

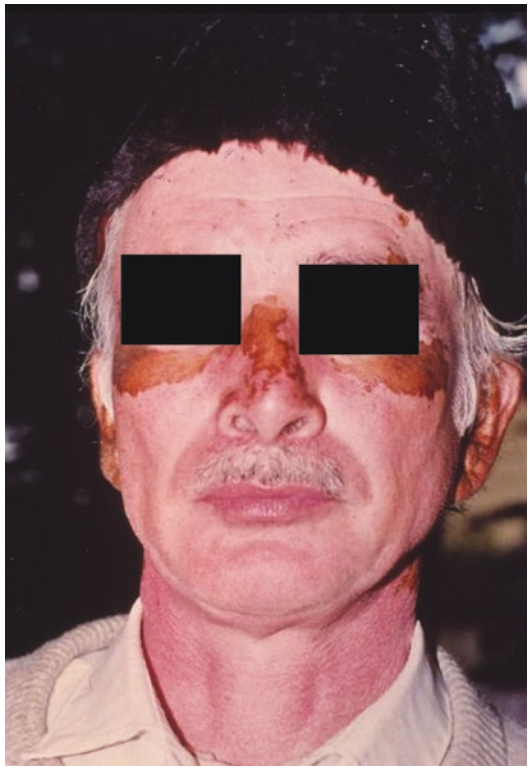


Fig. 5.5 A 65-year-old Indian male with vitiligo universalis and breakthrough repigmentation in a lupus pattern (Photograph courtesy of Prof Binod Khaitan, All India Institute of Medical Science, New Delhi, India)

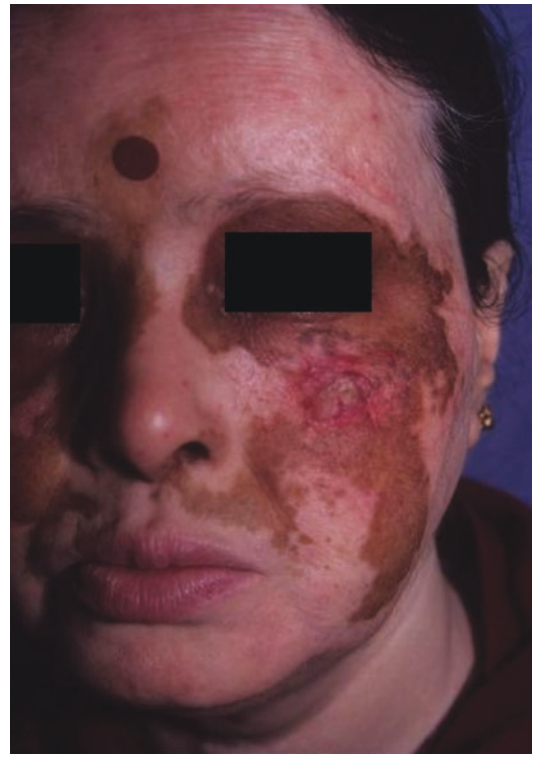


Fig. 5.7 An Indian woman with vitiligo universalis and photo-induced partial repigmentation, with a squamous cell carcinoma on the cheek. Carcinomas on vitiliginous skin are rare although sunburns are common (Photograph courtesy of Prof Binod Khaitan, All India Institute of Medical Science, New Delhi, India)

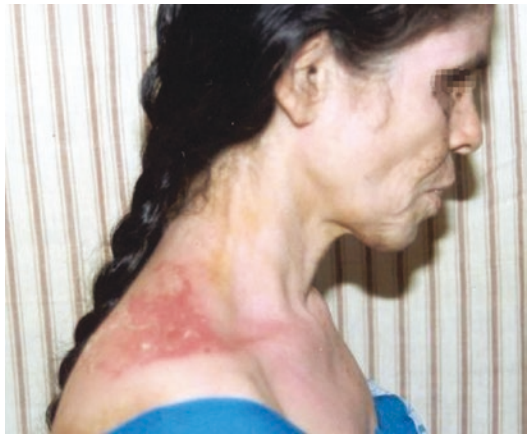


Fig. 5.6 A 58-year-old Sri Lankan woman with vitiligo universalis with an acute sunburn on the shoulders after exposure to strong sun while working in a paddy field

Occasionally before complete depigmentation, surrounding borders of the pigmented skin may show signs of inflammation, such as erythema. After the inflammation recedes, these areas also become depigmented. In one case of vitiligo (personal observation PK), a patient with limited, stable generalized vitiligo rapidly progressed to vitiligo universalis after an episode of diarrhoea and vomiting, following food poisoning. Similarly, pregnancy, physical injury, malignant melanoma, and emotional stress have been incriminated as 'triggers' for progression of vitiligo. But it is not clear whether these associations are true associations or chance occurrences [19]. Occasionally, chemical leukoderma, which starts focally, can spread all over the body. Following the topical use

of diphencyprone for extensive alopecia areata, extensive vitiligo has been reported to appear in areas beyond the contact areas [21]. Perifollicular breakthrough pigmentation on sun-exposed areas, especially the upper cheeks and exposed areas of forearms, is not uncommon (Figs. 5.4 and 5.5). Pigmentation around nipples may be lost or retained. The author (PK) has observed several cases of localized repigmentation in VU cases, after a secondary event, such as lichen planus or insect bite reactions. Dogra et al. have reported partial repigmentation of vitiligo universalis in a patient who was given dexamethasone and cyclophosphamide pulse therapy for pemphigus vulgaris [22]. This coincidental finding addresses an important aspect. Indeed, even in established VU, inactive but viable melanocytes are probably present and can be reactivated by adequate signals of the cellular environment. This confirms previous studies, where some viable melanocytes were found within established vitiliginous macules [23, 24]. Most of these melanocytes or precursors of melanocytes appear to be associated with hair follicles. This may be a reason why the perifollicular pigmentation is the most common type of breakthrough repigmentation in VU.

5.4.3 Influence of Previous Treatments

When a patient with VU presents to a given dermatologist, he or she may have already tried several medications. There may be skin atrophy and striae due to prolonged use of potent topical steroids. Monobenzyl ether of hydroquinone (MBEHQ) may have been used to depigment the residual pigmented spots (Chap. 37). This may sometimes cause a contact dermatitis. Breakthrough pigmentation may be a problem if proper sun protection has not been adhered to. The skin may appear yellowish if high doses of oral beta-carotene have been taken. Some may have applied artificial tanning substances (dyes) on the skin (Chap. 39). A few of these tanning substances may cause allergic contact dermatitis, but it is uncommon. Scalp hair, as well as beard or moustache ones, may have been dyed. Contact dermatitis due to PPD (*p*-phenylenediamine) containing hair dyes is not uncommon.

5.4.4 Autoimmune Diseases Associated with Extensive Vitiligo and Application to VU

Many autoimmune/autoinflammatory disorders and several other conditions have been reported in association with vitiligo (Chap. 13). These conditions may be found in patients with VU as well. These patients may have a family history of other autoimmune disorders, as well as a family history of NSV.

Some patients with Vogt-Koyanagi-Harada syndrome may also evolve into VU, and the limits between the two conditions are somewhat blurred. In this condition, visual defects, alopecia, and auditory and CNS symptoms may precede skin depigmentation. Often they present to the neurologist or ophthalmologist first, due to eye or meningeal symptoms.

In VU, other autoimmune disorders may evolve after the onset of vitiligo; therefore these patients have to be followed up with this in mind. In the author's experience, autoimmune thyroiditis and alopecia areata are the commonest associations.

5.4.5 Diagnosis

Usually there is no diagnostic difficulty in vitiligo universalis. Diagnosis can be made based on the history and the clinical features. Skin biopsy is not required unless for a research purpose. In some situations, some forms of oculocutaneous albinism may be confused with vitiligo, especially if the onset of disease or history is not known. Nystagmus and photophobia are not associations of vitiligo, but of oculocutaneous albinism. A skin biopsy is helpful in this setting to show the presence of non-functioning melanocytes in albinism.

5.4.6 Management

In simple NSV detailed investigations are not essential, but in VU several investigations are recommended (Table 5.1). Many patients with extensive vitiligo have serum antibodies against melanocytes [25], as well as antithyroid, anti-parietal cell, and anti-nuclear ones. Anti-melanocyte antibodies are not found in all cases of vitiligo, and these antibodies may be found in some other conditions as well (e.g. mucocutaneous candidiasis). Therefore, this test is not indicated as a routine test.

Once vitiligo universalis has fully developed, it is unrealistic to expect complete repigmentation. Management of VU patients is mostly directed at treating the residual patches of the pigmented skin, preventing breakthrough pigmentation and protecting from sun damage while addressing camouflage issues and psychosocial aspects (Table 5.2). Counselling is important before implementing complete depigmentation procedures [17, 26]. Occasionally, some patients are keen to attempt to retain pigmentation in some areas, notwithstanding extensive depigmentation in other areas. Q-switched ruby and Q-switched alexandrite lasers have proven to be useful in destroying residual pigmented macules in patients where topical bleaching agents have failed to depigment (Chap. 3.2.3).

Sun protection is important to avoid sunburns as well as to prevent unwanted breakthrough, spotty repigmentation. Some studies have revealed that wild-type p53 gene is upregulated in vitiligo patients [27]. This may have a protective effect with regard to sun-induced skin malignancy. Tumours, such as squamous cell carcinomas, on vitiliginous skin are very uncommon in dark-skinned individuals. However, it can occur rarely. Furthermore, as melanomas are a known association of vitiligoid depigmentation, if a suspicious new pigmented lesion appears, particularly in association with a pre-existing nevus, it should be excised and sent for histological evaluation.

Vitiligo universalis causes a huge psychological impact in dark-skinned individuals, and some patients may show signs of depression at the time of presentation [28]. In certain communities and cultures, VU would be even more problematic, patients being ostracized with regard to marriage and social contacts. Counselling and sometimes even psychiatric help may be needed, depending on emotional impact, particularly in dark-skinned individuals (Chaps. 15 and 42). Counselling the spouse and the family members may also be necessary in some situations. Occasionally, even in the fair-skinned individuals, vitiligo may cause major psychological problems, because sun-induced pigmentation occurs only in the unaffected areas. After understanding the disease process and after realization that no treatment is 100% effective in VU, some patients accept it and continue with their lives well.

Table 5.1 Recommended investigations in vitiligo universalis

Basic haematology	Full cell blood count
	Fasting blood sugar
Endocrinology	Free thyroxine
	TSH
	PTH
Immunology	Antithyroid antibodies
	Antinuclear factor
Others	Ophthalmological tests
	Audiological tests

Table 5.2 Management of residual pigmented areas and avoidance of breakthrough pigmentation

Chemical depigmentation	Physical depigmentation	Camouflage	Others
MBEHQ	Q-switched ruby laser	Oral beta-carotene	Counselling patient and/or family
	Alexandrite laser	Topical dyes for the skin	Psychiatric help
	NdYag laser	Topical dyes for scalp hair and eyebrows	Sun protection
			Surveillance versus autoimmune and neoplastic diseases

5.5 Conclusions

VU is probably the extreme severity end of the spectrum of NSV, and differentiation from VKH syndrome is not always easy. Genetic studies of families with vitiligo universalis in different ethnic groups would be helpful. Future research should focus on identifying and modifying the triggering factors which start the cascade of depigmentation process all over the skin.

Acknowledgements Dr. Prasad Kumarasinghe is grateful to Prof. Roy Chan, Medical Director, and Dr. Goh Boon Kee, Consultant Dermatologist, National Skin Centre, Singapore, for providing some of the VU photographs.

References

1. Ezzedine K, Diallo A, Léauté-Labrèze C, Seneschal J, Boniface K, Cario-André M, Prey S, Ballanger F, Boralevi F, Jouary T, Mossalayi D, Taieb A. Pre- vs. post-pubertal onset of vitiligo: multivariate analysis indicates atopic diathesis association in pre-pubertal onset vitiligo. Br J Dermatol. 2012;167:490–5.
2. Le Poole C. Vitiligo vulgaris. In: Nordlund J, editor. The pigmentary system. Malden, MA: Blackwell Publishing; 2006. p. 551–98.
3. Liu JB, Li M, Yang S, et al. Clinical profiles of vitiligo in China: an analysis of 3742 patients. Clin Exp Dermatol. 2005;30:327–31.
4. Zhang XJ, Liu JB, Gui JP, et al. Characteristics of genetic epidemiology and genetic models for vitiligo. J Am Acad Dermatol. 2004;51:383–90.
5. Ortonne JP, Mosher DB, Fitzpatrick TB. Vitiligo and other hypomelanoses of hair and skin. In: Parrish JA, Fitzpatrick TB, editors. Monographs in topics in dermatology. New York, NY: Plenum Press; 1983. p. 1–683.
6. Ortonne JP. Vitiligo and other disorders of hypopigmentation. In: Bolognia J, Jorizzo JL, Rapini RP, editors. Dermatology. New York, NY: Mosby; 2007. p. 913–38.
7. Gan EY, Cario-André M, Pain C, Goussot JF, Taïeb A, Seneschal J, Ezzedine K. Follicular vitiligo: a report of 8 cases. J Am Acad Dermatol. 2016;74:1178–84.
8. Chun WH, Hann SK. The progression of nonsegmental vitiligo: clinical analysis of 318 patients. Int J Dermatol. 1997;36:908–10.
9. Fargnoli MC, Bolognia JL. Pentachrome vitiligo. J Am Acad Dermatol. 1995;33:853–6.
10. Sehgal VN, Srivastava G. Vitiligo: compendium of clinico-epidemiological features. Indian J Dermatol Venereol Leprol. 2007;73:149–56.
11. Ivker R, Goldaber M, Buchness MR. Blue vitiligo. J Am Acad Dermatol. 1994;30:829–31.
12. Mchepange UO, Gao XH, Liu YY, Liu YB, Ma L, Zhang L, Chen HD. Vitiligo in North-Eastern China: an association between mucosal and acrofacial lesions. Acta Derm Venereol. 2010;90:136–40.
13. Silva de Castro CC, do Nascimento LM, Olandoski M, Mira MT. A pattern of association between clinical form of vitiligo and disease-related variables in a Brazilian population. J Dermatol Sci. 2012;65:63–7.
14. Agarwal S, Ojha A, Gupta S. Profile of vitiligo in Kumaun region of Uttarakhand, India. Indian J Dermatol. 2014;59:209. https://doi.org/10.4103/0019-5154.127706.
15. Attili VR, Attili SK. Acral vitiligo and lichen sclerosus - association or a distinct pattern?: a clinical and histopathological review of 15 cases. Indian J Dermatol. 2015;60:519.
16. Shah AS, Supapannachart N, Nordlund JJ. Acquired hypomelanotic disorders. In: Levine N, editor. Pigmentation and pigmentary disorders. Boca Raton, FL: CRS Press; 1993. p. 337–51.
17. Song MS, Hann SK, Ahn PS, et al. Clinical study of vitiligo: comparative study of Type A and Type B vitiligo. Ann Dermatol. 1994;6:22–30.
18. Tan WP, Goh BK, Tee SI, Kumarasinghe SPW. Clinical Profile of vitiligo in Singapore. 2nd Meeting of the Asian Society for Pigment Cell Research, July 2007, Singapore. Pigment Cell Res. 2007;20:253.. (abstract).
19. Hann SK, Nordlund JJ. Clinical features of generalized vitiligo. In: Hann S-K, Nordlund JJ, editors. Vitiligo. Oxford: Blackwell; 2000. p. 81–8.
20. Aydogan K, Turan OF, Onart S, et al. Audiological abnormalities in patients with vitiligo. Clin Exp Dermatol. 2006;31:110–3.
21. Pan JY, Goh BK, Theng C, Kumarasinghe SPW. Vitiligo as a reaction to topical treatment with diphencyprone. 2nd Meeting of the Asian Society for Pigment Cell Research, July 2007, Singapore. Pigment Cell Res. 2007;20:247.. (abstract).
22. Dogra S, Kumar B. Repigmentation in vitiligo universalis: role of melanocyte density, disease duration and melanocyte reservoir. Dermatol Online J. 2005;11:30.
23. Husain I, Vijayan E, Ramaiah A, et al. Demonstration of tyrosinase in the vitiligo skin of human beings by a sensitive fluorometric method as well as by 14C(U) L tyrosine incorporation into melanin. J Invest Dermatol. 1982;78:243–52.

24. Tobin DJ, Swanson NN, Pittlekow MR, et al. Melanocytes are not absent in lesional skin of long duration vitiligo. J Pathol. 2000;191:407–16.
25. Naughton GK, Eisenger M, Bystryn JC. Antibodies to normal human melanocytes in vitiligo. J Exp Med. 1983;158:246–51.
26. Kumarasinghe SPW. An optimistic approach to management of vitiligo. Ceylon Med J. 1995;40:94–6.
27. Schallreuter KU, Behrens-Williams S, Khaliq TP, et al. Increased epidermal functioning wild-type p53 expression in vitiligo. Exp Dermatol. 2003;12:268–77.
28. Mattoo SK, Handa S, Kaur I, et al. Psychiatric morbidity in vitiligo: prevalence and correlates in India. J Eur Acad Dermatol Venereol. 2002;16:573–8.

Segmental Vitiligo

6

Seung-Kyung Hann, Hsin-Su Yu, Cheng-Che Eric Lan, Ching-Shuang Wu, Yvon Gauthier, Laïla Benzekri, and Alain Taïeb

Contents

S.-K. Hann
Korea Institute of Vitiligo Research, Drs. Woo & Hann's skin clinic, Seoul, South Korea
e-mail: skhann@paran.com

H.-S. Yu · C.-C. E. Lan
Department of Dermatology, Kaohsiung Medical University, Kaohsiung, Taiwan
e-mail: dermyu@kmu.edu.tw; laneric@kmu.edu.tw

C.-S. Wu
Faculty of Biomedical Laboratory Science, Kaohsiung Medical University, Kaohsiung, Taiwan
e-mail: m785034@kmu.edu.tw

Y. Gauthier · A. Taïeb (✉)
Service de Dermatologie, Hôpital Saint-André, CHU de Bordeaux, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

L. Benzekri
Department of Dermatology, CHU Ibn Sina, Rabat, Morocco

Abstract

Segmental vitiligo (SV) has been historically opposed to vitiligo/nonsegmental vitiligo (NSV). However, evidence that the two forms are not mutually exclusive (delineation of mixed vitiligo) and share similar inflammatory features has challenged classical views over the last decade. SV belongs to the clinical spectrum of vitiligo, including inflammatory skin features. SV has usually an early onset and spreads rapidly in the affected segment. The hair follicle melanocytic compartment is frequently involved. Distribution in SV parallels that of other acquired pigmentation disorders such as nevus spilus, pointing out to some underlying developmental defect. Neurogenic influences have been highlighted by striking

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_6

clinical observations and experimental studies. Classification of SV is needed to establish a prognosis, and two recent classifications for cephalic and truncal SV have been proposed. Segmental vitiligo is a perfect model for studying repigmentation since it is a relative stable disease with limited active melanocyte loss. Early aggressive medical treatment of SV is currently recommended.

Key Points

- SV belongs to the clinical spectrum of vitiligo, including inflammatory skin features.
- SV has usually an early onset and spreads rapidly in the affected segment.
- The hair follicle melanocytic compartment is frequently involved.
- Distribution in SV parallels that of other acquired pigmentation disorders, such as nevus spilus, pointing out to some underlying developmental defect.
- Classification of SV is needed to establish a prognosis.
- Segmental vitiligo is a perfect model for studying repigmentation since it is a relative stable disease with limited active melanocyte loss.
- Early aggressive medical treatment of SV is currently recommended.

6.1 Historical Background and Controversies on Patterning

Segmental vitiligo (SV) has been historically opposed to vitiligo/nonsegmental vitiligo (NSV). However, evidence that the two forms are not mutually exclusive (Chap. 7) and share similar inflammatory features has challenged classical views over the last decade.

In 1977, Koga performed a sweat secretion stimulation test using physostigmine and accordingly reclassified vitiligo into nonsegmental type (type A) and segmental type (type B). He proposed that the nonsegmental type results from immunologic mechanisms, while segmental type results from dysfunction of the sympathetic nervous system in the affected skin and less prone to Koebner's phenomenon (Koga et al. 1977).

The controversy on the separation of SV from vitiligo/NSV has been fuelled by the interpretation of SV patterning, with two major options, namely, dermatomal/neural vs. blaschkolinear/developmental.

- For the dermatomal/neural hypothesis, it has been considered that SV follows partially or completely one or more dermatomes, similar to that commonly observed in herpes zoster for the trigeminal nerve dermatome [1–7] (Fig. 6.1a, b). However, in 64% of cases, depigmented macules do not follow a true dermatomal pattern. The depigmentation involves either partially each dermatome or overlaps two or three dermatomes especially when SV is located on the face. Many clinical observations of leukoderma have been reported in areas corresponding to local neurologic damage such as unilateral vitiligo, and poliosis located on the area innervated by both trigeminal and upper cervical nerves was reported in a child with viral encephalitis [8]; ipsilateral macules of vitiligo at the level of the upper arm, chest, buttocks, and sacrum were observed in a 31-year-old patient following trauma of the right brachial plexus [9], grayer hair on the left than on the right side after right cervical sympathectomy [10–12], and localized depigmentations similar to SV in patients with a spinal cord tumor or following nerve injury [13, 14].
- In addition to Koga's experiments, an abnormal effect of neurohormones and neuropeptides has been speculated to explain the chronic melanocyte loss in generalized vitiligo [10]. Electron microscopy has established that direct contact occurs between intraepidermal nerve endings and melanocytes [15]. Dystrophic changes in nerve trunks and nerve endings, as well as degenerative and regenera-

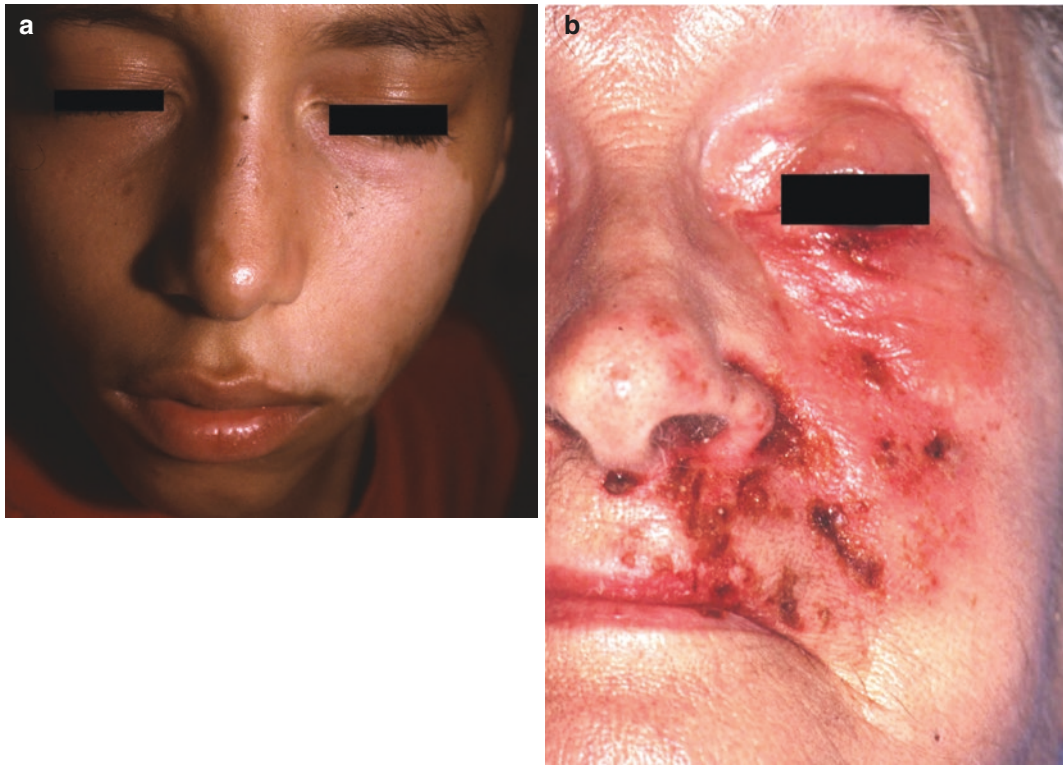


Fig. 6.1 Similar segmental distribution of SV and herpes zoster. SV corresponding strictly to V2 dermatome (**a**), herpes zoster with the same distribution (used with permission of JJ Morand) (**b**)

tive changes in terminal cutaneous nerves, have been found in vitiligo [15, 16]. Using antibodies against neuropeptide Y and calcitonin gene-related peptide (CGRP), several authors have been able to show an increased expression of these peptides in lesional and perilesional skin and frequently an increased number of CGRP-positive nerve fibers in involved skin [17]. Abnormalities in neuropeptides or other neurochemical mediators secreted by nerve endings could theoretically harm nearby melanocytes. The existence of a dermal adrenergic innervation to melanophores of teleost fish has been demonstrated. Electrical stimulation of nerve fibers of the skin causes lightening in the European turbot [18]. Application of epinephrine similarly leads to aggregation of melanin within melanophores in some fishes accompanied by lightening of skin color. Sympathetic denervation has been followed by the development of melanin pigment aggregation.

- Embryologically, melanocytes have the same origin as neural cells. They can react to many compounds that affect the nervous system. In frogs, acetylcholine, norepinephrine, epinephrine, and melatonin lighten dermal melanocytes [19]. In guinea pig skin [20], the spread of grafted melanocytes in denervated skin is statistically better than in nonoperated control sites. This could suggest that peripheral nerves could play a role in the regulation of melanocyte function especially for migration. A single subcutaneous injection of epinephrine into rats causes local depigmentation of the hairs, but the interpretation of this phenomenon is not straightforward, since it seems likely that vasoconstriction can induce ischemic depigmentation.

- In patients with vitiligo/NSV, local abnormalities related to possible neural-mediated aberrations have been reported. However, data are discordant. An increased adrenergic nerve-ending activity in vitiligo macules was suggested by Lerner [21]. Other authors came to the conclusion that there was a dominant cholinergic influence in vitiliginous skin compared to normal skin [22, 23]. Koga's classification proposes that SV could result from the dysfunction of sympathetic nerves in the affected area. On the basis of physostigmine-induced sweating response, a decreased responsiveness of SV was interpreted to be due to an increased cholinergic influence [24–26]. In SV lesions, the cutaneous blood flow was shown to be increased almost threefold compared to control normal skin, and a similar anomaly was shown to a lesser extent in vitiligo/NSV. A significant increase in cutaneous alpha- and beta-adrenoreceptor response was found in the segmental type suggesting that a dysfunction of the sympathetic nerves could exist [26–31]. As noted before, the catecholamine stimulation of adrenoreceptors may cause severe vasoconstriction. It is thus possible that hypoxia-induced oxidative stress at the epidermal level could lead to partial or total depigmentation of one or several dermatomes. However no changes in plasma catecholamine level or adrenoreceptor densities on blood cells have been so far reported [32].
- For the blaschkolinear/developmental hypothesis, the lines described by Alfred Blaschko in 1901 (Blaschko's lines, BL) were based on drawings of cutaneous nevoid lesions [33]. They have been revisited extensively by Happle [34] and considered to follow the dorsoventral development of cellular components of the skin. When visible, BL reflect an underlying somatic mosaicism demonstrated first in monogenic keratin disorders [35]. BL have been postulated to correspond to a migration pattern restricted to cells of ectodermal or neuroectodermal origin (like melanocytes) [36], but several observations of purely dermal diseases following this pattern mitigate this view [37]. The two types of lines (thin or large, see Fig. 6.2a, b) may somewhat correspond to the underlying cellular origin of somatic mosaicism, dermal disorders tending to form broad bands [38]. Other types of patterns which do not fit Blaschko's lines (checkerboard, phylloid, garment-type) are also observed in the dermatology clinic [39] and may concern the melanocytic system or other types of cells. Some blaschkolinear distribution patterns in SV are strikingly similar to that of epidermal nevi [40]. Mosaicism may sometimes also involve germline cell mutations, a fact which explains the coexistence of segmental and generalized patterns in some rare pedigrees [41]. The dermatomal distribution has been historically considered as reflecting better the distribution of SV and the underlying neural theory, but cases intersecting dermatomes without "filling" their theoretical territory of distribution were difficult to relate to this etiologic background. The sympathetic anomalies noted in favor of the neurogenic theory of SV may also be a confounding factor related to the absence of melanocytes which have been considered as "neurons of the skin" [42] and can release several neuromediators. The large blaschkolinear pattern, when isolated on a limb, can mimic a dermatomal distribution (Fig. 6.2c, d). On the face, the lines of Blaschko have been drawn more recently [43], and some cases of SV fit clearly better Blaschko's bands than dermatomal territories [38, 40] (Fig. 6.3). It has also been suggested that segmental vitiligo might follow acupuncture lines [44]. Another possibility is that the distribution of segmental vitiligo follows a so far unknown developmental pathway corresponding to a group of identical clonal cells. Differing characteristics of melanocyte survival and melanogenesis may influence the location/course of the lesions. In Fig. 6.4a, the 14-year-old male

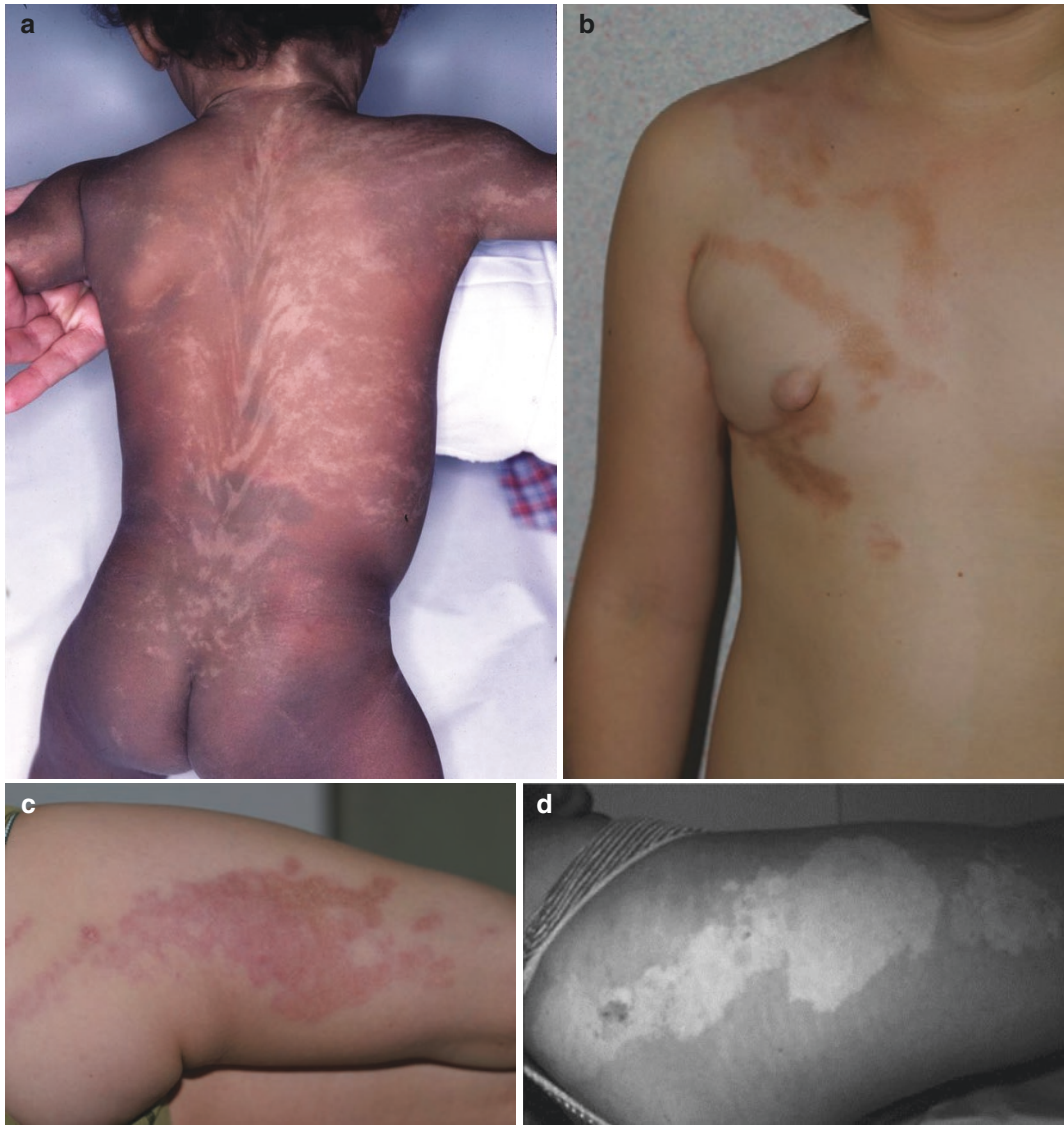


Fig. 6.2 Blaschko's bands vs. Blaschko's lines. Hypomelanosis of Ito (**a**) demonstrates nicely the thin blaschkolinear hypopigmented streaks originally described by Alfred Blaschko (courtesy of Dr Odile Enjolras) and large band blaschkolinear granuloma annulare (**b**) (reported by Morice-Picard et al. [37]); (**c**) shows same patient as in (**b**); note the difficulty to relate the pattern to Blaschko's bands (compare to **d**); segmental vitiligo of the thigh considered as following a dermatomal distribution (**d**)

patient had vitiligo around the left eye, while nevus spilus occurred in a symmetrical fashion. Both conditions appeared almost simultaneously several months before, suggesting a variant twin-spotting phenomenon [45]. The same cutaneous pattern may also occur in different diseases—segmental vitiligo (Fig. 6.4b) and nevus spilus (Fig. 6.4c). These findings suggest that clones of melanocytes with different functional characteristics exist in acquired pigmentary disorders. Melanocytes in nevus spilus can produce

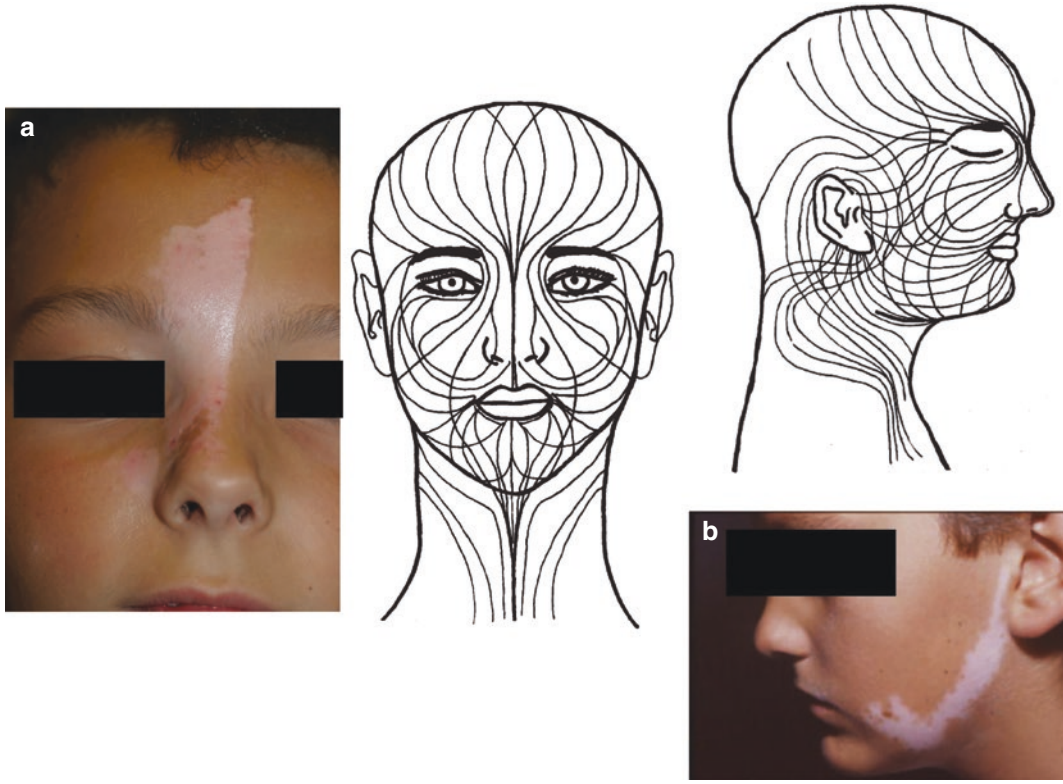


Fig. 6.3 Facial distribution of some cases of SV fits better Blaschko's lines than dermatomes. Two examples with the corresponding summary of lines by Happle and Assim, [43], From Taieb et al, [38], with permission

more melanin than normal and may have a normal lifespan. The type of melanocytes found in segmental vitiligo of the same location can be easily lost by an unknown mechanism. The exact nature of this phenomenon has not been fully elucidated but might reflect the embryonic migration pathway of a melanocyte colony with an inherited defect, which is not readily observable at birth or in the first months of life, thus suggesting an associated environmental trigger.

- The hypothesis of cutaneous mosaicism in SV favoring underlying developmental defect does not rule out other triggering factors, especially neurogenic ones. It does not also exclude the possibility of an inflammatory component extending locally beyond the area demarcated by the developmental lines, which has received recent confirmation [46–48] (see Chap. 32 for a more in-depth review of pathophysiology of SV and a modern convergent theory for SV).

6.2 Clinical and Pathological Features

Koga and Tango reported that SV affects more commonly the young and indicated a higher (27.9%) incidence in children SV develops before 30 years of age in 87.0% of cases and 41.3% before 10 years of age. The earliest reported onset was immediately after birth, whereas the latest was 54 years [3].

The typical lesion is not that different from the macule observed in nonsegmental vitiligo. The most common form is a totally amelanotic macule surrounded by the normal skin. The color

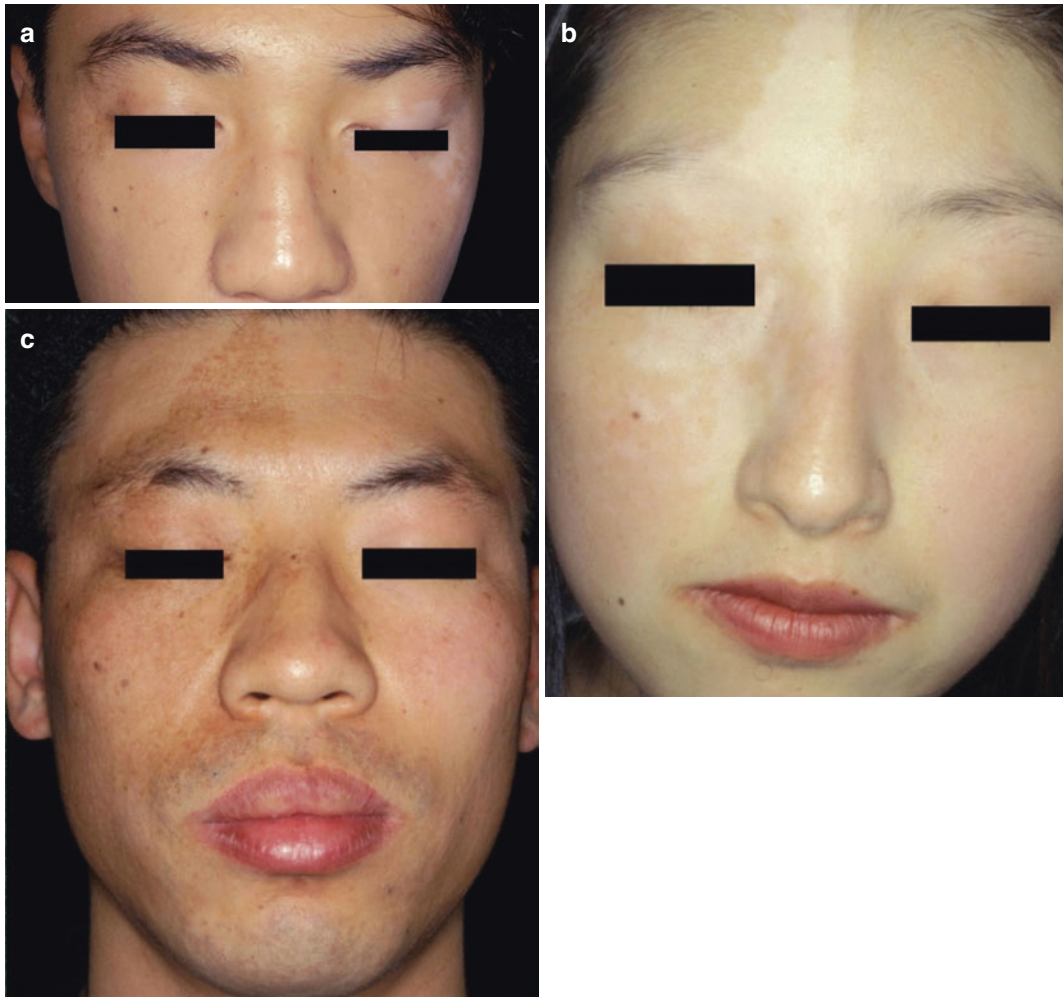


Fig. 6.4 (**a**) Segmental vitiligo and nevus spilus coexist symmetrically; same facial pattern in segmental vitiligo (**b**) and nevus spilus (**c**) in two different patients better to have b and c on the same horizontal level for comparison

of the macule is usually pure white or chalk white. However, as in vitiligo/NSV, a multichrome variation of hypopigmentation can be observed, and overall a less uniform depigmentation pattern was seen in SV compared to vitiligo/NSV when a decision was needed for grading a patch [49]. In some cases, such as on fair skin, the lesions are not easy to see under normal light but can be distinguishable with Wood's light examination. A dermatomal or pseudo dermatomal distribution has been suggested and fuelled the neural theory of SV, but common overlap involving several dermatomes occurs, and lesions may sometimes cross the midline (Fig. 6.5). In the majority of cases, depigmentation spreads within the segment in a short period of time and then stops. It leaves the skin segment partially or totally depigmented. It is rare for a patient with SV to progress to the generalized form. Such a phenomenon is now best recognized as the association of SV and NSV called "mixed vitiligo" [Chap. 1.5.3]. In this case the increased severity of SV versus vitiligo/NSV in response to therapy suggests that the dosing of

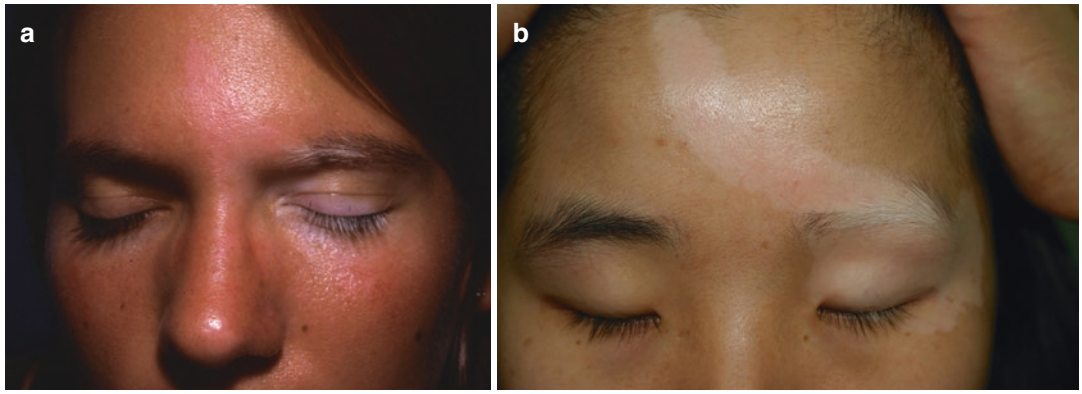


Fig. 6.5 Typical aspect of SV I-b of the face with poliosis of the eyebrow/eyelids crossing either slightly (**a**) or more markedly (**b**) the midline

the predisposing skin anomaly is augmented in the SV area (Chap. 7).

The head is involved in more than 50% of cases. In decreasing order of frequency, the trunk, the limbs (Fig. 6.6), the extremities, and the neck are common sites of involvement. In females, the neck is more frequently involved than the extremities. Lerner [10] reported that segmental vitiligo occurs as a single lesion in 75% of patients, a finding confirmed by Hann and Lee [50] who found that 87% of patients had a single lesion and more recently by Van Geel et al., who found 88% unilateral SV, 2% bilateral, and 15% mixed vitiligo cases in their series [51].

There is usually no preferential distribution between right and left sides of the body. Some patients have lesions in two different unilateral cutaneous segments. The most commonly involved is that sharing in part the distribution of the trigeminal nerve, a finding which was given as a major support of the neural theory of SV. Recent data indicate that no clinically recognizable pattern—non-dermatomal and non-blaschkoid—is common (77% of cases) and that facial involvement for SV is usually of later onset than extrafacial involvement (median 19 years vs. 6.5 years) [51].

A family history of vitiligo is present in approximately 12% of SV cases [51]. El Mofty & El Mofty [52] and Koga [1] suggested that SV is not significantly associated with other autoimmune disease. However, Park et al. [53] showed that about 9.5% of SV cases were associated with

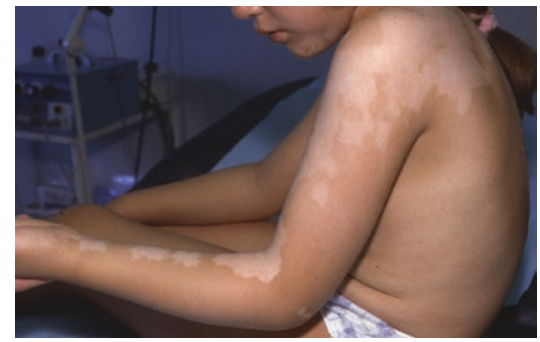


Fig. 6.6 SV of the left shoulder and upper limb

other diseases. Similarly, Hann and Lee [50] reported 6.7% of patients with associated disease either allergic or autoimmune. Looking at associated features to SV and vitiligo/NSV, including those pertaining to autoimmune diathesis, the global impression is more a continuum than two completely separate diseases [54]. Histopathology of recent progressing SV confirms the existence of infiltrating T cells [47] (Shin et al. 2016) - see also Chap. 32 [54].

6.3 Preferential Melanocytic Follicular Reservoir Involvement in SV

Body leukotrichia varies from 10% to more than 60% in the literature for all types of vitiligo (Chap. 9). It is striking that the follicular com-

partment of the melanocyte organ may be spared, while vitiligo affects the epidermal compartment. This dissociated behavior of epidermal and follicular melanocytes is very common in vitiligo/NSV. NSV affects both epidermal and follicular melanocytes in its early phase of disease as compared to vitiligo/NSV (Fig. 6.5, simultaneous involvement of the skin and eyelids/lashes).

Depigmentation of hairs (poliosis, leukotrichia) occurs more specifically in vitiliginous SV macules. Poliosis has been shown to occur in around half of cases of SV. If cases of body involvement of SV (for which this item is less accurately documented) had been excluded, the incidence of leukotrichia-associated SV would be even higher. Involvement is variable; a few to many hairs of a single macule may be depigmented. The eyebrow and eyelashes are commonly involved in SV of the upper face. Other involved hairy sites include the scalp, pubis, and axillae.

This particular tropism for hair melanocytes has therapeutic consequences. Repigmentation of hair is difficult to address with UV and other medical treatments. Transplantation of melanocytes is required for rapid and complete repigmentation (Chap. 36).

6.4 Classification

Knowledge about the exact spreading pattern and prognosis is important for both patients and doctors. So far cephalic and truncal distribution of SV have been systematically studied.

6.4.1 Classification of Cephalic SV

Based on a large (257 unambiguous cases) and careful Korean study, the distribution of segmental vitiligo on the face is classified into six subtypes (Fig. 6.7) [55].

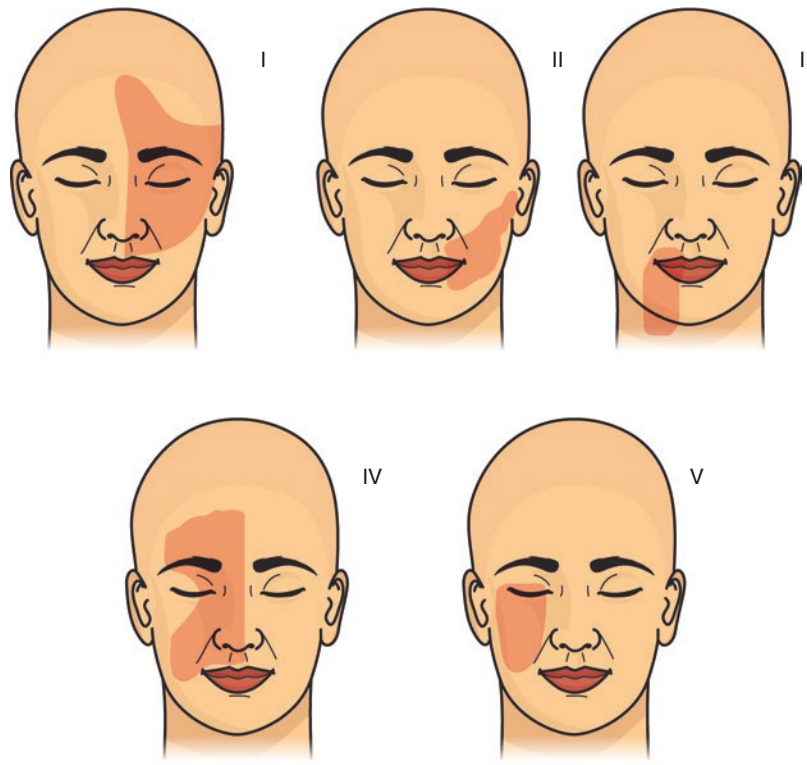


Fig. 6.7 Hann and coworkers revised classification of segmental vitiligo of the face

Typical lesions of **type I-a**, the most common type (28.8%), start on one side of the middle area of the forehead, cross the midline of the face around the glabella, and extend downward and laterally to the eyelid, nose, philtrum, and cheek of the contralateral side of the face without involving scalp hair. Spread to the neck can be seen rarely. Surprisingly, the left side of the face is more frequently involved (89% of cases). In **type I-b** (10.5%), lesions appear on the right or left side of the forehead with frequent involvement of scalp hair, with rare spread down to the periorbital area and eyebrows.

In **type II** (16%), the lesions begin at the angle of the mouth and then arch to the auricular area across the cheek or mandible. The degrees of curvature and lesion widths vary widely, and involvement of the upper neck area is common.

In **type III** (14.4%), lesions begin below the lower lip and spread down to include the chin and neck. Unlike type II, type III lesions usually involve medial rather than lateral aspects of the neck and rarely the upper chest.

In **type IV** (10.9%), all the lesions originate on the right side of the forehead and extend to the eyelids, nose, and cheek areas without crossing the midline at the forehead and glabella, unlike type I-a.

In **type V** (8.6%), lesions are confined to the right orbital area, although they could spread contiguously to the temporal area.

For **mixed types** (10.9%), the coincidence of type II and type III (15 cases) is the most common combination.

A suggestion to simplify this classification at the international level would be to combine in one type I-a and IV on the basis that crossing the midline is common in other developmental somatic mosaics affecting pigmentation, which would give four basic patterns for cephalic vitiligo. Another item to study further is the distribution of lesions in the lateral and posterior cephalic area, which is so far not taken sufficiently into account.

6.4.2 Classification of SV of the Trunk

The data were collected by the group of Ghent in 106 patients [56] and are summarized thereafter. The distribution on the trunk was classified into six patterns: (1) midline (3.8%), (2) shoulder (3.8%), (3) V-shape high on the thorax (2.6%), (4) V-shape low on the thorax 7 (6.6%), (5) band-like (17.9%), and (6) checkerboard (13.2%); 32.1% of cases could not be classified (Fig. 6.8). **Type 1** is a mid-central linear subtype that seems to be an extension of type III SV pattern on the face (previous section). It can cross the midline of the trunk.

Types 2, **3**, and **4** can extend to the ipsilateral arm, while type 6 may affect the upper leg. **Type 3** features a V shape to the lower midline, comparable with the down movement of the Blaschko's lines toward the ventral part of the trunk. A similar bowlike V pattern can sometimes be observed on the back, although in our cases this was always associated with an accompanying V-shaped pattern on the ventral side of the trunk. **Type 5** has a more horizontal band-shape distribution, mostly overlapping the lateral side of the trunk. **Type 6** is located on the lower part of the trunk under the waist, similar to the checkerboard pattern described by Happle [57]. In half of the cases, a combination of SV on both the front and back of the lower trunk was found, but the lateral side was spared in most instances.

In summary, truncal SV is frequently located at the ventral or ventral-lateral side of the trunk and rarely exclusively on the back. In contrast to the interpretation of SV patterning of the face [55], there is no left or right preferred distribution of certain subtypes. However, lesions of the left side of the trunk are observed in a younger age group.

6.5 Diagnosis, Course, and Special Locations

Most often SV patches remain unchanged for the rest of the patient's life after rapid initial spreading in the affected segment [50]. However, rarely it can progress again after being quiescent for several years (Fig. 6.9). When segmental vitiligo progresses, it usually spreads over the predicted segment. Early SV most often appears as a solitary oval-shaped white macule or as a patch that is difficult to differentiate from "focal" vitiligo until proven by a subsequent typical distribution

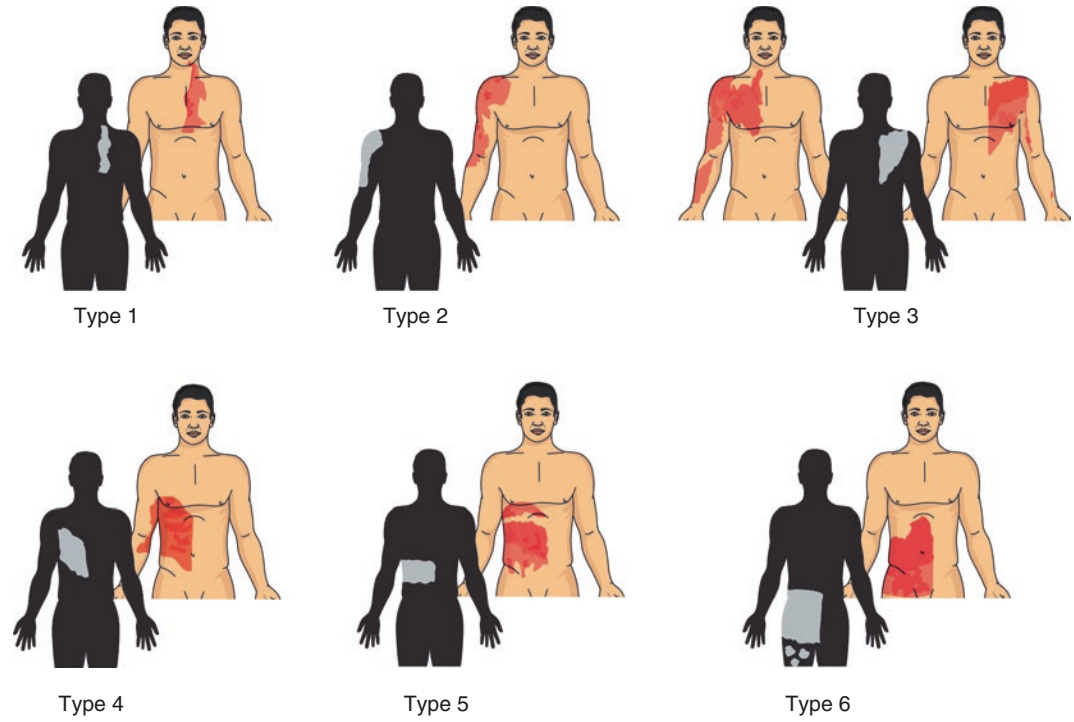


Fig. 6.8 Van Geel and coworkers classification of segmental vitiligo of the trunk

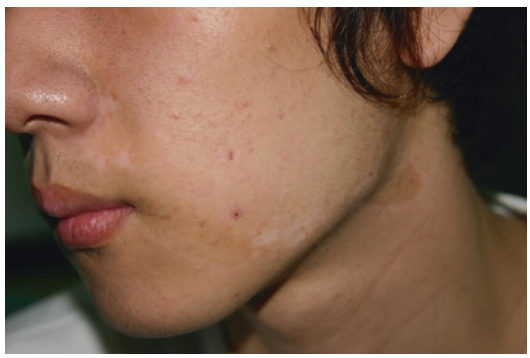


Fig. 6.9 Recurrence of vitiliginous lesions at the periphery of an autologously grafted SV site. The graft was done 10 years before the recurrence (corresponds to Hann's type III, see Fig. 6.7)

pattern. A white macule on the nipple or areola appearing as the initial lesion can be assumed to be an early manifestation of SV (Fig. 6.10a) [3]. Nipple or areolar involvement as the initial lesion in nonsegmental vitiligo is very rare and becomes bilateral later (Fig. 6.10b). If SV occurs bilaterally, following the same contralateral (or different) dermatomes, it may cause difficulties in defining vitiligo type (Fig. 6.11). It may at some point be confused with NSV or, for some locations, such as white patches of both legs, to piebaldism (Chap. 2). Lee and Hann [58] reported that 5 out of 240 patients, who had SV, exhibited two different depigmented segments on the same or opposite site of the body. The clinical course of bilateral SV seems to be the same as unilateral SV.

6.6 Repigmentation in Segmental Vitiligo and Phototherapies

Previous studies on vitiligo have focused on vitiligo/NSV, and very few studies have addressed specifically SV [1, 3, 40, 50, 52, 59]. In order to gain further insights on how to treat this disorder more effectively, a careful analysis of how differ-

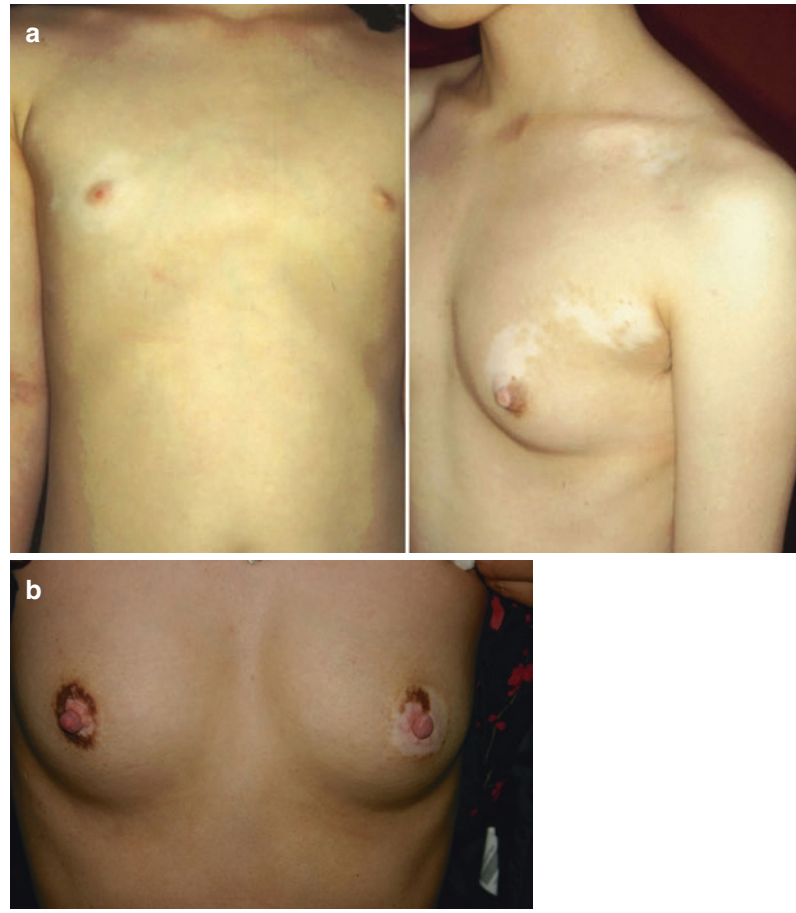


Fig. 6.10 (**a**) A white macule on the areola or nipple often progresses to segmental vitiligo (left panel early stage, difficult to predict; right panel, clear segmental involvement, different patient). (**b**) Bilateral involvement of the nipple in vitiligo/NSV

ent modalities induce vitiligo repigmentation would be invaluable. Segmental vitiligo is regarded as a stable disease once the initial melanocyte loss has ceased. Since the active destruction of melanocyte is no longer present, the recovery scheme of segmental vitiligo depends on (1) the activation, migration, and functional development of melanoblasts, (2) the proliferation and migration of functional melanocytes, and (3) the impact of epidermal keratinocytes on pigment cells [60].

The pattern for vitiligo repigmentation may take two forms: the follicular pattern and the diffuse pattern. The melanoblasts in the outer root sheath (ORS) of the hair follicle serve as the source for follicular repigmentation [61]. In the follicular pattern, recovery from vitiligo is initiated by the activation and proliferation of immature melanoblasts, followed by their upward migration onto the nearby epidermis to form the follicular pigment islands [62]. During this process, the melanoblasts mature morphologically and functionally and subsequently begin normal transfer of melanin to keratinocytes [63]. On the other hand, perilesional or diffuse patterns of repigmentation involve probably the migration of perilesional melanocytes toward the vitiligo macules or activation of inactive melanocytes within vitiligo lesions, respectively [64, 65]. Figure 6.12 summarizes the different repigmentation processes described above.

Epidermal melanocytes and follicular melanocytes express different antigens [66] providing further evidence of intrinsic differences between segmental and generalized vitiligo. Regardless of the initial damaging process, once the disease becomes stable, the most important cells involved in segmental vitiligo repigmentation besides

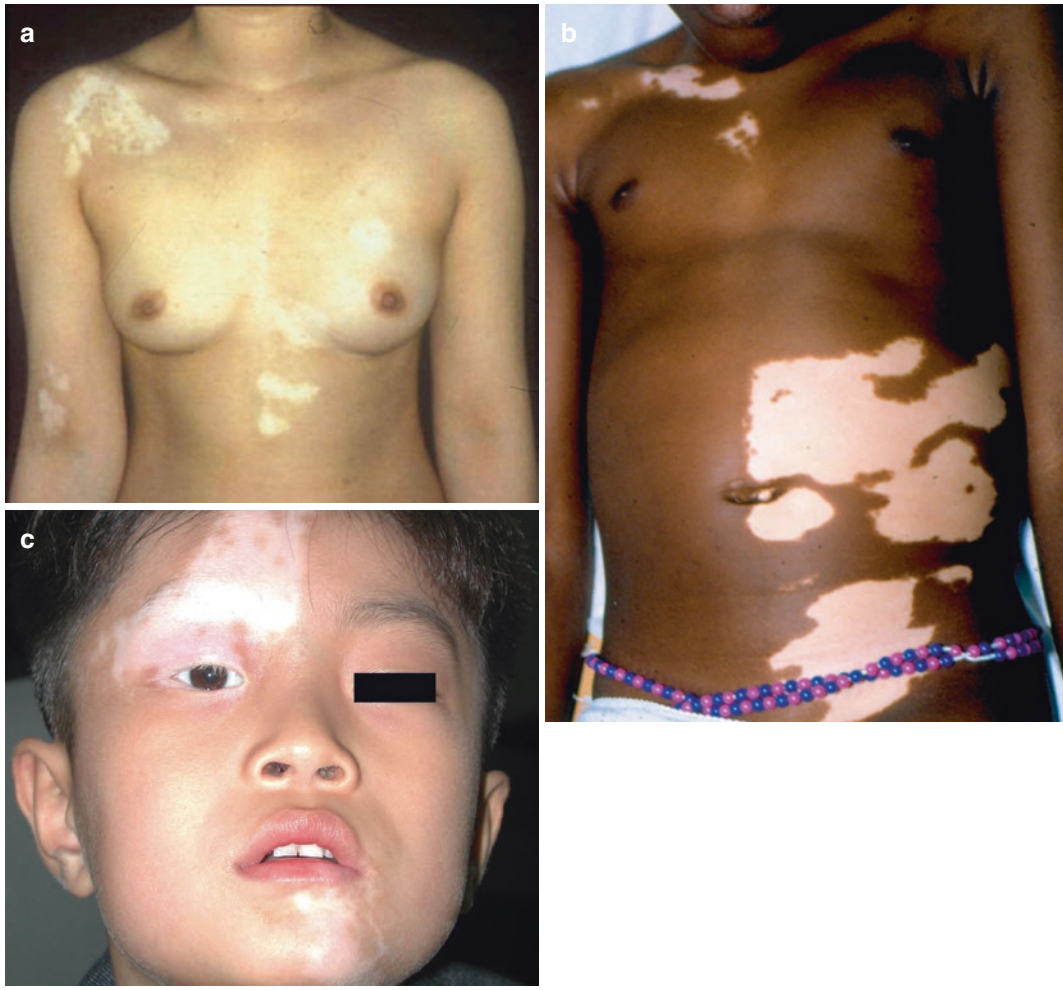


Fig. 6.11 Bilateral segmental vitiligo of the same distribution in Asian (**a**) and black (**b**) patients; bilateral facial vitiligo (**c**) bilateral facial SV I + IV

melanoblasts and melanocytes are keratinocytes, which play a crucial role in modulating epidermal pigmentation. Through their growth factors/mitogens, keratinocytes can modulate the biological behavior of neighboring melanocytes. Stem cell factor, basic fibroblast growth factor, endothelins, and nerve growth factor are among the documented factors that participate in this delicate relationship.

PUVA treatment has been shown to be more effective for treating vitiligo/NSV as compared to SV in a Saudi study [67]. The immunomodulating effects of PUVA are probably more important for active vitiligo/NSV and play a limited role in SV as active melanocyte loss is not a feature of established SV lesions [68]. The biological impact of PUVA on epidermal cells includes (1) limited stimulatory effect on keratinocytes; (2) increased matrix metalloproteinase (MMP)-2 activity, an important modulator for pigment cell motility, from melanocytes [69]; and (3) increased tyrosinase activity of melanocytes [70]. In addition, the photosensitizer 8-methoxypsoralen by itself can stimulate MMP-2 expression from melanoblasts [71]. These biological effects imparted by PUVA on epidermal cells provide a reasonable explanation for the therapeutic effect of PUVA on SV. Westerhof and Nieuweboer-Krobotova [72] reported that vitiligo patients receiving PUVA and NBUVB experienced a repigmentation rate of 46% and 67%,

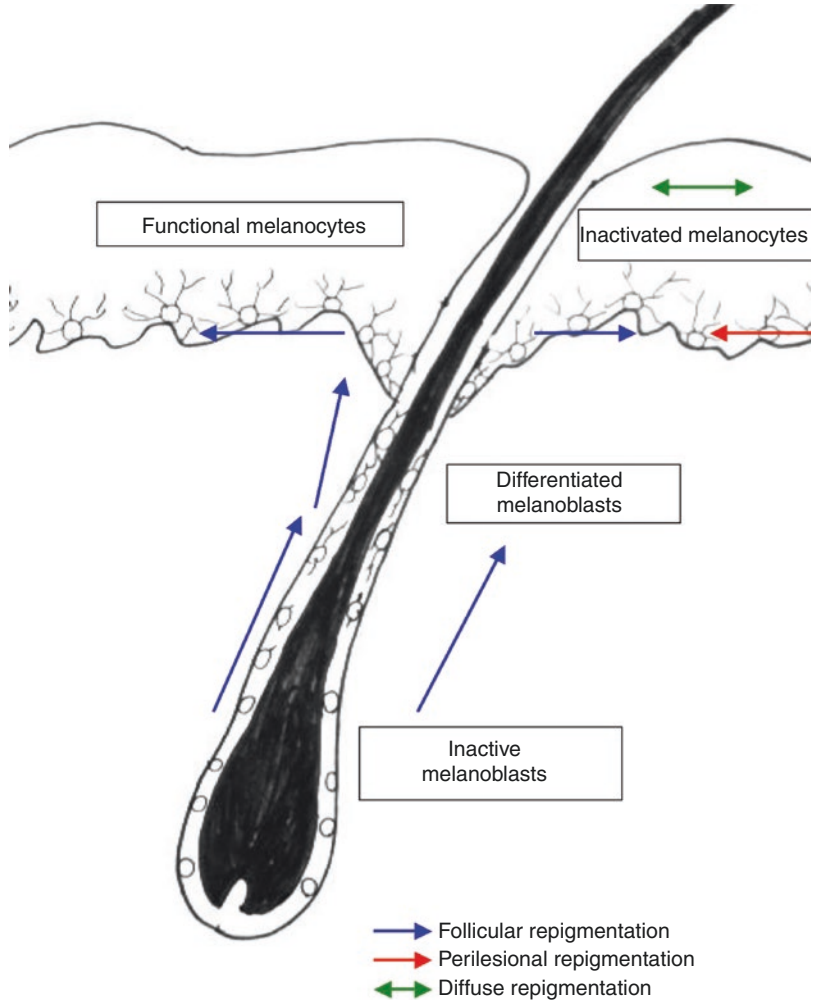


Fig. 6.12 Repigmentation scheme of vitiligo. The process of follicular pigmentation is shown in blue color. This repigmentation route involves activation, proliferation, migration, and functional development of immature melanoblasts residing on the outer root sheath of the hair follicle. Diffuse (green) and perilesional (red) patterns of repigmentation require activation of inactive melanocytes and migration of perilesional melanocytes, respectively. All three forms of pigmentation pattern may be observed in repigmenting vitiliginous skin

respectively, after 4 months of phototherapy. NBUVB is known to have immune modulatory effects including depleting skin infiltrating T cells and arresting maturation of epidermal dendritic cells [73]. In terms of biological effects, NBUVB irradiation affects several cellular targets: (1) it causes a direct stimulation of melanocyte locomotion via increased expressions of phosphorylated focal adhesion kinase, (2) it increases matrix metalloproteinase (MMP)-2 activity from melanocytes, (3) it causes indirect stimulation of melanocyte proliferation by increasing the production of basic fibroblast growth factor and endothelin from keratinocytes [69], and (4) it causes a direct stimulation of melanoblasts in terms of melanin formation and cell migration (Yu et al, unpublished data).

Comparing the in vitro effects of NBUVB and PUVA on epidermal cells, it is clear that both modalities have significant immunomodulatory effects, but NBUVB has more direct biological effects on epidermal cells in terms of supporting pigment cell migration, proliferation, and development. Therefore, according to the in vitro repigmentation model, NBUVB may provide better therapeutic efficacy for inducing repigmentation in SV [74], as compared to PUVA therapy.

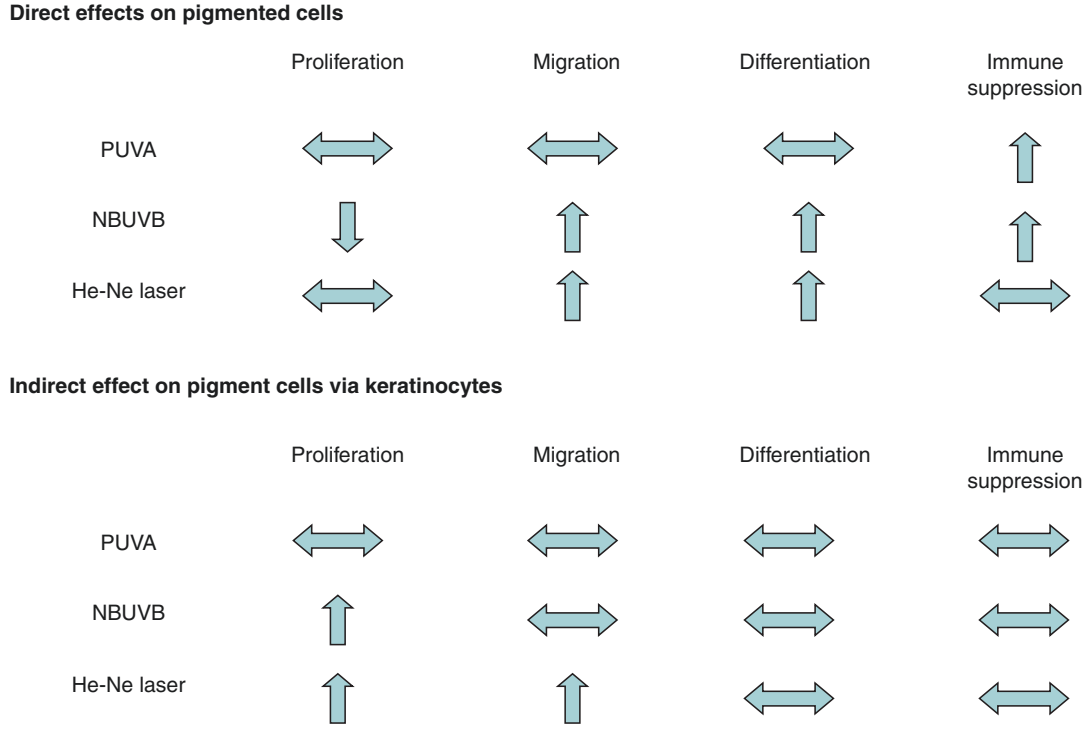


Fig. 6.13 Schematic diagram summarizing the mechanisms involved in PUVA, NBUVB, and laser red light-induced vitiligo repigmentation. PUVA treatment has significant immune modulatory effects on both melanocytes and cytotoxic immune cells. It also directly promotes melanocyte melanin formation and migrations but has limited biological effects on keratinocytes. Besides immune suppressive effects, NBUVB treatment has significant biological effects on pigment cells and keratinocytes. More specifically, NBUVB directly or vicariously via keratinocyte increases the proliferation and locomotion of melanocytes. In addition, NBUVB also stimulates melanogenesis and migration of immature melanoblasts. The Ne-He laser red light has also direct effects on melanoblasts. Abbreviations: *NBUVB* narrowband UVB, *KC* keratinocyte, *MC* melanocyte, *CTL* cytotoxic T lymphocyte, *MB* melanoblast

More recently, helium-neon (He-Ne) laser, which emits within visible red light, has been shown as a novel phototherapeutic modality for treating SV [75]. He-Ne laser promotes vitiligo repigmentation through (1) direct stimulation of melanocyte and melanoblast migration [75, 76], (2) direct stimulation of melanoblasts to undergo functional development [76], (3) increased secretion of nerve growth factor and basic fibroblast growth factor from keratinocytes, and (4) indirect stimulation of melanocyte growth via keratinocytes [75]. A summary of direct vs. indirect effect of phototherapies in repigmentation is shown in Fig. 6.13.

Tacrolimus ointment is a promising topical agent for treating SV. In vitro studies using epidermal cells have shown that tacrolimus (1) directly stimulates keratinocytes to release stem cell factor, (2) induces keratinocytes to promote the growth of both melanoblasts and melanocytes, (3) induces keratinocytes to upregulate MMP activities [77], and (4) directly induces pigmentation and migration of melanocytes [78]. Therefore, besides immunomodulatory effects of tacrolimus on immune cells, the direct biological effects of tacrolimus on epidermal cells probably account for its therapeutic effects on SV. Of interest is the effect of different therapies on the

cutaneous nervous system. Currently, in vitro data regarding this topic is lacking. However, it has been shown that He-Ne laser irradiation leads to improvement in nerve injury [79, 80] and topical tacrolimus induces neuropeptide release [81] and may lead to neuronal cell differentiation [82]. These reports suggest that neural modulatory effects may have a previously unrecognized role in the treatment of segmental vitiligo.

6.7 Treatment Overview and Perspectives

SV was previously known to be resistant to treatment. However, recent studies reported surprisingly good results. SV at an early stage has an excellent prognosis. Since the most frequently involved site of SV is the face, it can be easily detected and treated. Effective treatment modalities at an early stage are not standardized but include topical steroids, topical calcineurin inhibitors, or UV therapy, isolated or in combination (Fig. 6.14). Because SV often causes leukotrichia, it may resist standard medical therapies. Stable SV with leukotrichia can be cured successfully with epidermal grafting and subsequent UV treatment [83]. There may be also a possibility of activation and migration of epidermal melanocytes to the hair follicle. Overall, stable SV is a good indication for epidermal grafting and can be cured almost completely without recurrence (for a discussion of surgical therapies, see Chap. 3.2.3).

Although new strides have been made on treatment options for segmental vitiligo, the underlying molecular mechanisms inducing repigmentation must be elucidated for optimizing treatment outcomes. Another issue, which requires further attention, is the involvement of neurocutaneous interactions in the pathogenesis of vitiligo. Since skin homeostasis may be regulated by the peripheral neuropeptidergic nerve fibers [84], further work focusing on modulation of neurocutaneous unit may provide a key to more effective vitiligo treatment.

Segmental vitiligo is a perfect model for studying repigmentation since it is a relative stable disease with limited active melanocyte loss. Moreover, the cells involved in the repigmentation scheme of segmental vitiligo are readily available for in vitro experimentation. With recent advances in regenerative medicine, the use of melanocyte stem cells [85] may evolve from in vitro experimentations to in vivo reality as suggested by Yonetani et al. [86] (see Chap. 2.3.7).

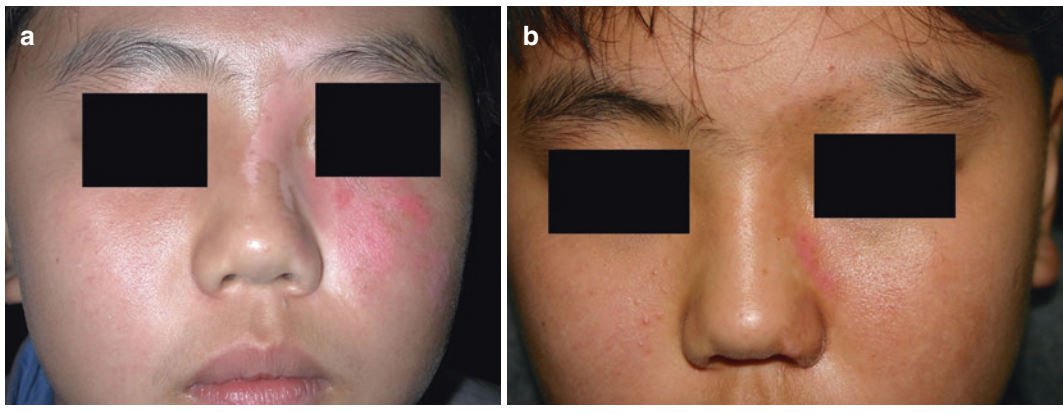


Fig. 6.14 Almost complete repigmentation of SV after 4 months of 308 nm excimer laser treatment (**a**, before; **b**, after) (corresponds to Hann's type I)

References

1. Koga M. Vitiligo: a new classification and therapy. Br J Dermatol. 1977;97:255–61.
2. Klippel M, Weill HP. Radicular distribution of vitiligo and nevi. Presse Med. 1922;30:388–90.
3. Koga M, Tango T. Clinical features and course of type A and type B vitiligo. Br J Dermatol. 1988;118:223–8.
4. Musumeci V. Sulla distribuzione radicolare della vitiligine. Minerva Dermatol. 1951;26:77–9.
5. Ortonne JP, Mosher DB, Fitzpatrick TB. Vitiligo and other hypomelanosis of hair and skin. New York, NY: Plenum Medical Book Company; 1983. p. 250–8.
6. Scholtz JR, Williamson C. Vitiligo in apparent neural distribution. Arch Dermatol Syphil. 1951;64:366–8.
7. Touraine A, Brizard A. La topographie radiculaire du vitiligo. Bull Soc Fr Dermotol Syphil. 1935;42:505–15.
8. Nelhaus G. Acquired unilateral vitiligo and poliosis of the head and subacute encephalitis with partial recovery. Neurology. 1970;20:965–74.
9. Costea V. Leucoderma patches in the course of traumatic paralysis of the brachial plexus in a subject with insular cavities. Act Dermat Venerol. 1961;2:161–6.
10. Lerner AB. Vitiligo. J Invest Dermatol. 1959;39:285–310.
11. Lerner AB. Sympathectomy and gray hair. Arch Dermatol. 1966a;93:235–6.
12. Lerner AB. Vitiligo and sympathectomy. Arch Dermatol. 1966b;94:269–78.
13. Ferriol L. Vitiligo et tumeurs neurologiques de la moelle. Rev Neurol Paris. 1905;13:282–6.
14. Tremiteria S. Vitiligo from spinal anesthesia. Rinazcenca medica. 1927;4:107–8.
15. Breatnach AS, Bors S, Willie L, et al. Electron microscopy of peripheral nerve terminals and marginal melanocytes in vitiligo. J Invest Dermatol. 1966;47:125–40.
16. Gauthier Y, Surlève-Bazeille JE. Ultrastructure des fibres nerveuses périphériques dermiques dans le vitiligo. Bull Soc Fr Dermatol Syphil. 1974;81:550–4.
17. Al'Abadie MS, Senior HJ, et al. Neuropeptide and neuronal marker studies in vitiligo. Br J Dermatol. 1994;131:160–5.
18. Jacobowitz DM, Laties AM. Direct adrenergic innervation of a teleost melanophore. Anat Rec. 1968;162:501–4.
19. McGuire J. Adrenergic control of melanocytes. Arch Dermatol. 1970;101:173–80.
20. Fabian G. The spread of black pigment of the denervated skin of the guinea pig. Acta Biol Acad Sci Hung. 1951;4:471–9.
21. Lerner AB. In: Kawanamura T, et al., editors. Neural control of pigment cells: biology of normal and abnormal melanocytes. Tokyo: University Park Press; 1971. p. 3–16.
22. Chanco-Turner ML, Lerner AB. Physiologic changes in vitiligo. Arch Dermatol. 1965;91:390–6.
23. Gauthier Y, Surlève-Bazeille JE, Gauthier O. Bilan de l'activité cholinestérasique dans le vitiligo: son interêt physiopathologique. Bull Soc Fr Dermatol Syphil. 1976;81:321.
24. Iyengar B. Modulation of melanocyte activity by acetylcholine. Acta Anat. 1989;136:139–41.
25. Jacklin NH. Depigmentation of the eyelids in eserine allergy. Am J Ophtalmol. 1965;59:890–902.
26. Schallreuter KU, Elwary SM, Gibbons NC, et al. Activation/desactivation of acetylcholinesterase by H^2O^2 more evidence for oxydative stress in vitiligo. Biochem Biophys Res Commun. 2004;315:502–8.
27. Bamshad J. COMT in Skin. J Invest Dermatol. 1964;43:111–3.
28. Bir LS, Aktan S. Sympathetic skin response in psoriasis and vitiligo. J Auton Nerv Syst. 1999;77(1):68–71.
29. Stajano C, Martirena H. Bilateral peripheral distribution of vitiligo according to nerve segments. Ac Fac Med Montevideo. 1926;11:563–81.
30. Touraine A, Picquart A. Maladie de Recklinghausen à pigmentation systématisée, vitiligo, pelade. Bull Soc F Dermtol Syphil. 1937;44:81–5.
31. Wu CS, Yu HS, Chang HR, et al. Cutaneous blood flow and adrenoceptor response increase in segmental-type vitiligo lesions. J Dermatol Sci. 2000;23:53–62.
32. Morrone A, Picardo M, De Luca C. Catecholamines and vitiligo. Pigm Cell Res. 1992;5:65–9.
33. Blaschko A. A "Die Nervenverteilung in der Haut in ihre Beziehung zu den Erkrankungen der Haut". Beilage zu den Verhandlungen der Deutschen Dermatologischen Gesellschaft VII Congress, Breslau, 1901
34. Happle R. Lyonization and the lines of Blaschko. Hum Genet. 1985;70:200–6.
35. Paller AS, Syder AJ, Chan YM, et al. Genetic and clinical mosaicism in a type of epidermal nevus. N Engl J Med. 1994;331:1408–15.
36. Taibjee SM, Bennett DC, Moss C. Abnormal pigmentation in hypomelanosis of Ito and pigmentary mosaicism: the role of pigmentary genes. Br J Dermatol. 2004;151:269–82.
37. Morice-Picard F, Boralevi F, Lepreux S, et al. Severe linear form of granuloma annulare along Blaschko's lines preceding the onset of a classical form of granuloma annulare in a child. Br J Dermatol. 2007;157:1056–8.
38. Taïeb A, Morice-Picard F, Jouary T, et al. Segmental vitiligo as the possible expression of cutaneous somatic mosaicism: implications for common non-segmental vitiligo. Pigment Cell Melanoma Res. 2008;21:646–52.
39. Happle R. [Patterns on the skin. New aspects of their embryologic and genetic causes, *German*] Hautarzt. 2004;55:960–1, 964–8.
40. Taieb A. Intrinsic and extrinsic pathomechanisms in vitiligo. Pigment Cell Res. 2000;13:41–7.
41. Nazzaro V, Ermacora E, Santucci B, Caputo R. Epidermolytic hyperkeratosis: generalized form in

children from parents with systematized linear form. Br J Dermatol. 1990;122:417–22.
42. Moellmann G, McGuire J, Lerner AB. Ultrastructure and cell biology of pigment cells. Intracellular dynamics and the fine structure of melanocytes with special reference to the effects of MSH and cyclic AMP on microtubules and 10-nm filaments. Yale J Biol Med. 1973;46:337–60.
43. Happle R, Assim A. The lines of Blaschko on the head and neck. J Am Acad Dermatol. 2001;44:612–5.
44. Bolognia JL, Orlow SJ, Glick SA. Lines of Blaschko. J Am Acad Dermatol. 1994;31:157–90.
45. Baba M, Akcali C, Seçkin D, Happle R. Segmental lentiginosis with ipsilateral nevus depigmentosus: another example of twin spotting? Eur J Dermatol. 2002;12:319–21.
46. van Geel NA, Mollet IG, De Schepper S, Tjin EP, Vermaelen K, Clark RA, Kupper TS, Luiten RM, Lambert J. First histopathological and immunophenotypic analysis of early dynamic events in a patient with segmental vitiligo associated with halo nevi. Pigment Cell Melanoma Res. 2010;23:375–84.
47. Shin J, Kang HY, Kim KH, Park CJ, Oh SH, Lee SC, Lee S, Choi GS, Hann SK. Involvement of T cells in early evolving segmental vitiligo. Clin Exp Dermatol. 2016;41:671–4.
48. Attili VR, Attili SK. Segmental and generalized vitiligo: both forms demonstrate inflammatory histopathological features and clinical mosaicism. Indian J Dermatol. 2013;58:433–8.
49. Taïeb A, Picardo M, VETF Members. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell Res. 2007;20:27–35.
50. Hann SK, Lee HJ. Segmental vitiligo: clinical findings in 208 patients. J Am Acad Dermatol. 1996;35:671–4.
51. van Geel N, De Lille S, Vandenhaute S, Gauthier Y, Mollet I, Brochez L, Lambert J. Different phenotypes of segmental vitiligo based on a clinical observational study. J Eur Acad Dermatol Venereol. 2011;25:673–8.
52. El Mofty AM, El Mofty M. Vitiligo: a symptom complex. Int J Dermatol. 1980;19:238–47.
53. Park K, Youn JL, Lee YS. A clinical study of 326 cases of vitiligo. Korean J Dermatol. 1988;26:200–5.
54. Ezzedine K, Diallo A, Léauté-Labrèze C, Mossalayi D, Gauthier Y, Bouchtnei S, Cario-André M, Seneschal J, Boralevi F, Jouary T, Taieb A. Multivariate analysis of factors associated with early-onset segmental and nonsegmental vitiligo: a prospective observational study of 213 patients. Br J Dermatol. 2011;165:44–9.
55. Kim DY, Oh SH, Hann SK. Classification of segmental vitiligo on the face: clues for prognosis. Br J Dermatol. 2011;164:1004–9.
56. van Geel N, Bosma S, Boone B, Speeckaert R. Classification of segmental vitiligo on the trunk. Br J Dermatol. 2014;170:322–7.
57. Happle R. Mosaicism in human skin. Understanding the patterns and mechanisms. Arch Dermatol. 1993;129:1460–70.
58. Lee HS, Hann SK. Bilateral segmental vitiligo. Ann Dermatol(Seoul). 1998;10:129–31.
59. Howitz J, Brodthagen H, Schwartz M, et al. Prevalence of vitiligo. Epidemiological survey on the Isle of Bornholm, Denmark. Arch Dermatol. 1977;113:47–52.
60. Yaar M, Gilchrest BA. Human melanocyte growth and differentiation: a decade of new data. J Invest Dermatol. 1991;97:611–7.
61. Staricco RG. Activation of the amelanotic melanocytes in the outer root sheath of the hair follicle following ultra violet rays exposure. J Invest Dermatol. 1962;39:163–4.
62. Cui J, Shen LY, Wang GC. Role of hair follicles in the repigmentation of vitiligo. J Invest Dermatol. 1991;97:410–6.
63. Yu HS. Melanocyte destruction and repigmentation in vitiligo: a model for nerve cell damage and regrowth. J Biomed Sci. 2002;9:564–73.
64. Bartosik J, Wulf HC, Kobayasi T. Melanin and melanosome complexes in long standing stable vitiligo-an ultrastructural study. Eur J Dermatol. 1998;8:95–7.
65. Silverberg NB, Lin P, Travis L, et al. Tacrolimus ointment promotes repigmentation of vitiligo in children: a review of 57 cases. J Am Acad Dermatol. 2004;51:760–6.
66. Tobin DJ, Bystryn JC. Different populations of melanocytes are present in hair follicles and epidermis. Pigment Cell Res. 1996;9:304–10.
67. Tallab T, Joharji H, Bahamdan K, et al. Response of vitiligo to PUVA therapy in Saudi patients. Int J Dermatol. 2005;44:556–8.
68. Khalid M, Mutjaba G. Response of segmental vitiligo to 0.05% clobetasol propionate cream. Int J Dermatol. 1998;37:705–8.
69. Wu CS, Yu CL, Wu CS, et al. Narrow-band ultraviolet-B stimulates proliferation and migration of cultured melanocytes. Exp Dermatol. 2004;13:755–63.
70. Kao CH, Yu HS. Comparison of the effect of 8-methoxypsoralen (8-MOP) plus UVA (PUVA) on human melanocytes in vitiligo vulgaris and in vitro. J Invest Dermatol. 1992;98:734–40.
71. Lei TC, Virador V, Yasumoto K, et al. Stimulation of melanoblast pigmentation by 8-methoxypsoralen: the involvement of microphthalmia-associated transcription factor, the protein kinase A signal pathway, and proteasome-mediated degradation. J Invest Dermatol. 2002;119:1341–9.
72. Westerhof W, Nieuweboer-Krobotova L. Treatment of vitiligo with UV-B radiation vs topical psoralen plus UV-A. Arch Dermatol. 1997;133:1525–8.
73. Ozawa M, Ferenczi K, Kikuchi T, et al. 312-nanometer ultraviolet B light (narrow-band UVB) induces apoptosis of T cells within psoriatic lesions. J Exp Med. 1999;189:711–8.
74. Anbar TS, Westerhof W, Abdel-Rahman AT, et al. Evaluation of the effects of NBUVB in both segmental and non-segmental vitiligo affecting different body sites. Photodermatol Photoimmunol Photomed. 2006;22:157–63.
75. Yu HS, Wu CS, Yu CL, et al. Helium Neon laser irradiation stimulates migration and proliferation in

melanocytes and induces repigmentation in segmental type vitiligo. J Invest Dermatol. 2003;120:56–64.
76. Lan CC, Wu CS, Chiou MH, et al. Low-energy helium-neon laser induces locomotion of the immature melanoblasts and promotes melanogenesis of the more differentiated melanoblasts: recapitulation of vitiligo repigmentation in vitro. J Invest Dermatol. 2006;126:2119–26.
77. Lan CC, Chen GS, Chiou MH, et al. FK506 promotes melanocyte and melanoblast growth and creates a favourable milieu for cell migration via keratinocytes: possible mechanisms of how tacrolimus ointment induces repigmentation in patients with vitiligo. Br J Dermatol. 2005;153:498–505.
78. Kang HY, Choi YM. FK506 increases pigmentation and migration of human melanocytes. Br J Dermatol. 2006;155:1037–40.
79. Khullar SM, Brodin P, Barkvoll P, et al. Preliminary study of low-level laser for treatment of longstanding sensory alteration in the inferior alveolar nerve. J Oral Maxillofac Surg. 1996;54:2–7.
80. Rochkind S, Rousso M, Nissan M, et al. Systemic effects of low-power laser irradiation on the peripheral and central nervous system, cutaneous wounds, and burns. Lasers Surg Med. 1989;9:174–82.
81. Ständer S, Ständer H, Seeliger S, et al. Topical pimecrolimus and tacrolimus transiently induce neuropeptide release and mast cell degranulation in murine skin. Br J Dermatol. 2007;156:1020–6.
82. Kano Y, Nohno T, Hasegawa T, et al. Immunosuppressant FK506 induces sustained activation of MAP kinase and promotes neurite outgrowth in PC12 mutant cells incapable of differentiating. Cell Struct Func. 2002;27:393–8.
83. Hann SK, Im S, Park YK, Hur W. Repigmentation of leukotrichia by epidermal grafting and systemic psoralen plus UV-A. Arch Dermatol. 1992;128:998–9.
84. Pavlovic S, Daniltchenko M, Tobin DJ, et al. Further exploring the brain-skin connection: stress worsens dermatitis via substance P-dependent neurogenic inflammation in mice. J Invest Dermatol. 2008;128:434–46.
85. Nishimura EK, Jordan SA, Oshima H, et al. Dominant role of the niche in melanocyte stem-cell fate determination. Nature. 2002;416:854–60.
86. Yonetani S, Moriyama M, Nishigori C, et al. In vitro expansion of immature melanoblasts and their ability to repopulate melanocyte stem cells in the hair follicule. J Invest Dermatol. 2008;128:408–20.

Mixed Vitiligo

7

Alain Taïeb

Contents

Abstract

By definition, mixed vitiligo (MV) is the association of a characteristic segmental involvement associated usually in a second step with the onset of bilateral vitiligo patches. The association of segmental vitiligo (SV) to vitiligo/nonsegmental vitiligo (vitiligo/NSV) suggests a continuum between the two subsets which were formerly opposed. A common mode of revelation is a segment resistant to phototherapy in a patient diagnosed previously as vitiligo/NSV. Halo nevi and leukotrichia at first consultation in segmental vitiligo are risk factors for the progression of SV to MV. In addition, this progression of SV to MV carries a stronger link if the segment is initially situated on the trunk. Combined somatic mosaicism and immune dysregulation may explain the continuum SV-vitiligo/NSV in the case of MV.

Key Points

- Mixed vitiligo (MV) is the association of SV to vitiligo/NSV.
- It suggests a continuum between the two subsets which were formerly opposed.
- A common mode of revelation of MV is a segment resistant to phototherapy in a patient diagnosed previously as vitiligo/NSV.
- Presence of halo nevi and leukotrichia at first consultation in segmental vitiligo is

A. Taïeb (✉)
Service de Dermatologie, Hôpital Saint-André, CHU de Bordeaux, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_7

a risk factor for the progression of SV to MV.
- Progression of SV to MV carries a stronger link if initial segmental involvement is situated on the trunk.
- Combined somatic mosaicism and immune dysregulation may explain the continuum SV-vitiligo/NSV in the case of MV.

7.1 Historical Background

Until the last decade, the opposition of SV and vitiligo/NSV was considered rather dogmatically. The association of SV and NSV was first suggested in 2003 by the description of a pediatric NSV case treated with ultraviolet (UV) B that left a recalcitrant segmental portion compatible with pre-existing SV [1]. Other observations [2–4] were subsequently reported, and the term "mixed vitiligo" (MV) proposed by Mulekar et al. to designate this particular form of the disease, followed by case series [5–11] was officially endorsed by international consensus [5]. By definition, MV is the association of a characteristic segmental involvement associated usually in a second step with the onset of bilateral vitiligo patches.

7.2 Clinical Features and Differential Diagnosis

Four case series have been published [3, 7, 10, 12] and case reports with therapeutic intervention [13–15] which permit to better delineate mixed vitiligo.

For the French Ezzedine et al. series [7], which comprises 19 children and adults extracted from a prospective cohort of 498 vitiligo (all types) in 3.5 years, the sex ratio is surprisingly skewed (male/female ratio, 1:4) but may just reflect that in hospital consultations for vitiligo in Europe, females predominate. Most patients had a disease onset before the age of 18 years and a segmental involvement of the trunk. Interestingly, the diagnosis of vitiligo/NSV localized to the trunk and the extremities was first made in three patients, and narrowband (NB) UVB phototherapy revealed the presence of a pre-existing thoracoabdominal SV undetected at the first visit that had poorly or not responded to the treatment. The patients further acknowledged the primary onset of this segmental involvement. A clear segmental blaschkolinear pattern was noted in two patients. A relevant history of axial skeletal abnormality (i.e., scoliosis) was found in four patients. In all patients SV preceded NSV with a delay ranging from 6 months to more than 24 months (Fig. 7.1).

For the Belgian series of van Geel et al. [10], 14 out of 141 patients with SV received a final diagnosis of MV. The aim of the authors was mostly to describe the pattern of segmental lesions. However, they made interesting additional observations concerning their MV cases: (1) rather late apparition of the nonsegmental involvement (3 years, 6 years, and 12 years, respectively, after the segmental lesion); (2) most generalized vitiligo lesions in the mixed vitiligo group were very mild (12/14 patients), and in two of these patients, differentiation from fully depigmented halo nevi was difficult; and (3) the age of onset was higher in patients with MV (median 22 years) compared with pure segmental vitiligo (median 13 years).

For the Italian pediatric study of Neri et al., 13 patients with MV were enrolled among 92 children affected by vitiligo in 5 years (59 NSV, 20 SV, 13 MV), with contrary to the Ezzedine et al. series a preponderance of male cases (*M*/*F* = 10:3). The mean age of onset was 7.5 years, and all cases showed a primary segmental involvement. Three patients showed scoliosis at clinical examination. Only two children had a segmental blaschkolinear pattern.

For the German study of Schallreuter et al. [3], extracted from an international group of 2411 patients (1033 male, 1378 female), 57 patients were diagnosed with SV (29 males/28 females; age of onset 1–47 years, mean age 15.4 years), and 76 patients had mixed vitiligo (mean age 16.0 years, range 1–61 years), a prevalence of 3.2% for MV. The mean age of the patients with SV was 15 years (range 9–19 years), while in the

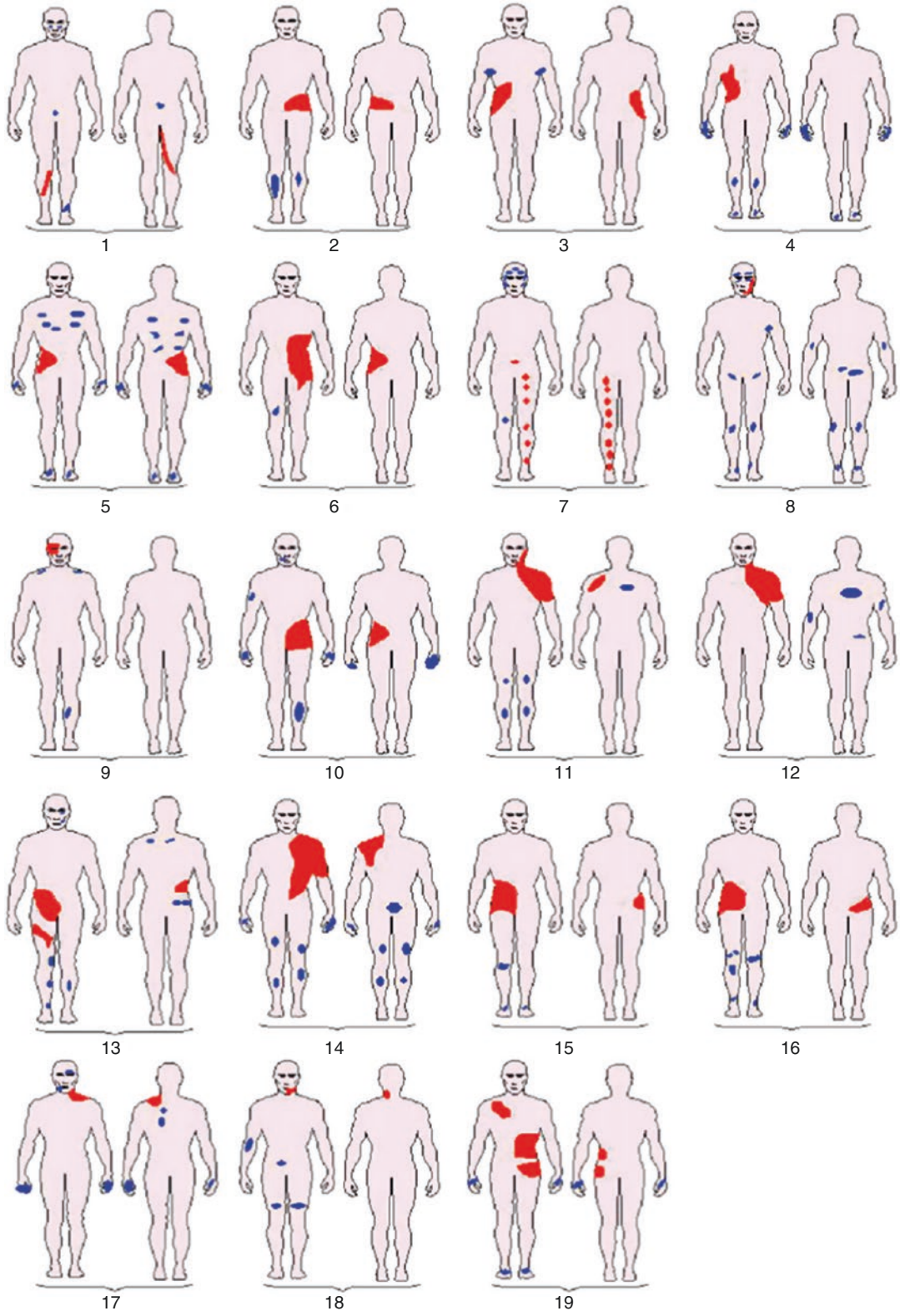


Fig. 7.1 Pattern of segmental (*red*) and nonsegmental (*blue*) involvement in 19 patients with mixed vitiligo

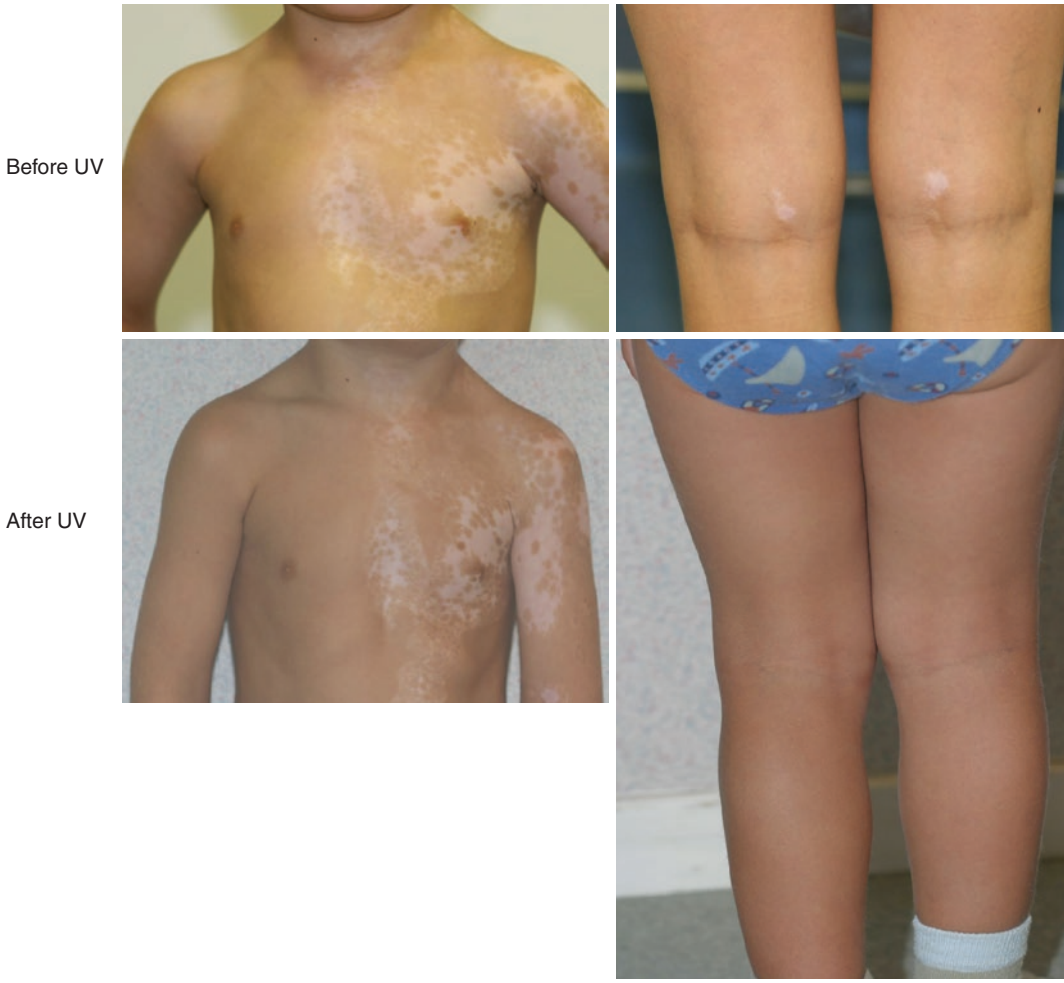


Fig. 7.2 Mixed vitiligo: better efficacy of NB UVB on nonsegmental involvement

MV group, the mean age was 25.5 years (range 7–57 years). This study shows in SV and MV accumulation of epidermal biopterin together with significantly decreased epidermal catalase and presence of H_2O_2 in the skin as in vitiligo/NSV, as well as a response to the combination of low-dose NB-UVB-activated pseudocatalase.

Based on these cumulated data, it appears that MV is a significant subset, especially in pediatric recruitments, and has most probably been underdiagnosed previously. A notable difference between SV in MV and SV in isolation is the limited number of cases of SV of the face and neck. The segment pattern may be blaschkolinear but only in a minority of cases. Response to UVB phototherapy is better in nonsegmental lesions (Fig. 7.2). In the case of grafted MV, the segmental type showed an excellent response after epidermal grafting, but it recurred in the grafted area repeatedly, suggesting that the segmental type may behave differently [14]. Concerning associated scoliosis, a possible neurogenic trigger in a topographically compatible dermatomal segment is possible but may also be coincidental.

Limited data exists concerning the pathology of segmental vs. nonsegmental lesions in MV. A recent study in a Japanese patient with hepatitis C and MV showed more prominent inflammation in the nonsegmental more recent lesion [13].

Other diagnoses should be ruled out before accepting the diagnosis of MV. This is particularly true for the other segmental hypomelanoses

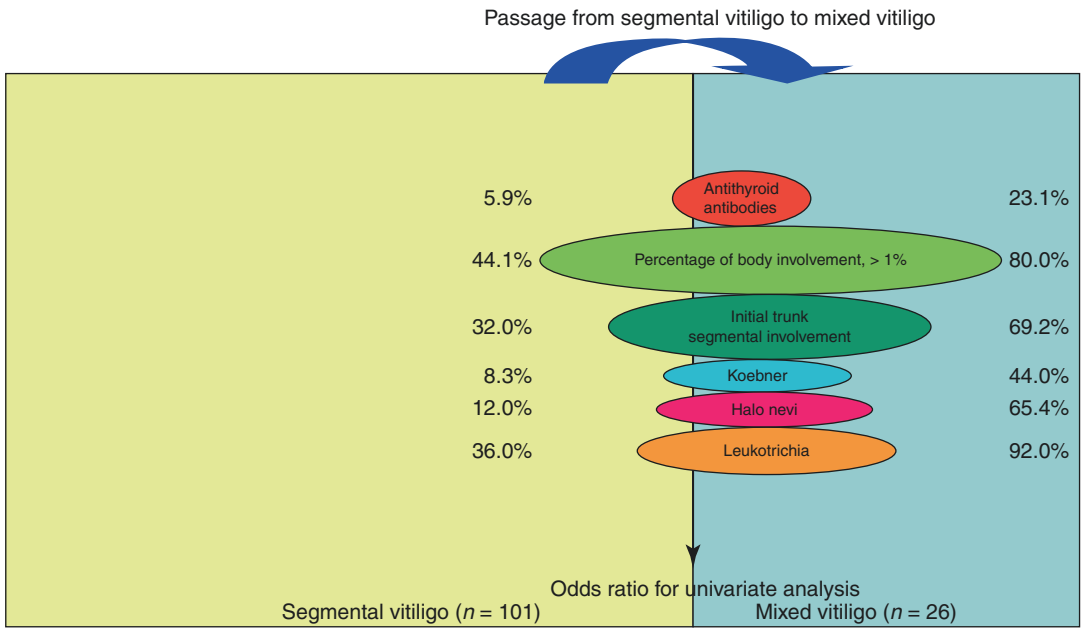


Fig. 7.3 Distribution and weighted contribution of selected features (odds ratios) according to the univariate analysis for segmental and mixed vitiligo

such as nevus depigmentosus that are generally present at birth or discovered in the first years of life but may be misdiagnosed at that time in patients with fair skin. Wood's lamp examination is usually sufficient to make a difference between SV and nevus depigmentosus (Chap. 1.2). Van Geel et al. noted that fully depigmented halo nevi may pose a problem if interpreted as a mild non-segmental involvement [11].

7.3 Risk Factors for Generalized Vitiligo in SV Patients

Given the established sequence SV first vitiligo/NSV later in MV patients, a study by Ezzedine et al. has compared 101 patients with SV and 26 who had disease which evolved from SV into MV. The age at disease onset in patients with segmental vitiligo was comparable in the two groups, as opposed to the van Geel study (9.2 ± 8.3 years for SV vs. 8.1 ± 7.3 years for MV). The presence of halo nevi, circulating antithyroid antibodies, and leukotrichia was noted more often in patients with mixed vitiligo (65.4%, 23.0%, and 92.0%, respectively) than those with segmental vitiligo (12.0%, 5.9%, and 36.0%, respectively). Finally, trunk involvement was confirmed as more frequent in mixed vitiligo compared with segmental vitiligo (69.2% and 32.0%, respectively). In the univariate analysis, segmental vitiligo located on the trunk, Koebner phenomenon, and the presence of circulating antithyroid antibodies were strongly linked with the progression of SV to MV, arguing for a more marked autoimmune background in MV. In multivariate analysis, initial percentage of body surface involvement of the segment of more than 1% and the presence of halo nevi and leukotrichia were found to be independent factors associated with the evolution of patients' disease from SV to MV [7] (Fig. 7.3).

7.4 Interpretations and Current Issues

7.4.1 Genetic Link SV-Vitiligo/NSV

The hypothesis that the difference in response to therapy of SV may be linked to the dosage of

underlying predisposing cutaneous defect involved in the SV area has previously been raised [16, 17]. This may relate to a possible mosaic expression in the skin of a major predisposing gene, following the model of monogenic disorders, where a loss of heterozygosity is found in type II mosaicism, whereas type I mosaicism results from a single dominant mutation [18]. The sequence (early SV, late NSV) may also reflect the role of a first cutaneous gene defect causing SV triggering a generalized immune response against cutaneous melanocytes supported by another immune-related gene defect. However, pedigrees showing the presence of cases with established SV in families with previous cases of NSV [17] reinforce the possibility of a mechanism close to type II mosaicism already demonstrated in monogenic skin disorders.

7.4.2 Halo Nevi as Markers of Vitiligo Diathesis and Progression to MV in SV

The strong link between the progression of SV to MV and the initial presence of halo nevi needs to be taken into consideration [6, 11]. Specifically, halo nevi may be considered as a clinical marker of cellular immune response against nevocytes, which may indirectly pertain to the process targeting normal melanocytes in vitiligo [6]. In MV, there might be a stronger natural cutaneous immunosurveillance as shown by the presence of halo nevi. The generalization of SV to MV might be initiated by an immune response first directed against nevocellular targets with collateral damage to surrounding melanocytes belonging to the same territory of lymphatic circulation, especially for truncal SV, as hypothesized for segmental vitiligo [9]. This may inversely suggest that the majority of SV without halo nevi patients have some counteracting protective mechanisms respective to the development of vitiligo/NSV and which explains the usual good take of autologous grafts in SV.

Some authors have proposed that halo nevi could be a risk factor for the development of vitiligo [19] and furthermore that halo nevi could be a clinical sign of vitiligo. However, some cases of extensive vitiligo clearly spare melanocytic nevi (Chap. 5). Whether or not SV with associated halo nevi can be classified as a special mixed type of vitiligo and whether or not the mild variant of nonsegmental lesions appearing in mixed vitiligo is based on the same pathomechanism determining generalized vitiligo/NSV need to be further investigated [11].

7.4.3 Leukotrichia and MV, Consequence of Loss of Immune Privilege?

The presence of leukotrichia at the time of first consultation of SV is one of the predictors of the evolution to MV. In vitiligo/NSV, repigmentation depends on available melanocytes from three possible sources, including the hair follicle, the border of vitiligo lesions, and unaffected melanocytes within depigmented areas ([20]; see Chap. 45). Among these sources, the hair follicle is the main source of melanocytes. In most cases of vitiligo/NSV, there is a preferential targeting of epidermal but not of follicular melanocytes arguing for different antigenic profiles between these two types of melanocytes. Some of this immunological difference is likely to reflect the fact that the follicular melanin unit resides in the immune-privileged proximal anagen hair bulb in contrast to the immunocompetent nature of the epidermal melanin unit [21] (see Chap. 9 and [11]).

7.4.4 Bilateral Mosaicism Hypothesis in Vitiligo/NSV

Mixed patterns of vitiligo are documented as the progression from localized SV to vitiligo/NSV. However, a phenomenon of segmentation of lesions (mirror images) occurring in vitiligo/NSV was observed in the Indian study of Attili and Attili [22] (Fig. 7.4) suggesting that mosaicism may be an underlying factor explaining this bilateral mirror pattern. The authors suggest that

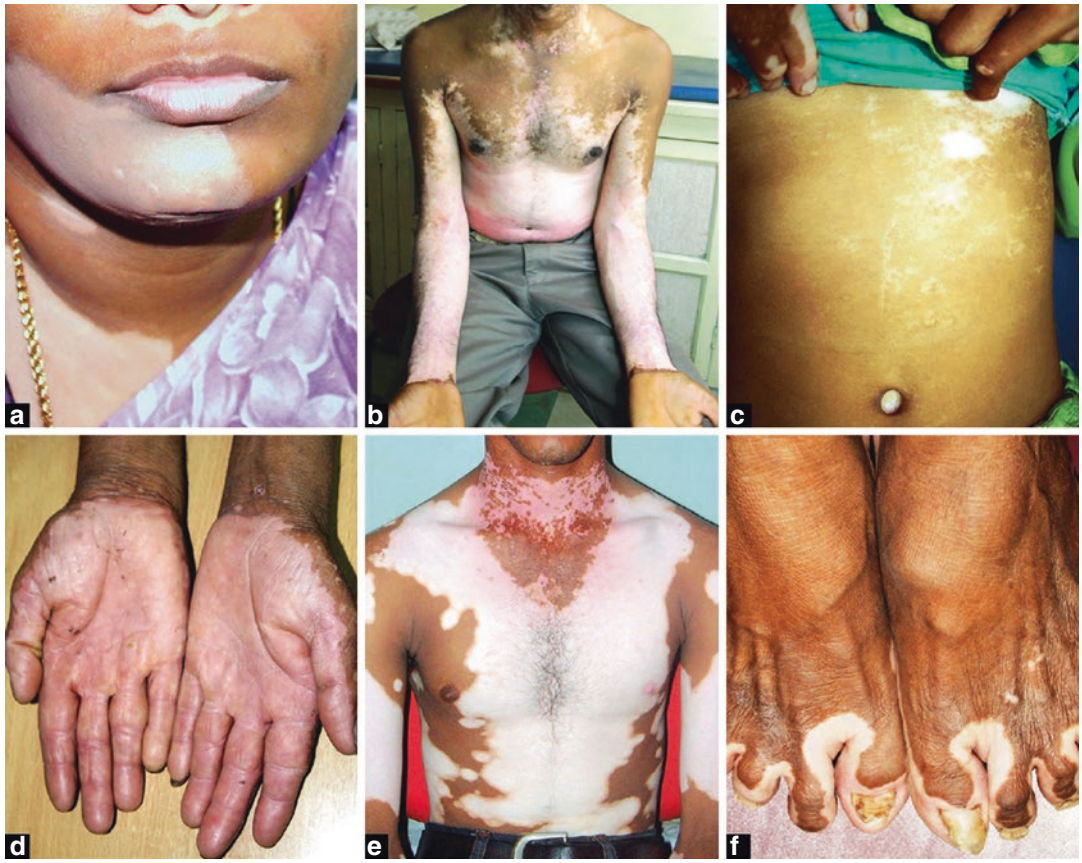


Fig. 7.4 Mirror patterns in vitiligo/NSV (from 22) The authors suggest that in vitiligo/NSV the sometimes striking symmetrical patterns correspond to an underlying developmental defect revealed by vitiligo

the multiplicity of underlying mosaic segments affected in vitiligo/NSV may not be appreciated when lesions do not extend up to the predetermined cutoff lines and may simply give an impression of bilateralism.

References

1. Gauthier Y, Cario André M, Taïeb A. A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? Pigment Cell Res. 2003;16:322–32.
2. Mulekar SV, Al Issa A, Asaad M, et al. Mixed vitiligo. J Cutan Med Surg. 2006;10:104–7.
3. Schallreuter KU, Krüger C, Rokos H, et al. Basic research confirms coexistence of acquired Blaschkolinear Vitiligo and acrofacial Vitiligo. Arch Dermatol Res. 2007;299:225–30.
4. Schallreuter KU, Krüger C, Würfel BA, et al. From basic research to the bedside: efficacy of topical treatment with pseudocatalase PC-KUS in 71 children with vitiligo. Int J Dermatol. 2008;47:743–53.
5. Ezzedine K, Lim HW, Suzuki T, Katayama I, Hamzavi I, Lan CC, Goh BK, Anbar T, Silva de Castro C, Lee AY, Parsad D, van Geel N, Le Poole IC, Oiso N, Benzekri L, Spritz R, Gauthier Y, Hann SK, Picardo M, Taieb A, Vitiligo Global Issue Consensus Conference Panelists. Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. Pigment Cell Melanoma Res. 2012a;25:E1–13.
6. Ezzedine K, Diallo A, Léauté-Labrèze C, Seneschal J, Mossalayi D, AlGhamdi K, Prey S, Bouchtnei S, Cario-André M, Boralevi F, Jouary T, Taieb A. Halo nevi association in nonsegmental vitiligo affects age at onset and depigmentation pattern. Arch Dermatol. 2012b;148:497–502.
7. Ezzedine K, Diallo A, Léauté-Labrèze C, Séneschal J, Prey S, Ballanger F, Alghamdi K, Cario-André M, Jouary T, Gauthier Y, Taieb A. Halo nevi and leukotrichia are strong predictors of the passage to mixed vitiligo in a subgroup of segmental vitiligo. Br J Dermatol. 2012c;166:539–44.

8. Schallreuter KU, Salem MA, Holtz S, Panske A. Basic evidence for epidermal H2O2/ONOO(-)-mediated oxidation/nitration in segmental vitiligo is supported by repigmentation of skin and eyelashes after reduction of epidermal H2O2 with topical NB-UVB-activated pseudocatalase PC-KUS. FASEB J. 2013;27:3113–22.
9. Van Geel N, Mollet IG, De Schepper S, et al. First histopathological and immunophenotypic analysis of early dynamic events in a patient with segmental vitiligo associated with halo nevi. Pigment Cell Melanoma Res. 2010;23:375–84.
10. Van Geel N, De Lille S, Vandenhaute S, et al. Different phenotypes of segmental vitiligo based on a clinical observational study. J Eur Acad Dermatol Venereol. 2011a;25:673–8.
11. Van Geel N, Speeckaert R, Lambert J, et al. Halo naevi with associated vitiligo-like depigmentations: pathogenetic hypothesis. J Eur Acad Dermatol Venereol. 2011b;25:673–8.
12. Neri I, Russo T, Piccolo V, Patrizi A. Mixed vitiligo in childhood: a study on 13 italian patients. J Eur Acad Dermatol Venereol. 2013;27:e136–47.
13. Hashimoto N, Tanemura A, Yamada M, Itoi S, Katayama I. Hepatitis C-related mixed type vitiligo in a patient with Ivemark syndrome. J Dermatol. 2014;41:185–6.
14. Lee DY, Kim PS, Lee JH. Mixed vitiligo treated by suction blister epidermal grafting: long-term follow up. J Dermatol (Seoul). 2009;36:672–3.
15. Oiso N, Kawada A. Superimposed segmental vitiligo (mixed vitiligo) with non-segmental vitiligo and segmental vitiligo along the narrow Blaschko lines (type 1a). J Dermatol. 2016;43:1388. https://doi.org/10.1111/1346-8138.13405.
16. Taieb A. Intrinsic and extrinsic pathomechanisms in vitiligo. Pigment Cell Res. 2000;13:41–7.
17. Taïeb A, Morice-Picard F, Jouary T, et al. Segmental vitiligo as the possible expression of cutaneous somatic mosaicism: implications for common non-segmental vitiligo. Pigment Cell Melanoma Res. 2008;21:646–52.
18. Koga M. Vitiligo: a new classification and therapy. Br J Dermatol. 1977;97:255–61.
19. Barona MI, Arrunategui A, Falabella R, Alzate A. An epidemiologic case-control study in population with vitiligo. J Am Acad Dermatol. 1995;33:621–5.
20. Parsad D, Pandhi R, Dogra S, Kumar B. Clinical study of repigmentation patterns with different treatment modalities and their correlation with speed and stability of repigmentation in 352 vitiliginous patches. J Am Acad Dermatol. 2004;50:63–7.
21. Ito T, Meyer KC, Ito N, Paus R. Immune privilege and the skin. Curr Dir Autoimmun. 2008;10:27–52.
22. Attili VR, Attili SK. Anatomical segmentations in all forms of vitiligo: a new dimension to the etiopathogenesis. Indian J Dermatol Venereol Leprol. 2016;82:379–88.

8 Clinically Inflammatory Vitiligo and Rare Variants

Alain Taïeb, Khaled Ezzedine, Julien Seneschal, and Ratnam Attili

Contents

Abstract

A particular form of vitiligo of clinically inflammatory vitiligo has been reported as "inflammatory vitiligo with raised borders" or marginal vitiligo and is associated with lichenoid infiltrates in the margins of progressing lesions. In pigmented progressing borders of common vitiligo/NSV and SV, similar features of lesser intensity have been demonstrated suggesting that most subtypes of vitiligo belong to the spectrum of a clinically silent chronic inflammatory skin disorder responsible for melanocyte loss. Clinically inflammatory vitiligo (CIV) has been sometimes associated with infectious or inflammatory diseases. Other rare variants considered in this chapter include follicular vitiligo, vitiligo/leukoderma guttata, and hypopigmented vitiligo/vitiligo minor. For both CIV and

A. Taïeb (✉) · K. Ezzedine · J. Seneschal
Service de dermatologie, Hôspital St André, CHU de Bordeaux, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr; khaled.ezzedine@chu-bordeaux.fr

R. Attili
Visakha Institute of Skin and Allergy, Visakhapatnam, India

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_8

hypopigmented vitiligo, biopsies (sometimes repeated) are needed to establish a firm diagnosis, in particular to exclude cutaneous T-cell lymphoma, a major differential diagnosis.

Key Points

- A rare form of clinically inflammatory vitiligo (CIV) reported as "inflammatory vitiligo with raised borders" or marginal vitiligo is associated with lichenoid infiltrates at the margins of progressing lesions.
- Histopathological findings in still pigmented progressing borders of vitiligo/NSV and SV may show less intense but similar features, suggesting that vitiligo could be considered as a clinically silent chronic inflammatory skin disorder.
- Clinically inflammatory vitiligo has been sometimes associated with other infectious or inflammatory diseases.
- Rare vitiligo variants include follicular vitiligo, vitiligo guttata, and hypopigmented vitiligo/vitiligo minor.
- For both CIV and hypopigmented vitiligo, biopsies (sometimes repeated) are needed to establish a firm diagnosis, in particular to exclude cutaneous T-cell lymphoma.

8.1 General Background

One of the striking features of vitiligo, when compared with other chronic skin conditions, is the absence of symptoms and signs of inflammation. In contrast with atopic dermatitis, which is characterized locally by a TH2 cytokine imbalance, where erythema and edema are common during flares, pruritus is not a classic vitiligo symptom. However, since this item has been included in the VETF evaluation form [1], some patients definitely report a mild pruritus preceding flares of depigmentation. Vitiligo contrasts also with psoriasis, which shows a predominant TH1-TH17 cytokine imbalance and where lesions are clinically very inflammatory, redness being mandatory in disease expression, with milder pruritus if present.

However, a particular form of vitiligo defined as "inflammatory vitiligo with raised borders" has been noticed long ago [2–4]. The clinical presentation of this particular form of vitiligo consists in depigmented patches with an erythematous micropapular edge. This condition is associated with inflammatory infiltrates in the margin of progressing lesions. In general, clinically inflammatory vitiligo (CIV) may occur in isolation but has unfrequently been associated with various disorders including infectious diseases [5–8], lichen sclerosus [9], atopic dermatitis [10], or Vogt-Koyanagi-Harada (VKH) disease [11, 12]. Several histological studies indicate that common vitiligo can also be microinflammatory at the progressing edge of depigmented lesions (Chap. 3). Other rare variants characterized by special clinical features most of them associated with microscopic inflammation are also discussed in this chapter (hypopigmented vitiligo, follicular vitiligo, vitiligo/leukoderma guttata/punctata).

8.2 Isolated Clinically Inflammatory Vitiligo

A few cases of CIV without documented association with other diseases have been reported [2–4, 13–20] (Fig. 8.1). In all reported cases, progressive lesions with erythema and fine scaling, with or without pruritus, are common features. Duration at diagnosis varied from

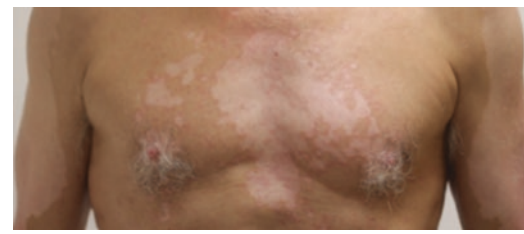


Fig. 8.1 Vitiligo with raised borders

2 months to 2 years. An erythematous raised border has been documented, coexisting or not with non-clinically inflammatory depigmented macules. Marginal hyperpigmentation surrounding hypopigmented patches has also been noticed. Inflammatory vitiligo is generally considered as a progressive (unstable) form since extensive involvement seems to be the rule, with improvement using topical corticosteroids, UV, or both. It seems to affect both sexes at any age, but most described cases concern adults. Interestingly, in almost half the patients, concomitant inflammatory and noninflammatory patches are observed. Based on histopathological studies, this unusual clinical manifestation can be interpreted as disease progression due to an inflammatory phase. CIV in segmental vitiligo was described in only one case [17].

8.3 Clinically Inflammatory Vitiligo Associated with Other Disorders

Inflammatory vitiligo has also been associated with specific disorders (Table 8.1). The clinically inflammatory stage of HIV-associated cases is different from CIV with raised borders (Chap. 1.5.8). It consists in nummular lichenoid lesions which result in complete pigment loss. Interestingly in this setting, the histopathological sequence shows a loss of melanocytic antigens preceding the definitive loss of pigment cells. Contrarily, in two cases of vitiligo occurring, respectively, in the setting of chronic hepatitis associated, respectively, with hepatitis C infection [8] and atopic dermatitis [10], clinical features were close to that of inflammatory vitiligo with raised borders. In these case reports, patients presented both inflammatory patches with raised border in recent lesions and noninflammatory older patches. Interestingly, Tsuruta et al. reported [11] a VKH patient with a 1-year history of a superimposed thin inflammatory raised erythema and plaque-type inflammatory erythema occurring in the setting of vitiligo/NSV of 20 years duration. Two particular features were noted: first, within patches of vitiligo with raised inflammatory borders, erythema was separated from the totally depigmented areas by an incompletely vitiliginous area; second, the presence of plaque-type erythema, which is uncommonly seen in the so-called inflammatory vitiligo [11]. Weisberg et al. [9] reported the case of a 41-year-old African-American woman who had a 6-month history of inflammatory segmental vitiligo that started on the left foot, then spread proximally up her leg to

Table 8.1 Inflammatory vitiligo in the setting of infectious or inflammatory diseases

Reference	Age/ gender of patient	Type of vitiligo	Associated disease	Pruritus	Duration of disease	Presence of other noninflammatory patches	Evolution
Tsuruta	48/M	NSV	Vogt-Koyanagi-Harada disease	Yes	1 year	Yes	Extensive
Sugita	31/M	?	Atopic dermatitis	Yes	5 years	?	Extensive
Tsuboi	38/M	NSV	Hepatitis C virus infection	Yes	2 years	Yes	Extensive
Weisberg Shin	41/W 45/W	SV NSV	Lichen sclerosus Sjögren's syndrome	Yes No	6 months 1 month	No ?	Extensive Regressive
Tanioka	53/W	NSV	Sjögren's syndrome	No	? 5 years for Sjögren's syndrome	Yes	Regressive under NBUVB therapy

her thigh, and then to her buttocks with an involvement of pubic hairs that turned white. Concomitantly, the patient had severe burning and itching in a clinically whitish pink skin of the vaginal area consistent with the diagnosis of lichen sclerosus [9]. Two reports point to a possible link with Sjögren's syndrome, with the presence of leukocytoclasis in addition to the T-cell lichenoid infiltrate [18, 21].

8.4 Differential Diagnosis

The diagnosis of annular inflammatory dermatoses, such as lupus and cutaneous T-cell lymphoma, needs to be ruled out in all cases by appropriate testing. For Sjögren's syndrome, the overlap needs clarification, and SSA/SSB testing should be performed.

A clinical presentation similar to that noted in HIV infection-associated inflammatory vitiligo may occur in patients with *graft versus host disease* (GVHD) following allogeneic hematopoietic cell transplantation (Fig. 8.2). The depicted GVHD patient presented with erythroderma followed by generalized depigmented macules affecting the whole body, clinically consistent with the diagnosis of vitiligo. In addition, he developed alopecia areata. One hypothesis is that GVHD may have triggered skin-targeted autoimmunity toward melanocytes (epidermal and follicular).

A peculiar annular lichenoid dermatitis named *annular lichenoid dermatitis of youth*, the clinical appearance of which can initially suggest diagnoses of morphea, mycosis fungoides, or annular erythema, may be mistaken for inflammatory vitiligo [22] (Fig. 8.3). Lesions consist of persistent asymptomatic erythematous macules and round annular patches with a red-brownish border and central hypopigmentation, mostly distributed on the groin and flanks. Histology reveals a lichenoid dermatitis with massive necrosis/apoptosis of the keratinocytes limited to the tips of rete ridges, in the absence of dermal sclerosis and epidermotropism of atypical lymphocytes. The infiltrate is composed mainly of memory CD4(+) CD30(−) T cells with few B cells and macrophages [22].

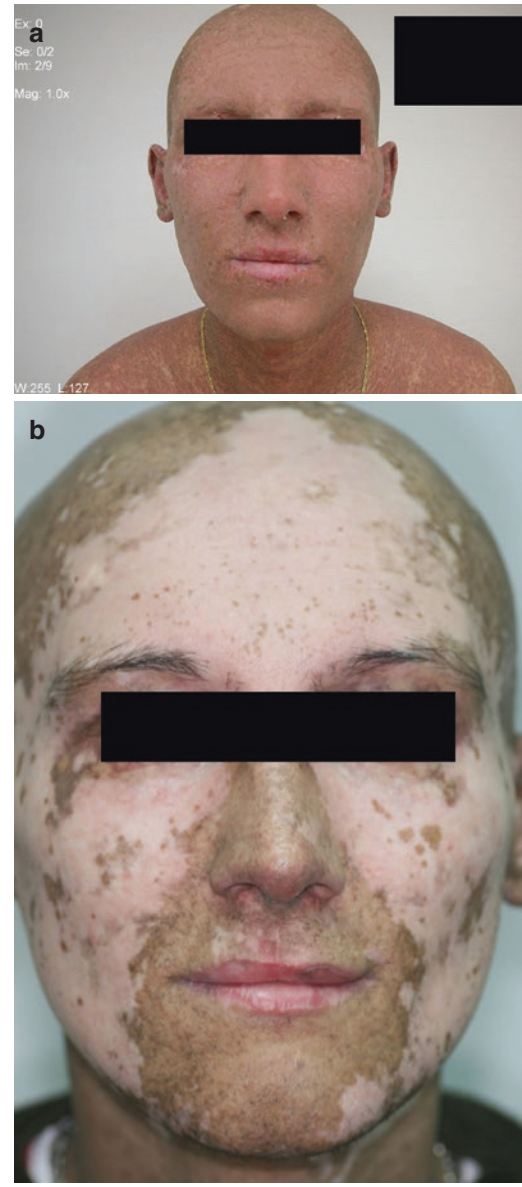


Fig. 8.2 Graft versus host disease at erythrodermic (**a**) and vitiligoid (**b**) phases (Courtesy M. Beylot-Barry)

8.5 Histological Features of Clinically Inflammatory Vitiligo vs. Common Clinically Noninflammatory Vitiligo

Histopathological examination in clinically inflammatory vitiligo should include biopsies taken from the inflammatory border. Whatever

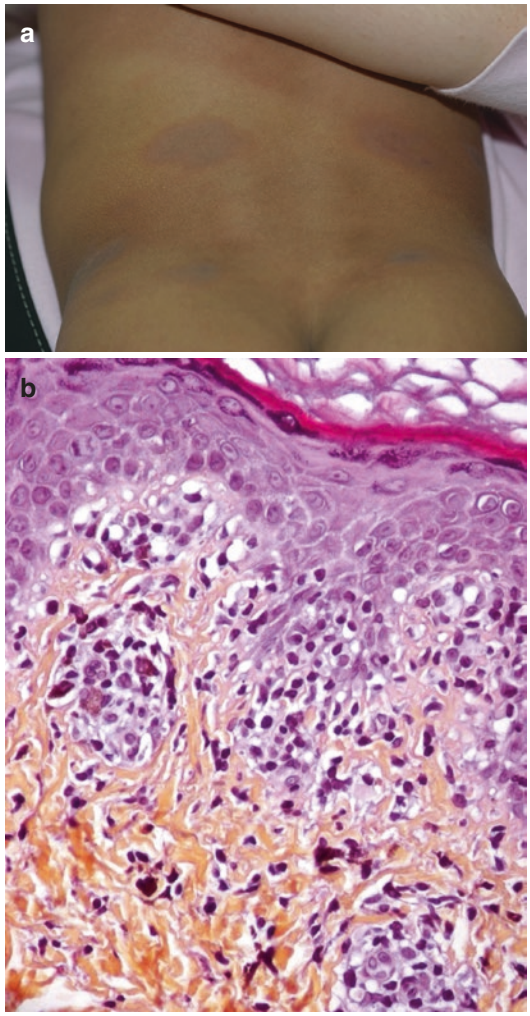


Fig. 8.3 ALDY (**a**) clinical features (**b**) histopathology

the case, vitiligo being associated or not with another disease, the perivascular mononuclear infiltrate with an associated lichenoid pattern is almost always present. In addition, degeneration of melanocytes and basal keratinocytes may be observed by ultrastructure. Immunohistochemistry may reveal CD8 cells and sparse CD4 cells. Table 8.2 summarizes published reports in case of associated conditions. Interestingly, Shin et al. have shown evidence of leukocytoclasis in two cases from Korea, one being associated with Sjögren's syndrome [18].

Histopathological studies of clinically noninflammatory vitiligo/NSV that include adjacent pigmented skin have shown inflammatory changes in the pigmented border with decreasing inflammation from the marginal pigmented areas to the fully depigmented vitiligo patches [20, 23–25]. The biopsy needs to be taken from hypopigmented fuzzy, confetti-like borders to maximize yield. Our experience based on rapidly progressing pediatric cases biopsied on clinically pigmented skin 0.5 cm of the visible border of depigmentation confirms this finding, and the intensity of lichenoid inflammation and sometimes epidermotropism may simulate mycosis fungoides (Chap. 3) (Fig. 8.4). Early biopsies taken in clinically noninflammatory SV show similar pathological features (Fig. 8.5) [23, 26].

8.6 Rare Variants

8.6.1 Follicular Vitiligo

Leukotrichia is considered as a marker of segmental vitiligo (Chap. 1.5.2). In vitiligo/NSV body hairs are usually spared although hair depigmentation may occur with disease progression. In 2011, a case of vitiligo with primary follicular involvement was presented at the VGICC in Bordeaux. It was named follicular vitiligo and classified after discussion as a rare variant within the spectrum of vitiligo/NSV (Chap. 2). The index case was a young black patient, who had primary involvement of the hair follicle melanocytic reservoir but limited interfollicular compartment involvement, manifesting in marked generalized hair whitening preceding the appearance of depigmented skin patches [27, 28] (Fig. 8.6a, b). A case series extracted from a prospective cohort of 1243 vitiligo patients was recently published to better delineate the clinical and histological characteristics of follicular vitiligo [29]. Concomitant alopecia areata of the scalp with leukotrichial regrowth was noted in one patient, and three patients had halo nevi. Histologic examination of a punch biopsy specimen taken from an area with both depigmented skin and leukotrichia demonstrated the presence of a discrete perifollicular infiltrate in the infundibular region of the hair follicle and an absence of melanocytes in both the basal layer of

Table 8.2 Histological and immunohistochemistry findings in patients with clinically inflammatory vitiligo and associated diseases

Reference	Age/ sex	Duration/progression of disease	Type of vitiligo	Localization	Histological findings	Immunohistochemistry	Associated disease	Treatment/evolution
Tsuruta	48/M	20 years/12 months	NSV	Entire body except face	Perivascular mononuclear infiltrate, lichenoid pattern	LFA-1, CD3, CD8, and rare CD4 and CD20	Vogt-Koyanagi-Harada disease	NA
Sugita	31/M	5 years/5 years	NSV	Limbs, trunk	Perivascular mononuclear infiltrate, lichenoid pattern	No melanocytes, CD4 and CD8 in raised border/CD8 outside the border	Atopic dermatitis	Topical corticosteroid/ improvement of erythema
Tsuboi	38/M	24 months/24 months	NSV	Buttock, axilla, inguinal, extremities	Degeneration of basal layer, perivascular mononuclear infiltrate	NA	Hepatitis virus C infection	Prednisolone 20 mg daily + PUVA/resolution of erythema + partial repigmentation
Weisberg	41/W	6 months/6 months	SV	Left foot, thigh, and buttock + Left genital area	Papular dermal sclerosis, Perivascular mononuclear infiltrate lichenoid pattern	NA	Lichen sclerosus	Topical corticosteroid/ involvement of erythema + partial repigmentation
Shin	45/W	1 month/?	NSV	Neck and arm	Basal vacuolization leukocytoclasia	Melan-A and Fontana-Masson no melanocyte or melanin pigment CD8 and myeloperoxidase stains positive	Sjögren's syndrome	Recovery with topical steroids

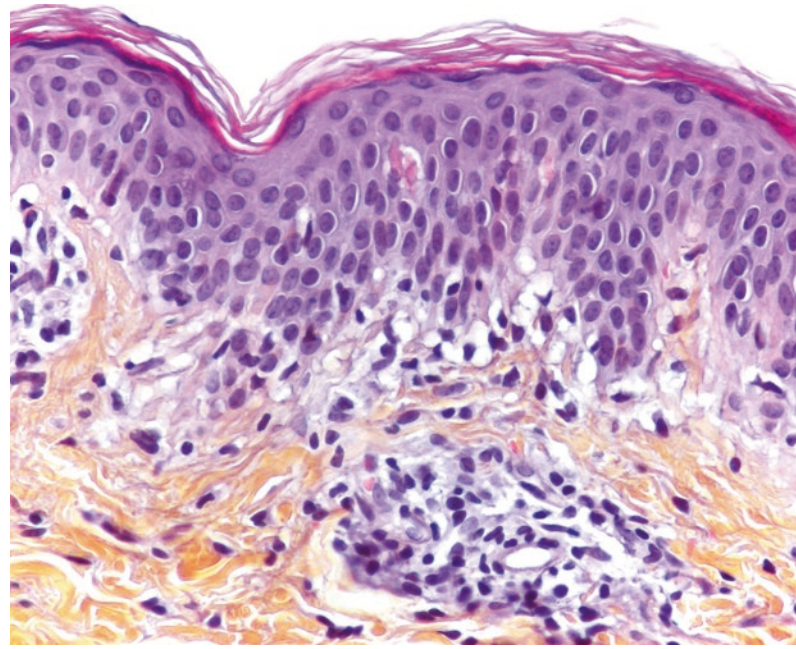


Fig. 8.4 Microscopic inflammation in progressive vitiligo/NSV, biopsy taken at the pigmented margin of a clinically noninflammatory lesion (HES ×400). The infiltrate is mostly composed of CD8+ cells (Chap. 1.4)

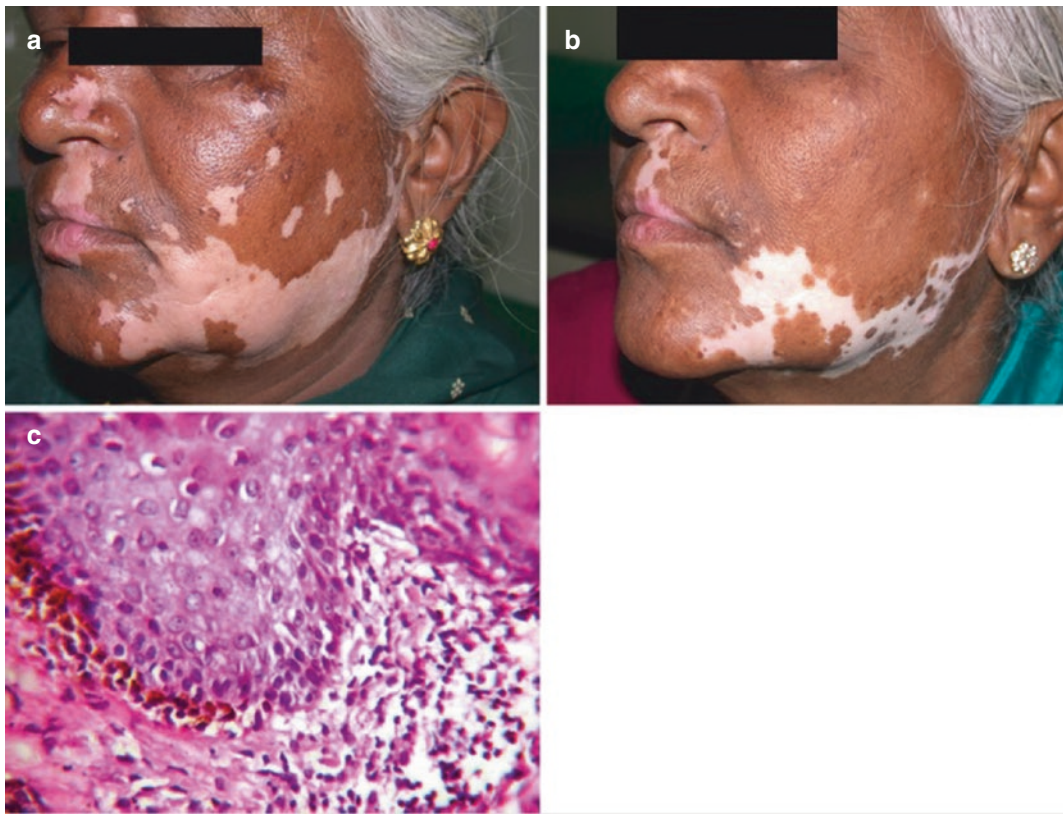


Fig. 8.5 Segmental vitiligo: recent onset lesion (3 months in **a**) and 4 months later (**b**). Microscopic lichenoid inflammation (**c**), on biopsy taken on pigmented skin across the midline (chin), at the margin of the lesion at stage depicted in (**a**) (Photographs Dr R Attili)

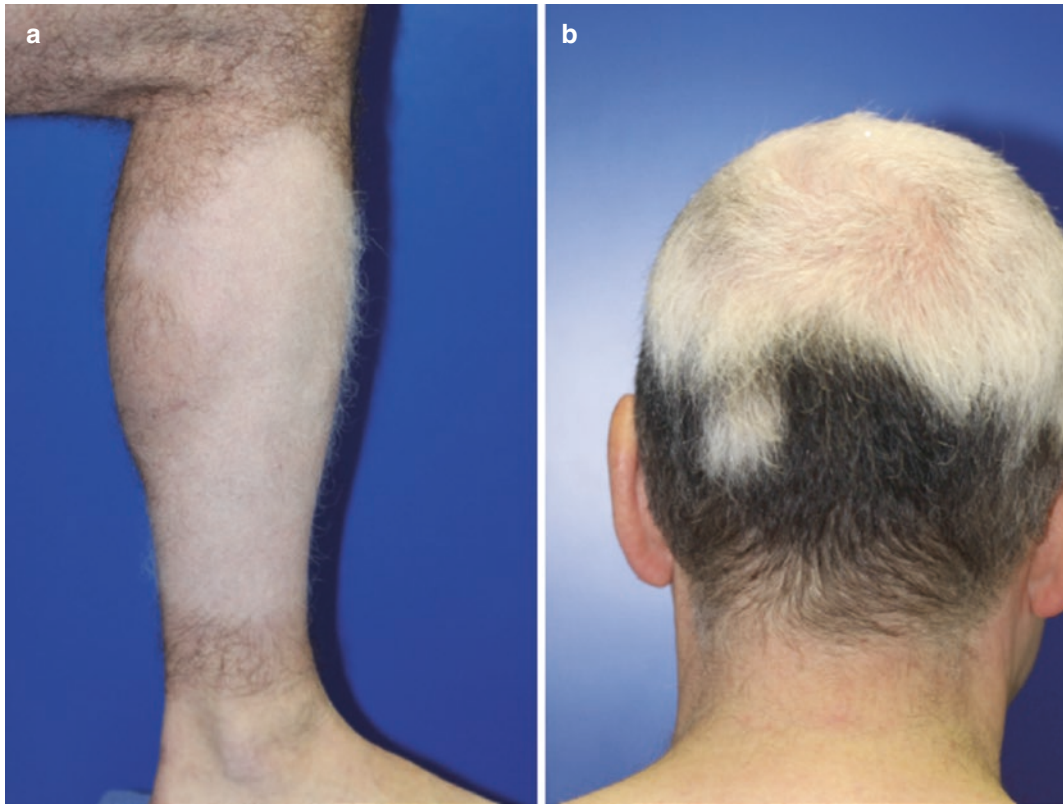


Fig. 8.6 Follicular vitiligo (**a**) leg with a patch of skin and hair depigmentation (**b**) scalp with a large patch of leucotrichia but normally pigmented underlying skin

the epidermis and the hair follicle with Melan-A staining. Presence of cells with concordant markers of mast cells in the perifollicular infiltrate was a surprising finding. It was speculated that follicular vitiligo might bridge vitiligo and alopecia areata and that the absence of follicular melanocytes and precursors may affect prognosis [29].

8.6.2 Hypopigmented Vitiligo/ Vitiligo Minor

Hypopigmented macules occur at onset of vitiligo/NSV or when vitiligo is unstable and spreading. Wood's lamp examination allows discrimination between hypopigmentation and completely depigmented lesions, which are highly suggestive of vitiligo. However, hypopigmentation should prompt consideration of differential diagnoses, mainly cutaneous T-cell lymphoma (CTCL) and various postinflammatory dermatoses (Chap. 1.2).

Hypochromic vitiligo/vitiligo minor is considered as a rare form of vitiligo, so far described only in dark-skinned individuals, characterized by the presence of hypopigmented lesions alone or associated with suggestive achromic macules, which arise without any preceding clinical inflammation. A recent international series of 24 patients has been published [30].

In this series, the diagnosis was made in adulthood, but one-third of patients had disease onset before the age of 20 years. The mean disease duration was 12.3 years (range 1–42). All patients had skin types V and VI. The pattern of distribution was highly similar in most of the patients with involvement of seborrhoeic areas, associated with multiple isolated hypopigmented macules predominantly involving the

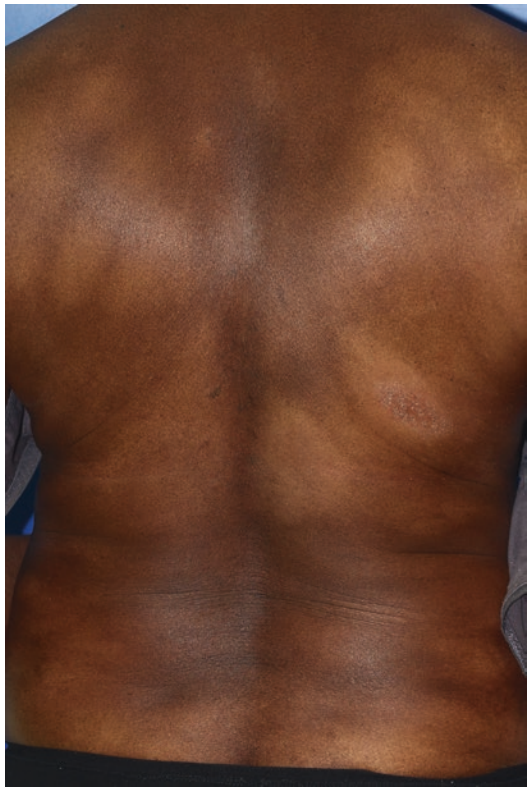


Fig. 8.7 Case diagnosed as hypopigmented vitiligo with a diagnosis of mycosis fungoides after longer follow-up

scalp, the limbs, and/or the trunk. None of the patients had clinical signs of inflammation, scaling or hypoesthesia, or a clear/pronounced skin infiltration. Leukotrichia was absent. Completely depigmented isolated macules were observed in only five patients (21%). Wood's lamp examination confirmed the hypopigmentation and the absence of fluorescence due to fungi. Twenty-one patients were treated with conventional vitiligo treatments, including narrowband ultraviolet B, PUVA, topical corticosteroids, and tacrolimus. Response to treatment was overall very poor.

Histopathology was performed in 18 patients. In all cases the patterns were similar, with an irregular decrease of melanin associated with a decrease in the number of melanocytes in hypopigmented areas, compared with the surrounding normal-looking skin. Inflammation was said not prominent in pathology reports, but unfortunately no systematic review of the biopsy material could be done. No atypical lymphocytes located at the dermo-epidermal interface were observed.

Of note, one of the published patients followed at Bordeaux University hospitals, who had serial biopsies, developed full-blown mycosis fungoides in the year following the publication of the paper (Fig. 8.7). The diagnosis of hypopigmented vitiligo remains thus very challenging and should be restricted to cases with long-term observation (more than 5 years) and serial negative biopsies for CTCL. Several recent observations point the difficulty of the differential [31–34].

8.6.3 Vitiligo Guttata/Punctata, Leukoderma Punctata, and Confetti Leukoderma

This entity was described by Falabella et al. in 13 patients with vitiligo (one segmental, four focal, eight generalized), most of them female children, who developed numerous punctate hypopigmented and achromic spots, 0.5–1.5 mm in diameter located primarily on the sun-exposed areas of the extremities, following treatment with PUVASOL [35]. Two of these patients improved subsequently, whereas the remaining patients showed a stable clinical course. Immunohistochemistry showed decreased numbers of functional melanocytes, marked reduction of melanin at Fontana-Masson stain, and ultrastructural studies demonstrated degenerative damage of keratinocytes and melanocytes of the type already reported in vitiligo. The phototoxic effect of PUVASOL therapy was suggested as a possible etiologic factor in these patients. The link vitiligo-guttata hypomelanosis needs further investigation especially if the association occurs [36], and biopsies are needed to make a definitive diagnosis. In particular cases of amyloidosis with confetti-like leukoderma have been described [37]. In addition, difficult to classify true punctate vitiligo lesions on photoexposed areas may occur even without a context of immunotherapy for melanoma or use of psoralens.

8.7 Summary and Concluding Remarks

Clinically inflammatory vitiligo is a rare clinical presentation of common vitiligo, described so far mostly in the context of vitiligo/NSV. When associated with other cutaneous diseases, it may correspond to a particular mode of onset of depigmentation triggered by local inflammation different from that of Sutton's (Chap. 9) or even Koebner's phenomena (Chap. 11). Overlap with Sjögren's syndrome cutaneous involvement or subacute lupus needs to be investigated. When associated with HIV infection, it has a clearly distinct clinical presentation for which the outcome of severe cutaneous inflammation leads to a vitiligo-like disorder, difficult to distinguish from true vitiligo/NSV. However, besides such rare observations, consistent histopathological findings in still pigmented progressing borders in NSV and SV [26] suggest that common vitiligo could be considered as a clinically silent chronic microinflammatory skin disorder, clinically visible forms being the emerged tip of an iceberg [38]. New basic science information has given clues to inflammation triggers in vitiligo such as inflammasome activation and iHSP 70 production [39–41]. Why the inflammation is usually silent remains a mystery. This could eventually relate either to a particular inflammatory cytokine profile of vitiligo, which would provoke minimal inflammation and pruritus, or to the loss of melanocyte as providers of mediators of an inflammatory response which would be lost in vitiligo, or to both phenomena. In this respect, the "neuron of the skin" view of the melanocyte needs probably to be reinvestigated more closely in terms of production of cytokines and neuropeptides mediating inflammatory skin responses.

For the rare variants discussed in this chapter, there is still a significant gap of knowledge to fill with new investigations. The links vitiligo-guttata hypomelanosis-UV exposure, vitiligo-CTCL, follicular vitiligo-alopecia areata are clearly important to consider in future studies.

References

1. Taïeb A, Picardo M, VETF Members. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell Res. 2007;20:27–35.
2. Buckley W, Lobitz W. Vitiligo with raised inflammatory border. Arch Derm Syph. 1953;67:316–20.
3. Garb J, Wise F. Vitiligo with raised borders. Arch Derm Syphilol. 1948;58:149–53.
4. Wise F. Leukoderma with inflammatory borders. Arch Derm Syph. 1942;45:218–9.
5. Duvic M, Rapini R, Hoots WK, et al. Human immunodeficiency virus-associated vitiligo: expression of autoimmunity with immunodeficiency? J Am Acad Dermatol. 1987;17:656–62.
6. Grandhe NP, Dogra S, Kumar B. Spontaneous repigmentation of vitiligo in an untreated HIV-positive patient. J Eur Acad Dermatol Venereol. 2006;20:234–5.
7. Niamba P, Traoré A, Taieb A. Vitiligo sur peau noire associée au VIH et repigmentation lors du traitement antiretroviral. Ann Dermatol Venereol. 2007;134:272–6.
8. Tsuboi H, Yonemoto K, Katsuoka K. Vitiligo with inflammatory raised borders with hepatitis C virus infection. J Dermatol. 2006;33:577–8.
9. Weisberg EL, Le LQ, Cohen JB. A case of simultaneously occurring lichen sclerosus and segmental vitiligo: connecting the underlying autoimmune pathogenesis. Int J Dermatol. 2008;47:1053–5.
10. Sugita K, Izu K, Tokura Y. Vitiligo with inflammatory raised borders, associated with atopic dermatitis. Clin Exp Dermatol. 2006;31:80–2.
11. Tsuruta D, Hamada T, Teramae H, et al. Inflammatory vitiligo in Vogt-Koyanagi-Harada disease. J Am Acad Dermatol. 2001;44:129–31.
12. Wong SS, Ng SK, Lee HM. Vogt-Koyanagi-Harada Disease: extensive vitiligo with prodromal generalized erythroderma. Dermatology. 1999;198:65–8.
13. Gunasekera N, Murphy GF, Sheth VM. Repigmentation of extensive inflammatory vitiligo with raised borders using early and aggressive treatment. Dermatology. 2015;230(1):11–5.
14. Michaëlsson G. Vitiligo with raised borders. Report of two cases. Acta Derm Venereol. 1968;48:158–61.
15. Ortonne JP, Baran R, Civatte J. Vitiligo with an inflammatory border. *A propos* of 2 cases with review of the literature (18 cases). Ann Dermatol Venereol. 1979;106:613–5.
16. Petit T, Cribier B, Bagot M, Wechsler J. Inflammatory vitiligo-like macules that simulate hypopigmented mycosis fungoides. Eur J Dermatol. 2003;17:410–2.
17. Swick BL, Walling HW. Depigmented patches with an annular inflammatory border. Clin Exp Dermatol. 2008;33:671–2.

18. Shin J, Lee JS, Kim MR, Kim do Y, Hann SK, Oh SH. New suggestive clue to the mechanism of vitiligo: inflammatory vitiligo showing prominent leukocytoclasis at the erythematous rim. J Dermatol. 2013;40(6):488–90.
19. Trikha R, McCowan N, Brodell R. Marginal vitiligo: an unusual depigmenting disorder. Dermatol Online J. 2014;21(3):pii: 13030.
20. Yagi H, Tokura Y, Furukawa Y, Takigawa M. Vitiligo with raised inflammatory borders: involvement of T cell immunity and keratinocytes expressing MHC class II and ICAM-1 molecules. Eur J Dermatol. 1997;7:19–22.
21. Tanioka M, Takahashi K, Miyachi YI. Narrowband ultraviolet B phototherapy for inflammatory vitiligo with raised borders associated with Sjögren's syndrome. Clin Exp Dermatol. 2009;34(3):418–20.
22. Annessi G, Paradisi M, Angelo C, et al. Annular lichenoid dermatitis of youth. J Am Acad Dermatol. 2003;49:1029–36.
23. Attili VR, Attili SK. Lichenoid inflammation in vitiligo-a clinical and histopathologic review of 210 cases. Int J Dermatol. 2008;47:663–9.
24. Hann SK, Park YK, Lee KG, et al. Epidermal changes in active vitiligo. J Dermatol. 1992;19:217–22.
25. Sharquie KE, Mehenna SH, Naji AA, Al-Azzawi H. Inflammatory changes in vitiligo: stage I and II depigmentation. Am J Dermatopathol. 2004;26:108–12.
26. Attili VR, Attili SK. Segmental and generalized vitiligo: both forms demonstrate inflammatory histopathological features and clinical mosaicism. Indian J Dermatol. 2013;58(6):433–8.
27. Ezzedine K, Amazan E, Seneschal J, Cario-André M, Léauté-Labrèze C, Vergier B, Boralevi F, Taieb A. Follicular vitiligo: a new form of vitiligo. Pigment Cell Melanoma Res. 2012a;25(4):527–9.
28. Ezzedine K, Amazan E, Séneschal J, Cario-André M, Léauté-Labrèze C, Vergier B, Boralevi F, Taieb A. Follicular vitiligo: a new form of vitiligo. Pigment Cell Melanoma Res. 2012b;25:527–9.
29. Gan EY, Cario-André M, Pain C, Goussot JF, Taïeb A, Seneschal J, Ezzedine K. Follicular vitiligo: a report of 8 cases. J Am Acad Dermatol. 2016;74(6):1178–84.
30. Ezzedine K, Mahé A, van Geel N, Cardot-Leccia N, Gauthier Y, Descamps V, Al Issa A, Ly F, Chosidow O, Taïeb A, Passeron T. Hypochromic vitiligo: delineation of a new entity. Br J Dermatol. 2015;172(3):716–21.
31. Gameiro A, Gouveia M, Tellechea Ó, Moreno A. Childhood hypopigmented mycosis fungoides: a commonly delayed diagnosis. BMJ Case Rep. 2014;2014:pii: bcr2014208306.
32. Ranawaka RR, Abeygunasekara PH, de Silva MV. Hypopigmented mycosis fungoides in type v skin: a report of 5 cases. Case Rep Dermatol Med. 2011;2011:190572.
33. Soro LA, Gust AJ, Purcell SM. Inflammatory vitiligo versus hypopigmented mycosis fungoides in a 58-year-old Indian female. Indian Dermatol Online J. 2013;4(4):321–5.
34. Tolkachjov SN, Comfere NI. Hypopigmented mycosis fungoides: a clinical mimicker of vitiligo. J Drugs Dermatol. 2015;14(2):193–4.
35. Falabella R, Escobar CE, Carrascal E, Arroyave JA. Leukoderma punctata. J Am Acad Dermatol. 1988;18(3):485–94.
36. Loquai C, Metze D, Nashan D, Luger TA, Böhm M. Confetti-like lesions with hyperkeratosis: a novel ultraviolet-induced hypomelanotic disorder? Br J Dermatol. 2005;153:190–3.
37. Verma S, Joshi R. Amyloidosis cutis dyschromica: a rare reticulate pigmentary dermatosis. Indian J Dermatol. 2015;60:385–7.
38. Taïeb A. Vitiligo as an inflammatory skin disorder: a therapeutic perspective. Pigment Cell Melanoma Res. 2012;25(1):9–13.
39. Levandowski CB, Mailloux CM, Ferrara TM, Gowan K, Ben S, Jin Y, McFann KK, Holland PJ, Fain PR, Dinarello CA, Spritz RA. NLRP1 haplotypes associated with vitiligo and autoimmunity increase interleukin-1β processing via the NLRP1 inflammasome. Proc Natl Acad Sci. 2013;110(8):2952–6.
40. Marie J, Kovacs D, Pain C, Jouary T, Cota C, Vergier B, Picardo M, Taieb A, Ezzedine K, Cario-André M. Inflammasome activation and vitiligo/nonsegmental vitiligo progression. Br J Dermatol. 2014;170(4):816–23.
41. Mosenson JA, Zloza A, Nieland JD, Garrett-Mayer E, Eby JM, Huelsmann EJ, Kumar P, Denman CJ, Lacek AT, Kohlhapp FJ, Alamiri A, Hughes T, Bines SD, Kaufman HL, Overbeck A, Mehrotra S, Hernandez C, Nishimura MI, Guevara-Patino JA, Le Poole IC. Mutant HSP70 reverses autoimmune depigmentation in vitiligo. Sci Transl Med. 2013;5(174):174ra28.

9 Halo Nevus, Leucotrichia and Mucosal Vitiligo

Anuradha Bishnoi and Davinder Parsad

Contents

Abstract

Halo nevus is defined as a well-defined, symmetrical, round or oval, hypopigmented, or depigmented rim/halo around a central melanocytic nevus. The size of nevus and ring can vary from few millimeters to centimeters. In general, the diameter of halo correlates with the diameter of the nevus.

Leukotrichia refers to whitening of the hair associated with a depigmented vitiligo patch. Leukotrichia, poliosis, and canities are all characterized by whitening of the hair. Poliosis represents a circumscribed collection of white hair. It may or may not be associated with whitening of the underlying skin. Vitiligo may present with both leukotrichia and poliosis, and midline frontal hair poliosis identical to

A. Bishnoi · D. Parsad (✉)
Department of Dermatology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_9

piebaldism can be seen in vitiligo. Canities refers to premature and generalized graying of hair in the scalp and beard.

The incidence of halo nevus and leukotrichia has been reported to be 4.4% and 12.3%, respectively.

In this chapter, we shall discuss about halo nevus, mucosal vitiligo and leucotrichia; and their significance in relation to vitiligo.

Key Points

- Halo nevi commonly develop in children or young adults having multiple melanocytic nevi.
- Careful examination of the central nevus is required to rule out melanoma, especially in elderly.
- Leukotrichia is considered to be a significant marker of segmental vitiligo.
- Presence of halo nevi and leukotrichia is considered to be a significant predictor of evolution from segmental vitiligo to mixed one.
- Presence of premature hair graying has been found to be an important risk factor for development of vitiligo in subsequent generations.

9.1 Clinical Features

Halo nevus refers to a well-defined, symmetrical, round or oval, hypopigmented or depigmented ring/halo around a central melanocytic nevus. The size of nevus and the depigmented ring can vary from few millimeters to centimeters. In general, the diameter of depigmented halo correlates with the diameter of the nevus.

9.1.1 Halo Nevi

Halo nevi [1] (Fig. 9.1) commonly develop in children or young adults having multiple melanocytic nevi. Incidence in individuals below 20 years of age is around 1% with mean age of onset being

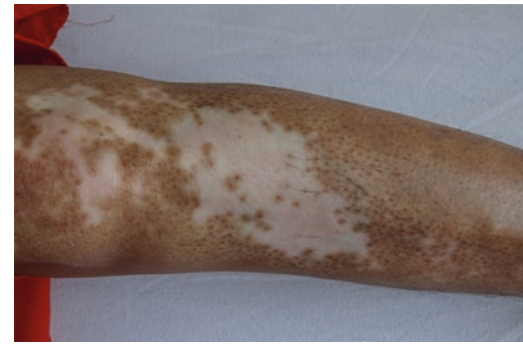


Fig. 9.1 Depigmented patch on shin. Some of the hair is still pigmented, while majority are depigmented

15 years [2]. Approximately 20% of those having halo nevi have vitiligo. Association with other autoimmune diseases like alopecia areata, Hashimoto's thyroiditis, celiac disease, and intense sun exposure has been reported [2]. Familial tendency to develop halo nevi has also been reported.

They commonly appear on the trunk, especially the upper back. It can occur in several types of melanocytic lesions, including acquired melanocytic nevus (junctional or dermal), congenital melanocytic nevus (CMN), dysplastic nevus, blue nevus, Spitz nevus, melanoma [3], and dermatofibroma.

Careful examination of the central nevus is therefore required to rule out melanoma, especially in elderly. Halo nevus around melanoma is generally irregular and asymmetrical as compared to other nevi [3].

In a recent study [4] evaluating the incidence of depigmentation in the presence of large CMN, it was observed that 8 of 92 patients developed depigmentation associated with nevus, with six developing depigmentation inside nevus, one developing halo nevi around a satellite nevus, and one developing vitiligo-like depigmentation.

In another report [5], the authors observed spontaneous repigmentation of periorbital vitiligo in a girl after excision of her CMN with a halo nevus around it.

Halo nevi have been found to be significantly associated with Turner's syndrome and HLA-Cw6 locus [6].

Four stages in its natural history have been described:

1. Development of depigmented/hypopigmented halo around the nevus
2. Development of hypopigmentation/depigmentation in the central nevus which becomes pinkish in color
3. Disappearance of the nevus
4. Disappearance of halo and subsequent repigmentation, which may take years to complete

Multiple nevi in different stages can be seen in the same individual.

Aouthmany M et al. [7] observed that 51% of the halo nevi demonstrate no change in the halo or nevus after an average of 4.2 years; 14.3% demonstrated partial nevus regression with persistence of the halo after an average of 6.7 years; 4.1% demonstrated complete involution of the nevus with persistent halo depigmentation after an average of 7.7 years; 8.2% demonstrated complete nevus involution with some repigmentation of the halo after an average of 11.8 years; and 22.4% demonstrated complete resolution of the nevus with complete repigmentation of the halo after an average of 7.8 years. The authors demonstrate that natural history of halo nevi is long, and patients should be educated about the same in order to avoid undue apprehension and surgical procedures for halo nevi.

9.1.2 Leukotrichia

Leucotrichia refers to whitening of hair associated with a depigmented vitiligo patch. Leucotrichia, poliosis and canities are all characterized by whitening of hair. Poliosis represents a circumscribed collection of white hair. It may or may not be associated with whitening of underlying skin. Vitiligo may present with both leucotrichia and poliosis, and midline frontal hair poliosis identical to piebaldism can be seen in vitiligo. Canities refers to premature and generalized graying of hair in scalp and beard.

Incidence varies in different studies, and overall, leukotrichia has been observed significantly more commonly in segmental vitiligo than non-segmental vitiligo (NSV). Also, the hair is affected quite early and prominently in segmental vitiligo (Fig. 9.2). A clinic-epidemiological study conducted in North India assessed 762 vitiligo patients and found the prevalence of leukotrichia to be 33.5% [8]. Another study carried out in Turkey found incidence of leukotrichia to be 3.38% in 148 patients [9].

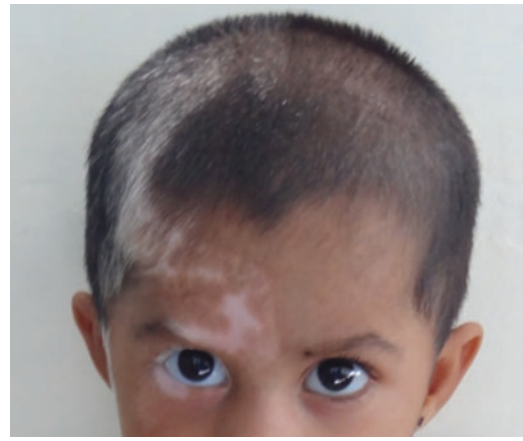


Fig. 9.2 Segmental vitiligo involving face. Leucotrichia and poliosis both are visible

Leukotrichia was found to be a significant marker of segmental vitiligo in a study assessing the characteristics of segmental vitiligo in 188 patients, with 162 patients (86.1%) having leukotrichia, and 70 of these had leukotrichia in all hair of the lesion, whereas 92 had both white and black hair in the lesion. In the same study, 12 patients did not have leukotrichia, and 122 patients had leukotrichia extending beyond the margins of vitiligo patch [10].

A study evaluated the percentage of leukotrichia in 82 patients with segmental vitiligo and found that all 82 patients had leukotrichia, though the percentage of white hair in the patch varied in different patients. While evaluating, the authors used digital microscope with 30× magnification for patches where they could not identify leukotrichia with the naked eye/magnifier. They also observed that due to underlying depigmentation in vitiligo patch, leukotrichia can be difficult to notice, especially if hair are vellus. But leukotrichia in segmental vitiligo involving terminal hair of the scalp, eyelashes, and eyebrows is

usually readily apparent and causes significant psychosocial distress by virtue of its location on exposed areas [11].

9.2 Pathogenesis

9.2.1 Halo Nevus

It was initially believed that halo nevus occurs due to immune response against dysplastic melanocytes developing within the nevus. But histopathology did not reveal dysplasia in the nevus component. It is now believed that it arises because of the destruction of nevus cells by cytotoxic T cells that get activated against specific antigens of normal nevus melanocytes. This cytotoxic T cell response can even eliminate the existing nevus as seen in stage 3 of normal evolution. A recent case report described the disappearance of a medium-sized CMN after formation of halo nevus around it [18].

Halo around the nevus is suggested to develop because of two mechanisms:

1. IFN-γ-mediated apoptosis of surrounding skin melanocytes
2. Direct destruction of surrounding skin melanocytes by T lymphocytes after they get activated against the antigens of nevus melanocytes

There are reports of partial regression of pigmented nevi without the formation of halo, but associated with generalized vitiligo [19].

9.2.2 Leukotrichia

Melanocytes are the dendritic cells derived from the neural crest that form melanin and provide the skin and hair with color. They are located in the basal layer of the epidermis and matrix of hair follicles. Melanocytes residing in hair follicles are larger, more dendritic, and metabolically more active than those present in the interfollicular area.

Melanocytes in hair follicle are present in the infundibulum, bulge and sub-bulge, and follicular bulb. The ones in the infundibulum and bulb resemble epidermal melanocytes and are polydendritic, pigmented, and DOPA (dihydroxyphenylalanine) positive, whereas those in bulge are bipolar, nonpigmented, and DOPA negative. Melanocytes in bulb near the dermal papilla are responsible for the pigmentation of the hair. Melanosomes produced by these melanocytes are taken up by keratinocytes of the hair shaft, resulting in pigmented hair fiber.

Follicular melanogenesis is strongly coupled to hair cycle, in contrast to epidermal melanogenesis, which is continuous. Anagen phase is associated with pigmentation of the hair, whereas initiation of catagen marks a decline in the function of melanocytes in the follicle. The regeneration of the follicular melanocytes in the next cycle results from migration of amelanotic melanocyte precursor stem cells located in the outer root sheath that have high regenerative potential [20–22]. It is generally presumed that melanocytes present in hair follicles are protected from immune damage by virtue of their protected niche inside hair follicles. But they can get targeted as well, resulting in white hair in a vitiligo patch, referred to as leukotrichia.

Gan and colleagues have reported eight cases of follicular vitiligo. These patients had classical depigmented vitiligo patches on the body, and in addition, there was depigmentation involving hair follicles without involvement of the skin underneath, resulting in leukotrichia on clinically normal skin. Histopathology showed loss of melanocytes from hair follicles, and the authors proposed that the entity "follicular vitiligo" might serve as the pathological link between alopecia areata and vitiligo [23].

Four patients developed vitiligo-like leukoderma with one having leukotrichia also, following psoralen and ultraviolet A (PUVA) phototherapy treatment for mycosis fungoides. Vitiligo patches had developed at the sites of mycosis fungoides plaques, and authors proposed that induction of cell-mediated immunity against tumor cells had resulted in cytotoxicity against melanocytes as well as causing vitiligo-like depigmentation [24].

Leukoderma and leukotrichia have been reported in two patients following PUVA treatment for GVHD. The authors proposed that

immune-mediated destruction of cells was responsible for both GVHD and associated leukoderma and leukotrichia [25]. Leukotrichia has been reported with nevus depigmentosus and discoid lupus erythematosus [26, 27]. The use of chloroquine has been reported to result in leukotrichia totalis [28].

Two patients having halo nevi were evaluated using portable digital microscope, and leukotrichia was visible in both, though it was not visible with the naked eye. The authors proposed that hair follicle melanocytes are also targeted in halo nevi formation, in association with epidermal melanocytes [29].

Poliosis in alopecia areata: Pigmented hair is preferentially affected in alopecia areata, and regrowing hair is initially white. It can result in circumscribed patch with sparse white hair causing poliosis. Various morphological changes have been observed that suggest active involvement of melanocytes in alopecia areata [20]. Colocalization of vitiligo and alopecia areata in a patch on the frontal scalp has been reported. The patch had depigmented base, suggestive of vitiligo; and the hair was relatively decreased in patch. Biopsy and immunohistochemistry revealed loss of melanocytes in the epidermis and melanocyte stem cells in the outer root sheath suggestive of vitiligo and peribulbar infiltrate suggestive of alopecia areata [30].

Poliosis can be idiopathic or can be seen in vitiligo, alopecia areata, halo nevi, Vogt-Koyanagi-Harada syndrome, piebaldism, Waardenburg syndrome, and many other hereditary and acquired conditions that result in abnormal melanocyte mobilization during embryogenesis or their destruction later on. It is usually described as a forelock of white hair on the frontal scalp, but can occur virtually anywhere on the body, resulting in a circumscribed collection of white hair.

Canities is defined as generalized graying of the hair. The pathogenesis of canities has been linked to oxidative stress that causes depletion of melanocytes and their precursor stem cells. The process is usually gradual and correlated well with chronological aging that results in a combination of depigmented and pigmented hair on scalp resulting in the so-called graying of the hair [31]. Premature canities is defined as the occurrence of white hair in an individual younger than 20 years in Whites, 25 years in Asians, and 30 years in Africans. Premature graying usually causes low self-esteem in the affected individual. Premature canities can be seen in some autoimmune disorders and premature aging syndromes, and can be isolated or idiopathic. Some studies have seen the association between canities and osteopenia and cardiovascular disorders [32]. Others have tried to identify underlying biochemical abnormalities like decreased ferritin and vitamin B12 and high-density lipoprotein cholesterol levels in individuals with premature canities [33]. Smoking, obesity, and family history of premature canities were seen to be associated with premature graying of the hair in another study [34].

9.3 Histopathology

9.3.1 Halo Nevus

Histology of the central nevus in a halo nevus reveals dense infiltration by antigen-presenting cells and lymphocytes around and inside the dermal component of the nevus (sometimes even inside the junctional component) and degenerative changes in the melanocytes like vacuolar changes and pyknotic nuclei. Histology of halo component reveals very few or absent lymphocytes.

Most of the infiltrating lymphocytes are CD8+ cytotoxic T lymphocytes [35]. Humoral response in the form of circulating antibodies against melanocytes has been observed, but this seems secondary to destruction of melanocytes by T cells, rather than a primary response.

Leukoderma associated with melanoma can be of three types [36]:

1. Associated with regression of melanoma, which is considered to be a poor prognostic factor since it implies increase in depth of melanoma. Depigmentation and white-red areas developing within a melanoma lesion characterize this type of leukoderma.
2. Halo nevus around melanoma.

3. Generalized leukoderma/vitiligo associated with primary melanoma elsewhere.

Halo nevus associated with nevus is characteristically more inflammatory and minimally fibrotic than regressing stage of melanoma, which is associated with significant fibrosis, more activated melanocytes, and rich cytokine milieu including more fibrogenic cytokines in melanoma component [37].

9.4 Association with Vitiligo

Presence of halo nevi and leukotrichia was found to be a significant predictor of evolution from segmental vitiligo to mixed vitiligo in a study [12–14].

Another study assessing the prognostic factors for progression of vitiligo observed that Koebner's phenomenon, positive family history, and mucosal involvement are poor prognostic factors and associated with significant progression of the disease. Leukotrichia, late age at onset, and longer duration of disease were not found to be associated significantly with progression of the disease [15].

Presence of premature hair graying was found to be an important risk factor for the development of vitiligo in subsequent generations [16].

In another study assessing antithyroid hormone autoantibodies in vitiligo, it was observed that anti-T3 antibodies are significantly associated with leukotrichia, and anti-T4 antibodies are associated with vitiligo duration and activity [17].

Since repigmentation in a vitiligo patch depends predominantly on the activity of hair follicle melanocytes, presence of leukotrichia is considered a bad prognostic marker by virtue of deficient melanocyte reservoir and decreased potential of repigmentation.

9.4.1 Halo Nevus

Relationship between halo nevus and vitiligo is unclear. Vitiligo and halo nevi can appear separately or together. Different studies report different prevalence of vitiligo developing in a patient having halo nevi or vice versa. Development of halo nevi is associated with less extensive vitiligo, although halo nevi have been described in both segmental and non-segmental vitiligo.

Presence of halo nevi and leukotrichia was found to be a significant predictor of evolution from segmental vitiligo to mixed vitiligo [12–14].

A retrospective study assessing 208 pediatric vitiligo patients observed that 55 (26%) had halo nevus, and presence of halo nevus was associated with male gender, generalized vitiligo, development of vitiligo at a later age, and statistically nonsignificant yet increased chances of repigmentation; but it was not found to be associated with increased rate of progression of the disease [38].

A case series of nine patients reported development of both halo nevi and vitiligo in patients having CMN [39].

A study [40] assessing the role of CMN in 1004 vitiligo patients found that presence of these nevi was associated with significantly increased incidence of halo phenomena, earlier age of onset of vitiligo, development of acral/joint lesions, and Koebner's phenomenon. This study found CMN in 3.3% of vitiligo patients and 1% of control patients. Halo nevi were seen in 30.3% of the cases and were positively associated with the diameter of the nevus. Presence of CMN was not found to be associated with progression of disease, family history, or autoimmune diseases.

Another case report [41] demonstrated the development of localized sacral and ankle vitiligo in a patient having halo nevus associated with CMN and spontaneous partial repigmentation in both halo nevi and vitiligo a few years later.

In a latent class analysis, it was observed that prepubertal onset of vitiligo (<12 years of age) was significantly associated with halo nevi, Koebner's phenomenon, atopic dermatitis, previous spontaneous repigmentation, family history of vitiligo, and early onset graying of hair [12, 42].

Van Geel et al. [43] propose that analogous to generalized depigmentation associated with melanoma, halo nevi can also trigger additional leukoderma-/vitiligo-like lesions, and this is an entity distinct from vitiligo with respect to lesion distribution, extent, and prognosis. They characterize this halo nevi-associated leukoderma and point out that these patients are younger and have asymmetric, limited-sized subtle lesions with ill-defined borders that show lack of progression and absence of Koebner's phenomenon, other autoimmune diseases and family history of vitiligo, have significantly larger number of halo nevi (>3) and temporal correlation of development of vitiligo-like lesions with halo nevi, and hence, proposed that halo nevi-associated leukoderma is a separate entity and has a distinct pattern from vitiligo per se.

In a prospective study evaluating 553 patients with non-segmental vitiligo (NSV), 130 patients with halo nevi (NSV-HN) and 423 without halo nevi (NSV) were compared. It was observed that presence of halo nevus is positively associated with family history of premature graying of the hair; trunk involvement; age of onset <18 years; phototypes I, II, and III; and depigmentation pattern (lesser staging, lesser leukotrichia), whereas no association was seen with hand and feet involvement and total affected area [13]. The authors proposed strong association between NSV-HN and premature hair graying and concluded that NSV-HN most commonly occurred on the trunk, had a central distribution, and spared the hand and feet, implying that NSV in isolation preferentially affects interfollicular epidermal melanocytes, whereas NSV-HN involves hair follicular melanocytes preferentially.

In another study by van Geel et al. [44] comparing halo nevi alone, NSV alone, and halo nevi with NSV, it was observed that presence of halo nevi in NSV is significantly associated with younger age of onset and reduced risk of autoimmune diseases. They also observed that in 61% of NSV cases, halo nevi preceded the development of vitiligo, and presence of halo nevi does not correlate with subtype, extent, and progression/activity of NSV. Halo nevi alone group had significantly less association with Koebner's phenomenon, associated autoimmune diseases and family history of vitiligo, and significant positive association with multiple nevi.

Patrizi et al. [45] studied the difference between 98 patients having halo nevi alone and 27 patients having halo nevi associated with vitiligo. They observed that two patients in halo nevi alone group developed vitiligo at follow-up period of >5 years (both had multiple halo nevi). While in those having both halo nevus and vitiligo, 11 patients developed both simultaneously, seven developed vitiligo first, and nine developed halo nevi first followed by vitiligo later (all of these nine patients had multiple halo nevi). They also observed that presence of both halo nevi and vitiligo was associated with multiple nevi and associated autoimmune diseases in patients and families. At follow-up period of 5 years, 18 of 52 available halo nevi-only patients developed multiple nevi. In vitiligo and halo nevi group, all nine patients where vitiligo was preceded by halo nevi had multiple nevi.

HLA typing was compared between NSV alone and that associated with halo nevi, and it was observed that both subsets have distinct HLA associations and might represent different entities with distinct pathogenesis [46].

A study [47] assessed the ultrastructural differences between mitochondria in the perilesional skin of halo nevi and vitiligo and found significant differences and thus concluded that both achromic lesions might actually have different pathogenic mechanisms.

Another study evaluated hydrogen peroxide concentration in halo of halo nevi and vitiligo and concluded on biochemical basis that these entities are distinct [48].

In a study comparing NSV and SV, it was observed that halo nevi are significantly more associated with NSV than SV [49].

Halo nevus has been associated with Vogt-Koyanagi-Harada syndrome [50].

9.5 Diagnosis

9.5.1 Halo Nevus and Leukotrichia

Diagnosis of halo nevus and leukotrichia is usually straightforward, and primarily clinical. Is usually straightforward, but Wood's lamp examination and dermatoscopy may be required to detect if clinical features are subtle, especially in lighter skin tones.

9.6 Treatment

9.6.1 Halo Nevus

Patient education about natural history of halo nevi and reassurance is required in majority of cases.

Non-cultured epidermal cell grafting has been successfully used in the treatment of 11 patients with halo nevi, and repigmentation thus achieved was maintained after a mean follow-up of 4 years. Excellent repigmentation was achieved in halo nevi [51].

Excimer laser has been successfully used in patients having facial halo nevi [52].

Individuals developing halo nevus after 40 years of age should be screened for melanoma (ocular, cutaneous, and mucosal).

9.6.2 Leukotrichia

Leukotrichia is difficult to treat. When affecting vellus hair, it might be difficult to appreciate leukotrichia on top of depigmented vitiligo patches. But when terminal hair of the scalp, beard, eyebrows, and eyelashes are affected, camouflage is also difficult. Even after repigmentation of a vitiligo patch with medical treatment, overlying leukotrichia might not improve and require transplantation of melanocytes.

A study employing *xenon chloride* excimer laser in vitiligo observed that presence of leukotrichia was associated with poor treatment outcomes in both segmental and non-segmental vitiligo alike. The authors also proposed that it might be beneficial to rule out leukotrichia affecting vellus hair using dermatoscopy, before initiating treatment, because of inherent poor response in such patients by virtue of minimum repigmentation potential [53].

Surgical treatment options like tissue grafts and follicular unit transplants have been used in treating leukotrichia. Eight patients with stable vitiligo patches having leukotrichia affecting the scalp, beard, and eyebrows were treated with split-thickness skin grafting, and it was observed that response was earlier (by 3 months) and greater (75–90% repigmentation) in eyebrows as compared to the scalp and beard, where it was late and lesser (6–9 months, 50–60%). Hair follicle melanocytes are responsible for perifollicular pattern of repigmentation observed in vitiligo. Narrowband ultraviolet B phototherapy results primarily in this pattern of repigmentation, by activation and migration of hair follicle melanocytes (anterograde repigmentation). But after split-thickness grafting, the epidermal repigmentation is followed by subsidence of leukotrichia, implying reverse migration of melanocytes from the repigmented epidermis to follicular ostia, outer root sheath, and bulb [54]. Follicular unit transplantation was done in stable vitiligo patients, and 11 of 46 patients with leukotrichia responded well [55].

Non-cultured cellular grafts were successfully employed in the treatment of leukotrichia in generalized and segmental vitiligo with good to excellent repigmentation (>50%) achieved after a period of 9–12 months, though initial response at 3 months was poor. Out of 11 cases in this study, nine had eyebrow leukotrichia and two had leg and scalp leukotrichia. The authors concluded that good to excellent repigmentation can be achieved in patients having leukotrichia with cellular grafts, thus obviating the need for hair transplantation [56, 57].

Eyebrow leukotrichia usually responds well to the treatment of vitiligo patch underneath, but response of eyelash leukotrichia is not that well to the treatment of periocular vitiligo and is a source for considerable psychological morbidity for the patient. Fifteen patients having eyelash vitiligo were treated with follicular unit transplantation from temporal scalp, and good to excellent response (>50% repigmentation) was

seen in 13 patients, whereas fair and poor response was seen in one patient each [58].

Leukotrichia is a part and parcel of vitiligo that has not been found to affect the progression of the disease, though it is a bad prognostic factor because of negligible potential for repigmentation in such patches. But new surgical techniques have shown considerable potential in repigmentation of leukotrichia. Further research in the field of canities might unfold new molecular targets that will help in deciphering new treatment modalities for leukotrichia and canities.

References

1. Rongioletti F, Cecchi F, Rebora A. Halo phenomenon in melanocytic nevi (Sutton's nevi). Does the diameter matter? J Eur Acad Dermatol Venereol. 2011;25:1231–2.
2. Pustisek N, Sikanic-Dugic N, Hirsl-Hecej V, Domljan ML. "Halo nevi" and UV radiation. Coll Antropol. 2010;34(Suppl 2):295–7.
3. Rubegni P, Nami N, Risulo M, Tataranno D, Fimiani M. Melanoma with halo. Clin Exp Dermatol. 2009;34:749–50.
4. Polat Ekinci A, Kilic S, Baykal C. Pigment loss in patients with large congenital melanocytic nevi: various clinical presentations documented in a large series. Pediatr Dermatol. 2016;33:307.
5. Wang K, Wang Z, Huang W. Resolution of vitiligo following excision of halo congenital melanocytic nevus: a rare case report. Dermatol Ther. 2016;29(3):145–7.
6. Brazzelli V, Larizza D, Martinetti M, Martinoli S, Calcaterra V, De Silvestri A, et al. Halo nevus, rather than vitiligo, is a typical dermatologic finding of turner's syndrome: clinical, genetic, and immunogenetic study in 72 patients. J Am Acad Dermatol. 2004;51:354–8.
7. Aouthmany M, Weinstein M, Zirwas MJ, Brodell RT. The natural history of halo nevi: a retrospective case series. J Am Acad Dermatol. 2012;67:582–6.
8. Agarwal S, Ojha A, Gupta S. Profile of vitiligo in kumaun region of uttarakhand, India. Indian J Dermatol. 2014;59:209.
9. Kalkanli N, Kalkanli S. Classification and comparative study of vitiligo in Southeast of Turkey with biochemical and immunological parameters. Clin Ter. 2013;164:397–402.
10. Khaitan BK, Kathuria S, Ramam M. A descriptive study to characterize segmental vitiligo. Indian J Dermatol Venereol Leprol. 2012;78:715–21.
11. Lee DY, Kim CR, Park JH, Lee JH. The incidence of leukotrichia in segmental vitiligo: implication of poor response to medical treatment. Int J Dermatol. 2011;50:925–7.
12. Ezzedine K, Diallo A, Leaute-Labreze C, Seneschal J, Prey S, Ballanger F, et al. Halo naevi and leukotrichia are strong predictors of the passage to mixed vitiligo in a subgroup of segmental vitiligo. Br J Dermatol. 2012a;166:539–44.
13. Ezzedine K, Diallo A, Leaute-Labreze C, Seneschal J, Boniface K, Cario-Andre M, et al. Pre- vs. post-pubertal onset of vitiligo: multivariate analysis indicates atopic diathesis association in pre-pubertal onset vitiligo. Br J Dermatol. 2012b;167:490–5.
14. Ezzedine K, Diallo A, Leaute-Labreze C, Seneschal J, Mossalayi D, AlGhamdi K, et al. Halo nevi association in nonsegmental vitiligo affects age at onset and depigmentation pattern. Arch Dermatol. 2012c;148:497–502.
15. Dave S, Thappa DM, Dsouza M. Clinical predictors of outcome in vitiligo. Indian J Dermatol Venereol Leprol. 2002;68:323–5.
16. Halder RM, Grimes PE, Cowan CA, Enterline JA, Chakrabarti SG, Kenney JA Jr. Childhood vitiligo. J Am Acad Dermatol. 1987;16:948–54.
17. Colucci R, Lotti F, Dragoni F, Arunachalam M, Lotti T, Benvenga S, et al. High prevalence of circulating autoantibodies against thyroid hormones in vitiligo and correlation with clinical and historical parameters of patients. Br J Dermatol. 2014;171:786–98.
18. Lee NR, Chung HC, Hong H, Lee JW, Ahn SK. Spontaneous involution of congenital melanocytic nevus with halo phenomenon. Am J Dermatopathol. 2015;37:e137–9.
19. Sotiriou E, Apalla Z, Panagiotidou D, Ioannides D. Partial pigmentary regression of a congenital nevocytic naevus without halo phenomenon followed by generalized vitiligo. J Eur Acad Dermatol Venereol. 2009;23:600–1.
20. Tobin DJ, Fenton DA, Kendall MD. Ultrastructural observations on the hair bulb melanocytes and melanosomes in acute alopecia areata. J Invest Dermatol. 1990;94:803–7.
21. Tobin DJ, Paus R. Graying: gerontobiology of the hair follicle pigmentary unit. Exp Gerontol. 2001;36:29–54.
22. Van Neste D, Tobin DJ. Hair cycle and hair pigmentation: dynamic interactions and changes associated with aging. Micron. 2004;35:193–200.
23. Gan EY, Cario-Andre M, Pain C, Goussot JF, Taieb A, Seneschal J, et al. Follicular vitiligo: a report of 8 cases. J Am Acad Dermatol. 2016;74:1178–84.
24. Mimouni D, David M, Feinmesser M, Coire CI, Hodak E. Vitiligo-like leucoderma during photochemotherapy for mycosis fungoides. Br J Dermatol. 2001;145:1008–14.
25. Williams JS, Mufti GJ, du Vivier AW, Salisbury JR, Creamer D. Leucoderma and leucotrichia in association with chronic cutaneous graft-versus-host disease. Br J Dermatol. 2008;158:172–4.
26. Dhar S, Kanwar AJ, Ghosh S. Leucotrichia in nevus depigmentosus. Pediatr Dermatol. 1993;10:198–9.
27. Kanwar AJ, Kaur S. Leucotrichia in discoid lupus erythematosus. Dermatology. 1992;184:232.

28. Linn HW. Leucotrichia totalis from chloroquine. Med J Aust. 1959;46(2):360–1.
29. Lee DY, Jung KD, Park JH, Lee JH, Yang JM, Lee ES. Halo naevus is associated with leucotrichia: use of portable digital microscopy. J Eur Acad Dermatol Venereol. 2009;23:1095–6.
30. Walker A, Mesinkovska NA, Boncher J, Tamburro J, Bergfeld WF. Colocalization of vitiligo and alopecia areata presenting as poliosis. J Cutan Pathol. 2015;42:150–4.
31. Pandhi D, Khanna D. Premature graying of hair. Indian J Dermatol Venereol Leprol. 2013;79:641–53.
32. Kocaman SA, Cetin M, Durakoglugil ME, Erdogan T, Canga A, Cicek Y, et al. The degree of premature hair graying as an independent risk marker for coronary artery disease: a predictor of biological age rather than chronological age. Anadolu Kardiyol Derg. 2012;12:457–63.
33. Chakrabarty S, Krishnappa PG, Gowda DG, Hiremath J. Factors Associated with Premature Hair Graying in a Young Indian Population. Int J Trichol. 2016;8:11–4.
34. Shin H, Ryu HH, Yoon J, Jo S, Jang S, Choi M, et al. Association of premature hair graying with family history, smoking, and obesity: a cross-sectional study. J Am Acad Dermatol. 2015;72:321–7.
35. Zeff RA, Freitag A, Grin CM, Grant-Kels JM. The immune response in halo nevi. J Am Acad Dermatol. 1997;37:620–4.
36. Naveh HP, Rao UN, Butterfield LH. Melanoma-associated leukoderma - immunology in black and white? Pigment Cell Melanoma Res. 2013;26:796–804.
37. Moretti S, Spallanzani A, Pinzi C, Prignano F, Fabbri P. Fibrosis in regressing melanoma versus nonfibrosis in halo nevus upon melanocyte disappearance: could it be related to a different cytokine microenvironment? J Cutan Pathol. 2007;34:301–8.
38. Cohen BE, Mu EW, Orlow SJ. Comparison of childhood vitiligo presenting with or without associated halo nevi. Pediatr Dermatol. 2016;33:44–8.
39. Stierman SC, Tierney EP, Shwayder TA. Halo congenital nevocellular nevi associated with extralesional vitiligo: a case series with review of the literature. Pediatr Dermatol. 2009;26:414–24.
40. van Geel N, Van Poucke L, Van de Maele B, Speeckaert R. Relevance of congenital melanocytic naevi in vitiligo. Br J Dermatol. 2015;172:1052–7.
41. Concha-Garzon MJ, Hernandez-Martin A, Faura-Berruga C, Davila-Seijo P, Torrelo A. Spontaneous partial repigmentation of halo nevi around congenital melanocytic nevus and vitiligo in a 13-year-old boy. Indian J Dermatol Venereol Leprol. 2014;80:69–70.
42. Ezzedine K, Le Thuaut A, Jouary T, Ballanger F, Taieb A, Bastuji-Garin S. Latent class analysis of a series of 717 patients with vitiligo allows the identification of two clinical subtypes. Pigment Cell Melanoma Res. 2014;27:134–9.
43. van Geel N, Speeckaert R, Lambert J, Mollet I, De Keyser S, De Schepper S, et al. Halo naevi with associated vitiligo-like depigmentations: pathogenetic hypothesis. J Eur Acad Dermatol Venereol. 2012;26:755–61.
44. van Geel N, Vandenhaute S, Speeckaert R, Brochez L, Mollet I, De Cooman L, et al. Prognostic value and clinical significance of halo naevi regarding vitiligo. Br J Dermatol. 2011;164:743–9.
45. Patrizi A, Bentivogli M, Raone B, Dondi A, Tabanelli M, Neri I. Association of halo nevus/i and vitiligo in childhood: a retrospective observational study. J Eur Acad Dermatol Venereol. 2013;27:e148–52.
46. de Vijlder HC, Westerhof W, Schreuder GM, de Lange P, Claas FH. Difference in pathogenesis between vitiligo vulgaris and halo nevi associated with vitiligo is supported by an HLA association study. Pigment Cell Res. 2004;17:270–4.
47. Ding GZ, Zhao WE, Li X, Gong QL, Lu Y. A comparative study of mitochondrial ultrastructure in melanocytes from perilesional vitiligo skin and perilesional halo nevi skin. Arch Dermatol Res. 2015;307:281–9.
48. Schallreuter KU, Kothari S, Elwary S, Rokos H, Hasse S, Panske A. Molecular evidence that halo in Sutton's naevus is not vitiligo. Arch Dermatol Res. 2003;295:223–8.
49. Ezzedine K, Diallo A, Leaute-Labreze C, Mossalayi D, Gauthier Y, Bouchtnei S, et al. Multivariate analysis of factors associated with early-onset segmental and nonsegmental vitiligo: a prospective observational study of 213 patients. Br J Dermatol. 2011;165:44–9.
50. Nordlund JJ, Albert D, Forget B, Lerner AB. Halo nevi and the Vogt-Koyanagi-Harada syndrome. Manifestations of vitiligo. Arch Dermatol. 1980;116:690–2.
51. van Geel N, Wallaeys E, Goh BK, De Mil M, Lambert J. Long-term results of noncultured epidermal cellular grafting in vitiligo, halo naevi, piebaldism and naevus depigmentosus. Br J Dermatol. 2010;163:1186–93.
52. Mulekar SV, Issa AA, Eisa AA. Treatment of halo nevus with a 308-nm excimer laser: a pilot study. J Cosmet Laser Ther. 2007;9:245–8.
53. Kim MS, Cho EB, Park EJ, Kim KH, Kim KJ. Effect of excimer laser treatment on vitiliginous areas with leukotrichia after confirmation by dermoscopy. Int J Dermatol. 2016;55:886–92.
54. Agrawal K, Agrawal A. Vitiligo: surgical repigmentation of leukotrichia. Dermatol Surg. 1995;21:711–5.
55. Thakur P, Sacchidanand S, Nataraj HV, Savitha AS. A study of hair follicular transplantation as a treatment option for vitiligo. J Cutan Aesthet Surg. 2015;8:211–7.
56. Al Jasser MI, Ghwish B, Al Issa A, Mulekar SV. Repigmentation of vitiligo-associated leukotrichia after autologous, non-cultured melanocyte-keratinocyte transplantation. Int J Dermatol. 2013;52:1383–6.
57. Gan EY, van Geel N, Goh BK. Repigmentation of leucotrichia in vitiligo with noncultured cellular grafting. Br J Dermatol. 2012;166:196–9.
58. Chatterjee M, Neema S, Vasudevan B, Dabbas D. Eyelash transplantation for the treatment of vitiligo associated eyelash leucotrichia. J Cutan Aesthet Surg. 2016;9:97–100.

Extra-Cutaneous Melanocytes

10

Tag S. Anbar, Rehab A. Hegazy, and Suzan Shalaby

Contents

Abstract

Besides their being in the skin, melanin and melanocytes are detected in the eye, stria vascularis of the cochlea in the ear, leptomeninges, substantia nigra, and locus coeruleus of the brain, heart, and lungs. In vitiligo, although melanocyte loss has been recognized as being mainly restricted to the skin, extra-cutaneous melanocyte involvement and subsequent extra-cutaneous site alterations have been detected. The knowledge of extra-cutaneous involvement may point toward considering vitiligo as a systemic disorder rather than just a cosmetic problem. Still, no clear guidelines on who deserve screening of their extra-cutaneous melanocyte sites in cases of vitiligo exist, an aim yearning to be fulfilled.

T. S. Anbar (✉)
Department of Dermatology and Andrology, Faculty of Medicine, Al Minya University, Al Minya, Egypt

R. A. Hegazy · S. Shalaby
Department of Dermatology, Faculty of Medicine, Cairo University, Cairo, Egypt

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_10

Key Points

- The extra-cutaneous melanocytes are getting more under the spotlight, with the ocular, otic, and cephalic melanocytes being still on the top list.
- Their entanglement in vitiligo acquired more proof over the years and may point toward considering vitiligo as a systemic disorder rather than just a cosmetic problem.
- Clear guidelines on who deserve screening of their extra-cutaneous melanocyte sites in cases of vitiligo need to be established.

10.1 Introduction

Even though melanocyte function is well established for pigmentation and photoprotection in cutaneous location, the existence and functions of pigment cells in extra-cutaneous sites should not be overlooked. Besides their being in the skin, melanin and melanocytes are detected in the eye [1], stria vascularis of the cochlea in the ear [2], leptomeninges [3], substantia nigra, and locus coeruleus of the brain [4], heart [5, 6], and lungs [7], and there is evidence that they operate even in unsuspected territories such as adipose tissue [8, 9] (Fig. 10.1). In such sites, melanocytes have been mostly recognized for providing physiologic functions important in organ development and maintenance [1].

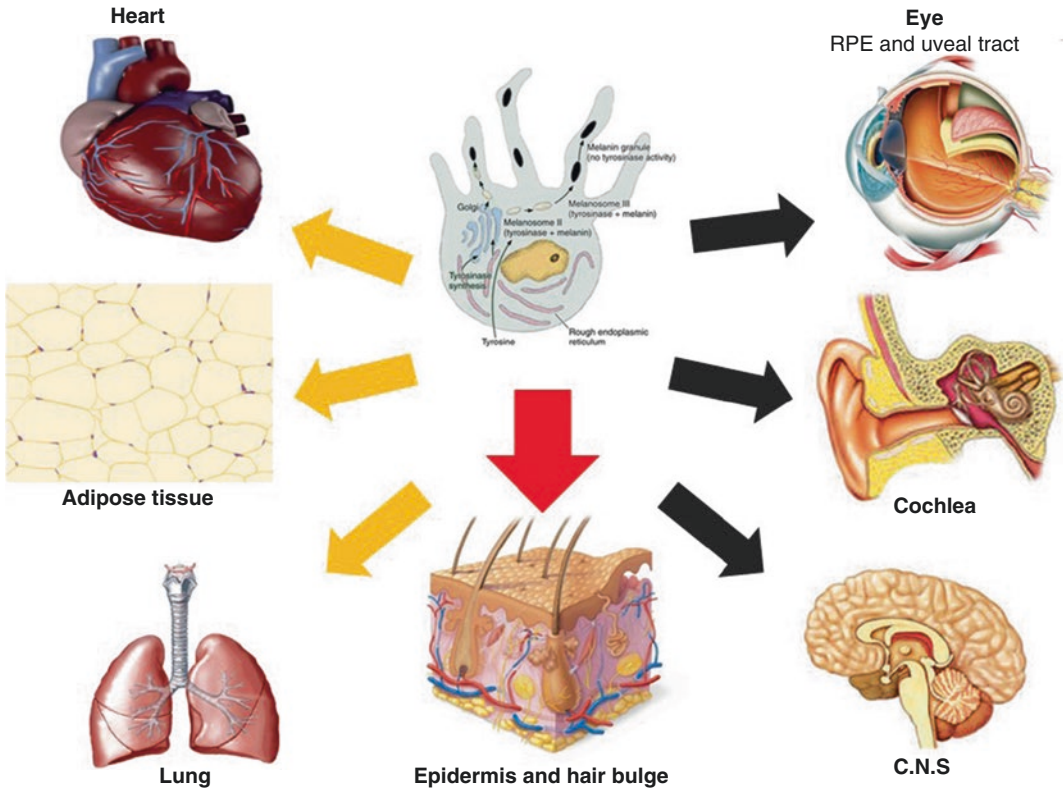


Fig. 10.1 Diagram showing the distribution of melanocytes in various human tissues, including the skin *(the main site)*, eye, ear, CNS *(well established)*, heart, adipose tissue, and lung *(more recently suggested)*

In vitiligo, although melanocyte loss has been recognized as being mainly restricted to the skin, extra-cutaneous melanocyte involvement and subsequent extra-cutaneous site alterations have been detected, particularly in the eyes and ears, resulting in a possible compromise to the function of those sensory organs [10].

10.2 Ocular Melanocytes

The presence of two totally separate populations of pigment-producing cells in one organ is unique to the eye [11]. The first population is present in the uveal tract, while the second exists in the retinal pigment epithelium (RPE) [12] (Fig. 10.2).

10.2.1 The Uveal Tract

The main contingent of the ocular melanocytes resides in the uveal tract [1], which also happens to be the site of most ocular melanomas [13]. The uveal tract is comprised of the choroid, ciliary body, and iris. The latter is responsible for the coloration of the eye which ranges from blue to dark brown, mainly owing to the amount of melanin in the iris melanocytes [14]. Originating from the neural crest, these melanocytes immigrate during embryonic development to the uveal tract.

The microenvironment of uveal melanocytes differs significantly from that of cutaneous melanocytes. The uveal tract is very highly vascularized, and the melanocytes of the choroid have ready access to blood-borne factors. This peculiar microenvironment allows the uveal melanocytes to get in physical contact with several cellular populations, including the fibroblasts that form the sclera and the vascular endothelial cells that form the choriocapillaris, and are typically attached to other melanocytes. This comes in contrast with the cutaneous interfollicular melanocytes that are distributed singly among clusters of keratinocytes [15].

Furthermore, the anterior structures of the eye that overlay the choroidal melanocytes absorb or scatter the majority of UV, visible, and infrared radiation found in sunlight, and the melanocytes of the choroid and ciliary body receive virtually no exposure to UV or visible light. Thus, the potential for direct photodamage is greatest for interfollicular cutaneous melanocytes, followed in decreasing order by follicular cutaneous

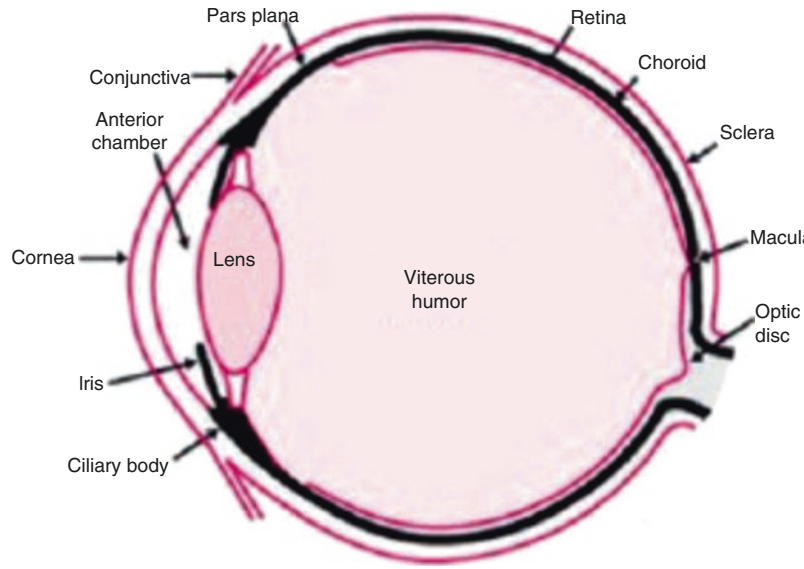


Fig. 10.2 Anatomy of the eye. Ocular melanocytes reside in two totally separate populations, namely, the uveal tract and the retinal pigment epithelium [25]

melanocytes and iris melanocytes [15–17]. Further discrepancies exist in the fact that unlike cutaneous melanocytes which distribute pigment to adjacent keratinocytes, uveal melanocytes retain the melanosomes they produce [18].

Until recently it was believed that melanin synthesis occurred continuously in interfollicular cutaneous melanocytes, cyclically during the anagen phase of the hair cycle in follicular cutaneous melanocytes, but only during prenatal development by uveal melanocytes [19]. However, because optic treatments with the prostaglandin analog latanoprost result in heterochromia and increased pigmentation of the iris, it became apparent that melanin synthesis can be induced in adult eyes [20, 21]. Further evidence that uveal melanocytes retain the potential for melanin synthesis in adults is apparent in the observations that (1) induction of tyrosinase activity by latanoprost in cultured adult human uveal melanocytes is blocked by the tyrosinase inhibitor 1-methyltyrosine, (2) the artificial melanin precursor [3H]-methimazole labels the uveal tract of pigmented adult DBA mice, and (3) tyrosinase activity is evident in the uveal tract of bovine eyes [22–24]. Thus, the potential for melanin synthesis appears to be present in adult ocular melanocytes.

10.2.2 Retinal Pigment Epithelium

Retinal pigment epithelium (RPE) cells are also derived from the neural tube; however, this unicellular layer does not migrate as neural crest cells, but rather develops directly from the neuroectoderm neural tube of the developing forebrain [25]. RPE is the brown monolayer of cells situated between the choroid and the retina proper, composed of cells joined by tight junctions and filled with pigment mainly melanin and lipofuscin. Pigment production in adult RPE cells remains a matter of debate. Some authors have postulated melanin continuous production throughout life; others suggest that it ceases at birth [11].

The RPE plays a critical role in the active phagocytosis of the photoreceptor outer segment membranes[26], which will make it clearer and still serves the meaning of its importance in vision [27]. Furthermore, its melanin—similar to the function of the rest of the ocular melanin—absorbs incident light that enters through the retina and prevents photons from bouncing about and restimulating or overstimulating the rods and cones [25].

10.2.3 Eye Changes in Vitiligo

Ocular pigmentary changes have been reported in patients with vitiligo; in fact, 18–50% of vitiligo patients exhibit focal hypopigmented lesions involving the iris, anterior chamber, and RPE/choroid, particularly the fundus, on ophthalmoscopic evaluation [27–31]. These pigmentary changes are frequently associated with vitiligo patches and macules involving the eyelids, in addition to poliosis of the eyebrows and eyelashes [27, 28]. Ocular findings in vitiligo are, however, not a constant finding [32].

In addition, recent studies demonstrated that patients with vitiligo may have more lenticular and retinal findings than normal [33] and can be more prone to the dry eye syndrome [33, 34]. The uveal tract has been also reported to be affected in vitiligo patients in the form of uveitis [10]. The relation between vitiligo and primary open-angle glaucoma has recently been investigated [35], and despite the small sample size, the authors concluded that the correlation between both diseases is not random. Furthermore, negative ocular electrophysiologic findings were demonstrated in vitiligo patients, a finding that positively correlated with the disease severity and duration [36].

Owing to the growing evidence linking between ocular disorders and vitiligo, a recommendation of complete ophthalmologic screening of patients treated for vitiligo, irrespective of their age, sex, affected area, localization, and duration of the disease, was issued [37].

10.3 Otic Melanocytes

Melanocytes are found in the stria vascularis of the cochlea. The stria vascularis forms the lateral wall of the scala media, which is an endolymph-filled chamber housing the principal sound-detecting component of the inner ear, i.e., the organ of Corti (Fig. 10.3). Otic melanocytes are of neural crest origin [38], and their embryonic development is under the regulation of Kit [39] and Sox 10 [40, 41]. Once established in the ear, otic melanocytes synthesize pigmented melanosomes until congested and then halt melanin synthesis [42].

The melanocytes of the stria vascularis are termed intermediate cells and provide functions that are necessary for normal hearing and endolymph maintenance [43, 44], and their loss results in deafness [2].

The melanin in the otic melanocytes acts as a free radical scavenger and a cation exchanger. Thereby, it has the ability to protect the cochlea by binding to ototoxic drugs, such as cisplatin and aminoglycosides, which may be deleterious to the sensory function of the cochlea [45]. Furthermore, several researches have highlighted the melanin involvement in the maintenance of calcium homeostasis, via calcium ion regulation in the inner ear, which may improve hearing [46–49]. Another interesting function is the role played by the melanin granules produced by the melanocytes of the inner ear in maintaining the body balance [50].

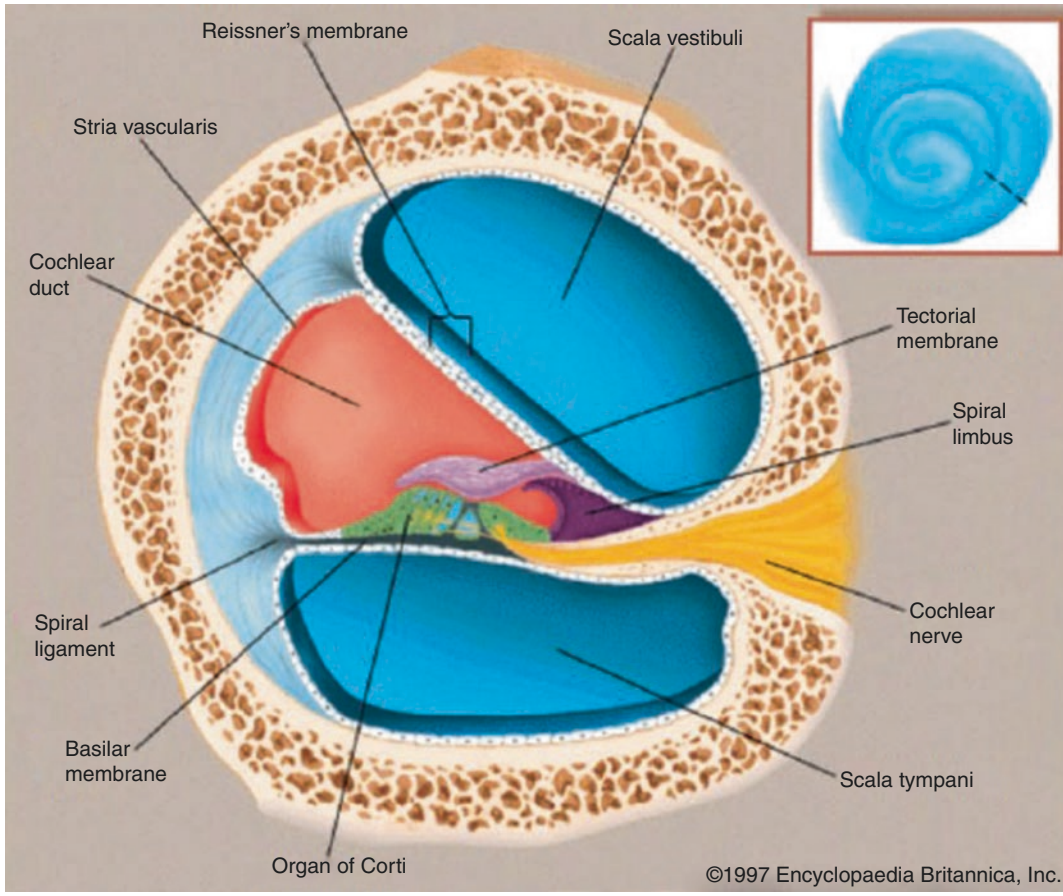


Fig. 10.3 Section of cochlea. Melanocytes are situated in the stria vascularis and modiolus of the cochlea [25]

10.3.1 Ear Changes in Vitiligo

Melanin—as shown above—may play a significant role in the establishment and/or maintenance of the structure and function of the auditory system and may modulate the transduction of the auditory stimuli by the inner ear [45–51]. Thereby, it has been hypothesized that the loss of melanocytes and the subsequent decrease in melanin production, which occurs in vitiligo, could lead to cochlear dysfunction and subsequently to sensorineural hearing loss [2]; this hypothesis was the focus of multiple studies. Although Escalente-Ugalde et al. [52] failed to find a significant association between vitiligo and hearing loss, other studies reported an incidence of 12.5–18.9% sensorineural hearing loss in vitiligo patients as evident in conventional pure tone audiograms [51, 53]. Moreover, vitiligo patients with normal hearing sensitivity showed evidence of subclinical cochlear pathology, evident by absent or abnormal otoacoustic emissions [54–56].

Brain stem auditory response abnormalities have been reported in 13–16% of vitiligo patients [57–59]. Significantly lower pure tone thresholds to high-frequency sounds were detected in vitiligo patients, particularly males, compared to the normal population [60]. Furthermore, a recent study [61] showed bilateral cochlear dysfunction in 60% of vitiligo patients with no significant difference between segmental and non-segmental types. Interestingly, in cases with segmental vitiligo, both ears were significantly affected compared to controls. Those findings opened new horizons toward the vital role of melanocytes and melanin in cochlear functions.

10.4 Cephalic Melanocytes

Cephalic melanocytes originate from the neural crest, and get distributed through the meninges covering the central nervous system (CNS), particularly within the ventrolateral leptomeninges overlying the pons and medulla oblongata [3]. They may have several neuroendocrine functions, through generating prostaglandin D_2 (PGD_2) and β-endorphin (endogenous opioid) [62]. Their involvement in sleep regulation has been suggested based on the facts that PGD_2 is a potent sleep-inducing substance [63], and opioid receptors are located in the nuclei that are active in sleep regulation [50]. Moreover, there are indications that a certain melanocyte-derived factor might be involved in controlling the central chemosensor that generates the respiratory rhythm [50].

The melanin in the cephalic melanocytes is formed from oxidation products of dopamine and cysteinyl-dopamine [64] and occurs only in two forms: neuromelanin and melanosomic melanin [65]. The distribution of these two types of melanin is different. Neuromelanin distribution occurs in the zona compacta of the substantia nigra, locus coeruleus, dorsal motor nucleus of the vagus, tegmentum of the brain stem, and scattered neurons in the roof of the fourth ventricle. Melanosomic melanin is normally found only in the leptomeninges, mostly concentrated in the leptomeninges over the ventral aspect of the medulla oblongata [66].

Melanosomic or true melanin is the same type of melanin as that seen in the skin. However, neuromelanin is chemically different from eumelanin and pheomelanin [67]. The functions of both melanosomic melanin and neuromelanin are not fully understood but have been considered to be involved in the protection of the neurological tissues against metals, such as iron [68], and potentially toxic organic and inorganic cations and free radical species [69, 70], in addition to their well-known role as light-absorbing photoprotectors [1]. A selective loss of dopaminergic neurons containing neuromelanin has been reported to be associated with Parkinson's disease [4].

10.4.1 Cephalic Changes in Vitiligo

It is unknown whether this population of melanocytes is lost in vitiligo. There have been very few anecdotal cases of patients with active vitiligo

developing concurrent severe headaches. It is conceivable that the destructions of the leptomeningeal melanocytes, probably by an accelerated immunologic response, may induce such a presentation [25]. Nevertheless, this finding is in need of further confirmation.

10.5 Other Extra-Cutaneous Melanocyte Populations

Other melanocyte populations attracted less attention, compared to the previously discussed ones. Nevertheless, their existence points to potential melanocyte and melanin functions that remain to be unraveled.

First are the cardiac melanocytes, which are located in the valves and septa [5, 6]. Cardiac melanocytes may originate from the same precursor population as skin melanocytes as they depend on the same signaling molecules known to be required for proper skin melanocyte development such as endothelin 3 [5], but their function in this location in humans so far remains obscure. They have been postulated to be involved in anti-inflammatory functions and in scavenging reactive oxygen species (ROS) [9].

There are no melanocytes recognized in the lungs; however melanin is seen in lymphangioleiomyomatosis (LAM), a rare disease in the lungs, in which muscle cells revert toward their developmental origins and express some melanocyte markers, such as tyrosinase, Pmel17, etc. The resulting production and accumulation of melanin in lung tissues are eventually lethal [7], exposing a dark side for melanin.

Melanin biosynthesis also takes place in the visceral adipose tissue of morbidly obese humans [8]. Hypothetically, the ectopic synthesis of melanin in the cytosol of obese adipocytes may serve as a compensatory mechanism to act as an anti-inflammatory factor and to reduce oxidative damage. During increases of cellular fat deposition, adipocytes become more exposed to endogenous apoptotic signals, especially reactive oxygen species, which could be counteracted by ectopically produced melanin. In addition, adipocytic melanin has been also suggested to suppress the secretion of proinflammatory molecules [8].

10.6 Extra-Cutaneous Melanocytes in Pigmentation-Related Syndromes

Hereditary pigmentary disorders, in addition to involving the skin, are associated with ocular pigmentary abnormalities, such as iris heterochromia or pigmentary changes in the fundus. However, ocular motility defects such as strabismus and nystagmus are more commonly encountered, pointing to a possible concomitant neurologic developmental defect. Meanwhile in albinos, the lack of melanin in the pigment epithelium during development is believed to be directly responsible for both the neurologic abnormality in the visual pathway and foveal hypoplasia [37].

Vogt-Koyanagi-Harada (VKH) syndrome, also known as uveo-meningo-encephalitic syndrome, is one of the acquired disorders of pigmentation [71] that is characterized by involvement of cutaneous, ocular, otic, and central nervous system melanocytes. Its frequent association with uveitis suggests an inflammatory cause for the cutaneous pigmentary changes [72]. Furthermore, an aberrant T cell-mediated immune response directed against self-antigens present in the melanocytes has been incriminated in the pathogenesis of the disease [73].

Two other rare syndromes exist in which the involvement of the extra-cutaneous melanocytes in pigmentary disorders is evident, namely, Waardenburg syndrome (WS) and Alezzandrini syndrome. WS was first described by an ophthalmologist [74] who described the association between depigmentation, deafness, neurological symptoms, and dysmorphology.

Alezzandrini syndrome, first described by Alezzandrini and Casala in 1959, is characterized by unilateral facial vitiligo and partial poliosis with ipsilateral retinal detachment that may be combined with hypoacusis [75].

10.7 Conclusion

By all means are the cutaneous melanocytes the most studied among their family members. However, the extra-cutaneous ones are getting more under the spotlight, with the ocular, otic, and cephalic melanocytes being still on the top list (Table 10.1). Their involvement in disorders that may involve pigmentation, sensory functions, autoimmunity, or malignancy warranted this attention. Their entanglement in vitiligo acquired more proof over the years and may point toward considering vitiligo as a systemic disorder rather than just a cosmetic problem. Still, no clear guidelines on who deserve screening of their extra-cutaneous melanocyte sites in cases of vitiligo exist, an aim yearning to be fulfilled.

Table 10.1 Extra-cutaneous melanocytes: types, differences, and changes in cases of vitiligo

Extra-cutaneous MC	Ocular		Otic	Cephalic
Site of melanocytes	– Uveal tract	– Retinal pigmented epithelium (RPE)	– Cochlea: stria vascularis (inner ear)	– Meninges overlying CNS
Melanocyte origin	– Neural crest Immigrate during embryonic development [1]	– Neural tube (Directly from the neuroectoderm of the developing forebrain)	– Neural crest under regulation of Kit and Sox 10 [38, 39]	– Neural crest [3]
Melanin synthesis	– Synthesis during prenatal life only [19] – Recent evidence show it might be inducible in adult life [20, 21]	– Debatable whether continuous synthesis of melanin throughout life or it ceases at birth	– Once established, melanocytes of cochlea start synthesis of melanosomes till congestion then stop [42]	– Neuromelanin first appears in 2–3-year-old human brains histologically and accumulates with aging [27]
Function of MC	– Color of the eye according to iris melanocytes [14]	– Phagocytosis – Retinoid metabolism – Phototransduction	– Hearing sensation [2] – Endolymph maintenance [2] – Free radical scavenger, protect cochlea against harmful drugs such as aminoglycosides [45] – Body balance mechanisms [50]	– Neuroendocrine function: PGD_2, β-endorphins [62] – Sleep regulation [50] – Protection of neural tissue against toxins [68]
Unique characters	– Two separate populations of melanocytes in the same organ [12] – Melanocytes are closely attached to each other unlike cutaneous melanocytes [1] – Melanocytes retain their melanosomes, no distribution to adjacent cells [18]		Melanocytes of cochlea are known as the intermediate cells [43, 44] and are scattered between the marginal cells (epithelial in origin) and basal cells (mesodermal in origin)	– Two types of melanin: 1. Neuromelanin 2. Melanosomic (only in leptomeninges) – Only melanosomic melanin similar to cutaneous melanin [65]
Changes in vitiligo	– Focal hypopigmented lesions in the iris, anterior chamber, and RPE/choroid [27–31] – Eyelid vitiligo lesions [30] – Poliosis (eyebrows and eyelashes) [27, 28], dry eye syndrome [33, 34] – Uveitis [10] – Negative ocular electrophysiologic findings correlating with disease severity and duration [36]		– Cochlear dysfunctions leading to sensorineural hearing loss [2, 51] – Subclinical cochlear pathology [54–56] – Absent or abnormal otoacoustic emission [54–56]	– Headaches in vitiligo patients due to immune response against leptomeningeal melanocytes [25]

References

1. Tolleson WH. Human melanocyte biology, toxicology, and pathology. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2005;23(2):105–61.. Review.
2. Tachibana M. Sound needs sound melanocytes to be heard. Pigment Cell Res. 1999;12:344–54.
3. Goldgeier MH, Klein LE, Klein-Angerer S, et al. The distribution of melanocytes in the leptomeninges of the human brain. J Invest Dermatol. 1984;82(3):235–8.
4. Zecca L, Tampellini D, Gatti A, et al. The neuromelanin of human substantia nigra and its interaction with metals. J Neural Transm. 2002;109:663–72.
5. Brito FC, Kos L. Timeline and distribution of melanocyte precursors in the mouse heart. Pigment Cell Melanoma Res. 2008;21:464–70.
6. Yajima I, Larue L. The location of heart melanocytes is specified and the level of pigmentation in the heart may correlate with coat color. Pigment Cell Melanoma Res. 2008;21:471–6.
7. Ferrans VJ, Yu ZX, Nelson WK, et al. Lymphangioleiomyomatosis (LAM). a review of clinical and morphological features. J Nippon Med Sch. 2000;67:311–29.
8. Randhawa M, Huff T, Valencia JC, et al. Evidence for the ectopic synthesis of melanin in human adipose tissue. FASEB J. 2009;23:835.
9. Brenner M, Vincent J. Hearing. "What are melanocytes really doing all day long…? : from the viewpoint of a keratinocyte: melanocytes – cells with a secret identity and incomparable abilities.". Exp Dermatol. 2009;18(9):799–819.
10. Mills MD, Albert DM. Ocular and otic findings in vitiligo. In: Hann SK, Nordlund JJ, editors. Vitiligo: a monograph on the basic and clinical sciences. Oxford: Blackwell Science; 2000.
11. Smith-Thomas L, Richardson P, Thody AJ. Human ocular melanocytes and retinal pigment epithelial cells differ in their melanogenic properties in vivo and in vitro. Curr Eye Res. 1996;15(11):1079–91.
12. Bulbul B, Baykara M, Ercan I, et al. Vitiligo and ocular findings: a study on possible. Assoc J Eur Acad Dermatol Venereol. 2006;20:829–33.
13. Boissy RE, Hornyak TJ. Extracutaneous melanocytes. In: Nordlund JJ, Boissy RE, Hearing VJ, editors. The pigmentary system: physiology and pathophysiology. Oxford: Blackwell Scientific; 2006.
14. Wakamatsu K, Hu DN, McCormick SA, et al. Characterization of melanin in human iridal and choroidal melanocytes from eyes with various colored irides. Pigment Cell Res. 2008;21:97–105.
15. Roberts JE. Ocular phototoxicity. J Photochem Photobiol B. 2001;64(2–3):136–43.
16. Sliney DH. How light reaches the eye and its components. Int J Toxicol. 2002;21(6):501–9.
17. Glickman RD. Phototoxicity to the retina: mechanisms of damage. Int J Toxicol. 2002;21(6):473–90.
18. Nordlund JJ, Boissy RE, Hearing VJ. The pigmentary system—physiology and pathophysiology. Oxford: Oxford University Press; 1998. p. 1106.
19. Boissy RE. The melanocyte. Its structure, function, and subpopulations in skin, eyes, and hair. Dermatol Clin. 1988;6(2):161–73.
20. Stjernschantz J, Alm A. Latanoprost as a new horizon in the medical management of glaucoma. Curr Opin Opthalmol. 1996;7(2):11–7.
21. Stjernschantz JW, Albert DM, Hu DN, et al. Mechanism and clinical significance of prostaglandin-induced iris pigmentation. Surv Ophthalmol. 2002;47(Suppl. 1):S162–75.
22. Drago F, Marino A, La Manna C. Alpha-methyl-p-tyrosine inhibits latanoprost induced melanogenesis in vitro. Exp Eye Res. 1999;68(1):85–90.
23. Nakazawa M, Tsuchiya M, Hayasaka S, et al. Tyrosinase activity in the uveal tissue of the adult bovine eye. Exp Eye Res. 1985;41(2):249–58.
24. Lindquist NG, Larsson BS, Stjernschantz J, et al. Age-related melanogenesis in the eye of mice, studied by microautoradiography of 3H-methimazole, a specific marker of melanin synthesis. Exp Eye Res. 1998;67(3):259–64.
25. Boissy RE. Non-skin melanocytes in vitiligo. In: Vitiligo. Berlin: Springer; 2010. p. 73–7.
26. Schubert HD. Structure and function of the neural retina. In: Yanoff M, Duker JS, editors. Ophthalmology. 3rd ed. Edinburgh: Mosby Elsevier, Elsevier Inc.; 2009. p. 511–21.
27. Cowan CL, Halder RM, Grimes PE, et al. Ocular disturbances in vitiligo. J Am Acad Dermatol. 1986;15:17–24.. 16 in book.
28. Albert DM, Nordlund JJ, Lerner AB. Ocular abnormalities occurring with vitiligo. Ophthalmology. 1979;86:1145–58.
29. Albert DM, Wagoner MD, Pruett RC, et al. Vitiligo and disorders of the retinal pigment epithelium. Br J Ophthalmol. 1983;67:153–6.
30. Biswas G, Barbhuiya JN, Biswas MC, et al. Clinical pattern of ocular manifestations in vitiligo. J Indian Med Assoc. 2003;101:478–80.
31. Bulbul Baskan E, Baykara M, Ercan I, et al. Vitiligo and ocular findings: a study on possible associations. J Eur Acad Dermatol Venereol. 2006;20:829–33.
32. Ayotunde A, Olakunle G. Ophthalmic assessment in black patients with vitiligo. J Natl Med Assoc. 2005;97:286–7.
33. Karadag R, Esmer O, Karadag AS, et al. Evaluation of ocular findings in patients with vitiligo. Int J Dermatol. 2016;55:351–5.
34. Dogan AS, Atacan D, Durmazlar SPK, et al. (2015) Evaluation of dry eye findings in patients with vitiligo. Pak J Med Sci. 2015;31(3):587–91.
35. Duplancić D, Rogosić V, Puizina-Ivić N, et al. Prognostic value of ophthalmic artery color Doppler sonography for progression to glaucoma in vitiligo patients. Acta Med Croatica. 2013;67(1):47–52.

36. Perossini M, Turio E, Perossini T, et al. Vitiligo: ocular and electrophysiological findings. G Ital Dermatol Venereol. 2010;145(2):141–9.
37. Park S, Albert DM, Bolognia JL. Ocular manifestations of pigmentary disorders. Dermatol Clin. 1992;10(3):609–22.
38. Hilding DA, Ginzberg RD. Pigmentation of the striavascularis. The contribution of neural crest melanocytes. Acta Otolaryngol (Stockh). 1977;84:24–37.
39. Mackenzie MA, Jordan SA, Budd PS, et al. Activation of the receptor tyrosine kinase kit is required for the proliferation of melanoblasts in the mouse embryo. Dev Biol. 1997;192:99–107.
40. Bondurand N, Pingault V, Goerich DE, et al. Interaction among SOX10, PAX3 and MITF, three genes altered in Waardenburg syndrome. Hum Mol Genet. 2000;9:1907–17.
41. Potterf SB, Gurumura M, Dunn KJ, et al. Transcription factor hierarchy in Waardenburg syndrome: regulation of MITF expression by SOX10 and PAX3. Hum Genet. 2000;107:1–6.
42. Cable J, Steel KP. Identification of two types of melanocyte within the striavascularis of the mouse inner ear. Pigment Cell Res. 1991;4:87–101.
43. Sage CL, Marcus DC. Immunolocalization of CIC-K chloride channel in strial marginal cells and vestibular dark cells. Hear Res. 2001;160(1–2):1–9.
44. Marcus DC, Wu T, Wangemann P, et al. KCNJ10 (Kir4.1) potassium channel knockout abolishes endocochlearpotential. Am J Physiol Cell Physiol. 2002;282(2):C403–7.
45. Momiyama J, Hashimoto T, Matsubara A, et al. Leupeptin, a calpain inhibitor, protect inner ear hair cells from aminoglycoside ototoxicity. Tohoku J Exp Med. 2006;209:89–97.
46. Furuta H, Luo L, Hepler K, Ryan AF. Evidence for differential regulation of calcium by outer versus inner hair cells: plasma membrane Ca-ATPase gene expression. Hear Res. 1998;123:w10–26.
47. Seagle B-LL, et al. Melanin photoprotection in the human retinal pigment epithelium and its correlation with light-induced cell apoptosis. Proc Natl Acad Sci U S A. 2005;102:25–30.
48. Nosanchuk JD, Casadevall A. Impact of melanin on microbial virulence and clinical resistance to antimicrobial compounds. Antimicrob Agents Chemother. 2006;11:3519–28.
49. Nosanchuk JD, Casadevall A. The contribution of melanin to microbial pathogenesis. Cell Microbiol. 2003;5:203–23.
50. Takeda K, Takahashi NH, Shibahara S. Neuroendocrine functions of melanocytes: beyond the skin-deep melanin maker. Tohoku J Exp Med. 2007;211:201–21.
51. Aydogan K, Turan OF, Onart S, et al. Audiological abnormalities in patients with vitiligo. Clin Exp Dermatol. 2006;31:110–3.
52. Escalente-Ugalde C, Poblano A, Montes de Oca E, et al. No evidence of hearing loss in patients with vitiligo. Arch Dermatol. 1991;127:1240.
53. Tosti A, Bardazzi F, Tosti G, et al. Audiologic abnormalities in cases of vitiligo. J Am Acad Dermatol. 1987;17:230–3.
54. Angrisani RM, Azevedo MF, Pereira LD, et al. A study on optoacoustic emissions and suppression effects in patients with vitiligo. Rev Bras Otorhinolaringol. 2009;75:111–5.
55. Bassiouny A, Faris S, El-Khousht M. Hearing abnormalities in vitiligo. Egypt J Otolaryngol. 1998;15:51–60.
56. Shalaby M, El-Zarea G, Nasar A. Auditory function in vitiligo patients. Egypt Dermatol Online J. 2006;2:7.
57. Dereymaeker AM, Fryns JP, Ars J, et al. Retinitis pigmentosa, hearing loss and vitiligo: report of two patients. Clin Genet. 1989;35:387–9.
58. Nikiforidis GC, Tsambaos DG, Karamitsos DS, et al. Abnormalities of the auditory brainstem response in vitiligo. Scand Audiol. 1993;22:97–100.
59. Orecchia G, Marelli MA, Fresa D, et al. Audiologic disturbances in vitiligo (letter to the editor). J Am Acad Dermatol. 1989;21:1317–8.
60. Ardic FN, Aktan S, Kara CO, et al. High-frequency hearing and reflex latency in patients with pigment disorder. Am J Otolaryngol. 1998;19:365–9.
61. Anbar TS, El-Badry MM, McGrath JA, et al. Most individuals with either segmental or non-segmental vitiligo display evidence of bilateral cochlear dysfunction. Br J Dermatol. 2015;172(2):406–11.
62. Takeda K, Yokoyama S, Aburatani H, et al. Lipocalin-type prostaglandin D synthase as a melanocyte marker regulated by MITF. Biochem Biophys Res Commun. 2006;339:1098–106.
63. Urade Y, Hayaishi O. Biochemical, structural, genetic, physiological, and pathophysiological features of lipocalin-type prostaglandin D synthase. Biochim Biophys Acta. 2000;1482:259–71.
64. Wakamatsu K, Fujikawa K, Zucca L, et al. The structure of neuromelanin as studied by chemical degrative methods. J Neurochem. 2003;86:1015–23.
65. Hirano A. Neurons and astrocytes. In: Davis RL, Robertson DM, editors. Textbook of neuropathology. 2nd ed. Baltimore, MD: Williams & Wilkins; 1991. p. 15–8.
66. Burger PC, Scheithauer BW, Vogel FS. Surgical pathology of the nervous system and its coverings, vol. 3. New York, NY: Churchill Livingstone; 1999. p. 115–7.
67. Zecca L, Fariello R, Riederer P, et al. The absolute concentration of nigralneuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease. FEBS Lett. 2002;510:216–20.
68. Zucca FA, Giaveri G, Gallorini M, et al. The neuromelanin of human substantia nigra: physiological and pathogenic aspects. Pigment Cell Res. 2004;17:610–7.
69. Rozanowska M, Sarna T, Land EJ, et al. Free radical scavenging properties of melanin interaction of eu- and pheo-melanin models with reducing and oxidizing radicals. Free Radic Biol Med. 1999;26(5–6):518–25.

70. Enochs WS, Petherick P, Bogdanova A, et al. Paramagnetic metal scavenging by melanin: MR imaging. Radiology. 1997;204(2):417–23.
71. Lucena DR, Paula JS, Silva GCM, et al. Síndrome de Vogt-Koyanagi-Harada incompletaassociada a HLA DRB1*01 emcriança de quatroanos de idade: relato de caso. Arq Bras Oftalmol. 2007;70:340–2.
72. Park HY, Perez JM, Laursen R, Hara M, et al. Protein kinase C-beta activates tyrosinase by phosphorylating serine residues in its cytoplasmic domain. J Biol Chem. 2009;274:16470–8.
73. Prignano F, Betts CM, Lotti T. Vogt-Koyanagi-Harada disease and vitiligo: where does the illness begin? J Electron Microsc (Tokyo). 2008;57(1):25–31.
74. Waardenburg PJ. A new syndrome combining developmental anomalies of the eyelids, eyebrows and nose root with pigmentary defects of the iris and head hair and with congenital deafness. Am J Hum Genet. 1951;3:195–253.
75. Andrade A, Pithon M. Alezzandrini syndrome: report of a sixth clinical case. Dermatology. 2011;222:8–9.

Koebner Phenomenon

11

Nanja van Geel and Reinhart Speeckaert

Contents

Abstract

Koebner phenomenon (KP) is a well-known entity in dermatology. It was first described in 1876 by Heinrich Koebner, a German dermatologist, as "the development of lesions at sites of specifically traumatized uninvolved skin of patients with cutaneous diseases". Since then, this isomorphic response has been reported in a several skin diseases, such as vitiligo, lichen planus, and psoriasis. In vitiligo a relation with disease activity has been suggested, as well as its possible negative influence on surgical treament results [1, 2].

Key Points

- Vitiligo, Koebner phenomenon, Triggers, Classification, Friction areas.

11.1 Introduction

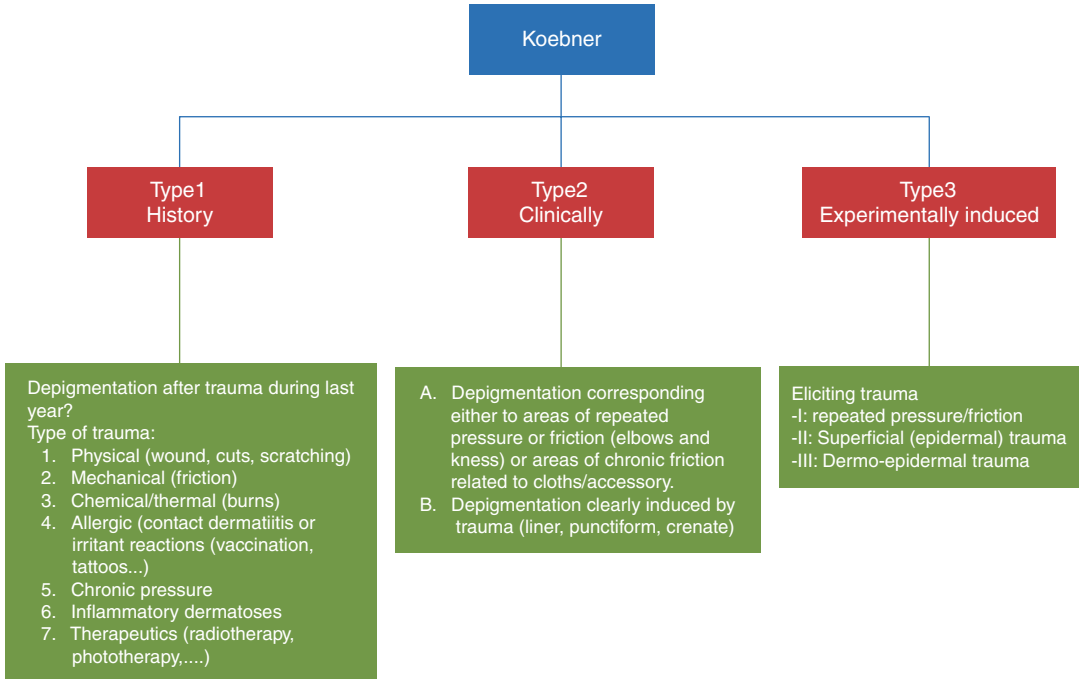
The reported incidence of KP in vitiligo ranges between 21% and 62% [2–4]. This wide range probably reflects different assessment methods to evaluate KP. In the majority of clinical trials, KP is assessed only by medical history, while a physical examination can give additional clinical information. KP is reported to occur more frequently in the non-segmental type of vitiligo than in the segmental form of vitiligo [5].

The Vitiligo European Task Force (VETF) group introduced a new assessment and classification method for the evaluation of KP in vitiligo. This classification includes three different sub types of Koebner's phenomenon: assessment by patient history (type 1), by clinical examination (type 2), and by lesions resulting from experimental induction (type 3) (Table 11.1) [2, 5].

N. van Geel, MD, PhD (✉) · R. Speeckaert
Department of Dermatology, Ghent University Hospital, Ghent, Belgium
e-mail: Nanja.vanGeel@UGent.be

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_11

Table 11.1 Proposed classification by the Vitiligo European Task Force group of Koebner phenomenon [2]



*van Geel N, Speeckaert R, Taieb A et al. Koebner's phenomenon in vitiligo: European position paper. Pigment Cell Melanoma Res 2011; **24**: 564–73. Permission still needed from Pigm Mel Research*

In KP type 2, clinical differentiation can be made between types 2A and 2B. In type 2A, leukoderma is present on any of the following areas corresponding either to areas of repeated pressure or friction (e.g., elbows and knees) or areas of chronic friction related to clothes or accessories (e.g., waistband, hips due to jeans, dorsal site of feet due to shoes, wrist site of a watch, etc.). In type 2B, the presence of linear, punctiform, or crenate depigmentations can be seen and is suggestive of previous injury [2].

With this new classification system, our group assessed the clinical significance of KP with respect to clinical profile, future disease course, and treatment response [5]. The results of this study suggested that KP, when assessed both by history and by clinical examination, can be used as a clinical parameter. Several significant differences were found between patients with and without signs of KP. It was established that the affected body surface area (BSA) was significantly higher when any KP subtype was present and that active disease was more frequently observed in KP1- and KP2B-positive patients. Patients with KP will also be more prone to develop further depigmentation [5].

Furthermore, the response on topical therapy was evaluated. In this observational study patients with any subtype of KP showed significantly less response to treatment than patients without KP. This negative relationship might be due to the external triggering event of KP, maintaining the pathogenesis of vitiligo [2, 5]. In a previous study, Njoo et al. already concluded that experimentally induced KP may function as a clinical indicator for assessing present disease activity and may predict responsiveness to therapy with topical steroids and UVA [6]. Experimentally induced Koebner phenomenon is however an invasive procedure and may worsen further evo-

lution, making it difficult to perform in routine clinical practice [5]. Moreover, our previous study results suggested that KP on friction areas (Type 2A) was associated with an increased prevalence of autoimmune disease [5].

Whether or not the incidence of KP is linked to other factors (e.g. ethnicity, skin phototype) [5]. Several investigators described its negative influence on the outcome of surgical treatments. Surgical treatment are suggested when lesions become therapy resistant and are clinically stable. However, the assessment of disease activity in vitiligo is still a matter of discussion [5, 7]. Attempts have been made to determine the possibility of success by using a mini-grafting test before a surgical intervention. Koebnerization at the donor site during this test is considered to be a warning sign about possible repigmentation failure after a surgical procedure and thus a contraindication for surgical treatment. Some researchers, however, noted depigmentation at the donor site but with appreciable perigraft pigment spread [7].

11.2 Etiopathogenesis

To date, the exact etiopathogenesis of Koebner phenomenon in vitiligo is still unknown. Most clinical trials and experiments on KP have been performed in patients with psoriasis, although some research has been performed in vitiligo. Multiple mechanisms including immunological, neural, and vascular factors leading to depigmentation have been suggested to play a role [2, 5]. Ueki [8] classified the pathophysiology of KP in different skin diseases in two particular steps, the first being the release of several common inflammatory factors (e.g., TNF-α, IL1, IL6, Hsp70, Hsp72, Hsp90, and ICAM-1). This release is triggered by environmental stimuli such as skin trauma. In the second step, a local flare of the skin disease is induced by disease-specific autoantigens [8]. In our European position paper with respect to Koebner phenomenon, we proposed a model of a two-step hypothesis (2) (Fig. 11.1). In this model multiple etiological mechanisms are integrated: cytotoxic melanocyte elimination (possibly enhanced by activated pDCs sensing self-DNA and LL37), increased oxidative stress, defective melanocyte adhesion, and deficiency of melanocyte growth factors [such as stem cell factor (SCF) and basic fibroblast growth factor (bFGF)]. In the first step, common inflammatory signals are released, and in the second step, multiple mechanisms are involved in the specific targeting of melanocytes.

Skin injuries, responsible for KP in vitiligo, may be of any kind. Physical, mechanical, chemical, allergic, or irritant reactions and even therapeutics like radiotherapy or phototherapy can cause this phenomenon. These stimuli are nonspecific and commonly induce inflammatory reactions. Sometimes the distinction between koebnerization and post-inflammatory hypopigmentation can be difficult, but in doubtful cases Wood's light examinationand and long-term follow-up can be helpful [2].

The possibility to actively induce koebnerization on a localized area makes it suitable and interesting for experimental models. In a pilot study, our group described the proof of concept of a new experimental vitiligo induction model, which allows both to investigate the pathophysiology of traumatic-induced depigmentation and to compare adequately the efficacy of treatments in a standardized and reproducible way [9].

11.3 Clinical Presentation

In patients with vitiligo, the lesions due to this Koebner phenomenon are clinically and histologically indistinguishable from vitiligo. These post-traumatic depigmented lesions can be present in different clinical forms. In some cases the depigmented macules are easy to recognize because of their artifactual or elongated linear shape. They correspond exactly to the traumatized areas. In areas of friction (type 2A) these lesions may occur with a border of intermediate pigmentation (trichrome vitiligo) [2].

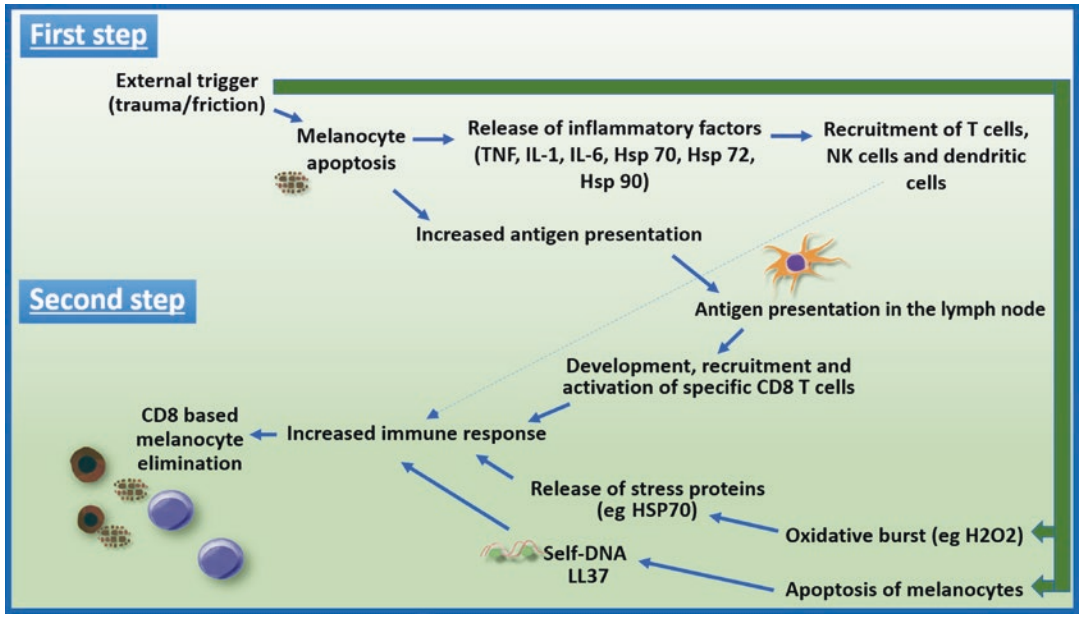


Fig. 11.1 Proposed model of the two-step hypothesis of the Koebner phenomenon in vitiligo (adapted from [2])

In a subgroup of patients, the lesions slightly exceed the dimension of trauma but still preserve the morphology of a Koebner reaction. Other patients state that depigmented lesions occur after trauma, while the depigmented area corresponds to classic vitiligo. It is suggested that the location of vitiligo lesions is related to areas of repeated friction, for example, due to washing, dressing, sports, and other occupational activity (Fig. 11.2). The chronicity of vitiligo can probably be explained by these koebnerization factors [2].

Manifestations of Koebner phenomenon can occur on the classical areas of vitiligo. The typical sites of involvement however seem to be related to the possibility of external injury to the skin. In this way locations including the hands, the lower arms and legs, are often involved. Areas of protected or shock-absorbing skin (e.g., haired scalp, palmar side of hands) are less prone to koebnerization. In vitiligo, some of the areas susceptible to koebnerization are in accordance with the typical distribution of KP observed in other inflammatory dermatoses, such as psoriasis. Other areas of chronic friction, pressure, or repeating movement (e.g., belt, tight underwear, perioral, etc.) are predominantly affected in patients with vitiligo [5].

Koebner phenomenon in patients with vitiligo should be differentiated from post-inflammatory hypopigmentation, a hypopigmentation following cutaneous trauma or inflammation. Post-inflammatory hypopigmentation is a common physiological phenomenon which may be caused by a partial loss of melanocytes or a deficient melanosome production and transfer. Moreover, in contrary to vitiligo, this skin condition is characterized by its temporary appearance. Wood's light examination will show a hypomelanotic skin because melanocytes are not totally absent in the affected area, whereas in vitiligo an amelanotic skin can be seen [2].

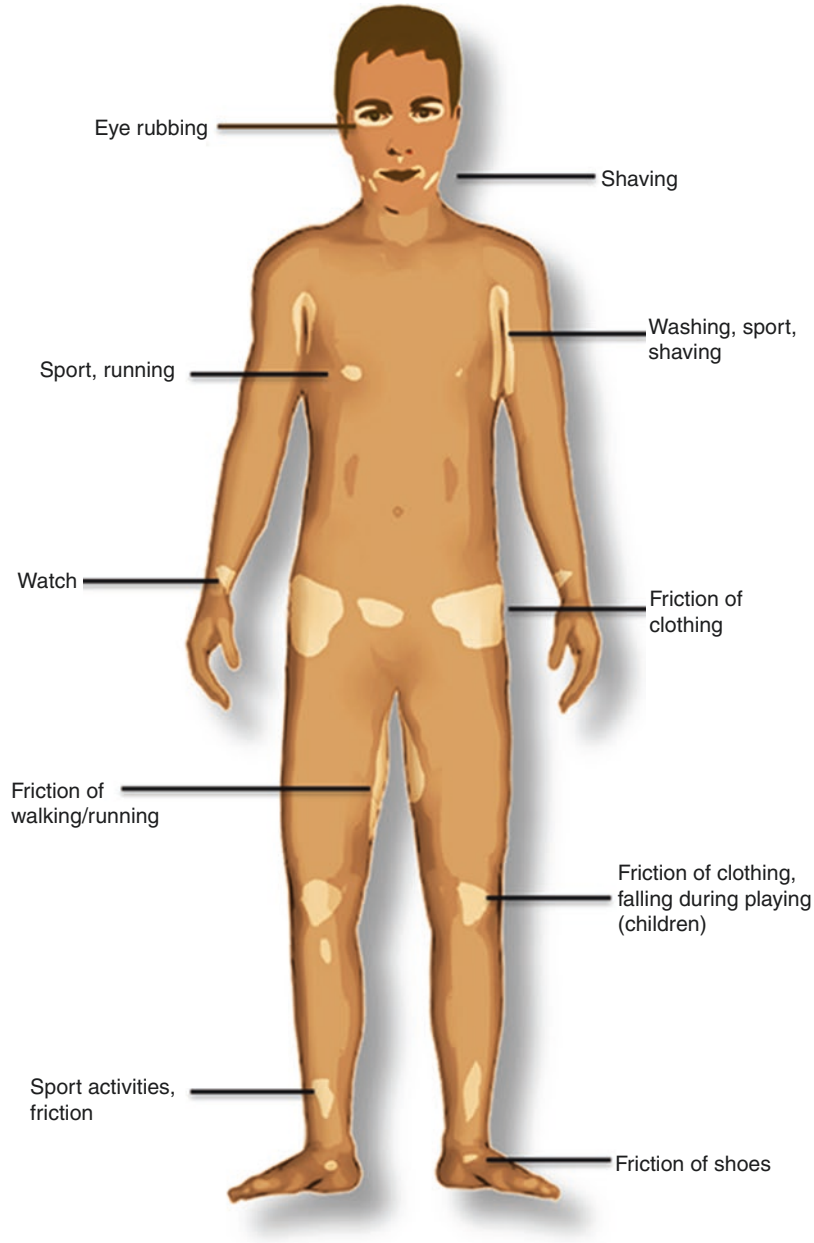


Fig. 11.2 Friction areas on the body

Although Koebner phenomenon in vitiligo has been defined and documented a lot, many unanswered questions still remain. More active research focusing on KP in vitiligo may not only lead to advances in our current knowledge on vitiligo but also help to create novel therapies against this chronic and often psychologically devastating skin disease.

References

1. Sagi L, Trau H. The Koebner phenomenon. Clin Dermatol. 2011;29:231–6.
2. van Geel N, Speeckaert R, Taieb A, et al. Koebner's phenomenon in vitiligo: European position paper. Pigment Cell Melanoma Res. 2011;24:564–73.
3. Mazereeuw-Hautier J, Bezio S, Mahe E, et al. Segmental and non-segmental childhood vitiligo has distinct clinical characteristics: a prospective observational study. J Am Acad Dermatol. 2010;62:945–9.
4. Barona MI, Arrunategui A, Falabella R, et al. An epidemiologic case-control study in a population with vitiligo. J Am Acad Dermatol. 1995;33:621–5.
5. van Geel N, Speeckaert R, De Wolf S, et al. Clinical significance of Koebner phenomenon in vitiligo. Br J Dermatol. 2012;167:1017–24.
6. Njoo MD, Das PK, Bos JD, et al. Association of the Koebner phenomenon with disease activity and therapeutic responsiveness in vitiligo vulgaris. Arch Dermatol. 1999;135:407–13.
7. Sanjeev V, Mulekar MD, Marwan Asaad MD, et al. Koebner phenomenon in vitiligo: not always an indication of surgical failure. Arch Dermatol. 2007;143:799–816.
8. Ueki H. Koebner phenomenon in lupus erythematosus with special consideration of clinical findings. Autoimmun Rev. 2005;4:219–23.
9. van Geel N, Speeckaert R, Mollet I, et al. In vivo vitiligo induction and therapy model: double-blind, randomized clinical trial. Pigment Cell Melanoma Res. 2012;25(1):57–65.

12 Environmental Triggers and Occupational/Contact Vitiligo

Charlotte Vrijman

Content

Abstract

Different factors, including the genetic ones, predispose an individual to developing vitiligo, but a trigger event initiates the actual depigmentation. The triggers are environmental factors that are encountered in everyday life. When the development of vitiligo is influenced by occupational exposures like chemical exposure, frequent physical trauma, or sun exposure, it is called occupational or contact vitiligo. Phenolic/catecholic derivatives are major chemicals known to be associated with vitiligo since they interfere in the melanin synthesis and induce oxidative stress. Other chemicals mentioned as causative agents are nickel, chrome, cobalt, leather, hair dye, cosmetics, and cleaning products, all allergens that also cause allergic contact dermatitis by contact hypersensitivity (CHS). Identification of provoking factors and therefore risk factors for vitiligo are important in preventing disease progression, although they are often not recognised due to unawareness of patients. Therefore, patients should be educated how to avoid these known risk factors.

Key Points

- The triggers for vitiligo are environmental factors that are encountered in everyday life.
- Epidemiological studies on provoking factors of vitiligo show percentages of work-related factors up to 11.5%.
- Phenolic/catecholic derivatives are major chemicals known to be associated with vitiligo. Monobenzone (monobenzyl ether of hydroquinone) is used as bleaching cream.
- 4-TBP is a resin in neoprene adhesives used in glues, rubber, and isolation material.
- Contact hypersensitivity might play a role in the initiation of contact vitiligo.

C. Vrijman (✉)
Department of Dermatology, Ziekenhuisgroep Twente, Hengelo, Netherlands
e-mail: c.vrijman@amc.uva.nl

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_12

Vitiligo is thought to develop in persons with a genetic predisposition that determines melanocyte fragility and susceptibility to precipitating environmental factors. Hence, genetic factors predispose an individual to developing vitiligo, but a trigger event initiates the actual depigmentation. These triggers are environmental factors that are encountered in everyday life. However, in many cases these initiation or aggravating factors have not been identified [1, 2]. In a Dutch cohort study, the majority of the patients with vitiligo did not mention provoking factors, probably because of unawareness of patients [2]. Of the ones who did mention a provoking factor, 25% was work related. Other studies on provoking factors of vitiligo show percentages of work-related factors up to 11.5% [1, 3]. These rates are probably underestimated due to difficulty of detection and also of unawareness of patients. Unfortunately, the epidemiological data of provoking factors, especially on the role of contact with chemicals, is scarce.

When the development of vitiligo is influenced by occupational exposures like chemical exposure, frequent physical trauma, or sun exposure, it is called occupational vitiligo [1, 4]. However, in literature other terms are also used for occupational vitiligo. It is frequently called contact vitiligo, chemically induced vitiligo, or chemical leukoderma which creates some confusion regarding terminology [4, 5]. Besides, the term occupational vitiligo is often used when the disease is initiated after exposure to chemicals that are toxic to melanocytes [1, 4]. The Vitiligo Global Issues Consensus Conference (VGICC) of 2011 concluded that the term contact vitiligo or chemically induced vitiligo requires clearer definition by epidemiological investigations in at-risk populations [6]. For this definition it is important to distinguish chemically induced vitiligo, with spread of leukodermic lesions beyond the original contact site even after cessation of the use of chemicals, from chemical leukoderma which remains confined to the site of contact [2, 4]. Contact vitiligo or chemically induced vitiligo has been suggested as a separate type of vitiligo [6]. However, commonly it behaves and reacts like non-segmental vitiligo and therefore could be seen as a non-segmental vitiligo with a known provoking factor rather than a distinctive type of vitiligo.

Phenolic/catecholic derivatives are major chemicals known to be associated with vitiligo [4, 7, 8]. There is experimental evidence that certain environmental chemicals, like phenolic/catecholic derivatives, are melanocytotoxic and can cause skin depigmentation [4, 9]. Phenolic compounds have structural similarities to tyrosine, the substrate for tyrosinase which plays an important role in the melanin synthesis [10]. These phenolic compounds serve as alternate substrates for the enzyme tyrosinase to be enzymatically converted into cytotoxic quinones and thus interfere in the melanin synthesis and induce oxidative stress [11]. Monobenzone (monobenzyl ether of hydroquinone) is such a phenolic derivate and is used as bleaching cream. Monobenzone can induce vitiligo in susceptible persons by specifically inducing melanocyte-directed autoimmunity [7]. Another phenolic derivate is 4-tertiarybutylphenol (4-TBP) also known as para-tertiarybutylphenol (PTBP). 4-TBP is a resin in neoprene adhesives used in glues, rubber, and isolation materials and often mentioned as a causative agent for vitiligo [1, 4, 12–14]. 4-TBP is known to be melanocytotoxic as it interferes with melanin synthesis as it acts as a competitive inhibitor of tyrosinase-related protein-1 (Tyrp1), a melanocyte-specific enzyme [15, 16]. In addition 4-TBP is able to induce oxidative stress in melanocytes and apoptotic cell death; it increases the immunogenicity of pigmented cells and can trigger melanocyte-specific autoimmunity [9, 15, 17–20]. Therefore, it is very likely that 4-TBP could induce vitiligo in susceptible persons. Other chemicals mentioned as causative agents are nickel, chrome, cobalt, leather, hair dye, cosmetics, and cleaning products, all allergens that also cause allergic contact dermatitis by contact hypersensitivity (CHS) [1, 3, 21]. CHS upon skin contact with haptens is characterized by local infiltration of CD4+ T-cells, followed by dampening of the response by the activation of regulatory T-cells producing IL-10 [22]. Though CD8+ T-cells play an important role in vitiligo, CD4+ T-cells are also found

in vitiligo skin [7, 20]. Hence, CHS might play a role in the initiation of contact vitiligo, although this obviously needs to be further elucidated.

In a Korean study to identify provoking factors of vitiligo especially in the work environment, the face and neck were found to be the most frequent areas exposed to vitiligo-provoking factors at work [1]. This was associated with frequent physical trauma of the face and neck and with people working in construction who are often exposed to sunlight. Hands were the second most commonly exposed area and were associated with frequent exposure to chemicals. Remarkably, wearing protective devices was associated with more vitiligo on hands and face, probably because of friction of these devices or the use of these devices in working environments more at risk for exposure to harmful materials [1]. It should be clear that it is very important to avoid exposure of provoking or aggravating factors. However, in advance it is often not known which individuals are susceptible for vitiligo. For that reason, industries in which potential dangerous chemicals are used should take precautions to protect all their employees from exposure. People themselves should also be aware of the risk of contact with chemicals and especially of contact with phenol and catechol derivatives.

Identifying provoking factors and therefore risk factors for vitiligo is important in preventing disease progression [1]. To date, there is no cure for vitiligo [23]. Despite treatment, in most cases of non-segmental vitiligo, flares lead to a gradual progression of depigmentation during life. Hence, prevention through early detection of risk factors is very important. Patients should be educated how to avoid these risk factors. Since many risk factors of vitiligo are still unclear, more research in this field is necessary.

References

1. Jeon IK, Park CJ, Lee MH, et al. A multicenter collaborative study by the Korean Society of Vitiligo about patients' occupations and the provoking factors of vitiligo. Ann Dermatol. 2014;26:349–56.
2. Vrijman C, Hosseinpour D, Bakker JG, et al. Provoking factors, including chemicals, in Dutch patients with vitiligo. Br J Dermatol. 2013;168:1003–11.
3. Ahn YS, Kim MG. Occupational skin diseases in Korea. J Korean Med Sci. 2010;25:S46–52.
4. Boissy RE, Manga P. On the etiology of contact/occupational vitiligo. Pigment Cell Res. 2004;17:208–14.
5. Ghosh S. Chemical leukoderma: what's new on etiopathological and clinical aspects? Ind J Dermatol. 2010;55:255–8.
6. Ezzedine K, Lim H, Suzuki T, et al. Revised classification/nomenclature of vitiligo and related issues: The Vitiligo Global Issues Consensus Conference. Pigment Cell Melanoma Res. 2012;25(3):E1–E13.
7. van den Boorn JG, Picavet DI, van Swieten PF, et al. Skin-depigmenting agent monobenzone induces potent T-cell autoimmunity toward pigmented cells by tyrosinase haptenation and melanosome autophagy. J Invest Dermatol. 2011;131:1240–51.
8. Toosi S, Orlow SJ, Manga P. Vitiligo-inducing phenols activate the unfolded protein response in melanocytes resulting in upregulation of IL6 and IL8. J Invest Dermatol. 2012;132:2601–9.
9. Yang F, Sarangarajan R, Le Poole IC, et al. The cytotoxicity and apoptosis induced by 4-tertiary butylphenol in human melanocytes are independent of tyrosinase activity. J Invest Dermatol. 2000;114:157–64.
10. Lerner AB. On the etiology of vitiligo and gray hair. Am J Med. 1971;51:141–7.
11. Webb KC, Eby JM, Hariharan V, et al. Enhanced bleaching treatment: opportunities for immune-assisted melanocyte suicide in vitiligo. Exp Dermatol. 2014;23:529–33.
12. Ebner H, Helletzgruber M, Hofer R, et al. Vitiligo from p-tert. butylphenol; a contribution to the problem of the internal manifestations of this occupational disease. Occup Environ Dermat. 1979;27:99–104.
13. Budde J, Stary A. Skin and systemic disease caused by occupational contact with p-tert-butylphenol. Occup Environ Dermat. 1988;36:17–9.
14. Bajaj AK, Gupta SC, Chatterjee AK. Contact depigmentation from free para-tertiary-butylphenol in bindi adhesive. Contact Dermatitis. 1990;22:99–102.
15. Manga P, Sheyn D, Yang F, et al. A role for tyrosinase-related protein 1 in 4-tert-butylphenol-induced toxicity in melanocytes: implications for vitiligo. Am J Pathol. 2006;169:1652–62.
16. Yang F, Boissy RE. Effects of 4-tertiary butylphenol on the tyrosinase activity in human melanocytes. Pigment Cell Res. 1999;12:237–45.
17. Hariharan V, Klarquist J, Reust MJ, et al. Monobenzyl ether of hydroquinone and 4-tertiary butyl phenol activate markedly different physiological responses in melanocytes: relevance to skin depigmentation. J Invest Dermatol. 2010;130:211–20.
18. Kroll TM, Bommiasamy H, Boissy RE, et al. 4-Tertiary butyl phenol exposure sensitizes human melanocytes to dendritic cell-mediated killing: relevance to vitiligo. J Invest Dermatol. 2005;124:798–806.
19. Mosenson JA, Eby JM, Hernandez C, et al. A central role for inducible heat-shock protein 70 in autoimmune vitiligo. Exp Dermatol. 2013;22:566–9.

20. van den Boorn JG, Konijnenberg D, Dellemijn TA, et al. Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. J Invest Dermatol. 2009;129:2220–32.
21. Ghosh S, Mukhopadhyay S. Chemical leucoderma: a clinico-aetiological study of 864 cases in the perspective of a developing country. Br J Dermatol. 2009;160:40–7.
22. Lecart S, Boulay V, Raison-Peyron N, et al. Phenotypic characterization of human CD4+ regulatory T cells obtained from cutaneous dinitrochlorobenzene-induced delayed type hypersensitivity reactions. J Invest Dermatol. 2001;117:318–25.
23. Ezzedine K, Eleftheriadou V, Whitton M, et al. Vitiligo. Lancet. 2015;386:74–84.

13

Vitiligo, Associated Disorders and Comorbidities (Autoimmune-Inflammatory Disorders, Immunodeficiencies, Rare Monogenic Diseases)

Julien Seneschal, Fanny Morice-Picard, and Alain Taïeb

Contents

Abstract

Vitiligo involves complex combinatorial factors, namely genetic predisposition, environmental triggers, metabolic abnormalities, and altered inflammatory and immune responses. The autoimmune and inflammatory theory is the leading hypothesis, and most vitiligo susceptibility loci identified studies encode immunomodulatory proteins. Therefore, vitiligo could be associated with other chronic autoimmune/inflammatory diseases, but also could occur in patients with inherited or acquired immunodeficiencies or patients with monogenic disorders that could impact immune response.

J. Seneschal (✉) · F. Morice-Picard · A. Taïeb
Department of Dermatology and Pediatric Dermatology, National Reference Center for Rare Skin Diseases, Saint-André Hospital, University of Bordeaux, Bordeaux, France
e-mail: julien.seneschal@chu-bordeaux.fr; alain.taieb@chu-bordeaux.fr

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_13

Key Points

- Vitiligo pathogenesis involves complex combinatorial mechanisms with prominent autoimmune component.
- Vitiligo is associated with other autoimmune/inflammatory chronic disorders such as thyroid disorders.
- Inherited or acquired immunodeficiencies are often associated with autoimmune disorders and vitiligo.
- Monogenic disorders including organ-specific autoimmune, mitochondrial, and breakage disorders can be associated with vitiligo suggesting potential mechanisms involved in vitiligo.

13.1 Introduction

Factors involved in the initiation of vitiligo remain still largely unknown, despite recent progresses. However, the most common hypothesis is that vitiligo is an autoimmune disorder, with involvement of both innate and adaptive immunities. Genome-wide associations (GWAS) have shown that 90% of vitiligo susceptibility loci encode components of the immune system, and some of them are shared with other autoimmune/inflammatory conditions [1, 2].

A contrario, a break in immune competence associated with inherited or acquired immunodeficiencies can lead to autoimmunity and vitiligo. HIV infection, the most common cause of acquired immunodeficiency, can be associated with vitiligo at onset or during the reconstitution of the immune system after antiviral therapies [3]. Among primary immunodeficiencies common variable immunodeficiency (CVID), which is associated with longer survival, could be associated with other autoimmune conditions and vitiligo.

Besides the association of vitiligo with inherited immunodeficiencies, monogenic disease involving organ-specific autoimmune, mitochondrial, and breakage disorders can present clinical symptoms of vitiligo. One interesting point is that some of them can be linked to autoimmunity. For example, patients with breakage disorders such as ataxia-telangiectasia and the Nijmegen breakage syndrome present immunodeficiency due to reduced immunoglobulin production and T-cell anomalies and symptoms of autoimmune disorders as vitiligo.

13.2 Common Autoinflammatory/Autoimmune Diseases Associated with Vitiligo

Vitiligo involves complex combinatorial pathogenic effects leading to the loss of melanocytes. Results of three large genome-wide association studies (GWAS, two in Caucasians and one in Chinese) show that vitiligo is involving numerous different susceptibility genes and that the great majority of these genes encode proteins associated with the immune system supporting the hypothesis of a deregulated immune response in vitiligo [4–10]. Many vitiligo susceptibility genes are shared with other autoimmune diseases, usually with the same high-risk alleles. For example, susceptibility loci HLA classes I and II, *PTPN22*, *CTLA4*, and *IL2RA* are also shared by type 1 diabetes, thyroid diseases, rheumatoid arthritis, alopecia areata, systemic lupus disease, and inflammatory bowel disease. Other susceptibility loci identified in vitiligo have also been identified in coeliac disease, psoriasis, or Addison's disease. Moreover other susceptibility genes such as vitamin D receptor or TSLP [11, 12], found to be associated with atopic diseases, have also been associated with vitiligo.

Recent epidemiological studies have been conducted to identify comorbid autoinflammatory/autoimmune diseases associated with vitiligo (Table 13.1). One was conducted in the US population [18] and analyzed results from a 10-year follow-up. This study showed that nearly 20% of patients with vitiligo have at least one comorbid autoimmune disease. The most commonly associated diseases were autoimmune thyroiditis (Hashimoto's thyroiditis or Graves' diseases) and alopecia areata. 12.3% of their

Table 13.1 Frequencies of autoimmune/inflammatory disorders associated with vitiligo

Comorbid autoimmune disorders (%)	Alkhateeb et al. [13] $n = 2078$	Liu J.B et al. [14] $n = 3742$	Sheth VM et al. [15] $n = 2441$ ns	Silverberg JI et al. [16] $n = 2273$ ns	Chen YT et al. [17] $n = 14{,}883$	Gill L et al. [18] $n = 1098$
Thyroid disease	17[a]	1.36[a]	11.8	23.8	1.56[a]	12.3[a]
Alopecia areata	1.1	0.32[a]	2.4	2.7	1.81[a]	3.8[a]
Psoriasis	1.0	0.11	7.6	2.6	2.75[a]	2.2
Inflammatory bowel disease	0.67[a]	NR	2.3	1.1	NR	0.7[a]
Coeliac disease	NR	NR	NR	0.8	0	NR
Pernicious anemia	1.78[a]	NR	NR	3.5	0.06	0.5[a]
Rheumatoid arthritis	0.67	0.32[a]	2.9	3.6	0.40	0.5
Systemic lupus disease	0.19[a]	0.05	2.2	0.7	0.28[a]	0.3[a]
Atopic dermatitis	NR	NR	NR	NR	7.98[a]	NR
Sjögren syndrome	0	NR	NR	0.8	0.36[a]	0.2[a]
Myasthenia gravis	0	NR	NR	NR	0.15[a]	0.2[a]
Diabetes mellitus type I	0.48	0.43	0.8	1.2	0.10	0.7

[a]Frequency found significant compared to controls or the frequency in the general population, *NR* None Reported

patients had thyroid disease. This study demonstrated a 15-fold increase in the prevalence of clinical thyroid disease in patients with vitiligo compared to the general population. This observation was associated with an increased percentage of patients with abnormal TSH level (18.6%) and positive antithyroglobulin or antithyroperoxydase antibodies. Another US study based on medical records in Massachusetts also identified thyroid disease as the most commonly associated condition in 11.8% of the patients [15]. These results confirm data previously obtained from a large survey of more than 2000 Caucasian patients with generalized vitiligo from North America and the UK [13] that found thyroid disease in 17% of their patients. However, data from Chinese populations showed different results. A large retrospective survey of 3742 patients with vitiligo in Mainland China [14] could not confirm the association of vitiligo with thyroid disease. Moreover, Cheng et al. investigated comorbid autoimmune disease in vitiligo patients in a Taiwanese population with a follow-up period of 4 years [17]. This study observed that thyroid disease was associated with vitiligo in only 1.56% of the population. More recently, a nationwide population-based study conducted in Korea that includes 73.336 vitiligo patients found a significant increased risk of autoimmune thyroid disorders as Hashimoto's thyroiditis (OR 1.609 (95% CI 1.437–1.802)) and Graves' disease (OR 2.610 (95% CI 2.319–2.938)) in vitiligo patients [19]. However despite this discrepancy between Caucasian and Asian populations, the need to screen patients with vitiligo for thyroid abnormalities remains clinically important.

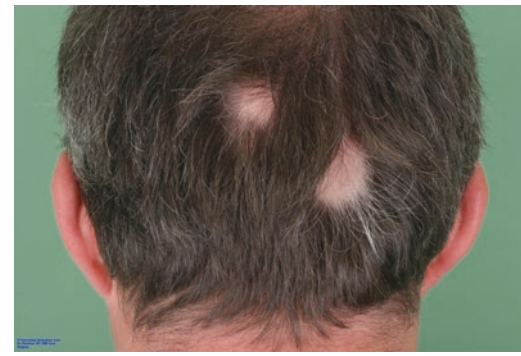


Fig. 13.1 Patches of alopecia areata with regrowing white hairs, which is common in this setting and not related to vitiligo. However, true vitiligo may coexist with alopecia areata

Gill et al. confirmed alopecia areata as another inflammatory disorder associated with vitiligo [18]. They found a prevalence of 3.8% corresponding to a 31-fold increase in the frequency of alopecia areata in patients with vitiligo compared to the general population (Fig. 13.1). A similar but weaker association was found in the

Taiwanese population (1.81%) [17] and in the Chinese population [14]. These results suggest similar pathomechanisms between these two diseases. In line with this hypothesis, we have recently reported a new entity called "follicular vitiligo" in which hair reservoir involvement generally precedes interfollicular involvement. These data suggest that this entity could be the missing link between alopecia areata and vitiligo [20].

Psoriasis, the most common chronic inflammatory skin disorder, has also been associated with vitiligo. Besides numbers of clinical cases published in the literature, reporting the coexistence of psoriasis and vitiligo, the recent Taiwanese observational study made by Chen et al. demonstrated a significant association of this condition with vitiligo [17]. However this association was not confirmed in other epidemiological studies.

Other chronic autoinflammatory or autoimmune diseases could also be linked to vitiligo but remain still in debate and need larger studies to confirm such association: inflammatory bowel diseases, systemic lupus disease, pernicious anemia, rheumatoid arthritis, type 1 diabetes, Guillain-Barré syndrome, morphea, myasthenia gravis, and Sjögren syndrome.

A number of epidemiological studies reported the association of atopic diseases with vitiligo. The largest study made in the US population found higher prevalence of atopic diseases in vitiligo compared with previously established prevalence in the general adult population for atopic dermatitis, asthma, hay fever, or food allergy [16]. Based on a survey including 2645 adults and pediatric patients with vitiligo, 61.8% of the patients reported a history of at least one atopic disease. Moreover atopic disease (including atopic dermatitis) was associated with vitiligo involving large body surface area, suggesting common genetic predisposition as already shown for vitamin D receptor or TSLP [11, 12]. Moreover the pro-inflammatory state of atopic dermatitis may predispose toward melanocyte loss, while scratching can influence the Koebner phenomenon important in the initiation of vitiligo disease. These results are consistent with other studies. A Chinese study of 6516 patients with vitiligo found a higher prevalence of asthma in familial probands [21]. Moreover in the Taiwanese study, 7.8% of the patients with vitiligo reported atopic dermatitis association [4].

Taken together, these studies indicate that vitiligo is epidemiologically associated with other common chronic diseases, such as autoimmune thyroiditis or alopecia areata, suggesting that pathologic variants in specific genes predispose to all these disorders. However, good genetic epidemiology studies are still lacking to understand the major discrepancies noted across populations of various ethnic backgrounds, including adequate control populations to check the validity of reported associations, which may reflect the simple coexistence of two disorders, vitiligo itself being not rare.

13.3 Immunodeficiencies

Combined or T-cell immunodeficiencies, inherited or acquired, are commonly associated with autoimmunity [22]. During frank losses of immunocompetence, autoimmune diseases, which are predominantly CD8 T-cell driven, predominate. Isolated reports of vitiligo associated with cellular or combined immunodeficiencies, especially during HIV infection, suggest a possible link to pathomechanisms involved in vitiligo. However, the initial clinical presentation, which leads to a vitiligoid condition, can be strikingly different from that of vitiligo. For example, Figs. 13.2 and 13.3 show a patient who presented with vitiligoid condition in the context of HIV infection. In this patient, depigmented lesions followed an actinic lichen planus-like condition on sun-exposed areas before spreading to the rest of the body.

Among primary immunodeficiencies, the most severe forms are usually lethal in the first years of life if cell or gene therapy is not available and a vitiligo phenotype is not clearly established in association. Worth of mention are ataxia-telangiectasia and the Nijmegen breakage syndrome which are leading indirectly to reduced immunoglobulin production and T-cell anomalies. Common variable immunodeficiency (CVID) is compatible with longer survival and is the most commonly primary inherited immunodeficiency encountered in clinical practice [23].

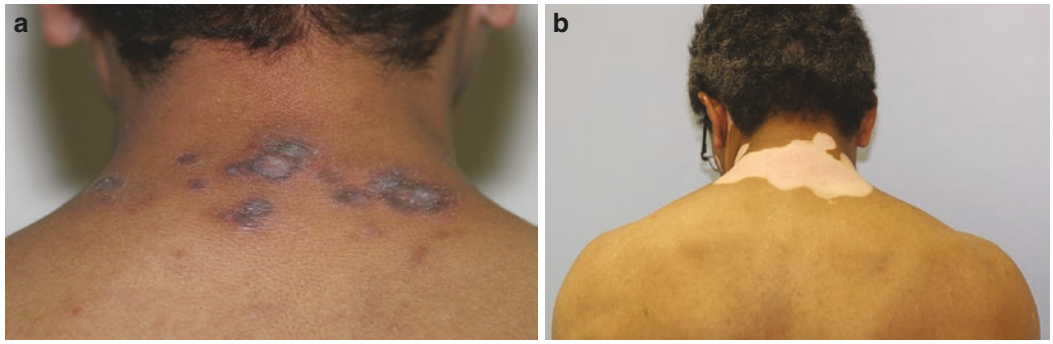


Fig. 13.2 HIV-associated vitiligo. (**a**) lichenoid annular dermatitis evolving into vitiligoid lesions undistinguishable from common vitiligo (**b**)

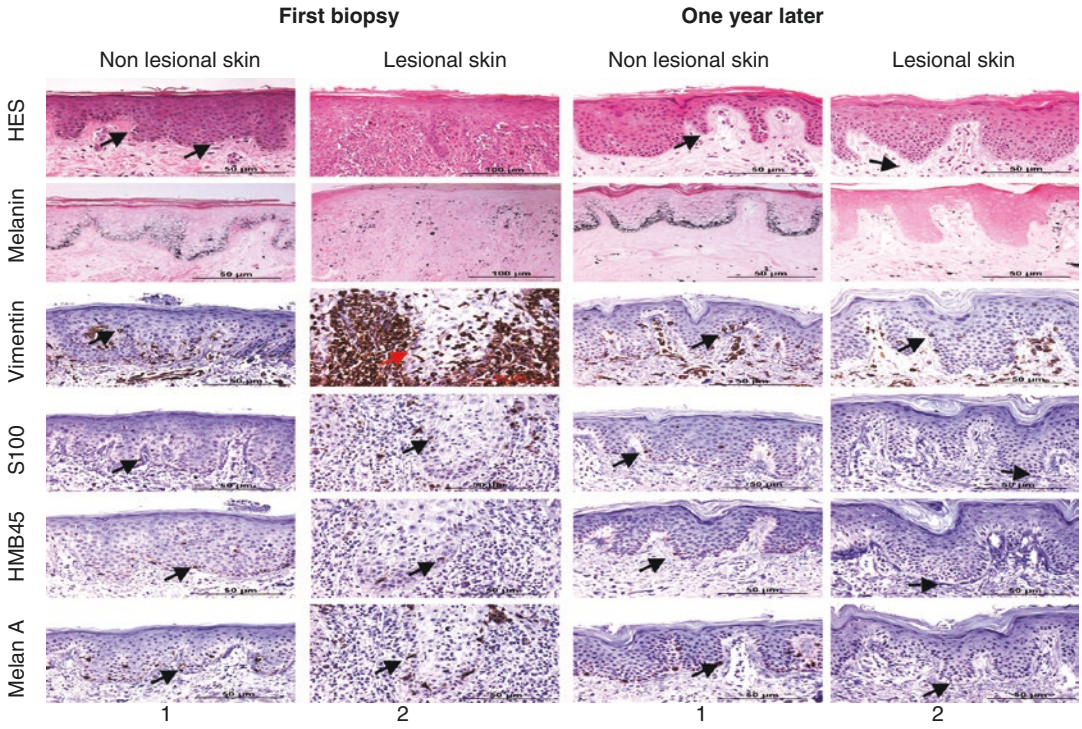


Fig. 13.3 Histopathologic sequence corresponding to the patient depicted in Fig. 13.2. Two biopsies were obtained from lesional and nonlesional skin. The procedure was repeated after a 1-year interval. *First biopsies (correspond to Fig. 13.2a)*: In nonlesional skin (1): melanocytes were present and expressed protein S100, HMB45, Melan A. Melanin was present in basal keratinocytes; in lesional skin (2): perivascular mononuclear infiltrate and lichenoid pattern. Melanocytes were present in the basal layer, and immunostaining for proteins S100, HMB45, Melan A was positive. Pigmentary incontinence was observed, and there was no melanin within keratinocytes. *Second biopsies, 1 year later (correspond to Fig. 13.2b)*: In nonlesional skin (1), no changes; in lesional skin (2), pigmentary incontinence. Melanocytes were still present as shown by vimentin staining although they did not express protein S100, HMB45, Melan A, and there was a loss of function (no melanin in keratinocytes)

Vitiligo could also result from anomalies of the innate immunity, but so far no inherited or acquired condition fitting a well-established innate immunity disturbance has been detected in this context. Oxidative stress dysregulation has been associated with immunosuppression, as in granulomatous disease [24], but also a link to vitiligo is not established.

Besides the commonest cause, HIV infection, other causes of secondary immunodeficiencies which can be associated with vitiligo include chronic undernutrition and other conditions including protein-losing enteropathy, nephrotic syndrome, and hematological malignancies.

13.3.1 Vitiligo, Acquired Immunodeficiency and Idiopathic CD4+ T-Cell Lymphocytopenia

Human immunodeficiency virus (HIV) infection is a common cause of immunodeficiency leading to CD4 T-cell depletion. Clinical consequences associate opportunistic infections and specific manifestations defining the acquired immunodeficiency syndrome (AIDS) stage. HIV infection is also associated with an early immune dysregulation. A few cases of vitiligo associated with HIV infection have been reported. The circumstances of occurrence of vitiligo are however variable if disease history, immune status, and history of exposition to highly active antiretroviral treatment (HAART) are taken into account. Three different situations have been observed, namely, (1) vitiligo revealing immunosuppression, (2) improvement of previous vitiligo during the course of immunodeficiency, and (3) modification of vitiligo presentation with antiretroviral treatment and/or immune restoration with inflammatory signs (described as "punctuate advanced erythematous margins") (Table 13.2).

Concerning vitiligo presenting as a manifestation of HIV infection, all patients reported by Duvic et al. in the 1980s were in an advanced AIDS stage [25]. The CD4 cell count available for three patients was less than 400 cells/mm^3. There was no information on the viral load.

Concerning the improvement of previous vitiligo, spontaneous repigmentation of vitiligo has been reported in an untreated HIV-positive patient. This patient had seroconverted for HIV infection 4 years before [26]. He noticed spontaneous and significant repigmentation of vitiligo lesions of 15-year duration. His CD4 cell count was at 390/mm^3 when repigmentation occurred. Contrary to the previous scenario, pigmentation has occurred following HAART. A first patient had generalized vitiligo onset associated with photosensitivity 2 years after the diagnosis of HIV infection [27]. Two years later, he was commenced on HAART, and the skin began to repigment. At vitiligo onset, the CD4 cell count was at 72/mm^3. When vitiligo began to repigment, the CD4 cell count was at 149. The improvement was attributed to the change in CD4 cell count. Another case reported in 2014 showed the discovery of HIV infection in the context of the development of severe alopecia areata and vitiligo in an 18-year-old man [29]. CD4 T cells count was as low as 9 cells/mm^3 and HIV viremia at 280,000 copies/ml. Interestingly under HAART, a complete response of the alopecia areata was observed as well as progressive repigmentation of the previously depigmented macules, concomi-

Table 13.2 HIV infection and vitiligo

Patient	Reference, year	Delay from HIV infection diagnosis to vitiligo onset	Vitiligo course
Patient 1	Duvic M et al. [25]	3 years	Repigmentation following ribavirin
Patient 2	Duvic M et al. [25]	Simultaneous	Unknown
Patient 3	Duvic M et al. [25]	14 months	No improvement following ribavirin
Patient 4	Duvic M et al. [25]	7 months	Unknown
Patient 5	Duvic M et al. [25]	Vitiligo preceded HIV infection for 14 years	Unknown
Patient 6	Grandhe NP et al. [26]	Vitiligo preceded HIV infection for 11 years	Spontaneous repigmentation 2 years following HIV infection diagnosis
Patient 7	Antony FC et al. [27]	1 year	Repigmentation following HAART
Patient 8	Niamba P et al. [28]	Simultaneous	Repigmentation following HAART
Patient 9	Nikolic DS et al. [29]	Simultaneous	Repigmentation following HAART
Patient 9	Personal data	Simultaneous	No repigmentation following HAART
Patient 10	Personal data	Simultaneous	No repigmentation following HAART

tantly with the control of HIV viremia and restitution of the immune system. The role of the immune reconstitution inflammatory syndrome (IRIS) was also discussed in this context.

The relation of vitiligo to IRIS which may occur in HIV-infected patients initiating antiretroviral therapy is intriguing. The mechanisms of these reactions have not been studied in detail but likely reflect an even broader spectrum of immunologic events that differ on the basis of not only the underlying antigenic trigger but also the specific nature of the immunosuppressive state. It may involve uncoupling of innate and acquired immune responses, restoration of exuberant pathogen-specific cellular responses, and defective or delayed regulatory responses. An excess of pro-inflammatory cytokines has also been associated with IRIS. It is probable that the presence of high self or nonself antigen in IRIS drives pro-inflammatory cytokine responses directly through stimulation of innate immune responses and indirectly when adaptive immunity recovers, leading to autoimmune response or excessive inflammatory responses to pathogens [3].

A patient with a long history of vitiligo associated with idiopathic CD4+ T-cell lymphocytopenia (ICTL) has been reported. ICTL is defined as a persistent depletion of peripheral blood CD4 cell count of less than 300 cells/mm^3 in the absence of either HIV infection or other known causes of immunodeficiency. ICTL has a variable clinical spectrum that ranges from patients exhibiting minimal symptoms to those who have died from opportunistic infections. In the Yamauchi et al. case report, in addition to the depletion of CD4+ T lymphocytes, the patient presented a depletion of CD8+ T lymphocytes. Vitiligo associated with ICTL could be tentatively explained as a shift in the balance of self-tolerance versus autoimmunity [30].

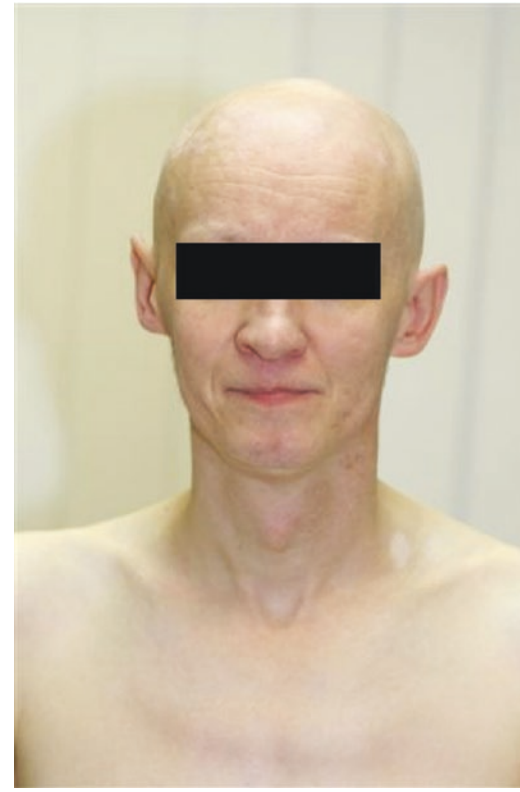


Fig. 13.4 CVID-associated vitiligo in a 34-year-old man. Lesions began at age 1 and expanded progressively over more than 30% of the body surface. Other clinical manifestations included Hashimoto's thyroiditis, alopecia areata, and recurrent nasopharyngeal and pulmonary infections

13.3.2 CVID

Common variable immunodeficiency (CVID) is the most common primary immunodeficiency. Among patients with CVID, up to 25% present with autoimmune events. The clinical phenotype of the condition is broad and heterogeneous. The autoimmune diseases associated include autoimmune thrombocytopenic purpura and autoimmune hemolytic anemia (frequently) and less commonly rheumatoid arthritis, vasculitis, and vitiligo. These autoimmune manifestations have suggested a genetic dysregulation provoking autoreactivity during B-cell development, with the production of multiple autoantibodies against various antigenic targets [31]. CVID can be caused by mutation in the TNFRSF13B gene located on chromosome 17, which encodes the transmembrane activator and CAML interactor (TACI). Figure 13.4 shows a patient with a very early onset of vitiligo (before the age of 1) that was further diagnosed with CVID and various autoimmune disorders including alopecia areata and Hashimoto's thyroiditis.

13.3.3 Complement Deficiencies

The arguments for a role of the complement cascade are mostly based on in vitro studies and theoretical speculations. However, heterozygous C4 deficiency has been linked to an increased risk for vitiligo [32]. Complement-activating anti-melanocyte antibodies have been implicated in vitiligo pathogenesis, with complement components directly involved in cell killing. Along this line, it has been speculated a role in vitiligo pathogenesis for a plasmatic protein called mannose-binding lectin (MBL). This protein recognizes mannose terminal residues of microbial glycoproteins or glycolipids and then activates the classic complement pathway without antibodies, through an associated serine protease [33].

13.3.4 Concluding Remarks

The link between immunodeficiencies and vitiligo remains poorly documented and understood. T-cell deficiencies and combined deficiencies such as CVID can be associated with vitiligo. The role of pure B-cell deficiencies and of innate immunity deficiencies is not as well established. From the clinical standpoint, it is important to consider—especially in the case of widespread vitiligo following a clinically inflammatory phase—an underlying T-cell immunosuppression and that triggered by HIV infection should be investigated in priority (Figs. 13.2 and 13.3).

13.4 Rare Inherited Diseases

13.4.1 The Interest of Studying Monogenic Disorders for the Understanding of Common NSV

The candidate gene approach has not been successful for vitiligo. However, the observation of relevant common features in monogenic disorders and complex multigenic ones may offer interesting clues, either for extracting a major genetic component in a complex disorder [34] or for looking at possible common pathogenetic pathways or disease markers. For vitiligo, even though the phenotype is easily recognizable in humans, there are limited human monogenic disease models (Table 13.3). Unfortunately, the "vitiligo" phenotype is usually rarely well documented in genetics publications. We have included only monogenic disorders with a confirmed ascertainment of this phenotype based on personal experience or literature review.

Animal models may be misleading because the human pigmentation system is the result of a very specific evolutional maturation. The discussion around separate human cutaneous melanocyte compartments (hair follicule and interfollicular glabrous skin) which underlies clinical differences between SV and NSV is probably relevant for the analysis of the various vitiligo-related phenotypes in monogenic disorders. Along the same line, the heritability of premature graying genes has been already addressed in some studies [37], but the definitive evidence of a link between the two phenotypes (premature graying and vitiligo) is still missing.

Some clearly distinct monogenic disorders have marked phenotypic overlap with vitiligo, which raise the question of a common pathogenic background. Piebaldism is an autosomal dominant disorder of melanocyte development characterized by ventral leucoderma/trichia due to *KIT* mutations which impair melanocyte precursor migration. Given the importance of its encoded protein c-Kit in the maintenance of viable melanocytes, the hypothesis of a direct genetic link to common vitiligo had to be raised but was excluded in genome-wide scans. An uncommon variant, the Val620Ala (1859T>C) mutation of the *KIT* gene, has been found in so-called progressive piebaldism leading to progressive loss of pigmentation as well as the progressive appearance of hyperpigmented macules also noted in trichrome vitiligo [38]. Another phenotype which is clinically related to vitiligo has been observed in a large Canadian family. Vitiligoid patches occurred at adolescence and progressed toward diffuse depigmentation. This "autosomal dominant vitiligo" was associated with a heterozygous -639G-T transversion identified in a highly conserved area in the promoter region of the *FOXD3*

Table 13.3 Monogenic disorders and NS vitiligo

Name OMIM entry Reference if not detailed in text	Inheritance/population	Gene/protein/antigen	Clinical symptoms/remarks
APS1 (autoimmune polyglandular syndrome, type I) / APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) 240300	Autosomal recessive (most cases) Finns, Iranian Jews, Sardinians	*AIRE* (autoimmune regulator gene)	Vitiligo not mandatory; autoimmunity to NALP5 frequent in case of hypoparathyroidism
APS 2/Schmidt syndrome 269200	Autosomal recessive or autosomal dominant with incomplete penetrance?	Unknown Linked to MHC class I on chromosome 6	No straightforward marker, vitiligo not mandatory
Ataxia-telangiectasia 208900	X-linked recessive	*ATM*/atm Spontaneous chromosomal instability with multiple rearrangements especially chromosomes 7 and 14 Chromosomal hypersensitivity to ionizing radiation and alkylating agents	Skin: vitiligo telangiectasias Granulomas Nonskin Elevated serum alpha fetoprotein
Nijmegen breakage syndrome 251260	Autosomal recessive Eastern European origin	8q21 *NBS 1*/nibrin Spontaneous chromosomal instability with multiple rearrangements, especially chromosomes 7 and 14 Chromosomal hypersensitivity to ionizing radiation and alkylating agents Radioresistant DNA synthesis	Skin: progressive vitiligo, cafe au lait spots, photosensitivity (?) Nonskin: facial dysmorphy, microcephaly++, mental retardation, immunodeficiency, autoimmune disorders, neoplasias
MELAS 540000	Mitochondrial	Genetically heterogeneous: mutations in MTTL1 (most common) and MTTQ, MTTH, MTTK, MTTS1, MTND1, MTND5, MTND6, and MTTS2	Vitiligo associated in 11% of cases studied with common Mt mutation 3243
Vitiligo-spasticity syndrome/spastic paraplegia with pigmentary abnormalities 270750 [35]	Autosomal recessive inbred Arab families	Mapped to 1q24-q32	Skin: vitiligo and lentigines of exposed areas (apparent at birth or in infancy); premature graying of body hair, canitia Nonskin: microcephaly, thin face, micrognathia, retrognathia Severe spastic paraplegia in childhood +++; mild cognitive impairment
Combined immunodeficiency with autoimmunity and spondylometaphyseal dysplasia 607944 [36]	Autosomal recessive or dominant	Unknown	Skin: vitiligo and hyperpigmented macules Nonskin: Spondylometaphyseal dysplasia + combined humoral and cellular immunodeficiency: recurrent infections (pneumonia, sinusitis, fulminant varicella) autoimmune disorders

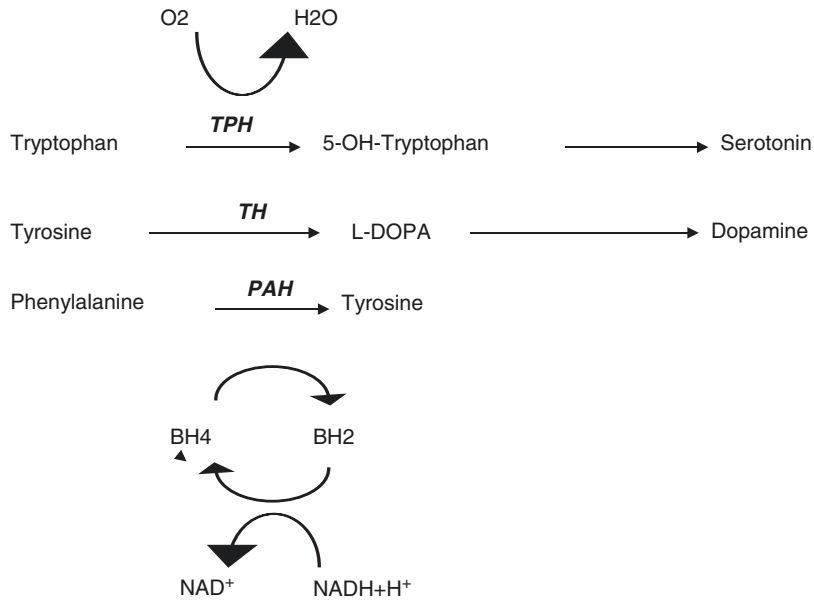


Fig. 13.5 The role of TPH, TH, and PAH in the biosynthesis of serotonin and dopamine. All three enzymes are dependent on tetrahydrobiopterin (BH_4) as a cofactor (NAD = nicotinamide adenine dinucleotide)

gene [39]. Functional expression studies of the gene variant indicated that it increased transcription in neural crest melanoblast precursors, possibly altering the differentiation profile of the melanoblast lineage.

Another interest of monogenic diseases which include organ-specific autoimmunity is the use of the monogenic disease as a model to identify autoantigens of importance in other disorders, such as vitiligo. APECED/APS1 is a relevant example. The gene causing APECED has been named autoimmune regulator (*AIRE*). It is predominantly expressed in some cells of the immune system and is thought to be involved in transcriptional regulation. Autoantibodies seem able to enter in target cells and neutralize enzymatic activities. APECED patients have autoantibodies against several enzymes involved in the biosynthesis of neurotransmitters. Autoantibodies against aromatic L-amino acid decarboxylase (AADC) in APS I patients are associated with the presence of autoimmune hepatitis and vitiligo [40].

TPH catalyzes the hydroxylation of tryptophan into 5-OH tryptophan and is the rate-limiting enzyme in the synthesis of serotonin. TH is the rate-limiting enzyme in the biosynthesis of catecholamines, where it converts tyrosine into L-DOPA. PAH is mainly expressed in the liver, where it catalyzes the conversion of phenylalanine into tyrosine. Mutations in PAH are the most common defect responsible for phenylketonuria, which is characterized by hair and skin pigment dilution. However, against this hypothesis, the vitiligo phenotype is not clearly associated in APECED patients with one subset of antibodies to tetrahydrobiopterin-dependent hydroxylases [41], and these have not been tested in common NSV or its autoimmune subsets. Similar to glutamic acid decarboxylase and AADC which are autoantigens in APECED, as well as enzymes of great importance in the synthesis of GABA, serotonin, and dopamine, it remains possible that the group of tetrahydrobiopterin-dependent hydroxylases has shared immunogenic properties or may play a role in the pathogenesis of vitiligo as in APECED (Fig. 13.5).

13.4.2 Discussion of Some Selected Monogenic Disorders

- Autoimmune Polyendocrine Syndromes

  Autoimmune polyendocrine syndromes (APS), also known as polyglandular autoimmune syndromes (PGA), are associated with an increased incidence of NS vitiligo. APS are

clinically and genetically heterogeneous, but they reflect multiple endocrine gland insufficiency caused by mainly humoral autoimmunity. APS-1 and APS-2 are the two major autoimmune polyendocrine syndromes. The APS-1/APECED gene *AIRE* is on 21q. The gene for Schmidt syndrome or APS-2 is not yet cloned. According to linkage studies, the *AIRE* gene is not associated with vitiligo coexisting with other autoimmune diseases in European-descent families. This does not exclude some common pathophysiological background (see discussion above).

– APS1/APECED

  Autoimmune polyglandular syndrome type I or APECED is characterized by the following major clinical symptoms: Addison's disease, hypoparathyroidism, and chronic mucocutaneous candidiasis. The spectrum of associated minor clinical diseases includes other autoimmune endocrinopathies (hypergonadotropic hypogonadism, insulin-dependent diabetes mellitus, autoimmune thyroid diseases, and pituitary defects), autoimmune or immunomediated gastrointestinal diseases, chronic active hepatitis, skin diseases (vitiligo and alopecia areata), ectodermal dystrophy including enamel and nail defects, keratoconjunctivitis, immunologic defects (cellular and humoral), asplenia, and cholelithiasis. The first manifestations occur commonly in childhood with the three main diseases developing in the first 20 years of life, but other accompanying diseases continue to appear until at least the fifth decade. In a majority of cases, candidiasis is the first clinical manifestation to appear, usually before the age of 5 years, followed by hypoparathyroidism (usually before the age of 10 years), and later by Addison's disease (usually before the age of 15 years). Overall, the three main components of APECED occur in chronologic order, but they are present together in only about one-third to one-half of the cases [42]. Vitiligo is more frequent in the APECED population but had until recently not been analyzed comprehensively to bring specific and useful information in the vitiligo field.

  The analysis of autoantibodies in APS I patients is a useful tool for establishing autoimmune manifestations of the disease as well as providing diagnosis in patients with suspected disease. For hypoparathyroidism, the most common autoimmune endocrinopathy of APECED, autoantibodies specific for NACHT leucine-rich repeat protein-5 (NALP5), has been shown in 49% of patients with APS1 and hypoparathyroidism [43]. NALP5 which is predominantly expressed in the cytoplasm of parathyroid chief cells appears to generate tissue-specific autoantibodies. Its role is under close investigation because of a suspected role of the innate immune system in triggering autoinflammation and autoimmunity [44]. Since NALP1 variants are associated with autoimmune vitiligo [45], this could be relevant to the vitiligo field.

– Schmidt (APS2) Syndrome

  Schmidt syndrome is characterized by the presence of autoimmune Addison's disease in association with either autoimmune thyroid disease or type 1 diabetes mellitus or both [42]. Chronic candidiasis is not present. APS2 may occur at any age and in both sexes but is most common in middle-aged females and is very rare in childhood. Alopecia areata, vitiligo, myasthenia gravis, pernicious anemia, and Graves' disease are frequently associated. Schmidt syndrome can be more specifically associated with interstitial myositis [46]. An association of HLA-B8 with APS2 has been found in three generations of a family [47].

– Immunodysregulation Polyendocrinopathy and Enteropathy X-Linked (IPEX; 304790)

  Interestingly, this more recently described X-linked recessive disease due to mutations in *FOXP3* which block the development of regulatory T cells has not yet been linked to vitiligo but induces multiple immune-related diseases, including TH2 predominant (atopic dermatitis) and

TH1 predominant (alopecia areata, type 1 diabetes) phenotypes. Further observations are necessary to exclude the alteration of this important pathway in the pathogenesis of immune vitiligo.

- Mitochondrial Disorders: MELAS (Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-Like Episodes) and MERRF (Myoclonic Epilepsy Associated with Ragged-Red Fibers) Syndromes

  These disorder belongs to the mitochondrial encephalomyopathies which include Leigh syndrome (LS; 256000), Kearns-Sayre syndrome (KSS; 530000), and Leber optic atrophy (535000) [48]. The most common clinical manifestation in these disorders is encephalomyopathy, because the nerve and muscle cells depend more heavily on aerobic energy production. However, any tissue or organ may be affected.

  The most frequent MELAS symptom is episodic sudden headache with vomiting and convulsions, which is found in 80% of cases in patients aged 5–15 years. Laboratory evidence of myopathy related to abnormal mitochondrial metabolism includes elevated resting serum lactate increased with exercise ragged-red fibers on muscle biopsy and subsarcolemmal pleomorphic mitochondria on electron microscopy [49]. The common mitochondrial causative mutations are mostly found in the muscle mtDNA molecules but can be found in other cell type responsible for the main symptoms. The variability of the clinical phenotype is partly due to mutation heteroplasmy, a condition where the normal genome and the mutant variant coexist in mitochondria [48]. An A to G transition at base pair 3243 in the $tRNA^{Leu(UUR)}$ gene in mtDNA is the most common molecular etiology of the MELAS syndrome, but other mutations have been described (Table 13.3).

  Vitiligo was associated in 11% of cases of MELAS bearing the common bp 3243 point mutation [50]. Interestingly, there is no reported evidence of melanocyte loss in this form of vitiligo but only decreased melanogenesis. Unfortunately, the melanocyte marker used in that study was the S100 protein which stains also Langerhans cells. The mitochondrial mutation was not studied in epidermal or skin cells [50]. In skeletal muscle, the biochemical defect is often segmental [51], suggesting a nonrandom distribution of mutant and wild-type mtDNAs within a muscle cell. A decreased activity of the mitochondrial respiratory chain may lead to a metabolic impairment within the epidermal melanin unit, which is consistent with previous hypotheses concerning the altered redox status in vitiligo [50]. Another possible molecular target is the transport of melanosomes via microtubules, which requires adenosine triphosphate (ATP). This is a possibility since all the mutations in mtDNA affect the respiratory chain and oxidative phosphorylation and may thus lead to a decreased amount of ATP in the cell. Mutations of mtDNA accumulate during normal aging. The most frequent mutation is a deletion, which is increased in photoaged skin. Oxidative stress in turn may play a major role in the generation of large-scale mtDNA deletions. Reactive oxygen species have been shown to be involved in the generation of aging-associated mtDNA lesions in human cells [52].

- Breakage Disorders: Ataxia-Telangiectasia and Nijmegen Breakage Syndrome
  - Ataxia-Telangiectasia (AT)

    Patients present in early childhood with progressive cerebellar ataxia and usually later develop conjunctival telangiectasias, progressive neurologic degeneration, recurrent infections, and mostly lymphoid malignancies. Lymphomas in AT patients tend to be of B-cell origin, whereas the leukemias tend to be of the T-cell type. Oculocutaneous telangiectasias typically develop between 3 and 5 years of age (Fig. 13.6). Vitiligo is a feature already mentioned [53, 54] but not emphasized in the context of major neurological problems.

    Elevated levels of alpha-fetoprotein and cytogenetic analysis may help confirm the diagnosis (7;14 translocations). Ataxia-telangiectasia mutated (ATM), the product

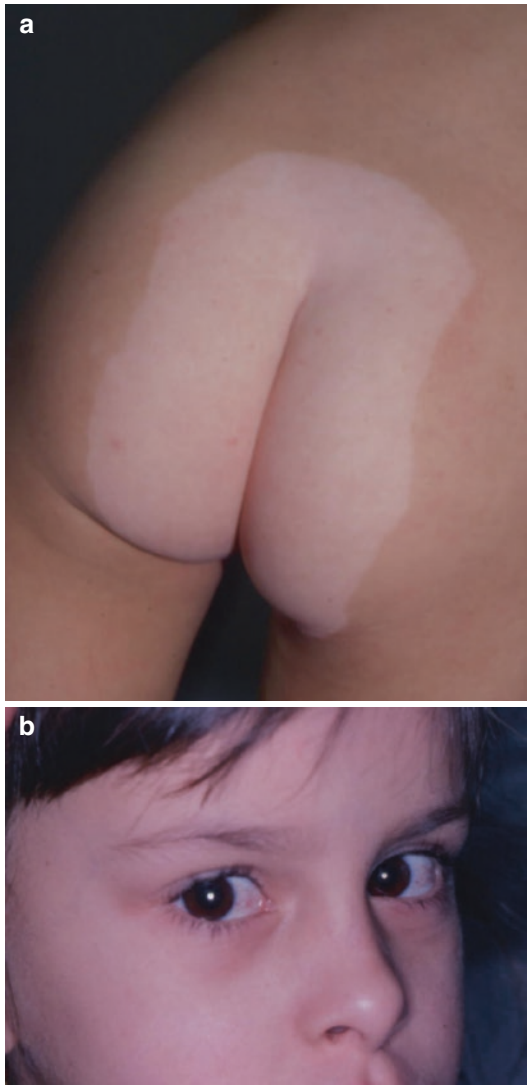


Fig. 13.6 Ataxia-telangiectasia manifested by vitiligo in the napkin area (**a**) and equatorial conjunctival telangiectasias (**b**)

of the mutated gene, is a 370-kDa protein member of the phospatidyl inositol 3-kinases superfamily. Increased recombination is a component of genetic instability in AT and may contribute to the cancer risk.

The phenotypic pleiotropy of AT may result from different tissue expressions of ATM targets. ATM plays an important role in the activation of the tumor suppressor gene product p53. In the skin, this may precipitate the apoptotic commitment of melanocytes in AT-related vitiligo. ATM interactions with beta-adaptin in the cytoplasm might mediate axonal transport and vesicle trafficking in the central nervous system and so account for the neuronal dysfunction and eventual neurodegeneration seen in ataxia-telangiectasia.

The previous pathways may be interesting for the immune and neural theories of vitiligo, but of considerable interest is that the AT phenotype seems to be a consequence, at least in part, of an inability to respond appropriately to oxidative damage {3} and that the absence of the ATM protein affects stem cells. Ionizing radiation oxidizes macromolecules and causes tissue damage through the generation of reactive oxygen species (ROS). When compared to normal human fibroblasts, AT dermal fibroblasts exhibit increased sensitivity to *t*-butyl hydroperoxide toxicity. These cells fail to show G1 to G2 phase checkpoint functions or to induce p53 in response to oxidative challenge. Cells derived from *ATM*-deficient mice, grow poorly in culture, are genetically instable and appear to undergo apoptosis more readily than control cells. In a mouse AT model, hair premature graying has been observed in heterozygous mice after a sublethal dose of ionizing radiation. Furthermore, ATM protein deficiency and telomere dysfunction seem to act together to impair cellular viability and cause adverse effects on stem/progenitor cell reserves.

– Nijmegen Breakage Syndrome (NBS)

This syndrome has been related to AT as a variant until more recent clear clinical and molecular evidence of a distinct etiology {34}. The gene product, nibrin, is member of the hMre11/hRad50 protein complex, suggesting that the gene is involved in DNA double-strand break repair. The immunological, cytogenetic, and cell biological findings in NBS closely resemble those described in AT (see Table 13.3). For the vitiligo phenotype, it is better documented (14/21 cases studied in

the international NBS study group). Telangiectasias are rare and café au lait spots common. Concerning the non-skin clinical aspects, however, NBS is more similar to Bloom syndrome (BS), which features also severe microcephaly with relatively preserved mental development. Unlike AT, neurological features are rare. NBS and BS lack the increased serum alpha-fetoprotein concentrations of AT.

Studies on the gene product suggest that deficiency in nibrin disrupts a common pathway that functions to sense or repair double-stranded DNA breaks. Clues to vitiligo pathogenesis need further specific studies in this disorder.

References

1. Spritz RA. Recent progress in the genetics of generalized vitiligo. J Genet Genomics. 2011;38:271–8.
2. Spritz RA. Six decades of vitiligo genetics: genome-wide studies provide insights into autoimmune pathogenesis. J Invest Dermatol. 2012;132:268–73.
3. Walker NF, Scriven J, Meintjes G, et al. Immune reconstitution inflammatory syndrome in HIV-infected patients. HIV AIDS (Auckl). 2015;7:49–64.
4. Chen JJ, Huang W, Gui JP, et al. A novel linkage to generalized vitiligo on 4q13-q21 identified in a genome-wide linkage analysis of Chinese families. Am J Hum Genet. 2005;76:1057–65.
5. Jin Y, Birlea SA, Fain PR, et al. Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. Nat Genet. 2012;44:676–80.
6. Jin Y, Birlea SA, Fain PR, et al. Variant of TYR and autoimmunity susceptibility loci in generalized vitiligo. N Engl J Med. 2010;362:1686–97.
7. Jin Y, Birlea SA, Fain PR, et al. Common variants in FOXP1 are associated with generalized vitiligo. Nat Genet. 2010;42:576–8.
8. Liang Y, Yang S, Zhou Y, et al. Evidence for two susceptibility loci on chromosomes 22q12 and 6p21-p22 in Chinese generalized vitiligo families. J Invest Dermatol. 2007;127:2552–7.
9. Traks T, Karelson M, Reimann E, et al. Association analysis of class II cytokine and receptor genes in vitiligo patients. Hum Immunol. 2016;77:375–81.
10. Jin Y, Andersen G, Yorgov D, et al. Genome-wide association studies of autoimmune vitiligo identify 23 new risk loci and highlight key pathways and regulatory variants. Nat Genet. 2016;48:1418–24.
11. Birlea SA, Jin Y, Bennett DC, et al. Comprehensive association analysis of candidate genes for generalized vitiligo supports XBP1, FOXP3, and TSLP. J Invest Dermatol. 2011;131:371–81.
12. Li K, Shi Q, Yang L, et al. The association of vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D levels with generalized vitiligo. Br J Dermatol. 2012;167:815–21.
13. Alkhateeb A, Fain PR, Thody A, et al. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. Pigment Cell Res. 2003;16:208–14.
14. Liu JB, Li M, Yang S, et al. Clinical profiles of vitiligo in China: an analysis of 3742 patients. Clin Exp Dermatol. 2005;30:327–31.
15. Sheth VM, Guo Y, Qureshi AA. Comorbidities associated with vitiligo: a ten-year retrospective study. Dermatology. 2013;227:311–5.
16. Silverberg JI, Silverberg NB. Association between vitiligo and atopic disorders: a pilot study. JAMA Dermatol. 2013;149:983–6.
17. Chen YT, Chen YJ, Hwang CY, et al. Comorbidity profiles in association with vitiligo: a nationwide population-based study in Taiwan. J Eur Acad Dermatol Venereol. 2015;29:1362–9.
18. Gill L, Zarbo A, Isedeh P, et al. Comorbid autoimmune diseases in patients with vitiligo: a cross-sectional study. J Am Acad Dermatol. 2016;74:295–302.
19. Bae JM, Lee JH, Yun JS, et al. Vitiligo and overt thyroid diseases: a nationwide population-based study in Korea. J Am Acad Dermatol. 2017;76:871.
20. Gan EY, Cario-Andre M, Pain C, et al. Follicular vitiligo: a report of 8 cases. J Am Acad Dermatol. 2016;74:1178–84.
21. Zhang Z, Xu SX, Zhang FY, et al. The analysis of genetics and associated autoimmune diseases in Chinese vitiligo patients. Arch Dermatol Res. 2009;301:167–73.
22. Liston A, Enders A, Siggs OM. Unravelling the association of partial T-cell immunodeficiency and immune dysregulation. Nat Rev Immunol. 2008;8:545–58.
23. Park MA, Li JT, Hagan JB, et al. Common variable immunodeficiency: a new look at an old disease. Lancet. 2008;372:489–502.
24. Brown KL, Bylund J, MacDonald KL, et al. ROS-deficient monocytes have aberrant gene expression that correlates with inflammatory disorders of chronic granulomatous disease. Clin Immunol. 2008;129:90–102.
25. Duvic M, Rapini R, Hoots WK, et al. Human immunodeficiency virus-associated vitiligo: expression of autoimmunity with immunodeficiency? J Am Acad Dermatol. 1987;17:656–62.
26. Grandhe NP, Dogra S, Kumar B. Spontaneous repigmentation of vitiligo in an untreated HIV-positive patient. J Eur Acad Dermatol Venereol. 2006;20:234–5.
27. Antony FC, Marsden RA. Vitiligo in association with human immunodeficiency virus infection. J Eur Acad Dermatol Venereol. 2003;17:456–8.
28. Niamba P, Traoré A, Taieb A. Vitiligo sur peau noire associée au VIH et repigmentation lors du

traitement antiretroviral. Ann Dermatol Venereol. 2007;134:272–3.
29. Nikolic DS, Viero D, Tije VC, et al. Alopecia universalis associated with vitiligo in an 18-year-old HIV-positive patient: highly active anti-retroviral therapy as first choice therapy? Acta Derm Venereol. 2014;94:116–7.
30. Yamauchi PS, Nguyen NQ, Grimes PE. Idiopathic CD4+ T-cell lymphocytopenia associated with vitiligo. J Am Acad Dermatol. 2002;46:779–82.
31. Knight AK, Cunningham-Rundles C. Inflammatory and autoimmune complications of common variable immune deficiency. Autoimmun Rev. 2006;5:156–9.
32. Westerhof W, d'Ischia M. Vitiligo puzzle: the pieces fall in place. Pigment Cell Res. 2007;20:345–59.
33. Onay H, Pehlivan M, Alper S, et al. Might there be a link between mannose binding lectin and vitiligo? Eur J Dermatol. 2007;17:146–8.
34. Palmer CN, Irvine AD, Terron-Kwiatkowski A, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet. 2006;38:441–6.
35. Blumen SC, Bevan S, Abu-Mouch S, et al. A locus for complicated hereditary spastic paraplegia maps to chromosome 1q24-q32. Ann Neurol. 2003;54:796–803.
36. Kulkarni ML, Baskar K, Kulkarni PM. A syndrome of immunodeficiency, autoimmunity, and spondylometaphyseal dysplasia. Am J Med Genet A. 2007;143:69–75.
37. Taieb A. Intrinsic and extrinsic pathomechanisms in vitiligo. Pigment Cell Res. 2000;13:41–7.
38. Richards KA, Fukai K, Oiso N, et al. A novel KIT mutation results in piebaldism with progressive depigmentation. J Am Acad Dermatol. 2001;44:288–92.
39. Alkhateeb A, Fain PR, Spritz RA. Candidate functional promoter variant in the FOXD3 melanoblast developmental regulator gene in autosomal dominant vitiligo. J Invest Derm. 2005;125:388–91.
40. Husebye ES, Gebre-Medhin G, Tuomi TM, et al. Autoantibodies against aromatic l-amino acid decarboxylase in autoimmune polyendocrine syndrome type I. J Clin Endocrinol Metab. 1997;82:147–50.
41. Ekwall O, Hedstrand H, Haavik J, et al. Pteridin-dependent hydroxylases as autoantigens in autoimmune polyendocrine syndrome type I. J Clin Endocrinol Metab. 2000;85:2944–50.
42. Betterle C, Dal Pra C, Mantero F, Zanchetta R. Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. Endocr Rev. 2002;23:327–64.
43. Alimohammadi M, Björklund P, Hallgren A, et al. Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. N Engl J Med. 2008;358:1018–28.
44. Taieb A. NALP1 and the inflammasomes: challenging our perception of vitiligo and vitiligo-related autoimmune disorders. Pigment Cell Res. 2007;20:260–2.
45. Jin Y, Mailloux CM, Gowan K, Riccardi SL, LaBerge G, Bennett DC, Fain PR, Spritz RA. NALP1 in vitiligo-associated multiple autoimmune disease. N Engl J Med. 2007;356:1216–25.
46. Heuss D, Engelhardt A, Gobel H, et al. Myopathological findings in interstitial myositis in type II polyendocrine autoimmune syndrome (Schmidt's syndrome). Neurol Res. 1995;17:233–7.
47. Eisenbarth GS, Wilson PW, Ward F, et al. The polyglandular failure syndrome: disease inheritance, HLA type, and immune function: studies in patients and families. Ann Intern Med. 1978;91:528–33.
48. Zeviani M, Muntoni F, Savarese N, et al. A MERRF/MELAS overlap syndrome associated with a new point mutation in the mitochondrial DNA tRNA(Lys) gene. Eur J Hum Genet. 1993;1:80–7.
49. Latkany P, Ciulla TA, Cacchillo PF, et al. Mitochondrial maculopathy: geographic atrophy of the macula in the MELAS associated A to G 3243 mitochondrial DNA point mutation. Am J Ophthalmol. 1999;128:112–4.
50. Karvonen SL, Haapasaari KM, Kallioinen M, et al. Increased prevalence of vitiligo, but no evidence of premature ageing, in the skin of patients with bp 243 mutation in mitochondrial DNA in the mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes syndrome (MELAS). Br J Dermatol. 1999;140:634–9.
51. Matsuoka T, Goto Y, Yoneda M, et al. Muscle histopathology in myoclonus epilepsy with ragged-red fibers (MERRF). J Neurol Sci. 1991;106:193–8.
52. Berneburg M, Grether-Beck S, Kürten V, et al. Singlet oxygen mediates the UVA-induced generation of the photoaging-associated mitochondrial common deletion. J Biol Chem. 1999;274:15345–9.
53. Cohen LE, Tanner DJ, Schaefer HG, et al. Common and uncommon cutaneous findings in patients with ataxia-telangiectasia. J Am Acad Dermatol. 1984;10:431–8.
54. Barlow C, Dennery PA, Shigenaga MK, et al. Loss of the ataxia-telangiectasia gene product causes oxidative damage in target organs. Proc Nat Acad Sci. 1999;96:9915–9.

14 Age and Vitiligo: Childhood, Pregnancy and Late-Onset Vitiligo

Steven Thng, Sai Yee Chuah, and Emily Yiping Gan

Contents

Abstract

Vitiligo usually begins in childhood or in young adults with some studies recording around half of patients with disease onset before the age of 20 years old. Childhood vitiligo tends to present more with segmental vitiligo and is associated with higher incidences of thyroid dysfunction as well as family history of autoimmune disease, significant psychological morbidity and quality of life (QOL) impairment in the patient and in the parent. Prepubertal onset of vitiligo associates with atopic dermatitis, halo naevi, family history of vitiligo and premature hair greying. When treating children with vitiligo, special considerations would include the need to coordinate treatments with school schedules and the significant psychosocial impact of the disease on children especially if they have visible lesions. Patients with vitiligo have possibly a higher incidence of worsening during pregnancy and 6 months' postpartum. Treatment of vitiligo during pregnancy is largely influenced by how treatment may affect

S. Thng (✉) · S. Y. Chuah · E. Y. Gan
National Skin Centre, Singapore, Singapore
e-mail: steventhng@nsc.com.sg

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_14

the foetus in utero, and treatment has to be balanced with possible adverse effects to foetus in utero. Late-onset vitiligo is an interesting subgroup of patients with vitiligo, and its prevalence ranges from 6.8% in Northern India to 14.7% in Singapore; they seem to progress faster than childhood vitiligo, and the disease progression is significantly associated with a stressful event.

Key Points

- Segmental vitiligo and associated thyroid dysfunction are frequent in childhood patients.
- Significant psychological morbidity and quality of life (QOL) impairment are reported in the patient and in the parent.
- Prepubertal onset of vitiligo associates with atopic dermatitis, halo naevi, family history of vitiligo and premature hair greying.
- In childhood treatment must take into account school schedules and psychosocial impact of the disease.
- Possible worsening of vitiligo has been noted during pregnancy and 6 months' post-partum.
- The prevalence of late-onset vitiligo ranges from 6.8% in Northern India to 14.7% in Singapore.
- The progress of late-onset vitiligo is faster than childhood vitiligo, and the disease progression is significantly associated with a stressful event.

14.1 Introduction

Many epidemiological studies around the world have surmised that vitiligo usually begins in childhood or in young adults [1] with some studies recording around half of patients with disease onset before the age of 20 years old [2]. While there are many studies looking at vitiligo in childhood and vitiligo in general, there are few publications that evaluate the differences in clinical characteristics, treatment response as well as disease progression of this disfiguring disease among different age groups and during pregnancy. This chapter will therefore examine the clinical characteristics of vitiligo through the ages, paying special attention to clinical presentation, disease progression, treatment response and considerations of childhood vitiligo, late-onset vitiligo and vitiligo in pregnancy.

14.2 Vitiligo in Childhood

Childhood vitiligo is not uncommonly encountered in the paediatric dermatology clinic. While recent reports show that the worldwide prevalence of childhood/adolescent vitiligo does not differ from adult vitiligo, ranging between 0.5% and 2% [3], from a clinical characteristic and treatment response perspective, vitiligo in childhood differs from adult-onset cases. In addition, there are specific considerations one needs to bear in mind when managing paediatric patients with vitiligo. These will be discussed in this segment.

14.2.1 Epidemiology of Vitiligo in Childhood

Nearly half of all cases of vitiligo begin before the age of 20 years [2] and one quarter before the age of 10 years [4]. The age at onset of vitiligo varies between studies, although many have reported that most cases are acquired early in life, between 4 and 12 years of age [5, 6]. There have been reports of patients with vitiligo lesions since birth [7, 8]; however the entity "congenital vitiligo" still remains controversial, as it is not clear whether these are patients with piebaldism or true vitiligo. In most reported paediatric case series, the majority of reported cases tend to be in girls [9, 10], with some studies reporting a prevalence of up to 60% in girls versus 40% in boys [6, 11–13]. However, there have also been other studies in the literature which do not show a gender predilection [14]. In a local study in Singapore examining 369 cases of childhood vitiligo over

5 years [52], 52.3% of our childhood vitiligo patients were girls, while 47.7% were boys with a mean age of onset of 8.9 years.

14.2.2 Clinical Characteristics of Vitiligo in Childhood

14.2.2.1 Classification of Vitiligo

Non-segmental (NSV) vitiligo is the most common type of vitiligo in children, of which the subtype generalised vitiligo (Fig. 14.1) occurs most frequently [6, 9, 11, 14, 15]. This is followed by focal and then segmental vitiligo [6, 9, 11, 14] (Fig. 14.2). Notwithstanding, segmental vitiligo tends to be more common in children compared to adults [5, 16] with a prevalence ranging from 4.6% to 32.5%. In our local study comprising 369 patients [52], 35% of patients (n = 129) had segmental vitiligo, of which two patients progressed to develop vitiligo vulgaris (mixed vitiligo) sometime later in the course of their disease.

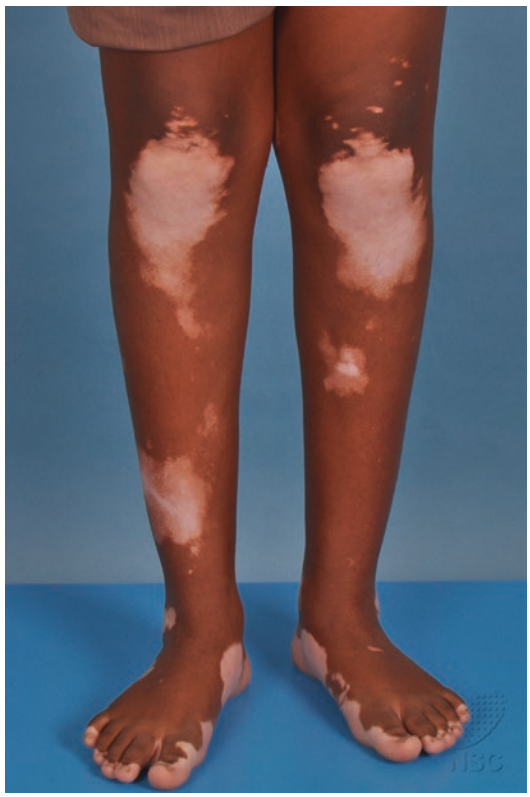


Fig. 14.1 Unstable vitiligo vulgaris in a 7 years old child

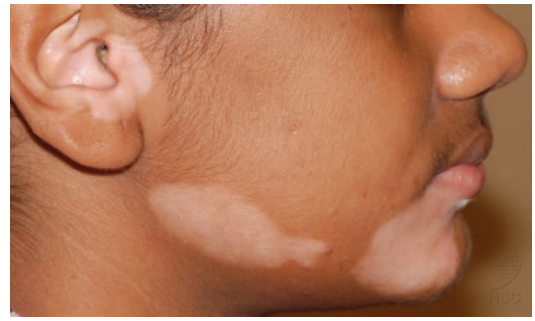


Fig. 14.2 Segmental Vitiligo in a paediatric patient

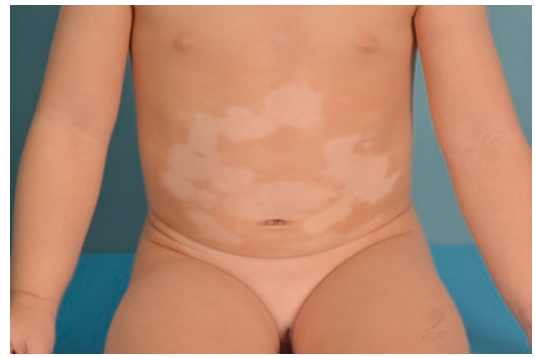


Fig. 14.3 Vitiligo vulgaris on the trunk and groin

14.2.2.2 Distribution of Vitiligo

The head and neck are the most common sites of onset of vitiligo [6, 9, 11, 15] in children. The perineum, especially the buttocks and perianal area, is a relatively frequent site of onset of non-segmental vitiligo in toddlers (Fig. 14.3), suggesting a role for koebnerisation triggered by use of nappies and hygiene care. In a large French study analysing children with non-segmental versus segmental vitiligo, Mazereeuw-Hautier et al. reported that children with non-segmental vitiligo had a greater number of lesions, a larger body surface area of involvement, higher incidence of Koebner phenomenon and more frequent progression of disease [17].

14.2.2.3 Disease Onset in Childhood

In a study comparing 679 patients [18] with pre- (before 12 years old) versus post-pubertal onset (after 12 years old) of vitiligo, atopic dermatitis

was often associated with or preceding prepubertal onset (odds ratio 2.42, $p = 0.006$), whereas thyroid disease or presence of thyroid antibodies was more frequent in post-pubertal onset (odds ratio 0.31, $p < 0.003$). Other factors associated with prepubertal onset include the presence of halo naevi, family history of vitiligo, premature hair greying and previous episode of spontaneous repigmentation. In contrast, factors associated with post-pubertal onset include stress as an initiating factor, personal history of thyroid disease and the acrofacial type of vitiligo [18]. A separate study by Nicolaidou et al., which also used 12 years old as the cut-off value for age at onset, found that the group with childhood-onset vitiligo had the head as the most frequent site of initial presentation, more cases of segmental vitiligo, a higher prevalence of allergic diseases and a lower prevalence of thyroid diseases compared to the later-onset group [19].

In a separate study of purely paediatric vitiligo patients, Mu et al. divided 208 children into early-onset (less than 3 years old) and later-onset (3–18 years old) groups. Early-onset vitiligo was associated with more extensive and progressive disease during the average 1.9 years of follow-up. There were no significant differences between the two groups in terms of repigmentation, vitiligo type, halo naevi, gender ratio or personal and family history of autoimmune diseases [20].

14.2.2.4 Other Clinical Features

Halo naevi occur more commonly in paediatric vitiligo patients than in adults with vitiligo [21]. Cohen et al. analysed 208 children with vitiligo and found 26% of them had halo naevi. Children with vitiligo and halo naevi were more likely to present with generalised vitiligo. There was however no significant association with percentage of body surface area affected or family history of vitiligo or autoimmune diseases [22]. Leukotrichia has been reported in 4–12% of patients with vitiligo [5, 6, 10]. Handa and Dogra reported it occurring most commonly in patients with generalised vitiligo (84%), followed by focal and segmental vitiligo [6].

Although children tend to scrape or scratch their elbows, knees and shins often, it is not clear whether koebnerisation occurs more frequently in children. In a series of 625 children with vitiligo, Handa and Dogra reported koebnerisation in 11% of patients [6]. In another study by Kayal et al., 83 children and 26 adult patients who had onset of vitiligo before 12 years of age were categorised as having childhood-onset vitiligo (COV). The Koebner phenomenon was found to occur in 37% of the COV group as compared to 29% of those with adult-onset vitiligo (186 patients) [11].

14.2.2.5 Treatment Response Characteristics and Disease Progression

In paediatric vitiligo, the repigmentation patterns differ from that in adults. Gan et al. reported that the most common pattern seen in children is the combined pattern (62%), followed by diffuse, marginal, perifollicular and lastly medium spotted repigmentation [23]. This is in contrast to adults, where the most common pattern is perifollicular. A possible explanation is that in children the melanocytic reservoirs have not yet been depleted or affected by other factors such as ageing, senescence of melanocyte precursors, cumulative sun exposure and sun-induced changes.

Longitudinal studies evaluating the evolution of childhood vitiligo are lacking. In one series, after approximately 1 year of follow-up, it was noted that vitiligo was clinically undetectable in only 5.5% of patients. More progression was noted in the group of children with non-segmental (23%) compared to segmental vitiligo (6%) [17].

14.2.3 Familial Background and Associated Diseases of Vitiligo in Childhood

14.2.3.1 Family History

A positive family history of vitiligo is elicited more often in children with vitiligo, although this

incidence varies considerably, from 3% up to 46% in different studies [5, 9, 24]. The presence of autoimmune disease within the family also tends to occur more frequently [5, 6]. Pajvani et al. reported that patients with vitiligo and an extended family history of vitiligo were significantly more likely to have an earlier age of onset of disease compared to those with a negative family history (odds ratio 3.7) [13]. Knowledge of this association can allow for closer monitoring of siblings, earlier detection and initiation of treatment.

14.2.3.2 Associated Conditions

Children with vitiligo are generally healthy; however, as with adult vitiligo, other autoimmune diseases can be associated, the most common being autoimmune thyroid disorders, which may be associated with thyroid dysfunction [25].

The risk for autoimmune thyroid disease increases with age and duration of disease, consistent with the findings of a systematic review where the median prevalence of autoimmune thyroid disease in children was 6.9%, with one-third found in adults (18.6%) [26]. Notwithstanding, it has been reported that Hashimoto's thyroiditis is 2.5 times more frequent among children and adolescents with vitiligo than in a healthy age- and sex-matched population. Iacovelli et al. showed that 11% of paediatric patients with vitiligo, particularly 16% of patients with non-segmental vitiligo, had thyroid abnormalities on testing [27]. In another study, Halder et al. found antithyroid antibodies in 12% of the 33 children with vitiligo who were screened [5]. Thyroid dysfunction has been reported to correlate with the specific type of vitiligo, namely, non-segmental as opposed to segmental vitiligo, but not with extension of lesions and localisation [27]. Vitiligo usually appears before the development of thyroid disease [28], and cases of subclinical hypo- or hyperthyroidism have been detected with thyroid testing [29]; therefore, it is prudent to screen children and adolescents with non-segmental vitiligo for thyroid dysfunction at presentation and during subsequent follow-up. Screening should include levels of thyroid-stimulating hormone (TSH), free thyroxine, anti-thyroglobulin and antithyroid peroxidase antibodies [28]. Antinuclear antibodies (ANA) have been detected in children with vitiligo, occurring in up to 5%, although other studies have not been typically consistent in finding positive ANA levels [24, 30]. Other autoimmune diseases which have been reported in children with vitiligo include alopecia areata, diabetes mellitus, Addison's disease and autoimmune polyglandular syndrome [6, 9].

14.2.4 Differential Diagnosis of Vitiligo in Childhood

In children, other common causes of hypomelanoses are postinflammatory hypopigmentation, naevus depigmentosus (for segmental vitiligo) and piebaldism. For cases with vulvar depigmentation, lichen sclerosus is also an important differential diagnosis that should be excluded. A thorough history and clinical examination including Wood's lamp examination would assist in making the right diagnosis.

14.2.5 Psychological Sequelae of Vitiligo in Childhood

Childhood vitiligo is associated with significant psychological morbidity and quality of life (QOL) impairment, both of which are present not only in the patient but also in the parent [31]. An affected body surface area of more than 25% is associated with greater impairment in QOL, self-consciousness, difficulty with friendships and schoolwork and teasing and bullying [32]. Teenagers aged 15–17 years seem to have the greatest QOL burden from vitiligo [32], and their emotional development may be affected especially if they have lesions in visible areas [33]. Cosmetic camouflage has been shown to have a positive impact on the QOL of this group of patients [34]. It is essential to screen for QOL impairment in both paediatric vitiligo patients and their parents and to incorporate this information into therapeutic decision-making.

14.2.6 Therapeutic Considerations of Vitiligo in Childhood

The approach to the treatment of childhood vitiligo largely mirrors that in an adult. First-line treatment includes topical corticosteroids, topical calcineurin inhibitors and topical vitamin D analogues. Other options are narrowband ultraviolet B phototherapy, targeted phototherapy such as 308 nm excimer light, surgical grafting and cosmetic camouflage. The use of systemic corticosteroids for unstable disease may be considered on a case-by-case basis. Specific considerations to make when treating children with vitiligo include the need to coordinate treatments with school schedules and the significant psychosocial impact of the disease on children especially if they have visible lesions.

Koh et al. reviewed 71 Asian patients aged 5–15 years who had received various modes of phototherapy for vitiligo and found that the response rates, defined as at least 50% repigmentation, with narrowband UVB (74%) and combined UVA1 and UVB phototherapy (67%) were marginally higher than those with the 308 nm excimer lamp phototherapy (53%) and paint psoralen-UVA photochemotherapy (52%) [35]. Paediatric patients with generalised vitiligo respond better to phototherapy than those with segmental vitiligo [35], consistent with results also reported in adult patients [36].

Vitiligo surgery is generally not preferred in children because the techniques are time consuming and require cooperation from the child in order to be successful. Mulekar et al. reported on a series of 25 children aged 4–16 years with segmental or focal vitiligo who underwent noncultured cellular grafting under sedation and local anaesthesia. Good to excellent repigmentation (≥65%) was reported in 77% and 83% of patients with segmental and focal vitiligo, respectively [37]. In a separate study comparing the results of children, adolescents and adults who underwent transplantation of autologous cultured melanocytes, Hong et al. reported no statistically significant difference in repigmentation among the three groups. Repigmentation of 50% or more was obtained in 83%, 95% and 84% of children, adolescents and adults, respectively, with a mean extent of repigmentation of 81%, 79% and 77% in the three respective groups [38]. Punch minigrafting has also been carried out with success in children [39]. With appropriate pre-surgical counselling of parents and child and willingness of the child to cooperate, surgical intervention for vitiligo is indeed a viable treatment option.

14.3 Vitiligo During Pregnancy

While the epidemiology and clinical characteristics of vitiligo in pregnant patients should not differ much from general population, the prognosis of vitiligo might be affected by the pregnancy, as pregnancy worsens certain autoimmune disease like systemic lupus erythematosus [40]. More importantly, there are important therapeutic considerations one has to be mindful of when treating pregnant patients with vitiligo, so as to ensure that the treatment does not affect the unborn foetus.

14.3.1 Clinical Characteristics of Vitiligo During Pregnancy

There has been a paucity of studies examining the prognosis of vitiligo during pregnancy as well as the pregnancy outcome in patients with vitiligo. In a study of 28 patients with vitiligo while pregnant [41], 17.54% noted a worsening of vitiligo during pregnancy with about 66.6% of patients remaining stable. In the same study, it was noted that 28% of patients experienced worsening of vitiligo in the 6 months after delivery [41].

A similar descriptive study was carried out by the authors at the Vitiligo Clinic in the National Skin Centre, Singapore, from January 2016 till April 2016 (unpublished data). The study included women who had been diagnosed with vitiligo before or during their pregnancies. A standardised questionnaire was employed to determine the clinical characteristics of vitiligo during pregnancy and in the 6 months postpartum. A total of 22 vitiligo patients participated in the survey. Of these, 70% recorded either

worsening of their vitiligo (40%) or onset of vitiligo during pregnancy (30%). All the patients who had vitiligo onset during pregnancy were primigravida. During the 6 months post-partum, about 36% of patients experienced worsening of vitiligo, while 10% noted an improvement.

The observed worsening of vitiligo during pregnancy and in the 6 months post-partum is interesting and should be studied further. Pregnancy is associated with considerable physiological stress and significant hormonal changes; in addition, most patients would stop their first-line topical therapies during pregnancy. Therefore, a confluence of these factors could account for the worsening of vitiligo during pregnancy.

With regard to effects of vitiligo on pregnancy outcomes, a study by Horev et al. [42] concluded that vitiligo is not associated with adverse pregnancy outcomes, and hence no special antenatal management considerations are required for this group of patients.

14.3.2 Therapeutic Considerations of Vitiligo During Pregnancy

The approach to the treatment of vitiligo during pregnancy is largely influenced by how the treatment may affect the unborn foetus. The benefits of any treatment have to be weighed against possible adverse effects on the unborn foetus. First-line treatment like topical corticosteroids, topical calcineurin inhibitors and topical vitamin D analogues are all classified by the FDA as Class C. (Risk cannot be ruled out. Human studies are lacking, and animal studies are either positive for foetal risk or lacking as well.) As such, most pregnant ladies are not keen to continue with the usual first-line topical treatment. The only treatment that has been deemed safe for use during pregnancy is NBUVB, and patients with vitiligo, especially those with unstable vitiligo, should be treated with NBUVB. However, a recent study on use of NBUVB in psoriasis patients seems to suggest that high cumulative NBUVB doses can decrease serum folate levels in patients with psoriasis treated with NBUVB [43]. In light of this, it is important to enforce folate supplementation in pregnant vitiligo patients undergoing NBUVB phototherapy. NBUVB phototherapy has also been shown to be safe during breastfeeding and should be offered to patients, especially since up to 36% of patients may experience worsening of their condition in the 6 months post-partum.

14.4 Late-Onset Vitiligo

There is limited published data on late-onset vitiligo. Furthermore, there is no standardised definition of late-onset vitiligo. Several authorities have placed the cut-off point of late-onset vitiligo to be after the age of 12 [19, 44], 20 [45], 30 [46], 40 [47] and 50 [48, 49]. Therefore, it is challenging to analyse the published data due to the heterogeneity of the study population.

14.4.1 Definition and Epidemiology of Late-Onset Vitiligo

In this chapter, we will arbitrarily adopt the definition by Dogra et al. [48], where late-onset vitiligo is defined as vitiligo that appears after the age of 50 years. In that particular case series, 2672 vitiligo patients were seen over a period of 10 years, and of these, a total of 182 patients (6.8%) were found to have late-onset vitiligo. The mean age at onset of disease was 55 years [48]. In a recent retrospective review done in the National Skin Centre, Singapore, out of 3134 vitiligo patients seen over a period of 5 years, 461 (14.7%) patients had late-onset vitiligo [53]. The mean age at onset was 59 years, similar to that reported by Dogra et al. Although a slight female preponderance has been reported [48], there is no significant gender difference in most studies including Singapore's series [48, 49, 53].

Childhood-onset vitiligo has more frequently been associated with a family history of vitiligo, as compared to late-onset vitiligo [19, 44, 47]. Notwithstanding, Dogra et al. reported that up to 16% of late-onset vitiligo patients had a family history of vitiligo, with first-degree relatives affected in 11.5% and second-degree

relatives affected in 4.4% of patients [48]. In our Singapore study, only 5.4% of late-onset patients had a family history of vitiligo [53]. It has been suggested that there may be two coexisting modes of inheritance for vitiligo, depending on the age at onset [50]. In early-onset vitiligo, the mode of inheritance has been reported to be dominant with incomplete penetrance, whereas in late-onset vitiligo, it is recessive and, hence, exposure to certain environmental factors is needed to trigger the presentation.

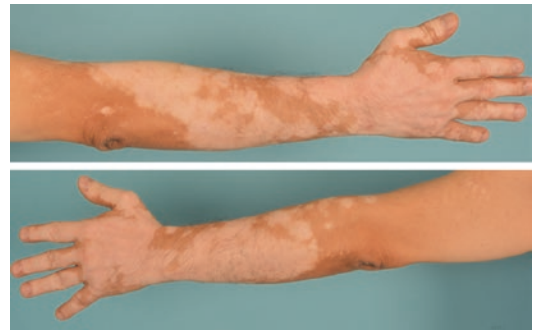


Fig. 14.4 Acral vitiligo in late onset vitiligo patient

14.4.2 Clinical Features, Treatment Response and Course of Disease

14.4.2.1 Clinical Features

Vitiligo vulgaris is the most common subtype of late-onset vitiligo [48, 49]. It is reported to account for 83.5% of cases of late-onset vitiligo seen by Dogra et al. [48] and 94.6% in the Singapore series [53]. Focal vitiligo was reported to be the second most commonly occurring subtype in the series by Dogra et al. [48], and this has been observed in our case series too; Esfandiarpour et al. [49], on the other hand, reported that the acrofacial subtype was the second most common presentation followed by focal vitiligo. Universal and pure mucosal vitiligo were the least common subtypes [48, 49]. Leukotrichia was found to be common in late-onset vitiligo with reports ranging from 47.3% [50] up to 77.8% [49]. Halo nevus is not commonly seen in late-onset vitiligo [48].

Although the head and neck was reported by Dogra et al. [48] to be the most common site of early involvement, other studies have found the main site of presentation for late-onset vitiligo to be the upper extremities [45, 47, 49] (Fig. 14.4). This is in contrast to childhood vitiligo where the lower extremities were affected more often [19, 45]. It has been postulated that such site predilection occurs because younger patients are more susceptible to lower extremity trauma sustained during play, whereas older patients holding desk jobs would exert pressure on their elbows at work and those who engage in household-related activities would tend to traumatise their hands and wrists more often [45]. Facial vitiligo has not been linked to specific age groups; however vitiligo in the perioral and beard areas in men and vitiligo in the axillae in women have been seen more often in older patients [45]. This may be accounted for by Koebner's phenomenon secondary to shaving in these areas [45, 48]. While the Koebner's phenomenon may explain the predilection for certain locations, it neither accounts for all age- and gender-related differences [45], nor does it explain the occurrence of vitiligo in locations without a history of trauma. Genome-wide association studies have given us more information on the genetic background of vitiligo, and the difference in the distribution pattern may reflect the underlying genetic predisposition rather than age at onset or gender factors [45].

Besides koebnerisation, psychological stress has been associated with vitiligo onset and progression in both childhood and late-onset vitiligo [19, 46, 49]. However, patients with late-onset vitiligo seem to progress faster than childhood vitiligo, and the disease progression is significantly associated with a stressful event [19, 46].

14.4.2.2 Associated Conditions

Vitiligo is associated with autoimmune diseases, and it was reported to be present in up to 21.4% of late-onset vitiligo patients [48, 49]. The most common associated autoimmune disease reported was diabetes mellitus followed by thyroid disease

and alopecia areata [47–49]. Other minor associated conditions included rheumatoid arthritis, pernicious anaemia and Addison's disease [47, 48]. The association of diabetes mellitus with late-onset vitiligo was reported to be between 15% [48] and as high as 69% [47]. Both diabetes mellitus and vitiligo are associated with HLA DR3 and HLA DR4. Hence, the occurrence of vitiligo in diabetic patients may be the result of autoimmune disturbance in the same patient affecting two organ systems. Therefore, it is recommended that all patients with late-onset vitiligo be screened for diabetes mellitus [47, 49].

Adult-onset vitiligo is less likely to be associated with a family history of autoimmune disease as compared to childhood vitiligo [19]. Family history of autoimmune diseases in adult-onset vitiligo was higher in first-degree relatives than in second-degree relatives (19.2% vs. 13.4%) [5, 47, 48]. In contrast, childhood vitiligo reported a higher family history of autoimmune disease in the extended family than in the immediate family [5, 48].

14.4.3 Therapeutic Considerations of Late-Onset Vitiligo

Studies comparing treatment response differences between late-onset and childhood vitiligo are lacking. However, it has been observed that late-onset vitiligo progresses slowly and is less responsive to treatment. Treatment with topical corticosteroids, calcineurin inhibitors and phototherapy remains the mainstay of treatment. Surgical treatment can be explored in stable disease, and systemic corticosteroids may be given for active disease as long as there are no contraindications resulting from age or other related diseases in this age group [48, 51].

Progression of late-onset vitiligo has been shown to be significantly associated with stressful events and Koebner's phenomenon [19, 48, 49]. Majority of these patients are concerned about halting disease progression and desire repigmentation of the exposed areas [48]. Therefore, it is important to counsel and educate these patients on how to avoid trauma and how to cope with stressful events.

The association of late-onset vitiligo with diabetes mellitus and thyroid disease has been reported to be significant [19, 47]. Screening for these conditions should be done appropriately.

References

1. Halder RM, Chappell JL. Vitiligo update. Semin Cutan Med Surg. 2009;28:86–92.
2. Lerner AB. Vitiligo. J Invest Dermatol. 1959;32:285–310.
3. Krüger C, Schallreuter KU. A review of the worldwide prevalence of vitiligo in children/adolescents and adults. Int J Dermatol. 2012;51:1206–12.
4. Howitz J, Brodthagen H, Schwartz M, Thomsen K. Prevalence of vitiligo: epidemiological survey on the isle of Bornholm, Denmark. Arch Dermatol. 1977;113:47–52.
5. Halder RM, Grimes PE, Cowan CA, Enterline JA, Chakrabarti SG, Kenney JA. Childhood vitiligo. J Am Acad Dermatol. 1987;16:948–54.
6. Handa S, Dogra S. Epidemiology of childhood vitiligo: a study of 625 patients from North India. Pediatr Dermatol. 2003;20:207–10.
7. Jain DK, Bhargava P, Mathur DK, Agarwal US, Bhargava R. Congenital familial acral vitiligo. Indian J Dermatol Venereol Leprol. 1997;63:193.
8. Kambhampati BN, Sawatkar GU, Kumaran MS, Parsad D. Congenital vitiligo: a case report. J Cutan Med Surg. 2016;20:354.
9. Al-Mutairi N, Sharma AK, Al-Sheltawy M, Nour-Eldin O. Childhood vitiligo: a prospective hospital-based study. Australas J Dermatol. 2005;46:150–3.
10. Jaisankar TJ, Baruah MC, Garg BR. Vitiligo in children. Int J Dermatol. 1992;31:621–3.
11. Kayal A, Gupta LK, Khare AK, Mehta S, Mittal A, Kuldeep CM. Pattern of childhood onset vitiligo at a tertiary care centre in south-west Rajasthan. Indian J Dermatol. 2015;60:520–6.
12. Marinho FS, Cirino PV, Fernandes NC. Clinical epidemiological profile of vitiligo in children and adolescents. An Bras Dermatol. 2013;88:1026–8.
13. Pajvani U, Ahmad N, Wiley A, Levy RM, Kundu R, Mancini AJ, Chamlin S, Wagner A, Paller AS. The relationship between family medical history and childhood vitiligo. J Am Acad Dermatol. 2006;55:238–44.
14. Hu Z, Liu JB, Ma SS, Yang S, Zhang XJ. Profile of childhood vitiligo in China: an analysis of 541 patients. Pediatr Dermatol. 2006;23:114–6.
15. Lin X, Tang LY, Fu WW, Kang KF. Childhood vitiligo in China: clinical profiles and immunological findings in 620 cases. Am J Clin Dermatol. 2011;12:277–81.
16. de Barros JC, Machado Filho CD, Abreu LC, de Barros JA, Paschoal FM, Nomura MT, Marques E, Martins LC. A study of clinical profiles of vitiligo in different ages: an analysis of 669 outpatients. Int J Dermatol. 2014;53:842–8.

17. Mazereeuw-Hautier J, Bezio S, Mahe E, Bodemer C, Eschard C, Viseux V, Labreze C, Plantin P, Barbarot S, Vabres P, Martin L, Paul C, Lacour JP, on behalf of the Groupe de Recherche Clinique en Dermatologie Pédiatrique (GRCDP). Segmental and nonsegmental childhood vitiligo has distinct clinical characteristics: a prospective observational study. J Am Acad Dermatol. 2010;62:945–9.
18. Ezzedine K, Diallo A, Léauté-Labrèze C, Seneschal J, Boniface K, Cario-André M, Prey S, Ballanger F, Boralevi F, Jouary T, Mossalayi D, Taïeb A. Pre- vs. post-pubertal onset of vitiligo: multivariate analysis indicates atopic diathesis association in prepubertal onset vitiligo. Br J Dermatol. 2012;167:490–5.
19. Nicolaidou E, Antoniou C, Miniati A, Lagogianni E, Matekovits A, Stratigos A, Katsambas A. Childhood- and later-onset vitiligo have diverse epidemiologic and clinical characteristics. J Am Acad Dermatol. 2012;66:954–8.
20. Mu EW, Cohen BE, Orlow SJ. Early-onset childhood vitiligo is associated with a more extensive and progressive course. J Am Acad Dermatol. 2015;73:467–70.
21. Ezzedine K, Diallo A, Léauté-Labrèze C, Seneschal J, Mossalayi D, AlGhamdi K, Prey S, Boutchnei S, Cario-André M, Boralevi F, Jouary T, Taïeb A. Halo nevi association in nonsegmental vitiligo affects age at onset and depigmentation pattern. Arch Dermatol. 2012;148:497–502.
22. Cohen BE, Mu EW, Orlow SJ. Comparison of childhood vitiligo presenting with or without associated halo nevi. Pediatr Dermatol. 2016;33:44–8.
23. Gan EY, Gahat T, Cario-André M, Seneschal J, Ezzedine K, Taïeb A. Clinical repigmentation patterns in paediatric vitiligo. Br J Dermatol. 2016;175:555. https://doi.org/10.1111/bjd.14635.
24. Prcic S, Djuran V, Mikov A, Mikov I. Vitiligo in children. Pediatr Dermatol. 2007;24:666.
25. Gey A, Diallo A, Seneschal J, Léauté-Labrèze C, Boralevi F, Jouary T, Taïeb A, Ezzedine K. Autoimmune thyroid disease in vitiligo: multivariate analysis indicates intricate pathomechanisms. Br J Dermatol. 2013;168:756–61.
26. Vrijman C, Kroon MW, Limpens J, Leeflang MMG, Luiten RM, van der Veen JPW, Wolkerstorfer A, Spuls PI. The prevalence of thyroid disease in patients with vitiligo: a systematic review. Br J Dermatol. 2012;167:1224–35.
27. Iacovelli P, Sinagra JL, Vidolin AP, Marenda S, Capitanio B, Leone G, Picardo M. Relevance of thyroiditis and of other autoimmune diseases in children with vitiligo. Dermatology. 2005;210:26–30.
28. Kakourou T, Kanaka-Gantenbein C, Papadopoulou A, Kaloumenou E, Chrousos GP. Increased prevalence of chronic autoimmune (Hashimoto's) thyroiditis in children and adolescents with vitiligo. J Am Acad Dermatol. 2005;53:220–3.
29. Cho SB, Kim JH, Cho S, Park JM, Park YK, Oh SH. Vitiligo in children and adolescents: association with thyroid dysfunction. J Eur Acad Dermatol Venereol. 2011;25:64–7.
30. Cho S, Kang HC, Hahm JH. Characteristics of vitiligo in Korean children. Pediatr Dermatol. 2000;17:189–93.
31. Amer AAA, Mchepange UO, Gao XH, Hong Y, Qi R, Wu Y, Cai Y, Zhai J, Chen HD. Hidden victims of childhood vitiligo: impact on parents' mental health and quality of life. Acta Derm Venereol. 2015;95:322–5.
32. Silverberg JI, Silverberg NB. Quality of life impairment in children and adolescents with vitiligo. Pediatr Dermatol. 2014;31:309–18.
33. Bilgic O, Bilgic A, Akis HK, Eskioğlu F, Kilic EZ. Depression, anxiety and health-related quality of life in children and adolescents with vitiligo. Clin Exp Dermatol. 2011;36:360–5.
34. Ramien ML, Ondrejchak S, Gendron R, Hatami A, McCuaig CC, Powell J, Marcoux D. Quality of life in pediatric patients before and after cosmetic camouflage of visible skin conditions. J Am Acad Dermatol. 2014;71:935–40.
35. Koh MJ, Mok ZR, Chong WS. Phototherapy for the treatment of vitiligo in Asian children. Pediatr Dermatol. 2015;32:192–7.
36. Anbar TS, Westerhof W, Abdel-Rahman AT, El-Khayyat MA. Evaluation of the effects of NB-UVB in both segmental and non-segmental vitiligo affecting body sites. Photodermatol Photoimmunol Photomed. 2006;22:157–63.
37. Mulekar SV, Al Eisa A, Delvi MB, Al Issa A, Al Saeed AH. Childhood vitiligo: a long-term study of localized vitiligo treated by noncultured cellular grafting. Pediatr Dermatol. 2010;27:132–6.
38. Hong WS, Hu DN, Qian GP, McCormick SA, Xu AE. Treatment of vitiligo in children and adolescents by autologous cultured pure melanocytes transplantation with comparison of efficacy to results in adults. J Eur Acad Dermatol Venereol. 2011;25:538–43.
39. Tsuchiyama K, Watabe A, Sadayasu A, Onodera N, Kimura Y, Aiba S. Successful treatment of segmental vitiligo in children with the combination of 1-mm minigrafts and phototherapy. Dermatology. 2016;232:237–41.
40. Jørgensen KT, Pedersen BV, Nielsen NM, Jacobsen S, Frisch M. Childbirths and risk of female predominant and other autoimmune diseases in a population-based Danish cohort. J Autoimmun. 2012;38(2-3):J81–7.
41. Delatorre G, Bruno C a, Chaves TP, Von Linsingen RF, de Castro CCS. A Study of the prognosis of vitiligo during pregnancy. Surg Cosmet Dermatol. 2013;5(1):37–9.
42. Horev A, Weintraub AY, Sergienko R, Wiznitzer A, Halevy S, Sheiner E. Pregnancy outcome in women with vitiligo. Int J Dermatol. 2011;50(9):1083–5.
43. El-Saie LT, Rabie AR, Kamel MI, Seddeik AK, Elsaie ML. Effect of narrowband ultraviolet B phototherapy on serum folic acid levels in patients with psoriasis. Lasers Med Sci. 2011;26(4):481–5. https://doi.org/10.1007/s10103-011-0895-0.

44. Ezzedine K, Le Thuaut A, Jouary T, et al. Latent class analysis of a series of 717 patients with vitiligo allows the identification of two clinical subtypes. Pigment Cell Melanoma Res. 2014;27(1):134–9.
45. Speeckaert R, van Geel N. Distribution patterns in generalized vitiligo. J Eur Acad Dermatol Venereol. 2014;28(6):755–62.
46. Kanwar AJ, Mahajan R, Parsad D. Effect of age at onset on disease characteristics in vitiligo. J Cutan Med Surg. 2013;17(4):253–8.
47. Al-Mutairi N, Al-Sebeih KH. Late onset vitiligo and audiological abnormalities: is there any association. Indian J Dermatol Venereol Leprol. 2011;77(5):571–6.
48. Dogra S, Parsad D, Handa S, et al. Late onset vitiligo: a study of 182 patients. Int J Dermatol. 2005;44(3):193–6.
49. Esfandiarpour I, Farajzadeh S. Clinical characteristics of late-onset vitiligo in an Iranian population. Dermatol Sinica. 2012;30:43–6.
50. Arcos-Burgos M, Parodi E, Salgar M, et al. Vitiligo: complex segregation and linkage disequilibrium analyses with respect to microsatellite loci spanning the HLA. Hum Genet. 2002;110:334–42.
51. Taieb A, Alomar A, Böhm M, et al. Guidelines for the management of vitiligo: the European Dermatology Forum consensus. Br J Dermatol. 2013;168(1):5–19.
52. Tay EY, Chong CLV, Chong WJJP, Gan YE, Chuah SY, Tan WDV, Thng TGS. Treatment outcomes of vitiligo in Asian children. Pediatr Dermatol. 2018;35(2):265–7.
53. Kong YL, Ching VHL, Chuah SY, Thng TG. Retrospective study on the characteristics and treatment of late-onset vitiligo. Indian J Dermatol Venereol Leprol. 2017;83(5):625.

Vitiligo and Skin of Color

15

Onyeka Obioha, Candrice Heath, and Pearl E. Grimes

Contents

Abstract

The prevalence of vitiligo does not differ with respect to different racial and ethnic groups; however the prevalence has been shown to vary in different regions of the world. Given the stark contrast between white patches and normal skin tone in black patients, vitiligo may have distressing psychological impacts in affected individuals and may adversely affect quality of life. Its impact on self-esteem and self-image may be profound. Additionally, the emotional impact on the female population and those with darker skin tones may be more profound. In some geographic areas, social stigma of having vitiligo is very high, and psychiatric morbidity is reported in nearly 75% of affected individuals. According to the psychological and social impact, even the management should be planned and discussed considering combinatory therapies as well as depigmenting approaches not frequently asked by fair-skin patients.

O. Obioha · C. Heath · P. E. Grimes (✉)
Vitiligo & Pigmentation Institute of Southern California, Los Angeles, CA, USA

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_15

Key Points

- The prevalence does not differ with respect to different racial and ethnic groups.
- Vitiligo may adversely affect quality of life mainly in dark-skin patients considering the stark contrast with unaffected skin.
- The path mechanism is the same in the fair- and dark-skin patients.
- Higher incidence of trichrome vitiligo and hypochromic and follicular vitiligo in darker racial ethnic groups.
- NB-UVB combined with afamelanotide appears to be highly effective in Fitzpatrick V–VI skin phototypes.

15.1 Introduction

Vitiligo affects 0.5–2% of the general population worldwide [1–3]. The prevalence does not differ with respect to different racial and ethnic groups; however the prevalence has been shown to vary in different regions of the world. An isolated village in Gujarat, India, has the highest reported prevalence of vitiligo at 8.8% [4]. In contrast, large population studies in China and Denmark report the prevalence to be much lower at 0.093% and 0.38%, respectively [5]. To date, there is no data that demonstrates there is a higher or lower incidence of vitiligo in skin of color.

15.2 Quality of Life (QOL)

Given the stark contrast between white patches and normal skin tone, vitiligo may have distressing psychological impacts in affected individuals and may adversely affect quality of life [6, 7]. Its impact on self-esteem and self-image may be profound [8, 9]. Additionally, the emotional impact on the female population and those with darker skin tones may be more profound [6, 10–12]. Ezzedine et al. concluded that regardless of skin type, dark or light, patients are equally stressed about having vitiligo [13]. However, the skin tone of subjects was uniquely associated with a few factors, as patients with darker skin tones were more likely to think that vitiligo had repercussions on physical appearance and felt burdened by the daily management of their vitiligo [13]. This is in contrast to the subjects with fair skin tones who worried more about a perceived increase risk of skin cancer [13]. A recent study assessed the psychosocial impact of vitiligo and acne compared to normal controls [14]. Irrespective of age, sex, and severity of their vitiligo or acne, quality of life was negatively impacted based upon the Dermatology Life Quality Index (DLQI) score [14]. Both groups had higher levels of social anxiety, depression, and general anxiety [14].

Commonly utilized dermatologic quality of life assessments, such as the Skindex-16 and Dermatology Life Quality Index, may not accurately capture all of the relevant information needed to further assess the true impact of vitiligo on quality of life [15]. Some studies have demonstrated little to no impact, which is a disconnect from what is expressed during patient interactions [15]. The questions on traditional generalized quality of life assessments contain symptom-focused questions about pruritus, scaling, and pain, all of which are usually absent in vitiligo [15]. Thus, several vitiligo-specific impact scoring systems have been published [16]. Investigators in India have also developed a vitiligo-specific quality of life measurement (VIS-22) that includes culturally specific topics by including questions which address social stigma, issues with marriage, the impact of others avoiding physical contact, and concerns about contagiousness [17, 18]. Such topics are crucial in addressing QOL in the Indian culture, since the social stigma of having vitiligo is very high and psychiatric morbidity is reported in nearly 75% of affected individuals [19]. Although many of these questions may not be relevant in other cultures, they are an example of conducting research and delivering patient care through the prism of cultural competence [20].

Lilly et al. developed and validated a new vitiligo-specific quality of life instrument (VitiQol) [21]. They studied 90 subjects, 65% of whom had Fitzpatrick photo skin types IV–

VI. VitiQol has also been validated in Brazilian Portuguese patients with vitiligo [22]. Despite the validation of this scale, other investigators continue to emphasize the need for an international vitiligo impact instrument consensus [15].

From a global perspective, regardless of race or ethnicity, patients with vitiligo are significantly impacted by their disease. In patients with dark skin tones, the contrast between their normally pigmented skin and depigmented skin may be dramatic, but in the senior author's opinion, even patients without a striking contrast may still have the same level of emotional disturbance which prompts them to seek care.

15.3 Etiology and Pathogenesis

There is no data in the literature to suggest or support that the etiology of vitiligo differs in different racial and ethnic groups.

Genetic studies support a non-Mendelian, multifactorial, polygenic inheritance pattern [23, 24]. Twenty-five percent to 50% of patients with vitiligo have affected relatives, and 6% of siblings have the disorder [24]. Thirty-six susceptibility loci have been identified for nonsegmental vitiligo [25]. Results from genetic studies demonstrate that there is significant genetic heterogeneity in susceptibility loci in different racial groups. In a genome-wide linkage analysis of 71 multiplex Caucasian families with generalized vitiligo in the United States and the United Kingdom, Fain and colleagues found a highly significant linkage of vitiligo with an autoimmune susceptibility locus on chromosome 1p31, suggesting that it represents a major susceptibility locus in the Caucasian population [23]. In contrast, a genome-wide analysis of 57 multiplex Chinese families identified the presence of a major susceptibility locus on chromosome 4q13-q21 [26]. In this Chinese sample, the authors found no overlap in linkage between the major susceptibility loci identified in white populations in previous genome-wide linkage studies [26]. Further studies are needed to fully elucidate the difference in susceptibility loci in different racial and ethnic groups.

The frequency of autoantibodies was compared between 70 black patients with vitiligo and 70 matched controls [27]. Both groups were screened for thyroid, antinuclear, parietal cell, smooth muscle, and mitochondrial antibodies. Antithyroidal antibodies were significantly increased in the population of affected black patients compared to controls. Other studies in Indian patients have documented an increase of thyroglobulin, thyroid peroxidase, and antinuclear antibodies in vitiligo patients [28, 36]. The presence of such antibodies in patients further substantiates the concept that autoimmune mechanisms contribute to vitiligo regardless of race and ethnicity.

Recent reports have investigated the role of Vitamin D in the pathogenesis of vitiligo. Vitamin D deficiency has been associated with other autoimmune disorders including multiple sclerosis, systemic lupus erythematosus, undifferentiated connective tissue disease, and rheumatoid arthritis [29]. Vitamin D has been shown to influence melanocyte activation, differentiation, and proliferation, as well as modulation of T-cell activation resulting in the suppression of proinflammatory cytokines [30]. Vitamin D exerts its effect via its nuclear hormone, Vitamin D receptor (VDR).

To date, many of the studies assessing the association between vitiligo and Vitamin D deficiency have been performed in people of color. In a Chinese population, Li et al. suggested that VDR polymorphisms may influence the risk of developing vitiligo by affecting the formation of 25-hydroxyvitamin D (25 (OH) D) [31]. In this study of 749 patients, a significantly decreased risk of vitiligo was found to be associated with VDR polymorphisms in the *BsmI-B*, *ApaI-A*, and *TaqI-t* alleles [31]. They also found significantly lower levels of 25 (OH) D in patients with vitiligo, as well as a dose-response relationship between decreased vitiligo risk and increased serum 25 (OH) D levels in subjects with the *ApaI* allele [31].

In a pilot study, Silverberg et al. assessed serum 25 (OH) D levels in 45 vitiligo patients and determined that vitiligo subjects with comorbid autoimmune disease and increasing Fitzpatrick phototype were more likely to have low serum levels of Vitamin D [32]. In another case-control study of 40 vitiligo subjects with Fitzpatrick skin phototypes III–IV, the authors reported lower serum 25 (OH) D levels in vitiligo

patients with autoimmune disease than in those without a comorbid autoimmune disorder [33].

Serum levels of Vitamin D and Vitamin D receptor expression in lesional and non-lesional skin were assessed in 30 Egyptian patients and 30 age- and gender-matched controls. Forty percent of the patients had insufficient levels and 27% had deficient levels. VDR-mRNA expression was significantly decreased in lesional versus non-lesional skin, further suggesting a role for Vitamin D as a contributor in the pathogenesis or progression of vitiligo [34]. Hence, Vitamin D may be a useful screening tool in patients with vitiligo.

15.4 Types of Vitiligo

The clinical subtypes of vitiligo are determined by the distribution and appearance of lesions on the skin. Common subtypes include generalized (vitiligo vulgaris), acral, acrofacial, and segmental vitiligo (Table 15.1). Studies suggest a higher incidence of trichrome vitiligo and hypochromic and follicular vitiligo in darker racial ethnic groups [35–40] (Figs. 15.1 and 15.2).

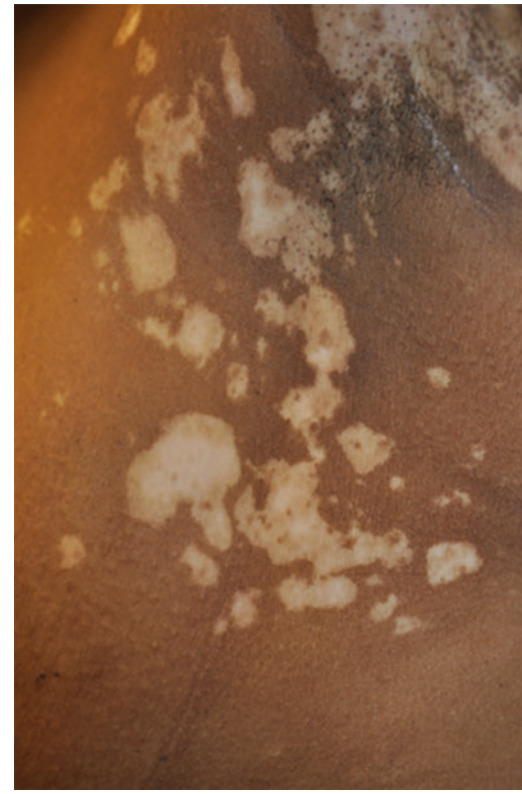


Fig. 15.1 Trichrome vitiligo axillary region. Note shades of white, tan, and normal skin

Table 15.1 Clinical subtypes of vitiligo

Subtypes of vitiligo	Distribution	Skin of color highlights
Generalized (vitiligo vulgaris)	Symmetric, generalized	No distinct features compared to other groups
Acral vitiligo	Extremities	
Acrofacial vitiligo	Extremities and face • Lip-tip subtype affects the lips and distal digits only	
Segmental vitiligo	Dermatomal pattern	
Trichrome vitiligo	Color of patches include white, brown, and normal skin color	Trichrome vitiligo most commonly occurs in those with darker skin hues
Follicular vitiligo	Primarily involves the hair follicles; leukotrichia of the hairs overlying vitiligo patches and normal skin	Common in skin of color
Hypochromic vitiligo	Hypopigmented patches in seborrheic distribution on the face, neck, and hypopigmented scalp macules; few or no depigmented macules or patches; no or poor response to standard vitiligo treatments; leukotrichia is absent	Reported cases have been exclusively in those with Fitzpatrick skin types V and VI

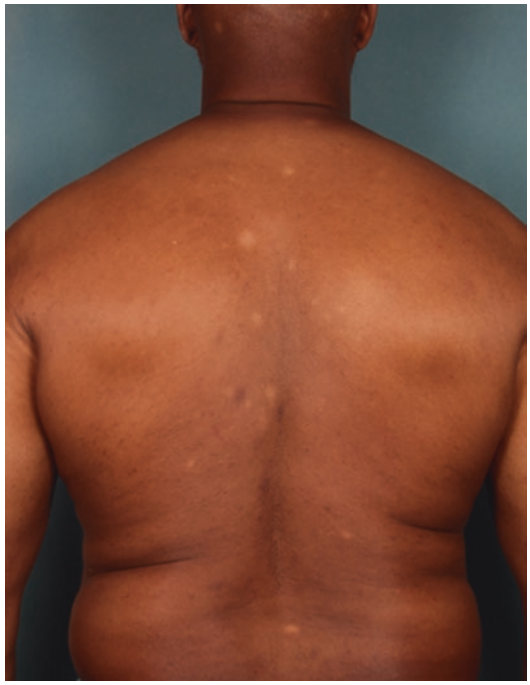


Fig. 15.2 Hypochromic vitiligo. Multiple hypopigmented lesions of the back

15.5 Treatment

The therapeutic objectives for vitiligo are stabilization of disease and repigmentation of vitiliginous skin lesions. Treatment requires an individualized approach based on patient expectations, disease severity, and progression of the disease. Current medical therapies include topical and systemic steroids, topical calcineurin inhibitors, narrowband UVB phototherapy, psoralen with ultraviolet A (PUVA), targeted phototherapy, nutritional vitamin supplementation, and calcipotriol. Of the aforementioned therapies, maximum repigmentation has been achieved with topical steroids, calcineurin inhibitors, and narrowband UVB phototherapy (Figs. 15.3 and 15.4). Emerging therapies include afamelanotide, a synthetic analog of naturally occurring alpha-melanocyte-stimulating hormone (α-MSH), bimatoprost, a prostaglandin F2-alpha analog, and prostaglandin E2.

Patients with skin of color have been found to exhibit enhanced treatment responses to vitiligo therapy compared to patients with lighter skin types. Silverberg and Silverberg conducted a retrospective chart review of adults and children with vitiligo treated using tacrolimus 0.003% and 0.1%. While tacrolimus was effective in all Fitzpatrick skin types, maximal repigmentation of body lesions occurred in individuals with skin types III and IV [41]. Studies show that photochemotherapy with PUVA achieves maximal repigmentation in patients with darker skin types compared to lighter skin types and in patients who reach erythema grade 2 [42, 43]. However, the risk of hyperpigmentation with repigmentation induced by PUVA is more common in darker skin types. In the senior author's experience, the hyperpigmentation fades either during or upon cessation of therapy.

Nicolaidou and colleagues found that darker skin (phototypes III–V) is a predictor of good response to narrowband UVB (NB-UVB) in patients with vitiligo [44]. In a study of 70 vitiligo patients, those with skin types III–V were significantly more likely to reach cosmetically acceptable repigmentation on the face after NB-UVB compared to patients with skin types I and II [44]. This enhanced treatment response was not seen with treatment of nonfacial sites.

The response to NB-UVB among different darker racial ethnic groups may also vary. In studies performed by Kanwar et al. evaluating the efficacy of NB-UVB in two different samples of Indian patients (all Fitzpatrick skin types IV to VI), the rates of cosmetically acceptable repigmentation were found to be 71.4% and 75% [45, 46]. Alternatively, results from a sample of Chinese subjects, skin types III and IV, reported a much lower rate of repigmentation, 12.5% [47]. This difference may be explained by skin phototype.

Grimes et al. and Lim et al. assessed the efficacy and safety of afamelanotide implants (a synthetic α-MSH analog) combined with NB-UVB phototherapy compared to NB-UVB monother-

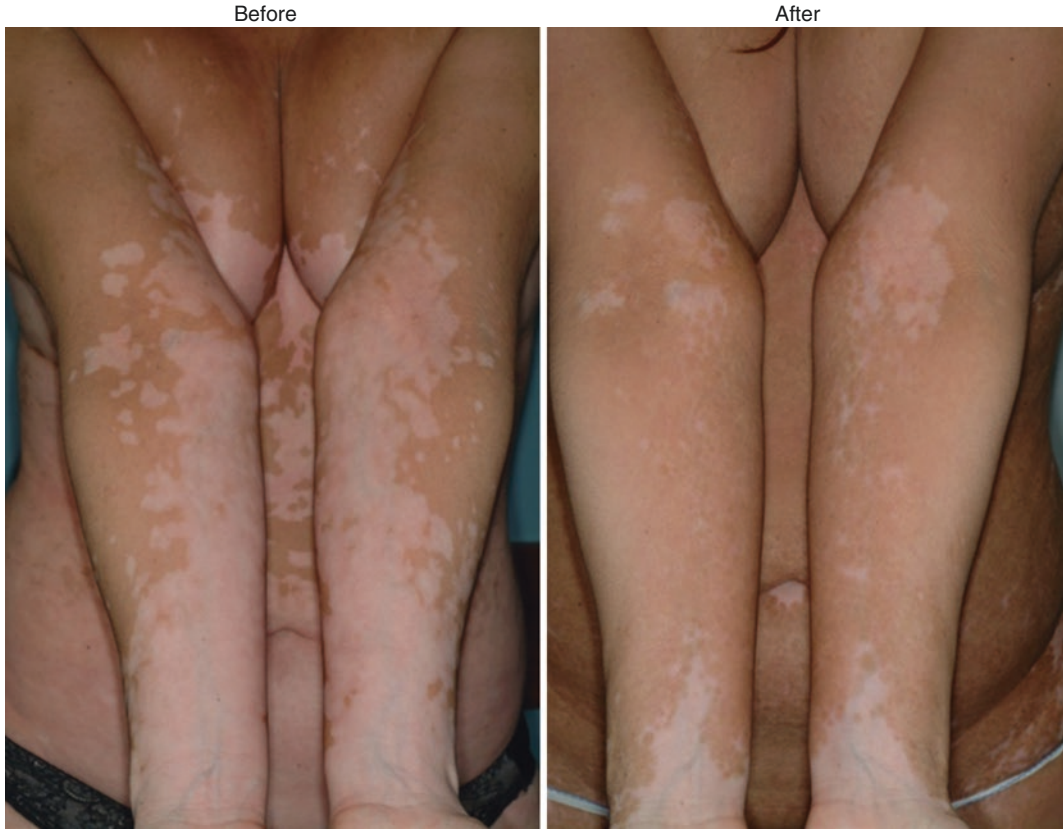


Fig. 15.3 Narrowband UVB phototherapy. Note repigmentation of the arms

apy for vitiligo [48, 49]. Fifty-five patients (28 combination group, 27 NB-UVB monotherapy) were included in this 6-month randomized multicenter trial [49]. Maximal repigmentation in the combination group was achieved in patients with Fitzpatrick SPT V and VI.

15.6 Depigmentation

Depigmentation therapy can be a viable therapeutic alternative in patients with extensive vitiligo affecting greater than 30–40% of body surface areas. In skin of color patients, many physicians may delay discussing depigmentation due to sensitivity and concerns for the perceived culturally driven self-image issues. In the senior author's opinion, patients with skin of color usually develop feelings of acceptance by the time discussions regarding depigmentation are broached. Some providers suggest or require patients to visit a psychologist or psychiatrist before depigmentation is started. The senior author has no patients of color who regretted their decision to depigment their skin.

Depigmentation can be achieved using topical phenolic compounds, laser therapy, and cryotherapy [50, 51]. However, monobenzyl ether of hydroquinone (MBEH), a phenol derivative, is the most commonly used depigmenting agent for vitiligo. The mechanism of action is not completely understood, but it has been shown to cause selective necrosis of non-follicular human melanocytes [52]. This may be due to its structural homology with tyrosine. Full depigmentation may require 4–12 months of therapy and generally takes longer in patients with darker skin [53, 54].

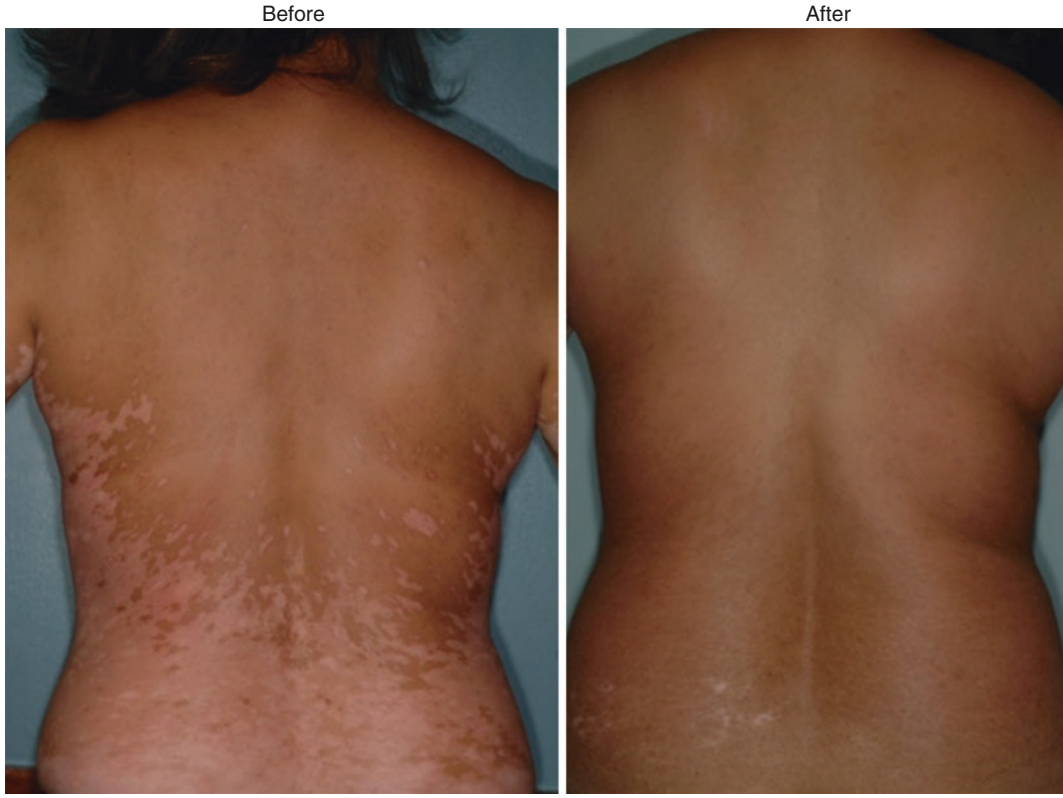


Fig. 15.4 Narrowband UVB phototherapy. Note significant repigmentation of the back

Depigmentation with MBEH is permanent in most cases and is histologically associated with loss of melanosomes and melanocytes; however spontaneous repigmentation can occur within weeks to years after discontinuation of therapy [54]. The mechanism of spontaneous repigmentation after therapy is not well characterized but may occur due to activation of the follicular melanocytes which are unaffected by MBEH [51].

In a retrospective study of 53 patients at a London hospital undergoing depigmentation, the majority had olive or darker skin [55]. The highest concentration MBEH tolerated was 20%. While 34% of the patients achieved marked but incomplete depigmentation, repigmentation was high in sun-exposed areas (78%). In the senior author's experience, in darker skin individuals who experience significant repigmentation after treatment, further depigmentation can be therapeutically challenging.

15.7 Conclusion

Our current database documents an equal incidence of vitiligo in all racial ethnic groups. However, the condition is most disfiguring in darker skin types given the contrast between the normal skin and white patches. The disorder is often psychologically devastating for darker skin types. No studies have reported differences in pathogenesis. However, multiple studies document enhanced repigmentation responses in skin of color.

References

1. Taieb A, Picardo M. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell Res. 2007;20:27–35.
2. Passeron T, Ortonne JP. Physiopathology and genetics of vitiligo. J Autoimmun. 2005;25(Suppl):63–8.

3. Alikhan A, Felsten LM, Daly M, et al. Vitiligo: a comprehensive overview Part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. J Am Acad Dermatol. 2011;65:473–91.
4. Valia AK, Dutta PK. IADVL text book and atlas of dermatology. Bombay: Bhalani Publishing House; 1996.
5. Krüger C, Schallreuter KU. A review of the worldwide prevalence of vitiligo in children/adolescents and adults. Int J Dermatol. 2012;51(10):1206–12.
6. Ezzedine K, Eleftheriadou V, Whitton M, van Geel N. Vitiligo. Lancet. 2015;386:74–84.
7. Ezzedine K, Pandya A. Time for a patient-oriented outcome in vitiligo: the vitiligo noticeability scale. Br J Dermatol. 2016;174:255–6.
8. Porter J. The psychological effects of vitiligo: response to impaired appearance. In: Hann SK, Nordlund JJ, editors. Vitiligo. Oxford, UK: Blackwell Science; 2000. p. 97.
9. Talsania N, Lamb B, Bewley A. Vitiligo is more than skin deep: a survey of members of the Vitiligo Society. Clin Exp Dermatol. 2010;35:736.
10. Pahwa P, Mehta M, Khaitan BK, et al. The psychosocial impact of vitiligo in Indian Patients. Indian J Dermatol Venereol Leprol. 2013;79:679.
11. Thompson AR, Clarke SA, Newell RJ, et al. Vitiligo linked to stigmatization in British South Asian women: a qualitative study of the experiences of living with vitiligo. Br J Dermatol. 2010;163:481.
12. Linthorst Homan MW, Spuls PI, de Korte J, et al. The burden of vitiligo: patient characteristics associated with quality of life. J Am Acad Dermatol. 2009;61:411.
13. Ezzedine K, et al. Living with vitiligo: results from a national survey indicate differences between skin phototypes. Br J Dermatol. 2015;173:607–9.
14. Salman A, Kurt E, Topcuoglu V, Demicray Z. Social anxiety and quality of life in vitiligo and acne patients with facial involvement: a cross-sectional controlled study. Am J Clin Dermatol. 2016;17:305–11.
15. Speeckaert R, Lambert J, van Geel N. Measuring the impact of vitiligo: behind the white spots. J Invest Dermatol. 2016;136:6–7.
16. Salzes C, Abadie S, Seneschal J, et al. The Vitiligo Impact Patient scale (VIPs): development and validation of a vitiligo burden assessment tool. J Invest Dermatol. 2016;136:52–8.
17. Krishna FS, Ramam M, Mehta M, et al. Vitiligo impact scale: an instrument to assess the psychological burden of vitiligo. Indian J Dermatol Venereal Leprol. 2013;79:205–10.
18. Gupta V, Sreenivas V, Mehta M, et al. Measurement of the Vitiligo Impact Scale-22 (VIS-22), a vitiligo-specific quality-of-life instrument. Br J Dermatol. 2014;171:1084–90.
19. Ramakrishna P, Rajni T. Psychiatric morbidity and quality of life in vitiligo patients. Indian J Psychol Med. 2014;36:302–3.
20. Taylor SC, Heath C. Cultural competence and unique concerns in patients with ethnic skin. J Drugs Dermatol. 2012;11:460–5.
21. Lilly E, Lu PD, Borovicka JH, et al. Development and validation of a vitiligo-specific quality of life instrument (VitiQoL). J Am Acad Dermatol. 2013;69:e11–8.
22. Boza JC, Kundu RV, Fabbrin A, Horn R, et al. Translation, cross-cultural adaptation and validation of the vitiligo-specific health-related quality of life instrument (VitiQoL) into Brazilian Portuguese. An Bras Dermatol. 2015;90:358–62.
23. Fain PR, Gowan K, LaBerge GS, et al. A genomewide screen for generalized vitiligo: confirmation of AIS1 on chromosome 1p31 and evidence for additional susceptibility loci. Am J Hum Genet. 2003;72:1560–4.
24. Alkhateeb A, Fain PR, Thody A, et al. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. Pigment Cell Res. 2003;16:208–14.
25. Czajkowski R, Męcińska-Jundziłł K. Current aspects of vitiligo genetics. Postepy Dermatol Alergol. 2014;31:247.
26. Chen H, Huang W, Gut JP, et al. *A novel linkage to generalized vitiligo* on *4q13-q21* identified in a genomewide linkage analysis of Chinese families. Am J Hum Genet. 2005;76(6):1057–65.
27. Grimes PE, Halder RM, Jones C, et al. Autoantibodies and their clinical significance in a black vitiligo population. Arch Dermatol. 1983;119:300.
28. Dash R, Mohapatra A, Man-Junathswamy BS. Antithyroid peroxidase antibody in vitiligo: a prevalence study. J Thyroid Res. 2015;2015:1–8.
29. Adorini L, Penna G. Control of autoimmune disease by the vitamin D endocrine system. Nat Clin Pract Rheumatol. 2008;4:404–12.
30. Brilea SA, Costin GE, Norris DA. Cellular and molecular mechanisms involved in the action of Vitamin D analogs targeting vitiligo depigmentation. Curr Drug Targets. 2008;9(4):345–59.
31. Li K, Shi Q, Yang L, Li X, Liu L, Wang L, et al. The association of vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D levels with generalized vitiligo. Br J Dermatol. 2012;167:815–21.
32. Silverberg JI, Silverberg AI, Malka E, Silverberg NB. A pilot study assessing the role of 25 hydroxy vitamin D levels in patients with vitiligo vulgaris. J Am Acad Dermatol. 2010;62:937–4.
33. Saleh HM, Abdel Fattah NS, Hamza HT. Evaluation of serum 25-hydroxyvitamin D levels in vitiligo patients with and without autoimmune diseases. Photodermatol Photoimmunol Photomed. 2013;29(1):34–40.
34. Doss RW, El-Rafail AA, Gohary YM, Rashed LA. Vitimin D receptor expression in vitiligo. Int J Dermatol. 2015;60:544–8.
35. Grimes PE. Vitiligo. In: Taylor SC, Kelly AP, Lim HW, Serrano AMA, editors. Taylor and Kelly's dermatology for skin of color. New York, NY: McGraw-Hill; 2016. p. 341–50.

36. Gan EY, Cario-André M, Pain C, Goussot JF, Taïeb A, Seneschal J, Ezzedine K. Follicular vitiligo: a report of 8 cases. J Am Acad Dermatol. 2015;74:1178–84.
37. Ezzedine K, Mahé A, van Geel N, Cardot-Leccia N, Gauthier Y, Descamps V, Al Issa A, Ly F, Chosidow O, Taïeb A, Passerson T. Hypochromic vitiligo: delineation of a new entity. Br J Dermatol. 2014;172:716–21.
38. Hann SK, Kim YS, Yoo JH, Chun YS. Clinical and histopathologic characteristics of trichrome vitiligo. J Am Acad Dermatol. 2000;42(4):589–96.
39. Di Chiacchio NG, Ferreira FR, de Alvarenga ML, Baran R. Nail trichrome vitiligo: a case report and literature review. Br J Dermatol. 2013;168(3):668–9.
40. Lee DY, Kim CR, Lee KH. Trichrome vitiligo in segmental type. Photodermatol Photoimmunol Photomed. 2011;27(2):111–2.
41. Silverberg JI, Silverberg NB. Topical tacrolimus is more effective for treatment of vitiligo in patiens of skin of color. J Drugs Dermatol. 2011;10:507–10.
42. Yones SS, Palmer RA, Garibaldinos TM, Hawk JL. Randomized double-blind trial of treatment of vitiligo: efficacy psoralen UVA therapy versus narrowband UVB therapy. Arch Dermatol. 2007;143:578–84.
43. Bhatnagar A, Kanwar AJ, Parsad D. Comparison of systemic PUVA and NB-UVB in the treatment of vitiligo: an open prospective study. J Eur Acad Dermatol Venereol. 2007;21:638–42.
44. Nicolaidou E, Antoniou C, Stratigos AJ, Stefanaki C, Katsambas AD. Efficacy, predictors of response, and long-term follow-up in patients with vitiligo treated with narrowband UVB phototherapy. J Am Acad Dermatol. 2007;56:274–8.
45. Kanwar AJ, Dogra S, Parsad D, Kumar B. Narrowband UVB for the treatment of vitiligo: an emerging effective and well tolerated therapy. Int J Dermatol. 2005;44:57–60.
46. Kanwar AJ, Dogra S. Narrow-band UVB for the treatment of generalized vitiligo in children. Clin Exp Dermatol. 2005;30:332–6.
47. Chen GY, Hsu MM, Tai HK, Chou TC, Tseng CL, Chang HY, et al. Narrow-band UVB treatment of vitiligo in Chinese. J Dermatol. 2005;32:793–800.
48. Grimes PE, Hamzavi I, Lebwohl M, Ortonne JP, Lim HW. JAMA Dermatol. 2013;149:68–73.
49. Lim HW, Grimes PE, Agbai O, et al. Afamelanotide and narrowband UV-B phototherapy for the treatment of vitiligo: a randomized multicenter trial. JAMA Dermatol. 2015;151(1):42–50.
50. Alghamdi KM, Kumar A. Depigmentation therapies for normal skin in vitiligo universalis. J Eur Acad Dermatol Venereol. 2011;25:749–57.
51. Gupta D, Kumari R, Thappa DM. Depigmentation therapies in vitiligo. Indian J Dermatol Venereol Leprol. 2012;78:49–58.
52. Hariharan V, Klarquist J, Reust M, et al. Monobenzylether of hydroquinone and 4-tertiary butyl phenol activate markedly different physiologic responses in melanocytes: relevance to skin depigmentation. J Invest Dermatol. 2010;130:211–20.
53. Mosher DB, Parrish JA, Fitzpatrick TB. Monobenzylether of hydroquinone. Br J Dermatol. 1977;97:669–79.
54. Grimes PE, Nashawati R. The Role of Diet and Supplements in Vitiligo Management. Dermatol Clin. 2017Apr;35(2):235–243..
55. Tan ES, Sarkany R. Topical monobenzyl ether of hydroquinone is an effective and safe treatment for depigmentation of extensive vitiligo; a retrospective cohort of 53 cases. Br J Dermatol. 2015;172:166.2–166.

16 Vitiligo-Like Lesions in Patients with Metastatic Melanoma Receiving Immunotherapies

Katia Boniface and Julien Seneschal

Contents

Abstract

The development of immunotherapies has remarkably improved the efficacy of treatment of patients with metastatic melanoma, as observed for example with immune checkpoint therapies targeting cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or programmed cell death-1 (PD-1). Nonetheless, reinstatement of an effective antitumor immune response also contributes to the development of immune-related adverse events, including vitiligo-like depigmentation. This review aims to discuss special considerations in vitiligo-like lesions occurring in patients receiving anti-PD-1 therapies from a clinical and physiopathological perspective.

Key Points

- Vitiligo-like lesions are one of the most frequent immune-related adverse events in melanoma patients receiving anti-PD-1 therapies.
- Vitiligo-like lesions occurring under anti-PD-1 are associated with clinical benefit and overall survival in patients with metastatic melanoma.
- Vitiligo-like lesions occurring in metastatic melanoma patients receiving anti-PD-1 harbor a strong type 1 skewed T cell phenotype.
- Vitiligo-like lesions in patients receiving anti-PD-1 clinically differ from vitiligo.

K. Boniface
1INSERM U1035, BMGIC, Immuno-dermatology team, University of Bordeaux, Bordeaux, France

J. Seneschal (✉)
1INSERM U1035, BMGIC, Immuno-dermatology team, University of Bordeaux, Bordeaux, France

Department of Dermatology and Pediatric Dermatology, National Reference Center for Rare Skin Diseases, Saint-André Hospital, University of Bordeaux, Bordeaux, France
e-mail: julien.seneschal@chu-bordeaux.fr

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_16

16.1 Introduction

Immunotherapy is poised to play a more central role in the treatment of metastatic melanoma. These therapies have shown variable success rated in the antitumor response. However, the use of these therapies is also associated with the development of several autoimmune side effects, including vitiligo-like lesions. Interestingly, development of vitiligo-like lesions (a clinically visible immune-related adverse event) has been correlated with increased survival in patients receiving immunotherapies [1]. Vitiligo-like lesions seem to appear frequently in patients receiving anti-programmed cell death-1 (PD-1) therapies, and recent data suggest that the pattern of depigmentation is different from spontaneously occurring vitiligo [2, 3].

16.2 General Background

Several immunotherapies have been evaluated in the treatment of metastatic melanoma including (1) immune stimulation with interferon (IFN)-alpha or interleukin (IL)-2; (2) vaccination strategies using vaccines based on dendritic cells, tumor cells, tumor antigenic peptides, and/or gene transfers; or (3) more recently, neutralizing antibodies directed against immunosuppressive checkpoints, such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or PD-1 or its ligand PD-L1 [4, 5]. Some of these strategies have shown clear antitumor activity in the treatment of advanced melanoma, suggesting a promising role for these novel immunotherapies in the treatment of metastatic melanoma. This was exemplified with the use of selective inhibitors of PD-1 (pembrolizumab, nivolumab), which have shown impressive clinical results in the treatment of metastatic melanoma, leading to an accelerated development and authorization [6, 7].

However, the use of immunotherapies is often associated with increased burden of toxicity, and some of them are immune-related adverse effects. Indeed, restoring effective tumor immunity requires the induction of the same responses that underlie autoimmunity. Among these autoimmune side effects, one is of particular interest in the context of melanoma: the occurrence of vitiligo-like lesions. It has been recently shown in a systemic review published by Teulings et al. [1] that while occurring in a low percentage of patients with melanoma treated with immunotherapy, vitiligo-like lesions were significantly associated with overall survival (HR 0.25; 95% CI 0.10–0.61). It has been estimated that the cumulative incidence of vitiligo-like lesions was 3.4% under immunotherapies. Richards et al. described first a positive correlation between vitiligo-like lesions with tumor regression in patients treated with sequences of chemotherapy (carmustine, dacarbazine, and cisplatin) and immunotherapy (IL-2 and IFN alfa-2) [8]. Boasberg et al. reported an incidence of vitiligo-like depigmentations of 43% in 49 patients with metastatic melanoma who were treated with injections of IL-2 after the induction of chemotherapy [9]. A positive correlation of autoimmune manifestations and clinical response in patients with metastatic melanoma who were treated with anti-CTLA4 antibodies has also been reported [10]. Of interest, vitiligo-like lesions in patients with metastatic melanoma treated with anti-PD-1 neutralizing antibodies occur more frequently than with other immunotherapies (further detailed below) [1]. Moreover, vitiligoid lesions are a much more common adverse event in melanoma immunotherapy compared with immunotherapy for other solid tumors [11, 12].

16.3 Special Considerations in Vitiligo-Like Lesions in Patients Receiving Anti-PD-1 Therapies

Vitiligo-like depigmentation is one of the most frequent dermatologic adverse events observed with the use of anti-PD-1 therapies in cancer patients, together with rash and pruritus [13, 14]. In patients with metastatic melanoma receiving anti-PD-1, occurrence of vitiligo-like depigmen-

tations ranges from 8% to 25%, which is about tenfold higher than the occurrence of vitiligo in the general population [7, 13, 15–18]. In line with these observations, clinical studies on patients with metastatic melanoma receiving anti-PD-1 therapies reported an objective response and overall survival benefit that were associated with development of vitiligo-like lesions in patients [2, 15, 19].

PD-1 is a well-known negative immune checkpoint receptor expressed by T cells. PD-L1/PD1 pathway causes T-cell apoptosis, anergy, and exhaustion. Therefore, PD-1 targeting on T cells with pembrolizumab or nivolumab will restore T-cell activation that will be involved in immune responses to tumor but will also lead to cytotoxic effects responsible for development of adverse events, as vitiligo-like lesions. Interestingly, all the patients receiving anti-PD-1 therapies that developed vitiligo-like lesions have been treated with anti-PD-1 for metastatic melanoma [13].

Histological analysis of vitiligo-like lesions in patients receiving anti-PD-1 showed a prominent CD8 T-cell infiltration and that the majority of these cells expressed the chemokine receptor CXCR3 and produced high levels of interferon-γ and tumor necrosis factor-α [3]. In line with these observations, PD-1 blockade induces the expression of the IFN-γ-inducible chemokine CXCL10 at the tumor site in mice [20], leading to the homing of $CXCR3^+$ T cells. Interestingly, it was recently shown in a mouse model of melanoma that CD8 T-cell responses to melanoma initiate autoimmune vitiligo and melanocyte destruction was required for robust CD8 T-cell-mediated antitumor response [21]. Therefore, the mechanistic correlation between vitiligo-like lesions and response to checkpoint therapies may shed light on the mechanisms of successful immune attack of melanoma cells and incidental attack of normal melanocytes. Microphthalmia-associated transcription factor (MITF) that induces the transcription of many genes important in pigment production and the maintenance of the melanocyte, both in melanoma and normal cells, could also play an important role. MITF is important for promoting survival of melanocytes after UV exposure and DNA damage. Indeed, the original signal that drives MITF is UV-induced DNA damage through p53, important for the promotion of melanocytes survival after UV exposure [22, 23]. Some of the MITF-regulated genes may contain tumor-specific neoantigens, and others may represent melanocyte lineage-specific antigens (such as melan A, gp100, and tyrosinase-related protein-2) [21, 24–26]. Therefore, activation of the immune system against MITF-associated epitopes with anti-PD-1 could result in destruction of melanoma and sometimes destruction of normal melanocytes.

Of interest is our recent observation that vitiligo-like depigmentation in patients receiving anti-PD-1 therapies occurs mainly on photoexposed areas associated with pre-existing solar lentigines, with a specific depigmentation pattern consisting of multiple flecked lesions, without the involvement of a Koebner phenomenon (Fig. 16.1) [3]. A case report also described the disappearance of pigmented skin lesions with anti-PD-1 in a patient with metastatic melanoma, including naevi, seborrheic keratoses, and solar lentigines [27]. These clinical observations contrast with spontaneously occurring vitiligo characterized by white patches symmetrically distributed, and most patients develop lesions on repetitive friction sites. In addition, no history of vitiligo of other autoimmune diseases were reported by patients receiving anti-PD-1 therapies, suggesting that vitiligo-like depigmentation in these patients is clinically distinct from vitiligo.

Therefore, melanocyte loss in vitiligo-like depigmentation occurring in patients receiving anti-PD-1 therapies appears to mainly result from melanocyte destruction, while in vitiligo two main mechanisms have been involved: destruction by the immune system and detachment from the basal layer of the epidermis [28, 29]. Moreover, the occurrence of vitiligo-like lesions mainly on previously sun-exposed areas supports the possible link between MITF and the immune response reinforced by immune checkpoint therapies. Hence, the mechanisms involved in melanocyte loss in spontaneously occurring vitiligo and vitiligo-like lesions in patients receiving anti-PD-1 therapies might be different, and future

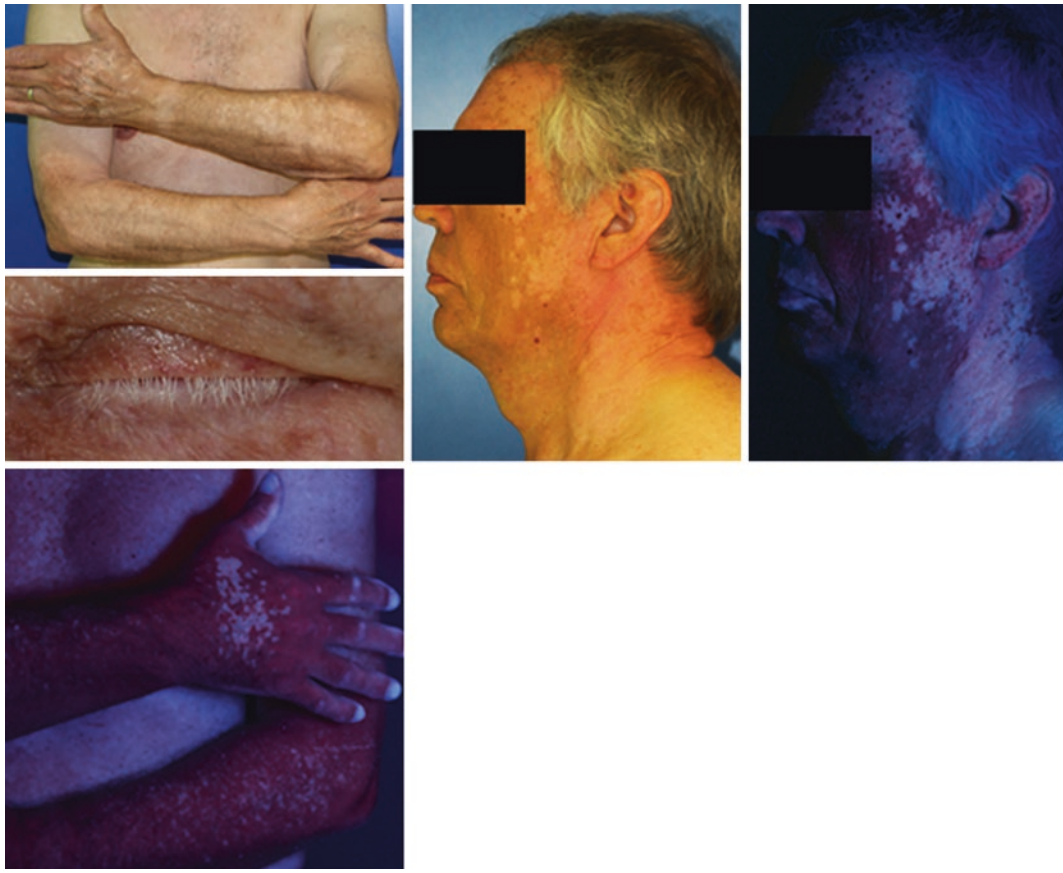


Fig. 16.1 Clinical pattern of depigmentation in patients receiving anti-PD-1 therapies

studies should in depth characterize and compare the molecular pathways involved in melanocyte disappearance in these two conditions.

References

1. Teulings HE, Limpens J, Jansen SN, Zwinderman AH, Reitsma JB, Spuls PI, et al. Vitiligo-like depigmentation in patients with stage III-IV melanoma receiving immunotherapy and its association with survival: a systematic review and meta-analysis. J Clin Oncol. 2015;33(7):773–81.
2. Hua C, Boussemart L, Mateus C, Routier E, Boutros C, Cazenave H, et al. Association of vitiligo with tumor response in patients with metastatic melanoma treated with pembrolizumab. JAMA Dermatol. 2016;152(1):45–51.
3. Larsabal M, Marti A, Jacquemin C, Rambert J, Thiolat D, Dousset L, et al. Vitiligo-like lesions occurring in patients receiving anti-programmed cell death-1 therapies are clinically and biologically distinct from vitiligo. J Am Acad Dermatol. 2017;76(5):863–70.
4. Achkar T, Tarhini AA. The use of immunotherapy in the treatment of melanoma. J Hematol Oncol. 2017;10(1):88.
5. Anguille S, Smits EL, Lion E, van Tendeloo VF, Berneman ZN. Clinical use of dendritic cells for cancer therapy. Lancet Oncol. 2014;15(7):e257–67.
6. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372(4):320–30.
7. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med. 2015;372(26):2521–32.
8. Richards JM, Mehta N, Ramming K, Skosey P. Sequential chemoimmunotherapy in the treatment of metastatic melanoma. J Clin Oncol. 1992;10(8):1338–43.
9. Boasberg PD, Hoon DS, Piro LD, Martin MA, Fujimoto A, Kristedja TS, et al. Enhanced survival

associated with vitiligo expression during maintenance biotherapy for metastatic melanoma. J Invest Dermatol. 2006;126(12):2658–63.
10. Bertrand A, Kostine M, Barnetche T, Truchetet ME, Schaeverbeke T. Immune related adverse events associated with anti-CTLA-4 antibodies: systematic review and meta-analysis. BMC Med. 2015;13:211.
11. Rosenberg SA, White DE. Vitiligo in patients with melanoma: normal tissue antigens can be targets for cancer immunotherapy. J Immunother Emphasis Tumor Immunol. 1996;19(1):81–4.
12. Sibaud V, David I, Lamant L, Resseguier S, Radut R, Attal J, et al. Acute skin reaction suggestive of pembrolizumab-induced radiosensitization. Melanoma Res. 2015;25(6):555–8.
13. Belum VR, Benhuri B, Postow MA, Hellmann MD, Lesokhin AM, Segal NH, et al. Characterisation and management of dermatologic adverse events to agents targeting the PD-1 receptor. Eur J Cancer. 2016;60:12–25.
14. Sibaud V, Meyer N, Lamant L, Vigarios E, Mazieres J, Delord JP. Dermatologic complications of anti-PD-1/PD-L1 immune checkpoint antibodies. Curr Opin Oncol. 2016;28(4):254–63.
15. Freeman-Keller M, Kim Y, Cronin H, Richards A, Gibney G, Weber JS. Nivolumab in resected and unresectable metastatic melanoma: characteristics of immune-related adverse events and association with outcomes. Clin Cancer Res. 2016;22(4):886–94.
16. Goldinger SM, Stieger P, Meier B, Micaletto S, Contassot E, French LE, et al. Cytotoxic cutaneous adverse drug reactions during anti-PD-1 therapy. Clin Cancer Res. 2016;22(16):4023–9.
17. Hwang SJ, Carlos G, Wakade D, Byth K, Kong BY, Chou S, et al. Cutaneous adverse events (AEs) of anti-programmed cell death (PD)-1 therapy in patients with metastatic melanoma: a single-institution cohort. J Am Acad Dermatol. 2016;74(3):455–61.e1.
18. Sanlorenzo M, Vujic I, Daud A, Algazi A, Gubens M, Luna SA, et al. Pembrolizumab cutaneous adverse events and their association with disease progression. JAMA Dermatol. 2015;151(11):1206–12.
19. Nakamura Y, Tanaka R, Asami Y, Teramoto Y, Imamura T, Sato S, et al. Correlation between vitiligo occurrence and clinical benefit in advanced melanoma patients treated with nivolumab: a multi-institutional retrospective study. J Dermatol. 2017;44(2):117–22.
20. Peng W, Liu C, Xu C, Lou Y, Chen J, Yang Y, et al. PD-1 blockade enhances T-cell migration to tumors by elevating IFN-gamma inducible chemokines. Cancer Res. 2012;72(20):5209–18.
21. Byrne KT, Cote AL, Zhang P, Steinberg SM, Guo Y, Allie R, et al. Autoimmune melanocyte destruction is required for robust CD8+ memory T cell responses to mouse melanoma. J Clin Invest. 2011;121(5):1797–809.
22. Hsiao JJ, Fisher DE. The roles of microphthalmia-associated transcription factor and pigmentation in melanoma. Arch Biochem Biophys. 2014;563:28–34.
23. Levy C, Khaled M, Fisher DE. MITF: master regulator of melanocyte development and melanoma oncogene. Trends Mol Med. 2006;12(9):406–14.
24. Kawakami Y, Suzuki Y, Shofuda T, Kiniwa Y, Inozume T, Dan K, et al. T cell immune responses against melanoma and melanocytes in cancer and autoimmunity. Pigment Cell Res. 2000;13(Suppl 8):163–9.
25. Wang RF, Appella E, Kawakami Y, Kang X, Rosenberg SA. Identification of TRP-2 as a human tumor antigen recognized by cytotoxic T lymphocytes. J Exp Med. 1996;184(6):2207–16.
26. Yee C, Thompson JA, Roche P, Byrd DR, Lee PP, Piepkorn M, et al. Melanocyte destruction after antigen-specific immunotherapy of melanoma: direct evidence of t cell-mediated vitiligo. J Exp Med. 2000;192(11):1637–44.
27. Wolner ZJ, Marghoob AA, Pulitzer MP, Postow MA, Marchetti MA. A case report of disappearing pigmented skin lesions associated with pembrolizumab treatment for metastatic melanoma. Br J Dermatol. 2018;178:265.
28. Gauthier Y, Cario Andre M, Taieb A. A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? Pigment Cell Res. 2003;16(4):322–32.
29. Sandoval-Cruz M, Garcia-Carrasco M, Sanchez-Porras R, Mendoza-Pinto C, Jimenez-Hernandez M, Munguia-Realpozo P, et al. Immunopathogenesis of vitiligo. Autoimmun Rev. 2011;10(12):762–5.

Evaluation, Assessment, and Scoring

17

Alain Taïeb and Mauro Picardo

Contents

Key Points

- At the first visit enough time is needed for a clinical evaluation.
- A checklist of important items is useful.
- The VETF assessement and scoring system covers most important items.
- For scoring, extent, staging, and progression dimensions need to be covered.
- Wood's lamp examination is important in light skin for all scoring dimensions.
- Subjective items need to be considered, a global visual analog scale as used in VETF is usually sufficient in clinical routine.

A. Taïeb
Service de Dermatologie, Hôpital St André, CHU de Bordeaux, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

M. Picardo (✉)
Cutaneous Physiopathology and CIRM, San Gallicano Dermatological Institute, IFO, Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

17.1 Introduction

The first contact with a patient coming with a previous diagnostic of vitiligo is of major importance. He/she says generally that previous doctors have not been keen to engage on a conventional

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_17

doctor-patient relationship about their disease, which they said was benign but out of therapeutic reach. This common approach denies the status of patient and explains the long search for alternative sources of cure. Secondly, it is important to check the accuracy of the diagnosis (Chap. 2), because in a subset of patient, the depigmenting disease has been labelled loosely "vitiligo" for some reason, and patients are grateful to obtain a diagnosis, if not a cure. This is commonly the case in children consulting for segmental hypopigmentation (Chap. 14).

There is a current lack of consensus in methods of assessment of vitiligo, which makes it generally impossible to compare the outcomes of different studies of the same treatment and to perform meta-analyses [1]. In order to address these problems, the Vitiligo European Task Force (VETF) was founded in 2003 during the ESPCR meeting in Ghent with three initial goals: (1) the proposal of a consensus definition of the disease (Chap. 2), (2) the design of biometric tools to assess disease severity/stability, and if possible (3) the derivation of a consensus scoring system. The second and third step are still ongoing, but following a method already used for the SCORAD index in atopic dermatitis [2], an evaluation form was discussed and gradually improved at several meetings which included the input of patient's support groups and tested in patients with generalized vitiligo in a dozen of academic medical centers. Secondly, the most relevant descriptive variables were selected for severity assessment and prognosis. Following this step, the VETF held a workshop in Rome to test and validate the evaluation sheet on a series of patients [3]. This chapter is written following the remarks and criticism, which have followed the publication of this report [3]. An updated and simplified version has been produced more recently [4].[1]

[1]The last version is also available online at http://content.nejm.org/cgi/content/full/360/2/160/DC1.

17.2 Step-by-Step Evaluation

It is necessary to take enough time for a first visit, which includes a comprehensive clinical evaluation. The time required is roughly 20 min (an identical extra time is needed for counseling and replying to questions). For lightly pigmented patients, a dark room is needed for Wood's lamp examination (Fig. 17.1). The help of a standardized sheet as available in the appendix allows not to miss some issues and to secure the relevant information for routine clinical work or research, which is summarized in Table 17.1. It includes the analysis of the Koebner phenomenon (Chap. 11) is of particular interest for prevention [5]. A question about vitiligo on genitals is on the VETF checklist because it causes a strong embarrassment to patients. Itch preceding lesions may correspond to the micro-inflammatory nature of the disease. This symptom was mentioned by the patient's support groups. However, since common disorders such as atopic dermatitis may coexist, especially in children, the exact value of this symptom needs more in-depth investigations, and questioning should address a topographic relation to the vitiligo patches.

Family history of vitiligo and/or premature hair graying is currently routinely part of the assessment, as well as personal and family history of thyroid disease and the presence of thyroid autoantibodies and autoimmune diseases. Hair graying is defined as more than 50% of white/gray hairs before the age of 40. This item is generally more difficult to assess in the female ancestry. It is commonly found in pedigrees of vitiligo patients [3, 6] and suggests the role of an aging defect in the pigmentary system (Chap. 9). For the personal/family history of chronic possible autoimmune/inflammatory disorders, Table 17.2 indicates some hints to ask questions, because the related diseases are not always known by nonprofessionals. Clinical examination should guide the need of specialized investigations (see below). Halo nevi, considered as a marker of cellular autoimmunity (Chap. 9), are assessed by history and at examination. A global quality of life assessment is recommended ("how does vitiligo cur-

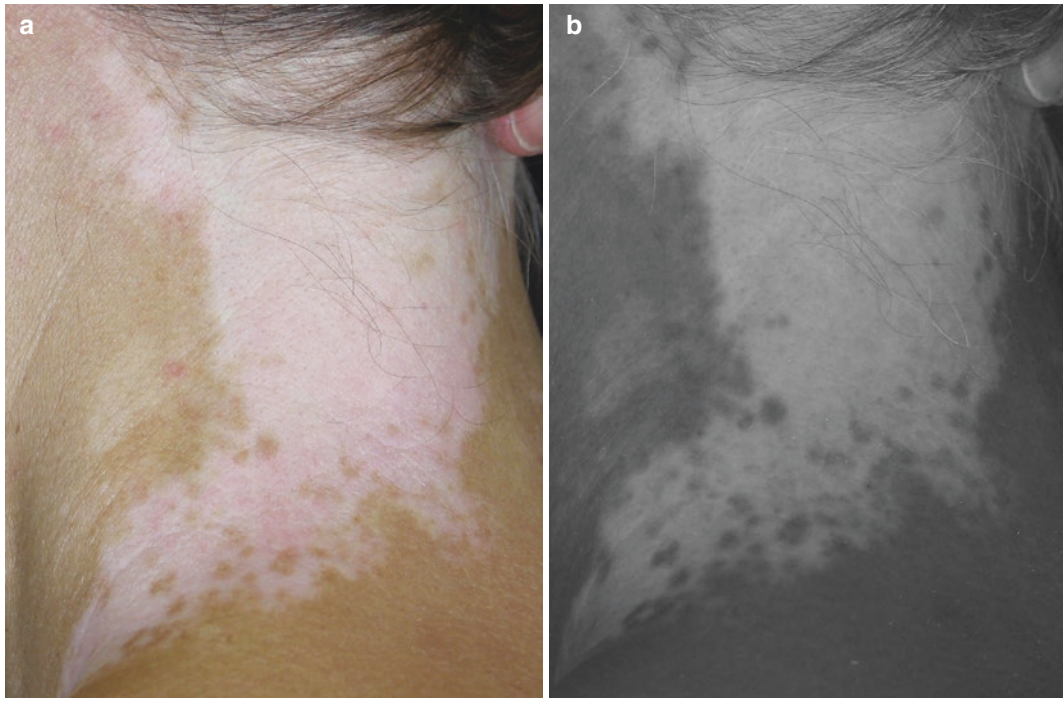


Fig. 17.1 Wood's lamp examination: it is best performed in a completely dark room. The examiner should adapt to the darkness for at least 30 s before starting examining the patient. Note the bright reflection of white patches under Wood's lamp (**b**) and details on intermediate pigment tones to compare with (**a**). A magnifying lens incorporated to the lamp is useful to analyze (terminal and vellus) hair pigmentation

Table 17.1 Evaluation checklist for NSV (adapted from Taieb and Picardo [3])

Subject features	Disease features	Family	Interventions
Phototype (Fitzpatrick's skin type)	Duration	Premature hair graying	Type and duration of previous treatments including opinion of patient on previous treatments (list): useful/not useful
Ethnic origin	Activity, based on patient's opinion (progressive, regressive, stable over the last 6 months)	Vitiligo (detail if needed in family tree)	Current treatment(s)
Age at onset	Previous episodes of repigmentation, and if yes, spontaneous or not (details)		Other diseases and treatments (list)
Stress/anxiety levels	Koebner phenomenon on scars or following mechanical trauma		
Halo nevus	Itch before flares		
History of autoimmune diseases: if yes, which type	Thyroid disease, if yes detail, including the presence of thyroid autoantibodies		
Global QoL assessment (10 cm analog scale) "how does vitiligo currently (last week) affect your everyday life"	Vitiligo on genitals	History of autoimmune diseases (detail if needed in family tree)	
	Clinical photographs and if possible UV light photographs		

rently (last week) affects your everyday life" or "how much have you been bothered by the white spots last week" assessed on a visual analog scale). The impact of stress on disease onset/progression is also taken into account. This form has been designed for NSV but can serve also for segmental or unclassified forms of the disease.

17.3 Associated Disorders and Laboratory Workup

Because of the magnitude of the association with autoimmune thyroid disease with NSV (25% in our recruitment), the assessment of thyroid function (thyrotropin, thyroid hormones) and presence of antibodies to thyroid peroxidase (TPO)/thyroglobulin should be routinely performed. A specific follow-up is needed when Hashimoto's thyroiditis and Graves' disease are diagnosed with referral to an endocrinologist. Associated autoimmune disease frequencies are apparently not similar according to ethnic background [7, 8]. Some data emphasize the need of a larger preventive screening because vitiligo may precede the onset of organ-specific autoimmune diseases [9]. If a personal and/or family history of autoimmune/auto-inflammatory disorders is detected (Table 17.2), broadening the etiologic investigations more specifically should be considered. In the context of familial autoimmune syndromes (Chap. 13) such as autoimmune polyendocrine syndrome (APS), anti-21-hydroxylase autoantibodies (Addison's) and anti-NALP 5 antibodies (hypoparathyroidism) [10] can be looked for. For precursors to diabetes, antibodies can be searched against GAD65 and ICA512. A specialized advice is strongly suggested.

Table 17.2 Assessment of chronic autoimmune/inflammatory diseases

Ask by name	Ask by symptoms or treatments
Alopecia areata	Chronic diarrhea
Psoriasis, psoriatic arthritis	Arthritis
Lupus	Hair loss
Scleroderma	Chronic bowel disease
Lichen planus, lichen sclerosus	Chronic skin disease
Rheumatoid arthritis	
Ulcerative colitis	Insulin
Crohn's disease	Thyroxin
Biermer's (pernicious) anemia	(Hydro)cortisone
Addison's disease	Vitamin B12
Celiac disease	

17.4 Skin Biopsy

A skin biopsy is usually not requested for common NSV, except when another diagnosis cannot be ruled out (Chap. 2). However, this point of view needs probably to be challenged. Since skin inflammation in vitiligo seems clinically not detectable (Chap. 8), this simple test might allow a more precise "inflammatory" staging of vitiligo in the close future (pending the implementation of less invasive tools). The finding of a micro-inflammatory border in active NSV (Chap. 3)—and/or of other local markers of disease activity—might become useful to make more appropriate therapeutic decisions.

17.5 Scoring

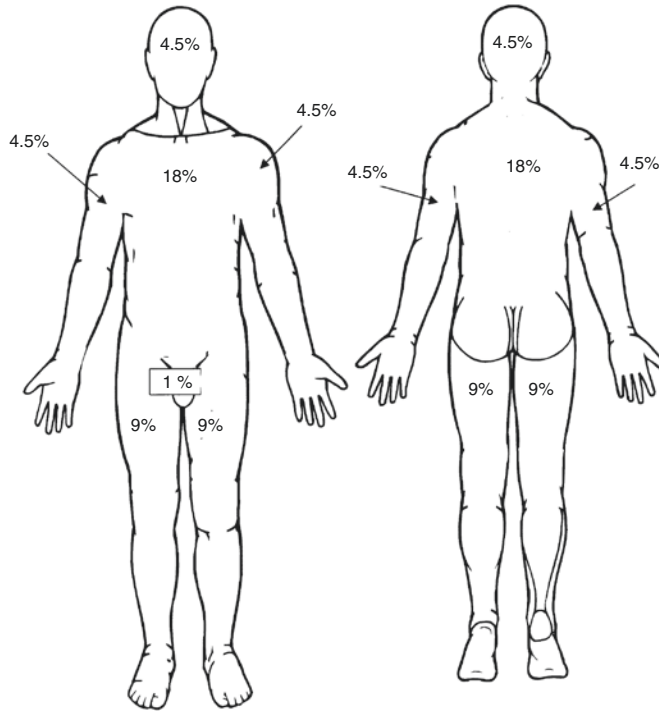
Vitiligo treatments have been previously analyzed using the proportion of treated patients who achieve a specified degree of repigmentation, usually >50% for a "good" response [11], which is much lower than patient's expectations. A quantitative score has been recently proposed [12]. The VETF has partially validated a clinical method of assessment which combines analysis of extent (rule of nines), grading of depigmentation, and progression. Figure 17.2 details the scoring system. Several problems have been raised during the patient's session [3]. For staging, color of the selected patch is clearly not homogeneous, especially in SV. Staging chosen by investigators generally reflects the worst stage and not the most representative stage. This poses problems when a few white hairs are present in association with skin repigmentation, and more than 30% is required for the worst stage in the revised scoring system [4]. Another difficulty was related to the need to magnify the lesions to assess hairs,

a

Area	% Area	Staging* (0–4)	Spreading* (−1+1)
Head and neck (0–9%)			
Trunk (0–36%)			
Arms (0–18%)			
Legs (0–36%)			
Hands and feet			
Totals (0–100%)		0–20	(−5 +5)

*Largest patch in each area

b



c

Fig. 17.2 Scoring system (adapted from Taieb and Picardo [4]). The system assesses three dimensions of the disease (extent, staging, spreading/progression), which are summarized in a table for practical purposes (Part **a** of panel). To assess **extent** (Part **b**), it is useful to refer to the patient's palm including digits, which averages 1% of body surface area. We recommend to draw the patches and mark the evaluated patches on figure, if necessary with a higher magnification for detail; if child under 5, the head and neck total 18% and legs 13.5% each and no change in other parts. If any, indicate halo nevi on the graph. **Staging** of vitiligo is based on the assumption that repigmentation requires melanocytes to be still present either in the epidermis or in the hair follicle (reservoir) to repigment the skin. Repigmentation patterns reflect this assumption: homogeneous diffuse repigmentation occurs when melanocytes remain in the interfollicular epidermis and either marginal (from the border of the patch) or perifollicular (melanocyte precursors migrating from the hair follicle). Based on this, four stages (0–3) (Part **c**) are distinguished from normal pigmentation (0) to complete depigmentation (3), based on the assessment of the largest patch in each territory. Intermediate stages are defined as follows: stage 1 means incomplete depigmentation; stage 2 means complete depigmentation (may include hair whitening in a minority of hairs, less than 30%). If stage 3 persists after attempts of medical therapy, this would indicate a need for surgical treatment. **Spreading** is introduced to include a dynamic dimension, since rapidly progressive vitiligo needs urgent intervention to stabilize the disease. +1 means additional patches in a given area or demonstrated ongoing repigmentation using Wood's lamp in light skin-colored patients: 0 means stable disease, and −1 means observed ongoing depigmentation

especially vellus hairs. Wood's lamp equipment for vitiligo assessment should include a magnifying lens. Analysis of spreading (progressive/stable/regressive) was the most difficult item in a blind (non-patient influenced) test. The view already expressed in textbooks that concavity versus convexity as related to the general shape of a patch may predict progression versus regression is probably an indication to take with caution. Perifollicular repigmentation may occur with progressing marginal depigmentation. Partial depigmentation in a border of a patch may be interpreted as repigmentation. Surprisingly, at the Rome workshop [3], the investigator's opinion was right in the majority of cases if the patient's opinion was chosen as the gold standard. Overall, it was felt that this item should be graded more accurately using the patient's opinion.

The concordance between investigators using the same assessment grid was measurable, but it seems reasonable to predict that the results can be improved if some interactive training is available. There are probably, as noted for atopic dermatitis workshops [13], intrinsic high and low scorer profiles which can be minimized when the causes of variability between observers have been identified.

17.6 Interobserver Variability

As tested at a workshop with patients [3], the interobserver agreement was acceptable for the three items, namely, extent, staging, and spreading, and individual scorer's profiles could be defined. Black and white digitized photographs (Fig. 17.3) were chosen as references for

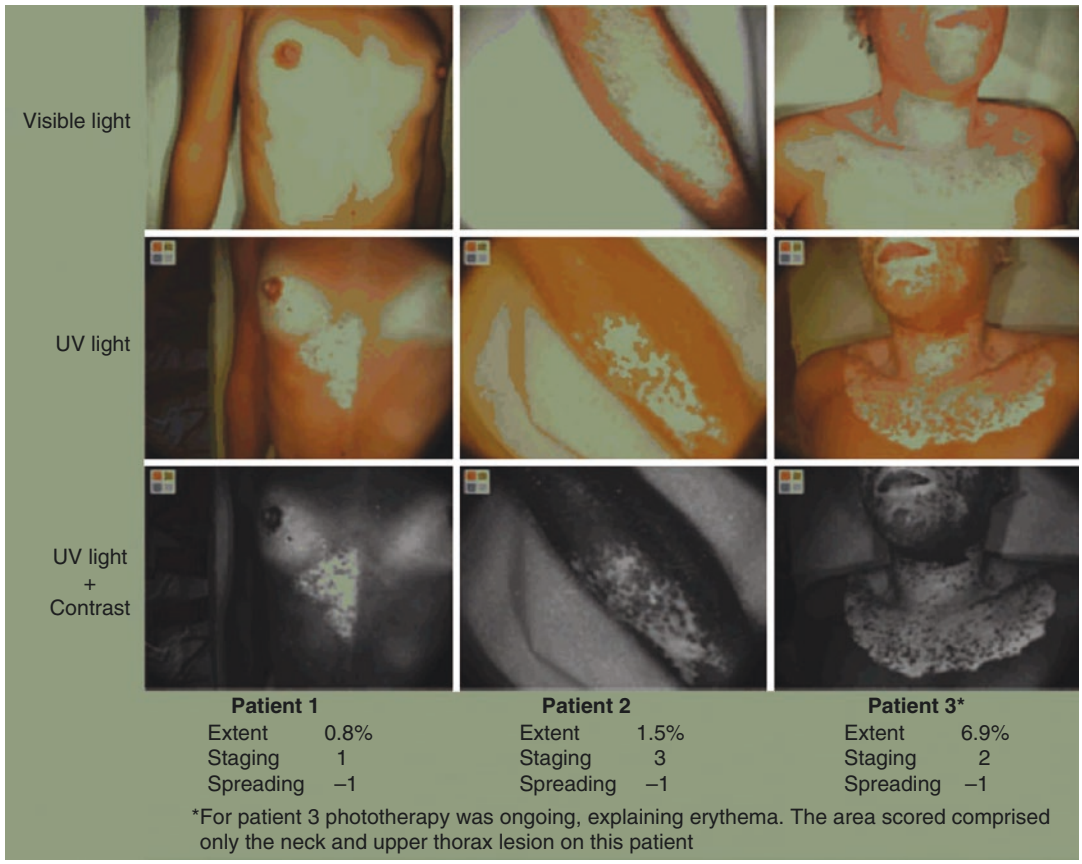


Fig. 17.3 Examples of assessments in three patients using the "ideal observer" method. This "ideal observer" is a virtual investigator, assigning the reference mean area involved and the modal value (mode) for the staging and spreading variables. The underlying assumption is that the value expressed by the majority of specialists is most likely to be the true value, as there is no gold standard

the discussion of data. However, it was noted that erythema (in patients treated with phototherapy) will appear gray in digitized black and white images.

To check the validity of the data gathered, concordance analysis was carried out based on the "ideal observer" method (Table 17.3). The investigators tended to underestimate staging 2 for staging 1, which resulted in being overestimated (27 indications against 10 in part a of Table 17.3). The frequency of assigning incorrect values decreased with staging: 40% for stage 1, 34% for stage 2, and 30% for stages 3 and 4, respectively. Table 17.3 (part b) shows a wide dispersion of data for spreading. Based on these data, it seems easier to identify regression (identified in 72.5% of cases) even if one investigator assigned progression instead of regression than stability and progression. For extent (Table 17.3 part c), the investigators' findings were very close, because 92% of the evaluations were within a range of 1% of the mean value. Denominators were important to make a good judgment, as larger areas such as trunk and legs are more difficult to score. Image analysis may help in different settings (clinical research mostly) to reduce interobserver variability.

Table 17.3 Interobserver variability

	Panelist scores				
	1	**2**	**3**	**4**	**Tot**
Staging					
1	6	1	3	0	10
2	17	46	6	1	70
3	4	7	28	1	40
4	0	0	3	7	10
Total	27	54	40	9	130
	Panelist scores				
	−1	**0**	**1**		
Spreading					
−1	29	10	1		40
0	11	27	12		50
1	11	8	21		40
Total	51	45	34		130
Extent	**±0.2%**	**±0.5%**	**±1%**		
Head and neck	17 (85%)	19 (95%)	20 (100%)		20
Arms	11 (55%)	13 (65%)	20 (100%)		20
Leg	13 (43%)	17 (57%)	29 (97%)		30
Trunk	23 (38%)	36 (60%)	51 (85%)		60
	64 (49%)	85 (65%)	120 (92%)		130

The ideal observer method was used for staging, K ¼ 0.499; SE ¼ 0.059 ($P < 0.00001$); spreading, K ¼ 0.388; SE ¼ 0.064 ($P < 0.0001$); and extent. The reference values correspond to the modal (staging and spreading) or mean (extent) score of the 10 panelists

17.7 Correlations Between Assessment Variables

Possible correlations between the three main assessment variables (extent, staging, and spreading) have been investigated. Some association was suggested between high staging and limited extent of vitiligo, when assessment was limited to only one patch. Similarly, larger patches tended to be assumed by investigators to spread less than smaller patches, but the difference was not significant here, and a wide variability in spreading assessment makes an interpretation of this item difficult. Stage 2 increased with extent, and stages 3 and 4 were mainly assigned to areas >1% [3].

17.8 Subjective Items

Response to disfiguring diseases is affected by basic ego strength [14]. Thus, psychological factors should be taken seriously into account in the global care of vitiligo patients. Quality of life impact (Chap. 18) is generally considered overall as moderate in vitiligo, but the patient's phototype, cultural background, and gender may influence the differences in data reported [15]. The perceived severity of vitiligo is explained mainly by the patients' personality and their psychological features and less significantly by the clinical objective criteria. Perceived severity of the illness and patient's psychological features such as trait depression are predictors of the patients' QoL. Patients who tend to be more anxious in their daily life and who have a poor self-esteem perceive their illness as more severe even if vitiligo is less extensive [15]. A higher prevalence of alexithymia and depression or anxiety was found in vitiligo patients as compared with the general population [16].

There are now some specific arguments for including a psychological support in the care of vitiligo patients including consideration of their personality and their difficulty of living with this disease (Chap. 18). Consequently, some factors which are easy to assess such as perceived severity and patient's personality (trait anxiety, trait depression, trait self-esteem) could be included in the assessment and management of this chronic disfiguring disease. A simple perceived severity scale is clearly useful in clinical practice as a screening tool. However, the development of a vitiligo specific QoL scale would probably be helpful.

References

1. Whitton ME, Ashcroft DM, Barrett CW, Gonzalez U. Interventions for vitiligo. Cochrane Database Syst Rev. 2006;(1):CD003263.
2. Anonymous. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. Dermatology. 1993;186:23–31.
3. Taïeb A, Picardo M, VETF Members. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell Res. 2007;20:27–35.
4. Taieb A, Picardo M. Clinical practice. Vitiligo. N Engl J Med. 2008;360:160–9.
5. Gauthier Y. The importance of Koebner's phenomenon in the induction of vitiligo vulgaris lesions. Eur J Dermatol. 1995;5:704–8.
6. Halder RM, Grimes PE, Cowan CA, et al. Childhood vitiligo. J Am Acad Dermatol. 1987;16:948–54.
7. Alkhateeb A, Fain PR, Thody A, et al. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. Pigment Cell Res. 2003;16:208–14.
8. Liu JB, Li M, Yang S, et al. Clinical profiles of vitiligo in China: an analysis of 3742 patients. Clin Exp Dermatol. 2005;30:327–31.
9. Betterle C, Caretto A, De Zio A, et al. Incidence and significance of organ-specific autoimmune disorders (clinical, latent or only autoantibodies) in patients with vitiligo. Dermatologica. 1985;171:419–23.
10. Alimohammadi M, Björklund P, Hallgren A, et al. Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. N Engl J Med. 2008;358:1018–28.
11. Westerhof W, Nieuweboer-Krobotova L. Treatment of vitiligo with UV-B radiation vs topical psoralen plus UV-A. Arch Dermatol. 1997;133:1525–8.
12. Hamzavi I, Jain H, McLean D, et al. Parametric modeling of narrowband UV-B phototherapy for vitiligo using a novel quantitative tool: the Vitiligo Area Scoring Index. Arch Dermatol. 2004;140:677–83.
13. Kunz B, Oranje AP, Labreze L, et al. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. Dermatology. 1997;195:10–9.
14. Porter JR, Beuf AH, Lerner A, Nordlund J. Psychological reaction to chronic skin disorders: a study of patients with vitiligo. Gen Hosp Psychiatry. 1979;1:73–7.
15. Kostopoulou P, Jouary T, Quintard B, et al. Objective vs subjective factors in the psychological impact of vitiligo: the experience from a French referral center. Br J Dermatol. 2009;161(1):128.
16. Sampogna F, Raskovic D, Guerra L, et al. Identification of categories at risk for high quality of life impairment in patients with vitiligo. Br J Dermatol. 2008;159:351–9.

Quality of Life

18

Anuradha Bishnoi and Davinder Parsad

Contents

Abstract

Vitiligo can induce considerable psychosocial stress and psychiatric comorbidity. Then, it is important to recognize and manage the psychological component not only to improve coping but also to obtain a better treatment response.

The training in assertiveness, relaxation skills and help in building self-confidence would have substantial effects on quality of life as well as treatment outcomes. In fact, social and psychological well-being increase when patients with facial disfigurement are helped to develop social skills and to confront their difficulties. There is need for a standardized quality of life evaluation tool which can quantify the psychosocial stress of vitiligo subject and which could be used for treatment evaluation.

Key Points

- Vitiligo can have a staggering effect on the psychosocial well-being of an individual, especially in those with darker phototypes and lesions on exposed sites.
- Various health-related quality of life measurement tools are now available, with those specifically designed to measure the impact of vitiligo.
- An enhanced awareness amongst the masses about this common disease shall help to bring down the stigmatization associated with vitiligo.

18.1 Introduction and Historical Perspective

Vitiligo has a major impact on the quality of life of patients, many of whom feel distressed and stigmatized by their condition [1–3].

A. Bishnoi · D. Parsad (✉)
Department of Dermatology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_18

In ancient Indian texts, vitiligo was referred by the name kilas (in Sanskrit, kil - white, as - throw or cast away), meaning one who throws away the colour. Since ancient times, patients of vitiligo have suffered the same physical and mental abuses as lepers of that age, and they were considered to have 'shweta kustha'. Vitiligo is particularly disfiguring for people with dark skin and carries such a social stigma in Indian society that patients are sometimes considered unmarriageable [4]. Society greets vitiligo patients in as much the same way as it does anyone else who appears to be different. In some parts of India, they are still stared at or subjected to whispered comments, antagonism, insult or isolation. A woman with vitiligo may face numerous social problems and experience great difficulties in getting married. If vitiligo develops after marriage, it grounds for divorce by the husband. In some parts of India, disease is often considered as a punishment by God, presumably caused by unconscious feelings of guilt. Patients are subjected to various dietary restrictions like avoidance of milk, fish, citrus fruits, etc. [5].

18.2 Modern Psychological Studies

Porter et al. [6, 7] in the late 1970s brought the psychosocial effects of vitiligo to the attention of dermatologists. A questionnaire survey among 62 vitiligo patients in a hospital-based outpatient setting indicated that two-thirds felt embarrassed by the disease, more than half of them felt ill at ease, and a majority of patients felt anxious, concerned and worried about the disease itself. They also studied the effect of vitiligo on sexual relationship and found that embarrassment during sexual relationship was especially frequent for men with vitiligo [8]. Many patients chose adapted clothing and used large amounts of cosmetics in order to hide the skin lesions. Although 80% perceived their friends and family as supportive, strangers expressed less understanding, and patients felt uncomfortable meeting them. Salzer and Schallreuter [9] reported that 75% found their disfigurement moderately or severely intolerable. An important component of stigma and stigmatization is the impossibility of concealing the affected skin parts and consequent visibility. In a recent study, stigmatization experienced by vitiligo patients was found psychologically relevant since patients with visible lesions experienced a higher level of stigmatization [10].

Appearance of skin can condition an individual's self-image, and any pathological alteration can have psychological consequences [11]. Patients often develop negative feelings about their skin, which are reinforced by their experiences over a number of years. Most patients with vitiligo report embarrassment, which can lead to a low self-esteem and social isolation [12]. Facial vitiligo lesions may be particularly embarrassing, and the frustration of resistant lesions over exposed part of hands and feet can lead to anger and disillusionment. Particularly in teenagers, mood disturbances including irritability and depression are common. Patients with vitiligo are extensively sensitive to the way others perceive them, and they will often withdraw, because they anticipate being rejected. Sometimes, strangers and even close friends can make extremely hurtful and humiliating comments. The impact of such factors is profound subjecting them to emotional distress, interference with their employment or use of tension-lessening, oblivion-producing substances such as alcohol [13]. Severe depression has been known to lead to suicide attempts [14]. Vitiligo can also result in problems in interpersonal relations and induce depression and frustration. In a study from India, vitiligo has been shown to be associated with high psychiatric morbidity [12]. One fourth of vitiligo patients attending a specialized clinic was found to have psychiatric morbidity, and a diagnosis of adjustment disorder was made in the majority of cases. Psychiatric morbidity was significantly correlated with dysfunction arising out of illness [12].

18.3 Socio-economic and Educational Consequences

Patients often suffer financial loss because they have to take time off from work to attend hospital appointments to perform treatments such as photo-

therapies. Lesions in exposed sites can adversely affect a person's chances of getting a job at interview and so restrict career choices. Vitiligo beginning in childhood can be associated with significant psychological trauma that may have long lasting effects on personal self-esteem [15]. Children with vitiligo usually avoid sport or restrict such activities and often lose vital days from school.

18.4 Quality of Life Evaluation

Most of the studies have used Dermatology Life Quality Index (DLQI), a widely validated questionnaire that is easy to use and allows comparison between several skin disorders. However, there is a need for a uniformly acceptable scale which can more specifically quantify psychosocial stress associated with this disease. Moreover, this type of quality of life measures shall be useful as tool to assess treatment effectiveness or to compare treatment outcome.

Although a limited number of studies have paid attention to the psychosocial effects of vitiligo, they point towards an appreciable psychosocial impact on those afflicted. Kent and Al' Abadie [16] found that the stigmatization experience accounted for 39% of the variance in the quality of life of vitiligo patients. On the other hand, self-esteem, a number of symptoms on the distress checklist, race and general health accounted for only 12% of the variance in quality of life.

There may be a relationship between stress and vitiligo since psychological stress can increase levels of neuroendocrine hormones, leading to a damage of melanocytes in the skin, and affect the immune system altering the level of neuropeptides [17]. Recently, increased levels of neuropeptide-Y were shown in the plasma and skin tissue fluids of patients with vitiligo [18]. Liu et al. [19] studied the occurrence of cutaneous nerve endings and neuropeptides in vitiligo vulgaris and suggested that emotional trauma and stressful life events can cause large adrenal secretions and this can result in acute onset of vitiligo.

Because of the possible connection between stress and exacerbation of vitiligo [20], psychological and psychotherapeutic interventions may be helpful. In a study of 150 vitiligo patients, we assessed the nature and extent of the social and psychological difficulties associated with the disease and their impact on treatment outcome by using Dermatology Life Quality Index [21]. Our study clearly demonstrated that patients with high DLQI scores responded less favourably to a given therapeutic modality. Recently, many vitiligo-specific quality of life scoring systems have been developed like vitiligo impact scale (VIS) [22], VIS-22 [23, 24], vitiligo specific quality of life (vitiQoL) [25, 26] and vitiligo impact patient scale [27]. All these are well validated and correlate well with other general skin disease quality of life scores (like DLQI, Skindex), while catering specifically to the need of measuring quality-of-life impact of vitiligo, which extends much beyond the visible depigmentation.

These results suggest that additional psychological approaches may be particularly helpful in these patients. In a preliminary study by Papadopoulos et al. [28], it has been shown that counselling can help to improve the body image, self-esteem and quality of life of patients with vitiligo as well as it may have a positive effect on course of the disease. To conclude, vitiligo can have a tremendous effect on all spheres of one's lives. A major part of this effect arises because of the lack of knowledge about this disease in masses and the resultant stigmatization. Through initiatives like world vitiligo day (celebrated each year on 25th June), and other information and education activities, the knowledge regarding vitiligo should be actively spread amongst the general population. That shall bring a positive change in the manner vitiligo is perceived by laymen.

References

1. Bolognia JL, Pawelek JM. Biology of hypopigmentation. J Am Acad Dermatol. 1988;19:217–55.
2. Lerner AB. Vitiligo. J Invest Dermatol. 1959;32:285–310.
3. Lerner AB, Nordlund JJ. Vitiligo. What is it? Is it important? JAMA. 1978;239:1183–7.

4. Fitzpatrick TB. The scourge of vitiligo. Fitzpatrick's J Clin Dermatol. 1993;1:68–9.
5. Parsad D, Dogra S, Kanwar AJ. Quality of life in patients with vitiligo. Health Qual Life Outcomes. 2003;1:58.
6. Porter JR, Beuf AH, Nordlund JJ, Lerner AB. Personal responses to vitiligo. Arch Dermatol. 1978;114:1348–85.
7. Porter JR, Beuf AH, Nordlund JJ, Lerner AB. Psychological reaction to chronic skin disorders. A study of patients with vitiligo. Gen Hosp Psychiatry. 1979;1:73–7.
8. Porter J, Beuf A, Lerner A, et al. The effect of vitiligo on sexual relationship. J Am Acad Dermatol. 1990;22:221–2.
9. Salzer B, Schallreuter K. Investigations of the personality structure in patients with vitiligo and a possible association with catecholamine metabolism. Dermatology. 1995;190:109–15.
10. Schmid-Ott G, Kunsebeck HW, Jecht E, Shimshoni R, et al. Stigmatization experience, coping and sense of coherence in vitiligo patients. J Eur Acad Dermatol Venereol. 2007;21:456–61.
11. Savin J. The hidden face of dermatology. Clin Exp Dermatol. 1993;18:393–5.
12. Mattoo SK, Handa S, Kaur I, et al. Psychiatric morbidity in vitiligo: prevalence and correlates in India. J Eur Acad Dermatol Venereol. 2002;16:573–8.
13. Ginsburg IH. The psychological impact of skin diseases: an overview. Dermatol Clin. 1996;14:473–84.
14. Cotterill JA, Cunliffe WJ. Suicide in dermatological patients. Br J Dermatol. 1997;137(2):246–50.
15. Hill-Beuf A, Porter JDR. Children coping with impared appearance. Social and psychologic influences. Gen Hosp Psychiatry. 1984;6:294–300.
16. Kent G, Al' Abadie M. Factors affecting responses on dermatology life quality index items among vitiligo sufferers. Clin Exp Dermatol. 1996;21:330–3.
17. Al'Abadie MSK, Kent G, Gawkrodger DJ. The relationship between stress and the onset and exacerbation of psoriasis and other skin conditions. Br J Dermatol. 1994;130:199–203.
18. Tu C, Zhao D, Lin X. Levels of neuropeptide-Y in the plasma and skin tissue fluids of patients with vitiligo. J Dermatol Sci. 2001;27:178–82.
19. Liu PY, Bondesson L, Löntz W, Johansson O. The occurrence of cutaneous nerve endings and neuropeptides in vitiligo vulgaris: a case-control study. Arch Dermatol Res. 1996;288:670–5.
20. Picardi A, Pasquini P, Cattaruzza MS, et al. Stressful life events, social support, attachment security and alexithymia in vitiligo. A case-control study. Psychother Psychosom. 2003;72:150–8.
21. Parsad D, Pandhi R, Dogra S, et al. Dermatology Life Quality Index score in vitiligo and its impact on the treatment outcome. Br J Dermatol. 2003;148:373–4.
22. Krishna GS, Ramam M, Mehta M, Sreenivas V, Sharma VK, Khandpur S. Vitiligo impact scale: an instrument to assess the psychosocial burden of vitiligo. Indian J Dermatol Venereol Leprol. 2013;79:205–10.
23. Gupta V, Sreenivas V, Mehta M, Khaitan BK, Ramam M. Measurement properties of the Vitiligo Impact Scale-22 (VIS-22), a vitiligo-specific quality-of-life instrument. Br J Dermatol. 2014;171:1084–90.
24. Gupta V, Sreenivas V, Mehta M, Ramam M. What do Vitiligo Impact Scale-22 scores mean? Studying the clinical interpretation of scores using an anchor-based approach. Br J Dermatol. 2018.
25. Lilly E, Lu PD, Borovicka JH, Victorson D, Kwasny MJ, West DP, et al. Development and validation of a vitiligo-specific quality-of-life instrument (VitiQoL). J Am Acad Dermatol. 2013;69:e11–8.
26. Catucci Boza J, Giongo N, Machado P, Horn R, Fabbrin A, Cestari T. Quality of life impairment in children and adults with vitiligo: a cross-sectional study based on dermatology-specific and disease-specific quality of life instruments. Dermatology. 2016;232:619–25.
27. Salzes C, Abadie S, Seneschal J, Whitton M, Meurant J-M, Jouary T, et al. The Vitiligo Impact Patient scale (VIPs): development and validation of a Vitiligo Burden Assessment Tool. J Invest Dermatol. 2016;136:52–8.
28. Papadopoulos L, Bor R, Legg C. Coping with the disfiguring effects of vitiligo: a preliminary investigation into the effects of cognitive-behaviour therapy. Br J Med Psychol. 1999;72:385–96.

19 Defining the Disease: Editor's Synthesis

Alain Taïeb and Mauro Picardo

Contents

Abstract

The initial clinical evaluation of a vitiligo patient is a step frequently neglected. Some new variants have been described only recently such as mixed and follicular vitiligo, and the frontiers of the disease challenge an unambiguous delineation of the disease (e.g. vitiligoid depigmentation after cancer immunotherapies, hypochromic vitiligo...). Major progresses have been made to understand heritability, but more epidemiological data are needed to address in particular environmental factors, natural history, and comorbidities to confirm on a large scale a protection against cancer. At this time point, available clinical data do not support a significant involvement of non-skin melanocytes in vitiligo/NSV.

Major initial determinants are probably situated directly in the skin and activated in a subset of patients by largely unknown stressors. Vitiligo/NSV can be considered as a marker of an auto-inflammatory/autoimmune diathesis, and microinflammation is central to its pathophysiology. The skewing of the immune system towards vitiligo seems of benefit to fight skin cancer and possibly internal cancer. If inflammation and autoimmunity are pivotal in all subset of vitiligo, other factors are involved predisposing to premature melanocyte ageing.

Key Points

- A good clinical assessment is important for optimal management.
- Epidemiology is largely uncovered, but convergent evidence suggests that increased immunosurveillance in vitiligo limits the risk of cancer..

A. Taïeb (✉)
Service de Dermatologie, Hôpital St André, CHU de Bordeaux, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

M. Picardo
Cutaneous Physiopathology and CIRM, San Gallicano Dermatological Institute, IRCCS, Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_19

- Clinical data suggest possible variable melanocyte targets according to subtype of vitiligo.
- Associated diseases/comordidities need to be assessed and may provide pathophysiological insight.
- Mapping of segmental vitiligo has begun and may help predicting affected territories.
- Histopathology is needed for difficult cases.

19.1 Clinical Assessment Is Important

The initial evaluation of a vitiligo patient is a step which is frequently neglected, as witnessed by the only recent delineation of a mixed form of vitiligo combining SV and vitiligo/NSV, of follicular vitiligo and of frontier disorders such as vitiligoid depigmentation under cancer immunotherapies and hypochromic vitiligo. It should include a thorough history taking and examination including Wood's lamp or a monochromatic 365 nm lamp in a dark cabinet for all low phototype individuals. Clinical evaluation yields important information for routine management purposes, such as detection of disease progression or stabilization, but also for asking relevant clinical research questions. Assessing coping and quality of life issues should not be forgotten. In particular, the role of stress has been emphasized by some studies and patients' support groups. There is a need to clarify this issue of stress acting as a trigger factor or more importantly as influencing disease progression, because it could open new perspectives for management. The evaluation of the impact of the disease on non-skin melanocytes (eye, ear, heart, central nervous system) is not necessary for common vitiligo/NSV. For vitiligo universalis, there is a frontier with Vogt-Koyanagi-Harada (VKH) syndrome, which is unclear, and those patients should be evaluated more carefully for associated autoimmunity in this respect.

19.2 A Need for Epidemiological Studies and Deep Phenotyping

The spectrum of clinical manifestations in vitiligo is limited, but simple items such as variations in age at onset and extension or pattern of disease and natural course with possible spontaneous repigmentation suggest a complex and mysterious interplay between host and environment. Incidence or prevalence rates seem stable, without major in changes observable in a short time span, as noted for some chronic either Th1 or Th2 predominant diseases, which have been the basis of the hygiene hypothesis, suggesting that a westernized life style changes our microbial environment and influence the population risk for chronic inflammatory disorders [1]. However, informative studies such as repeated twin studies have not been performed in vitiligo as in atopic dermatitis [2]. Contrary to the large data collected to understand the genetics of vitiligo, more epidemiological data are clearly needed to better delineate the role of environmental factors, natural history, as well as comorbidities. The access to large databases is now empowering research with new resources, and the possibility to access to deep phenotyping with standardized digital photographic systems may fill some of the current gaps in disease definition. Very recent evidence using insurance claims database suggest that vitiligo is protective against cancer.

19.3 Variable Melanocytic Targets According to Clinical Subtypes

Clinical aspects suggest different cellular targets/territories according to subtypes of vitiligo as shown for the marked preference of leukotrichia in SV. The mucosal pigmentary system seems to be involved more frequently in patients of dark complexion, but this aspect needs more accurate studies in other populations.

Data gathered in vitiligo studies worldwide do not support the concept of a common

involvement of non-skin melanocytes (with the exception of the oral and genital mucosae and vitiligo universalis), contrary to what is found in Vogt Koyanagi Harada syndrome which targets ocular, auditory and central nervous system melanocytes [3]. However some fulminant forms of vitiligo which are classified universalis may involve more generalized melanocytic targets and other types of organ autoimmunity. Vitiligo/NSV is in most instances a chronic skin condition with major initial determinants situated directly in the skin and which might be activated in a subset of patients by largely unknown stressors.

19.4 Lessons from Associated Diseases and Rare Syndromic Cases

History assessment of patients underlines two facts: the association in 20% of cases with a personal or familial history of autoimmune/autoinflammatory disorders where vitiligo is a marker of an autoimmune diathesis, and the association in 11% of patients with a family history of premature hair greying, which witnesses a visible premature senescence of the pigmentary system [4]. Syndromic vitiligo cases occurring in monogenic heritable diseases underline possible similar pathophysiological pathways which belong either to the immune diathesis, with more emphasis on the humoral responses (APECED), or defects in the oxidative stress and ageing process (MIDAS syndrome, ataxia telangiectasia and Nijmegen breakage syndrome). The immune-mediated destruction of naevus cells (halo naevus) and the rare clinically inflammatory onset of vitiligo, as well as vitiligo changes in VKH, indicate clearly that inflammation and cell-mediated immunity are important in a subset of patients. Interestingly, the recent emphasis on pruritus in vitiligo may correspond to a far more common micro-inflammatory component which is not clinically symptomatic. Microinflammation might be related to increased immune surveillance with the benefit of a decreased risk for skin cancer.

19.5 Predictive assessment of Vitiligo

For the common and disfiguring facial involvement in segmental vitiligo, the possible prediction of territories involved after the detection of the initial macule is important. There is clearly a need of establishing a good database including patients of various ethnic backgrounds to settle this important issue. Similar studies are needed for other body locations. The patterns of extension in vitiligo/NSV, if predictable, deserve also a better attention for prognosis and management issues. The speed of progression is also an important parameter of assessment which needs a better quantitative validation of clinical symptoms such as Koebner phenomenon, fuzzy borders of patches and pinpoint depigmentation around lesions. Noninvasive techniques of assessment such as in vivo confocal microscopy may help the clinician, if more widely available. The use of new scoring systems [5] combined with standardized photography may help acquiring robust data for deep phenotyping and predictive assessment.

19.6 Suboptimal Use of Pathology to Assess Vitiligo

Pathology has been considered as providing limited information on the nature and course of the disease, because diagnosis was easy to make on clinical grounds. Vitiligo, based on the microscopic examination of long lasting NSV depigmented macules, was synonym of an epidermis without pigment cells. As discussed in this section, the study of the progressing but still pigmented edges of vitiligo/NSV lesions (or with pinpoint depigmentation) and of distant normal-looking skin has provided evidence of microdepigmentation and microinflammation. The systematic pathology assessment of SV for evidence of microinflammation is still lacking, but evidence has accumulated over the last decade that the onset of SV has histologically inflammatory features. This new vision raises several questions: (1) If vitiligo is inflammatory pathologically, why is it usually not clinically? Erythema and

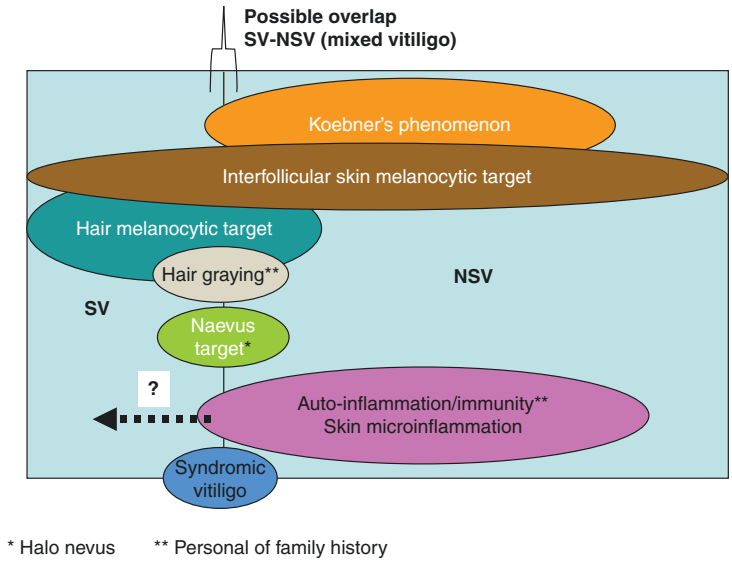


Fig. 19.1 Possible research angles of attack based on clinical findings in vitiligo. SV and NSV can overlap (mixed vitiligo). The epidermal melanocytic target is equally shared between SV and NSV, but the hair melanocytic target is skewed towards SV. The history of hair greying and the presence of halo naevus seem equally shared between the two main types (to be confirmed by larger studies). The Koebner phenomenon and personal/familial history of auto-inflammation/autoimmunity seem mostly associated with NSV. Syndromic vitiligo may share some pathways influencing either survival or immune loss of melanocyte with common nonsyndromic vitiligo (SV and NSV). The question of microinflammation for SV needs additional studies

pruritus are uncommon findings in the setting of even rapidly progressing vitiligo, suggesting a unique pattern of responsiveness or a so far unexpected active role of melanocytes in symptomatic inflammation in nondepigmenting disorders. Is microinflammation primary, or secondary to some basic imbalance in pigment cell homeostasis? Whatever the answers, the growing evidence of microinflammation in vitiligo which is now considered as a memory T cell disease has considerably changed our conception of the disease and of its management [6].

19.7 Summary

In summary, Fig. 19.1 proposes a synthetic vision of the vitiligo clinical data. There are several points which need clarification. Is there a common predisposition shared for developing cellular immune response against naevus cells in SV and NSV, halo naevus being a distinct phenomenon [7] associated to the two forms? Can SV, at least at onset, be inflammatory as it is the rule in vitiligo/NSV? Some simple clinical and histological studies are needed to address the problem.

This section has provided clinical definitions, classification and methods of assessment based whenever possible on the largest consensus. The clinically based definitions given have been intended to be useful for the practitioner and the clinical researcher. We clearly need also better definitions and monitoring of disease activity/stability, which may include evidence of nonclinically graded inflammation with skin biopsies or less invasive skin/blood markers. More accurate and standardized methods of assessment need to be developed for specific needs including clinical trials. A basic science understanding of vitiligo should represent soon an available alternative to this clinically based approach in the context of developing translational research. The next section will thus review in vivo and in vitro clinical and experimental data which form the basis of our current understanding of vitiligo.

References

1. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med. 2002;347:911–20.
2. Schultz Larsen F. Atopic dermatitis: a genetic-epidemiologic study in a population-based twin sample. J Am Acad Dermatol. 1993;28:719–23.
3. Bae JM, Chung KY, Yun SJ, Kim H, Park BC, Kim JS, Seo SH, Ahn HH, Lee DY, Kim YC, Park HJ, Kim M. Markedly reduced risk of internal malignancies in patients with Vitiligo: a nationwide population-based cohort study. J Clin Oncol. 2019;37(11):903–11.
4. Taïeb A, Picardo M, Members VETF. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell Res. 2007;20:27–35.
5. van Geel N, Bekkenk M, Lommerts JE, Ezzedine K, Harris J, Hamzavi I, Eleftheriadou V, Picardo M, Taieb A, Prinsen CAC, Wolkerstorfer A, Speeckaert R. The Vitiligo Extent Score (VES) and the VESplus are responsive instruments to assess global and regional treatment response in patients with vitiligo. J Am Acad Dermatol. 2018;79(2):369–71. https://doi.org/10.1016/j.jaad.2017.12.070. Epub 2018.
6. Boniface K, Jacquemin C, Darrigade A-S, Dessarthe B, Martins C, Boukhedouni N, Vernisse C, Grasseau A, Thiolat D, Rambert J, Lucchese F, Bertolotti A, Ezzedine K, Taieb A, Seneschal J. Vitiligo skin is imprinted with resident memory CD8 T cells expressing CXCR3. J Invest Dermatol. 2018;138(2):355–64.
7. Schallreuter KU, Kothari S, Elwary S, et al. Molecular evidence that halo in Sutton's naevus is not vitiligo. Arch Dermatol Res. 2003;295:223–8.

Part II

Understanding the Disease

Pathophysiology Overview

20

Mauro Picardo

Contents

Thanks to the advances in technology over the past few years, there has been an ongoing and in-depth study of the genes that make up the human being as a whole and more specifically those involved in pathologies and diseases affecting the general population. So far, over 50 different vitiligo susceptible genes have been identified [1–3]. Gaining a better understanding of the genes involved in vitiligo may in the future lead to a better approach in treating vitiligo and might even allow for disease prevention in genetically susceptible individuals.

Although the advances in genetics are ongoing and have definitely shed new light on the pathology of this disorder, many of the genes associated with vitiligo are also associated with other autoimmune disorders. There is increasing evidence showing a combination of environmental and genetic factors involved in the pathogenesis of vitiligo. Studies on twins have clearly demonstrated that there is only a 23% concordance of vitiligo susceptible genes in monozygotic twins, suggesting that epigenetics might play a more important role. Environmental factors can induce epigenetic alterations in patients, which can affect gene expression involved in inflammation, immune tolerance and melanocyte homeostasis, eventually leading to an active immune response and melanocyte destruction [4].

A unified approach to the understanding of the pathophysiology of vitiligo has not yet been completely defined, but melanocyte loss is certainly the pathological hallmark of vitiligo.

The loss of functional melanocytes may be attributed to multiple mechanisms including metabolic abnormalities, oxidative stress, generation of inflammatory mediators, cell detachment, and autoimmune response.

It seems that in subjects with a genetic predisposition to develop autoimmune, there is an intrinsic defect within melanocytes which triggers the phenomena. The intracellular oxidative stress generated can lead to a local inflammatory reaction with the activation of an innate immune process and subsequent generation of cell-specific cytotoxic immune responses. The final step is a progressive loss of melanocytes with skin depigmentation [5].

The role of oxidative stress is supported by the evidence of increased levels of reactive oxygen

M. Picardo (✉)
Cutaneous Physiopathology and CIRM, San Gallicano Dermatological Institute, IRCCS, Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_20

species (ROS) in lesional and non-lesional skin both in vitro and in vivo, which lead to an impaired expression and/or activity of the antioxidant system. Moreover, epidermis appears to be overall characterized by a deregulation of the biopterin metabolism [6].

The redox alteration of membrane lipid may affect lipid rafts with subsequent modification of the membrane receptor function as well as the housing of the proteins ensuring the electron transfer and the ATP production in the mitochondria.

The imbalance of the prooxidant/antioxidant status has been indicated as the basis of the increased sensitivity of vitiligo melanocytes to external prooxidant stimuli and, on a long-term exposure, as the cause of generation of a presenescent status [7].

The possible detachment of melanocytes, observed at the borders of lesions, may be related to the stress-mediated damage possibly explaining the Koebner phenomenon (KP). Mitochondrial dysfunction, reported in vitiligo cells, has been related to cell damage observed in other degenerative diseases and may possibly account for metabolic stress.

Some of the alterations observed in melanocytes have been described in other cells of vitiligo skin suggesting that the degenerative phenomenon has a more generalized involvement. Keratinocytes and fibroblasts present an intracellular oxidative stress that could be the basis for an altered secretion of soluble growth factors supporting melanocyte survival and homeostasis [8, 9].

Finally, stressed melanocytes may initiate immune responses through several mechanisms. The role of autoimmunity in vitiligo is supported by the association with other autoimmune diseases, the presence of antibodies against melanocytes, the association with polymorphisms at immune loci, the presence of prominent T-cell perilesional infiltrates, and cytokine expression [10].

The mechanisms that initiate autoimmunity likely begin with the activation of innate immune populations that sense exogenously or endogenously induced stress signals released from the melanocyte. Melanocyte-specific, cytotoxic CD8+ T cells will then act in the destruction of melanocytes. CD8+ lesional T cells from patient skin biopsies produce IFN-γ and TNF-α, among other cytokines. IFN-γ is also expressed within lesions, suggesting the importance of this cytokine in driving autoreactive T-cell responses involved in the destruction of melanocytes. Functional studies in a mouse model of vitiligo confirmed the critical role of the IFN-γ-CXCL10-CXCR3 axis in both the progression and maintenance of depigmentation in vitiligo [11].

The role of T regulatory cells in controlling autoimmune responses is likely based on functional experiments in mouse models as well as observations made in vitiligo patients; however, clear evidence of their function is still lacking [12].

A large number of genes found to confer risk for vitiligo implicate both innate and adaptive immune pathways, confirming the role of autoimmunity in vitiligo pathogenesis. However, polymorphisms in nonimmune genes have also been identified, including the melanocyte-specific genes tyrosinase and the melanocortin 1 receptor96. While these may serve as antigens recognized by T cells, they also participate in melanin production, which may contribute to the generation of stress within the cells.

The pathogenic mechanisms underlying segmental and non-segmental vitiligo were thought to be distinct due to their different clinical patterns. However, recent data indicate overlapping inflammatory pathogenesis for segmental and non-segmental vitiligo. Both are caused by a multistep process, which includes an initial release of proinflammatory cytokines and neuropeptides, triggered by external or internal injury, with a subsequent vascular dilatation and immune response. Conventionally, the possible pathogenetic mechanisms for segmental vitiligo include neuronal mechanisms, somatic mosaicism, and microvascular skin homing of immune cells. Immunohistochemical data are rather scarce, and significant lymphocytic infiltrates have only occasionally been observed. However, increasing evidence has been published on a possible autoimmune/auto-inflammatory destruction of segmental vitiligo. Currently, it remains

ambiguous whether the inflammatory process is based on a deregulated immune system or can be regarded as a secondary event to cellular abnormalities in the epidermis.

A cell-mediated immune response, characterized by CD8 and CD4 infiltrates around dermoepidermal junction, is involved in the early phases of segmental vitiligo with associated halo nevi. As regards the immune involvement, it has been suggested that the midline delineation in unilateral lesion could represent the migration pattern of cytotoxic T cells from specific lymph nodes along the microvascular system via homing receptors. According to the literature, these homing receptors can have a unique unilateral homing code.

Similarly to that hypothesized for non-segmental vitiligo, a combinatory three-step theory was proposed. The theory of cutaneous mosaicism does not exclude the neuronal theory, as a neurogenic factor can still be one of the initial triggers to induce the whole process in its early stage. The first step includes the release of inflammatory factors, neuropeptides, and catecholamines, which can be considered as the triggering factor. This triggering factor leads to a small inflammatory response at a site where melanocytes are more vulnerable to immune-mediated destruction (possibly due to somatic mosaicism) (step 2). Subsequently, an antimelanocyte-specific response develops in the draining lymph node, and specific T cells migrate by binding to their vascular adhesion receptors toward the segmental vitiligo area, causing the characteristic skin depigmentations [13].

Some of the alterations observed in melanocytes have also been described in other skin cells, suggesting that vitiligo leads to generalized degeneration. Keratinocytes and fibroblasts also exhibit oxidative stress, phosphorylation of p38, an overexpression of p53, and a senescent phenotype. These could be the basis for altered secretion of soluble growth factors supporting melanocyte survival and homeostasis.

Reported keratinocytes and fibroblasts could be altered in vitiligo skin showing some aging phenotypes [9, 14].

The evolution of the experimental approach to vitiligo has provided new in vivo and in vitro models. In vitro models are useful in the investigation of the differences between normal cells and vitiligo cells. The in vitro approach has evolved over time, and now new techniques involving a limited use of biological material are proving very interesting. Furthermore, through in vitro studies, one can generate melanocyte-specific silencing or overexpression of modified genes. The possible development of vitiligo models using normal cells will bypass the difficulty of culturing vitiligo cells which is currently the main limiting factor in studying vitiligo in vitro.

The use of animal models has been essential in understanding the importance of the different elements affecting vitiligo such as genetic susceptibility, immune system, and environmental factors. Studies have been carried out on both spontaneous autoimmune vitiligo (Smyth line chicken) and induced autoimmune vitiligo (in mice). The first includes all the same clinical and biological manifestations seen in human vitiligo, while the latter provides defined in vivo models useful in examining the loss of melanocytes and developing new treatments. Together, the study of these two models is necessary to delineate the complex mechanisms involved in vitiligo [15–17]. The results from these studies can provide new insights into the prevention, treatment, and management of vitiligo in humans.

References

1. Spritz RA, Andersen GH. Genetics of vitiligo. Dermatol Clin. 2017;35(2):245–55. https://doi.org/10.1016/j.det.2016.11.013.
2. Shen C, Gao J, Sheng Y, Dou J, Zhou F, Zheng X, Ko R, Tang X, Zhu C, Yin X, Sun L, Cui Y, Zhang X. Genetic susceptibility to vitiligo: GWAS approaches for identifying vitiligo susceptibility genes and loci. Front Genet. 2016;7:3. https://doi.org/10.3389/fgene.2016.00003.
3. Spritz RA. Six decades of vitiligo genetics: genome-wide studies provide insights into autoimmune pathogenesis. J Invest Dermatol. 2012;132(2):268–73. https://doi.org/10.1038/jid.2011.321.
4. Alkhateeb A, Fain PR, Thody A, Bennett DC, Spritz RA. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. Pigment Cell Res. 2003;16(3):208–14.

5. Picardo M, Dell'Anna ML, Ezzedine K, Hamzavi I, Harris JE, Parsad D, Taieb A. Vitiligo. Nat Rev Dis Primers. 2015;1:15011. https://doi.org/10.1038/nrdp.2015.11.
6. Schallreuter KU, Moore J, Wood JM, Beazley WD, Gaze DC, Tobin DJ, Marshall HS, Panske A, Panzig E, Hibberts NA. In vivo and in vitro evidence for hydrogen peroxide (H2O2) accumulation in the epidermis of patients with vitiligo and its successful removal by a UVB-activated pseudocatalase. J Investig Dermatol Symp Proc. 1999;4(1):91–6.
7. Bellei B, Pitisci A, Ottaviani M, Ludovici M, Cota C, Luzi F, Dell'Anna ML, Picardo M. Vitiligo: a possible model of degenerative diseases. PLoS One. 2013;8(3):e59782. https://doi.org/10.1371/journal.pone.0059782.
8. Lee AY. Role of keratinocytes in the development of vitiligo. Ann Dermatol. 2012;24(2):115–25. https://doi.org/10.5021/ad.2012.24.2.115.
9. Kovacs D, Bastonini E, Ottaviani M, Cota C, Migliano E, Dell'Anna ML, Picardo M. Vitiligo skin: exploring the dermal compartment. J Invest Dermatol. 2018;138:394. https://doi.org/10.1016/j.jid.2017.06.033.. pii: S0022-202X(17)33029-4.
10. Boniface K, Seneschal J, Picardo M, Taïeb A. Vitiligo: focus on clinical aspects, immunopathogenesis, and therapy. Clin Rev Allergy Immunol. 2018;54:52. https://doi.org/10.1007/s12016-017-8622-7.
11. Harris JE, Harris TH, Weninger W, Wherry EJ, Hunter CA, Turka LA. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-γ for autoreactive $CD8^+$ T-cell accumulation in the skin. J Invest Dermatol. 2012;132(7):1869–76. https://doi.org/10.1038/jid.2011.463.
12. Dwivedi M, Kemp EH, Laddha NC, Mansuri MS, Weetman AP, Begum R. Regulatory T cells in vitiligo: implications for pathogenesis and therapeutics. Autoimmun Rev. 2015;14(1):49–56.
13. van Geel N, Speeckaert R. Segmental vitiligo. Dermatol Clin. 2017;35(2):145–50. https://doi.org/10.1016/j.det.2016.11.005.
14. Becatti M, Prignano F, Fiorillo C, Pescitelli L, Nassi P, Lotti T, Taddei N. The involvement of Smac/DIABLO, p53, NF-kB, and MAPK pathways in apoptosis of keratinocytes from perilesional vitiligo skin: protective effects of curcumin and capsaicin. Antioxid Redox Signal. 2010;13(9):1309–21. https://doi.org/10.1089/ars.2009.2779.
15. Shi F, Kong BW, Song JJ, Lee JY, Dienglewicz RL, Erf GF. Understanding mechanisms of vitiligo development in Smyth line of chickens by transcriptomic microarray analysis of evolving autoimmune lesions. BMC Immunol. 2012;13:18. https://doi.org/10.1186/1471-2172-13-18.
16. Rashighi M, Agarwal P, Richmond JM, Harris TH, Dresser K, Su MW, Zhou Y, Deng A, Hunter CA, Luster AD, Harris JE. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. Sci Transl Med. 2014;6(223):223ra23. https://doi.org/10.1126/scitranslmed.3007811.
17. Eby JM, Kang HK, Klarquist J, Chatterjee S, Mosenson JA, Nishimura MI, Garrett-Mayer E, Longley BJ, Engelhard VH, Mehrotra S, Le Poole IC. Immune responses in a mouse model of vitiligo with spontaneous epidermal de- and repigmentation. Pigment Cell Melanoma Res. 2014;27(6):1075–85. https://doi.org/10.1111/pcmr.12284.

21 Methods to Study Vitiligo: Noninvasive Techniques and In Vivo Reflectance Confocal Microscopy

Hee Young Kang and Marco Ardigò

Contents

Abstract

Standardized methodologies for describing and classifying vitiligo and for assessing the effect of treatments are needed to be developed. Currently, there are many noninvasive techniques available for diagnosis and assessment of vitiligo. Objective measurements of vitiligo area using digital image analysis are available. Noninvasive instruments such as reflectance spectroscopy or dermoscopy are methods to characterize and study vitiligo lesion. The Vitiligo European Task Force (VETF) recommends Wood's light examination as a diagnostic tool in evaluating vitiligo to assess staging and spreading in a selected area. More reliable and quantitative measures to augment clinical judgment of vitiligo assessment include image analysis of digital photographs. The digital image analysis system can overcome the inevitable differences between observers, which are intrinsic to a visual grading method, and is advisable for clinical trials on vitiligo to objectively assess repigmentation in limited lesions (Linthorst Homan MW et al. J Eur Acad Dermatol Venereol 27(2):e235–238, 2013). However, this technique needs to be properly validated.

H. Y. Kang (✉)
Department of Dermatology, Ajou University School of Medicine, Suwon, Korea
e-mail: hykang@ajou.ac.kr

M. Ardigò
San Gallicano Dermatological Institute, IRCCS, Rome, Italy
e-mail: marco.ardigo@ifo.gov.it

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_21

Limitations of this method include as the technique is complex and laborious, it is only feasible for monitoring limited areas of vitiligo and less feasible in daily practice. Mexameter measurement provides a clinically accessible and straightforward means of increasing diagnostic accuracies in hypopigmentary disorders. In vivo reflectance confocal microscopy (RCM) is a real-time, repetitive imaging tool that provides noninvasive images at a nearly histological resolution. RCM could be used in the therapeutic monitoring and evaluation of the evolution of vitiligo. Limitations of RCM include that the instrument is expensive and the RCM imaging can still be time-consuming especially for the systematic examination of lesion area. Also, this machine requires a group of technicians to operate it and an expert to analyze the images.

Key Points

- The extension and severity of vitiligo guide prognosis and therapeutic choices. However, wide variations exist both in assessment rules and interpretation of their use, making intra- and inter-observer variations unavoidable.
- Wood's light examination remains a traditional tool in measuring the extent and progression of the disease.
- A variety of clinical scoring systems and objective assessment with image analysis of digital photographs is used to evaluate the treatment outcome in terms of repigmentation.
- Several instruments such as reflectance spectroscopy or dermoscopy could be useful in characterizing and studying the vitiligo lesion.
- In vivo reflectance confocal microscopy is a promising tool for characterizing melanocytes loss in untreated and treated vitiligo lesions.

21.1 Introduction

Standardized methodologies for describing and classifying vitiligo and for assessing the effect of treatments are needed to be developed. Currently, there are many noninvasive techniques available for diagnosis and assessment of vitiligo. Wood's light examination has been used to measure the extent and progression of the disease. Visual comparison of vitiligo lesion using photographs and the parametric measurement of vitiligo severity such as VASI (vitiligo area scoring index) or VIDA (vitiligo disease activity) score were introduced to measure the extent of depigmentation both at baseline and following therapeutic interventions. Objective measurements of vitiligo area using digital image analysis are available. Noninvasive instruments such as reflectance spectroscopy or dermoscopy are methods to characterize and study vitiligo lesion. Reflectance confocal microscopy offers a great potential for noninvasive assessment of vitiligo. All these techniques have been reported to be useful in the diagnosis or assessment of vitiligo lesions.

21.2 Wood's Light Examination

Wood's lamp emits long-wave UV radiation in a band between 320 and 400 nm with a peak at 365 nm [1] (Fig. 21.1a). The light source is a high-pressure mercury arc fitted with a compound filter made of barium silicate and 9% nickel oxide, the "Wood's filter." The UV radiation penetrates the epidermis, where it is attenuated by melanin; upon entering the dermis, it stimulates fluorescence emission by collagen bundles (Fig. 21.1b). Part of the emitted fluorescence is directed toward the surface of the skin, but it is attenuated by hemoglobin in the capillaries and melanin in the epidermis. This procedure enhances the epidermal pigmentation variations that are not apparent under visible light and improves the assessment of the extent of pigment abnormalities. Ideally, the lamp should be allowed to warm up for about 1 min and the

examination should be performed in a very dark room. In depigmented skin lesions of vitiligo, there is reduced or no epidermal melanin, and hence there is a window through which a light-induced autofluorescence of dermal collagen can be seen. The lesion appears bright blue-white due to autofluorescence (Fig. 21.1c, d). The results of Wood's light examination can be recorded through UV photography. The Vitiligo European Task Force (VETF) recommends Wood's light examination as a diagnostic tool in evaluating vitiligo to assess staging and spreading in a selected area [2]. All patients with vitiligo of lower phototypes should be examined under both visible and Wood's light. On the other hand, the technique examination is less valuable in dark-skinned patients in whom hypopigmented lesions can be easily perceived by the naked eye.

21.3 Clinical Scoring/Grading Methods

Reliable outcome measures are important to compare studies and to assess the changes over time. A variety of scoring systems is used to evaluate the treatment outcome in terms of repigmentation [3]. Most methods used in clinical practice are subjective, as they involve subjective assessment by a clinician to grade the degree of repigmentation. Commonly, serial photographs either with

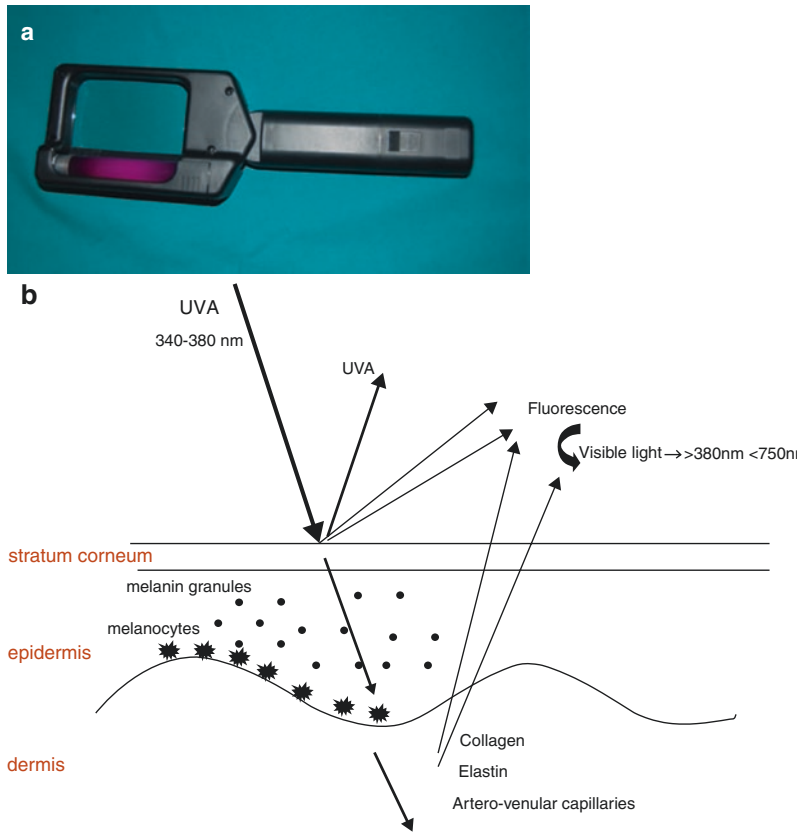


Fig. 21.1 (**a**) Wood's lamp. The envelope of the bulb is composed of a deep *bluish-purple* glass called Wood's glass, a nickel oxide-doped glass, which blocks almost all visible light above 400 nm. In this instrument a magnifying glass is present. (**b**) The UV radiation penetrates the epidermis, where it is attenuated by melanin; upon entering the dermis, it stimulates fluorescence emission by collagen bundles. The light emitted, even if absorbed by the epidermal melanin, exits the skin and will reach the eye of the observer. (**c**, **d**) Wood's light examination of depigmented patches on the hands of a patient with vitiligo. Standard lighting (**c**) and Wood's light lighting (**d**)

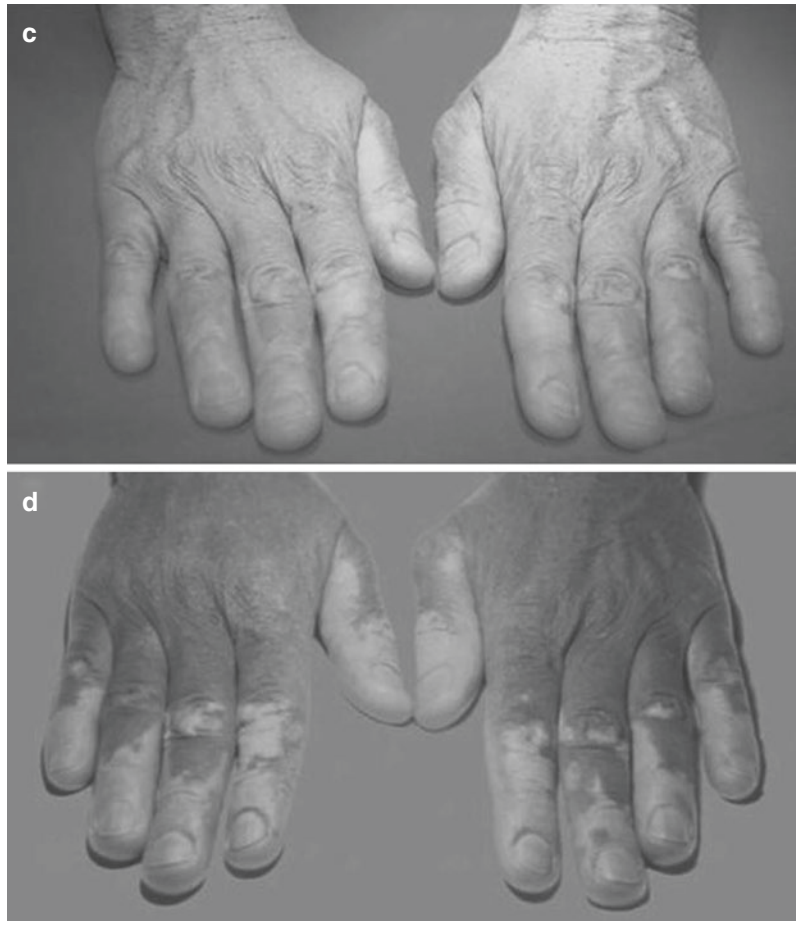


Fig. 21.1 (continued)

visible light or a Wood's lamp is used to compare the current lesion with its size in the past. A global assessment scale (GAS) is currently one of the most commonly used methods for evaluating treatment response. It is a four or five ordinal scoring system based on repigmentation percentage within a lesion over time categories (e.g., 1% to 25%, 26% to 50%, 51% to 75%, >75%). The "rule of nines" developed for calculating percentage surface area burns has been proposed by the VETF [4]. The "rule of nines" assumes that the head/neck, each arm, each leg, and the four trunk quadrants each compose 9% of the total body surface area, leaving 1% for the genitalia. In the "flat hand = 1%" method, a flat hand represents 1% of the total body surface area. Both methods are subjective and are based on visual assessments [3].

Several parametric methods such as vitiligo area severity index (VASI) and vitiligo disease activity (VIDA) score were introduced as quantitative methods to standardize outcome measurements in vitiligo. The VASI is a standardized, sensitive method to measure the extent and percentage of de-/repigmentation [4]. In the VASI, the patient's body is divided into five separate and mutually exclusive regions: hands, upper extremities, trunk, lower extremities, and feet. For each body region, the VASI is determined by the product of the area of vitiligo in hand units (which was set at 1% per unit) and the extent of depigmentation within each hand unit-measured patch (possible values of 0%, 10%, 25%, 50%, 75%, 90%, or 100%). The total body VASI is then calculated using the following formula by

considering the contributions of all body regions (possible range, 0–100): VASI = *P* (all body size) (hand units) · (depigmentation). It was suggested that VASI is a valid quantitative clinical tool that can be used to evaluate vitiligo parametrically. However, this method has a subjective component as it involves the physician deciding the amount of pigmentation and the area of involvement. The VIDA is a six-point scale for assessing vitiligo stability over time. It depends on patient's own reports of disease activity [5]. Active vitiligo involves either the expansion of existing lesions or the appearance of new lesions. Grading is as follows: VIDA score +4, activity lasting 6 weeks or less; +3, activity lasting 6 weeks to 3 months; +2, activity lasting 3–6 months; +1, activity lasting 6–12 months; 0, stable for 1 year or more; and −1, stable with spontaneous repigmentation for 1 year or more. A low VIDA score indicates less vitiligo activity.

Point-counting method has been proposed and may provide a simple alternative in measuring the surface area of vitiligo lesions [6]. In this method, the lesion borders are marked with an ordinary ballpoint pen, and a piece of paper is immediately placed over the lesion. For each lesion, the copied borders of the projection areas are enhanced by redrawing the contours with a pen. To estimate the number of points, a transparent sheet that has a point (+) printed on it is randomly superimposed on the lesion projection area (Fig. 21.2). The number of intersections hitting the area of interest is counted. The total area of each lesion is estimated by multiplying the representative area of a point on a grid according to the total number of points counted for the lesion. The reliability of the point-counting method has been tested against image analysis and has shown to be statistically significant in measuring surface areas [6].

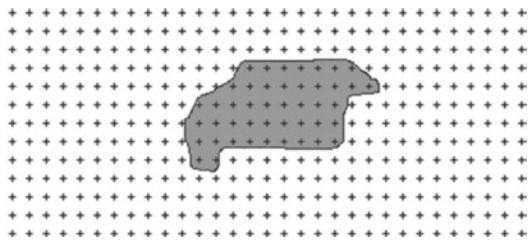


Fig. 21.2 Point-counting method. The total area of each lesion is estimated by multiplying the representative area of a point on grid by total number of points counted for the lesion

21.4 Photograph (Light/UV) and Digital Image Analysis

More reliable and quantitative measures to augment clinical judgment of vitiligo assessment include image analysis of digital photographs. Objective measurements of vitiligo area using digital image analysis have been introduced. Van Geel et al. [7] first published a computerized digital image analysis system for the objective assessment of vitiligo lesions in transplantation studies, followed by other groups that have used a digital image analysis system in evaluating vitiligo treatments. Digital photograph images are taken under standardized conditions for lighting, position, and exposure time. The images are analyzed using a commercial image analysis software such as Image-Pro Plus 4.5 (Media Cybernetics, Rockville, MD) [6], AutoCAD 2000 (Autodesk, Inc, San Rafael, CA, USA) [8], or Corel Draw 9.0 (Corel Corporation, Ottawa, Ontario, Canada) [9] or other novel software programs that combine standard image-processing techniques which can compute the surface area of a pre-outlined depigmented lesion or by planimetry (manual tracing of lesions onto transparent graph paper) [10–14]. The technique is based on principal component analysis and independent component analysis which converts three different spectral bands in a skin image into an image that represents skin area based only on melanin and hemoglobin. This technique allows the effective segmentation of vitiligo skin lesion areas and normal skin and consistently maintains high sensitivity, specificity, and accuracy values for all images. Whereas the results of the digital and clinical assessments with a global assessment scale (GAS) were comparable, patients' ratings diverged [10]. The digital image analysis system can overcome the inevitable differences between observers, which are intrinsic to a visual grading method, and is advisable for clinical trials on vit-

iligo to objectively assess repigmentation in limited lesions [10]. However, this technique needs to be properly validated.

Limitations of this method include as the technique is complex and laborious, it is only feasible for monitoring limited areas of vitiligo and less feasible in daily practice. There also exists potential difficulty in assessing larger lesions or lesions over curved surface as any photograph is a two-dimensional (2D) projection of a three-dimensional (3D) surface. Therefore, it is subject to errors due to the inability to estimate accurately the area from a photograph. Furthermore, perifollicular repigmentation or other complex patterns are difficult to catch using boundary tracing system. The emerging technique of taking whole-body 3D images followed by 3D image analysis to quantify the vitiligo-affected body surface area (BSA) of the subjects was recently introduced [15]. In this study, for the known surface area reference, 2D image analysis resulted in lesion area of 14.98 cm^2, whereas 3D image analysis resulted in a lesion area of 19.50 cm^2. It was concluded that 2D image analysis of contoured surfaces can underestimate lesion area by more than 20%, whereas 3D image analysis accounts for the changes in relative contour and has an associated error of <1%.

21.5 Reflectance Spectroscopy

Skin color is predominantly determined by pigments such as hemoglobin, melanin, bilirubin, and carotene. Oxyhemoglobin and deoxyhemoglobin absorb specifically between 540 and 547 nm. Melanin heavily absorbs all wavelengths but demonstrates a monotonic increase toward shorter wavelengths. Objective skin color-measuring instruments have been reported to provide a reproducible and sensitive means of quantifying small skin color differences [16]. It determines color by measuring the intensity of reflected light of specific wavelengths. Two types of skin reflectance instruments are available currently for the determination of skin color: a tristimulus colorimeter using the L*, a*, b* color system and the narrowband simple reflectance meters using the erythema/melanin indices. It was reported that moderate to high significant linear correlations could be established between the L*, a*, b* color parameters and the erythema/melanin indices [16]. Reflectance tristimulus colorimeter converts reflectance data into three color values, expressed in the L*, a*, b* color system. L* indicates luminance and describes the relative lightness ranging from 0 (black) to 100 (white). The a* indicates the balance between green (negative value) and red (positive value), and b* represents the balance between blue (negative value) and yellow (positive value). Different handheld tristimulus reflectance colorimeters are commercially available for the measurement of skin color, and the Chroma Meter CR 200 or CR 300 (Minolta Osaka, Japan) has become popular (Fig. 21.3a). Colorimetric examination of vitiligo patients revealed that the perilesional skin near to the vitiligo spot is not normal [17] (Fig. 21.3b). L* values decreased significantly in relation to increasing distance from the vitiligo spot, and the skin near vitiligo spot was lighter than normal skin as far as 5 cm from the vitiligo spot. In contrast, the pigmentation index (b*) gradually increased from lesional to perilesional to normal skin. Furthermore, the comparison of the b* value between the normal skin as far as 5 cm from the nearest vitiligo spot was significantly higher than perilesional skin. This finding suggested that the use of colorimetric method in vivo makes perceivable slight color variations that the eyes are not able to distinguish differences where there are gradual changes as in the perilesional skin of vitiligo lesion. In another study, skin color measurement was performed with colorimeter after excimer laser treatment [18]. The L* and a* values of the vitiligo lesions before laser treatment were significantly different from those after laser treatment. The repigmentation scores were correlated significantly with the vitiligo a* values after treatments.

Narrowband simple reflectance meters measure “melanin index” (MI) and an “erythema index” (EI). Each index increases as the skin becomes more pigmented or erythematous, respectively. There are several commercially

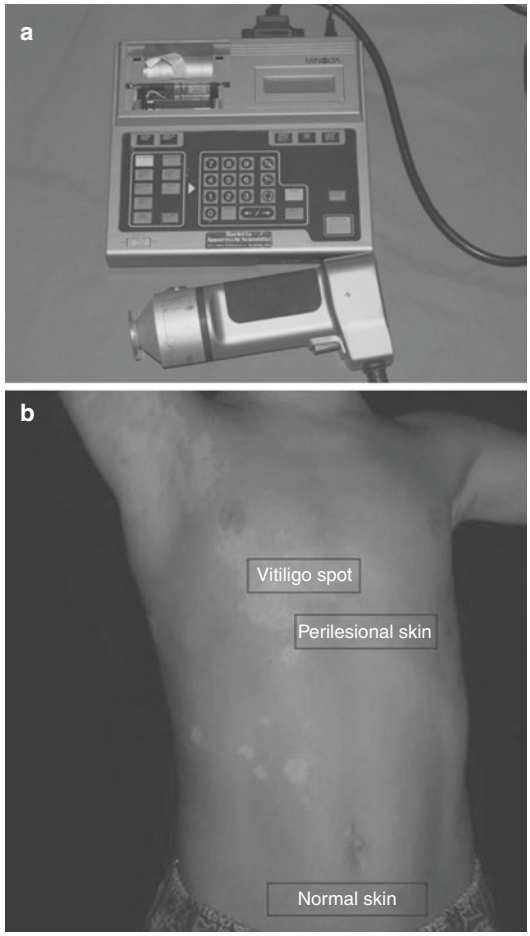


Fig. 21.3 (**a**) Minolta CR-200 Chroma Meter. (**b**) Colorimetric examination was performed with the lesional skin, the perilesional skin, and the normal skin as far as 5 cm from the nearest vitiligo spot in a young patient. L*, a*, b* color parameters were measured

available instruments that measure MI and EI such as DermaSpectrometer from Cortex (Denmark) and Mexameter from Courage-Khazaka (Germany). The usefulness of a Mexameter for discriminating vitiligo with nevus depigmentosus was shown [19]. The mean relative melanin index (RMI) was calculated by % melanin index (MI) of an affected lesion/MI of a symmetrically located normally pigmented area ×100. The RMI of nevus depigmentosus lesions was significantly higher than that of vitiligo lesions. No nevus depigmentosus lesions had an RMI of <50%. These results suggested that the Mexameter measurement provides a clinically accessible and straightforward means of increasing diagnostic accuracies in hypopigmentary disorders.

21.6 Dermoscopy

Digital epiluminescence dermoscopy is employed to examine pigmented lesions. Dermoscopy is useful for finding leukotrichia that is clinically common in patients with segmental vitiligo (Fig. 21.4). In a study using digital microscopy to detect tiny and thin vellus hairs unnoticeable with the naked eye, all 82 patients with segmental vitiligo had leukotrichia [20]. The examination of white hairs with a digital microscope might be useful for the prediction of treatment outcome and decision of treatment modalities. Confirming the presence of leukotrichia in patients with vitiligo before excimer laser treatment with dermoscopy was thought to be helpful in predicting the response to treatment [21]. In this study, 92.9% of patients with leukotrichia achieved <50% repigmentation. Various dermoscopic findings are associated with stability of vitiligo [22]. These include reduced pigmentary network, absent pigmentary network, reversed pigmentary network, perifollicular hyperpigmentation, and perilesional hyperpigmentation in the evolving vitiligo lesions; a white glow was present in 90% of patients.

21.7 In Vivo Reflectance Confocal Microscopy

In vivo reflectance confocal microscopy (RCM) is a real-time, repetitive imaging tool that provides noninvasive images at a nearly histological resolution [23]. The basic concept of confocal microscopy is the selective collection of light from the focused spot of the skin according to the different reflectance indexes of the different skin structures (Fig. 21.5). A confocal microscope consists of a light source, condenser and objective lenses, and a detector. The light source, near-infrared wavelength laser beam, illuminates a specific point within skin, and reflected light is

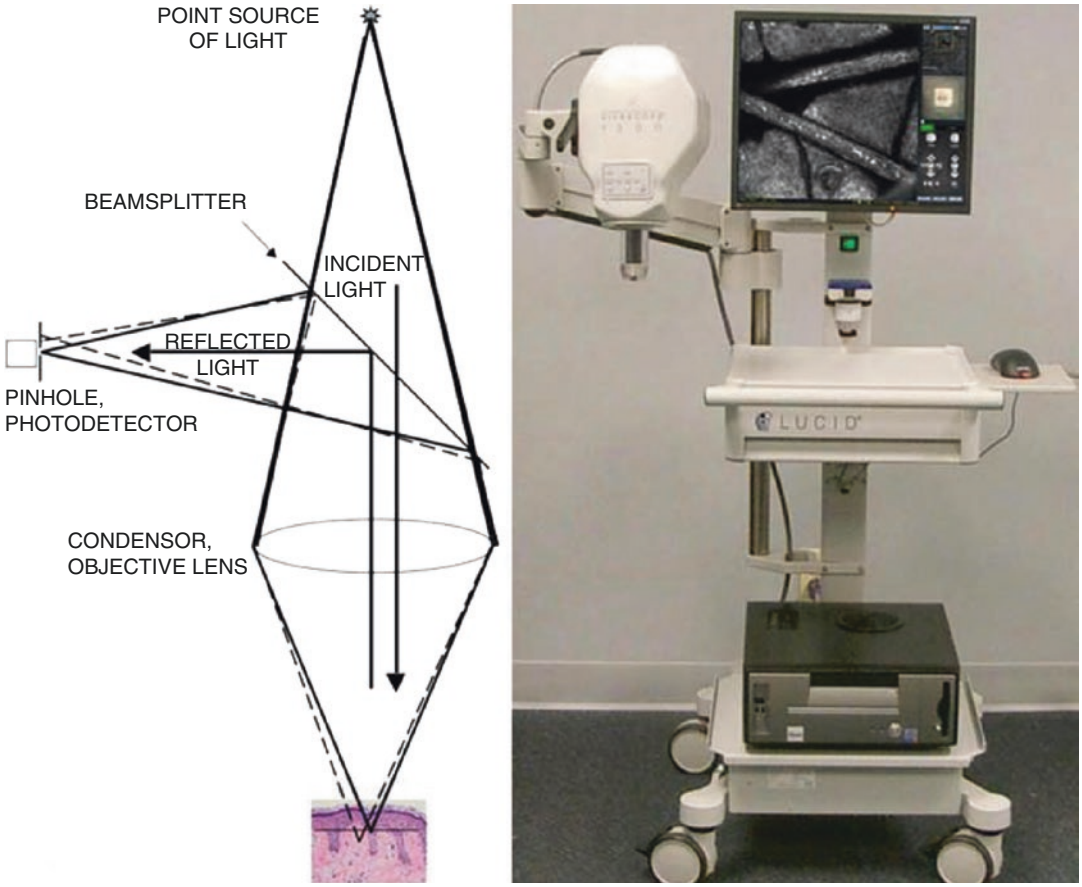


Fig. 21.4 The digital microscopy detects tiny and thin vellus hairs unnoticeable with the naked eye in segmental vitiligo (Courtesy of Pr. DY Lee)

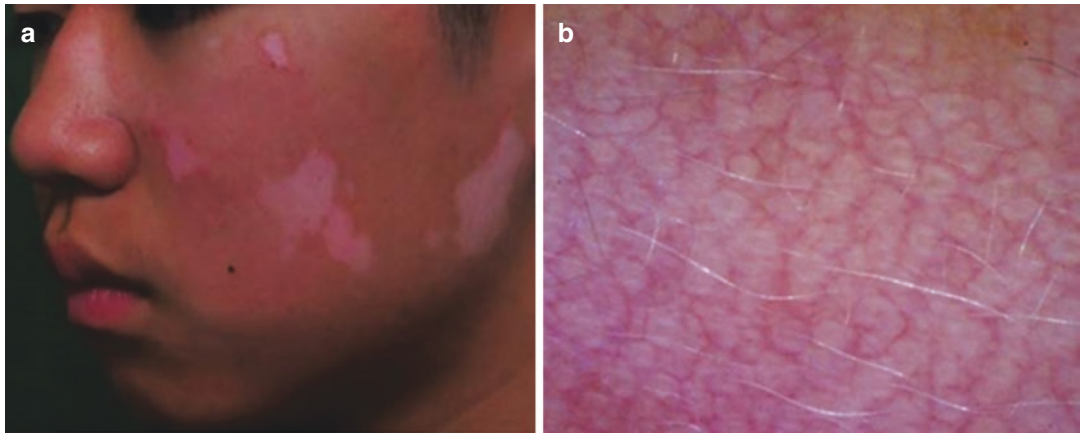
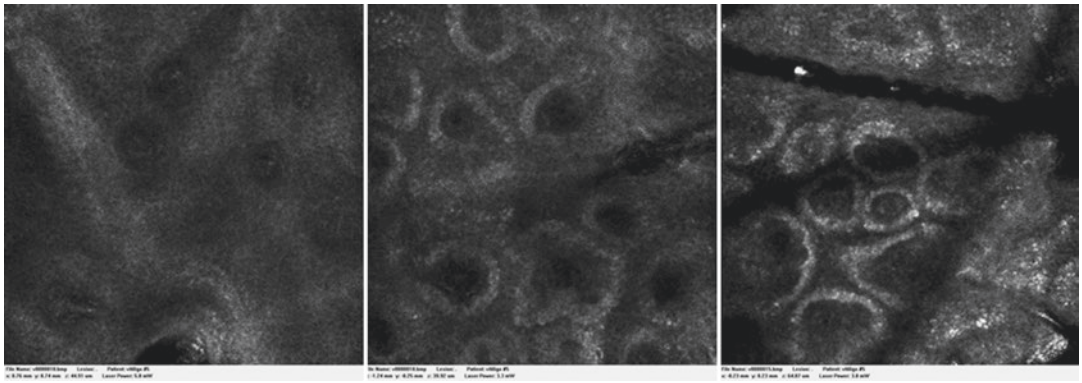


Fig. 21.5 Reflectance confocal microscope optical function (**a**) and commercial device Vivascope® (**b**)

collected through a pinhole in the detector. The illuminated spot is then scanned horizontally producing black and white images from the stratum corneum to the upper dermis with an imaging



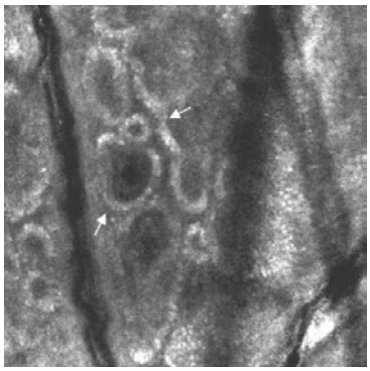


Fig. 21.6 In vivo reflectance confocal microscope (RCM) imaging of vitiligo. (**a**) RCM images of lesional skin show no bright cells and the disappearance of the normal papillary ring at the level of basal layer. The brightness of the basal cells in perilesional skin is lighter than distant normal skin. (**b**) So-called half rings or scalloped border-like rings (arrows)

depth of up to a maximum of 250 μm. Highly reflective skin components including melanin, collagen, and keratin appear bright (white) in RCM images. Because melanin is the strongest endogenous contrast in the skin, RCM is particularly suitable to analyze pigmented lesions and consequently pigmentary disorders.

The RCM examination allows the identification of characteristic features of vitiligo and repigmented skin during treatment [24] (Fig. 21.6). In normally pigmented skin, RCM images from the dermo-epidermal junction appear as bright cells grouped around dermal papilla defining characteristic ring structures composed of keratinocytes and melanocytes surrounding the upper dermis and "papillary rings." RCM examination of vitiligo lesions shows a disappearance of papillary rings at the basal layer level and no evidence of melanocytes. Perilesional normal skin shows intact papillary rings, but the brightness of cells was significantly reduced compared to distant normal skin. Distant normal skin showed no difference with controls. So-called half rings or scalloped border-like rings were observed in distant normal skin but also normal skin of control (Fig. 21.6d–f). Ardigo et al. [25] suggested that this change could derive from an incomplete distribution of pigment along the basal layer because of an initial and progressive disappearing of melanocytes or to a congenital defective melanocyte distribution. Another group suggested that the incomplete papillary rings are observed in the active lesions of vitiligo [26]. During UVB treatment, RCM examination shows large dendritic melanocytes located close to hair follicles, in the epidermis of perilesional skin, and of repigmentation islands (Fig. 21.7). These findings suggested that RCM

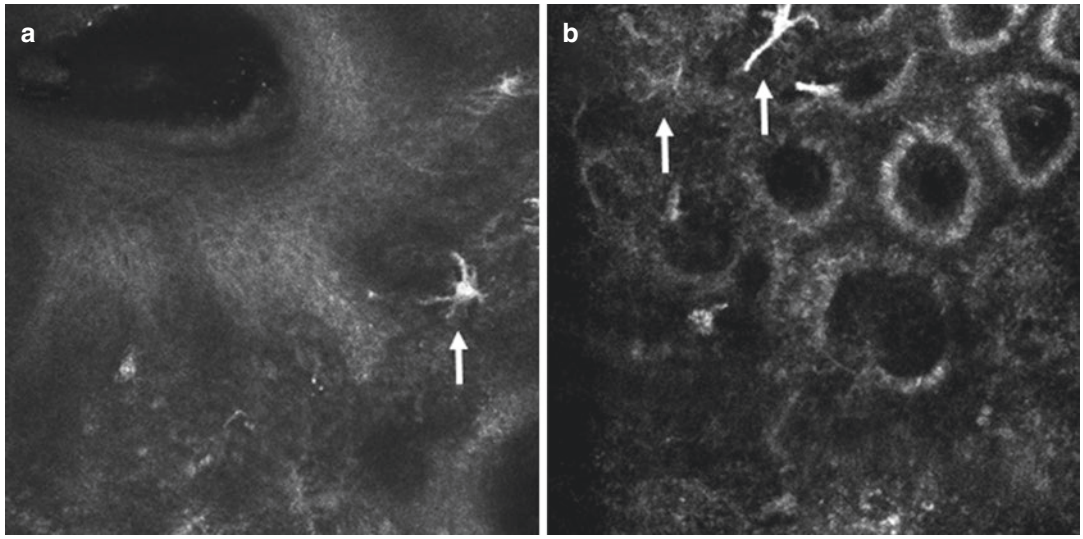


Fig. 21.7 RCM imaging of vitiligo during UVB therapy showing bright dendritic melanocytes (arrows) (**a**) around a hair follicle, (**b**) at the basal layer of perilesional skin

could be used in the therapeutic monitoring and evaluation of the evolution of vitiligo.

RCM might be useful to determine stability of vitiligo [27]. In the active stage of vitiligo, the RCM examination reveals that the bright dermal papillary rings lose their integrity or totally disappear; border between vitiligo lesion and normal skin becomes unclear, and highly refractile cells that referred to infiltrated inflammatory cells could be seen within the papillary dermis at the edge of the lesions. Differently, in the stable stage of vitiligo, RCM shows a complete loss of melanin in lesional skin and a clear border between lesional and normal skin. In the cited study [27], the RCM images were comparable with vitiligo staging based on vitiligo disease activity (VIDA) scoring.

RCM might serve as a useful instrument in noninvasively differentiating vitiligo and other hypopigmentary disorders such as nevus depigmentosus, nevus anemicus, or postinflammatory hypopigmentation in vivo [28, 29]. In the lesional skin of nevus depigmentosus, the content of melanin and the brightness of dermal papillary rings decrease, and the melanin in adjacent normal appearing skin of nevus depigmentosus shows no change. This differs from the active stage of vitiligo which may show part of the dermal papillary rings disappear or the brightness of melanin in the part of dermal papillary rings decrease [26].

Limitations of RCM include that the instrument is expensive and the RCM imaging can still be time-consuming especially for the systematic examination of lesion area. Also, this machine requires a group of technicians to operate it and an expert to analyze the images [3].

21.8 Conclusion

There are many noninvasive techniques available for the diagnosis and assessment of vitiligo. Wood's light examination remains a traditional tool in measuring the extent and progression of the disease. A variety of clinical scoring systems and objective assessment with image analysis of digital photographs is used to evaluate the treatment outcome in terms of repigmentation. However, to date, there is no standardized method for measuring vitiligo lesions. The lack of standardization is responsible for the high variability in vitiligo assessment. Objective, noninvasive methods for measuring and monitoring the extent

of vitiligo skin compared to the surrounding normal skin are needed. Several instruments such as reflectance spectroscopy or dermoscopy could be useful in characterizing and studying the vitiligo lesion. In vivo reflectance confocal microscopy is a promising tool for characterizing the nature of melanocytes loss in untreated and treated vitiligo lesions.

References

1. Asawanonda P, Taylor CR. Wood's light in dermatology. Int J Dermatol. 1999;38(11):801–7.
2. Taieb A, Picardo M. The definition and assessment of vitiligo: a consensus report of the VitiligoEropean Task Force. Pigment Cell Res. 2007;20(1):27–35.
3. Alghamdi KM, Kumar A, Taïeb A, et al. Assessment methods for the evaluation of vitiligo. J Eur Acad Dermatol Venereol. 2012;26(12):1463–71.
4. Hamzavi I, Jain H, McLean D, et al. Parametric modeling of narrowband UV-B phototherapy for vitiligo using a novel quantitative tool: the Vitiligo Area Scoring Index. Arch Dermatol. 2004;140(6):677–83.
5. Bhor U, Pande S. Scoring systems in dermatology. Indian J Dermatol Venereol Leprol. 2006;72(4):315–21.
6. Aydin F, Senturk N, Sahin B, et al. A practical method for the estimation of vitiligo surface area: a comparison between the point counting and digital planimetry techniques. Eur J Dermatol. 2007;17(1):30–2.
7. VanGeel NAC, Vander Haeghen YMSJ, Ongenae K, et al. A new image analysis system useful for surface assessment of vitiligo lesions in transplantation studies. Eur J Dermatol. 2004;14(3):150–5.
8. Marrakchi S, Bouassida S, Meziou TJ, et al. An objective method for the assessment of vitiligo treatment. Pigment Cell Melanoma Res. 2008;21(1):106–7.
9. Sanclemente G, Garcia JJ, Zuleta JJ, et al. A double-blind, randomized trial of 0.05% betamethasone vs. topical catalase/dismutase superoxide in vitiligo. J Eur Acad Dermatol Venereol. 2008;22(11):1359–64.
10. Linthorst Homan MW, Wolkerstorfer A, Sprangers MA, et al. Digital image analysis vs. clinical assessment to evaluate repigmentation after punch grafting in vitiligo. J Eur Acad Dermatol Venereol. 2013;27(2):e235–8.
11. Nugroho H, Ahmad Fadzil MH, Shamsudin N, et al. Computerised image analysis of vitiligo lesion: evaluation using manually defined lesion areas. Skin Res Technol. 2013;19(1):e72–7.
12. Oh TS, Lee O, Kim JE, et al. Quantitative method for measuring therapeutic efficacy of the 308 nm excimer laser for vitiligo. Skin Res Technol. 2012;18(3):347–55.
13. Shamsudin N, Hussein SH, Nugroho H, et al. Objective assessment of vitiligo with a computerised digital imaging analysis system. Australas J Dermatol. 2015;56(4):285–9.
14. Sheth VM, Rithe R, Pandya AG, et al. A pilot study to determine vitiligo target size using a computer-based image analysis program. J Am Acad Dermatol. 2015;73(2):342–5.
15. Kohli I, Isedeh P, Al-Jamal M, et al. Three-dimensional imaging of vitiligo. Exp Dermatol. 2015;24(11):879–80.
16. Clarys P, Alewaeters K, Lambrecht R, et al. Skin color measurements: comparison between three instruments: the Chromameter(R), the DermaSpectrometer(R) and the Mexameter(R). Skin Res Technol. 2000;6(4):230–8.
17. Brazzelli V, Muzio F, Antoninetti M, et al. The perilesional skin in vitiligo: a colorimetric in vivo study of 25 patients. Photodermatol Photoimmunol Photomed. 2008;24(6):314–7.
18. Noborio R, Nakamura M, Yoshida M, et al. Monotherapy for vitiligo using a 308-nm xenon-chloride excimer laser: colorimetric assessment of factors that influence treatment efficacy. J Dermatol. 2012;39(12):1102–3.
19. Park ES, Na JI, Kim SO, et al. Application of a pigment measuring device--Mexameter--for the differential diagnosis of vitiligo and nevus depigmentosus. Skin Res Technol. 2006;12(4):298–302.
20. Lee DY, Kim CR, Park JH, et al. The incidence of leukotrichia in segmental vitiligo: implication of poor response to medical treatment. Int J Dermatol. 2011;50(8):925–7.
21. Kim MS, Cho EB, Park EJ, et al. Effect of excimer laser treatment on vitiliginous areas with leukotrichia after confirmation by dermoscopy. Int J Dermatol. 2016;55:886. https://doi.org/10.1111/ijd.12972.
22. Thatte SS, Khopkar US. The utility of dermoscopy in the diagnosis of evolving lesions of vitiligo. Indian J Dermatol Venereol Leprol. 2014;80(6):505–8.
23. Kang HY, Bahadoran P, Ortonne JP. Reflectance confocal microscopy for pigmentary disorders. Exp Dermatol. 2010;19(3):233–9.
24. Kang HY, le Duff F, Passeron T, et al. A noninvasive technique, reflectance confocal microscopy, for the characterization of melanocyte loss in untreated and treated vitiligo lesions. J Am Acad Dermatol. 2010;63(5):e97–100.
25. Ardigo M, Malizewsky I, Dell'anna ML, et al. Preliminary evaluation of vitiligo using in vivo reflectance confocal microscopy. J Eur Acad Dermatol Venereol. 2007;21(10):1344–50.
26. Lai LG, Xu AE. In vivo reflectance confocal microscopy imaging of vitiligo, nevus depigmentosus and nevus anemicus. Skin Res Technol. 2011;17(4):404–10.
27. Li W, Wang S, Xu AE. Role of in vivo reflectance confocal microscopy in determining stability in

vitiligo: a preliminary study. Indian J Dermatol. 2013;58(6):429–32.
28. Pan ZY, Yan F, Zhang ZH, et al. In vivo reflectance confocal microscopy for the differential diagnosis between vitiligo and nevus depigmentosus. Int J Dermatol. 2011;50(6):740–5.
29. Xiang W, Xu A, Xu J, et al. In vivo confocal laser scanning microscopy of hypopigmented macules: a preliminary comparison of confocal images in vitiligo, nevus depigmentosus and postinflammatory hypopigmentation. Lasers Med Sci. 2010;25(4):551–8.

Animal Models

22

Gisela F. Erf and I. Caroline Le Poole

Contents

Abstract

Vitiligo is a non-communicable, multifactorial pigmentation disorder. Autoimmunity has been identified as a major etiological factor in the postnatal loss of epidermal melanocytes in the skin of vitiligo patients. As with other tissue-specific autoimmune diseases, genetic predisposition to vitiligo development may be manifested in altered responsiveness of tissue cells and immune system components to endogenous and exogenous environmental factors, leading to immunorecognition and immune system-mediated loss of melanocytes. To understand the etiology and pathogenic mechanisms driving disease onset and progression, and to develop

G. F. Erf (✉)
Division of Agriculture, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR, USA
e-mail: gferf@uark.edu

I. C. Le Poole
Robert H. Lurie Comprehensive Center, Department of Microbiology and Immunology, North Western University, Chicago, IL, USA
e-mail: caroline.lepoole@northwestern.edu

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_22

effective treatment and prevention strategies for autoimmune vitiligo, appropriate animal models are required. In this context, experimental animal models that naturally develop vitiligo would more closely reflect the complex nature of the disorder in humans than experimental models where the autoimmune disease was induced. Animal models with truly naturally occurring autoimmune disease are rare. However, for autoimmune vitiligo, the Smyth line of chicken was established as a highly relevant, spontaneous model for both basic and translational research, as it displays the entire spectrum of clinical and biological manifestations of autoimmune vitiligo in humans. While there is no natural vitiligo mouse model, induction of vitiligo in mice by generation of melanocyte-specific immune responses proved to be an invaluable approach to dissect cellular and molecular mechanisms involved in the autoimmune depigmentation and repigmentation processes. Together, research using the natural and induced animal models will provide the critical knowledge needed to understand, treat, and prevent autoimmune vitiligo.

Key Points

- Chronic autoinflammatory/autoimmune disorders typically are multifactorial in nature, requiring several components such as genetic susceptibility, immune system components, and environmental factors for expression. Appropriate experimental animal models have become an essential tool to delineate and dissect the relative contributions of these components.
- The spontaneous autoimmune vitiligo described in the Smyth line chicken recapitulates the entire spectrum of clinical and biological manifestations of human vitiligo, providing a unique opportunity to examine etiology, progression, prevention, and treatment of the disease.
- Induction of autoimmune vitiligo in the mouse by generation of endogenous melanocyte-specific immune responses, adoptive transfer of melanocyte-specific T cells, or introduction of antigen-specific T cell receptor genes provides defined in vivo models to examine melanocyte loss and develop vitiligo treatment strategies.

22.1 The Multifactorial Nature of Autoimmune Disorders: Application to Vitiligo

Autoimmunity has been identified as a major etiological factor in vitiligo, although many other factors including infections, stress, neural abnormalities, aberrant melanocyte function, and genetic susceptibility have been implicated [1–8]. This multifactorial nature of vitiligo involving genetic susceptibility and disease-precipitating factors in addition to immunopathology is in itself in accordance with the general concepts described for tissue-specific autoimmune disease. In tissue-specific autoimmune diseases such as vitiligo, the genetic susceptibility is frequently associated with an inherent target cell defect that predisposes the target cell to immunorecognition and may include aberrant immune activity at various levels (e.g., dendritic cells/macrophages, T cells, and B cells). The autoimmune destruction of cells has been found to be associated with a lack of regulatory function within the immune system, heightened immune activity, and altered responsiveness of immune components and target cells to endogenous and exogenous factors. The role of environmental factors in the development of autoimmune disease is also multifaceted and may include infections by microbes, exposure to chemicals, and a wide array of other stress factors that can provoke an autoimmune response to the target cells [1, 5, 9–11]. Unfortunately, the relative contributions of these components in organ-specific autoimmune disease cannot be easily delineated and dissected, especially in human patients when they become apparent only after the clinical manifestation of the disease.

22.2 Naturally Occurring Animal Models of Vitiligo

In order to understand the initial etiology and pathogenic events leading to the onset and progression of autoimmune disease, appropriate animal models are required. In this context, experimental animal models that naturally develop the autoimmune disease would reflect the situation in humans more closely than experimental models where the autoimmune disease was induced. In biomedical research, the mouse has become the most studied animal model, due in part to its short-generation time, its small size, and the extensive availability of genetically defined strains of mice, research reagents, and research procedures. Murine models for spontaneous autoimmune disease are, however, rare, and there is currently no mouse model that develops spontaneous autoimmune vitiligo [9, 10, 12].

For vitiligo research, several animal models of naturally occurring vitiligo were identified and reviewed [9, 13–15]. These included the Smyth line of chicken, the water buffalo, the Sinclair pig, the gray horse, the barred rock chicken, and the vitiligo mouse. Of these models, the Smyth line chicken [9, 14, 16] and the water buffalo [17, 18] best reflect naturally occurring vitiligo; vitiligo in the Sinclair pig [19–21] and the gray horse [22, 23] is generally preceded by melanoma and is the result of immune system-mediated melanoma regression; the barred rock chicken [24–26] represents a model of natural melanocyte loss associated with cellular stress; and the vitiligo mouse (C57Bl/J6-vit/vit) [27–29] is no longer studied as a model for vitiligo because the progressive loss of fur pigmentation was found to be due to a mutation of the microphthalmia-associated transcription factor gene, a mutation not associated with human vitiligo [30]. Of all the naturally occurring animal models for vitiligo, only the Smyth line chicken continues to be studied extensively, and, as outlined later, it is currently the only animal model for spontaneous autoimmune vitiligo that has been demonstrated to recapitulate the entire spectrum of clinical and biological manifestations of the human disease.

22.2.1 The Smyth Line Chicken Model of Spontaneous Autoimmune Vitiligo

The Smyth line (SL) chicken is characterized by a spontaneous, vitiligo-like, post-hatch loss of melanin-producing pigment cells (melanocytes) in feather and choroidal tissue (Fig. 22.1, Table 22.1). SL vitiligo (SLV) occurs in approximately 80–95% of hatch mates, with about 70% of those affected expressing complete depigmentation in adulthood (>20 weeks of age). There are many similarities between SL and human vitiligo. Both are characterized by autoimmune destruction of melanocytes, usually first seen during adolescence and early adulthood. In both SL chickens and humans, pigmentation loss may be either partial or complete. Remelanization of amelanotic tissue occurs, although severe pigment loss and remelanization are more frequent in the chicken. In addition to vitiligo, SL chickens exhibit uveitis, often resulting in blindness (5–15%), and have associated autoimmune diseases such as hypothyroidism (4–8%) and an alopecia areata-like feathering defect (2–3%) [9, 12, 16, 32]. Similarly, in humans it is not uncommon to find thyroidal and other autoimmune diseases associated with vitiligo. Moreover, SL vitiligo, like human vitiligo, is a multifactorial disorder involving a genetic component (manifested in part as an inherent melanocyte defect; e.g., abnormal melanosome membranes), an immune system component (melanocyte-specific cell-mediated immunity), and environmental triggers (e.g., herpesvirus of turkey; HVT) [9, 12].

Continuous research on SL autoimmune vitiligo for over more than 35 years has not only established the many similarities between human and SL vitiligo but also has positioned the SL chicken as a highly suitable intermediate model for translational research on vitiligo treatment and prevention strategies. Key characteristics of the SL chicken that make it a highly relevant and suitable model for fundamental studies on the etiology, progression, and pathology of autoimmune vitiligo as well as for development of treatment and prevention therapies are summarized in Table 22.1.

The origin, development, and characteristics of this animal model have been reviewed extensively [9, 12, 16]. Highlights and recent developments

Fig. 22.1 Spontaneous autoimmune vitiligo in the Smyth line (SL) chicken model. (**a**) SL (yellow) and parental BL (blue) chicks hatch with normal pigmentation; (**b**) 6-week-old juvenile SL male and female with normal pigmentation; (**c**) young adult males, (**d**) females, and (**e**) growing feathers (GF) with various extents of depigmentation; (**e**) GF consist of a column (8–10 mm by 2 mm) of living tissue (pulp) surrounded by a sheath from which the barbs emerge; the pulp consists of an inner dermis, the side and top of which are enclosed by an epidermal layer; (**f**) the epidermis (E) of the bottom 2–3 mm (barb ridge) of a GF is modified containing melanocytes with their cell bodies facing the dermis (D) and dendrites extending along columns of keratinocytes; (**g**) normal barb ridge with melanocytes and pigmented keratinocytes; and (**h**) barb ridge in active vitiligo; brown cells are CD8+ T cells associated with damaged melanocyte cell bodies and dendrites; note: almost no pigment in keratinocytes

regarding this animal model that support its relevance for both basic and translational research on autoimmune vitiligo will be provided below.

22.2.1.1 The Genetics Basis of Smyth Line Autoimmune Vitiligo

The SL and control lines of chicken were developed by Dr. J. Robert Smyth, Jr. at the University of Massachusetts, Amherst, MA [16]. The mutant SL (previously known as the delayed-amelanotic (DAM) chicken) was developed from one female hatched in 1971 from a non-pedigreed mating of the Massachusetts Brown line (BL) [33]. The ability to develop a line of chickens with a predictably high incidence of vitiligo starting with one vitiliginous hen clearly demonstrates the heritability of this disorder.

Table 22.1 Spontaneous autoimmune vitiligo in the Smyth line chicken: unique opportunities for fundamental and translational research

Production	Robert Smyth, Poultry Geneticist, University of Massachusetts, Amherst, MA
Maintenance	Gisela F. Erf, Avian Immunologist, Division of Agriculture, University of Arkansas, Fayetteville, AR, USA
Features	Vitiligo-like, autoimmune, post-hatch loss of pigmentation in feathers and eye
Onset	6–14 weeks of age
Incidence	80–95% by 20 weeks (young adult)
Severity	Erratic to complete loss of pigmentation
Model	Availability of MHC-matched ($B^{101/101}$) control lines of chickens, including the parental Brown line, from which the Smyth line was developed (<2% incidence of vitiligo), and the Light-brown Leghorn chicken (vitiligo resistant)
Unique features	Recapitulates the entire spectrum of clinical and biological manifestations of the human disease
	Autoimmune vitiligo development is truly spontaneous and involves immune system mechanisms that parallel those established in human vitiligo
	Spontaneously occurring, associated autoimmune diseases (e.g., autoimmune thyroiditis, uveitis, alopecia) observed in some SL individuals also reflect the complexity and true nature of this autoimmune disease
	The target tissue (growing feather) is easily accessible and can be sampled prior to and throughout the development of SL vitiligo in the same individual
	The growing feather is a skin derivative with dermal and epidermal layers; melanocyte is located in the epidermis in close association with keratinocytes. Unlike hair, the growing feather is an immunologically active tissue (Fig. 22.1)
	The growing feather is an accepted skin test site (in vivo test tube) to monitor cellular/tissue responses to injected test materials [31]
	The progenitor pool of melanocytes is not affected by the melanocyte-specific autoimmune response, and repigmentation has been demonstrated
	The strong direct association of herpesvirus of turkey (HVT) administration at hatch and the incidence of vitiligo expression (incidence: >80% with HVT, <20% without HVT) allow for examination of disease-precipitating/protective factors in genetically susceptible individuals

The genetic basis of autoimmune vitiligo and line-associated traits has long been described as being under the control of multiple autosomal genes [16, 34]. Next-generation whole-genome resequencing identified various potential genetic markers with amino acid changes that may play a role in SLV development. Based on bioinformatic analysis of the genome data, SNPs were found to include genes involved in dermatological diseases/conditions. Furthermore, intermolecular gene network analysis revealed that candidate genes identified in SLV play a role in networks centered on protein kinases, phosphatase, ubiquitinylation, and amyloid production [35]. These studies provide new insight and direction in identifying genetic components underlying the susceptibility of SL chickens to develop SLV.

While SL and parental BL are closely related [36] and both are considered vitiligo susceptible, only a few (<2%) BL chickens develop the disorder compared to 80–95% of SL chickens. The incidence of vitiligo in the BL however dramatically increased to 71% when $B^{101/101}$ BL chickens were treated with the DNA methylation inhibitor 5-azacytidine [37]. These observations confirmed genetic susceptibility in the BL and epigenetic regulation of vitiligo expression. It should be noted that the 5-azacytidine treatment did not trigger vitiligo in Light-brown Leghorn (LBL) chickens, which supports their use as the vitiligo-resistant pigmentation control [37].

22.2.1.2 Immune System Involvement in Smyth Line Autoimmune Vitiligo

Several studies have provided evidence supporting the role of the immune system in the pathology of SLV. Destruction of melanocytes is preceded by increased levels of circulating

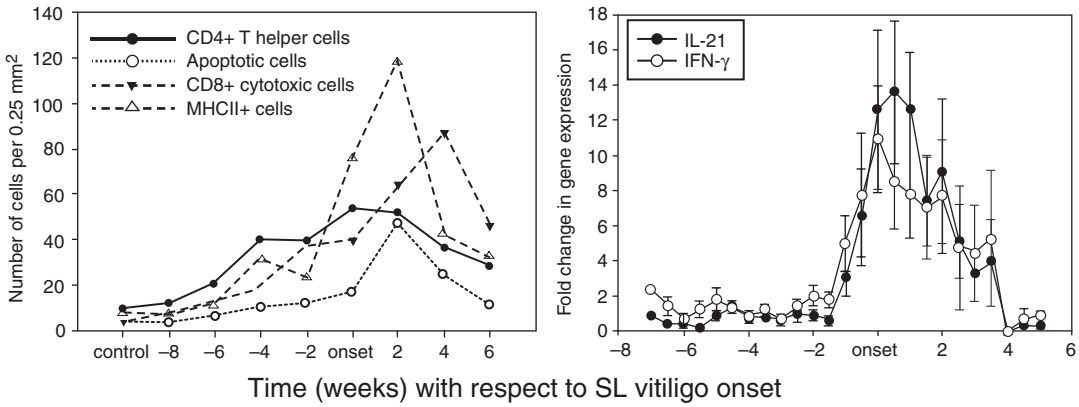


Fig. 22.2 T cell infiltration, MHC class II expression, melanocyte apoptosis, and IFN-γ and IL-21 mRNA expression in Smyth line growing feathers (GF) prior to and throughout the development of vitiligo. Left: growing feathers were collected from Smyth line chickens every 2 weeks. GF were snap-frozen for immunohistochemical and tunnel staining and tissues analyzed by bright-field microscope. Right: GF were collected twice per week and RNA isolated for qRT-PCR (mean ± SEM). Because age of onset differed among birds, data were expressed with respect to visible onset of vitiligo for both studies

inflammatory cells and the infiltration of the feather pulp and barb ridge by macrophages and lymphocytes. Prior to onset of vitiligo, the infiltrate consisted primarily of T-helper cells (CD4+), but with onset of vitiligo and active melanocyte destruction, cytotoxic T cells (CD8+) predominated (Fig. 22.2) [9, 38–40]. Melanocyte death was shown to occur by apoptosis, apparently induced by cytotoxic T cells [41]. Overall, the immunopathology of SLV described earlier is very similar to observations in affected skin of vitiligo patients [6] and supports the involvement of cell-mediated immune mechanisms in melanocyte destruction. In SL chickens, direct evidence for a role of cell-mediated immunity in the development of SLV was provided by immunosuppression studies and by in vivo demonstration of anti-melanocyte cell-mediated immunity in vitiliginous but not in non-vitiliginous SL and control chickens [42, 43].

Targeted cytokine gene expression (RNA) analysis of growing feathers collected prior to and throughout SLV development revealed a Th1 cytokine signature, whereby IFN-γ expression was accompanied by high expression of IL-10 and IL-21 (Fig. 22.2) [44]. The observation of IL-21 expression near onset and during SLV progression is particularly interesting, especially since IL-21 has been implicated in a number of autoimmune diseases [45–47]. Additionally, as shown by microarray transcriptome analysis [48], IL-21 receptor expression is similarly increased in growing feathers within 2 weeks before SLV onset and throughout active autoimmune loss of melanocytes.

Differential gene expression analysis at the transcriptome level using a 44K microarray also confirmed the complex nature of SLV [48]. For this study, gene expression comparisons were made between growing feather samples collected from the parental BL control chickens, from SL chickens that did not develop vitiligo, and from SL chickens at various stages of SLV development (within 2 weeks before visible SLV onset, during active SLV, and 2 weeks after complete depigmentation). Functional and network analyses of differently expressed genes highlighted innate and adaptive immunity (both cell-mediated and humoral), as well as neuronal involvement, apoptosis, cellular stress, and melanocyte function. Moreover, the time course approach used in this study led to important new insights into events leading to SLV onset and provided a more precise window in time to study the etiology of SLV [44, 48].

Vitiliginous SL chickens have melanocyte-specific autoantibodies that have been shown to bind to chicken as well as human melanocytes

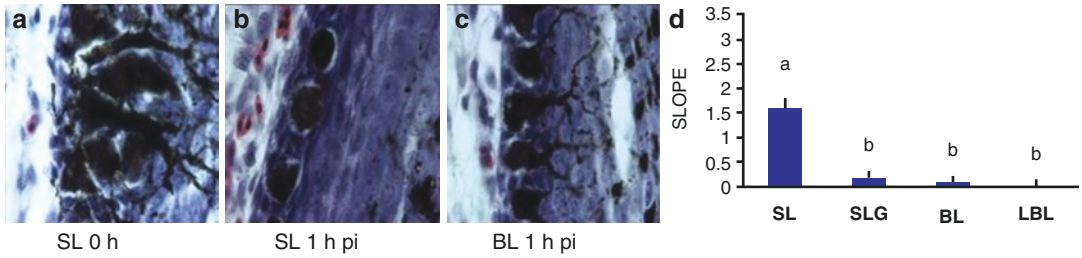


Fig. 22.3 Response of SL and BL melanocytes to 4-TBP (4-tertiary butyl phenol; 10 μL of 0.15 mg/mL) injection into the feather pulp. (**a**) Vehicle-injected SL feather. (**b**) 1-h postinjection (pi) SL melanocytes retracted their dendrites, whereas those of BL controls (**c**) did not. (**d**) Production of oxidative radicals (kinetic 2′,7′-dichlorofluorescein diacetate assay) by pigmented feather tips cultured with 200 μM 4-TBP (n = 8; mean ± SEM; SL, Smyth line; SLG, SL with gray shanks; SLG rarely express vitiligo; BL, Brown line parental control; LBL, Light-brown Leghorn normal control)

and to be specific to tyrosine-related protein-1 (TRP1) and heat-shock proteins (HSP), but their role in the etiology of SLV has not been established [14, 49, 50]. Maternal autoantibodies to melanocytes have been shown to be transferred from the hen to her chick via the egg but disappeared from the chick's circulation within 10 days of hatch, and their transfer was not correlated with vitiligo expression [51].

22.2.1.3 Inherent Melanocyte Alterations in Smyth Line Autoimmune Vitiligo

Previous studies by Smyth and co-workers describe the presence of a competent pigment system in SL chicks at hatch (Fig. 22.1a). The earliest abnormalities within SL melanocytes, prior to visible onset of SLV, were irregularly shaped melanosomes containing pigmented membrane extensions, hyperactive melanization, and selective autophagocytosis of melanosomes [52–55]. Similar degenerative processes were also observed in vitro in embryo-derived SL melanocytes, including heightened lipid peroxidation and catalase activity [55, 56]. Exposure of SL and control melanocytes to 4-TBP, a phenolic compound known to initiate stress in human melanocytes [57], also revealed heightened sensitivity of SL melanocytes [58, 59]. Specifically, in vivo injection of 4-TBP into the dermis of pigmented growing feathers resulted in melanocyte detachment and dendrite retraction at 1 and 3.5 h postinjection, with signs of recovery by 6 h and full restoration of melanocyte morphology 3 days after 4-TBP injection. Exposure of melanocyte containing tissue sections (feather tips), as well as feather-derived and embryo-derived melanocytes to 4-TBP, resulted in increased reactive oxygen species generation in SL compared to BL cultures (Fig. 22.3).

These in vivo and ex vivo observations on the responses of melanocytes to 4-TBP in the chicken model further supports aberrant ability of SL melanocytes to respond to cellular stress. However, as shown through immunosuppression studies, the inherent melanocyte sensitivity alone is not sufficient to cause SLV without a functioning immune system, but it appears to play a role in provoking a melanocyte-specific autoimmune response [53, 60–63]. Morphological and biological melanocyte defects/alterations have also been reported in vitiligo patients and non-SL animal models for vitiligo. In both humans and animals, cultured vitiligo melanocytes grow more slowly than normal melanocytes, are more dependent on antioxidants in the medium, and present structural alterations [7, 24, 25, 55, 64–68].

22.2.1.4 Role of Environmental Factor in Smyth Line Vitiligo Expression

In addition to heightened sensitivity of SL melanocytes to stress, we reported the role of environmental factors, specifically vaccination with live turkey herpesvirus (HVT) at hatch, in the expression of SLV [69]. Without HVT, the incidence of

SLV is <20%, but with HVT, the incidence is generally >80%. HVT is an alpha-herpesvirus commonly used in commercial chicken production as a vaccine to protect chickens from Marek's disease caused by serotype 1 Marek's disease viruses (MDV-1). HVT is a non-oncogenic serotype 3 MDV isolated from turkeys that causes only minor inflammatory lesions. And like other MDV, it exhibits strong tropism for feather follicles, where it infects the feather follicle epithelium [70, 71]. Additional studies on the role of HVT in SLV revealed that killed HVT had no effect on the expression of SLV. Therefore, the ability of HVT to cause infection must be of importance. Administration of other live virus vaccines at hatch (i.e., Newcastle disease virus, NDV; infectious bronchitis virus, IBV), instead of HVT, did not trigger the expression of SLV, suggesting that viral infection and associated antiviral immune activity, as such, are not responsible for triggering expression of SLV. Unlike HVT, NDV and IBV do not translocate to the feather; hence the presence of HVT where melanocytes are located may be the key to its effect on SLV expression [9]. Lastly, comparison of HVT-vaccinated and non-HVT-vaccinated SL and parental control BL chicks revealed heightened cell-mediated immune activity to HVT in SL compared to BL chicks [72].

Based on these observations, we hypothesize that the translocation of the HVT infection to the feather epithelium brings antiviral immune activity to the feather where the melanocytes are present. The resulting local antiviral cell-mediated immune activity in the melanocytes' environment causes alterations in the already inherently defective melanocytes that result in their recognition by the immune system leading to the development of melanocyte-specific immune activity and autoimmune destruction of melanocytes.

22.2.1.5 Associated Autoimmune Diseases in Smyth Line Vitiligo

Like in humans, SLV can be associated with other autoimmune diseases, including Hashimoto's thyroiditis, an alopecia areata-like feathering defect, and uveitis/blindness associated with melanocyte destruction in the choroid [16, 32].

Autoimmune hypothyroiditis: Hypothroidism is consistently observed in every hatch of SL chickens. The observation of autoimmune thyroiditis in the current Smyth line population varies from year to year with an average incidence of early (3–6 weeks) severe hypothyroidism in approximately 5% of SL hatch mates. The incidence of thyroiditis can be increased by selection. Moreover, extensive mononuclear cell infiltration can be observed in thyroids of fully developed adult SL chickens with vitiligo [9].

Uveitis, impaired vision and blindness: Impaired vision and blindness, like hypothyroidism, is also observed in every SL population, even with the exclusion of vision-impaired breeders. Unlike hypothyroidism, ocular impairment is strongly associated with vitiligo development [16]. In an ongoing study [73, 74], the incidence of blindness increased from the usual 3–5% to nearly 40% among offspring from vision-impaired or blind parents. To examine the phenotype of choroid-infiltrating leukocytes, eyes were collected from SL chickens and age-matched parental BL and LBL controls at 1 and 4 weeks of age (before SLV and impaired vision) and between 8 and 12 weeks of age, when SL chickens exhibited vitiligo and partial (PB) or complete (B) blindness. Immunohistochemical staining of frozen eye sections revealed that melanocyte loss in choroids was associated with mononuclear leukocyte infiltration similar to that observed in vitiliginous growing feathers, consisting of T cells (CD4+, CD8+, gamma-delta), IgM, B cells, macrophages, and MHC II+ cells. In eyes from birds with impaired vision, gross morphology of the retinal pigmented epithelium (RPE) was intact, whereas in birds judged to be blind, the RPE was damaged and replaced by the inflammatory infiltrate [74]. Target gene expression profile revealed the same signature cytokines as those reported for vitiliginous growing feathers, with the exception of a more prominent increase in IL-6 in eye tissue [73]. Our observations further confirm those summarized by Smyth [75] that impaired vision in SLV resembles induced uveitis and other ocu-

lar diseases in humans, especially Vogt-Koyanagi-Harada syndrome and sympathetic ophthalmia (result of penetrating eye injury), both of which are autoimmune diseases associated with vitiligo.

Alopecia areata-like feathering defect: The alopecia areata-like feathering defect is rarely seen before adult hood in the current populations, but patchy loss and/or abnormal feather development can be observed on the neck and back in 20–25% of SL adults with vitiligo.

22.2.1.6 Other Useful Characteristics of the Avian Model of Naturally Occurring Vitiligo

The avian model has many other useful characteristics for biomedical research, including access to embryos and embryo-derived target cells, availability of age-matched multiple siblings from multiple families, relatively short-generation time, ease of maintenance, and convenient body size (large enough for easy blood sampling, small enough for convenient housing and handling). Additionally, research tools, reagents, and advances in chicken immunology continue to be generated at a fast pace, especially since the chicken genome has been sequenced [76].

22.2.2 Water Buffalo

Vitiligo is also known to occur spontaneously in water buffalos (Fig. 22.4). Histological, histochemical, and ultrastructural analyses of biopsies taken from involved and uninvolved skin from two female buffalos revealed cytological aberrations of melanocytes similar to those reported in humans and other vitiligo models [17]. A recent field survey of water buffalo in India found 286 out of 45,043 buffalo examined had depigmented patches (0.64% prevalence). Histological analysis of biopsies taken from five adult female vitiliginous buffalos confirmed the presence of patchy leukocyte infiltration in perilesional skin but not in non-lesional skin. The distribution of lesions and the progressive nature of depigmentation in skin of vitiliginous water buffalo also paralleled observations in human vitiligo [18]. These observations together with the similarities between human and buffalo epidermis, and the fact that spontaneous vitiligo is observed in an outbred population, make the water buffalo a suitable intermediate model for treatment testing, particularly when test material will be topically applied [17, 18].

Fig. 22.4 Water buffalo with vitiligo (Courtesy of Dr F. Roperto, University of Naples Federico II, Naples, Italy)

22.3 Experimental Mouse Models of Induced Autoimmune Vitiligo

While no truly spontaneous mouse model for human vitiligo has been identified, several approaches have been used in mice to induce depigmentation that models human vitiligo. These induced mouse models for vitiligo are important in addressing mechanistic aspects of the role of cellular stress and inflammation and the nature of autoimmune responses identified in the human system. Moreover, the ability to effectively dissect the contribution of individual components, pathways, and mechanisms underlying depigmentation and melanocyte loss is essential for the development of treatment and prevention strategies for the disease. In fact, some of the induced vitiligo mouse models emerged from efforts to induce effective immune responses to

melanoma where vitiligo was observed as a side effect of immunotherapy. Methods of vitiligo induction in mice focus to a large extent on targeting melanocyte differentiation antigens by inducing endogenous melanocyte differentiation antigen-specific immune responses, adoptive transfer of melanocyte antigen-specific T cells, introduction of melanocyte antigen-specific T cell receptor (TCR) genes into the genome of mice, or various combinations thereof.

22.3.1 Induction of Vitiligo in Mice Through Generation of Endogenous Melanocyte-Specific Immune Responses

Experimental mouse models of melanoma have provided insight into the mechanisms regulating immunity and tolerance to pigment cells. Successful approaches to generate an immune response to melanoma in mice (e.g., C57BL/6 with black fur) included vaccination with viruses or plasmids that carry DNA coding for melanocyte differentiation antigens. Depending on immunization protocols, tumor (melanoma) immunity was often accompanied by autoimmunity manifested in vitiligo-like depigmentation of the fur. Ensuing efforts to uncouple tumor immunity and autoimmunity formed the basis for the development of murine vitiligo models. One promising approach to achieving active immunity to melanocyte antigens and vitiligo-like hair depigmentation in mice involved preparation of microscopic gold particles coated with plasmid DNA and bombardment of the skin with these particles using a gene gun [77, 78]. However, while this approach was effective in breaking tolerance and developing self-reactive immune system components, reliable induction of autoimmune vitiligo in the mouse model involved additional considerations. For example, in genetic immunization studies, TRP2-specific CD8+ T cells and antibodies, as well as, vitiligo were successfully produced with cDNA encoding xenogeneic human TRP2 or modified murine TRP2 peptide, but not with cDNA encoding murine TRP2 [79]. Similar observations that xenogenicity and/or alteration of melanocyte differentiation antigens contributed to their immunogenicity were also made by others [75, 80–83]. In addition to finding the most immunogenic forms of the melanocyte self-antigens, these studies further emphasized the importance of local tissue/cellular stress in targeting the autoimmune response to the skin. Melanocytes may be completely ignored by activated TRP2-specific CD8+ cytotoxic T lymphocytes unless an inflammatory environment is established in the target tissue.

In vitiligo, a wide variety of stress factors have been reported to provoke an autoimmune response to melanocytes ranging from chemicals, overexposure to sunlight (UV), skin injury, infection, and emotional stress [2, 84–86]. Cells under stress will produce stress proteins such as HSP which serve a protective function during the cell-stress episode. HSP70 was shown to enhance dendritic cell uptake of antigen and to be an ideal adjuvant for antitumor vaccines, and HSP70 has been implicated in precipitating vitiligo in susceptible individuals [2, 87]. Based on these observations, we [88] developed a reproducible in vivo mouse model of autoimmune vitiligo by combining gene gun vaccination with eukaryotic expression plasmids encoding melanocyte differentiation antigens (e.g., hTRP2) with human- or mouse-derived HSP70i (inducible HSP70 isoform). Inclusion of HSP70i, independent of origin, was found to accelerate depigmentation in this model. Interestingly, the progressive depigmentation induced by HSP70i involved areas not directly exposed to the stress. Depigmentation was accompanied by the induction of prolonged antibody responses to HSP70i and correlated with T cell-mediated cytotoxicity toward targets loaded with TRP2 peptide [88]. In follow-up studies, the observation that HSP70i administration alone was sufficient to induce vitiligo in vitiligo-prone mice (described below) was perhaps the most important finding in support of HSP70i as a critical player in vitiligo expression [89]. Considering that HSP70i has since been established as an important link between cellular stress and development of a melanocyte-specific autoimmune attack and that HSP70i overexpression is also observed in the skin of vitiligo patients [89–91], this mouse

model provides a relevant in vivo system to examine the role of cellular stress in immunorecognition of different melanocyte proteins. Moreover, this model can be extended to assess the ability of other environmental factors to precipitate or protect from the induction of a melanocyte-specific autoimmune attack. In fact, it was shown that mutant HSP70i interferes with immune activation and vitiligo development in this mouse model [90].

You et al. [92] reported another mouse vitiligo model based on the induction of an endogenous immune response to melanocyte differentiation antigens. Addressing the previously established importance of skin inflammation, they administered immunogenic TRP2-related peptide 180–188 together with lipopolysaccharide (LPS) and cytosine-phosphate-guanosine oligodeoxynucleotides (CpG), which are microbial products with known immunostimulatory activity. TRP2 peptide, LPS, and CpG were injected subcutaneously into the rear footpad for primary immunizations and intradermally into the tail skin for booster vaccinations. Immunized mice developed epidermal depigmentation in the tail skin without hair involvement. CD8+ IFN-γ+ T cells dominated the leukocyte infiltration into the tail skin, and the development of depigmented skin lesions coincided with TRP2 peptide-specific CD8+ T cell effector functions. Interestingly, priming vaccinations were sufficient to break tolerance, but melanocyte killing did not occur without the intradermal booster injections into the tail. While it is not clear why skin depigmentation occurred in the absence of tail hair pigmentation when self-antigen was introduced together with microbial products, this phenomenon distinguishes this model from other vaccination models of vitiligo [92].

A mouse model of induced vitiligo that is solely based on melanocyte stress employed the well-known FDA-approved phenolic bleaching compound monobenzone (MBEH) [93]. When MBEH was topically applied to the skin, depigmentation was not only observed at the application sites but also at nonexposed sites (Fig. 22.5). The severity of lesions was dependent on drug dosage. Histological examination revealed loss of epidermal melanocytes and perilesional accumulation of CD8+ cells. The application of the same treatment to Rag-1 knockout mice, which lack mature lymphocytes required for specific immune responses, resulted in hair depigmentation at the application site, but not at distant sites. Therefore in addition to direct cytotoxic effects of MBEH on melanocytes, MBEH is able to trigger endogenous melanocyte-specific immune responses resulting in progressive vitiligo. Similar observations were made in melanoma studies where administration of MBEH together with imiquimod and CpG induced a robust melanoma antigen-specific response and vitiligo-like depigmentation of the fur [94]. Considering that MBEH was shown to make melanocytes more vulnerable to cytotoxic lysis and promote development of melanocyte antigen-specific immunity in a variety ways [91, 95], the MBEH model may be more reflective of activation mechanisms leading to autoimmunity in human vitiligo than the vaccination models.

22.3.2 Induction of Vitiligo in TCR Transgenic Hosts or by Adoptive Transfer of Transgenic T Cells

Gene-transfer technology has been applied to generate mice with increased frequency of melanocyte reactive T cells. When genes encoding genetic information for a T cell receptor are inserted into the genome of a mouse, all mature T cells in the periphery will be expressing the single transgenic TCR. Hence, T cells in TCR transgenic mice only recognize one antigen peptide in association with self-MHC molecules. The need for TCR to be specific to both, portions of the antigen peptide and polymorphic region of MHC molecules, and the stringent endogenous selection procedures imposed on developing T cells to ensure tolerance to self-antigens further complicate the design of the gene-transfer approach. However, several T cell clones with a melanocyte differentiation antigen-specific TCR have been isolated from mice subjected to melanoma immunotherapy. The use of the TCR genes from these T cell clones in the generation of TCR transgenic

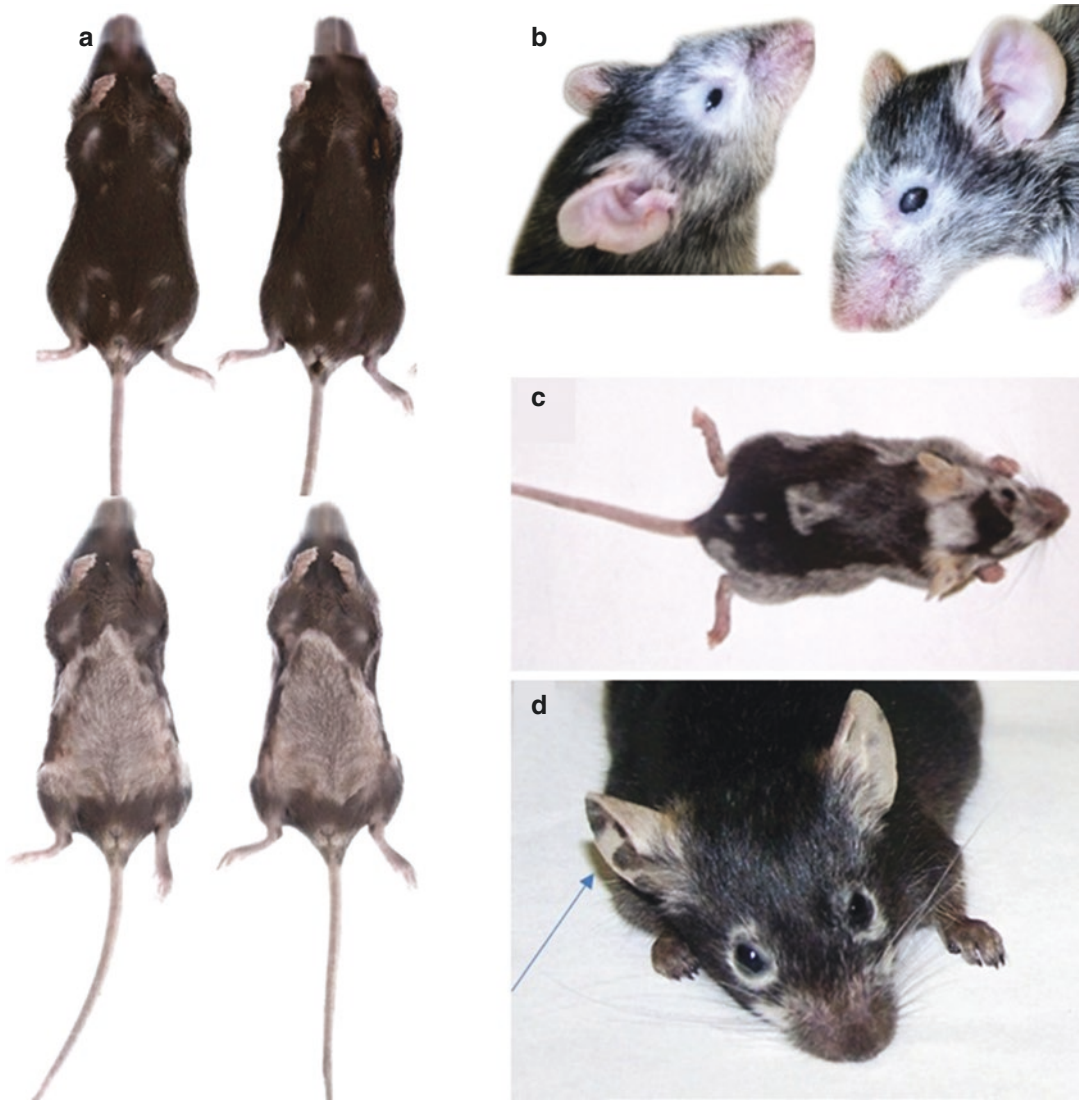


Fig. 22.5 Induced vitiligo mouse models. (**a**) C57BL/6 mice before and after 21 days of treatment with topical 1 M MBEH treatment; (**b**) h3T-A2 vitiligo mouse with normal (left) and cloudy eye; (**c**) 9.5-week-old male Vitesse mouse with vitiligo; (**d**) Vitesse mouse with repigmentation (Courtesy of Dr. Jonathan Eby and Dr. Caroline Le Poole, Loyola University, Chicago, IL)

mice has led to several mouse strains with transgenic T cells that survive and function in the periphery of the host. These autoreactive, transgenic T cells provide important insight into mechanisms involved in activating the autoimmune response and melanocyte loss in the transgenic host. Additionally, when used in adoptive transfer experiments, their contributions to autoimmune vitiligo can be explored in hosts with various levels of immunodeficiency, gene deletions, or normal, diverse immune systems. By crossing the TCR transgenic mice with other mouse strains with desired genetic characteristics, mouse models can be engineered to address a multitude of scientific questions [96].

In recent years the TCR transduction approach has led to various TCR transgenic mouse models that reflect T cell-mediated melanocyte-specific

autoimmunity. T cells from these mouse strains differ in TCR specificity, affinity, MHC restriction, co-receptors, phenotype, and stimulatory requirements, but all are able to induce hair depigmentation [82, 97–99]. The hair depigmentation observed in murine models has been a distinguishing feature between mouse and human vitiligo, whereby human vitiligo involves loss of epidermal melanocytes in the skin and is only rarely accompanied by hair depigmentation. Incorporation of the Krt14-Kitl* (aka as the K14-SCF) mouse strain [100], which has black skin and black hair, helped address the hair versus skin depigmentation concerns in mouse vitiligo studies [101, 102]. In the Krt14-kitl* mouse, localization of melanocytes in the skin is due to induced keratinocyte expression of transgenic stem cell factor (SCF or Kit ligand, kitl). Targeted expression of SCF to epidermal keratinocytes in mice was achieved by introducing a SCF transgene that codes for membrane-bound SCF (kitl*) and is under the control of the human keratin 14 (K14) promoter. Expression of the membrane-bound SCF resulted in the maintenance of a population of melanocytes within the interadnexal epidermis in mice, the same location where melanocytes and melanin are found in human skin [100].

Pmel-1 mouse: One of the first TCR transgenic mouse strains used for studies on autoimmune vitiligo is the pmel-1 mouse, which has CD8+ T cells with TCR specific for melanocyte differentiation antigen Pmel17 (gp100) [75, 82]. Despite large numbers of gp100-specific T cells in the pmel-1 mouse, B16 melanoma grew normally, and there was no loss of normal melanocytes. Even adoptive transfer of large numbers of transgenic pmel-1 T cells was not effective in reducing tumor growth without additional T cell stimulation. As shown by Antony et al. [103], optimal results were obtained when CD8+ pmel-1 T cells were transferred into Rag-1-deficient or sublethally irradiated mice and provided with in vivo activation signals in the form of gp100 expressing viruses and IL-2 or CD4 T cells that had been depleted of regulatory T cells (Tregs). Rag-1-deficient mice treated with pmel-1 T cells, vaccine and IL-2 or Th cells, developed profound autoimmunity 5 weeks after adoptive transfer. Hair depigmentation started periorbitally and spread in random fashion. Uveitis was also noted and found to be dependent on IL-2 or Th presence. They concluded that naturally occurring Th cells can help bring self-reactive CD8+ T cells out of their functionally tolerant state through an IL-2-dependent mechanism but require the absence of naturally occurring Tregs to be effective.

Krt14-Kitl mouse-pmel-1 T cell adoptive transfer model*: Harris et al. [102] have taken the optimized adoptive transfer protocol for pmel-1 T cells a step further and successfully applied it to the development of a mouse model of vitiligo with focused skin depigmentation while sparing the hair. In this model, pmel-1 mice were crossed with mice that express GFP in both CD4+ and CD8+ T cells to be able to isolate and track CD8+ pmel-1 cells. Purified naïve GFP+CD8+ pmel-1 T cells were then transferred into sublethally irradiated Krt14-Kitl* hosts which have black skin and black hair [100]. On the day of transfer, Krt14-Kitl* hosts were also infected i.p. with 10^6 pfu of recombinant vaccinia virus (rVV) that expressed human pmel17, a potent antigenic stimulus for the murine CD8+ pmel-1 T cells [82]. With all three treatments, vitiligo appeared 4–5 weeks after transfer [102]. Depigmentation was observed in the skin of ears, rear footpads, tail, and, less commonly, the nose; occasionally the trunk skin under the hair was affected, as were the front footpads and genitals. Depigmentation was initially patchy but often progressed to confluence, involving the entire epidermal surface, while depigmentation of the hair was not observed, even months after transfer. Cutaneous depigmentation was associated with mononuclear cell infiltration into the epidermis, including aggregates and single pmel-1 (GFP+CD8+) T cells. IFN-γ from CD8+ pmel-1 T cells was observed within 2 weeks.

All aspects of vitiligo described above for the induced Krt14-Kitl*-pmel-1 adoptive transfer model paralleled observations in the skin from human vitiligo patients. To demonstrate the utility of this model in the development of vitiligo treatments, the role of IFN-γ expression was

more closely examined. Blocking IFN-γ with neutralizing antibody significantly abrogated depigmentation, even when treatment was initiated 2 weeks after vitiligo development and skin-specific accumulation of autoreactive CD8+ T cells was greatly diminished [102]. Based on further explorations in both humans and the mouse model [104], the IFN-γ/CXCL10/CXCR3 pathway was identified as promising treatment strategy for vitiligo, which is actively being pursued using this mouse model [105–107].

FH mouse: The FH TCR transgenic mouse vitiligo model was developed by Engelhard and collaborators [97]. It is based on the recognition of a melanocyte protein tyrosinase, specifically HLA-A*0201-restricted epitope Tyr 369, by CD8+ T cells. To engineer a mouse that can process and present the murine homologue of this Tyr369 epitope, transgenic C57BL/6 mice were produced that express recombinant class I MHC molecule AAD, which contains the peptide-binding region of human HLA-A*0201 linked to the CD8-binding domain of murine H-2D^{d}. Subsequent transfer of genes for a tyr369-specific, ADD-restricted TCR (called FH) into ADD transgenic mice resulted in a double-transgenic mouse with tyr369-specific CD8+ T cells capable of recognizing the tyr369 melanocyte peptide in the context of endogenous ADD. The FH T cells did not undergo central tolerance, and the FH TCR was expressed on most peripheral CD8+ T cells of both albino and tyrosinase+ ADD+ mice. All AAD+, tyrosinase+ FH transgenic mice developed progressive depigmentation of epidermis and hair follicles. The spatial and temporal development of vitiligo displayed a depigmentation pattern similar to that observed in the human disease, with bilateral head and facial areas affected in juveniles and depigmentation extending over the rest of the body later in adults. Vitiligo was entirely dependent on CD8+ T cells, while CD4+ T cells exerted a negative regulatory effect, presumably due to the presence of Tregs. Importantly, CD8+ T cells were pervasively present in the skin in the steady state without inducing vitiligo in most areas. Both IFN-γ and T cell chemokine receptor CXCR3 were found to be necessary for disease expression. Ablation of these genes also resulted in scarce CD8+ T cell infiltration into the skin.

TRP1 transgenic mouse: The transgenic mouse generated by Muranski et al. [99] is characterized by CD4+ T cells expressing a MHC class II-restricted TCR specific for endogenous melanocyte differentiation antigen TRP1 (or gp75). While the role of CD4+ melanocyte differentiation antigen-specific T cells in autoimmune vitiligo is not clear, the transfer of TRP1-specific CD4+ T cells (TRP1 T cells) from these mice into Rag-1-deficient mice or sublethally irradiated TRP1-expressing mice resulted in rapid depigmentation of the hair. A combination of ex vivo and in vivo differentiation and activation studies using the TRP1 T cells revealed that Th subsets with different polarization defining cytokine profiles and different abilities to migrate and infiltrate tissues could be generated. In melanoma studies, Th17-polarized TRP1 T cells were found to be more effective than Th1 TRP1 T cells in eliciting tumor rejection. However, effects of the Th17-polarized cells were completely abrogated when IFN-γ-depleting antibodies were included in this model, suggesting that Th17-mediated responses of the TRP1 transgenic T cells were highly dependent on IFN-γ-based mechanisms.

TrpHEL mouse: The TrpHEL model developed by Lambe et al. [108] also involves CD4+ T cells that mediate autoimmune disease with striking similarities to human vitiligo and Vogt-Koyanagi-Harada syndrome. In this model a hen egg lysozyme (HEL) peptide is expressed as a neoantigen in melanocytes under the control of the TRP2 promoter, and T cells are MHC class II-restricted CD4+ T cells with transgenic TCR specific for HEL peptide. Advantages of using the well-defined HEL model antigen as a melanocyte neoantigen is the enhanced ability to track autoreactive CD4+ T cells from their development in the thymus to their involvement in spontaneous autoimmune disease.

h3T-A2 mouse: Mehrotra et al. [98] developed transgenic mice with a high-affinity tyrosine peptide (368–376)-specific TCR isolated from an unusual MHC class I (HLA-A2)-restricted

tumor-infiltrating human CD4+ T cell (h3T). T cells expressing the h3T TCR transgenes were positively selected whether they expressed CD8 or CD4, but, when allowed to grow up in mice with the HLA-A2 transgene, the transgenic TCR was primarily expressed on CD3+CD4-CD8- double-negative T cells. However, in the h3T single- and the h3T-HLA-A2 double-transgenic (h3T-A2) mice, all three types of T cells (CD4+, CD8+, or double-negative) were functional without vaccination or cytokine support. h3T-A2 transgenic mice spontaneously developed hair depigmentation from an early age and exhibited eye pathology that progressed with age (Fig. 22.5b). Similar to depigmentation in the skin, visual impairment and interruption of the RPE were associated with infiltration by transgenic T cells and macrophages. Addressing the mechanism regulating autoimmunity in this model in combination with gene-knockout mice, Chatterjee et al. [109] confirmed a central role of IFN-γ in the observed vitiligo development. In the absence of IFN-γ (i.e., h3T-A2-IFN-γ-/- mice), increased presence of Tregs was noted. When Tregs were deleted by employing anti-CD25 antibody, the depigmentation phenotype was fully restored in h3T-A2-IFN-γ-/- mice. In the absence of IFN-γ and Tregs, development of depigmentation appeared to be mediated in part by other inflammatory cytokines such as IL-17 and IL-22. Efforts to increase the Treg population in the skin of the h3T-A2 mouse model resulted in lasting remission of vitiligo.

Vitesse mouse: We used the h3T-A2 mouse strain to develop the Vitesse mouse [101]. The Vitesse mouse is a triple transgenic carrying the inter-epidermal pigmentation transgene Krt14-Kitl* in addition to the h3T-A2 genes. In the Vitesse model, spontaneous skin depigmentation preceded symmetrical and sharply demarcated patches of graying hair by 5–7 weeks, at which time pigment loss from ears and extremities was essentially complete (Fig. 22.5c). Vitesse mice reached maximum depigmentation earlier and at higher levels than h3T-A2 mice (30 weeks vs. 40 weeks; 83% vs. 65% depigmentation, respectively). Depigmentation occurred spontaneously and predictably without exogenous cytokine or antigen administration and was again found to be associated with IFN-γ and to a lower but significant extent with IL-17 responses. Gradually appearing patches of repigmenting skin were also observed (Fig. 22.5d), and repigmentation was associated with increased presence of Tregs. The significant Th17 responses observed in the epidermal depigmentation in the Vitesse mouse are particularly interesting considering the pure Th1 responses observed in the h3T-A2 model, which has the same TCR as the Vitesse mouse but no epidermal pigmentation [101]. Hence, the Vitesse mouse offers unique opportunity to dissect autoimmune activities involved in depigmentation and repigmentation processes in cutaneous vitiligo.

22.3.3 Summary on Induced Mouse Models

Collectively, the induced mouse models of vitiligo each confirm observations in human vitiligo and can contribute to our understanding of various aspects of autoimmune vitiligo. Opportunities exist to apply the strength of each model to answer different questions, although there are clearly overlapping opportunities between the models. Mechanisms of breaking tolerance to melanocyte antigens are best pursued in models of endogenously induced immune response, while adoptive transfer of autoreactive cells yields important insight into mechanisms of recruitment and activation of cells with known antigen specificity. Models with T cells expressing a transgenic TCR with specificity for one melanocyte differentiation antigen peptide provide insight into T cell development, selection, differentiation, and regulation of events leading to spontaneous depigmentation. All of these mouse model systems have the potential to find answers to mechanisms of melanocyte killing in vitiligo. Studying the autoimmune loss of melanocytes in the skin of mice, rather than the hair, may more faithfully reflect mechanisms underlying the cutaneous depigmentation observed in humans, although membrane-bound

SCF also is known to exert immune- and melanocyte-altering effects [101]. The study of autoimmune depigmentation of the fur in mice has made significant contributions to our understanding of the etiology and pathology of autoimmune vitiligo and revealed important parallels to human disease. However, considering the immune privilege of hair, induction of follicular melanocyte destruction may require more focused and powerful immune responses to break tolerance. And, in the context of repigmentation therapy, the much greater density of hair follicles in murine compared to human skin may present difficulty in interpreting the effectiveness of potential repigmentation treatments [101]. Lastly, one needs to keep the complexity of spontaneous autoimmune disease in mind, which cannot be fully recreated by genetic engineering of T cell specificity.

22.4 Concluding Remarks

Vitiligo is a multifactorial disease requiring animal models to delineate and dissect the components and mechanisms involved in this complex disease. Excellent animal models of both spontaneous and induced autoimmune vitiligo are available to the vitiligo research community for in vivo research on mechanisms leading to the expression and progression of vitiligo as well as for the development of treatment and prevention strategies.

Past and current research efforts have clearly established the Smyth line chicken model as an appropriate and suitable animal model of vitiligo. Currently, the Smyth line chicken model is the only animal model of spontaneous autoimmune vitiligo shown to recapitulate the entire spectrum of clinical and biological manifestations of the human disease. The Smyth line chicken model together with the induced autoimmune vitiligo mouse models provides the necessary tools to delineate and dissect the mechanisms involved in this complex disease. Insight gained from these models can then be translated into strategies for vitiligo prevention, treatment, and management in humans.

References

1. Harris JE. Cellular stress and innate inflammation in organ-specific autoimmunity: lessons learned from vitiligo. Immunol Rev. 2016;269:11–25.
2. Le Poole IC, Luiten RM. Autoimmune etiology of generalized vitiligo. In: Nickoloff BJ, Nestle FO, editors. Current directions in autoimmunity: dermatologic immunity, vol. 10. Basel: Karger; 2008. p. 227–43.
3. Le Poole IC, Das PK, van den Wijngaard RMJGJ, et al. Review of the etiopathomechanism of vitiligo: a convergence theory. Exp Dermatol. 1993;2:145–53.
4. Nordlund JJ, Lerner AB. Vitiligo-it is important. Arch Dermatol. 1982;118:5–8.
5. Picardo M, Dell'Anna ML, Ezzedine K, et al. Vitiligo. Nat Rev Dis Primers. 2015;1:15011.
6. Rezaei N, Gavalas NG, Weetman AP, et al. Autoimmunity as an aetiological factor in vitiligo. J Eur Acad Dermatol Venereol. 2007;21:865–76.
7. Schallreuter KU, Wood JM, Berger J. Low catalase levels in the epidermis of patients with vitiligo. J Invest Dermatol. 1991;97:1081–5.
8. Spritz RA. The genetics of generalized vitiligo and associated autoimmune diseases. Pigment Cell Res. 2007;20:271–8.
9. Erf GF. Autoimmune diseases of poultry. In: Schat KA, Kaspers B, Kaiser P, editors. Avian immunology. London: Elsevier; 2014.
10. Krishnamoorthy G, Holz A, Wekerle H. Experimental models of spontaneous autoimmune disease in the central nervous system. J Mol Med. 2007;85:1161–73.
11. National Institutes of Health Autoimmune Diseases Coordinating Committee. Autoimmune diseases research plan. In: Progress in autoimmune disease. Bethesda, MD: Research NIH; 2005.
12. Wick G, Andersson L, Hala K, et al. Avian models with spontaneous autoimmune diseases. Adv Immunol. 2006;92:71–117.
13. Boissy RE, Lamoreux ML. Animal models of an acquired pigmentary disorder-vitiligo. Prog Clin Biol Res. 1988;256:207–18.
14. Erf GF (2010) Animal models. In: Vitiligo, Picardo M A. Taieb, 205-218 Springer, Berlin.
15. Essien KI, Harris JE. Animal models of vitiligo: matching the model to the question. Dermatol Sinica. 2014;32:240–7.
16. Smyth JR Jr. The Smyth chicken: a model for autoimmune amelanosis. Poult Biol. 1989;2:1–19.
17. Cerundolo R, De Caprariis D, Esposito L, et al. Vitiligo in two water buffaloes: histological, histochemical, and ultrastructural investigations. Pigment Cell Res. 1993;6:23–8.
18. Singh V, Motiani R, Singh A, et al. Water Buffalo (Bubalus bubalis) as a spontaneous animal model of vitiligo. Pigment Cell Melanoma Res. 2016;29:465. https://doi.org/10.1111/pcmr.12485.

19. Berkelhammer J, Ensign BM, Hook RR, et al. Growth and spontaneous regression of swine melanoma: relationship of in vitro leukocyte reactivity. J Natl Cancer Inst. 1982;68:461–8.
20. Misfeldt ML, Grimm DR. Sinclair miniature swine: an animal model of human melanoma. Vet Immunol Immunopathol. 1994;43:167–75.
21. Richerson JT, Burns RP, Misfeldt ML. Association of uveal melanocyte destruction in melanoma-bearing swine with large granular lymphocyte cells. Invest Ophthalmol Vis Sci. 1989;30:2455–60.
22. Gebhart W, Niebauer G. Connections between pigment loss and melanogenesis in gray horses of the Lipizzaner breed. Yale J Biol Med. 1977;50:45.
23. Naughton GK, Mahaffey M, Bystryn J-C. Antibodies to surface antigens of pigmented cells in animals with vitiligo. Proc Soc Exp Biol Med. 1986;181:423–6.
24. Bowers RR, Harmon J, Prescott S, et al. Fowl model for vitiligo: genetic regulation on the fate of the melanocytes. Pigment Cell Res Suppl. 1992;2:242–8.
25. Bowers RR, Lujan J, Biboso A, et al. Premature avian melanocyte death due to low antioxidant levels of protection: fowl model for vitiligo. Pigment Cell Res. 1994;7:409–18.
26. Bowers RR, Nguyen B, Buckner S, et al. Role of anti-oxidants in the survival of normal and vitiliginous avian melanocytes. Cell Mol Biol. 1999;45:1065–74.
27. Boissy RE, Moellmann GE, Lerner AB. Morphology of melanocytes in hair bulbs and eyes of vitiligo mice. Am J Pathol. 1987;127:380–8.
28. Lamoreux ML, Boissy RE, Womack JE, et al. The vit gene maps to the mi (microphthalmia) locus of the laboratory mouse. J Hered. 1992;83:435–9.
29. Lerner AB, Shiohara T, Boissy RE, et al. A possible mouse model for vitiligo. J Invest Dermatol. 1986;87:299–304.
30. Tripathi RK, Flanders DJ, Young TL, et al. Microphthalmia-associated transcription factor (MITF) locus lacks linkage to human vitiligo or osteoporosis: an evaluation. Pigment Cell Res. 1999;12:187–92.
31. Erf GF, Ramachandran IR. The growing feather as a dermal test-site: comparison of leukocyte profiles during the response to *Mycobacterium butyricum* in growing feathers, wattles, and wing webs. Poult Sci. 2016;95:2011–122.
32. Smyth JR Jr, McNeil M. Alopecia areata and universalis in the Smyth chicken model for spontaneous autoimmune vitiligo. J Invest Dermatol Symp Proc. 1999;4:211–5.
33. Smyth JR Jr, Boissy RE, Fite KV. The DAM chicken: a model for spontaneous postnatal cutaneous and ocular amelanosis. J Hered. 1981;72:150–6.
34. Kerje S, Ek W, Asahlquist A-S, Ekwall O, Erf G, Carlborg Ö, Andersson L, Kämpe O. Genetic mapping of loci underlying vitiligo in the Smyth line chicken model. Pigment Cell Melanoma Res. 2011;24:831.
35. Jang H-M, Erf GF, Rowland KC, et al. Genome resequencing and bioinformatics analysis of SNP containing candidate genes in the autoimmune vitiligo Smyth line chicken model. BMC Genomics. 2014;15:707.
36. Sreekumar GP, Smyth JR Jr, Ponce de Leon FA. Molecular characterization of the Smyth chicken sublines and their parental controls by RFLP and DNA fingerprint analysis. Poult Sci. 2001;80:1–5.
37. Sreekumar GP, Erf GF, Smyth JR Jr. 5-Azacytidine treatment induces autoimmune vitiligo in the parental control strains of the Smyth line chicken model for autoimmune vitiligo. Clin Immunol Immunopathol. 1996;81:136–44.
38. Erf GF, Smyth JR Jr. Alterations in blood leukocyte populations in Smyth line chickens with autoimmune vitiligo. Poult Sci. 1996;75:351–6.
39. Erf GF, Trejo-Skalli AV, Smyth JRJ. T cells in regenerating feathers of Smyth line chickens with vitiligo. Clin Immunol Immunopathol. 1995;76:120–6.
40. Shresta S, Smyth JR Jr, Erf GF. Profiles of pulp infiltrating lymphocytes at various times throughout feather regeneration in Smyth line chickens with vitiligo. Autoimmunity. 1997;25:193–201.
41. Wang X, Erf GF. Apoptosis in feathers of Smyth line chickens with autoimmune vitiligo. J Autoimmun. 2004;22:21–30.
42. Falcon DM, Dienglewicz RL, Erf GF. Monitoring of leukocyte infiltration responses to melanocytes injected into growing feathers of Smyth line chickens with autoimmune vitiligo. Pigment Cell Melanoma Res. 2015;28:627.
43. Wang X, Erf GF. Melanocyte-specific cell mediated immune response in vitiliginous Smyth line chickens. J Autoimmun. 2003;21:149–60.
44. Shi F, Erf GF. IFN-gamma, IL-21 and IL-10 co-expression in evolving autoimmune vitiligo lesions of Smyth line chickens. J Invest Dermatol. 2012;132:642–9.
45. Leonard WJ, Zeng R, Spolski R. Interleukin 21: a chemokine/cytokine receptor system that has come of age. J Leukoc Biol. 2008;84:348–56.
46. Liu S, Lizée G, Lou Y, et al. Il-21 synergizes with IL-7 to augment expansion and anti-tumor function of cytotoxic T cells. Int Immunol. 2007;19:1213–21.
47. van Belle TL, Nierkens S, Arens R, et al. Interleukin-21 receptor-mediated signals control autoreactive T cell infiltration in pancreatic islets. Immunity. 2012;36:1060–72.
48. Shi F, Kong B-W, Song JJ, Lee JY, Dienglewicz RL, Erf GF. Understanding mechanisms of spontaneous autoimmune vitiligo development in the Smyth line chicken model by transcriptomic microarray analysis of evolving lesions. BMC Immunol. 2012;13:18.
49. Austin LM, Boissy RE. Mammalian tyrosinase-related protein-1 is recognized by autoantibodies from vitiliginous Smyth chickens. An avian model for human vitiligo. Am J Pathol. 1995;146:1529–41.

50. Searle EA, Austin LM, Boissy YL, et al. Smyth chicken melanocyte autoantibodies: cross-species recognition, in vivo binding, and plasma membrane reactivity of the antiserum. Pigment Cell Res. 1993;6:145–57.
51. Erf GF, Lockhart BR, Griesse RL, et al. Circulating melanocyte-specific autoantibodies and feather-infiltrating lymphocytes in young Smyth line chickens prior to visible onset of vitiligo. Pigment Cell Res. 2003;16:420–1.
52. Boissy RE, Smyth JR Jr, Fite KV. Progressive cytologic changes during the development of delayed feather amelanosis and associated choroidal defects in the DAM chicken line. Am J Pathol. 1983;111:197–212.
53. Boissy RE, Lamont SJ, Smyth JR Jr. Persistence of abnormal melanocytes in immunosuppressed chickens of the autoimmune "DAM" line. Cell Tissue Res. 1984;235:663–8.
54. Boissy RE, Moellmann G, Smyth JR Jr. Melanogenesis and autophagocytosis of melanin within feather melanocytes of delayed amelanotic (DAM) chickens. Pigment Cell. 1985;1:731–9.
55. Boissy RE, Moellmann G, Trainer AT, et al. Delayed-amelanotic (DAM or Smyth) chicken: melanocyte dysfunction in vivo and in vitro. J Invest Dermatol. 1986;86:149–56.
56. Erf GF, Wijesekera HD, Lockhart BR, et al. Antioxidant capacity and oxidative stress in the local environment of feather-melanocytes in vitiliginous Smyth line chickens. Pigment Cell Res. 2005;18:69.
57. Manga P, Sheyn D, Yang F, et al. A role for tyrosinase-related protein 1 in 4-tert-butylphenol-induced toxicity in melanocytes: implications for vitiligo. Am J Pathol. 2006;169:1652–62.
58. Dong L, Dienglewicz RL, Erf GF. The response of melanocytes to 4-tertiary butylphenol is related to vitiligo susceptibility in the Smyth line chicken model for autoimmune vitiligo. Pigment Cell Melanoma Res. 2010;23:717.
59. Dong L, Dienglewicz RL, Erf GF. Divergent gene-expression profiles in 4-TBP-injected growing feathers of vitiligo-prone Smyth- and control chickens. Pigment Cell Melanoma Res. 2012;25:696–7.
60. Boyle ML III, Pardue SL, Smyth JR Jr. Effects of corticosterone on the incidence of amelanosis in Smyth delayed amelanotic line chickens. Poult Sci. 1987;66:363–7.
61. Fite KV, Pardue S, Bengston L, et al. Effects of cyclosporine in spontaneous, posterior uveitis. Curr Eye Res. 1986;5:787–96.
62. Lamont SJ, Smyth JR Jr. Effect of bursectomy on development of a spontaneous postnatal amelanosis. Clin Immunol Immunopathol. 1981;21:407–11.
63. Pardue SL, Fite KV, Bengston L, et al. Enhanced integumental and ocular amelanosis following termination of cyclosporine administration. J Invest Dermatol. 1987;88:758–61.
64. Boissy RE, Liu YY, Medrano EE, et al. Structural aberration of the rough endoplasmic reticulum and melanosome compartmentalization in long-term cultures of melanocytes from vitiligo patients. J Invest Dermatol. 1991;97:395–404.
65. Bowers RR, Gatlin JE. A simple method for the establishment of tissue culture melanocytes from regenerating fowl feathers. In Vitro Cell Dev Biol. 1985;21:39–44.
66. Le Poole IC, Boissy RE, Sarangarajan R, et al. PIG3V, an immortalized human vitiligo melanocyte cell line, expresses dilated endoplasmic reticulum. In Vitro Cell Dev Biol Anim. 2000;36:309–19.
67. Medrano EE, Nordlund JJ. Successful culture of adult human melanocytes obtained from normal and vitiligo donors. J Invest Dermatol. 1990;95:441–5.
68. Puri N, Phil M, Mojandar M, et al. In vitro growth characteristics of melanocytes obtained from adult normal and vitiligo subjects. J Invest Dermatol. 1987;88:434–8.
69. Erf GF, Bersi TK, Wang X, et al. Herpesvirus connection in the expression of autoimmune vitiligo in Smyth line chickens. Pigment Cell Res. 2001;14:40–6.
70. Calnek BW, Witter RL. Marek's disease. In: Calnek BW, Barnes HJ, Beard CW, Reid WM, Yoder Jr HW, editors. Diseases of poultry. Ames, IA: Iowa State University Press; 1991.
71. Holland MS, Mackenzie CD, Bull RW, et al. Latent turkey herpesvirus infection in lymphoid, nervous, and feather tissues of chickens. Avian Dis. 1998;42:292–9.
72. Erf GF, Johnson JC, Parcells MS, et al. A role of turkey herpesvirus in autoimmune Smyth line vitiligo. In: Schat KA, editor. Current progress on avian immunology research. Jacksonville, FL: American Association of Avian Pathologists; 2001. p. 226–31.
73. Huett W, Byrne KA, Sorrick J, et al. Uveitis and blindness in Smyth line chickens with autoimmune vitiligo: expression of cytokine- and melanogenesis-related-genes in eyes before and during loss of choroidal melanocytes. Pigment Cell Melanoma Res. 2015;28:627.
74. Sorrick J, Dienglewicz RL, Erf GF. Uveitis and blindness in Smyth line chickens with autoimmune vitiligo: immunopathology associated with melanocyte loss in the eye. Pigment Cell Melanoma Res. 2013;26:769.
75. Overwijk WW, Tsung A, Irvine KR, et al. gp100/pmel 17 is a murine tumor rejection antigen: induction of "self"-reactive, tumoricidal T cells using high-affinity, altered peptide ligand. J Exp Med. 1998;188:277–86.
76. International Chicken Polymorphism Map Consortium. A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. Nature. 2004;432:717–22.
77. Tang DC, DeVit M, Johnston SA. Genetic immunization is a simple method for eliciting an immune response. Nature. 1992;356:152–4.
78. Tüting T, Storkus WJ, Falo LD Jr. DNA immunization targeting the skin: molecular control of adaptive immunity. J Invest Dermatol. 1998;111:183–8.

79. Steitz J, Wenzel J, Gaffal E, et al. Initiation and regulation of CD8+ T cells recognizing melanocytic antigens in the epidermis: implications for the pathophysiology of vitiligo. Eur J Cell Biol. 2004;83:797–803.
80. Bowne WB, Srinivasan R, Wolchok JD, et al. Coupling and uncoupling of tumor immunity and autoimmunity. J Exp Med. 1999;190:1717–22.
81. Engelhorn ME, Guevara-Patiño JA, Merghoub T, et al. Mechanisms of immunization against cancer using chimeric antigens. Mol Ther. 2008;16:773–81.
82. Overwijk WW, Theoret MR, Finkelstein SE, et al. Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. J Exp Med. 2003;198:569–80.
83. Weber LW, Bowne WB, Wolchok JD, et al. Tumor immunity and autoimmunity induced by immunization with homologous DNA. J Clin Invest. 1998;102:1258–64.
84. Boissy RE, Manga P. On the etiology of contact/occupational vitiligo. Pigment Cell Res. 2004;17:208–14.
85. Kroll TM, Bommiasamy H, Boissy RE, et al. 4-Tertiary butyl phenol exposure sensitizes human melanocytes to dendritic cell-mediated killing, relevance to vitiligo. J Invest Dermatol. 2005;124:798–806.
86. Namazi MR. Neurogenic dysregulation, oxidative stress, autoimmunity, and melanocytorrhagy in vitiligo: can they be interconnected? Pigment Cell Res. 2007;20:360–3.
87. Overwijk WW, Lee DS, Irvine KR, et al. Vaccination with a recombinant vaccinia virus encoding "self" antigen induces autoimmune vitiligo and tumor destruction in mice: requirement for CD4(+) T lymphocytes. Proc Natl Acad Sci U S A. 1999;96:2982–7.
88. Denman CJ, McCracken J, Hariharan V, et al. HSP70i accelerates depigmentation in a mouse model of autoimmune vitiligo. J Invest Dermatol. 2008;128:2041–8.
89. Mosenson JA, Zloza A, Klarquist J, et al. HSP70i is a critical component of the immune response leading to vitiligo. Pigment Cell Melanoma Res. 2012;25:88–98.
90. Mosenson JA, Eby JM, Hernandez C, et al. A central role for inducible heat-shock protein 70 in autoimmune vitiligo. Exp Dermatol. 2013;22:566–9.
91. Mosenson JA, Flood K, Klarquist J, et al. Preferential secretion of inducible HSP70 by vitiligo melanocytes under stress. Pigment Cell Melanoma Res. 2014;27:209–20.
92. You S, Cho Y-H, Byun J-S, et al. Melanocyte-specific CD8+ T cells are associated with epidermal depigmentation in a novel mouse model of vitiligo. Clin Exp Immunol. 2013;174:28–44.
93. Zhu Y, Wang S, Xu A. A mouse model of vitiligo induced by monobenzone. Exp Dermatol. 2013;22:482–501.
94. van den Boorn JG, Konijnenberg D, Tjin EP, et al. Effective melanoma immunotherapy in mice by the skin-depigmenting agent monobenzone and the adjuvants imiquimod and CpG. PLoS One. 2010;5:e10626.
95. Westerhof W, Manini P, Napolitano A, et al. The haptenation theory of vitiligo and melanoma rejection: a close-up. Exp Dermatol. 2011;20:92–6.
96. Manga P, Orlow SJ. Engineering a new mouse model for vitiligo. J Invest Dermatol. 2012;132:1752–5.
97. Gregg RK, Nichols L, Chen Y, et al. Mechanisms of spatial and temporal development of autoimmune vitiligo in tyrosinase specific TCR transgenic mice. J Immunol. 2010;184:1909–17.
98. Mehrotra S, Al-Khami AA, Klarquist J, et al. A coreceptor-independent transgenic human TCR mediates anti-tumor and anti-self immunity in mice. J Immunol. 2012;189:1627–38.
99. Muranski P, Boni A, Antony PA, et al. Tumor-specific TH17-polarized cells eradicate large established melanoma. Blood. 2008;112:362–73.
100. Kunisada T, Lu S-Z, Yoshida H, et al. Murine cutaneous mastocytosis and epidermal melanocytosis induced by keratinocyte expression of transgenic stem cell factor. J Exp Med. 1998;187:1565–73.
101. Eby J, Kang H-K, Klarquist J, et al. Immune responses in a mouse model of vitiligo with spontaneous epidermal de- and repigmentation. Pigment Cell Melanoma Res. 2014;27:1075–785.
102. Harris JE, Harris TH, Weninger W, et al. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-γ for autoreactive CD8+ T cell accumulation in the skin. J Invest Dermatol. 2012;132:1869–76.
103. Antony PA, Piccirillo CA, Akpinarli A, et al. CD8+ T cell immunity against a tumor/self-antigen is augmented by CD4+ T helper cells and hindered by naturally occurring T regulatory cells. J Immunol. 2005;174:2591–601.
104. Rashighi M, Agarwal P, Richmond JM, et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. Sci Transl Med. 2014;6:223ra23.
105. Agarwal P, Rashighi M, Essien KI, et al. Simvastatin prevents and reverses depigmentation in a mouse model of vitiligo. J Invest Dermatol. 2015;135:1080–8.
106. Harris JE. IFN-γ in vitiligo, is it the fuel or the fire? Acta Derm Venereol. 2015;95:643–4.
107. Rashighi M, Harris JE. Interfering with the IFN-γ/CXCL10 pathway to develop new targeted treatments for vitiligo. Ann Transl Med. 2015;3:343–7.
108. Lambe T, Leung JCH, Bouriez-Jones T, et al. CD4 cell-dependent autoimmunity against a melanocyte neoantigen induces spontaneous vitiligo and depends upon Fas-Fas ligand interactions. J Immunol. 2006;177:3055–62.
109. Chatterjee S, Eby J, Al-Khami AA, et al. A quantitative increase in regulatory T cells controls development of vitiligo. J Invest Dermatol. 2014;134:1285–94.

In Vitro Study of Vitiligo

23

Maria Lucia Dell'Anna and Muriel Cario-André

Contents

M. L. Dell'Anna (✉)
Laboratory of Cutaneous Physiopathology, San Gallicano Dermatological Institute, IFO, Rome, Italy
e-mail: marialucia.dellanna@ifo.gov.it

M. Cario-André
Inserì U876, Centre de référence des maladies rares de la peau, Université V Segalen Bordeaux 2, Bordeaux, France
e-mail: muriel.cario-andre@dermatol.u-bordeaux2.fr

Abstract

The study of vitiligo has been approached by several different perspectives. Accordingly to the progressive improvement of the technological support to the research and discovery of new fields in the biological world, even researchers who focused on vitiligo gained new opportunities. An unsolved question regards

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_23

how and where to analyze the possible pathomechanisms underlying the vitiligo onset and progression. A clear-cut position against the best in vitro approach is aleatory. It is surely customary to use the primary cell cultures to perform functional studies; however, the in vitro assays with primary cell cultures are indissolubly affected by the intrinsic selection of the more "aggressive" or best cells among the overall tissue bulk. Moreover, the media usually used to select and grow the primary cells are optimized to burst their proliferative or differentiative ability, probably overcoming the intrinsic features of the isolated cells. The first requirement of a researcher is to maximize yield of a bioptic sample, but this requirement affects per se the quality of the resulting cultures that may no longer reflect the initial cellular asset of an individual. This initial reflection did not intend to attack the studies currently performed with primary cell cultures, but it just aims to alert about the possible intrinsic bias.

Key Points

- Vitiligo cells are obtained from lesional or non-lesional skin of vitiligo patients. The specific composition of the growth factor cocktail will affect the proliferative or differentiative signature as well as growth rate and the delay between isolation and standard growth.
- Considering that physiologically the cells aren't seeded on plastic or glass but they grow in a 3D space, biomaterials, including hydrogels, have been developed to investigate the complex world of the live cells.
- The flow cytometric approach permits a quali-quantitative multiparametric and time-dependent analysis.
- Metabolomic research will provide a fingerprinting of the metabolic activities related to immune deregulation and degenerative process, possibly merging the vitiligo profile with those known and described in other diseases and health population.
- The complexity of cell culture models varies from monolayers for adherent cells or suspensions for nonadherent cells to 2D or 3D hydrogel-supported cultures, to cocultures, or to epidermal reconstructs (3D model).
- 3D hydrogel cultures, cocultures, and epidermal reconstructs allow the study of paracrine or contact cell-cell effects.
- The tests on nonepidermal nonadherent cells, such as peripheral blood mononuclear cells (PBMC), expand the view, going beyond melanogenesis-associated metabolisms.
- The in vitro studies on hair follicle melanocytes help understand the maturation and differentiation of melanocytes and to unveil the defective steps of growth and migration in melanocyte precursors during the repigmentation.

23.1 Cell Isolation and Culture

Melanocytes and keratinocytes can be cultured from both the skin and hair follicle. Indeed, epidermal stem cells occur in the basal layer of the epidermis and within hair follicles, where keratinocyte stem cells are situated in the bulge area, whereas those for melanocytes are found in the sub-bulge area.

23.1.1 Isolation and Culture of Skin Melanocytes and Keratinocytes

Vitiligo cells are obtained from lesional or non-lesional skin of vitiligo patients. The method for the isolation of primary melanocytes or keratinocytes is similar for both vitiligo and normal skin samples. Split-thickness skin samples are cut in small pieces and trypsinized. Trypsin disrupts the epidermis above the basal layer, and it is neutralized with fetal calf serum or trypsin-soybean inhibitor. The epidermis is removed, and the basal layer is scraped to dissociate melanocytes and

Table 23.1 Cocktails of growth factors used for melanocyte culture

	commercial	Boissy 1991 [2]	Kroll 2005 [3]	Richmond 2005 [4]	Medrano 1990 [1]	Cario-Andrè 2007 [5]	Smith 2005 [6]	Greatens 2005 [7]
BPE	0.2%	30 μg/mL	13 μg/mL	30 μg/mL	30 μg/mL	140 μg/mL	30 μg/mL	30 μg/mL
FBS	0.5%	5%	4%	10%	5%	3%	5%	4%
bFGF	3 ng/mL	0.6 ng/mL	0.6 ng/mL	0.6 ng/mL	0.3 ng/mL	–	0.6 ng/mL	0.6 ng/mL
Eparin	3 μg/mL	–	–	–	–	–	–	–
Hydrocrtisone	0.18 μg/mL	0.5 μg/mL	–	0.5 μg/mL	0.5 μg/mL	1.75 μM	0.5 μg/mL	0.5 μg/mL
Insulin	5 μg/mL	5 μg/mL	5 μg/mL	5 μg/mL	5 μg/mL	20 μg/mL	5 μg/mL	5 μg/mL
Vitamin E	–	1 μg/mL	1 μg/mL	1 μg/mL	1 μg/mL	–	1 μg/mL	1 μg/mL
Transferrin	5 μg/mL	5 μg/mL	–	5 μg/mL	5 μg/mL	–	5 μg/mL	5 μg/mL
PMA	10 ng/mL	8 nM	8 nM	8 nM	–	–	8 nM	8 nM
Catalase	–	20 μg/mL	–	20 μg/mL	–	–	–	–
MSH	–	–	–	–	–	–	10^{-8} M	–
Endothelin1	–	–	–	–	–	–	10^{-9} M	–
Medium	M254	MCDB153	M154	MCDB153	MCDB153	MCDB153	M154	MCDB153

basal keratinocytes. Cells are seeded at a density of 200,000/cm^2 for melanocytes and 100,000/cm^2 for keratinocytes culture [1]. Usually, the culture media for melanocytes are M2 medium (PromoCell), M254 (Gibco), or MCDB153 (Sigma), MGM4 BulletKit (Lonza) added with specific growth factors. Several different handmade growth factor cocktails for culture are used (Tables 23.1 and 23.2). The specific composition of the growth factor cocktail will affect the proliferative or differentiative signature as well as growth rate and the delay between isolation and standard growth. Moreover, the presence of α-MSH analog or precursor could influence the effect of some in vitro treatments. The culture media used for keratinocytes are CellnTechn-07 (Chemicon), MCDB153 (Sigma), and M154 (Gibco) with the appropriate growth factors. Cells at passage 2–3 can be used to perform functional studies, to reconstruct the epidermis, or to start cocultures for studies regarding cellular network.

23.1.2 Isolation and Culture of Skin Fibroblasts

After obtaining keratinocyte and melanocyte suspensions, the scraped dermis is cut in small pieces and incubated with collagenase allowing the fibroblasts to exit from extracellular matrix. Fibroblasts are cultured in DMEM supplemented with 10% fetal calf serum.

23.1.3 Isolation and Culture of Hair Follicle Melanocytes and Keratinocytes

Scalp specimens are cut into small pieces, and the epidermis and upper 1 mm of the dermis are carefully removed with a scalpel [2]. Hair follicles are isolated by incubating the tissue in Eagle's minimal essential medium (EMEM) containing dispase and then collagenase. The released hair follicles are washed repeatedly with PBS until hair follicles appear pure by microscopic examination. Single-cell suspensions are then obtained by treatment with trypsin-EDTA [3]. Hair follicle melanocyte (HFM) cultures are established using media supplemented with either artificial mitogens or natural melanocyte mitogens as follows. Contaminating fibroblasts, when present, are eliminated by treating the cultures with 150 μg/mL Geneticin (G418) sulfate [4]. Follicular keratinocytes are separated from the HFM cultures by differential trypsinization. The identity of the isolated cells is confirmed by immunophenotyping with the melanocyte lineage-specific marker NKI/beteb against glycoprotein100 (gp100) [5, 6]. Hair follicle keratinocytes (HFK) are established by preparing single-cell suspensions from isolated hair follicles. At the primary culture stage, follicular melanocytes are first selectively trypsinized from the culture. This step is carried out under microscopic observation. Remaining keratinocytes are then switched to the keratinocyte-

specific medium, which does not support melanocyte growth.

23.1.3.1 Transgenesis

Since it is difficult to have enough cells from vitiligo patients to test all hypothesis, we can, according to genetic data, modulate susceptible genes using overexpressing or silencing vectors such as CCN3 (homeostasis of melanocyte), DDR1 (adhesion of melanocyte to basal membrane), and cadherin (cell adhesion) [7, 8]. After the first or the second passage, cells are incubated with viral particles at MOI (multiplicity of infection) 10 in a small volume of serum-free medium. After 6–16 h, fresh SVF-containing medium is added to complete the volume to usual one (e.g., in 25 cm flasks, transduction is made in 0.5 mL serum-free medium, and then 3 SVF medium is added). Medium is changed after 48 h, and percentage of transduction if fluorescent reporter gene (RFP, GFP, YFP) is present in the vector is estimated 72 h after transduction to avoid overestimation due to pseudo-transduction. To obtain 100% transduction, an antibiotic resistance gene such as puromycin-resistance gene can be added in the vector construct. Otherwise cells can be selected by flow cytometry to obtain around 95% transduction.

23.1.4 Next-Generation Cultures

Growing estimation is recently gained by the extracellular environment role on cellular behavior. The network between mechanical, chemical, and structural aspects of the environment heavily affects the cell function. Considering that physiologically the cells aren't seeded on plastic or glass but they grow in a 3D space, biomaterials, including hydrogels, have been developed to investigate the complex world of the live cells. Most of the functional data on cultured cells arise from 2D support, such as flat stiff materials (polystyrene and glass), determining per se flattened shape, aberrant polarization, loss of differentiation, and altered response to drugs. Hydrogels, that are water-swollen networks of polymers, are developed to overcome these problems by mirroring, more than standard stiff supports, the physiological extracellular matrix. Currently, several different types of hydrogel are available by market, both natural and synthetic (or mixed), characterized by specific physical and chemical properties. BD Biosciences, Baxter, Johnson & Johnson, Sigma, ProNova, and BioTime, Inc. are the major vendors of the different hydrogels for both 2D and 3D cultures. Accordingly to the different physical features, each hydrogel is more or less suitable, based on epitope accessibility or stability and protein/RNA recovery, for microscopy, flow cytometry, or molecular studies [9].

23.1.5 Isolation and Freezing of Peripheral Blood Mononuclear Cells (PBMC)

The peripheral blood mononuclear cells (PBMC) have been analyzed in vitiligo in order to characterize their immunological status, the capability to recognize specific melanocyte antigens, the cytotoxic effects toward melanocyte or melanoma cell lines, redox status, and response to DNA damage [10–12]. The isolation of vitiligo PBMC is performed through the stratification onto Ficoll density gradient, which allows separating mononuclear from polynuclear and red cells. The PBMC localize at interface between serum and Ficoll, whereas the polynucleates, after centrifugation, go to the bottom of the tube. After recovery, PBMC are washed twice with saline solution (NaCl 0.9%) and used as planned. The procedure should be carefully performed (short time, less than 30 min, between blood withdrawal and PBMC isolation; gentle manipulation) in order to avoid any physical stress able to affect vitiligo PBMC independently of in vitro test. Theoretically, vitiligo PBMC may be used even after freezing in DMSO/serum mixture. However, our experience suggests avoiding freezing when ROS generation or some functional membrane-dependent parameters will be assayed. On the other hand, the cellular pellet, stored at −80 °C, can be used for enzymatic analysis.

23.2 In Vitro Reconstructed Epidermis

As early as 1979, Prunieras et al. [13] have demonstrated that it is possible to obtain a fully differentiated epidermis in vitro by simply raising

keratinocytes up to the air–liquid interface. Apparently, the interface with air stimulates synthesis of profilaggrin by keratinocytes and thus the appearance of the granular phenotype when keratohyalin granules develop [14]. The epidermis can be reconstructed using various supports: dead de-epidermized dermis (DDD) [13], gel of collagen (EpiSkin®), lattices including fibroblasts and collagen [15], lattices including fibroblasts and collagen-glycosaminoglycan-chitosan [16], human fibrous sheet [17], and basal inert substrates such as porous filters [18]. Otherwise fibroblasts can colonize dermal matrix used in clinic as dermal regeneration template such as Integra® and MatriDerm™ (European patent EP 3072535 A1).

The reconstructed epidermis has been perfected first by adding melanocytes [19] and secondly Langerhans cells [20], but models with Langerhans cells have not been tested in vitiligo experiments. The reconstructed epidermis with keratinocytes and melanocytes on DDD reproduces the epidermal melanin unit (EMU) and is suitable to study pigmentation [21].

23.2.1 Preparation of Dead De-epidermized Dermis

Human dermis is obtained from plastic surgery specimen from normal adults, mostly breast reduction specimen. Skin samples are thinned, cut into very small pieces, and incubated at 37 °C in Hank's balanced salt solution until epidermis can be removed without excessive scraping. After removal, the dermis is rinsed in 70 °C ethanol and submitted to two cycles of freezing-thawing and stored in Hank's balanced salt solution at −20 °C until use.

23.2.2 Epidermal Reconstruction

Melanocytes and keratinocytes (from normal or non-lesional vitiligo skin) at passages 2 or 3 are seeded in an incubation chamber placed on the epidermal side of DDD at 4×10^5 cells/cm^2 at a melanocyte/keratinocyte ratio of 1:20 (5%) for normal melanocytes [21] or transduced melanocytes (Ricard et al. 2012, Wagner et al. 2015) and 1:20 or 1:10 (5–10%) for vitiligo melanocytes since vitiligo melanocytes have a defective adhesion [22, 23]. Twenty-four hours after seeding, the incubation chamber is removed, and the DDD is immersed for 3 days. The DDD are shifted to the air–liquid interface for 8 days before functional studies (Fig. 23.1). The model can be improved by seeding fibroblasts in an incubation chamber placed on the dermal side of DDD 72 h before seeding keratinocytes and melanocytes.

23.3 Functional Studies

23.3.1 Functional Studies of NSV Cells Using Monolayers: Melanocytes, Keratinocytes, and Fibroblasts

Cultures can be used to characterize the phenotype of vitiligo melanocytes as compared to control pigment cells under various treatments, such as UV or pharmacological agents. The cells are usually seeded the day before to obtain 60–70% confluency on the day of treatment. Various techniques can be used to observe melanocyte behavior. Direct observation by microscopy on fixed and specifically stained melanocytes (melan-A, DOPA, c-kit, S-100, HMB-45) gives information on shape, dendriticity, and pigmentation. DOPA staining often is used as alternative method to overcome the poor melanocyte number and not enough to allow spectrophotometric melanin content measure. Vitiligo melanocyte and keratinocyte cultures can be tested in vitro in order to determine their specific susceptibility to noxious stimuli or to physiological growth factors. UVB, cumene hydroperoxide, and *tert*-butylphenol are the most used stimuli [19, 24–31]. Cell proliferation and mortality can be assessed through MTT test, manual cell count, annexin V/propidium staining, DNA ladder, or caspase profile. In addition, the morphology and modality of melanosome transfer in vitro can be studied using atomic force microscopy, which allows to estimate and quantize the measure and the distribution of the dendrites including internal melanosome distribution and arrangement [31–33]. Culture on 3T3 feeder layer induces the formation of colonies of keratinocytes which vary

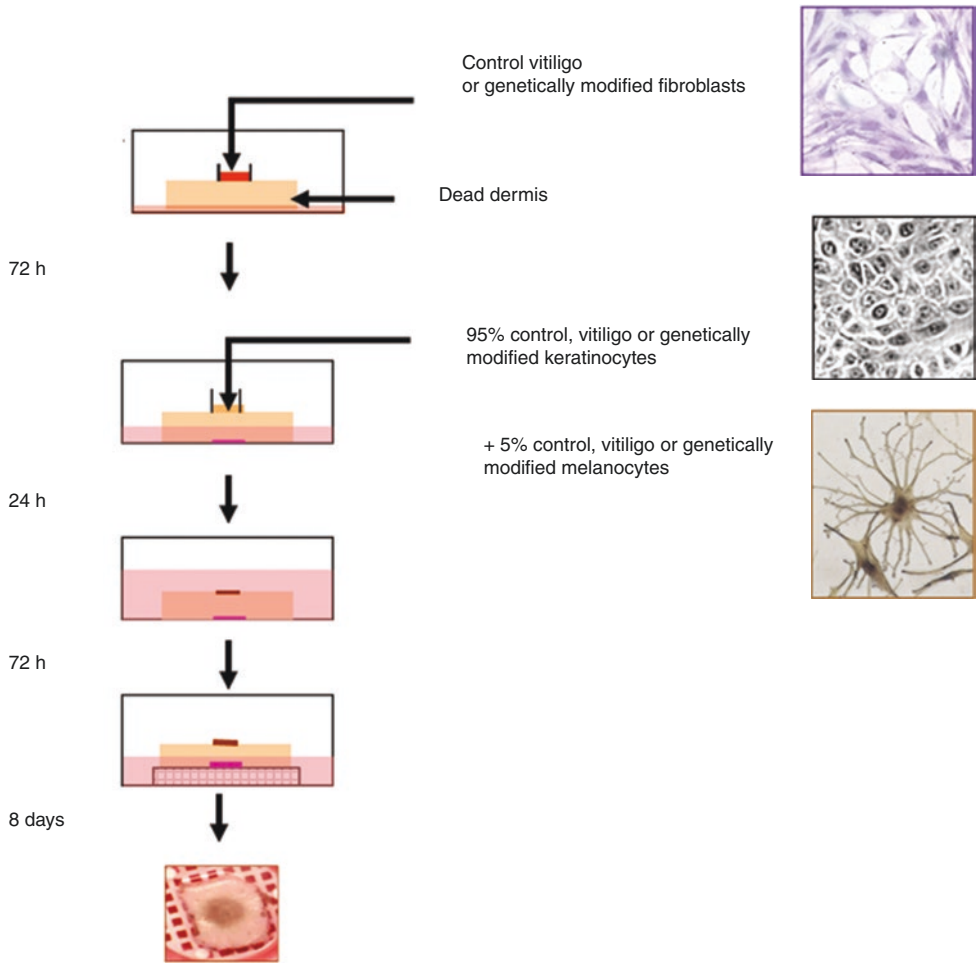


Fig. 23.1 Schematic protocol for epidermal reconstruction

in size according to the state of cell proliferation, differentiation, or senescence. Lesional NSV keratinocytes are characterized by a lower proliferative potential, as indicated by a shorter in vitro life span. Moreover, the expression of p16, PCNA, p53, and p63 markers differs between lesional and non-lesional cells. Lesional keratinocytes show a lower level of the senescence marker p16 and a higher level of the melanocyte growth factor SCF [34].

23.3.2 Functional Studies Using Genetically Modified Cell Culture in Monolayer

Genetically modified cells in monolayer can be useful to verify if putative gene can reproduce some key features of diseased cells (adhesion, dendricity for melanocytes) but sometimes in monolayer cells do not express protein they express in tissue. For example, monolayer melanocytes do not express cadherin; thus silencing of cadherin has no effect on their behavior in culture. Several genes are implicated in cell adhesion, but their silencing may have adverse effect; silencing of DDR1 (collagen IV receptor) has no effect on plastic adhesion, whereas silencing of CCN3 (melanocyte's homeostasis) is lethal for melanocytes (Fig. 23.2).

23.3.3 Functional Studies of NSV Cells by Next-Generation Approach

Cultures can be also used to perform functional studies based on epigenome editing. This branch

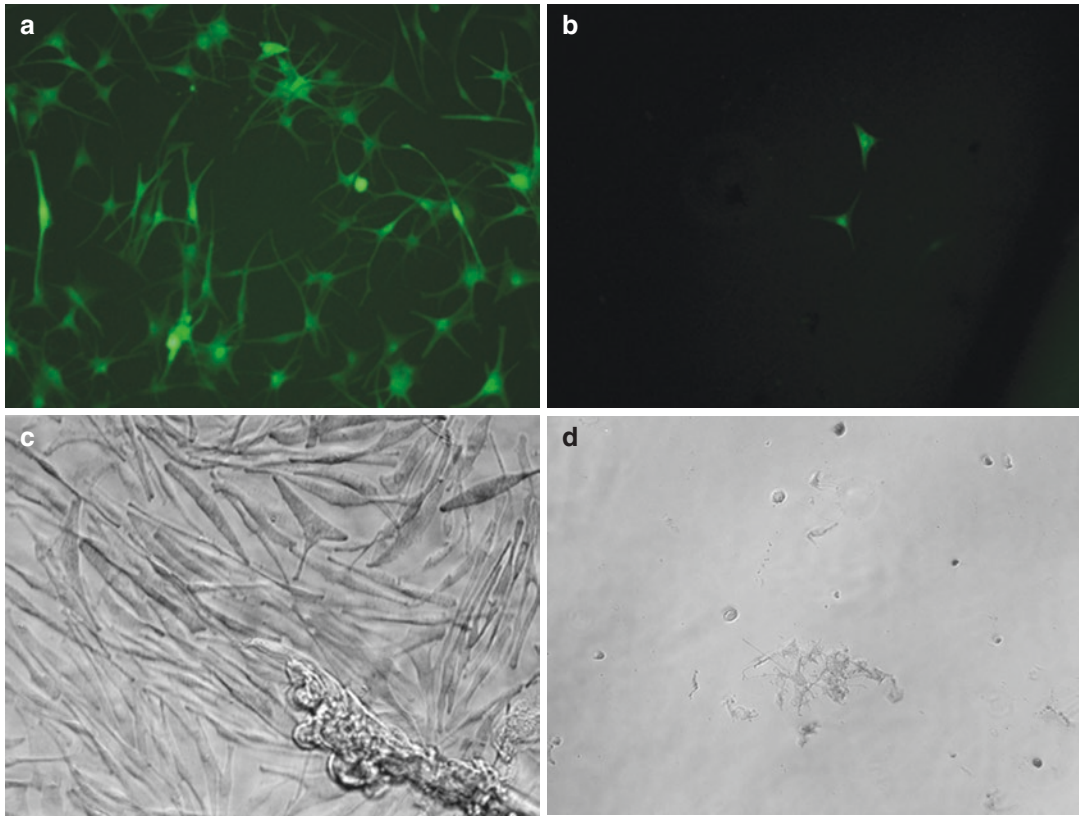


Fig. 23.2 Effect of inhibition of CCN3 on melanocytes. Expression of GFP in melanocytes transduced with a vector coding shCCN3 and GFP at day 5 post-transduction (**a**) and day 12 post-transduction (**b**). Brigthfield analysis at day 12 of untransduced melanocytes (**c**) and corresponding melanocytes transduced with vector coding shCCN3 and puromycin resistance and treated with puromycin to obtain 100% transduction (**d**)

of biology refers to directed alteration of chromatin marks at specific genomic loci by Epi effectors aiming to durable gene regulation with application in basic research and clinics. Targeted deposition or removal of chromatin modifications is a powerful approach for functional studies. It includes histone posttranslational modifications, DNA methylation, and hydroxymethylation, which in concert regulate the gene expression. The available profile of chromatin modifications yields thousands of global-scale epigenomic maps. In preclinical studies, epigenome editing will give rise to a fine mapping of the regulatory mechanisms leading to health/diseased process. It is integrating part of interdisciplinary field of *synthetic biology*. It represents new and interesting avenues to treat metabolic diseases, possibly including vitiligo, characterized by aberrant signaling pathways through a causative therapy. However, some questions are still open: which modifications have a causal role in governing processes such as transcription or alternative splicing? Which modifications lead to stable and heritable changes in chromatin status? Which chromatin behavior affects the maintenance of such modifications? Is the specificity per se able to provide clinical applications [35]?

Recently, a single-cell epigenomics approach was developed by combining several different high-throughput techniques allowing studies on cytosine modification, protein-DNA interaction, chromatin structure, and 3D organization [36].

23.3.4 Functional Studies Using Reconstructed Epidermis

Monolayer cultures and coculture [37] are useful to study vitiligo melanocytes and keratinocyte direct (cell–cell contact) or indirect interaction

(soluble factors/culture insert), but they do not reproduce the tridimensional interactions of cells of the EMU. Epidermal reconstructs, which reproduce the EMU and basal membrane attachment, are thus a handful "in vivo-like" model. Indeed, reconstructions of different levels of complexity can be prepared (reconstructs with keratinocytes alone, keratinocytes and melanocytes, keratinocytes, melanocytes, and fibroblasts), including chimeric reconstructs (normal vs. pathological cells or genetically modified cells). According to the initial reflection, the analysis of the behavior of melanocytes and keratinocytes is done in a more physiological environment than that of monolayer cultures and cocultures and allows to test conveniently compounds which are suspected to be implicated in vitiligo etiology or susceptible to improve the attachment and survival of melanocytes upon the basal layer. An example of epidermal reconstructs tested with epinephrine, norepinephrine, dopamine, hydrogen peroxide, or vitiligo sera is illustrated in Fig. 23.3. Our main results using this model [23] were the following: reconstructs made with melanocytes from nonlesional generalized vitiligo skin have a significantly reduced number of basal layer melanocytes, whereas the presence of vitiligo keratinocytes enhances this effect. Similarly, reconstructs made with melanocytes silenced for CCN3 have less basal melanocytes and more detached melanocytes than their normal counterpart (Ricard et al. 2012). Vitiligo sera may induce melanocyte detachment independently of the disease activity or extent. Hydrogen peroxide induces melanocyte detachment in reconstructs containing vitiligo melanocytes or melanocytes silenced for E-cadherin (Wagner et al. 2015) and normal keratinocytes, but not in normal controls. Finally, epinephrine, but not norepinephrine, allows melanocyte detachment. Epidermal reconstructs are useful to address several research questions such as: Is the melanocyte primarily affected? Is the cellular environment important (keratinocytes, fibroblasts)? Are other (soluble) factors implicated in the development of vitiligo? However, this model has some limitation since we have not yet been able to introduce, for instance, Langerhans cells or other immune cells to study their implication in vitiligo etiology; the study of topical molecules to improve vitiligo treatment is less easy than that of soluble factors and that long-term studies (more than 3 weeks) are not possible, since there is no renewal of the basal layer. Moreover, the analysis of intracellular signaling and membrane assessment per cell can be just on single-cell-type culture, when cell sorting is not available.

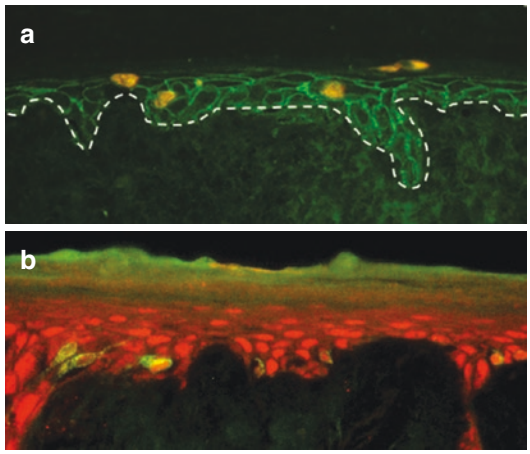


Fig. 23.3 Detection of melanocyte in genetically modified reconstructed epidermis. (**a**) Detachment of melanocyte (red) in reconstructed epidermis made with melanocytes silenced for E-cadherin. Double staining of cadherin (green) and melanocyte (red). Detached melanocyte (arrow). (**b**) Detachment of melanocyte silenced for CCN3 (green with absence of red nuclear staining) (arrow) and basal location of CCN3-positive melanocyte (green with red nuclear staining) (arrow head). Double staining of CCN3 (red) and melanocyte (green)

23.4 Analytic Techniques

23.4.1 Fluorescence-Based Assays

Fluorochrome-conjugated monoclonal or polyclonal antibodies are widely applied to visualize and quantify the expression of some surface or internal melanocyte markers [20, 38–40]. The first data were derived by immunohistochemistry methods on frozen and paraffin-embedded sections, followed by fluorescence and confocal microscopy on slide-cultured cells. Phenotypic characterization of cultured vitiligo melanocytes with respect to control cells was carried out. A reduced expression of c-Kit and ET-1 receptors, tyrosinase, and MITF was found in vitiligo

melanocytes with pattern progressively varying from the edge of the white spot to the non-lesional area [39]. The flow cytometric approach permits a quali-quantitative multiparametric and time-dependent analysis. Membrane and intracellular staining together with the analysis of the physical and time parameters permits the structural and functional characterization of the melanocytes and their sorting for further cultures. The current flow cytometers can analyze up to 16 parameters. Besides the antibody-based approach, the cytomic one has been used to detect in vitiligo melanocytes the intracellular ROS production (DCFH-DA or dhRho123 staining), the membrane lipo-peroxidation [41] (BODIPY581/591 staining), and the content and the transmembrane cardiolipin distribution (NAO fluorescence pattern) [24]. The flow cytometer may be also used for fluorescence resonance energy transfer (FRET) analysis. Cells are stained with nonyl acridine orange (NAO) (donor) and MitoTracker Orange, a dye with high affinity for mitochondrion proteins and voltage sensible (acceptor). The mitochondrial mass and the polarization state of the inner mitochondrial membrane can be monitored using FRET index based on NAO FL1 (green fluorescence) decrease and NAO FL2 (red fluorescence) increase from single to double-labeled tubes [42]. The limitation associated with flow cytometry is that it is sample consuming. Several new approaches, based on "fluo world," are now available. Most of these innovative technologies have not yet been applied to the study of vitiligo, but they open promising perspectives. The laser scanning cytometer (LSC) permits slide-based cytometry (SBC) and the hyperchromatic approach. Its potential application in vitiligo study arises from its intrinsic features: nonconsumptive (unlike the flow cytometer), iterative restaining, differential photobleaching (fluorochromes differentiated on the basis of their specific photostability), and photoactivation (for nanoparticles or photo-caged dyes). A single cell can be reanalyzed, whereas the information gained per specimen is only limited by the number of available antibodies and sterical hindrance [43]. The LSC when combined with fine-needle sampling (FNS) may be used to monitor the cell structural and functional modifications subsequent to in vitro treatment, where FNS further reduces the amount of sample needed for the analysis. The SBC is a significant advance research in the measurement of short-lived processes in adherent cells and small samples, two crucial features of vitiligo samples. New light microscope architecture is provided by iMIC, which integrates time-lapse studies, FRET measurements, laser microdissection, and slit-scan confocal measurements within coherent illumination (340–680 nm).

23.4.2 Proteomic

Mass spectrometry in conjunction with free-flow electrophoresis of sucrose density gradient has allowed the identification of early-stage melanosome proteins [44]. Two-dimensional differential image-gel electrophoresis (2D-DIGE) and liquid chromatography tandem-mass spectrometry (LC-MS/MS) allow the analysis and identification of the proteic components of the organelles of melanocytes with melanosomes at different maturation stages [45]. Calreticulin, a soluble Ca^{2+}-binding chaperone protein, is involved together with calnexin in the folding of newly synthesized proteins and glycoproteins (including tyrosinase) and for quality control pathways in endoplasmic reticulum. The LC-MS/MS analysis has highlighted that calreticulin expression is dependent on the maturation stage of melanocytes. Even if the proteomic assay has been so far carried out on murine healthy melanocytes, the scenario possibly designed by this approach may be crucial for the maturation process leading from nonpigmented melanocytes to pigmented melanocytes in vitiligo. Starting from the consideration of vitiligo as metabolic multifactorial disease, an analysis of the systemic proteomic profile will support its characterization. The proteomic study of the body fluids (plasma, serum, saliva) should start from the preliminary remotion of albumin and other major represented proteins, also taking into account how and when removing them. The difference in subcellular derivation (plasma membrane, cytoplasm, mitochondria) has been related to the nature/severity of the stimulus. Innovative or traditional disease markers should be as possible tissue- and disease-specific. An excellent

database reference is provided by the Human Protein Atlas (HPA), also including data on mRNA coding for the same proteins. The albumin depletion can be carried out by 2DE with IEF or sequential precipitation steps. Major plasma/serum proteins can be removed, in order to improve the sensitivity of proteomic protocols, by hydrophobic interaction chromatography (HIC) before 2DE. Different companies provide validated columns, including Agilent, R&D Systems, and Sigma. A crucial question should be answered: that related to the cost-effectiveness ratio of the devices. An alternative perspective to depletion of high-abundance proteins is the enrichment of low-abundance ones by means of their relative concentration, according to a process named "protein equalization" and based on saturable and specific interaction to a high diversity binding sites present on chromatographic beads. The library provides dozen of millions of hexapeptides able to interact with most of proteins in any proteome. The analysis can be performed by SELDI. Some protein modification such as glycosylation correlates with specific pathological conditions, and differently glycosylated proteins can be identified on the basis of the pI and Mr on 2DE assay. During inflammatory process some featuring proteins are released in the plasma/serum according to acute and chronic phase of the inflammation itself. The summary of the most relevant inflammatory markers is provided by Cytokine & Cells Online Pathfinder Encyclopedia (COPE) website at www.copewithcytokines.org. An interesting integration to the above atlas is provided by http://wallace.uab.es/multitask providing database collecting hundreds of characterized *moonlighting* multitasking proteins, involved in different functions [46]. Recently, proteomic approach was applied to formalin-fixed paraffin-embedded tissue [47].

23.4.3 Metabolomics/Lipidomics

Metabolomics is an emerging field of biological science, which considers the highthroughput characterization of small compounds (>1500 Da) representing the final products of cellular metabolism and mirroring the chemical fingerprint of an organism at a precise point. It aims to identify and quantify the metabolites to correlate their changes with pathological conditions or with drug intake. Accordingly, metabolomics is one of the building blocks of systems biology. There are two general analytical approaches in metabolomics analysis: targeted and untargeted. Whereas the targeted approach considers a well-defined and known set of metabolites, the second one aims to define the quantitative modification among the analyzed populations (fold change) without standard references and allows the study of unidentified metabolites. The untargeted approach is also called metabolite fingerprinting. However, it is relevant to remember that according to the different used instrument and method, the final metabolite spectra may change. In any case metabolomic study generates a complex set of data requiring specialized analysis based on cheminformatics, bioinformatics, and statistics expertise.

As regards the specific vitiligo application, metabolomic research will provide a fingerprinting of the metabolic activities related to immune deregulation and degenerative process, possibly merging the vitiligo profile with those known and described in other diseases and health population. The separation techniques are based on gas chromatography, high-performance liquid chromatography, and capillary electrophoresis; the detection techniques usually are done by mass spectrometry or nuclear magnetic resonance. Each approach shows different and specific sensitivity and methodological limitations. Mass spectrometry is the most widely used approach in tandem with chromatography separation method; it is sensitive and selective [48].

Serum lipidomics is a global profiling of lipid molecular species. Lipidomics, defined as the large-scale study of pathways and networks of cellular lipids, is an emerging and rapidly expanding research field. It is often necessary to evaluate a wide range of molecular species and lipid classes to gain insights into pathophysiology. In the future, research in lipidomics will expand to include interactions of lipids with lipids, proteins, and other cellular components. Mass spectroscopy, nuclear magnetic resonance, and fluorescence spectroscopy have played a crucial role in lipid characterization, identification, and quantization [49]. The study of vitiligo pathogenesis should actually gain a relevant burst from the metabolomics approach.

23.4.4 Transcriptomic

Recently, oligonucleotide-based microarrays have been used to explore the pattern of gene expression of vitiligo melanocytes. Interestingly, the most upregulated genes are related to the network of endosome and lysosome organelles. The next step was to analyze the various clusters of the differentially expressed genes. This approach can represent the basis for further in-depth analyses to better clarify the complex vitiligo pathomechanisms [50].

23.5 Concluding Remarks

Cell culture models are useful to investigate the differences between normal and vitiligo cells and to test potential treatment options in a cell-focusing approach. New analytic techniques using a limited amount of biological material are very promising. Beyond the study of vitiligo pathomechanisms or therapies using unmodified patient's cells, the in vitro approach can be also adapted to generate melanocyte-specific silencing or overexpression of putative target genes or, a further step, to design animal models using melanocyte-specific expression of modified genes. The study of various issues pertaining to vitiligo including the role and regulation of transcription factors, organelle genesis, intracellular transport, stem cell maintenance, and senescence can be envisaged [51]. This step will allow the development of vitiligo models using normal cells bypassing the difficulty to culture vitiligo cells, which is currently the limiting factor for studying vitiligo in vitro.

References

1. Medrano EE, Nordlund JJ. Successful culture of adult human melanocytes obtained from normal and vitiligo donors. J Invest Dermatol. 1990;95:441–5.
2. Kauser S, Thody AJ, Schallreuter KU, et al. β-Endorphin as a regulator of human hair follicle melanocyte biology. J Invest Dermatol. 2004;123:184–95.
3. Tobin DJ, Colen SR, Bystryn JC. Isolation and long term culture of human hair-follicle melanocytes. J Invest Dermatol. 1995;104:86–9.
4. Halaban R, Alfano FD. Selective elimination of fibroblasts from cultures of normal human melanocytes. In Vitro. 1984;20:447–50.
5. Na GY, Paek SH, Park BC, et al. Isolation and characterization of outer root sheath melanocytes of human hair follicles. Br J Dermatol. 2006;155:902–9.
6. Vennegor C, Hageman P, Van Nouhuijs H, et al. A monoclonal antibody specific for cells of the melanocyte lineage. Am J Pathol. 1988;130:179–92.
7. Ricard AS, Pain C, Daubos A, et al. Study of CCN3 (NOV) and DDR1 in normal melanocytes and vitiligo skin. Exp Dermatol. 2012;21:411–6.
8. Wagner RY, Luciani F, Cario-André M, et al. Altered E-cadherin levels and distribution in melanocytes precede clinical manifestations of vitiligo. J Invest Dermatol. 2015;135:1810–9.
9. Caliari SR, Burdick JA. A practical guide to hydrogels for cell culture. Nat Methods. 2016;13:405.
10. Dell'Anna ML, Maresca V, Briganti S, et al. Mitochondrial impairment in peripheral blood mononuclear cells during the active phase of vitiligo. J Invest Dermatol. 2001;117:908–13.
11. Donmez-altuntas H, Sut Z, Ferahbas A, et al. Increased micronucleus frequency in phytohaemagglutinin-stimulated blood cells of patients with vitiligo. J Eur Acad Dermatol Venereol. 2008;22:162–7.
12. Giovannelli L, Bellandi S, Pitozzi V, et al. Increased oxidative DNA damage in mononuclear leukocytes in vitiligo. Mutat Res. 2004;556:101–6.
13. Prunieras M, Regnier M, Schlotterer M. [New procedure for culturing human epidermal cells on allogenic or xenogenic skin: preparation of recombined grafts]. Ann Chir Plast. 1979;24:357–362.
14. Poumay Y, Coquette A. Modelling the human epidermis in vitro: tools for the basic and applied research. Arch Dermatol Res. 2007;298:361–9.
15. Bell E, Sher S, Hull B, et al. The reconstitution of living skin. J Invest Dermatol. 1983;81:2s–10s.
16. Black AF, Bouez C, Perrier E, et al. Optimization and characterization of an engineered human skin equivalent. Tissue Eng. 2005;11:723–33.
17. Lee DY, Lee JH, Yang JM, et al. A new dermal equivalent: the use of dermal fibroblast culture alone without exogenous materials. J Dermatol Sci. 2006;43:95–104.
18. Rosdy M, Bjorklund MG, Asplud A, et al. Terminal epidermal differentiation of human keratinocytes grown in chemically defined medium on inert filter substrates at the hair-liquid interface. J Invest Dermatol. 1990;95:409–14.
19. Jimbow K, Chen H, Park JS, et al. Increased sensitivity of melanocytes to oxidative stress and abnormal expression of tyrosinase-related protein in vitiligo. Br J Dermatol. 2001;144:55–65.
20. Régnier M, Staquet MJ, Schmitt D, et al. Integration of Langerhans cells into a pigmented reconstructed human epidermis. J Invest Dermatol. 1997;109:510–2.
21. Cario-André M, Bessou S, Gontier E, et al. The reconstructed epidermis with melanocytes: a new tool to study pigmentation and photo protection. Cell Mol Biol. 1999;45:931–42. Review (Erratum in: Cell MolBiol (2000); 446:489).
22. Bessou S, Surlève-Bazeille JE, Sorbier E, Taïeb A. Ex vivo reconstruction of the epidermis with melano-

cytes and the influence of UVB. Pigment Cell Res. 1995;8:241–9.
23. Cario-André M, Pain C, Gaythier Y, et al. The melanocythorragic hypothesis of vitiligo tested on pigmented, stressed, reconstructed epidermis. Pigment Cell Res. 2007;20:385–93.
24. Dell'Anna ML, Ottaviani M, Albanesi V, et al. Membrane lipid alterations as a possible basis for melanocyte degeneration in vitiligo. J Invest Dermatol. 2007;127:1226–33.
25. Ivanova K, van der Wijngaard R, Gerzer R, et al. Nonlesional vitiliginous melanocytes are not characterized by an increased proneness to nitric oxide-induced apoptosis. Exp Dermatol. 2005;14:445–53.
26. Kroll TM, Bommiasamy H, Boissy RE, et al. 4-tertiary butylphenol exposure sensitizes human melanocytes to dendritic cell-mediated killing: relevance to vitiligo. J Invest Dermatol. 2005;124:798–806.
27. Lee AY, Kim NH, Choi WI, et al. Less keratinocyte derived factors related to more keratinocyte apoptosis in depigmented than normally pigmented suction-blistered epidermis may cause passive melanocyte death in vitiligo. J Invest Dermatol. 2005;124:976–83.
28. Maresca V, Roccella M, Roccella F, et al. Increased sensitivity to peroxidative agents as a possible pathogenetic factor of melanocyte damage in vitiligo. J Invest Dermatol. 1997;109:310–3.
29. Yang F, Boissy RE. Effects of 4-tertiary butylphenol on the tyrosinase activity in human melanocytes. Pigment Cell Res. 1999;12:237–45.
30. Yang F, Sarangarajan R, Le Poole IC, et al. The cytotoxicity and apoptosis induced by 4-tertiary butylphenol inhuman melanocytes are independent of tyrosinase activity. J Invest Dermatol. 2000;114:157–64.
31. Zhang RZ, Zhu WY, Xia MY, et al. Morphology of cultured human epidermal melanocytes observed by atomic force microscopy. Pigment Cell Res. 2004;17:62–5.
32. Boissy RE, Liu YY, Medrano EE, et al. Structural aberration of the rough endoplasmic reticulum and melanosome compartmentalization in long-term cultures of melanocytes from vitiligo patients. J Invest Dermatol. 1991;97:395–404.
33. Bondanza S, Maurelli R, Paterna P, et al. Keratinocytes cultures from involved skin in vitiligo patients show an impaired in vitro behaviour. Pigment Cell Res. 2007;20:288–300.
34. Van den Wijngaard RMJGJ, Aten J, Scheepmaker A, et al. Expression and modulation of apoptosis regulatory molecules in human melanocytes: significance in vitiligo. Br J Dermatol. 2000;143:573–81.
35. Kungolovaski G, Jeltsch A. Epigenome editing: state of the art, concepts, and perspectives. Trends Genet. 2016;32:101–13.
36. Clark SJ, Lee HL, Smallwood SA, Kelsey G, Reik W. Single-cell epigenomics: powerful new methods for understanding gene regulation and cell identity. Genome Biol. 2016;17:72–81.
37. Eves PC, Beck AJ, Shard AG, et al. A chemically defined surface for the co-culture of melanocytes and keratinocytes. Biomaterials. 2005;26:7068–81.
38. Graham A, Westerhof W, Thody AJ. The expression of a-MSH by melanocytes is reduced in vitiligo. Ann N Y Acad Sci. 1999;885:470–3.
39. Kitamura R, Tsukamoto K, Harada K, et al. Mechanisms underlying the dysfunction of melanocytes in vitiligo epidermis: role of SCF/KIT protein interactions and the downstream effector, MITF-M. J Pathol. 2004;202:463–75.
40. Norris A, Todd C, Graham A, et al. The expression of the c-kit receptor by epidermal melanocytes maybe reduced in vitiligo. Br J Dermatol. 1996;134:299–306.
41. Dell'Anna ML, Urbanelli S, Mastrofrancesco A, et al. Alterations of mitochondria in peripheral blood mononuclear cells of vitiligo patients. Pigment Cell Res. 2003;16:553–9.
42. Dykens JA, Fleck B, Ghosh S, et al. High-throughput assessment of mitochondrial membrane potential in situ using fluorescence resonance energy transfer. Mitochondrion. 2002;1:461–73.
43. Tellez CS, Davis DW, Prieto VG, et al. Quantitative analysis of melanocytic tissue array reveals inverse correlation between activator protein-2 alpha and protease-activated receptor-1 expression during melanoma progression. J Invest Dermatol. 2007;127:387–93.
44. Ouvry-Patat SA, Torres MP, Quek HH, et al. Free-flow electrophoresis for top-down proteomics by Fourier transform ion cyclotron resonance mass spectrometry. Proteomics. 2008;8:2798–808.
45. Kawase A, Kushimoto T, Kawa Y, et al. Proteomic analysis of immature murine melanocytes at different stages of maturation: a crucial role for calreticulin. J Dermatol Sci. 2008;49:43–52.
46. Gianazza E, Miller I, Palazzolo L, Parravicini C, Eberini I. With or without you-proteomics with or without major plasma/serum proteins. J Proteomics. 2016;140:62–80.
47. O'Rourke MB, Padula MP. Analysis of formalin-fixed paraffin-embedded (FPPE) tissue via proteomic techniques and misconceptions of antigen retrieval. Biotechniques. 2016;60:229–38.
48. Cambiaghi A, Ferrario M, Masseroli M. Analysis of metabolomic data: tools, current strategies and future challenges for omics data integration. Brief Bioinform. 2017;18:498–510.
49. Han X. Potential mechanisms contributing to sulfatide depletion at the earliest clinically recognizable stage of Alzheimer's disease: a tale of shotgun lipidomics. J Neurochem. 2007;103:171–9.
50. Stromberg S, Bjorklund MG, Asplud A, et al. Transcriptional profiling of melanocytes from patients with vitiligo vulgaris. Pigment Cell Melanoma Res. 2008;21:162–71.
51. Goding CR. Melanocytes: the new black. Pigment Cell Res. 2007;39:275–9.

Genetics

24

Richard A. Spritz

Contents

Abstract

Large-scale epidemiological surveys have shown that most cases of vitiligo occur sporadically, though about 15–20% of patients report one or more affected relatives. The rationale for genetic studies of vitiligo susceptibility is that underlying genes are involved in mediating disease causation, either increasing or decreasing risk (protective). Three different general approaches have been used to identify genes that mediate vitiligo susceptibility: the *candidate gene* approach, the *genome-wide* approach, and the *gene expression* approach. Extensive experience has proven that the only analytic approach that produces verified discovery of bona fide disease genes is the genome-wide approach. Retrospective analyses of candidate gene studies have shown that the vast majority of claimed candidate gene associations represent false-positives. Accordingly, the candidate gene approach is no longer considered valid for de novo disease gene discovery and is reserved for confirmatory studies only. Similarly, almost all genes that exhibit major expression differences between disease and non-disease states turn out to not correspond to causal genes, but instead represent secondary effects, and thus likewise gene expression studies have generally not led to the discovery of genes that are causal for complex diseases. Indeed, none of the genes initially suggested on the basis of the expression approach now appear to be involved in vitiligo causation at all. In contrast, genome-wide

R. A. Spritz (✉)
Human Medical Genetics and Genomics Program, University of Colorado School of Medicine, Aurora, CO 80045, USA

Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO 80045, USA
e-mail: Richard.Spritz@ucdenver.edu

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_24

genetic analyses, particularly genome-wide association studies (GWAS), have proven a remarkably robust approach to disease gene discovery, yielding findings that are highly reproducible and which, in aggregate, have provided dramatic advances in understanding the biological basis of many different complex diseases, including vitiligo. Reported candidate gene associations and expression difference findings that are not observed in well-powered GWAS of the same population are not now considered to be valid indications of disease-causal genes and thus will not be discussed here.

Key Points

- Several different approaches have been taken to identify vitiligo susceptibility genes. It has become clear that the most reliable method to identify bona fide causal disease genes for "complex diseases," such as vitiligo, is the genome-wide association study (GWAS), which has become the "gold standard" for disease gene identification. In contrast, the great majority of genes suggested on the basis of candidate gene studies have proven to be false-positives.
- To date, approximately 50 vitiligo susceptibility genes have been identified by GWAS. These genes encode a network of proteins that regulate aspects of the immune system, the cellular apoptosis, the melanocyte, and the interface between the melanocyte and the immune system.
- Vitiligo susceptibility genes that encode melanocyte components or melanocyte regulators have also been associated with melanoma, involving exactly the same genetic variants but the opposite alleles. This genetically opposite relationship suggests that vitiligo might represent a dysregulated mechanism of immune surveillance for melanoma.
- Vitiligo is epidemiologically associated with a number of other autoimmune diseases, both in vitiligo patients themselves and in their close relatives. This epidemiologic association has a genetic basis, as many vitiligo susceptibility genes have also been shown to contribute to these other autoimmune diseases.

24.1 Genetic Epidemiology

Large-scale epidemiological surveys have shown that most cases of vitiligo occur sporadically, though about 15–20% of patients report one or more affected relatives [1]. Rarely, large multi-generation families segregate vitiligo in patterns that suggest autosomal dominant [2] or autosomal recessive [3] inheritance with incomplete penetrance. More typically, however, familial aggregation of vitiligo cases occurs in a non-Mendelian pattern. Hafez [4] and Das [5, 6] suggested polygenic, multifactorial inheritance and estimated vitiligo heritability at 46% [6] to 72% [4]. Subsequent analyses similarly suggested polygenic, multifactorial inheritance [1, 7–13], with approximately 50% heritability [13]. In general, the frequency of vitiligo is approximately equal in males and females, when the female sex bias present in most vitiligo clinical case series is controlled for appropriately [14].

Strong evidence for genetic factors in the pathogenesis of vitiligo comes from studies of patients' close relatives. Among Caucasians of European origin (EUR), the risk of vitiligo to a patient's siblings is about 6.1% [1], a 16-fold increase over the approximately 0.38% prevalence of vitiligo in at least one EUR population

[15]. The overall risk of vitiligo to patients' first-degree relatives is 7.1% in EUR, 6.1% in Indo-Pakistanis, and 4.8% in USA Hispanic/Latinos [1], with lower risks to more distant relatives. Generally similar results have come from studies of Han Chinese (CHN) families [13]. Furthermore, in a study of vitiligo in EUR monozygotic twins, the concordance for vitiligo was 23% [1], more than 60 times the general population risk of 0.38%, and almost four times the 6.1% risk of vitiligo to probands' siblings, providing additional strong support for a complex genetic basis of vitiligo risk.

Additional evidence for a genetic component to vitiligo susceptibility comes from age-of-onset data: among unselected (mostly sporadic) EUR vitiligo patients, the mean age of vitiligo onset is 24.2 years [16], but among patients in families with multiple relatives affected by vitiligo, the mean age of onset is significantly earlier, 21.5 years [14]. Earlier age of disease onset in more "familial" cases and diminishing disease risk with increasing genetic distance from a vitiligo case are typical characteristics of a polygenic disorder, and formal genetic segregation analyses have indicated that vitiligo is a complex trait, with multiple contributory genetic loci [10, 12, 13].

While epidemiologic and twin studies thus indicate that genes play an important role in vitiligo pathogenesis, nongenetic, environmental triggers must also be very important. Identical twins share all of their genes, and incomplete heritability, limited twin concordance and typical adult onset underscore the apparent role of environmental triggers. While many different environmental risk factors for vitiligo have been suggested, epidemiologic data definitively supporting a role any of these in typical vitiligo remain very limited. Many experts believe that skin damage (Koebnerization) perhaps with low-level infection may be a disease trigger in many cases.

Epidemiologic studies have also shown that vitiligo is strongly associated with other autoimmune diseases and that this association has a genetic basis. Vitiligo is a component of the APECED (APS1; OMIM #240300) and "Schmidt" (APS2; OMIM %269200) multiple autoimmune disease syndromes (additional suggested "autoimmune polyendocrine syndrome" categories [17] are not widely accepted). More importantly, about 10–20% of vitiligo patients develop other concomitant autoimmune diseases, as first observed by Thomas Addison in 1855 [18]. Many retrospective studies have shown that vitiligo is epidemiologically associated with increased prevalence of autoimmune thyroid disease [1, 19, 20], adult-onset type 1 diabetes [1], systemic lupus erythematosus [1], pernicious anemia [1, 21, 22], Addison's disease [1, 23], and perhaps alopecia areata [24, 25]. These same diseases also occur at increased frequency in vitiligo patients' first-degree relatives, regardless of whether or not those relatives have vitiligo themselves [1]. Families with multiple cases of vitiligo have even higher frequencies of these autoimmune diseases, in both vitiligo patients and their siblings [14], indicating even greater genetic susceptibility to autoimmune diseases in such "multiplex" families than in typical singleton vitiligo patients. Generally similar results have come from retrospective studies of vitiligo patients in India [26, 27] and Nigeria [28].

Taken together, these findings suggest that pathologic variants in specific genes predispose to a specific subset of autoimmune diseases that includes vitiligo, autoimmune thyroid disease, rheumatoid arthritis, adult-onset autoimmune diabetes mellitus, pernicious anemia, systemic lupus erythematosus, Addison's disease, and perhaps others (Fig. 24.1). As described below, large-scale genetic studies have borne this out, as many susceptibility genes are shared among these autoimmune diseases, in different combinations, sometimes involving the same high-risk gene variants.

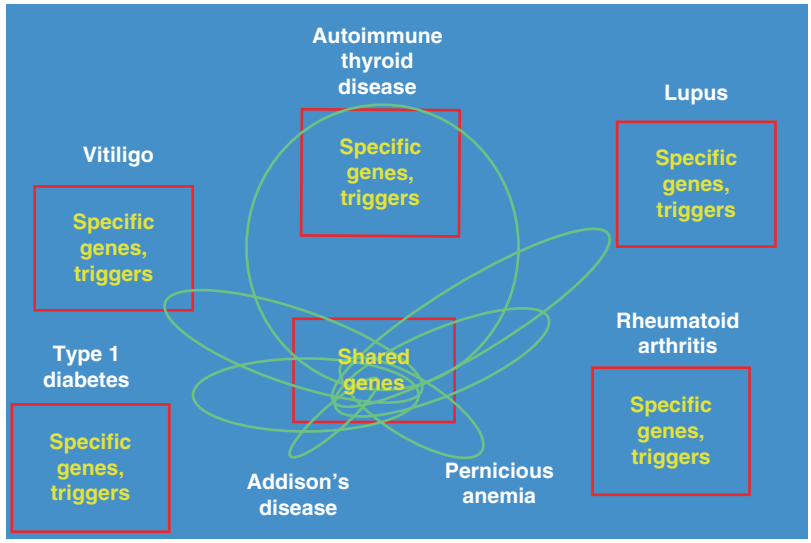


Fig. 24.1 Shared genetic relationships among epidemiologically associated autoimmune diseases. Elevated prevalence of autoimmune thyroid disease, rheumatoid arthritis, adult-onset autoimmune diabetes mellitus, pernicious anemia, systemic lupus erythematosus, Addison's disease, and perhaps other autoimmune diseases in both vitiligo patients and their first-degree relatives (even those without vitiligo) suggests that these autoimmune diseases share some susceptibility genes that increase risk to all or some of these diseases, as well as disease-specific susceptibility genes and likely environmental triggers

24.2 Identification of Vitiligo Susceptibility Genes

The rationale for genetic studies of vitiligo susceptibility is that underlying genes are involved in mediating disease causation, either increasing or decreasing risk (protective). Three different general approaches have been used to identify genes that mediate vitiligo susceptibility: the *candidate gene* approach, the *genome-wide* approach, and the *gene expression* approach. Extensive experience has proven that the only analytic approach that produces verified discovery of bona fide disease genes is the genome-wide approach. Retrospective analyses of candidate gene studies have shown that the vast majority of claimed candidate gene associations represent false-positives [29, 30]. Accordingly, the candidate gene approach is no longer considered valid for de novo disease gene discovery and is reserved for confirmatory studies only. Similarly, almost all genes that exhibit major expression differences between disease and non-disease states turn out to not correspond to causal genes, but instead represent secondary effects, and thus likewise gene expression studies have generally not led to the discovery of genes that are causal for complex diseases. Indeed, none of the genes initially suggested on the basis of the expression approach now appear to be involved in vitiligo causation at all. In contrast, genome-wide genetic analyses, particularly genome-wide association studies (GWAS), have proven a remarkably robust approach to disease gene discovery, yielding findings that are highly reproducible and which, in aggregate, have provided dramatic advances in understanding the biological basis of many different complex diseases, including vitiligo [31, 32]. Reported candidate gene associations and expression difference findings that are not observed in well-powered GWAS of the same population are not now considered to be valid indications of disease-causal genes and thus will not be discussed here.

24.2.1 The Candidate Gene Approach

Candidate gene studies typically test for nonrandom genetic association of specific DNA sequence variants in specific genes thought to

perhaps be involved in susceptibility to vitiligo on the basis of a priori biological hypotheses. While the variants tested are usually unlikely to be causal for disease, they are assumed to at least be in linkage disequilibrium with true pathological variants. The candidate gene approach obviously is limited to testing hypotheses involving already known biological candidate genes; it cannot discover completely novel genes or pathways. The most common study design is the case-control analysis, in which allele or genotype frequencies are assayed and compared between ethnically matched unrelated cases and ethnically matched unrelated controls. While the case-control study design seems simple, this approach is highly subject to false-positive errors due to imperfect ethnic matching of cases versus controls, population admixture stratification, inadequate statistical power and statistical fluctuation, and inadequate correction for multiple testing. Retrospective analysis of published "genetic associations" has shown that over 95% represent such false-positives, complicated by bias toward publishing apparently positive results [29, 30]. Conversely, most published negative genetic association studies fail to consider the statistical power of the analysis to detect a genetic effect of a given magnitude, which must be considered in evaluating a negative result.

Only three of the very many published candidate gene associations reported for vitiligo now appear to be valid: *HLA*, *PTPN22*, and *CTLA4*. The earliest genetic studies of vitiligo were case-control genetic association analyses of genes in the major histocompatibility complex (MHC), carried out by typing various MHC markers in patients with various different vitiligo phenotypes versus in controls, from many different populations [11, 33–41]. In general, these studies have found no consistent association between the occurrence of vitiligo and specific *HLA* alleles. However, Foley and coworkers [36] found association between vitiligo and class II HLA-DR4 (now known to represent *HLA-DRB1*04* alleles), the first true genetic association reported for vitiligo. Subsequently, a meta-analysis of multiple studies found association of vitiligo with class I *HLA-A2* [37]. These associations have subsequently been validated by robust GWAS analyses in multiple different ethnic populations around the world. Moreover, genetic associations of vitiligo with *HLA* class I and class II alleles appear to be particularly important in patients from families with various other vitiligo-associated autoimmune diseases, many of which are themselves associated with genetic variation in the MHC class I or class II gene regions.

The first genetic association of vitiligo outside of the MHC was reported by Kemp and coworkers [42] in the candidate gene *CTLA4*, which encodes a T-cell co-receptor involved in regulation of T-cell activation. *CTLA4* is also associated with several other autoimmune diseases that are epidemiologically associated with vitiligo, and indeed, *CTLA4* association is strongest in vitiligo cases with other concomitant autoimmune diseases [43, 44]. While vitiligo association with *CTLA4* is inconsistent in non-EUR populations, this association has been confirmed by GWAS in EUR [45].

The only other valid candidate gene association for vitiligo was also reported by Kemp and coworkers [46], with *PTPN22*, which encodes LYP protein tyrosine phosphatase. Like *CTLA4*, *PTPN22* also shows genetic association with many different autoimmune diseases. Again, genetic association of *PTPN22* with vitiligo in the EUR population has been observed in many other studies [47, 48], and has been confirmed by GWAS, but has not been observed in most studies of non-EUR populations. Thus, *HLA* class II, *CTLA4*, and *PTPN22* are key loci that underlie both genetic susceptibility to vitiligo and also epidemiologic association of vitiligo with other autoimmune diseases, at least in the EUR population (Fig. 24.2).

24.2.2 The Genome-Wide Approach

Genome-wide genetic approaches scan the entire genome to identify genetic markers that flag genomic regions that may contain disease susceptibility genes. Because genome-wide approaches are based on genomic location only, findings are not limited by known (or surmised) biology, and these approaches thus offer the possibility of discovering entirely new disease

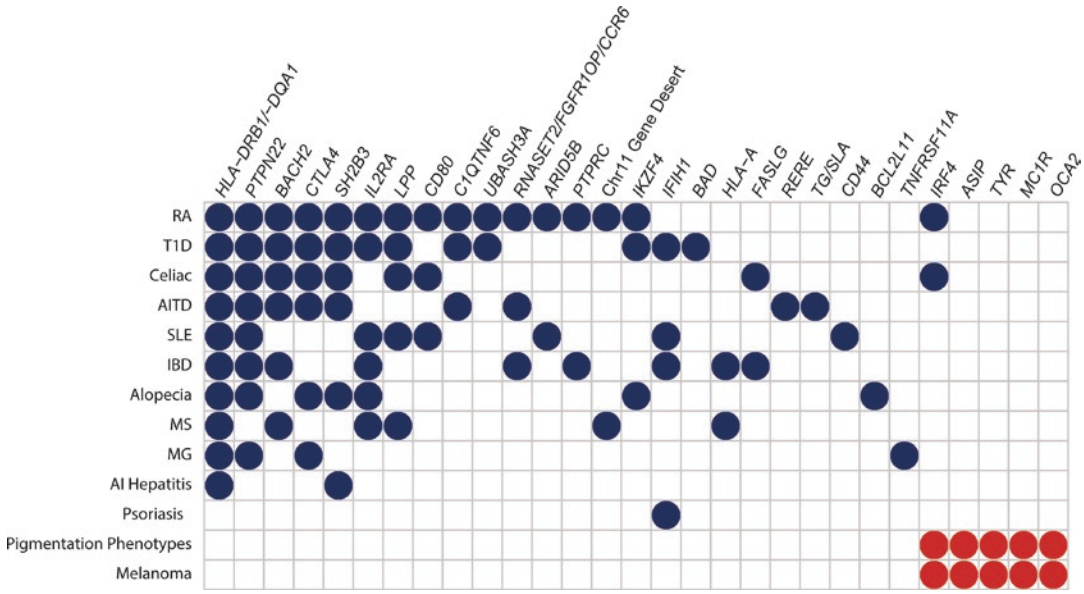


Fig. 24.2 Shared genetic associations of vitiligo with other autoimmune diseases and with pigmentation and melanoma phenotypes. Blue circles denote genetic associations shared between vitiligo and other autoimmune diseases. Red circles denote genetic associations shared between vitiligo and normal pigmentary variation phenotypes, as well as malignant melanoma. All associations shown were identified by GWAS and meet standard criterion for genome-wide significance ($P < 5 \times 10^{-8}$); claimed associations based solely on candidate gene association studies are not included. AI hepatitis, autoimmune hepatitis; AITD, autoimmune thyroid disease (Graves' disease and Hashimoto thyroiditis); IBD, inflammatory bowel disease; MG, myasthenia gravis; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T1D, type 1 diabetes mellitus. Reprinted from [32] with permission

susceptibility genes that highlight entirely new pathways to disease. Moreover, genome-wide approaches allow for measurement of and control for underlying population stratification and other biases and for appropriate correction for multiple testing and thus are far less subject to false-positive artifacts than are other approaches to disease gene discovery.

There are two quite different genome-wide approaches to disease gene discovery, which are applicable in different circumstances and which likely highlight different types of genetic loci and underlying causal variants. *Genome-wide linkage studies* compare the genomes of affected members of "multiplex families" with multiple cases of a given disease, versus relatives without the disease. Such families are not typical, and indeed they may segregate relatively rare genetic variants that have large effects, perhaps different than those in more typical singleton cases. *Genome-wide association studies* (GWAS) analyze the genomes of large numbers of such typical singleton cases, comparing allele or genotype frequencies at hundreds of thousands or millions of so-called single-nucleotide polymorphisms (SNPs) across the genome versus in large collections of controls. GWAS thus are well-suited to analyses of typical cases and predominantly detect relatively common disease susceptibility genes and variants, though approaches to detect uncommon variants are improving rapidly. GWAS have been particularly fruitful in studies of vitiligo, which has proven highly tractable to this powerful approach [31, 32].

24.2.2.1 Genome-Wide Linkage Studies

The first genome-wide findings relevant to vitiligo came from genetic linkage studies of systemic lupus erythematosus that detected a locus on chromosome 17p13, called *SLEV1*, in EUR multiplex lupus families that included at least one

relative with vitiligo [49]. *SLEV1* was subsequently confirmed by linkage analysis of EUR multiplex vitiligo families with various other autoimmune diseases [50], and fine-mapping in vitiligo families ultimately identified the underlying gene as *NLRP1* (previously termed *NALP1*) [51, 52]. NLRP1 is a key regulator of the innate immune system, particularly Langerhans cells and dendritic cells. In response to bacterial "pathogen-associated molecular patterns" (PAMPs), possibly including muramyl dipeptide [53], NALP1 directs assembly of a "NLRP1-inflammasome," which then activates the interleukin-1β (IL-1β) inflammatory pathway [54], which recruits responses by the adaptive immune system. Next-generation DNA sequencing has shown that the most common high-risk *NLRP1* haplotype carries multiple amino acid substitution variants, resulting in enhanced activation of IL-1β in both basal and stimulated states [55]. Together, these findings suggest that activation of the IL-1β pathway in response to bacterial components sensed by skin-resident innate immune receptors might be one trigger for vitiligo.

An additional genome-wide linkage study investigated a unique, very large, multigeneration EUR family in which vitiligo and other autoimmune diseases were inherited as an apparent autosomal dominant trait with incomplete penetrance. Vitiligo in this family was mapped to a locus termed *AIS1*, in chromosome segment 1p31.3-p32.2, whereas susceptibility to other autoimmune diseases in the context of a co-inherited *AIS1* mutation was mapped to a region of chromosome 6 that included the MHC [2]. Detailed studies of genes in the *AIS1* region of chromosome 1p in this family identified a promoter variant in *FOXD3* that increases activity of the FOXD3 transcriptional promoter by 50% [16]; recently, another *FOXD3* promoter variant associated with vitiligo has also been found that increases *FOXD3* transcription [56]. FOXD3 is an embryonic transcription factor that regulates differentiation and development of neural crest melanoblasts and some mesodermal elements, including pancreatic islet cells. The *FOXD3* promoter variant in this family increases transcription in transfected permissive cells by 50% and in vivo might thus interfere with melanoblast/melanocyte differentiation or survival, predisposing to vitiligo [16].

Genome-wide linkage analyses of smaller multiplex vitiligo families in both EUR and CHN populations detected a number of additional linkage signals, for most of which specific corresponding genes have not yet been identified. In EUR families, in addition to *NLRP1* on chromosome 17p, additional vitiligo linkage signals were detected on chromosomes 7p13–q21 (termed *AIS2*), 8p12 (termed *AIS3*), 9q22, 11p15, 13q33, 19p13, and 22q11 [57, 58]. In CHN families, genetic linkage studies detected a different set of linkage signals, at chromosomes 1p36, 4q13-q21, 6p21-p22, 6q24-q25, 14q12-q13, and 22q12 [59, 60]. Association analysis of several candidate genes within the 22q12.1-q12.3 linkage peak suggested that the corresponding gene may be *XBP1* [61], which encodes a transcription factor important in immune cells. The remaining vitiligo linkage signals have not yet been specifically identified, and it remains uncertain which represent true vitiligo susceptibility loci versus false-positives.

24.2.2.2 Genome-Wide Association Studies (GWAS)

Without question, the most effective approach to identifying susceptibility genes for vitiligo has been the GWAS approach [31, 32], as has likewise been the case for almost all other complex diseases. To date, GWAS have identified approximately 50 chromosomal loci that appear to be involved in vitiligo pathogenesis, with high reproducibility between studies, both within and in some instances even across major ethnic categories. About two-thirds of these chromosomal loci have been resolved to specific genes, and for about half of those, the specific causal sequence variants and pathobiology have been elucidated, providing a working framework of vitiligo pathogenesis for the first time.

The first GWAS of vitiligo was carried out in a EUR "special population," an isolated village in a mountainous region of Romania in which there is a remarkably high prevalence of vitiligo, as

well as some of the other autoimmune diseases with which vitiligo is epidemiologically associated [3]. This GWAS identified significant association with a SNP located on distal chromosome 6q, which in the context of this relatively low-resolution GWAS was interpreted as representing *SMOC2* [62]. In fact, however, this SNP is located immediately adjacent to a robust vitiligo association signal in the *RNASET2-FGFR1OP-CCR6* region detected in three subsequent GWAS of vitiligo conducted in the EUR population [45, 63–65], as well as another carried out in CHN [66]. Interestingly, this locus is also in close proximity to *IDDM8*, a linkage and association signal reported in genetic analyses of both type 1 diabetes mellitus and rheumatoid arthritis.

Indeed, analyzed together in meta-analyses, the three GWAS of vitiligo conducted in EUR have detected a total of 48 confirmed vitiligo susceptibility loci [45, 63–65] (Table 24.1).

Table 24.1 Vitiligo susceptibility genes identified and confirmed by GWAS

Chromosome	Gene or locus	Protein	Function
1p36.23	*RERE*	Arginine-glutamic acid dipeptide repeats	Regulator of apoptosis
1p13.2	*PTPN22*	Protein tyrosine phosphatase, nonreceptor type 22	Alters responsiveness of T-cell receptors
1q24.3	*FASLG*	FAS ligand	Regulator of immune apoptosis
1q31.3-q32.1	*PTPRC*	Protein tyrosine phosphatase, receptor type C	Regulator of T- and B-cell antigen receptor signaling
2p16.1	*PPP4R3B*	Protein phosphatase 4, regulatory subunit 3B	Unknown
2q13	*BCL2L11*	BCL2-like 11	Regulator of apoptosis in thymocyte negative selection
2q24.2	*IFIH1*	Interferon induced with helicase C domain 1	Innate immune dsRNA receptor
2q33.2	*CTLA4*	Cytotoxic T-lymphocyte-associated protein 4	T-lymphocyte checkpoint regulator
2q37.3	*FARP2-STK25*	?	?
3p24.3	*UBE2E2*	Ubiquitin-conjugating enzyme E2 E2	Protein ubiquitination pathway; damage response
3p13	*FOXP1*	Forkhead box protein P1	Transcriptional regulator of B-cell development
3q13.33	*CD80*	T-lymphocyte activation antigen CD80	T-cell costimulatory signal
3q27.3-q28	*LPP*	Lipoma-preferred partner	Unknown
3q29	*FBXO45-NRROS*	?	?
4q24	*PPP3CA*	Serine/threonine protein phosphatase 2B catalytic subunit alpha isoform	T-cell calcium-dependent, calmodulin-stimulated protein phosphatase
6p25.3	*IRF4*	Interferon regulatory factor 4	Transcriptional activator in immune cells and melanocytes
6p25.2	*SERPINB9*	Serpin B9 Association	Endogenous inhibitor of granzyme B
6p21.3	*HLA-A*	HLA class I histocompatibility antigen, A	Presents peptide antigens to the immune system
6p21.3	*HLA-DRB1—HLA-DQA1*	HLA histocompatibility antigens, DRB1 and DQA1	Present peptide antigens to the immune system
6q15	*BACH2*	BTB domain and CNC homolog 2	Transcriptional activator regulator of apoptosis
6q27	*RNASET2-FGFR1OP-CCR6*	?	?
7p14.3	*CPVL*	Probably serine carboxypeptidase CPVL	Inflammatory protease; trims antigens for presentation

Table 24.1 (continued)

Chromosome	Gene or locus	Protein	Function
8q24.22	*SLA*	Src-like adapter	Regulator of T-cell antigen receptor signaling
9q33.3	*NEK6*	NIMA-related serine/threonine protein kinase NEK6	Regulator of apoptosis
10p15.1	*IL2RA*	Interleukin-2 receptor subunit alpha	IL2 receptor regulates regulatory T-cells
10q21.2	*ARID5B*	AT-rich interactive domain-containing protein 5B	Transcriptional coactivator
10q22.3	*ZMIZ1*	Zinc finger MIZ domain-containing protein 1	Possible PIAS-family transcriptional or sumoylation regulator
10q25.3	*CASP7*	Caspase7	Apoptosis executioner protein
11p13	*CD44*	CD44 antigen	Regulator of FOXP3 expression
11q13.1	*PPP1R14B-PLCB3-BAD-GPR137-KCNK4-TEX40-ESRRA-TRMT112-PRDX5*	?	?
11q14.3	*TYR*	Tyrosinase	Melanocyte melanogenic enzyme; vitiligo autoantigen
11q21	Gene desert	?	?
12q13.2	*PMEL*	Premelanosome protein PMEL Expression analysis	Melanocyte melanosomal type 1 transmembrane glycoprotein
12q13.2	*IKZF4*	Zinc finger protein Eos	Transcriptional repressor; regulates *FOXP3* transcription in regulatory T-cells
12q24.12	*SH2B3*	SH2B adapter protein 3	Links T-cell receptor activation signal to phospholipase C-gamma-1, GRB2, and phosphatidylinositol 3-kinase
13q14.11	*TNFSF11*	Tumor necrosis factor ligand superfamily member 11	T-cell cytokine that binds to TNFRSF11A and TNFRSF11B
14q12	*GZMB*	Granzyme B	Apoptosis executioner protein of cytotoxic T-cells
15q12-q13.1	*OCA2-HERC2*	Oculocutaneous albinism 2	Melanocyte melanogenic protein; vitiligo autoantigen
16q24.3	*MC1R*	Melanocortin 1 receptor	Melanocyte melanogenic protein; vitiligo autoantigen
17q21.2	*KAT2A-HSPB9-RAB5C*	?	?
18q21.33	*TNFRSF11A*	Tumor necrosis factor receptor superfamily member 11A	Regulates interactions between T-cells and dendritic cells
19p13.3	*TICAM1*	TIR domain-containing adapter molecule 1	TLR3/TLR4 adapter; mediates NFkappa-B and interferon-regulatory factor (IRF) activation; induces apoptosis
19q13.33	*SCAF1-IRF3-BCL2L12*	?	?
20q11.2	*RALY-ASIP*	Agouti signaling protein	Regulator of melanocytes via MC1R
20q13.13	*PTPN1*	Tyrosine-protein phosphatase, nonreceptor type 1	Dephosphorylates JAK2 and TYK2 kinases cellular response to interferon?
21q22.3	*UBASH3A*	Ubiquitin-associated and SH3 domain-containing protein A	Promotes accumulation of activated T-cell receptors on T-cell surface
22q12.3	*C1QTNF6*	Complement C1q tumor necrosis factor-related protein 6	Unknown
22q13.2	*ZC3H7B-TEF*	?	?
Xp21.3-p21.2	*IL1RAPL1*	Interleukin-1 receptor accessory protein-like 1	Unknown
Xp11.23	*CCDC22-FOXP3-GAGE*	?	?

? indicates that specific gene (and corresponding function) has not yet been defined among those listed

Those loci with the largest individual effects, as measured by odds ratios (ORs), include the MHC class I (*HLA-A*) and class II (*HLA-DRB1-DQA1*) regions, *CPVL*, *ASIP*, and *TYR*. Altogether, these associations account for approximately 22.5% of vitiligo heritability in the EUR population [45]. Fine-mapping and functional analyses have identified many of the specific genes that underlie these associations, as well as many of the specific causal gene variants, providing deep insight into pathobiological pathways, mechanisms of disease susceptibility, and even potential targets for new therapies. As has also been found for most other complex diseases, the majority of vitiligo causal variants appear to reside in gene regulatory regions, which may prove more tractable as potential drug targets than missense mutations that directly alter protein structure and function.

Additionally, several GWAS of vitiligo have been carried out in Asian populations, in general detecting a subset of vitiligo susceptibility loci identified in EUR. In the CHN Han and Uygur populations, complex association signals were detected in the MHC and the *RNASET2-FGFR1OP-CCR6* regions [66], which were also detected in EUR, as well as *PMEL* [67] and *ZMIZ1* [68], which thus far have only been associated with vitiligo in CHN (Table 24.1). A smaller GWAS of vitiligo conducted in Japanese detected only an association in the MHC class I (*HLA-A*) region [69], and another conducted in the Indo-Pakistani population detected only an association in the MHC class II (*HLA-DRB1-DQA1*) region [70]. A very small GWAS of vitiligo in Koreans detected no significant association signals, almost certainly because of inadequate sample size and thus insufficient statistical power [71].

An alternative analysis of the EUR GWAS data considered vitiligo age of onset as a complex trait [72]. This analysis detected significant association with the MHC class II (*HLA-DRB1-DQA1*) region, indicating that this function may influence occurrence of vitiligo via mediating the effects of environmental triggers that may initiate or propagate the disease process at various times during a susceptible individual's life.

24.3 Opposite Genetic Relationship Between Vitiligo and Melanoma

An early discovery from GWAS analyses of EUR subjects [45, 63–65] was that a number of vitiligo susceptibility genes encode melanocyte proteins or regulate melanocyte function and thus affect expression of melanocyte proteins. These include *TYR*, encoding tyrosinase; *OCA2*, encoding a melanocyte melanogenic protein; *MC1R*, encoding the melanocortin 1 receptor; *IRF4*, encoding a key melanocytic transcription factor; and *ASIP* and *PPARGC1B*, both of which encode paracrine regulators of melanocyte gene expression. TYR, OCA2, and MC1R additionally constitute important vitiligo autoantigens. All of these loci have thus far only been associated with vitiligo in the EUR population.

Unexpectedly, in each case the vitiligo-associated SNPs at these loci were protective, and in each case the same SNPs are also associated with increased susceptibility to melanoma but with association to the opposite allele. Thus, for these loci and associated variants, vitiligo and melanoma risk constitute genetic opposites. This inverse genetic relationship almost certainly mirrors a corresponding inverse biological relationship. One possibility is that vitiligo may represent dysregulation of a normal mechanism of immune surveillance against melanoma [50, 73]. This would be consistent with both the 75% reduction in melanoma incidence among patients with pre-existing vitiligo [74, 75] and prolonged survival of melanoma patients who develop vitiligo during immunotherapy [76].

24.4 Current Understanding

The purpose for identifying genes involved in vitiligo pathogenesis is that such genes are causal. This thus provides a solid basis for the longer-term goals of understanding the pathobiology of the disease, facilitating rational choice of new targets for drugs or other treatment modalities, and enabling genetic-based classification of patients for specific therapies and eventually pre-

symptomatic disease prevention. It is clear that genetic studies of vitiligo have gone a long way toward enabling all three of these long-term goals.

As shown in Table 24.1, genetic studies have to date identified approximately 50 different genetic susceptibility loci for vitiligo, mostly in the EUR population. Most of these loci have been resolved to specific corresponding genes; this vitiligo "parts list" identified by gene discovery provides a solid basis for understanding vitiligo pathobiology. Furthermore, for many of these genes, specific causal gene variants have been identified (e.g., *HLA-A*, *HLA-DRB1-DQA1*, *GZMB*, *TYR*, *PTPN22*, *IFIH1*, *NLRP1*, and others). For vitiligo, as for other complex diseases, the great majority of causal variants appear to be regulatory in nature [45], rather than structural, which may prove advantageous for novel pharmacologic interventions.

Almost all identified vitiligo susceptibility genes encode proteins that play roles in immunoregulation, apoptosis, or melanocyte function, consistent with the fact that vitiligo is an autoimmune disease. These proteins furthermore highlight a series of pathways and processes that comprise a "blueprint" for vitiligo pathobiology [58] (Fig. 24.3).

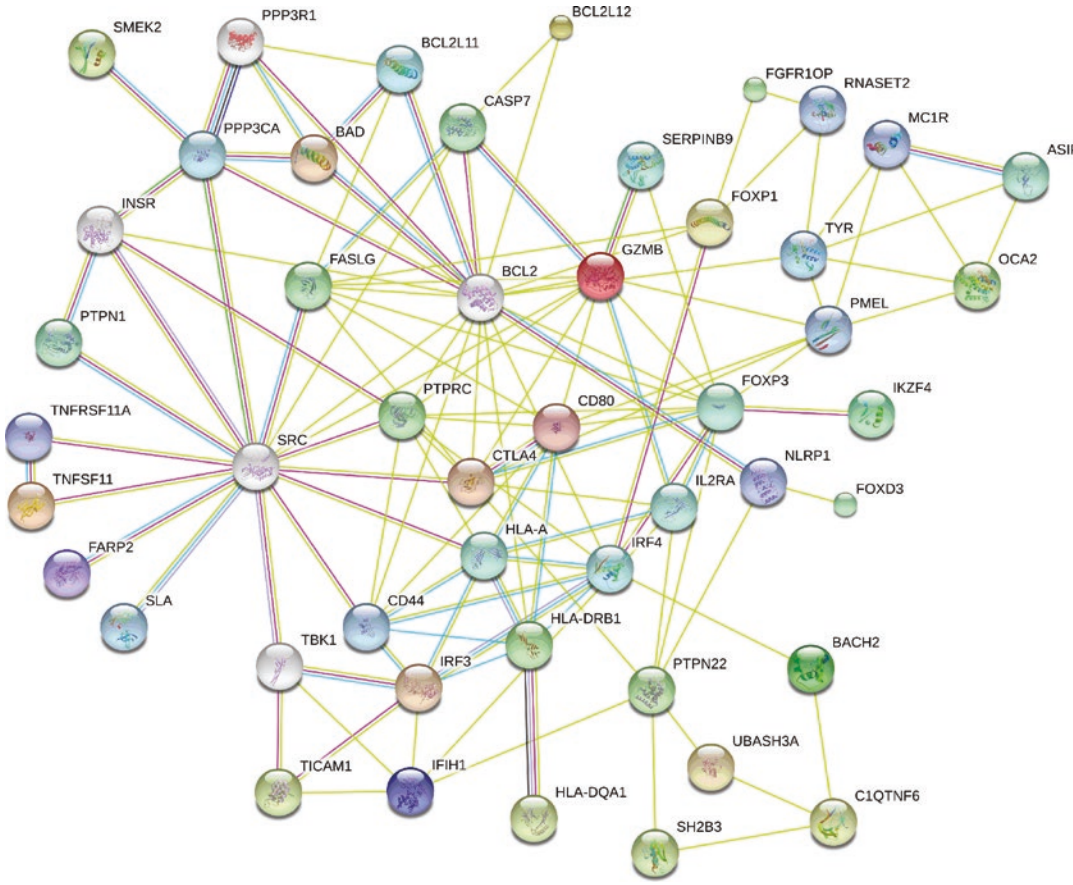


Fig. 24.3 Bioinformatic functional interaction network of proteins encoded by genes at confirmed vitiligo susceptibility loci. Unsupervised functional interaction network analysis was carried out using STRING v10.5 (http://string-db.org/) [77], using each protein as a node, and permitting ≤5 second-order interactions to maximize connectivity. Nodes that shared no edges with other nodes were excluded. Edge colors are as defined by STRING: teal, interactions from curated databases; purple, experimentally determined interactions; green, gene neighborhood; blue, databases; red, gene fusions; dark blue, gene co-occurrence; pale green, text-mining; black, co-expression; lavender, protein homology. SMEK2 is an alternative name for PPP4R3B

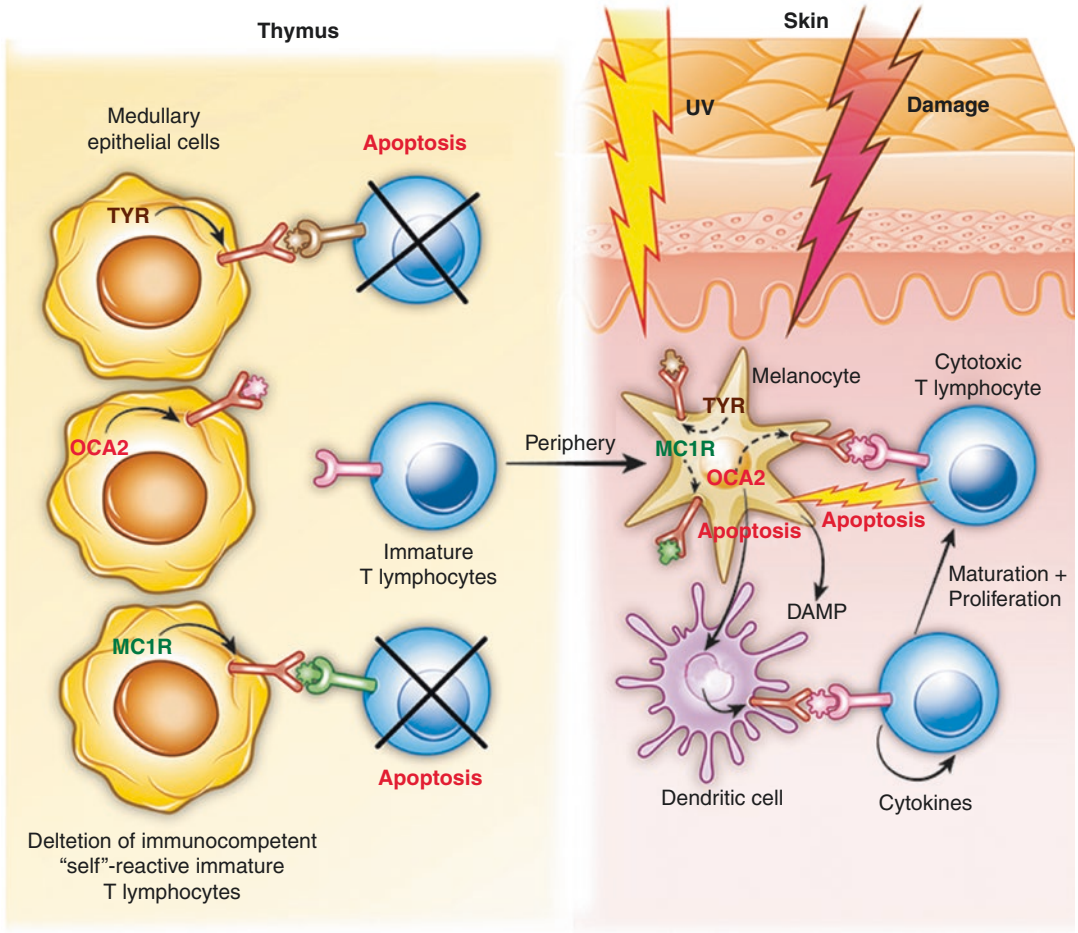


Fig. 24.4 A general framework of vitiligo pathogenesis. During embryonic development, a T-cell repertoire is selected by positive selection of immunocompetent immature T-lymphocytes in the cortex of the thymus. Immunocompetent T-cells that recognize self-antigens expressed by thymus medullary epithelial cells undergo negative selection and apoptosis. Immunocompetent immature T-cells that do not encounter a cognate self-antigen then exit the thymus and enter the peripheral circulation. Subsequently, in the skin, many or most cases of vitiligo likely initiate with skin damage, often induced by ultraviolet (UV) exposure or trauma (Koebnerization). Damaged melanocytes apoptose and release molecules that act as damage-associated molecular patterns (DAMPs), which stimulate activation of local dendritic cells. Dendritic cells engulf melanocyte proteins, which are degraded in the proteasome, and peptide fragments that act as antigens are presented by HLA class II molecules on the dendritic cell surface. Immature T-cells that express cognate T-cell receptors bind these self-antigens and are activated to express costimulatory molecules that result in cell proliferation and differentiation into CD8+ effector cytotoxic T-cells, with the assistance of CD4+ T-helper cells. The resultant activated cytotoxic T-cells recognize and bind the cognate self-antigen presented by HLA class I molecules on the melanocyte surface, assisted by interaction of FAS ligand on the T-cell and FAS on the target melanocyte. The cytotoxic T-cell then elaborates granzyme B and perforin, which induce apoptosis of the target melanocyte. Almost all of these processes involve proteins that are encoded by genes associated with genetic susceptibility to vitiligo. Reprinted from [32] with permission

While incomplete, and some respects perhaps even incorrect, this vitiligo "parts list" and "blueprint" begin to outline what might be considered a vitiligo "instruction manual" (Fig. 24.4). In the thymus, some immunocompetent immature T-lymphocytes that can recognize melanocyte self-antigens escape deletion, entering the circulation; perhaps this is part of

an advantageous system of immune surveillance for nascent melanomas. Subsequently, skin damage and perhaps cell death induces release of damage-associated molecular patterns (DAMPs) that activate skin-resident dendritic cells to take up melanocyte protein fragments. These are then degraded in the proteasome and are presented on the dendritic cell surface as antigens by HLA class II molecules. Immature T-cells expressing cognate T-cell receptors bind these melanocyte self-antigens and differentiate into CD8+ effector cytotoxic T-cells. These activated cytotoxic T-cells then recognize and bind the cognate self-antigen presented by HLA class I molecules on the surface of melanocytes, which are killed by the actions of granzyme B and perforin.

This level of understanding opens avenues toward developing novel approaches to vitiligo treatment, gene-based personalized medicine, and perhaps even disease prevention in genetically susceptible individuals. Significant challenges will be to identify particular genetic combinations that constitute major pathways toward disease risk and which perhaps represent distinct biological pathways that define different subgroups of vitiligo patients. A further challenge will be to design specific treatments that target these different patient subgroups. Nevertheless, it is clear that, from the standpoint of understanding vitiligo pathobiology, we are the "end of the beginning" thanks, in large part, to recent advances in vitiligo gene discovery.

Acknowledgments This work was supported by grants AR056292 and AR065951 from the National Institutes of Health.

References

1. Alkhateeb A, Fain PR, Thody A, et al. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their relatives. Pigment Cell Res. 2003;16:208–14.
2. Alkhateeb A, Stetler GL, Old W, et al. Mapping of an autoimmunity susceptibility locus (*AIS1*) to chromosome 1p31.3-p32.2. Hum Mol Genet. 2002;11:661–7.
3. Birlea SA, Fain PR, Spritz RA. A Romanian population isolate with high frequency of vitiligo and associated autoimmune diseases. Arch Dermatol. 2008;144:310–6.
4. Hafez M, Sharaf L, El-Nabi SMA. The genetics of vitiligo. Acta Dermatovener (Stockh). 1983;63:249–51.
5. Das SK, Majumder PP, Chakraborty R, et al. Studies on vitiligo. I. Epidemiological profile in Calcutta, India. Genet Epidemiol. 1985;2:71–8.
6. Das SK, Majumder PP, Majumdar TK, et al. Studies on vitiligo. II. Familial aggregation and genetics. Genet Epidemiol. 1985;2:255–62.
7. Bhatia PS, Mohan L, Pandey ON, et al. Genetic nature of vitiligo. J Dermatol Sci. 1992;4:180–4.
8. Carnevale A, Zavala C, Castillo VD, et al. Analisis genetico de 127 families con vitiligo. Rev Invest Clin. 1980;32:37–41.
9. Majumder PP, Das SK, Li CC. A genetical model for vitiligo. Am J Hum Genet. 1988;43:119–25.
10. Majumder PP, Nordlund JJ, Nath SK. Pattern of familial aggregation of vitiligo. Arch Dermatol. 1993;129:994–8.
11. Mehta NR, Shah KC, Theodore C, et al. Epidemiological study of vitiligo in Surat area, South Gujarat. Indian J Med Res. 1973;61:145–54.
12. Nath SK, Majumder PP, Nordlund JJ. Genetic epidemiology of vitiligo: multilocus recessivity cross-validated. Am J Hum Genet. 1994;55:981–90.
13. Sun X, Xu A, Wei X, et al. Genetic epidemiology of vitiligo: a study of 815 probands and their families from south China. Int J Dermatol. 2006;45:1176–81.
14. Laberge G, Mailloux CM, Gowan K, et al. Early disease onset and increased risk of other autoimmune diseases in familial generalized vitiligo. Pigment Cell Res. 2005;18:300–5.
15. Howitz J, Brodthagen H, Schwartz M, et al. Prevalence of vitiligo: epidemiological survey of the Isle of Bornholm, Denmark. Arch Dermatol. 1977;113:47–52.
16. Alkhateeb A, Fain PR, Spritz RA. Candidate functional promoter variant in the *FOXD3* melanoblast developmental regulator gene in autosomal dominant vitiligo. J Investig Dermatol. 2005;125:388–91.
17. Neufeld M, Maclaren NK, Blizzard RM. Two types of autoimmune Addison's disease associated with different polyglandular autoimmune (PGA) syndromes. Medicine (Baltimore). 1981;60:355–62.
18. Addison T. (1855). On the constitutional and local effects of disease of the suprarenal capsules. In: A collection of the published writing of the late Thomas Addison, M.D., physician to Guy's Hospital. New Sydenham Society, London 1868. 1855. Med Classics. 1937;2:244–93.. Reprinted.
19. Cunliffe WJ, Hall R, Newell DJ, et al. Vitiligo, thyroid disease and autoimmunity. Br J Dermatol. 1968;80:135–9.
20. Schallreuter KU, Lemke R, Brandt O, et al. Vitiligo and other diseases: coexistence or true association? Dermatology. 1994;188:269–75.

21. Dawber RP. Integumentary associations of pernicious anemia. Br J Dermatol. 1970;82:221–3.
22. Grunnet I, Howitz J. Vitiligo and pernicious anemia. Arch Dermatol. 1979;101:82–5.
23. Zelissen PM, Bast EJ, Croughs RJ. Associated autoimmunity in Addison's disease. J Autoimmun. 1995;8:121–30.
24. Sharma VK, Dawn G, Kumar B. Profile of alopecia areata in Northern India. Int J Dermatol. 1996;35:22–7.
25. Sharma VK, Kumar V, Dawn G. A clinical study of childhood alopecia areata in Chandigarh, India. Pediatr Dermatol. 1996;13:372–7.
26. Handa S, Kaur I. Vitiligo: clinical findings in 1436 patients. J Dermatol. 1999;26:653–7.
27. Handa S, Dogra S. Epidemiology of childhood vitiligo: a study of 625 patients from north India. Pediatr Dermatol. 2003;20:207–10.
28. Onunu AN, Kubeyinje EP. Vitiligo in the Nigerian African: a study of 351 patients in Benin City, Nigeria. Int J Dermatol. 2003;42:800–2.
29. Hirschhorn JN, Lohmueller K, Byrne E. A comprehensive review of genetic association studies. Genet Med. 2002;4:45–61.
30. Ioannidis JPA, Tarone R, McLaughlin JK. The false-positive to false-negative ratio in epidemiologic studies. Epidemiology. 2011;22:450–6.
31. Shen C, Gao J, Sheng Y, et al. Genetic susceptibility to vitiligo GWAS approaches for identifying vitiligo susceptibility genes and loci. Front Genet. 2016;7:3.
32. Spritz RA, Andersen G. Genetics of vitiligo. Dermatol Clin. 2017;35:245.
33. Ando I, Chi HI, Nakagawa H, et al. Difference in clinical features and HLA antigens between familial and non-familial vitiligo of non-segmental type. Br J Dermatol. 1993;129:408–10.
34. Arcos-Burgos M, Parodi E, Salgar M, et al. Vitiligo: complex segregation and linkage disequilibrium analyses with respect to microsatellite loci spanning the HLA. Hum Genet. 2002;110:334–42.
35. Finco O, Cuccia M, Martinetti M, et al. Age of onset in vitiligo: relationship with HLA supratypes. Clin Genet. 1991;39:48–54.
36. Foley LM, Lowe NJ, Misheloff E, et al. Association of HLA-DR4 with vitiligo. J Am Acad Dermatol. 1983;8:39–40.
37. Liu JB, Li M, Chen H, et al. Association of vitiligo with HLA-A2: a meta-analysis. J Eur Acad Dermatol Venereol. 2007;21:205–13.
38. Schallreuter KU, Levenig C, Kühnl P, et al. Histocompatibility antigens in vitiligo: Hamburg study on 102 patients from northern Germany. Dermatology. 1993;187:186–92.
39. Tastan HB, Akar A, Orkunoglu FE, et al. Association of HLA class I antigens and HLA class II alleles with vitiligo in a Turkish population. Pigment Cell Res. 2004;17:181–4.
40. Xia Q, Zhou WM, Liang YH, et al. MHC haplotypic association in Chinese Han patients with vitiligo. J Eur Acad Dermatol Venereol. 2006;20:941–6.
41. Zamani M, Spaepen M, Sghar SS, et al. Linkage and association of HLA class II genes with vitiligo in a Dutch population. Br J Dermatol. 2001;145:90–4.
42. Kemp EH, Ajjan RA, Waterman EA, et al. Analysis of a microsatellite polymorphism of the cytotoxic T-lymphocyte antigen-4 gene in patients with vitiligo. Br J Dermatol. 1999;140:73–8.
43. Birlea SA, LaBerge GS, Procopciuc LM, et al. *CTLA4* and generalized vitiligo: two genetic association studies and a meta-analysis of published data. Pigment Cell Melanoma Res. 2009;22:230–4.
44. Blomhoff A, Kemp EH, Gawkrodger DJ, et al. CTLA4 polymorphisms are associated with vitiligo, in patients with concomitant autoimmune diseases. Pigment Cell Res. 2005;18:55–8.
45. Jin Y, Andersen G, Yorgov D, et al. Genome-wide association studies of autoimmune vitiligo identify 23 new risk loci and highlight key pathways and regulatory variants. Nat Genet. 2016;48:1418–24.
46. Cantón I, Akhtar S, Gavalas NG, et al. A single-nucleotide polymorphism in the gene encoding lymphoid protein tyrosine phosphatase (PTPN22) confers susceptibility to generalised vitiligo. Genes Immun. 2005;6:584–7.
47. Laberge G, Birlea SA, Fain PR, et al. The *PTPN22* -1858C>T (R620W) functional polymorphism is associated with generalized vitiligo in the Romanian population. Pigment Cell Melanoma Res. 2008;21:206–8.
48. Laberge G, Fain PR, Bennett DC, et al. Family-based association analysis of generalized vitiligo confirms association with *PTPN22* but not *CTLA4*. J Investig Dermatol. 2008;128:1757. https://doi.org/10.1038/sj.jid.5701233.
49. Nath SK, Kelly JA, Namjou B, et al. Evidence for a susceptibility gene, *SLEV1*, on chromosome 17p13 in families with vitiligo-related systemic lupus erythematosus. Am J Hum Genet. 2001;69:1401–6.
50. Spritz RA. The genetics of generalized vitiligo autoimmune pathways and an inverse relationship with malignant melanoma. Genome Med. 2010;2:78.
51. Jin Y, Birlea SA, Fain PR, et al. Genetic variations in *NALP1* are associated with generalized vitiligo in a Romanian population. J Investig Dermatol. 2007;127:2558–62.
52. Jin Y, Mailloux CM, Gowan K, et al. *NALP1* in vitiligo-associated multiple autoimmune disease. New Engl J Med. 2007;356:1216–25.
53. Faustin B, Lartigue L, Bruey J-M, et al. Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation. Mol Cell. 2007;25:713–24.
54. Bruey J-M, Bruey-Sedano N, Luciano F, et al. Bcl-2 and Bcl-X_L regulate proinflammatory caspase-1 activation by interaction with NALP1. Cell. 2007;129:45–56.

55. Levandowski CB, Mailloux CM, Ferrara TM, et al. *NLRP1* haplotypes associated with vitiligo and autoimmunity increase interleukin-1β processing via the NLRP1 inflammasome. Proc Natl Acad Sci U S A. 2013;110:2952–6.
56. Schunter JA, Löffler D, Wiesner T, et al. A novel *FoxD3* variant is associated with vitiligo and elevated thyroid autoantibodies. J Clin Endocrinol Metab. 2015;100:E1335–42.
57. Fain PR, Gowan K, LaBerge GS, et al. A genomewide screen for generalized vitiligo: confirmation of *AIS1* on chromosome 1p31 and evidence for additional susceptibility loci. Am J Hum Genet. 2003;72:1560–4.
58. Spritz RA, Gowan K, Bennett DC, et al. Novel vitiligo susceptibility loci on chromosomes 7 (*AIS2*) and 8 (*AIS3*), confirmation of *SLEV1* on chromosome 17, and their roles in an autoimmune diathesis. Am J Hum Genet. 2004;74:188–91.
59. Chen JJ, Huang W, Gui JP, et al. A novel linkage to generalized vitiligo on 4q13-q21 identified in a genomewide linkage analysis of Chinese families. Am J Hum Genet. 2005;76:1057–65.
60. Liang Y, Yang S, Zhou Y, et al. Evidence for two susceptibility loci on chromosomes 22q12 and 6p21-p22 in Chinese generalized vitiligo families. J Investig Dermatol. 2007;127:2552–7.
61. Ren Y, Yang S, Xu S, et al. Genetic variation of promoter sequence modulates *XBP1* expression and genetic risk for vitiligo. PLoS Genet. 2009;5:e1000523.
62. Birlea SA, Gowan K, Fain PR, et al. Genome-wide association study of generalized vitiligo in an isolated European founder population identifies *SMOC2*, in close proximity to *IDDM8*. J Investig Dermatol. 2009;130:798–803.
63. Jin Y, Birlea SA, Fain PR, et al. Variant of *TYR* and autoimmunity susceptibility loci in generalized vitiligo. New Engl J Med. 2010;362:1686–97.
64. Jin Y, Birlea SA, Rain PR, et al. Common variants in *FOXP1* are associated with generalized vitiligo. Nat Genet. 2010;42:576–8.
65. Jin Y, Birlea SA, Fain PR, et al. Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. Nat Genet. 2012;44:676–81.
66. Quan C, Ren YQ, Xiang LH, et al. Genome-wide association study for vitiligo identifies susceptibility loci at 6q27 and the MHC. Nat Genet. 2010;42:614–8.
67. Tang XF, Zhang Z, Hu DY, et al. Association analyses identify three susceptibility loci for vitiligo in the Chinese Han population. J Invest Dermatol. 2013;133:403–10.
68. Sun Y, Zuo X, Zheng X, et al. A comprehensive association analysis confirms *ZMIZ1* to be a susceptibility gene for vitiligo in Chinese population. J Med Genet. 2014;51:345–53.
69. Jin Y, Hayashi M, Fain PR, et al. Major association of vitiligo with *HLA-A*02:01* in Japanese. Pigment Cell Melanoma Res. 2015;28:360–2.
70. Birlea SA, Ahmad FJ, Uddin RM, et al. Association of generalized vitiligo with MHC class II loci in patients from the Indian subcontinent. J Invest Dermatol. 2013;133:1369–72.
71. Cheong KA, Kim NH, Noh M, et al. three new single nucleotide polymorphisms identified by a genome-wide association study in Korean patients with vitiligo. J Korean Med Sci. 2013;28:775–9.
72. Jin Y, Birlea SA, Fain PR, et al. Genome-wide analysis identifies a quantitative trait locus in the MHC class II region associated with generalized vitiligo age of onset. J Investig Dermatol. 2011;13:1138–312.
73. Das PK, van den Wijngaard RMJGJ, Wankowicz-Kalinska A, et al. A symbiotic concept of autoimmunity and tumour immunity: lessons from vitiligo. Trends Immunol. 2001;22:130–6.
74. Paradisi A, Tabolli S, Didona B, et al. Markedly reduced incidence of melanoma and nonmelanoma skin cancer in a nonconcurrent cohort of 10,040 patients with vitiligo. J Am Acad Dermatol. 2014;71:1110–6.
75. Teulings HE, Oerkamp M, Ceylan E, et al. Decreased risk of melanoma in patients with vitiligo a survey among 1307 patients and their partners. Br J Dermatol. 2013;168:162–71.
76. Nakamura Y, Tanaka R, Teraoto Y, et al. Correlation between vitiligo occurrence and clinical benefit in advanced melanoma patients treated with nivolumab: a multi-institutional retrospective study. J Dermatol. 2017;44:117. https://doi.org/10.1111/1346-8138.13520.
77. Szklarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res. 2017;45:D362–8.

Epigenetics

25

Li Zhou, Henry W. Lim, and Qing-Sheng Mi

Contents

Abstract

Even though the pathogenesis of vitiligo is not completely understood, the most compelling etiology of vitiligo involves a combination of environmental and genetic factors that contribute to autoimmune melanocyte destruction. Large-scale epidemiological studies have shown that about 15–20% of vitiligo patients have one or more affected first-degree relatives. The familial aggregation takes a non-Mendelian pattern that suggests a polygenic, multifactorial inheritance for vitiligo. Recent genome-wide association studies (GWAS) to investigate the genetics of vitiligo have resulted in the identification of multiple vitiligo susceptibility genes (Birlea et al., J Invest Dermatol 130:798–803, 2010; Quan et al., Nat Genet 42:614–618, 2010), many of which are shared by other autoimmune diseases. These evidences strongly support the significance of genetic factors contributing to one's risk for vitiligo. Nevertheless, the concordance for generalized vitiligo in monozygotic twins is only 23%, which suggests that environmentally induced

L. Zhou (✉) · H. W. Lim · Q.-S. Mi
Henry Ford Immunology Program, Department of Dermatology, Henry Ford Hospital, Detroit, MI, USA
e-mail: lzhou1@hfhs.org; hlim1@hfhs.org; qmi1@hfhs.org

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_25

epigenetic factors must also play an important role, and may be even more important than genetics.

Epigenetics is the study of hereditable patterns of gene expression without changes in the DNA sequence. Epigenetic regulations in response to environmental changes play a critical role in determining gene function and activities. Disorders of epigenetic processes, which involve DNA methylation, histone modification, and noncoding RNA expression, were found to be associated with the pathogenesis of various diseases. Over the past few years, great progress has been made in identifying related epigenetic mechanisms in autoimmune disease development, including vitiligo, to bridge the gap between environmental and genetic factors. These emerging studies provide new insights into not only clinical biomarkers for diagnosis and disease progression but also novel targets for potential epigenetic therapeutic strategies. In this chapter, we summarize the epigenetic regulations in melanocyte biology, immune tolerance regulation, as well as related epigenetic alterations in autoimmune vitiligo.

Key Points

- Epigenetics is the study of hereditable patterns of gene expression without changes in the DNA sequence.
- The concordance for generalized vitiligo in monozygotic twins is only 23%, which suggests that environmentally induced epigenetic factors must also play an important role and may be even more important than genetics.
- Recent studies identified upregulation of miR-224-3p and miR-4712-3p and downregulation of miR-3940-5p in the PBMCs of NSV patients.
- The mean genomic DNA methylation levels were significantly higher in vitiligo patients.
- Histone modifications play critical roles in the biological processes of the immune system as well as the development of autoimmune diseases.
- Involvement of epigenetic regulations, especially miRNAs and DNA methylation, in melanocyte development, function, and immune tolerance regulation.

25.1 miRNAs in Melanogenesis and Vitiligo

25.1.1 miRNA Biogenesis and Function

MicroRNAs are 22-nucleotide, single-stranded RNA that modulates the stability and translation of mRNAs posttranscriptionally. To date, more than 2500 mature miRNAs have been described in humans [1]. In the past decade, miRNAs have been recognized as an important component of epigenetic regulation and a new layer of gene regulation impacting cellular development, physiology, and disease development [2]. In addition, miRNAs represent the best-characterized class of noncoding RNAs in terms of both expression and function.

As shown in Fig. 25.1, miRNAs are first transcribed as long, polyadenylated primary precursors (pri-miRNA) by RNA polymerase II. They are cleaved into a hairpin-shaped 70–100-nucleotide precursor (pre-miRNA), by Drosha (a nuclear RNase III), which functions in a complex that contains DGCR8, a dsRNA-binding protein. The resulting pre-miRNAs are then exported to the cytoplasm by exportin-5. In the cytoplasm, Dicer (another RNase III) in association with a dsRNA-binding protein partner called TRBP, or PACT in humans, processes pre-miRNAs into a 19–24-nucleotide miRNA duplex

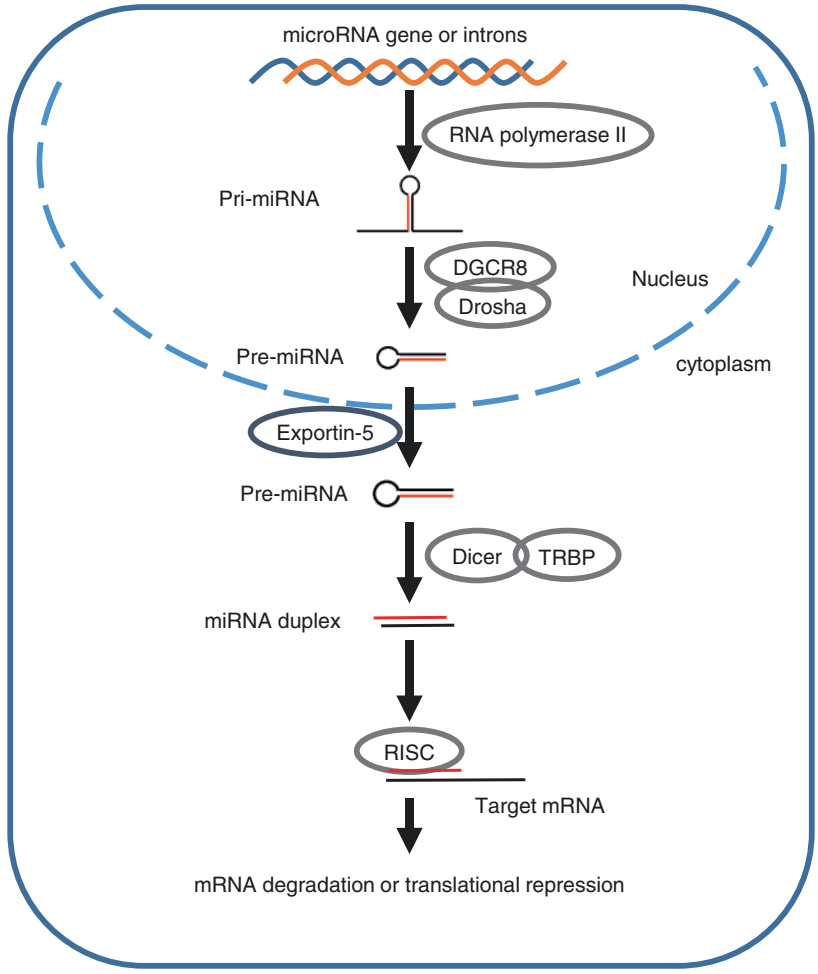


Fig. 25.1 MicroRNA biogenesis in animal cells. miRNAs are first transcribed as long, polyadenylated primary transcripts (pri-miRNA) by RNA polymerase II from specific miRNA genes. Pri-miRNAs are processed into precursor miRNA (pre-miRNA) stem-loops by the nuclear RNase III enzyme Drosha and its partner DGCR8. The pre-miRNA is then actively transported to the cytoplasm by exportin-5 and is further processed into ~22-nucleotide duplexes by the cytoplasmic RNase III enzyme Dicer in association with a dsRNA-binding protein partner TRBP. The final step of miRNA maturation is the selective loading of the functional strand of the small RNA duplex onto the RNA-induced silencing complex (RISK). Mature miRNAs then guide the RISK to cognate target mRNA and repress target gene expression by either destabilizing target mRNAs or repressing their translation

by removing the terminal loop. One strand of the resulting duplex is bound by Argonaut to form the RNA-induced silencing complex (RISC), which targets mRNA for regulation [3, 4]. Nucleotides 2–7 of the mature miRNA sequence create the "seed region" which primarily specifies the mRNA that the miRNA will bind. It is now becoming apparent that base pairing outside the seed region provides a further layer of specificity [5]. miRNAs control the expression of specific genes, typically by base paring to the 3′ untranslated region (3′UTR) of target mRNAs to mediate repression of that gene either by mRNA destabilization, translational inhibition, or both. It is predicted that a single miRNA may target, on average, more than a hundred mRNAs, and over 60% of human protein-coding genes contain miRNA-binding sites within their 3′UTRs [2, 4].

25.1.2 miRNAs in Melanogenesis

Microphthalmia-associated transcription factor (MITF), a master regulator of melanogenesis and melanocyte function, is a key transcription factor controlling genes linked to pigmentation. Recent studies have shown the clear interrelationship between miRNAs and MITF, further indicating the potential involvement of miRNAs in skin pigmentation.

As a central regulator of microRNA biogenesis, Dicer converts pre-miRNAs to fully functional mature miRNAs. In melanocytes, Dicer expression was found to be under direct transcriptional regulation by MITF. MITF binds and activates a conserved regulatory element upstream of Dicer's transcriptional start site upon melanocyte differentiation. Meanwhile, targeted deletion of Dicer is lethal to melanocytes [6]. These observations highlight a central mechanism underlying melanocyte-specific miRNA regulation and the critical role of miRNAs in melanocyte differentiation and survival.

Nevertheless, multiple recent studies have shown strong evidence of the regulatory role of individual miRNAs on MITF and other related molecules controlling melanogenesis activities and skin pigmentation. In a search for miRNAs directly regulating MITF expression, Guo and colleagues identified miR-218 as a novel candidate for direct action on MITF expression [7]. Ectopic miR-218 expression dramatically reduced MITF expression, suppressed tyrosinase activity, and induced depigmentation in murine immortalized melan-a melanocytes. Furthermore, miR-218 also suppressed melanogenesis in human pigmented skin organotypic culture (OCT) and human primary skin melanocytes through the repression of MITF [7]. Transgenic mice overexpressing miR-137 developed a range of coat color changes from dark black to light color. Molecular analyses of the transgenic mice showed decreased expression of the major target gene MITF and its downstream molecules, including TYR, TYRP1, and TYRP2, which indicate the regulatory role of miR-137 in melanogenesis and coat color control [8]. A recent study on the role of miRNAs in determining fish skin color by Yan et al. indicated that miR-429 is a potential regulator of skin pigmentation. They showed that miR-429 directly regulates expression of Foxd3 by targeting its 3′-UTR, which repress the transcription of MITF and its downstream genes, such as TYR, TYRP1, or TYRP2 [9].

In addition to the intra-melanocyte localized miRNA-mediated melanogenesis regulation, a recent study by Kim and colleagues showed that miR-675, a long noncoding RNA H19-generated miRNA from keratinocytes, could be released extracellularly and delivered to neighboring melanocytes, in which miR-675 reduces the expression of MITF through direct binding to its 3′UTR [10]. These results suggest that miR-675 derived from keratinocytes could be involved in H19-stimulated melanogenesis through targeting.

UVR is the major environmental inducing factor of the melanogenesis process upregulating a network of genes involved in the pigmentation process. To identify miRNAs interfering with the pigmentary process, Dynoodt and colleagues performed miRNA profiling on mouse melanocytes after three consecutive treatments involving forskolin and solar-simulated UV (ssUV) irradiation [11]. Within the 16 differentially expressed miRNAs in treated melan-a cells, a 15-fold downregulation of miR-145 was detected. The expression of miR-145 is inversely correlated with the levels of Sox9, Mitf, Tyr, Trp1, Myo5a, Rab27a, and Fscn1. The direct targeting of Myo5a by miR-145 was confirmed in mouse and human melanocytes [11]. These results suggest a key role for miR-145 in regulating melanogenesis.

25.1.3 miRNAs in the Regulation of Immune Tolerance

Through extensive use of microarray hybridization and small RNA library sequencing in combination with next-generation sequencing approaches, investigators have identified lineage- and developmental stage-specific miRNA expression profiles in a variety of immune cell types

[12, 13], which indicate the relevance of miRNAs to immune cell development and function. CD4+CD25+Foxp3+ regulatory T (Treg) cells and invariant natural killer T (iNKT) cells are both critical immune regulators and play a pivotal role in the maintenance of self-tolerance and immunity. Numerical and functional defects of Treg and iNKT cells have been linked to the development of a variety of autoimmune diseases, including vitiligo [14–16]. Our recent clinical study on NSV showed decreased iNKT cell numbers and a trend toward altered iNKT cell phenotype in PBMCs of active NSV patients compared to age-, gender-, and race-matched healthy controls, suggesting the potential involvement of iNKT cells in the pathogenesis of vitiligo [17]. Using Treg-specific Dicer or Drosha deletion mouse models, three individual groups almost simultaneously identified the critical role of miRNAs in Treg lineage stability and function [18–20]. Tregs with miRNA deletion showed impaired peripheral homeostasis, eradicated suppression function, and interrupted Treg cell lineage stability, which ultimately lead to the fatal early onset of lymphoproliferative syndrome in these mouse models [18–20]. Using bone marrow- and thymus-specific Dicer deletion mouse models, studies from our laboratory and others demonstrate the role of miRNA in iNKT cell development and function [21–23]. A substantial reduction of iNKT cell numbers was observed in both thymus and peripheral immune organs from Dicer KO mice. Furthermore, iNKT cells with Dicer deletion showed a significantly interrupted capacity of activation and cytokine secretion [22, 23]. The critical involvement of miRNAs in regulatory immune cell development, homeostasis, and function further emphasize the potential role of miRNAs in maintaining immune tolerance and the pathogenesis of autoimmune diseases including vitiligo.

25.1.4 Dysregulated miRNAs in Vitiligo

The critical roles of miRNAs in both melanogenesis and immune tolerance regulation strongly suggest the potential involvement of miRNAs in vitiligo development. To explore the potential relationship between miRNA dysregulation and vitiligo development, we compared serum miRNA expression profiles in patients with active NSV and matched healthy controls. Thirty-one miRNAs showed significantly different expression levels (>2-fold changes, $P < 0.05$) between the two groups, while a panel of three miRNAs (miR-16, miR-19b, and miR-720) [24] appeared to be the best serum biomarkers to distinguish NSV patients from healthy controls, with the area under the curve (AUC) value of 0.97 [25]. In addition, the downregulation of miR-574-3p is correlated with disease severity [25]. Furthermore, we also evaluate the serum miRNA expression profiles from an autoimmune vitiligo mouse model, in which TRP-1-specific CD4+ T cells from TRP-1 CD4+ TCR-transgenic mice were adoptively transferred into Rag1KO host [26]. Twenty miRNAs showed significantly different expressions between vitiligo and control mice, among which miR-146 [27] and miR-191 [28] were significantly upregulated in the serum of both vitiligo mouse and NSV patients [25, 26]. Our studies raise the possibility that miRNAs may be involved in NSV development and could serve as promising biomarkers for differential diagnosis, progression, and therapy response of vitiligo. In a recent study, Wang and colleagues evaluated the miRNA expression profile in peripheral blood mononuclear cells (PBMCs) of patients with NSV and healthy controls and identified the upregulation of miR-224-3p and miR-4712-3p and downregulation of miR-3940-5p in the PBMCs of NSV patients [29]. Mansuri et al. further analyzed the miRNA expression profiles of lesional, nonlesional skin from NSV patients and healthy skin from control subjects with the aim to detect the potential role of skin microenvironmental miRNAs in the development of vitiligo. In addition to the changed miRNA levels in lesional skin compared to healthy skin, nonlesional skin from NSV patient showed dysregulated miRNA expression compared to healthy skin, and some miRNAs showed common dysregulations in both lesional and nonlesional

Table 25.1 MicroRNAs potentially involved in vitiligo

Changed miRNAs	Subjects/tissues	Biological function	References
miR-16, miR-19b, miR-720	NSV patients and healthy controls/serum	Potential serum biomarkers, may be related to Th1 response regulation	[24, 25]
miR-574-3p	NSV patients and healthy controls/serum	May be associated with NSV lesion severity	[25]
miR-146a, miR-191	NSV patients and healthy controls/serum, $Rag1^{-/-}$ vitiligo mouse and control/serum	Potential serum biomarkers, may contribute to TNFa over production; proliferation, survival of melanocytes	[25–28]
miR-224-3p, miR-4712-3p, miR-3940-5p	NSV patients and healthy controls/PBMCs	Potential biomarker, potential role in regulating inflammatory cytokine production	[29]
miR-135a, mijR-183, miR-30a-3p, miR-487a	NSV patient lesional, nonlesional and helthy control skin	Potential role of initiation and progression of disease	[30]
miR-196a-2	NSV patients and healthy control tissues	rs11614913 miR-196a-2 CC-allele associated with lower risk of vitiligo	[31]
miR-25	Human melanocytes and keratinocytes	Promote the degeneration of melanocytes by targeting MITF and inhibiting SCF, bFGF	[32]

skin in NSV patients [30]. These results indicate the potential role of miRNAs in the initiation as well as progression of vitiligo and that dysregulated miRNAs might be one of the mechanisms for the increased susceptibility of vitiligo patients to environmental triggers in disease development.

To investigate the contribution of genetic variations in miRNAs to the development of vitiligo, Huang et al. found a significantly lower risk of vitiligo was associated with the rs11614913 miR-196a-2 CC genotype. As the target of miR-196a-2, TYRP1 gene expression was downregulated by the rs11614913 C allele in miR-196a-2, which lowered the levels of intracellular reactive oxygen species (ROS) and reduced early apoptosis in human melanocytes in response to H_2O_2 treatment [31]. Interestingly, studies from the same group showed that oxidative stress induced the overexpression of miR-25 in both melanocytes and keratinocytes, and upregulated miR-25 could not only promote the degeneration of melanocytes by targeting MITF but also inhibit the production and secretion of SCF and bFGF from keratinocytes, thus impairing their paracrine protective effect on the survival of melanocytes [32]. These results indicate that oxidative stress-induced miR-25 overexpression may be potentially involved in vitiligo development [32] (Table 25.1).

25.2 DNA Methylation Alterations in Vitiligo

25.2.1 DNA Methylation

DNA methylation is a dynamic process involving methylation and demethylation events in different regions of genome, which is crucially important for embryogenesis, cellular proliferation, and differentiation [33, 34]. DNA methylation is catalyzed and maintained by the DNA methyltransferase (DNMT) family which is composed of a group of enzymes, including DNMT1, DNMT3a, DNMT3b, and DNMT3L. In mammals, DNMTs catalyze the donation of a methyl group to cytosine (C) and guanine (G) dinucleotides [35]. In the human genome, there are regions with enriched CpG dinucleotides, which are called CpG islands. The human genome contains about 30,000 CpG islands, 50–60% of which are located in the promoter region of genes [36]. It is believed that methylation of CpG in the

promoter region leads to the silencing of gene expression by interfering with transcription activator binding and/or recruitment of chromatin repressor complexes through the binding of methyl-CpG-binding domain (MBD) proteins, which leads to tightly condensed chromatin around the gene promoter, blocking accessibility to transcriptional activators and thereby inhibiting the gene transcription [36, 37]. In addition to CPG islands, recent genome-wide DNA methylation studies reveal that non-CpG methylation in both the intragenic and intergenic sites is also important for regulation of differential gene expression [38]. Demethylation could be either passive or active. Passive demethylation is induced by inhibition of DNMTs, providing the basis for treatments with the aim of erasing abnormal hypermethylation [39], while active demethylation could depend on the activity of cytosine deaminases occurring predominantly in cell differentiation and associated with immune cell activation [40].

25.2.2 DNA Methylation in Vitiligo Animal Models

In 1996, Sreekumar et al. reported that DNA methylation inhibitor 5-azacytidine (5-azaC) induced autoimmune vitiligo in vitiligo-susceptible but normally pigmented chicken stains, which are parental control strains of the Smyth Line chicken model, due to loss of skin melanocytes [41]. In this model, both T cells and B cells are involved in the pathogenesis of the disease. Interestingly, chronic low-dose administration of 5-azaC also increased the incidence of autoimmune thyroiditis in a chicken model for Hashimoto's autoimmune thyroiditis [42], which supports the interrelationship between vitiligo and autoimmune thyroiditis observed in humans. It is possible that DNA hypomethylation caused by 5azaC may turn on certain genes that induce autoreactivity in specific T and/or B cells. This suggests a role for demethylation of genes in the pathogenesis of these autoimmune diseases in genetically susceptible individuals.

25.2.3 DNA Methylation in Human Vitiligo

To investigate the potential involvement of epigenetic changes in the development of human vitiligo, Lu's group examined genomic and gene-specific DNA methylation levels as well as mRNA levels of DNAMTs, MDB proteins in peripheral blood mononuclear cells (PBMCs), from vitiligo patients and controls [43]. Compared to healthy controls, the mean genomic DNA methylation levels were significantly higher in vitiligo patients, which is consistent with some other organ-specific autoimmune diseases such as psoriasis and type 1 diabetes mellitus [44, 45]. However, some systemic autoimmune diseases, such as systemic lupus erythematosus (SLE) and systemic sclerosis (SSC), showed global hypomethylation in CD4+ T cells compared to controls [46, 47]. These results indicate that hypermethylation may be important in organ-specific immune activation. In addition, DNMT1, the enzyme for maintaining DNA methylation, was significantly increased, while some MDB proteins including MBD1, MBD3, MBD4, and MeCP2 were also significantly upregulated in PBMCs from vitiligo patients, which is consistent with the global hypermethylation identified in vitiligo PBMCs. More interestingly, the expression levels of MBD1 and MBD3 were positively correlated with overall methylation levels in PBMCs of vitiligo patients, suggesting that increased MBD1 and MBD3 may contribute to global hypermethylation and the development of vitiligo [43].

IL-10 is an anti-inflammatory cytokine. It is capable of inhibiting synthesis of pro-inflammatory cytokines and is critically involved in regulatory T cell-mediated immune regulation. Since IL-10 is particularly sensitive to alterations in methylation status [48, 49] and vitiligo patient PBMCs have decreased IL-10 transcription [43], the DNA methylation status of an IL-10 enhancer region was evaluated. The enhancer region within intron 4 of IL-10 containing eight CG pairs was hypermethylated in vitiligo PBMCs, and the overall methylation levels of this region are negatively correlated with IL-10 mRNA transcription,

which suggests that DNA hypermethylation within IL-10 gene may contribute to decreased IL-10 expression in vitiligo PBMCs [43]. Nevertheless, future studies in exploring the gene-specific DNA methylation patterns are needed to determine the specific role of DNA methylation dysregulation in the pathogenesis of vitiligo. These information may supply not only novel markers for vitiligo clinical management but also potential epigenetic therapies for vitiligo in adjusting DNA methylation.

25.3 Histone Modifications

The nucleosome is a repeating subunit that consists of 146 base pairs of chromatin in a double helix wrapped around a protein core composed of histone octamers H2A, H2B, H3, and H4. As another major source of epigenetic alteration, histone posttranslational modifications, such as acetylation, methylation, ubiquitination, and phosphorylation, could modulate the chromatin structure, influencing its accessibility to transcription factors at gene promoters and enhancers, therefore modulating related gene expression. Among these processes, acetylation and methylation have been the most extensively studied ones.

25.3.1 Mechanisms of Histone Acetylation and Histone Methylation

Histone acetylation and deacetylation are of great importance in gene regulation. Histone acetylation is catalyzed by histone acetyltransferases (HATs), adding acetyl group on lysine residues in the N-terminal tail and promoting a more open chromatin structure which links to active genes. In contrast, histone deacetylation catalyzed by histone deacetylases (HDACs) removes the acetyl groups, causing the DNA to wrap around nucleosomes more tightly, thereby repressing gene expression [50].

Histone methylation, which adds a methyl group to the histone subunit, is catalyzed by histone methyltransferases (HTMs), while demethylation occurs as a result of interaction of histone demethylase (HDMs). As a major contributor of epigenetic modification, histone methylation regulates fundamental processes such as gene transcription and DNA repair [51]. Unlike acetylation, the effects of histone methylation on gene regulation depend on the position of residues modified or the number of methyl groups present, resulting in either transcription activation or suppression [52].

25.3.2 Histone Modification in Autoimmune Diseases

Accumulated evidence from recent studies has indicated that histone modifications play critical roles in the biological processes of the immune system as well as the development of autoimmune diseases. Dysregulated immune homeostasis, activation, and inflammatory cytokine production are associated with aberrant histone and chromatin modification [53, 54]. Type 1 diabetes (T1D) is an organ-specific autoimmune disease and shares multiple susceptible genes with vitiligo. Recent evidence suggests that aberrant histone modifications are involved in T1D pathogenesis. The expression of histone deacetylase gene, a key regulator of histone modification, is reduced in CD4+ T cells from T1D patients [55].

By screening histone modification variations at known T1D susceptible genes in blood cells, increased H3K9 acetylation (H3K9Ac) upstream regions of HLA-DRB1 and HLA-DQB1 within the IDDM1 locus in T1D monocytes from T1D. These histone acetylation changes could be the cause of the upregulated *HLA-DRB1* and *HLA-DQB1* gene expression, which is highly associated with T1D [56]. Furthermore, in lymphocytes of T1D patients, altered H3K9me2 was identified in subset of genes which are involved in autoimmune and inflammation-related pathways, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), transforming growth factor-beta (TGF-β), nuclear factor-kappaB (NFκB), toll-like receptor (TLR), and interleukin-6 (IL-6) [57]. In patients with rheumatoid arthritis (RA),

synovial fibroblasts show increased histone methyltransferase EZH2 which could silence secreted frizzled-related protein 1 (SFRP1) gene expression, thereby abrogating its inhibitory role on aberrant collagen deposition [58]. These studies provide evidence of a novel association between autoimmune diseases and altered histone modifications of key genes that are components of disease-related biological pathways.

Nevertheless, the association of global or gene-specific histone modification patterns with the development vitiligo remains to be explored. These future studies will not only further elucidate the epigenetic mechanisms of vitiligo development but also provide potential novel therapeutic targets for the treatment of this disease.

25.3.3 Chromatin Modification in Melanocyte Differentiation

Microphthalmia-associated transcription factor (MITF) not only regulates the survival and proliferation of melanocytes but also promotes the transcription of several pigmentation genes. A comprehensive analysis of the MITF "interactome" in melanoma cells revealed the nucleosome remodeling factor (NURF) complex associates with MITF [59]. More recent studies showed that NURF acts downstream of MITF in melanocytes and co-regulate gene expression. In vivo, mice lacking the NURF subunit BPTF in the melanocyte lineage show premature graying due to deficiency in generating mature melanocytes from the adult stem cell population [60]. Therefore, chromatin remodeling by NNURF appears to be essential for the transition of the transcriptionally quiescent stem cell to the differentiated state. Furthermore, NURF subunits, SMARCA5 and BPTF, were also involved in controlling epidermal stem cell differentiation [61]. Despite extensive characterization of the biochemical properties of the NURF complex and its subunits, the detailed biological functions of NURF complex in mammals and its relationship to the development and maintenance of vitiligo remain to be explored.

25.4 Concluding Remarks

Growing amount of evidences suggest that gene function depends on not only DNA sequences but also the epigenetic modifications, including DNA methylation, histone modification, noncoding RNA, and their cross talks, which are thought to bridge the gap between environment and genetics. Different from genetic factors, epigenetic alterations vary among different tissues and cell types. Each cell type could be characterized by a particular epigenome that are associated with a specific gene expression profile. In addition, different environmental factors exposed could also contribute to their distinction in epigenetic alteration and disease pathogenesis. These epigenetic specificities manifested in different diseases, and even individual patients with the same disease will supply the novel clinical biomarkers for disease diagnosis, prognosis, and progression monitoring. Furthermore, epigenetic alterations are potentially reversible, which supply novel targets for potential epigenetic therapeutic treatment and the more personalized application of treatment for autoimmune diseases in the future.

Similar to other autoimmune diseases, vitiligo is a polygenic, multifactorial disease resulting from the complex interactions of genetic and environmental factors that ultimately contribute to melanocyte destruction. As shown in Fig. 25.2, [62–67] genetic polymorphisms, mutations predispose individuals with dysregulated immunosurveillance against the melanocytic system. In these individuals, environmental factors induce epigenetic alterations including miRNA level, DNA methylation, and histone modification. These alterations then affect the expression of related genes involved in immune tolerance, inflammation, and melanocyte homeostasis and function as summarized in this chapter, which eventually lead to active immune response and melanocyte destruction. Even though epigenetic studies in vitiligo are still in its infancy, accumulated studies have already shown clear evidence of the critical involvement of epigenetic regulations, especially miRNAs and DNA methylation, in melanocyte development, function, and

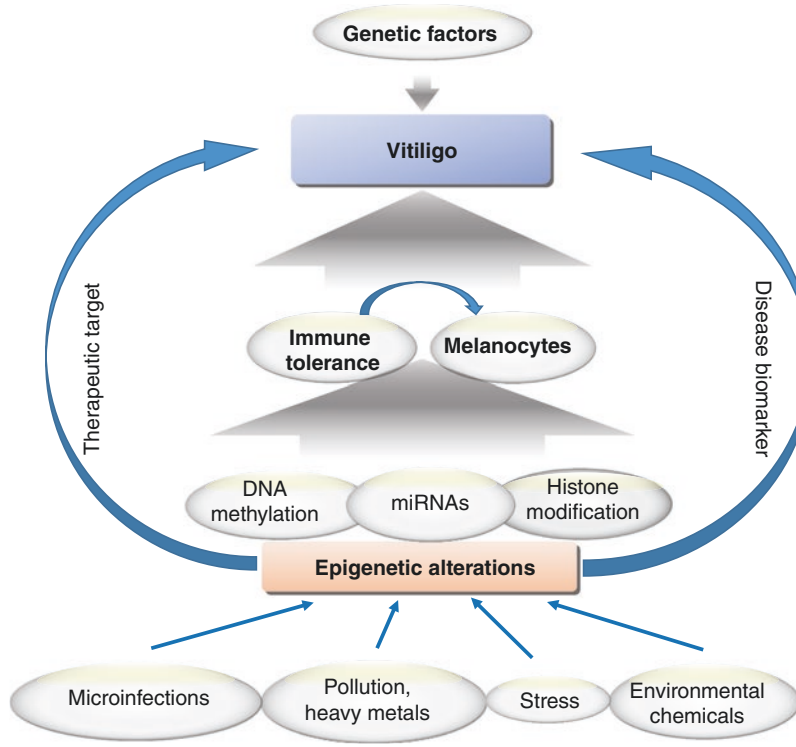


Fig. 25.2 Schematic representation of environmental factor-mediated epigenetic modifications in the development of autoimmune vitiligo. In these genetically predisposed individuals, environmental factors, such as stress, chemicals, and microinfections, induce epigenetic alterations including miRNA level, DNA methylation, and histone modification. These alterations then affect the expression of related genes involved in immune tolerance, inflammation, and melanocyte homeostasis and function, which ultimately lead to melanocyte destruction

immune tolerance regulation. Continued studies in epigenetic mechanisms on vitiligo development will definitely result in more promises in not only understanding the pathogenesis but also the novel diagnostic and tailored preventive and therapeutic strategies for vitiligo.

References

1. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 2014;42:D68–73.
2. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem. 2010;79:351–79.
3. Pasquinelli AE. MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. Nat Rev Genet. 2012;13:271–82.
4. Siomi H, Siomi MC. Posttranscriptional regulation of microRNA biogenesis in animals. Mol Cell. 2010;38:323–32.
5. Shin C, Nam J-W, Farh KK-H, et al. Expanding the microRNA targeting code: functional sites with centered pairing. Mol Cell. 2010;38:789–802.
6. Levy C, Khaled M, Robinson KC, et al. Lineage-specific transcriptional regulation of DICER by MITF in melanocytes. Cell. 2010;141:994–1005.
7. Guo J, Zhang JF, Wang WM, et al. MicroRNA-218 inhibits melanogenesis by directly suppressing microphthalmia-associated transcription factor expression. RNA Biol. 2014;11(6):732–41.
8. Dong C, Wang H, Xue L, et al. Coat color determination by miR-137 mediated down-regulation of microphthalmia-associated transcription factor in a mouse model. RNA (New York, NY). 2012;18:1679–86.
9. Yan B, Liu B, Zhu CD, et al. microRNA regulation of skin pigmentation in fish. J Cell Sci. 2013;126:3401–8.
10. Kim NH, Choi SH, Kim CH, et al. Reduced MiR-675 in exosome in H19 RNA-related melanogenesis via MITF as a direct target. J Invest Dermatol. 2014;134:1075–82.
11. Dynoodt P, Mestdagh P, Van Peer G, et al. Identification of miR-145 as a key regulator of the pigmentary process. J Invest Dermatol. 2013;133:201–9.
12. Belver L, Papavasiliou FN, Ramiro AR. MicroRNA control of lymphocyte differentiation and function. Curr Opin Immunol. 2011;23:368–73.
13. O'Connell RM, Rao DS, Chaudhuri AA, et al. Physiological and pathological roles for microRNAs in the immune system. Nat Rev Immunol. 2010;10:111–22.
14. Berzins SP, Smyth MJ, Baxter AG. Presumed guilty: natural killer T cell defects and human disease. Nat Rev Immunol. 2011;11:131–42.
15. Dwivedi M, Helen Kemp E, Laddha NC, et al. Regulatory T cells in vitiligo: implications for

pathogenesis and therapeutics. Autoimmun Rev. 2015;14:49–56.
16. Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. Nat Immunol. 2008;9:239–44.
17. Zhou L, Li K, Shi YL, et al. Systemic analyses of immunophenotypes of peripheral T cells in non-segmental vitiligo: implication of defective natural killer T cells. Pigment Cell Melanoma Res. 2012;25:602–11.
18. Chong MMW, Rasmussen JP, Rudensky AY, et al. The RNAseIII enzyme Drosha is critical in T cells for preventing lethal inflammatory disease. J Exp Med. 2008;205:2005–17.
19. Liston A, Lu L-F, O'Carroll D, et al. Dicer-dependent microRNA pathway safeguards regulatory T cell function. J Exp Med. 2008;205:1993–2004.
20. Zhou X, Jeker LT, Fife BT, et al. Selective miRNA disruption in T reg cells leads to uncontrolled autoimmunity. J Exp Med. 2008;205:1983–91.
21. Fedeli M, Napolitano A, Wong MPM, et al. Dicer-dependent microRNA pathway controls invariant NKT cell development. J Immunol. 2009;183:2506–12.
22. Seo KH, Zhou L, Meng DM, et al. Loss of microRNAs in thymus perturbs invariant NKT cell development and function. Cell Mol Immunol. 2010;7:447–53.
23. Zhou L, Seo KH, He HZ, et al. Tie2cre-induced inactivation of the miRNA-processing enzyme Dicer disrupts invariant NKT cell development. Proc Natl Acad Sci U S A. 2009;106:10266–71.
24. Jiang S, Li C, Olive V, et al. Molecular dissection of the miR-17-92 cluster's critical dual roles in promoting Th1 responses and preventing inducible Treg differentiation. Blood. 2011;118:5487–97.
25. Shi Y-L, Weiland M, Li J, et al. MicroRNA expression profiling identifies potential serum biomarkers for non-segmental vitiligo. Pigment Cell Melanoma Res. 2013;26:418–21.
26. Shi Y-L, Weiland M, Lim HW, et al. Serum miRNA expression profiles change in autoimmune vitiligo in mice. Exp Dermatol. 2014;23:140–2.
27. Pauley KM, Satoh M, Chan AL, et al. Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. Arthritis Res Ther. 2008;10:1–10.
28. Mueller DW, Rehli M, Bosserhoff AK. miRNA expression profiling in melanocytes and melanoma cell lines reveals miRNAs associated with formation and progression of malignant melanoma. J Investig Dermatol. 2009;129:1740–51.
29. Wang Y, Wang K, Liang J, et al. Differential expression analysis of miRNA in peripheral blood mononuclear cells of patients with non-segmental vitiligo. J Dermatol. 2015;42:193–7.
30. Mansuri MS, Singh M, Dwivedi M, et al. MicroRNA profiling reveals differentially expressed microRNA signatures from the skin of patients with nonsegmental vitiligo. Br J Dermatol. 2014;171:1263–7.
31. Huang Y, Yi X, Jian Z, et al. A single-nucleotide polymorphism of miR-196a-2 and vitiligo: an association study and functional analysis in a Han Chinese population. Pigment Cell Melanoma Res. 2013;26:338–47.
32. Shi Q, Zhang W, Guo S, et al. Oxidative stress-induced overexpression of miR-25: the mechanism underlying the degeneration of melanocytes in vitiligo. Cell Death Differ. 2016;23:496–508.
33. Ehrlich M, Lacey M. DNA methylation and differentiation: silencing, upregulation and modulation of gene expression. Epigenomics. 2013;5:553–68.
34. Guo H, Zhu P, Yan L, et al. The DNA methylation landscape of human early embryos. Nature. 2014;511:606–10.
35. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet. 2003;33(Suppl):245–54.
36. Costello JF, Plass C. Methylation matters. J Med Genet. 2001;38:285–303.
37. Fuks F, Hurd PJ, Wolf D, et al. The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. J Biol Chem. 2003;278:4035–40.
38. Maunakea AK, Nagarajan RP, Bilenky M, et al. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature. 2010;466:253–7.
39. Fritz EL, Papavasiliou FN. Cytidine deaminases: AIDing DNA demethylation? Genes Dev. 2010;24:2107–14.
40. Fritz EL, Rosenberg BR, Lay K, et al. A comprehensive analysis of the effects of the deaminase AID on the transcriptome and methylome of activated B cells. Nat Immunol. 2013;14:749–55.
41. Sreekumar GP, Erf GF, Smyth JR Jr. 5-Azacytidine treatment induces autoimmune vitiligo in parental control strains of the Smyth line chicken model for autoimmune vitiligo. Clin Immunol Immunopathol. 1996;81:136–44.
42. Schauenstein K, Csordas A, Kromer G, et al. In-vivo treatment with 5-azacytidine causes degeneration of central lymphatic organs and induces autoimmune disease in the chicken. Int J Exp Pathol. 1991;72:311–8.
43. Zhao M, Gao F, Wu X, et al. Abnormal DNA methylation in peripheral blood mononuclear cells from patients with vitiligo. Br J Dermatol. 2010;163:736–42.
44. Stefan M, Zhang W, Concepcion E, et al. DNA methylation profiles in type 1 diabetes twins point to strong epigenetic effects on etiology. J Autoimmun. 2014;50:33–7.
45. Zhang P, Su Y, Chen H, et al. Abnormal DNA methylation in skin lesions and PBMCs of patients with psoriasis vulgaris. J Dermatol Sci. 2010;60:40–2.
46. Lei W, Luo Y, Lei W, et al. Abnormal DNA methylation in CD4+ T cells from patients with systemic lupus erythematosus, systemic sclerosis, and dermatomyositis. Scand J Rheumatol. 2009;38:369–74.
47. Wu H, Zhao M, Tan L, et al. The key culprit in the pathogenesis of systemic lupus erythematosus: aberrant DNA methylation. Autoimmun Rev. 2016;15:684–9.

48. Szalmas A, Banati F, Koroknai A, et al. Lineage-specific silencing of human IL-10 gene expression by promoter methylation in cervical cancer cells. Eur J Cancer (Oxford, England: 1990). 2008;44:1030–8.
49. Zhao M, Tang J, Gao F, et al. Hypomethylation of IL10 and IL13 promoters in CD4+ T cells of patients with systemic lupus erythematosus. J Biomed Biotechnol. 2010;2010:931018.
50. Kouzarides T. Chromatin modifications and their function. Cell. 2007;128:693–705.
51. Bannister AJ, Kouzarides T. Reversing histone methylation. Nature. 2005;436:1103–6.
52. Scharf AN, Imhof A. Every methyl counts – epigenetic calculus. FEBS Lett. 2011;585(13):2001–7.
53. Zhang Z, Zhang R. Epigenetics in autoimmune diseases: pathogenesis and prospects for therapy. Autoimmun Rev. 2015;14:854–63.
54. Zhao M, Wang Z, Yung S, et al. Epigenetic dynamics in immunity and autoimmunity. Int J Biochem Cell Biol. 2015;67:65–74.
55. Orban T, Kis J, Szereday L, et al. Reduced CD4+ T-cell-specific gene expression in human type 1 diabetes mellitus. J Autoimmun. 2007;28:177–87.
56. Miao F, Chen Z, Zhang L, et al. Profiles of epigenetic histone post-translational modifications at type 1 diabetes susceptible genes. J Biol Chem. 2012;287:16335–45.
57. Miao F, Smith DD, Zhang L, et al. Lymphocytes from patients with Type 1 diabetes display a distinct profile of chromatin histone H3 lysine 9 dimethylation: an epigenetic study in diabetes. Diabetes. 2008;57:3189–98.
58. Trenkmann M, Brock M, Gay RE, et al. Expression and function of EZH2 in synovial fibroblasts: epigenetic repression of the Wnt inhibitor SFRP1 in rheumatoid arthritis. Ann Rheum Dis. 2011;70:1482–8.
59. Laurette P, Strub T, Koludrovic D, et al. Transcription factor MITF and remodeller BRG1 define chromatin organisation at regulatory elements in melanoma cells. eLife. 2015;4 https://doi.org/10.7554/eLife.06857.
60. Koludrovic D, Laurette P, Strub T, et al. Chromatin-remodelling complex NURF is essential for differentiation of adult melanocyte stem cells. PLoS Genet. 2015;11:e1005555.
61. Mulder KW, Wang X, Escriu C, et al. Diverse epigenetic strategies interact to control epidermal differentiation. Nat Cell Biol. 2012;14:753–63.
62. Alikhan A, Felsten LM, Daly M, et al. Vitiligo: a comprehensive overview. Part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. J Am Acad Dermatol. 2011;65:473–91.
63. Alkhateeb A, Fain PR, Thody A, et al. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. Pigment Cell Res. 2003;16:208–14.
64. Birlea SA, Gowan K, Fain PR, et al. Genome-wide association study of generalized vitiligo in an isolated European founder population identifies SMOC2, in close proximity to IDDM8. J Invest Dermatol. 2010;130:798–803.
65. Quan C, Ren YQ, Xiang LH, et al. Genome-wide association study for vitiligo identifies susceptibility loci at 6q27 and the MHC. Nat Genet. 2010;42:614–8.
66. Spritz RA. The genetics of generalized vitiligo and associated autoimmune diseases. Pigment cell Res. 2007;20:271–8.
67. Spritz RA. Six decades of vitiligo genetics: genome-wide studies provide insights into autoimmune pathogenesis. J Invest Dermatol. 2012;132:268–73.

Melanocyte Homeostasis in Vitiligo

26

Véronique Delmas and Lionel Larue

Contents

Abstract

To understand the pathophysiology of pigmentary disorders, such as vitiligo, it is essential to understand the development and homeostasis of melanocytes, the cells that produce the melanin pigment. In this chapter, we will concentrate on classical melanocytes that give rise to skin colour that can be lost in depigmented region of vitiligo patients. We address the origins of melanocytes during development, the homeostasis of these cells and their renewal during the course of life. We focus on the early alterations of melanocyte homeostasis that appeared before the depigmentation of the skin in vitiligo patients and highlight the key role of E-cadherin, the main cell-cell adhesion molecule that links melanocyte to the surrounding keratinocytes in the epidermis.

Key Points

- In vitiligo patients, alteration of melanocyte homeostasis leads to the reduction of basal melanocytes in normal pigmented skin.
- Detached basal melanocytes are replaced until exhaustion of surrounding melanocyte stem cells.

V. Delmas · L. Larue (✉)
INSERM U1021, Normal and Pathological Development of Melanocytes, Institut Curie, PSL Research University, Orsay, France
e-mail: veronique.delmas@curie.fr; lionel.larue@curie.fr

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_26

- Alteration of the level/activity of E-cadherin in basal melanocytes is an early event of vitiligo pathophysiology.
- The level/activity of E-cadherin is reduced according to the genetic status and/or the environment (oxidative and/or mechanical stresses).

26.1 Introduction

To understand the pathophysiology of pigmentary disorders, such as vitiligo, it is essential to understand the features and homeostasis of melanocytes, the cells that produce the pigment, melanin. Melanocytes are specified at an early stage of neural crest development during embryonic development [1]. Neural crest cells (NCC) are a temporary cell population unique to vertebrates that arise from the embryonic dorsal ectodermal cell layer and give rise to diverse cell lineages, including melanocytes, craniofacial cartilage and bone, smooth muscle, peripheral and enteric neurons, and glia. NCC are originally located in the dorsal part of the embryo and subsequently migrate to their ultimate destination, acquiring their various properties, including differentiation into pigment cells. In the human body, melanocytes are found in the skin, as well as the iris, inner ear, brain, heart, and fatty tissue [2, 3]. Melanocytes in the epidermis synthesize melanin and transfer this pigment to the surrounding keratinocytes; skin melanocytes are considered to be "classical melanocytes." The other melanocytes found throughout the body do not transfer their melanin to surrounding cells and are classified as "nonclassical melanocytes." The function of nonclassical melanocytes and the associated melanin are mostly unknown [2]. However, we do know the function of some of them, melanocytes located in the inner ear are essential for hearing, and those located in heart valves are involved in their stiffness.

In this chapter, we will concentrate on classical melanocytes that give rise to skin color and lost in vitiligo. Pigmentation of the eyes can also be affected in this disease, but we will not address this issue. Skin melanocytes are found in the dermis and the epidermis. Most are found in the epidermis where they are located in different structures (hair follicles, sweat glands, and sebaceous glands) and dispersed throughout the stratum. Skin melanocytes produce different sets of proteins depending on their location. Better knowledge of the origins and various specificities of these melanocyte populations can lead to a better understanding of the different types of vitiligo, including segmental vitiligo.

26.2 Origin of Skin Melanocytes and Skin Colonization

26.2.1 Cellular Origin and Migratory Pathway of Melanocyte Progenitors

The embryonic precursor cells of melanocytes, melanoblasts, originate from neural crest cells (NCC), a transient cell population arising from the dorsal part of the neural tube. In humans, melanoblasts actively proliferate during development, concomitant with specification and migration. Melanoblast specification starts 8 weeks after conception. Dorsolateral migration of melanoblasts occurs throughout the mesenchymal dermis between the 5th and 12th weeks of embryonic development.

The mechanisms associated with melanoblast specification, proliferation, and migration during development have been little studied in humans for obvious reasons. Most information has been generated from studies on avian (chicken and quail), fish (zebrafish), and mammalian (rat and mouse) embryos. Melanoblasts specify, proliferate, and first migrate from the vagal region and then progressively further toward the anterior and posterior extremities.

In the regions where somites are present, melanoblasts migrate between the somites and the ectoderm from the dorsal region toward the belly. This pathway is called the dorsolateral pathway. In the cephalic region, lacking somites,

melanoblasts migrate laterally under the ectoderm. Melanoblasts from different parts of the body thus migrate and interact with various cells, extracellular matrices, and/or microenvironments. As a consequence, melanoblasts receive information that can vary, differentially influencing their potential biological properties or predispositions.

Furthermore, although most melanoblasts follow the dorsolateral pathway, some emerge from the dorsoventral pathway, in between the neural tube and the somites [4]. It has been observed in several species that the growing nerves projecting through the body serve as a niche for progenitor cells, including Schwann cell precursors (SCPs), which give rise to Schwann cells and melanocytes [1]. SCPs are derived from neural crest cells that delaminate before the dorsolateral migration of the melanoblasts. These cells initially follow the dorsoventral pathway, between the neural tube and somites and not between the somites and ectoderm. SCP and Schwann cells, once differentiated, remain in close contact with the nerves. In some cases, SCPs lose their contact with nerves and may acquire the melanocytic fate. These melanocytes colonize the dorsal and lateral body walls and largely influence limb pigmentation. Some patients with segmental vitiligo have depigmentation that is confined to innervation zones. It is possible that dorsoventral melanocytes are at the origin of this type of depigmentation. Many signaling molecules (ligands) are required during all stages of melanocyte development [5]. These ligands educate neural crest cells to specify the melanocytic fate and instruct melanoblasts to proliferate, migrate, survive, and home (final destination), prior to terminal differentiation into pigmented melanocytes. The currently best-documented signaling pathways implicated in the development of the melanocyte lineage arising from the dorsolateral pathway are the EDN3/EDNRB (endothelin 3 ligand with its endothelin receptor B), WNT/β--catenin (Wnt1/3A with one of its mediators, β-catenin), and SCF/KIT (stem cell factor with its transmembrane tyrosine kinase receptor) signaling pathways [6]. The signaling pathways involved in the development of melanocytes of the dorsoventral pathway are poorly understood. The SCP signals for melanoblast specification are not fully characterized, but various proteins may play a role in regulating the cell fate of SCPs, including NRG/ERBB3 (neuregulin and its receptor tyrosine kinase 3), IGFI-IGFII/IGF1R (insulin-like growth factor and its receptor), and PDGF/PDGFR (platelet-derived growth factor and its receptor) [7].

The difference(s), if any, between dorsolateral and dorsoventral melanocytes remain(s) unknown. For example, it is unclear whether these two cell types have the exact same function and whether they colonize the body in a uniform manner. These questions are of clear importance to better understanding melanomagenesis and pigmentation problems, including vitiligo.

26.2.2 Migration of Melanoblasts to the Skin and "the Cadherin Code"

After specification, dorsolateral melanoblasts migrate through the dermis and enter the epidermis by crossing the basement membrane. Once in the epidermis, melanoblasts remain in contact with the basement membrane via the stratum of the epidermis. In mice, melanoblasts are evenly distributed throughout the epidermis of the embryo [8]. This suggests that melanoblasts communicate between each other using soluble factors/receptors and/or keratinocytes to maintain a defined and dynamic distance apart from each other. From embryonic day E15.5, melanoblasts migrate toward the matrix of the nascent hair follicles, a process that is stimulated by SCF. Just prior birth, melanoblasts of the hair follicles are found in the forming hair bulge and hair bulb. The hair bulge is located in the permanent portion of the hair follicle and contains the niche of the keratinocyte stem cells (KSCs) and melanocyte stem cells (McSCs). The presence of McSCs is required for melanocyte renewal. After induction of the McSCs, they proliferate and migrate toward the forming hair bulb [9]. The cells making up this transient cell population are called TAC (transit amplifying cells). Once the

cells arrive at the hair bulb, they start to terminally differentiate into mature melanocytes and express genes encoding enzymes required for melanin production, including Tyr (tyrosinase) and Tyrp1 (tyrosinase-related protein 1), and differentiate into mature melanocytes by birth. During the entire developmental process, the environment surrounding the melanoblasts varies dramatically: melanoblasts are first surrounded by the mesenchyme of the dermis, then keratinocytes of the epidermis, and finally the various cells of the hair follicle, including those of the hair bulge, root sheath, and hair bulb. During this process, melanoblasts must adapt their behavior based on external information (direct cell-cell interactions, soluble factors, and, potentially, exosomes) and their interactions with the extracellular matrix; their adhesive properties must be strictly regulated, both temporally and spatially.

Cadherins constitute a superfamily of cell adhesion molecules that have been implicated in cell-cell recognition and interaction, cell sorting, cell transformation, and histogenesis [10]. Classical cadherins are Ca^{2+}-dependent cell-cell adhesion molecules. They are transmembrane proteins comprising an extracellular domain, a membrane-spanning region, and a highly conserved cytoplasmic portion. The extracellular domain is composed of five subdomains (EC), each of approximately 110 amino acids. Classical cadherins are subdivided into two groups (type I and type II) depending upon their sequence characteristics [11]. Type I cadherins include E-cadherin (E-cad or Cdh1), N-cadherin (N-cad or Cdh2), P-cadherin (P-cad or Cdh3), and R-cadherin (R-cad or Cdh4). Type II cadherins include cad5 to cad12 (Cdh5 to Cdh12). Cell-cell adhesion is mediated by homophilic interactions between the extracellular domains of cadherins on adjacent cells. Cadherins are anchored within cells by dynamic associations with catenins, which link them to actin filaments. The cytoplasmic domain of cadherins directly interacts with either β-catenin or plakoglobin (γ-catenin). β-catenin or plakoglobin binds to actin filaments via α-catenin. Cadherins are not only involved in cell-cell adhesion but also cell signaling, morphogenesis, and histogenesis [12]. The signaling pathways and gene regulation associated with the morphogenetic and histogenetic functions of cadherins are not clear, but β-catenin is known to be involved. Indeed, β-catenin is involved in cell-cell adhesion, signal transduction, and the control of gene expression [13]. β-Catenin activity is tightly regulated by its tyrosine and serine/threonine phosphorylation status, depending on specific kinases and phosphatases and the transduction pathways activated by soluble factors, such as Wnt, EGF, and IGF-II.

Melanoblasts dynamically express distinct cadherins during development [14]. For example, E- and P-cadherins are weakly expressed in migrating melanoblasts in the dermis, but E-cadherin expression increases 200-fold when the cells enter the epidermis and interact with E-cadherin-expressing keratinocytes [15]. The weak expression of E-cadherin in the dermis may prevent self-aggregation and premature colonization of the epidermis. Melanoblast entry into the hair follicles leads to a reduction in the level of E-cadherin and an increase in that of P-cadherin (Fig. 26.1). Melanoblasts in the dermis may differentiate into melanocytes, producing melanin, but their melanosomes are not transferred to the surrounding cells. Dermal melanocytes express N-cadherin as the surrounding fibroblast, possibly allowing their interaction [14]. In conclusion, these dynamic changes in cadherin expression appear to aid the melanoblasts in adapting to their local environment in the skin. In mice, melanoblast-specific loss of E-cad leads to a reduction in the number of epidermal interfollicular melanoblasts due to a proliferative defect of these cells and partial loss of the pigmentation of the epidermis without any effect on coat color, as P-cad is the major cadherin in follicular melanocytes [16]. No coat color phenotype has been reported in mice lacking P-cadherin. The inactivation of both genes in melanocytes has not been reported yet to evaluate the importance of E- and P-cadherin for hair follicle melanocyte function. In conclusion, E-cadherin expression in melanoblasts is required specifically for the establishment of an adequate number of epidermal melanocytes.

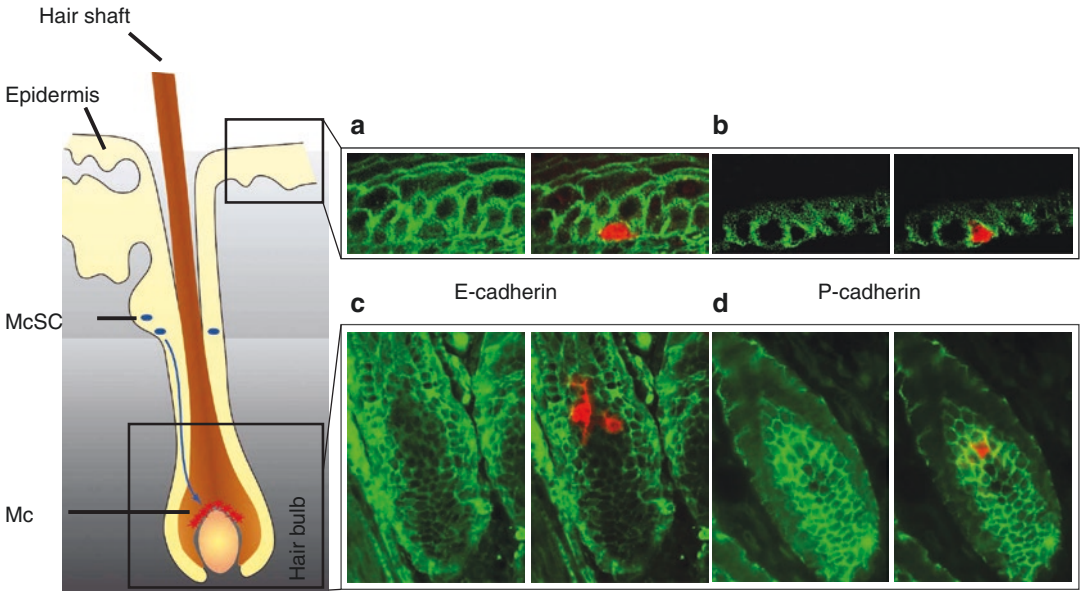


Fig. 26.1 Cadherin expression in mouse epidermis and hair follicles. Immunostaining of tail skin of 10-day-old Dct::LacZ mice using anti-E- (**a**, **c**) or P-cadherin (**b**, **d**) (green) and anti-β-galactosidase (red) antibodies. The Dct::LacZ melanocytes expressed β-galactosidase, allowing the detection of individual melanocytes using anti-β-galactosidase antibody. Epidermal and hair bulb melanocytes express both E- and P-cadherin. However the relative ratio of these two type 1 cadherins is inversed: epidermal melanocytes express more E-cadherin and less P-cadherin than hair bulb melanocytes

26.2.3 Melanoblast Skin Distribution and Differentiation

Once melanoblasts have reached their final destinations, they differentiate into melanocytes. The distribution of melanocytes differs depending on the species. In humans, melanocytes are mostly located in the basal layer of the epidermis, whereas they are found mostly in hair follicles in animals with fur, such as rodents. In humans, melanocytes have been identified within fetal epidermis as early as 50 days of gestation. The precise mechanisms that control the organization and number of melanoblasts in the epidermis remain unknown, but the initial step is clearly the distribution of migrating melanoblasts in the dermis and their ability to cross the basement membrane from the dermis to epidermis [8]. Dermal melanocytes normally decrease in number during gestation and virtually disappear by birth, whereas epidermal melanocytes continue to proliferate and start producing mature melanosomes, in which melanin synthesis takes place. Epidermal melanocytes transfer melanin through their dendrites to the surrounding cells, the keratinocytes, by a mechanism, which is not fully understood.

26.3 Homeostasis of Melanocytes

26.3.1 The Epidermal Melanin Unit

To understand pigmentation of the skin and the factors that affect it, it is necessary to focus on the intricate cellular and molecular interactions between melanocytes and keratinocytes. Epidermal interfollicular melanocytes are surrounded by keratinocytes, with which they are in contact via their dendrites, at a ratio of approximately 30–40 keratinocytes per

melanocyte. This association has been called the epidermal melanin unit [17]. The cell body of the melanocyte sits on the basal lamina, and its dendrites come into contact with keratinocytes as far away as the mid-stratum spinosum. The number of keratinocytes between two basal melanocytes varies mostly from 3 to 8, with an average of 6 (Fig. 26.2 and [16]). The first value (30–40 keratinocytes/melanocytes) must be considered in three dimensions, whereas the second value (3–8 keratinocytes/melanocytes) must be considered in two dimensions. Although the density of epidermal melanocytes varies per square millimeter in the various regions of the body, the number of epidermal melanocytes in human skin of all types is essentially constant at an average of 1500 melanocytes per square millimeter of the human epidermis. The major determinant of normal skin color is the activity of the melanocytes, i.e., the quantity and quality of pigment production, and its transfer to surrounding keratinocytes, not their density [18]. However, this mostly holds true only for normal, healthy skin; the number of melanocytes in the epidermis must be sufficient to provide skin pigmentation, which is clearly affected in vitiligo. Melanized keratinocytes are constantly shed and renewed, but not the melanocytes, which remain mostly at the basal membrane, with 5% in the suprabasal layer [16]. Dividing melanocytes are very infrequently observed in adult epidermis, consistent with the low number of melanocytes lost during shedding [19]. After 30 years of age, 10–20% of epidermal melanocytes are lost every decade [20].

Melanocytes, keratinocytes, and dermal fibroblasts communicate with each other via secreted factors and cell-cell contact. The cross talk of the various signaling pathways between these cells is a complex network that controls melanocyte homeostasis. Melanocyte-stimulating hormone (α-MSH), endothelins (EDN), basic fibroblast growth factor (β-FGF), nerve growth factors (NGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), steel factor (SCF), leukemia inhibitory factor (LIF), hepatocyte growth factor (HGF), transforming growth factor beta (TGFβ), and Jagged1/2 are keratinocyte-derived factors that regulate melanocyte proliferation and differentiation [9, 21]. The production of some of these factors is stimulated by ultraviolet radiation (UVR) exposure and can be influenced by chemical compounds, drugs, and/or stress.

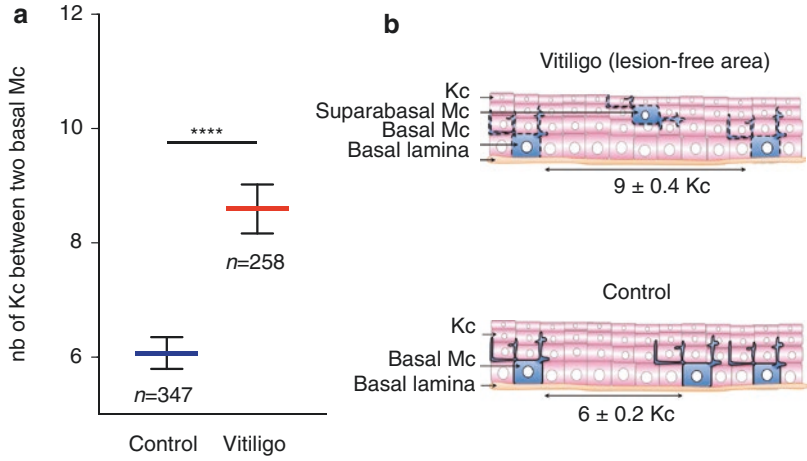


Fig. 26.2 Alteration of melanocyte-keratinocyte distribution in the epidermis of non-lesional skin of vitiligo patients. (**a**) Means of the number of keratinocytes (Kc) between two melanocytes (Mc) are represented by color horizontal bars (blue for control and red for vitiligo), and standard errors are indicated in black: mean 6 ± 0.3 for controls, 9 ± 0.4 for vitiligo. A minimum of 2220 basal keratinocytes was analyzed for each group, and a total of 347 and 258 counts were performed for controls and vitiligo skin sections. (**b**) Schematic of a control and vitiligo epidermis

26.3.2 Epidermal Melanocyte Renewal

Melanocyte stem cell (McSC) has been identified in the bulge area of hair follicles as a reservoir for the replenishment of melanocytes that are lost as adults [9]. McSC in mice appears to be similar to the amelanotic population in the lower permanent portion of the human hair follicle. Histologically, McSC can be identified by their specific cell shape and localization in hair follicles. No specific molecular marker of McSC has been discovered to date, but these cells are Dct-positive and Ki-67-negative, retain BrdU, and have very low levels of KIT and MITF (microphthalmia-associated transcription factor).

Repigmentation in vitiligo patients is often observed at the hair follicles before spreading out to generate continuous coloring to the skin. This observation supports the idea that McSC migrates from the hair follicles to the basal layer and differentiates into mature epidermal melanocytes (for more information see Chaps. 30 and 31 please check). Regions of skin in patients with vitiligo that lack hair follicles, such as the palms of the hands, can occasionally become repopulated by melanocytes. This indicates that stem cell niches are not only present in hair follicles but also in other skin structures such as sweat or sebaceous glands and the dermis. A subpopulation of dermal stem cells is able to migrate to the basement membrane of the epidermis and differentiate into melanocytes [22]. The dermal melanocyte stem cells were negative for E-cadherin and N-cadherin, whereas they acquired E-cadherin expression upon contact with epidermal keratinocytes. The other source of melanocytes is present in sweat glands of volar skin both in humans and mice and can provide differentiated melanocytes to the epidermis [23]. Although there is clear evidence that epidermal melanocytes can be replenished from McSC from the hair bulge, it is unknown whether melanocyte stem cells from the dermis and sweat/sebaceous glands could serve as a source of epidermal melanocytes for vitiligo repigmentation.

Despite their potential for renewal, the McSC population is limited, and their number declines during aging, resulting in graying hair in both humans and mice. In older humans and mice, pigmented melanocytes are present in the bulge region of the outer root sheath and are associated with McSC depletion [24]. It will be of great interest to analyze whether pigmented melanocytes can be detected in vitiligo patients in the bulge of hair follicles as an indicator of McSC depletion. The connection between the loss of epidermal melanocytes and McSC exhaustion in vitiligo requires further investigation.

26.4 Melanocyte Stability and Vitiligo

26.4.1 The Epidermal Melanin Unit in Vitiligo

The epidermal melanin unit is totally disturbed in depigmented areas of vitiligo patients as no melanocytes (or very few) are present and morphological abnormalities can also be detected in surrounding keratinocytes. Intriguingly, changes in melanocyte distribution in the basement membrane are found in normal pigmented skin of vitiligo patients located far from the depigmentated area. Indeed, the number of keratinocytes between two basal melanocytes is higher in vitiligo pigmented epidermis than in individuals without vitiligo, average of nine instead of six keratinocytes (Fig. 26.2 and [16]). Thus, the epidermal melanin unit in normal pigmented skin of vitiligo patients is modified but insufficiently to have a visible effect on skin pigmentation. Furthermore, the reduction of basal melanocytes in vitiligo is associated with an increase in the number of suprabasal melanocytes relative to those in a control population: 5% versus 25% [16].

26.4.2 E-Cadherin and Vitiligo

The reduced number of basal melanocytes associated with an increase of suprabasal

melanocytes in vitiligo leads to a number of specific questions and hypotheses.

- What is the mechanism that induces the presence of suprabasal melanocytes?
- Why are these basal melanocytes not renewed by melanocyte stem cells?
- Are these suprabasal melanocytes alive?

The presence of suprabasal melanocytes implies a cell adhesion defect in basal melanocytes that is corroborated by altered levels of E-cadherin at the membrane of melanocytes of normal pigmented skin of vitiligo patients (Fig. 26.3 and [16]). An inverse correlation between the location of the melanocytes in the upper layer of the epidermis and the homogeneous staining of E-cadherin at the membrane of melanocytes has been established, indicating that a reduction of E-cadherin in melanocytes correlates with melanocyte detachment. Furthermore, when the reduction of E-cadherin on melanocytes is associated with oxidative or mechanical stress, melanocyte detachment is induced in mice and in human epidermal reconstructed skin [16]. These data are in agreement with the melanocytorrhagy hypothesis (see chapter 31, is it still in?) proposing that melanocytic detachment occurs in response to mechanical trauma [25]. Interestingly, a polymorphism of the E-cadherin gene was recently shown to be associated with vitiligo, providing further evidence of a role for E-cadherin in the etiology of this disease [26]. Another E-cadherin gene polymorphism (39 bp away from that identified for vitiligo) is associated with Crohn's disease [27]. These two SNPs are located in intron 3 of the E-cadherin gene (*Cdh1*), between two exons encoding the portions of the precursor protein responsible for preventing protein aggregation before trafficking to the plasma membrane. Accurate processing of the precursor domain is required for E-cadherin targeting to the membrane. The SNPs associated with the Crohn's

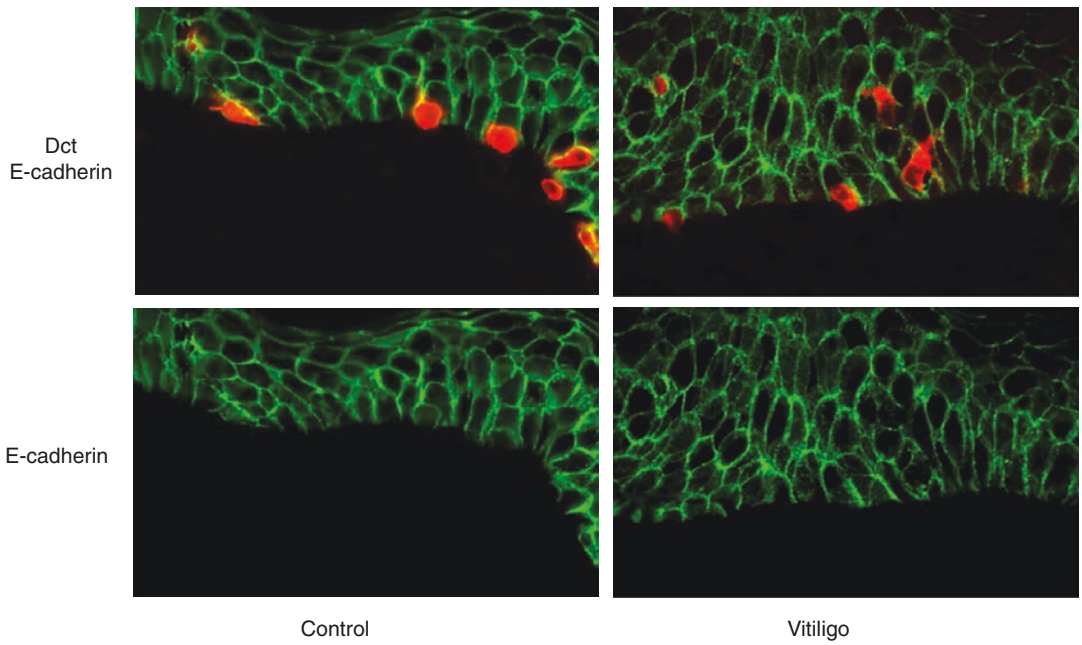


Fig. 26.3 E-cadherin membranous staining is altered in non-lesional skin of vitiligo patients. Immunofluorescence staining of E-cadherin (green) in normal skin or non-lesional skin of vitiligo patients. Melanocytes were stained with antibodies directed against Dct (red). Images are merged to visualize E-cadherin staining in melanocytes more clearly. Note the presence of suprabasal melanocytes in vitiligo epidermis

disease result in the production of a truncated mRNA, probably due to an alternative splicing, altering E-cadherin levels at the plasma membrane [27]. Of note, some patients have both vitiligo and Crohn's disease (see Chap. 13, please check). Therefore, the SNP associated with Crohn's disease results in reduced E-cadherin levels at the plasma membrane of epithelial cells, similar to the alteration of E-cadherin levels seen at the melanocyte plasma membrane in vitiligo. It is possible that the effect of E-cadherin variants associated with the unfolded protein response pathway, present in the stressed cells of both diseases, results in reduced protein levels at the plasma membrane. Importantly, the loss of E-cadherin initially found only in melanocytes

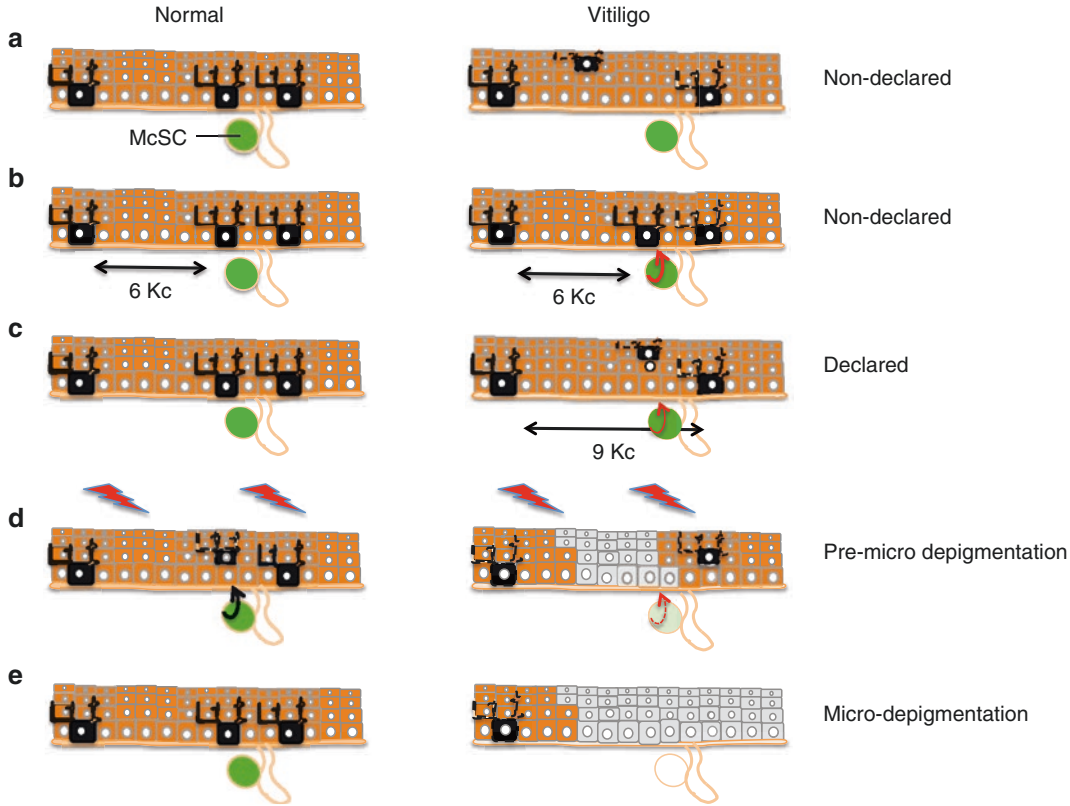


Fig. 26.4 Schematic of vitiligo initiation. Proposed mechanisms leading to early loss of pigmentation in vitiligo. (**a**, **b**) Upper panels show the preclinical vitiligo phase (non-declared): melanocytes have primary combinatory intrinsic defects leading to reduction of membranous E-cadherin in melanocytes and subsequently detachment and transepidermal loss of these cells. (**c**) The detached melanocytes are not necessarily replaced (average of nine keratinocytes between two melanocytes instead of six in the controls—"normal") indicating a defect in melanocyte renewal from the melanocyte stem cells (McSCs) located in the niche of the epidermal appendage (e.g., hair bulge). Skin remains normally pigmented even though the vitiligo is declared. (**d**) This panel shows the triggering phase after mechanical or chemical stresses (red arrows). The detachment of melanocytes increases (due to the loss of E-cadherin), and the renewal of the cells at the basal membrane becomes not sufficient for normal pigmentation (pre-micro-depigmentation). After stresses melanocytes can be seen in the suprabasal layer of control skin but are replaced efficiently by the McSC. (**e**) The lower panel shows the late phase of vitiligo initiation where the skin is depigmented due to the absence of melanocyte. The intrinsic defects in the melanocytes combined with environmental stresses lead ultimately to the total depletion of melanocytes due to exhaustion of McSC in the most severe case and create a micro-depigmentation. The vitiligo progression is certainly followed by an autoimmune response participating and accelerating importantly melanocyte destruction

in normally pigmented skin is subsequently seen also in melanocytes and keratinocytes in and around the depigmented lesions [28]. Altered E-cadherin levels in the epidermis (mainly keratinocytes) could impair epithelial integrity and permeability, consistent with the epidermal barrier defects observed in vitiligo patients and the known association with atopic dermatitis [29]. It may be informative to analyze E-cadherin levels in other E-cadherin expressing cells involved in shared diseases frequently associated with vitiligo, such as follicular cells of the thyroid. Concerning melanocyte renewal, the depletion of the melanocyte reservoir in vitiligo could be due to the death of McSC by an autoimmune response or exhaustion of the McSC population not adapted to rapid renewal or with a limited life span [30]. A possible scenario is that changes in membrane E-cadherin levels in melanocytes, leading to the detachment of basal melanocytes, occur early in the pathogenesis of vitiligo but remain silent until melanocyte renewal becomes a limiting factor for normal skin pigmentation. Finally, half of the suprabasal melanocytes appear to be apoptotic in the epidermis of vitiligo patients. This could lead to the release of danger signals important for the activation of the immune system. A model for the early steps of the pathogenesis of vitiligo focusing on a defect of cell adhesion as an initiating factor is proposed in Fig. 26.4. Further functional studies are necessary to determine whether E-cadherin could serve as a predictive factor/marker for the resistance to stressful conditions in vitiligo patients.

Although the structure of the epidermal melanin unit is known, its establishment during development, maintenance throughout life, and alterations in pathological conditions are still undocumented. Therapeutic actions that would maintain the integrity of the epidermal unit by reinforcing the adhesion of basal melanocytes would certainly be beneficial in the context of vitiligo.

Acknowledgments We thank all members of the laboratory, especially F. Luciani, A. Rubod, R. Wagner, and C. Grill who participated actively in the experimental work. This work was supported by the Ligue Nationale Contre le Cancer, Société Française de Dermatologie (SFD), INCa, and ITMO Cancer and is under the program "Investissements d'Avenir" launched by the French Government and implemented by ANR LabEx CelTisPhyBio (ANR-11-LBX-0038 and ANR-10-IDEX-0001-02 PSL).

References

1. Petit V, Larue L. Any route for melanoblasts to colonize the skin! Exp Dermatol. 2016;25:669–73.
2. Colombo S, Berlin I, Delmas V, Larue L. Classical and nonclassical melanocytes in vertebrates. In: Borovanský J, Riley PA, editors. Melanins melanosomes biosynthesis, biogenesis, physiological, pathological functions. Weinheim: Willey-Blackwell; 2011.
3. Gudjohnsen SAH, Atacho DAM, Gesbert F, Raposo G, Hurbain I, Larue L, Steingrimsson E, Petersen PH. Meningeal melanocytes in the mouse: distribution and dependence on Mitf. Front Neuroanat. 2015;9:149.
4. Adameyko I, Lallemend F, Aquino JB, Pereira JA, Topilko P, Müller T, Fritz N, Beljajeva A, Mochii M, Liste I, et al. Schwann cell precursors from nerve innervation are a cellular origin of melanocytes in skin. Cell. 2009;139:366–79.
5. Costin G-E, Hearing VJ. Human skin pigmentation: melanocytes modulate skin color in response to stress. FASEB J. 2007;21:976–94.
6. Larue L, de Vuyst F, Delmas V. Modeling melanoblast development. Cell Mol Life Sci. 2013;70:1067–79.
7. Van Raamsdonk CD, Deo M. Links between Schwann cells and melanocytes in development and disease. Pigment Cell Melanoma Res. 2013;26:634–45.
8. Luciani F, Champeval D, Herbette A, Denat L, Aylaj B, Martinozzi S, Ballotti R, Kemler R, Goding CR, De Vuyst F, et al. Biological and mathematical modeling of melanocyte development. Development. 2011;138:3943–54.
9. Osawa M. Melanocyte stem cells. In: StemBook. Cambridge, MA: Harvard Stem Cell Institute; 2009.
10. Niessen CM, Leckband D, Yap AS. Tissue organization by cadherin adhesion molecules: dynamic molecular and cellular mechanisms of morphogenetic regulation. Physiol Rev. 2011;91:691–731.
11. Nollet F, Kools P, van Roy F. Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members. J Mol Biol. 2000;299:551–72.
12. Vleminckx K, Kemler R. Cadherins and tissue formation: integrating adhesion and signaling. BioEssays. 1999;21:211–20.
13. Aktary Z, Bertrand JU, Larue L. The WNT-less wonder: WNT-independent β-catenin signaling. Pigment Cell Melanoma Res. 2016;29:524–40.
14. Pla P, Moore R, Morali OG, Grille S, Martinozzi S, Delmas V, Larue L. Cadherins in neural crest cell development and transformation. J Cell Physiol. 2001;189:121–32.

15. Nishimura EK, Yoshida H, Kunisada T, Nishikawa SI. Regulation of E- and P-cadherin expression correlated with melanocyte migration and diversification. Dev Biol. 1999;215:155–66.
16. Wagner RY, Luciani F, Cario-André M, Rubod A, Petit V, Benzekri L, Ezzedine K, Lepreux S, Steingrimsson E, Taieb A, et al. Altered E-cadherin levels and distribution in melanocytes precede clinical manifestations of vitiligo. J Invest Dermatol. 2015;135(7):1810–9.
17. Jimbow K, Quevedo WC, Fitzpatrick TB, Szabo G. Some aspects of melanin biology: 1950–1975. J Invest Dermatol. 1976;67:72–89.
18. Glimcher ME, Kostick RM, Szabo G. The epidermal melanocyte system in newborn human skin. A quantitative histologic study. J Invest Dermatol. 1973;61:344–7.
19. Jimbow K, Roth SI, Fitzpatrick TB, Szabo G. Mitotic activity in non-neoplastic melanocytes in vivo as determined by histochemical, autoradiographic, and electron microscope studies. J Cell Biol. 1975;66:663–70.
20. Whiteman DC, Parsons PG, Green AC. Determinants of melanocyte density in adult human skin. Arch Dermatol Res. 1999;291:511–6.
21. Hirobe T. How are proliferation and differentiation of melanocytes regulated? Pigment Cell Melanoma Res. 2011;24(3):462–78.
22. Li L, Fukunaga-Kalabis M, Yu H, Xu X, Kong J, Lee JT, Herlyn M. Human dermal stem cells differentiate into functional epidermal melanocytes. J Cell Sci. 2010;123:853–60.
23. Okamoto N, Aoto T, Uhara H, Yamazaki S, Akutsu H, Umezawa A, Nakauchi H, Miyachi Y, Saida T, Nishimura EK. A melanocyte--melanoma precursor niche in sweat glands of volar skin. Pigment Cell Melanoma Res. 2014;27:1039–50.
24. Nishimura EK, Granter SR, Fisher DE. Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. Science. 2005;307:720–4.
25. Gauthier Y, Cario-Andre M, Lepreux S, Pain C, Taïeb A. Melanocyte detachment after skin friction in non lesional skin of patients with generalized vitiligo. Br J Dermatol. 2003;148:95–101.
26. Tarlé RG, Silva de Castro CC, do Nascimento LM, Mira MT. Polymorphism of the E-cadherin gene CDH1 is associated with susceptibility to vitiligo. Exp Dermatol. 2015;24:300–2.
27. Muise AM, Walters TD, Glowacka WK, Griffiths AM, Ngan B-Y, Lan H, Xu W, Silverberg MS, Rotin D. Polymorphisms in E-cadherin (CDH1) result in a mis-localised cytoplasmic protein that is associated with Crohn's disease. Gut. 2009;58:1121–7.
28. Kim N-H, Lee A-Y. Reduced aquaporin3 expression and survival of keratinocytes in the depigmented epidermis of vitiligo. J Invest Dermatol. 2010;130:2231–9.
29. Liu J, Man WY, Lv CZ, Song SP, Shi YJ, Elias PM, Man MQ. Epidermal permeability barrier recovery is delayed in vitiligo-involved sites. Skin Pharmacol Physiol. 2010;23:193–200.
30. Taïeb A. Intrinsic and extrinsic pathomechanisms in vitiligo. Pigment Cell Res. 2000;13(Suppl 8):41–7.

Oxidative Stress and Intrinsic Defects

27

Mauro Picardo and Maria Lucia Dell'Anna

Contents

Abstract

Overall, experimental data support intrinsic damage and a close link between oxidative stress and immune responses. For example, histological analysis of developing lesions shows NALP1 (NACHT, LRR, and PYD domain-containing protein 1), IL-1, and catalase expression; moreover, the expression levels of heme oxygenase 1 in plasma have been linked to the activity phase of the disease and to IL-2 levels. All of these factors are involved both in stress responses and triggering of innate immunity. In summary, genetic, experimental, and clinical studies have revealed important pathways in the pathogenesis of vitiligo and have identified targets for the development of new therapies.

Our unified view considers the intrinsic defect in melanocytes as the initial event. In this model, oxidative stress in the melanocytes leads to a local inflammatory response and the activation of innate immune processes, which, in subjects with a genetic predisposition to develop autoimmunity, generate melanocyte-specific cytotoxic immune responses.

M. Picardo (✉)
Cutaneous Physiopathology and CIRM, San Gallicano Dermatological Institute, IRCCS, Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

M. L. Dell'Anna
Laboratory of Cutaneous Physiopathology, San Gallicano Dermatological Institute IFO, Rome, Italy
e-mail: marialucia.dellanna@ifo.gov.it

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_27

Key Points

- ROS are crucial and essential mediators of the intracellular signal transduction. On the other hand, when the intracellular ROS level is excessive and uncontrolled, an overall cellular impairment takes place, as that we suggest to occur in vitiligo.
- ROS also lead to impaired expression or activity of the antioxidant system.
- The imbalance of the prooxidants and antioxidant status in vitiligo has been indicated to cause increased sensitivity of melanocytes to external prooxidant stimuli and, over time, to induce a presenescent status.
- Probably as a consequence of the intracellular oxidative stress, they overexpress p53 and some of its target genes.
- Mitochondria are key in mediating melanocyte dysfunction: inhibitors of mitochondrial transition pores reduce ROS levels and cell death, transmembrane potential is lost, cardiolipin pattern and respiratory chain complex expression are altered, and the mitochondrial mass is increased.

27.1 Introduction

Vitiligo is characterized by the loss of functional melanocytes, and multiple mechanisms might contribute to this loss, including metabolic abnormalities, oxidative stress, generation of inflammatory mediators, cell detachment, and autoimmune responses [1–3]. The overall contribution of each of these processes is still under debate. Metabolic alterations are central to current concepts in pathophysiology. In our opinion, they induce an increased generation of reactive oxygen species and susceptibility to mild exogenous stimuli in the epidermis. This produces a biological senescent phenotype of skin cells; leads to the release of innate immune molecules, which trigger autoimmunity; and ultimately causes dysfunction and death of melanocytes.

27.2 Oxidative Stress

ROS are physiologically produced during usual metabolic cellular activities and they play a double role. According to a correct balanced redox status, at micromolar concentration, ROS are crucial and essential mediators of the intracellular signal transduction. On the other hand, when the intracellular ROS level is excessive and uncontrolled, an overall cellular impairment takes place, as that we suggest to occur in vitiligo [4–14].

Melanocytes are intrinsically exposed to high level of toxic compounds because during the melanin synthesis, potentially noxious intermediates are produced. In addition, melanocytes, due to their anatomical localization, are specifically exposed to UV irradiation and physical toxic agents. The early hypotheses regarding the involvement of the oxidative stress, named autocytotoxic and neurogenic, have suggested that biochemical alterations leading to the intra- or extracellular generation of free radicals and other toxic intermediates can induce melanocyte degeneration [9–16].

The attention to the potential pathogenetic role of the catecholamines comes from clinical data, which suggest that psychophysical stress (relative death, school tests, work's problems, etc.) can be associated with the onset or the worsening of the clinical manifestation of vitiligo and by the morphological demonstration of the presence of nerve ending close to melanocytes. Increased plasma level of dopamine and norepinephrine, as well as of the urinary metabolites homovanillic acid and vanillylmandelic acid, in early and active phase of the disease has been reported [6, 15–23]. A direct toxic mechanism may be due to the toxicity of the quinone and semiquinone moieties and oxyradicals generated by the oxidation of the catecholamines, and an indirect mechanism may be related to the vasoconstriction associated with norepinephrine release and the subsequent production of free radicals. Moreover, an alteration of the epidermal and systemic metabolic synthetic pathway of biopterins, required for the catecholamine synthesis, as well as serotonin and melatonin, possibly due to a genetic polymorphism, has been

reported. The alteration could lead to the accumulation of the intermediated product 7-tetrahydrobiopterin, capable of inhibiting melanin synthesis and toxic for melanocytes.

Besides the occurrence of an initial defect of the activity or expression of the antioxidant components, ROS also lead to impaired expression or activity of the antioxidant system. Epidermal catalase levels are low, probably as a result of H_2O_2-mediated deactivation of the nicotinamide adenine dinucleotide (NADH)-binding site of the enzyme, as catalase mRNA expression is unchanged. Other antioxidant enzymes, including thioredoxin reductase and thioredoxin, glutathione peroxidase, glutathione reductase, superoxide dismutases, and the repair enzymes methionine sulfoxide reductases A and B, are also altered in vitiligous skin, which indicates ROS generation causes widespread alteration of the antioxidant system [6, 7, 9–11].

Oxidative stress compromises the function of other cellular proteins and membrane lipids. One of the affected proteins is tyrosine-related protein (TRP)-1, which is important for melanin synthesis. Oxidative-driven modification of the TRP-1-calnexin complex can lead to reduced TRP-1 stability with subsequent production of toxic melanin intermediates. Moreover, the dysregulation of some metabolic pathways, even not strictly related to the melanogenetic activity, has been considered [2].

Modification and inactivation of acetylcholinesterase further promote and maintain skin oxidative damage. Redox alterations of membrane lipids affect lipid rafts, which compromises the function of membrane receptors and electron transfer and ATP production in mitochondria [2].

In vitiligo, melanocytes may be thus the theater of the loss of the redox balance because of the increased generation of free radical and dangerous metabolites or because of a defective antioxidant pattern [7, 24].

This imbalance of the prooxidants and antioxidant status in vitiligo has been indicated to cause increased sensitivity of melanocytes to external prooxidant stimuli and, over time, to induce a pre-senescent status. Stress-dependent melanocyte detachment has been observed at the borders of lesions. This finding possibly explains the Koebner phenomenon (i.e., the induction of new lesions by minor skin trauma) (Chap. 1.5.7) observed in patients with vitiligo [25, 30].

27.3 Metabolic Profile in Non-lesional Skin

Considering that a general intrinsic defect occurs in vitiligo cells, the biological phenotype and metabolic properties of cells derived from normal-appearing skin might help to clarify pathogenesis. Melanocytes from non-lesional skin show aberrant signal transduction, including hyperactivation of mitogen-activated protein kinase (MAPK) and cAMP response element-binding protein (CREB), and modifications of their membrane lipids. Moreover, probably as a consequence of the intracellular oxidative stress, they overexpress p53 and some of its target genes. This expression induces a senescence-associated secretory phenotype (SASP), which is characterized by the production of interleukin (IL)-6, matrix metalloproteinase 3, cyclooxygenase-2, and insulin-like growth factor-binding proteins 3 and 7 [31, 32]. This pre-senescent profile could be melanocyte-specific, which might explain the absence of clinically manifest aging of the entire skin. A possible hypothesis could be that mitochondrion-driven degenerative damage leads to the specific impairment or loss of melanocytes alone. p53 might facilitate DNA damage repair, which maintains genome stability, and can induce the production of *POMC* (encoding pro-opiomelanocortin) which can modulate, along SIRT1 (NAD-dependent protein deacetylase sirtuin-1) and FOXO1 (forkhead box protein O1), the energy balance and cell metabolic processes.

Recently, the ROS-mediated reduced activity of MITF, the transcription factor controlling the melanin synthesis pathway, has been proposed. MITF is usually phosphorylated and activated by receptor-associated kinases allowing the subsequent production of the melanogenetic enzymes. To bind M-Box and then promote the transcription of tyrosinase, TRP-1, and DCT, MITF needs the phosphorylation in Ser73 and

Ser49. However, the oxidation of MITF can compromise the melanogenetic pathway, as well as the cell-cycle progression (p16INK4A-dependent) and cell survival (Bcl-2-dependent). Finally, the oxidative damage of MITF may affect even its ability to antagonize TNF-a activity and to bind 6BH4, further contributing to the oxidative stress. The compromised assembly of the TRP-1 in a multiproteic complex may affect the stability of the TRP-1 itself with subsequent production of the melanin toxic intermediates. Moreover, the chemical compounds may allow the increased membrane expression of hsp70, favoring the activation of the dendritic cells and of the immune system.

An altered intracellular membrane-dependent signal transduction may take place in vitiligo melanocytes, possibly affecting the response to growth factors and dangerous stimuli. A marked membrane lipoperoxidation, together with loss and dislocation of the cardiolipin across the mitochondrial membrane, has been described in cultured vitiligo melanocytes, which could be the cause of the increased mitochondrial ROS production and of the possible reduced response to the specific growth factors.

The oxidative-mediated damage has been suggested to be involved even in the alteration of other epidermal cells, compromising their survival/proliferative potential, and also production of melanocyte-specific growth factors. Lesional keratinocytes, in fact, can present vacuolar degeneration, which has been associated with the increased exposure to H_2O_2, and produce an inadequate amount of the specific melanocyte growth factors, including membrane-bound SCF, which could lead to melanocyte apoptosis (Chap. 2.3.8). Moreover, keratinocytes themselves, following the exposure to prooxidant stimuli, can produce and release high amount of proinflammatory cytokines, such as IL-6, IL-1a, and TNF-a, leading to lymphocyte recruitment. However, TNF-a was reported to directly interfere with some mitochondrial activities through the production of peroxides (including hydrogen peroxide) leading to a mitochondria-dependent cell death or, at least, to the activation of the inflammatory genes, through the nuclear translocation of NF-kB [33–39].

27.4 Mitochondria

Mitochondria have been suggested as a key source of ROS as they mediate energy production and oxidative stress-related aging and control apoptosis in healthy cells. How mitochondrial defects impact metabolism and cell fate in vitiligo is under debate. Several lines of evidence suggest that mitochondria are key in mediating melanocyte dysfunction: inhibitors of mitochondrial transition pores reduce ROS levels and cell death, transmembrane potential is lost, cardiolipin pattern and respiratory chain complex expression are altered, and the mitochondrial mass is increased.

In other degenerative diseases, mitochondrial dysfunction has been connected to cell damage. To date, a role for the Bcl-2 protein family as modulators of cell survival, metabolism, and mitochondrial dynamics is emerging. Downstream, cytochrome C participates both in electron transfer and ATP production and activates caspases during apoptosis. Sirtuins are another example of the cross talk between metabolism and cell fate. Damaged mitochondria modulate sirtuin 1, phosphoinositide 3-kinase/Akt, protein kinase A, mTOR (mammalian target of rapamycin), and then nuclear PGC1 (PPARγ coactivator 1), CREB (cyclic AMP-responsive element-binding protein), and FOXO-1 restoring cellular metabolic normal status. As some of the key steps linking mitochondria to cell fate are defective in vitiligo cells, this evaluation of cell metabolism can illuminate our understanding of vitiligo. Altered arrangement of the ETC proteins affects the ATP level and subsequent mitochondria-nucleus cross talk by activating some key mediators, including PGC1α, resulting in increased compensatory mitochondrial mass and potentially reverting the metabolic impairment [40–50]. Moreover, in some chronic degenerative processes, the increase in mitochondrial content is an adaptive mechanism to meet the increased energy demand [41]. The inability of vitiligo cells to further cope with the energetic demand provides the metabolic basis for the pre-senescent phenotype progress [31].

27.5 The Systemic Oxidative Stress

In red blood cells and peripheral blood mononuclear cells (PBMC), a defective activity of catalase and GPx, associated with an increased production of lipid peroxidation by-products, has been described. Melanocytes and PBMC show altered antioxidant pattern, with low activity of catalase and glutathione peroxidase and high activity of SOD and xanthine oxidase, associated with the presence of index of lipid peroxidation. The alteration of the antioxidant apparatus in peripheral blood cells has been suggested to be due to the H_2O_2 produced inside the epidermis and systemically distributed by the blood. However, the ROS production could take place inside the nonepidermal cells, independently on melanocyte-specific metabolisms. In fact, the increased ROS production in PBMC has been reported to be associated with some mitochondrial alterations, such as the loss of the transmembrane potential, the cardiolipin loss, or dislocation. Moreover, the increased ROS production can be inhibited by drugs acting on the mitochondrial transition pores, such as cyclosporin A. In addition, the concentration of the shuttle enzyme MDH, physiologically ensuring the adequate level of NADH inside the mitochondria, was reported to be defective. The last alteration could be associated with the impairment of the mitochondrial activity leading to the ROS production. Finally, the DNA oxidative damage in PBMC has been described indicating that the site of production may be intracellular [11, 17].

27.6 The Possible Genetic Background

The expression of the genes of the FOXD3 pathway (SLUG, Wnt-2, SOX9, SOX10), as well as of calreticulin, GGA1, and MATP (the latest two genes, codifying for proteins involved in the trafficking of melanosome proteins, regulate the post-Golgi vesicle-mediated transport), has been found to be differently modulated in vitiligo. On this basis, the accumulation of toxic melanin intermediates may be due to an impaired melanosome transport, inner coating of the melanosome membranes, or defective expression of proteins regulating melanogenic enzyme synthesis. A catalase polymorphism (T/C SNP), leading to an incorrect subunit assembly, was reported in Caucasian population. A reduced expression of VIT1 (22q11) could account for an altered G/T mismatch repair, calcium homeostasis, and protein degradation. In acrofacial vitiligo, COMT polymorphism (G/A, val/met) could be responsible for a thermo-sensibility of the enzyme with a subsequent high production of quinones. However, the true value of the different genetic studies has to be further confirmed, because they are frequently carried out on a limited number of subjects and without validation by other authors [2, 8, 51, 52].

References

1. Le Poole IC, Das PK, van den Wijngaard RM, Bos JD, Westerhof W. Review of the etiopathomechanism of vitiligo: a convergence theory. Exp Dermatol. 1993;4:145–53.
2. Schallreuter KU, et al. Vitiligo pathogenesis: autoimmune disease, genetic defect, excessive reactive oxygen species, calcium imbalance, or what else? Exp Dermatol. 2008;2:139–140; discussion 139–160.
3. Dell'Anna ML, Picardo M. A review and a new hypothesis for non-immunological pathogenetic mechanisms in vitiligo. Pigment Cell Res. 2006;5:406–11.
4. Liu L, et al. Promoter variant in the catalase gene is associated with vitiligo in Chinese people. J Invest Dermatol. 2010;11:2647–53.
5. Sravani PV, et al. Determination of oxidative stress in vitiligo by measuring superoxide dismutase and catalase levels in vitiliginous and non-vitiliginous skin. Indian J Dermatol Venereol Leprol. 2009;3:268–2671.
6. Schallreuter KU, Wood JM, Berger J. Low catalase levels in the epidermis of patients with vitiligo. J Invest Dermatol. 1991;97:1081–5.
7. Maresca V, et al. Increased sensitivity to peroxidative agents as a possible pathogenic factor of melanocyte damage in vitiligo. J Invest Dermatol. 1997;3:310–3.
8. Bulut H, et al. Lack of association between catalase gene polymorphism (T/C exon 9) and susceptibility to vitiligo in a Turkish population. Genet Mol Res. 2011;4:4126–32.
9. Kostyuk VA, et al. Dysfunction of glutathione S-transferase leads to excess 4-hydroxy-2-nonenal and H(2)O(2) and impaired cytokine pattern in cultured keratinocytes and blood of vitiligo patients. Antiox Redox Signal. 2010;5:607–20.

10. Vafaee T, Rokos H, Salem MM, Schallreuter KU. In vivo and in vitro evidence for epidermal H2O2-mediated oxidative stress in piebaldism. Exp Dermatol. 2010;10:883–7.
11. Ozturk IC, Batcioglu K, Karatas F, Hazneci E, Genc M. Comparison of plasma malondialdehyde, glutathione, glutathione peroxidase, hydroxyproline and selenium levels in patients with vitiligo and healthy controls. Indian J Dermatol. 2008;3:106–10. https://doi.org/10.4103/0019-5154.39577.
12. Dell'Anna ML, et al. Membrane lipid alterations as a possible basis for melanocyte degeneration in vitiligo. J Invest Dermatol. 2007;5:1226–33.
13. Jimbow K, Chen H, Park JS, Thomas PD. Increased sensitivity of melanocytes to oxidative stress and abnormal expression of tyrosinase-related protein in vitiligo. Br J Dermatol. 2001;1:55–65.
14. Boissy RE, Manga P. On the etiology of contact/occupational vitiligo. Pigment Cell Res. 2004;3:208–14.
15. Hasse S, Gibbons NC, Rokos H, Marles LK, Schallreuter KU. Perturbed 6-tetrahydrobiopterin recycling via decreased dihydropteridine reductase in vitiligo: more evidence for H2O2 stress. J Invest Dermatol. 2004;2:307–13.
16. Schallreuter KU, Elwary SM, Gibbons NC, Rokos H, Wood JM. Activation/deactivation of acetylcholinesterase by H2O2: more evidence for oxidative stress in vitiligo. Biochem Biophys Res Commun. 2004;2:502–8.
17. Dell'Anna ML, et al. Membrane lipid defects are responsible for the generation of reactive oxygen species in peripheral blood mononuclear cells from vitiligo patients. J Cell Physiol. 2010;1:187–93.
18. Le Poole IC, van den Wijngaard RM, Westerhof W, Das PK. Tenascin is overexpressed in vitiligo lesional skin and inhibits melanocyte adhesion. Br J Dermatol. 1997;2:171–8.
19. Wagner R, et al. Altered e-cadherin levels and distribution in melanocytes precedes clinical manifestations of vitiligo. J Invest Dermatol. 2015. https://doi.org/10.1038/jid.2015.25.
20. Gauthier Y, Cario-Andrè M, Lepreux S, Pain C, Taieb A. Melanocyte detachment after skin friction in non lesional skin of patients with generalized vitiligo. Br J Dermatol. 2003;148:95–101.
21. Rokos H, Beazley WD, Schallreuter KU. Oxidative stress in vitiligo: photo-oxidation of pterins produces H(2)O(2) and pterin-6-carboxylic acid. Biochem Biophys Res Commun. 2002;4:805–11.
22. Moore J, Wood JM, Schallreuter KU. Evidence for specific complex formation between alpha-melanocyte stimulating hormone and 6(R)-L-erythro-5,6,7,8-tetrahydrobiopterin using near infrared Fourier transform Raman spectroscopy. Biochemistry. 1999;46:15317–24.
23. Schallreuter KU, et al. Epidermal H(2)O(2) accumulation alters tetrahydrobiopterin (6BH4) recycling in vitiligo: identification of a general mechanism in regulation of all 6BH4-dependent processes? J Invest Dermatol. 2001;1:167–74.
24. Bellei B, et al. Vitiligo: a possible model of degenerative diseases. PLoS One. 2013;3:e59782.
25. Salem MMAEL, Shalbaf M, Gibbons NCJ, Chavan B, Thornton JM, Schallreuter KU. Enhanced DNA binding capacity on up-regulated epidermal wild-type p53 in vitiligo by H2O2-mediated oxidation: a possible repair mechanism for DNA damage. FASEB J. 2009;23:3790–807.
26. Xavier JM, Morgado AL, Solá S, Rodrigues CM. Mitochondrial translocation of p53 modulates neuronal fate by preventing differentiation-induced mitochondrial stress. Antiox Redox Signal. 2014;21:1009–24.
27. Dell'Anna ML, et al. Alterations of mitochondria in peripheral blood mononuclear cells of vitiligo patients. Pigment Cell Res. 2003;16:553–9.
28. Dell'Anna ML, Maresca V, Briganti S, Camera E, Falchi M, Picardo M. Mitochondrial impairment in peripheral blood mononuclear cells during the active phase of vitiligo. J Invest Dermatol. 2001;117:908–13.
29. Nakagawa T, Guarente L. SnapShot: sirtuins, NAD, and aging. Cell Metab. 2014;20:192.
30. Imai S, Guarente L. NAD+ and sirtuins in aging and disease. Trends Cell Biol. 2014;24:464–71.
31. Shulyakova N, Sidorova-Darmos E, Fong J, Zhang G, Mills LR, Eubanks JH. Over-expression of the Sirt3 sirtuin protects neuronally differentiated PC12 cells from degeneration induced by oxidative stress and trophic withdrawal. Brain Res. 2014. pii:S0006-8993(14)01161-5.
32. Vega-Naredo I, Cunha-Oliveira T, Serafim TL, Sardao VA, Oliveira PJ. Analysis of pro-apoptotic protein trafficking to and from mitochondria. Methods Mol Biol. 2015;1241:163–80.
33. Green DR, Galluzzi L, Kroemer G. Cell biology. Metabolic control of cell death. Science. 2014;345:1250256.
34. Martel C, Wang Z, Brenner C. VDAC phosphorylation, a lipid sensor influencing the cell fate. Mitochondrion. 2014. pii: 1400100-7.
35. Basak NP, Roy A, Banerjee S. Alteration of mitochondrial proteome due to activation of Notch1 signaling pathway. J Biol Chem. 2014;11:7320–34.
36. de Moura MB, Uppala R, Zhang Y, Van Houten B, Goetzman ES. Overexpression of mitochondrial sirtuins alters glycolysis and mitochondrial function in HEK293 cells. PLoS One. 2014;9:e106028.
37. Dai SH, et al. Sirt3 attenuates hydrogen peroxide-induced oxidative stress through the preservation of mitochondrial function in HT22 cells. Int J Mol Med. 2014;34:1159–68.
38. Wu YT, Wu SB, Wie YH. Roles of sirtuins in the regulation of antioxidant defense and bioenergetic function of mitochondria under oxidative stress. Free Radic Res. 2014;48:1070–84.
39. Giblin W, Skinner ME, Lombard DB. Sirtuins: guardians of mammalian healthspan. Trends Genet. 2014;30:271–86.

40. Prignano F, et al. Ultrastructural and functional alterations of mitochondria in perilesional vitiligo skin. J Dermatol Sci. 2009;54:157–67.
41. Bondanza S, et al. Keratinocyte cultures from involved skin in vitiligo patients show an impaired in vitro behaviour. Pigment Cell Res. 2007;20:288–300.
42. Bastonini E, Kovacs D, Ottaviani M, Dell'Anna ML, Picardo M. Vitiligo: focusing on the dermal compartment. OP at XII International Pigment Cell Conference, 4–7 September 2014, Singapore, abstract book p970; 2014.
43. Zhang CF, et al. Suppression of autophagy dysregulates the antioxidant response and causes premature senescence of melanocytes. J Invest Dermatol. 2014; https://doi.org/10.1038/jid.2014.439.
44. Ainger SA, et al. DCT protects human melanocytic cells from UVR and ROS damage and increases cell viability. Exp Dermatol. 2014; https://doi.org/10.1111/exd.12574.
45. Lee A-Y, Kim N-H, Choi W-I, Youm Y-H. Less keratinocyte-derived factors related to more keratinocyte apoptosis in depigmented than normally pigmented suction-blistered epidermis may cause passive melanocyte death in vitiligo. J Invest Dermatol. 2005;124:976–83.
46. Cario-André M, Pain C, Gauthier Y, Casoli V, Taoeb A. In vivo and in vitro evidence of dermal fibroblasts influence on human epidermal pigmentation. Pigment Cell Res. 2006;19:434–42.
47. Imokawa G. Autocrine and paracrine regulation of melanocytes in human skin and in pigmentary disorders. Pigment Cell Res. 2004;17:96–100.
48. Shi Y, Luo LF, Liu XM, Zhou Q, Xu SZ, Lei TC. Premature graying as a consequence of compromised antioxidant activity in hair bulb melanocytes and their precursors. PLoS One. 2014;9:e93589. https://doi.org/10.1371/journal.pone.0093589.
49. Kim J, et al. p53 induces skin aging by depleting Blimp1+ sebaceous gland cells. Cell Death Dis. 2014;5:e1141. https://doi.org/10.1038/cddis.2014.87.
50. Laddha NC, et al. Role of oxidative stress and autoimmunity in onset and progression of vitiligo. Exp Dermatol. 2014;5:352–3.
51. Mosenson JA, et al. Mutant HSP70 reverses autoimmune depigmentation in vitiligo. Science Transl Med. 2013;5:174ra128.
52. Yu R, et al. Transcriptome analysis reveals markers of aberrantly activated innate immunity in vitiligo lesional and non-lesional skin. PLoS One. 2012;7:e51040.

Immunity/Immunopathology

28

Kirsten C. Webb, Steven W. Henning,
and I. Caroline Le Poole

Contents

Abstract

For appreciable time, the pathophysiology leading to ultimate melanocyte destruction remained uncertain. This is because skin-infiltrating T cells involved in melanocyte loss are few in number and are only observed in actively depigmenting skin; thus, such infiltrates are easily overlooked. Moreover, T cells were more difficult to distinguish before antibodies became readily available for immunohistology. Ample support exists for autoimmunity as a chief etiopathological factor in vitiligo: susceptible individuals exhibit polymorphisms in immune regulatory genes which promote autoimmunity in the cutaneous microenvironment; additionally,

K. C. Webb
Division of Dermatology, Department of Medicine, Loyola University Chicago, Maywood, IL, USA

S. W. Henning
Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL, USA

I. C. Le Poole (✉)
Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL, USA

Department of Dermatology, Microbiology and Immunology, Northwestern University, Chicago, IL, USA
e-mail: caroline.lepoole@northwestern.edu

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_28

vitiligo has a well-established association with other autoimmune diseases. Among the possibly involved cell populations, Langerhans cells might contribute to depigmentation on-site (perhaps through continued melanocyte antigen presentation to cytotoxic T cells), thereby preventing viability of any melanocytes attempting to repopulate the skin. The HSP-native protein complexes can trigger a local immune response directed at the cells from which the native proteins originate. Upon melanocyte stress and subsequent HSP70i release, antigen-presenting cells will recruit an initial cohort of melanocyte-reactive T cells that produce IFN-γ upon antigen recognition. This would lead to CXCL10 production and further recruitment to the epidermis. The absence of Tregs in vitiligo skin is likewise best explained by differential chemokine expression in lesional skin, mainly involving CCL22.

Key Points

- Progressive depigmentation in vitiligo relies on skin-infiltrating cytotoxic T cells specific for melanocyte self-antigens.
- Melanocytes in perilesional skin are also sensitive to self-reactive antibodies.
- Regulatory T cells (Tregs) are present in decreased numbers in vitiligo-affected skin.
- Stress proteins including inducible heat shock protein 70 (HSP70i) link initial skin trauma to the adaptive immune responses that follow.
- Interferon-gamma (IFN-γ) signaling through the JAK-STAT pathway drives vitiligo pathogenesis by recruiting cytotoxic T cells to the skin.
- Stress protein upregulation, activation of antigen-presenting cells, recruitment of melanocyte-reactive T cells, and a paucity of regulatory T cells are intertwined phenomena that lie at the heart of vitiligo pathogenesis.
- Promising immunotherapeutic strategies are being considered for vitiligo treatment including modified HSP70 delivery, chemokine receptor blockade, JAK-STAT inhibitor application, adoptive Treg transfer, and anti-cytokine therapies.
- Vitiligo development is considered a positive prognostic factor in melanoma, as it serves as a marker of generation of robust anti-melanocyte tumor responses.

28.1 Establishment of Vitiligo as an Autoimmune Disease Entity

The disease course of vitiligo is variable, and it affects between 0.5% and 1% of the population [1]. The clinical finding of depigmented skin patches correlates with the finding of nearly complete absence of melanocytes histologically [2, 3]. This observation prompted studies that would explain disease etiology in terms of melanocyte destruction rather than suppression of pigmentation. For an appreciable time, the pathophysiology leading to ultimate melanocyte destruction remained uncertain. This is because skin-infiltrating T cells involved in melanocyte loss are few in number and are only observed in actively depigmenting skin; thus, such infiltrates are easily overlooked. Moreover, T cells were more difficult to distinguish before antibodies became readily available for immunohistology [4, 5], and, initially, most attention was directed toward possible humoral involvement in vitiligo [6, 7]. Observations including the transmission of disease through adoptive transfer of antibodies [8, 9], as well as reduced surface expression of factors such as decay-accelerating factor (DAF) that otherwise protects from complement-mediated destruction, also led to antibodies being considered as the culprits in melanocyte destruction [10]. For the morphology of cultured melanocytes required for such studies, see Fig. 28.1. However, as the target antigens identified by these studies are not necessarily expressed in the cell membrane, anti-melanocyte

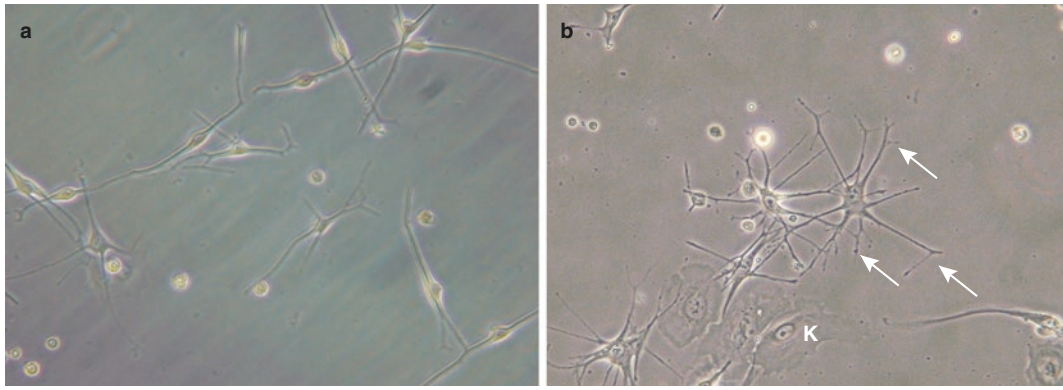


Fig. 28.1 Dendritic morphology of recently plated melanocytes in culture. Cells were isolated from (C) neonatal foreskin or (V) a scalp biopsy from a vitiligo patient by overnight enzymatic treatment and plated in replete media. Note that vitiligo melanocytes exhibit multiple, branched dendrites (arrows) in contrast to the more bipolar morphology of healthy neonatal melanocytes. *K* keratinocytes

antibodies were ultimately considered an epiphenomenon of the disease. Since B cell activation and T cell activation are intimately related and mutually supportive [11–13], renewed attention to B cell and antibody involvement is to be expected.

While many environmental factors can contribute to disease precipitation, the consensus reached by the Vitiligo European Task Force (VETF) is that autoimmunity contributes to all cases of vitiligo [14]. Interestingly, this includes segmental vitiligo [15], which was initially considered a developmental defect [16]. Vitiligo likely manifests itself when certain environmental factors affect individuals with a genetic predisposition for the condition. Interestingly, many of the proposed vitiligo susceptibility genes have immune modulatory functions, including interleukin-2 receptor alpha chain (IL2RA), ubiquitin-associated and SH3 domain-containing A protein (UBASH3A), and C1q and tumor necrosis factor-related protein (C1QTNF6) [17]. Human leukocyte antigens (HLA)-A2 [17, 18] and HLA-DR4 [17, 19] have also been linked with vitiligo development. Indeed, MHC involvement is a hallmark of autoimmunity [20]. In the following sections, we describe the interplay between the cutaneous microenvironment and immunologic factors that lead to ultimate melanocyte destruction in vitiligo.

28.2 Cellular Immunity in Vitiligo

Ample support exists for autoimmunity as a chief etiopathological factor in vitiligo. As discussed above, susceptible individuals exhibit polymorphisms in immune regulatory genes which promote autoimmunity in the cutaneous microenvironment. Additionally, vitiligo has a well-established association with other autoimmune diseases [6], the most common of which is Hashimoto's thyroiditis [21]. In fact, this association is common enough to warrant checking antithyroid antibodies, including antithyroid peroxidase antibody and anti-thyroglobulin levels, in addition to baseline thyroid-stimulating hormone (TSH) levels in newly diagnosed vitiligo patients [22, 23]. Other associated autoimmune diseases include alopecia areata, diabetes mellitus type I, pernicious anemia, and Addison's disease [21] (see Chap. 13). Some of the more pertinent findings supporting contributory autoimmune mechanisms in vitiligo involve the inflammatory infiltrates consistently identified in newly vitiligo-afflicted skin. Specifically, lymphocytic infiltrates exhibiting a decreased ratio of $CD4^+/CD8^+$ T cells have been identified in perilesional areas of patients with active depigmentation [24, 25]. The cytotoxic ($CD8^+$) T cells present in perilesional areas are activated [26] and are located in close proximity to melanocytes and melanocyte fragments in the basal layer of the epidermis

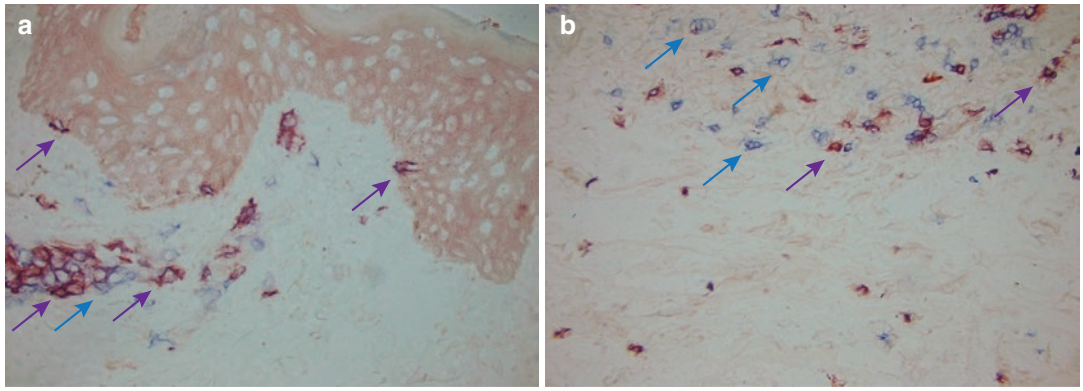


Fig. 28.2 T cells in vitiligo and melanoma. Sections of (**a**) depigmenting vitiligo skin and (**b**) a melanoma tumor metastasis were stained with antibodies to CD3 (blue) and CD8 (red). Note infiltrates consisting of primarily CD8+ T cells in vitiligo skin with some cytotoxic T cells approaching the basal layer of the epidermis. A larger proportion of the tumor-infiltrating T cells belongs to the $CD4^+$ subset of helper and regulatory T cells (blue)

(Fig. 28.2). Notably, their presence correlates with melanocyte disappearance [25]. These cytotoxic T cell infiltrates are found in perilesional skin of vitiligo patients [24], suggesting a role in melanocyte destruction. Indeed, these skin-infiltrating T cells are reactive with melanocyte self-antigens [27, 28]. Circulating melanocyte antigen-specific T cells have been found in increased levels in vitiligo patients as well [29], but, given their location, the skin-homing T cells are of greater pathogenic significance. Further supporting the role of antigen-specific T cells in vitiligo pathogenesis is their epidermal tropism [30, 31].

28.3 Antigen-Presenting Cells in Vitiligo

The development of cutaneous immune responses begins with antigen-presenting cells (APCs) residing in the epidermis and dermis. Indeed, these APCs ultimately promote T cell activation, which, in turn, begets both further T cell responses (cellular immunity), as well as B cell activation and antibody formation (humoral immunity). Numerous types of professional antigen-presenting cells reside in the skin, including macrophages, Langerhans cells (LC), and dermal dendritic cells [32]. Dendritic cells (DCs) play a particularly important role in cutaneous immune responses by patrolling for pathogens. Pathogenic signals such as microbial peptides interact with receptors on resident dendritic cells, resulting in DC maturation and antigen presentation. This maturation involves a marked increase in a number of MHC class II and costimulatory molecules on the DC cell surface [33] and allows DCs to process antigens and activate T cells efficiently [34]. Two important receptor families which commonly stimulate DC maturation are the toll-like receptors (TLRs) and tumor necrosis factor (TNF) receptors [35–39]. Dendritic cells then endocytose, process, and present antigenic peptides on the cell surface in the context of major histocompatibility complex (MHC) molecules [40]. After migrating to skin-draining lymph nodes, they interact with, activate, and recruit cytotoxic and helper T cells specific to the antigens presented [41, 42]. Thus, antigen-presenting cells govern the type of helper T cell response elicited. Through cross-presentation, melanocyte-specific antigens displayed via MHC class I molecules give rise to the cytotoxic T cell cohort known to mediate melanocyte loss in vitiligo. Interestingly, a viral trigger may exist in some cases of vitiligo [43].

Langerhans cells are epidermal antigen-presenting cells that form the first line of defense against foreign antigens entering via the skin. They can stimulate $CD4^+$ T cells [44] and are largely responsible for inducing contact hypersensitivity reactions [45]. Sensitization to contact allergen was not observed in Langerhans cell-deficient skin [46]. The role of Langerhans cells in vitiligo is not as clearly established. Interestingly,

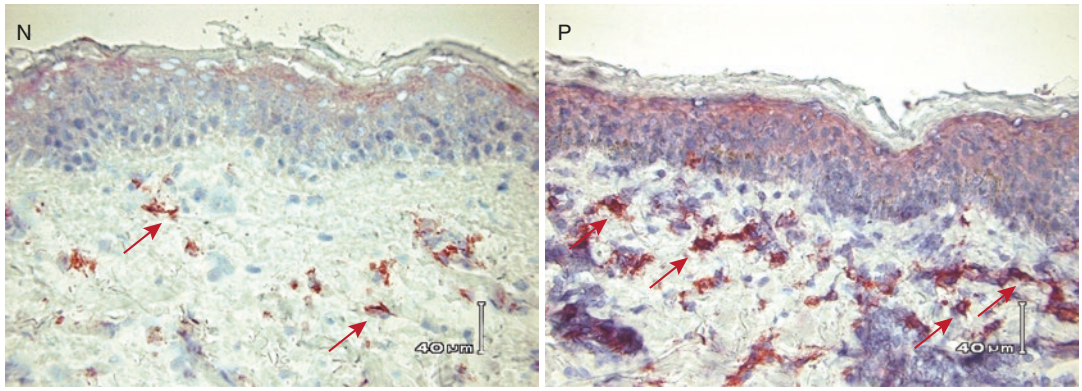


Fig. 28.3 Abundance of CD36⁺ cells in perilesional vitiligo skin. Overexpression of the thrombospondin receptor CD36 has been associated with monocyte-to-macrophage differentiation and activation of the phagocytic process. CD36 can be expressed by other cells including thrombocytes, endothelial cells, and melanocytes, yet the morphology and location of the majority of cells observed in the dermis of this patient's non-lesional and perilesional skin (detectable as a red AEC precipitate, red arrows) support the reported abundance of macrophages in depigmenting vitiligo skin (P) as compared to non-lesional skin (N)

Langerhans cells reposition themselves, settling into the basal layer of the epidermis in melanocyte-depleted vitiliginous skin [47]. Furthermore, repigmentation in response to treatment can be associated with Langerhans cell depletion [48]. Thus, Langerhans cells might contribute to depigmentation on-site (perhaps through continued melanocyte antigen presentation to cytotoxic T cells), thereby preventing viability of any melanocytes attempting to repopulate the skin. This model could be likened to a delayed type hypersensitivity response, whereby the melanocyte self-antigens represent the "contact allergen." Indeed, peptide-pulsed Langerhans cells can stimulate and expand CD8⁺ MART-I specific T cells [44]. However, as Langerhans cells are relatively inefficient at processing antigen [49, 50], their actual role in recruiting melanocyte-reactive T cells and initiating disease remains to be firmly established. Macrophages have been implicated in autoimmune disease development [51], and diabetes will not develop in macrophage-deficient mice [52]. Macrophages may likewise be pathogenic in vitiligo as they consistently infiltrate depigmenting perilesional vitiligo skin (Fig. 28.3), and their appearance correlates with melanocyte loss [24, 25]. However, the role of macrophages in vitiligo has not been clearly established [53]. Macrophages may phagocytize already apoptotic melanocytes or may actively contribute to melanocyte destruction. Once activated by interferon-gamma (IFN-γ) [54], macrophages secrete proinflammatory cytokines. Among these, TNF-α can contribute to melanocyte apoptosis [55]. Moreover, phagocytic macrophages [56] generate proinflammatory cytokines, such as IL-8, when engulfing apoptotic cells [57]. They may also serve as APCs to promote continued activation of autoreactive T cells. Taken together, there is ample cause for further studies into the pathogenic role of macrophages in vitiligo.

28.4 Heat Shock Proteins in Immune Activation

Heat shock proteins (HSPs) play an integral role in the development of several autoimmune diseases, including vitiligo [58, 59]. Expression of HSPs is induced by stress [60, 61]. HSPs bind native proteins to prevent misfolding and cellular apoptosis [62]. In addition to facilitating cellular self-preservation, these HSP-native protein complexes can also trigger a local immune response directed at the cells from which the native proteins originate [63]. Once the HSP-protein complexes are released into the microenvironment, they bind receptors located on antigen-presenting cells [64, 65]. Surrounding APCs subsequently process the bound peptides and cross-present

them to CD8+ T cells [66], eliciting immune responses toward the chaperoned antigens. Inducible heat shock protein 70 (HSP70i) can also be secreted from live cells under stress as a "chaperokine" [67]. HSP70 and other stress proteins can activate dendritic cells and subsequent T cell responses, leading to autoimmunity. HSP70 mediates development of autoimmune diabetes in mice [68] and likely plays a role in other autoimmune diseases as well. Overexpression of inducible HSP70 is sufficient to precipitate vitiligo disease [69], and vitiligo does not develop in mice that lack expression of HSP70i [70]. Importantly, the stress protein is overexpressed in vitiligo skin [71, 72]. These findings support a critical role for HSP70i in T cell-mediated melanocyte destruction.

Stress exposures that precipitate and worsen vitiligo include trauma to the affected area (koebnerization) [73], psychological stress [74], UV irradiation (oxidative stress) [75], and exposure to bleaching phenols [76]. HSP70 is upregulated in melanocytes after exposure to bleaching phenols [77], which have a well-established role in precipitating and worsening depigmentation [76, 78]. Other heat shock proteins are less likely to induce vitiligo as they are released only after cell death [79]. HSP70i is unique in that it is secreted by viable cells under stress, including melanocytes [80–82], which are ultimately later killed as the result of HSP70i-mediated cellular immune activation.

28.5 T Cell Subset Imbalance in Vitiligo

Infiltrates with decreased CD4+/CD8+ T cell ratios are found in actively depigmenting vitiligo patient skin [24, 25, 83]. T cells infiltrating vitiligo skin were shown to express the cutaneous lymphocyte antigen (CLA), a skin-homing receptor [25]. Circulating T cells in vitiligo patients are reactive with several melanocyte-specific antigens, including gp-100, tyrosinase, and Melan-A/MART-1 [83, 85]. Moreover, a T cell receptor reactive with the gp100 antigen has been characterized and cloned from patient skin [86]. T cells derived from vitiligo patient skin secrete predominantly type I cytokines in response to melanocytes, again indicative of cytotoxic T cell involvement [28].

In addition to increased numbers of skin-homing effector T cells [83], decreased skin homing of their inhibitory counterparts, the regulatory T cells (Tregs), is observed [87]. Others have reported a decreased abundance of Tregs in the circulation [88] or mentioned that Treg function may be reduced among circulating Tregs [89], but such deficiencies are expected to accompany more systemic autoimmune consequences. Decreased numbers or impaired function of Tregs have been established in systemic autoimmune disease [90]. This deficiency can well explain why tolerance to self-antigens is inadequately maintained [91]. The T cell imbalance in vitiligo skin thus creates the perfect environment for depigmentation. Destruction of melanocytes in the skin not only results in clinical depigmentation but also leads to a large number of resident memory T cells capable of recognizing melanocytic self-antigens [92, 93]. These self-reactive T cells have been found to persist in the skin long after melanocyte destruction [93]. This might well explain disease relapse in patients following periods of repigmentation.

28.6 T Cell Trafficking

In order for vitiligo to develop, melanocyte-specific cytotoxic T cells must migrate toward the epidermis in response to chemoattractant cytokines. The presence of IFN-γ is crucial for active depigmentation to ensue [94]; it induces the expression of the Th1 chemokine, CXCL10 [95], which is produced by numerous cell types, including keratinocytes, macrophages, fibroblasts, neutrophils [95, 96], and, importantly, activated T cells [97, 98]. Upon melanocyte stress and subsequent HSP70i release, antigen-presenting cells will recruit an initial cohort of melanocyte-reactive T cells that produce IFN-γ upon antigen recognition. This would lead to CXCL10 production and further T cell recruitment to the epidermis. Indeed, neutralization of CXCL10 prevented depigmentation and promoted repigmentation in mice [99]. Aside from T cells, CXCL10 can recruit monocytes/

macrophages and natural killer (NK) cells [96]. Yet, the primary contribution of CXCL10 to vitiligo development lies in skin recruitment of self-reactive T cells expressing its receptor CXCR3, as found in vitiligo patient skin [99].

The absence of Tregs in vitiligo skin is likewise best explained by differential chemokine expression in lesional skin. Cells expressing the Treg chemoattractant CCL22 are present in markedly decreased numbers in vitiligo as compared to control skin [82]. CCL22 is secreted by macrophages and dendritic cells [100]. As the CCL22 receptor, CCR4, is expressed in similar amounts by circulating Tregs from patients and controls, the decreased amounts of CCL22 may be primarily responsible for the significant underrepresentation of Tregs in vitiligo skin.

28.7 Cytokines in Vitiligo Skin

Activated perilesional vitiligo T cells secrete cytokines and chemokines, which either propagate immune mechanisms or contribute directly or indirectly to melanocyte destruction. Vitiligo development is critically dependent on IFN-γ in mouse models of vitiligo [94, 101]. Similarly, T cells derived from vitiligo patient skin secrete IFN-γ in response to melanocytes, again indicative of a type 1 cytokine response and cytotoxic T cell involvement [28]. IFN-γ-responsive effector chemokines, including CXCL9, CCL5, and especially CXCL10, are reportedly elevated in the vitiligo skin [102] and can serve to facilitate T cell trafficking toward resident melanocytes. IFN-γ also increases T cell expression of CXCR3, activates macrophages, and increases ICAM expression on endothelial cells [54, 103–105]. Each of these factors can facilitate melanocyte destruction. Importantly, IFN-γ also inhibits Treg generation through STAT1-signaling [106]. However, in mice knockout for IFN-γ, spontaneous depigmentation was restored when Tregs were depleted from the circulation [107], supporting the concept that IFN-γ involvement is not the sole pathogenic cytokine in vitiligo progression.

TNF-α contributes to vitiligo pathogenesis by supporting cytotoxic T cell development [108] and enhancing IFN-γ secretion [109]. TNF-α also inhibits melanocyte proliferation in vivo [110] and promotes melanocyte apoptosis in vitro [111]. However, its presence is not required for disease development, as vitiligo development ensued in TNF-α knockout mice [107]. Cytotoxic T cell-mediated apoptosis occurs through either the perforin-granzyme pathway [112] or the Fas/Fas ligand pathways [112–114]. Because melanocytes proved resistant to Fas ligand-mediated cell death [115], and most perilesional T cells in vitiligo skin are perforin and granzyme-B immunoreactive [25], this has implicated perforin-granzyme-mediated cell death in vitiligo. However, mice knockout for perforin can still develop vitiligo [100, 107], as can granzyme knockout mice [115]. This implies that neither mechanism is exclusively responsible for depigmentation.

IL-17 is a cytokine with controversial involvement in vitiligo pathogenesis. IL-17 is produced by T cells and natural killer cells [116] and is increased both in vitiligo patient serum and in the lesional skin [117]. Furthermore, IL-17A mRNA and IL-17A$^+$ T cells were present in increased quantities in perilesional compared to non-lesional vitiligo skin [118]. In mice, adoptive transfer of Th17 cells induced vitiligo, supporting a possible role for their cytokines, including IL-17, in vitiligo pathogenesis. However, a role for IFN-γ cannot be ruled out [119]. Thus, focusing on the blockade of production or action of the latter cytokine may be of greater utility.

28.8 Additional Players in Cellular Immunity

Importantly, melanocytes from vitiligo patients are increasingly sensitive to cellular stress, as evidenced by dilation of the endoplasmic reticulum [120] and increased levels of oxidative byproducts [121–123]. When melanocytes release stress signals into the microenvironment (reactive oxygen species and HSP70i), this activates pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) [124]. In addition to induction of adaptive immune responses via dendritic cell activation, there is evidence to support that HSP70 also stimulates local innate immune responses. Indeed, HSP70$^+$ exosomes were found

to stimulate activation and migration of natural killer (NK) cells in vitro [125]. This likely translates to human disease, as patients with vitiligo were found to have increased amounts of natural killer cells in both lesional and non-lesional skin compared to their non-vitiligo counterparts [126]. This likely renders vitiligo patients capable of generating robust innate immune responses. Interestingly, NKs taken from lesional skin of vitiligo patients expressed high levels of granzyme B [126], the serine protease that can mediate melanocyte destruction. Inflammatory dendritic cells are also increased in vitiliginous skin [59, 72] and are induced by HSP70i in vitro [127]. Gene transcription evaluation in Smyth chickens that develop spontaneous vitiligo revealed increased transcription of innate immune response genes in response to oxidative stress [128], further supporting the role of innate immune responses in vitiligo.

The role of humoral immunity in vitiligo pathogenesis may be less contributory than cellular immune responses, but it likely fuels disease progression. Vitiligo patients have elevated anti-melanocyte antibody titers [29, 129]. Such antibodies could mediate cytotoxicity toward melanocytes [130]. Moreover, B cells may be involved in propagating T cell activation [11–13] to drive depigmentation. A noteworthy observation to discriminate between causative and bystander roles for autoantibodies is that of rarely reported congenital vitiligo [131, 132], where depigmentation was postulated to result from transplacental transfer of melanocyte-specific IgG antibodies in utero.

28.9 Opportunities for Immunotherapy

- *Phototherapy*
- Ultraviolet radiation (UVR) has immunosuppressive properties and is utilized to treat many skin conditions including psoriasis, atopic dermatitis, cutaneous T cell lymphoma, and vitiligo [133]. Immunosuppression is mediated by keratinocyte secretion of Th2 cytokines, primarily IL-10 and IL4 [134]. Specifically, IL-10 appears to decrease the ability of APCs to activate Th1 cells [134], inhibits development of delayed type hypersensitivity responses [135], and decreases T cell production of type I cytokines, including IL-12, IL-8, and interferon-γ [136]. Importantly, UVB was shown to inhibit phosphorylation of STAT-1, inhibiting IFN-γ signaling [137]. Narrowband UVB (nb-UVB) also markedly increases lesional and perilesional abundance of the Treg transcription factor FoxP3 [138], implying that nb-UVB increases the number of Tregs in the cutaneous environment. This likely contributes to the observed treatment success of nb-UVB in the clinic [139]. The immunosuppressive properties of UVR are evidenced by blunted T cell immune-mediated responses as well as decreased immune surveillance of UV-induced skin cancers following exposure [140–144].
- In addition to its immunosuppressive effects, UVR has direct effects on melanocyte function. Specifically, UVR increases melanosome accumulation and transfer to keratinocytes [145]; treatment with UVR also achieves clinical repigmentation. Narrowband UVB resulted in increased keratinocyte release of bFGF and ET-1, which induce melanocyte proliferation; nb-UVB also resulted in increased melanocyte expression of $p125^{FAK}$ and MMP-2, which enhance melanocyte migration [146]. Therefore, UVR may induce melanocyte stem cell proliferation, differentiation, and migration from their reservoir site upon exposure. The source of repigmenting melanocytes is presumably the hair follicle, which would clinically explain the appearance of perifollicular repigmentation as the first sign of treatment response in UVR-treated vitiligo patients. Inactive (Dopa-negative, non-melanin-producing) melanocyte stem cells are preserved in the outer root sheath of the hair follicles of vitiligo skin [147] and do not yet express the target molecules recognized by pathogenic T cells. When clinical repigmentation was noted, the first signs microscopically were an increased number of melanocytes in the outer root sheath of the hair follicle, migrating toward the epidermis and undergoing maturation [147]. UVA and nb-UVB

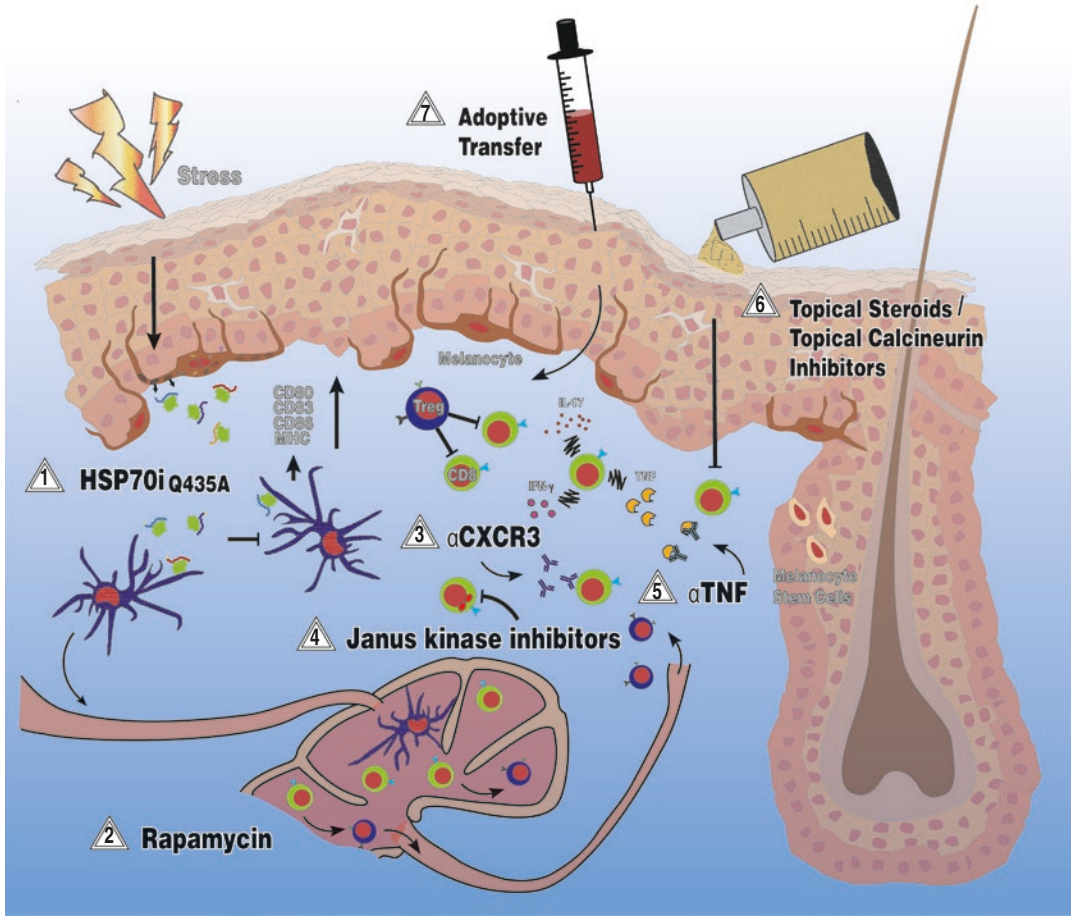


Fig. 28.4 Immunotherapeutic approaches for vitiligo. Stressed melanocytes can secrete inducible HSP70. (1) Activation of dendritic cells can be inhibited by overexpressing a modified version of inducible heat shock protein, namely, $HSP70i_{Q435A}$. Migratory dendritic cells transporting melanocyte-specific antigens can activate and recruit T cells from draining lymph nodes. (2) However, rapamycin can favor the development of Tregs over inflammatory IL-17-producing T cells and prevent effector T cell development and recruitment. (3) By introducing inhibitors of chemokine receptors such as anti-CXCR3, migration of effector T cells to the skin can be avoided. When encountering their target antigens presented by dendritic cells or upon arrival in the skin, T cells become activated. (4) Such T cell activation can be inhibited by JAK inhibitors. Within the skin, activated T cells will secrete cytokines that can promote ongoing autoimmunity. (5) Inhibitors of type 1 cytokines can neutralize such immune enhancement. The local environment is conducive to effector responses in the absence of sufficient regulatory T cells. (6) By topical application of steroids or calcineurin inhibitors, regulatory responses are encouraged, and autoimmune responses can be brought to a halt in early stages of disease. (7) Adoptive transfer of Tregs can similarly counterbalance ongoing effector responses. In preclinical models, most therapeutics not only halt depigmentation but also allow for subsequent repigmentation of the skin if a reservoir of stem cells is available

(311 nm) and excimer laser (308 nm) have all been employed and have each met with varying degrees of success in treating vitiligo patients [148, 149]. While phototherapy is an efficacious and well-tolerated treatment option for vitiligo [139], it carries some limitations including limited accessibility to patients and its blunted efficacy in more resilient body sites. Therefore, development of additional immunotherapies is prudent. The principles of UV-based and other immunotherapeutic strategies are depicted in Fig. 28.4.

- *Modified HSP70*
- To be successful, treatment should both halt ongoing melanocyte destruction and stimulate repigmentation. HSP70 lends way

to melanocyte destruction by activating dendritic cells, which subsequently leads to T cell recruitment and an anti-melanocyte immune response. $HSP70i_{Q435A}$ is a variant of inducible HSP70i of potential therapeutic benefit in vitiligo treatment [71] (=Sci. Transl. Med. 2013). Vitiligo-prone mice treated with DNA encoding $HSP70i_{Q435A}$ exhibited markedly reduced depigmentation [69]. This treatment is currently being applied to Sinclair swine with human-like skin and vitiligo lesions that develop in response to regressing melanomas [148]. In this application, the gene gun has been replaced by needle-less injectors, which are more translatable to use in the clinic and which are less traumatic than the gene gun delivery method [149]. The latter is important in vitiligo given its tendency to koebnerize. Such studies as well as further safety testing will be have to be completed before a clinical trial can be considered. By preventing dendritic cell activation, autoimmunity is prevented, and repigmentation can occur in the absence of infiltrating T cells.

- *Inhibiting T cell recruitment*
- Effector T cells engage the chemokine receptor, CXCR3, and its ligand, CXCL10, to migrate to the skin [99, 101, 152]. Recently, serum CXCL10 levels have been found to correlate with progressive disease, whereas levels decreased following successful treatment [153]. It follows that one proposed treatment strategy includes blocking the chemokine receptor, CXCR3, from binding its ligand [154]. This intervention has proven efficacious in vitiligo mice in preventing and reversing disease [99]. CXCL10 is secreted by cytotoxic T cells in vitiligo skin [97, 98]. Though anti-CXCL10 monoclonal antibodies have been tested in treating rheumatoid arthritis and ulcerative colitis [155, 156], their efficacy has not yet been tested in vitiligo patients. It is possible that chemokine depletion will be relatively less successful due to its redundancy with, in particular, CXCL11. Overall, however, interfering with effector T cell homing offers an exciting therapeutic approach in vitiligo.
- *JAK inhibitors*
- Janus kinases (JAKs) are tyrosine kinases which transmit extracellular cytokine signals to the intracellular space via their association with cytokine receptors [157]. Through their activation of the STAT transcription pathway (JAK-STAT pathway) [158], the JAK kinases mediate signaling of type I cytokines and interferons [159–161]. This ultimately affects lymphocyte activation and proliferation [160, 162, 163]. There are four mammalian JAK kinases [160, 161]. JAK 1 and 2 are clinically relevant to vitiligo, as they mediate IFN-γ signal transduction [164, 165]. Transcription of IFN-γ-inducible genes leads to CXCL10 production [166]. A JAK 1/3 inhibitor is currently FDA-approved for the treatment of moderate to severe rheumatoid arthritis. Oral treatment has also been successfully used for alopecia areata [167] and psoriasis [168], and systemically administered treatment can prevent alopecia areata in mice [169]. Randomized controlled trials are required to further define their utility and safety, but given the mechanistic relevance of JAK inhibition to vitiligo pathogenesis, their use to treat vitiligo holds promise.
- An exciting new treatment proposal entails the use of lipid-lowering statins to treat vitiligo. HMG-CoA reductase inhibitors can target a different aspect of the JAK-STAT signaling pathway than the JAK inhibitors, by inhibiting STAT1 activation [170]. Indeed, statins can prevent progression of vitiligo and promote repigmentation in mice [171] and likewise promoted repigmentation in a vitiligo patient [172].
- *Treg-based therapy*
- The paucity of skin-homing regulatory T cells [87] promotes a local environment whereby cytotoxic T cells may freely attack melanocytes. Cutaneous Treg recruitment fails in vitiligo patients due to decreased levels of the Treg chemokine CCL22 [87]. In fact, replenishing CCL22 was sufficient to overcome depigmentation [173]. Vitiligo-prone mice [174] exhibited reduced depigmentation following adoptive Treg transfer [107]. Animals experienced prolonged inhibition of depig-

mentation, while transferred Tregs migrated to the skin [107]. Likewise, vitiligo was successfully treated with rapamycin, which promoted Treg development [107, 175]. When administered with all-trans retinoic acid, rapamycin was able to confer both expansion of Tregs and biological stability of such Tregs in an inflammatory environment [176]. Rapamycin as monotherapy also conferred protection against depigmentation for up to 6 weeks after cessation of therapy in mice, highlighting its stabilizing effects on expanded Tregs [107]. Thus, creating a quantitative increase in Tregs in the skin via adoptive Treg transfer or rapamycin administration may serve as a potentially efficacious treatment modality in halting progressive vitiligo in humans.

- *Cytokine inhibition*
- The presence of minute immune infiltrates in depigmenting vitiligo skin warrants the application of antibodies to neutralize the effects of cytokines generated by activated lymphocytes. IFN-γ-neutralizing antibodies were shown to markedly reduce self-reactive cytotoxic T cell accumulation in the skin and to prevent depigmentation in a mouse model of vitiligo [94]. In the absence of IFN-γ, T cell activation is prevented, as is IFN-γ-induced melanocyte apoptosis [177]. Furthermore, melanocytes will downregulate the expression of MHC, including MHC class II [178]. TNF-α likely contributes to active depigmentation via its activation of $CD8^+$ T cells. This is supported by the finding that TNF-α inhibition has been successful in halting progressive vitiligo [179–181], in which $CD8^+$ T cells play an active role [25]. Paradoxically, some individuals developed de novo vitiligo when treated with anti-TNF-α agents. This may be explained by the fact that TNF-α can also increase Treg activity; the scale may be tipped in favor of depigmentation as a result of Treg depletion in these exceptional cases [182].

28.10 Vitiligo and Melanoma T Cell Responses

After decades without marked progress, several immune-based treatments are currently emerging for the treatment of malignant melanoma. Their efficacy is often associated with depigmentation of the skin. The development of vitiligo is a positive prognostic factor in melanoma patients [183]. This is likely the case because the presence of vitiligo denotes efficient anti-melanocyte T cell responses against shared tumor and normal melanocyte antigens [184], including MART-1 and gp100 [185]. A comparison of T cell infiltrates in vitiligo and melanoma tissue is shown in Fig. 28.2. Meanwhile, a large retrospective cohort study revealed that patients with vitiligo have a threefold decreased lifetime risk of developing melanoma [186]. Adoptive transfer of cytotoxic T cells specific for melanoma differentiation antigens has been associated with vitiligo development [31, 174, 187], again supporting a pathogenic role of autoimmune T cells in vitiligo. Interestingly, the presence of normal or increased levels of regulatory T cells in the skin of melanoma patients is associated with less effective clearing of melanoma tumors [188]; depleting these Tregs is required in order for sufficient cytotoxic T cell-mediated melanocyte destruction to ensue [93]. As vitiligo represents autoimmune melanocyte destruction in the skin, dermatologists must consider the origin of generation of these responses. While vitiligo often does not have a readily identifiable trigger by history, the appearance of this entity beyond adolescence might be a harbinger of the body generating immune responses against an underlying melanoma. This concept is similar to the clinical implications of halo nevi as a potential marker of dysplastic nevi or malignant melanocytic neoplasms [189, 190]. Therefore, new-onset vitiligo in this age group warrants a total body skin exam to screen for melanoma..

References

1. Taieb A, Picardo M. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell Res. 2007;20:27–35.
2. Kim YC, Kim YJ, Kang HY, et al. Histopathologic features in vitiligo. Am J Dermatopathol. 2008;30:112–6.
3. Le Poole IC, van den Wijngaard RMJGJ, Westerhof W, et al. Presence or absence of melanocytes in vitiligo lesions: an immunohistochemical investigation. J Invest Dermatol. 1993;100:816–22.
4. Buckley WR, Lobitz WC Jr. Vitiligo with a raised inflammatory border. Arch Dermatol Syph. 1953;67:316–20.
5. Ishii M, Hamada T. Ultrastructural studies of vitiligo with inflammatory raised borders. J Dermatol. 1981;8:313–22.
6. Kemp EH, Waterman EA, Weetman AP. Immunological pathomechanisms in vitiligo. Expert Rev Mol Med. 2001;3:1–22.
7. Park YK, Kim NS, Hann SK, et al. Identification of autoantibody to melanocytes and characterization of vitiligo antigen in vitiligo patients. J Dermatol Sci. 1996;11:111–20.
8. Hara I, Takechi Y, Houghton AN. Implicating a role for immune recognition of self in tumor rejection: passive immunization against the brown locus protein. J Exp Med. 1995;182:1609–14.
9. Takechi Y, Hara I, Naftzger C, et al. A melanosomal membrane protein is a cell surface target for melanoma therapy. Clin Cancer Res. 1996;2:1837–42.
10. Venneker GT, Vodegel RM, Okada N, et al. Relative contributions of decay acceleration factor (DAF), membrane cofactor protein (MCP) and CD59 in the protection of melanocytes from homologous complement. Immunobiology. 1998;198:476–84.
11. Damle NK, Doyle LV, Grosmaire LS, et al. Differential regulatory signals delivered by antibody binding to the CD28 (Tp44) molecule during the activation of human T lymphocytes. J Immunol. 1988;140:1753–61.
12. Damle NK, Linsley PS, Ledbetter JA. Direct helper T cell-induced B cell differentiation involves interaction between T cell antigen CD28 and B cell activation antigen B7. Eur J Immunol. 1991;21:1277–82.
13. Lesslauer WF, Koning T, Ottenhoff M, et al. T90/44 (9.3 antigen). A cell surface molecule with a function in human T cell activation. Eur J Immunol. 1986;16:1289.
14. Ezzedine K, Lim HW, Suzuki T, et al. Reviewed classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. Pigment Cell Melanoma Res. 2012;25:E1–E13.
15. Van Geel NA, Mollet IG, De Schepper S, et al. First histopathological and immunophenotypic analysis of early dynamic events in a patient with segmental vitiligo associated with halo nevi. Pigment Cell Melanoma Res. 2010;23:375–84.
16. Taieb A, Morice-Picard F, Jouary T, et al. Segmental vitiligo as the possible expression of cutaneous somatic mosaicism: implications for common non-segmental vitiligo. Pigment Cell Melanoma Res. 2008;21:646–52.
17. Jin Y, Birlea SA, Fain PR, et al. Variant of TYR and autoimmunity susceptibility loci in generalized vitiligo. N Engl J Med. 2010;362:1686–97.
18. Liu JB, Li M, Chen H, et al. Association of vitiligo with HLA-A2: a meta-analysis. J Eur Acad Dermatol Venereol. 2007;21:205–13.
19. Foley LM, Lowe NJ, Misheloff E, et al. Association of HLA-DR4 with vitiligo. J Am Acad Dermatol. 1983;8:39–40.
20. Fernando MM, Stevens CR, Walsh EC, et al. Defining the role of the MHC in autoimmunity: a review and pooled analysis. PLoS Genet. 2008;4:e1000024.
21. Akay BN, Bozkir M, Anadolu Y, et al. Epidemiology of vitiligo, associated autoimmune diseases and audiological abnormalities: Ankara study of 80 patients in Turkey. J Eur Acad Dermatol Venereol. 2010;24:1144–50.
22. Daneshpazhooh M, Behjati J, Akhyani M, et al. Anti-thyroid peroxidase antibody and vitiligo: a controlled study. BMC Dermatol. 2006;6:3.
23. Hegedüs L, Heidenheim M, Gervil M, et al. High frequency of thyroid dysfunction in patients with vitiligo. Acta Derm Venereol. 1994;74:120–3.
24. Le Poole IC, van den Wijngaard RMJGJ, Westerhof W. Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. Am J Pathol. 1996;148:1219–28.
25. Van den Wijngaard R, Wankowicz-Kalinska A, Le Poole C, et al. Local immune response in skin of generalized vitiligo patients. Destruction of melanocytes is associated with the prominent presence of CLA+ T cells at the perilesional site. Lab Investig. 2000;80:1299–309.
26. Wu J, Zhou M, Wan Y, et al. CD8+ T cells from vitiligo perilesional margins induce autologous melanocyte apoptosis. Mol Med Rep. 2013;7:237–41.
27. Steitz J, Wenzel J, Gaffal E, et al. Initiation and regulation of CD8+ T cells recognizing melanocytic antigens in the epidermis: implications for the pathophysiology of vitiligo. Eur J Cell Biol. 2004;83:797–803.
28. Wańkowicz-Kalińska A, van den Wijngaard RM, Tigges BJ, et al. Immunopolarization of CD4+ and CD8+ T cells to Type-1-like is associated with melanocyte loss in human vitiligo. Lab Investig. 2003;83:683–95.
29. Ogg GS, Dunbar PR, Romero P, et al. High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. J Exp Med. 1998;188:1203–8.
30. Van den Boorn JG, Konijnenberg D, Dellemijn TA, et al. Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. J Invest Dermatol. 2009;129:2220–32.

31. Yee C, Thompson JA, Roche P, et al. Melanocyte destruction after antigen-specific immunotherapy of melanoma: direct evidence of T cell mediated vitiligo. J Exp Med. 2000;192:1637–44.
32. Mathers AR, Larrgenia AT. Professional antigen-presenting cells of the skin. Immunol Res. 2006;36:127–36.
33. Mellman I, Steinman RM. Dendritic cells: specialized and regulated antigen processing machines. Cell. 2001;106:255–8.
34. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annu Rev Immunol. 2003;21:685–711.
35. Akbari O, Panjwani N, Garcia S, et al. DNA vaccination: transfection and activation of dendritic cells as key events for immunity. J Exp Med. 1999;189:169–78.
36. Bonifaz L, Bonnyay D, Mahnke K, et al. Efficient targeting of protein antigens to the dendritic cell receptor DEC205 in the steady state leads to antigen presentation on MHC class I products and peripheral CD8+ T cell tolerance. J Exp Med. 2002;196:1627–38.
37. Hawiger D, Inaba K, Dorsett Y, et al. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J Exp Med. 2001;194:769–80.
38. Liu K, Iyoda T, Saternus M, et al. Immune tolerance following delivery of dying cells to dendritic cells in situ. J Exp Med. 2002;196:1091–7.
39. Sparwasser T, Vabulas RM, Villmow B, et al. Bacterial CpG-DNA activates dendritic cells in vivo: T helper cell-independent cytotoxic T cell responses to soluble proteins. Eur J Immunol. 2000;30:3591–7.
40. Guermonprez P, Valladeau J, Zitvogel L, et al. Antigen presentation and T cell stimulation by dendritic cells. Annu Rev Immunol. 2002;20:621–67.
41. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. Annu Rev Immunol. 2000;18:767–811.
42. Nestle FO, Banchereau J, Hart D. Dendritic cells: on the move from bench to bedside. Nat Med. 2001;7:761–5.
43. Grimes PE, Sevall JS, Vojdani A. Cytomegalovirus DNA identified in skin biopsy specimens of patients with vitiligo. J Am Acad Dermatol. 1996;35:21–6.
44. Klechevsky E, Morita R, Liu M, et al. Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. Immunity. 2008;29:497–510.
45. Toews GB, Bergstresser PR, Streilein JW. Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. J Immunol. 1980;124:445–53.
46. Dumay O, Karam A, Vian L, et al. Ultraviolet AI exposure of human skin results in Langerhans cell depletion and reduction of epidermal antigen-presenting cell function: partial protection by a broad-spectrum sunscreen. Br J Dermatol. 2001;144:1161–8.
47. Birbeck MS, Breathnach AS, Everall JD. An electron microscope study of basal melanocytes and high-level clear cells (Langerhans cells) in vitiligo. J Invest Dermatol. 1961;37:51.
48. Kao C-H, Yu H-S. Depletion and repopulation of Langerhans cells in nonsegmental type vitiligo. J Dermatol. 1990;17:287–96.
49. Inaba KG, Schuler MD, Witmer J, et al. The immunologic properties of purified Langerhans cells: distinct requirements for the stimulation of unprimed and sensitized T lymphocytes. J Exp Med. 1986;164:605.
50. Schuler G, Steinman RM. Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells in vitro. J Exp Med. 1985;161:526.
51. Krausgruber T, Blazek K, Smallie T, et al. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. Nat Immunol. 2011;12:231–8.
52. Jun HS, Yoon CS, Zbytnuik L, et al. The role of macrophages in T cell-mediated autoimmune diabetes in nonobese diabetic mice. J Exp Med. 1999;189:347–58.
53. Richmond JM, Frisoli ML, Harris JE. Innate immune mechanisms in vitiligo: danger from within. Curr Opin Immunol. 2013;25:676–82.
54. Schroder KP, Hertzog PJ, Ravasi T, et al. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004;75:163–89.
55. Soma T, Ogo M, Suzuki J, et al. Analysis of apoptotic cell death in human hair follicles in vivo and in vitro. J Invest Dermatol. 1998;111:948–54.
56. Van Furth R. Development and distribution of mononuclear phagocytes. In: Gallin JI, Goldstein IM, Snyderman R, editors. Inflammation. New York: Raven Press; 1992. p. 325.
57. Kurosaka K, Watanabe N, Kobayashi Y. Production of proinflammatory cytokines by resident tissue macrophages after phagocytosis of apoptotic cells. Cell Immunol. 2001;211:1–7.
58. Rajaiah R, Moudgil KD. Heat-shock proteins can promote as well as regulate autoimmunity. Autoimmun Rev. 2009;8:388–93.
59. Mosenson JA, Eby JM, Hernandez C, et al. A central role for inducible heat-shock protein 70 in autoimmune vitiligo. Exp Dermatol. 2013;22:566–9.
60. Määttänen P, Gehring K, Bergeron JJ, Thomas DY. Protein quality control in the ER: the recognition of misfolded proteins. Semin Cell Dev Biol. 2012;21:500–11.
61. Welch NJ. Heat shock proteins functioning as molecular chaperones: their roles in normal and stressed cells. In: Molecular chaperones. Netherlands: Springer; 1993. p. 71–7.
62. Benbrook DM, Long A. Integration of autophagy, proteasomal degradation, unfolded protein response and apoptosis. Exp Oncol. 2012;34:286–97.
63. Calderwood SK, Stevenson MA, Murshid A. Heat shock proteins, autoimmunity, and cancer treatment. Autoimmune Dis. 2012;2012:486069.

64. Binder R, Han D, Srivastava PK. CD91: a receptor for the heat shock protein gp96. Nat Immunol. 2000;1:51.
65. Srivastava PK, Udono H, Blachere NE, et al. Heat shock proteins transfer peptides during antigen processing and CTL priming. Immunogenetics. 1994;39:93–8.
66. Basu S, Binder RJ, Suto R, et al. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-κB pathway. Int Immunol. 2000;12:1539–46.
67. Mambula SS, Calderwood SK. Heat shock protein 70 is secreted from tumor cells by a nonclassical pathway involving lysosomal endosomes. J Immunol. 2006;177:7849–57.
68. Millar DG, Garza KM, Odermatt B, et al. Hsp70 promotes antigen-presenting cell function and converts T-cell tolerance to autoimmunity in vivo. Nat Med. 2003;9:1469–76.
69. Denman CJ, McCracken J, Hariharan V, et al. HSP70i accelerates depigmentation in a mouse model of autoimmune vitiligo. J Invest Dermatol. 2008;128:2041–8.
70. Mosenson JA, Zloza A, Klarquist J, et al. HSP70i is a critical component of the immune response leading to vitiligo. Pigment Cell Melanoma Res. 2012;25:88–98.
71. Abdou AG, Maraee AH, Reyad W. Immunohistochemical expression of heat shock protein 70 in vitiligo. Ann Diag Pathol. 2013;17:245–9.
72. Mosenson JA, Zloza A, Nieland JD, et al. Mutant HSP70 reverses autoimmune depigmentation in vitiligo. Sci Transl Med. 2013;5:174ra28.
73. Sanghavi SA, Dongre AM, Khopkar US. Koebnerization and generalized spread of vitiligo following radiotherapy. Indian Dermatol Online J. 2013;4:147.
74. Picardi A, Pasquini P, Cattaruzza MS, et al. Stressful life events, social support, attachment security and alexithymia in vitiligo. Psychother Psychosom. 2003;72:150–8.
75. Maresca V, Roccella M, Roccella F, et al. Increased sensitivity to peroxidative agents as a possible pathogenic factor of melanocyte damage in vitiligo. J Invest Dermatol. 1997;109:310–3.
76. Boissy RE, Manga P. On the etiology of contact/occupational vitiligo. Pigment Cell Res. 2004;17:208–14.
77. Kroll TM, Bommiasamy H, Boissy RE, et al. 4-Tertiary butyl phenol exposure sensitizes human melanocytes to dendritic cell-mediated killing: relevance to vitiligo. J Invest Dermatol. 2005;124:798–806.
78. Van den Boorn JG, Picavet DI, van Swieten PF, et al. Skin-depigmenting agent monobenzone induces potent T-cell autoimmunity toward pigmented cells by tyrosinase hapentization and melanosome autophagy. J Invest Dermatol. 2011;131:1240–51.
79. Strbo N, Podack ER. Secreted heat shock protein 96-Ig: an innovative vaccine approach. Am J Reprod Immunol. 2008;59:407–16.
80. Asea A. Mechanisms of HSP72 release. J Biosci. 2007;32:579–84.
81. Vega VL, Rodríguez-Silva M, Frey T, et al. Hsp70 translocates into the plasma membrane after stress and is released into the extracellular environment in a membrane-associated form that activates macrophages. J Immunol. 2008;180:4299–307.
82. Mosenson JA, Flood K, Klarquist J, et al. Preferential secretion of inducible HSP70 by vitiligo melanocytes under stress. Pigment Cell Melanoma Res. 2014;27:209–20.
83. Dwivedi M, Laddha NC, Arora P, et al. Decreased regulatory T-cells and CD4+/CD8+ ratio correlate with disease onset and progression in patients with generalized vitiligo. Pigment Cell Melanoma Res. 2013;26:586–91.
84. Mandelcorn-Monson RL, Shear NH, Yau E, et al. Cytotoxic T lymphocyte reactivity to gp100, MelanA/MART-1, and tyrosinase, in HLA-A2-positive vitiligo patients. J Invest Dermatol. 2003;121:550–6.
85. Palermo B, Campanelli R, Garbelli S, et al. Specific cytotoxic T lymphocyte responses against Melan-A/MART1, tyrosinase and gp100 in vitiligo by the use of major histocompatibility complex/peptide tetramers: the role of cellular immunity in the etiopathogenesis of vitiligo. J Invest Dermatol. 2001;117:326–32.
86. Klarquist J, Eby JM, Henning SW, et al. Functional cloning of a gp100-reactive T-cell receptor from vitiligo patient skin. Pigment Cell Melanoma Res. 2016;29(3):379–84.
87. Klarquist J, Denman CJ, Hernandez C, et al. Reduced skin homing by functional Treg in vitiligo. Pigment Cell Melanoma Res. 2010;23:276–86.
88. Lili Y, Yi W, Ji Y, et al. Global activation of CD8+ cytotoxic T lymphocytes correlates with an impairment in regulatory T cells in patients with generalized vitiligo. PLoS One. 2012;7:e37513.
89. Lin M, Zhang BX, Shen N, et al. Regulatory T cells from active non-segmental vitiligo exhibit lower suppressive ability on CD8+ CLA+ T cells. Eur J Dermatol. 2014;24:676–82.
90. Cvetanovich GL, Hafler DA. Human regulatory T cells in autoimmune diseases. Curr Opin Immunol. 2010;22:753–60.
91. Baecher-Allan C, Hafler DA. Human regulatory T cells and their role in autoimmune disease. Immunol Rev. 2006;212:203–16.
92. Boniface K, Dessarthe B, Vernisse C, et al. Vitiligo is enriched with population of skin T cells expressing a resident memory phenotype. J Invest Dermatol. 2015;135:S76.
93. Byrne KT, Cote AL, Zhang P, et al. Autoimmune melanocyte destruction is required for roust CD8+ memory T cell responses to mouse melanoma. J Clin Invest. 2011;121:1797–809.

94. Harris JE, Harris TH, Weninger W, et al. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-γ for autoreactive CD8+ T-cell accumulation in the skin. J Invest Dermatol. 2012;132:1869–76.
95. Luster AD, Ravetch JV. Biochemical characterization of a gamma interferon-inducible cytokine (IP-10). J Exp Med. 1987;166:1084–97.
96. Groom JR, Luster AD. CXCR3 ligands: redundant, collaborative and antagonistic functions. Immunol Cell Biol. 2011;89:207–15.
97. Biddison WE, Taub DD, Cruikshank WW, et al. Chemokine and matrix metalloproteinase secretion by myelin proteolipid protein-specific CD8+ T cells: potential roles in inflammation. J Immunol. 1997;158:3046–53.
98. Gattass CR, King LB, Luster AD, et al. Constitutive expression of interferon gamma-inducible protein 10 in lymphoid organs and inducible expression in T cells and thymocytes. J Exp Med. 1994;179:1373–8.
99. Rashighi M, Agarwal P, Richmond JM, et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. Sci Transl Med. 2014;6:223ra23.
100. Kimura S, Tanimoto A, Wang KY, et al. Expression of macrophage-derived chemokine (CCL22) in atherosclerosis and regulation by histamine via the H2 receptor. Pathol Int. 2012;62:675–83.
101. Gregg RK, Nichols L, Chen Y, et al. Mechanisms of spatial and temporal development of autoimmune vitiligo in tyrosinase-specific TCR transgenic mice. J Immunol. 2010;184:1909–17.
102. Antonelli A, Ferrari SM, Fallahi P. The role of the Th1 chemokine CXCL10 in vitiligo. Ann Transl Med. 2015;3:S1.
103. Hubbard AK, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) expression and cell signaling cascades. Free Radic Biol Med. 2000;28:1379–86.
104. Moser B, Wolf M, Walz A, et al. Chemokines: multiple levels of leukocyte migration control. Trends Immunol. 2004;25:75–84.
105. Nakajima C, Mukai T, Yamaguchi N, et al. Induction of the chemokine receptor CXCR3 on TCR-stimulated T cells: dependence on the release from persistent TCR-triggering and requirement for IFN-γ stimulation. Eur J Immunol. 2002;32:1792–801.
106. Caretto D, Katzman SD, Villarino AV, et al. Cutting edge: the Th1 response inhibits the generation of peripheral regulatory T cells. J Immunol. 2010;184:30–4.
107. Chatterjee S, Eby JM, Al-Khami AA, et al. A quantitative increase in regulatory T cells controls development of vitiligo. J Invest Dermatol. 2014;134:1285–94.
108. Ranges GE, Figari IS, Espevik T, et al. Inhibition of cytotoxic T cell development by transforming growth factor beta and reversal by recombinant tumor necrosis factor alpha. J Exp Med. 1987;166:991–8.
109. Scheurich P, Thoma B, Ricer U, et al. Immunoregulatory activity of recombinant human tumor necrosis factor (TNF)-alpha: induction of TNF receptors on human T cells and TNF-alpha-mediated enhancement of T cell responses. J Immunol. 1987;138:1786–90.
110. Swope VB, Abdel-Malek Z, Kassem LM, et al. Interleukins 1α and 6 and tumor necrosis factor α are paracrine inhibitors of human melanocyte proliferation and melanogenesis. J Invest Dermatol. 1991;96:180–5.
111. Kim NH, Jeon S, Lee HJ, et al. Impaired PI3K/Akt activation-mediated NF-κB inactivation under elevated TNF-α is more vulnerable to apoptosis in vitiliginous keratinocytes. J Invest Dermatol. 2007;127:2612–7.
112. Froelich CJ, Dixit VM, Yang X. Lymphocyte granule-mediated apoptosis: matters of viral mimicry and deadly proteases. Immunol Today. 1998;19:30–6.
113. Lowin B, Hahne M, Mattmann C, et al. Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathway. Nature. 1994;370:650–2.
114. Nagata S. Apoptosis by death factor. Cell. 1997;88:355–65.
115. Rivoltini L, Radrizzani M, Accornero P, et al. Human melanoma-reactive $CD4^+$ and $CD8^+$ CTL clones resist Fas ligand-induced apoptosis and use Fas/Fas ligand-independent mechanisms for tumor killing. J Immunol. 1998;161:1220–30.
116. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. N Engl J Med. 2009;361:888–98.
117. Bassiouny DA, Shaker O. Role of interleukin-17 in the pathogenesis of vitiligo. Clin Exp Dermatol. 2011;36:292–7.
118. Wang CQ, Cruz-Inigo AE, Fuentes-Duculan J, et al. Th17 cells and activated dendritic cells are increased in vitiligo lesions. PLoS One. 2011;6:e18907.
119. Muranski P, Boni A, Antony PA, et al. Tumor-specific Th17-polarized cells eradicate large established melanoma. Blood. 2008;112:362–73.
120. Boissy RE, Liu YY, Medrano EE, et al. Structural aberration of the rough endoplasmic reticulum and melanosome compartmentalization in long-term cultures of melanocytes from vitiligo patients. J Invest Dermatol. 1991;97:395–404.
121. Koca R, Armutcu F, Altinyazar HC, et al. Oxidant-antioxidant enzymes and lipid peroxidation in generalized vitiligo. Clin Exp Dermatol. 2004;29:406–9.
122. Schallreuter KU, Moore J, Wood JM, et al. In vivo and in vitro evidence for hydrogen peroxide (H_2O_2) accumulation in the epidermis of patients with vitiligo and its successful removal by a UVB-activated pseudocatalase. J Investig Dermatol Symp Proc. 1999;4:91–6.
123. Shalbaf M, Gibbons NC, Wood JM, et al. Presence of epidermal allantoin further supports oxidative stress in vitiligo. Exp Dermatol. 2008;17:761–70.
124. Chen GY, Nuñez G. Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol. 2010;10:826–37.

125. Gastpar R, Gehrmann M, Bausero MA, et al. Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. Cancer Res. 2005;65:5238–47.
126. Yu R, Broady R, Huang Y, et al. Transcriptome analysis reveals markers of aberrantly activated innate immunity in vitiligo lesional and non-lesional skin. PLoS One. 2012;7:e51040.
127. Richmond JM, Frisoli ML, Harris JE. Innate immune mechanisms in vitiligo: danger from within. Curr Opin Immunol. 2013;25:676–82.
128. Shi F, Kong BW, Song JJ, et al. Understanding mechanisms of vitiligo development in Smyth line of chickens by transcriptomic microarray analysis of evolving autoimmune lesions. BMC Immunol. 2012;13:18.
129. Ongenae K, Van Geel N, Naeyaert JM. Evidence for an autoimmune pathogenesis of vitiligo. Pigment Cell Res. 2003;16:90–100.
130. Norris DA, Kissinger RM, Naughton GM, et al. Evidence for immunologic mechanisms in human vitiligo: patients' sera induce damage to human melanocytes in vitro by complement-mediated damage and antibody-dependent cellular cytotoxicity. J Invest Dermatol. 1988;90:783–9.
131. Chandra S, Kumar A, Singh KK, et al. Congenital vitiligo. Indian J Dermatol Venereol Leprol. 1992;58:339.
132. Kedward AL, Gawkrodger DJ. Congenital stable symmetrical type vitiligo in a patient whose mother developed vitiligo during pregnancy. Eur J Dermatol. 2008;18:353.
133. Yashar SS, Gielczyk R, Scherschun L, et al. Narrowband ultraviolet B treatment for vitiligo, pruritus, and inflammatory dermatoses. Photodermatol Photoimmunol Photomed. 2003;19:164–8.
134. Ullrich SE. Mechanism involved in the systemic suppression of antigen-presenting cell function by UV irradiation: keratinocyte-derived IL-10 modulates antigen-presenting cell function of splenic adherent cells. J Immunol. 1994;152:3410–6.
135. Rivas JM, Ullrich SE. The role of IL-4, IL-10, and TNF-alpha in the immune suppression induced by ultraviolet radiation. J Leukoc Biol. 1994;56:769–75.
136. Weichenthal M, Schwarz T. Phototherapy. How does UV work? Photodermatol Photoimmunol Photomed. 2005;21:260–6.
137. Aragane Y, Kulms D, Luger TA, et al. Downregulation of interferon-g-activated STAT1 by ultraviolet light. Proc Natl Acad Sci U S A. 1997;94:11490–5.
138. Hegazy RA, Fawzy MM, Gawdat HI, et al. T helper 17 and Tregs: a novel proposed mechanism for NB-UVB in vitiligo. Exp Dermatol. 2014;23:283–6.
139. Scherschun L, Kim JJ, Lim HW. Narrow-band ultraviolet B is a useful and well-tolerated treatment for vitiligo. J Am Acad Dermatol. 2001;44:999–1003.
140. Cooper KD, Oberhelman L, Hamilton T, et al. UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: relationship to dose, CD1a-DR+ epidermal macrophage induction, and Langerhans cell depletion. Proc Natl Acad Sci U S A. 1992;89:8497–501.
141. Fisher MS, Kripke ML. Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carginogenesis. Proc Natl Acad Sci U S A. 1977;74:1688–92.
142. Hersey P, Haran G, Hasic E, et al. Alteration of T cell subsets and induction of suppressor T cell activity in normal subjects after exposure to sunlight. J Immunol. 1983;131:171–4.
143. Noonan FP, Fabo EC, Kripke ML. Suppression of contact hypersensitivity by UR radiation and its relationship to UV-induced suppression of tumor immunity. Photochem Photobiol. 1981;34:683–9.
144. Ullrich SE, Azizi E, Kripke ML. Suppression of the induction of delayed-type hypersensitivity reactions in mice by a single exposure to ultraviolet radiation. Photochem Photobiol. 1986;43:633–8.
145. Virador VM, Muller J, Wu X, et al. Influence of α-melanocyte-stimulating hormone and ultraviolet radiation on the transfer of melanosomes to keratinocytes. FASEB J. 2002;16:105–7.
146. Wu CS, Yu CL, Wu CS, et al. Narrow-band ultraviolet-B stimulates proliferation and migration of cultured melanocytes. Exp Dermatol. 2004;13:755–63.
147. Cui J, Shen LY, Wang GC. Role of hair follicles in the repigmentation of vitiligo. J Invest Dermatol. 1991;97:410–6.
148. Casacci M, Thomas P, Pacifico A, et al. Comparison between 308-nm monochromatic excimer light and narrowband UVB phototherapy (311–313 nm) in the treatment of vitiligo–a multicentre controlled study. J Eur Acad Dermatol Venereol. 2007;21:956–63.
149. El-Zawahry BM, Bassiouny DA, Sobhi RM, et al. A comparative study on efficacy of UVA1 vs. narrow-band UVB phototherapy in the treatment of vitiligo. Photodermatol Photoimmunol Photomed. 2012;28:84–90.
150. Hook RR Jr, Berkelhammer J, Oxenhandler RW. Melanoma: Sinclair swine melanoma. Am J Pathol. 1982;108:130–3.
151. Logomasini MA, Stout RR, Marcinkowski R. Jet injection for the needle-free administration of compounds, vaccines, and other agents. Int J Pharm Compd. 2013;17:270–80.
152. Mohan KE, Cordeiro M, Vaci C, et al. CXCR3 is required for migration to dermal inflammation by normal and in vivo activated T cells: differential requirements by CD4 and CD8 memory subsets. Eur J Immunol. 2005;35:1702–11.
153. Wang XX, Wang QQ, Wu JQ, et al. Increased expression of CXCR3 and its ligands in patients with vitiligo and CXCL10 as a potential clinical marker for vitiligo. Br J Dermatol. 2016;174(6):1318–26.
154. Wijtmans M, Verzijl D, Leurs R, et al. Towards small-molecule CXCR3 ligands with clinical potential. ChemMedChem. 2008;3:861–72.
155. Mayer L, Sandborn WJ, Stepanov Y, et al. Anti-IP-10 antibody (BMS-936557) for ulcerative colitis: a phase II randomised study. Gut. 2014;63:442–50.
156. Yellin M, Paliienko I, Balanescu A, et al. A phase II, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of MDX-1100, a fully human anti-CXCL10 monoclonal

antibody, in combination with methotrexate in patients with rheumatoid arthritis. Arthritis Rheum. 2012;64:1730–9.
157. Di Lernia V. Targeting the IFN-gamma/CXCL10 pathway in lichen planus. Med Hypotheses. 2016;92:60–1.
158. O'Shea JJ, Gadina M, Schreiber RD. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell. 2002;109:S121–31.
159. Darnell JE Jr. STATs and gene regulation. Science. 1997;277:1630–5.
160. Leonard WJ, O'Shea JJ. Jaks and STATs: biological implications. Annu Rev Immunol. 1998;16:293–322.
161. Leonard WJ. Type I cytokines and interferons and their receptors. In: Paul WE, editor. Fundamental immunology. 4th ed. Philadelphia, PA: Lippincott Raven; 1999. p. 741–74.
162. Ghoreschi K, Gadina M. JAKpot! New small molecules in autoimmune and inflammatory. Exp Dermatol. 2014;23:7–11.
163. Lindstrom TM, Robinson WH. A multitude of kinases – which are the best targets in treating rheumatoid arthritis? Rheum Dis Clin N Am. 2010;36:367–83.
164. O'Shea JJ, Holland SM, Staudt LM. JAKs and STATs in immunity, immunodeficiency, and cancer. N Engl J Med. 2013;368:161–70.
165. Rodig SJ, Meraz MA, White JM, et al. Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. Cell. 1998;93:373–83.
166. Bach EA, Aguet M, Schreiber RD. The IFN gamma receptor: a paradigm for cytokine receptor signaling. Annu Rev Immunol. 1997;15:563–91.
167. Craiglow BG, King BA. Killing two birds with one stone: oral tofacitinib reverses alopecia universalis in a patient with plaque psoriasis. J Invest Dermatol. 2014;134:2988–90.
168. Mamolo C, Harness J, Tan H, et al. Tofacitinib (CP-690,550), an oral Janus kinase inhibitor, improves patient-reported outcomes in a phase 2b, randomized, double-blind, placebo-controlled study in patients with moderate-to-severe psoriasis. J Eur Acad Dermatol Venereol. 2014;28:192–203.
169. Xing L, Dai Z, Jabbari A, et al. Alopecia areata is driven by cytotoxic T lymphocytes and is reversed by JAK inhibition. Nat Med. 2014;20:1043–9.
170. Zhao Y, Gartner U, Smith FJ, et al. Statins downregulate K6a promoter activity: a possible therapeutic avenue for pachyonychia congenita. J Invest Dermatol. 2011;131:1045–52.
171. Agarwal P, Rashighi M, Essien KI, et al. Simvastatin prevents and reverses depigmentation in a mouse model of vitiligo. J Invest Dermatol. 2015;135:1080–8.
172. Noel M, Gagne C, Bergeron J, et al. Positive pleiotropic effects of HMG-CoA reductase inhibitor on vitiligo. Lipids Health Dis. 2004;3:7.
173. Eby JM, Kang HK, Tully ST, et al. CCL22 to activate treg migration and suppress depigmentation in vitiligo. J Invest Dermatol. 2015;135:1574–80.
174. Mehrotra S, Al-Khami AA, Klarquist J. A coreceptor-independent transgenic human TCR mediates anti-tumor and anti-self-immunity in mice. J Immunol. 2012;189:1627–38.
175. Daniel C, Wennhold K, Kim HJ, et al. Enhancement of antigen-specific Treg vaccination in vivo. Proc Natl Acad Sci U S A. 2010;107:16246–51.
176. Scotta C, Esposito M, Fazekasova H, et al. Differential effects of rapamycin and retinoic acid on expansion, stability and suppressive qualities of human CD4(+)CD25(+)FOXP3(+) T regulatory cell subpopulations. Haematologica. 2013;98:1291–9.
177. Yang L, Wei Y, Sun Y, et al. Interferon-gamma inhibits melanogenesis and induces apoptosis in melanocytes: a pivotal role of CD8+ cytotoxic T lymphocytes in vitiligo. Acta Derm Venereol. 2015;95:664–71.
178. Al Badri AM, Foulis AK, Todd PM, et al. Abnormal expression of MHC class II and ICAM-1 by melanocytes in vitiligo. J Pathol. 1993;169:203–6.
179. AlGhamdi KM, Khurrum H, Rikabi A. Worsening of vitiligo and onset of new psoriasiform dermatitis following treatment with infliximab. J Cutan Med Surg. 2011;15:280–4.
180. Kim NH, Torchia D, Rouhani P, et al. Tumor necrosis factor-α in vitiligo: direct correlation between tissue levels and clinical parameters. Cutan Ocul Toxicol. 2011;30:225–7.
181. Rigopoulos D, Gregoriou S, Larios G, et al. Etanercept in the treatment of vitiligo. Dermatology. 2007;215:84–5.
182. Webb KC, Tung R, Winterfield LS, et al. Tumour necrosis factor-α inhibition can stabilize disease in progressive vitiligo. Br J Dermatol. 2015;173:641–50.
183. Quaglino P, Marenco F, Osella-Abate S, et al. Vitiligo is an independent favourable prognostic factor in stage III and IV metastatic melanoma patients: results from a single-institution hospital-based observational study. Ann Oncol. 2010;21:409–14.
184. Das PK, van den Wijngaard RM, Wankowicz-Kalinska A, et al. A symbiotic concept of autoimmunity and tumour immunity: lessons from vitiligo. Trends Immunol. 2001;22:130–6.
185. Irvine DJ, Purbhoo MA, Krogsgaard M, et al. Direct observation of ligand recognition by T cells. Nature. 2002;419:845–9.
186. Teulings HE, Overkamp M, Ceylan E, et al. Decreased risk of melanoma and nonmelanoma skin cancer in patients with vitiligo: a survey among 1307 patients and their partners. Br J Dermatol. 2013;168:162–71.
187. Sakai C, Kawakami Y, Law LW, et al. Melanosomal proteins as melanoma-specific immune targets. Melanoma Res. 1997;7:83–95.
188. Turk MJ, Guevara-Patiño JA, Rizzuto GA, et al. Concomitant tumor immunity to a poorly immunogenic melanoma is prevented by regulatory T cells. J Exp Med. 2004;200:771–82.
189. Pellegrini JR, Wagner RF Jr, Nathanson L. Halo nevi and melanoma. Am Fam Physician. 1984;30:157–9.
190. Reed RJ, Webb SV, Clark WH, et al. Minimal deviation melanoma (halo nevus variant). Am J Surg Pathol. 1990;14:53–68. Ficabore ndipidias ilis unt

29 Cytokines, Growth Factors, and POMC Peptides

Markus Böhm, Katia Boniface, and Silvia Moretti

Contents

Abstract

The epidermis and its main constituents, keratinocytes, produce a vast repertoire of cytokines, including interleukins (IL), growth factors, colony-stimulating factors, and chemokines. Under normal circumstances most of them are not synthesized or not released, but a number of external stimuli and stressors, e.g., infections, chemicals, trauma, or ultraviolet radiation, are capable of inducing production and release of such molecules from keratinocytes. IL-6 and TNF are paracrine inhibitors of human melanocyte proliferation and melanogenesis, eliciting a dose-dependent decrease in tyrosinase activity of cultured normal human melanocytes and inhibiting melanocyte proliferation, while IFN-γ impedes maturation of the key organelle melanosome by concerted regulation of pigmentation genes. Interestingly, a higher expression of these cytokines has been demonstrated by various authors in the affected skin of vitiligo patients at both protein and transcript levels.

Key Points

- IL-6 and TNF are paracrine inhibitors of human melanocyte proliferation and melanogenesis.
- IFN-γ impedes maturation of the key organelle melanosome and induces in normal human keratinocytes the expression of IL-33.

M. Böhm (✉)
Department of Dermatology, University of Münster, Münster, Germany
e-mail: bohmm@uni-muenster.de

K. Boniface
INSERM U1035, BMGIC, Immunodermatology team, ATIP-AVENIR, Université de Bordeaux, Bordeaux, France

S. Moretti
Division of Clinical Preventive and Oncologic Dermatology, University of Florence, Florence, Italy

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_29

- CXCL10 was shown to be critical for progression and maintenance of depigmentation.
- GM-CSF and SCF upregulate proteins required both for proliferation and melanogenesis, such as tyrosinase, TRP-1, and TRP-2.
- Reduced expression of SCF could result from keratinocyte apoptosis and might be responsible for passive melanocyte death.

29.1 Alterations in Proinflammatory Cytokines

The epidermis and its main constituents, keratinocytes, produce a vast repertoire of cytokines, including interleukins (IL), growth factors, colony-stimulating factors, and chemokines. Under normal circumstances most of them are not synthesized or not released, but a number of external stimuli and stressors, e.g., infections, chemicals, trauma, or ultraviolet radiation, are capable of inducing production and release of such molecules from keratinocytes [1].

Human keratinocytes indeed can synthesize a number of inflammatory cytokines under proper stimuli, such as IL-1α, IL-6, tumor necrosis factor (TNF), interferon (IFN)-γ, and more recently IL-18, which exert inflammatory and noninflammatory effects [2, 3].

The abovementioned cytokines deserve special attention in vitiligo since they have been found to be involved in its pathogenesis. IL-6 and TNF are paracrine inhibitors of human melanocyte proliferation and melanogenesis, eliciting a dose-dependent decrease in tyrosinase activity of cultured normal human melanocytes and inhibiting melanocyte proliferation [4], while IFN-γ impedes maturation of the key organelle melanosome by concerted regulation of pigmentation genes [5]. Interestingly, a higher expression of these cytokines has been demonstrated by various authors in the affected skin of vitiligo patients at both protein and transcript levels [6–12], but the exact mechanism explaining how these molecules can affect pigmentation in vitiligo is not fully understood. It seems likely that inflammatory cytokines play a complex role in vitiligo pathogenesis, based both on their inflammatory properties and paracrine activity on melanocytes.

Besides their anti-melanogenic properties, IL-1α, IL-6, and TNF can also induce the expression of adhesion molecules (in particular, ICAM-1) on melanocyte membrane, thus promoting further lymphocyte recruitment that can play a role in melanocyte destruction [13].

TNF can interfere with some mitochondrial activities through the production of peroxides, including hydrogen peroxide, possibly leading to a mitochondria-dependent cell death [14, 15]. In vitiligo epidermis, besides the high TNF-α levels, an impaired nuclear factor (NF)-κBNF activation has been described, and in TNF-treated cultured normal human keratinocytes, the inhibition of NF-kB activation resulted in keratinocyte apoptosis [9]. These data suggest that NF-κB dysfunction may promote TNF-α-dependent apoptosis in keratinocytes from vitiligo lesions and underline the pivotal role of this cytokine in the epidermal cell impairment of vitiligo patients. The close involvement of this cytokine in vitiligo is also strengthened by its significant reduction in vitiligo lesions undergoing repigmentation after topical tacrolimus treatment [10].

The proinflammatory properties of TNF stem from its activation of the proinflammatory cytokines IL-1 and IL-6 [16] and of numerous nuclear transcription factors, most importantly NF-κB, which is involved in maintaining inflammatory responses [17]. But TNF may possess also anti-inflammatory properties, since it induces activation and proliferation of T regulatory cells (Tregs) in vivo [18], so that TNF-α can be considered to potentially play both dangerous and protective roles in vitiligo, by stimulating the cytotoxic T lymphocytes that are detrimental to melanocytes and activating Tregs [19].

It has been reported that increased IFN-γ in the skin plays a direct role in vitiligo pathogenesis by inducing melanocyte death [20] and that

persistent IFN-γ treatment induces viability loss, apoptosis, cell cycle arrest, and senescence in melanocytes [21]. IFNγ also seems important for maintaining epidermal pigmentation homeostasis [22]. However, this cytokine can affect pigmentation also through the interaction with other cytokines. In particular, IL-18, a member of the IL-1 cytokine family, is released after UVB exposure and is capable of increasing melanogenesis [23]; such an effect is inhibited by IFN-γ treatment [24]. In addition, IFN-γ inhibits the UVB-induced expression of protease-activated receptor-2, which is a key mediator of melanosome transfer, expressed in keratinocytes [24]; PAR-2 is in turn reduced in vitiligo epidermis [25]. Furthermore, IFN-γ induces in normal human keratinocytes the expression of IL-33, another member of the IL-1 cytokine family [26], which has been demonstrated to be increased in lesional skin and serum of vitiligo patient; IL-33 was able to reduce expression of both stem cell factor (SCF) and basic fibroblastic growth factor (bFGF) and to increase the expression of both IL-6 and TNF in primary keratinocytes [27]; these data support the concept of a vicious network of anti-melanogenic cytokines in vitiligo. More recently, vitiligo pathogenesis has been attributed also to keratinocyte IFN-γ-induced chemokines which have been demonstrated to promote T lymphocyte recruitment to the epidermis where melanocytes reside [28]. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-γ for autoreactive $CD8^+$ T-cell accumulation in the skin [29]. Skin of vitiligo patients and will contribute to the recruitment of CXCR3-expressing T cells [28, 30]. In addition, CXCL10 was shown to be critical for progression and maintenance of depigmentation in vivo in a mouse model of autoimmune vitiligo [28]. IFN-α, produced by plasmacytoid dendritic cells in vitiligo skin, can also induce the release of these two chemokines by epidermal cells, providing an initial signal for $CXCR3^+$ T-cell recruitment during initiation of the disease [31]. Lastly, the release of CXCL16 by keratinocytes and CXCL12, and CCL5 by melanocytes in vitiligo under oxidative stress, could be important for the recruitment of T cells [32, 33].

Concerning IL-1, it is known that human keratinocytes constitutively express inflammasome proteins together with pro-IL-1α and pro-IL-1β [34, 35]; IL-1b activation in human epidermis can occur via an alternative mechanism involving stratum corneum kallikrein 7, a serine protease specifically expressed in keratinizing squamous epithelia [36]. NLR (nucleotide-binding domain and leucine-rich repeat containing) family, pyrin domain-containing protein 1 (NLRP1) is a key protein of the inflammasome complex which activates the proinflammatory cytokine IL-1β. In perilesional skin of vitiligo patients with active disease, NLRP1 and IL-1β expression were found to be increased and associated with a mostly T-cell inflammatory infiltrate [12], suggesting that inflammation represents a crucial moment in vitiligo progression. Furthermore, IL-β has been reported to inhibit melanocyte activity via microphthalmia transcription factor (MITF) regulation [37].

IL-6 is a proinflammatory cytokine that has been proposed to link the environmental stressors and autoimmunity in vitiligo pathogenesis; in fact oxidative stress may have a role in vitiligo onset, while autoimmunity contributes to progression. It was reported that IL-6 and IL-8 are upregulated in melanocytes after the activation of the unfolded protein response (UPR). This response is activated by the accumulation of misfolded peptides derived by the exposure of melanocytes to damaging phenols, which are able to induce oxidative stress, thus resulting in harmful redox disruptions [38]. In vitiligo, impaired function of Tregs that is associated with a widespread activation of cytotoxic T lymphocytes has been described [39], and in psoriasis IL-6 allows cytotoxic T cells to escape from Treg suppression [40]. Thus the upregulation of IL-6 production by melanocytes under oxidative stress through the UPR activation [38] could reduce Treg modulation, leading to the activation of the immune response against melanocytes [41]. It was also demonstrated that subtoxic levels of hydrogen peroxide (H_2O_2) induce expression of IL-6 in epidermal melanocytes, and both H_2O_2 and IL-6 have been reported to be elevated in vitiligo lesions; H_2O_2-induced overexpression of IL-6 by

melanocytes may be another molecular linkage for the oxidative stress and autoimmune/inflammatory reactions in vitiligo [42].

Recently, it was proposed that the proinflammatory cytokine IL-17 A secreted by the Th17 cells infiltrating vitiligo skin can induce IL-1β, IL-6, and TNF production in skin resident cells such as keratinocytes and fibroblasts, suggesting the presence of mutual cytokine signaling between skin resident cells and accumulating inflammatory cells [43]. Interestingly, in vitro analysis demonstrated that the expression of MITF and downstream genes was downregulated in melanocytes by treatment with the abovementioned cytokines [43, 44]. Nonetheless, IL-17 on its own had little effect on melanogenesis and would rather act indirectly on pigmentation through upregulation of the inflammatory cytokines cited above. The presence of a cytokine network induced by IL-17A secreted by Th17 cells infiltrating vitiligo skin may represent another mechanism underlying vitiligo pathogenesis but still needs further investigation.

29.2 Disturbances in Growth Factor Expression

In human skin, homeostasis is controlled by either endocrine or paracrine communication via soluble factors including hormones, growth factors, and cytokines or by intercellular communication via cell-cell and cell-matrix adhesion and gap junctional intercellular communication [45, 46].

At present evidence exists that melanogenic paracrine cytokine networks occur between melanocytes and other types of skin cells, including keratinocytes and fibroblasts, which regulate melanocytes function [47]. Keratinocytes have been reported to stimulate the proliferation, melanogenesis, or dendritogenesis of epidermal melanocytes in normal skin and/or in UVB-irradiated skin [48]. In fact, after colonizing in the epidermis during development, melanocytes may require keratinocyte-derived factors for their proliferation and differentiation.

In human nerve growth factors are produced and released by keratinocytes and stimulate melanogenesis and dendritogenesis of melanocytes [49]. This binding activates a cascade of intracellular signals that lead to transient increase in microphthalmia-associated transcription factor (Mitf) gene expression, which in turn result in upregulation of tyrosinase, tyrosinase-related protein (TRP)-1, and TRP-2 (or dopachrome tautomerase, DCT) to allow melanin synthesis [50]. Endothelin (ET)-1 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are further keratinocyte-derived factors that regulate the proliferation and melanogenesis/dendritogenesis of melanocytes in UVB- or UVA-irradiated human skin [51–53]. Moreover, SCF is also produced by keratinocytes in a membrane-bound form. It stimulates proliferation and dendritogenesis [54] of epidermal melanocytes in the presence of dibutyryl adenosine 3′:5′-cyclic monophosphate (dbcAMP), via the binding to its specific receptor c-kit [55]. ET-1 (through signaling pathway of protein kinase C) [56], GM-CSF (via activation of signal transducers and activators of transcription STAT-1, STAT-3, and STAT-5 or mitogen-activated protein (MAP) kinase) [57, 58], and SCF (through activation of MAP kinase pathway) [48] upregulate proteins required both for proliferation and melanogenesis, such as tyrosinase, TRP-1, and TRP-2. Keratinocyte-derived b-FGF is well known to stimulate proliferation of epidermal melanocytes in the presence of dbcAMP [59]. It can also act in synergy with ET-1 but also MGF to enhance melanogenesis and proliferation of epidermal melanocytes [60].

Less is known about the influence of dermal-derived cytokines/growth factors on epidermal melanocytes. Human fibroblasts seem to induce a decrease of epidermal pigmentation in chimeric human epidermal reconstructs [61], although they can secrete melanogenic growth factors such as SCF or hepatocyte growth factor (HGF) which are mitogens for human melanocytes in vitro and in vivo [47, 48]. Some data suggest that upregulation of HGF plays a role in skin homeostasis after UVA irradiation [62].

Overall, the imbalance of keratinocyte-derived cytokines described by some authors in vitiligo

epidermis suggests an involvement of epidermal cytokines in the pathogenesis of vitiligo. The results did not appear univocal, since some melanocyte-stimulating cytokines have been described as decreased [5, 6, 11, 63, 64] or increased [59] in lesional epidermis, possibly due to different techniques, diverse choices of controls, and possibly dissimilar selections of vitiligo patients. However, a critical evaluation of the findings reported in literature suggests that SCF is actually reduced in depigmented lesions, as observed in vivo at both protein and transcript levels [5, 6, 11, 63]. In fact keratinocytes in the depigmented lesions compared to the normally pigmented counterpart seem to be more prone to apoptosis [65, 66] and incapable of producing adequate amounts of SCF for melanocyte survival [63]. In vitro functional studies have shown that apoptosis of cultured normal human keratinocytes is associated to a concentration-dependent decreased production of SCF mRNA and protein and that deprivation of SCF or keratinocyte feeder in the culture medium induces a marked decrease in melanocytes as a result of apoptosis [63]. These data strongly suggest that in vitiliginous keratinocytes a reduced expression of SCF could result from keratinocyte apoptosis and might be responsible for passive melanocyte death. Further support to the role of keratinocyte-derived SCF in regulating melanocyte activity and possible implication in vitiligo is also given by additional in vitro data, showing that proliferation of cultured melanocytes is enhanced by tacrolimus-treated keratinocyte supernatant, whose SCF concentration increases dose-dependently with tacrolimus treatment [67].

Concerning other melanogenic cytokines, a substantial decrease of GM-CSF and bFGF protein levels [5, 6, 63] and a reduced expression of ET-1 [11] and bFGF [68] transcripts have been demonstrated in the depigmented epidermis in vitiligo patients. In addition, a failure in ET-1 production from vitiligo keratinocytes in response to ultraviolet-B irradiation has been described [68].

Dermis can probably contribute to vitiligo pathogenesis at least in part, since dermal fibroblasts secrete various growth factors which are important for skin pigmentation. In explants cultures of fibroblasts from vitiligo dermis, a higher gene expression of senescence markers p16, p21, and hp1 has been demonstrated at mRNA and protein level in lesional dermis. Senescence in vitiligo fibroblasts can decrease the secretion of growth factors and cytokines produced by fibroblasts which may lead to the melanocyte death [69, 70].

29.3 Dysfunction of the Proopiomelanocortin System

The proopiomelanocortin (POMC)-derived peptides include α-, β-, and γ-melanocyte-stimulating hormones (α-, β-, and γ-MSH), adrenocorticotropin (ACTH), as well as β-endorphin (β-ED). These peptides are players of the classical neuroendocrine hypothalamic-pituitary-adrenal (HPA) stress axis which importantly is also installed in the skin as an analogon [71]. Accordingly, epidermal keratinocytes but also several other cutaneous cell types express POMC and process this precursor into POMC-derived peptides which not only induce melanogenesis and/or melanocyte proliferation [72–74]. Importantly, melanocortin peptides have additional biological actions including immunomodulation and cytoprotection against genotoxic stress and oxidative damage [75–78] due to expression of functional melanocortin receptors (MCRs) in skin cells and cells of the immune system. Several studies have thus addressed the question as to whether the POMC system in vitiligo is altered.

Early studies revealed that the peripheral blood levels of some POMC-derived peptides and their circadian rhythm are altered. Accordingly, plasma levels of β-ED were higher in vitiligo patients than in healthy individuals. Moreover, the circadian rhythm of this POMC peptide was lost in patients with vitiligo [79]. Increased β-ED plasma levels in vitiligo were subsequently confirmed in another study with more patients [80] indicating an increased level of stress mediated by the classical neuroendocrine HPA axis or by increased cutaneous

production of β-ED in the skin of vitiligo patients. However, in a more recent study on 40 matched normal individuals, vitiligo patients had a significantly lower median α-MSH plasma level but a higher ACTH plasma level. In contrast, the levels of morning serum cortisol were not altered in vitiligo patients [81].

With regard to the cutaneous POMC system, it was reported that the immunoreactive amounts of α-MSH in lesional and non-lesional epidermis of vitiligo are less than in normal epidermis. Subsequent immunohistochemical studies on skin biopsies (lesional, perilesional, and non-lesional) from vitiligo patients and control subjects further supported a reduction in the level of α-MSH in lesional and perilesional skin, especially within the melanocytes [82]. Similar to α-MSH a reduction in the number of melanocytes staining for PC1 and PC2 in vitiligo skin both in non-lesional and perilesional skin compared with control skin was seen [82]. Additional studies revealed that epidermal immunoreactivity for both α-MSH and β-ED immunoreactivity is significantly reduced while that of ACTH is increased in vitiliginous skin [83]. In light of increased amounts of hydrogen peroxide in vitiligo skin, it was proposed that oxidation of epidermal POMC peptides leads to structural changes that may alter antibody epitope recognition. Based on FT-Raman spectroscopy and computer modeling ACTH, α-MSH and β-ED appear to be redox sensitive resulting in the generation of Met sulfoxide when exposed to hydrogen peroxide. In accordance with this, treatment of α-MSH and β-ED with hydrogen peroxide in vitro reduced the melanotropic activity of this POMC-derived peptide [83]. In support of this concept, treatment of vitiligo patients with pseudocatalase increased immunoreactivity of both α-MSH in repigmented skin of patients with vitiligo [83]. Subsequent studies demonstrated that not only POMC-derived peptides but also furin convertase, a member of the PC family of POMC-processing enzymes [84], could be a target for oxidative stress-induced damage in vitiligo [85]. Furin convertase was found to be expressed in both normal human melanocytes and keratinocytes in culture at RNA and protein level. In support of the former studies, furin convertase expression was found to be significantly lower in both lesional and non-lesional skin of untreated patients with progressive vitiligo compared with healthy controls. Antibody-antigen recognition was not affected by exposure of furin protein to hydrogen peroxide. However, treatment with hydrogen peroxide in vitro resulted in a 55% reduction in Ca^{2+} binding [85].

Expression of the POMC system was also assessed in a more quantitative manner in full-thick skin biopsies from vitiligo patients using quantitative real-time RT-PCR [86, 87]. In one study on Estonian vitiligo, patients POMC mRNA levels were somewhat lower in lesional skin than in control skin. In contrast, the relative mRNA amounts of both MC-1R and also MC-4R were found to be slightly elevated in non-lesional skin of vitiligo compared with lesional skin and control skin [86]. In a more recent study involving a similar cohort in size (n = 40), POMC and MC1R mRNA amounts were lower in lesional skin compared with non-lesional skin and control skin from sex- and age-matched controls [87]. A statistically significant positive correlation between lesional levels of POMC and MC1R, as well as between non-lesional levels of POMC and MC1R in the patients, was found in this study. On the other hand, a statistically significant negative correlation between the lesional and non-lesional levels of POMC, as well as between the lesional and non-lesional levels of MC1R in the patients, was detected [87].

Therefore, it appears that based on immunohistochemical studies, quantitative mRNA expression analysis and in vitro studies on the action of POMC-derived peptides as well as on the function of POMC-processing enzymes the cutaneous POMC system are dysfunctional. Consequences of this deviation could be not only impaired melanogenesis in melanocytes but also an increased susceptibility to cell death or apoptotic signals including oxidative stress. Moreover, impairment of the POMC system may render melanocytes to an immune attack.

In this context it is interesting that however no evidence has been found in antibodies interfering with the activity of the melanocortin-1 receptor [88].

In addition to the aforementioned alterations, there is emerging evidence that genetic abnormalities contribute to the overall dysfunction of the POMC system in vitiligo. Two genes of the POMC system, *MC1R* and also agouti signaling protein (*ASIP*), the latter encoding a natural MC1R/MC4R antagonist, have been in the focus of such studies. Importantly, the human *MC1R* gene is unusually polymorphic with more than 100 variant alleles, some of which are associated with red hair, fair skin, freckling, and poor or even absent tanning in response to UV irradiation [89].

No significant changes in the allele frequency of *ASIP* polymorphisms have been detected so far in at least two smaller studies [90, 91]. Regarding MC1R partially controversial findings have been reported which may be due to ethnic variations in the patient cohorts, the methodology, and the power (sample size) of the studies. In a Hungarian cohort of 108 vitiligo patients, the C478T single nucleotide polymorphism of *MC1R* had a significant difference in allele frequency in fair-skinned vitiligo patients and in fair-skinned healthy controls with a higher allele frequency in the control group suggesting that Arg160Trp amino acid change is protective against vitiligo [90]. Importantly, *MC1R* has been confirmed as a novel risk locus based on a third genome-wide association study (GWAS3) of vitiligo in European subjects, with augmentation of GWAS1 and GWAS2 controls, genome-wide imputation, and meta-analysis of all three vitiligo GWAS, followed by an independent replication study. The combined analyses included 4680 vitiligo cases and 39,568 controls. MC1R was among eight other loci that showed evidence for independent association, for a total 22.5% of vitiligo heritability [92]. The MC1R is thus among 50 vitiligo susceptibility genes; most of them encoding immune and apoptotic regulators, with some associated with other autoimmune diseases, and several melanocyte regulators.

References

1. Uchi H, Terao H, Koga T, et al. Cytokines and chemokines in the epidermis. J Dermatol Sci. 2000;24:S29–38.
2. Schwartz T, Luger TA. Effect of UV irradiation on epidermal cell cytokine production. J Photochem Photobiol. 1989;B4:1–13.
3. Cho D, Seung Kang J, Hoon Park J, et al. The enhanced IL-18 production by UVB irradiation requires ROI and AP-1 signaling in human keratiocyte cell line (HaCaT). Biochem Biophys Res Commun. 2002;298:289–95.
4. Swope VB, Abdel-Malek Z, Kassem ML, et al. Interleukin 1a and 6 and tumor necrosis factor – are paracrine inhibitors of human melanocyte proliferation and melanogenesis. J Invest Dermatol. 1991;96:180–5.
5. Natarajan V, Ganju P, Singh A, et al. IFN-g signaling maintains skin pigmentation homeostasis through regulation of melanosome maturation. Proc Natl Acad Sci U S A. 2014;111:2301–6.
6. Moretti S, Spallanzani A, Amato L, et al. Vitiligo and epidermal microenvironment: possible involvement of keratinocyte-derived cytokines. Arch Dermatol. 2002;138:273–4.
7. Moretti S, Spallanzani A, Amato L, et al. New insight into the pathogenesis of vitiligo: imbalance of epidermal cytokines at sites of lesions. Pigment Cell Res. 2002;15:87–92.
8. Birol A, Kisa U, Kara F, et al. Increased tumor necrosis factor alpha (TNF-a) and interleukin 1 alpha (IL-1a) levels in the lesional skin of patients with non segmental vitiligo. Int J Dermatol. 2007;45:992–3.
9. Kim NH, Jeon S, Lee HJ, Lee AY. Impaired PI3K/Akt activation-mediated NF-kB inactivation under elevated TNF-a is more vulnerable to apoptosis in vitiliginous keratinocytes. J Invest Dermatol. 2007;127:2612–7.
10. Grimes PE, Morris R, Avaniss-Aghajani F, et al. Topical tacrolimus therapy for vitiligo: therapeutical responses and skin messenger RNA expression of proinflammatory cytokines. J Am Acad Dermatol. 2004;51:52–61.
11. Moretti S, Fabbri P, Baroni G, Berti S, Bani D, Berti E, Nassini R, Lotti T, Massi D. Keratinocyte dysfunction in vitiligo epidermis: cytokine microenvironment and correlation to keratinocyte apoptosis. Histol Histopathol. 2009;24:849–57.
12. Marie J, Kovacs D, Pain C, et al. Inflammasome activation and vitiligo/non segmental vitiligo progression. Br J Dermatol. 2014;170:816–23.
13. Morelli JG, Norris DA. Influence of inflammatory mediators and cytokines on human melanocytes function. J Invest Dermatol. 1993;100(Suppl):191S–5S.
14. Haycock JW, Rowe SJ, Cartledge S, et al. α-Melanocyte stimulating hormone reduces impact of proinflammatory cytokine and peroxide-generated

oxidative stress on keratinocytes and melanoma cell lines. J Biol Chem. 2000;275:15629–36.
15. Dell'Anna ML, Picardo M. A review and a new hypothesis for non-immunological pathogenetic mechanism in vitiligo. Pigment Cell Res. 2006;19:406–11.
16. Tracey D, Klareskog I, Saso EH, et al. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. Pharmacol Ther. 2008;117:244–79.
17. Blanco P, Palucka AK, Pascual V, Banchereau J. Dendritic cells and cytokines in human inflammatory and autoimmune diseases. Cytokine Growth Factors Rev. 2008;19:41–52.
18. Biton J, Boissier MC, Bessis N. TNF-alpha: activator or inhibitor of regulator T cells? Joint Bone Spine. 2012;79:119–23.
19. Webb KB, Tung R, Winterfield LS, et al. Tumor necrosis factor-a inhibition can stabilize disease in progressive vitiligo. Br J Dermatol. 2015;173:641–50.
20. Yang L, Wei Y, Sun Y, et al. Interferon-gamma inhibits melanogenesis and induces apoptosis in melanocytes: a pivotal role of CD8+ cytotoxic T lymphocytes in vitiligo. Acta Derm Venereol. 2015;95:664–70.
21. Wang S, Zhou M, Lin F, et al. Interferon-g induces senescence in normal human melanocytes. PLoS One. 2014;9(3):e93232.
22. Natarajan VT, Ganju P, Singh A, et al. IFN-γ signaling maintains skin pigmentation homeostasis through regulation of melanosome maturation. Proc Natl Acad Sci U S A. 2014;111(6):2301–6.
23. Zhou J, Shang J, Song J, Ping F. Interlekin-18 augments growth ability of primary human melanocytes by PTEN inactivation through the AKT/NFkB pathway. Int J Biochem Cell Biol. 2013;45:308–16.
24. Zhou J, Ling J, Wang Y, et al. Cross-talk between interferon-gamma and interleukin-18 in melanogenesis. J Photochem Photobiol. 2016;163:133–43.
25. Moretti S, Nassini R, Prignano F, Pacini A, Materazzi S, Naldini A, Simoni A, Baroni G, Pellerito S, Filippi I, Lotti T, Geppetti P, Massi D. Protease-activated receptor-2 down-regulation is associated to vitiligo lesions. Pigment Cell Melanoma Res. 2009;22:335–8.
26. Meephansa J, Tsuda H, Komine M, et al. Regulation of IL-33 expression by IFN-g and tumor necrosis factor-a in normal human keratinocytes. J Invest Dermatol. 2012;132:2593–600.
27. Li P, Ma H, Han D, Mou K. Interleukin-33 affects cytokine production by keratinocytes in vitiligo. Clin Exp Dermatol. 2015;40:163–70.
28. Richmond JM, Bangari DS, Essien KI, et al. Keratinocyte-derived chemokines orchestrate T-cell positioning in the epidermis during vitiligo and may serve as biomarkers of the disease. J Invest Dermatol. 2017;137:350–8.
29. Harris JE, Harris TH, Weninger W, et al. J Invest Dermatol. 2012;132(7):1869–76.
30. Rashighi M, Argawal P, Richmond JM, et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. Sci Transl Med. 2014;6:223ra23.
31. Bertolotti A, Boniface K, Vergier B, et al. Type I interferon signature in the initiation of the immune response in vitiligo. Pigment Cell Melanoma Res. 2014;27(3):398–407.
32. Jacquemin C, Rambert J, Guillet S, et al. HSP70 potentiates interferon-alpha production by plasmacytoid dendritic cells: relevance for cutaneous lupus and vitiligo pathogenesis. Br J Dermatol. 2017. https://doi.org/10.1111/bjd.15550.
33. Li S, Zhu G, Yang Y, et al. Oxidative stress drives CD8(+) T-cell skin trafficking in patients with vitiligo through CXCL16 upregulation by activating the unfolded protein response in keratinocytes. J Allergy Clin Immunol. 2016. pii: S0091-6749(16)31277-5. https://doi.org/10.1016/j.jaci.2016.10.013.
34. Rezk AF, Kemp DM, El-Domyati M, et al. Misbalanced CXCL12 and CCL5 chemotactic signals in vitiligo onset and progression. J Invest Dermatol. 2017;137(5):1126–34. https://doi.org/10.1016/j.jid.2016.12.028.
35. Feldmeyer L, Keller M, Niklaus G, et al. The inflammasome mediates UVB-induced activation and secretion of interleukin-1b by keratinocytes. Curr Biol. 2007;17:1140–5.
36. Lundqvist EN, Egelrud T. Biologically active, alternatively processed interleukin-1beta in psoriatic scales. Eur J Immunol. 1997;27:2165–71.
37. Kholmanskikh O, Van Baren N, Brasseur F, et al. Interleukins 1 alpha and 1 beta secreted by some melanoma cell lines strongly reduce expression of MITF-M and melanocyte differentiation antigens. Int J Cancer. 2010;127:1625–36.
38. Toosi S, Orlow SJ, Manga P. Vitiligo-inducing phenols activate the unfolded protein response in melanocytes resulting in upregulation of IL-6 and IL-8. J Invest Dermatol. 2012;132:2601–9.
39. Lili Y, Yi W, Ji Y, et al. Global activation of CD8+ cytotoxic T lymphocytes correlates with an impairment in regulatory T cells in patients with generalized vitiligo. PLoS One. 2012;7:e37513.
40. Goodman WA, Levine AD, Massari JV, et al. IL-6 signaling in psoriasis prevents immune suppression by regulatory T cells. J Immunol. 2009;183:3170–6.
41. Passeron T, Ortonne JP. Activation of the unfolded protein response in vitiligo: the missing link? J Invest Dermatol. 2012;132:2502–4.
42. Yao L, Hu DN, Chen M, Li SS. Subtoxic levels hydrogen-peroxide-induced expression of interleukin-6 by epidermal melanocytes. Arch Dermatol Res. 2012;304:831–8.
43. Wang CQ, Akalu YT, Suarez-Farinas M, et al. IL-17 and TNF synergistically modulate cytokine expression while suppressing melanogenesis: potential relevance to psoriasis. J Invest Dermatol. 2013;133(12):2741–52. https://doi.org/10.1038/jid.2013.237.
44. Kotobuki Y, Tanemura A, Yang L, et al. Dysregulation of melanocyte function by Th17-related cytokines: significance of Th17 cell infiltration in autoimmune vitiligo vulgaris. Pigment Cell Melanoma Res. 2011;25:219–30.
45. Haass NK, Herlyn M. Normal human melanocyte homeostasis as a paradigm for understand-

ing melanoma. J Invest Dermatol Symp Proc. 2005;10:153–63.
46. Haass NK, Smalley KSM, Herlyn M. Adhesion, migration and communication in melanocytes and melanoma. Pigment Cell Res. 2005;18:150–9.
47. Imokawa G. Autocrine and paracrine regulation of melanocytes in human skin and in pigmentary disorders. Pigment Cell Res. 2004;17:96–110.
48. Hirobe T. Role of keratinocytes-derived factors involved in regulating the proliferation and differentiation of mammalian epidermal melanocytes. Pigment Cell Res. 2004;18:2–12.
49. Yaar M, Grossman K, Eller M, et al. Evidence for nerve growth factor-mediated paracrine effects in human epidermis. J Cell Biol. 1991;115:821–8.
50. Tachibana M. MITF: a stream flowing for pigment cells. Pigment Cell Res. 2000;13:230–40.
51. Hara M, Yaar M, Gilchrest BA. Endothelin-1 of keratinocytes origin is a mediator of melanocyte dendricity. J Invest Dermatol. 1995;105:744–8.
52. Imokawa G, Yada Y, Miyagishi M. Endothelins secreted from human keratinocytes are intrinsic mitogens for human melanocytes. J Biol Chem. 1992;267:24675–80.
53. Imokawa G, Yada Y, Kimura M, et al. Granulocyte/macrophage colony-stimulating factor is an intrinsic keratinocyte-derived growth factor for human melanocytes in UVA-induced melanosis. Biochem J. 1996;313:625–31.
54. Grichnick JM, Burch JA, Burchette J, et al. The SCF/KIT pathway plays a critical role in the control of normal human melanocyte homeostasis. J Invest Dermatol. 1998;111:233–8.
55. Geissler EN, Ryan MA, Housman DE. The dominant-white spotting (*W*) locus of the mouse encodes the c-kit proto-oncogene. Cell. 1988;55:185–92.
56. Imokawa G, Kobayashi T, Miyagishi M, et al. The role of endothelin-1 in epidermal hyperpigmentation and signalling mechanisms of mitogenesis and melanogenesis. Pigment Cell Res. 1997;10:218–28.
57. Mui ALF, Wakao H, O'Farrell AM, et al. Interleukin-3, granulocyte-macrophage colony stimulating factor and interleukin-5 transduce signals through two STAT5 homologs. EMBO J. 1995;14:1166–75.
58. Wang Y, Morella KK, Ripperger J, et al. Receptors for interleukin-3 (IL-3) and growth hormone mediate an IL-6-type transcriptional induction in the presence of JAK2 or STAT3. Blood. 1995;86:1671–9.
59. Halaban R, Langdom R, Birchall N, et al. Basic fibroblastic growth factor from human keratinocytes is a natural mitogen for melanocytes. J Cell Biol. 1988;107:1611–9.
60. Böhm M, Moellmann G, Cheng E, Zhao B, Wagner S, Alvarez-Franco M, Sassone-Corsi P, Halaban R. Identification of p90RSK as the probable 133-Ser-CREB kinase in human melanocytes. Cell Growth Diff. 1995;6:291–302.
61. Cario-André M, Pain C, Gauthier Y, et al. In vivo and in vivo evidence of dermal fibroblasts influence on human epidermal pigmentation. Pigment Cell Res. 2006;19:434–42.
62. Mildner M, Mlitz V, Gruber F, et al. Hepatocyte growth factor establishes autocrine and paracrine feedback loops the protection of skin cells after UV irradiation. J Invest Dermatol. 2007;127:2637–44.
63. Lee AY, Kim NH, Choi WI, et al. Less keratinocyte-derived factors related to more keratinocyte apoptosis in depigmented than normally pigmented suction-blisters epidermis may cause passive melanocyte death in vitiligo. J Invest Dermatol. 2005;124:976–83.
64. Bondanza S, Maurelli R, Paterna P, et al. Keratinocyte cultures from involved skin in vitiligo patients show an impaired in vitro behaviour. Pigment Cell Res. 2007;20:288–300.
65. Kitamura R, Tsukamoto K, Harada K, et al. Mechanisms underlying the dysfunction of melanocytes in vitiligo epidermis: role of SCF/KIT protein interactions and its downstream effector, MITF-M. J Pathol. 2004;202:463–75.
66. Lee AY, Youm YH, Kim NH, et al. Keratinocytes in the depigmented epidermis of vitiligo are more vulnerable to trauma (suction) than the keratinocytes in the normally pigmented epidermis, resulting in their apoptosis. Br J Dermatol. 2004;151:995–1003.
67. Lan CCE, Chen GS, Chiou MH, et al. FK506 promotes melanocyte and melanoblast growth and creates a favourable milieu for cell migration via keratinocytes: possible mechanisms of how tacrolimus ointment induces repigmentation in patients with vitiligo. Br J Dermatol. 2005;153:498–505.
68. Seif El Nasr H, Shaker OG, Fawzi MM, et al. Basic fibroblastic growth factor and tumour necrosis factor alpha in vitiligo and other hypopigmented disorders: suggestive possible therapeutic targets. J Eur Acad Dermatol Venereol. 2013;27:103–8.
69. Takata T, Tarutani M, Sano S. A failure in endothelin-1 production from vitiligo keratinocytes in response to ultraviolet B irradiation. J Dermatol Sci. 2013;71:210–2.
70. Rani S, Bhardwaj S, Srivastava N, et al. Senescence in the lesional fibroblasts of non-segmental vitiligo patients. Arch Dermatol Res. 2017; https://doi.org/10.1007/s00403-016.1713-0.
71. Slominski A, Wortsman J, Luger T, Paus R, Solomon S. Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. Physiol Rev. 2000;80:979–1020.
72. Abdel-Malek Z, Swope VB, Suzuki I, Akcali C, Harriger MD, Boyce ST, Urabe K, Hearing VJ. Mitogenic and melanogenic stimulation of normal human melanocytes by melanotropic peptides. Proc Natl Acad Sci U S A. 1995;92:1789–93.
73. Kauser S, Schallreuter KU, Thody AJ, Gummer C, Tobin DJ. Regulation of human epidermal melanocyte biology by beta-endorphin. J Invest Dermatol. 2003;120:1073–80.
74. Kauser S, Thody AJ, Schallreuter KU, Gummer CL, Tobin DJ. Beta-endorphin as a regulator of human hair follicle melanocyte biology. J Invest Dermatol. 2004;123:184–95.

75. Böhm M, Wolff I, Scholzen TE, Robinson SJ, Healy E, Luger TA, Robinson S, Healy E, Schwarz T, Schwarz A. Alpha-melanocyte-stimulating hormone protects from ultraviolet radiation-induced apoptosis and DNA damage. J Biol Chem. 2005;280:5795–802.
76. Böhm M, Luger TA, Tobin DJ, Garcia-Borron JC. Melanocortin receptor ligands: new horizons for skin biology and clinical dermatology. J Invest Dermatol. 2006;126:1966–75.
77. Brzoska T, Luger TA, Maaser C, Abels C, Böhm M. Alpha-melanocyte-stimulating hormone and related tripeptides: biochemistry, antiinflammatory and protective effects in vitro and in vivo, and future perspectives for the treatment of immune-mediated inflammatory diseases. Endocr Rev. 2008;29:581–602.
78. Kokot A, Metze D, Mouchet N, Galibert MD, Schiller M, Luger TA, Böhm M. Alpha-melanocyte-stimulating hormone counteracts the suppressive effect of UVB on Nrf2 and Nrf-dependent gene expression in human skin. Endocrinology. 2009;150:3197–206.
79. Mozzanica N, Villa ML, Foppa S, Vignati G, Cattaneo A, Diotti R, Finzi AF. Plasma alpha-melanocyte-stimulating hormone, beta-endorphin, met-enkephalin, and natural killer cell activity in vitiligo. J Am Acad Dermatol. 1992;26:693–700.
80. Caixia T, Daming Z, Xiran L. Levels of beta-endorphin in the plasma and skin tissue fluids of patients with vitiligo. J Dermatol Sci. 2001;26:62–6.
81. Pichler R, Sfetsos K, Badics B, Gutenbrunner S, Auböck J. Vitiligo patients present lower plasma levels of alpha-melanotropin immunoreactivities. Neuropeptides. 2006;40:177–83.
82. Graham A, Westerhof W, Thody AJ. The expression of alpha-MSH by melanocytes is reduced in vitiligo. Ann N Y Acad Sci. 1999;885:470–3.
83. Spencer JD, Gibbons NC, Rokos H, Peters EM, Wood JM, Schallreuter KU. Oxidative stress via hydrogen peroxide affects proopiomelanocortin peptides directly in the epidermis of patients with vitiligo. J Invest Dermatol. 2006;127:411–20.
84. Seidah NG, Benjannet S, Hamelin J, Mamarbachi AM, Basak A, Marcinkiewicz J, Mbikay M, Chretien M, Marcinkiewicz M. The subtilisin/kexin family of precursor convertases. Emphasis on PC1, PC2/7B2, POMC and the novel enzyme SKI-1. Ann N Y Acad Sci. 1999;885:57–74.
85. Spencer JD, Gibbons NC, Böhm M, Schallreuter KU. The Ca^{2+}–binding capacity of epidermal furin is disrupted by H_2O_2-mediated oxidation in vitiligo. Endocrinology. 2008;149:1638–45.
86. Kingo K, Aunin E, Karelson M, Philips MA, Rätsep R, Silm H, Vasar E, Soomets U, Kõks S. Gene expression analysis of melanocortin system in vitiligo. J Dermatol Sci. 2007;48:113–22.
87. Nagui NA, Mahmoud SB, Abdel Hay RM, Hassieb MM, Rashed LA. Assessment of gene expression levels of proopiomelanocortin (POMC) and melanocortin-1 receptor (MC1R) in vitiligo. Australas J Dermatol. 2017;58(2):e36–9.
88. Agretti P, De Marco G, Sansone D, Betterle C, Coco G, Dimida A, Ferrarini E, Pinchera A, Vitti P, Tonacchera M. Patients affected by vitiligo and autoimmune diseases do not show antibodies interfering with the activity of the melanocortin 1 receptor. J Endocrinol Investig. 2010;33:784–8.
89. Pérez Oliva AB, Fernéndez LP, Detorre C, Herráiz C, Martínez-Escribano JA, Benítez J, Lozano Teruel JA, García-Borrón JC, Jiménez-Cervantes C, Ribas G. Identification and functional analysis of novel variants of the human melanocortin 1 receptor found in melanoma patients. Hum Mutat. 2009;30:811–22.
90. Széll M, Baltás E, Bodai L, Bata-Csörgo Z, Nagy N, Dallos A, Pourfarzi R, Simics E, Kondorosi I, Szalai Z, Tóth GK, Hunyadi J, Dobozy A, Kemény L. The Arg160Trp allele of melanocortin-1 receptor gene might protect against vitiligo. Photochem Photobiol. 2008;84:565–71.
91. Na GY, Lee KH, Kim MK, Lee SJ, Kim DW, Kim JC. Polymorphisms in the melanocortin-1 receptor (MC1R) and agouti signaling protein (ASIP) genes in Korean vitiligo patients. Pigment Cell Res. 2003;16:383–7.
92. Jin Y, Andersen G, Yorgov D, Ferrara TM, Ben S, Brownson KM, Holland PJ, Birlea SA, Siebert J, Hartmann A, Lienert A, van Geel N, Lambert J, Luiten RM, Wolkerstorfer A, Wietze van der Veen JP, Bennett DC, Taïeb A, Ezzedine K, Kemp EH, Gawkrodger DJ, Weetman AP, Kõks S, Prans E, Kingo K, Karelson M, Wallace MR, McCormack WT, Overbeck A, Moretti S, Colucci R, Picardo M, Silverberg NB, Olsson M, Valle Y, Korobko I, Böhm M, Lim HW, Hamzavi I, Zhou L, Mi QS, Fain PR, Santorico SA, Spritz RA. Genome-wide association studies of autoimmune vitiligo identify 23 new risk loci and highlight key pathways and regulatory variants. Nat Genet. 2016;48:1418–24.

30 Regenerating Melanocytes: Current Stem Cell Approaches with Focus on Muse Cells

Mari Dezawa, Kenichiro Tsuchiyama, Kenshi Yamazaki, and Setsuya Aiba

Contents

Abstract

Muse cells are recently found endogenous non-tumorigenic pluripotent stem cells that reside in connective tissue of various organs including the dermis and in the bone marrow. They are collectable as cells positive for stage-specific embryonic antigen (SSEA)-3, a pluripotent surface marker, from tissues, and are expandable in vitro. Other than SSEA-3, they express Oct3/4, Nanog and Sox, other pluripotent genes. Notably, they are able to differentiate into cells representative of all three germ layers from single cells and are self-renewable, suggesting their pluripotency. Muse cells collected from human dermal fibroblasts (dermal-Muse cells) were shown

M. Dezawa (✉)
Department of Stem Cell Biology and Histology, Tohoku University Graduate School of Medicine, Sendai, Japan
e-mail: mdezawa@med.tohoku.ac.jp

K. Tsuchiyama · K. Yamazaki · S. Aiba
Department of Dermatology, Tohoku University Graduate School of Medicine, Sendai, Japan

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_30

to efficiently differentiate into melanin-producing functional melanocytes by treating them with ten factors. Functions of melanocytes induced from Muse cells (Muse melanocytes) were comparable to that of primary human melanocytes. Melanin-producing ability of human Muse melanocytes was retained when they were incorporated into human-colored three-dimensional (3D) cultured skin and even after transplantation of the 3D-cultured skin into the back of immunodeficient mice. Since Muse cells are non-tumorigenic and harvestable from easy accessible sources such as skin biopsy and dermal fibroblasts, Muse melanocytes are beneficial for both industrial and clinical uses.

Key Points

- Muse cells are non-tumorigenic endogenous pluripotent stem cells.
- Muse cells express pluripotency markers such as transcription factors Oct3/4, Sox2, and Nanog, as well as pluripotent surface marker, stage-specific embryonic antigen (SSEA)-3.
- Muse cells show characters relevant to pluripotent stem cells; they are able to generate cells representative of all three germ layers from a single cell.
- Muse cells distribute sporadically in the connective tissue of various organs and do not look like associated with a structural niche.

30.1 Introduction

Since melanocytes are tightly incorporated into the basal layer of the epidermis, their isolation is not easy, and therefore primary human melanocyte culture with high purity is generally hard to establish.

Autologous human melanocytes are potential cell therapy for vitiligo [1, 2] but are not for general use because they are difficult to culture and amplify into large scale in vitro. Embryonic stem (ES) and induced pluripotent stem (iPS) cells are pluripotent cells that have high ability to produce melanocytes [3–8]. However, the ethical problems of obtaining ES cells [9, 10] and the risk of tumorigenesis for both ES and iPS cells are obstacles to clinical use [11–14]. Melanocytes have also been induced from dental pulp stem cells (DPSCs), a kind of mesenchymal stem cell (MSC); however, induction efficiency is not high because MSCs, including DPSCs, are crude heterogeneous population, and subpopulation truly responsible for melanocyte differentiation is not identified [15, 16].

This chapter mainly focuses on multilineage-differentiating stress-enduring (Muse) cells, recently found novel type of non-tumorigenic pluripotent stem cells that are collectable from human adult skin, adipose tissue, umbilical cord, and the bone marrow [17–22]. The technique newly developed was to generate functional melanin-producing melanocytes from dermal-Muse cells by using certain cytokine induction. Induced Muse melanocytes were demonstrated to function as authentic melanocytes both in vitro and in vivo. Of note, three-dimensional (3D) human cultured skin in which Muse melanocytes were incorporated has been produced by DS Pharma Biomedical Co., Ltd. This technique is innovative because melanocytes, hard to be establish in culture and expanded with retaining their original properties, can be stably supplied with reasonable scale for industrial and clinical use.

30.2 Background of Muse Cells

Muse cells, first reported in 2010 by Dezawa et al., are non-tumorigenic endogenous pluripotent stem cells [18]. They reside in connective tissue of various kinds of organs and in the bone marrow, and thus they are somatic stem cells [23] (Fig. 30.1).

Somatic stem cells were believed not to cross the boundaries between the three germ layers. For example, neural stem cells generate neurons and glial cells but not other lineage cells such as cardiomyocytes or hepatocytes

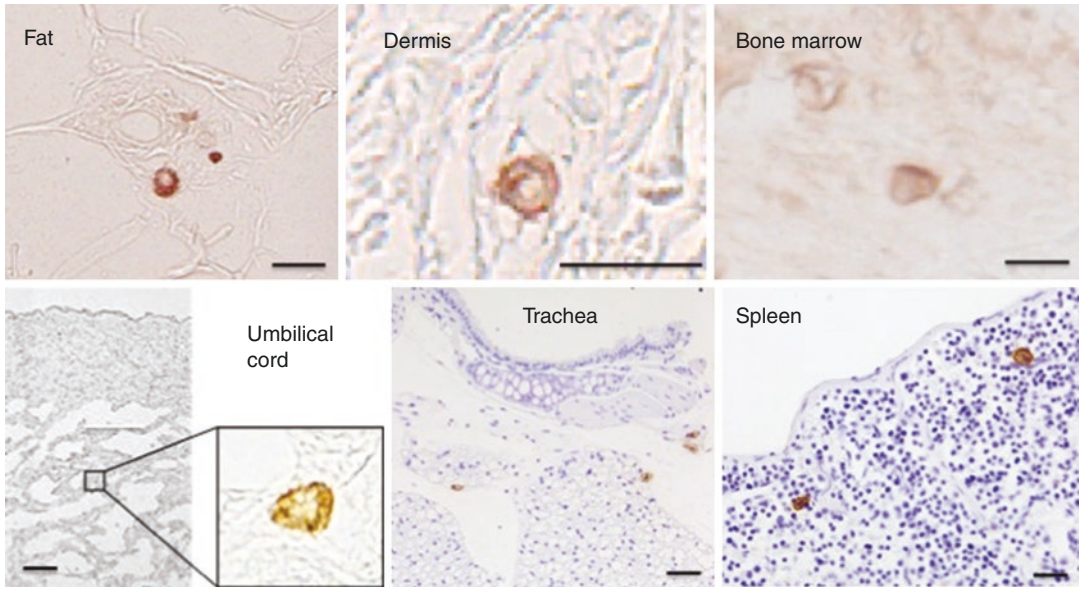


Fig. 30.1 Muse cells in adult tissues. SSEA-3(+) Muse cells, detected as HRP+ brown color coded, located in connective tissue of the human fat tissue, dermis, umbilical cord, trachea, and spleen. Muse cells also located in the bone marrow cavity. Scale bar = 50 μm. (Pictures adapted from Dezawa, (2016) *Cell Transplant*, https://doi.org/10.3727/096368916X690881)

[24]. Similarly, hematopoietic stem cells are specified stem cells for generating hematopoietic cells and not for other kinds of cells [25]. However, this dogma was questioned by the discovery of mesenchymal stem cells (MSCs) [26, 27]. MSCs are harvestable from mesenchymal tissues such as the bone marrow (BM), adipose tissue, umbilical cord, and dental pulp [27, 28]. They were reported to differentiate into cells representative of all three germ layers by either gene introduction or cytokine induction into endothelial cells [29], cardiac muscle cells [30], skeletal muscle cells [31], hepatocytes [32], neuronal cells [33], and peripheral glial cells [34], as pluripotent-like. The differentiation efficiency of MSCs, however, was generally low, suggesting that a small subpopulation was responsible for this phenomenon [28]. In fact, MSCs are population that are generally harvested simply as adherent cells from mesenchymal tissues, so that they are actually heterogeneous [27].

Muse cells were initially found from cultured mesenchymal cells, dermal fibroblasts, and BM-MSCs, as stress-enduring cells that have pluripotency [18]. Although in low frequency, BM-MSCs cultured in general culture media without any cytokines or reagents (alpha-minimum essential medium or Dulbecco's minimum essential medium supplied with 10% fetal bovine serum) spontaneously formed cell clusters which are very similar to embryoid bodies that are formed by embryonic stem (ES) cells in suspension culture [18]. Such clusters are occasionally pigmented and have hair-like structures (Fig. 30.2a). Interestingly, cells positive for each of the ectodermal, endodermal, and mesodermal markers were detected as mixture in the clusters, suggesting that MSCs contain a small subpopulation of ES-like cells [18, 23].

Generally, tissue stem cells are stress-tolerant, and while normally dormant, they are activated by stimuli such as stress [35]. We incubated BM-MSCs for over 16 h in trypsin solution containing no nutrients but only with digestive enzymes in HEPES buffer. This treatment substantially increased the ratio of SSEA-3+ cells, a surface marker for ES cells, while simultaneously eliminating cells other than the target, allowing us to identify the Muse cells [18]. The

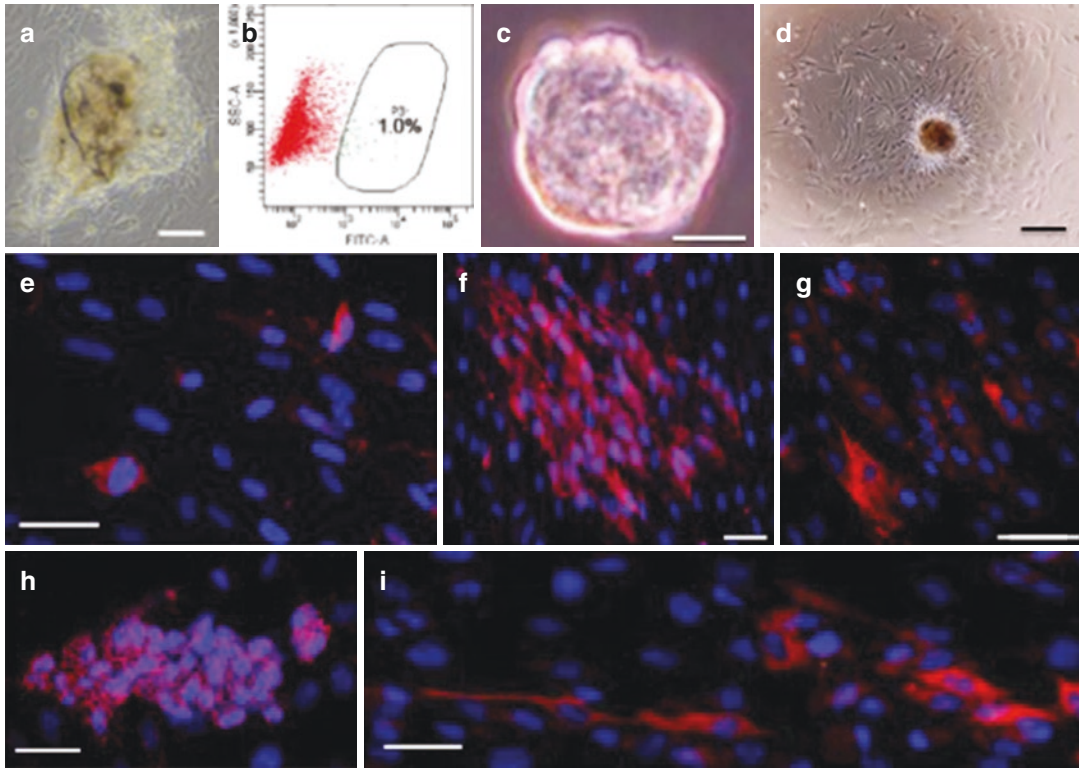


Fig. 30.2 (**a**) Human MSCs spontaneously form clusters similar to ES cell-derived embryoid bodies. (**b**) An example of cell sorting of SSEA-3(+) cells from human dermal fibroblasts. (**c**) After isolating Muse cells by fluorescence-activated cell sorting based on SSEA-3, single-Muse cells were subjected to single-cell suspension culture to form characteristic clusters which are very similar to the embryoid bodies formed by human ES cells in suspension. (**d**) Cells expanded out from the adhered adherent cluster, when the cell clusters were transferred onto gelatin culture. (**e**–**i**) The cells that expanded from the cluster on gelatin culture contained cells positive for (**e**) cytokeratin 7 (endodermal marker), (**f**) alpha-fetoprotein (endodermal), (**g**) desmin (mesodermal), (**h**) smooth muscle actin (mesodermal), and (**i**) neurofilament (ectodermal). Bars: **a**, **d** = 100 μm, **c** = 20 μm, and **e**–**i** = 50 μm. (Pictures adapted and modified with permission from Y. Kuroda et al. (2010), 2010 Proceedings of the National Academy of Sciences and with permission from Wakao et al. [22], and 2011 Proceedings of the National Academy of Sciences)

same strategy was also reproduced in fibroblasts (Fig. 30.2b), as well as in adipose-derived MSCs and umbilical cord-MSCs, showing that Muse cells are not confined to BM-MSCs but are generally contained in mesenchymal cultured cells and mesenchymal tissues [17–22] (Fig. 30.1). These SSEA-3+ cells were further shown to form cluster which is very similar to ES cell-derived embryoid body when cultured in single-cell suspension (Fig. 30.2c), suggesting that spontaneous formation of embryoid body-like clusters in general culture of BM-MSCs are to be delivered from SSEA-3+ Muse cells.

30.3 Characteristics of Muse Cells

- *SSEA-3 as a Muse cell marker*

  Muse cells express pluripotency markers such as transcription factors Oct3/4, Sox2, and Nanog, as well as pluripotent surface marker, stage-specific embryonic antigen (SSEA)-3. SSEA-3 is an antibody that recognizes glycolipid on cell surface and was first identified in mouse-fertilized egg, eight-cell stage cells, and epiblast stem cells and later was found to be expressed in human ES cells [36, 37]. Thus, SSEA-3 is relevant to pluripotent stem cells.

Currently, Muse cells are collected as SSEA-3+ cells from various sources [23]. Comparison of the gene expression between human ES/induced pluripotent stem cells (iPS) and human Muse cells reveals that the "expression pattern" of pluripotency genes is very similar between Muse and ES/iPS cells, whereas the "expression level" is moderate in Muse cells compared to ES/iPS cells [22].

Muse cells show characters relevant to pluripotent stem cells; they are able to generate cells representative of all three germ layers from a single cell, and this triploblastic differentiation ability can be reproduced over generations, demonstrating that the triploblastic differentiation ability is self-renewable [18, 21].

- *Sources of Muse cells*

MSCs are suggested to locate closely to the subendothelial region of small blood vessels [38], while Muse cells distribute sporadically in the connective tissue of various organs and do not look like associated with a structural niche [23]. Therefore, Muse cells are collectable from organs; however, such sources are not practical for both clinical and industrial uses. Sources that are easy accessible without ethical issues and fully harvestable would be mesenchymal tissues. Fibroblasts can be obtained from patients and donors by skin biopsy. In the fibroblasts, both adult dermal and foreskin fibroblasts, 1~5% of SSEA-3+ Muse cells are reported to be contained [20, 22]. Besides, fibroblasts are one of the most accessible cells from commercial sources. In fact, 2 commercially obtained fibroblasts contain Muse cell as several % of total proportion [20, 22].

In the bone marrow (BM), Muse cells reside in the BM cavity at a ratio of nearly 1:3000 cells, i.e., comprising ~0.03% of the mononucleated cell fraction [18], and different from those in connective tissue in other organs, Muse cells exist as clusters in the BM cavity [39]. In adipose tissue, a culture of 15 cm^3 (4 cm × 9 cm size) of subcutaneous adipose tissue for 3 weeks yielded ~3 × 10^7 adipose-MSCs which contained 7~8%, namely, ~two million of SSEA-3+ Muse cells, that were collectable [21].

Doubling time of Muse cells is nearly the same as that of fibroblasts, ~1.3 day/cell division. Therefore, if we collect 30 ml bone marrow aspirate, ~one million Muse cells are harvestable within 3 days [18, 23], indicating that they are practical source for clinical and industrial uses.

- *Pluripotency of Muse cells*

Pluripotent stem cells are defined as cells that can generate cells of all three germ layers and are self-renewable. As described below, Muse cells have both abilities at a single-cell level.

Differentiation ability of ES cells into triploblastic lineages is confirmable by transferring embryoid bodies formed from ES cells onto gelatin-coated culture plates to let them spontaneously differentiate. As mentioned above, Muse cells form embryoid body-like cluster when they were cultured in single-cell suspension [18] (Fig. 30.2c). When these single-Muse cell-derived clusters were transferred to gelatin-coated culture to allow the cells to expand from adhered clusters (Fig. 30.2d), cells positive for markers representative of all three germ layers, namely, neurofilament (ectodermal), smooth muscle actin (mesodermal), and alpha-fetoprotein (endodermal), are recognized in expanded cells [18, 21] (Fig. 30.2e–i). Because this differentiation is generated not by cytokine induction but spontaneously, single-Muse cells were shown to have triploblastic differentiation ability. Not only do Muse cells differentiate spontaneously, but they also differentiate at a high rate (~80–95%) into hepatocyte- and neural-lineage cells as well as into adipocytes when the proper cocktail of cytokines is supplied [21, 22].

Muse cells exhibit self-renewal of pluripotency: single-Muse cell-derived clusters generated cells positive for endodermal, mesodermal, and ectodermal markers, namely, MAP-2, GATA6, alpha-fetoprotein, and NKX2.5, in gelatin-coated culture dish [18, 21]. Other clusters were individually transferred to adherent culture and allowed to proliferate for a certain period, after which they underwent a second

round of single-cell suspension in culture to generate second-generation single-Muse cell-derived clusters. The second-generation cluster also showed triploblastic differentiation. This experimental cycle was repeated several times and clusters from each step exhibited triploblastic differentiation at the single-cell level, demonstrating self-renewability of Muse cell triploblastic differentiation ability [18, 21].

Notably, as SSEA-3+ Muse cells directly collected from human bone marrow aspirate showed expression of Oct3/4, Nanog, and Sox2 and self-renewability of triploblastic differentiation, pluripotency of Muse cells is not the resultant of artificial modification of their original characters in vitro manipulation [18].

Since Muse cells are naturally existing somatic stem cells, they generate non-Muse cells during proliferation by asymmetric cell division [18]. Even if Muse cells are purified 100% by cell sorting, they will gradually produce non-Muse cells by random asymmetrical cell division during proliferation. In this manner, proportion of Muse cells gradually decrease, reaching to one to several percent plateau of total cells, which corresponds to the proportion of Muse cells in general fibroblasts and BM-MSCs [23].

- *Non-tumorigenicity of Muse cells*

  Consistent with the fact that Muse cells reside in adult normal tissue, they are non-tumorigenic. In fact, the pattern of gene expression of cell cycle-related factors, namely, tumorigenic factors, differs between ES/iPS and Muse cells [22]. Generally, those factors are highly expressed in ES/iPS cells, while very low expression is observed in Muse cells, and the level and pattern are similar to that in somatic cells, namely, non-Muse cells (Fig. 30.4) [28].

  Notably, telomerase activity, an indicator of tumorigenic activity, is very low in Muse cells compared with iPS and Hela cells and is rather similar to that in non-Muse cells [21, 22]. In fact, when human BM- and adipose-derived Muse cells were transplanted into testes of immunodeficient mice, no teratoma formation was recognized for up to 6 months [21, 22]. Therefore, Muse cells are considered to have a low risk of tumorigenesis.

- *Other characters of Muse cells*

  The two unique characters of Muse cells that are not recognized in other types of pluripotent stem cells are their ability to perceive damage signals and autonomously home into damaged sites when administered intravenously and to spontaneously differentiate in vivo into cells compatible with the homing tissue after integration. Intravenously injected human Muse cells were demonstrated to home into the liver of fulminant hepatitis and muscle degeneration models of immunodeficient (SCID) mice, respectively, and efficiently differentiated spontaneously into albumin-producing hepatocytes and dystrophin-positive skeletal muscle cells after integration, replenished new cells, and contributed to eventual tissue repair [18]. Locally injected Muse cells also showed remarkable effects. While skin ulcers of a diabetes mellitus model are known to be intractable, subcutaneous injected human Muse cells incorporated as dermal and epidermal cells and repaired skin defect in Nod SCID-diabetes mellitus mice [40]. In a rat stroke model, human Muse cells injected into the cortex spontaneously differentiated into neuronal cells and extended neurites incorporated into pyramidal tract to recover motor function [41].

30.4 Basic Difference Between Muse Cells and Remainder of Cells, Non-Muse Cells, in Fibroblasts and BM-MSCs

Cells other than Muse cells in fibroblasts or BM-MSCs, namely, non-Muse cells, sharply contrast with Muse cells in many points. Non-Muse cells are SSEA-3-negative, and their expression level of pluripotency genes is generally very low or under detection level [22]. Methylation level of pluripotency marker is consistent; promoter of Oct3/4 and Nanog in non-Muse cells is mostly methylated, while those of Muse cells are more demethylated [22].

While non-Muse cells are able to differentiate into osteocytes, cartilage cells, and adipocytes in the presence of certain sets of induction

cytokines, their differentiation rate is substantially lower than that of Muse cells, and the time required for differentiation is longer than that required for the differentiation of Muse cells [21, 42]. Most importantly, non-Muse cells are unable to cross the lineage boundaries between mesoderm, where they originally belong to, to ectoderm or endoderm. Even under cytokine inductions, non-Muse cells do not differentiate into hepatocytes and neuronal cells although they show only partial intracellular responses to cytokine induction [21, 42].

Non-Muse cells do not exhibit tissue repair effect; most importantly, they do not remain in the host-damaged tissues after administration and thus do not differentiate nor replenish lost cells [18, 41, 43, 44].

For these reasons, utilization of purified Muse cell population rather than crude MSCs or fibroblasts is rational for generating purposive cells and tissue regeneration.

30.5 Comparison of Differentiation Propensity of Muse Cells from Different Sources

To examine whether Muse cells derived from different tissues have different differentiation propensities, gene expression of ectodermal-, endodermal-, and mesodermal-lineages in Muse cells derived from the BM, dermal fibroblasts, and adipose tissue was compared [21].

Ectodermal-related genes were generally higher in both dermal-Muse and BM-Muse cells than in adipose-Muse cells, and particularly microphthalmia-associated transcription factor (MITF) and KIT, genes relevant to melanocytes, were expressed in dermal-Muse cells [42]. On the other hand, mesodermal-lineage genes were generally higher in adipose-Muse cells than in dermal- and BM-Muse cells; for example, SP7, osteogenic factor, and Pax7, muscle stem cell marker, were only detected in adipose-Muse cells. In endodermal-lineage genes, BM-Muse cells demonstrated the highest levels, particularly genes pertinent to hepatocyte- and pancreatic-lineages [21].

While pluripotency is the same among dermal-, BM-, and adipose-Muse cells, their differentiation propensity seemed different from each other. This suggests that the appropriate tissue source should be selected based on the target cell type and that dermal-Muse cells are the proper candidate for generating melanocytes.

30.6 Generation of Melanocytes from Human Dermal-Muse Cells

The Wnt3a, endothelin-3 (ET-3), stem cell factor (SCF), basic fibroblast growth factor (b-FGF), and cAMP inducers (such as cholera toxin and TPA) are known to promote the expression of transcription factors PAX3, SOX10, CREB, and LEF1 through intracellular signaling [45–47]. These four transcription factors regulate the promoter of MITF-M, one of the MITF variants specific for melanocytes, and have a crucial role in melanocyte differentiation. Ascorbic acid stimulates the activity and synthesis of tyrosinase [48], which plays an important role in functional melanocyte. Dexamethasone promotes the generation of melanocytic cells from mouse ES cells [7].

Based on this knowledge, melanocyte induction system was set as follows: human dermal-Muse cells were seeded at a density of 10,000 cells per 6-well plate and cultured for 1 day in α-MEM without serum. The cells were then cultured in differentiation medium containing 0.05 M dexamethasone, insulin-transferrin-selenium (ITS), 1 mg/ml linoleic acid-bovine serum albumin, 30% low-glucose DMEM, 20% MCDB-201 medium, 10^{-4} M L-ascorbic acid, 50% DMEM conditioned by L-Wnt3a cells that contain Wnt3a, 50 ng/ml SCF, 10 nM ET-3, 20 pM cholera toxin, 50 nM 12-*O*-tetradecanoyl-phorbol 13-acetate (TPA), and 4 ng/ml b-FGF. Cells were maintained in this differentiation medium for 6 weeks with medium exchange every 2 days. Cultures were passaged when cells reached ~80% confluency [42].

The morphology of the Muse cells started to change and cells with dendrites appeared within 3 weeks (Fig. 30.3). Cell size was reduced by 5 weeks, and by 6 weeks the cells had morphology

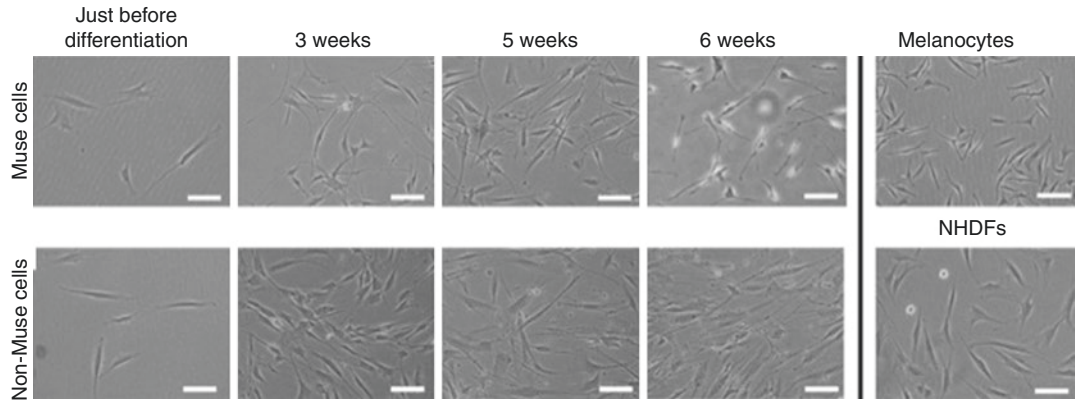


Fig. 30.3 Generation of Muse melanocytes. Phase contrast microscopic images of Muse and non-Muse cells during melanocyte induction. Muse melanocytes at 6 weeks are similar to human primary melanocytes (melanocytes), while non-Muse cells at 6 weeks are more similar to original fibroblasts (NHDF). Scale bar = 50 μm. (Pictures adapted from Tsuchiyama, K. et al. (2013). *J Invest Dermatol, 133*(10), 2425–2435, https://doi.org/10.1038/jid.2013.172)

very similar to that of human melanocytes (Fig. 30.3). Muse cells which originally express MITF and KIT newly expressed tyrosinase-related protein 1 (TRP-1) and gp100 at 3 weeks, dopachrome tautomerase (DCT) at 5 weeks, and finally tyrosinase at 6 weeks, at which point they become pigment-producing functional melanocytes that are positive for the L-DOPA reaction (Fig. 30.4). Expressions of tyrosinase, gp100, and MITF were not only recognized in gene level but were also confirmed by protein level, immunocytochemistry. These results demonstrated that cells with characteristics very close to human primary melanocytes were induced from human dermal-Muse cells [42].

Among ten factors used for melanocytes induction, essential factors were searched. Fang et al. described a combination of Wnt3a, ET-3, and SCF that is sufficient to induce melanocytes from pluripotent stem cells [3]. When Muse cells were cultured in medium lacking any of those three factors, expression of melanocyte-related markers was not recognized, and morphology of cells remained fibroblast-like [42]. Reversely, when Muse cells were cultured only with those three factors, cells expressed almost all of essential melanocyte markers, MITF, KIT, TRP-1, DCT, and gp100, but not tyrosinase, one of the most important enzymes for melanocyte function, suggesting that while these three factors play a central role in differentiation into melanocytes, dermal-Muse cells need additional differentiation-enhancing factors such as cholera toxin, TPA, and linoleic acid [42].

30.7 Reaction of Non-Muse Dermal Fibroblasts Against Melanocyte Induction

Muse cells and non-Muse cells have fundamental differences; Muse cells originally express a subset of pluripotency genes, but those are not expressed in non-Muse cells [22]. Non-Muse cells show lower ratio of differentiation into osteocytes and adipocytes by cytokine induction, while they did not show ectodermal or endodermal differentiation even under the presence of cytokines [21]. Similarly, the reaction of non-Muse cells to melanocyte induction was different from that of Muse cells.

Human dermal-non-Muse cells newly expressed TRP-1 at 3 weeks, while its expression was not sustained for a longer period and eventually returned to negative by 5 weeks, with the cells reverting back to the gene expression pattern of fibroblasts, showing a fibroblast-like morphology (Figs. 30.3 and 30.4). DCT and gp100 were never expressed for the entire period of induction. While MITF signal was detected in non-Muse cell-derived cells in RT-PCR level at 6 weeks, the protein expression level was not high enough to

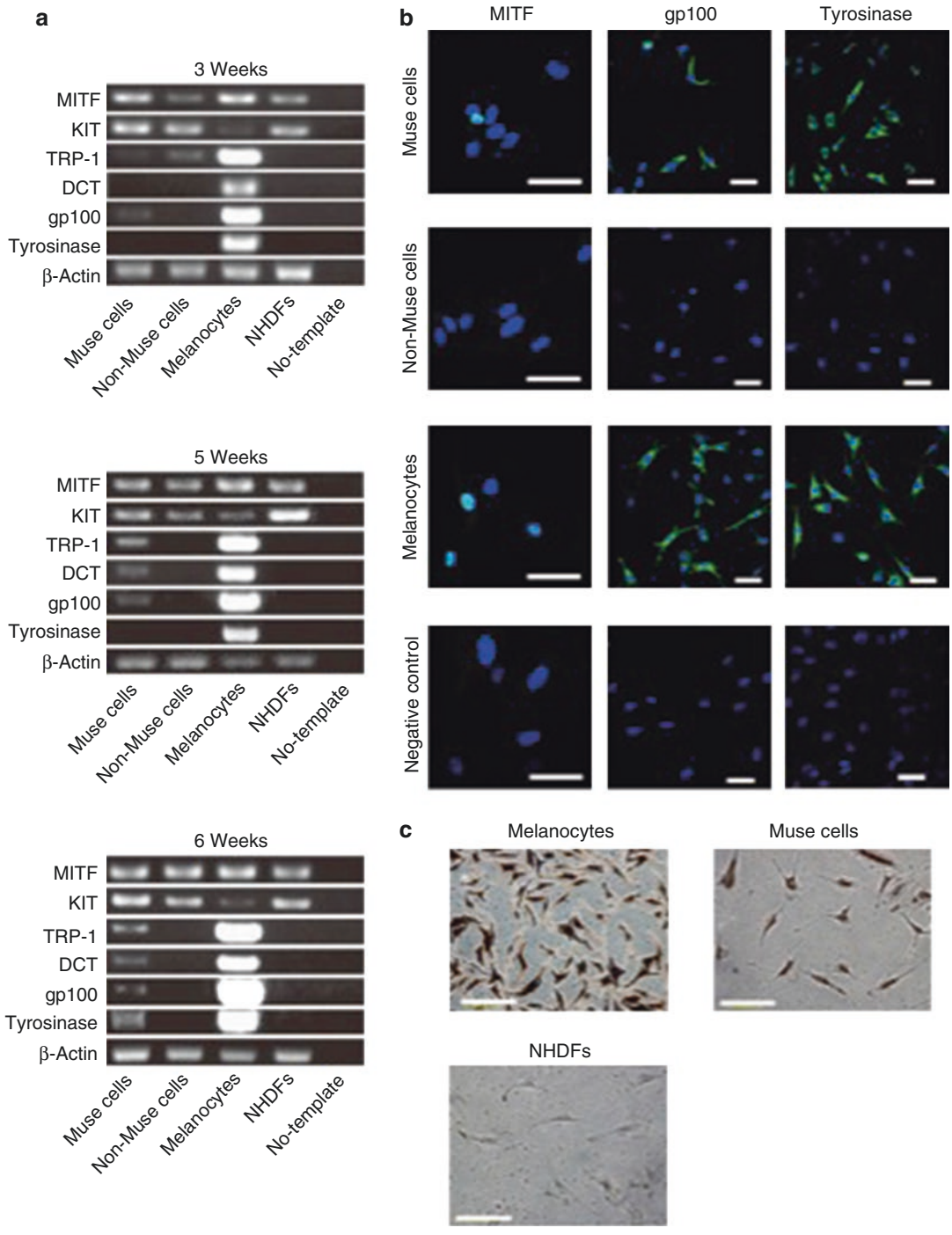


Fig. 30.4 Melanocyte marker expression in Muse melanocytes. (**a**) RT-PCR of melanocyte marker in Muse cells, non-Muse cells, human primary melanocytes (melanocytes), human dermal fibroblasts (NHDFs), and non-template at 3, 5, and 6 weeks. (**b**) Immunocytochemistry for MITF, gp100, and tyrosinase in immunocytochemical analysis of the melanocyte markers MITF, gp100, and tyrosinase in Muse and non-Muse cells at 6 weeks after differentiation. The positive control was human melanocytes, and the negative control was naive Muse cells without primary antibody. Scale bars = 50 mm. (**c**) L-DOPA reaction assay of Muse melanocytes (6 weeks), human melanocytes (positive control), and naive NHDFs (negative control). Scale bars = 100 mm. (Pictures adapted from Tsuchiyama, K. et al. (2013). *J Invest Dermatol, 133*(10), 2425–2435, https://doi.org/10.1038/jid.2013.172)

be detected by immunocytochemistry (Figs. 30.3 and 30.4). Consequently, none of non-Muse cells were positive for these melanocyte markers, and this suggested that non-Muse cells responded partially to melanocyte induction [42]. Therefore, there is rationality for purified Muse cells to be used for efficient generation of melanocytes.

30.8 Generation of Human-Colored 3D-Cultured Skin

Before evaluation of Muse melanocytes in vivo, their function was validated in vitro. If they truly acquired melanocyte properties, they would successfully integrate into the place where authentic melanocytes normally reside and express functional markers in the 3D-cultured skin, which provides visible system to evaluate functional property of Muse melanocytes. There is another benefit for producing 3D-cultured skin system; animal skins covered with dense hairs show very different histological structure from that of human, and thus animal experiment has limitation for safety test. Therefore, an in vitro assay system that mimics human skin is highly demanded.

Human skin is comprised of the dermis which mainly contains fibroblasts and matrix, such as collagen, and the epidermis which is mainly composed of keratinocytes. Ideally, 3D-cultured skin should show integration of melanocytes at correct position, the basal to second layer of the epidermal keratinocyte layer, and a dermal papilla-like structure. For this, human fibroblasts, human keratinocytes, and collagen type 1 were prepared along with Muse melanocytes.

A gel layer was created comprising collagen type 1 and human dermal fibroblasts (3.5×10^5 cells/ml), to mimic the dermis, and cultured for ~3 days (Fig. 30.5). After making the dermal

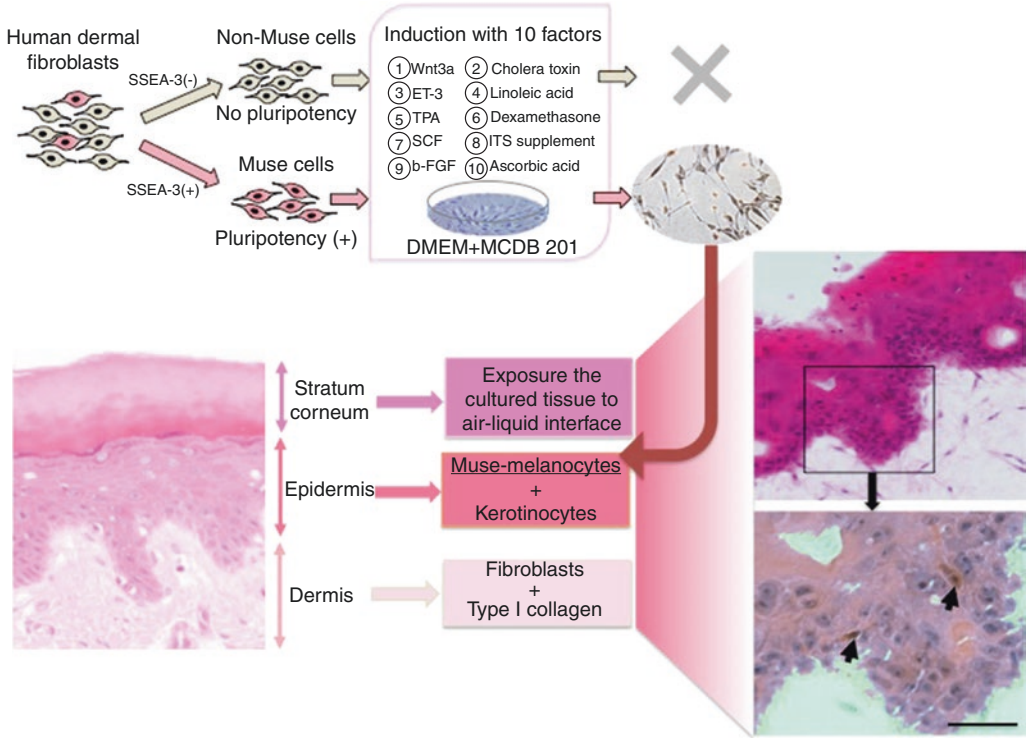


Fig. 30.5 Outline of melanocyte generation from dermal-Muse cells. Human dermal fibroblasts are source of Muse cells. SSEA-3(+) Muse cells and SSEA-3(−) non-Muse cells were separated, and each population was subjected to induction with ten factors. Muse cells successfully generated melanin-producing melanocytes, while non-Muse cells did not. 3D-cultured skin was constructed by incorporating Muse melanocytes into basal layer of the stratum corneum. Scale bar = 50 μm. (Pictures adapted and modified from Tsuchiyama, K. et al. (2013). *J Invest Dermatol, 133*(10), 2425–2435, https://doi.org/10.1038/jid.2013.172)

equivalent, the epidermal layer was placed onto the dermal equivalent by mixing Muse melanocytes: keratinocytes at 1:2.5. They were then incubated in Humedia KG2 medium (Kurabo) for 5 days with a gradually increasing Ca^{2+} concentration from 0.15 to 1.5 mM. For the final step, 3D-cultured skins were cultivated for another 7 days at an air-liquid interface to accelerate keratinization [42] (Fig. 30.5).

As a consequence, 3D skins showed dermal-papilla-like structure, placing pigmented Muse melanocytes in the basal to second layer of the epidermis (Fig. 30.5). In addition, cells positive for MITF, tyrosinase, TRP-1, gp100, and S100 were all identified in the basal layer of epidermal layer, and Fontana–Masson staining revealed the presence of melanin in the epidermis of both cultures (Fig. 30.6). When Muse melanocytes were replaced with other cell types, such as keratinocytes or non-Muse cells, no pigmented cells or cells positive for melanocyte-related markers and Fontana–Masson staining were observed (Fig. 30.6). These suggest that Muse melanocytes could integrate into the basal layer of epidermal layer of 3D human cultured skin with sustaining melanocyte function [42].

Even though Muse cells show spontaneous differentiation into the cells compatible to the tissue, they homed in in vivo disease models [18, 41, 43, 44]; naive Muse cells did not spontaneously differentiate into melanocyte property nor did they spontaneously become positive for melanocyte markers or Fontana–Masson staining when supplied to artificial 3D-cultured skin [42]. This result indicated that naive Muse cells do not spontaneously differentiate into melanocytes in vitro even if they are integrated into the epidermal layer of 3D-cultured skin, and thus, induction of Muse cells into melanocytes is required before construction of 3D human cultured skin.

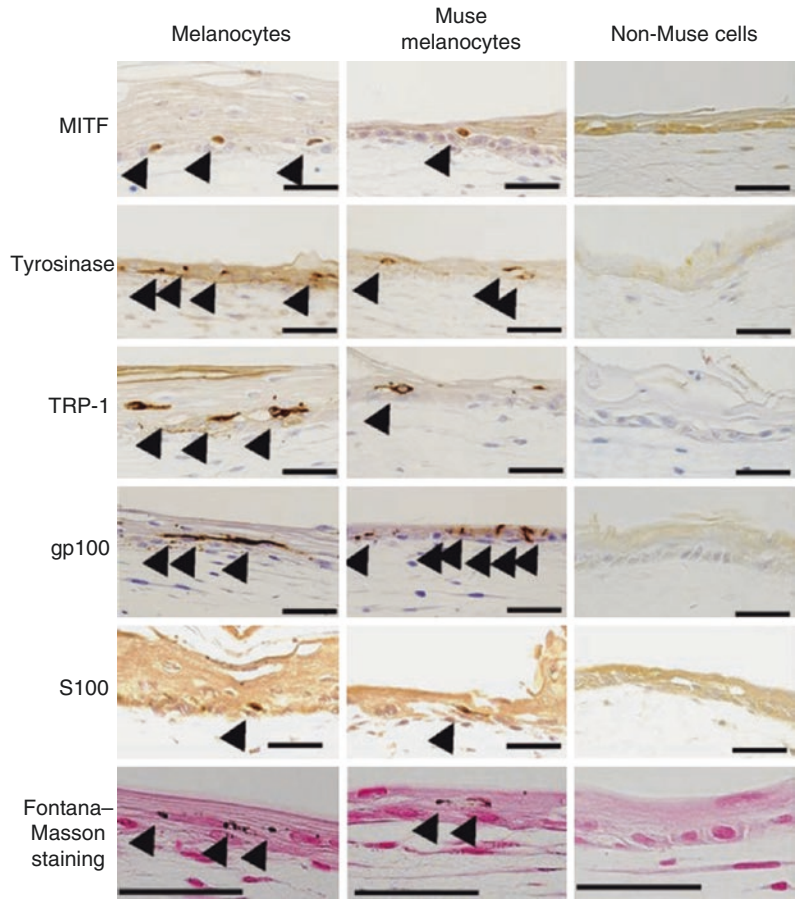


Fig. 30.6 Melanocyte maker expression in 3D skin culture. Immunohistochemical analysis of MITF, tyrosinase, TRP-1, gp100, and S-100 and the Fontana–Masson staining of the 3D-cultured skin. Scale bars = 50 μm. (Pictures adapted from Tsuchiyama, K. et al. (2013). *J Invest Dermatol, 133*(10), 2425–2435, https://doi.org/10.1038/jid.2013.172)

30.9 Functional Evaluation of Muse Melanocytes In Vivo

To investigate whether Muse melanocytes can survive and maintain their melanocyte functions in vivo, 3D-cultured skin, in which human Muse melanocytes were incorporated, was transplanted onto the back skin of severe combined immune deficiency (SCID) mice. 3D-cultured skin containing human melanocytes and 3D-cultured skin containing only keratinocytes were transplanted as positive and negative controls, respectively (Fig. 30.7). Histologic evaluation revealed that skin grafts with Muse melanocytes contained brown color Muse melanocytes located in the basal layer, and immunohistochemical analysis demonstrated that they were positive for MITF, tyrosinase, TRP-1, gp100, and S100, the same as grafted human melanocytes (Fig. 30.7). Furthermore, Muse melanocytes and the neighboring keratinocytes were positive for Fontana–Masson staining, as seen in the 3D human skin culture with human primary melanocytes (Fig. 30.7). Following transplantation of 3D-cultured skin containing GFP-labeled Muse melanocytes, GFP-positive Muse melanocytes were confirmed to be located within the grafted skin, demonstrating that these GFP-positive cells were transplanted cells and not derived from the host [42]. Double staining for the melanocyte marker S100 and the proliferative marker Ki-67 revealed the proliferation capacity of Muse melanocytes; 9.5% of Muse melanocytes expressed both Ki-67 and S100 [42]. In the negative control, the skin graft did not appear pigmented, and no pigmented cells were observed histologically.

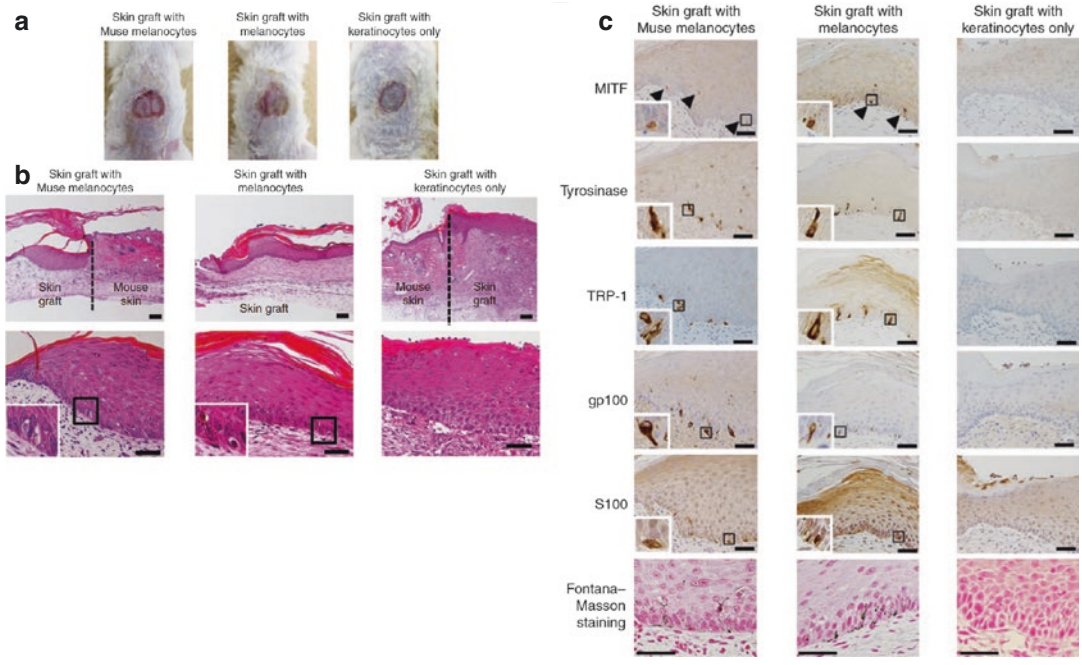


Fig. 30.7 Functional characterization of 3D skin culture in combined immunodeficient (SCID) mouse. (**a**) Macroscopic observation of skin grafts containing Muse melanocytes, human melanocytes, and keratinocytes only 10 days after transplantation. (**b**) Hematoxylin and eosin staining of each skin graft. Scale bars 100 μm (upper panels) and 50 mm (lower panels). (**c**) Immunohistochemical analysis of MITF, tyrosinase, TRP-1, gp100, S100, and the Fontana–Masson staining in the skin grafts, showing that 3D skin culture maintain melanocyte function of incorporated Muse melanocytes. Scale bars = 50 μm. (Pictures adapted from Tsuchiyama, K. et al. (2013). *J Invest Dermatol, 133*(10), 2425–2435, https://doi.org/10.1038/jid.2013.172)

These findings indicated that Muse melanocytes homed to the basal layer of the epidermis, produced melanin, and delivered it to the neighboring keratinocytes in vivo.

30.10 Other Stem Cells as Source for Melanocytes

It has been reported previously that other pluripotent cells, such as ES cells and iPS cells, can be differentiated into melanocytes [3, 5–8, 49]. However, the potential clinical applications of melanocytes derived from pluripotent ES cells or iPS cells have faced several hurdles. There continue to be ethical controversies surrounding the use of ES cells, and for iPS cells, inefficient generation and the presence of integrated transgenes are significant limitations, although alternatives to viral vectors or the administration of analogous proteins or chemical compounds for cell reprogramming may ultimately address these problems. In addition, it is known that the response to differentiation cues is variable for some iPS cell lines.

Multipotent stem cells are reported to reside in skin dermis. Toma et al. reported that skin-derived precursors (SKPs) existing in human foreskin tissue are multipotent stem cells [50]. SKPs are derived from neural crest cells residing in the skin, and the putative niche of SKPs is in human hair papilla [51]. SKPs express a stem cell marker Sox2 and have self-renewal ability and express Snail and Slug as characteristic markers. Since Muse cells don't express Snail and Slug primarily, SKPs and Muse cells are different populations though both of them are isolated from the dermis. While SKPs are derived from neural crest cells, SKPs do not express melanocyte markers such as DCT nor do they differentiate into both neural and mesodermal progeny [52, 53]. On the other hand, dermis-derived stem cells (DSCs), which are also isolated from human foreskins, differentiate into melanocytes [54]. DSCs are isolated from spheres formed from dermis-derived single cells grown in HESCM4 medium, which is sufficient to maintain human embryonic stem cells in an undifferentiated state in the absence of feeder cells. The dermal stem cells, grown as three-dimensional spheres, displayed a capacity for self-renewal and expressed NGFRp75, nestin, and OCT4 but not melanocyte markers. Under conditions favoring melanocyte formation as defined with human ES cells and HFSCs [3, 55], some of the attached DSCs develop dendritic processes and express the melanocyte markers MITF, DCT, S100, and HMB45. Thus at least a couple of multipotent stem cells reside in human skin, and Muse cells and DSCs differentiate to melanocytes in certain conditions in vitro.

30.11 Future Perspectives

By full utilization of Muse cells, the technique to generate melanin-producing functional melanocytes and the basis for colored 3D human cultured skin that mimics human skin tissue could be both developed. DS Pharma Biomedical Co., Ltd. applied this technique for industrial use and now producing "POCA® human 3D 'HADA' (High-performance and Advanced Dermal Assay)." The system is beneficial particularly for safety test for validation of cosmetics and drugs, since animal experiment has limitation for different histological structure of the skin. Since Muse melanocytes are incorporated into the 3D skin culture, validation for adverse effect that may cause vitiligo is expected to be enforceable.

Muse cells are non-tumorigenic endogenous pluripotent stem cells that show high differentiation ability across oligolineage boundaries from mesodermal to endodermal or ectodermal. Melanocyte differentiation is one of their abilities. Notably, the current technique does not require exogenous gene introduction for converting dermal-Muse cells into functional melanocytes, lowering hurdles for application to clinical use. For example, patients' skin biopsy-derived Muse cells could be converted into Muse melanocytes and transplanted for autologous treatment of vitiligo, a possibility to be validated in a future study.

References

1. Fioramonti P, Onesti MG, Marchese C, Carella S, Ceccarelli S, Scuderi N. Autologous cultured melanocytes in vitiligo treatment comparison of two techniques to prepare the recipient site: erbium-doped yttrium aluminum garnet laser versus dermabrasion. Dermatol Surg. 2012;38(5):809–12. https://doi.org/10.1111/j.1524-4725.2012.02354.x.
2. van Geel N, Ongenae K, Naeyaert JM. Surgical techniques for vitiligo: a review. Dermatology. 2001;202(2):162–6. https://doi.org/10.1159/000051626.
3. Fang D, Leishear K, Nguyen TK, Finko R, Cai K, Fukunaga M, et al. Defining the conditions for the generation of melanocytes from human embryonic stem cells. Stem Cells. 2006;24(7):1668–77. https://doi.org/10.1634/stemcells.2005-0414.
4. Motohashi T, Aoki H, Yoshimura N, Kunisada T. Induction of melanocytes from embryonic stem cells and their therapeutic potential. Pigment Cell Res. 2006;19(4):284–9. https://doi.org/10.1111/j.1600-0749.2006.00317.x.
5. Nissan X, Larribere L, Saidani M, Hurbain I, Delevoye C, Feteira J, et al. Functional melanocytes derived from human pluripotent stem cells engraft into pluristratified epidermis. Proc Natl Acad Sci U S A. 2011;108(36):14861–6. https://doi.org/10.1073/pnas.1019070108.
6. Ohta S, Imaizumi Y, Okada Y, Akamatsu W, Kuwahara R, Ohyama M, et al. Generation of human melanocytes from induced pluripotent stem cells. PLoS One. 2011;6(1):e16182. https://doi.org/10.1371/journal.pone.0016182.
7. Yamane T, Hayashi S, Mizoguchi M, Yamazaki H, Kunisada T. Derivation of melanocytes from embryonic stem cells in culture. Dev Dyn. 1999;216(4–5):450–8. https://doi.org/10.1002/(SICI)1097-0177(199912)216:4/5<450::AID-DVDY13>3.0.CO;2-0.
8. Yang R, Jiang M, Kumar SM, Xu T, Wang F, Xiang L, et al. Generation of melanocytes from induced pluripotent stem cells. J Invest Dermatol. 2011;131(12):2458–66. https://doi.org/10.1038/jid.2011.242.
9. Knoppers BM, Bordet S, Isasi R. The human embryo: ethical and legal aspects. Methods Mol Biol. 2009;550:281–305. https://doi.org/10.1007/978-1-60327-009-0_18.
10. Manzar N, Manzar B, Hussain N, Hussain MF, Raza S. The ethical dilemma of embryonic stem cell research. Sci Eng Ethics. 2013;19(1):97–106. https://doi.org/10.1007/s11948-011-9326-7.
11. Ben-David U, Benvenisty N. The tumorigenicity of human embryonic and induced pluripotent stem cells. Nat Rev Cancer. 2011;11(4):268–77. https://doi.org/10.1038/nrc3034.
12. Fong CY, Gauthaman K, Bongso A. Teratomas from pluripotent stem cells: a clinical hurdle. J Cell Biochem. 2010;111(4):769–81. https://doi.org/10.1002/jcb.22775.
13. Goldring CE, Duffy PA, Benvenisty N, Andrews PW, Ben-David U, Eakins R, et al. Assessing the safety of stem cell therapeutics. Cell Stem Cell. 2011;8(6):618–28. https://doi.org/10.1016/j.stem.2011.05.012.
14. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature. 2007;448(7151):313–7. https://doi.org/10.1038/nature05934.
15. Paino F, Ricci G, De Rosa A, D'Aquino R, Laino L, Pirozzi G, et al. Ecto-mesenchymal stem cells from dental pulp are committed to differentiate into active melanocytes. Eur Cell Mater. 2010;20:295–305.
16. Stevens A, Zuliani T, Olejnik C, LeRoy H, Obriot H, Kerr-Conte J, et al. Human dental pulp stem cells differentiate into neural crest-derived melanocytes and have label-retaining and sphere-forming abilities. Stem Cells Dev. 2008;17(6):1175–84. https://doi.org/10.1089/scd.2008.0012.
17. Heneidi S, Simerman AA, Keller E, Singh P, Li X, Dumesic DA, et al. Awakened by cellular stress: isolation and characterization of a novel population of pluripotent stem cells derived from human adipose tissue. PLoS One. 2013;8(6):e64752. https://doi.org/10.1371/journal.pone.0064752.
18. Kuroda Y, Kitada M, Wakao S, Nishikawa K, Tanimura Y, Makinoshima H, et al. Unique multipotent cells in adult human mesenchymal cell populations. Proc Natl Acad Sci U S A. 2010a;107(19):8639–43. https://doi.org/10.1073/pnas.0911647107. 0911647107 [pii].
19. Kuroda Y, Wakao S, Kitada M, Murakami T, Nojima M, Dezawa M. Isolation, culture and evaluation of multilineage-differentiating stress-enduring (Muse) cells. Nat Protoc. 2013;8(7):1391–415. https://doi.org/10.1038/nprot.2013.076.
20. Liu Q, Zhang RZ, Li D, Cheng S, Yang YH, Tian T, et al. Muse cells, a new type of pluripotent stem cell derived from human fibroblasts. Cell Reprogram. 2016;18(2):67–77. https://doi.org/10.1089/cell.2015.0085.
21. Ogura F, Wakao S, Kuroda Y, Tsuchiyama K, Bagheri M, Heneidi S, et al. Human adipose tissue possesses a unique population of pluripotent stem cells with nontumorigenic and low telomerase activities: potential implications in regenerative medicine. Stem Cells Dev. 2014;23(7):717–28. https://doi.org/10.1089/scd.2013.0473.
22. Wakao S, Kitada M, Kuroda Y, Shigemoto T, Matsuse D, Akashi H, et al. Multilineage-differentiating stress-enduring (Muse) cells are a primary source of induced pluripotent stem cells in human fibroblasts. Proc Natl Acad Sci U S A. 2011;108(24):9875–80. https://doi.org/10.1073/pnas.1100816108.
23. Dezawa M. Muse cells provide the pluripotency of mesenchymal stem cells: direct contribution of Muse cells to tissue regeneration. Cell Transplant. 2016. https://doi.org/10.3727/096368916X690881.

24. Gage FH. Mammalian neural stem cells. Science. 2000;287(5457):1433–8.
25. Weissman IL, Shizuru JA. The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases. Blood. 2008;112(9):3543–53. https://doi.org/10.1182/blood-2008-08-078220.
26. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet. 1970;3(4):393–403.
27. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284(5411):143–7.
28. Kuroda Y, Kitada M, Wakao S, Dezawa M. Bone marrow mesenchymal cells: how do they contribute to tissue repair and are they really stem cells? Arch Immunol Ther Exp. 2011;59(5):369–78. https://doi.org/10.1007/s00005-011-0139-9.
29. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells. 2004;22(3):377–84. https://doi.org/10.1634/stemcells.22-3-377.
30. Qayyum AA, Haack-Sorensen M, Mathiasen AB, Jorgensen E, Ekblond A, Kastrup J. Adipose-derived mesenchymal stromal cells for chronic myocardial ischemia (MyStromalCell Trial): study design. Regen Med. 2012;7(3):421–8. https://doi.org/10.2217/rme.12.17.
31. Dezawa M, Ishikawa H, Itokazu Y, Yoshihara T, Hoshino M, Takeda S, et al. Bone marrow stromal cells generate muscle cells and repair muscle degeneration. Science. 2005;309(5732):314–7. https://doi.org/10.1126/science.1110364.
32. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, et al. Bone marrow as a potential source of hepatic oval cells. Science. 1999;284(5417):1168–70.
33. Dezawa M, Kanno H, Hoshino M, Cho H, Matsumoto N, Itokazu Y, et al. Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. J Clin Invest. 2004;113(12):1701–10. https://doi.org/10.1172/JCI20935.
34. Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H. Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. Eur J Neurosci. 2001;14(11):1771–6.
35. Kingham PJ, Kalbermatten DF, Mahay D, Armstrong SJ, Wiberg M, Terenghi G. Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. Exp Neurol. 2007;207(2):267–74. https://doi.org/10.1016/j.expneurol.2007.06.029. S0014-4886(07)00257-9 [pii].
36. Fox NW, Damjanov I, Knowles BB, Solter D. Stage-specific embryonic antigen 3 as a marker of visceral extraembryonic endoderm. Dev Biol. 1984;103(1):263–6.
37. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282(5391):1145–7.
38. Galderisi U, Giordano A. The gap between the physiological and therapeutic roles of mesenchymal stem cells. Med Res Rev. 2014;34(5):1100–26. https://doi.org/10.1002/med.21322.
39. Hori E, Hayakawa Y, Hayashi T, Hori S, Okamoto S, Shibata T, et al. Mobilization of pluripotent multilineage-differentiating stress-enduring cells in ischemic stroke. J Stroke Cerebrovasc Dis. 2016. https://doi.org/10.1016/j.jstrokecerebrovasdis.2015.12.033.
40. Kinoshita K, Kuno S, Ishimine H, Aoi N, Mineda K, Kato H, et al. Therapeutic potential of adipose-derived SSEA-3-positive muse cells for treating diabetic skin ulcers. Stem Cells Transl Med. 2015;4(2):146–55. https://doi.org/10.5966/sctm.2014-0181.
41. Uchida H, Morita T, Niizuma K, Kushida Y, Kuroda Y, Wakao S, et al. Transplantation of unique subpopulation of fibroblasts, muse cells, ameliorates experimental stroke possibly via robust neuronal differentiation. Stem Cells. 2016;34(1):160–73. https://doi.org/10.1002/stem.2206.
42. Tsuchiyama K, Wakao S, Kuroda Y, Ogura F, Nojima M, Sawaya N, et al. Functional melanocytes are readily reprogrammable from multilineage-differentiating stress-enduring (muse) cells, distinct stem cells in human fibroblasts. J Invest Dermatol. 2013;133(10):2425–35. https://doi.org/10.1038/jid.2013.172.
43. Katagiri H, Kushida Y, Nojima M, Kuroda Y, Wakao S, Ishida K, et al. A distinct subpopulation of bone marrow mesenchymal stem cells, muse cells, directly commit to the replacement of liver components. Am J Transplant. 2016;16(2):468–83. https://doi.org/10.1111/ajt.13537.
44. Yamauchi T, Kuroda Y, Morita T, Shichinohe H, Houkin K, Dezawa M, et al. Therapeutic effects of human multilineage-differentiating stress enduring (MUSE) cell transplantation into infarct brain of mice. PLoS One. 2015;10(3):e0116009. https://doi.org/10.1371/journal.pone.0116009.
45. Dong L, Li Y, Cao J, Liu F, Pier E, Chen J, et al. FGF2 regulates melanocytes viability through the STAT3-transactivated PAX3 transcription. Cell Death Differ. 2012;19(4):616–22. https://doi.org/10.1038/cdd.2011.132.
46. Kondo T, Hearing VJ. Update on the regulation of mammalian melanocyte function and skin pigmentation. Expert Rev Dermatol. 2011;6(1):97–108. https://doi.org/10.1586/edm.10.70.
47. Steingrimsson E, Copeland NG, Jenkins NA. Melanocytes and the microphthalmia transcription factor network. Annu Rev Genet. 2004;38:365–411. https://doi.org/10.1146/annurev.genet.38.072902.092717.
48. Lee SA, Son YO, Kook SH, Choi KC, Lee JC. Ascorbic acid increases the activity and synthesis

of tyrosinase in B16F10 cells through activation of p38 mitogen-activated protein kinase. Arch Dermatol Res. 2011;303(9):669–78. https://doi.org/10.1007/s00403-011-1158-4.
49. Motohashi T, Aoki H, Chiba K, Yoshimura N, Kunisada T. Multipotent cell fate of neural crest-like cells derived from embryonic stem cells. Stem Cells. 2007;25(2):402–10. https://doi.org/10.1634/stemcells.2006-0323.
50. Toma JG, McKenzie IA, Bagli D, Miller FD. Isolation and characterization of multipotent skin-derived precursors from human skin. Stem Cells. 2005;23(6):727–37. https://doi.org/10.1634/stemcells.2004-0134.
51. Fernandes KJ, McKenzie IA, Mill P, Smith KM, Akhavan M, Barnabe-Heider F, et al. A dermal niche for multipotent adult skin-derived precursor cells. Nat Cell Biol. 2004;6(11):1082–93. https://doi.org/10.1038/ncb1181.
52. Joannides A, Gaughwin P, Schwiening C, Majed H, Sterling J, Compston A, et al. Efficient generation of neural precursors from adult human skin: astrocytes promote neurogenesis from skin-derived stem cells. Lancet. 2004;364(9429):172–8. https://doi.org/10.1016/S0140-6736(04)16630-0.
53. Toma JG, Akhavan M, Fernandes KJ, Barnabe-Heider F, Sadikot A, Kaplan DR, et al. Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol. 2001;3(9):778–84. https://doi.org/10.1038/ncb0901-778.
54. Li L, Fukunaga-Kalabis M, Yu H, Xu X, Kong J, Lee JT, et al. Human dermal stem cells differentiate into functional epidermal melanocytes. J Cell Sci. 2010;123.(Pt 6:853–60. https://doi.org/10.1242/jcs.061598.
55. Yu H, Fang D, Kumar SM, Li L, Nguyen TK, Acs G, et al. Isolation of a novel population of multipotent adult stem cells from human hair follicles. Am J Pathol. 2006;168(6):1879–88. https://doi.org/10.2353/ajpath.2006.051170.

Other Defects/Mechanisms

31

Maria Lucia Dell'Anna and Mauro Picardo

Contents

Abstract

Vitiligo pathogenesis still represents a puzzle whose pieces are not all at the same level of knowledge and interest among the scientist people. The most popular aspects currently considered include genetic, inflammatory, autoimmune, oxidative, and metabolic alterations, even if the individual contribution of each of these alterations is still unclear. The role of local environment, detachment process and emerging pathways needs renewed attention. These pieces of vitiligo puzzle represent a possible link between the most studied mechanisms and all three together are supporting each other.

Key Points

- Overexpression of senescence-associated markers has been described in the entire non-lesional epidermis.
- ECM proteins contribute to melanocyte adhesion to the basement membrane.
- Vitiligo fibroblasts appeared flattened and enlarged, resembling senescent cells.
- An increased expression of CCN3 in keratinocytes in lesional skin was the most prominent feature.
- Decreased activation of the WNT pathway in vitiligo.
- WNT/β-catenin pathway was found to have a key role in UVB-induced melanocyte stem cell differentiation.

31.1 Local (Dermal) Environment

Increasing evidence suggest the presence of cellular and functional abnormalities encompassing other cutaneous cell populations in both lesional

M. L. Dell'Anna · M. Picardo (✉)
Cutaneous Physiopathology, San Gallicano Dermatological Institute, IFO, Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_31

and non-lesional skin [1]. These abnormalities include the overexpression of senescence-associated markers distributed to the entire non-lesional epidermis [2] and redox imbalance and senescence markers in cultured lesional keratinocytes [3, 4]. Dermal components, via extracellular matrix (ECM) proteins and mesenchymal cells, exert an important role in the regulation of melanocyte homeostasis. ECM proteins contribute to melanocyte adhesion to the basement membrane. Fibroblasts release growth factors and messengers that are part of a paracrine signaling network which includes melanocytes. Most of these mediators, such as hepatocyte growth factor (HGF), stem cell factor (SCF), keratinocyte growth factor (KGF), and neuregulin-1 act as pro-melanogenic factors favoring melanocyte growth, differentiation, migration, and survival. An altered expression of growth factors controlling melanocyte homeostasis has been observed in several pigmentary disorders including vitiligo [5–7]. However, only few reports point to a specific involvement of the dermis in vitiligo, and most of the data are focused on lesional skin. Upregulation of the anti-adhesive ECM protein tenascin and of DKK1 has been demonstrated in lesional dermis [8, 9]. By contrast, the expression and release of KGF by lesional fibroblasts are reduced [10].

Most vitiligo fibroblasts appeared flattened and enlarged, resembling senescent cells [6, 11]. Vitiligo fibroblasts also exhibited increased actin stress fibers, ROS content, and p53 expression. The p53-responsive stress gene was also significantly induced in vitiligo cells.

High levels of intracellular ROS favor the conversion of fibroblasts into transdifferentiated α-SMA-positive myofibroblasts.

The ECM glycoprotein fibronectin and the intermediate filament-associated protein vimentin, both associated with myofibroblast differentiation and known to be induced by ROS and in senescent cells, were also up-modulated in vitiligo, as well as the mesenchymal-derived factor DKK1 [12].

Oxysterols may be involved in the induction of the myofibroblast phenotype by contributing to the unbalanced redox status in the dermis and favoring over time the induction of premature senescence. The deregulated dermal-epidermal network may also account for the below synthesized melanocytorrhagy.

31.2 Detachment

The hypothesis of a chronic detachment and transepidermal loss of melanocytes is usually named melanocytorrhagy. In this theory, the defective adhesion of melanocytes is the predisposing factor, which may or may not lead to further immunomediated inflammatory steps. Interactions between melanocytes and basement membrane are mediated by integrins and those between melanocytes and keratinocytes by cadherins in association with β-catenin [13].

Integrin expression seems not affected in NSV, and down-modulation of a6 integrin in melanocytes does not alter their localization in skin reconstructs. However, an increased expression of CCN3 in keratinocytes in lesional skin was the most prominent feature, whereas melanocytes remaining in perilesional vitiligo skin did not express CCN3. In addition, epidermal expression of DDR1 and collagen IV were very weak as compared to normal control skin [14].

According to the studies on dermal involvement, these data may also suggest a dermal factor influencing collagen IV deposition in vitiligo and a related possibly compensatory effect of dermal-producing CCN3 cells. CCN2, CCN3, and CCN5 are the three most highly expressed transcripts in the dermis, and CCN3 is expressed in the cytoplasm of fibroblast-like cells and endothelial cells of the papillary dermis. In skin fibroblasts, CCN3 works synergistically with TGF-β to upregulate PAI-1 expression to participate in cutaneous wound healing. CCN2, which is expressed in melanocytes, induces ROS production in fibroblasts, and CCN3 interacts with CCN2. Altogether, these data suggest that the CCN proteins in skin may influence in a complex manner not only melanocyte adhesion to the basal layer and the composition of the papillary dermis but also melanocyte survival and differentiation. Accordingly, abnormal deposition of another

extracellular matrix protein, tenascin, and a fibroblastic influence on epidermal pigmentation have been demonstrated. Downregulation of a6 integrin in melanocytes does not alter their localization in reconstructs, whereas CCN3 production by melanocytes upregulates the DDR1 adhesion receptor for collagen IV [15–21].

31.3 WNT Pathways

WNT/β-pathway contributes to the differentiation of stem cells in melanocytes. Functional analyses support the impact of the decreased activation of the WNT pathway in vitiligo, as decreased expression of LEF1 and decreased activity of the LEF1/TCF promoter were observed after oxidative stress, and differentiation of resident stem cells was induced in pre-melanocytes following ex vivo stimulation with WNT activators. The WNT pathway is known to regulate E-cadherin expression, and interestingly, recent data showed decreased expression of E-cadherin across melanocyte membranes in vitiligo patients, leading to decreased adhesiveness of these cells to the basal layer under oxidative and mechanical stress, linking this pathway to the suggested melanocytorrhagy. Oxidative stress decreases WNT pathway activity in melanocytes and keratinocytes. WNT ligands and LEF1 are first decreased in both melanocytes and keratinocytes; yet, decreased levels of LEF1 and CDH3 persist in vitiligo skin ex vivo despite the augmentation of WNT probably owing to compensation of the impaired pathway. In vitiligo lesions devoid of melanocytes, the keratinocytes are presumably responsible for the production of WNT proteins. Oxidative stress may negatively impact the differentiation of melanocytes in vitiligo skin. Treatment with WNT agonists or GSK3β inhibitors induces increased expression of melanocyte markers, triggering the differentiation of resident melanocyte stem cells, not only in the hair follicles but also in the dermis, in pre-melanocytes expressing PAX3 and DCT. Recently, the WNT/β-catenin pathway was found to have a key role in UVB-induced melanocyte stem cell differentiation. Within the skin, the secretion of WNT mainly by keratinocytes and melanocytes contributes to the differentiation of stem cells in melanocytes [22].

References

1. Picardo M, Dell'Anna ML, Ezzedine K, Hamzavi I, Harris JE, Parsad D, et al. Vitiligo. Nat Rev Dis Primers. 2015;1:15011.
2. Bellei B, Pitisci A, Ottaviani M, Ludovici M, Cota C, Luzi F, et al. Vitiligo: a possible model of degenerative diseases. PLoS One. 2013;8(3):e59782.
3. Bondanza S, Maurelli R, Paterna P, Migliore E, Giacomo FD, Primavera G, et al. Keratinocyte cultures from involved skin in vitiligo patients show an impaired in vitro behaviour. Pigment Cell Res. 2007;20(4):288–300.
4. Kostyuk VA, Potapovich AI, Cesareo E, Brescia S, Guerra L, Valacchi G, et al. Dysfunction of glutathione S-transferase leads to excess 4-hydroxy-2-nonenal and H(2)O(2) and impaired cytokine pattern in cultured keratinocytes and blood of vitiligo patients. Antioxid Redox Signal. 2010;13(5):607–20.
5. Kitamura R, Tsukamoto K, Harada K, Shimizu A, Shimada S, Kobayashi T, et al. Mechanisms underlying the dysfunction of melanocytes in vitiligo epidermis: role of SCF/KIT protein interactions and the downstream effector, MITF M. J Pathol. 2004;202(4):463–75.
6. Kovacs D, Cardinali G, Aspite N, Cota C, Luzi F, Bellei B, et al. Role of fibroblast-derived growth factors in regulating hyperpigmentation of solar lentigo. Br J Dermatol. 2010;163(5):1020–7.
7. Lee AY, Kim NH, Choi WI, Youm YH. Less keratinocyte-derived factors related to more keratinocyte apoptosis in depigmented than normally pigmented suction-blistered epidermis may cause passive melanocyte death in vitiligo. J Invest Dermatol. 2005;124(5):976–83.
8. Le Poole IC, van den Wijngaard RM, Westerhof W, Das PK. Tenascin is overexpressed in vitiligo lesional skin and inhibits melanocyte adhesion. Br J Dermatol. 1997;137(2):171–8.
9. Oh SH, Kim JY, Kim MR, Do JE, Shin JY, Hann SK. DKK1 is highly expressed in the dermis of vitiligo lesion: is there association between DKK1 and vitiligo? J Dermatol Sci. 2012;66(2):163–5.
10. Purpura V, Persechino F, Belleudi F, Scrofani C, Raffa S, Persechino S, et al. Decreased expression of KGF/FGF7 and its receptor in pathological hypopigmentation. J Cell Mol Med. 2014;18(12):2553–7.
11. Briganti S, Flori E, Bellei B, Picardo M. Modulation of PPARγ provides new insights in a stress induced premature senescence model. PLoS One. 2014;9(8):e104045.

12. Kuang HB, Miao CL, Guo WX, Peng S, Cao YJ, Duan EK. Dickkopf-1 enhances migration of HEK293 cell by beta-catenin/E-cadherin degradation. Front Biosci (Landmark Ed). 2009;14:2212–20.
13. Wagner RY, Luciani F, Cario-André M, Rubod A, Petit V, Benzekri L, et al. Altered E-cadherin levels and distribution in melanocytes precede clinical manifestations of vitiligo. J Invest Dermatol. 2015;135(7):1810–9.
14. Ricard AS, Pain C, Daubos A, Ezzedine K, Lamrissi-Garcia I, Bibeyran A, Guyonnet-Dupérat V, Taieb A, Cario-André M. Study of CCN3 (NOV) and DDR1 in normal melanocytes and vitiligo skin. Exp Dermatol. 2012;21(6):411–6.
15. Akiba S, Chiba M, Mukaida Y, Sato T. Involvement of reactive oxygen species and SP-1 in fibronectin production by oxidized LDL. Biochem Biophys Res Commun. 2003;310(2):491–7.
16. Desmoulière A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol. 1993;122(1):103–11.
17. Miyazaki M, Gohda E, Kaji K, Namba M. Increased hepatocyte growth factor production by aging human fibroblasts mainly due to autocrine stimulation by interleukin-1. Biochem Biophys Res Commun. 1998;246(1):255–60.
18. Nishio K, Inoue A, Qiao S, Kondo H, Mimura A. Senescence and cytoskeleton: overproduction of vimentin induces senescent-like morphology in human fibroblasts. Histochem Cell Biol. 2001;116(4):321–7.
19. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. I. Paracrine cells important in health and disease. Am J Phys. 1999;277(1 Pt 1):C1–9.
20. Waldera Lupa DM, Kalfalah F, Safferling K, Boukamp P, Poschmann G, Volpi E, et al. Characterization of skin aging-associated secreted proteins (SAASP) produced by dermal fibroblasts isolated from intrinsically aged human skin. J Invest Dermatol. 2015;135(8):1954–68.
21. Yamaguchi Y, Itami S, Watabe H, Yasumoto K, Abdel-Malek ZA, Kubo T, et al. Mesenchymal-epithelial interactions in the skin: increased expression of dickkopf1 by palmoplantar fibroblasts inhibits melanocyte growth and differentiation. J Cell Biol. 2004;165(2):275–85.
22. Regazzetti C, Joly F, Marty C, Rivier M, Mehul B, Reiniche P, Mounier C, Rival Y, Piwnica D, Cavalié M, Chignon-Sicard B, Ballotti R, Voegel J, Passeron T. Transcriptional analysis of vitiligo skin reveals the alteration of WNT pathway: a promising target for repigmenting vitiligo patients. J Invest Dermatol. 2015;135(12):3105–14.

Pathophysiology of Segmental Vitiligo

32

Nanja van Geel, Carole Van Haverbeke, and Reinhart Speeckaert

Contents

Abstract

Segmental vitiligo is characterized by its early onset, rapid stabilization and unilateral distribution (Chap. 6). Prevalences between 3.5% and 20.5% of all patients with vitiligo have been reported [1]. Many studies across decades and all over the world have attempted to clarify the pathogenesis behind it, but the exact pathophysiology of segmental vitiligo remains unclear.

Key Points

- Segmental vitiligo, Unilateral, Mosaicism

N. van Geel, MD, PhD (✉) · R. Speeckaert, MD, PhD
Department of Dermatology, Ghent University Hospital, Ghent, Belgium
e-mail: Nanja.VanGeel@UGent.be

C. Van Haverbeke, MD
Department of Pathology, Ghent University Hospital, Ghent, Belgium

Presence of inflammatory infiltrates in progressing borders of non-segmental vitiligo is a well-demonstrated finding, and for long autoimmunity has been considered as a key factor in vitiligo because of the clear association of the disorder with a personal or familial autoimmune diathesis, which targets in particular the thyroid gland. However, this typical association with other autoimmune diseases is significantly less in segmental vitiligo [2].

For those reasons, both types were generally considered to be separate entities because of the significant differences in disease presentation, evolution and associated diseases [1, 2]. However, pathological evidence of inflammation in segmental vitiligo was described in the first edition of this book, as well as a case report published in 2012. This case presented an exceptional combination of segmental vitiligo, alopecia areata, psoriasis and halo naevus. These disorders are supposed to be inflammation-driven conditions, strengthening the

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_32

likelihood of an immune-mediated pathophysiology in segmental vitiligo [3, 4].

More recently, more evidence became available that segmental and non-segmental vitiligo are not two completely separate entities but could represent variants of the same disease spectrum. In particular, the systematic analysis of segmental vs. non-segmental forms in children and the observation of mixed vitiligo (combination of segmental and non-segmental vitiligo, Chap. 1.5.3) are supporting this new view.

Until today, several theories for the pathogenesis of segmental vitiligo have been suggested, including neural mechanisms, somatic mosaicism and microvascular skin homing, whether or not leading to an autoimmune destruction of melanocytes.

32.1 Neural Mechanisms

In 1959, Lerner et al. proposed the 'neural theory', a theory based on the suggestion that segmental vitiligo is following the course of dermatomes [5]. Therefore, neural mechanisms have been assumed to play a role in the pathogenesis of this type of vitiligo (Fig. 32.1) [1]. This theory was also supported by cases of depigmentation in areas corresponding to local neurological damage (e.g. in subacute encephalitis, spinal cord tumour or following trauma) [6]. Besides these findings, physiological abnormalities associated with sympathetic nerve function were also described in lesional skin of patients with segmental vitiligo (e.g. acetylcholine activity, increased catecholamine and neuropeptide release) [7]. In a study of Wu et al., an increased cutaneous blood flow compared to the contralateral normal skin was demonstrated along with a significantly increased α- and β-adrenoreceptor response in segmental vitiligo lesions. However, this reaction might also be explained as a bystander effect of inflammation instead of a triggering factor. More recently, the exact distribution of segmental vitiligo was evaluated more in detail. A study on the clinical profile of patients with segmental vitiligo demonstrated that the distribution of segmental vitiligo lesions was in most cases not dermatomal, as previously assumed [8].

32.2 Somatic Mosaicism

Due to the observation that the majority of segmental vitiligo lesions did not exactly fit within the borders of dermatomal lines, a second theory has been suggested. In an observational study,

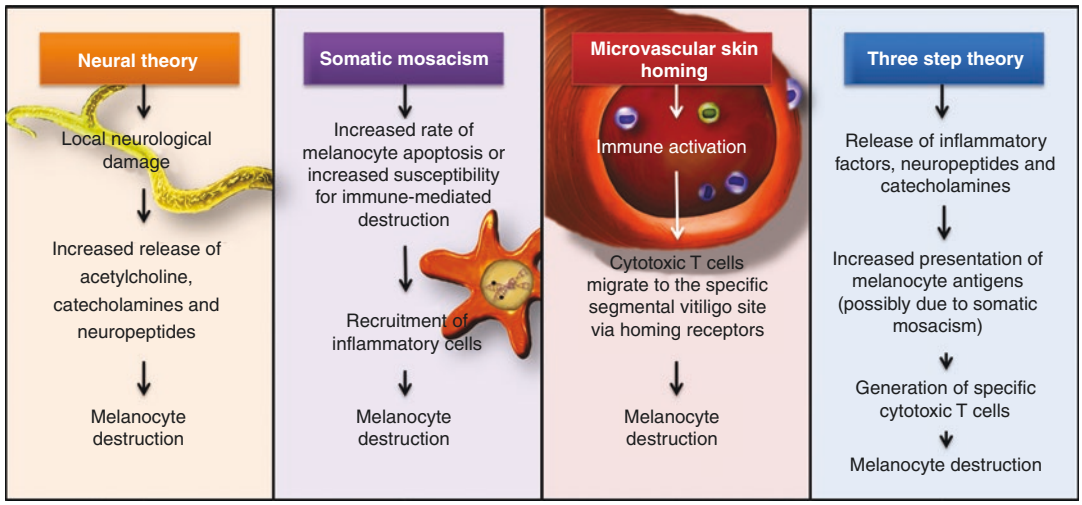


Fig. 32.1 Neural, somatic mosaicism and microvascular skin-homing hypotheses for segmental vitiligo and three-step theory of several possible pathophysiological mechanisms leading to clinical outcome of segmental vitiligo (adapted from figure included in Brit J Derm 2012 166:240–6)

the pattern of segmental vitiligo resembled in some cases a Blaschkoid distribution, while other segmental vitiligo lesions corresponded to other patterns of mosaicism (Fig. 32.1) [9]. Striking similarities were found in this study with other mosaic skin disorders, in particular segmental lentiginosis—speckled lentiginous nevus—as also noted previously by Hann (Chap. 1.5.2) and verrucous epidermal naevi [9]. A typical recurring 'segmental vitiligo pattern' was observed that can fit within the theory of mosaicism, but is not yet included in the six subtypes of mosaicism as described by Happle et al. [1, 9, 10]. Pigmentary mosaicism has been suggested to represent the migration pattern of skin cells during embryogenesis. When visible, pigmentary mosaicism (e.g. Blaschkoid lines) reflects an underlying mosaicism of different cell lines [2]. This mosaic distribution has been observed in several pigmentation disorders such as Blaschkoid hypopigmentation, epidermal naevi and naevus depigmentosus [1, 9]. So far, convincing data at the molecular level are still missing. A clinical observation, supporting this theory, is the superior long-term take of epidermal cellular grafting in segmental vitiligo lesions compared with inferiour results in patients with generalized vitiligo. It indicates that the transplanted cells of autologous donor skin may be genetically affected as well in the generalized type of vitiligo and not in the isolated type of segmental vitiligo [1].

32.3 Microvascular Skin Homing

Another hypothesis on the pathogenesis of segmental vitiligo suggests that the midline delineation in unilateral lesions could represent the migration pattern of cytotoxic T cells from specific lymph nodes along the efferent microvascular system via homing receptors (Fig. 32.1). The observation of halo naevi in patients with segmental vitiligo may support this theory. Patients most often mention that the appearance of halo naevi occurs prior to the development of the segmental vitiligo lesions, suggesting a clonal expansion of melanocyte-specific T lymphocytes in the regional lymph node [1]. A study in 2010 provides evidence that a melanocyte-specific CD8+ T-lymphocyte-mediated immune response was involved in the early phases of segmental vitiligo [3]. It seems however unlikely that this theory of skin-homing receptors can entirely explain the specific disease pattern of segmental vitiligo. Furthermore, no other inflammatory skin disease has been described showing a similar pattern. Melanoma-associated depigmentation after vaccination (often delivered by subcutaneous or intradermal injection) follows a more generalized vitiligo-like pattern and not a segmental pattern that could be expected according to the theory of unilateral skin homing [1].

32.4 Conclusion

The pathophysiology of segmental vitiligo remains controversial, and several hypotheses lack a clear cascade of events explaining various aspects of this disease. However, it should be emphasized that the different theories of segmental vitiligo do not exclude each other. Moreover, the existence of several aetiopathological pathways or different underlying initial triggers is likely. These different pathways might all subsequently activate skin immune or inflammatory responses leading to melanocyte destruction. Our group proposed a three-step theory, including the different pathophysiological mechanisms leading to the clinical presentation of segmental vitiligo, providing hereby a more integrated view into the current evidence on segmental vitiligo [1]. This three-step theory includes possible reactions related to neural mechanism, somatic mosaicism, microvasular skin homing and an antimelanocyte-specific immune response. It explains the typical distribution pattern and different prognosis of segmental vitiligo, compared with general vitiligo [11].

Although this three-step theory is based on limited evidence, it summarizes the different proposed aetiopathogenetic mechanisms for segmental vitiligo into one model. Future research needs to shed more light on this hypothesis, and the exact pathogenesis of segmental vitiligo is yet to figure out.

References

1. Van Geel N, Mollet I, Brochez L, et al. New insights in segmental vitiligo: case report and review of theories. Br J Dermatol. 2012;166:240–6.
2. Taïeb A, Morice-Picard F, Jouary T, et al. Segmental vitiligo as the possible expression of cutaneous somatic mosaicism: implications for common non-segmental vitiligo. Pigment Cell Melanoma Res. 2008;21:646–52.
3. van Geel NA, Mollet IG, De Schepper S, et al. First histopathological and immunophenotypic analysis of early dynamic events in a patient with segmental vitiligo associated with halo nevi. Pigment Cell Melanoma Res. 2010;23:375–84.
4. Shin J, Kang HY, Kim KH, Park CJ, Oh SH, Lee SC, Lee S, Choi GS, Hann SK. Involvement of T cells in early evolving segmental vitiligo. Clin Exp Dermatol. 2016;41(6):671–4. https://doi.org/10.1111/ced.12852.
5. Mohammed GF, Gomaa AH, Al-Dhubaibi MS. Highlights in pathogenesis of vitiligo. World J Clin Cases. 2015;3:221–30.
6. Nelhaus G. Acquired unilateral vitiligo and poliosis of the head and subacute encephalitis with partial recovery. Neurology. 1970;20:965–74.
7. Wu CS, Yu HS, Chang HR, et al. Cutaneous blood flow and adrenoceptor response increase in segmental-type vitiligo lesions. J Dermatol Sci. 2000;23:53–62.
8. Zhou J, Zhong Z, Li J, Fu W. Motor nerve conduction velocity is affected in segmental vitiligo lesional limbs. Int J Dermatol. 2016;55(6):700–5. https://doi.org/10.1111/ijd.13171.
9. van Geel N, Speeckaert R, Melsens E, et al. The distribution pattern of segmental vitiligo: clues for somatic mosaicism. Br J Dermatol. 2013;186:56–64.
10. Happle R. Mosaicism in human skin. Understanding the patterns and mechanisms. Arch Dermatol. 1993;129:1460–70.
11. van Geel N, De Lille S, Vandenhaute S, et al. Different phenotypes of segmental vitiligo based on a clinical observational study. J EurAcad Dermatol Venereol. 2010;25:673–8.

Editor's Synthesis

33

Mauro Picardo and Alain Taïeb

Contents

We have summarized in Table 33.1 the current status of our understanding of vitiligo pathomechanisms. We have tried to divide it into major fields and levels of evidence. Clearly, in vivo evidence in human disease comes first. In vitro work, which relies on cultures, can be viewed more cautiously because of artifacts caused by culture conditions, even though appropriate controls are used. Some animal models are probably relevant, but definitive evidence is lacking.

M. Picardo (✉)
Cutaneous Physiopathology and CIRM,
San Gallicano Dermatological Institute, IRCCS,
Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

A. Taïeb
Service de Dermatologie, Hôpital St André, CHU de Bordeaux, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

Concerning clinical, epidemiological, and pathological data reviewed in Sect. 33.1, which should have provided a solid basis for the understanding of the disease, we have already noticed intriguing weaknesses in the vitiligo field. Indeed, the cooperation among research groups has been only recently developing, and variable definitions of type or stage of the disease (Chap. 1.2.1) led to difficulties in the interpretation of some in vivo data, such as the importance of skin inflammation in the disease. There has been also a rampant temptation (conscious or not) to erase some aspects, which were not considered as consistent with the pathophysiological point of view defended by individual authors.

In the following sections, we highlight some gaps in our understanding, underline possible unifying scenario, and propose, based on Sect. 33.2

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_33

Table 33.1 Summary of some aspects of our current understanding of vitiligo

	Level of evidence		
Field	Good	Moderate	Limited
Cell biology	• Loss of pigment cells in epidermal/follicular compartments, melanocyte structural and functional anomalies, including friction-induced detachment in vivo • Keratinocyte anomalies (structural and in culture) • Reduced keratinocyte-derived melanocyte growth factors (e.g., SCF, αMSH, ET1)	• Loss of melanocyte adhesion • Melanocyte senescence and stem cell compartment involved • Fibroblast functional defects • PBMC defects • Mitochondrial impairment • Defective activity of receptors for growth factors • MITF-M deficiency • Propopiomelanocortin system alterations (primary or secondary)	• Involvement of non-skin melanocytes? (not completely settled) • Apoptosis of melanocytes • TNF-induced disease • Stratum corneum activation of inflammasome or other melanocyte-targeting pathways to explain Koebner's phenomenon
Genetics	• Polygenic background • NALP 1 genetic variants in NSV associated with autoimmune diseases	• Causative inflammasome functional alteration • Other causative gene variants or gene-modifying variants in subsets of patients (AIRE, CMH haplotypes, oxidative stress regulators, etc.)	• Several candidate gene associations (approaches not validated/replicated) • Mitochondrial genome defects (not fully tested) • Abnormal expression of genes of the FOXD3 pathway (to confirm) • Protective MC1-R alleles • Predisposing skin-related genetic anomaly (applies mostly to segmental vitiligo)
Biochemistry	• Catechols metabolism anomalies • Antioxidant systems anomalies • Biopterin pathway anomalies	• Membrane-associated defects, including mitochondria defects • Role of environmental toxic compounds (phenol, catechols, etc.)? (more epidemiologic studies needed)	• Melanogenesis defect exclusively
Immunology	• Humoral (serum) and cellular (in situ) immune responses to pigment cell proteins • T-cell lichenoid infiltrates in progressive NSV, mostly CD8+	• Relevant cellular targets of immune responses not clearly established • Autoimmunity to melanin catabolites • Viral infection (direct) • Innate immunity defect to bacterial infections (NALP1?)	• Contagious disease (but role of microorganisms not ruled out; see Role of Herpesvirus in the Smyth Chicken Model of Vitiligo (Chap. 2.2.4) • Injury activation of the skin innate immune system • Deficient clearance of apoptotic fragments

review of data, a hierarchy of possible events occurring upstream of melanocyte loss in the vitiligo puzzle (Fig. 33.1).

33.1 Do All Supposed Mechanisms of Melanocyte Loss Occur In Vivo?

Table 33.1 indicates a wide possible range of events, which may provoke melanocyte loss. Interestingly, some popular theories, such as the apoptotic demise of melanocytes, are not yet substantiated. In nonsegmental vitiligo, there is now ample evidence that melanocytes of non-clinically affected areas can be morphologically or functionally altered in favor of either an intrinsic abnormality, which could be the basis of the Koebner's phenomenon, or of a systemic low-key immune disturbance targeting the cutaneous melanocytic system, or both. This point is crucial for a better understanding and will need more in vivo studies using skin biopsies to look at early changes in the disease. The experimental

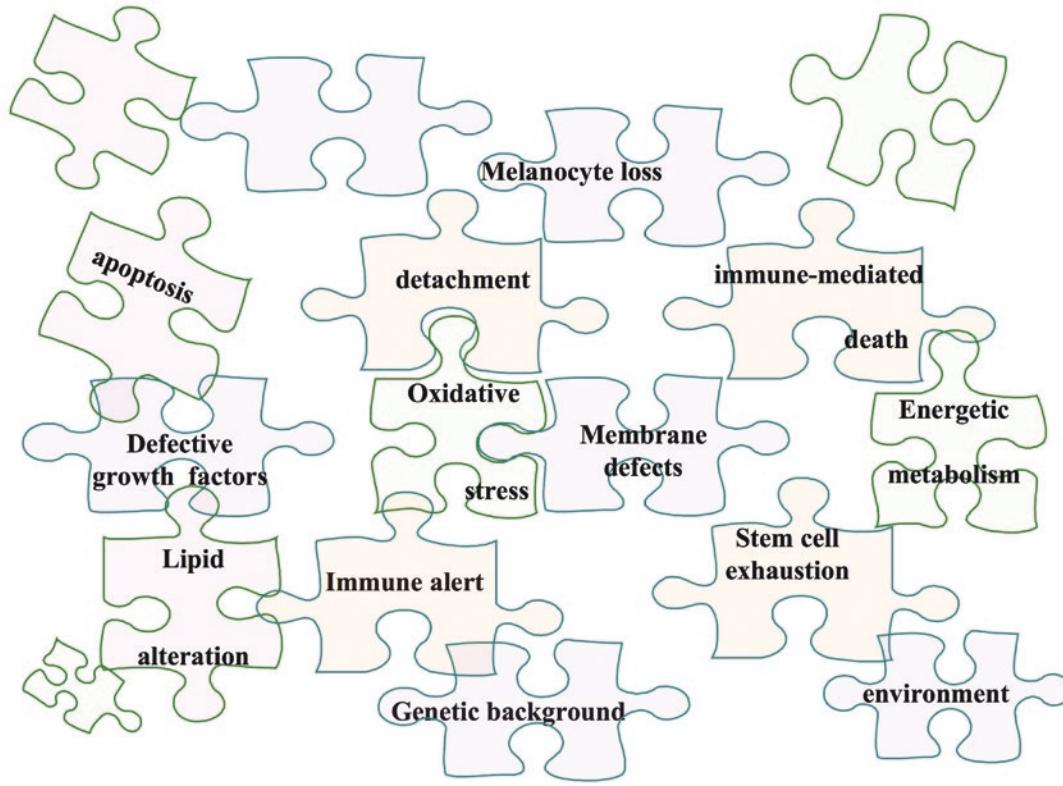


Fig. 33.1 The organized puzzle of the pathogenetic factors

provocation of the Koebner's phenomenon, as indicated in Chap. 2.2.2.1, may be a valuable tool to detect the chronology of events in vitiligo and especially a link between trauma and inflammation.

33.2 An Enlarged Vision of the Skin Melanogenic Unit May Apply to Vitiligo

If melanocytes are the undisputed target of the disease, other skin cells affect their function and survival. The conventional skin pigmentation unit was the epidermal melanin unit of Fitzpatrick and Breathnach [1]. A dermal-epidermal melanin unit was more recently defended [2]. Based on current knowledge of vitiligo pathophysiology, the skin melanogenic unit should include, besides melanocytes, keratinocytes, fibroblasts, and possibly cutaneous nerve endings of the considered unit. Langerhans cells could also play a role as pivotal cells of the innate immune system, being envisioned as maintaining the immune surveillance of the pigmentary system in case of local danger. Progresses in the knowledge of the epidermal and dermal network governing the release of specific cytokines and growth factors open new perspectives in the pathogenesis and therapy of vitiligo, if the loss of pigment cells can be first stopped.

33.3 Melanocyte Stemness and Vitiligo

Until now, the attention has been mostly focused on resident epidermal melanocytes. However, exchanges between hair follicles and interfollicular compartments have been well demonstrated for melanocytes (Chaps. 1.3.6 and 2.2.10). The melanoblasts of the hair follicle niche, mostly studied in the mouse, differentiate and migrate to the hair bulb while maintaining a reserve of

undifferentiated cells inside the bulge [3]. The niche allows thus the continuous production of new melanocytes avoiding cell exhaustion and senescence. Vitiligo may represent a localized senescence process through a mechanism similar to the "free radical-mediated graying" [4, 5], associated with a progressive melanocyte loss from the hair bulb. The presence, or not, of a functioning follicular reservoir may also be the critical factor for a successful melanocyte graft besides loss through the Koebner's phenomenon. Usually, a minority of melanocyte stem cells matures into differentiated and migrating melanocytes, whereas the bulk of them remains quiescent. The exhaustion of the quiescent stock will affect the next melanocyte production. The ability to respond to specific growth factors, such as SCF, may determine the continuous production of differentiated and functioning epidermis-committed melanocytes. At this regard, Sect. 33.2 has provided several arguments indicating that the c-Kit/SCF axis appears to be broken in vitiligo (Chap. 2.2.8). Vitiligo may be considered as a possible model for the study of stemness and senescence processes.

33.4 The Somatic Mosaicism Hypothesis for SV and Deductions for NSV

The recently demonstrated association of SV and NSV has provided new insights in the pathogenesis of vitiligo [6]. The main reasoning behind is that still unknown genetic predisposing factors may affect first the skin pigmentary system. The activation of the skin immune/inflammatory responses would come second leading to a more severe expression of disease. This scenario is somewhat derived from that proposed for another common skin disorder, atopic dermatitis, for which a skin barrier genetic dysfunction comes first and may produce direct inflammation [7] and engages in a facultative second step the immune system into an "allergic" Th2-dependent pathway in a subset of patients. This two-phase model is consistent with the common presentation of NS vitiligo as an isolated chronic cutaneous disorder in the majority of patients. Since friction-/pressure-prone areas are frequently initially affected, it would be tempting to speculate that the primary skin anomaly affects the upper layers of the epidermis to explain the Koebner's phenomenon; it would explain nicely the subsequent epidermal activation of innate immunity-based mechanisms via stratum corneum activation of inactive IL1beta precursors [8]. Pressure may also affect directly basal epidermal layer biology if an abnormal adhesion of melanocytes comes first to explain their detachment [9]. Also as envisaged above is a possible genetic cause limiting survival and self-renewal of epidermal and follicular melanocytes, the latter being hit earlier by the disease in SV as compared to NSV [6]. The clear association of NSV to familial hair graying also favors a genetic background affecting melanocyte survival. The expression of several genes influencing such pathways is indeed modified in NSV [10].

33.5 Membrane Lipids as Possible Culprits

The lipidomics approach to cellular signaling indicates that lipids may be considered as "signaling molecules" between extracellular events and cell adaptive response [11]. Alterations involving lipid metabolism may lead to modifications of the structure of the membranes, affecting the sensitivity to prooxidant agents, modulating the intracellular redox status (Chap. 2.2.6), and favoring the release of haptened lipids, which are, in turn, able to activate the immune system. Moreover, the altered membrane may participate to the propagation of the oxidative damage, responsible for a high level of intracellular ROS, which are, in turn, also capable to affect the structure and activity of the POMC-derived peptides. This phenomenon might be the basis for the reduction of αMSH in vitiligo epidermis. The responses to the specific growth factors, as well as the processes underlying adhesion and survival events, could also be impaired by

the defective metabolism of lipid messengers. The topical application of sphingolipids-mediated restoration of Mitf expression and repigmentation in a mouse model of hair graying is in accordance with this view [4, 5].

33.6 The Innate Immunity Hypothesis

Besides the possible exposure of lipid-protein complexes with antigenic properties, apoptosis or other mechanisms of cellular alteration may activate the immune surveillance through the release of membrane fragments. Based on the finding of NALP variants in immune NSV [12], it has been hypothesized that such cellular debris or apoptotic fragments could activate the NALP1 inflammasome complex, activating in turn the IL1 pathway and alerting the immune system. In addition, hsp70 production related to membrane damage (Chap. 2.2.7.4) has been widely associated to the activation of antigen-presenting cells. The immune-mediated damage, strongly suggested by a wide range of clinical and experimental data (Chaps. 2.2.3.2, 2.2.4, and 2.2.5), could be thus the most prominent modality of loss of genetically compromised melanocytes best demonstrated during flares or acceleration phases of the disease.

33.7 Concluding Remarks

Looking at the most recent experimental approaches, Le Poole et al. convergence theory [13] can tentatively be updated and featured as a pyramid where melanocyte loss is the tip of an enormous puzzle, with a predisposing genetic background at the basement level (Fig. 33.1).

A variable genetic background (level 1) could, under the influence of also variable and still poorly understood environmental triggering factors (arrows), activate several pathogenic pathways (level 2), occur through different mechanisms (level 3) and, according to the intensity of the stimuli, all may concur to melanocyte loss (level 4).

For unravelling the mysteries of the basement level 1, the genetic basis of vitiligo, besides the conventional approaches on blood DNA, SV considered as a common skin genetic mosaic revealed postnatally would be a good model. SV is candidate disease to explore as a proof of principle for a new gene discovery strategy in multigenic disorders with organ specificity [6, 14]. For a good angle of attack of the puzzle-like pyramid, we still lack a definitive argument coming from either a clinical or a basic science perspective. The following section will deal with those uncertainties for the currently limited available therapeutic options.

References

1. Fitzpatrick TB, Breathnach AS. The epidermal melanin unit system. Dermatol Wochenschr. 1963;147:481–9.
2. Cario-André M, Pain C, Gauthier Y, et al. In vivo and in vitro evidence of dermal fibroblasts influence on human epidermal pigmentation. Pigment Cell Res. 2006;19:434–42.
3. Yonetani S, Moriyama M, Nishigori C, et al. In vitro expansion of immature melanoblasts and their ability to repopulate melanocyte stem cells in the hair follicle. J Invest Dermatol. 2008;128:408–20.
4. Picardo M. Lipid-mediated signalling and melanocyte function. Pigment Cell Melanoma Res. 2009;22:152–3.
5. Saha B, Singh SK, Mallik S, et al. Sphingolipid-mediated restoration of Mitf expression and repigmentation in vivo in a mouse model of hair graying. Pigment Cell Melanoma Res. 2009;22:205–18.
6. Taïeb A, Morice-Picard F, Jouary T, et al. Segmental vitiligo as the possible expression of cutaneous somatic mosaicism: implications for common non-segmental vitiligo. Pigment Cell Melanoma Res. 2008;21:646–52.
7. Taieb A. Hypothesis: from epidermal barrier dysfunction to atopic disorders. Contact Dermatitis. 1999;41:177–80.
8. Taieb A. NALP1 and the inflammasomes: challenging our perception of vitiligo and vitiligo-related autoimmune disorders. Pigment Cell Res. 2007;20:260–2.
9. Gauthier Y. The importance of Koebner's phenomenon in the induction of vitiligo vulgaris lesions. Eur J Dermatol. 1995;5:704–8.
10. Strömberg S, Björklund MG, Asplund A, et al. Transcriptional profiling of melanocytes from patients with vitiligo vulgaris. Pigment Cell Melanoma Res. 2008;21:162–71.

11. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. Nat Rev Mol Cell Biol. 2008;9:139–50.
12. Jin Y, Mailloux CM, Gowan K, et al. NALP1 in vitiligo-associated multiple autoimmune disease. N Engl J Med. 2007;356:1216–25.
13. Le Poole IC, Das PK, van den Wijngaard RM, et al. Review of the etiopathomechanism of vitiligo: a convergence theory. Exp Dermatol. 1993;2:145–53.
14. Happle R. Superimposed segmental manifestation of polygenic skin disorders. J Am Acad Dermatol. 2007;57:690–9.

Part III

Treating the Disease

Management Overview

34

Alain Taïeb and Mauro Picardo

Contents

Abstract

Vitiligo psychosocial impact is essential when considering management issues, as well as previous messages given to the patient. Patients should be informed that (1) vitiligo is a chronic/relapsing disorder, (2) repigmentation is a slow process, and (3) reactivation of the disease in different body regions or the reappearance of lesions in treated ones may occur, which gives a rationale for a maintenance treatment. Initial assessment should focus on overall severity and course of disease profile, with special attention to possible aggravating environmental factors and associated autoimmune diseases, in particular thyroid disease. The first target of therapy is in most cases to stop disease progression of the disease, best achieved by combined approaches phototherapy, systemic and local drug intervention. Narrowband UVB is the most useful repigmenting regimen, with the current development of home phototherapy devices. Potent topical corticosteroid or topical calcineurin inhibitor therapy may be used first line for localized disease; on the face, calcineurin inhibitors are currently preferred because of potential side effects of prolonged application of steroids and their good safety profile. In addition to psychological support, offering training to camouflage techniques is particularly helpful in dark-skinned patients for facial/hand lesions. Cellular transplantation or grafting is an option in specialized centers for patients who have stable and limited

A. Taïeb (✉)
Hôpital Saint-André Service de Dermatologie, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

M. Picardo
Cutaneous Physiopathology and CIRM, San Gallicano Dermatological Institute, IRCCS, Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_34

lesions that are refractory to other therapy. Depigmenting techniques are difficult to handle and concern a minority of patients.

Key Points

- Vitiligo psychosocial impact is essential when considering management issues.
- Patients should be informed that (1) vitiligo is a chronic/relapsing disorder, (2) repigmentation is a slow process, and (3) reactivation of the disease in different body regions or the reappearance of lesions in treated ones may occur, which gives a rationale for a maintenance treatment.
- Initial assessment should focus on overall severity and course of disease profile, including aggravating environmental factors and associated autoimmune diseases.
- The first target of therapy is to stop disease progression, best achieved by combined approaches phototherapy, systemic and local drug intervention.
- Narrowband UVB is the most useful repigmenting regimen.
- Potent topical corticosteroid or topical calcineurin inhibitor therapy may be used first line for localized disease.
- In addition to psychological support, offering training to camouflage techniques is particularly helpful in dark-skinned patients for facial/hand lesions.
- Cellular transplantation or grafting is an option in specialized centers for patients who have stable and limited lesions that are refractory to other therapy.
- Depigmenting techniques are difficult to handle and concern a minority of patients.

34.1 Management-Oriented Evaluation

Before discussing management with the patient, a thorough assessment is needed. An assessment form has been produced by the Vitiligo European Task Force (VETF), which summarizes major history taking and examination items [1]. Skin color and ability to tan (phototype), disease duration, and disease activity are important decision management items, as well as the patient psychological profile and coping with the disease. In some vitiligo/NSV patients, an "acceleration phase" occurs with a rapid disease progression in a few weeks/months which needs a more urgent intervention, such as minipulse therapy. Other useful clinical management items include previous episodes of repigmentation and type, duration, and usefulness of previous treatments. Overall, estimating body surface area (e.g., rule of 9 using VETF scoring system; see below) and dividing by duration of disease give a rough but useful profile of disease progression. The analysis of the Koebner phenomenon and occupations is of particular interest for prevention [2]. A simple scoring system of the Koebner's phenomenon has been introduced in the clinic [3]. A question about vitiligo on genitals is included because it causes a strong embarrassment to patients. A global quality of life assessment is recommended ("how does vitiligo currently affects your everyday life", measured over the last week) assessed on a visual analog scale, which is a coarse but useful indicator of coping with the disease. The patient's personality and perceived severity of the disease are predictors of quality of life impairment [4]. Validated HRQoL scales have been published and can be filled by patients in the waiting room [5].

Because of the frequent association of vitiligo/NSV with autoimmune thyroid disease, especially Hashimoto's thyroiditis, it is recommended to measure on an annual basis the thyrotropin level in patients with antibodies to thyroid peroxidase, which may precede overt thyroiditis [6]. Any suggestive manifestations of organ-specific autoimmune diseases should prompt appropriate investigations [7]. The index of suspicion should be raised when a personal and/or family history of autoimmune/auto-inflammatory disorders is obtained.

34.2 Treatment Overview

Vitiligo treatments have so far been analyzed using the proportion of treated patients who achieve a specified degree of repigmentation, usually starting at >50% for a "good" response.

"Good" for this level of skin repigmentation is indeed debatable in a patient-oriented view because satisfaction needs, in addition to stopping disease progression, complete restauration of pigmentation, especially in visible areas. This point has been recently examined in depth by the VGICC [8]. The VETF has proposed a simple method of assessment which combines analysis of extent, grading of depigmentation, and progression [1]. Clinical photographs and if possible UV-light photographs are needed for accurate monitoring of repigmentation.

For stopping progression, besides UV therapies, systemic steroids have been evaluated mostly in open studies and seem to arrest disease progression [9–12]. Commonly used repigmentation therapies for vitiligo that are supported by data from randomized trials (RCT) include UV light (whole-body irradiation or UV targeted to lesions) and topical agents (corticosteroids, calcineurin inhibitors, calcipotriol). Camouflaging and depigmenting (in widespread disease) are the other current options. Table 34.1 outlines a stepwise treatment approach divided by type of vitiligo and extent, which needs modulation by visibility, age, and coping. A zero line is always possible, meaning no treatment if the disease is not bothering the patient. Maintenance therapy is validated for facial lesions and intermittent tacrolimus use [13].

Table 34.1 General outline of management for vitiligo in adults

Type of vitiligo	Usual management
Segmental and limited vitiligo/ NSV <2–3% body surface involvement	*First line*: Avoidance of triggering factors, local therapies (corticosteroids, calcineurin inhibitors) combined with localized NB-UVB therapy, especially excimer monochromatic lamp or laser, or home NB-UVB phototherapy *Second line*: Consider surgical techniques if repigmentation cosmetically is unsatisfactory on visible areas
Vitiligo/NSV >3% BSA	*First line*: Usual initial combination total body NB-UVB with systemic/ topical therapies, including reinforcement with localized UVB therapy (see text) *Second line*: Consider surgical techniques in nonresponding areas especially with high cosmetic impact *Third line*: Consider depigmentation techniques in nonresponding widespread (>50%) or highly visible recalcitrant facial/hand vitiligo

(Revised from Taieb A, Gauthier Y. Combining surgical and medical therapies. In: Surgical management of Vitiligo, ed. by Gupta, Olsson and Ortonne. Wiley Blackwell 2006. pp. 267–272)

A no treatment option (zero line) can be considered in patients with a fair complexion after discussion. For children, phototherapy is limited by feasibility in the younger age group and surgical techniques rarely proposed before prepubertal age. There is no current recommendation validated to the case of rapidly progressive vitiligo, non-stabilized by UV or combined therapy. Camouflage, psychological support, and maintenance treatment are indicated in all cases

34.2.1 UV Treatments

The currently preferred treatment in adults and compliant children with vitiligo/NSV is narrow-band UVB (NB-UVB), which delivers peak emission at 311 nm [14]. The color match of repigmented skin is excellent, but the response rate remains low based on patient expectations. With twice-weekly NB-UVB treatments for 1 year, 48–63% of patients with NSV repigment 75% of the affected areas [15]. At least 3 months and preferably 4 months of treatment is warranted before identifying a patient as a nonresponder, and approximately 9–12 months of treatment is usually required to achieve the maximal repigmentation. Unfortunately, access to this treatment is frequently limited in some areas due to low availability or long distances to treatment centers. Home phototherapy devices are thus more convenient and suitable for small extent disease [16]. The optimal dose may differ at different sites, and an option is shielding the area with the lower MED after reaching its optimal dose while continuing to expose higher MED areas until optimal dosing [15]. There is no apparent relationship between the degree of initial depigmentation and the response to NB-UVB treatment [14], but the duration of disease is inversely correlated with the degree of treatment-induced repigmentation [15]. The best results are achieved on the face, followed by the trunk and limbs. The poorest outcomes

have been noted for hands and feet lesions that at best show a moderate response. Relapses are common at all sites; around 60–70% of patients resume depigmentation in areas repigmented by treatment within 1 year whether the regimen is PUVA or NB-UVB [17].

Responses of segmental vitiligo to narrow-band UVB are at best limited when the same NB-UVB therapy is applied for 6 months [15]. Earlier and more aggressive treatment schemes are thus advisable. The use of targeted high-fluency UVB (excimer laser or monochromatic excimer lamp, both at 308 nm) which may reach deeper targets such as amelanotic melanocytes of the hair follicle, and also avoid irradiation of uninvolved skin, may improve outcomes, as well as combined approaches. Results are also promising in cases of vitiligo/NSV involving limited areas [4, 18–35]. Red light helium-neon laser phototherapy has also been reported as promoting repigmentation in SV [36].

34.2.2 Topical Therapies and Combined Therapies

Topical therapies are not suitable for widespread NSV but may be effective in cases with more localized disease (including SV). Combined treatments are frequently considered when phototherapy alone does not show efficacy after 3–4 months or in an attempt to accelerate response and reduce cumulative UV exposure [37]. As compared with PUVA which determines a predominant perifollicular pattern of repigmentation, topical corticosteroids (and topical calcineurin inhibitors—TCI—such as tacrolimus and pimecrolimus) exhibit a diffuse type, which is faster but less stable [33]. Based on a meta-analysis, class 3 (potent) topical corticosteroids achieve more than 75% repigmentation in 56% of patients [22]. TCI are preferred for face and neck lesions, because they do not cause skin atrophy. The efficacy of TCI is enhanced by exposure to UV radiation delivered by high-fluency UVB devices [35] but not clearly by conventional NB-UVB [28]. The concerns about the risk of cutaneous or even extracutaneous cancer provoked by topical calcineurin inhibitors have been disproportionate [38, 39]. Calcineurin inhibitors can promote repigmentation without immunosuppression [25]. The combined use of UV and calcineurin inhibitors is not yet fully approved but recommended by the VETF/EDF/EADV/UEMS guidelines [40]. Alternative sources of light such as the helium-neon laser (red light) need more development [26]. Topical corticosteroids can also increase the efficacy of UVB [41]. Topical calcipotriene RCT studies indicate limited or no effect in isolation and a possible minor enhancer effect on repigmentation in combination with UV or topical corticosteroids [19].

34.2.3 Surgery

Surgical methods such as minigrafting, which consists in transplanting punch grafts from an autologous donor site [21]—currently less used when other options are possible—or cellular transplantation using autologous epidermal cell suspensions containing melanocytes [23] or ultrathin epidermal grafts [31] or a combination of cellular transplantation and ultrathin grafting, are used in cases of focal/segmental vitiligo if medical approaches fail. Several variants have been recently developed. UV irradiation is frequently associated [42]. Patients with vitiligo/NSV are considered good candidates for surgical techniques depending on availability and cost, if their disease is stable (over the preceding year) [3] and has a limited extent (2–3% of body surface area). Contrary to SV, in which the grafted cells come from apparently disease-free areas, the survival of living but potentially abnormal transplanted melanocytes is less predictable in vitiligo/NSV. Koebnerization limits efficacy (hands in particular). Based on a RCT, after a strict preoperative selection for disease stability in vitiligo/NSV, cellular transplantation plus UV results in repigmentation of at least 70% of the treated area [42].

34.2.4 Camouflage and Depigmentation

Topical camouflaging products mask esthetic skin disfigurement on a transient, semipermanent, or

permanent basis (tattoos). Benefits can be obtained by the skilled use of corrective cosmetics. For dihydroxyacetone (DHA), the most used self tanner, the higher the concentration, the better the response observed particularly in darker phototypes [10]. Chemical or laser depigmentation can definitely be a choice in a small subset of carefully selected patients, but results are variable both in efficacy and duration [30].

34.2.5 Other Therapies

The use of topically applied antioxidants has not yet been supported by long-term follow-up studies [40]. Yet, the topical application of the synthetic analog of the catalase (PC-KUS) has been proposed as consistent therapy for the NSV both in adults and children. Reports investigating the efficacy of natural health products (Chinese herbs, plant extracts, vitamins) for vitiligo exist but are of poor methodological quality and contain significant reporting flaws [43]. An RCT supports a moderate adjunctive effect of *Polypodium leucotomos* to NB-UVB [29] and another that of systemic antioxidants in addition to NB-UVB [20]. *Ginkgo biloba* as a monotherapy [34] is not recommended.

34.2.6 Counseling

The involvement of a psychologist or psychiatrist may be helpful in patients who experience difficulty coping with the diagnosis. Sunscreens are needed in case of real risk of sunburn on non-photoprotected skin, but not on a routine basis, because moderate sun exposure (heliotherapy) is a good substitute to UV therapies. Photoadaptation exists in depigmented vitiligo skin [11, 44]. Repeated frictions for applying sunblockers without real sunburn risk may be more detrimental than beneficial. It is important to make time available to discuss photoprotection issues vs. therapy by UV light in the vitiligo clinic, because this is an important source of confusion for the patients.

34.3 Evidence-Based Guidelines

General guidelines for adults and children have been elaborated by the British Association of Dermatologists [24] and guidelines for surgery by the IADVL Dermatosurgery Task Force [32]. A treatment algorithm has been also proposed based on evidence-based medicine principles [27]. In addition to the Indian and Japanese guidelines, the European VETF/EDF guidelines have been published in 2011 [40] and are currently in revision. Beyond guidelines, personalized/stratified strategies have been proposed [45].

References

1. Taïeb A, Picardo M, VETF Members. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell Res. 2007;20:27–35.
2. Diallo A, Boniface K, Jouary T, Seneschal J, Morice-Picard F, Prey S, Cario-André M, Mazereeuw-Hautier J, Taieb A, Ezzedine K. Development and validation of the K-VSCOR for scoring Koebner's phenomenon in vitiligo/non-segmental vitiligo. Pigment Cell Melanoma Res. 2013;26:402–7.
3. Ezzedine K, Lim HW, Suzuki T, Katayama I, Hamzavi I, Lan CC, Goh BK, Anbar T, Silva de Castro C, Lee AY, Parsad D, van Geel N, Le Poole IC, Oiso N, Benzekri L, Spritz R, Gauthier Y, Hann SK, Picardo M, Taieb A, Vitiligo Global Issue Consensus Conference Panelists. Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. Pigment Cell Melanoma Res. 2012;25:E1–13.
4. Kostopoulou P, Jouary T, Quintard B, Marques S, Boutchnei S, et al. Objective vs subjective factors in the psychological impact of vitiligo: the experience from a French referral center. Br J Dermatol. 2009;161(1):128–33.
5. Salzes C, Abadie S, Seneschal J, Whitton M, Meurant JM, Jouary T, Ballanger F, Boralevi F, Taieb A, Taieb C, Ezzedine K, The Vitiligo Impact Patient Scale (VIPs). Development and validation of a vitiligo burden assessment tool. J Invest Dermatol. 2016;136:52–8.
6. Gey A, Diallo A, Seneschal J, Léauté-Labrèze C, Boralevi F, Jouary T, Taieb A, Ezzedine K. Autoimmune thyroid disease in vitiligo: multivariate analysis indicates intricate pathomechanisms. Br J Dermatol. 2013;168:756–61.
7. Betterle C, Caretto A, De Zio A, et al. Incidence and significance of organ-specific autoimmune disorders (clinical, latent or only autoantibodies) in patients with vitiligo. Dermatologica. 1985;171:419–23.

8. Gan EY, Eleftheriadou V, Esmat S, Hamzavi I, Passeron T, Böhm M, Anbar T, Goh BK, Lan C-CE, Lui H, Ramam M, Raboobee N, Katayama I, Suzuki T, Parsad D, Seth V, Lim H, van Geel N, Mulekar S, Harris J, Wittal R, Benzekri L, Gauthier Y, Kumarasinghe P, Thng STG, de Castro CCS, Abdallah M, Vrijman C, Bekkenk M, Seneschal J, Pandya A, Ezzedine K, Picardo M, Taïeb A, on behalf of the VGICC. Repigmentation in vitiligo: position paper of the Vitiligo Global Issues Consensus Conference (VGICC). Pigment Cell Melanoma Res. 2017;30(1):28–40.
9. Radakovic-Fijan S, Fürnsinn-Friedl AM, Hönigsmann H, et al. Oral dexamethasone pulse treatment for vitiligo. J Am Acad Dermatol. 2001;44:814–7.
10. Rajatanavin N, Suwanachote S, Kulkollakarn S. Dihydroxyacetone: a safe camouflaging option in vitiligo. Int J Dermatol. 2008;47:402–6.
11. Rivard J, Hexsel C, Owen M, Strickland FM, et al. Photoadaptation of vitiliginous skin to targeted ultraviolet B phototherapy. Photodermatol Photoimmunol Photomed. 2007;23:258–60.
12. Seiter S, Ugurel S, Tilgen W, et al. Use of high-dose methylprednisolone pulse therapy in patients with progressive and stable vitiligo. Int J Dermatol. 2000;39:624–7.
13. Cavalié M, Ezzedine K, Fontas E, Montaudié H, Castela E, Bahadoran P, Taïeb A, Lacour JP, Passeron T. Maintenance therapy of adult vitiligo with 0.1% tacrolimus ointment: a randomized, double blind, placebo-controlled study. J Invest Dermatol. 2015;135:970–4.
14. Westerhof W, Nieuweboer-Krobotova L. Treatment of vitiligo with UV-B radiation vs topical psoralen plus UV-A. Arch Dermatol. 1997;133:1525–8.
15. Anbar TS, Westerhof W, Abdel-Rahman AT, et al. Evaluation of the effects of NB-UVB in both segmental and non-segmental vitiligo affecting different body sites. Photodermatol Photoimmunol Photomed. 2006;20(22):157–63.
16. Eleftheriadou V, Thomas K, Ravenscroft J, Whitton M, Batchelor J, Williams H. Feasibility, double-blind, randomised, placebo-controlled, multi-centre trial of hand-held NB-UVB phototherapy for the treatment of vitiligo at home (HI-Light trial: Home Intervention of Light therapy). Trials. 2014;15:51.
17. Yones SS, Palmer RA, Garibaldinos TM, et al. Randomized double-blind trial of treatment of vitiligo: efficacy of psoralen-UV-A therapy vs Narrowband-UV-B therapy. Arch Dermatol. 2007;143:578–84.
18. Casacci M, Thomas P, Pacifico A, et al. Comparison between 308-nm monochromatic excimer light and narrowband UVB phototherapy (311–313 nm) in the treatment of vitiligo--a multicentre controlled study. J Eur Acad Dermatol Venereol. 2007;2:956–63.
19. Chiavérini C, Passeron T, Ortonne JP. Treatment of vitiligo by topical calcipotriol. J Eur Acad Dermatol Venereol. 2002;16:137–8.
20. Dell'Anna ML, Mastrofrancesco A, Sala R, et al. Antioxidants and narrow band-UVB in the treatment of vitiligo: a double-blind placebo controlled trial. Clin Exp Dermatol. 2007;32:631–6.
21. Falabella R. Treatment of localized vitiligo by autologous minigrafting. Arch Dermatol. 1988;124:1649–55.
22. Forschner T, Buchholtz S, Stockfleth E. Current state of vitiligo therapy--evidence-based analysis of the literature. J Dtsch Dermatol Ges. 2007;5:467–75.
23. Gauthier Y, Surleve-Bazeille JE. Autologous grafting with noncultured melanocytes: a simplified method for treatment of depigmented lesions. J Am Acad Dermatol. 1992;26(2 Pt 1):191–4.
24. Gawkrodger DJ, Ormerod AD, Shaw L, Mauri-Sole I, et al. Guideline for the diagnosis and management of vitiligo. Br J Dermatol. 2008;159:1051–76.
25. Lan CC, Chen GS, Chiou MH, et al. FK506 promotes melanocyte and melanoblast growth and creates a favourable milieu for cell migration via keratinocytes: possible mechanisms of how tacrolimus ointment induces repigmentation in patients with vitiligo. Br J Dermatol. 2005;153:498–505.
26. Lan C, Wu C, Chen G, et al. Helium-neon laser and topical tacrolimus combination therapy: novel treatment option for vitiligo without additional photocarcinogenic risks. J Eur Acad Dermatol Venereol. 2009;23(3):344–5.
27. Lim HW, Hexsel CL. Vitiligo: to treat or not to treat. Arch Dermatol. 2007;143:643–6.
28. Mehrabi D, Pandya AG. A randomized, placebo-controlled, double-blind trial comparing narrowband UV-B plus 0.1% tacrolimus ointment with narrowband UV-B plus placebo in the treatment of generalized vitiligo. Arch Dermatol. 2006;142:927–9.
29. Middelkamp-Hup MA, Bos JD, Rius-Diaz F, et al. Treatment of vitiligo vulgaris with narrow-band UVB and oral Polypodium leucotomos extract: a randomized double-blind placebo-controlled study. J Eur Acad Dermatol Venereol. 2007;21:942–50.
30. Njoo MD, Vodegel RM, Westerhof W. Depigmentation therapy in vitiligo universalis with topical 4-methoxyphenol and the Q-switched ruby laser. J Am Acad Dermatol. 2000;42(5 Pt 1):760–9.
31. Olsson MJ, Juhlin L. Epidermal sheet grafts for repigmentation of vitiligo and piebaldism, with a review of surgical techniques. Acta Derm Venereol. 1997;77:463–6.
32. Parsad D, Gupta S, IADVL Dermatosurgery Task Force. Standard guidelines of care for vitiligo surgery. Indian J Dermatol Venereol Leprol. 2008;74(Suppl):S37–45.
33. Parsad D, Pandhi R, Dogra S, et al. Clinical study of repigmentation patterns with different treatment modalities and their correlation with speed and stability of repigmentation in 352 vitiliginous patches. J Am Acad Dermatol. 2004;50:63–7.
34. Parsad D, Pandhi R, Juneja A. Effectiveness of oral Ginkgo biloba in treating limited, slowly spreading vitiligo. Clin Exp Dermatol. 2003;28:285–7.
35. Passeron T, Ostovari N, Zakaria W, et al. Topical tacrolimus and the 308-nm excimer laser: a syner-

gistic combination for the treatment of vitiligo. Arch Dermatol. 2004;140:1065–9.
36. Yu HS, Wu CS, Yu CL, et al. Helium-neon laser irradiation stimulates migration and proliferation in melanocytes and induces repigmentation in segmental-type vitiligo. J Invest Dermatol. 2003;120:56–64.
37. Westerhof W, Nieuweboer-Krobotova L, Mulder PG, et al. Left-right comparison study of the combination of fluticasone propionate and UV-A vs. either fluticasone propionate or UV-A alone for the long-term treatment of vitiligo. Arch Dermatol. 1999;135:1061–6.
38. Luger T, Boguniewicz M, Carr W, Cork M, Deleuran M, Eichenfield L, Eigenmann P, Fölster-Holst R, Gelmetti C, Gollnick H, Hamelmann E, Hebert AA, Muraro A, Oranje AP, Paller AS, Paul C, Puig L, Ring J, Siegfried E, Spergel JM, Stingl G, Taieb A, Torrelo A, Werfel T, Wahn U. Pimecrolimus in atopic dermatitis: consensus on safety and the need to allow use in infants. Pediatr Allergy Immunol. 2015;26:306–15.
39. Rustin MH. The safety of tacrolimus ointment for the treatment of atopic dermatitis: a review. Br J Dermatol. 2007;157:861–73.
40. Taieb A, Alomar A, Böhm M, Dell'Anna ML, De Pase A, Eleftheriadou V, Ezzedine K, Gauthier Y, Gawkrodger DJ, Jouary T, Leone G, Moretti S, Nieuweboer-Krobotova L, Olsson MJ, Parsad D, Passeron T, Tanew A, van der Veen W, van Geel N, Whitton M, Wolkerstorfer A, Picardo M, Vitiligo European Task Force (VETF); European Academy of Dermatology and Venereology (EADV); Union Europeénne des Médecins Spécialistes (UEMS). Guidelines for the management of vitiligo: the European Dermatology Forum consensus. Br J Dermatol. 2013;168:5–19.
41. Sassi F, Cazzaniga S, Tessari G, Chatenoud L, Reseghetti A, Marchesi L, Girolomoni G, Naldi L. Randomized controlled trial comparing the effectiveness of 308-nm excimer laser alone or in combination with topical hydrocortisone 17-butyrate cream in the treatment of vitiligo of the face and neck. Br J Dermatol. 2008;159:1186–91.
42. Van Geel N, Ongenae K, De Mil M. Double-blind placebo-controlled study of autologous transplanted epidermal cell suspensions for repigmenting vitiligo. Arch Dermatol. 2004;140:1203–8.
43. Szczurko O, Boon HS. A systematic review of natural health product treatment for vitiligo. BMC Dermatol. 2008;8(1):2.
44. Caron-Schreinemachers AL, Kingswijk MM, Bos JD, et al. UVB 311 nm tolerance of vitiligo skin increases with skin phototype. Acta Derm Venereol. 2005;85(1):24–6.
45. Anbar TS, Hegazy RA, Picardo M, Taieb A. Beyond vitiligo guidelines: combined stratified/personalized approaches for the vitiligo patient. Exp Dermatol. 2014;23(4):219–23. https://doi.org/10.1111/exd.12344. Review.

Medical Therapies

35

Alain Taïeb

Contents

Abstract

Medical therapies of vitiligo have a twofold aim: stopping inflammation and regenerating pigment cells. So far, the first aim has been generally fulfilled for short periods with courses of systemic corticosteroids or with only topical anti-inflammatory drugs for limited vitiligo. Maintenance treatment for long term control of microinflammation remains more problematic since this issue has not been addressed convincingly in large studies. The second aim relies mostly on phototherapies, which can also participate to the control of microinflammation. The most effective medical approaches combine anti-inflammatory and regenerative strategies. After psoriasis and atopic dermatitis, new targeted and semi targeted

A. Taïeb (✉)
Service de Dermatologie, Hôpital St André, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_35

therapies (such as JAK inhibitors) are currently in development for vitiligo and alopecia areata.

Key Points

- Topical corticosteroids (TCS) and topical calcineurin inhibitors (TCI) are commonly used as a first line treatment in limited vitiligo.
- TCS and TCI stabilize active progressing lesions and can promote repigmentation in combination with UV.
- Topical calcineurin inhibitors are preferred on the face.
- In extrafacial locations, potent TCS are first choice and should be given not more than once daily with a discontinuous chronic application regimen to avoid skin atrophy.
- TCS could be a useful adjunctive treatment after skin grafts, and can increase UV repigmentation efficacy.
- Controversy still exists about the efficacy of TCI alone to induce repigmentation on strictly UV protected areas.
- The therapeutic effect of vitamins and antioxidants is at best limited and seems to be obtained only in combinatory treatment with phototherapy or topical steroids.
- The safety of prolonged administration of topical and systemic antioxidants requires further evaluation.
- Week end oral minipulses of corticosteroids (OMP) is a well tolerated regimen proposed for stabilizing progressive vitiligo.
- Optimal duration of OMP therapy to stop vitiligo progression is situated between 3 and 6 months.
- Systemic immunosuppressants and immunomodulators including biologics and JAK inhibitors have been tested on a too limited scale in vitiligo to make firm conclusions.

35.1 Topical Therapies

35.1.1 Topical Corticosteroids

Wietze Van der Veen, Bas S. Wind, and Alain Taïeb

35.1.1.1 General Background

Currently, topical glucocorticosteroids (TCS) or topical calcineurin inhibitors (TCI) are the usual first line treatments of vitiligo. TCS have been used widely as topical and sometimes intralesional therapy in vitiligo since their introduction in dermatology in the 1950s. However, based on current criteria for clinical trials, the studies are of poor quality.

The mechanism of glucocorticoid (GC) action at the gene level can be divided into transrepression, which is responsible for a large number of desirable anti-inflammatory and immunomodulating effects; and transactivation, which is associated with frequently occurring side effects as well as with some immunosuppressive activities [1].

TCS have the well-known anti-inflammatory effects of GC, useful to control the inflammatory phase of vitiligo which is well demonstrated at the margin of lesions. TCS, when used in combination with tretinoin and hydroquinone in the treatment of the hypermelanoses, suppress the biosynthetic and secretory functions of melanocytes, and thus melanin production. Used alone, they are also depigmenting agents, and the loss of pigment or delay in pigmentation induced may help the migration of pigment cells from the follicular compartment towards the epidermis during repigmentation.

35.1.1.2 Studies

Most patients included in published studies had vitiligo/NSV and the duration of the intervention was limited. The use of a highly potent (clobetasol) or potent (betamethasone) topical steroid can repigment NSV but only in a limited proportion of cases in the small published series [2, 3]. Clayton [2] found 15–25% repigmentation in 10/23 subjects and >75% in 2/23 (the other 11 showed no response); Kandil [3] found 90–100%

repigmentation in 6/23 subjects and 25–90% in 3 (with 6 showing 'beginning' repigmentation). Clayton [2] found that all steroid users had skin atrophy with clobetasol, a highly potent topical steroid (used for 8 weeks), whilst Kandil [3] noted hypertrichosis in two subjects and acne in three subjects, related to 4 months use of the potent topical steroid, betamethasone.

Topical fluticasone (a potent TCS) alone or combined with UV-A has been studied in 135 adults [4]. Fluticasone used alone for 9 months induced a mean repigmentation of only 9% (compared to UV-A alone of 8%) whereas the combination of fluticasone and UV-A induced a mean repigmentation of 31%; no steroid atrophy was noted in steroid users.

In a meta-analysis on non-surgical therapies in vitiligo [5] of data extracted of three RCT on potent [3, 6, 7] and two RCT's on very potent corticosteroids [2, 6], 75% repigmentation was found in one third of the patients treated with potent corticosteroids, while no significant differences were found between very potent corticosteroids and their placebo. In larger non-randomized patient series however, potent and very potent TCS appeared to be equally effective (more than 75% repigmentation) in about 55% of the patients. As in all topical therapies, corticosteroids achieve the best results on sun-exposed areas like face and neck [4, 5]. Other indications for successful treatment are dark skin [8] and lesions of short duration [9] (Fig. 35.1).

In children, the topical use of the highly potent steroid clobetasol dipropionate was noted to induce better repigmentation than PUVAsol alone, >50% repigmentation in 15/22 (vs. 4/23 for PUVAsol) used for 6 months, but 6 steroid users developed skin atrophy [10]. Another study in 20 children over an 8-week period compared topical clobetasol and tacrolimus and found 41% repigmentation for clobetasol vs. 49% repigmentation for tacrolimus [11, 12].

Westerhof et al. [4] demonstrated a three times higher repigmentation rate in vitiligo patients treated with fluticasone propionate (FP) combined with UVA as compared to patients treated with either UVA or FP alone. A synergic effect of UVB or calcipotriol plus TCS is possible based on clinical observations [13]. The 308 excimer laser was also shown to be synergic with hydrocortisone 17-butyrate [9]. Carbon Dioxide laser dermabrasion or fractional CO_2 laser plus TCS and narrowband ultraviolet B has been proposed for refractory vitiligo [14, 15].

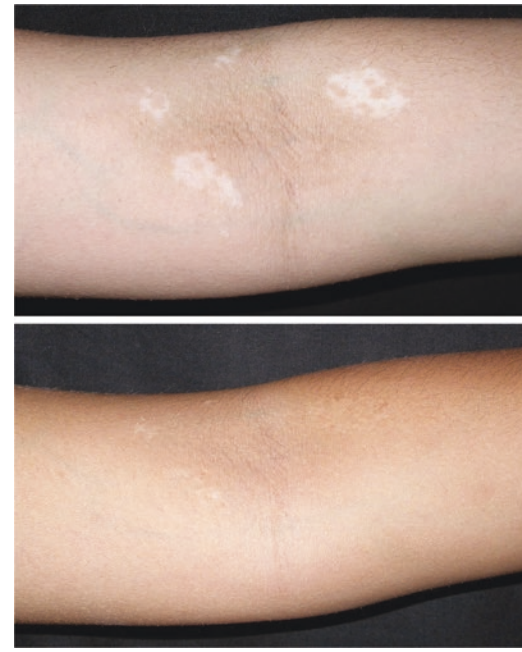


Fig. 35.1 Patient treated with betamethasone: before (up) and after (down) the therapy

35.1.1.3 Application Scheme

There is a general agreement to use TCS, whatever their indication, on the basis of once daily application, to limit side effects. Optimal duration for vitiligo is not established, as well as the use of a continuous vs. discontinuous regimen [16]. The amount used should be monitored (number of tubes/month is the most manageable like in atopic dermatitis). Potent TCS should be used first. When no results are seen after 3 months of sincere trial we suggest either to try very potent TCS if no side effects have been noted or to stop the medication.

35.1.1.4 Side Effects

Side effects like atrophy have been described in 14% of cases with very potent TCS as compared to 2% with potent TCS [1]. Systemic absorption

is a concern on thin skin. Children with head and/or neck affected areas are eight times more likely to have an abnormal cortisol levels compared with children affected in other body areas [17]. For local therapy of vitiligo potent TCS should therefore be preferred over very potent TCS that have more side effects. The lower classes of local corticosteroids have not demonstrated benefit.

A major improvement in the therapeutic index of GCs has been made with the development of compounds that display only local activity. This limited activity is due to the instability of the compounds that markedly reduces undesired systemic effects. For example, mometasone furoate, methylprednisolone aceponate and budesonide locally inhibit pro-inflammatory cytokines and chemokines very effectively with negligible systemic effects.

Selective modulation of GR action is a promising concept for separation of anti-inflammatory effects from side effects, which has led to the design of qualitatively new drugs, such as selective glucocorticoid receptor agonists (SEGRAs). These innovative steroidal or non-steroidal molecules induce transrepression, while transactivation processes are less affected [1]. Limitation of atrophic side effects by combination with retinoids (etretinate) seems also promising for long term treatments [18].

35.1.2 Topical Calcineurin Inhibitors

N. van Geel, B. Boone, I. Mollet and J. Lambert

35.1.2.1 General Background

The topical calcineurin inhibitors (TCI)—tacrolimus ointment 0.1% and 0.03% and pimecrolimus cream 1% —have been specifically developed for the treatment of inflammatory skin diseases, and approved for the short term and intermittent long term treatment of atopic dermatitis in several countries [19–24]. In contrast to TCS, they do not have the risk of local side effects, such as skin atrophy, telangiectasia, and glaucoma after prolonged use. Therefore they are preferentially used in areas more susceptible to these side effects, such as head and neck region, flexures and genital area [25, 26]. Furthermore, because of the limited percutaneous penetration, significant systemic absorption has not been reported following normal use [27]. However, they are more expensive than topical steroids, and the black box warning issued by the FDA in 2006 (see below) because of fear of increased skin cancer risk in case of combination with UV treatments has limited their use in atopic dermatitis and vitiligo.

Tacrolimus and pimecrolimus are topical ascomycin immunomodulating macrolactams, and act as calcineurin inhibitors, affecting the activation/maturation of T-cells, and subsequently inhibit the production of various Th1 and Th2 type of cytokines (IL-2, IL-3, IL-4, IL-5, IL-10, GM-CSF, TNFα and IFNγ). This has led to speculate that this mechanism may interfere with the autoimmune/inflammatory mediated loss of melanocytes in vitiligo lesions. The inhibition of TNFα production was suspected by some authors to be especially important in vitiligo, as TNFα can inhibit melanocyte proliferation and melanogenesis, and that it can induce ICAM-1 expression on melanocytes, through which T-lymphocyte induced destruction of melanocytes may occur [28]. In addition, in vitro evidence of a direct interaction between tacrolimus and keratinocytes has been obtained, creating a favourable milieu for melanocytic growth and migration by inducing the release of stem cell factor (SCF) and enhancing matrix metalloproteinase (MMP)-9 activity [29]. Furthermore, Kang and Choi [30] demonstrated that tacrolimus could even directly stimulate melanogenesis and migration of cultured human melanocytes.

35.1.2.2 Studies

Since 2002 the beneficial effect of TCI has been reported for patients with vitiligo. The first clinical trial regarding the efficacy of TIMs was carried out in six patients with generalized vitiligo. Five out of six patients achieved >50% repigmentation of their treated areas using topical tacrolimus for 1–5 months [31].

Many reports have followed this initial trial, investigating mainly tacrolimus, and since 2003

Table 35.1 Case reports and clinical studies on tacrolimus in the treatment of vitiligo

Reference	Study design	*N*	Study duration	Treatment regimen	Results
[32]	Open pilot study	6	4–6 months	0.1% Tacrolimus	Repigmentation in 5/6 patients. More than 25% repigmentation in 3/6 in UV protected areas
[33]	Open pilot study	12	8 months	0.1% tacrolimus twice daily	Good to excellent repigmentation in 50% of patients
[34]	Open pilot study	25 children	12 weeks	0.03% tacrolimus twice daily	Complete repigmentation in 57.9% of patients, best results in face and hear bearing sites
[21]	Retrospective review	57 children	3 months	0.03% or 0.1% tacrolimus once or twice daily	At least partial repigmentation in 84% of patients, best results in head and neck region
[35]	Open prospective study	23	24 weeks	0.1% tacrolimus twice daily	Varying levels in 89% of patients, best result head and neck region
[20]	Randomized, double-blind, comparative trial	20 children	2 months	0.1% tacrolimus versus 0.05% clobetasol propionate	Tacrolimus almost as effective as clobetasol propionate on several body locations
[22]	Case report	3	2–4 months	0.1% tacrolimus twice daily	Complete repigmentation in 100% of patients
[36]	Open pilot study	15	1.5–9.5 months	0.1% tacrolimus twice daily (+ sunlight exposition in some patients)	At least partial repigmentation in 87% of patients on several body locations
[28]	Open pilot study	6	1–5 months	0.03% or 0.1% tacrolimus twice daily	Moderate to excellent repigmentation in 83% of patients
[37]	Case report	1	18 months	0.1% tacrolimus twice daily	90% repigmentation in face and scalp region

also pimecrolimus (Tables 35.1 and 35.2). Unfortunately, only few randomized trials have been published, some of them in combination with UVB (Table 35.3) and only one study compared pimecrolimus and tacrolimus in vitiligo (Table 35.4).

35.1.2.3 Tacrolimus and Pimecrolimus Monotherapy

One randomized, double blind, left-right comparative trial has been conducted and showed that tacrolimus is effective similarly to clobetasol propionate 0.05% [12]. This study has been confirmed in children [34].

A double-blind vehicle controlled study, evaluating the efficacy and safety of pimecrolimus cream 1% predominantly on extremities of 20 vitiligo patients did not show efficacy on the actively treated site, possibly because all the treated lesions were localized on the extremities with 9 out of 20 (45%) on the back of the hands [43].

35.1.2.4 Combination Therapy

Two studies demonstrated that addition of topical tacrolimus to excimer laser therapy can improve laser efficacy [42, 49]. In another study narrow band UVB phototherapy was combined with tacrolimus ointment in 110 vitiligo patients [40]. The authors observed more than 50% of repigmentation in 42% of the lesions. The repigmentation rate appeared strictly related to the site: an improvement of >50% was obtained for lesions located on the face (83%), followed by the limbs (68%) and trunk (53.5%) compared to lesions on both extremities and genital areas, where responses were more disappointing. In a randomized, pla-

Table 35.2 Case reports and clinical studies on pimecrolimus in the treatment of vitiligo

Reference	Study design	*N*	Study duration	Treatment regimen	Results
[38]	Case report	1	5 months	1% pimecrolimus versus calcipotriol cream	Significant improvement with both, but mainly with pimecrolimus of facial lesions
[39]	Case report	2 children	3–4 months	1% pimecrolimus twice daily	Almost complete repigmentation of eyelid and genital lesions
[12]	Retrospective study	8	11 months	1% pimecrolimus twice daily	Mean percentage repigmentation in face 72.5%
[40]	Open prospective study	26	6	1% pimecrolimus twice daily	Repigmentation in 57.7% of lesions. Mean repigmentation of 62% head and neck region
[41]	Open prospective study	30	12 weeks	1% pimecrolimus twice daily	Repigmentation in 57.7% of lesions. Best results face and truck (mean repigmentation 31% and 36% respectively)
[42]	Open prospective study	19	6 months	1% pimecrolimus once daily	>25% repigmentation in 68% of patients
[43]	Randomized, placebo-controlled, double-blind trial	20	6 months	1% pimecrolimus twice daily versus Placebo	No significant difference and effect on extremities/hands
[11]	Comparartive prospective, non blind trial	10	2 months	1% pimecrolimus versus 0.05% clobetasol propionate	Comparable rate of repigmentation in non facial areas
[29]	Case report	1	5 months	1% pimecrolimus	Percentage of repigmentation: >90%

Table 35.3 Topical calcineurin inhibitors in combination with UVB

Reference	Study design	*N*	Study duration	Treatment regimen	Results
[44]	Open prospective study	110	16 weeks	0.03–0.1% tacrolimus once daily + UVB	Repigmentation of >50% in 42% of lesions, best results in face (83%)
[45]	Randomized, placebo-controlled, double-blind trial	8	12 weeks	0.1% tacrolimus + UVB versus placebo + UVB	No statistically significant difference in non facial areas
[46]	Comparative prospective, non blind, pilot study	9	12 weeks	0.1% tacrolimus + UVB versus 0.1% tacrolimus	No repigmentation with tacrolimus monotherapy on UV protected areas
[25]	Comparative, prospective, randomized, intra-individual study	14	12 weeks	0.1% tacrolimus + excimer laser versus excimer laser	Combination therapy is superior to excimer laser monotherapy in UV-resistant areas
[30]	Prospective, double-blind, placebo-controlled study	8	10 weeks	0.1% tacrolimus + excimer laser versus placebo + excimer laser	Significantly greater degree of repigmentation with combination therapy on elbows and knees
[47]	Case report	1	4 months	0.1% tacrolimus + UV-B	Repigmentation 95% on face
Majid	Left-right controlled study	8	6 months	0.1% tacrolimus + UVB vs. 0.1% tacrolimus	Earlier and better repigmentation with combination therapy

Table 35.4 Comparative studies between tacrolimus and pimecrolimus

Reference	Study design	*N*	Study duration	Treatment regimen	Results
[48]	Case report	1	18 months	1% pimecrolimus versus 0.1% tacrolimus under overnight occlusion	Tacrolimus site 88% and pimecrolimus 73% site repigmentation

cebo-controlled, double-blind study of Mehrabi and Pandya [50], narrow-band-UVB therapy with and without tacrolimus ointment was compared in eight patients. They did not demonstrate an additional effect of tacrolimus. However, it has to be mentioned that this study was underpowered ($n = 8$) to detect significant differences. Besides, the evaluated lesions were all located on non-facial areas.

35.1.2.5 Interpretation

TCI seem to affect repigmentation differently according to the anatomical location of the lesions. Most studies show beneficial results mainly in the head and neck region. This may be explained by the greatest density of hair follicles in these areas and thus of melanocyte reservoir. Besides, the influence of a reduced epidermal thickness might be of importance as this facilitates penetration of large molecules into the skin. Moreover, the head and neck region is a sun-exposed areas and UV therapy is a well-known treatment option of vitiligo. Probably, UV light exposure during TCI treatment seems to play an important or even synergistic role [41]. Sardana et al. [31] reported that there is sufficient evidence that tacrolimus monotherapy is useful to produce repigmentation, as illustrated by their two cases. However, it is still not known whether monotherapy with tacrolimus ointment requires longer periods to induce repigmentation as compared to combined treatment with UVB. A correlation between the repigmentation rate and patients' age or duration of the disease has not been consistently demonstrated. According to the literature, the results in children using TCI seem to be comparable to those obtained in adult patients [12, 22, 26, 51]. Generally, therapeutic options in childhood vitiligo patients are more limited, and TCI are a potential therapeutic alternative with a good benefit:risk ratio.

35.1.2.6 Application Scheme

Data about the most effective treatment scheme using TCI in vitiligo are still missing. In the available vitiligo studies the application frequency is varying between once and twice daily. Twice daily gives better results [37]. Duration of treatment mentioned in the studies ranged from 10 weeks to 18 months. Good results are usual for facial vitiligo, in vitiligo/NSV and SV (Figs. 35.2 and 35.3). Information about the minimal or ideal

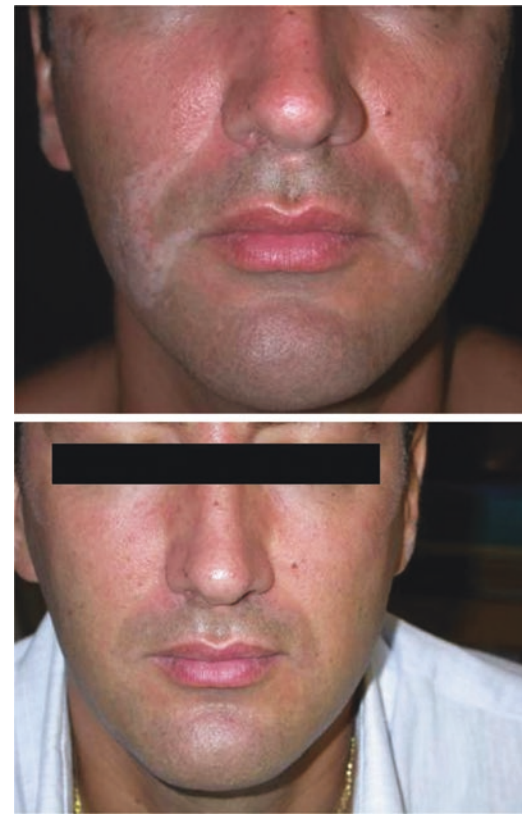


Fig. 35.2 Before (up) and after (down) tacrolimus tratment

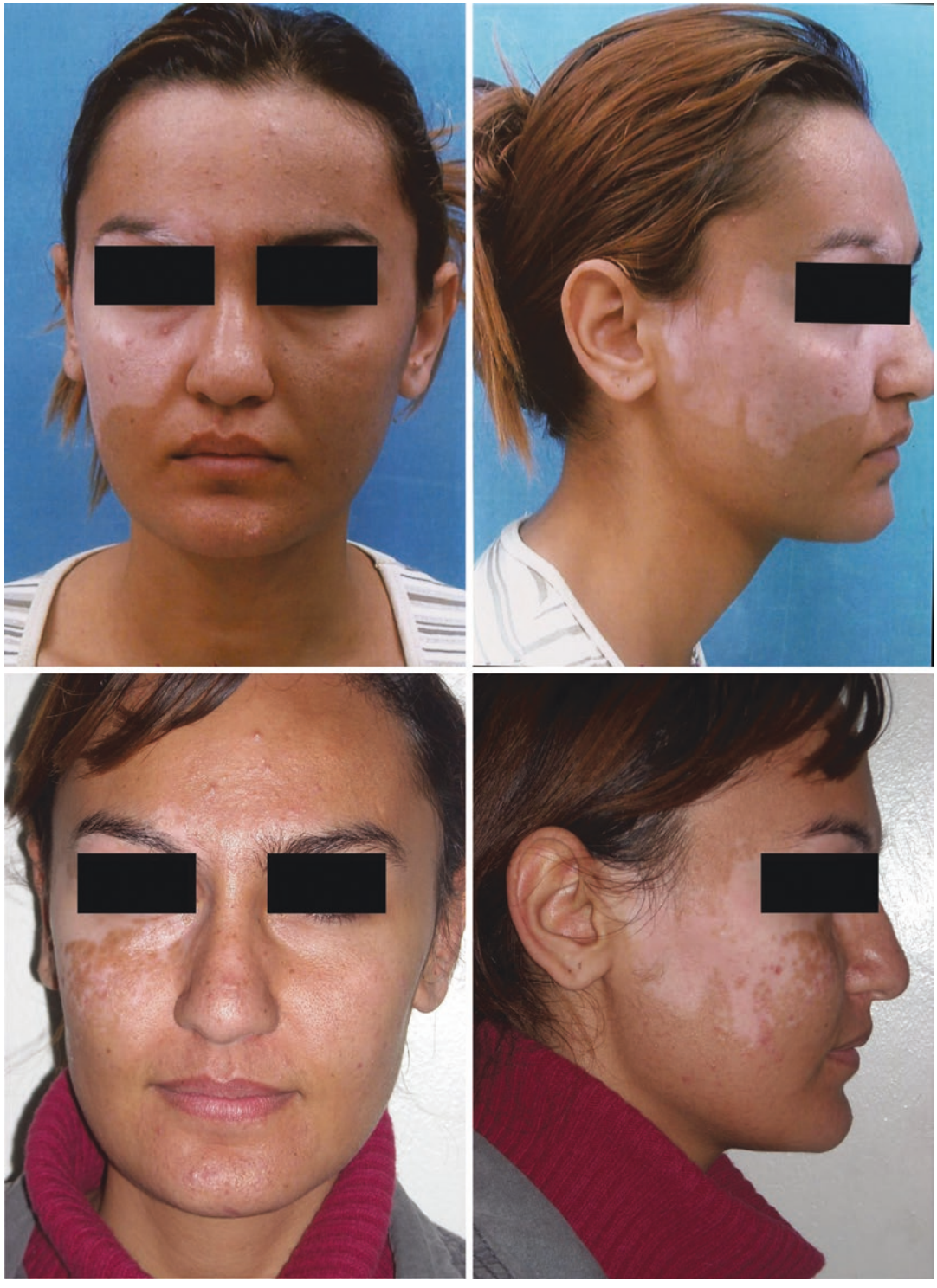


Fig. 35.3 Patients treated with tacrolimus. Segmental vitiligo patient treated with topical tacrolimus for 6 months (courtesy Dr A. Salhi)

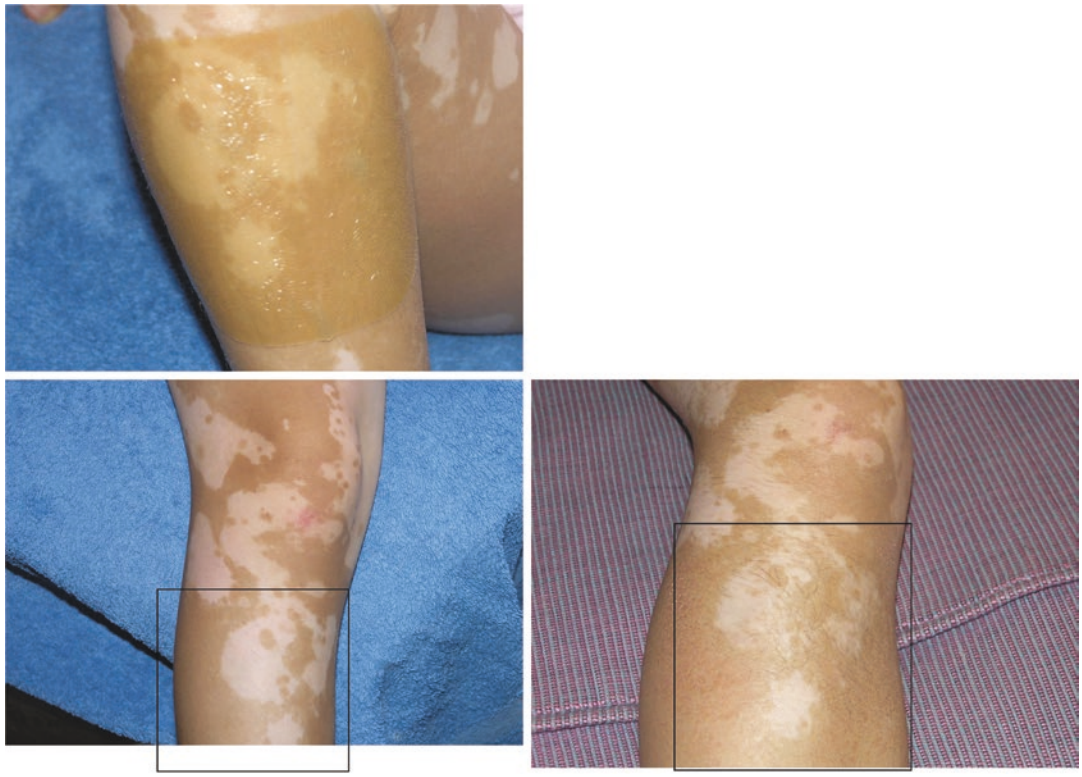


Fig. 35.4 Before and after tacrolimus under occlusion (hydrocolloid dressing) for 7 weeks

treatment period in vitiligo is not available. However, intermittent use after repigmentation, on the model of proactive treatment of atopic dermatitis, has proven to be helpful to limit relapse in a controlled study [47]. Repeated application may limit Koebner's phenomenon [52]. Furthermore, long-term results about the course of disease after treatment interruption are missing or incomplete. Interestingly, the additional overnight occlusion of 0.1% tacrolimus with polyurethane and hydrocolloid foils can lead to a good repigmentation on the extremities after a previous response failure when used without occlusion [53]. It was mentioned that the hydrocolloid dressings might be more suitable for improving trans-cutaneous penetration compared to polyurethane dressings, as they produce a much stronger water-binding capacity in the stratum corneum (Fig. 35.4). In general most vitiligo specialists prefer for long term control of the disease to use TCI on the face and neck because of good efficacy and limited side effects as compared with potent TCS, and use potent or very potent TCS intermittently on other body sites, optimally as combination therapies with natural or UV light.

35.1.2.7 Side Effects

The most common reported side effects for TCI within the first days of treatment are local application reactions such as burning sensation, pruritus, and erythema. However, the incidence of this side effect is lower in vitiligo patients than in the published atopic dermatitis studies. This is probably due to the presence of an intact epidermis in vitiligo patients versus the altered skin barrier of the atopic dermatitis patient. Treatment with TCI has not been associated with a statistically significant increase of skin infections or systemic infections in patients with atopic dermatitis [45, 54, 55].

A tacrolimus induced hyperpigmentation in a vitiligo lesion of the infraorbital area has been

related to sun exposure. The hyperpigmentation was temporary, with reappearance of depigmentation within 1 month of discontinuing topical tacrolimus application. Temporary tacrolimus induced perilabial lentiginose has also been observed [35]. Less frequently reported side effects in the face are acne, hypertrichosis and rosaceiform eruptions [54].

Since January 2006 a black box warning for tacrolimus and pimecrolimus was announced by the FDA, because of concerns of potential safety risks including skin cancer and lymphoma. However there is up till now no evidence of a causal relationship between the sporadically reported lymphomas and the use of a topical calcineurin inhibitor. It should be noted that most studies with TIM's are conducted in patients with atopic dermatitis, in which the barrier function of the skin is disturbed. In vitiligo, the barrier function of the skin is normal which results in a lesser degree of penetration of TIMs. So far the use of TIMs has not been reported to be associated with significant systemic immunosuppression or increased risk for skin cancer and other malignancies in clinical vitiligo trials [45].

35.1.3 Other Topical Therapies

35.1.3.1 Vitamin D Analogues

Mauro Picardo

The idea that vitamin D analogues may be considered an option for the treatment of the vitiligo arose from the casual observation of the occurrence of cutaneous perilesional hyperpigmentation after local application on psoriatic plaques in combination with phototherapy. Starting from this clinical evidence, several in vitro and in vivo data supported the therapeutic role of the calcipotriol and tacalcitol for vitiligo [56–60]. Skin cells, including keratinocytes, melanocytes, and dermal fibroblasts possess vitamin D receptors (VDR), the activation of which induces the expression of several genes associated with proliferation, differentiation and immune responses. Exposure of human melanocytes to vitamin D promotes tyrosinase activity and melanogenesis, together with the up-regulation of c-Kit. Morphological modifications are detected in number and length of the dendrites. For keratinocytes, vitamin D induces differentiation, decreased proliferation and release of the pro-inflammatory cytokines IL-8 and IL-6, as well as increased production of IL-10. In vitiligo skin, both melanocytes and keratinocytes have been shown to have an alteration of Ca^{2+} uptake and a decreased intracellular concentration of Ca^{2+}, which could compromise melanogenesis by increasing the level of reduced thioredoxin, which inhibits tyrosinase [56].

Vitamin D3 analogues have been reported to interfere with intracellular Ca^{2+} fluxes, cause G1 block, induce cKit upregulation, and inhibit TNFα, IL8, and RANTES production. The functional consequences of these activities affect cell survival, proliferation and differentiation processes, including melanogenesis. Moreover, vitamin D analogues are believed to target the immune response interfering with activated T cells and inhibit the expression of several cytokine genes, such as those encoding TNFα and IFNγ.

Vitamin D3 analogues have been mainly proposed as a supporting therapy during PUVA, NB-UVB, or excimer (308 nm) phototherapy [61–63].

Initially, open studies reported repigmentation of vitiligo lesions using calcipotriol, a synthetic analogue of calcitriol [1,25(OH)2D3] with PUVAsol or solar exposure. Subsequent studies reported contradictory results. The combination of calcipotriol and PUVA was reported as more effective than PUVA alone (70% versus 52% of the patients obtained mild-moderate repigmentation), in particular in initiating repigmentation [64–66], also suggesting the up-regulation of c-kit expression as possible melanocyte-mediated mechanism of action. However, a right/left comparative open study did not shown that the addition of calcipotriol increased the response rate to PUVA [67].

Some studies have suggested the enhancement of NB-UVB phototherapy when calcipotriol or tacalcitol was locally applied (Fig. 35.5) before or after UVB exposure [68–70], providing earlier pigmentation with lower total UVB dosage, thus

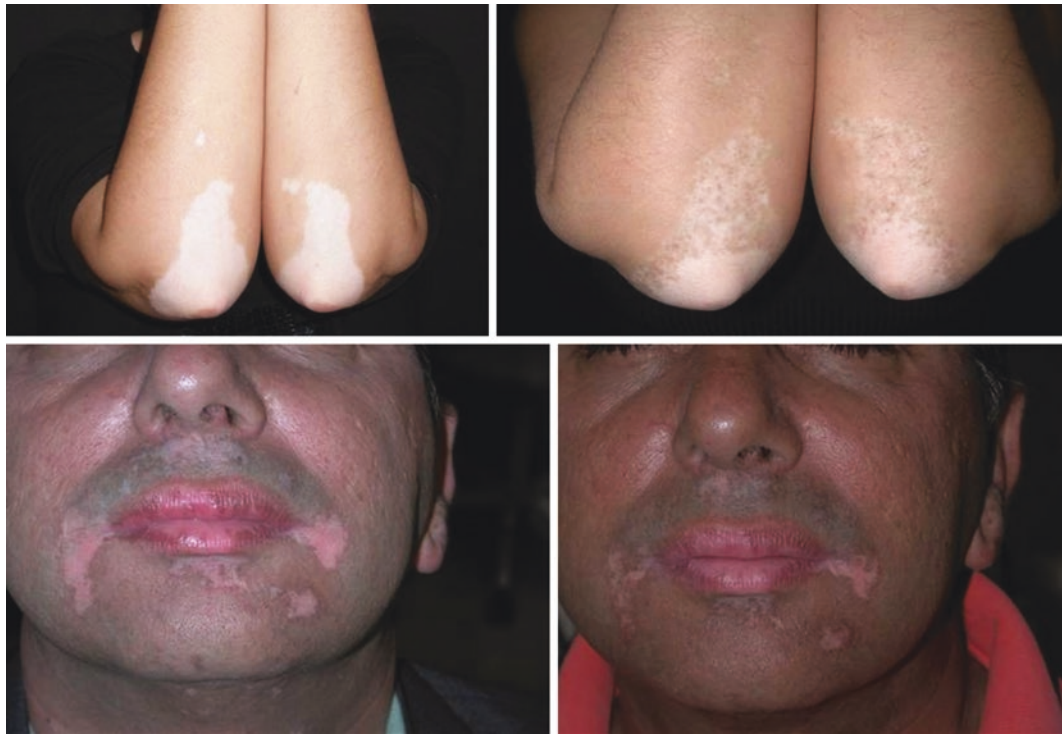


Fig. 35.5 Two patients, with different localization of the lesions, treated with tacalcitol plus NB-UVB

reducing the cost and the duration of the treatments. However two single blinded left-right clinical studies and one RCT did not show any improvement in treatment outcome when adding topical calcipotriol [71].

Another synthetic analogue of vitamin D, topical tacalcitol [1-24$(OH)_2$D3], in combination with bi-weekly NB-UVB phototherapy, improved the extent of pigmentation and increased the rate of response as compared to phototherapy alone in a left-right RCT. Another RCT using tacalcitol in combination with monochromatic excimer light (308 nm MEL) confirmed that tacalcitol improved the efficacy of phototherapy, achieving earlier pigmentation with lower dosage [72]. However the true effectiveness of the combinatory treatment, it is still considered controversial because other studies failed to find improvement when various sources of UVB phototherapies were associated with vitamin D3 analogues [73, 74]. Moreover, a vehicle-controlled RCT performed on 80 patients reported that the combination of tacalcitol with heliotherapy has no additional advantage when compared to heliotherapy alone [75].

However, the published trials are mainly based on small numbers of enrolled patients and different phototherapy protocols were implemented, characterized by different temporal sequences and modality of vitamin D3 analogue application, and variable/different period of treatment-washout before starting the tested therapy, so that a comparison of the results is not easy. The most recent studies of vitamin D analogues-UVB combined treatment conducted in India gave also opposite results [76, 77]. In any case, most of the studies reported best results when lesions were localized on the face or on the trunk.

Small studies and isolated case-reports described initially the effectiveness of calcipotriol or tacalcitol in children [78, 79]. Calcipotriol has been evaluated in combination with corticosteroids (betamethasone dipropionate) in

Table 35.5 Summary of the studies

Vitamin D analogue	UV	Study	Administration	Duration	Effectiveness	Reference
Calcipotriol	No	Children (18)	2/day	4–6 months	Yes	[79]
Tacalcitol	Sun	Case-report children	1/day	1 month	Yes	[78]
Calcipotriol	PUVA	Adults (26)	2/day	3–9 months	Yes	[83]
Tacalcitol	NB-UVB	Randomized assessor-blind adults (32)	2/week UV ± 1/day cream	6 months	Yes	[82]
Tacalcitol	308 nm MEL	Single-blind adults (38)	1/week MEL ± 2/day cream	12 sessions	Yes	[62]
Tacalcitol	Sun	Double-blind randomized placebo controlled (80 adults)	1/day	16 weeks	No	[63]
Calcipotriol	NB-UVB	Comparative prospective, 40 adults	3/week UV ± 2/day cream	30 sessions	No	[84]
Calcipotriol	No	Left-right open (24 adults)	1/day	3–6 months	No	[85]

randomized trials involving also some pediatric subjects. Good results (80% of success) in terms of onset, degree, and stability of repigmentation were obtained with the combinatory therapy [80, 81]. A possible benefit of the association was to limit atrophogenic effect of TCS. The most frequent side effects in all studies was mild to moderate erythema or itching, a typical symptom associated with the application of vitamin D analogues [82] (Table 35.5).

35.1.3.2 Antioxidants

Mauro Picardo and Maria Lucia Dell'Anna

General Background for Topical and Systemic Antioxidant Therapy in Vitiligo

The therapeutic approach with antioxidants originated from the description of the occurrence of the oxidative stress and possible vitamin deficiency in vitiligo patients, involving melanocytes and other epidermal as well as non-epidermal cells [86, 87]. Increased production of H_2O_2, biopterins and catecholamines, defective expression and/or activity of the antioxidant enzymes catalase and glutathione peroxidase in addition to lipid peroxidation are the major metabolic alterations reported in the literature [86–88]. The occurrence of a redox unbalance, both at epidermal and systemic level, has been described in vitiligo patients (Chap. 31). Usually, the redox unbalance is caused by a hyperproduction of ROS not associated with the increased activity of the adequate detoxifying apparatus. In vitro and clinical data suggests that several different cellular metabolisms may lead to the unwanted production of radical species, possibly associated with the lipoperoxidative processes. The high lipid content of the cellular and mitochondrial membranes seems to play a relevant role in the propagation of the peroxidative events, mainly the mitochondrial ones due to their strictly physical association with the main cellular source of free radicals. Moreover, some studies indicate that the reactive species, released as side-products of specific epidermal metabolisms, can diffuse systemically, and can target any cell type [89].

Antioxidants form an integrated network inside the cells, and the activity of each compound depends on the presence and function of the rest of the antioxidant molecules. The participating enzymes and non enzymatic molecules act as scavengers of the free radicals and are able to reduce the oxidised compounds inside the

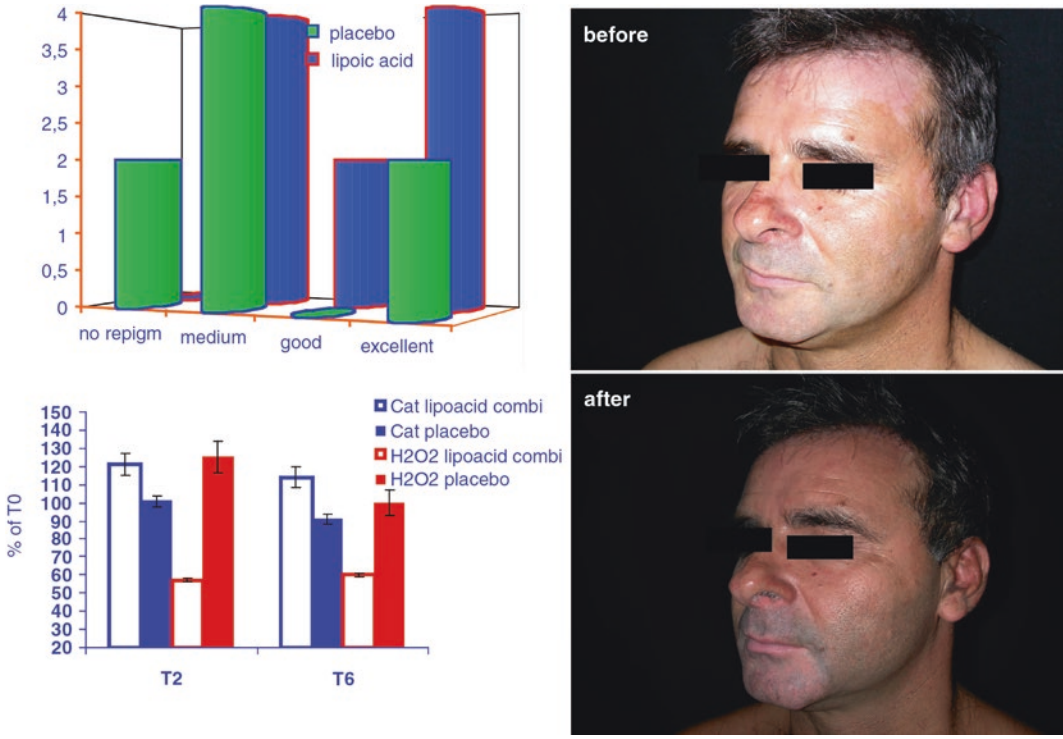


Fig. 35.6 Modification of oxidative stress in patients treated with NB-UVB alone (placebo) or in combination with lipoacid. Clinical response of a patients treated with a pool of antioxidants

network, allowing the restoration of a correct redox balance. The system glutathione/glutathione peroxidase, catalase, superoxide dismutases, α-lipoic acid, vitamin E, and vitamin C are the main components of this antioxidant network. α-Lipoic acid is a lipophilic and hydrophilic compound acting as a fatty acids peroxyl and hydroxyl radical scavenger, lipoxygenase inhibitor and glutathione synthesis promoter. Moreover, α-lipoic acid is involved in recycling vitamins C and E. Vitamin C is a hydrophilic antioxidant; whereas vitamin E is a lipophilic free-radical scavenger and inhibits lipid peroxidation helping to maintain membrane integrity. According to the physiological redox-based system, the most effective therapeutic approach should provide a balanced pool of antioxidant molecules, with the aim of restoring the correct intracellular network (Fig. 35.6).

35.1.4 Topical

35.1.4.1 Pseudocatalase

This antioxidant treatment containing pseudocatalase and calcium chloride was initially suggested by Schallreuter and colleagues [88]. Pseudocatalase is a low molecular weight coordination complex [bis-manganese III-EDTA-$(HCO_3\text{-})_2$] able to produce O_2 and H_2O from H_2O_2 at a rate higher than catalase. Solar or UVB exposure is required for the chemical activation of pseudocatalase [90] but not for the therapeutic effects, since the amount of UVB administered (0.15–0.3 J/cm^2 per session, two to three times a week) is lower than that used during conventional phototherapic protocols in association with low UVB doses [88]. A group of 33 vitiligo patients were treated for 15 months and repigmentation was found excellent in 90% of the subjects, mainly on the face and dorsum. The clinical

improvement has been related to the reduction of epidermal H_2O_2 level. Subsequently, the combination pseudocatalase plus Dead Sea climatotherapy—dead sea water contains metals able to activate pseudocatalase—has been shown to shorten the time required for the onset of repigmentation (10 days versus some weeks) in nearly all the patients treated [91], as compared to UVB-activated pseudocatalase. The recovery in the epidermal enzymatic activities of catalase, tetrahydrobiopterin dehydratase, acetylcholinesterase, and dihydropteridine reductase (Chap. 2.3.4) which can be inactivated by the oxidation of constitutive aminoacids such as methionine, cysteine, tryptophan [11, 92], has been reported after the treatment. The clinical validity of the pseudocatalase regimen has been debated, because large scale double-blinded versus placebo studies have not been published and since data have not been confirmed by others authors [90, 93, 94]. The effectiveness of pseudocatalase, however, seems to be dependent on the formulation of the cream, and the PC-KUS® brand is reported to be successful whereas different pseudocatalase preparations have been used by other groups. PC-KUS® has been also successfully tested in pediatric population and a retrospective study carried out on 71 children, 61 with diffuse and 10 with segmental disease. The progression of the disease was stopped, without side effects and more than 75% of repigmentation was observed in 93% of the patients, associated with the H_2O_2 removal by epidermis. Only feet and hands lesions appeared to be poorly responsive [95]. The effectiveness of the treatment was found independent of disease duration and skin phototype.

Vitix®. A formulation containing an extract of *Cucumis melo* vehiculated by beads of palm oil (Vitix®) has been proposed for the treatment of vitiligo. Indeed, the antioxidant properties of the extract evaluated in an in vitro model, was attributed mainly to superoxide dismutase, glutathione reductase and catalase activities [96]. The application of the gel, twice daily, with a simultaneous sun exposure for 30 min or NB-UVB phototherapyhas been reported to induce repigmentation after 1–3 months [97]. Yet, others failed to confirm both the ability of the gel to remove H_2O_2 from the epidermis and the clinical effectiveness, when compared to pseudocatalase cream [98]. Another negative small trial has been reported [99].

35.2 Immunosuppressive Systemic Therapies

Infammation and dysimmunity definitely play a role in vitiligo, which has suggested to try systemically various immunomodulators including corticosteroids, immunosuppressants, and more recently biologics and targeted small molecules such as JAK inhibitors.

35.2.1 Corticosteroids

Davinder Parsad and Dipankar De

35.2.1.1 General Background

Though topical steroids are used extensively in the management of vitiligo, studies on systemic steroids have been sparingly reported in the literature. The cause of this discrepancy is not known. Systemic steroids can arrest the activity of the disease, if they are used in sufficient doses for a sufficient period of time [100, 101]. They are in general not effective in repigmenting stable vitiligo. Moreover side effects associated with long-term use of daily systemic corticosteroids may act as deterrent against their common use.

Pulse therapy refers to the administration of large (supra-pharmacologic) doses of drugs in an intermittent manner to enhance the therapeutic effect and reduce the side effects of a particular drug [102]. The credit of first use of corticosteroids in pulse form goes to Kountz and Cohn [103] who used it to prevent renal graft rejection. Subsequently, pulse corticosteroids have been used in various dermatological as well as non-dermatological indications. Oral minipulse (OMP), i.e. intermittent administration of betamethasone/dexamethasone was pioneered in India by Pasricha et al. [101]. They first used OMP in vitiligo. Subsequently, OMP has been used successfully in various other dermatoses like extensive alopecia areata [104],

cicatricial alopecia, extensive/bullous lichen planus [105], trachyonychia [106], infantile periocular haemangioma [107] etc. As the indications of use of OMP suggest, it is understandable that in dermatoses where steroid therapy is effective, OMP may be used, maintaining efficacy and cutting down on side effects.

35.2.1.2 OMP Studies

In the first reported study on OMP in vitiligo by Pasricha et al. [101], betamethasone or dexamethasone, both corticosteroids with a long half-life (36–54 h), were given as a single oral dose of 5 mg on 2 consecutive days per week. This dose of steroids was decided upon arbitrarily. Progression of the disease was arrested in 91% of the patients. A degree of repigmentation was observed in a proportion of patients and the side effects were either not significant or altogether absent.

In the subsequent trial of 40 patients—36 with progressive and 4 with static disease—the same OMP regimen was used [108]. In children, the dose was proportionately reduced. In adults who did not respond to the standard dose of corticosteroids, the dose was increased to 7.5 mg/day then reduced to 5 mg/day when disease progression was arrested. Within 1–3 months of starting treatment, 89% patients with progressive disease stabilized, while within 2–4 months, repigmentation was observed in 80% of total patient cohort. The area of repigmentation continued to progress as treatment continued, though none of the patient achieved complete repigmentation. Seventeen of 40 (42%) had at least one side effect, though they were not significant. The side effects profile included weight gain, dysgeusia, headache, transient mild weakness, acne, mild puffiness of the face alone, perioral dermatitis, herpes zoster, glaucoma and amenorrhoea. The authors presumed that the repigmentation observed was spontaneous and thus varied from patient to patient and lesion to lesion [108]. Subsequently Kanwar et al. [109] assessed the efficacy of OMP in vitiligo. They used dexamethasone in a dose of 5 mg/day on 2 consecutive days per week and the dose was halved in children 16 years of age or younger. Of 37 patients included with actively spreading disease, 32 were evaluable at the end of the study. 43.8% had mild to moderate repigmentation without appearance of new lesions. The pigmentation appeared in majority of patients within 15 weeks of starting treatment. No side effects were reported [109]. In the study by Radakovic-Fijan et al. [110], 29 patients, 25 with progressive disease and 4 with stable disease were included. The daily dose of dexamethasone was significantly increased to 10 mg/day and the treatment was continued for a maximum period of 24 weeks. In addition, plasma cortisol and corticotrophin levels were measured to detect hypothalamopituitary-adrenal axis suppression before and up to 6 days after the dexamethasone pulse in the first and fourth weeks of treatment in 14 patients. Disease activity was arrested in 88% of patients with progressive disease after an average treatment period of 18.2 weeks. Marked repigmentation was observed in 6.9% and moderate or slight repigmentation in 10.3%, while 72.4% had no response in repigmentation. A tendency towards better treatment results was observed with an increasing number of pulses. Side effects were observed in 69% patients, which included weight gain, insomnia, agitation, acne, menstrual disturbances, and hypertrichosis. Plasma cortisol and corticotrophin levels, though markedly decreased after one pulse, returned to normal before starting the next pulse. The authors observed that ethnic background may have an impact on therapeutic response [110].

35.2.1.3 Interpretation and Recommendations

OMP with either betamethasone or dexamethasone can arrest progression of spreading disease. However, it is not usually suitable alone for repigmentation of vitiligo lesions. In addition, in patients with fast spreading vitiligo, disease progression is not stopped immediately. There are no RCT confirming that either speed or magnitude of response to phototherapy and photochemotherapy in patients with generalized fast spreading vitiligo might be potentiated by concomitant administration of OMP. As noted before, the dosage of dexamethasone used in OMP has been arbitrarily chosen. In the majority of the studies, a dose of 5 mg every day for 2 consecutive days per week has been used. For those who not respond, 7.5 mg/day may be used, then reduced to 5 mg/day if disease progression is

arrested. The lower OMP regimen 2.5 mg for 2 consecutive days is however sufficient in the majority of cases [111]. The drug can be preferably given on weekends to increase the compliance as short therm side effects may be troublesome in some. If the 5 mg/day dose is used, the drug can be gradually tapered off over 6 months, after desired response is achieved. However, when a lower dose is used (2.5 mg/day), the treatment can be stopped abruptly without any noticeable manifestation of hypothalamo-pituitary-adrenal axis suppression. Minocycline has been tested as an alternative with less side effects to OMP in progressive vitiligo, and shown to have similar stabilizing effects [112]. Concerning stable vitiligo, NB-U.V.B plus OMP and Nb-U.V.B alone were found as expected to be clinically superior over OMP alone [113].

35.2.2 Immunosuppressants/Biologics

Markus Böhm

35.2.2.1 Rationale for the Use of Systemic Immunomodulators

As described in Chap. 2.3.5 there is a large body of data indicating that vitiligo is an immune-mediated inflammatory disease. This concept is further corroborated by the beneficial effects from anti-inflammatory and/or immunomodulatory treatment, e.g. therapy with corticosteroids or topical calcineurin inhibitors. In this view, different systemic imunomodulators have been proposed as an alternative to corticosteroids for the treatment of progressive vitiligo.

35.2.2.2 Systemic Immunosuppressants

Low dose cyclophosphamide has been proposed in the early 1980s. Cyclophosphamide inhibits the production of antibodies by B lymphocytes [114]; the rationale for its use in vitiligo derived from the experience in other autoimmune skin diseases, such as pemphigus, and was supported by the identification of circulating melanocyte-specific antibodies in the serum of affected patients. A good response was seen at the dosage of 50 mg twice a day in about the 27% of the cases, while no response was observed in 33% of the treated patients. Haematological toxicity, hair loss and nausea have been reported as common side effects during the treatment, limiting its use [115, 116].

Low dose azathioprine (at the maximal dosage of 50 mg/day) in association with PUVA has been also proposed. Azathioprine is able to inhibit the cellular immune response and is widely used in inflammatory bowel disorders and in rheumatology, and in dermatology for autoimmune dermatoses, including pemphigus, at the dosage of 50–100 mg/day. A study performed on 60 patients randomized to receive either azathioprine (0.6–0.75 mg/kg/day) in association with PUVA or PUVA therapy alone, has demonstrated a potential synergic effect of the two approaches, but true positive results have been demonstrated only in a minority of the subjects, with the limitation of lack of validated, standardized measures for vitiligo assessment [117].

Starting from the pathogenetic concept of a systemic over-activation of cellular immunity in vitiligo, systemic cyclosporine has been anecdotally used in patients with diffuse disease, at the dosage of 5 mg/kg/daily with difficult to interpret responses. Vogt-Koyanagi-Harada patients have benefited cyclosporine for eye involvement but vitiligoid changes have not been assessed precisely. Considering the kidney and liver toxicity of cyclosporine, experience remains limited [118].

Methotrexate is now widely used in chronic inflammatory skin diseases but has not been much investigated in vitiligo. A study in unstable vitiligo has shown comparable stabilizing effects of 10 mg methothrexate/week to low dose OMP over 6 months [119].

35.2.2.3 Biologics

The clinical experience in vitiligo of biologics introduced in dermatology for the treatment of

psoriasis is limited. They include anti-TNF-α targeted therapies (etanercept, infliximab, adalimumab) and efalizumab, the latter being an antibody that binds to the CD11a subunit of LFA-1.

Anti-IFN-γ Strategy

The pioneering concepts and preclinical observations of Skurkovich et al. deserve to be summarized [120, 121]. These scientists were amongst the first who proposed not only to remove certain types of IFNs but also TNF-α to treat various autoimmune diseases. Based on their longstanding research on the pathogenetic role of proinflammatory cytokines in immune-mediated inflammatory diseases, Skurkovich et al. initially proposed that IFN-γ should be removed in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, type I diabetes, psoriasis vulgaris, alopecia areata or vitiligo [122]. In this context it is worth mentioning that IFN-γ mRNA levels have been shown increased in lesional and in adjacent uninvolved skin of patients with vitiligo [28]. It is also well known that systemic administration of IFN-α can induce or aggravate vitiligo [123–125] although improvement of preexisting vitiligo under pegylated IFN-α-2A was reported in a patient with hepatitis C [126]. In a small preclinical case series, Skurkovich et al. injected polyclonal IFN-γ antibodies (both IgG and/or F(ab′)2 antibody fragments) into patients with various Th-1-mediated autoimmune diseases [122]. The protein concentration of these antibodies was about 33 mg/ml with an IFN-γ neutralizing capacity of more 66 μg/ml [127]. Four patients with vitiligo (12–14 years old) received intradermal injections of F(ab′)2 fragments (titer: 24×10^3 IU/ml) generated from goat antibodies to human IFN-γ [128]. Aliquots of 0.1 ml of the antibody were given perilesionally for 10 days. All treated patients experienced sustained erythema after 3 days of therapy followed by development of small, slightly infiltrated pinkish papules in the depigmented areas. On day 10, the authors observed loss of the well-defined borders between the normal and depigmented skin in all treated patients [128]. Intramuscular injections of the IFN-γ antibody were also performed. A gradual diminishment of the border between the depigmented area and normal skin was seen [122, 127]. In addition, the authors reported on the clearance of vitiligo in another patient with autoimmune polyglandular syndrome (APS)-4, i.e. vitiligo and alopecia areata [129]. It is unclear for how long systemic anti-IFN-γ therapy was given in the latter individual. Moreover, the above preclinical study lacks detailed description of patient's characteristics as well as follow-up data.

The group of John Harris using a transgenic mouse model reported that the IFN-γ-induced chemokine CXCL10 is expressed in lesional skin from vitiligo patients, and that it is critical for the progression and maintenance of depigmentation in this model [130]. Following Skurkovich, they hypothesized that targeting IFN-γ signaling might be an effective new treatment strategy. Activation of signal transducer and activator of transcription 1 (STAT1) is required for IFN-γ signaling and other studies revealed that simvastatin, an FDA-approved cholesterol-lowering medication, inhibited STAT1 activation in vitro. They found that simvastatin both prevented and reversed depigmentation in their mouse model of vitiligo, and reduced the number of infiltrating autoreactive CD8(+) T cells in the skin [131]. Unfortunately a subsequent trial of simvastatin in human vitiligo failed [132]. The recent development of JAK inhibitors (below) is an alternative to block the JAK-STAT signalling pathway in vitiligo.

AntiTNF-α Approach

Since TNF-α protein expression and immunoreactivity is elevated in lesional skin of patients with vitiligo [133, 134] it is not surprising that anti-TNF-α drugs had been investigated in this indication. However, anti-TNF-α therapy, in accordance with its potential to trigger other autoimmune phenomena (such as alopecia areata and lupus erythematosus-like syndromes) can induce de novo vitiligo. Accordingly, a 61-year-old Caucasian suffering from rheumatoid arthritis was reported to develop vitiligo lesions on the

dorsa of the hands 6 months after intravenous therapy with infliximab at 3 mg/kg [129]. Infliximab was not discontinued and the patient's vitiligo was subsequently treated with *Polypodium leucotomos* extracts and topical pseudocatalase to regain 50% of his pigmentation. In another case report, a 66-year-old white man receiving adalimumab for the treatment of his psoriasis is described [135]. Within 4 months of 40 mg adalimumab administered subcutaneously every other week his psoriasis had cleared. However, depigmented skin was noticed in those areas previously affected by psoriasis but not in unaffected areas. A skin biopsy specimen from the depigmented skin confirmed the presence of vitiligo. Concomitant psoriasis and vitiligo is already described, with the common occurrence of koebnerization (Chap. 1.5.7). Regarding the therapeutic efficacy of TNF-α antibodies in patients with pre-existing vitiligo the data are limited and controversial. Rigopoulos et al. [136] assessed the therapeutic potential of etanercept in a small open-label pilot study consisting of four male patients with vitiligo vulgaris (mean age: 29.3; mean duration of vitiligo: 7.5 months). All patients had progressive disease with development of new lesions within the previous 3 months. Most of the patients had an involvement of extremities including hands and feet. All of the patients had not received any treatment for vitiligo for the last 2 months. The treatment protocol consisted of weekly injections of etanercept (50 mg subcutaneously) for 12 weeks followed by 25 mg of etanercept weekly for another 4 weeks. Treatment success was evaluated photographically and photometrically. Although the overall tolerability was good none of the patients had any repigmentation. However, no aggravation of their vitiligo was noticed. The authors suggested that monotherapy with TNF-α antibodies should not be considered as a treatment option for vitiligo. In another case report, vitiligo improvement was observed in a patient with ankylosing spondylitis under infliximab treatment [135]. A 24-year-old male with ankylosing spondylitis since the age of 18 and generalized vitiligo for 11 years received 350 mg infliximab intravenously at weeks 0, 2 and 6, and then every other week for 10 months. With this treatment the patient's disease activity score and functional indices for arthritis improved as well as his vitiligo: 6 months after infliximab spreading of two vitiligo spots at both axilla and pretibial areas was halted. In addition, several other vitiligo spots (located on the trunk, finger joints, face and pretibial areas) revealed partial repigmentation or even disappeared. In accordance with the established role of TNF-α as an inhibitor of melanocyte proliferation [137] and melanogenesis [138] these data would suggest some potential of antiTNF-α agents in the treatment of vitiligo, as shown in other reports suggesting a possible stabilizing effect [139, 140].

T Cell Skin Recruitment Blocking Strategy

Two reports have described a beneficial effect of efalizumab in vitiligo as a T cell targeted recombinant antibody binding to the CD11a subunit of LFA-1 [141, 142]. As a consequence of such an immunomodulatory approach by an anti-LFA-1 antibody, the interaction between LFA-1 and intercellular adhesion molecule (ICAM)-1 expressed by numerous activated resident skin cells is blocked. Expression of ICAM-1 had previously been detected in perilesional melanocytes around active vitiligo patches [143]. Regarding expression of LFA-1 in vitiligo patients, it was shown that LFA-1 immunoreactivity in leukocytes is higher in minigrafts of non-responders than in responders [144].

In the first report, a 52-year-old male Surinamese Hindustani man with plaque psoriasis and universal vitiligo received efalizumab [142]. He had been suffering from vitiligo since more than 10 years. His previous anti-psoriatic therapies had included ultraviolet (UV) light and psoralen, methotrexate, cyclosporine and fumaric acid. Six weeks after starting subcutaneous efalizumab at 1 mg/kg once per week the patient developed spotty repigmentation in vitiliginous areas of his face. Facial repigmentation continued in a perifollicular pattern under efalizumab

treatment for 6 further weeks but treatment had to be terminated due to the deterioration of his psoriasis. Subsequently, facial vitiligo became worse. In the second report, Fernandez-Obregon [141] reported on a 43-year-old male Hispanic with a history for vitiligo vulgaris for more than 10-years and plaque psoriasis for 5 years. Before starting efalizumab he had received topical high-potency steroids, anthralin, and UVB phototherapy. However, UVB therapy was not tolerated and his vitiligo deteriorated. Upon systemic acitretin he developed pruritus and therapy had to be stopped. Efalizumab was started at 0.7 mg/kg and thereafter continued at 1 mg/kg/week. At the time when the patient's psoriasis improved (6–8 weeks after beginning with efalizumab) his vitiligo also showed some improvement, especially on his trunk and lower extremities. Later, the patient received systemic methyprednisolone followed by methotrexate upon which his vitiligo continued to improve.

These two reports suggested some therapeutic effect of efalizumab even in long standing vitiligo vulgaris, but the product has been withdawn because of the risk of progressive multifocal encephalopathy by reactivation of JC virus infection.

Multicytokine Blockade: JAK Inhibitors

The Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway is a ubiquitous intracellular signaling network. Janus kinases (named after the two-faced Roman god) are a particular group of tyrosine kinases that associate with cytokine receptors. When cytokines bind to their receptor, JAKs (denoted as JAK1, JAK2, Tyk2 etc.) associated with different receptor units cross-phosphorylate each other on tyrosine residues. The receptors are then phosphorylated by JAKs, creating phosphotyrosine-docking sites for SH2-containing proteins, in this case a specific group of proteins called STATs. Activation of each STAT depends on the type of cytokine. In most cases, cytokine receptors are composed of multiple subunits, each of which binds to one of the four JAK family members. The major TH1 cytokine IFNγ uses JAK1 and JAK2, whereas the major TH17 cytokine IL-23 activates JAK2 and Tyk2, and the major TH2 cytokines IL4 and IL13 activate JAK1 and 3.

Selective JAK-inhibitors have anti-inflammatory properties and have been approved in several countries for the treatment of rheumatoid arthritis (tofacitinib, baricitinib) and myelofibrosis or polycythemia vera (ruxolitinib). Currently both topical [145] and systemic administration of JAK inhibitors is under investigation following very promising preliminary observations [146, 147].

35.2.2.4 Concluding Remarks

Studies with classic immunosuppressants, such as methotrexate and azathioprine, are limited. No study has been reported with mycophenolate mofetil. As in many reported vitiligo intervention, a better differentiation of interest on stopping disease progression vs. promoting repigmentation would be useful. Combination therapies with UV need to be studied for the second type of outcome.

For biologics, neutralization of IFN-γ, TNF-α and LFA-1, limited data regarding efficacy have been reported. Antibodies against IFN-γ are not yet routinely available whereas off-label use of TNF-α antibodies in vitiligo is possible. However, TNF-α antibodies should be considered with great caution. First, they may trigger de novo development of vitiligo. The data on the use of anti-TNF-α antibodies in pre-existing vitiligo are controversial regarding the efficacy of these agents with no or only a limited repigmentary response at best, but combined treatment with UV needs further investigation. Recent evidence that vitiligo infiltrating memory T cells produce both IFN-γ and TNF-α would suggest a dual blockade to be helpful [148]. The promising current development of JAK inhibitors may fill the gap. Among other therapeutic possibilities, the apparent beneficial use of efalizumab, no longer available, suggests that blocking T cell entry in the skin or derived strategies may help. Local administration of biologics or targeted drugs such as JAK inhibitors could become an alternative option in selected vitiligo patients in future.

35.3 Other Systemic Therapies

Several alternative treatments using supplementation with antioxidants and vitamins have been proposed [149].

35.3.1 Vitamin Supplementation

Vitamin B12 and folic acid have been found to be decreased in sera of vitiligo patients, even in absence of specific clinical manifestations [150, 151], but the initial mechanism accounting for the reduction has been poorly evaluated. Folic acid and vitamin B12 derivatives interact in the one-carbon cycle and the folate form *N-N*-methylene tetrahydrofolate gives the methyl group to homocysteine producing, in a vitamin B12-dependent manner, methionine. This enzymatic reaction determines the level of homocysteine, and the depigmentation may be due to the deficient methionine synthesis and homocysteine build-up. Taking into account that homocystinuria can cause pigmentary dilution, characterized by fair skin and hair [152], and that the alteration of the homocysteine metabolism may be related to the catalase polymorphism, is interesting to point out that in vitiligo patients the serum level of homocysteine has been found increased and positively correlated with the activity of the disease [152]. The pteridine in folic acid might refund the pteridine deficiency, which inhibits the melanogenesis by lowering tyrosine; indeed, the pteridine from folic acid may stop the altered recycling of the reduced pterines (6BH4 and 7BH4) proved in vitiligo epidermis. Finally, the addition of vitamin B12 and folic acid may support the UV-induced stimulation of melanocyte stem cells, through a mechanism independent on the serum level of folic acid [150]. An open study indicated that vitamin-induced repigmentation occurs mainly in patients with vitiligo more recent than 10 years, independently on the activity of the disease [150].

The combination folic acid and vitamin B12 was investigated first with promising results in 1992 in the USA in an open study in association with vitamin C [151]. Another trial was conducted in Sweden, involving 100 patients treated with folic acid (5 mg) and vitamin B12 (1 mg) for 3 months and advised to expose their skin to sun. Repigmentation was observed in nearly half 52 the treated patients [153].

Low vitamin D levels have been associated with several autoimmune diseases. Although no convincing evidence has been published of a vitamin D deficiency in vitiligo, the topical rhododendrol induced vitiligo observed epidemically in Japan responded to oral vitamin D in association with phototherapy [154].

It is known that *para-aminobenzoic acid* (PABA) induces hair and skin darkening, not specifically in vitiligo but in patients treated for different diseases. The trial conducted in vitiligo patients indicated that 1000 mg/day, together with vitamin C and B12 and folic acid, enhanced the pigmentation in 12 out of 20 enrolled patients [151]. However, this study was limited by the low number of enrolled subjects as well as by the inconsistent outcome measures.

35.3.2 L-Phenylalanine

Four trials evaluated the effectiveness of L-phenylalanine (50–100 mg/kg up to 18 months) even as supporting therapy of UVA or UVB phototherapy, or of other approaches. All studies reported beneficial effects (30–90% of repigmentation in 25–60% of the patients) even if they show high patients dropout or inconsistent outcome measures [153, 155, 156].

35.3.3 Systemic Antioxidants

Vitamin E. A clinical trial evaluated the effects of vitamin E on the recovery of skin lipid peroxidation induced by PUVA treatment in a group of 30 patients [86]. The study reported 75% of repigmentation in 60% of the patients treated with PUVA and vitamin E whereas the same percentage of repigmentation was obtained in 40% out

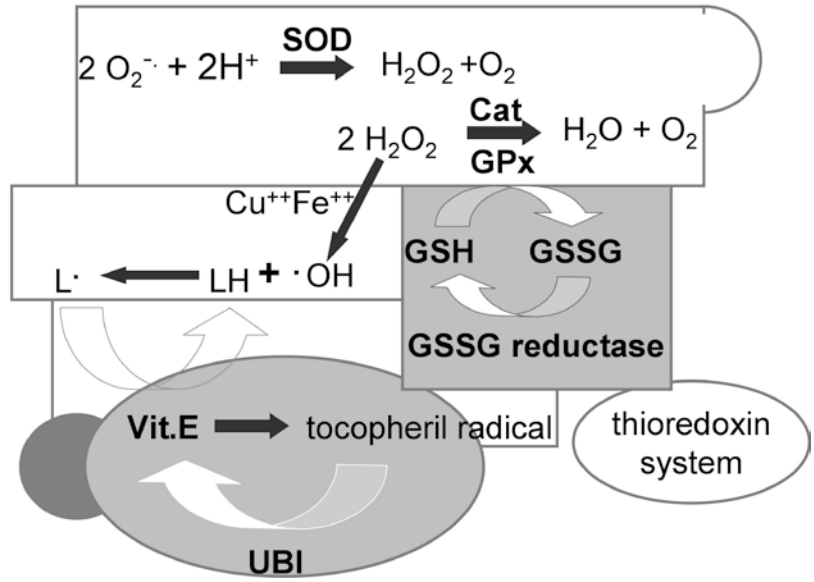


Fig. 35.7 A semplified schematic representation of the enzymatic and non-enzymatic antioxidant network

of 15 subjects exposed to PUVA only. The clinical result was associated with a decreased level of lipoperoxidation products.

Balanced pool. Taking into account the mechanisms underlying the correct synthesis and recycling of the antioxidant network, the administration of a balanced pool of antioxidants and molecules involved in their recycling has been proposed [157]. According to that, a pool of α-lipoic acid, vitamin E and C, has been evaluated in a double-blind placebo controlled trial, enrolling 35 patients, in order to test its role in reducing the UV dosage and in improving the rate of repigmentation [157]. The oral intake of the balanced pool promoted the effectiveness of the NB-UVB phototherapy, increasing the extent of repigmentation and lowering the UV dosage. At the same time the activity of the catalase was partially restored and the intracellular level of ROS decreased. Excellent and good repigmentation was reported in 40% and 20% of antioxidant-treated patients respectively (versus 22% and 10% respectively in patients exposed to NB-UV alone). Clinical data have been also confirmed by digital photography performed at the beginning and at two different subsequent time points of the study (Fig. 35.7).

Polypodium leucotomos. A further approach has been performed with *Polypodium leucotomos* extract, a fern native to the tropical and subtropical regions of the Americas, with history of use as a folk remedy in Honduras. The presence of phenolic derivatives, accounts for the antioxidant and immunomodulatory activities [150, 158]. *Polypodium leucotomos*, administrated both locally and systemically, quenches free radicals and counteracts the lipoperoxidation. In addition, a shift of the lymphocytes with the Th1 phenotype versus the Th2 one, and lower serum levels of TNFα and IFNγ were observed. Two double-blinded placebo controlled trials have suggested its effectiveness. The association with PUVA increases the percentage of patients with more than 50% of repigmentation [152]. A clear trend of repigmentation of neck and head in patients treated with *Polypodium leucotomos* during NB-UVB phototherapy (44% versus 27% out of the 50 enrolled patients) has been described in the second study [159], supported by digital photography performed at the beginning and at two different subsequent time points of the study.

The oral intake of antioxidants do not interfere with other drugs or other diseases (such as thyroiditis, diabetes, hypertension possibly occurring in vitiligo patients). Even if long-term

follow-up studies have not been still published, the oral intake of antioxidants is not associated with the occurrence of side effects, allowing thus their repeated and prolonged administration. However, recent data have raised some concerns for cancer prevention, following very long time of treatment with antioxidants in normal individuals [160]. Even if a protective effect has been reported in subjects with lower antioxidant levels in the blood, no definitive recommendations on their intake can be made at this point.

35.3.4 Afamelanotide

Afamelanotide is a potent and longer-lasting synthetic analogue of naturally occurring α-MSH known to induce tanning of the skin. Several studies have demonstrated defects in the melanocortin system in patients with vitiligo, including decreased circulating and lesional skin levels of α-MSH.

To evaluate the efficacy and safety of combination therapy for generalized vitiligo consisting of afamelanotide implant and NB-UV-B phototherapy, stable or slowly progressive vitiligo patients were randomized to combination therapy (n = 28) vs. NB-UV-B monotherapy (n = 27). After 1 month of NB-UV-B phototherapy, 16 mg of afamelanotide was administered subcutaneously to the combination therapy group monthly for 4 months while NB-UV-B phototherapy continued; the other group continued to receive NB-UV-B monotherapy. The study showed a response in the combination therapy group which was superior to that in the NB-UV-B monotherapy group at day 56. For the face and upper extremities, a significantly higher percentage of patients in the combination therapy group achieved repigmentation, and at earlier times.

Notable adverse events included erythema in both groups and minor infections and nausea in the combination therapy group. Patients with phototypes IV to VI in the combination therapy group had improvement in the Vitiligo Area Scoring Index at days 56 and 84 but no significant difference was noted in patients with SPT III [161]. Despite these encouraging results, the drug has not been developed further for vitiligo.

References

1. Schake H, Rehwinkel H, Asadullah K, Cato AC. Insight into the molecular mechanisms of glucocorticoid receptor action promotes identification of novel ligands with an improved therapeutic index. Exp Dermatol. 2006;15:565–73.
2. Clayton R. A double-blind trial of 0.05% clobetasol propionate in the treatment of vitiligo. Br J Dermatol. 1977;96:71–3.
3. Kandil E. Treatment of vitiligo with 0.1% betamethasone 17-valerate in isopropyl alcohol-a double-blind trial. Br J Dermatol. 1974;91:457–60.
4. Westerhof W, Nieuweboer-Krobotova L, Mulder PGH, et al. Left-Right comparison study of the combination of fluticasone propionate and UV-A vs either fluticasone propionate or UV-A alone for the long-term treatment of vitiligo. Arch Dermatol. 1999;135:1061–6.
5. Njoo MD, Spuls PI, Bos JD, et al. Nonsurgical repigmentation therapies in vitiligo. Meta-analysis of the literature. Arch Dermatol. 1998;134:1532–40.
6. Bleehen SS. The treatment of vitiligo with topical steroids. Light and electronmicroscopic studies. Br J Dermatol. 1976;94(S12):43–50.
7. Koopmans-van Dorp B, Goedhart-van Dijk B, Neering H, et al. Treatment of vitiligo by local application of betamethasone 17-valerate in a dimethyl sulfoxide cream base. Dermatologica. 1973;146:310–4.
8. Kumari J. Vitiligo treated with topical clobetasol propionate. Arch Dermatol. 1984;120:631–5.
9. Schaffer JV, Bolognia JL. The treatment of hypopigmentation in children. Clin Dermatol. 2003;21:296–310.
10. Khalid M, Mujtaba G, Haroon TS. Comparison of 0.05% clobetasol propionate cream and topical Puvasol in childhood vitiligo. Int J Dermatol. 1995;34:203–5.
11. Coskun B, Saral Y, Turgut D. Topical 0.05% clobetasol propionate versus 1% pimecrolimus ointment in vitiligo. Eur J Dermatol. 2005;15:88–91.
12. Lepe V, Moncada B, Castanedo-Cazares JP, et al. A double-blind randomized trial of 0.1% tacrolimus vs 0.05% clobetasol for the treatment of childhood vitiligo. Arch Dermatol. 2003;139:581–5.
13. Kumaran MS, Kaur I, Kumar B. Effect of topical calcipotriol, betamethasone dipropionate and their combination in the treatment of localized vitiligo. J Eur Acad Dermatol Venereol. 2006;20:269–73.
14. Bayoumi W, Fontas E, Sillard L, Le Duff F, Ortonne JP, Bahadoran P, Lacour JP, Passeron T. Effect of a preceding laser dermabrasion on the outcome of combined therapy with narrowband ultravio-

let B and potent topical steroids for treating non-segmental vitiligo in resistant localizations. Br J Dermatol. 2012;166(1):208–11. https://doi.org/10.1111/j.1365-2133.2011.10564.x.
15. Lee L, Wu Y, Li L, Sun Y, Qiu L, Gao XH, Chen HD. Triple combination treatment with fractional CO_2 laser plus topical betamethasone solution and narrowband ultraviolet B for refractory vitiligo: a prospective, randomized half-body, comparative study. Dermatol Ther. 2015;28:131–4.
16. Geraldez CB, Gutierrez GT. A clinical trial of clobetasol propionate in Filipino vitiligo patients. Clin Ther. 1987;9:474–982.
17. Kwinter J, Pelletier J, Khambalia A, et al. High-potency steroid use in children with vitiligo: a retrospective study. J Am Acad Dermatol. 2007;56:236–41.
18. Kwon HB, Choi Y, Kim HJ, Lee AY. The therapeutic effects of a topical tretinoin and corticosteroid combination for vitiligo: a placebo-controlled, paired-comparison, left-right study. J Drugs Dermatol. 2013;12:e63–7.
19. Fleischer AB, Ling M, Eichenfield L, et al. Tacrolimus ointment for the treatment of atopic dermatitis is not associated with an increase in cutaneous infections. J Am Acad Dermatol. 2002;47:562–70.
20. Kostovic K, Pasic A. New treatment modalities for vitiligo: focus on topical immunomodulators. Drugs. 2005;65:447–59.
21. Qureshi AA, Fischer MA. Topical calcineurin inhibitors for atopic dermatitis: balancing clinical benefit and possible risks. Arch Dermatol. 2006;142:633–7.
22. Silverberg NB, Lin P, Travis L, et al. Tacrolimus ointment promotes repigmentation of vitiligo in children: a review of 57 cases. J Am Acad Dermatol. 2004;51:760–6.
23. Soter NA, Fleischer AB, Webster GF, et al. Tacrolimus ointment for the treatment of atopic dermatitis in adult patients: part II, safety. J Am Acad Dermatol. 2001;44:S39–46.
24. Wolff K. Pimecrolimus 1% cream for the treatment of atopic dermatitis. Skin Therapy Lett. 2005;10:1–6.
25. Mayoral FA, Vega JM, Stavisky H, et al. Retrospective analysis of pimecrolimus cream 1% for treatment of facial vitiligo. J Drugs Dermatol. 2007;6:517–21.
26. Souza Leite RM, Craveiro Leite AA. Two therapeutic challenges: periocular and genital vitiligo in children successfully treated with pimecrolimus cream. Int J Dermatol. 2007;46:986–9.
27. Allen A, Siegfried E, Silverman R, et al. Significant absorption of topical tacrolimus in 3 patients with Netherton syndrome. Arch Dermatol. 2001;137:747–50.
28. Grimes PE, Morris R, Avaniss-Aghajani E, et al. Topical tacrolimus therapy for vitiligo: therapeutic responses and skin messenger RNA expression of proinflammatory cytokines. J Am Acad Dermatol. 2004;51:52–61.
29. Lan CC, Chen GS, Chiou MH, et al. FK506 promotes melanocyte and melanoblast growth and creates a favourable milieu for cell migration via keratinocytes: possible mechanisms of how tacrolimus ointment induces repigmentation in patients with vitiligo. Br J Dermatol. 2005;153:498–505.
30. Kang HY, Choi YM. FK506 increases pigmentation and migration of human melanocytes. Br J Dermatol. 2006;155:1037–40.
31. Sardana K, Bhushan P, Kumar Garg V. Effect of tacrolimus on vitiligo in absence of UV radiation exposure. Arch Dermatol. 2007;143:119–20.
32. Ormerod AD. Topical tacrolimus and pimecrolimus and the risk of cancer: how much cause for concern? Br J Dermatol. 2005;153:701–5.
33. Almeida P, Borrego L, Rodriguez-Lopez J, et al. [Vitiligo. Treatment of 12 cases with topical tacrolimus]. Actas Dermosifiliogr. 2005;96:159–163.
34. Ho N, Pope E, Weinstein M, Greenberg S, Webster C, Krafchik BR. A double-blind, randomized, placebo-controlled trial of topical tacrolimus 0.1% vs. clobetasol propionate 0.05% in childhood vitiligo. Br J Dermatol. 2011;165:626–32.
35. Gan EY, Taïeb A. Unwanted lentigines after topical tacrolimus for vitiligo. Australas J Dermatol. 2017;58(4):e259–60.
36. Sendur N, Karaman G, Sanic N, Savk E. Topical pimecrolimus: a new horizon for vitiligo treatment? J Dermatolog Treat. 2006;17:338–42.
37. Radakovic S, Breier-Maly J, Konschitzky R, Kittler H, Sator P, Hoenigsmann H, Tanew A. Response of vitiligo to once- vs. twice-daily topical tacrolimus: a controlled prospective, randomized, observer-blinded trial. J Eur Acad Dermatol Venereol. 2009;23:951–3.
38. Boone B, Ongenae K, Van Geel N, et al. Topical pimecrolimus in the treatment of vitiligo. Eur J Dermatol. 2007;17:55–61.
39. Seirafi H, Farnaghi F, Firooz A, et al. Pimecrolimus cream in repigmentation of vitiligo. Dermatology. 2007;214:253–9.
40. Castanedo-Cazares JP, Lepe V, Moncada B. Repigmentation of chronic vitiligo lesions by following tacrolimus plus ultraviolet-B-narrow-band. Photodermatol Photoimmunol Photomed. 2003;19:35–6.
41. Ostovari N, Passeron T, Lacour JP, Ortonne JP. Lack of efficacy of tacrolimus in the treatment of vitiligo in the absence of UV-B exposure. Arch Dermatol. 2006;142:252–3.
42. Passeron T, Ostovari N, Zakaria W, et al. Topical tacrolimus and the 308-nm excimer laser: a synergistic combination for the treatment of vitiligo. Arch Dermatol. 2004;140:1065–9.
43. Dawid M, Veensalu M, Grassberger M, Wolff K. Efficacy and safety of pimecrolimus cream 1% in adult patients with vitiligo: results of a randomized, double-blind, vehicle-controlled study. J Dtsch Dermatol Ges. 2006;4:942–6.
44. Fai D, Cassano N, Vena GA. Narrow-band UVB phototherapy combined with tacrolimus ointment in vitiligo: a review of 110 patients. J Eur Acad Dermatol Venereol. 2007;21:916–20.

45. Luger T, Boguniewicz M, Carr W, Cork M, Deleuran M, Eichenfield L, Eigenmann P, Fölster-Holst R, Gelmetti C, Gollnick H, Hamelmann E, Hebert AA, Muraro A, Oranje AP, Paller AS, Paul C, Puig L, Ring J, Siegfried E, Spergel JM, Stingl G, Taieb A, Torrelo A, Werfel T, Wahn U. Pimecrolimus in atopic dermatitis: consensus on safety and the need to allow use in infants. Pediatr Allergy Immunol. 2015;26:306–15.
46. Mayoral FA, Gonzalez C, Shah NS, Arciniegas C. Repigmentation of vitiligo with pimecrolimus cream: a case report. Dermatology. 2003;207:322–3.
47. Cavalié M, Ezzedine K, Fontas E, Montaudié H, Castela E, Bahadoran P, Taïeb A, Lacour JP, Passeron T. Maintenance therapy of adult vitiligo with 0.1% tacrolimus ointment: a randomized, double blind, placebo-controlled study. J Invest Dermatol. 2015;135:970–4.
48. Grimes PE, Soriano T, Dytoc MT. Topical tacrolimus for repigmentation of vitiligo. J Am Acad Dermatol. 2002;47:789–91.
49. Kalek AZ, Spencer JM, Phelps RG. Combined excimer laser and topical tacrolimus for the treatment of vitiligo: a pilot study. Dermatol Surg. 2004;30:130–5.
50. Mehrabi D, Pandya AG. A randomized, placebo-controlled, double-blind trial comparing narrowband UV-B Plus 0.1% tacrolimus ointment with narrowband UV-B plus placebo in the treatment of generalized vitiligo. Arch Dermatol. 2006;142:927–9.
51. Kanwar AJ, Dogra S, Parsad D. Topical tacrolimus for treatment of childhood vitiligo in Asians. Clin Exp Dermatol. 2004;29:589–92.
52. Van Geel N, Speeckaert R, Mollet I, De Schepper S, De Wolf J, Tjin EP, Luiten RM, Lambert J, Brochez L. In vivo vitiligo induction and therapy model: double-blind, randomized clinical trial. Pigment Cell Melanoma Res. 2012;25:57–65.
53. Hartmann A, Brocker EB, Hamm H. Occlusive treatment enhances efficacy of tacrolimus 0.1% ointment in adult patients with vitiligo: results of a placebo-controlled 12 month prospective study. Acta Derm Venereol. 2008;88:474–9.
54. Bakos L, Bakos RM. Focal acne during topical tacrolimus therapy for vitiligo. Arch Dermatol. 2007;143:1223–4.
55. DDe D, Kanwar AJ. Tacrolimus-induced hyperpigmentation in a patch of vitiligo. Skinmed. 2008;7:93–4.
56. Birlea SA, Costin GE, Norris DA. Cellular and molecular mechanisms involved in the action of vitamin D analogs targeting vitiligo depigmentation. Curr Drug Targets. 2008;9:345–59.
57. Guilhou JJ. The therapeutic effects of vitamin D3 and its analogues in psoriasis. Expert Opin Investig Drugs. 1998;7:77–84.
58. Ortonne JP, Kaufmann R, Lecha M, Goodfield M. Efficacy of treatment with calcipotriol/betamethasone dipropionate followed by calcipotriol alone compared with tacalcitol for the treatment of psoriasis vulgaris: a randomised, double-blind trial. Dermatology. 2004;209:308–13.
59. Takahashi H, Ibe M, Kinouchi M, et al. Similarly potent action of 1,25-dihydroxyvitamin D3 and its analogues, tacalcitol, calcipotriol, and maxacalcitol on normal human keratinocyte proliferation and differentiation. J Dermatol Sci. 2003;31:21–8.
60. Watabe H, Soma Y, Kawa Y, et al. Differentiation of murine melanocyte precursore induced by 1,25 dihydroxyvitamin D3 is associated with the stimulation of endothelin B receptor expression. J Invest Dermatol. 2002;119:583–9.
61. Gawkrodger DJ, Ormerod AD, Shaw L, et al. Guideline for the diagnosis and management of vitiligo. Br J Dermatol. 2008;159:1051–76.
62. Lotti T, Buggiani G, Troiano M, et al. Targeted and combination treatments for vitiligo. Comparative evaluation of different current modalities in 458 subjects. Dermatol Ther. 2008;21(1):s20–6.
63. Parsad D, Saini R, Nagpal R. Calcipotriol in vitiligo: a preliminary study. Pediatr Dermatol. 1999;16:317–20.
64. Cherif F, Azaiz MI, Ben Hamida A, et al. Calcipotriol and PUVA as treatment for vitiligo. Dermatol Online J. 2003;9:4.
65. Ermis O, Alpsoy E, Cetin L, Yilmaz E. Is the efficacy of psoralen plus ultraviolet A therapy for vitiligo enhanced by concurrent topical calcipotriol? A placebo-controlled double-blind study. Br J Dermatol. 2001;145:472–5.
66. Katayama I, Ashida M, Maeda A, et al. Open trial of topical tacalcitol [1alpha24(OH)2D3] and solar irradiation for vitiligo vulgaris: upregulation of cKit mRNA by cultured melanocytes. Eur J Dermatol. 2003;13:372–6.
67. Baysal V, Vildirim M, Erel A, Kesici D. Is the combination of calcipotriol and PUVA effective in vitiligo? J Eur Acad Dermatol Venereol. 2003;17:299–302.
68. Goktas EO, Aydin F, Senturk N, et al. Combination of narrow band UVB and topical calcipotriol for the treatment of vitiligo. J Eur Acad Dermatol Venereol. 2006;20:553–7.
69. Kullavanijaya P, Lim HW. Topical calcipotriene and narrowband ultraviolet B in the treatment of vitiligo. Photodermatol Photoimmunol Photomed. 2004;20:248–51.
70. Leone G, Pacifico A, Iacovelli P, et al. Tacalcitol and narrow-band phototherapy in patients with vitiligo. Clin Exp Dermatol. 2006;31:200–5.
71. Ada S, Sahin S, Boztepe G, et al. No additional effect of topical calcipotriol on narrow-band UVB phototherapy in patients with generalized vitiligo. Photodermatol Photoimmunol Photomed. 2005;21:79–83.
72. Lu-yan T, Wen-wen F, Lei-hong X, et al. Topical tacalcitol and 308-nm monochromatic excimer light: a synergistic combination for the treatment of vitiligo. Photodermatol Photoimmunol Photomed. 2006;22:310–4.
73. Goldinger SM, Dummer R, Schmid P, et al. Combination of 308-nm xenon chloride excimer laser and topical calcipotriol in vitiligo. J Eur Acad Dermatol Venereol. 2007;21:504–8.

74. Hartmann A, Lurz C, Hamm H, et al. Narrow-band UVB311 nm vs. broad-band UVB therapy in combination with topical calcipotriol vs. placebo in vitiligo. Int J Dermatol. 2005;44:736–42.
75. Rodriguez-Martìn M, Garcia Bustinduy M, Saez Rodriguez M, Noda Cabrera A. Randomized, double-blind clinical trial to evaluate the efficacy of topical tacalcitol and sunlight exposure in the treatment of adult nonsegmental vitiligo. Br J Dermatol. 2009;160:409–14.
76. Khullar G, Kanwar AJ, Singh S, Parsad D. Comparison of efficacy and safety profile of topical calcipotriol ointment in combination with NB-UVB vs. NB-UVB alone in the treatment of vitiligo: a 24-week prospective right-left comparative clinical trial. J Eur Acad Dermatol Venereol. 2015;29(5):925–32.
77. Sahu P, Jain VK, Aggarwal K, Kaur S, Dayal S. Tacalcitol: a useful adjunct to narrow band ultraviolet-B phototherapy in vitiligo. Photodermatol Photoimmunol Photomed. 2016. https://doi.org/10.1111/phpp.12265.
78. Amano H, Abe M, Ishikawa O. First case report of topical tacalcitol for vitiligo repigmentation. Pediatr Dermatol. 2008;25:262–4.
79. Gargoom AM, Duweb GA, Elzorghany AH, et al. Calcipotriol in the treatment of childood vitiligo. Int J Clin Pharmacol Res. 2004;24:11–4.
80. Kumaran MS, Kaur I, Kumar B. Effect of topical calcipotriol, betamethasone dipropionate and their combinattion in the treatment of localized vitiligo. J Eur Acad Dermatol Venereol. 2006;20:269–73.
81. Travis LB, Silverberg NB. Calcipotriene and corticosteroids combination therapy for vitiligo. Pediatr Dermatol. 2004;21(4):495–8.
82. Leone G, Pacifico A. Profile of clinical efficacy and safety of topical tacalcitol. Acta Biomed. 2005;76:13–9.
83. Ameen M, Exarchou V, Chu AC. Topical calcipotriol as monotherapy and in combination with psoralen plus ultraviolet A in the treatment of vitiligo. Br J Dermatol. 2001;145:476–9.
84. Arca E, Tastan HB, Erbil AH, et al. Narrow-band ultraviolet B as monotherapy and in combination with topical calcipotriol in the treatment of vitiligo. J Dermatol. 2006;33:338–43.
85. Chiaverini C, Passeron T, Ortonne JP. Treatment of vitiligo by topical calcipotriol. J Eur Acad Dermatol Venereol. 2002;16:137–8.
86. Akyol M, Celik VK, Ozcelik S, et al. The effects of vitamin E on the skin lipid peroxidation and the clinical improvement in vitiligo patients treated with PUVA. Eur J Dermatol. 2002;12:24–6.
87. Dell'Anna ML, Mastrofrancesco A, Sala R, et al. Anrtioxidants and narrow band-UVB in the treatment of vitiligo: a double-blind placebo controlled trial. Clin Exp Dermatol. 2007;32:631–6.
88. Schallreuter KU, Wood JM, Lemke KR, et al. Treatment of vitiligo with a topical application of pseudocatalase and calcium in combination with short-term UVB exposure: a case study on 33 patients. Dermatology. 1995;190:223–9.
89. Dell'Anna ML, Ottaviani M, Kovacs D, et al. Energetic mitochondrial failing in vitiligo and possible rescue by cardiolipin. Sci Rep. 2017;7(1):13663.
90. Naini FF, Shooshtari AV, Ebrahimi B, Molaei R. The effect of pseudocatalase/superoxide dismutase in the treatment of vitiligo: a pilot study. J Res Pharm Pract. 2012;1:77–80.
91. Schallreuter KU, Moore J, Behrens-Williams S, et al. Rapid initiation of repigmentation in vitiligo with dead sea climatotherapy in combination with pseudocatalase (PC-KUS). Int J Dermatol. 2002;41:482–7.
92. Gibbons NCJ, Wood JM, Rokos H, et al. Computer simulation of native epidermal enzyme structure in the presence and absence of hydrogen peroxide (H_2O_2): potential and pitfalls. J Invest Dermatol. 2006;126:2576–82.
93. Bakis-Petsoglou S, Le Guay JL, Wittal R. A randomized, double-blinded, placebo-controlled trial of pseudocatalase cream and narrowband ultraviolet B in the treatment of vitiligo. Br J Dermatol. 2009;161:910–7.
94. Patel DC, Evans AV, Hawk JL. Topical pseudocatalase mousse and narrowband UVB phototherapy is not effective for vitiligo: an open single-center study. Clin Exp Dermatol. 2002;27:641–4.
95. Schallreuter KU, Kruger C, Wurfel C, et al. From basic research to the bedside: efficacy of topical treatment with psudocatalase PC-KUS in 71 children with vitiligo. Int J Dermatol. 2008;47:743–53.
96. Vouldoukis I, Lacan D, Kamate C, et al. Antioxidant and antinflammatory properties of a Cucumis melo LC. extract rich in superoxido dismutase activity. J Ethnopharmacol. 2004;94:67–75.
97. Khemis A, Ortonne JP. Comparative study of a vegetable extract with superoxide dismutase and catalase (Vitix®) plus selective UVB phototherapy versus excipient plus selective UVB phototherapy in the treatment of vitiligo vulgaris. Les Nouv Dermatol. 2004;23:45–6.
98. Schallreuter KU, Rokos H. Vitix®- a new treatment for vitiligo? Int J Dermatol. 2005;44:969–70.
99. Yuksel EP, Aydin F, Senturk N, Canturk T, Turanli AY. Comparison of the efficacy of narrow band ultraviolet B and narrow band ultraviolet B plus topical catalase-superoxide dismutase treatment in vitiligo patients. Eur J Dermatol. 2009;19:341–4.
100. Farah FS, Kurban AK, Chaglassian HT. The treatment of vitiligo with psoralens and triamcinolone by mouth. Br J Dermatol. 1967;79:89–91.
101. Pasricha JS, Seetharam KA, Dashore A. Evaluation of five different regimes for the treatment of vitiligo. Indian J Dermatol Venereol Leprol. 1989;55:18–21.
102. Pasricha JS. Pulse therapy as a cure for autoimmune diseases. Indian J Dermatol Venereol Leprol. 2003;69:323–8.
103. Kountz SL, Cohn R. Initial treatment of renal allografts with large intrarenal doses of immunosuppressive drugs. Lancet. 1969;1:338–40.

104. Pasricha JS, Kumrah L. Alopecia totalis treated with oral mini-pulse (OMP) therapy with betamethasone. Indian J Dermatol Venereol Leprol. 1996;62:106–9.
105. Joshi A, Khaitan BK, Verma KK, Singh MK. Generalized and bullous lichen planus treated successfully with oral mini-pulse therapy. Indian J Dermatol Venereol Leprol. 1999;65:303–4.
106. Mittal R, Khaitan BK, Sirka CS. Trachyonychia treated with oral minipulse therapy. Indian J Dermatol Venereol Leprol. 2001;67:202–3.
107. Verma K, Verma KK. Infantile periocular haemangioma treated with two days in a week betamethasone oral mini pulse therapy. Indian J Pediatr. 2001;68:355–6.
108. Pasricha JS, Khaitan BK. Oral mini-pulse therapy with betamethasone in vitiligo patients having extensive or fast-spreading disease. Int J Dermatol. 1993;32:753–7.
109. Kanwar AJ, Dhar S, Dawn G. Oral minipulse therapy in vitiligo. Dermatology. 1995;190:251–2.
110. Radakovic- Fijan S, Firnsinn-Friedl AM, Honigsmann H, et al. Oral dexamethasone pulse treatment for vitiligo. J Am Acad Dermatol. 2001;44:814–7.
111. Kanwar AJ, Mahajan R, Parsad D. Low-dose oral mini-pulse dexamethasone therapy in progressive unstable vitiligo. J Cutan Med Surg. 2013;17:259–68.
112. Singh A, Kanwar AJ, Parsad D, Mahajan R. Randomized controlled study to evaluate the effectiveness of dexamethasone oral minipulse therapy versus oral minocycline in patients with active vitiligo vulgaris. Indian J Dermatol Venereol Leprol. 2014;80:29–35.
113. El Mofty M, Essmat S, Youssef R, Sobeih S, Mahgoub D, Ossama S, Saad A, El Tawdy A, Mashaly HM, Saney I, Helal R, Shaker O. The role of systemic steroids and phototherapy in the treatment of stable vitiligo: a randomized controlled trial. Dermatol Ther. 2016. https://doi.org/10.1111/dth.12384.
114. Cara CJ, Pena AS, Sans M, et al. Reviewing the mechanism of action of thiopurine drugs: towards a new paradigm in clinical practice. Med Sci Monit. 2004;10:247–54.
115. Gokhale BB. Cyclophosphamide and vitiligo. Int J Dermatol. 1979;18:92.
116. Gokhale BB, Parakh AP. Cyclophosphamide in vitiligo. Indian J Dermatol. 1983;28:7–10.
117. Radmanesh M, Saedi K. The efficacy of combined PUVA and low-dose azathioprine for early and enhanced repigmentation in vitiligo patients. J Dermatolog Treat. 2006;17:151–3.
118. Mahmoud BH, Hexsel CL, Hamzavi IH. An update on new and emerging options for the treatment of vitiligo. Skin Therapy Lett. 2008;13:1–6.
119. Singh H, Kumaran MS, Bains A, Parsad D. A randomized comparative study of oral corticosteroid minipulse and low-dose oral methotrexate in the treatment of unstable vitiligo. Dermatology. 2015;231:286–90.
120. Skurkovich SV, Klinova EG, Eremkina EI, Levina NV. Immunosuppressive effect of an anti-interferon serum. Nature. 1974;247:551–2.
121. Skurkovich S, Skurkovich B, Bellanti JA. A unifying model of the immunoregulatory role of the interferon system: can interferon produce disease in humans? Clin Immunol Immunopathol. 1987;43:362–73.
122. Skurkovich S, Skurkuvich B (2006) Inhibition of IFN-γ as a method of treatment of various autoimmune diseases, including skin disease. In: Numerof R, Dinarello CA, Asadullah K (edts.), Cytokines as potential therapeutic targets for inflammatory skin diseases. Ernst Schering Research Foundation Workshop 58, Springer, Berlin, 1-27.
123. Anbar TS, Abdel-Rahman AT, Ahmad HM. Vitiligo occurring at site of interferon-alpha 2b injection in a patient with chronic viral hepatitis C: a case report. Clin Exp Dermatol. 2007;33:503.
124. Bernstein D, Reddy KR, Jeffers L, Schiff E. Canities and vitiligo complicating interferon therapy for hepatitis C. Am J Gastroenterol. 1995;90:1176–7.
125. Simsek H, Savas C, Akkiz H, Telatar H. Interferon-induced vitiligo in a patient with chronic viral hepatitis C infection. Dermatology. 1996;193:65–6.
126. Taffaro M, Pyrsopoulos N, Cedron H, et al. Vitiligo improvement in a hepatitis C patient after treatment with PEG-interferon alpha-2a and ribavirin: a case report. Dig Dis Sci. 2007;52:3435–7.
127. Skurkovich B, Skurkovich S. Anti-interferon-gamma antibodies in the treatment of autoimmune diseases. Curr Opin Mol Ther. 2003;5:52–7.
128. Skurkovich S, Korotky NG, Shaova NM, Skurkovich B. Successful anti-IFN therapy of alopecia, vitiligo, and psoriasis. Clin Immunol. 2002;103:S103.
129. Ramírez-Hernández M, Marras C, Martínez-Escribano JA. Infliximab-induced vitiligo. Dermatology. 2005;210:79–80.
130. Rashighi M, Agarwal P, Richmond JM, Harris TH, Dresser K, Su MW, Zhou Y, Deng A, Hunter CA, Luster AD, Harris JE. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. Sci Transl Med. 2014;6:223ra23.
131. Agarwal P, Rashighi M, Essien KI, Richmond JM, Randall L, Pazoki-Toroudi H, Hunter CA, Harris JE. Simvastatin prevents and reverses depigmentation in a mouse model of vitiligo. J Invest Dermatol. 2015;135:1080–8.
132. Vanderweil SG, Amano S, Ko WC, Richmond JM, Kelley M, Senna MM, Pearson A, Chowdary S, Hartigan C, Barton B, Harris JE. A double-blind, placebo-controlled, phase-II clinical trial to evaluate oral simvastatin as a treatment for vitiligo. J Am Acad Dermatol. 2017;76:150–1.
133. Birol A, Kisa U, Kurtipek GS, et al. Increased tumor necrosis factor alpha (TNF-alpha) and interleukin 1 alpha (IL1-alpha) levels in the lesional skin of patients with nonsegmental vitiligo. Int J Dermatol. 2006;45:992–3.

134. Moretti S, Spallanzani A, Amato L, et al. New insights into the pathogenesis of vitiligo: imbalance of epidermal cytokines at sites of lesions. Pigment Cell Res. 2002;15:87–92.
135. Smith DI, Heffernan MP. Vitiligo after the resolution of psoriatic plaques during treatment with adalimumab. J Am Acad Dermatol. 2008;58:S50–1.
136. Rigopoulos D, Gregoriou S, Larios G, et al. Etanercept in the treatment of vitiligo. Dermatology. 2007;215:84–5.
137. Swope VB, Abdel-Malek Z, Kassem LM, Nordlund JJ. Interleukins 1 alpha and 6 and tumor necrosis factor-alpha are paracrine inhibitors of human melanocyte proliferation and melanogenesis. J Invest Dermatol. 1991;96:180–5.
138. Martínez-Esparza M, Jiménez-Cervantes C, Solano F, et al. Mechanisms of melanogenesis inhibition by tumor necrosis factor-alpha in B16/F10 mouse melanoma cells. Eur J Biochem. 1998;255:139–46.
139. Alghamdi KM, Khurrum H, Taieb A, Ezzedine K. Treatment of generalized vitiligo with anti-TNF-α agents. J Drugs Dermatol. 2012;11:534–9.
140. Webb KC, Tung R, Winterfield LS, Gottlieb AB, Eby JM, Henning SW, Le Poole IC. Tumour necrosis factor-α inhibition can stabilize disease in progressive vitiligo. Br J Dermatol. 2015;173:641–50.
141. Fernandez-Obregon AC. Clinical management with efalizumab of a patient with psoriasis and comorbid vitiligo. J Drugs Dermatol. 2008;7:679–81.
142. Wakkee M, Assen YJ, Thio HB, Neumann HA. Repigmentation of vitiligo during efalizumab. J Am Acad Dermatol. 2008;59:S57–8.
143. Al Badri AM, Foulis AK, Todd PM, et al. Abnormal expression of MHC class II and ICAM-1 by melanocytes in vitiligo. J Pathol. 1993;169:203–6.
144. Abdallah M, Abdel-Naser MB, Moussa MH, et al. Sequential immunohistochemical study of depigmenting and repigmenting minigrafts in vitiligo. Eur J Dermatol. 2003;13:548–52.
145. Joshipura D, Alomran A, Zancanaro P, Rosmarin D. Treatment of vitiligo with the topical Janus kinase inhibitor ruxolitinib: a 32-week open-label extension study with optional narrow-band ultraviolet B. J Am Acad Dermatol. 2018;78:1205–7.
146. Craiglow BG, King BA. Tofacitinib citrate for the treatment of vitiligo: a pathogenesis-directed therapy. JAMA Dermatol. 2015;151:1110–2.
147. Liu LY, Strassner JP, Refat MA, Harris JE, King BA. Repigmentation in vitiligo using the Janus kinase inhibitor tofacitinib may require concomitant light exposure. J Am Acad Dermatol. 2017;77:675–82.
148. Boniface K, Jacquemin C, Darrigade AS, Dessarthe B, Martins C, Boukhedouni N, Vernisse C, Grasseau A, Thiolat D, Rambert J, Lucchese F, Bertolotti A, Ezzedine K, Taieb A, Seneschal J. Vitiligo skin is imprinted with resident memory CD8 T cells expressing CXCR3. J Invest Dermatol. 2018;138:355–64.
149. Cohen BE, Elbuluk N, Mu EW, Orlow SJ. Alternative systemic treatments for vitiligo: a review. Am J Clin Dermatol. 2015;16:463–74.
150. Juhlin L, Olsson MJ. Improvement of vitiligo after oral treatment with vitamin B12 and folic acid and the importance of sun exposure. Acta Derm Venereol. 1997;77:460–2.
151. Montes LF, Diaz ML, Lajous J, et al. Folic acid and vitamin B12 in vitiligo: a nutritional approach. Cutis. 1992;50:39–42.
152. Shaker OG, El-Tahlawi SMR. Is there a relationship between homocysteine and vitiligo? Br J Dermatol. 2008;159:720–4.
153. Tjioe M, et al. Treatment of vitiligo vulgaris with narrow band UVB (311 nm) for one year and the effect of addition of folic acid and vitamin B12. Acta Derm Venereol. 2002;82:369–72.
154. Watabe A, Yamasaki K, Asano M, Kanbayashi Y, Nasu-Tamabuchi M, Terui H, Furudate S, Kakizaki A, Tsuchiyama K, Kimura Y, Ito Y, Kikuchi K, Aiba S. Efficacy of oral cholecalciferol on rhododendrol-induced vitiligo: a blinded randomized clinical trial. J Dermatol. 2018;45:456–62.
155. Cormane RH, et al. Phenylalanine and UVA light for the treatment of vitiligo. Arch Dermatol Res. 1985;277:126–30.
156. Rojas-Urdaneta JE, Poleo-Romero AG. Evaluation of an antioxidant and mitochondria-stimulating cream formula on the skin of patients with stable common vitiligo. Investig Clin. 2007;48:21–31.
157. Colucci R, Dragoni F, Conti R, Pisaneschi L, Lazzeri L, Moretti S. Evaluation of an oral supplement containing Phyllanthus emblica fruit extracts, vitamin E, and carotenoids in vitiligo treatment. Dermatol Ther. 2015;28:17–21.
158. Parsad D, Pandhi R, Juneja A. Effectiveness of oral Gingko biloba in treating limited, slowly spreading vitiligo. Clin Exp Dermatol. 2002;28:285–7.
159. Middelkamp MA, Bos JD, Riuz-Diaz F, et al. Treatment of vitiligo vulgaris with narrow-band UVB and oral Polypodium leucotomos extract: a randomized double-blind placebo-controlled study. J Eur Acad Dermatol Venereol. 2007;21:942–50.
160. Hercberg S, Ezzedine K, Guinot C, et al. Antioxidant supplementation increases the risk of skin cancers in women but not in men. J Nutr. 2007;137:2098–105.
161. Lim HW, Grimes PE, Agbai O, Hamzavi I, Henderson M, Haddican M, Linkner RV, Lebwohl M. Afamelanotide and narrowband UV-B phototherapy for the treatment of vitiligo: a randomized multicenter trial. JAMA Dermatol. 2015;151:42–50.

Surgical Therapies

36

Boon Kee Goh

Contents

Abstract

Cellular grafting for vitiligo has evolved since its inception. Non-cultured cellular grafting has superseded cultured techniques as the preferred modus operandi in clinical practice. The reasons are straightforward: it can be completed in a few hours, does not require the use of xenobiotics (except trypsin) or culture media and is of significantly lower costs. Although it does not offer the possibility of cell expansion and cryopreservation for future transplant, this can be supplanted by harvesting a new piece ultrathin split skin graft. Under proper training, harvesting an ultrathin split skin graft (not beyond the papillary dermis) is fast and results in no or minimal scarring. Innovative methods to simplify non-cultured cellular grafting have also been successfully achieved.

Melanocytes can now be extracted not only from the epidermis for transplantation but from outer root sheath of hair follicles.

Non-cultured cellular grafting has proven to be a safe and effective surgical treatment for stable vitiligo, substantiated by various long-term studies over the last two decades. The imposition of strict legislative regulation on this technique is unwarranted and will only drive up cost and deprive patients' access to this innovative procedure.

B. K. Goh (✉)
Skin Physicians Pte Ltd, Mount Elizabeth Medical Centre, Singapore, Singapore

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_36

Key Points

- Non-cultured cellular grafting has superseded cultured techniques as the preferred modus operandi in clinical practice for stable vitiligo.
- It is not time-consuming and does not require the use of xenobiotics (except trypsin) or culture media and is of significantly lower costs.
- Under proper training, harvesting an ultrathin split skin graft (not beyond the papillary dermis) is fast and results in no or minimal scarring.
- Innovative methods (the "6-well" and "4-well" plate techniques) to simplify non-cultured cellular grafting have also been successfully achieved, including using the body as the natural incubator.
- Melanocytes can now be extracted from the outer root sheath of the hair follicles.
- The imposition of strict legislative regulation on this technique is unwarranted.

36.1 Cellular Grafting for Vitiligo

Over the last two decades, cellular grafting for vitiligo has achieved significant progress. This surgical approach is indicated for stable vitiligo refractory to medical therapy. The procedure involves harvesting epidermal cells from autologous donor skin and transplanting them (with or without prior selective cultivation) onto vitiliginous recipient sites (Fig. 36.1). Unlike tissue grafting, where the treatment area is limited by the size or number of tissue grafts, cellular grafting allows treatment of achromic skin manifold larger than the donor area. It also has the capability of repigmenting leukotrichia in vitiligo, possibly due to retrograde migration of transplanted melanocytes into the hair follicles [1, 2].

Cellular grafting can be broadly categorized into (a) cultured melanocyte or epidermal cell transplantation (cultured cellular grafting) and (b) non-cultured transplantation of epidermal cells or outer root sheath hair follicle cells (non-cultured cellular grafting) (Fig. 36.2). The key advantage of in vitro culture is that the cells can

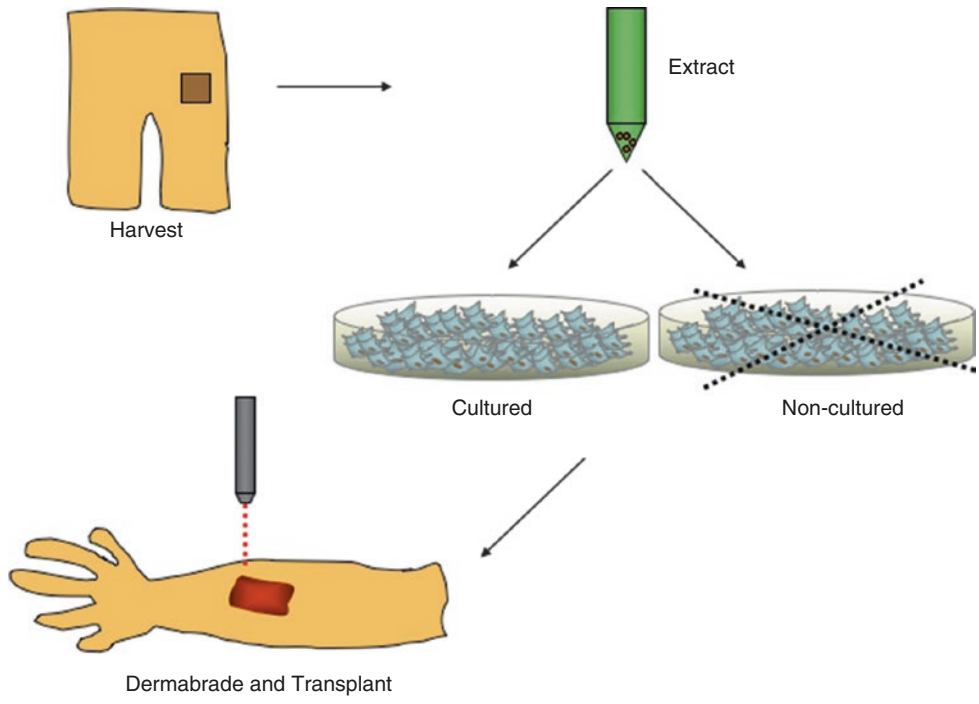


Fig. 36.1 Cellular grafting involves harvesting epidermal cells from autologous donor skin and transplanting them (with or without prior selective cultivation) onto vitiliginous recipient sites

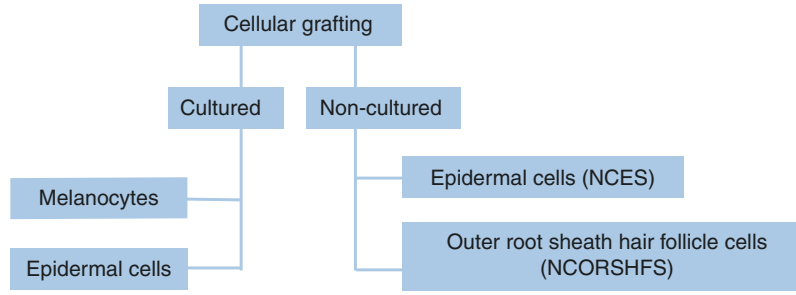


Fig. 36.2 Types of cellular grafting for vitiligo

be expanded and cryopreserved for future use. However, the legislative restriction on cultivating cells for clinical use has limited the widespread application of cultured cellular grafting.

36.2 Cultured Cellular Grafting

36.2.1 Cultured Melanocyte Transplantation

Until the 1980s, it had not been feasible to cultivate melanocytes in vitro in large quantities. An important reason was the preferential overgrowth of keratinocytes over melanocytes in culture. It was not until 1982 that a breakthrough occurred with the introduction of melanocyte mitogens. Eisinger and Marko selectively cultivated human melanocytes from neonatal foreskin and adult skin by introducing 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and cholera toxin (CT) into the culture medium [3]. Subsequent enhancement was achieved by the addition of isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor, which works synergistically with TPA [4].

By supplementing culture medium with these mitogens, cultivation of large quantities of melanocytes became a reality. These advances opened up the possibility of culturing human melanocytes for clinical use. The first translational experiment was undertaken by Lerner and colleagues, who cultivated melanocytes from a patient with piebaldism using a medium containing TPA, IBMX, CT, bovine serum and pituitary extract. By injecting these cells into the achromic skin (raised by suction blisters) of the patient, successful melanocyte engraftment and repigmentation of piebaldism were achieved [5].

In 1988, it was found that basic fibroblast growth factor (bFGF) is an important keratinocyte-derived factor influencing melanocyte survival and proliferation, a natural mitogen for melanocytes [6]. By using a defined medium that includes bFGF, it became feasible to cultivate human melanocytes in vitro without TPA (a tumour promoter) and fetal calf serum (FCS). In 1993, Olsson and Juhlin successfully expanded melanocytes in culture with a medium free of phorbol esters, pituitary extract and serum. The medium they used was PC-1 (Ventrex, USA), supplemented with bFGF, dibutyryl cyclic adenosine monophosphate and antibiotics. They transplanted the cultured cells (1000–2000 melanocytes/mm^2) onto superficially dermabraded achromic skin of ten patients with vitiligo, out of which nine had good cosmetic outcome [7]. Further evaluation of this technique was reported a year later. By excluding geneticin in the culture medium (which suppresses proliferation of keratinocytes and fibroblasts), Lontz et al. transplanted 16 patients with stable or active vitiligo with cultured melanocytes. Of them, 8 had 50% to beyond 90% repigmentation in their grafted sites, while the remaining 8 had less than 50%. The major factor that influenced outcome was neither the age of the patient nor the disease activity but anatomic location of the recipient sites. The face and trunk achieved the best repigmentation outcome, while the fingers and elbows were most refractory. Ultrastructural examination of the transplanted site revealed that it resembled that of an uninvolved healthy skin, although the melanocytes were positioned slightly higher among basal keratinocytes. The investigators concluded that this procedure was a viable therapeutic option for vitiligo when conventional treatments had failed [8].

Besides having the advantage of expanding melanocytes from a small skin biopsy, cultured cellular grafting offers the attractive option of cryopreserving excess cells for future use. The successful reimplantation of cryostored melanocytes was reported in four patients with vitiligo in 1994 [9]. Cultured melanocytes were preserved in a cryoprotectant, consisting of 8% dimethyl sulphoxide (DMSO) and newborn calf serum, and were frozen for 6–12 months in −70 °C. The melanocyte survival after thaw was around 70%. The value of cryopreservation is that it allows regrafting of incompletely repigmented vitiligo after the initial transplant or regrafting of large achromic areas after successful test grafts.

Over time, further progress was made in cultured melanocyte transplantation. De-epithelization of the recipient site had traditionally been achieved by cryotherapy, suction blister or dermabrasion. These interventions work well for vitiligo areas that are accessible. But for delicate anatomic sites (such as eyelids and nasal folds) and irregular lesions, they may be impractical [10]. Although costs may be a concern, laser ablation using Erbium-YAG or carbon dioxide (CO_2) lasers offers a superior alternative, because of excellent precision over margin and depth [11].

Cumulative data on the outcome of cultured melanocyte transplantation in the treatment of vitiligo also began to emerge. Olsson and Juhlin reported their series on 100 vitiligo patients. Using the surgical approach they previously described, 72 patients had good to excellent repigmentation (40 patients had repigmentation rates exceeding 94%; and 32 achieved repigmentation rates between 65% and 94%).The best results were seen in patients with stable vitiligo for at least 1–2 years. Fingers, elbows and knees were particularly refractory to repigmentation. Colour mismatch was initially common but improved over 6–8 months. No significant scarring was reported, and repigmentation remained unchanged in patients followed up for 1 and 2 years [12]. Chen and colleagues reported their series consisting of 120 patients followed up over a 5-year period [13]. They divided their cohort into three groups: stable localized vitiligo (i.e. segmental or focal) (n = 80), stable generalized vitiligo (n = 26) and active generalized vitiligo (n = 14). Patients' melanocytes were cultivated in Hu16 medium, which consisted of Ham's F12 mixture (Gibco, USA), supplemented with recombinant human bFGF, CT, IBMX, 20% FCS and gentamicin. The bFGF concentration was much higher than that used by Olsson and Juhlin (20 ng/ml vs. 5 ng/ml), based on the premise that lipoproteins in the serum bind bFGF and lower the growth factor's attachment to its receptors. After ablating the recipient sites with CO_2 laser, the melanocyte suspension was applied to the denuded areas at a density of 600–1000 cells/mm^2, followed by a silicone gauze dressing. The patients were followed up for 6–66 months. The outcome of transplantation was impressive, and disease stability was the main outcome determinant: 90–100% repigmentation was recorded in 84% of cases in the stable localized vitiligo group, 54% in the stable generalized group and 0% in the active generalized group ($p < 0.01$). Age, gender and size of lesion did not influence the outcome of transplantation, while the effect of anatomical location could not be evaluated statistically because most of the treated areas were in the head and neck.

36.2.2 Cultured Epidermal Sheet Transplantation

Instead of selectively cultivating melanocytes through special media, epidermal cells (keratinocytes and melanocytes) can be cultured into layered sheets and used in repigmenting stable vitiligo. The basis of using cultured epidermal grafts stems from its long-standing application in the treatment of burns and chronic non-healing wounds. When cultured epidermal grafts are transplanted into full-thickness burns that have been excised to the fascia (hence devoid of local melanocyte reservoir), repigmentation can be observed after re-epithelization, indicating that melanocytes in the graft have repopulated the recipient site [14, 15].

In 1989, Brysk and colleagues reported their experience using culturing epidermal cells for the

treatment of stable vitiligo [16]. Autologous epidermal cells were harvested from shave biopsies of normally pigmented skin and were cultured in MCDB-153 medium (Clonetics, USA). This TPA-free medium supports clonal proliferation of keratinocytes and melanocytes, but not fibroblasts. The cells were seeded onto collagen-coated membranes, and after 50–70% confluence, the composite was transplanted as an autologous graft onto dermabraded vitiliginous sites. Re-epithelization was complete in 2 weeks, and repigmentation was evident after 4 weeks. The investigators extended this technique in treating four vitiligo patients, of whom three had 40–90% repigmentation of grafted areas at 1-year follow-up, and the colour match with the surrounding skin was good [17].

Cultivation of melanocyte-bearing epidermal grafts was also successfully carried out by Falabella and colleagues [18]. Autologous melanocytes and keratinocytes were cocultured in Eagles' minimal essential medium with Hanks' balanced salt solution, supplemented by fetal bovine serum, hydrocortisone, glutamine and antimicrobials. Instead of using a feeder layer to promote epidermal differentiation, adjustment of pH and calcium concentration was utilized. The pH of the culture medium was adjusted to 7.2, and after achieving keratinocyte confluency, the calcium concentration was switched from 1.2 to 3.0 mmol/l. Epidermal sheets were obtained after 21 days of plating and were released from the substratum by dispase. They were then attached to a supporting layer of petroleum gauze and transplanted to the recipient sites (denuded by cryotherapy) of three patients with segmental or focal vitiligo. Satisfactory repigmentation was clinically evident, and recolonization of melanocytes in the grafted sites was demonstrated by electron microscopy. Following this pilot success, the investigators extended the procedure in treating eight patients with generalized vitiligo and one patient with segmental vitiligo [19]. In this trial, the calcium concentration used in promoting keratinocyte differentiation was 1.8 mmol/l. Five out of the nine patients achieved at least 60% repigmentation at 1–2 years of follow-up. Failure of or poor repigmentation was attributed to loss of initial vitiligo stability and difficulty in securing the grafts over joints and acral sites.

Cultured epidermis is delicate to handle, and its manipulation can affect cell viability and graft survival. This problem can be mitigated by growing epidermal cells on a chemically defined surface, forming a composite sheet that is easy to handle. Examples of such biomaterial supports include collagen-coated membranes and acrylic acid-coated silicone sheets. In 1998, Andreassi and colleagues assessed the utility of a flexible membrane made up of a biopolymer of hyaluronic acid esterified by benzyl alcohol [20]. This membrane is perforated with a regular array of laser-drilled micropores for the seeding of cells and larger pores for drainage. After culturing keratinocytes on 3T3 fibroblast feeder layer, they were seeded onto the membrane. Autologous transplants using this composite epidermal sheet were carried out in 11 patients with facial and truncal vitiligo. No side effects were reported, and 5 of the patients had almost complete repigmentation. Epidermal cells can also be grown on silicone sheets coated with acrylic acid, prepared by plasma polymerization. Successful transfer of melanocyte-bearing epidermal sheets was successfully carried out in human wound bed equivalents [21–23].

36.3 Non-cultured Cellular Grafting

36.3.1 Non-cultured Epidermal Cell Suspension Transplantation (NCES)

Although cultured cellular grafting offers the possibility of cryopreserving excess cells for future transplantation, it is not without drawbacks. Cell cultures are expensive to maintain and time-consuming, as it takes several weeks for cultured cells to reach confluence. The procedure also requires highly qualified personnel and involves the use of culture medium and xenobiotics, which invokes the imposition of good manufacturing practice (GMP) standards. This

essentially restricts the treatment to highly specialized centres or industries. An alternative is to transplant autologous epidermal cells in the same surgical setting without first expanding the cells in culture. Large vitiliginous areas, five- to tenfold larger than the size of the donor skin, can still be treated in an outpatient setting and the entire procedure completed just over a few hours.

This form of transplantation, non-cultured cellular grafting, was pioneered by Gauthier and Surleve-Bazeille in 1992 [24]. Donor skin was harvested from the occipital scalp and incubated in cold trypsin for 18 h, after which the epidermis was separated from the dermis using a pair of fine forceps. Epidermal cells were extracted and suspended in PBS. The cellular suspension was subsequently injected into blisters raised by liquid nitrogen at the recipient areas of segmental and focal vitiligo as well as naevus depigmentosus. In 8 out of 12 patients treated, a repigmentation rate of more than 70% was achieved. Six years later, Olsson and Juhlin published a comparable technique using a basal cell layer enriched suspension [25]. The donor skin, however, was harvested from the gluteal region, and the time for trypsinization was reduced to 50 min, which made it possible to complete the entire surgical procedure within the same day. The cell suspension was directly applied onto dermabraded vitiligo lesions, before securing it with a thin collagen film, moistened gauze and Tegaderm. Out of 17 patients treated, 12 with vitiligo vulgaris had a repigmentation rate of at least 80%, while the remaining 5 patients with segmental vitiligo had complete repigmentation.

These techniques, however, have several practical problems. Blisters are particularly difficult to raise by liquid nitrogen on bony eminences and can be associated with leakage of cell suspension. Significant hypopigmentation can also result from cryo-damage of melanocytes. The fluidity of the cell suspension can also lead to significant "run-off" over contoured surfaces and result in loss of viable cells. Van Geel et al. mitigated this problem of "run-off" by increasing the viscosity of the cell suspension using hyaluronic acid (Alcon, France) [26]. Turning the suspension into a gel-like paste did not impair engraftment of the cells; all four treated patients with stable vitiligo achieved more than 80% repigmentation. Instead of using cryotherapy to prepare the recipient bed, carbon dioxide laser was utilized. The advantage of using laser ablation was mentioned in the earlier section; notably it confers better speed, precision and depth control. Finally, trypsin is not accepted by the regulatory agency of some EU members as it is an enzyme classified together with restriction enzymes for DNA. Consequently, the mechanical dissociation should be currently preferred.

Because wound healing on its own can theoretically induce repigmentation by activation of stem cell reservoirs and associated pathways, it is important to clarify if the repigmentation of leukoderma by cellular grafting is contributed mainly by the engraftment of the transplanted cells. The seminal paper that addressed this was the elegant work done by Van Geel et al. [27]. In this double-blind placebo-controlled trial, 33 paired vitiligo lesions of 28 patients were treated either with epidermal cell suspension enriched with hyaluronic acid or the vehicle fluid alone. The transplantation of epidermal cells resulted in repigmentation of at least 70% in 77% of the treated areas at 12 months, whereas none of the placebo group achieved this (and at any time point). The investigators concluded that repigmentation is caused by engrafted melanocytes, rather than postinflammatory reaction induced by epidermal injury or a response to previously failed phototherapy.

Long-term follow-up studies on autologous NCES transplantation concur that it is a safe procedure and is effective in repigmenting vitiligo that is stable. Many reports have been published since 2002. In general, segmental vitiligo shows the best repigmentation outcome and retention of colour, followed by focal vitiligo. For vitiligo vulgaris, the average repigmentation does not exceed 50% in long-term evaluations. The key reason is attributed to the resurgence of vitiligo activity or that the condition has not been completely stable over time. The role of postsurgical phototherapy or sun exposure has also been evaluated; it is reasonable to conclude that the ultraviolet light exposure

enhances the speed of the repigmentation but does not affect the final repigmentation outcome [28].

Olsson and Juhlin were the first group who reported their transplantation experience using basal cell layer suspension in patients treated in the preceding 1–7 years [29]. For segmental vitiligo and piebaldism, all patients achieved and retained 95–100% repigmentation; for focal vitiligo, the repigmentation was 100%. However, for generalized vitiligo, the intervention achieved only an average repigmentation of 49%. Mulekar reported his results in the treatment of segmental (n = 49) and focal (n = 15) vitiligo for the preceding 1–5 years, using the surgical technique similar to that of Olsson and Juhlin. Segmental vitiligo achieved better repigmentation outcomes compared to focal vitiligo; 84% of patients with segmental vitiligo achieved at least 95% repigmentation, compared to 73% of those with focal vitiligo. In both types of vitiligo, the repigmentation was retained until the end of the respective follow-up period, and no adverse effects were reported [30]. These two reports, however, did not address several important outcome measurements and other factors that influence final results, such as time interval to achieve final repigmentation, colour matching and the role of phototherapy or sun exposure post-grafting.

Van Geel et al. addressed these concerns in their long-term follow-up study: percentage of repigmentation was assessed in 82 patients, and long-term outcomes were evaluated retrospectively by 54 patients using a questionnaire up to 7 years post-grafting. Their surgical technique was largely similar to that of Olsson and Juhlin, with the exception that hyaluronic acid was added to increase the viscosity of the epidermal cell suspension [31]. In their study, NCES achieved a high percentage of repigmentation, with more than 75% repigmentation achieved in 71% of patients, which was maintained during the follow-up in the majority of patients. Those who lost the repigmentation were patients (7%) with generalized vitiligo. Corresponding to previous reports, best results (mean repigmentation of 85–91%) were obtained in segmental vitiligo (n = 30), piebaldism (n = 3) and halo naevi (n = 5), whereas generalized (n = 33) and mixed (n = 5) vitiligo fared less well (mean repigmentation of 70% and 37%, respectively). Final repigmentation was achieved within a mean of 10 months post-grafting; however, a perfect colour match (between treated areas and surrounding normal skin) was seldom achieved. Although 80% of the patients had some colour mismatch, the outcome was not disturbing to a majority of them.

Our team evaluated the 12- and 60-month outcome of a large multi-racial cohort of vitiligo patients (n = 177) following NCES transplantation [28]. Several practical issues, additional to previous reports, were addressed: the role of post-grafting phototherapy, the comparison of collagen dressing versus hyaluronic acid in reducing "run-off" and the time interval to repigmentation among different ethnicities. We adopted the transplant technique described by Van Geel et al. but replaced hyaluronic acid with collagen dressing as the biomaterial to reduce "run-off" in a proportion of patients. At 12-month follow-up, good to excellent repigmentation (i.e. >50% repigmentation) was seen in 88% of patients with segmental vitiligo, significantly higher than 71% of patients with non-segmental vitiligo. For patients who were followed up to 60 months, 83% of them retained >75% repigmentation. This underscores again that NCES is effective in repigmenting vitiligo, and segmental vitiligo fares the best. Significantly more patients treated with collagen dressing (Neuskin-F®, Eucare Pharmaceuticals, India) (82%) achieved good to excellent repigmentation compared to those who received hyaluronic acid (63%). The advantage of using collagen dressing is that the biomaterial is highly pliable and adheres well to the wound bed after applying a small aliquot of cell suspension. It prevents "run-off" on contoured surfaces better than hyaluronic acid. Interestingly, post-grafting targeted phototherapy did not significantly affect repigmentation outcome at 12 months. We believe that the final repigmentation rests upon the successful engraftment of transplanted melanocytes (and other epidermal cells) and is unaffected by UVB stimulation. In other words, UVB stimulation may speed up repigmentation but does not alter

the final outcome. Although unpublished, we also observed that the first signs of post-grafting repigmentation are faster in Indian patients (within 4 weeks) than our Chinese (around 6–8 weeks) and Caucasian (around 8–12 weeks) patients. The technique of NCES adopted by our group (including the surgical pearls) is described in Annex A and Figs. 36.3a–g and 36.4.

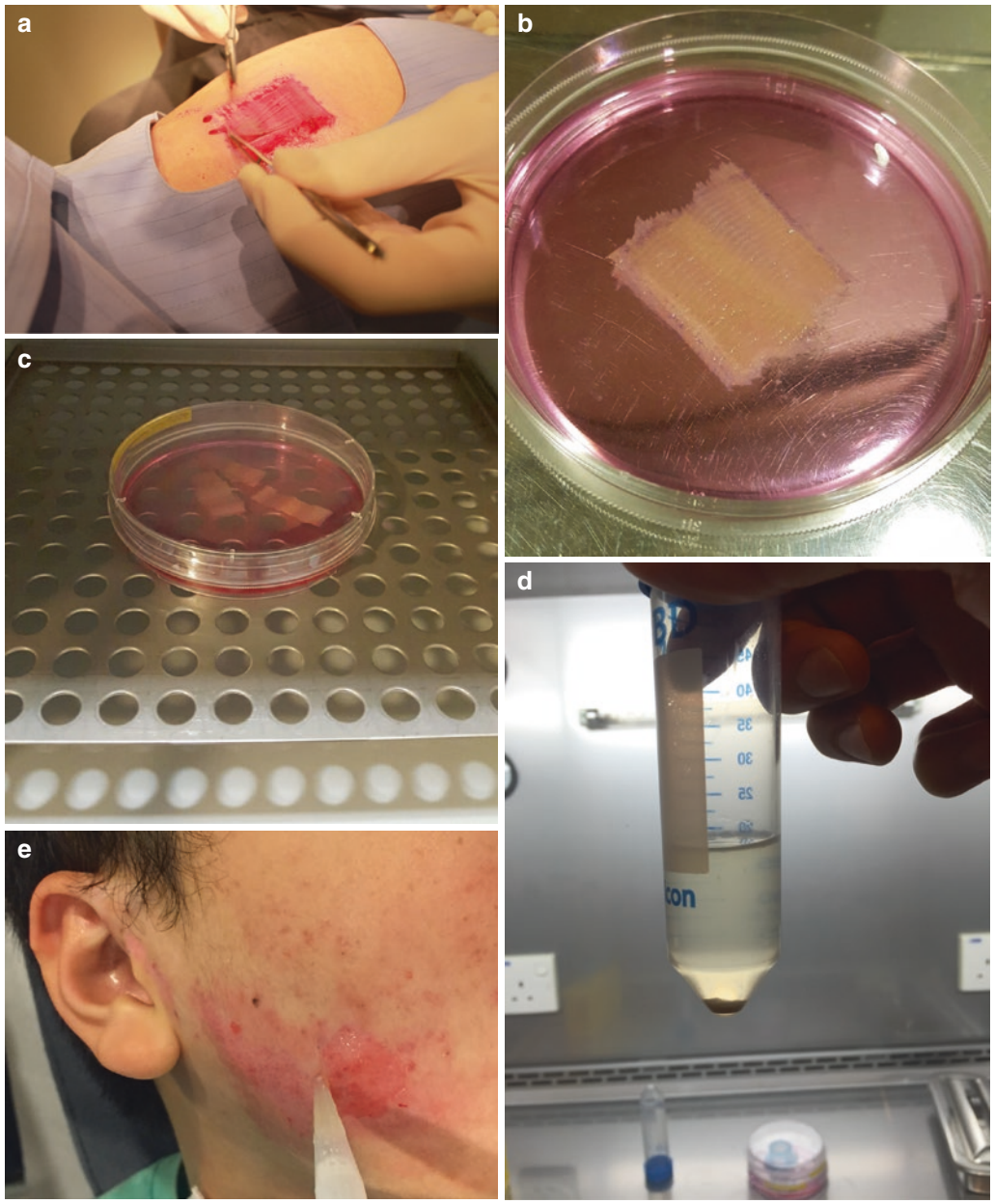


Fig. 36.3 (**a**) Harvesting an ultrathin epidermal graft using a silver dermatome. (**b**) An ultrathin epidermal graft in a petri dish containing 0.25% trypsin-EDTA. (**c**) The graft is cut into smaller pieces and incubated in 0.25% trypsin-EDTA at 37 °C. (**d**) Completion of cellular extraction: a dense cell pellet at the base of the Falcon tube after centrifugation. (**e**) Application of cell suspension onto laser-ablated vitiliginous recipient site. (**f**) Placing collagen sheets to hold cell suspension in place and prevent "run-off". (**g**) Securing suspension and collagen sheets using Hypafix dressings

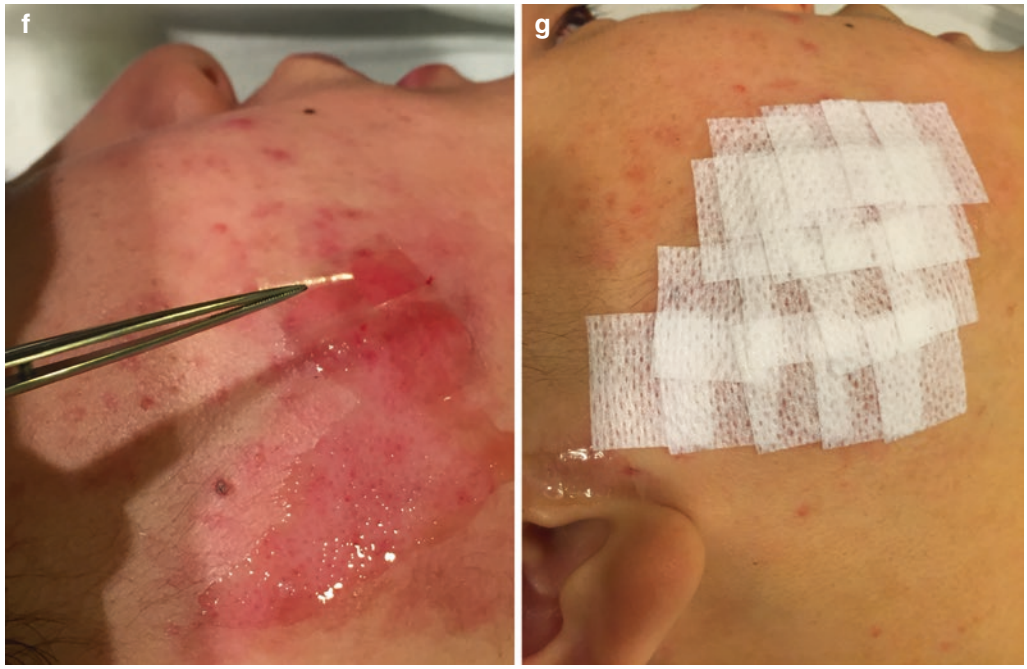


Fig. 36. 3 (continued)

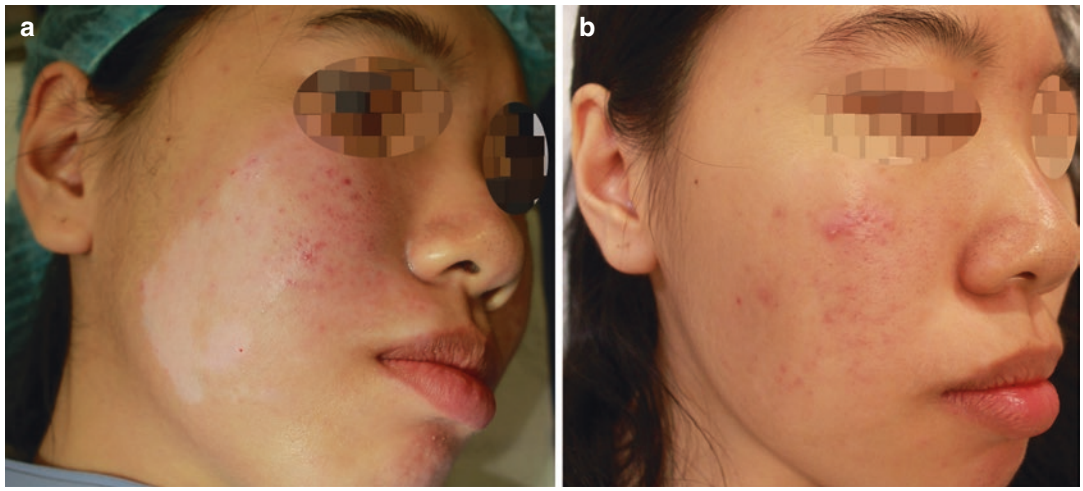


Fig. 36.4 (**a** and **b**) Successful repigmentation of segmental vitiligo after cellular grafting

36.4 Factors Influencing Outcome in NCES

From the above discussion, it is clear that for NCES to be successful, the selection criteria of patients have to be strict. A surgical intervention is indicated only if a patient with vitiligo has failed medical treatments and phototherapy. The vitiligo has to be stable (preferably more than a year) before surgical transplant, and the patient does not display Koebner phenomenon. The ability to meet patients' expectation also has to be carefully assessed before surgery. In addition, the concentration of melanocytes transplanted and

the presence of inflammation in the recipient sites also influence surgical outcomes.

Rao et al. studied the clinical, biochemical and immunological factors determining stability of disease in patients with generalized vitiligo undergoing surgical transplantation [32]. The success rates were highest among patients whose vitiligo is clinically stable for 2 years or more. Poor repigmentation after transplantation was associated with shorter disease stability (13.2 ± 14.3 vs. 56.6 ± 52.4 months) and higher percentages of $CD8^+$ (3.0 ± 2.1 vs. 1.0 ± 1.4) and $CD45RO^+$ (1.7 ± 2.4 vs. 0) cells in the lesional biopsy. These findings suggest that the presence of an inflammatory milieu at the recipient site portends disease instability and is associated with poor surgical success.

There is a dearth of studies determining the optimal concentration of melanocytes required for successful NCES. Olsson and Juhlin postulated that a melanocyte count of 190/mm^2 was sufficient for adequate repigmentation [25]. In a randomized prospective study, Tegta et al. compared the efficacy of two different dilutions of melanocytes in achieving early and acceptable repigmentation [33]. In one group, the epidermal cell suspension was prepared from a donor graft one-third the size of the recipient area, while in the other group, the graft was one-fifth that of the recipient area. The former group received a higher density of melanocytes (231 ± 27 cells/mm^2) and had significantly greater extent repigmentation (50% patients achieving >75% repigmentation) than the latter group with lower melanocyte density (154 ± 27 cells/mm^2) (none of the patients achieved >75% repigmentation). The investigators concluded that the minimum number of cells to produce acceptable repigmentation is in the range of 210–250/mm^2. What defines as an "acceptable" repigmentation rate is subject to debate, and practitioners should reflect if 50% of subjects failing to achieve >75% repigmentation is an acceptable outcome. It is important to note that melanocyte density harvested from the donor graft in this study was low (694–771/mm^2) compared to published numbers (1700 ± 139/mm^2; thigh area) [34], and the numbers were even lower in the final cell suspension (231 ± 27/mm^2). In other words, there was a significant attrition of cells during the harvesting process. It is reasonable to conclude instead that a higher donor-recipient area ratio offers a better repigmentation (1:3 fares better than 1:5) and a transplant density of 210–250 melanocytes/mm^2 provides >75% repigmentation in half of the treated cases.

36.5 Simplified Non-cultured Cellular Grafting

Although NCES transplantation is effective in repigmenting stable vitiligo, the technique requires extraction of epidermal cells that involves multiple stages and reliance on a laboratory space and equipment (such as a laminar flow chamber and centrifuge machine). This largely limits the use of this technique to academic institutions. Over the last decade, ways to simplify this procedure have been explored. ReCell® kit (Avita Medical, Australia) is a portable battery-operated cell-harvesting device that was initially developed for the treatment of superficial burns through epidermal cell transplantation. Sodium lactate is used as the delivery fluid. During the treatment of burns, it was observed that the resultant healing was accompanied by repigmentation, which led to the use of this device for treatment of stable vitiligo. In a small pilot study involving ten lesions in five patients with stable vitiligo (one segmental and four generalized vitiligo), Mulekar et al. evaluated the effectiveness of the ReCell® kit in repigmentation compared to conventional NCES transplantation [35]. The results were comparable: with ReCell®, two lesions showed 100%, one 65%, one 40% and one 0% repigmentation, while with NCES, three showed 100%, one 30% and one 0% repigmentation. Another conclusion that can be drawn from this study, which is useful and important in practice, is that it is unnecessary to use a melanocyte medium as a carrier fluid for the cell suspension. Sodium lactate or PBS suffices, as the recipient wound bed provides serum and adequate nutrients to neutralize the effects of trypsin and support the survival and proliferation of the

transplanted epidermal cells. However commercial kits like ReCell® have a certain drawback, and that is cost. These devices are expensive, and affordability becomes an important limiting factor for their routine use.

To circumvent this issue, our team developed an inexpensive, simplified grafting technique that obviates the need of a laboratory set-up [36]. The term "6-well plate technique" was coined. It makes use of a 6-well tissue culture plate in the preparation of the epidermal cell suspension. Moving in a clockwise manner, soybean trypsin inhibitor (Sigma) is added into the first well, while PBS is added into the second to fourth wells. The fifth well is left empty, and a 40-μm cell strainer is placed in the sixth well (Fig. 36.5). After harvesting the autologous donor tissue, the ultrathin split skin graft is cut into smaller pieces in a petri dish and incubated in trypsin-EDTA 0.25% for 40 min at 37 °C. The "trypsinized" tissues are subsequently placed in the first well, where the effects of trypsin are neutralized by soybean trypsin inhibitor (Fig. 36.5a). The tissues are then rinsed in PBS in the next three wells and placed in the empty fifth well. Using a pair of fine forceps, the epidermis is separated from the dermis of each tissue piece in this well, and the tissue surfaces are scraped to mechanically dislodge the epidermal cells (Fig. 36.5b). PBS is then added, and suspension is mixed evenly by aspirating several times with a pipetter. This suspension is then withdrawn from the fifth well and dispensed into the sixth well through the microfilter to remove residual tissue debris. The filtrate, containing the final cell suspension, is then applied to laser-ablated recipient sites. Using this cell extraction process, we treated two patients with segmental vitiligo, two with focal vitiligo and one with piebaldism. All treated lesions repigmented, and the percentage of repigmentation at 6 months ranged between 65% and 92%. This "6-well plate" set-up differs from commercially available devices in a few ways. It does not have a built-in automated heater; this can easily be circumvented by warming the harvested skin tissue in a petri dish containing trypsin using a small benchtop portable incubator. Soybean trypsin inhibitor is used to neutralize trypsin, but it can be replaced by Ringer's lactate solution. The cost of this set-up is only a fraction of that in commercial devices, making it an appealing and inexpensive alternative.

Kumar et al. simplified this technique further by using a four-compartment petri dish [37]. The basic principles, however, remain the same. Instead of using a separate petri dish, the harvested donor tissue is placed in the first compartment of the petri dish containing trypsin-EDTA 0.25%. This is then incubated for an hour at 37 °C. The tissue is subsequently rinsed in PBS in the next two compartments to remove residual

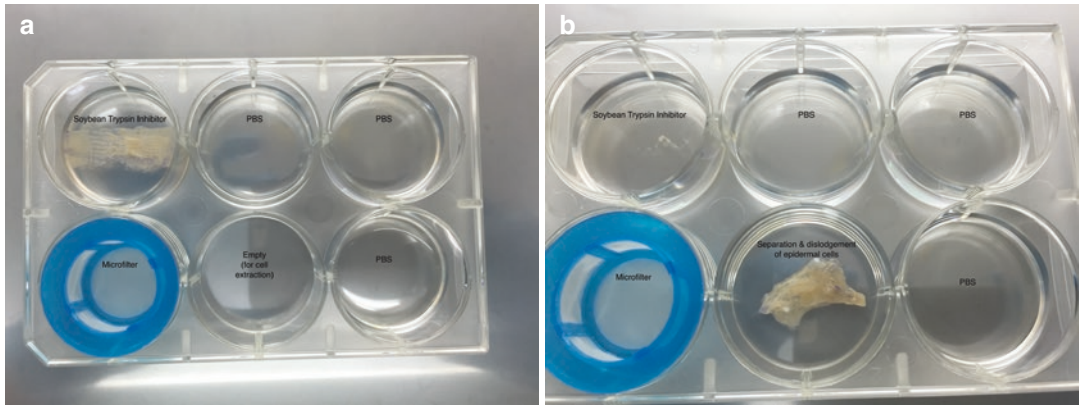


Fig. 36.5 (**a**) Using a 6-well culture plate to extract epidermal cells. Trypsinized ultrathin skin graft is washed in soybean trypsin inhibitor, before rinsing in PBS (second to fourth wells). (**b**) Separation of the epidermis and dermis, followed by mechanical dislodgement of epidermal cells (by scraping), takes place in the fifth well

trypsin. In the fourth compartment, the epidermis of the tissue is separated from the dermis using a pair of fine forceps and the epidermal cells dislodged. Tissue debris are removed using forceps, and PBS is added and the suspension mixed by aspirating several times using a sterile syringe. The final suspension is then applied to dermabraded vitiliginous recipient sites. Six patients (four with segmental and two with focal vitiligo) were treated with this simplified NCES transplantation. The repigmentation was 90–100% in four of the patients and more than 75% in two of them at 16 weeks. Although the effects of trypsin may not be fully removed by PBS rinses (thereby impairing cell viability), this is overcome by serum present on the recipient wound bed after dermabrasion.

In the absence of an incubator, cold trypsinization can take place instead [24]. This can be done by placing the petri dish containing the donor tissue in trypsin 0.25% in a refrigerator at 4 °C overnight. Gupta et al. [38], however, devised an ingenious way of harnessing the human body as a natural incubator and as a "mini-laboratory." Cell separation and harvesting are performed inside suction blisters induced on a patient's thigh (Fig. 36.6). After a suction blister has been raised using standard procedures (Fig. 36.6a), the blister fluid is aspirated using a 24G hypodermic needle attached to a 2-ml syringe. This needle is introduced into the blister cavity through the adjacent skin via a subcutaneous route (Fig. 36.6b,c). With the needle still in place, the syringe is then replaced with another syringe containing 1 ml of 0.25% trypsin, which is instilled into the blister cavity (Fig. 36.6d). The needle is removed, and the solution is left in the blister cavity for 45 min, during which epidermal cells (melanocytes and keratinocytes) will begin to dislodge to form a cell suspension. This suspension is then aspirated and transferred to a petri dish (Fig. 36.6e). The roof of the blister is subsequently excised, placed in the same petri dish, and its dermal surface scraped to mechanically dislodge residual basal cells. The final suspension is then applied to dermabraded patches of recipient vitiligo. The excised blister roof is returned to the donor site to serve as a biological dressing. The investigators treated five patients with stable vitiligo (three NV and two SV), all of whom had excellent repigmentation (Fig. 36.6f). A key concern with this procedure is the safety of trypsin, incubating within the suction blister. Recombinant trypsin is noted to be safe by the investigators, and its minute quantity, even if absorbed, is neutralized promptly by serum. Because trypsin breaks intercellular epidermal bonds, it has no effects on dermal tissue, and the healing of the donor site was observed to be unaffected.

These techniques simplify NCES transplantation, obviating the need of a laboratory set-up and reagents such as soybean trypsin inhibitor. It makes the procedure more cost-effective and accessible in a clinic setting. Although the total number of viable cells extracted may arguably not be as high as conventional methods per unit tissue, the repigmentation rates have been shown to be acceptable. Future studies, comparing the two methods (i.e. simplified vs. conventional), will be helpful in determining if there are significant differences in cell densities and viability and in clinical outcomes.

36.6 Non-cultured Outer Root Sheath Hair Follicle Cell Suspension (NCORSHFS) Transplantation

Besides the epidermis, melanocytes can be extracted from human hair follicles. The hair follicle contains a rich reservoir of melanocytes and their precursors. Melanocyte-lineage antigens and c-Kit-positive cells are localized in the outer root sheath (ORS) of the infundibulum and midfollicle, as well as in the hair bulb matrix [39]. The perifollicular connective tissue sheath and papilla are also potential sources of mesenchymal stem cells. Unlike the epidermal-melanin unit which has 1 melanocyte to every 36 keratinocytes, the follicular-melanin unit has a significantly higher ratio of 1:5 [40]. In addition, melanocytes in anagen hair bulb are functionally more active and have remarkable synthetic

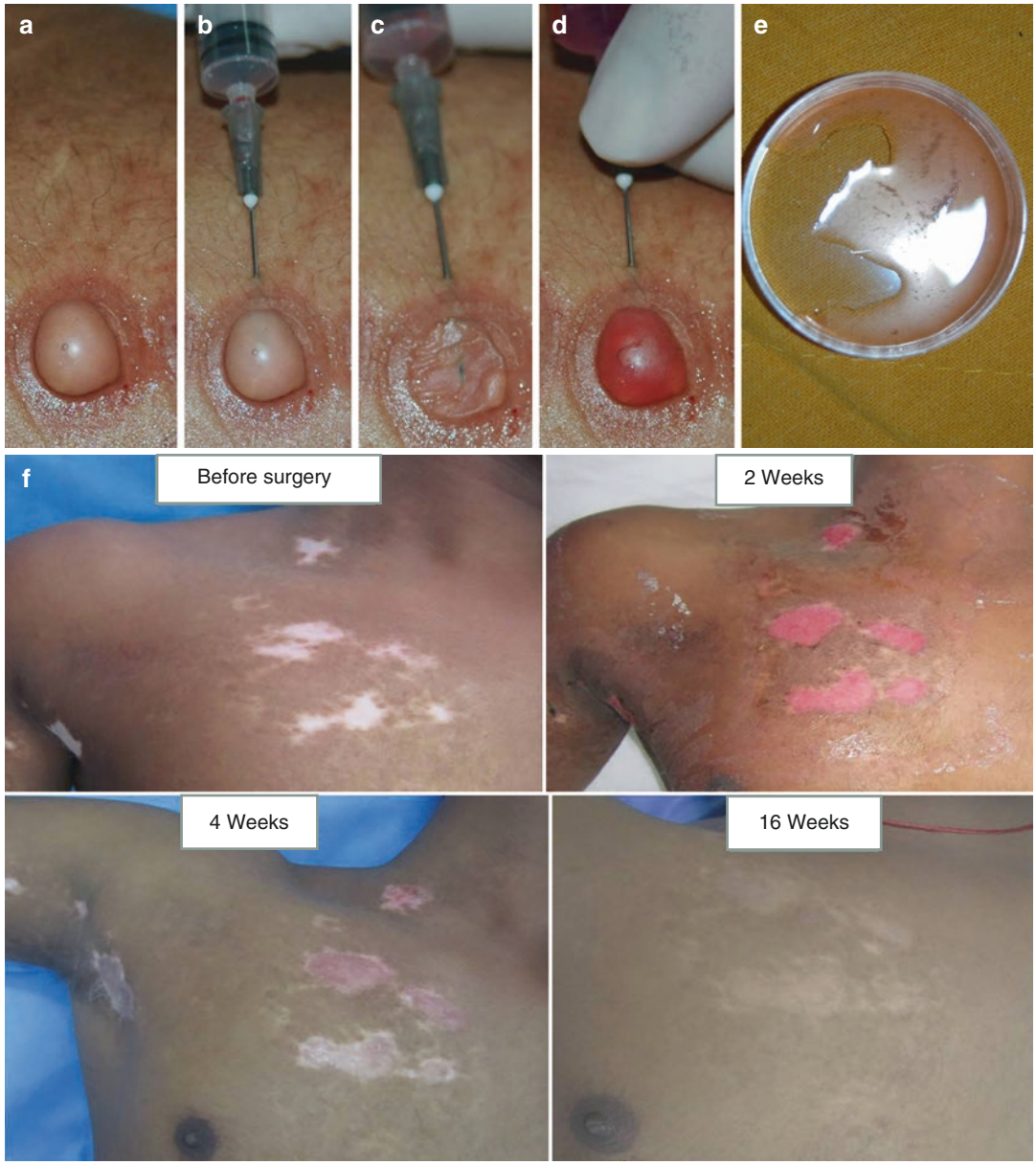


Fig. 36.6 In-vivo technique of harvesting epidermal cells for cellular grafting (Courtesy of Dr. Somesh Gupta)

capacity in comparison to epidermal melanocytes [41]. These physiological features make hair follicle a more attractive source of melanocytes for cell-based therapies in vitiligo.

The use of hair follicle cells for repigmenting stable vitiligo was first explored by Vanscheidt and Hunziker [42]. In small case series, the investigators derived single-cell suspension from "plucked" hair follicles for grafting stable vitiligo. Three out of five patients they treated had almost complete (>90%) repigmentation. Although this technique is simple, non-invasive and highly accessible, the cellular yield from plucked hairs can be low and inadequate for transplantation. Kumar et al. evaluated the yield of CD200+ cells (a marker for hair follicle bulge

stem cells) derived from plucked hairs compared with follicular unit extraction (FUE); the latter has significantly higher yield. They concluded that the stem cell niche is partially lost in follicular plucking [43].

Using FUE of anagen hairs, Mohanty et al. extracted cells from the outer root sheaths and grafted vitiligo lesions with the suspension [44]. The technique they adopted is as follows (Fig. 36.7). Anagen hairs were selected from the occipital scalp and trimmed to 2 mm in length. Field block anaesthesia was administered using 2% lignocaine. The follicular units were extracted by rotating a 1-mm biopsy punch in the direction of each hair follicle to the level of the mid-dermis. The follicular unit was pulled out using a follicle-holding forceps; transected hairs were discarded. Fifteen to 25 follicular units were extracted and collected in a medium containing Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, USA) supplemented by antibiotics. In the laboratory, these units were washed in PBS and then incubated in 0.25% trypsin–0.05% EDTA (Gibco, USA) at 37 °C for 90 min to separate ORS cells. After every 30 min, the extracted hair follicles were placed in a new tube of

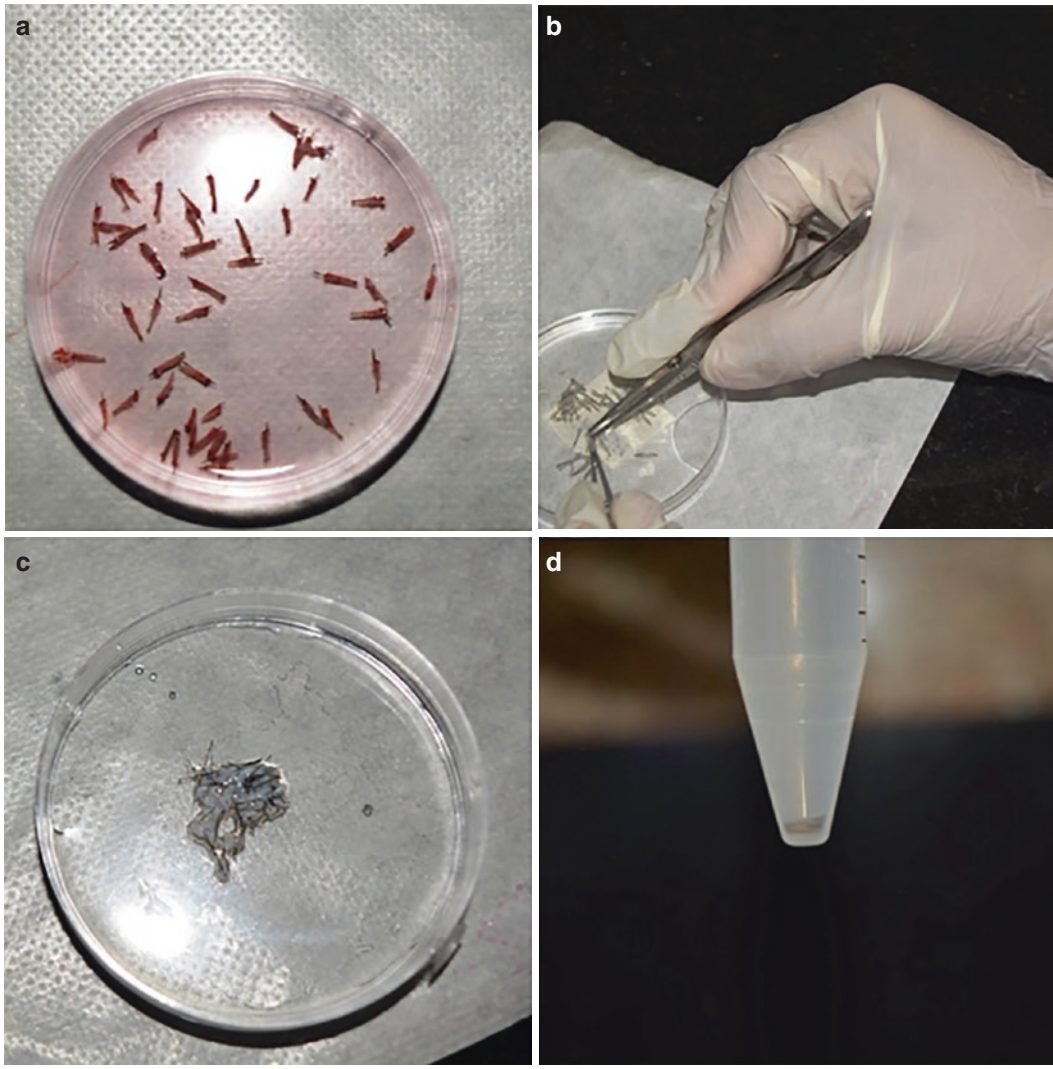


Fig. 36.7 Extraction of outer root sheath cells for cellular grafting (Courtesy of Dr. Somesh Gupta)

trypsin-EDTA and the reaction in the previous tube aborted with soybean trypsin inhibitor (Sigma-Aldrich, USA). Post-trypsinization only keratinous hair shafts remained, which were discarded. The cell suspension of all three tubes were combined in a single tube and filtered through a 70-μm cell strainer. The filtrate was centrifuged for 5 min at 1000 rpm to obtain a cell pellet, which was subsequently resuspended in a small amount of DMEM, before application onto dermabraded vitiligo patches, and covered with collagen dressings (Neuskin-F®, Eucare Pharmaceuticals, India). Fourteen patients (3 SV and 11 NSV), stable for at least 3 months, were treated; out of them, 9 patients achieved >75% repigmentation. The mean percentage of repigmentation was 65.7 ± 36.7%. Similar to previous studies, segmental vitiligo fares better than non-segmental vitiligo; and patients with at least a year of clinical stability have significantly better repigmentation than those with less than a year (78.6 ± 29.1% vs. 18.3 ± 16.1%, $p = 0.02$).

These results were reproducible by other investigators. Vinay et al. evaluated the repigmentation rates of NCORSHFS transplantation in 30 patients with 60 lesions of stable vitiligo. They also assessed the factors that determine the treatment outcome [45]. Twenty-one out of 60 (35%) lesions achieved a repigmentation rate of >75%, and out of them, 10 had >90% (or "excellent") repigmentation. Concurring with previous reports, segmental vitiligo fared the best in that 33% of segmental vitiligo lesions achieved excellent repigmentation, compared to 25% of focal vitiligo and only 4% of generalized vitiligo. The investigators found that optimal (>75%) repigmentation was associated with a higher number of melanocytes (mean = 1187 vs. 796 cells/cm^2) as well as a higher number of hair follicle stem cells (mean = 756 vs. 494 cells/cm^2) transplanted. The presence of dermal inflammation in the vitiliginous recipient site was associated with poor repigmentation, due to its deleterious effects on transplanted cells.

Although there are physiological advantages of using ORS melanocytes over epidermal melanocytes for vitiligo transplant, the repigmentation outcome is not superior to that in NCES transplantation in practice. Furthermore, FUE (followed by ORS melanocyte extraction) is technically more laborious and time-consuming compared to harvesting a single piece of split skin epidermal graft. In a randomized study, Singh et al. compared the treatment outcome in patients with stable vitiligo treated with NCORSHFS and NCES methods [46]. Although not statistically significant, excellent (90–100%) and good (>75%) repigmentation were achieved in more of the treated areas using NCES (83% and 92%, respectively) than those with NCORSHFS (65% and 78%). Furthermore, patients in the NCES group were significantly more satisfied with the outcome than those in the NCORSHFS group.

Annex A: Surgical Protocol for NCES (Adopted and Modified from [27])

1. The first step is always the most important. It is crucial to harvest an *ultrathin* split skin graft. We usually choose the left lateral hip as the donor site, and the size is one-third to one-fifth the vitiligo area to be transplanted. The donor site is cleansed with chlorhexidine 4% and outlined with skin marking ink, before performing an anaesthetic field block using 2% lignocaine. A silver dermatome is used to harvest a split skin graft not beyond the papillary dermis, which can be gauged by the translucency of the graft (Fig. 36.3a). Too thick a graft will impede trypsinization and can lead to scarring of the donor site. This is a skill to be acquired and a learning curve. It requires the help from an assistant in stretching the skin taut (using, e.g. a sterilized wooden spatula), so that the surface is as flat as possible. Sterile liquid paraffin provides good lubrication for the dermatome to glide on the donor skin surface.
2. The split skin graft is placed in a sterile tray and washed in normal saline, before placing it in a petri dish containing 10 ml of trypsin-EDTA 0.25%, pre-warmed to 37 °C (Fig. 36.3b). The graft is then cut into smaller pieces, and the petri dish is covered and placed in an incubator, set at 37 °C, for 30 min (Fig. 36.3c).

3. After incubation, the petri dish containing the donor tissue is placed in a laminar flow chamber, wherein the extraction of epidermal cells takes place. The lid of the petri dish is lifted, inverted and placed on the benchtop of the chamber. The tissue pieces are removed from trypsin and placed on the inverted lid. Here, using fine forceps, the epidermis is peeled away from the dermis and their surfaces scraped with the forceps to dislodge the epidermal cells including melanocytes.
4. The tissue-cell mixture is then collected and placed into a 50 ml Falcon tube containing 10 ml of PBS. The tube is capped and the suspension mixed by shaking and twirling tube for 30 s. In so doing, the cells will be further dislodged and suspended in the PBS.
5. The suspension is decanted into a petri dish, and 10 ml of soybean trypsin inhibitor is added to neutralize trypsin. The tissue debris are physically removed and discarded from the mixture. The neutralized suspension is then pipetted into a 50-ml Falcon tube, through a 40-μm filter, to remove the residual tissue debris.
6. The filtrate, containing the cell suspension, is then centrifuged at 120 × *g* for 5–8 min. Epidermal cells will settle to the bottom of the tube as a pellet (Fig. 36.3d). The supernatant is discarded, and the cell pellet is resuspended in 2–5 ml of PBS (depending on the size of the pellet and the area of recipient site).
7. The final suspension is then applied onto laser-ablated or dermabraded vitiliginous recipient sites. The depth of ablation of the recipient area is gauged by pinpoint bleeding. The application of the cell suspension is carried out using a pipetter which offers good control of the amount dispensed. A small amount is applied to avoid run-off, controlled by surface tension of the fluid (Fig. 36.3e). Collagen dressing (Neuskin-F, Eucare, India), cut into small pieces, is then applied to hold the suspension in place (Fig. 36.3f), and the area is further secured with Hypafix dressings (Smith & Nephew, UK) (Fig. 36.3g).
8. The dressings are kept in place for 5–7 days before removal. Post-grafting phototherapy is not required in our experience. Patients can expose the recipient sites to sunlight daily for 5–10 min.
9. If the grafting is successful, signs of repigmentation are usually evident by 4 weeks in Indian, 8 weeks in Chinese (Fig. 36.4a, b) and 12 weeks in Caucasian patients.

References

1. Hartmann A, Broecker EB, Hamm H. Repigmentation of skin and hairs in stable vitiligo by transplantation of autologous melanocytes in fibrin suspension. J Eur Acad Dermatol Venereol. 2008;22:624–6.
2. Gan EY, van Geel N, Goh BK. Repigmentation of leucotrichia in vitiligo with noncultured cellular grafting. Br J Dermatol. 2012;166:196–9.
3. Eisinger M, Marko O. Selective proliferation of normal human melanocytes in vitro in the presence of phorbol ester and cholera toxin. Proc Natl Acad Sci U S A. 1982;79:2018–22.
4. Halaban R, Ghosh S, Duray P, Kirkwood JM, Lerner AB. Human melanocytes cultured from naevi and melanomas. J Invest Dermatol. 1986;87:95–101.
5. Lerner A, Halaban R, Klaus SN, Moellman GE. Transplantation of human melanocytes. J Invest Dermatol. 1987;89:219–24.
6. Halaban R, Langdon R, Birchall N, et al. Basic fibroblast growth factor from human keratinocytes is a natural mitogen for melanocytes. J Cell Biol. 1988;107:1611–9.
7. Olsson MJ, Juhlin L. Repigmentation of vitiligo by transplantation of cultured autologous melanocytes. Acta Derm Venereol. 1993;73:49–51.
8. Löntz W, Olsson MJ, Moellmann G, Lerner AB. Pigment cell transplantation for the treatment of vitiligo: a progress report. J Am Acad Dermatol. 1994;30:591–7.
9. Olsson MJ, Moellman G, Lerner AB, Juhlin L. Vitiligo: repigmentation with cultured melanocytes after cryostorage. Acta Derm Venereol. 1994;74:226–8.
10. Kaufmann R, Greiner D, Kippenberger S, Bernd A. Grafting of in vitro cultured melanocytes onto laser-ablated lesions of vitiligo. Acta Derm Venereol. 1998;78:136–8.
11. Dover JS, Hruza G. Lasers in skin resurfacing. Australas J Dermatol. 2000;41:72–85.
12. Olsson MJ, Juhlin L. Transplantation of melanocytes in vitiligo. Br J Dermatol. 1995;132:587–91.
13. Chen YF, Yang PY, Hu DH, Kuo FS, Hung CS, Hung CM. Treatment of vitiligo by transplantation of cultured pure melanocyte suspension: analysis of 120 cases. J Am Acad Dermatol. 2004;51:68–74.
14. O'Connor NE, Mulliken JB, Banks-Schlegel S, Kehinde O, Green H. Grafting of burns with cultured

epithelium prepared from autologous epidermal cells. Lancet. 1981;1:75–8.
15. Compton CC, Gill JM, Bradford DA, Regauer S, Gallico GG, O'Connor NE. Skin regenerated from cultured epithelial autografts on full-thickness burn wounds from 6 days to 5 years after grafting. Lab Investig. 1989;60:600–12.
16. Brysk MM, Newton RC, Rajaraman S, et al. Repigmentation of vitiliginous skin by cultured cells. Pigment Cell Res. 1989;2:202–7.
17. Plott RT, Brysk MM, Newton RC, Raimer SS, Rajaraman S. A surgical treatment for vitiligo: autologous cultured-epithelial grafts. J Dermatol Surg Oncol. 1989;15:1161–6.
18. Falabella R, Escobar C, Borrero I. Transplantation of *in vitro*-cultured epidermis bearing melanocytes for repigmenting vitiligo. J Am Acad Dermatol. 1989;21:257–64.
19. Falabella R, Escobar C, Borrero I. Treatment of refractory and stable vitiligo by transplantation of *in vitro* cultured epidermal autografts bearing melanocytes. J Am Acad Dermatol. 1992;26:230–6.
20. Andreassi L, Pianigiani E, Andreassi A, Taddeucci P, Biagioli M. A new model of epidermal culture for the surgical treatment of vitiligo. Int J Dermatol. 1998;37:595–8.
21. Eves PC, Beck AJ, Shard AG, MacNeil S. A chemically defined surface for the co-culture of melanocytes and keratinocytes. Biomaterials. 2005;26:7068–81.
22. Beck AJ, Phillips J, Smith-Thomas L, Short RD, Mac Neil S. Development of a plasma-polymerised surface suitable for the transplantation of keratinocyte-melanocyte cocultures for patients with vitiligo. Tissue Eng. 2004;9:1123–1131.
23. MacNeil S, Eves P, Beck A, Gawkrodger D. Practical issues of delivering cultured melanocytes to vitiligo patients. Proceedings of the 19th International Pigment Cell Conference, VA, USA; 2005.
24. Gauthier Y, Surleve-Bazeille J. Autologous grafting with noncultured melanocytes: a simplified method for treatment of depigmented lesions. J Am Acad Dermatol. 1992;26:191–4.
25. Olsson MJ, Juhlin L. Leucoderma treated by transplantation of a basal cell layer enriched suspension. Br J Dermatol. 1998;138:644–8.
26. Van Geel N, Ongenae K, De Mil M, Naeyaert JM. Modified technique of autologous noncultured epidermal cell transplantation for repigmenting vitiligo: a pilot study. Dermatol Surg. 2001;27:873–6.
27. Van Geel N, Ongenae K, De Mil M, Haeghen YV, Vervaet C, Naeyaert JM. Double-blind placebo-controlled study of autologous transplanted epidermal cell suspensions for repigmenting vitiligo. Arch Dermatol. 2004;140:1–6.
28. Gan EY, Kong YL, Tan WD, Thng ST, Goh BK. Twelve-month and sixty-month outcomes of noncultured cellular grafting for vitiligo. J Am Acad Dermatol. 2016;75:564–71.
29. Olsson MJ, Juhlin L. Long-term follow-up of leucoderma patients treated with transplants of autologous cultured melanocytes, ultrathin epidermal sheets and basal cell layer suspension. Br J Dermatol. 2002;147:893–904.
30. Mulekar SV. Long-term follow-up study of segmental and focal vitiligo treated by autologous, noncultured melanocyte- keratinocyte cell transplantation. Arch Dermatol. 2004;140:1211–5.
31. Van Geel N, Wallaeys E, Goh BK, De Mil M, Lambert J. Long-term results of noncultured epidermal cellular grafting in vitiligo, halo naevi, piebaldism and naevus depigmentosus. Br J Dermatol. 2010;163:1186–93.
32. Rao A, Gupta S, Dinda AK, et al. Study of clinical, biochemical and immunological factors determining stability of disease in patients with generalized vitiligo undergoing melanocyte transplantation. Br J Dermatol. 2012;166:1230–6.
33. Tegta GR, Parsad D, Majumdar S, Kumar B. Efficacy of autologous transplantation of noncultured epidermal suspension in two different dilutions in the treatment of vitiligo. Int J Dermatol. 2006;45:106–10.
34. Inger R, Hans R. An estimation of the melanocyte mass in humans. J Invest Dermatol. 1983;81:278–81.
35. Mulekar SV, Ghwish B, Al Issa A, Al Eisa A. Treatment of vitiligo lesions by ReCell® vs. conventional melanocyte–keratinocyte transplantation: a pilot study. Br J Dermatol. 2008;158:45–9.
36. Goh BK, Chua XM, Chong KL, De Mil M, Van Geel NAC. Simplified cellular grafting for treatment of vitiligo and piebaldism: the "6-well plate" technique. Dermatol Surg. 2010;36:203–7.
37. Kumar R, Parsad D, Singh C, Yadav S. Four compartment method: a simplified and cost-effective method of noncultured epidermal cell suspension for the treatment of vitiligo. Br J Dermatol. 2014;170:581–5.
38. Gupta S, Sahni K, Tembhre MJ, Mathur S, Sharma VK. A novel point-of-care in vivo technique for preparation of epidermal cell suspension for transplantation in vitiligo. J Am Acad Dermatol. 2015;72:e65–6.
39. Randall VA, Jenner TJ, Hibberts NA, et al. Stem cell factor/c-Kit signalling in normal and androgenetic alopecia hair follicles. J Endocrinol. 2008;197:11–23.
40. Tobin DJ, Paus R. Graying: gerontobiology of the hair follicle pigmentary unit. Exp Gerontol. 2001;36:29–54.
41. Legue E, Sequeira I, Nicolas JF. Hair follicle renewal: authentic morphogenesis that depends on a complex progression of stem cell lineages. Development. 2010;137:569–77.
42. Vanscheidt W, Hunziker T. Repigmentation by outer-root-sheath-derived melanocytes: proof of concept in vitiligo and leucoderma. Dermatology. 2009;218:342–3.
43. Kumar A, Gupta S, Mohanty S, Bhargava B, Airan B. Stem cell niche is partially lost during follicular plucking: a preliminary pilot study. Int J Trichol. 2013;5(2):97–100.

44. Mohanty S, Kumar A, Dhawan J, Sreenivas V, Gupta S. Noncultured extracted hair follicle outer root sheath cell suspension for transplantation in vitiligo. Br J Dermatol. 2011;164:1241–6.
45. Vinay K, Dogra S, Parsad D, Kanwar AJ, Kumar R, Minz RW, Saikia UN. Clinical and treatment characteristics determining therapeutic outcome in patients undergoing autologous non-cultured outer root sheath hair follicle cell suspension for treatment of stable vitiligo. J Eur Acad Dermatol Venereol. 2015;29:31–7.
46. Singh C, Parsad D, Kanwar AJ, Dogra S, Kumar R. Comparison between autologous noncultured extracted hair follicle outer root sheath cell suspension and autologous non cultured epidermal cell suspension in the treatment of stable vitiligo: a randomized study. Br J Dermatol. 2013;169:287–93.

Depigmenting Therapies

37

Abdulrahman Aljamal, Mohammed Aljamal, Sanjeev Mulekar, and Aleissa Ahmed

Contents

Abstract

Depigmentation may be suggested when other possible treatment failed and when the lesions are perceived as disfiguring. Chemical or physical approaches are available, depending on the site and the extent of the lesions. Hydroquinone and phenol derivatives are the most appreciated chemical depigmenting agents, whereas lasers and cryotherapy may be relevant alternatives even if the last ones must be applied in hospital.

Key Points

- Only patients with extensive vitiligo should be treated, when other possible therapies failed.
- Incomplete or trichrome repigmentation may cause more disfigurement.
- MBEH is the most potent and mainstay depigmenting agent, and it can induce depigmetation at distant sites.
- Repigmentation occurs in MBEH because it often does not destroy follicular melanocytes.
- 4MP is as effective as MBEH, but the side effects like skin irritation are less

A. Aljamal · M. Aljamal · S. Mulekar · A. Ahmed (✉)
The National Center of Vitiligo and Psoriasis, Riyadh, Kingdom of Saudi Arabia

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_37

common and less severe. It required longer treatment.

- Lasers are fast, effective, and safe with short follow-up durations.
- Patches with positive Koebner respond to cryosurgery.

37.1 Introduction

The depigmenting approach is quite recent, deriving from the observation of unwanted depigmenting action of the phenol derivatives [1]. On the basis of this clinical observation, the researchers aimed to define the possible mechanisms of action of this class of compounds. The first suggested target was the enzyme tyrosinase, and the capability of different phenol derivatives to act as alternative substrate of the enzyme or as competitive inhibitor was evaluated. Consequently, it was hypothesized that this class of substances, or some of them, may be used for the treatment of the skin disorders due to hyperpigmentation or melanocyte hyperproliferation. Structural studies have indicated the role of the position and of the type of substitutes in the phenolic ring to allow the compound to be hydroxylated or oxidated by tyrosinase [2]. Hydroquinone (HQ) belongs to the phenol/catechol class of chemical agents. HQ inhibits tyrosinase through the interaction with the copper at the active site, as well as decreases the amount of intracellular glutathione and induces the production of oxygen-reactive species. HQ acts as alternative substrate, according to most part of phenol/catechol compounds, because it is similar to tyrosine. The enzyme can thus oxidize HQ without generating the pigment. In addition, the produced quinones are able to react with the sulfhydryl residues of the proteins generating oxidative damage and affecting the cell growth. The oxidative damage, involving both lipids and proteins of the cellular membranes, may thus account for the depigmenting action. Functional studies have demonstrated that HQ and other phenolic compounds, such as tert-buthyl-phenol, may act even through different mechanisms, including the oxidation of TRP1, and by interfering with RNA and DNA synthesis. HQ has been identified as the main depigmenting agent, whereas among the several phenolic derivatives, the monobenzyl ether of hydroquinone (MBEH) appeared as the more handfuls one. In this chapter, we will review and compared various established and potential depigmentation agents and emerging therapies that can be used in extensive and universal vitiligo (Figs. 37.1 and 37.2).

37.2 Ideal Depigmenting Agent

An ideal depigmenting agent should have a potent, rapid, and selective effect on melanocytes; it should lead to permanent depigmentation and should be nontoxic with least side effects [3]. Presently, there is no ideal agent for depigmentation.

37.3 Patient Selection

The first step in depigmentation is to choose an appropriate patient. Only patients with extensive vitiligo should be treated, and this should be done only after trying other possible therapies. The patient should be informed that these therapies are a potent depigmenting agent and not used a cosmetic purpose [1, 2]. The subjects with high skin phototypes (V and VI), have a disfiguring - especially when exposed areas are involved (face or the hands), may be candidate to depigmentation. Moreover, incomplete or trichrome repigmentation (e.g., when using UV light) may cause more disfigurement. The patients should be informed that the repigmentation might occur in vitiligo lesions, causing further depigmenting cycles. Patients must be informed that these treatments lead to a definitive irreversible depigmentation.

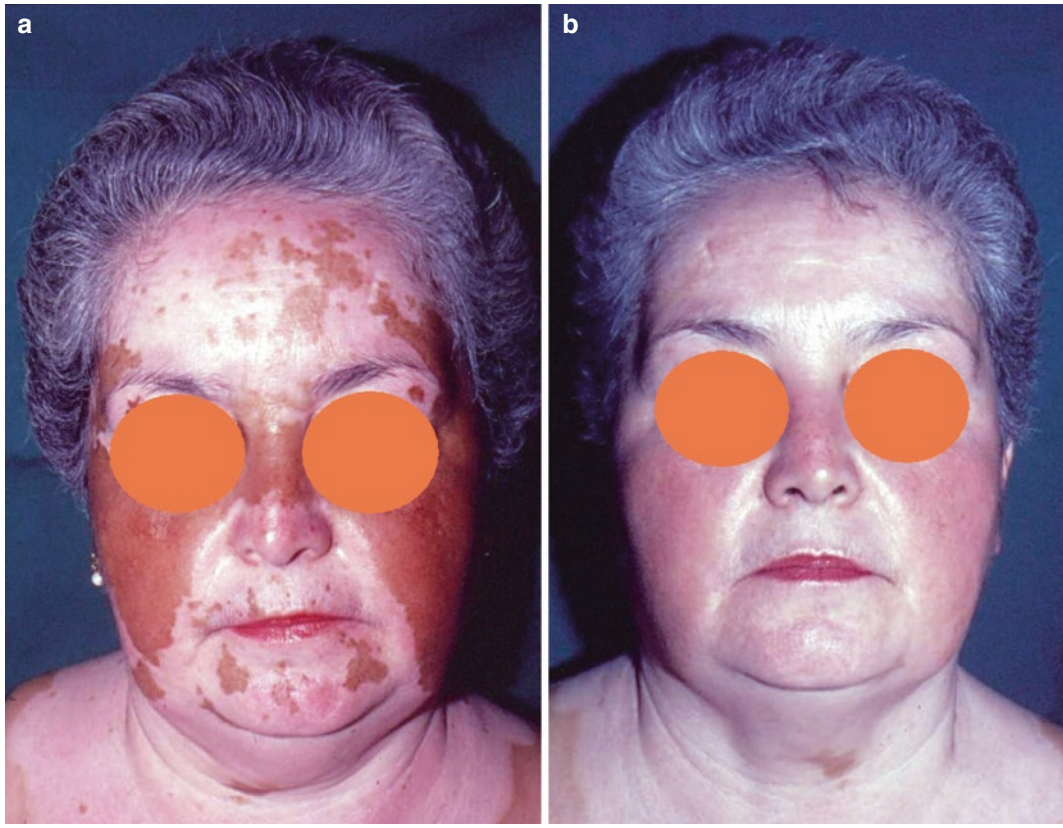


Fig. 37.1 Residual patches of pigmentation (**a**) Results after monobenzone therapy (courtesy prof. Falabella)

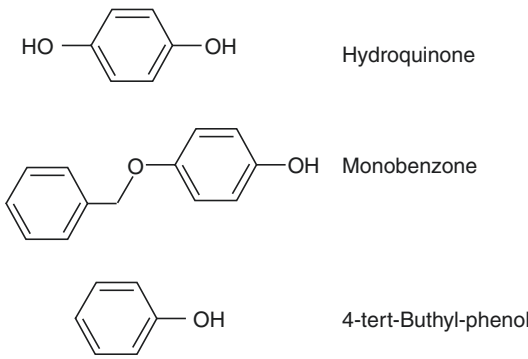


Fig. 37.2 Chemical structure of some hydroquinone-related compounds with depigmenting activity

Depigmentation therapy should be avoided in children less than 12 years of age [4]. Younger patients should be given the option of repigmentation [5]. Table 37.1 shows the patients in whom depigmenting therapy should be considered, and Table 37.2 depicts the possible current approaches.

Table 37.1 Patients in whom depigmenting therapy should be considered

1. Vitiligo involving >50% of BSA
2. Patients with progressive disease and fail to regimentation therapy
3. Vitiligo involving face and hands which affect severally patient quality of life
4. Vitiligo on area resistant to treatments, e.g., hands, malar area

37.4 Agents for Depigmentation

37.4.1 Monobenzyl Ether of Hydroquinone (MBEH)

MBEH is a derivate of hydroquinone, also known as monobenzone or *p*-(benzyloxy) phenol. It is the most potent and mainstay depigmenting agent. It is the only drug USFDA approved for depigmentation in vitiligo [3].

Table 37.2 Summary of depigmenting agents

	Mechanism of action	Dose/concentration	Outcome	Side effect	Remarks
MBEH	After converting to quinone, it induces melanocyte death	10%, 20%, 30%, 40% Once to twice daily	1 month	Dermatitis, pruritus, xerosis, conjunctival melanosis, and distal depigmentation	USFDA approved Patient use it at home
Tretinoin with MBEH	Inhibition of enzyme glutathione *S*-transferase in melanocytes	0.025–0.1%	10 days	Erythema, desquamation, dermatitis, and distressing hyperpigmentation	With combination you can avoid high concentration of MBEH
4-Methoxyphenol	Like MBEH	20% cream	4–12 months	Skin irritation but less than MBEH	Take longer time than MBEH
88% phenol solution	Compromise melanocytic activity	88%	Two sessions, 45 days apart	Scar, dyschromia, high-dose is toxic	Cheap, no complications in experienced hands
Laser	Selectively targeting melanocytes for destruction by photothermolysis	Q-switched lasers like 1064, 755, 694 and 532 nm	Might require multiple sessions	Painful; requires local anesthesia	Rapid result Costly Only in clinic
Cryotherapy	Intracellular ice formation leads to irreversible melanocyte damage	Liquid nitrogen for 10–20 s	4 weeks	Permanent scarring, if performed aggressively Pain, edema, bullae	Only small area treated Cheap On clinic
Imatinib	Tyrosine kinase inhibitor	800 mg/day for 6 months by the systemic route	4 weeks	Periorbitaledema, fluid retention, nausea, emesis, diarrhea and myelosuppression	
Imiquimod	Stimulation of the innate immune response and cell-mediated adaptive immunity	5% cream	3 months	Burning, itching and pain at the target site	Depigmentation not involve untreated area
Diphencyprone	Immunomodulatory action	0.0001%	10.5 months	Local eczema with blistering, regional lymphadenopathy and contact urticaria	

37.4.1.1 Mechanism of Action

There are many theories on how MBEH causes depigmentation. These include the following [6]:

1. Reacting with tyrosinase as the key enzyme during melanin synthesis forming quinone. The quinone formed fastens with cysteine found in tyrosinase proteins (sulfhydryl (-SH) group) to form hapten-carrier compounds. This means that formation of neo-antigens in the tyrosinase proteins enables a systemic, melanocyte destruction and an inflammatory reaction.
2. MBEH produces reactive oxygen species (ROS), for example, peroxide, by inducing cellular oxidative stress in the unprotected pigmented cells. This in turn leads to lysosomal degradation of melanosomes by autophagy. Additionally, there is interference of the melanosome structure and membranes. Henceforth, the major histocompatibility complex (MHC) class I and II routes and initiation of melanocyte Ag-specific T-cell responses cause an increase in surface expression of melanosomal antigens.
3. Generation of ROS contributes to immune response resulting from the release of exosomes containing tyrosinase and MART-1 antigen.
4. MBEH-exposed skin presents with rapid and persistent innate immune activation. Moreover, MBEH is a contact-sensitizer inducer of a type IV delayed-type hypersensitivity response against the quinone-hapten. However, this only occurs if there is production of pro-inflammatory cytokines such as interleukin (IL)-1b and IL-18 by the Langerhans cells or keratinocytes.

37.4.1.2 Treatment Procedure

The application of MBEH is usually done by the patient at home. First, test spot is advised over a normal pigmented skin on the forearm to assess the development of contact dermatitis. If there is no contact dermatitis, the cream can be used on the face or areas of top priority and then move in stages for low-priority areas. Different concentrations of MBEH can be used at one time, for example, 5% on the neck, 10% on the face, and 20% on the arms and legs. There are some patients who fail to lighten with 20% MBEH over a course of 3–4 months; in these patients, the concentration of MBEH can be increased to 30% and then, if there is no response over a similar time period, to 40%. Concentrations of 30% and 40% MBEH have been used primarily on the extremities, especially the elbows and knees. Concentrations greater than this are not recommended [5].

When the vitiligo has been stable for years, a longer duration of therapy and higher concentrations of MBEH may be required [5].

Gradually, depigmentation occurs over a period of 4–12 months [5]. Depigmentation is mostly irreversible and histologically associated with loss of melanosomes and melanocytes [3].

37.4.1.3 Precautions

Patients should always be informed about some precautions while using MBEH:

1. Application of MBEH at one site can lead to loss of pigment at distant body sites, i.e., application of MBEH to the arm may result in loss of pigment on the face [4].
2. The pigment loss is permanent.
3. Avoid application over areas close to the eye [5].
4. Avoid skin-to-skin contact with another person because it can cause a decrease in pigmentation at the site of contact in the other person.
5. Daily use of sunscreens with a sun protection factor (SPF) of 30 or more throughout the year is essential to prevent repigmentation as well as sunburn reactions [4].
6. Follicular repigmentation can occur spontaneously or after sun exposure over the exposed areas of face and forearms. It may vary from as soon as therapy is discontinued [7] to months and years [5]. Repigmentation occurs in MBEH because it often does not destroy follicular melanocytes. One theory is that there is a lack of penetration of the MBEH to the level of the hair matrices, while a second theory is that two populations of melanocytes exist in the skin, the population more resistant

to vitiligo as well as to depigmentation therapy residing in the hair follicle. Hair, eyebrows, and eyelashes may be resistant to depigmentation for the same reason [5].

37.4.1.4 Side Effects

Irritant contact dermatitis, more than allergic, can develop mainly in areas of pigmented rather than vitiliginous skin [8]. If this happens, MBEH is withheld, and open wet dressings are applied to the affected area along with topical steroids. When the dermatitis subsides, MBEH can be reconstituted at a lower concentration of 5%. Frequency of application is also reduced and gradually advanced as tolerated. Mixing MBEH with emollients at the time of application may also decrease irritant reactions [5]. Other side effects include exogenous ochronosis [9], unmasking of telangiectasias and phlebectasias on the lower extremities [5], pruritus, xerosis, erythema, rash, edema, conjunctival melanosis, and distant depigmentation [4]. Risk of carcinogenesis cannot be ruled out, and hence it is banned from European Union since 2001 in cosmetics [10].

37.4.1.5 How to Increase the Efficacy of Monobenzyl Ether of Hydroquinone?

MBEH alone may need to be used occasionally even beyond 1 year to maintain depigmentation. MBEH therapy can be combined with all-trans retinoic acid (ATRA) to enhance its depigmenting and melanocytotoxic effects via inhibition of enzyme glutathione *S*-transferase in melanocytes. This way, contact dermatitis can be avoided by using high concentrations of 40% MBEH. However, combination of ATRA-MBEH did not affect hair pigmentation in animal studies [11].

37.4.2 Monomethyl Ether of Hydroquinone/4-0 Methoxyphenol

This compound is a phenol derivative and is also known as *p*-hydroxyanisole (HA) or mequinol [3].

37.4.2.1 Mechanism of Action

This is similar to that of MBEH. Through a dose-dependent response manner, melanocytes in the hair follicles may also be affected by 4-methoxyphenol (MP), but because of their deeper localization, these melanocytes are less susceptible to the compound compared with the epidermal melanocytes [3].

37.4.2.2 Treatment Procedure

The compound can be used in 20% concentration in an oil/water cream base. Patients are instructed to first apply the cream on a normal pigmented test spot (as big as 5 cm^2) to observe whether an allergic reaction would occur in the next 48 h. Patients with a negative allergic reaction are allowed to apply the cream on the remaining pigmented skin areas twice daily until complete depigmentation is observed [12]. The effectiveness of 4MP has been correlated with the duration of use of the cream; the longer the cream was used, the better the results [3].

Combination product of 2% 4-hydroxyanisole (mequinol) and 0.01% tretinoin was tested in a double-blind multicenter study and was found to significantly improve solar lentigenes and related hyperpigmented lesions of the face and hands after a twice-daily application of up to 24 weeks [3].

37.4.2.3 Side Effects

Like MBEH, 4MP also produces side effects like mild burning or itching, irregular leukoderma, contact dermatitis, ochronosis, and risk of carcinogenesis cannot be ruled out [10]. Protection from sunlight is necessary or repigmentation risk is high [3, 10].

37.4.2.4 4-Methoxyphenol vs. Monobenzyl Ether of Hydroquinone

4MP is as effective as MBEH, but the side effects like skin irritation are less common and less severe. Melanocytotoxic properties of 4MP are comparable with those of MBEH. However, compared with MBEH cream, a disadvantage of 4MP is the longer time required prior to the onset of visible depigmentation (between 4 and

12 months), whereas it was previously reported that depigmentation with MBEH may already be evident after 1 month [3].

37.4.3 88% Phenol Solution

Phenol is inexpensive and has been used topically for chemical peelings. A non-occluded 88% phenol application in a small area (area <20% of face or neck area) does not demand necessary care as with Baker-Gordon's phenol formula (40–50% phenol). This is because 88% phenol, being more concentrated, rapidly coagulates epidermis thus itself stopping its own further percutaneous penetration; on the other hand, Baker-Gordon's formula, being more dilute, does not coagulate epidermis, hence showing enhanced penetration and consequently systemic absorption [13].

37.4.3.1 Mechanism of Action

All phenol compounds are toxic to melanocytes. Transient or definite hypopigmentation is a feature of phenol cauterizing, and this is due to the development of a melanocytic incapacity to normally synthesize melanin, i.e., phenol does not cause melanocyte destruction; rather, it compromises its activity [13]. On the other hand, other depigmenting agents, such as hydroquinone and MBEH, destroy melanocytes.

Protein coagulation is observed in the epidermis immediately after application of 88% phenol solution. If phenol is re-applied, depth will be greater, with the capacity of reaching reticular dermis [3].

37.4.3.2 Treatment Procedure

First the skin is cleaned with gauzes soaked with alcohol. The use of a swab moistened with phenol is used to treat small areas, till cutaneous frosting occurs. The patient feels a burning sensation for approximately 60 s, which gradually decreases in intensity, and it can last from minutes to hours. After two sessions, 45 days apart, total elimination of residual pigmented areas is noticed. No signs of repigmentation have been seen till after 1½ year of therapy [13].

37.4.3.3 Post-procedure Care

Delicate cleaning with saline and use of antibiotic ointment with steroids of mild to moderate potency and sun blocks should be done. Antiviral use is indicated for patients with a history of herpes simplex.

37.4.3.4 Side Effects

In experienced hands, 88% phenol solution does not produce any complications. However, sometimes 88% phenol solution produces complications such as non-esthetic scar formation, dyschromia, and development of herpetic eczema. High-dose phenol usage is toxic, so it should not be applied over large areas. Its cellular uptake is both rapid and passive because of its lipophilic character and signs of systemic toxicity develop soon after exposure. Phenol's main target organs are the liver, kidney, respiratory, and cardiovascular systems. Cardiovascular shock, cardiac arhythmias, and bradycardia, as well as metabolic acidosis, have been reported within 6 h of skin peeling procedures with phenol. Repigmentation may occur if patients do not protect themselves properly from ultraviolet radiation [13].

37.4.3.5 88% Phenol vs. Monobenzyl Ether of Hydroquinone

With phenol, the side effects are lesser. It can be used in areas where MBEH is not available. It is a cheap, practical product, with no complications in experienced hands [13].

37.4.4 Laser Therapy

Lately, laser therapies have been recommended for vitiligo depigmentation due to their fast, effective, and safe nature with short follow-up durations.

They have been reported to be more effective for positive Koebner phenomenon vitiligo patients [14].

Additionally, lasers are advocated for MBEH and other bleaching agent failure cases more so on areas such as the face where short-term rapid depigmentation is required. Moreover, to overcome side effects of topical therapies, e.g., itch-

ing, redness, burning, high failure rates with only partial depigmentation/possible repigmentation, and long treatment follow-up, laser has been documented to overcome these disadvantages. Additionally risks of scar formation are minimized with laser therapies [12].

37.4.5 Q-Switched Ruby (QSR) Laser (694 nm)

Njoo et al. reported a successful vitiligo depigmentation QSR laser [12]. They noted that for extremities big confluent pigment areas, topical therapy can be used first with combined therapy giving better results but with risks of rapid depigmentation as early as 1 or 2 weeks after therapy.

37.4.5.1 Mechanism of Action

Laser therapies have been demonstrated to be very effective in identifying the melanocytes that cause destruction and therefore resulting to depigmentation. QSR lasers in particular have wavelengths between 600 and 800 nm, which are known to prompt selective photothermolysis for pigmented lesions due to their faster absorption by melanin. Moreover, QSR laser duration of pulse energy is known to be shorter than melanosomes relaxation time thereby releasing no energy to the surrounding tissues [3].

37.4.5.2 Treatment Procedure

QSR laser releases energy pulses of 694 nm in wavelength, within 25–28 ns in 1–1.2 Hz frequency [12]. Depending on the skin type, a 5 mm to the pigmented spot can be administered pulse energy of 0–10 J/cm^2, which can be varied to 10–40 J/cm^2 accordingly [14]. Initially, a 5 cm^2 test spot is treated and clinical depigmentation evaluated after 8 weeks. Where depigmentation is noted, more laser treatment is done until total depigmentation is achieved. In cases of no depigmentation, laser treatment is stopped. For pain management, laser procedure is done under eutectic mixture of 25 mg/g lidocaine (EMLA) and prilocaine each.

However, a maximum of 80 cm^2 is allowed per session with the area treated covered with sterile gauze and patients advised to avoid sunlight exposure for a period of 6 weeks. For larger affected areas, multiple lasers are performed at 2–4-week intervals to achieve total depigmentation [12].

37.4.5.3 Tanning of Skin Prior to Using Q-Switched Ruby Laser

Selective photothermolysis targets activated melanocytes that are triggered by tanning of the skin. Henceforth, performing QSR laser after tanning can permanently destroy activated melanocytes. Kim et al. [7] reported no repigmentation after 1 year follow-up where QSR was performed after tanning.

37.4.6 Q-Switched Alexandrite Laser (755 nm)

In a case study by Rao and Fitzpatrick [15], a 68-year-old woman had 18 sessions of QSR laser and 20% MBEH application in 5 years with a residual pigmentation clearance failure. It was reported that there was noted repigmentation within 3 months of QSR laser session. Henceforth, ten sessions (mean per session, 3.4–6.0 J/cm^2, 3–4 mm, 484–1636 pulses) of Q-switched alexandrite (QSA) laser (755 nm, 50–100 ns) were performed to the resistant pigmented patches with discontinuation of topical MBEH therapy. A notable clearance of all treated sites was noted within 22 months and minimal repigmentation at 12 months follow-up.

QSA 755 nm has more advantages compared to the 694 nm QSR due to its faster frequency that helps in rapid therapy and improved results from its higher tissue penetration [15].

However, there are other potential Q-switched lasers that can selectively destruct melanocytes which include neodymium-yttrium aluminum garnet (Nd:YAG) laser (1064 nm) and the frequency-doubled Nd:YAG laser (532 nm) [3].

37.4.6.1 Side Effects

The main disadvantage is that the procedure is painful requiring local anesthesia. Additionally,

this treatment is expensive as it can only be done in a clinic setting. There is also possible failure in removing pigmented patches even after several treatment months because follicular repigmentation due to movement of perifollicular melanocytes to the epidermis showing that they were not completely destroyed by laser treatment. This condition is known as Koebner phenomenon. Patients with active vitiligo respond better to laser treatments compared to those with stable vitiligo. Henceforth, patients who are Koebner negative relapse [12].

37.4.7 Cryotherapy

When rapid depigmentation is desirable, physical agents like cryotherapy and lasers work faster than bleaching agents.

37.4.7.1 Mechanism of Action

Vitiligo patches with positive Koebner responds well to cryosurgery. Intracellular ice formation leads to irreversible tissue damage. In this aspect, melanocytes are more sensitive to cryotherapy damage in comparison with other epidermal cells. The degree of damage depends on the rate of cooling and minimum temperature achieved. Inflammation develops within 24 h of treatment, further contributing to destruction of lesion through immunologically mediated mechanisms. Mild freezing leads to a dermoepidermal separation, which is useful in treating epidermal lesions.

37.4.7.2 Treatment Procedure

Spot testing by a single freeze-thaw cycle is done, and when the edema and erythema subside, the patches are treated with cryotherapy 3–6 weeks later. Both CO_2 and liquid N_2 can be used. A 2-cm flat-topped and round cryoprobe is held approx 40 mm from the skin surface. The whole patch can be frozen with a single freeze-thaw cycle from the periphery and then by forming successive rows inward. Procedure should be terminated when a narrow (<1 mm) frost rim forms around the periphery of the cryoprobe. The rim can develop within 10–20 s by a cryogun connected to a container with barometric pressure above 80 kg/cm^2. For lesions around the orbits or uneven areas of the nose, cyoprobes with smaller diameters may be required. No more than one freeze-thaw cycle is advised. However, another study has used two freeze-thaw cycles also [16].

After a week, a depigmented, unscarred, slightly atrophic, and erythematous smooth area appears. The best cosmetic result is obtained 4 weeks after cryotherapy. The depigmentation is permanent, although more than one session may be required for partially depigmented lesions, with 4–6 weeks intervals, before complete depigmentation occurs [10].

In those with more extensive pigmented patches, cryotherapy can be performed in several sessions 1–3 weeks apart in order to prevent discomfort to the patient. Spot cryotherapy can be used for areas which repigment.

37.4.7.3 Advantage

Cryotherapy has been suggested to depigment MBEH-resistant skin. The procedure requires no anesthesia and can be performed in an outpatient department. No dressing, sedatives, or antibiotics are required. Preparation time is short and inexpensive. The risk of infection is low and wound care is minimal. This method is simple, easy to perform, safe, efficacious, and cost effective. Depigmentation developed by cryotherapy is permanent and without scarring if performed by experienced dermatologists. Many patients prefer a single short-term procedure than applying an expensive compound for 10 months or more with unpredictable effects and a considerable failure rate.

37.4.7.4 Disadvantage

A qualified experienced person is required for this, and hence treatment is hospital based. Also, cryotherapy is suitable for small lesions, and a single sitting cannot be utilized for depigmenting extensive areas unlike lasers.

37.4.7.5 Side Effects

Immediate side effects include edema, pain, and bulla formation. If cryotherapy is performed aggressively, it can lead to permanent scarring. Cryotherapy should be used by experienced person.

37.4.8 Imatinib

Skin depigmentation (whitening/generalized hypopigmentation) from imatinib first reported in patients of chronic myeloid leukemia [17]. Skin became darker during the discontinuation period and began lightening again once imatinib mesylate treatment was resumed.

37.4.8.1 Mechanism of Action

It is postulated that imatinib mesylate, being a tyrosine kinase inhibitor, may interfere with the production of melanin, resulting in decreased pigmentation of the skin. After patients begin receiving imatinib mesylate, hypopigmentation can be observed within 12 weeks. It is difficult to define the onset exactly, because the change is gradual. An ethnic and/or genetic basis has also been considered [17].

37.4.8.2 Side Effects

The side effects of imatinib mesylate are periorbital edema, fluid retention, weight gain, musculoskeletal pain, headache, nausea, diarrhea, and myelosuppression. In addition, a number of dermatological side effects have been documented, such as follicular mucinosis, erythroderma, and lichenoid eruption. Imatinib mesylate can also induce local or generalized hyperpigmentation. Severe congestive cardiac failure is an uncommon but recognized side effect of imatinib. Imatinib in children can delay normal growth, although a proportion will experience catch-up growth during puberty [3].

37.4.9 Imiquimod

Imiquimod is a novel imidazoquinoline immune response modifier, frequently used for topical treatment of anogenital warts and basal cell carcinomas [18].

37.4.9.1 Mechanism of Action

Imiquimod increases production of pro-inflammatory cytokines, mainly interferon (IFN)-α, tumor necrosis factor (TNF)-α, and IL-6, IL-8, IL-10, and IL-12, all of which augment the type 1 helper T-cell (TH1) response which is found to be prominent in the pathogenesis of vitiligo [3]. Imiquimod also stimulates CD8 cells to become cytotoxic and enhances antigen presentation [19]. Recently, it was reported that human melanocytes express toll-like receptor 7 (TLR7). When applied topically, imiquimod binds to TLR7 followed by stimulation of various cytokines, which induce the abovementioned T lymphocytic response [20]. Imiquimod also has a direct action on melanocytes via apoptosis of melanocytes. This action is related to reduction of expression of Bcl-2 and/or an increase in the proapoptotic stimulus (cytotoxic T lymphocytes, natural cytotoxic T cells/killer cells, granzymes B, Fas, TNF, Bax, etc.) [21].

Therefore, it is possible that imiquimod may cause elimination of melanocytes by direct influence on cells as well as inducing acquired immunity indirectly, thus inducing vitiligo-like hypopigmented lesions.

37.4.9.2 Method of Application

Five percent imiquimod use may be followed by erythema which gradually turns to depigmented patches over a period of 3 months. No repigmentation has been seen till 6 months after the depigmentation. Also, depigmentation did not extend to areas that had not been treated with imiquimod [22]. The depigmenting effects of imatinib and imiquimod have only been reported in a few studies, and randomized control trials are lacking. Hence, further studies are required on these agents and other similar molecules before they can be used as mainstream depigmenting agents.

37.4.9.3 Side Effects

The most common side effects of imiquimod are burning, itching, pain, erythema, erosions, and scabbing/crusting at the target site which occur more frequently with twice-daily application [3].

37.4.10 Diphencyprone (DPCP)

Topical application of DPCP when used for the treatment of alopecia areata was found to produce depigmentation as part of its side effects. Duhra and Foulds [23] reported a case of

alopecia totalis in whom sensitization therapy with topical DPCP was commenced. There was marked reaction with erythema and edema on the forearm after 3 days, but the scalp manifested only slight macular erythema. The forearm reaction subsided after 2 weeks and was replaced 6 weeks later by a depigmented patch. Similar depigmented areas appeared on the nape of the neck and the midline of the back. These remained unchanged for 2 years after discontinuing DPCP therapy. Electron microscopy and incubation with dopa in affected skin revealed an absence of melanosomes and melanocytes.

DPCP-induced vitiligo is rare and may represent a Koebner phenomenon in predisposed individuals. Vitiligo can develop even with DPCP concentrations as low as 0.0001% [23].

37.4.10.1 Side Effects

Adverse effects include local eczema with blistering, regional lymphadenopathy, hyperpigmentation, hypopigmentation, and vitiligo [18].

References

1. Nordlund JJ. Depigmentation for the treatment of extensive vitiligo. In: Hann SK, Nordlund JJ, editors. Vitiligo. Lucon: Blackwell Science; 2000. p. 207–13.
2. Solano F, Briganti S, Picardo M, et al. Hypopigmenting agents: an updated review on biological, chemical and clinical aspects. Pigment Cell Res. 2006;19:550–71.
3. Alghamdi KM, Kumar A. Depigmentation therapies for normal skin in vitiligo universalis. J Eur Acad Dermatol Venereol. 2011;25:749–57.
4. Drake LA, Dinehart SM, Farmer ER, Goltz RW, Graham GF, Hordinsky MK, et al. Guidelines of care for vitiligo. J Am Acad Dermatol. 1996;35:620–6.
5. Bolognia JL, Lapia BK, Somma S. Depigmentation therapy. Dermatol Ther. 2001;14:29–34.
6. Van doon Boorn JG, Melief CJ, Luiten RM. Monobenzone induced depigmentation: from enzymatic blockade to autoimmunity. Pigment Cell Melanoma Res. 2011;24:673–9.
7. Kim YJ, Chung BS, Choi KC. Depigmentation therapy with Q-switched ruby laser after tanning in vitiligo universalis. Dermatol Surg. 2001;27:969–70.
8. Lyon CC, Beck MH. Contact hypersensitivity to monobenzyl ether of hydroquinone used to treat vitiligo. Contact Dermatitis. 1998;39:132–56.
9. Charlin R, Barcaui CB, Kac BK, Soares DB, Rabello Fonseca R, Azulay-Abulafia L. Hydroquinone induced exogenous ochronosis: a report of four cases and usefulness of dermoscopy. Int J Dermatol. 2008;47:19–23.
10. Radmanesh M. Depigmentation of the normally pigmented patches in universal vitiligo patients by cryotherapy. J Eur Acad Dermatol Venereol. 2000;14:149–52.
11. Kasraee B, Fallahi MR, Ardekani GS, Doroudchi G, Omrani GR, Handjani S, et al. Retinoic acid synergistically enhances the melanocytotoxic and depigmenting effects of MBEH in black guinea pigs skin. Exp Dermatol. 2006;15:509–14.
12. Njoo MD, Vodegel RM, Westerhof W. Depigmentation therapy in vitiligo universalis with topical 4methoxyphenol and the Q-switched ruby laser. J Am Acad Dermatol. 2000;42:760–9.
13. Zanini M. Depigmentation therapy for generalized vitiligo with topical 88% phenol solution. An Bras Dermatol. 2005;80:415–6.
14. Thissen M, Westerhof W. Laser treatment for further depigmentation in vitiligo. Int J Dermatol. 1997;36:3868.
15. Rao J, Fitzpatrick RE. Use of the Q-switched 755 nm alexandrite laser to treat recalcitrant pigment after depigmentation therapy for vitiligo. Dermatol Surg. 2004;30:1043–5.
16. Di Nuzzo S, Masotti A. Depigmentation therapy in vitiligo universalis with cryotherapy and 4-hydroxyanisole. Clin Exp Dermatol. 2010;35:215–6.
17. Leong KW, Lee TC, Goh AS. Imatinib mesylate causes hypopigmentation in the skin. Cancer. 2004;100:2486–7.
18. Halder RM, Taliaferro SJ. Vitiligo. In: Wolff K, Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Lefell DJ, editors. Fitzpatrick's dermatology in general medicine, vol. 1. 7th ed. New York: McGraw Hill; 2008. p. 61622.
19. Zirvi TB, Costarelis G, Gelfand JM. Vitiligo-like hypopigmentation associated with imiquimod treatment of genital warts. J Am Acad Dermatol. 2005;52:715–6.
20. Kang HY, Park TJ, Jin SH. Imiquimod, a toll-like receptor 7 agonist, inhibits melanogenesis and proliferation of human melanocytes. J Invest Dermatol. 2009;129:243–6.
21. Kim CH, Ahn JH, Kang SU, Hwang HS, Lee MH, Pyun JH, et al. Imiquimod induces apoptosis of human melanocytes. Arch Dermatol Res. 2010;302:301–6.
22. Senel E, Seckin D. Imiquimod-induced vitiligo-like depigmentation. Indian J Dermatol Venereol Leprol. 2007;73:422–3.
23. Duhra P, Foulds IS. Persistent vitiligo induced by diphencyprone. Br J Dermatol. 1990;123:415–6.

38 Combined/Sequential/Integrated Therapies for Vitiligo

Thierry Passeron

Contents

Abstract

Vitiligo is a challenging disorder to treat, and to date no treatment provides truly satisfactory results. However, from molecules that target the immune system to therapies that stimulate the proliferation and differentiation of melanocytes and/or melanocyte progenitors and others that fight against radical species or bring new melanocytes to the affected areas, we have many strategies to repigment vitiligo patches. Association of surgical procedures with phototherapy was the first to demonstrate that combination approaches could further increase repigmentation rates. The interest of combining medical approaches is now also well demonstrated in several prospective randomized studies. This is mainly true for the association of topical treatments, such as calcineurin inhibitor or topical corticosteroids, with phototherapy. Other interesting associations are those between antioxidants and lasers, but the data remain limited. New strategies have been also reported aiming to reduce the risk of relapse after achieving repigmentation. The development in the near future of

T. Passeron (✉)
University Côte d'Azur, Department of Dermatology,
University Hospital of Nice,
Nice Cedex 3, France

University Côte d'Azur, INSERM U1065,
team 12, C3M, Nice, France
e-mail: passeron@unice.fr

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_38

targeted immune treatments but also new topical drugs aiming to enhance the differentiation of melanocyte stem cells should lead to new and hopefully more effective combination or sequential protocols adapted to the severity and the activity of each vitiligo.

Key Points

- Combination approaches were first reported associating surgical procedures and phototherapy.
- Increasing data have emphasized the interest of combining also medical approaches for enhancing repigmentation rates.
- Recent data also reported effective strategy for decreasing the risk of relapses after achieving effective repigmentation.

38.1 Combination of Surgical Therapies and Phototherapy

Surgical therapies of vitiligo such as punch or blister or split thickness grafting or more recently transplanted autologous melanocyte or epidermal cell suspensions have been the first therapeutic options to be associated with phototherapy. In 1983, Bonafé et al., first reported the combined use of skin grafts followed with psoralen plus ultraviolet A (PUVA) therapy [1]. The combination of several surgical therapies with PUVA has been then further studied and remains today the best studied combination in vitiligo treatment. Indeed, several studies have shown that adjunction of PUVA following surgical treatment enhances the repigmentation rate of vitiligo patches [2–10]. Most of authors add phototherapy several weeks after the surgical procedures (usually 3 or 4 weeks after) and use standard phototherapy protocols for vitiligo treatment. The interest of combining phototherapy and surgical procedures has been proven in a prospective, randomized, double-blind study that clearly showed that autologous transplanted epidermal cell suspensions followed by narrowband UVB (NB-UVB) or PUVA were clearly superior to phototherapy alone for repigmenting vitiligo [11].

Combination of NB-UVB with surgical therapies has been less studied but also enhances repigmentation [11–13]. One prospective randomized intra-individual study performed on a limited number of patients ($n = 14$) found no difference between NB-UVB and excimer laser after punch grafting [14]. To the best of our knowledge, there is no direct comparison between NB-UVB and PUVA as a synergic agent for surgical therapies available in the literature. A series of five patients who failed to repigment after the combination of grafting plus PUVA has been reported to finally repigment when the surgical procedure was combined with NB-UVB [15]. Although interesting, this report is not sufficient to prove the superiority of NB-UVB in this indication, and further studies are required.

38.2 Combination of Surgical Therapies and Corticosteroids

Although most combined approaches using surgical therapies have been performed with phototherapy, topical and systemic steroids have also been evaluated. In a prospective study performed on 50 vitiligo patients, the combination of punch grafting with topical steroids (fluocinolone acetonide 0.1%) has been shown to be as effective as punch grafting followed by PUVA [2].

An open series and a case report have suggested that low doses of oral steroids might be beneficial in addition to surgical procedures [16, 17]. This has to be confirmed in a controlled trial.

38.3 Combination of Phototherapy and Topical Steroids

The combined use of UVA and topical steroids has been studied by the Westerhof group. In a prospective, randomized, controlled, left–right comparison study, it was shown that the combination

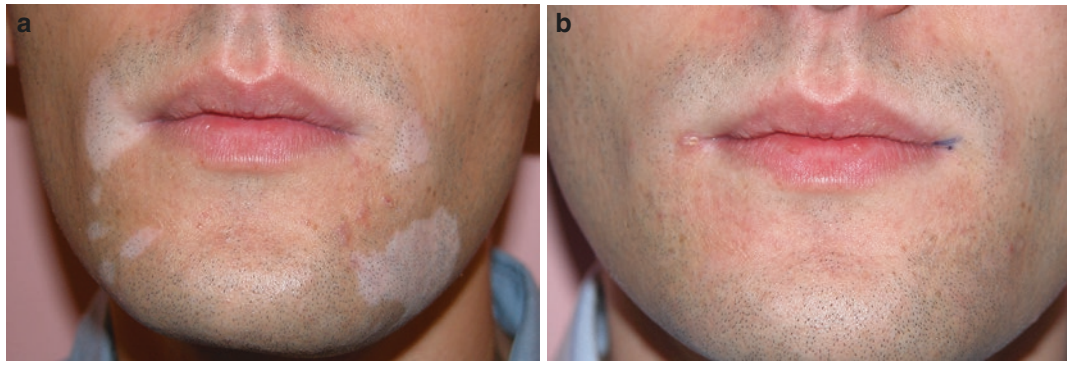


Fig. 38.1 (**a**) Vitiligo patches of the face. (**b**) Clinical aspect after 12 weeks of twice-weekly 308 nm excimer laser combined with daily application of desonide 0.05% cream

of UVA and fluticasone propionate was much more effective than UVA or topical steroid alone [18]. Lately, topical hydrocortisone 17-butyrate cream combined to 308 nm excimer laser was found in a prospective randomized trial to be more effective in the treatment of vitiligo of the face and neck compared to excimer laser alone [19]. However, studies having assessed the association of topical steroids to NB-UVB or excimer laser/light remain limited preventing to draw definitive conclusions as emphasized recently by meta-analyses of the literature (Fig. 38.1) [20, 21].

38.4 Combination of Phototherapy and Topical Calcineurin Inhibitors (TCI)

TCI such as tacrolimus and pimecrolimus have demonstrated their effectiveness for treating vitiligo, but the best results are achieved in sun-exposed areas and especially on the face [22–26]. Treatment with such drugs in monotherapy for other areas provides disappointing results [27]. Two studies first evaluated if the combination of 308 nm excimer laser and topical tacrolimus could be synergistic. They have compared the efficiency of 308 nm excimer combined with tacrolimus ointment to 308 nm excimer laser monotherapy [28] or associated with placebo ointment [29]. In both cases, a total of 24 sessions were evaluated, and tacrolimus ointment was applied twice a day. The results were similar and showed a greater efficiency with the combined treatment as compared to laser monotherapy. Tolerance was good, and side effects were limited to constant erythema, sticking due to ointment and rare bullous lesions. These results have been confirmed by many others, showing that tacrolimus and pimecrolimus used topically achieve greater results when combined to excimer laser/light but also to NB-UVB [30–34]. Studies performed in the pediatric population also underline the efficacy of the combination of topical tacrolimus or pimecrolimus with NB-UVB or excimer laser in children suffering from vitiligo [35, 36]. Recent meta-analysis of the literature on the combination of NB-UVB or excimer laser/light confirmed the superiority of combining TCI to these phototherapies compared to phototherapy alone [20, 21, 37].

The potential increased risk of skin cancers promoted by the association of two immunosuppressive treatments remains to be taken in consideration. However, reassuring data on the use of TCI have been reported [38, 39]. Moreover, penetration of high quantities of calcineurin inhibitors can mostly be observed when used over large surfaces in atopic dermatitis patients where the skin barrier is altered, which is not the case for vitiligo skin. TCI have been used for vitiligo alone or combined with phototherapy for more than 10 years without any indication of risk. Taken together these data are very reassuring concerning the use of TCI combined with UV

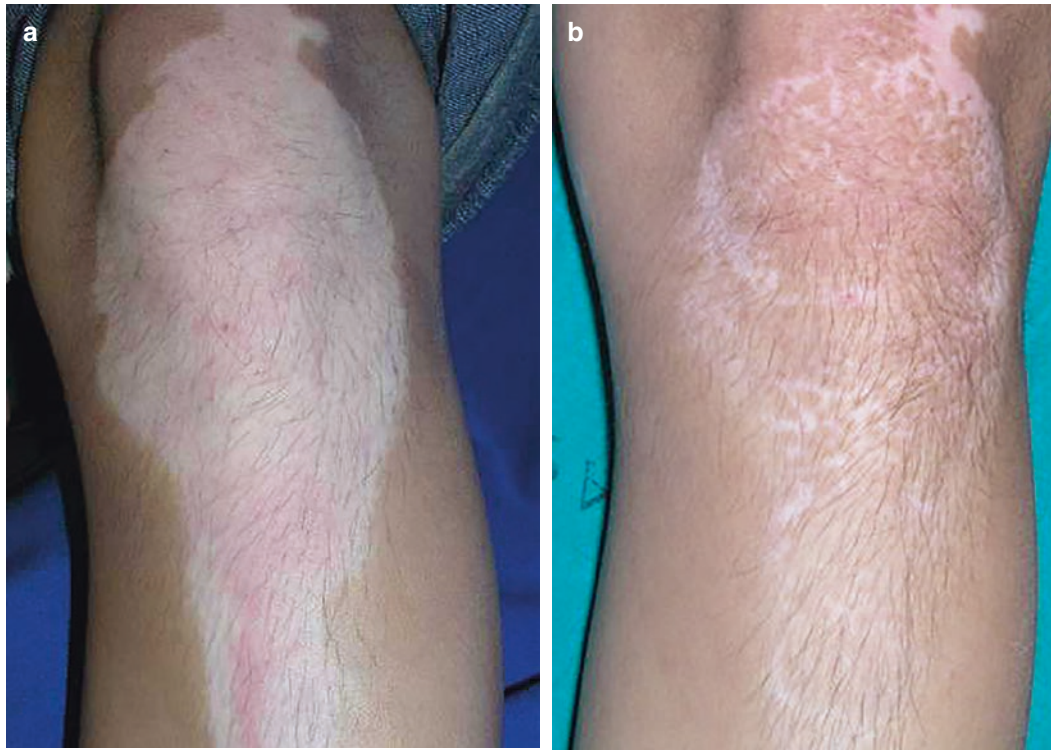


Fig. 38.2 (**a**) Vitiligo of the leg and knee before treatment. (**b**) Clinical aspect after 12 weeks of twice-weekly 308 nm excimer laser combined with twice-daily application of 0.1% tacrolimus ointment

exposures in vitiligo patients; however, a total follow-up of 20–25 years may be required to be completely reassured concerning a potential increased risk of skin cancers. Thus, the risk–benefit ratio needs to be discussed with patients when TCI are proposed in combination to phototherapy or sun exposures (Fig. 38.2).

38.5 Combination of Phototherapy and Topical Vitamin D

The occurrence of repigmentation of vitiligo in patients treated with calcipotriol (a vitamin D3 analogue) for psoriasis has suggested that it might be efficacious in treating vitiligo. It appears now quite clear that calcipotriol in monotherapy is useless for treating vitiligo [40]. The use of topical vitamin D with sun, PUVA, or NB-UVB provided controversial data [41–53]. Recent meta-analysis of the literature couldn't find sufficient evidence to support the interest of combining phototherapy to vitamin D analogues [20, 21]. Thus, accordingly to the existing data, combination approaches with phototherapy should clearly prefer the use of topical steroids or TCI.

38.6 Combination of Phototherapy and Antioxidants

Oxidative stress has been shown to be involved in the pathogenesis of vitiligo. Pseudocatalase has the ability to remove hydrogen peroxide and so could be interesting in the treatment of vitiligo. The combination of topical pseudocatalase with UVB has shown very promising results in a pilot non-RCT study (complete repigmentation on the face and the dorsum of the hands in 90% of patients) [54]. Unfortunately, these results were

not confirmed in another study [55]. Finally, a prospective randomized placebo-controlled study showed that pseudocatalase cream does not add any incremental benefit to NB-UVB alone [56].

Oral antioxidant supplementation was also reported to increase the effectiveness of UVB phototherapy in a prospective double-blind placebo-controlled study [57]. Although this study concerned a relative small number of patients with only 80% having completed the entire treatment, the results are certainly encouraging. This potential interest of oral antioxidants was also supported by a prospective double-blind trial that studied the combination of *Polypodium leucotomos* extract with NB-UVB [58]. Better repigmentation was observed with combination of *Polypodium leucotomos* extract and UV, but the difference was slight and only observed in a subgroup of patients. Of note, no study has been published since 2007 using *Polypodium leucotomos* extract in combination to phototherapy. Thus, despite the demonstrated role of the oxidative stress in the pathophysiology of vitiligo, further studies are mandatory to confirm the interest of oral antioxidants combined with phototherapy.

38.7 Combination Therapies Using Dermabrasion or Fractional Ablative Lasers

Skin ablation with mechanical superficial dermabrasion combined with topical application of 5% 5-fluorouracil (5-FU) was introduced in 1983. The supposed mechanism of action is mainly based on the combination of epidermal removal and induction of irritation mediated by 5-FU, which through the production of inflammatory cytokines and prostaglandins stimulates melanocyte migration and proliferation. In a prospective trial, 50 adult subjects with 64 symmetrical lesions of non-segmental vitiligo were enrolled [59]. One side was treated with ER:YAG laser ablation, followed by 5-FU application before simultaneous NB-UVB therapy of both sides for a maximum period of 4 months. The overall response to therapy was better using the combination therapy with 50 lesions (78%) achieving a moderately or marked repigmentation compared to 14 lesions (22%) of similar effectiveness in the monotherapy group ($p < 0.0001$). Quite surprisingly, only moderate pain during ablation or at sites of 5-FU application was reported in all cases. As 5-FU, dermabrasion and UVB were combined in this study, the respective role of the 5-FU and the dermabrasion in potentiating the UVB is difficult to determine. The impact of the laser-assisted dermabrasion on the repigmentation of vitiligo was assessed in a prospective randomized study performed on symmetrical patches of vitiligo located only in difficult-to-treat areas (such as bony prominences and extremities) [60]. Twenty patients were treated with a combination of topical steroids and NB-UVB for 3 months. On the side that was randomly assigned, a pretreatment with an erbium laser-assisted dermabrasion was performed. The criteria of success were defined as a repigmentation of at least 50% 1 month after the end of the treatment. Two patients were lost to follow-up, and a total of 24 symmetrical lesions were treated. The combination with a preceding dermabrasion provided significant better results with a rate of success of 46% compared to 8% in the group with only topical steroids and UVB ($p < 0.0001$). However, side effects were important with high pain, delayed healing and two hypertrophic scars in the group using dermabrasion. These side effects strongly limit the usefulness of this combination approach in daily practice, but the results clearly show the beneficial role of a preceding dermabrasion. The mechanisms involved in this enhancing effect remain to be elucidated.

A similar approach but using fractional ablative CO_2 laser was then conducted in a prospective randomized trial [61]. Ten patients with symmetrical vitiligo patches were treated with NB-UVB with one side also receiving two sessions of CO_2 fractional ablative laser at 2 months interval. Two months after the end of the treatment, the combination approach was more effective with three patches achieving

moderated or marked improvement compared to none with UVB alone ($p = 0.034$). Although the enhancing repigmentation rate appears to be much lower with fractional CO_2 laser compared to laser-assisted dermabrasion, the tolerance was much better without significant side effects using the fractional ablative laser. These results were corroborated in a small prospective trial showing that fractional CO_2 laser and sun provide better results compared to sun exposure alone [62]. More recently a prospective randomized intra-individual study was conducted on 27 patients with 27 pair-lesions of non-segmental vitiligo located on hands [63]. All lesions received NB-UVB phototherapy and 0.05% clobetasol propionate cream, and one side was randomly assigned to receive also fractional CO_2 laser (ten sessions at 1-week interval). One patient was lost to follow-up, and six vitiliginous lesions (23.1%) in combination group achieved good to excellent repigmentation compared with one lesion (3.9%) in group with topical steroid and NB-UVB ($p = 0.065$). Interestingly, a prospective intra-individual study performed on extremities and/or bony prominences of 25 vitiligo patients showed that topical steroids associated to fractional CO_2 laser and NB-UVB is more effective than fractional CO_2 and phototherapy without steroids [64]. These studies support the interest of combining the three approaches (laser, phototherapy and topical treatment) to achieve optimal results. However, studies performed on larger population are still required to confirm the effectiveness of fractional ablative lasers in potentiating repigmentation in vitiligo and to determine the optimal modalities of the sessions.

Interestingly, the holes made by these fractional ablative lasers but also by microneedles have been shown, using an ex vivo model, to successfully deliver melanocytes into the skin [65]. A pilot study recently suggested that ablative fractional lasers could be used to prepare the bed grafting before using epidermal suspension suggesting that such laser procedure could be also of interest for surgical treatments of vitiligo (Fig. 38.3) [66].

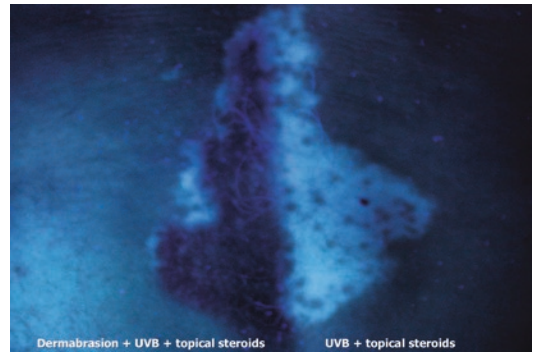


Fig. 38.3 Vitiligo lesion of the back under Wood's lamp examination after 12 weeks of NB-UVB and daily application of topical steroids. Left side has received a pretreatment with an erbium laser-assisted dermabrasion compared to right side that did not received any pretreatment

38.8 Maintenance Therapy

After successful repigmentation, the rate of relapse in vitiligo patches is about 40% [67]. In atopic dermatitis proactive treatment with topical steroids or calcineurin inhibitors has demonstrated their efficacy to decrease flares of the disease [68]. In a two-centre, prospective randomized study, the use of bi-weekly application of 0.1% tacrolimus ointment was compared to placebo [69]. Thirty-five patients with 72 non-segmental vitiligo lesions who achieved at least 75% of repigmentation after phototherapy, topical treatment or a combination approach were included. After 6 months 40% of lesions showed depigmentation in the placebo group compared to 9.7% with the tacrolimus ($p = 0.0075$). The tolerance was good, and the side effects were limited to transient erythema and stinging or burning sensations. This study shows that twice-weekly applications of 0.1% of tacrolimus are effective for decreasing vitiligo relapses.

Accordingly to the data available in atopic dermatitis and the comparable efficacy of topical steroids and TCI for treating vitiligo, it may be hypothesized that topical steroids could also be effective for preventing vitiligo relapse. Many questions remain. How long should this preventive treatment be continued? Are applications

three times a week more effective than only two and thus could further reduce the risk of relapse? We actually proposed this maintenance treatment only in patients with active vitiligo or patients who already had relapses after having achieved repigmentation, and we continue this proactive approach for at least 6 months without any sign of disease activity. However, further studies are clearly required to answer to these questions.

38.9 Conclusions

There are three aims that need to be reached for the optimal care of vitiligo patients. First halting the disease progression, then allowing complete repigmentation of lesional areas and finally preventing relapses. Combination approaches have now clearly showed their interest in treating vitiligo. The usefulness of associating treatments is demonstrated for surgical and medical approaches. Due to the difficulties for achieving full repigmentation and the length of treatments that often requires 6–24 months to provide optimal results, such combination approaches appear of great interest in most vitiligo patients. However, the current approaches mainly aim to repigment vitiligo lesions. Strategies to prevent or at least to reduce the relapses have only been reported recently, and data remain limited. Hopefully, recent advances in the understanding of the pathophysiology of vitiligo foster new therapeutic opportunities letting us hope that we could succeed reaching these three aims. Thus, the demonstrated role of the INFγ/JAK/CXCL10 pathway in the depigmentation process of vitiligo suggests that targeting this pathway might provide very effective approaches for halting disease progression and possibly to prevent relapses [70]. Similarly, the demonstration of an impaired WNT signaling pathway in vitiligo lesions that alters the differentiation of melanocyte stem cells suggests that topical agents stimulating this pathway could help in repigmenting vitiligo lesions including in very difficult areas such as the extremities of the hands and feet [71]. Finally, the development of markers of the severity and the activity of the disease [72, 73] should also help us to choose the more suitable treatments that could be used in combination or sequentially depending on each individual case.

Take-Home Pearls

- Combination approaches have now clearly showed their interest in treating vitiligo. The usefulness of associating treatments is demonstrated for surgical and medical approaches.
- Best evidences are reported with phototherapy (PUVA, NB-UVB, excimer laser/light) associated with surgical procedures or with topical calcineurin inhibitors.
- The use of 0.1% tacrolimus ointment twice weekly is the first approach demonstrated to reduce the risk of relapse after achieving repigmentation of vitiligo lesions.
- Recent advances in the understanding of vitiligo pathophysiology lead to great hope using new therapeutic strategies for halting disease progression or repigmenting vitiligo lesions that could be used in association or sequentially depending on each patient and the current course of his disease.

References

1. Bonafe JL, Lassere J, Chavoin JP, et al. Pigmentation induced in vitiligo by normal skin grafts and PUVA stimulation: a preliminary study. Dermatologica. 1983;166:113–6.
2. Barman KD, Khaitan BK, Verma KK. A comparative study of punch grafting followed by topical corticosteroid versus punch grafting followed by PUVA therapy in stable vitiligo. Dermatol Surg. 2004;30:49–53.
3. Hann SK, Im S, Bong HW, et al. Treatment of stable vitiligo with autologous epidermal grafting and PUVA. J Am Acad Dermatol. 1995;32:943–8.
4. Hann SK, Im S, Park YK, et al. Repigmentation of leukotrichia by epidermal grafting and systemic psoralen plus UV-A. Arch Dermatol. 1992;128:998–9.
5. Lee AY, Jang JH. Autologous epidermal grafting with PUVA-irradiated donor skin for the treatment of vitiligo. Int J Dermatol. 1998;37:551–4.
6. Shenoi SD, Srinivas CR, Pai S. Treatment of stable vitiligo with autologous epidermal grafting and PUVA. J Am Acad Dermatol. 1997;36:802–3.
7. Skouge J, Morison WL. Vitiligo treatment with a combination of PUVA therapy and epidermal autografts. Arch Dermatol. 1995;131:1257–8.

8. Skouge JW, Morison WL, Diwan RV, et al. Autografting and PUVA. A combination therapy for vitiligo. J Dermatol Surg Oncol. 1992;18:357–60.
9. Suga Y, Butt KI, Takimoto R, et al. Successful treatment of vitiligo with PUVA-pigmented autologous epidermal grafting. Int J Dermatol. 1996;35:518–22.
10. Tsukamoto K, Osada A, Kitamura R, et al. Approaches to repigmentation of vitiligo skin: new treatment with ultrasonic abrasion, seed-grafting and psoralen plus ultraviolet A therapy. Pigment Cell Res. 2002;15:331–4.
11. van Geel N, Ongenae K, De Mil M, et al. Double-blind placebo-controlled study of autologous transplanted epidermal cell suspensions for repigmenting vitiligo. Arch Dermatol. 2004;140:1203–8.
12. Lahiri K, Malakar S, Sarma N, et al. Repigmentation of vitiligo with punch grafting and narrow-band UV-B (311 nm)--a prospective study. Int J Dermatol. 2006;45:649–55.
13. Pianigiani E, Risulo M, Andreassi A, et al. Autologous epidermal cultures and narrow-band ultraviolet B in the surgical treatment of vitiligo. Dermatol Surg. 2005;31:155–9.
14. Linthorst Homan MW, Spuls PI, Nieuweboer-Krobotova L, et al. A randomized comparison of excimer laser versus narrow-band ultraviolet B phototherapy after punch grafting in stable vitiligo patients. J Eur Acad Dermatol Venereol. 2012;26:690–5.
15. Lahiri K, Malakar S, Sarma N, et al. Inducing repigmentation by regrafting and phototherapy (311 nm) in punch grafting failure cases of lip vitiligo: a pilot study. Indian J Dermatol Venereol Leprol. 2004;70:156–8.
16. Lee KJ, Choi YL, Kim JA, et al. Combination therapy of epidermal graft and systemic corticosteroid for vitiligo. Dermatol Surg. 2007;33:1002–3.
17. Mulekar SV. Stable vitiligo treated by a combination of low-dose oral pulse betamethasone and autologous, noncultured melanocyte-keratinocyte cell transplantation. Dermatol Surg. 2006;32:536–41.
18. Westerhof W, Nieuweboer-Krobotova L, Mulder PG, et al. Left-right comparison study of the combination of fluticasone propionate and UV-A vs. either fluticasone propionate or UV-A alone for the long-term treatment of vitiligo. Arch Dermatol. 1999;135:1061–6.
19. Sassi F, Cazzaniga S, Tessari G, et al. Randomized controlled trial comparing the effectiveness of 308-nm excimer laser alone or in combination with topical hydrocortisone 17-butyrate cream in the treatment of vitiligo of the face and neck. Br J Dermatol. 2008;159:1186–91.
20. Bae JM, Hong BY, Lee JH, et al. The efficacy of 308-nm excimer laser/light (EL) and topical agent combination therapy versus EL monotherapy for vitiligo: a systematic review and meta-analysis of randomized controlled trials (RCTs). J Am Acad Dermatol. 2016;74:907–15.
21. Li R, Qiao M, Wang X, et al. Effect of narrow band ultraviolet B phototherapy as monotherapy or combination therapy for vitiligo: a meta-analysis. Photodermatol Photoimmunol Photomed. 2017;33(1):22–31.
22. Grimes PE, Soriano T, Dytoc MT. Topical tacrolimus for repigmentation of vitiligo. J Am Acad Dermatol. 2002;47:789–91.
23. Lepe V, Moncada B, Castanedo-Cazares JP, et al. A double-blind randomized trial of 0.1% tacrolimus vs 0.05% clobetasol for the treatment of childhood vitiligo. Arch Dermatol. 2003;139:581–5.
24. Smith DA, Tofte SJ, Hanifin JM. Repigmentation of vitiligo with topical tacrolimus. Dermatology (Basel, Switzerland). 2002;205:301–3.
25. Tanghetti EA. Tacrolimus ointment 0.1% produces repigmentation in patients with vitiligo: results of a prospective patient series. Cutis. 2003;71:158–62.
26. Travis LB, Weinberg JM, Silverberg NB. Successful treatment of vitiligo with 0.1% tacrolimus ointment. Arch Dermatol. 2003;139:571–4; discussion 3.
27. Ostovari N, Passeron T, Lacour JP, et al. Lack of efficacy of tacrolimus in the treatment of vitiligo in the absence of UV-B exposure. Arch Dermatol. 2006;142:252–3.
28. Passeron T, Ostovari N, Zakaria W, et al. Topical tacrolimus and the 308-nm excimer laser: a synergistic combination for the treatment of vitiligo. Arch Dermatol. 2004;140:1065–9.
29. Kawalek AZ, Spencer JM, Phelps RG. Combined excimer laser and topical tacrolimus for the treatment of vitiligo: a pilot study. Dermatol Surg. 2004;30:130–5.
30. Castanedo-Cazares JP, Lepe V, Moncada B. Repigmentation of chronic vitiligo lesions by following tacrolimus plus ultraviolet-B-narrow-band. Photodermatol Photoimmunol Photomed. 2003;19:35–6.
31. Esfandiarpour I, Ekhlasi A, Farajzadeh S, et al. The efficacy of pimecrolimus 1% cream plus narrow-band ultraviolet B in the treatment of vitiligo: a double-blind, placebo-controlled clinical trial. J Dermatolog Treat. 2009;20:14–8.
32. Fai D, Cassano N, Vena GA. Narrow-band UVB phototherapy combined with tacrolimus ointment in vitiligo: a review of 110 patients. J Eur Acad Dermatol Venereol. 2007;21:916–20.
33. Stinco G, Piccirillo F, Forcione M, et al. An open randomized study to compare narrow band UVB, topical pimecrolimus and topical tacrolimus in the treatment of vitiligo. Eur J Dermatol. 2009;19:588–93.
34. Tanghetti EA, Gillis PR. Clinical evaluation of B Clear and Protopic treatment for vitiligo. Lasers Surg Med. 2003;32:37.
35. Dayal S, Sahu P, Gupta N. Treatment of childhood vitiligo using tacrolimus ointment with narrow-band ultraviolet B phototherapy. Pediatr Dermatol. 2016;33(6):646–51.
36. Hui-Lan Y, Xiao-Yan H, Jian-Yong F, et al. Combination of 308-nm excimer laser with topical

pimecrolimus for the treatment of childhood vitiligo. Pediatr Dermatol. 2009;26:354–6.
37. Dang YP, Li Q, Shi F, et al. Effect of topical calcineurin inhibitors as monotherapy or combined with phototherapy for vitiligo treatment: a meta-analysis. Dermatol Ther. 2016;29:126–33.
38. Doelker L, Tran C, Gkomouzas A, et al. Production and clearance of cyclobutane dipyrimidine dimers in UV-irradiated skin pretreated with 1% pimecrolimus or 0.1% triamcinolone acetonide creams in normal and atopic patients. Exp Dermatol. 2006;15:342–6.
39. Tran C, Lübbe J, Sorg O, Doelker L, Carraux P, Antille C, Grand D, Leemans E, Kaya G, Saurat JH. Topical calcineurin inhibitors decrease the production of UVB-induced thymine dimers from hairless mouse epidermis. Dermatology. 2005;211(4):341–7.
40. Chiaverini C, Passeron T, Ortonne JP. Treatment of vitiligo by topical calcipotriol. J Eur Acad Dermatol Venereol. 2002;16:137–8.
41. Ada S, Sahin S, Boztepe G, et al. No additional effect of topical calcipotriol on narrow-band UVB phototherapy in patients with generalized vitiligo. Photodermatol Photoimmunol Photomed. 2005;21:79–83.
42. Anke Hartmann CL, Hamm H, Bröcker E-B, Hofmann UB. Narrow-band UVB311 nm vs. broadband UVB therapy in combination with topical calcipotriol vs. placebo in vitiligo. Int J Dermatol. 2004;44(9):736–42.
43. Arca E, Tastan HB, Erbil AH, et al. Narrow-band ultraviolet B as monotherapy and in combination with topical calcipotriol in the treatment of vitiligo. J Dermatol. 2006;33:338–43.
44. Baysal V, Yildirim M, Erel A, et al. Is the combination of calcipotriol and PUVA effective in vitiligo? J Eur Acad Dermatol Venereol. 2003;17:299–302.
45. Ermis O, Alpsoy E, Cetin L, et al. Is the efficacy of psoralen plus ultraviolet A therapy for vitiligo enhanced by concurrent topical calcipotriol? A placebo-controlled double-blind study. Br J Dermatol. 2001;145:472–5.
46. Goktas EO, Aydin F, Senturk N, et al. Combination of narrow band UVB and topical calcipotriol for the treatment of vitiligo. J Eur Acad Dermatol Venereol. 2006;20:553–7.
47. Goldinger SM, Dummer R, Schmid P, et al. Combination of 308-nm xenon chloride excimer laser and topical calcipotriol in vitiligo. J Eur Acad Dermatol Venereol. 2007;21:504–8.
48. Khullar G, Kanwar AJ, Singh S, et al. Comparison of efficacy and safety profile of topical calcipotriol ointment in combination with NB-UVB vs. NB-UVB alone in the treatment of vitiligo: a 24-week prospective right-left comparative clinical trial. J Eur Acad Dermatol Venereol. 2015;29:925–32.
49. Kullavanijaya P, Lim HW. Topical calcipotriene and narrowband ultraviolet B in the treatment of vitiligo. Photodermatol Photoimmunol Photomed. 2004;20:248–51.
50. Leone G, Pacifico A, Iacovelli P, et al. Tacalcitol and narrow-band phototherapy in patients with vitiligo. Clin Exp Dermatol. 2006;31:200–5.
51. Lu-yan T, Wen-wen F, Lei-hong X, et al. Topical tacalcitol and 308-nm monochromatic excimer light: a synergistic combination for the treatment of vitiligo. Photodermatol Photoimmunol Photomed. 2006;22:310–4.
52. Oh SH, Kim T, Jee H, et al. Combination treatment of non-segmental vitiligo with a 308-nm xenon chloride excimer laser and topical high-concentration tacalcitol: a prospective, single-blinded, paired, comparative study. J Am Acad Dermatol. 2011;65:428–30.
53. Parsad D, Saini R, Verma N. Combination of PUVAsol and topical calcipotriol in vitiligo. Dermatology (Basel Switzerland). 1998;197:167–70.
54. Schallreuter KU, Wood JM, Lemke KR, et al. Treatment of vitiligo with a topical application of pseudocatalase and calcium in combination with short-term UVB exposure: a case study on 33 patients. Dermatology (Basel, Switzerland). 1995;190:223–9.
55. Patel DC, Evans AV, Hawk JL. Topical pseudocatalase mousse and narrowband UVB phototherapy is not effective for vitiligo: an open, single-centre study. Clin Exp Dermatol. 2002;27:641–4.
56. Bakis-Petsoglou S, Le Guay JL, Wittal R. A randomized, double-blinded, placebo-controlled trial of pseudocatalase cream and narrowband ultraviolet B in the treatment of vitiligo. Br J Dermatol. 2009;161:910–7.
57. Dell'Anna ML, Mastrofrancesco A, Sala R, et al. Antioxidants and narrow band-UVB in the treatment of vitiligo: a double-blind placebo controlled trial. Clin Exp Dermatol. 2007;32:631–6.
58. Middelkamp-Hup MA, Bos JD, Rius-Diaz F, et al. Treatment of vitiligo vulgaris with narrow-band UVB and oral Polypodium leucotomos extract: a randomized double-blind placebo-controlled study. J Eur Acad Dermatol Venereol. 2007;21:942–50.
59. Anbar TS, Westerhof W, Abdel-Rahman AT, et al. Effect of one session of ER:YAG laser ablation plus topical 5Fluorouracil on the outcome of short-term NB-UVB phototherapy in the treatment of non-segmental vitiligo: a left-right comparative study. Photodermatol Photoimmunol Photomed. 2008;24:322–9.
60. Bayoumi W, Fontas E, Sillard L, et al. Effect of a preceding laser dermabrasion on the outcome of combined therapy with narrowband ultraviolet B and potent topical steroids for treating nonsegmental vitiligo in resistant localizations. Br J Dermatol. 2012;166:208–11.
61. Shin J, Lee JS, Hann SK, et al. Combination treatment by 10 600 nm ablative fractional carbon dioxide laser and narrowband ultraviolet B in refractory nonsegmental vitiligo: a prospective, randomized half-body comparative study. Br J Dermatol. 2012;166:658–61.
62. Helou J, Maatouk I, Obeid G, et al. Fractional laser for vitiligo treated by 10,600 nm ablative fractional carbon dioxide laser followed by sun exposure. Lasers Surg Med. 2014;46:443–8.

63. Vachiramon V, Chaiyabutr C, Rattanaumpawan P, et al. Effects of a preceding fractional carbon dioxide laser on the outcome of combined local narrowband ultraviolet B and topical steroids in patients with vitiligo in difficult-to-treat areas. Lasers Surg Med. 2016;48:197–202.
64. Li L, Wu Y, Sun Y, et al. Triple combination treatment with fractional CO2 laser plus topical betamethasone solution and narrowband ultraviolet B for refractory vitiligo: a prospective, randomized half-body, comparative study. Dermatol Ther. 2015;28:131–4.
65. Regazzetti C, Alcor D, Chignon-Sicard B, et al. Micro holes for delivering melanocytes into the skin: an ex vivo approach. Pigment Cell Melanoma Res. 2016;29:481–3.
66. Silpa-Archa N, Griffith JL, Williams MS, et al. Prospective comparison of recipient-site preparation with fractional carbon dioxide laser vs. dermabrasion and recipient-site dressing composition in melanocyte-keratinocyte transplantation procedure in vitiligo: a preliminary study. Br J Dermatol. 2016;174:895–7.
67. Nicolaidou E, Antoniou C, Stratigos AJ, et al. Efficacy, predictors of response, and long-term follow-up in patients with vitiligo treated with narrowband UVB phototherapy. J Am Acad Dermatol. 2007;56:274–8.
68. Schmitt J, von Kobyletzki L, Svensson A, et al. Efficacy and tolerability of proactive treatment with topical corticosteroids and calcineurin inhibitors for atopic eczema: systematic review and meta-analysis of randomized controlled trials. Br J Dermatol. 2011;164:415–28.
69. Cavalie M, Ezzedine K, Fontas E, et al. Maintenance therapy of adult vitiligo with 0.1% tacrolimus ointment: a randomized, double blind, placebo-controlled study. J Invest Dermatol. 2015;135:970–4.
70. Rashighi M, Agarwal P, Richmond JM, et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. Sci Transl Med. 2014;6:223ra23.
71. Regazzetti C, Joly F, Marty C, et al. Transcriptional analysis of vitiligo skin reveals the alteration of WNT pathway: a promising target for repigmenting vitiligo patients. J Invest Dermatol. 2015;135:3105–14.
72. Richmond JM, Bangari DS, Essien KI, et al. Keratinocyte-derived chemokines orchestrate T cell positioning in the epidermis during vitiligo and may serve as biomarkers of disease. J Invest Dermatol. 2016;137(2):350–8.
73. Wang XX, Wang QQ, Wu JQ, et al. Increased expression of CXCR3 and its ligands in patients with vitiligo and CXCL10 as a potential clinical marker for vitiligo. Br J Dermatol. 2016;174:1318–26.

Camouflage

39

Alida DePase and Thomas Jouary

Contents

Abstract

It is only recently that camouflage has been recognised as being a medical intervention, when there are no other satisfactory options to really help the patient. Non permanent techniques such as self tanners should be usually preferred. The Koebner's phenomenon can be observed in areas where an adherent camouflage needs an intense frictional washing to pull it off. Cosmetic tattoo may be suitable for depigmented lips, especially in black people, and for depigmented nipples.

A. DePase
Associazione Ricerca Informazione per la Vitiligine (ARIV), Cernusco Lombardone, LC, Italy

T. Jouary (✉)
Service de Dermatologie, Hôpital Saint André, CHU de Bordeaux, Bordeaux, France
e-mail: thomas.jouary@ch-pau.fr

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_39

Key Points

- It is only recently that camouflage has been recognized as being a medical intervention, when there are no other satisfactory options to really help the patient.
- It is important to discuss with the patient his or her lifestyle to choose products (self-tanners, cover creams, etc.) suitable for his/her case.
- Camouflage can improve the quality of life of vitiligo patients.

39.1 Introduction

The World Health Organization defines "health" as a physical and psychological well-being. Considering all physical and psychological aspects of vitiligo, dermatologists should suggest a therapy when feasible but should also recommend psychological approaches and the use of camouflage [1–7]. Benefits can be obtained by the skilled use of corrective cosmetics which are presented in the following chapter.

39.2 Why Camouflage and Cosmetic Rehabilitation Are Needed

Camouflage refers to a range of special products, specially developed to disguise aesthetic skin disfigurement of any kind, requiring special application techniques. Cosmetic rehabilitation is about encouraging patients to achieve a positive image of the self.

Vitiligo is a disfiguring disease, pervasive to the patient. In an attempt to cope, patients may drastically change their way of life. They choose clothes with the sole aim of covering the patches. They feel obliged of wearing long sleeves and long trousers even in the hottest summer. Patients presenting with face and hand lesions demonstrate usually a higher impairment of their quality of life and self-body image than others.

The expressed fear of many vitiligo patients is that that the disease may spread to visible areas. Indeed, patients with visible vitiligo are usually seeking for a treatment more actively than others. They are logically searching help first for patches in the visible areas and only secondly for areas of lesser aesthetic importance.

Standard and experimental vitiligo treatment options are demanding from the psychological point of view and may be disappointing for both patient and dermatologist. Moreover repigmentation is often partial and relapses may occur after stopping treatment. The treatment of hand and face lesions is particularly difficult, because of Koebnerization (Chap. 2.2.2.1). Segmental vitiligo of the face starts frequently in childhood. At this age, melanocyte cell grafts are rarely considered for ethical reasons and because some repigmentation may appear spontaneously in the future. In all these situations, when classical medical treatments fail or cannot be considered, efficient camouflage techniques should be proposed to the patient.

39.3 Camouflage as a Medical Intervention?

Dermatologists as a rule don't know much about camouflage; in the past, they have been dismissive of what they considered merely make-up. They are uninformed about the wide range of available camouflage products and the different techniques of application. They are equally unaware of the benefits that can be obtained by the skilled use of corrective cosmetics, such as self-tanners, stains, dyes, whitening lotions, tinted cover creams, compact, liquid and stick foundations, fixing powders, fixing sprays, cleansers, semi-permanent and permanent tattoos and dyes for facial and head white hair.

It is only recently that camouflage has been recognized as being equally worthy of consideration as a medical intervention, when there are no other satisfactory options to really help the patient and when the disease is recalcitrant to all standard and alternative therapies.

Table 39.1 Self-tanner and cover creams: application method

Facilities	Preparative phase	Cleaning
Room or bathroom at medium-low temperature (sweating is not good)	Cotton washcloth to exfoliate the skin	Soap
Band or tie to keep hair off the face		Skin lotion (feet, ankles, knees, elbows, hands, wrists) for areas with fine lines
Fingernail scrub brush		
Baby wipes to remove the unwanted trace		
Latex gloves		
Sponge paintbrush (for self-tanner on the back)		
Dark-coloured bathing suits (for whole body)		
Loose outfit (during the product drying)		
Dark T-shirt		
Moisturizer (for the subsequent period)		

Camouflage consultations have been developed in some dermatology departments. The aim of these consultations is to educate patients about camouflage (Table 39.1 details the application method of commonly used products). These multidisciplinary consultations, composed of camouflage trained nurses, dermatologists and camouflage specialized persons, have had a favourable impact on patients. Camouflage therapy or education means also to enable patients to apply the cosmetics themselves. It is worthwhile when the patient can master the correct procedure with the products chosen and the various techniques of application. Patients should also be informed on where and how to obtain camouflage products that suit their individual needs.

Candidates for vitiligo camouflage are patients of both genders and of all ethnic origins and ages. As with all patients, it is important for the camouflage practitioner to learn about their prior history, current medical situation, emotional state and attitude towards camouflage. The patient's ability and desire to perform the various camouflage techniques should be discussed, and the patient should be asked whether he/she has experienced allergic reactions to cosmetics in general in the past. It is important to discuss with the patient his or her lifestyle to choose products (self-tanners, cover creams, etc.) suitable for his/her case.

39.4 A Brief History of Camouflage

Since ancient times, an unblemished face has universally been considered a symbol of beauty and therefore sought after at all costs. In ancient Roman times, camouflage was used by the slaves who had gained their freedom, became rich, and were determined to leave their past behind. This consisted of an ochre-coloured paste made of clay and helped to cover the mark made on their forehead with a hot iron, the ignominious stigma of the slave. Quinto Sereno Sammonico, doctor of the second century AC, in the Chapter Cutis et faciei vitiis propellendis of his Liber Medicinalis, proposes a remedy to eliminate freckles: "Invida si maculat faciem lentigo decoram nec prodesse valent naturae dona benignae, erucam atque acidum laticem simul inline mali Saepiolae cineres ex ossibus omnia levant…" (If the horrible ephelides spoil the skin, spoiling its natural beauty, spread a lotion made of vinegar and rocket on the skin). Until the early twentieth century, make-up in general was used only by the rich, theatre actors or prostitutes. It was only in the twentieth century, with the birth of the film industry, that camouflage and make-up in general became a must for film stars. Products resistant to the effect of stage lighting were requested for actors with imperfections to hide. After the First World

War, many soldiers came back from the front severely burnt or disfigured, and camouflage was a necessary blessing for them. The make-up and camouflage era was born, and mass production for the general public became a reality.

39.5 Camouflage Controlled Studies

Clinical research on camouflage in vitiligo is very limited given the practical importance of this field. A Cochrane database review in 2006 did not find published trial in this field [8]. Some authors report the efficacy and safety of dihydroxyacetone (DHA) in healthy volunteers and vitiligo patients. These open and/or retrospective studies have compared different DHA concentrations in patients of various phototypes. The higher the concentration, the better the response observed particularly in darker phototypes [8]. Only one study demonstrated the positive impact of self-tanning interventions on the quality of life in a cohort of vitiligo patients [9]. These studies were principally conducted with DHA-derived products which are described in detail thereafter.

39.6 Self-Tanning Creams, Lotions and Sprays

Self-tanners in gel, cream, lotion or spray give the skin a brown colour that resembles a natural tan and normally lasts 3–5 days. The tanning of the skin develops in about 3–24 h after the application. Instant colour self-tanners, available from some manufacturers, thanks to a colour guide, give a tanned colour instantly. These products are popular among those who cannot or do not like sun exposure and can be considered camouflage products as they disguise depigmentation successfully. Marketed for about 40 years, at first self-tanners were not successful because they provided a yellowish and uneven colour, while today the latest formulas give excellent aesthetic results. Unluckily they are not suitable for coloured people, and the best results are in Caucasians of phototypes I to III.

The active ingredient is dihydroxyacetone (DHA), a sugar that reacts with the proteins of the stratum corneum and gives a tan resembling the solar UV-induced tan. This is due to the so-called "Maillard's" reaction, after the author who studied the chemical reaction that goldens the crust of bread in the oven. Recent studies have demonstrated that DHA reacts totally and only with the first cells with which it comes into contact, and therefore it remains on the surface of the stratum corneum, until it is eliminated with the normal turnover of the epidermis. Preparations containing DHA are stable between pH 4 and pH 6. At neutral pH, brown grumes are formed inactivating pigmentation. It has been noticed that the presence on the skin or in the product of little organic or inorganic molecules may alter the DHA colouring capability. Traces of metals such as iron, titanium, zinc or alpha-hydroxy acids such as lactic acid can inactivate the product. So to avoid the frequent risk of lack of uniformity, a good rule is not to utilize self-tanners after using creams with zinc or titanium or after washing the skin with alkaline or lactic acid-based soaps largely present in products being defined by manufacturers as "at physiologic pH" [1]. They can be used throughout the year, they are waterproof, and the fake tan developed does not stain clothes or sheets. However, sea water makes them fade away quickly, while swimming pool water does not.

No sunless tanner currently available contains adequate sunscreen, so sun shielding products may be applied during the day, and moisturizers as well, but only after the desired colour intensity has been obtained. Before applying self-tanners, it is advisable to gently rub the skin with a very soft brush to eliminate dead cells, especially on elbows, knees and knuckles, in order to obtain an even skin colour. The skin should be perfectly dry. It is advisable not to apply these products during the hot hours of the day in summer, because excessive sweat can result in uneven application and may prevent the active substance to develop colour properly. Moreover, the application on eyebrows or near the forehead hairline should be avoided. Only a small quantity of the product should be applied first, and if the desired colour is not achieved, it is possible to intensify with additional daily applications. If too much of the product is used, the result will be unnatural. Particular spare amount should be applied on the face and neck, because this part of the skin takes

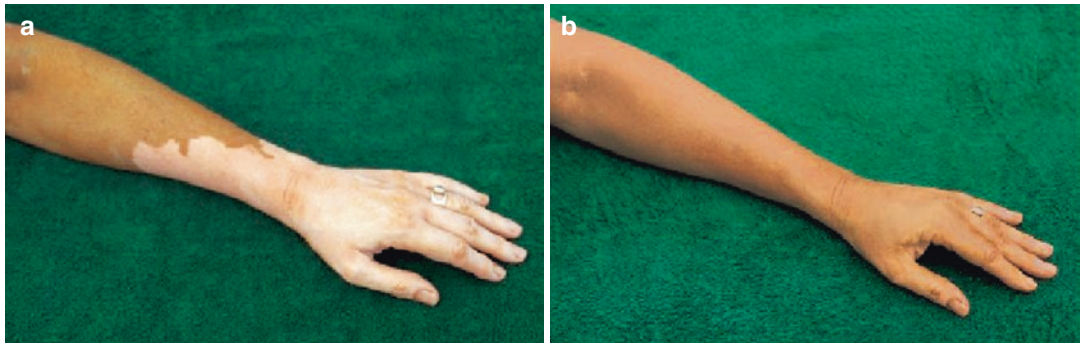


Fig. 39.1 (**a** and **b**) Camouflaging of hands and forearms

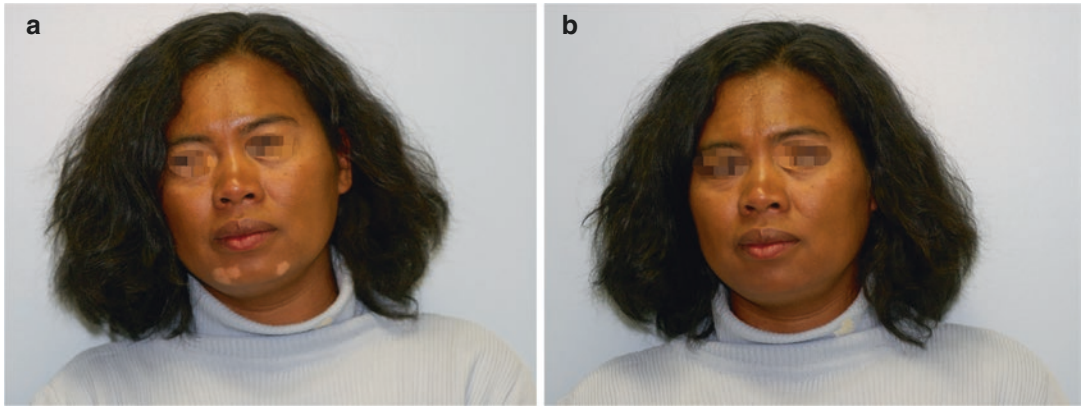


Fig. 39.2 (**a**–**b**) Camouflaging of facial vitiligo in one dark-skinned patient

to self-tanners quite well. If the hair is short, the product should be applied behind the ears.

For small-sized vitiligo lesions, it is advisable to use a self-tanner in lotion (not in cream) with a q-tip. The lotion is spread from the centre of the lesion towards the outside, up to 1–2 mm from the lesion edge. A different technique is to spread the product over the entire area, for instance, hands and arms, including the normal pigmented areas and subsequently repeat over only the white areas using a q-tip dipped in the product (Figs. 39.1 and 39.2).

Most instructions for sunless tanners mention rub it well until it is absorbed, but this takes too much time and the hands will become orange. Instead, the product should be applied quickly but thoroughly, spreading in a circular motion to avoid streaking. Finally, after applying the product, washing for 3 h should be avoided. Any sweat-inducing physical activity sould be avoied for 1 h: no tight jeans, belts, shoes and bras for 1 h, if the products have been used on the body.

39.7 Cover Creams, Foundations and Sticks

Highly pigmented creams come in compact, liquid or stick formulations and are available from several manufacturers in a wide range of natural skin colours. They are lightweight and easy to apply, usually free of contact allergens, but dermatitis and allergic reactions may occur, due to fragrances and preservatives present in some brands. The texture is denser than traditional foundation creams used by the general public, as their aim is to provide effective cover. They may contain up to 50% mineral oils and wax. The texture, different from normal foundation products, is also due to titanium dioxide, used as a thickening and shielding agent, offering sun protection,

Table 39.2 Cover cream application

Cover cream	Applied with the ball of the middle finger and smoothed over the discoloured area with a light-pressing motion. A flat brush can be used to feather out the outer edges of the thick, opaque cover cream solution until it is so well-blended to become undetectable
Fixing powder	Colourless powder to stabilize the foundation, applied with a small cotton pad. This procedure makes the application waterproof and resistant to smudges and friction. A few minutes for the talc to be absorbed, then the excess is dusted off
Fixing spray	It maintains the corrective make-up for a whole day. Vaporized at a distance of 40 cm, it creates, thanks to its silicon and polymers formula, an elastic film that guarantees coverage

while the colours are provided by iron oxides. Blended to complement the individual's particular skin colour, they can conceal disfiguring vitiligo patches of exposed areas. Suitable for men, women and children, their waterproof characteristics allows shower and swimming. When they are applied on the face, they should be removed every day. It is crucial to choose one's basic colour and then the various shades that can vary indefinitely, according to the different parts of the body, to the seasons or simply from one day to another. Sometimes it is necessary to mix more colours of different brands.

For a correct application, the skin should be cleaned. Any previous make-up has to be gently removed. The patient has to learn proper make-up removal techniques, that is, gentle movements to avoid the Koebner phenomenon. Using a sample palette of cover creams, two or three shades can be mixed, to achieve the desired colour match. More than three colours will make the procedure too complicated and expensive. An optimal blend of colours should be created, and when applied, this should match the colour of the surrounding skin as closely as possible (Table 39.2).

39.8 Vitiligo of the Lips

Transfer-resistant lip colour lasting up to 12 h of wear, in matte formulas, is available in many shades that duplicate the natural colour of the lips, suitable also for male patients. They are an alternative to lip tattoos. These very popular cosmetics are easily available in almost all department stores and pharmacies.

39.9 Leukotrichia

Leukotrichia can affect visible areas on the beard, moustaches, eyebrows and eyelashes. In these cases it is advisable to dye the white hair in a hairdressing salon the first time, and subsequently the dyeing can be practiced at home with products formulated for these delicate areas.

39.10 Permanent and Semi-permanent Camouflage

Also referred to as micro-pigmentation, cosmetic tattooing and dermal pigmentation are techniques that require an experienced technician, qualified to offer these services. Results vary according to the skill of the practitioner and his/her experience with colours. Cosmetic tattoo may be suitable for depigmented lips, especially in black people, and for depigmented nipples. As regards other vitiligo areas, results may be very disappointing. Pigments especially formulated for cosmetic application are implanted beneath the epidermis into the dermal layer, by microinsertion. Topical anaesthetics are used to minimize discomfort, allergic reactions are rare, and sterile needles and surgical gloves are used for each procedure. Semi-permanent camouflage, or cosmetic tattooing, differs from traditional tattooing, where permanent skin inks and dyes are inserted into the skin. Camouflage can be called permanent if compared to normal make-up, but as it will fade with time, which usually lasts from 2–5 years, it is actually "semi-permanent".

39.11 Precautions of Use

Some precautions should be considered when a camouflage technique is used or proposed to the patients. First, the patient should be informed that camouflage has to be removed smoothly to

avoid intensive friction by using washcloths. This is of importance as the Koebner phenomenon is observed in areas where an adherent camouflage needs an intense frictional washing to pull it off (Chap. 2.2.2.1). Similarly, fixing powder should be used cautiously and avoided whenever possible. Smooth, liquid and light instant colour self-tanner and stains should be preferred for these reasons.

Another point of importance is the risk with permanent camouflage. Indeed, vitiligo course is highly unpredictable. Even after many years, stable large patches can resolve spontaneously. Giving these considerations, permanent camouflage and tattoos should be considered with particular caution. The development of a depigmented patch around an area previously treated with these techniques can lead to inaesthetic results. Thus, if performed in vitiligo, the colour of the tattoo has to be very close to the natural pigmentation of the patient, which is very difficult. Since the colour of the tattoo is definitive and does not follow the UV-induced tanning changes, the contrast between tattooed skin and natural pigmented areas may cause problems.

39.12 Conclusion: Camouflage as a Balm for "Bruised" Souls

Camouflage increases the patient's confidence and improves his/her quality of life. It is readily accepted by women, who, unlike children and men, may be already accustomed to use make-up products. But both men and adolescents can easily learn how to apply the products.

There are many ways to conceal small or large areas of vitiligo and the natural result increases the patient's confidence. No more embarrassing questions or intrusive staring, wearing a short-sleeved shirt or shorts in the summer, no hands hidden in pockets and greeting with a handshake without fear, these are some of what "camouflage therapy" can do to improve the quality of life of vitiligo patients.

Take-Home Messages

- Non-permanent techniques such as self-tanners should be usually preferred.
- The Koebner phenomenon can be observed in areas where an adherent camouflage needs an intense frictional washing to pull it off.
- Cosmetic tattoo may be suitable for depigmented lips, especially in black people, and for depigmented nipples.

References

1. DePase A. Importanza del Camouflage in Pazienti con Vitiligine. In: Lotti T, editor. La Vitiligine, nuovi Concetti e Nuove Terapie. Milano: Utet; 2000.
2. DePase A. La Voce dei Pazienti. In: EBD evidence based dermatology. Milano: Masson; 2003.
3. DePase A, Naldi L. Vitiligo, some reflections on clinical research in a rather elusive disorder Centro Studi Gised. Bergamo: Ospedali Riuniti; 2004.
4. DePase A. Vitiligo, au-delà de la maladie I. In: Ortonne JP, editor. Cutis and psyche. France; 2004.
5. DePase A. The view of the cosmetologist. In: Lotti T, Hercegova L, editors. Vitiligo: problems and solutions. New York: Marcel Dekker Inc.; 2004.
6. DePase A. Il Camouflage, Chapter 69. In: Naldi L, Rebora A, editors. Dermatologia Basata sulle prove di Efficacia. Milano: Masson; 2006.
7. Ongenae K, Dierckxsens L, Brochez L, et al. Quality of life and stigmatization profile in a cohort of vitiligo patients and effect of the use of camouflage. Dermatology. 2005;210:279–85.
8. Whitton ME, Ashcroft DM, Barrett CW, González U. Interventions for vitiligo. Cochrane Database Syst Rev. 2006;(1):CD003263.
9. Rajatanavin N, Suwanachote S, Kulkollakarn S. Dihydroxyacetone: a safe camouflaging option in vitiligo. Int J Dermatol. 2008;47:402–6.

Further Reading

http://www.skin-camouflage.net
http://www.redcross.org.uk/standard.asp?id=49354

Photoprotection Issues

40

Alessia Pacifico, Giovanni Leone,
and Mauro Picardo

Contents

Key Points

- Using Fitzpatrick's system, the physician can estimate the relative risk for each phototype of developing acute and chronic changes related to UV exposure.
- Stratum corneum protects against UV mainly because of reflection of radiation, absorption of the radiant energy and internal scattering.
- Melanin is a potent UV absorber and a protective factor against UV radiation.
- Hyperkeratosis and epidermal hyperplasia would develop in vitiligo exposed to UV to compensate for the lack of pigment.
- Antioxidant status may account for the SPT-related differences which is the antioxidant status of the skin.
- Low incidence of actinic damage, basal and squamous cell carcinomas in vitiligo could be due to a protective function of upregulated wild-type p53.
- For photoprotection in sunny and tropical climates, avoidance of peak sunny hours and clothing is the major step.
- In patients with skin types >III usually two different sunscreens (the first one on the vitiliginous lesions with SPF around 15 and another one with SPF 50+ on surrounding healthy skin) are recommended.

A. Pacifico · G. Leone (✉)
Phototherapy Unit, San Gallicano Dermatological Institute, IFO, Rome, Italy
e-mail: gleone@ifo.it

M. Picardo
Cutaneous Physiopathology and CIRM, San Gallicano Dermatological Institute, IRCCS, Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_40

40.1 Normal and Vitiligo Skin UV Sensitivity

A number of mechanisms have been developed during evolution to protect human skin from excessive UV radiation. To classify the susceptibility of human subjects to develop erythema following UV exposure, Fitzpatrick's system differentiates six sun-reactive skin phototypes (SPT) [1] (Table 40.1). In this system, the capacity to tan is equally important to help to categorize individuals of any colour or ethnic background. Using this method, the physician can estimate the relative risk of developing acute and chronic changes related to UV exposure. A study by Carrettero-Mangolis and Lim confirms that there is a correlation between minimal erythema dose (MED) and SPT, i.e. the higher the SPT, the higher the MED [2].

There is evidence that the stratum corneum (SC) protects against UV mainly because of reflection of radiation, absorption of the radiant energy and internal scattering. In the palms where the SC is very thick, the minimal erythema dose is 16 times higher than on the back. It was observed that in areas stripped from SC, sunburn develops more easily than in the unstripped areas of the skin [3].

Melanin is a potent UV absorber, and thus skin pigmentation is another natural protective factor against UV radiation. It is already well known that pigmented skin is more resistant to sunburn than poorly pigmented skin [4, 5]. Delayed effects of chronic UV exposure, such as development of skin cancer and photoageing are also more pronounced in individuals with fair skin colour [6]. Skin responds to repeated exposure by tanning, especially in skin types III and IV who tan easily and burn rarely, which is the result of melanogenesis and associated SC thickening. A study performed by Cario-André et al. assessed the photoprotective role of melanocytes in the epidermis and studied the effects of UV B on epidermis reconstructed with and without melanocytes. To address more specifically the role of melanin in fair-skinned individuals, experiments were done with cells obtained from human skin of low skin types (II–III). In order to study the effect of constitutive melanin and possibly that of newly synthetized melanin precursors, a single dose of UVB (4–5 MED) was administered to reconstruct, and the effects were then monitored over the first 24 h. The results clearly showed that low phototype melanocytes protect epidermal basal cells against UVB-induced apoptosis as well as necrosis and may thus preserve epidermis integrity after UVB irradiation. On the other hand, such melanocytes do not seem to have a protective role against DNA damage and consequently may not prevent skin cancer [7].

Table 40.1 Fitzpatrick SPT classification

SPT I	Always burns, never tans
SPT II	Burns easily, tans minimally
SPT III	Burns moderate, tans gradually to light brown
SPT IV	Burns minimally, alias tans well to moderate brown
SPT V	Rarely burns, tans profuse to dark
SPF VI	Never burns, deeply pigmented

In a recent study by Yamaguchi et al. DNA damage and apoptosis in different skin types before and after UV exposure have been reviewed. Their results, accordingly with other published reports, indicate that UV-induced DNA damage is more effectively prevented in darker skin types. This paper also demonstrated that rates of repair of DNA damage may differ greatly among different individuals and that UV-induced apoptosis is significantly greater in darker skin. These results suggest that UV damaged cells are more efficiently removed in darker skin [8].

In patients with vitiligo, it is generally assumed that lesional skin of SPT I–VI would be very sensitive to UV radiation owing to the fact that pigment is absent in such lesions, but it has been recently established that there are several mechanisms that contribute to modulate UV sensitivity in vitiliginous skin. Depigmented area of vitiligo patients does not contain pigment and thus protection against UV is afforded by the SC and the rest of epidermis. It might thus be suspected that hyperkeratosis and epidermal hyperplasia would develop in vitiligo exposed to UV to compensate for the lack of pigment. The importance of hyperplasia in photoprotection was validated in studies looking at vitiligo skin which is characterized by a lack of melanocytes. Gniadecka et al. assessed solar simulated radia-

tion (SSR) MED on amelanotic and adjacent normally pigmented skin of 14 vitiligo patients. Erythema and melanin content were quantified using a reflectance device and SC thickness was determined from frozen skin samples. These authors reported that the predominant protective factor in both vitiligo and pigmented skin is the SC where it accounted for 57% of the total photoprotection in pigmented skin. Repeated exposures to this skin was shown to elicit a protection factor of 15 in the absence of melanocytes. In contrast, selective activation of melanocytes by UVA that does not induce SC thickening only gave a protection factor of 2–3. Gniadecka et al. suggested that SC accounted for over two-thirds of photoprotection observed in normal skin and hence was far more significant in this role than induced tanning [9]. The significance of SC in photoprotection may be greater in fair-skinned individuals than in pigmented individuals especially in vitiligo skin where the SC may represent the only source of protection. However, studies performed by Sheehan et al. did not support a significant photoprotective role for stratum corneum thickening [10]. The different results obtained by Gniadecka and Sheehan may be due to several variables. It is known in fact that UVR sensitivity vary between body sites on the same person.

Kaidbey et al. investigating epidermal UVR transmission in black skin (skin type VI), as well as Caucasian skin (skin types I–III), found that in skin types I, II and III, the SC is the main site of UVR screening, absorbing over 50% of the incident radiation. However, samples were taken from previously sun-exposed sites (abdominal skin) [4]. Kaidbey and Kligman also reported in an earlier study that melanogenesis (without appreciable thickening of the SC) induced by repeated UVA exposure afforded a protection factor of 2–3 [11].

40.2 Photoadaptation of Vitiliginous Skin to UV Irradiation

A recent retrospective study showed that most patients with vitiligo treated with narrow band UVB (NB UVB) phototherapy did not develop phototoxicity in their skin despite increasing doses of UV radiation [12]. Thus, these patients developed photoadaptation, a frequently observed phenomenon. Photoadaptation has been described in different SPT, and it is probably due to both pigmentary and non-pigmentary influences. Several studies have indicated that factors involved in photoadaptation include hyperkeratosis, acanthosis, melanogenesis as well as an unknown factor that may be due to DNA repair or an immune related process. Photoadaptation was described by Oh et al. as a "number of changes occurred that are adaptive, in the sense that they result in a diminished future response to equivalent doses of radiation" [13]. One of the methods to measure photoadaptation is MED phototesting.

In phototherapy protocols, vitiliginous skin has been always classified as Fitzpatrick's SPT I or II because of the lack of pigment. Caron-Schreinmachers et al. have recently showed that by elicitation of the MED on areas of vitiliginous skin, UVR sensitivity varied with total body skin type even in skin without pigment. Unlike previous studies where we only had information about the relation between MED and SPT from normal skin of different SPT, in this study it has been proved that there is a linear relationship between the SPT of non-affected skin of vitiligo patient and the sensitivity to NB UVB irradiation of lesional skin (Fig. 40.1) [14]. Since melanocytes are absent or scarcely present in lesional vitiligo skin, this difference in photosensitivity cannot be due to melanin, but this protection must be based on other mechanisms. We know that epidermis thickness increases after UV irradiation. In the study performed by Caron-Schreinmachers et al., however, patients' skin had not been exposed to UV for at least 3 months, and tests were carried out in regions that had not been exposed to sun so skin thickening could not be responsible for the observed differences. One mechanism that possibly accounts for the SPT-related differences is the antioxidant status of the skin. It has been already well established that antioxidants provide photoprotection. Bessou-Touya et al. have shown that melanocytes of Caucasian subjects, which have a higher content of unsaturated fatty acids in their cell membrane, are more prone to the

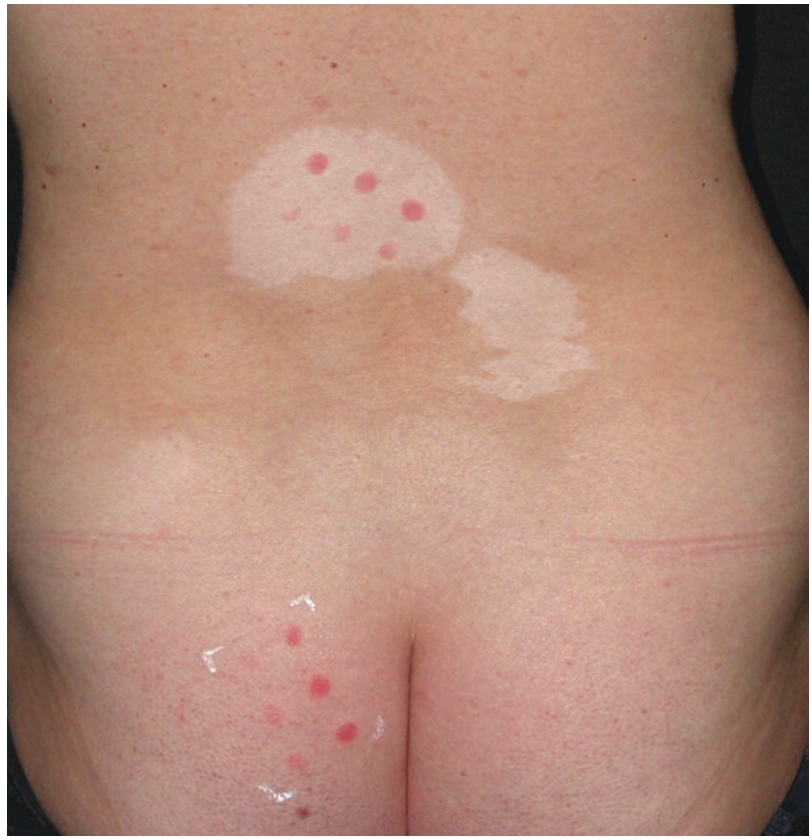


Fig. 40.1 MED on lesional and nonlesional skin

peroxidative effects of UV light and that keratinocytes participate in photoprotection via phototype-dependent antioxidant enzyme activities [15]. Picardo et al. demonstrated that there are significant differences in antioxidant status in normal skin between people with high (III–V) and low (I–II) SPT [16]. Possibly the same differences in antioxidant status between the different SPT exist also in vitiligo skin.

Overall the studies performed indicate that photoadaptation occurs following UVR exposure also in depigmented areas of vitiligo subjects and that the normally pigmented skin phototype should be taken in account when a photoprotection strategy (or phototherapy) is recommended.

40.3 Vitiligo and Skin Cancer

Sun exposure is the main cause of photocarcinogenesis, photoageing and photosensitivity. Unprotected exposure to ultraviolet radiation is a major causal factor in the development of skin cancer. Non-melanoma skin cancers (NMSC) are initiated for the most part by chronic sunlight exposure and can readily be produced by experimental exposure to ultraviolet radiation in animal models [17, 18]. Ultraviolet B (UVB) is the major active waveband region that causes direct photochemical damage to DNA, from which gene mutations arise. Unlike UVB, ultraviolet A (UVA) could have more indirect effects on DNA via the generation of reactive oxygen species. In contrast with NMSC, cutaneous melanoma is more commonly associated with sporadic burning exposure to sunlight, especially early in life, but the wavelengths responsible have not been clearly identified. There are several indications that UVA might have an important role in the pathogenesis of melanoma [19]. However, this involvement has recently been questioned, since only UVB could induce melanoma in a transgenic mouse model [20].

The action spectrum for UV-induced tanning and erythema are almost identical. Indirect evidence suggests that UVA has a greater role in long-term sun damage than it does in acute effects such as sunburn or vitamin D synthesis, which are overwhelmingly attributable to UVB [21, 22].

Considering that patients with vitiligo have patches lacking pigment and that a group of patients develop the disease during childhood, it would be expected that these subjects with the involvement of the head, neck and hands, which are sites at constant risk of sun damage, should develop an increased risk for NMSC [23]. A review of the literature indicates an even lower risk of NMSC. The rarity of NMSC in vitiligo was noted earlier in a study by Calanchini-Postizzi and Frank on 23 patients who had a mean duration of vitiligo of 15.1 years. These investigators found only three actinic keratoses in light-exposed vitiligo patches in those 23 patients. In addition, the number of "sunburn cells", commonly considered as the morphological sign of UV damage to keratinocytes, was found significant lower compared to healthy controls [13]. Schallreuter et al. more recently hypothesized that the low incidence for actinic damage and basal and squamous cell carcinomas as documented in vitiligo could be due to a protective function of upregulated wild-type p53 induced by the constant H_2O_2 stress existing in all the epidermis of vitiligo patients [24].

The association between vitiligo and melanoma is interesting because both diseases affect melanocytes and that immunological mechanisms play a part in both conditions. Different clinical studies report the connection between malignant melanoma and vitiligo, and also several authors suggest that the appearance of depigmentation during the course of malignant melanoma or its treatment with interferon can be considered a good prognostic sign [25–27].

Thus a series of arguments indicate that either the chronic adaptation to UV stress via increased antioxidant responses or reinforced disease-driven immunosurveillance against melanocyte antigens may naturally protect vitiligo patients. However, large-scale epidemiological studies are needed to clarify this important issue.

40.4 Practical Photoprotection

All patients with vitiligo, but particularly those with fair skin, should have a photoprotective counselling adapted to their phototype, environmental exposure risk and therapy plan. Indeed, repigmentation needs UV stimulation, and an exceedingly high photoprotection could be counterproductive. However, for photoprotection in sunny and more risky areas such as tropical climates, avoidance of peak sunny hours and clothing are the major steps. It is thus most important to explain to the patient the importance of wearing sun-protective clothing (such as wide brimmed hats) to help prevent tanning and sunburns. Sunblockers come as second line. Sunscreen that provides protection from both UVA and UVB should be used. Besides helping to protect the skin from sunburn and resulting koebnerization and long-term damage of susceptible depigmented areas, sunscreens also minimize tanning, which makes the contrast between normal and depigmented skin less noticeable [28]. A waterproof, broad-spectrum sunscreen with a sun protection factor of at least 15 should be used on all exposed skin, pigmented and depigmented. Particular attention should be given to the formulation of the sunscreen to allow easy spreading (and removal if needed) without risk of skin trauma (Koebner's phenomenon).

Sunscreen act by one of two mechanisms, either by absorbing UV rays or by blocking or/and scattering these rays. Chemical sunscreens function by absorbing UVB and/or UVA. Nowadays, protection against both UVA and UVB is very common, and as a result, sunscreens often contain a mixture of light-absorbing chemicals. Sunscreen's efficacy in absorbing UVB is measured by the sun-protective factor (SPF). UVB absorbers have been commonly used worldwide for decades, whereas most UVA and broadband absorbers have been developed in recent years. Since a sunscreen has to protect against the entire UV spectrum, different filters have to be combined in the same product. The cinnamates (2-ethyl *p*-methoxycinnamate) are by far the most popular UVB absorbers in both the USA and Europe, and they are used in combination with other UVB absorbers to achieve a

high SPF. The second most popular filters during the recent past are camphor derivatives. Salicylates and para-aminobenzoic acid (PABA) and its derivatives are among the oldest commercially available UVB filters, and they are still used worldwide. The increasing need for broadband agents and improved photostability has led to the introduction of a new generation of filters, including methylene bis-benzotriazolyl tetramethylbutylphenol (Tinosorb M) and bisethylhexyloxyphenol methoxyphenyl triazine (Tinosorb S), both manufactured by CIBA Specialty (Basel, Switzerland), as well as terephthalylidene dicamphor sulfonic acid (Mexoryl SX) and drometrizole trisiloxane (Mexoryl XL), produced by L'Oreal (Clichy, France). The mexoryls and tinosorbs are not licensed in the USA and Japan [29].

Physical sunscreens act by blocking or scattering UV rays. Micronized formulations of zinc oxide and titanium dioxide are gaining in popularity. Mixtures of these minerals, along with chemical sunscreens, have led to a marked reduction in the transmission of both UVB and UVA. In particular, the use of broad-spectrum sunscreens alone limiting tan contrast may be an effective therapy in vitiligo patients with skin type I or II. Many of these agents are also used in cosmetic products such as eye shadow, foundation and powders. Zinc oxide, titanium dioxide, talc, kaolin and calamine are examples of physical agents. In the past, formulations containing these agents were often opaque, and as a result, patients found them cosmetically unacceptable. More recently, brown colours, such as iron oxide, were added as ingredients; not only does this make the physical sunscreen more appealing cosmetically, it also helps to scatter the UV rays making the formulation more effective.

It is recommended that the sunscreen be reapplied approximately every 90 min. In patients with darker skin types (>III), we usually recommend the use of two different sunscreens: the one on the vitiliginous lesions with SPF around 15 and another one with SPF 50+ on surrounding healthy skin, if possible with a high UVA protection factor. We find this strategy particularly useful in minimizing the contrast between healthy and vitiliginous skin and nevertheless allowing part of the UV spectrum to stimulate pigmentation on affected skin [30].

References

1. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol. 1988;124:869–71.
2. Carretero-Magolis C, Lim HW. Correlation between skin types and minimal erythema dose in narrow band UVB (TL-01) phototherapy. Photodermatol Photoimmunol Photomed. 2001;17:244–6.
3. Pathak MA, Fitzpatrick TB. The role of natural photoprotective agents in human skin. In: Pathak MA, Harber LC, Seljl M, Kukita A, editors. Sunlight and man, normal and abnormal photobiologic responses. Tokyo: University of Tokyo Press; 1974. p. 725–50.
4. Kaidbay KH, Agin PP, Sayre RM, Kligman AM. Photoprotection by melanin-a comparison of black and Caucasian skin. J Am Acad Dermatol. 1979;1:249–60.
5. McFadden AW. Skin disease in the Cuna Indians. Arch Dermatol. 1961;84:1013–23.
6. Taylor CR, Stern RS, Leyden JJ, Gilchrest BA. Photoaging/photodamage and photoprotection. J Am Acad Dermatol. 1990;22:1–15.
7. Cario-André M, Pain C, Gall Y, et al. Studies on epidermis reconstructed with and without melanocytes: melanocytes prevent sunburn cell formation but not appearance of DNA damaged cells in fair skinned Caucasians. J Invest Dermatol. 2000;115:193–9.
8. Yamaguchi Y, Beer JZ, Hearing VJ. Melanin mediated apoptosis of epidermal cells damaged by ultraviolet radiation: factors influencing the incidence of skin cancer. Arch Dermatol Res. 2008;300:s43–50.
9. Gniadecka M, Wulf HC, Mortensen NN, Poulsen T. Photoprotection in vitiligo and normal skin. A quantitative assessment of the role of stratum corneum, viable epidermis and pigmentation. Acta Derm Venereol. 1996;76:429–32.
10. Sheehan JM, Potten CS, Young AR. Tanning in human skin types II and III offers modest photoprotection against erythema. Photochem Photobiol. 1998;68:588–92.
11. Kaidbay KH, Kligman AM. Sunburn protection by longwave ultraviolet radiation induced pigmentation. Arch Dermatol. 1978;114:46–8.
12. Hamzavi I, Deleon S, Yue K, Murakawa G. Repigmentation does not affect tolerance to NB UVB light in patients with vitiligo. Photodermatol Photoimmunol Photomed. 2004;20:117.
13. Oh C, Hennessy A, Ha T, et al. The time course of photoadaptation and pigmentation studies using a novel method to distinguish pigmentation from erythema. J Invest Dermatol. 2004;123:965–72.

14. Caron-Schreinemachers ALDB, Kingswijk MM, Bos JD, Westerhof W. UVB 311 nm tolerance of vitiligo skin increases with skin photo type. Acta Derm Venereol. 2005;85:24–6.
15. Bessou-Touya S, Picardo M, Maresca V, et al. Chimeric human epidermal reconstructs to study the role of melanocytes and keratinocytes in pigmentation and photoprotection. J Invest Dermatol. 1998;111:1103–8.
16. Picardo M, Maresca V, Eibenschutz L, et al. Correlation between antioxidants and phototypes in melanocytes cultures. A possible link of physiologic and pathologic relevance. J Invest Dermatol. 1999;113:424–5.
17. Dumaz N, van Kranen HJ, de Vries A, et al. The role of UVB light in skin carcinogenesis through the analysis of p53 mutations in squamous cell carcinomas of hairless mice. Carcinogenesis. 1997;18:897–904.
18. Madan V, Hoban P, Strange RC, et al. Genetics and risk factors for basal cell carcinoma. Br J Dermatol. 2006;154(Suppl 1):5–7.
19. Wang SQ, Setlow R, Berwick M, et al. Ultraviolet A and melanoma: a review. J Am Acad Dermatol. 2001;44:837–46.
20. De Fabo EC, Noonan FP, Fears T, Merlino G. Ultraviolet B but not Ultraviolet A radiation initiates melanoma. Cancer Res. 2004;64:6372–6.
21. Lim HW, Naylor M, Honigsman H, et al. American Academy of Dermatology consensus conference on UVA protection of sunscreens: summary and recommendations. J Am Acad Dermatol. 2001;44:505–8.
22. Morison WL. Clinical practice. Photosensitivity. N Engl J Med. 2004;350:1111–7.
23. Calanchini-Postizzi E, Frank E. Long term actinic damage in sun exposed vitiligo and normally pigmented skin. Dermatologica. 1987;174:266–71.
24. Schallreuter KU, Behrens-Williams S, Khaliq TP, et al. Increased epidermal functioning wild-type p53 expression in vitiligo. Exp Dermatol. 2003;12:268–77.
25. Buljan M, Situm M, Lugovic L, Vucic M. Metastatic melanoma and vitiligo: a case report. Acta Dermatovenerol Croat. 2006;14:100–3.
26. Gogas H, Ioannovich J, Dafni U, et al. Prognostic significance of autoimmunity during treatment of melanoma with interferon. N Engl J Med. 2006;354:709–18.
27. Michail M, Wolchock J, Goldberg SM, et al. Rapid enlargement of a malignant melanoma in a child with vitiligo vulgaris after application of topical tacrolimus. Arch Dermatol. 2008;144:560–1.
28. Halder RM, Brooks HL. Medical therapies for vitiligo. Dermatol Ther. 2001;14:1–6.
29. Palm MD, O'Donoghue MN. Update on photoprotection. Dermatol Ther. 2007;20:360–76.
30. Paro Vidolin A, Barbieri, L, Aurizi C, Marcassoli LG, Leone G. Photoprotection in vitiligo patients: a new approach. Poster n. 13 Abstracts of the Vitiligo International Symposium (VIS), Detroit, 2018;9–10.

Treating the Disease: Age, Gender, Ethnic Skin, and Specific Locations

41

Savita Yadav and M. Ramam

Contents

Abstract

Vitiligo treatment has to be tailored to every individual after considering several individual features, including age, gender, and ethnic skin type as well as the specific location of patches. Vitiligo affects people of all age groups though the highest incidence is seen in the second and third decades of life. Treatment options are limited in childhood vitiligo because some systemic therapies are contraindicated, phototherapy is difficult to administer, there are age-specific adverse effects of different medicines, and, finally, there are difficulties in undertaking surgical treatment in children. In spite of all these limitations, response to medical as well as surgical treatments is generally stated to be better in children compared to adults. Vitiligo in older patients does not usually produce as much distress as in young people since the psychosocial burden is considerably less at this stage of life when getting married and securing employment are not of concern. The social stigma and cosmetic disfigurement of vitiligo affect both men and women, but the burden is significantly heavier in women, particularly in communities where marriages are arranged by families. Treatment options and response are similar in men and women. Vitiligo in subjects with darker skin poses particular problems because of the greater visibility and disfigure-

S. Yadav · M. Ramam (✉)
Department of Dermatology & Venereology, All India Institute of Medical Sciences, New Delhi, India

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_41

ment along with the widespread prejudices and taboos associated with the disease in these communities. The location of patches is an important determinant of response with vitiligo on the hands and feet, knees and elbows, and the mucosae responding poorly to all current treatment modalities. Choice of surgical technique, dressing, and postoperative care and instructions are guided by the site affected.

Key Points

- Treatment should be defined for the specific patient based on features related to the disease and the individual.
- Treatment options are somewhat limited in childhood vitiligo, but response to medical as well as surgical treatments is generally stated to be better in children compared to adults.
- Vitiligo is usually less distressing in older subjects as the psychosocial burden is low.
- The social stigma and cosmetic disfigurement of vitiligo affect both men and women, but the burden is significantly heavier in women.
- Greater visibility and societal responses pose serious problems in subjects with dark skin, particularly in communities where vitiligo is stigmatized.
- Acral, periorificial, and mucosal vitiligo respond poorly to treatment.
- Choice of surgical technique is guided by the site and total area affected.

41.1 Introduction

Vitiligo is a common cutaneous disorder of depigmentation affecting around 1% of world population [1–4]. It carries a significant social stigma which has a great impact on personal and social life [5, 6]. Research over the last decade, at both molecular and clinical level, has led advances in the arena of medical and surgical treatment. Though we are some way from a cure for this disease, it has become possible to provide efficacious treatment to most patients with the presently available range of options.

Vitiligo treatment has to be tailored to every individual after considering several demographic and disease factors. In this chapter, we will address the effect of age, gender, and ethnic skin type as well as the specific location of patches on choice of intervention and the treatment response.

41.2 Age

Vitiligo affects people of all age groups though the highest incidence is seen in the second and third decade of life. The disease commonly starts in childhood or young adulthood [7, 8]. There are rare reports of congenital vitiligo as well [9]. Expectedly, the psychological impact of the disease is marked in adolescence, a stage at which concern about personal appearance is high leading to difficulty in coping with the stigma of disease [10].

Vitiligo starts in the first decade of life in about a fourth of all patients [11]. There is a slight female preponderance in children compared to adults. The commonest morphological type is vitiligo vulgaris (generalized vitiligo) followed by focal, segmental, and acrofacial vitiligo [11, 12]. Vitiligo in children has certain features that are different from adult vitiligo. The proportion of focal and segmental vitiligo is higher in children compared to adults. It is usually more extensive and associated with a higher proportion of familial disease (12–35% compared to 6.25–30% in general) [13]. Koebnor phenomenon, halo nevi, and leukotrichia also appear to be more frequent in children [11, 12, 14, 15]. Trauma-prone sites like knee, elbow, and hands are frequently involved. Childhood vitiligo can result in significant psychological trauma in the form of teasing by others, loss of self-esteem, avoidance of social gatherings, and anxiety.

41.2.1 Treatment of Vitiligo in Childhood

Before initiating treatment, a detailed discussion regarding the nature of disease, its course, treatment options, and expectations is necessary. Treatment options are limited in childhood vitiligo because some systemic therapies are contraindicated, phototherapy is difficult to administer, there are age-specific adverse effects of different medicines, and, finally, there are difficulties in undertaking surgical treatment in children. In spite of all these limitations, response to medical as well as surgical treatments is generally stated to be better in children compared to adults [16].

41.2.1.1 Medical Treatment

Topical steroids form the first-line therapy for both adults and children with vitiligo by virtue of good efficacy and low cost with side effects that are mainly local. Potent topical corticosteroids can be safely used for short durations in limited disease involving less than 20% body surface area [17–19]. Two studies of the efficacy of potent topical corticosteroids conducted primarily in children showed that more than 50% repigmentation could be achieved in 34% and 68% of patients [20, 21].

Clobetasol propionate was found to be more effective than calcipotriol [22]. Kose et al. noted marked to complete improvement (75–100%) in 8 out of 20 patients treated with clobetasol and in none of the patients treated with calcipotriol. There was no to minimal improvement (0–50%) in 18 out of 22 patients receiving calcipotriol and in 7 out of 22 patients receiving clobetasol. Potent topical corticosteroids showed better results than with tacrolimus, though this difference was not statistically significant [17].

The main limitations of topical corticosteroid therapy are adverse effects which can be both local and systemic. Local side effects are commoner and include thinning of skin, telangiectasia, and striae. These can be minimized by regular examination of skin and by alternating potent with less potent corticosteroids every 6–8 weeks or by providing steroid free intervals of 4–6 weeks after using potent topical corticosteroids for 4–6 weeks [19]. Perilesional hypopigmentation (Fig. 41.1) is another side effect of potent topical corticosteroids irrespective of indication but is of particular significance in vitiligo as this adverse effect may be misinterpreted to be a sign of disease activity or failure of treatment. We have found that stopping the potent topical corticosteroid leads to a reversal of this side effect in a few weeks. Resumption of therapy with a less potent topical corticosteroid keeps it from happening again. In fact, we now initiate therapy with less potent preparations to forestall the development of this distressing adverse effect.

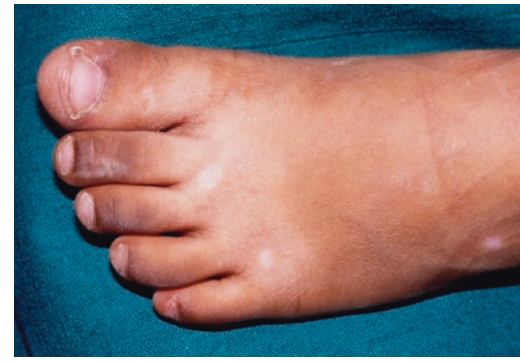


Fig. 41.1 Hypopigmentation around macules of vitiligo following the use of a potent topical corticosteroid

When topical corticosteroids are used for prolonged periods over large surfaces, especially on the head and neck, there may be suppression of the hypothalamo-pituitary axis. Kwinter et al. [23] found abnormal cortisol levels in 29% of tested children and recommended assays of early morning plasma cortisol levels at baseline and every 4 weeks in children treated with moderate- to high-potency topical corticosteroids. However, this testing schedule may be burdensome to undertake in clinical practice, and a more practical approach would be to use topical corticosteroids cautiously and for short durations when treating children with extensive disease.

Topical tacrolimus has been found effective in the treatment of vitiligo in children, both in the strengths of 0.03% and 0.1% [17, 24–26]. In our practice, we almost exclusively use the higher

strength, applied once daily. Silverberg et al. treated vitiligo in 57 children with tacrolimus (0.03% and 0.1%) with good response. Vitiligo on the head and neck was more likely to respond and also showed more complete repigmentation, and twice daily dosing was found to be more efficacious. A randomized trial comparing tacrolimus 0.1% ointment with clobetasol 0.05% ointment found the mean percentage repigmentation with these agents was 41.3% and 49.3%, respectively [17]. Ho et al. found similar results with an intermittent regimen of clobetasol and a continuous regimen of tacrolimus administration for 6 months for both facial and non-facial vitiligo [27]. On the face, successful repigmentation (>50% improvement) was noted in 58% of patients in both the clobetasol and tacrolimus groups. On non-facial sites, successful repigmentation was noted in 39% and 23% of patients in the clobetasol and tacrolimus groups, respectively. Even though it is significantly more expensive than topical corticosteroids, topical tacrolimus can be considered as the primary treatment option for pediatric vitiligo at sites such as the face and flexures where the skin is prone to topical corticosteroid-induced atrophy. In addition, it could be used as an alternative to topical corticosteroids at other sites. The repigmentation induced by tacrolimus is often darker than the surrounding normal skin and may take several weeks to normalize. Other side effects are uncommon.

Pimecrolimus was used in 60 children in combination with microdermabrasion to enhance penetration. Three vitiliginous patches in the same anatomical location were chosen for treatment in each patient [28]. The patches were treated with pimecrolimus 1% cream alone for 10 days or pimecrolimus 1% cream application for 1 day after microdermabrasion or placebo application for 10 days. Clinical response (>50% repigmentation) was seen in 60%, 32%, and 1.7% patients in the microdermabrasion, pimecrolimus, and placebo group, respectively.

Calcipotriol showed some efficacy in an open label trial with 11 (77.8%) of 14 patients who completed the study showing some improvement [29]. However, a randomized study comparing its efficacy with clobetasol found calcipotriol had a weak effect. Calcipotriol and calcitriol are therefore not recommended as monotherapy [22]. Travis and Silverberg treated 12 children with topical corticosteroids in the morning and calcipotriene in the evening. Ten (83.3%) patients repigmented and no adverse effects were seen. This may suggest a role for calcipotriol in combination with topical corticosteroids, but most workers now do not use vitamin D derivatives in the treatment of vitiligo [30].

41.2.1.2 Phototherapy

Phototherapy can be considered for the treatment of extensive vitiligo involving more than 20% of the skin surface and for less extensive but refractory disease. Narrow-band ultraviolet B (NB-UVB) is preferred for phototherapy in children as it is well tolerated and effective. Phototherapy is best administered in a UV chamber when there is extensive involvement. Data on the maximum dose of NB-UVB that can be administered safely is lacking, but ordinarily it is given for up to 1 year. If further treatment is necessary, a phototherapy-free period is recommended followed by limited exposure [31]. Njoo et al. treated 51 children with a mean body surface area involvement of 15% with twice weekly NB-UVB therapy for a maximum period of 1 year. Following treatment, 80% of children had stabilization of disease, and 53% of children had greater than 75% repigmentation. Vitiligo on the hands, feet, fingers, toes, and bony prominences did not repigment well. Minor side effects observed with NB-UVB therapy included itching, erythema, and xerosis. No photoallergic or phototoxic reactions have been reported with NB-UVB, and there does not appear to be an increased risk of malignancy. However, there are some practical problems with NB-UVB therapy in children. First, the child has to be accompanied by an adult to the clinic every time thus increasing the cost of treatment. Second, some children may not be comfortable standing alone in the phototherapy chamber. Also, long-term safety data in children exposed to NB-UVB during the early years of life is not available.

Systemic photochemotherapy either as psoralen and ultraviolet A (PUVA) therapy administered in a phototherapy chamber or with sunlight (PUVASOL) is contraindicated in children below 12 years of age because of the unknown long-term effects of oral psoralen, increased risk of carcinogenesis, and premature aging of skin [32, 33]. However, this restriction may not be applicable in children with brown skin who have a 70-time lower risk of sunlight-induced cutaneous malignancies.

Children with localized disease refractory to medical treatment can be offered locally directed photochemotherapy or phototherapy. Topical PUVA or PUVASOL therapy is an option for localized disease [21]. A response rate of 53% was observed with paint PUVA photochemotherapy in children [34]. Topical PUVA is generally less preferred over NB-UVB because of the risk of unpredictable phototoxic reactions, perilesional hyperpigmentation, increased contrast between lesional and pigmented skin, and the need for longer treatment though these can be minimized by careful use of the modality and detailed counseling of caregivers [31]. Recently, handheld NB-UVB units have become available and can be used to treat localized vitiligo without whole-body exposure. Excimer laser has also been used similarly and found to be effective in children, more so when combined with topical calcineurin inhibitors or corticosteroids which help to reduce the cumulative UV dose [35–38]. Cho et al. found that children respond better than adults to excimer light and did not show any significant adverse effects [39].

In a retrospective review of different modes of phototherapy in children, Koh et al. observed that patients not responding to one mode may be responsive to other modes [34].

Some guiding principles should be kept in mind when using phototherapy in children, irrespective of the type of treatment [31]. First, parts of the body which are not affected should be covered during treatment. Second, areas which have repigmented should be protected in subsequent sessions. Third, genitals should be shielded as they invariably do not respond to phototherapy. Fourth, exposure to natural sunlight should be avoided as far as possible, and sunscreen should be used on photoexposed parts.

41.2.1.3 Surgical Treatment

The last two decades have seen a lot of work in the surgical management of stable vitiligo. Several surgical techniques have been described (see Chap. 3.2.3), but our focus here is on the role of surgery in stable childhood vitiligo. There is a dearth of literature on this subject, and studies specifically addressing children are scarce. Most studies on surgical treatment include either adults alone or have a mixed study population of children as well as adults, but some conclusions can be drawn.

There are several practical problems when operating on children. First, the appropriate type of anesthesia should be administered; otherwise, the child may not cooperate during the procedure. The type of anesthesia required will depend upon the size and site of area to be treated, age, and maturity of the child and type of surgery. In general, it is preferable to give some sedative prior to surgery, use topical anesthetic creams under occlusion as far as possible, and avoid injectable anesthetic agents. Also, parents need to be carefully taught what to do and what to avoid following surgery to ensure good results.

Mulekar et al. [40] reported satisfactory results (more than 65% repigmentation) with the non-cultured epidermal cell suspension (NCES) technique in 80% of treated children and adolescents and have suggested that this technique can be considered the first choice to treat clinically stable segmental vitiligo in children [41]. As in adults, focal and segmental vitiligo in children respond better to surgery than generalized vitiligo in the amount, spread, and homogeneity of repigmentation.

As in adults, small stable patches can be managed with either mini-punch grafting (MPG) or suction blister epidermal grafting (SBEG) [42]. In children, mini-punch grafting has some advantages over suction blister epidermal grafting as the former procedure is quicker and does not need require lying in a fixed position for long and the chances of post-procedure graft displacement

are less than with suction blister epidermal grafting. Studies suggest that repigmentation is faster, and there is greater peri-graft spread of pigment in young patients compared to old, with both techniques [16, 43]. Some have postulated that this may be due to a greater release of cytokines in young patients following the injury of grafting, which then stimulates melanocytes to proliferate and migrate briskly [44].

Melanocytes from younger persons proliferate better in in vitro cultures than those from older people [45, 46]. These in vitro data suggest that autologous melanocyte culture and transplantation should give good results in children. In a clinical study, Hong et al. treated vitiligo in 12 children, 20 adolescents, and 70 adults with cultured pure melanocyte transplantation with a mean extent of repigmentation of 80.7%, 78.9%, and 76.6%, respectively, though this difference was not statistically significant. These results indicate that the technique is a useful option in children with refractory stable disease covering an extensive area [47]. Acceptability of this technique is higher among parents as a large area of vitiligo is treated from a small donor area.

To conclude, surgical techniques lead to good results in children with stable vitiligo but require detailed planning of both the procedure and anesthesia along with good postoperative care by parents to achieve the best outcome.

41.2.1.4 Treatment of Psychosocial Aspects of Disease

Besides medical and surgical treatment, counseling of the child and parents is important as the disease course is chronic and repigmentation takes time. Sometimes, the help of professional counselors/psychiatrists may be necessary in case preliminary assessment reveals significant psychological distress in the child and/or parents.

41.2.2 Vitiligo in Older Adults

Vitiligo in older patients may produce as much distress as in younger people, but more often the psychosocial burden is considerably less at this stage of life when getting married and securing employment are not of concern. The stigma of vitiligo may affect the marriageability of their offspring, and they may worry about spreading the disease to young grandchildren. Education and counseling may suffice for some patients. Camouflage, even as the only intervention, is more acceptable at this stage of life. It is undertaken in order to overcome the extended effects of social stigma on children and other members of the family. Localized disease can be addressed with topical corticosteroids or calcineurin inhibitors. For those who seek more aggressive therapy for extensive disease, photo(chemo)therapy is a good option as it avoids the adverse effects of immunosuppressive systemic agents in patients who may have other comorbidities such as diabetes mellitus and hypertension.

41.3 Gender

Vitiligo occurs with almost equal frequency in men and women though some studies suggest a mild female preponderance [48–50]. The social stigma and cosmetic disfigurement of vitiligo affect both men and women, but the burden is significantly heavier in women, particularly in communities where marriages are arranged by families. This may lead to pressure on the dermatologist to treat girls and women more aggressively. Most studies indicate that the response to medical and surgical treatment is similar in men and women. Women and their families may require greater psychosocial support.

41.4 High Phototypes

The prevalence of vitiligo varies from 0.1% to 4% worldwide [51–55]. There is some data to suggest that the disease is more frequent in people with darker skin and begins earlier in life [3, 48, 56]. Vitiligo vulgaris is the most common type in Southeast Asia, and most studies from this region indicate that the disease occurs with equal frequency in men and women [48, 57–61].

Vitiligo in darker skin types poses particular problems because of the greater visibility and disfigurement along with the widespread prejudices and taboos associated with the disease in these communities, including the confusion of vitiligo and leprosy in some [62, 63]. Patients and their families report facing social ostracism, difficulties in getting a job, and formidable barriers to getting married [64].

Because of the social stigma, patients want every small patch treated and resist suggestions to forgo treatment for small patches on covered areas, for example. Leukotrichia in the absence of adjacent skin involvement is more visible in darker skin, and often patients demand treatment for this. Even when people have immigrated to countries where the social stigma of vitiligo is not as burdensome, they continue to respond to it in the manner of their communities of origin.

The various medical, surgical, and phototherapy treatment options are similar for people with darker skin as for other skin types. There are minor variations in protocols and differences in the response rates that we will discuss in this section.

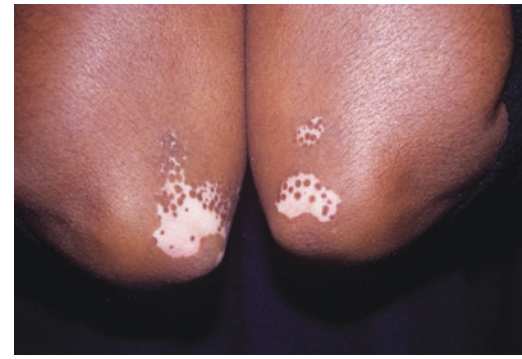


Fig. 41.2 Follicular repigmentation in vitiligo

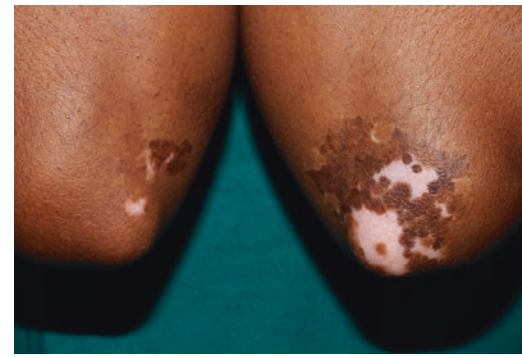


Fig. 41.3 Hyperpigmentation following photochemotherapy

41.4.1 Medical Treatment

Topical corticosteroids steroids and tacrolimus can be safely used, and the response is similar to that in other skin types [65]. Vitiligo on the head and neck responds best, while there is a relatively poorer response on the trunk and limbs. The pattern of repigmentation is usually diffuse on the head and neck, while vitiligo on the trunk and extremities shows both diffuse and follicular patterns (Fig. 41.2) of repigmentation with roughly equal frequency. Tacrolimus is safe for long-term use, but caution needs to be exercised while using topical steroids. In darker skin types, corticosteroid-induced telangiectasias and atrophy are less obvious, so these adverse effects must be looked for carefully and treatment modified when they are noted.

Photo(chemo)therapy is effective in producing repigmentation in vitiligo [66]. When used in darker skin types, there are special considerations to keep in mind. Both oral and topical psoralen should be dosed appropriately as the response is dose-related to a great extent. While inadequate doses will result in a poor response, overdosing of oral psoralen or using a higher strength of topical psoralen solution leads to repigmentation that is excessively dark (Fig. 41.3). When sunlight is used for photochemotherapy, the duration of light exposure should be increased gradually, and patients should be encouraged to take treatment at the same time of day as the intensity of sunlight varies at different times of day. Patches that are completely repigmented can be covered when exposing to light to prevent further darkening. As the risk of cutaneous malignancy is relatively low in dark skin, photochemotherapy can be given for longer periods. Response to photo(chemo) therapy tends to be better than in those with lighter skin [67]. Conversely, as pigmented skin tans easily, this may heighten the contrast between unaffected skin and vitiligo leading to an apparent worsening of disease.

Lastly, depigmented patches on exposed skin should be protected from sunlight to avoid phototoxicity.

Some studies have compared the different photo(chemo)therapeutic techniques in pigmented skin. A study comparing PUVA therapy administered using light chambers with PUVAsol reported quicker onset and greater total repigmentation and greater improvement in quality-of-life with PUVA at the end of 9 months of treatment [66]. Another study comparing NB-UVB therapy with systemic PUVA therapy found that the median repigmentation achieved at the end of 6 months was similar, 45% with NB-UVB and 40% with PUVA. However, the color match was better in the NB-UVB group, while side effects were more frequent with PUVA (57%) than with NB-UVB (7.4%) [68].

41.4.2 Surgery

Several different surgical techniques including mini-punch grafting, ultrathin split-thickness skin grafting, suction blister grafting, and non-cultured epidermal cell suspension (Fig. 41.4a, b) were first developed and are regularly used in darker skin patients. We would like to present the differential response to treatment in this particular skin type. For somewhat larger patches, non-cultured epidermal suspension (NCES) is a good surgical approach which showed better results than suction blister grafting in an Indian study. Budania et al. [69] reported excellent repigmentation (90–100%) in 71% (20 out of 28 patches) of the lesions in the NCES group compared to 27% (7 out of 26 patches) of the lesions in the SBEG group at the end of the study period of 16 weeks [69]. Color match and pattern of repigmentation were similar in the two groups. Initial repigmentation was faster in vitiligo treated with suction blister grafts, but pigment spread and homogeneity of repigmentation were better with non-cultured epidermal suspension. Hyperpigmentation is a common adverse event in darker skin types, both at the donor and the recipient site, and this was the most common adverse event when treating with suction blister grafting (SBG) [43].

Leukotrichia responds poorly to medical treatment. It is amenable to surgical treatment to some extent provided the disease is stable. Holla et al. retrospectively analyzed the improvement in leukotrichia in patients who had undergone NCES for vitiligo [70]. They found improvement in leukotrichia in 88% of 42 treated patches. Improvement was faster in body hair than pubic hair and hair on the face and scalp. Initial repigmentation was observed as early as 2 months on the trunk and extremities with almost complete improvement in body hair at 6 months.

Some workers have used tattooing with dark colored dyes to camouflage refractory vitiligo in individuals with dark skin. This technique is reported to work well for patches over the nipple, areola, lip, genitalia, and scalp where a good color match can be achieved with proper selection

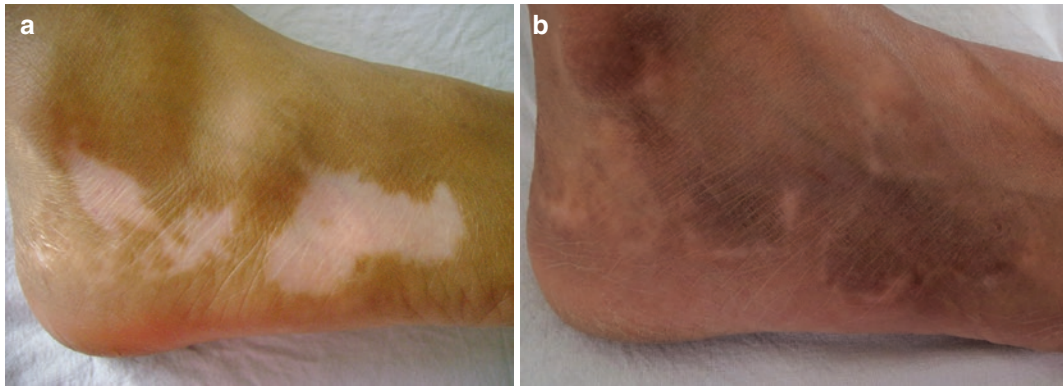


Fig. 41.4 Vitiligo (**a**) before and (**b**) after grafting with non-cultured epidermal cell suspension

of dye color and appropriate technique. The procedure can be done under local anesthesia using medical grade tattooing dye and an electrical tattooing machine [71]. The right shade is made by mixing the basic pigment and matching with the color of the surrounding skin. Results are immediate and permanent and the procedure is inexpensive. However there are problems with leaching of dye, color mismatch, change in shade over time, and the risk of infection. For these reasons, tattooing is no longer a recommended treatment technique.

41.4.3 Vitiligo Phobia

The fear of developing vitiligo prompts consultations for a variety of hypopigmented macules in communities where vitiligo is stigmatized. Dermatologists should remember that the treatment of such patients is incomplete unless it is clearly stated that the disease is not vitiligo, even if the patient has not explicitly asked the question. Similarly, patients who have vitiligo should be reassured that every hypopigmented macule they notice on their skin does not represent an extension of the disease.

41.5 Specific Locations

Apart from type of vitiligo and extent of disease, response to treatment depends to a great extent on the site of lesions. Vitiligo on the head and neck and hair-bearing areas responds more rapidly and completely to treatment [17, 18, 48] On the other hand, vitiligo on the hands and feet, bony prominences (knees, elbows, ankles), and mucosa responds poorly to both surgical and medical treatment.

We describe below the issues related to treatment of vitiligo at particular body sites.

Periocular vitiligo can be easily managed with medical treatment alone in most patients. Repigmentation tends to be brisk and complete at this site. Topical steroids (low to mid-potency) or calcineurin inhibitors (tacrolimus and pimecrolimus) are effective options. When using topical steroids, patients should be closely monitored for cutaneous atrophy. Ophthalmological adverse effects such as glaucoma and cataract are uncommon but must be kept in mind. Patients with eyelid vitiligo undergoing phototherapy in a UV chamber for more extensive vitiligo can just keep the eyes closed to protect them and avoid using goggles which would prevent the beneficial effects of UV light on eyelid skin.

Sometimes, in refractory lesions, surgical management is warranted. Non-cultured epidermal suspension (NCES) is a good technique at this site as it does not require immobilization and dressing of eyelids and also provides the best color match. However, since the eyelids are a curved surface, when pouring the suspension over the recipient site, the head should be positioned such that the runoff of the suspension is minimal. Suction blister epidermal grafting (SBEG) also provides a reasonably good color match, and graft uptake tends to be very good because of the rich vascularity of this area. A disadvantage is the need for postoperative dressing and immobilization of the eyelid for 1 week. Tissue glue can be used to prevent the displacement of grafts. Mini-punch grafting (MPG) is preferred less as the skin is very thin over the eyelid making it technically challenging and cobble stoning, and polka dot appearance can compromise the results.

Recipient-site preparation is difficult in eyelid vitiligo. When administering anesthesia, the skin should be infiltrated enough to make it tumescent, and injection into the eyelid margin should be avoided to prevent damage to eyelash roots. Laser dermabrasion is preferred as it allows more precise control, while mechanical or motorized dermabrasion is difficult at this site as there is no underlying bony support. Care must be exercised to avoid injury to the eye during vitiligo surgery.

Perioral vitiligo is relatively resistant to treatment. Medical management is similar to that of eyelid vitiligo but is usually less effective. In addition, the role of recurrent herpes labialis in producing and perpetuating depigmentation needs to be considered. If recurrences are frequent, suppressive antiviral therapy may help to control both herpes and vitiligo.

Localized phototherapy with a handheld NB-UVB therapy device may be tried but is unlikely to be helpful. Surgical treatment may be considered if the disease is stable. Like the eyelid, the lip is a curved surface, and runoff of the suspension has to be avoided during the noncultured epidermal suspension (NCES) technique by proper positioning of the head. Suction blister epidermal grafting (SBEG) on the lip runs a risk of graft displacement. This can be avoided by immobilizing grafts with fibrin glue [72] and surgical tapes [73], restricting oral intake to liquids during the first few postoperative days, and restricting the movement of perioral muscles. A study comparing mini-punch grafting (MPG) and SBEG concluded that both techniques were effective, but MPG gave a better color match. Cobble stoning was the most common adverse event with MPG and hyperpigmentation with SBEG [74].

Postoperative care is imperative irrespective of the technique in order to achieve good results as the operated area is affected by brushing, eating, talking, and drinking. The patient should be instructed to take only liquids through a straw or using a vessel with a spout for the first 2 days and a semisolid diet for the next 5 days. Every effort should be made to keep the area clean and dry and movement of the perioral muscles (as in talking) avoided, as far as possible.

Genital vitiligo can have profound psychosexual effects on the patient and/or the partner. Topical calcineurin inhibitors are the preferred treatment at this site [75]. Topical steroids (low to mid-potency) can also be used for short durations. However, the treatment response tends to be poor [76]. The response to phototherapy is also not good. Surgery is relatively difficult compared to other body sites but has been attempted [77, 78].

Elbows, knees, hands, and feet respond poorly to all treatment modalities: medical, surgical, and phototherapy. In an attempt to find an explanation for the poor response, Esmat et al. [79] took biopsies from vitiliginous and perilesional skin on the trunk and proximal limbs and compared them with similar biopsies from acral vitiligo in 20 patients, before and after PUVA therapy. They found that factors such as inherent low melanocyte density, lower melanocyte stem cell reservoirs, and lower baseline epidermal stem cell factor may possibly be playing a role. Most patients get <25% or 25–50% repigmentation with medical treatment and/or phototherapy, and complete repigmentation is hardly ever achieved. Many authors have tried combination therapies in order to get better results (Table 41.1).

In the study by Bayoumi et al. [81], laser dermabrasion enhanced the treatment response of vitiligo over resistant sites to combination therapy with steroids and phototherapy. Complete repigmentation was seen in 16.7% of lesions on the dermabraded side but in none of the lesions on the control side. Unfortunately, side effects were common in the dermabrasion group and made this a less preferred option in spite of the good results. Dermabrasion possibly enhances the biological UV impact by increasing the penetration of UV rays as well as topical steroids, enhancing production of inflammatory cytokines with a pro-pigmenting action and removing affected keratinocytes.

Surgery (NCECS and SBEG) can be tried but repigmentation is not as good as at other sites [84]. Postoperative immobilization is difficult in these areas, and complications such as delayed healing and secondary infection are more common. For small patches, punch grafting is preferred and gives good results even on acral areas. Gupta et al. reported good results with suction blister epidermal grafting (SBEG) on acral areas [43]. Results may be enhanced by using PUVA-exposed epidermal grafts [85].

Scalp vitiligo is more obvious when the overlying hair is white. It can be managed with a topical corticosteroid lotion that may be easier to use than a cream or ointment. Localized phototherapy with NB-UVB combs may be helpful. If leukotrichia is not extensive, plucking out the white hair is a simple and rapidly effective solution. White hair may regrow but can be plucked out again.

Table 41.1 Different combination treatments tried to treat vitiligo at resistant sites

S No	Author and year	Study design	Patient no.	Results	Comments
1	Garg et al. [80]	Treated all patches with combination of microdermabrasion (2 weekly, 1–5 sittings) and topical 5% 5-FU twice daily	40 vitiligo patches in 22 patients	50% patches showed marked repigmentation after 1 or max 4 sessions of microdermabrasion	Only minor adverse effects
2	Bayoumi et al. [81]	Randomized left and right comparative study done to find the effect of ErYAG laser dermabrasion on response to treatment with combination of topical steroid and phototherapy	18	At least 50% repigmentation achieved in 50% and 4.2% patches in active and control group, respectively	Laser dermabrasion improved the treatment response significantly but had side effects like delayed healing, hypertrophic scar, and pain
3	Li et al. [82]	Active side—CO_2 laser dermabrasion along with topical steroid and phototherapy. Control side—CO_2 laser dermabrasion and phototherapy	25 patients with symmetrical stable resistant vitiligo patches	44% patches in active group achieved more than 50% repigmentation which was higher than the control group	Combination therapy works better in resistant patches
4	Mohamed et al. [83]	Group I—5-FU Group II—CO_2 laser Group III—combination	68 adult patients with symmetric acral vitiligo	50% patches in Group III achieved Grade 4 repigmentation	Only minor adverse effects

References

1. Nordlund J. The epidemiology and genetics of vitiligo. Clin Dermatol. 1997;15:875–8.
2. Sehgal VN, Srivastava G. Vitiligo: compendium of clinic-epidemiological features. Indian J Dermatol Venereol Leprol. 2007;73:149–56.
3. Burns T, Breathnach S, Cox N, Griffiths C. Rook's textbook of dermatology, vol. II. 7th ed. Oxford: Blackwell Science; 2004. pp. 32.57–32.59.
4. Howitz J, Brodthagen H, Schwartz M, Thomsen K. Prevalence of vitiligo. Arch Dermatol. 1977;113:47–52.
5. Ramakrishna P, Rajni T. Psychiatric morbidity and quality of life in vitiligo patients. Indian J Psychol Med. 2014;36:302–3.
6. Parsad D, Pandhi R, Dogra S, et al. Dermatology Life Quality Index score in vitiligo and its impact on the treatment outcome. Br J Dermatol. 2003;148:373–4.
7. Wolff K, Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ. Fitzpatrick's dermatology in general medicine, vol. I. 7th ed. New York: McGraw Hill; 2007. p. 616–21.
8. James WD, Berger TG, Elston DM. Andrews diseases of the skin: clinical dermatology. 10th ed. Philadelphia, PA: Saunders Elsevier; 2006. p. 860–3.
9. Kedward AL, Gawkrodger DJ. Congenital stable symmetrical type vitiligo in a patient whose mother developed vitiligo during pregnancy. Eur J Dermatol. 2008;18(3):353.
10. Catucci Boza J, Giongo N, Machado P, Horn R, Fabbrin A, Cestari T. Quality of life impairment in children and adults with vitiligo: a cross-sectional study based on dermatology-specific and disease-specific quality of life instruments. Dermatology. 2016;232(5):619–25.
11. Handa S, Dogra S. Epidemiology of childhood vitiligo: a study of 625 patients from north India. Pediatr Dermatol. 2003;20:207–10.
12. Isenstein AL, Morrell DS, Burkhart CN. Vitiligo: treatment approach in children. Pediatric Ann. 2009;38(6):339–44.
13. Shajil EM, Agrawal D, Vagadia K, Marfatia YS, Begum R. Vitiligo: clinical profiles in Vadodara, Gujarat. Indian J Dermatol. 2006;51:100–4.
14. Cho S, Kang H, Hahm J. Characteristics of vitiligo in Korean children. Pediatr Dermatol. 2000;17:189–93.
15. Halder R, Grimes P, Cowan C, Enterline J, Chakabarti S, Kenney J. Childhood vitiligo. J Am Acad Dermatol. 1987;16:948–54.
16. Kato H, Furuhashi T, Ito E, Kaneko N, Nakamura M, Watanabe S, Shintani Y, Maeda A, Yamaguchi Y, Morita A. Efficacy of 1-mm minigrafts in treating

vitiligo depends on patient age, disease site and vitiligo subtype. J Dermatol. 2011;38:1140–5.
17. Lepe V, Moncada B, Castaneda-Cazares J, Torres-Alvarez M, Ortiz C, Torres-Rubalcalva A. A double-blind randomized trial of 0.1% tacrolimus vs 0.05% clobetasol for the treatment of childhood vitiligo. Arch Dermatol. 2003;139:581–5.
18. Cockayne S, Messenger A, Gawkrodger D. Vitiligo treated with topical corticosteroids: children with head and neck involvement respond well. J Am Acad Dermatol. 2002;46:964–5.
19. Schaffer J, Bolognia J. The treatment of hypopigmentation in children. Clin Dermatol. 2003;21:296–310.
20. Khalid M, Mujtaba G. Response of segmental vitiligo to 0.05% clobetasol propionate cream. Int J Dermatol. 1998;37:705–8.
21. Khalid M, Mujtaba G, Haroon TS. Comparison of 0.05% clobetasol propionate cream and topical Puvasol in childhood vitiligo. Int J Dermatol. 1995;34:203–5.
22. Kose O, Riza GA, Kurumlu Z, Erol E. Calcipotriol ointment versus clobetasol ointment in localized vitiligo: an open, comparative clinical trial. Int J Dermatol. 2002;41:616–8.
23. Kwinter J, Pelletier J, Khambalia A, Pope E. High-potency steroid use in children with vitiligo: a retrospective study. J Am Acad Dermatol. 2007;56:236–41.
24. Grimes P, Soriano T, Dytoc M. Topical tacrolimus for repigmentation of vitiligo. J Am Acad Dermatol. 2002;47:789–91.
25. Silverberg N, Lin P, Travis L, Farely-Li J, Mancini A, Wagner A, Chamlin S, Paller A. Tacrolimus ointment promotes repigmentation of vitiligo in children: a review of 57 cases. J Am Acad Dermatol. 2004;51(3):760–6.
26. Kanwar A, Dogra S, Parsad D. Topical tacrolimus for treatment of childhood vitiligo in Asians. Clin Exp Dermatol. 2004;29:589–92.
27. Ho N, Pope E, Weinstein M, Greenberg S, Webster C, Krafchik BR. A double-blind, randomized, placebo controlled trial of topical tacrolimus 0.1% vs clobetasol propionate 0.05% in childhood vitiligo. Br J Dermatol. 2011;165:626–32.
28. Farajzadeh S, Daraei Z, Esfandiarpour I, Hosseini SH. The efficacy of pimecrolimus 1% cream combined with microdermabrasion in the treatment of non segmental childhood vitiligo: a randomized placebo-controlled study. Pediatr Dermatol. 2009;26:286–91.
29. Gargoom AM, Duweb GA, Elzorghany AH, Benghazil M, Bugrein OO. Calcipotriol in the treatment of childhood vitiligo. Int J Clin Pharmacol Res. 2004;24:11–4.
30. Travis LB, Silverberg NB. Calcipotriene and corticosteroid combination therapy for vitiligo. Pediatr Dermatol. 2004;21:495–8.
31. Njoo MD, Bos JD, Westerhof W. Treatment of generalized vitiligo in children with narrow-band (TL-01) UVB radiation therapy. J Am Acad Dermatol. 2000;42(2 Pt 1):245–53.
32. Drake LA, Dinehart SM, Farmer ER, Holtz RW, Graham GF, Hordinsky MK, et al. Guidelines of care for vitiligo. J Am Acad Dermatol. 1996;35:620–6.
33. Antoniou C, Katsambas A. Guidelines for the treatment of vitiligo. Drugs. 1992;43:490–8.
34. Koh MJ, Mok ZR, Chong WS. Phototherapy for the treatment of vitiligo in Asian children. Pediatr Dermatol. 2015;32:192–7.
35. Hadi S, Spencer J, Lebwohl M. The use of the 308 nm excimer laser for the treatment of vitiligo. Dermatol Surg. 2004;30(7):983–6.
36. Passeron T, Ostovari N, Zakaria W, et al. Topical tacrolimus and the 308 nm excimer laser: a synergistic combination for the treatment of vitiligo. Arch Dermatol. 2004;140(9):1065–9.
37. Sassi F, Cazzaniga S, Tessari G, et al. Randomized controlled trial comparing the effectiveness of 308 nm excimer laser alone or in combination with topical hydrocortisone 17-butyrate cream in the treatment of vitiligo of the face and neck. Br J Dermatol. 2008;159:1186–91.
38. Yang HL, Huang XY, Yong FJ, Rong L. Combination of 308-nm excimer laser with topical pimecrolimus for the treatment of childhood vitiligo. Pediatr Dermatol. 2009;26:354–6.
39. Cho S, Zheng Z, Park YK, Roh MR. The 308-nm excimer laser: a promising device for treatment of childhood vitiligo. Photodermatol Photoimmunol Photomed. 2011;27:24–9.
40. Mulekar SV, Al Eisa A, Delvi MB, et al. Childhood vitiligo: a long-term study of localized vitiligo treated by noncultured cellular grafting. Pediatr Dermatol. 2009;27:132–6.
41. Mulekar SV, Isedeh P. Surgical interventions for vitiligo: an evidence-based review. Br J Dermatol. 2013;169(Suppl 3):57–66.
42. Gupta S, Kumar B. Epidermal grafting for vitiligo in adolescents. Pediatr Dermatol. 2002;19:159–62.
43. Gupta S, Kumar B. Epidermal grafting in vitiligo: influence of age, site of lesion, and type of disease on outcome. J Am Acad Dermatol. 2003;49:99–104.
44. Halaban R. The regulation of normal melanocyte proliferation. Pigment Cell Res. 2000;13:4–14.
45. Abdel-Malek ZA, Swope VB, Nordlund JJ, Medrano EE. Proliferation and propagation of human melanocytes in-vitro are affected by donor age and anatomical site. Pigment Cell Res. 1994;7:116–22.
46. Gilchrist BA, Vrabel MA, Flynn E, Szabo G. Selective cultivation of human melanocytes from newborn and adult epidermis. J Invest Dermatol. 1984;83:370–6.
47. Hong WS, Hu DN, Qian GP, McCormick SA, Xu AE. Treatment of vitiligo in children and adolescents by autologous cultured pure melanocytes transplantation with comparison of efficacy to results in adults. J Eur Acad Dermatol Venereol. 2011;25:538–43.
48. Handa S, Kaur I. Vitiligo: clinical findings in 1436 patients. J Dermatol. 1999;26:653–7.

49. Koranne RV, Sehgal VN, Sachdeva KG. Clinical profile of vitiligo in North India. Indian J Dermatol Venereol Leprol. 1986;52:81–2.
50. Sarin RC, Kumar AS. A clinical study of vitiligo. Indian J Dermatol Venereol Leprol. 1977;43:300–14.
51. Lerner AB. Vitiligo. J Invest Dermatol. 1959;32:285–310.
52. Lerner AB, Nordlund JJ. Vitiligo. What is it? Is it important? JAMA. 1978;239:1183–7.
53. Howitz J, Brodthagen H, Schwartz M, Thomsen K. Epidemiological survey on the Isle of Bornholm, Denmark. Arch Dermatol. 1977;113:47–52.
54. Bolognia JL, Pawelek JM. Biology of hypopigmentation. J Am Acad Dermatol. 1988;19:217–55.
55. Mosher DB, Fitzpatrick TB, Hori Y, Ortonne JP. Disorders of pigmentation. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, editors. Dermatology in general medicine. New York: McGraw Hill; 1993. p. 903.
56. Shah H, Mehta A, Astik B. Clinical and sociodemographic study of vitiligo. Indian J Dermatol Venereol Leprol. 2008;74:701.
57. Dogra S, Parsad D, Handa S, Kanwar AJ. Late onset vitiligo: a study of 182 patients. Int J Dermatol. 2005;44:193–6.
58. Akrem J, Baroudi A, Aichi T, Houch F, Hamdaoui MH. Profile of vitiligo in the south of Tunisia. Int J Dermatol. 2008;47:670–4.
59. Mchepange UO, Gao XH, Liu YY, Liu YB, Ma L, Zhang L, Chen HD. Vitiligo in North-Eastern China: an association between mucosal and acrofacial lesions. Acta Derm Venereol. 2010;90:136–40.
60. Arýcan O, Koç K, Ersoy L. Clinical characteristics in 113 Turkish vitiligo patients. Acta Dermatovenerol Alp Panonica Adriat. 2008;17:129–32.
61. Hu Z, Liu JB, Ma SS, Yang S, Zhang XJ. Profile of childhood vitiligo in China: an analysis of 541 patients. Pediatr Dermatol. 2006;23:114–6.
62. Koronne RV, Sachdevo KG. Vitiligo. Int J Dermatol. 1998;27:676–81.
63. Porter J, Beuf AH, Nordlund JJ, AB L. Response to cosmetic disfigurement: patients with vitiligo. Cutis. 1987;39:493–4.
64. Pahwa P, Mehta M, Khaitan BK, Sharma VK, Ramam M. The psychosocial impact of vitiligo in Indian patients. Indian J Dermatol Venereol Leprol. 2013;79:679–85.
65. Xu AE, Zhang DM, Wei XD, Huang B, Lu LJ. Efficacy and safety of tarcrolimus cream 0.1% in the treatment of vitiligo. Int J Dermatol. 2009;48:86–90.
66. Singh S, Khandpur S, Sharma VK, Ramam M. Comparison of efficacy and side-effect profile of oral PUVA vs. oral PUVA sol in the treatment of vitiligo: a 36-week prospective study. J Eur Acad Dermatol Venereol. 2013;27:1344–51.
67. Park KK, Liao W, Murase JE. A review of monochromatic excimer light in vitiligo. Br J Dermatol. 2012;167:468–78.
68. Sapam R, Agrawal S, Dhali TK. Systemic PUVA vs. narrowband UVB in the treatment of vitiligo: a randomized controlled study. Int J Dermatol. 2012;51:1107–15.
69. Budania A, Parsad D, Kanwar AJ, Dogra S. Comparison between autologous noncultured epidermal cell suspension and suction blister epidermal grafting in stable vitiligo: a randomized study. Br J Dermatol. 2012;167:1295–301.
70. Holla AP, Sahni K, Kumar R, Kanwar A, Mehta S, Parsad D. Repigmentation of leukotrichia due to retrograde migration of melanocytes after noncultured epidermal suspension transplantation. Dermatol Surg. 2014;40:169–75.
71. Singh AK, Karki D. Micropigmentation: tattooing for the treatment of lip vitiligo. J Plast Reconstr Aesthet Surg. 2010;63:988–91.
72. Kim H, Kang JN, Hwang SH, Seo JK, Sung HS. Fibrin glue fixation for suction blister epidermal grafting in two patients with stable vitiligo. Ann Dermatol. 2014;26:751–4.
73. Laxmisha C, Thappa DM. Surgical Pearl: surgical tape for dressing of epidermal grafts in lip vitiligo. J Am Acad Dermatol. 2005;53:498–9.
74. Babu A, Thappa DM, Jaisankar TJ. Punch grafting versus suction blister epidermal grafting in the treatment of stable lip vitiligo. Dermatol Surg. 2008;34:166–78.
75. Souza Leite RM, Craveiro Leite AA. Two therapeutic challenges: periocular and genital vitiligo in children successfully treated with pimecrolimus cream. Int J Dermatol. 2007;46:986–9.
76. Fai D, Cassano N, Vena GA. Narrow–band UVB phototherapy combined with tacrolimus ointment in vitiligo: a review of 110 patients. J Eur Acad Dermatol Venereol. 2007;21:916–20.
77. Mulekar SV, Al Issa A, Al Eisa A, Asaad M. Genital vitiligo treated by autologous, noncultured melanocyte-keratinocyte cell transplantation. Dermatol Surg. 2005;31:1737–9.
78. Matsuzaki K, Chiyokura T, Kumagai N. Special considerations when grafting cultured epithelial sheets in male genital vitiligo. Dermatol Surg. 2016;42:128–30.
79. Esmat SM, El-Tawdy AM, Hafez GA, Zeid OA, Abdel Halim DM, Saleh MA, Leheta TM, Elmofty M. Acral lesions of vitiligo: why are they resistant to photochemotherapy? J Eur Acad Dermatol Venereol. 2012;26:1097–104.
80. Garg T, Chander R, Jain A. Combination of microdermabrasion and 5-fluorouracil to induce repigmentation in vitiligo: an observational study. Dermatol Surg. 2011;37:1763–6.
81. Bayoumi W, Fontas E, Sillard L, Le Duff F, Ortonne JP, Bahadoran P, Lacour JP, Passeron T. Effect of a preceding laser dermabrasion on the outcome of combined therapy with narrow band ultraviolet B and potent topical steroids for treating nonsegmental

vitiligo in resistant localizations. Br J Dermatol. 2012;166:208–11.
82. Li L, Wu Y, Li L, Sun Y, Qiu L, Gao XH, Chen HD. Triple combination treatment with fractional CO2 laser plus topical betamethasone solution and narrowband ultraviolet B for refractory vitiligo: a prospective, randomized half-body, comparative study. Dermatol Ther. 2015;28:131–4.
83. Mohamed HA, Mohammed GF, Gomaa AH, Eyada MM. Carbon dioxide laser plus topical 5-fluorouracil: a new combination therapeutic modality for acral vitiligo. J Cosmet Laser Ther. 2015;17:216–23.
84. Mulekar SV, Al Issa A, Al Eisa A. Treatment of vitiligo on difficult-to treat sites using autologous noncultured cellular grafting. Dermatol Surg. 2009;35:66–71.
85. Suga M, Butt KI, Takimoto R, et al. Successful treatment of vitiligo with PUVA pigmented autologous epidermal grafting. Int J Dermatol. 1996;35:518–22.

Psychological Interventions

42

Panagiota Kostopoulou and Alain Taïeb

Contents

Abstract

A low self-esteem and high levels of perceived stigma seem to be important factors for quality of life impairment in vitiligo patients. A simple visual analog scale from 0 to 10 with the notice 'How much does your skin disease bother you currently?' helps to measure coarsely how patients feel about their disease independently of medical findings. Patients with high perceived severity are the best targets of psychological interventions. Cognitive-behavioural therapy might help improve the quality of life, self-esteem and perceived body image.

Key Points

- A low self-esteem and high levels of perceived stigma seem to be important factors for quality of life impairment in vitiligo patients.
- A simple visual analog scale from 0 to 10 with the notice 'How much does your skin disease bother you currently?' helps to measure coarsely how patients feel about their disease independently of medical findings.
- Patients with high perceived severity are the best targets of psychological interventions.
- Cognitive-behavioural therapy might help improving the quality of life, self-esteem and perceived body image.

P. Kostopoulou · A. Taïeb (✉)
Service de Dermatologie, Hôspital St André, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_42

42.1 Why Psychological Support Is Important

Although vitiligo does not lead to severe physical illness, patients experience a variable degree of psychosocial impairment. The psychological impact of vitiligo has been shown in different studies all over the world. Patients with vitiligo suffer from poor body image, low self-esteem and social isolation, caused by feelings of embarrassment, and they experience a considerable level of disability [1–7]. The prevalence of psychiatric morbidity associated with vitiligo ranges from 25% to 30% in Western Europe [2, 8] and from 56% to 75% in India [3, 9, 10]. A low self-esteem and high levels of perceived stigma seem to be important factors for quality of life impairment in vitiligo patients [9, 11–16]. The majority of patients with vitiligo found their disfigurement moderately or severely intolerable [14], and most of them said that vitiligo had affected their lives recently [9]. Psychological interviews with patients at our department confirm that vitiligo has an important impact on their daily lives. Visible or not directly visible lesions influence not only their personal relationships and their professional career but also their social life. Patients said they avoided daily activities and different social events in order to protect themselves from embarrassing comments [17]. In the same study, we found that perceived severity of the disease and patient's personality are important factors to consider when assessing the psychological impact of vitiligo. If self-body image is more influenced by gender, perceived severity is more influenced by patient's personality than by objective criteria of the disease (Fig. 42.1) [17].

42.2 Screening Patients in Need of Psychological Support

Objective criteria like percentage of the body area affected and staging score [15] are important but not enough for patient's assessment and follow-up. A psychologically oriented interview is essential in order to evaluate the perceived impact of the skin disorder. This perceived impact is important to tailor the management to the patients' needs and difficulties. A simple visual analog scale from 0 to 10 with the notice 'How much does your skin disease bother you currently?' helps to measure coarsely how patients feel about their disease independently of medical findings. A simple questionnaire of quality of life such as the DLQI [18, 19] which is very easy and rapid to use can be also be used to measure patients' daily difficulties associated to their skin disorder.

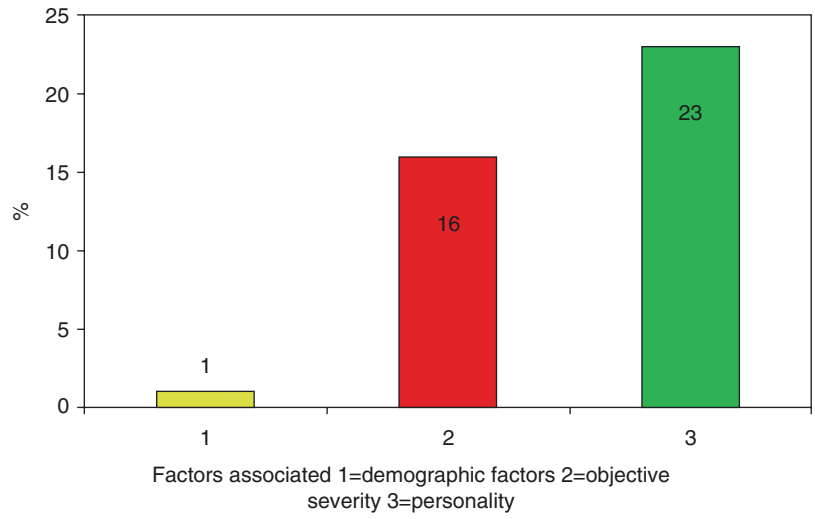


Fig. 42.1 Relative importance of factors affecting the variance of perceived severity

42.3 Psychological Interventions

Different types of psychological support and help can be proposed. Most published articles concentrate on the type of psychological difficulties and the factors that influence them, but do not mention specific interventions. The only psychological intervention published in vitiligo is that of Papadopoulos et al. [12], who have used a cognitive-behavioural therapy. They indicated it can help improve the quality of life, self-esteem and perceived body image. They also suggested that cognitive-behavioural therapy may influence the progression of the condition itself. Even if their findings are based to a very small sample of patients and if their conclusions are difficult to acknowledge without reservation, this work offers new perspectives. Dermatologists might consider adding psychosocial interventions to standard medical treatment [13].

In addition, based on our experience, a simple psychological interview and a supportive attitude can also be beneficial. Patients are happy to have the opportunity to express difficulties resulting from their disease. Being understood and listened to is a part of the global management of vitiligo. Sometimes, a simple discussion with a professional can help the patient becoming free from the complexes caused by the disease and embarrassing comments he has endured. Throughout this process, patients may move from a state of discomfort to a greater acceptance of their image. During the interview, we can also evaluate the patient socio-professional context and the stigmatisation caused by the disorder either at work or during daily activities. Different strategies to be accepted and to better enjoy social life can be proposed. Accordingly, a multidisciplinary team should manage, mainly at first visit, the patient. Patients who can benefit a psychological follow-up can receive more attention and may just feel better because of that. An objective is that patients' negative thoughts related to the disease should be gradually replaced by other more positive based on their personality and qualities. By doing so, patients should improve their self-esteem and socialisation.

42.4 Concluding Remarks

Psychological support has primarily the intention to help the patients express their psychological suffering and the negative feelings associated with the disease. It is also aimed at helping the patients to accept themselves as they are, with or without the disease. In case of beneficial impact of the intervention, patients may be able not only to resume their activities and to participate in their social life without feeling handicapped by their skin disorder but also to develop their own personality without being restricted by disease considerations [16, 20–26].

References

1. Firooz A, Bouzari N, Fallah N, et al. What patients with vitiligo believed about their condition. Int J Dermatol. 2004;43:811–4.
2. Kent G, Al Abadie M. Factors affecting responses on dermatology life quality index items among vitiligo sufferers. Clin Exp Dermatol. 1996;21:330–3.
3. Matoo SK, Handa S, Kaur I, et al. Psychiatric morbidity in vitiligo: prevalence and correlates in India. J Eur Acad Dermatol Venereol. 2002;16:573–8.
4. Porter JR, Beuf AH, Lerner A, Nordlund J. Psychosocial effect of vitiligo: a comparison of vitiligo patients with 'normal' control subjects, with psoriasis patients and patients with other pigmentary disorders. J Am Acad Dermatol. 1986;15:220–4.
5. Porter JR, Beuf AH, Lerner A, Nordlund J. Psychological reaction to chronic skin disorders: a study of patients with vitiligo. Gen Hosp Psychiatry. 1979;1:73–7.
6. Porter JR, Beuf AH, Lerner A, Nordlund J. The effect of vitiligo in sexual relationships. J Am Acad Dermatol. 1990;22:221–2.
7. Sampogna F, Raskovic D, Guerra L, et al. Identification of categories at risk for high quality of life impairment in patients with vitiligo. Br J Dermatol. 2008;159:351–9.
8. Picardi A, Abeni D, Melchi CF, et al. Psychiatric morbidity in dermatological outpatients: an issue to be recognised. Br J Dermatol. 2000;143:983–91.
9. Kent G, Al'Abadie M. Psychologic effects of vitiligo: a critical incident analysis. J Am Acad Dermatol. 1996;35:895–8.
10. Matoo SK, Handa S, Kaur I, et al. Psychiatric morbidity in vitiligo and psoriasis: a comparative study from India. J Dermatol. 2002;28:424–32.
11. Harlow B, Poyner T, Finlay AY, Dykes PJ. Impaired quality of life of adults with skin disease in primary care. Br J Dermatol. 2000;143:979–82.

12. Papadopoulos L, Bor R, Legg C. Coping with the disfiguring effects of Vitiligo: a preliminary investigation into the effects of Cognitive-Behavioural Therapy. Br J Med Psychol. 1999;72:385–96.
13. Picardi A, Abeni D. Can Cognitive-Behavioural Therapy help patients with vitiligo? Arch Dermatol. 2001;137:786–8.
14. Salzer B, Schallreuter KU. Investigation of the personality structure in patients with vitiligo and a possible association with catecholamine metabolism. Dermatology. 1995;190:109–15.
15. Taieb A, Picardo M. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell Res. 2007;20:27–35.
16. Bonotis K, Pantelis K, Karaoulanis S, et al. Investigation of factors associated with health-related quality of life and psychological distress in vitiligo. J Dtsch Dermatol Ges. 2016;14(1):45–9.
17. Kostopoulou P, Jouary T, Quintard B, et al. Objective vs subjective factors in the psychological impact of vitiligo: the experience from a French referral centre. Br J Dermatol. 2009;161(1):128–33.
18. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI). A simple practical measure for routine clinical use. Clin Exp Dermatol. 1994;19:210–6.
19. Finlay AY. Quality of Life Measurement in Dermatology: a practical guide. Br J Dermatol. 1997;136:305–14.
20. Shah R, Hunt J, Webb TL, Thompson AR. Starting to develop self-help for social anxiety associated with vitiligo: using clinical significance to measure the potential effectiveness of enhanced psychological self-help. Br J Dermatol. 2014;171(2):332–7.
21. Chan MF, Chua TL. The effectiveness of therapeutic interventions on quality of life for vitiligo patients: a systematic review. Int J Nurs Pract. 2012;18(4):396–405.
22. Eleftheriadou V, Whitton ME, Gawkrodger DJ, et al. Vitiligo priority setting partnership. Future research into the treatment of vitiligo: where should our priorities lie? Results of the vitiligo priority setting partnership. Br J Dermatol. 2011;164(3):530–6.
23. Wang KY, Wang KH, Zhang ZP. Health-related quality of life and marital quality of vitiligo patients in China. J Eur Acad Dermatol Venereol. 2011;25(4):429–35.
24. Thompson AR, Clarke SA, Newell RJ, Gawkrodger DJ, Appearance Research Collaboration (ARC). Vitiligo linked to stigmatization in British South Asian women: a qualitative study of the experiences of living with vitiligo. Br J Dermatol. 2010;63(3):481–6.
25. Ongenae K, Van Geel N, De Schepper S, Naeyaert JM. Effect of vitiligo on self-reported health-related quality of life. Br J Dermatol. 2005;152:1165–72.
26. Whitton M, Pinart M, Batchelor JM, Leonardi-Bee J, Gonzalez U, Jiyad Z, Eleftheriadou V, Ezzedine K. Evidence-based management of vitiligo: summary of a Cochrane systematic review. Br J Dermatol. 2016;174(5):962–9. https://doi.org/10.1111/bjd.14356. Epub 2016 Mar 25. Review.

Patients' Perspectives

43

Jean-Marie Meurant

Contents

Abstract

It is important to take into account the major psychological effects of vitiligo in direct sufferers and their immediate family (parents of children, siblings, etc.) towards treatment. Above all, the seriousness of the disease should not be minimised by doctors and/or sufferers' friends and family because doing so entails the risk of increasing the risk of isolation, depression, and self-harm, as well as weakening the doctor-patient relationship and the ability to educate the patient to follow their treatment against vitiligo and diagnosed comorbidities. Announcing a diagnosis of vitiligo may entail psychological support being arranged for sufferers, and their families, soon after the announcement, by clinical psychologists specialising in skin diseases. In addition, implementing individual or group psychological support such as support groups and/or corrective makeup workshops in individual or group sessions, assisted by professionals and clinical psychologists, is recommended in

J.-M. Meurant (✉)
Association Française du Vitiligo, Paris, France
e-mail: jean-marie.meurant@afvitiligo.com

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_43

order to help sufferers cope with the difficult situations which can often arise. The use of phototherapy devices at home is recommendable for better treatment monitoring and long-term efficacy, in view of work and transport considerations. There is a need to educate patients about favourable sun exposure conditions, recommending sunscreen creams and solar-protective clothing to avoid being "shut away", as witnessed by a large number of vitiligo sufferers. The awareness of the risks of depigmentation caused by repeated rubbing should be raised. A global care perspective enables better compliance, allowing sufferers to become less withdrawn and thus avoiding loss of self-esteem, resulting in better inclusion in family, school, and professional life.

Providing information about any local vitiligo sufferers' associations is important: these are useful points of contact for the various players in the healthcare system, overcoming the isolation of sufferers, speaking on their behalf, and moderating their statements by providing objective information and serving as credible references.

Key Points

- Take into account the major psychological effects of vitiligo; direct sufferers and their immediate family (parents of children, siblings, etc.) towards treatment.
- Above all, the seriousness of the disease should not be minimised by doctors and/or sufferers' friends and family; doing so entails the risk of increasing the risk of isolation, depression, and self-harm, as well as weakening the doctor-patient relationship or "therapeutic alliance" and the ability to educate the patient to follow their treatment against vitiligo and diagnosed comorbidities.
- Announcing a diagnosis of vitiligo may entail psychological support being arranged for sufferers, and their families, soon after the announcement, by clinical psychologists specialising in skin diseases.
- Implement, or direct patients towards, transitional care schemes pending therapy solutions, in the form of individual or group psychological support such as support groups and/or corrective makeup workshops in individual or group sessions, assisted by professionals and clinical psychologists, in order to help sufferers cope with the difficult situations which can often arise.
- Recommend the use of phototherapy devices at home for better treatment monitoring and long-term efficacy, in view of work and transport considerations.
- Educate patients about favourable sun exposure conditions, recommending sunscreen creams and solar protective clothing to avoid being "shut away", the experience of a large number of vitiligo sufferers. Raise awareness of the risks of depigmentation caused by repeated rubbing.
- Arrange global care to enable treatment to be followed better, enabling sufferers to become less withdrawn and thus avoiding loss of self-esteem, resulting in better inclusion in family, school, and professional life.
- Provide information about any local vitiligo sufferers' associations: these are useful points of contact for the various players in the healthcare system, overcoming the isolation of sufferers, speaking on their behalf, and moderating their statements by providing objective information and serving as credible references.

43.1 Introduction

Vitiligo sufferers very frequently say that their future as a human being is at stake, that their life is at risk of collapse, and that their relationships with others have radically changed. Sufferers are

ashamed of having "patches" and become withdrawn and afraid of others' gaze; they suffer from inner feelings of guilt that undermine and may even seriously damage their self-esteem.

The suffering entailed by this state of affairs may be exacerbated by a number of factors:

- The sufferer's environment being steeped in the exaggerated cults of beauty and health.
- Beliefs and rites entailing the risk of not being able to marry because of depigmentation, being forced to divorce, being rejected, etc.

The following are aggravating factors: having a dark skin type, being in frequent contact with the public, and fragile personal circumstances. Any major life changes (house move, change of school, job, etc.) can further exacerbate these situations.

43.2 The Contrasting Perceptions of Doctors and Patients

The appearance of depigmented areas is almost systematically a traumatic experience for sufferers, as well as for their friends and family in many cases. This trauma may be assessed or deemed to be unwarranted and exaggerated by the examining physician or the sufferer's partner. This in no way changes the way in which sufferers see themselves, their suffering, or the distress that consumes and isolates them.

Worse still, playing down or ignoring the painful feelings of sufferers increases their distress and their feelings of guilt and shame.

The increasing, deliberate visibility of "different" models who suffer from vitiligo (such as Winnie Harlow in Canada, Tysca in France, and April Star in the USA) and the use of their services by PR agencies, particularly in fashion shows, should not obscure the reality experienced by the vast majority of sufferers, afflicted with depigmentation, who cannot or can no longer put up with their skin, have to endure the stigma of other people looking at them, and feel excluded—all of which is reinforced by a lack of care and satisfactory medical solutions.

Sufferers vent their despair on social media, weep in doctors' surgeries, break down in support groups, lock themselves up at home, cut themselves off from the world, and gradually slip into depression: this can lead to self-harm and attempted suicide.

Vitiligo is not simply a disease that affects the appearance. It is a full-blown auto-immune disease with its related comorbidities and harmful effects on the psyche; it therefore has serious psychological repercussions.

As it loses its pigment, the skin—the protective envelope for the inner self—creates a sensation of "flaws" and "holes" in this protection, weakening the person as a whole. Vitiligo also has comorbidities, and thus is not simply a weakness, but rather an overall deficiency of the whole person, causing isolation, depression, and risks of suicide.

A large number of parents, pregnant mothers, and women who want a baby ask questions about whether they are to blame for having passed on vitiligo or indeed whether they might pass on their vitiligo or other related diseases. The serious thing about vitiligo is that contrary to a non-visible disease, depigmentation is terribly and constantly visible on the face and hands, making the sufferer prey to stigma at all times.

43.3 The Case for Providing Holistic Care for Sufferers

Sufferers who attend a medical consultation for their vitiligo have previously been through a lot, both personally and in private, experiencing anxiety about the disease and its development combined with an overwhelming fear of the diagnosis. Almost systematically, sufferers will have already carried out Internet searches which reinforce their anxiety about a potential lack of treatment.

Hesitations about the diagnosis and fear caused by the announcement of the disease lead patients into web searches that end up making them more vulnerable still.

Sufferers are constantly searching on the Internet. They go back and forth between medical sites and sufferers' associations, as well as

websites where certain solutions are presented as providing definitive treatment (from the Dead Sea, Cuba, Tunisia, plant-based, aloe vera, etc.). Each time, the sufferer will attempt to find a comforting solution to bring an end to their tireless quest for healing. This never-ending search leads them to web surfing with all the related financial costs and risks (purchasing products with untested effects, exotic treatments, travel, etc.).

Vitiligo sufferers arrive in doctors' surgeries with high levels of anxiety. Sufferers have very high expectations, which are vital in terms of their conscious and subconscious self-projection into a decidedly "patchy" future, one that will revolutionise their lives.

The physician that sees the sufferer is often unable to provide a direct response in terms of treatment; this makes it difficult for them to address the sufferer's expectations.

A brief, swift consultation (involving a short time spent looking at the affected skin, so short it may even be perceived as being non-existent) will be experienced as a failure; it is as if the disease, what the patient has to say, and what they feel are not being taken into account.

The patient may well understand that there are legitimate limits to the extent of possible treatment, but this will once again bring them face to face with the fact that they must suffer alone and confront them once more with the rejection they feel and the stigma they regularly experience.

Sufferers are therefore often tense and sometimes aggressive. As suffering human beings, they may be subject to outbursts of tears and/or various types of decompensation: these are difficult for physicians to cope with, given the technical and economic considerations of surgeries, be they private or public, even in countries with well-developed healthcare systems.

In this context, many physicians report that they find relations with vitiligo sufferers difficult. They are only too aware of how the patient is affected, the limited treatment possibilities, and the need to refer them to systems which are beyond their own dermatology specialisation, and so they prefer to minimise the extent to which they are confronted with this pathology, given the lack of clear and/or alternative solutions.

There needs to be a radical shift from analysis conducted solely in terms of the medical issue of skin depigmentation and the reactions it causes.

43.4 The Skin and the Sufferer: Two Responses to Two Distinct Issues

The diseased organ should not obscure the patient themselves. Depigmented skin must not conceal the desperate sufferer, who is in dire need of assistance, if other more serious damage is to be avoided. The latter is far costlier for the community around the sufferer and their family (withdrawal, academic difficulties, the consequences of stigma and bullying, isolation, unemployment, episodes of depression, suicide, etc.). This is also the case for the comorbidities associated with vitiligo: these require long-term treatment in a climate of trust between the patient and their physician (the main comorbidities currently known are thyroid disorders, autoimmune diabetes, pernicious anaemia, Addison's disease, psoriasis, rheumatoid arthritis, lichen planus, coeliac disease, and alopecia).

43.5 Trust, the Doctor-Patient Relationship, and Phototherapy

The treatments which may be proposed to vitiligo sufferers are long term, lasting several months: these are often felt to be a bind in busy schedules, due to their frequency during the week, and often require non-negligible or even long journey times.

The demands of phototherapy sessions on sufferers are clear: leaving their work or classes, having to make the journey to the doctor's surgery or hospital, etc. The impact of these considerations may cause them to give up on their treatment for a time or even permanently.

Some sufferers may have already bought phototherapy devices for the face and hands on the Internet, to make it easier for them to follow their treatment.

The possibility of prescribing home use of phototherapy systems rented from recognised professionals should be examined during the course of the doctor-patient relationship.

Procedures need to be identified; they include educating the patient by means of guidelines and clear instructions for use, with regular monitoring of the treatment by the prescribing physician. Checking the proper use of appliances and exposure duration, together with the ease of use of home devices, clearly favours treatment being followed and increases its chances of being successful.

In 2006, the *Journal of Cutaneous Medicine and Surgery* (the official publication of the Canadian Dermatology Association) published a study [1] in this respect which concludes as follows: "Narrow-band ultraviolet B home phototherapy was found to be an effective form of therapy for photoresponsive diseases, when compared to the therapies delivered in hospitals. It is safe and presents few side effects when patients receive appropriate guidelines, teaching, and follow-ups. Not only is it convenient; it also provides effective savings for patients who are unable to attend the hospital owing to time, travel, and interference with work schedule. All patients on home therapy were satisfied with their treatment, plan to continue it, and recommend it to others in similar situations".

Some may be concerned that patients may not use the appliances correctly or that family members may misuse them; however, the latter concern also applies for any drugs kept in family medicine cabinets, despite which it is not an overriding issue in such cases. Vitiligo sufferers are no different from other responsible patients and should be trusted accordingly.

43.6 The Case for Care Schemes

The progress of vitiligo research (aetiology, potential avenues for treatment, clinical trials, etc.) and the time required to develop therapeutic and drug-based solutions mean that the organisation of transitional care schemes is called for. These can reduce the psychological impacts, as well as their consequences for sufferers and their friends and families, and provide communication resources and solutions to mitigate the withdrawal and the stigma to which sufferers are very often subjected.

Psychological care, preferably immediately or alternatively at a later date, during consultations, clearly appears to be called for, in order to meet the following three needs:

- A medical need, with consultations focusing on being attentive to the appearance and development of the disease, the diagnosis of the form of vitiligo present, potential solutions, the treatment choices to be made, precautions to be taken (moisturising, sunscreen, monitoring for related diseases), and referral to global and individual psychological care.
- A psychological need, with the swift provision of psychological care (this may even be immediate in certain hospitals), giving an opportunity for the sufferer's private thoughts to be expressed, as well as their experience of the disease and its effects, its impact on their life and those of their friends and family, the professional and/or academic consequences, its effect on self-esteem, etc. This care must evaluate whether additional referrals should be made in coordination with the GP and/or the physician who made the diagnosis and is treating the vitiligo.
- A need to control healthcare costs by providing overall care for the patient, in order to avoid the all-too-frequent pitfall that consists in going from doctor to doctor to get as many opinions as possible, leading to Internet surfing and the risk of falling into the various traps of miracle solutions, unverified doses that may pose risks to the patient's health, and costly travel and/or treatment.

43.7 Existing Transitional Care

Transitional care should not be restricted simply to individual psychological care, even though this is one of the fundamental aspects for recovering self-esteem and reducing the risks of depression. Drug-based treatments suited to the most difficult

situations must be prescribed by specialists or GPs.

Individual psychological care tends to maintain the sufferer in isolation, as do individual corrective makeup sessions.

Dedicated support groups for vitiligo sufferers and group corrective makeup workshops allow individuals to be less isolated and to discuss their experiences and feelings. They can see that other men and women are suffering too and are able to talk about best practices and tips together.

43.7.1 Psychological Care

Vitiligo sufferers often need to be listened to and expect advice over and above specific treatment advice for the areas of depigmented skin.

In hospitals, consultation with a psychologist may sometimes be tied in with dermatology departments; this can make patients attending consultations aware of the need to benefit from this kind of care and follow-up. This is however rare in hospitals and in private practices.

Patients are disoriented by the diagnosis and are greatly sometimes even deeply distressed.

Sufferers often interpret oxidative stress in the melanocytes as meaning "you are too stressed" and thus feel they are being blamed for their condition.

Guilt contributes to a loss of self-esteem and leads to withdrawal; this may even become serious depression.

A personal interview with a clinical psychologist is one form of care. This allows the patient to work on expressing their fears and anxiety and relate their personal journey with the disease and its impact.

However, faced with the many questions surrounding the disease, other people's opinions, and so on, the possibility of offering additional care in the form of a support group overseen by clinical psychologists is a desirable one.

Support groups (or focus groups, e.g. work and vitiligo, children and vitiligo, etc.) allow discussion that relieves the patient from the weight of their experience as they share feelings and emotions. At last, they feel that they are acknowledged and are no different from others. Support groups should be in addition to individual therapy, rather than a replacement; individual therapy may open the way for this additional support.

Support groups overseen by clinical psychologists allow sufferers to escape from their isolation by allowing them to discuss the disease and their coping practices and begin the process of resilience. Sympathetic and benevolent listening by other sufferers can help restore self-confidence.

Social events and other meetings organised by associations, in particular by people suffering from the same disease (after-work evenings, themed days, holidays, etc.), are all ways of combating isolation, particularly in view of the discussions initiated by such events. They also facilitate recognition of the extent to which the disease is burdensome on sufferers. The presence of clinical psychologists is also advisable, to facilitate and encourage benevolent expression.

43.7.2 Corrective Makeup

The way others see sufferers of the disease, in particular when the depigmented zones affect the face and hands, is experienced as a stigma, even if people are only looking out of compassion or curiosity ("Is it contagious?", "Does it hurt?", etc.). The gaze of others is perceived as apportioning blame for being unlike other people. This is more particularly the case in countries in which skin phototypes are dark and/or in jobs in contact with the public, as well as in situations in which individuals are especially vulnerable in terms of self-image and how other people look at them (teenagers, young adults, etc.).

It is therefore appropriate to arrange psychological care (or provide additional care) in the form of actions aimed at lessening the difference in colour between pigmented and depigmented zones.

Individual corrective makeup advice from a professional makeup artist, or from tutorials to be found on social media, can improve patients'

quality of life by providing immediate solutions which they can implement. However, this personal advice or Internet viewing has the drawback of maintaining sufferers seeking solutions in difficult, isolated situations.

Corrective makeup workshops have four goals:

- Making patients less isolated, by giving them the opportunity to meet other individuals who have the same skin disease, enabling them to discuss their experience and the coping strategies they use, share information about research, etc., as well as hear from other sufferers whose kind words will reassure other participants.
- Giving reasoned advice and answering questions about skin care, sunscreens, etc. Such workshops offer an opportunity for a direct presentation and explanation of preconceived ideas about sun exposure, the Koebner phenomenon, and rubbing.
- Experimenting with a wide range of both specific and non-specific products for correcting depigmented zones in order to find the "best" shade, in the participant's appraisal, for the correction they wish to benefit from. The sole arbiter of the effectiveness of the skin correction is the way individual patients see themselves, even though the opinions of other patients present clearly play a moderating role, in that they are deemed to be more impartial than individuals who do not suffer from vitiligo.
- Providing participants with a greater degree of autonomy by giving them their very own opportunity to experiment with techniques during the makeup workshop, benefit from personal advice dispensed by professional corrective makeup artists and beauticians, and choose suitable products from among those presented (the actual availability of the latter in stores or on the Internet should be checked beforehand).

  These workshops should be run according to a clearly defined protocol and be supervised by one or two clinical psychologists and two corrective makeup artists.

To meet differing expectations, specialised workshops can be arranged for children, teenagers, and dark skin phototypes; advanced workshops can also be organised. Workshops now tend to be mixed, with increasing numbers of men registering.

Each participant can come with a friend or family member; the latter's presence will provide reassurance for them, encourage them to attend, and allow them to hear what the other participants have to say about the difficulty of living with a visible disease.

Participants are almost 100% satisfied. At one of the French Vitiligo Association makeup workshops, the following comment was made at the end of the session: "Thanks to you, I've got my wife back". This comment embodies the extent of the changes that can take place—and which are perceived by both participants and their families.

43.7.3 Sunscreen Cover

Although vitiligo sufferers do not need to avoid the sun altogether, they do need to protect themselves regularly all year round, not simply during holidays in the sun.

Sunscreen creams and UV protective clothing are vital aspects to be included in advice, as well as in the financial cover provided by social security systems.

43.8 Patient/Sufferers' Associations

Over the past few years, vitiligo patients' associations have become more organised, creating informational websites and a presence on social media, as well as taking part in public health commissions and other similar bodies.

Associations managed by sufferers for sufferers are becoming helpful points of contact for the various players in the healthcare system (researchers, organisations of healthcare professionals, pharmaceutical firms, ministries and government bodies, etc.), speaking on behalf of

sufferers about their concerns and priorities, with a view to promoting and contributing to more efficient action and research.

Associations' familiarity with the disease, the ability to mobilise sufferers, and credibility in the eyes of sufferers are all assets for researchers when constructive collaboration is put into place for the purposes of research.

Sufferers' associations also act as moderators of their message, offering a detached way of informing sufferers via online communications, hotlines, answers to emails, and information meetings organised on their initiative.

Patients' and sufferers' associations are genuine sources of resilience.

They allow sufferers to escape from isolation and make them active participants in their own treatment—and potentially, their healing. Sufferers are no longer helpless victims; instead, they become informed and can help others thanks to their own experience as victims, thereby becoming resource individuals. Such an achievement is an outstanding way of overcoming their suffering.

43.9 Conclusion

Sufferers are in the position of anxiously awaiting a therapeutic solution.

Minimisation of the disease ("it's not serious!"), the dismissal of vitiligo as being solely a question of appearance ("it's only skin deep!"), the paucity of the therapeutic solutions offered, and the perceived lack of compassion all increase the risk of withdrawal and major depressive episodes. It is therefore appropriate to provide assistance through transitional solutions, throughout the journey of vitiligo patients, not simply from a dermatological point of view but holistically.

The skin must be treated—but the human who has it on and who lives inside it must not be forgotten.

Reference

1. Haykal K-A, DesGroseilliers J-P. Are narrow-band ultraviolet B home units a viable option for continuous or maintenance therapy of photoresponsive diseases? J Cutan Med Surg. 2006;10(5):234–40.

44 Discussion on Empirical, Traditional, and Alternative Treatments

Mauro Picardo

Contents

Abstract

Alternative treatments, such as traditional Chinese products, plant-derived photosensitizing agents, and herbal and vitamin supplements, can certainly improve therapeutic outcomes in vitiligo.

Traditional Chinese medicine is currently attracting interest in dermatological research looking at the possible development of new drugs. This approach is supported by the political strategies of Western medicine community toward China.

The pro-melanogenic effect may be related to antioxidant or anti-inflammatory properties; however, the main limitation of the published studies concerns the non-complete list of the used products as well as their chemical composition. Similarly, the effectiveness of melagenin is still lacking the knowledge of the underlying mechanisms of repigmentation. On the basis of the in vitro study on immunomodulatory action, aspirin and statins may be considered for the treatment.

More controlled studies should be performed before considering most of the new emerging options based on unconventional drug or combinatory approaches.

M. Picardo (✉)
Cutaneous Physiopathology and CIRM, San Gallicano Dermatological Institute, IRCCS, Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_44

Key Points

- The pro-melanogenic effect of products by traditional Chinese medicine may be related to antioxidant or anti-inflammatory properties.
- The main limitation of the published studies concerns the non-complete list of the used products as well as their chemical composition.
- Melagenin, aspirin, or statins may act through an immunomodulatory action.
- Dermabrasion associated with 5FU may be applied in stable vitiligo.
- More controlled studies should be provided, and increased knowledge of the underlying mechanism of action should support the use of these new approaches.

44.1 Introduction

Clinical evidence has shown that alternative treatments, such as traditional Chinese products, plant-derived photosensitizing agents, and herbal and vitamin supplements, can certainly improve therapeutic outcomes in vitiligo. Some prospective controlled, double-blind, and randomized studies have been performed to identify mechanisms of action and potential adverse effects supporting the efficacy and safety of these products. A recent review article identified 27 studies involving alternative treatments for vitiligo including prospective controlled trials, prospective uncontrolled trials, and retrospective studies [1].

44.2 Chinese Traditional Products

Traditional Chinese medicine refers to a system of medical practice originated in ancient China more than 2000 years ago and based on empirical evidence and theories. Although traditional Chinese medicine was widely practiced in Asian populations such as China, Hong Kong, Taiwan, and Singapore, many non-Asian countries have, only in the last decades, recognized the usefulness of this traditional practice in improving health and providing supplementary options for patients looking for an alternative approach to their care.

Traditional Chinese medicine is currently attracting interest in dermatological research looking at the possible development of new drugs. This approach is supported by the political strategies of Western medicine community toward China.

The use of herbal products and vitamin supplements as enhancers or modulators of melanogenesis is becoming particularly popular. This pro-melanogenic effect may be related to antioxidant or anti-inflammatory properties; however, the main limitation of the published studies concerns the non-complete list of the used products as well as their chemical composition.

An early randomized controlled study performed by Jin et al. in more than 200 subjects described a complete or more than 60% repigmentation in 13% and 17% of patients treated with undefined mixture of Chinese herbs [2]. The results were carried out by comparing herbal treatment versus oral corticosteroids (15 mg/day, decreased to 5 mg every 2–4 weeks), psoralen (topical, 30%), or corticosteroids combined with herbal products. After 2 months of treatment, corticosteroids were more effective than herbal mixture since 31% and 14% of patients, respectively, showed an excellent and a good (more than 60%) repigmentation. However, the combination of corticosteroids and herbal drugs resulted in the best treatment since it produced a complete repigmentation in 31% and more than 60% in 14% of treated patients.

A further study described the occurrence of complete or good (more than 60%) repigmentation in 95% of patients treated with Xiaobai mixture, which is an aqueous extract of walnut, red flower, black sesame, black beans, zhi bei fu ping, lu lu tong, and plums (1 ml contains 0.1 g of raw medication). The mixture has been administered (160 ml) every day for 3 months [3].

Many issues surrounding the efficacy of traditional Chinese medicine remain unresolved.

The main criticism is the absence of high-quality clinical trials performed with sufficient number of patients and well-characterized products [4]. Moreover, none of the published treatments have been unequivocally confirmed.

Therefore, the use of such alternative treatments may be considered as a supplement of established therapies since biochemical and molecular mechanisms underlying the effect on vitiligo are clearly unknown. A recent review and meta-analysis examined 48 studies that assessed phototherapy in combination with oral Chinese herbal medicine in the treatment of vitiligo, and they reported that combination therapy resulted in a higher repigmentation rate than phototherapy alone [5]. Nevertheless, there is limited available evidence of long-term follow-up and poor methodological of the trials.

44.3 Plant-Derived Extracts

Some plant-derived substances like khellin, *Polypodium leucotomos*, and *Ginkgo biloba* have been proposed in the management of vitiligo.

Khellin is an extract of the Mediterranean fruit *Ammi visnaga*, and it is structurally similar to psoralens used in PUVA. Khellin has been shown to provide benefit alone and in combination with UVA or UVB phototherapy, but its systemic use may be limited by potential side effects such as liver toxicity, nausea, and orthostatic hypotension [6, 7]. Therefore, research interest focused on topical application of khellin within liposomes in combination with phototherapy [8]. Hofer et al. reported the results obtained from 28 patients with generalized vitiligo treated at least for 3 months with 100 mg of khellin and UVA. Forty-one percent of patients showed 70% repigmentation, and nausea was experienced by 29% of patients. Moreover, long-term follow-up demonstrated no instances of actinic damage or skin cancer.

A study investigated the topical application effectiveness of *Cucumis melo* extract [9] in combination with UVB, but no differences toward the placebo cream have been detected.

Some studies evaluated the effect of oral administration of *Ginkgo biloba* and *Polypodium leucotomos* in combination with photo- or photochemotherapy chapter 44) to improve antioxidant activities of the skin.

44.4 Melagenin

The extract of the human placenta named "melagenin" has been tested, and the first use was in 1991 [10]. However, the underlying mechanisms of repigmentation induced by melagenin have not been thoroughly clarified. A study evaluated the efficacy of topical melagenin for repigmentation in 22 child patients with vitiligo treated twice daily with 1.2 mg/ml aqueous melagenin in combination with 20 min of infrared exposure [11]. The general effective rate of melagenin-infrared combination treatment was 45.5%, and the treatment has shown minimal side effects. Some in vitro studies suggested that melagenin is extremely rich of bioactive molecules since human placental extracts contain keratinocyte growth factor [12], endothelin-1 [13], and sphingolipids [14]. Melagenin may act on pigmentation by modulating melanocyte proliferation and melanogenesis. The melanoblast cell line NCCmelb4M5 proliferates and differentiates after melagenin treatment as demonstrated by increased expression of c-KIT, tyrosinase, and MITF [15].

44.5 Aspirin

Acetylsalicylic acid (aspirin) has been reported to have free radical scavenging property [16] and to be a potent antioxidant in many oxidative stress-related diseases [17]. Previously, Zailaie reported that acetylsalicylic acid was beneficial to vitiligo patients by inhibiting melanocyte lipid peroxidation and increasing cell proliferation [18]. The mechanism of action has been attributed to the inhibition of leukotriene synthesis and to the induction of catalase/glutathione peroxidase activities [19]. Moreover, the author reported

a reduction of soluble IL2 receptor (sIL2R) concentration of the serum IL1β, IL-6, IL-8, and TNFα levels as well as of anti-melanocyte antibodies [20, 21]. Recently, Jian and co-workers demonstrated that pretreatment with acetylsalicylic acid protects human melanocytes against H_2O_2-induced oxidative stress via Nrf2-driven transcriptional activation of HO-1 [22] and inhibition of HO-1 expression abrogates acetylsalicylic acid protective effects. Therefore, although more preclinical and clinical research is needed, aspirin might represent a promising new therapeutic agent for vitiligo.

44.6 Statins

Statins are inhibitors of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme of the cholesterol synthesis pathway. In 2004, Noel et al. described an unusual case of regression of vitiligo in a patient treated with statins [23]. The clinical effectiveness has been related to immunomodulatory and anti-inflammatory activities of this family of compounds through the inhibition of TNFα, IL-6, and IL-8 release and of the LFA1 and ICAM1 expression. Moreover, statins are also able to in vitro inhibit the expression of MHC class II molecules contributing thus to the immune suppression [24]. A different possible mechanism of action has been related to the interference with the phosphatidylinositol-3-kinase/Akt signal transduction pathway [25].

A recent study reported the ability of simvastatin, an HMG-CoA reductase inhibitor approved by the Food and Drug Administration for the treatment of hypercholesterolemia, to both prevent and reverse established depigmentation in a mouse model of vitiligo by reducing the number of infiltrating autoreactive $CD8^+$ T cells in the skin and IFN-γ production [26]. Based on these in vitro and in vivo studies, a clinical trial for patients with vitiligo treated with high-dose simvastatin would be useful to determine whether simvastatin may be a safe, targeted treatment option for patients with vitiligo.

44.7 Dermabrasion Combined with 5-Fluorouracil

Application of 5-fluorouracil (5-FU) after mechanical dermabrasion, as a treatment for vitiligo, was introduced by Tsuji and Hamada in 1983 [27], and its efficacy was successively confirmed by other authors [28]. Only patients with stable disease and limited extension of the manifestations can be treated because of the risk of Koebner phenomenon induced by the treatment. Successful repigmentation of areas devoid of hair follicles was first reported by Anbar after ER:YAG laser skin ablation followed by topical application of 5-FU and Nb-UVB phototherapy [29], but it was difficult to understand why a topical drug, such as 5-FU, well known for its antimitotic activity, might improve the proliferation and migration of melanocytes. The mechanism of action is mainly based on the combination of epidermal removal and induction of irritation mediated by 5FU, which through the production of inflammatory cytokines and prostaglandins stimulates melanocyte migration and proliferation. Moreover, based on histological findings obtained by Gauthier et al. on guinea pig skin, it was possible to propose a possible "scenario" including several successive events, allowing us to give an explanation for successful repigmentation after skin ablation combined with 5-FU [30]. As was demonstrated in vitro, 5-FU can exert a selective cytotoxic effect on different types of epidermal cells. Melanocytes seem to be less vulnerable than keratinocytes to 5-FU, and their ability to proliferate in cultures appears to be preserved [31]. After dermabrasion followed immediately by application of 5-FU ointment for 2 days, many keratinocytes implicated in the new epithelialization are chemically damaged. Consequently, a strong inflammatory reaction is seen, and the healing is considerably delayed. On day 15, some degenerative keratinocytes located in the lower layers still persist, but due to the persistence of a local edema, the intercellular spaces of the basal layer remain very enlarged for a long time. Active melanocytes migrate through these enlarged intercellular spaces from the pigmented to the achromic epidermis.

During this period, inflammatory mediators such as leukotrienes C4 and D4 (LTC4 and LTD4) are locally released, which could stimulate melanocyte proliferation and migration [32]. Moreover, during epidermis remodeling, keratinocytes synthesize and secrete metalloproteinases, which are involved in the degradation of the extracellular matrix. These favorable conditions, which persist for a long time, could explain the successful migration of melanocytes from the pigmented to the adjacent achromic area both in guinea pig and vitiligo skin.

44.8 Others

Sporadic clinical and in vitro evaluations have been performed to select drugs able to interfere with the physiological intracellular signal transduction pathways affecting melanocyte proliferation/migration/differentiation. Among those, the secreted phospholipase A_2, which is a component of the bee venom, has been considered [33]. When tested in vitro, bee venom was able to promote melanocyte proliferation, dendriticity, migration, and tyrosinase activity probably by modulating intracellular signal transduction pathways related to PKA, Erk, and PI3K/Akt. The water extract (0.1 mg/ml) of *Piper nigrum*, and its main alkaloid piperine, has been demonstrated to promote in vitro melanocyte proliferation, probably mediated by the activation of PKC [34].

Several beneficial effects in terms of repigmentation response have been reported following the use of prostaglandins in the treatment of vitiligo. Parsad et al. [35] and Kapoor et al. demonstrated efficacy of topical PGE_2 in the treatment of localized stable vitiligo. The application of a gel containing PGE_2 gives rise to repigmentation, mainly on the face, after 6 months of therapy [36].

On the other hand, Anbar et al. evaluated the effects of latanoprost, a $PGF_{2\alpha}$ analogue used in the treatment of glaucoma, to induce skin pigmentation in 22 patients with vitiligo [37], and they compared the results with those obtained by using narrow-band ultraviolet B (NB-UVB). Moreover, the combined effect for achieving better results has been also assessed. After 3 months of treatment, repigmentation degree of treated lesions has been measured. Latanoprost was found to be better than placebo and comparable with NB-UVB in inducing skin pigmentation. This effect was enhanced by the addition of NB-UVB exposure.

References

1. Cohen BE, Elbuluk N, Mu EW, Orlow SJ. Alternative systemic treatments for vitiligo: a review. Am J Clin Dermatol. 2015;16:463–74.
2. Jin QX, Wj M, Zs D, et al. Clinical efficacy observation of combined treatment with Chinese traditional medicine and western medicine for 407 cases of vitiligo. Biomed Res. 1983;12:9–11.
3. Liu ZJ, Xiang YP. Clinical observation on treatment of vitiligo with xiaobai mixture. Chinese J Integr Trad Western Med. 2003;23:596–8.
4. The Cochrane Collaboration. 2014. http://www.cochrane.org/.
5. Chen YJ, Chen YY, Wu CY, Chi CC. Oral Chinese herbal medicine in combination with phototherapy for vitiligo: a systematic review and meta-analysis of randomized controlled trials. Complement Ther Med. 2016;26:21–7.
6. Hofer A, Kerl H, Wolf P. Long-term results in the treatment of vitiligo with oral khellin plus UVA. Eur J Dermatol. 2001;11:225–9.
7. Ortel B, Tanew A, Honigsmann H. Treatment of vitiligo with khellin and ultraviolet A. J Am Acad Dermatol. 1988;18:693–701.
8. De Leeuw J, Assen YJ, van der Beek N, et al. Treatment of vitiligo with khellin liposomes, ultraviolet light and blister roof transplantation. J Eur Acad Dermatol Venereol. 2011;25:74–81.
9. Khemis A, Ortonne JP. Comparative study of vegetable extracts possessing active superoxide dismutase and catalase (Vitix) plus selective UVB phototherapy versus an excipient plus selective UVB phototherapy in the treatment of common vitiligo. Nouvelles Dermatologiques. 2004;23:45–6.
10. Suite M, Quamina DB. Treatment of vitiligo with topical melagenine - a human placental extract. J Am Acad Dermatol. 1991;24:1018–9.
11. Xu AE, Wei XD. Topical melagenin for repigmentation in twenty-two child patients with vitiligo on the scalp. Chin Med J. 2004;117:199–201.
12. Chiu ML, O'Keefe EJ. Placental keratinocyte growth factor: partial purification and comparison with epidermal growth factor. Arch Biochem Biophys. 1989;269:75–85.
13. Wilkes BM, Susin M, Mento PF. Localization of endothelin-1-like immunoreactivity in human placenta. J Histochem Cytochem. 1993;41:535–41.

14. Pal P, Roy R, Datta PK, et al. Hydroalcoholic human placental extracts: skin pigmenting activity and gross chemical composition. Int J Dermatol. 1995;34:61–6.
15. Zhao D, Li Y, Wang P, et al. Melagenin modulates proliferation and differentiation of melanoblasts. Int J Mol Med. 2008;22:193–7.
16. Maniglia FP, Costa JA. Effects of Acetylsalicylic acid usage on inflammatory and oxidative stress markers in hemodialysis patients. Inflammation. 2015. https://doi.org/10.1007/s10753-015-0244-8.
17. Berg K, Langaas M, Ericsson M, et al. Acetylsalicylic acid treatment until surgery reduces oxidative stress and inflammation in patients undergoing coronary artery bypass grafting. Eur J Cardiothorac Surg. 2013;43:1154–63.
18. Zailaie MZ. Short- and long-term effects of acetylsalicylic acid treatment on the proliferation and lipid peroxidation of skin cultured melanocytes of active vitiligo. Saudi Med J. 2004;25:1656–63.
19. Zailaie MZ. The effect of acetylsalicylic acid on the release rates of leukotrienes B4 and C4 from cultured melanocytes of active vitiligo. Saudi Med J. 2004;25:1439–44.
20. Zailaie MZ. Decreased proinflammatory cytokine production by peripheral blood mononuclear cells from vitiligo patients following aspirin treatment. Saudi Med J. 2005;26:799–805.
21. Zailaie MZ. Aspirin reduces serum anti-melanocyte antibodies and soluble interleukin-2 receptors in vitiligo patients. Saudi Med J. 2005;26:1085–92.
22. Jian Z, Tang L, Yi X, et al. Aspirin induces Nrf2-mediated transcriptional activation of haem oxygenase-1 in protection of human melanocytes from H_2O_2-induced oxidative stress. J Cell Mol Med. 2016;20:1307–18.
23. Noel M, Gagné C, Bergeron J, et al. Positive pleiotropic effects of HMG-CoA reductase inhibitor on vitiligo. Lipids Health Dis. 2004;3:7–11.
24. Namazi MR. Statins: novel additions to the dermatologic arsenal? Exp Dermatol. 2004;13:337–9.
25. Neuhaus O, Strasser-Fachs S, Fazekas F, et al. Statins as immunomodulators – comparison with interferon-β1b in MS. Neurology. 2002;59:990–7.
26. Agarwal P, Rashighi M, Essien KI, et al. Simvastatin prevents and reverses depigmentation in a mouse model of vitiligo. J Invest Dermatol. 2015;135:1080–8.
27. Tsuji T, Hamada T. Topically administered fluorouracil in vitiligo. Arch Dermatol. 1983;119:722–7.
28. Sethi S, Mahajan BB, Gupta RR, Ohri A. Comparative evaluation of therapeutic efficacy of dermabrasion, dermabrasion combined with topical 5% 5-fluorouracil cream, and dermabrasion combined with topical placentrex gel in localized stable vitiligo. Int J Dermatol. 2007;46:875–9.
29. Anbar TS, Westerhof W, Abdel-Rahman AT, et al. Effect of one session of ERG:YAG laser ablation plus topical 5Fluorouracil on the outcome of short-term NB-UVB phototherapy in the treatment of non-segmental vitiligo: a left-right comparative study. Photodermatol Photoimmunol Photomed. 2008;24:322–9.
30. Gauthier Y, Anbar T, Lepreux S, et al. Possible mechanisms by which topical 5-Fluorouracil and dermabrasion could induce pigment spread in vitiligo skin: an experimental study. ISRN Dermatol. 2013;2013:852497. https://doi.org/10.1155/2013/852497.
31. Tsuji T, Karasek MA. Differential effects of 5-fluorouracil on human skin melanocytes and malignant melanoma cells in vitro. Acta Derm Venereol. 1986;66:474–8.
32. Morelli JG, Yohn JJ, Lyons MB, et al. Leukotrienes C4 and D4 as potent mitogens for cultured human neonatal melanocytes. J Investig Dermatol. 1989;93:719–22.
33. Jeon S, Kim NH, Koo BS, et al. Bee venom stimulates human melanocyte proliferation, melanogenesis, dendriticity and migration. Exp Mol Med. 2007;39:603–13.
34. Lin Z, Liao Y, Venkatasamy R, et al. Amides from Piper nigrum L. with dissimilar effects on melanocytes proliferation in vitro. Pharm Pharmacol. 2007;59:529–36.
35. Parsad D, Pandhi R, Dogra S, et al. Topical prostaglandin analog (PGE_2) in vitiligo-a preliminary study. Int J Dermatol. 2002;41:942–5.
36. Kapoor R, Phiske MM, Jerajani HR. Evaluation of safety and efficacy of topical prostaglandin E2 in treatment of vitiligo. Br J Dermatol. 2009;160:861–3.
37. Anbar TS, El-Ammawi TS, Abdel-Rahman AT, Hanna MR. The effect of Latanoprost on vitiligo: a preliminary comparative study. Int J Dermatol. 2015;54:587–93.

Beyond Guidelines

45

Tag S. Anbar, Rehab A. Hegazy, and Amira A. Eid

Contents

Abstract

The therapeutic plan must start from the shared decision of patient and medical doctor, taking into account that repigmentation, depigmentation, camouflage, or a combinatory approach should depend on localization, extent, and duration of the lesions. In repigmentation option, the available melanocytes useful for migration have to be evaluated, as well as in depigmentation or camouflage ones. An algorithm will support the physician in the modulation of the therapeutic plan.

T. S. Anbar (✉)
(in memory) Department of Dermatology and Andrology, Faculty of Medicine, Al Minya University, Al Minya, Egypt

R. A. Hegazy
Department of Dermatology, Faculty of Medicine, Cairo University, Cairo, Egypt

A. A. Eid
Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_45

Key Points

- Discuss the current clinical profile and the expected outcome, as well as the possible therapeutic options (repigmentation, depigmentation, camouflage, or combined), at the first visit.
- Stabilization is a continuous process going hand in hand with repigmentation and depigmentation.
- Flexibility in changing the plan of therapy according to the patient's response is a must.
- Follow-up does not only give an option of treating vitiligo in its early stages, but it also can help through Wood's light examination in controlling the disease in its subclinical state.

45.1 Introduction

The therapeutic plan must start from the shared decision of patient and treating physician. Accordingly, based on the clinical data and the expected sources of melanocytes for repigmentation in each individual lesion, the decision of repigmentation, depigmentation, and/or camouflage is taken after a thorough explanation to the patient; keeping in mind that this plan will be a joint doctor-patient decision. We propose the use of a simple drawing that will not only facilitate in the treatment description to the patient, but also it will help the doctor in subsequent follow-ups at a glance.

45.2 Repigmentation

Over the years, a number of treatment guidelines for vitiligo have been published, aiming to provide an approach to the available therapeutic modalities for vitiligo management [1–3]. Whether spontaneous or medically induced, five main repigmentation patterns have been reported in vitiligo lesions, namely, marginal, perifollicular, diffuse, combined [4], and medium spotted, which has been recently described in children in areas with scanty or no hair follicles [5]. Regardless of the pattern, repigmentation depends on the presence of a new generation of functional melanocytes recruited to the affected area from one or more of the following sources [6]:

1. *Follicular melanocytes*
   Melanocyte stem cells were detected in the bulge area residing in the niche, from where they usually migrate to differentiate into melanocytes. However, under certain circumstances they can migrate upwards and differentiate into melanocytes in the basal layer of the epidermis [7–9]. This *vertical migration* of melanocytes is thought to be responsible for the perifollicular pattern of repigmentation. In addition to the bulge, a secondary possible melanocyte germ was described in the infundibulum following the treatment with narrowband ultraviolet B [10].
2. *Marginal melanocytes*
   They provide the source for the marginal repigmentation pattern, where active perilesional melanocytes may gradually migrate toward the lesion and produce melanin, the so-called horizontal melanocyte migration. Unfortunately, they are only responsible for repigmentation of the peripheral 2–3 mm of the lesion, which alone is clearly not enough to induce repigmentation in large lesions [11].
3. *Other possible sources*
   In vitiligo, amelanotic, DOPA-negative melanocytes are spared [12]. Small amelanotic melanocytes containing abnormal melanosomes [13], as well as Melan-A+ cells [14], have been detected in epidermis of patients with long-standing vitiligo. Reactivation of those amelanotic melanocytes could possibly be the reason behind the diffuse type of pigmentation seen in vitiligo. Furthermore, repigmentation that occasionally occurs in areas devoid of hair follicles such as the palms and soles raised the possibility of the presence of melanocyte precursors or epidermal stem cells related to melanocytes in glabrous skin [15, 16]. However, this deduction awaits further research.

To achieve repigmentation, any treatment plan should target the available melanocyte sources. Owing to the speculative and contradictory data concerning interfollicular epidermal

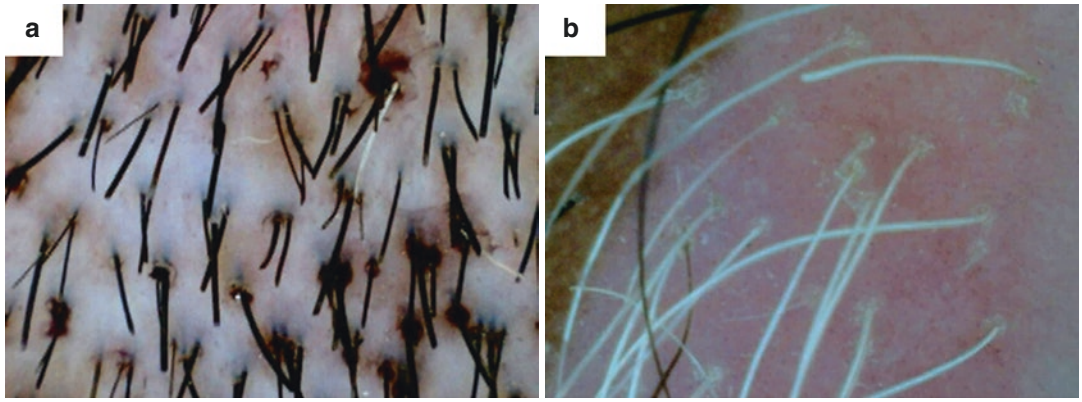


Fig. 45.1 The effect of narrowband stimulation on vitiligo skin. (**a**) Perifollicular pigmentation in lesions with black hair. (**b**) Erythema in lesions with white hair

stem cells and melanocyte precursors, only follicular and marginal melanocytes will be addressed in our repigmentation approach. Several factors can influence the decision of whether to target the follicular, or marginal melanocytes, or both in the treatment plan, including:

(a) *Anatomical factors*

Repigmentation is dependent on functioning melanocytes migrating to the lesion and producing melanin. Given the fact that follicular melanocytes represent the main source for repigmentation, areas devoid of hair, such as mucous membranes, palms, and soles, will depend mainly on the horizontal migration of marginal melanocytes toward the lesion resulting in marginal repigmentation, which is of clinical value in small lesions only. Similarly, areas with scanty hair distribution, for example, the flexor aspect of the wrist, the dorsum of the foot, and periungual skin, will exhibit marginal repigmentation in addition to minimal perifollicular repigmentation, which will result in scattered small pigmented macules of negligible clinical value. A third clinical scenario is encountered in areas with numerous pigmented hairs such as the face and trunk. Since the outcome of the vertical migration outweighs the outcome of horizontal one, it can be deduced that the degree of repigmentation will be largely dependent on the density of pigmented hairs in such lesions [17].

(b) *Pathological factors*

Since the hair follicle is considered the main reservoir of melanocytes, cases with leukotrichia are probably associated with melanocyte reservoir exhaustion resulting in the absence of amelanotic melanocytes in the bulge area [18]. As a result, narrowband stimulation which usually results in perifollicular pigmentation in lesions with black hair will only cause erythema in lesions with leukotrichia without any pigmentary changes related to these white hairs (Fig. 45.1a, b). On the other hand, the presence of pigmented hairs in the lesion carries a good prognostic sign, but is not a 100% guarantee for repigmentation. The duration of the lesion is inversely proportional to the presence of these cells in black hair, thus the early intervention is mandatory [18, 19].

Moreover, the size of the lesion, the hair density in the lesion, as well as the duration of vitiligo will affect the choice of therapy.

Larger numbers of pigmented hairs as well as shorter disease durations are generally associated with better results [18]. The size of the lesion influences the choice of the technique used to induce repigmentation. Logically, targeted phototherapy, excimer laser, or topical medications can be of value if small areas are affected; meanwhile if large or multiple small areas are affected, the choice of whole-body phototherapy with or without topical medications will be more feasible [20].

In case of absence or scarcity of pigmented hairs in vitiligo lesions, marginal melanocytes become the primary melanin provider in the repigmentation process. Owing to their limited (2–3 mm) migratory capacity, the size of the lesion in this condition represents a major challenging factor. The resultant improvement in repigmentation will be considered marked in small lesions and poor in large affected areas (Fig. 45.2a–e). Consequently, horizontal migration is not enough

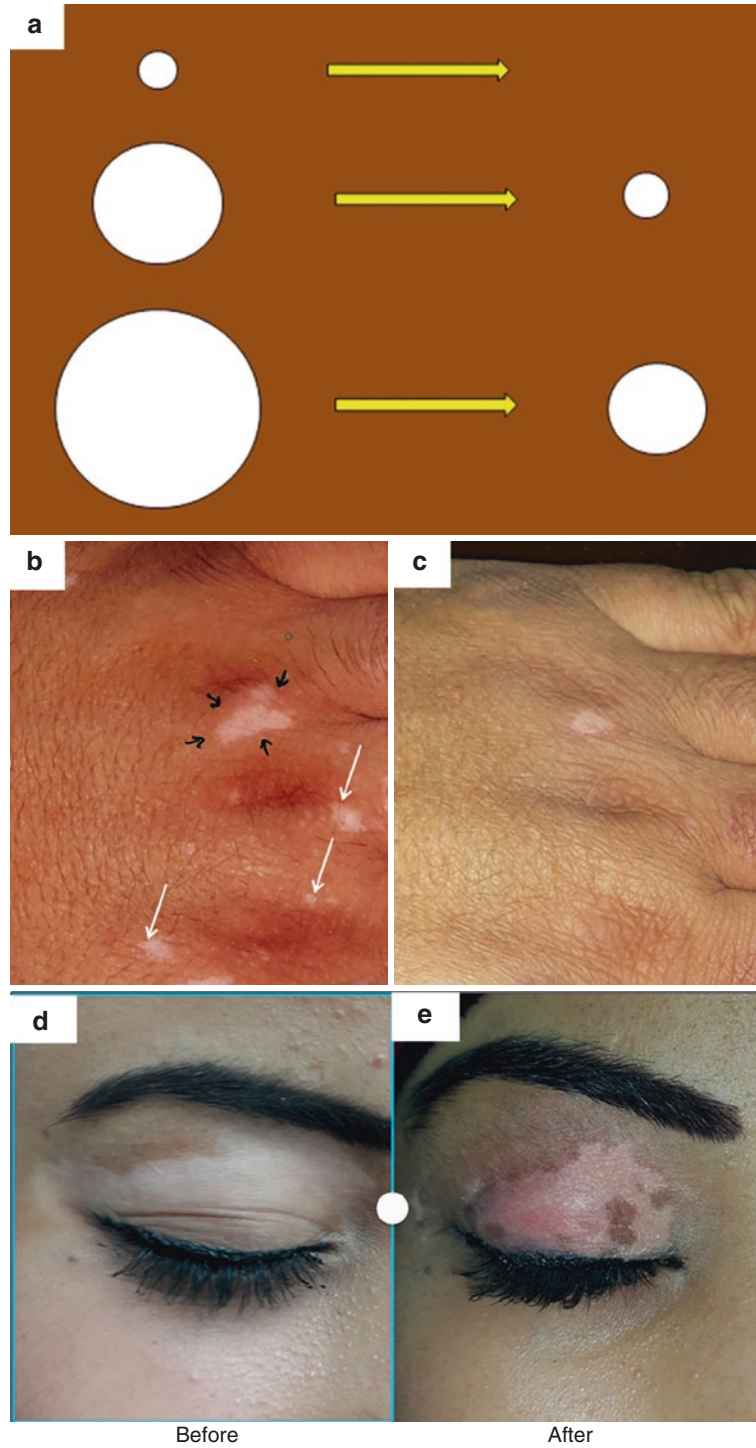


Fig. 45.2 (**a**) Diagrammatic illustration of the different degrees of improvement in relation to the size of the lesion. (**b** and **c**) Complete repigmentation in small lesions (white arrows) and moderate improvement of slightly larger lesions (black arrows). (**d** and **e**) Poor response in a large lesion. N.B. The difference in response in the three given examples occurred despite the same size of resultant marginal repigmentation (courtesy of Dr. Mohamed T. Anbar)

to repigment lesions more than 1 cm^2 [20, 21]. Enhancing migration of marginal melanocytes and accordingly pigment spread can be achieved by different modalities such as phototherapy [22], topical tacrolimus [23], and 5-FU applied on skin ablated by Erbium-YAG laser [24, 25]. The clinical success of the latter technique in the treatment of small areas with scanty hair such as periungual areas (Fig. 45.3a–f) is believed to be due to keratinocytes degeneration and local releases of inflammatory mediators. This in turn will be responsible for melanocyte stimulation, proliferation, and migration through the enlarged intracellular spaces from the normally pigmented to the achromic epidermis, eventually leading to repigmentation [11].

In lesions with an expected minimum therapeutic impact for either source of melanocytes, the need for melanocytes from remote sources,

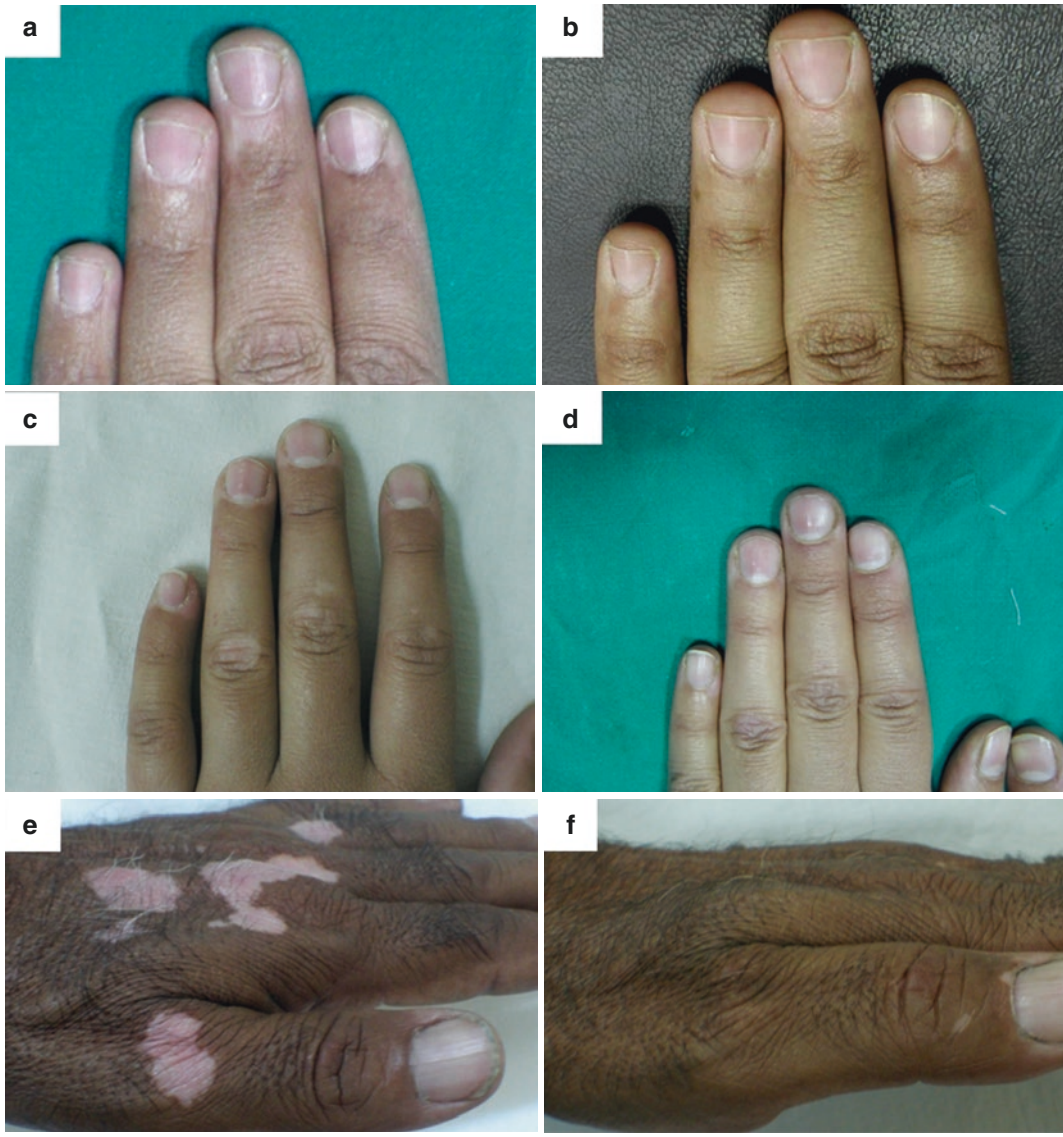


Fig. 45.3 Erbium-YAG laser followed by 5-FU in vitiligo

i.e., surgery, arises. Such decision is taken in most cases during the first visit after careful analysis of the repigmentation influential factors. In some patients surgery would be their only therapeutic line, for example, a single stable lesion. In other instances, similar lesions can be present accompanying medically treatable lesions, in whom it is more advisable to start with the medical treatment and then continue with surgery. This would ultimately aid in gaining the patient's confidence and improving stabilization chances. To complete the picture, one must state that not uncommonly does the surgical decision impose itself during the course of therapy, for example, in case of an un-expected poor response in one or more lesions receiving phototherapy together with topical medications.

The timing of surgery is an important factor that affects the success of the operation, as the stability of the lesion is the mainstay of success. Furthermore, taking into account the size of the lesion is crucial for the decision of surgery, considering the need for an adequate donor area.

Apart from psychological effect, the delay in surgery does not affect the outcome of the operation, as opposed to the delay in the medical intervention that decreases the opportunity of success as described above.

Though different from vitiligo/NSV in various aspects, segmental vitiligo can also be assessed using the same approach, keeping in mind the short time for leukotrichia development hence the importance of very early medical intervention, otherwise surgery or camouflage will be the only hope.

45.3 Depigmentation

Although when this decision could be adopted is not clearly agreed upon, depigmentation by chemical or physical means may be considered as a therapeutic option when vitiligo involves >50% of the body surface area of patients with refractory vitiligo [1]. Monobenzyl ether of hydroquinone 20% (MBEH) [1, 26], 4-methoxyphenol, and 88% phenol are capable of inducing chemical depigmentation [27]. Physical depigmentation can be achieved using Q-switched ruby laser (QSR 694 nm) [28], Q-switched alexandrite laser (QSA 755 nm) [29], Q-switched Nd-YAG laser [30], and cryotherapy [31].

45.4 Camouflage

Camouflage as a therapeutic option can be considered if cosmetically acceptable repigmentation or depigmentation cannot be achieved. It can also be of value during the course of treatment to conceal the lesion until the final outcome of therapy is reached. Camouflage in vitiligo can be temporary or permanent. Temporary options include self-tanning agents, dyes, tinted cover creams, liquid and stick foundation, and fixing powders and creams, whereas the permanent options include micro-pigmentation or tattooing. Temporary methods are generally preferred over the permanent ones, which should be used with caution given the unpredictable nature of the disease and the metallic nature of the injected pigments [32, 33].

45.5 Combination Between Repigmentation, Depigmentation, and/or Camouflage

We can face situations necessitating combinations between the abovementioned therapeutic lines, for example, a patient with a history of long-standing vitiligo affecting the trunk and hands, and with concern about the newly developing facial lesions, as well as the unsightly remaining colored islands on the dorsum of the hands. In such a situation, we can use camouflage or extend the scope of depigmentation to entail a localized approach to the hands; meanwhile, the recent facial lesions can be repigmented. The choice between depigmentation and camouflage in the management of his hands will depend on the original skin color of the patient and the patient's desire. In patients with dark skin phototypes (V and VI), depigmentation of the remaining pigmented island on the hands will yield marked contrast between the hands and face that

will be clearly apparent revealing the nature of the patient's condition. In such a situation, the patient's preference plays a vital role in the decision; if having a uniform skin color is more important to him than the resultant contrast, then the decision of depigmentation will be possible. On the other hand, if this contrast will be unacceptable to the patient, then a decision of camouflage will be made. An important advantage of depigmentation over camouflage is that it is done once, and it does not require daily or every other day care like camouflage, but again the patients' preference is what matters at the end, and the doctor's role is to explain and clarify. Needless to say, ensuring disease stability is essential regardless the patient's decision.

45.6 Stabilization Throughout the Process of Repigmentation/ Depigmentation

Vitiligo is considered stable if there are no new lesions, no increase in size of pre-existing lesions, and no Koebner phenomenon over a certain period. However, there is no consensus even on the duration to define a stable lesion [34].

Determining the stability of a lesion might appear to be a simple task, but this is far from true because all the abovementioned criteria of stability depend mainly on the patient history, which was recently proved to be of questionable value compared to objective methods [35]. In this context, the question of the reliability of our clinical evaluation is raised, in other words, does what we see clinically reflect what is going on beneath the surface?

In the absence of a disease process, constitutive skin color is determined by melanosomes synthesized by epidermal melanocytes that are then transferred to neighboring keratinocytes, [36], aiding us to expect the sequence of events occurring during vitiligo activity. It could be assumed that the affected areas will pass through three stages, each of which has its histopathological and clinical characteristics. In the *first* stage, regardless of the underlying pathogenic mechanism, the melanocytes are lost after being attacked by lymphocytes [37]; hence, no more melanin is transferred to the keratinocytes. Despite the fact that this step exhibits histopathological activity in the form of the inflammatory infiltrate, there is no clinical evidence of what is occurring; thus, a biased decision of stability can be taken. The *second* stage soon follows, in which the amount of melanin in the epidermis will gradually decrease due to lack of melanosome production, as well as the continuous turnover of keratinocytes. The decrease in melanin might not be clinically evident even by Wood's light examination, but further progress of the process will lead to the *final* stage where clinically visible hypo- and eventually depigmented lesions are present [6, 38]. Interestingly enough, by the time the lesion becomes clinically evident in the third stage, the underlying inflammatory process might have already subsided; hence, our decision of disease activity might be inaccurate. In other words, clinically stable lesions or even normal skin may be the site of activity in the first stage; meanwhile the increase in lesion size or even the appearance of new lesions in the third stage may be associated with cessation of the activity process.

It was recently reported that amelanotic lesions with well-defined borders are clinical markers of stability, while disease activity should be considered on finding hypomelanotic lesions with ill-defined borders [39, 40]. Meanwhile the increased expression of CXCL10 [41] and the deficiency of E-cadherin [42] were found to be preclinical markers of vitiligo activity. Despite these valuable findings, the distinction between stability and activity is still not crystal clear; and until more accurate biochemical and clinical markers of disease activity are well established, the combined subjective data supplied by the patient history together with the objective evaluation through careful lesion examination, photos, and planimetry [43] will still be the only practical methods of evaluation in our hands.

Stabilization during repigmentation and depigmentation is quite different. In cases requiring depigmentation, stabilization is achieved primarily through photo-protective measures. However in cases in which repigmentation is the plan, stabilization is more complicated and can be

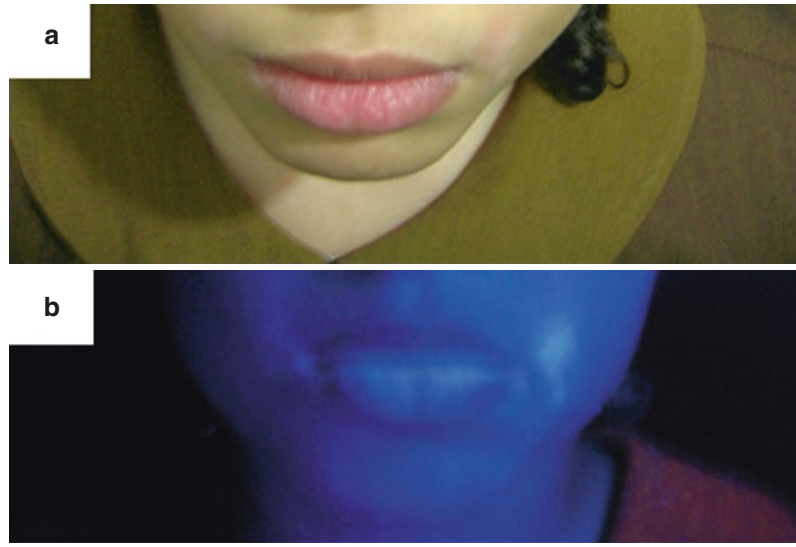


Fig. 45.4 Value of Wood's light in follow-up

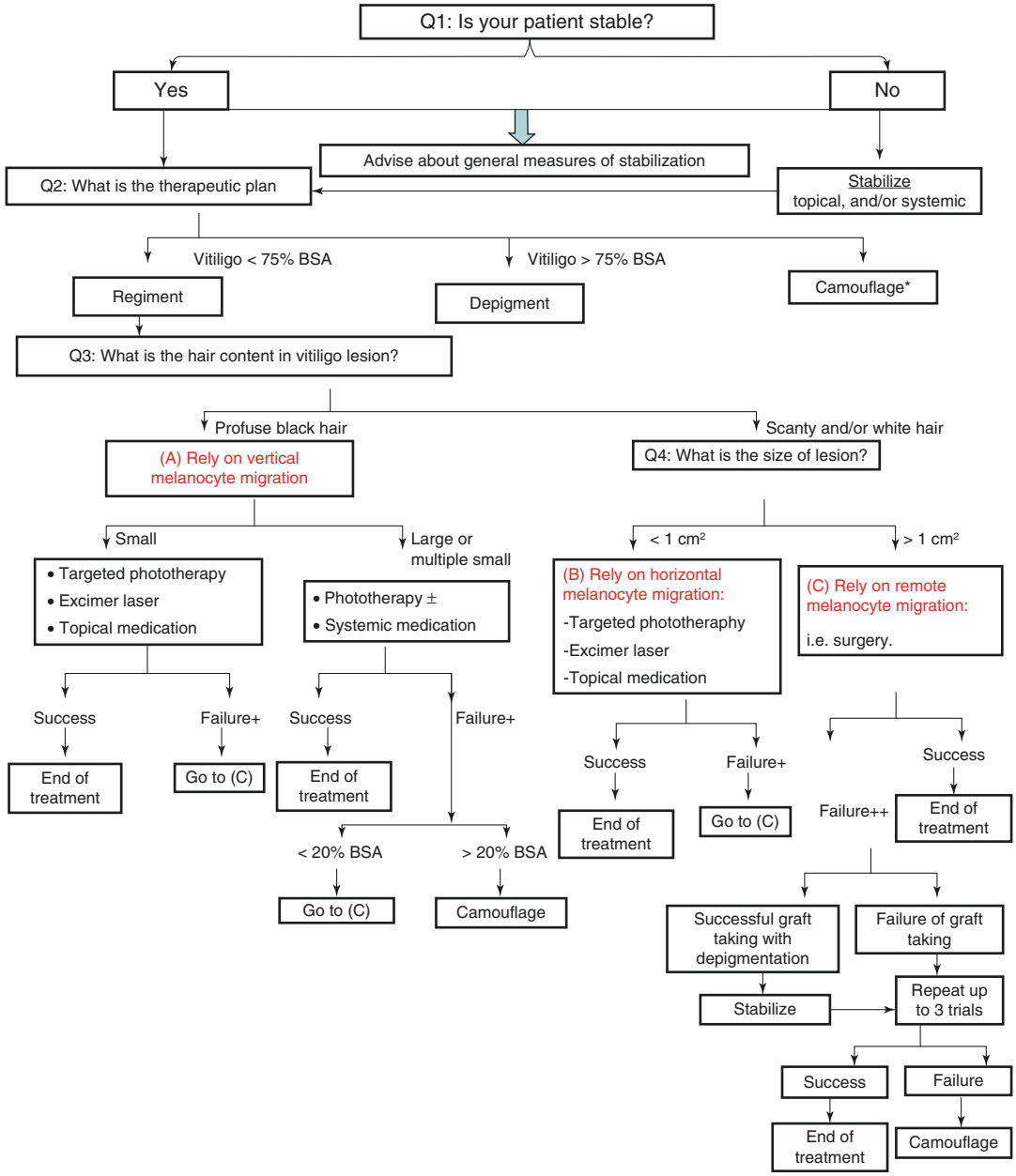
achieved by general, local, or systemic measures. *General measures* include avoiding Koebner phenomenon [44], photoprotection, and controlling psychological stress. Patient education on how to avoid trauma, whether at home or at work, after the discussion of his daily activities, together with avoiding the use of rough or sharp objects in his clothes [45], is integral to this process. *Topical measures* were assumed to be of benefit in the stabilization process if applied on extensions of lesions existing in naturally aggressive sites such as the periungual areas or after cure of a lesion if relapse is highly expected on discontinuation of treatment [20]. This assumption was recently proved when maintenance therapy with tacrolimus 0.1% twice weekly was reported to be effective in preventing depigmentation of vitiligo lesions [46]. However, topical measures are not applicable for stabilization of newly occurring lesions, since their site cannot be predicted. *Systemic measures* include systemic corticosteroids, phototherapy (NB-UVB or excimer laser), systemic antioxidants, immunosuppressants, and biologics [47–49]. They can control the progression of existing lesions together with the prevention of the development of new lesions.

45.7 Follow-Up

Our role is not restricted to reaching the most acceptable color to the patient, but it extends to include follow-up visits. These follow-up visits can be regular every 6 months or situational as in case of the patient's suspicion about the appearance of new lesions or after vacations in sunny climates that can pose a threat to the patients. The use of Wood's light in the follow-up visits is mandatory because it can help detect early lesions not yet discernible by the naked eye especially in children where dealing with subclinical vitiligo (Fig. 45.4a, b) is easier and less stressful.

45.8 Conclusion

This personalized and stratified approach is summarized in an algorithm (Fig. 45.5) [20], which offers a step-by-step guide from the patient's first visit onwards. Its individualized outcome-oriented plan is flexible in dealing with the mandatory modifications that imposes itself due to different patients' responses.



• It is done when repigmentation and depigmentation are not affordable and/or not accepted by the patient. It might be applied hand in hand with both options.

•+; Failure is considered when the treatment modality yields no response in a duration of 3 monts, or shows a non-satisfactoy improvement for 6 months, keeping in mind the individual side effects of each modality (similar to the parameters of treatment discontinuation applied after NBUVB treatment).8

•++; Failure of surgery occurs either due to improper attachment of donor tissue to the recipient area, or due to loss of pigment from the donor area due to disease activity.

•BSA; body surface area, ±; with or without

Fig. 45.5 An algorithm depicting the personalized stratified approach for management of vitiligo [20]

References

1. Gawkrodger DJ, Ormerod AD, Shaw L, et al. Guideline for the diagnosis and management of vitiligo. Br J Dermatol. 2008;159:1051–76.
2. Madigan LM, Al-Jamal M, Hamzavi I. Exploring the gaps in the evidenced-based application of narrowband-UVB for the treatment of vitiligo. Photodermatol Photoimmunol Photomed. 2016;32:66–80.
3. Meredith F, Abbott R. Vitiligo: an evidence-based update. Report of the 13th Evidence Based Update Meeting, 23 May 2013, Loughborough, U.K. Br J Dermatol. 2014;170:565–70.
4. Parsad D, Pandhi R, Dogra S, et al. Clinical study of repigmentation patterns with different treatment modalities and their correlation with speed and stability of repigmentation in 352 vitiliginous patches. J Am Acad Dermatol. 2004;50:63–7.
5. Gan EY, Gahat T, Cario-Andre M, et al. Clinical repigmentation patterns in paediatric vitiligo. Br J Dermatol. 2016; https://doi.org/10.1111/bjd.14635.
6. Anbar TS, El-Sawy AE, Attia SK, et al. Patterns of repigmentation in two cases of hypopigmented type of vitiligo. Photodermatol Photoimmunol Photomed. 2009;25:156–8.
7. Ferguson B, Kunisada T, Aoki H, et al. Hair follicle melanocyte precursors are awoken by ultraviolet radiation via a cell extrinsic mechanism. Photochem Photobiol Sci. 2015;14:1179–89.
8. Mull AN, Zolekar A, Wang YC. Understanding melanocyte stem cells for disease modeling and regenerative medicine applications. Int J Mol Sci. 2015;16:30458–69.
9. Nishimura EK. Melanocyte stem cells: a melanocyte reservoir in hair follicles for hair and skin pigmentation. Pigment Cell Melanoma Res. 2011;24:401–10.
10. Goldstein NB, Koster MI, Hoaglin LG, et al. Narrow band ultraviolet B treatment for human vitiligo is associated with proliferation, migration, and differentiation of melanocyte precursors. J Invest Dermatol. 2015;135:2068–76.
11. Gauthier Y, Anbar T, Lepreux S, et al. Possible mechanisms by which topical 5-Fluorouracil and dermabrasion could induce pigment spread in vitiligo skin: an experimental study. ISRN Dermatol. 2013;2013:852497. https://doi.org/10.1155/2013/852497.
12. Yu HS. Melanocyte destruction and repigmentation in vitiligo: a model for nerve cell damage and regrowth. J Biomed Sci. 2002;9:564–73.
13. Tobin DJ, Swanson NN, Pittelkow MR, et al. Melanocytes are not absent in lesional skin of long duration vitiligo. J Pathol. 2000;191:407–16.
14. Kubanov A, Proshutinskaia D, Volnukhin V, et al. Immunohistochemical analysis of melanocyte content in different zones of vitiligo lesions using the Melan-A marker. Acta Dermatovenerol Alp Pannonica Adriat. 2016;25:5–9.
15. Davids LM, du Toit E, Kidson SH, et al. A rare repigmentation pattern in a vitiligo patient: a clue to an epidermal stem-cell reservoir of melanocytes? Clin Exp Dermatol. 2009;34:246–8.
16. Falabella R. Vitiligo and the melanocyte reservoir. Indian J Dermatol. 2009;54:313–8.
17. Hamzavi I, Jain H, McLean D, et al. Parametric modeling of narrowband UV-B phototherapy for vitiligo using a novel quantitative tool: the Vitiligo Area Scoring Index. Arch Dermatol. 2004;140:677–83.
18. Anbar TS, Abdel-Raouf H, Awad SS, et al. The hair follicle melanocytes in vitiligo in relation to disease duration. J Eur Acad Dermatol Venereol. 2009;23:934–9.
19. Scherschun L, Kim JJ, Lim HW. Narrow-band ultraviolet B is a useful and well-tolerated treatment for vitiligo. J Am Acad Dermatol. 2001;44:999–1003.
20. Anbar TS, Hegazy RA, Picardo M, et al. Beyond vitiligo guidelines: combined stratified/personalized approaches for the vitiligo patient. Exp Dermatol. 2014;23:219–23.
21. Drake LA, Dinehart SM, Farmer ER, et al. Guidelines of care for vitiligo. American Academy of Dermatology. J Am Acad Dermatol. 1996;35:620–6.
22. Wu CS, Yu CL, Lan CC, et al. Narrow-band ultraviolet-B stimulates proliferation and migration of cultured melanocytes. Exp Dermatol. 2004;13:755–63.
23. Jung H, Oh ES. FK506 positively regulates the migratory potential of melanocyte-derived cells by enhancing syndecan-2 expression. Pigment Cell Melanoma Res. 2016. https://doi.org/10.1111/pcmr.12480.
24. Anbar T, Westerhof W, Abdel-Rahman A, et al. Treatment of periungual vitiligo with erbium-YAG-laser plus 5-flurouracil: a left to right comparative study. J Cosmet Dermatol. 2006;5:135–9.
25. Anbar TS, Westerhof W, Abdel-Rahman AT, et al. Effect of one session of ER:YAG laser ablation plus topical 5Fluorouracil on the outcome of short-term NB-UVB phototherapy in the treatment of non-segmental vitiligo: a left-right comparative study. Photodermatol Photoimmunol Photomed. 2008;24:322–9. https://doi.org/10.1111/j.1600-0781.2008.00385.x.
26. Tan ES, Sarkany R. Topical monobenzyl ether of hydroquinone is an effective and safe treatment for depigmentation of extensive vitiligo in the medium term: a retrospective cohort study of 53 cases. Br J Dermatol. 2015;172:1662–4.
27. AlGhamdi KM, Kumar A. Depigmentation therapies for normal skin in vitiligo universalis. J Eur Acad Dermatol Venereol. 2011;25:749–57.
28. Komen L, Zwertbroek L, Burger SJ, et al. Q-switched laser depigmentation in vitiligo, most effective in active disease. Br J Dermatol. 2013;169:1246–51.
29. Rao J, Fitzpatrick RE. Use of the Q-switched 755-nm alexandrite laser to treat recalcitrant pigment after depigmentation therapy for vitiligo. Dermatol Surg. 2004;30:1043–5.

30. Majid I, Imran S. Depigmentation therapy with Q-switched Nd:YAG laser in universal vitiligo. J Cutan Aesthet Surg. 2013;6:93–6.
31. van Geel N, Depaepe L, Speeckaert R. Laser (755 nm) and cryotherapy as depigmentation treatments for vitiligo: a comparative study. J Eur Acad Dermatol Venereol. 2015;29:1121–7.
32. Hossain C, Porto DA, Hamzavi I, et al. Camouflaging agents for vitiligo patients. J Drugs Dermatol. 2016;15:384–7.
33. Kaliyadan F, Kumar A. Camouflage for patients with vitiligo. Indian J Dermatol Venereol Leprol. 2012;78(1):8–15.
34. Ezzedine K, Lim HW, Suzuki T, et al. Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. Pigment Cell Melanoma Res. 2012;25:E1–13.
35. Ukoha UT, Sosa JJ, Hynan LS, et al. Assessment of vitiligo severity: patient-reported estimates are not accurate. Br J Dermatol. 2015;173:1325–6.
36. Quevedo WCJ, Holstein TJ. General biology of mammalian pigmentation. In: Nordlind JJ, Boissy RE, Hearing VJ, King RA, Oetting WS, Ortonne J, editors. The pigmentary system. 2nd ed. Oxford: Blackwell Publishing Ltd; 2006. p. 63–90.
37. Gauthier Y, Cario Andre M, Taieb A. A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? Pigment Cell Res. 2003;16:322–32.
38. Ortonne J, Passeron T. Vitiligo and other disorders of hypopigmentation. In: Bolognia JL, Jorizzo JL, Schaffer JV, editors. Dermatology. 3rd ed. Philadelphia: Elsevier; 2012. p. 1023–48.
39. Benzekri L, Gauthier Y, Hamada S, et al. Clinical features and histological findings are potential indicators of activity in lesions of common vitiligo. Br J Dermatol. 2013;168:265–71.
40. Benzekri L, Hmamouchi I, Gauthier Y. Possible patterns of epidermal melanocyte disappearance in nonsegmental vitiligo: a clinicopathological study. Br J Dermatol. 2015;172:331–6.
41. Regazzetti C, Joly F, Marty C, et al. Transcriptional analysis of vitiligo skin reveals the alteration of WNT pathway: a promising target for repigmenting vitiligo patients. J Invest Dermatol. 2015;135:3105–14.
42. Wagner RY, Luciani F, Cario-Andre M, et al. Altered E-cadherin levels and distribution in melanocytes precede clinical manifestations of vitiligo. J Invest Dermatol. 2015;135:1810–9.
43. Rogers LC, Bevilacqua NJ, Armstrong DG, et al. Digital planimetry results in more accurate wound measurements: a comparison to standard ruler measurements. J Diabetes Sci Technol. 2010;4:799–802.
44. van Geel N, Speeckaert R, De Wolf J, et al. Clinical significance of Koebner phenomenon in vitiligo. Br J Dermatol. 2012;167:1017–24.
45. El-Din Anbar T, Abdel-Rahman AT, El-Khayyat MA, et al. Vitiligo on anterior aspect of neck in Muslim females: case series. Int J Dermatol. 2008;47:178–9.
46. Cavalie M, Ezzedine K, Fontas E, et al. Maintenance therapy of adult vitiligo with 0.1% tacrolimus ointment: a randomized, double blind, placebo-controlled study. J Invest Dermatol. 2015;135:970–4.
47. Bhatnagar A, Kanwar AJ, Parsad D, et al. Psoralen and ultraviolet A and narrow-band ultraviolet B in inducing stability in vitiligo, assessed by vitiligo disease activity score: an open prospective comparative study. J Eur Acad Dermatol Venereol. 2007;21:1381–5.
48. Radakovic-Fijan S, Furnsinn-Friedl AM, Honigsmann H, et al. Oral dexamethasone pulse treatment for vitiligo. J Am Acad Dermatol. 2001;44:814–7.
49. Taieb A, Alomar A, Bohm M, et al. Guidelines for the management of vitiligo: the European Dermatology Forum consensus. Br J Dermatol. 2012;168:5–19.

Editor's Synthesis and Perspectives 46

Alain Taïeb and Mauro Picardo

Contents

Abstract

EBM guidelines have many limitations in a field like vitiligo but can help to fight charlatanism and harmful interventions. Inflammation/autoimmunity are the major targets to stop disease progression. Combination therapies using systemic/topical antiinflammatory-immunosuppressant drugs with UVB therapy are the current mainstay approach for vitiligo. More targeted immunosuppressants such as Jak inhibitors are currently in clinical development Novel approaches should promote melanocyte survival and stability within the basal membrane zone of the epidermis.

Regenerative medicine is the next important development because of the exhaustion of spontaneous regeneration especially in acral locations.

Key Points

- EBM guidelines have many limitations in a field like vitiligo but can help to fight charlatanism and harmful interventions.
- Inflammation/autoimmunity are the major targets to stop disease progression.
- Combination therapies using systemic/topical antiinflammatory-immunosuppressant drugs with UVB therapy are the current mainstay approach for vitiligo.
- More targeted immunosuppressants such as Jak inhibitors are currently in clinical development.
- Novel approaches should promote melanocyte survival and stability within the basal membrane zone of the epidermis.
- Regenerative medicine is the next important development because of the exhaustion of spontaneous regeneration especially in acral locations.

A. Taïeb (✉)
Hôpital Saint-André Service de Dermatologie, Bordeaux, France
e-mail: alain.taieb@chu-bordeaux.fr

M. Picardo
Cutaneous Physiopathology and CIRM, San Gallicano Dermatological Institute, IRCCS, Rome, Italy
e-mail: mauro.picardo@ifo.gov.it

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_46

Table 46.1 General outline of management for vitiligo

Type of vitiligo	Usual management
Segmental and limited nonsegmental <2–3% body surface involvement	*First line*: Avoidance of triggering factors, local therapies (corticosteroids, calcineurin inhibitors) combined with NB-UVB therapy, Excimer monochromatic lamp if available or home phototherapy. Treatment to monitor closely over 6 to 12 months before going to second line. *Second line*: Consider systemic intervention (see below) if disease flare during first line treatment, and total body NB-UVB treatment. *Third line*: Consider surgical techniques if repigmentation is cosmetically unsatisfactory on visible areas
Nonsegmental	*First line*: Avoidance of triggering/aggravating factors. Stabilization with NB-UVB therapy, at least 3 months. Optimal duration at least 9 months if response. Combination with systemic/topical therapies, including reinforcement with localized UVB therapy, possible *Second line*: Consider systemic steroids (e.g. 3–4 month minipulse therapy) or immunosuppressants if rapidly progressing disease or absence of stabilization under NB-UVB. New molecules especially Jak inhibitors in development. *Third line*: Consider surgical techniques in non-responding areas especially with high cosmetic impact. However, Koebner phenomenon limits the persistence of grafts. Relative contraindication in areas such as dorsum of hands *Fourth line*: Consider depigmentation techniques (hydroquinone monobenzyl ether or 4-methoxyphenol alone or associated with Q-switch ruby laser) in non-responding widespread (>50%) or highly visible recalcitrant facial/hands vitiligo

A no treatment option (zero line) can be considered in patients with a fair complexion after discussion. For children, phototherapy is limited by feasibility in the younger age group and surgical techniques rarely proposed before prepubertal age. There is no current recommendation applicable to the case of rapidly progressive vitiligo, not stabilized by UV therapy, but classic immunosuppressants and Jak inhibitors can be started. For all subtypes of disease or lines of treatment, psychological support and counselling including access to camouflage instructors are needed

46.1 From EBM Guidelines to Clinical Practice

The major interest of EBM reviews and meta-analyses is to point to potentially harmful interventions or those with limited interest. Given the importance of charlatanism in the vitiligo field, counselling patients to avoid some therapies of dubious efficacy is indeed a major step.

As partly stated in the Cochrane review [1], there are many limitations to derive a valuable algorithm of treatment for all vitiligo patients based on RCTs. First, RCTs are rare and often lacking important methodological steps or details. Second, studies have often been conducted in heterogeneous groups in terms of vitiligo duration or progression, if not mixing localized, segmental and nonsegmental forms. Third, confounding factors are many, e.g. light exposure in long-term interventions for which light sources may influence outcome, nutritional intake if antioxidant status is considered or awareness on limitation of the Koebner phenomenon which is rarely taken into account.

Nevertheless, a stepwise treatment approach divided by type of vitiligo and extent, which needs modulation by visibility, age and coping, is outlined in Table 46.1 [2]. A zero line is always possible, meaning no treatment if the disease is not bothering the patient. The environmental factors (occupation, Koebner's phenomenon, sustained stress or anxiety) should be always discussed in the management plan. This stepwise approach should be considered as a proposal based mostly on EBM data. However, there is much room for modulation and innovation based on this scheme.

46.2 Perspectives

Part II of this book has put the emphasis on numerous potential therapeutic resources, based on a better understanding of the crucial steps of melanocyte

mechanisms of disappearance and repigmentation schemes. For a common disorder like vitiligo, there are probably subtypes in terms of mechanisms of melanocyte loss, but the best documented is of inflammatory/immune origin, with the activation of memory T cells during flares [3]. Currently, based on these principles, a more aggressive anti-inflammatory therapy is becoming more widely accepted for stopping disease progression and promote repigmentation in combination with UV light. If the initial step preceding inflammation comes from a local predisposition of melanocytes to attach poorly to the basement membrane, a more precise description of the basic impairment is obviously needed to improve melanocyte stability. Another possible therapeutic target is an underlying impairment of melanocyte survival mechanisms, and enhancing the Wnt/betacatenin pathway is a possibility to explore in addition to the MSH analogs [4]. The issue of self-renewal (stemness) aptitude of melanocytes has been raised especially for SV, which clearly benefits from autologous grafting [3]. It is yet unclear if this is a real issue in vitiligo/NSV and if this is related to the cellular environment rather than directly to melanocyte themselves, as suggested by Guerra [5].

When melanocyte loss has been stopped, therapy tries to address repigmentation. New repigmenting therapies are emerging such as He-Ne lasers [6] and prostaglandin E2 [7]. The field of regenerative medicine is booming and applications to vitiligo are expected. The use of melanocyte precursors in the hair follicle is promising, as well as the clinical development of mesenchymal stem cells. If we can better stimulate the emigration of those stem cells towards the epidermis and understand why they usually stop migrating when becoming pigmented, a major step should be achieved. There is also the issue of dormant residual (dedifferentiated) melanocytes which could be resuscitated in interfollicular zones and opening some new therapeutic avenues. A better appreciation of this issue is clearly needed to consider newer possibilities for medical treatments. Surgical treatments for limited and disfiguring disease have dramatically improved. Newer technologies derived from progenitors or reprogrammed skin cells [8] will probably further increase our surgical possibilities of intervention.

References

1. Whitton M, Pinart M, Batchelor JM, Leonardi-Bee J, Gonzalez U, Jiyad Z, Eleftheriadou V, Ezzedine K. Evidence-based management of vitiligo: summary of a Cochrane systematic review. Br J Dermatol. 2016;174(5):962–9. https://doi.org/10.1111/bjd.14356. Epub 2016 Mar 25. Review. PubMed PMID: 26686510.
2. Taieb A, Alomar A, Böhm M, Dell'anna ML, De Pase A, Eleftheriadou V, Ezzedine K, Gauthier Y, Gawkrodger DJ, Jouary T, Leone G, Moretti S, Nieuweboer-Krobotova L, Olsson MJ, Parsad D, Passeron T, Tanew A, van der Veen W, van Geel N, Whitton M, Wolkerstorfer A, Picardo M, Vitiligo European Task Force (VETF); European Academy of Dermatology and Venereology (EADV); Union Europe´enne des Me´decins Spe´cialistes (UEMS). Guidelines for the management of vitiligo: the European Dermatology Forum consensus. Br J Dermatol. 2013;168(1):5–19. https://doi.org/10.1111/j.1365-2133.2012.11197.x. Epub 2012 Nov 2. PubMed PMID: 22860621.
3. Boniface K, Seneschal J. Vitiligo as a skin memory disease: The need for early intervention with immunomodulating agents and a maintenance therapy to target resident memory T cells. Exp Dermatol. 2019; https://doi.org/10.1111/exd.13879. [Epub ahead of print] Review. PubMed PMID: 30636075.
4. Lim HW, Grimes PE, Agbai O, Hamzavi I, Henderson M, Haddican M, Linkner RV, Lebwohl M. Afamelanotide and narrowband UV-B phototherapy for the treatment of vitiligo: a randomized multicenter trial. JAMA Dermatol. 2015;151(1):42–50. https://doi.org/10.1001/jamadermatol.2014.1875. PubMed PMID: 25230094.
5. Bondanza S, Maurelli R, Paterna P, et al. Keratinocyte cultures from involved skin in vitiligo patients show an impaired in vitro behaviour. Pigment Cell Res. 2007;20:288–300.
6. Lan CC, Wu CS, Chiou MH, et al. Low-energy helium-neon laser induces locomotion of the immature melanoblasts and promotes melanogenesis of the more differentiated melanoblasts: recapitulation of vitiligo repigmentation in vitro. J Invest Dermatol. 2006;126:2119–26.
7. Kapoor R, Phiske MM, Jerajani HR. Evaluation of safety and efficacy of topical prostaglandin E2 in treatment of vitiligo. Br J Dermatol. 2009;160:861–3.
8. Takahashi K, Tanabe K, Ohnuki M. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–72.

Correction to: Vitiligo

Mauro Picardo and Alain Taïeb

Correction to:
M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5

Owing to an unfortunate oversight a preliminary version of the book was inadvertently published. The most important corrections are:

1. Permission for using the table 11.1 had been acquired from Pigment Cell Melanoma Res (not Pigm Mel Research). Hence, caption for Table 11.1 has been corrected to "van Geel N, Speeckaert R, Taieb A et al. Koebner's phenomenon in vitiligo: European position paper. Pigment Cell Melanoma Res 2011; 24: 564–73. With permissions from Pigment Cell Melanoma Res (not Pigm Mel Research)"
2. Dr. Gisela F. Erf was inadvertently omitted as a contributor to chapter 28. The correct order of authors is as follows:
   Webb, K. C., S. W. Henning, G. F. Erf, and I. C. Le Poole
3. An outdated version of text had been used in section 35.1.2 in chapter 35 "Topical calcineurin inhibitors" and the correct version is below.

Topical calcineurin inhibitors

J.E. Lommerts, A. Wolkerstorfer, N. van Geel, M.W. Bekkenk

Background

The topical calcineurin inhibitors (TCI)- tacrolimus ointments 0.1% and 0.03% and pimecrolimus cream 1%- have been specifically developed for the treatment of atopic dermatitis, but are used for a wide range of inflammatory disorders. In contrast to topical corticosteroids (TCS), TCI do not have the risk of local

The online versions of these chapters can be found at
https://doi.org/10.1007/978-3-319-62960-5_11
https://doi.org/10.1007/978-3-319-62960-5_28
https://doi.org/10.1007/978-3-319-62960-5_35
https://doi.org/10.1007/978-3-319-62960-5_36
https://doi.org/10.1007/978-3-319-62960-5

M. Picardo (✉)
Cutaneous Physiopathology & CIRM, San Gallicano Dermatological Institute, Rome, Italy

A. Taïeb
Hôpital Saint André, Service de Dermatologie Adulte et Pédiatrique, INSERM U 1035, Université de Bordeaux, Bordeaux, France

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_47

side effects related to skin atrophy, telangiectasia, and glaucoma after prolonged use. Therefore they are preferentially used in areas more susceptible to these side effects, such as head and neck region.[1, 2] Furthermore, because of the limited percutaneous penetration through intact skin, significant systemic absorption has not been reported following normal use.[3] Tacrolimus and pimecrolimus are topical immunomodulating agents (derivative of ascomycin macrolactam), that inhibit calcineurin. By inhibiting the release of various Th1 and Th2 type cytokines, such as TNFα it can influences the activation and maturation i of T-cells. This has led to speculate that this mechanism may interact with the process leading to melanocytes loss in lesional skin of vitiligo lesions. The inhibition of TNFα production was suspected by some authors to be especially important in vitiligo, as TNFα can inhibit melanocyte proliferation and melanogenesis, and that it can induce ICAM-1 expression on melanocytes, through which T- lymphocyte induced destruction of melanocytes may occur.[4] In addition, in vitro studies[5, 6] also have shown stimulating impact of TCI on melanocytic migration and growth.

Efficacy

Monotherapy with TCI

To date, several clinical studies have investigated the efficacy of topical monotherapy with either pimecrolimus or tacrolimus in vitiligo.[4, 7–22] In a randomized controlled trial, Ho et al.[22] showed that tacrolimus 0.1% ointment twice daily was effective after 6 months in approximately 60% of lesions on the face and in 23% of lesions on the rest of the body. Furthermore, Radakovic et al.[21] showed moderate tot excellent responses (>25% repigmentation) in 27% of the patients after tacrolimus 0.1% ointment 1-2 times daily. In another randomized clinical trial tacrolimus 0.1% ointment was shown to be as effective as 0.05% fluticasone proprionate cream after a follow-up duration of 6 months.[20] Kose et al.[19] compared pimecrolimus 1% cream twice daily with mometasone cream 0.1% once daily in childhood vitiligo. In 45% of patients treated with pimecrolimus a decrease in lesion size was found and in 3 patients complete remission was achieved. Dawid et al.[18] investigated in a randomized clinical trial the lesion size of vitiligo patients treated with either pimecrolimus 1% cream or placebo and found a decrease of 90 mm2 vs 220 mm2, respectively, however this difference was not significant. Pimecrolimus 0.1% cream under occlusion once daily with or without microdermabrasion was more effective than placebo in the study of Farajzadeh et al.[17]. Several case series also published the efficacy of topical tacrolimus and pimecrolimus in vitiligo. [4, 7–10, 12–16] Lepe et al. assessed the efficacy of topical tacrolimus 0.1% vs topical 0.05% clobetasol proprionate in childhood vitiligo and after 2 months mean repigmentation of 41.3% and 49.3% were found, respectively[11] Data about the most effective treatment scheme using TCI in vitiligo are still missing. In the available vitiligo studies the application frequency is varying between once and twice daily for both pimecrolimus and tacrolimus application. Twice daily application of tacrolimus seems to be more effective than once daily.[21, 23] Several studies have shown that the efficacy of topical pimecrolimus and tacrolimus is higher when applied on the face than on other locations.[4, 7, 9–11, 15, 19, 21, 22, 24] Duration of treatment ranged in most of the studies from several weeks to 1.5 years and information about the minimal or ideal treatment period in vitiligo is not available. However, intermittent use after repigmentation, on the model of proactive treatment of atopic dermatitis, has proven to be helpful to limit relapse in a controlled study[25] and repeated application may limit Koebner's phenomenon[26].

Combination therapy of TCI and phototherapy

To enhance repigmentation in vitiligo lesions the combination of TCI and phototherapy is frequently used in daily practice as the combination therapy is thought to play a synergistic role.[27] Furthermore, in the past years several studies[24, 28–32] showed the efficacy of combination therapy of TCI and phototherapy. In a randomized clinical trial, Esfandiarpour et al.24 compared the efficacy of narrowband UVB phototherapy three times weekly with and without pimecrolimus 1% cream. After 3 months of treatment the combination of narrowband UVB phototherapy with topical pimecrolimus application was significantly more effective than nar-

rowband UVB alone. Dayal et al. 28 showedtthat combination of narrowband UVB with tacrolimus 0.03% was significantly more effective than phototherapy alone in childhood vitiligo. Furthermore, a meta-analysis showed that lesions located on the face and neck show better results with combination of narrowband UVB phototherapy and TCI than narrowband UVB phototherapy alone.[33] This improved efficacy of the combination was found for both ≥50% repigmentation (RR = 1.40, 95% CI 1.08-1.81) and ≥75% repigmentation (RR = 1.88, 95% CI 1.10-3.20) In a randomized placebo-controlled study of Mehrabi et al.[31], narrow-band -UVB therapy with and without tacrolimus ointment was compared and no additional effect of tacrolimus was found. However, a low number of study participants (n=8) was included and the evaluated lesions were all located on non-facial areas. Systematically congregated evidence in the systematic review of Bae et al.[34] showed that the combination of 308-nm excimer laser therapy with either topical pimecrolimus or tacrolimus application is more effective than excimer laser therapy alone (4 studies, relative risk 1.93, 95% confidence interval 1.28-2.91).[34] These results were confirmed in childhood vitiligo in a recent randomized controlled trial of Li et al.[35]

Side effects

The most common reported side effects for TCI within the first days of treatment are local reactions such as burning sensation, pruritus, and erythema. This seems to be less frequently reported compared to atopic dermatitis.[17, 20, 30] A possible explanation for the latter might be the difference in skin barrier between both diseases. Based on some studies in atopic dermatitis no significant increased risk for skin or systemic infections could be demonstrated.[36–39] Tacrolimus-induced lentigines and hyperpigmentation in vitiligo lesions of the infraorbital area are rare side effects that have been related to sun- and UV-exposure.[38, 40] Less frequently reported side effects in the face are acneiform eruptions and hypertrichosis.[39] The FDA announced a black box warning in 2006 for the application of tacrolimus and pimecrolimus, including concerns of potential safety issues (e.g. risk for skin cancer and lymphoma). So far the use of TCI has not been reported to be associated with significant systemic immunosuppression or increased risk for skin cancer and other malignancies in clinical vitiligo trials. Moreover, in contrast to atopic dermatitis the barrier of the skin in vitiligo is not disturbed, so less absorption can be assumed. Furthermore, a recent study found that in atopic dermatitis in more than 25,000 person-years of follow-up no association was found between occurrence of malignancy and use of TCI.[41] Therefore, the risk on skin cancer and other malignancy in vitiligo after application of TCI is considered to be very low. However, in vitiligo the TCI are often used in combination with UV therapy. This may lead to a potentially higher risk for skin cancer and conclusive data on the safety on the combination therapy of TCI and phototherapy is still lacking.

Interpretation and recommendations

Although the quality of the evidence for the efficacy of TCI in vitiligo is limited, topical pimecrolimus and tacrolimus are widely recommended in national and international guidelines for vitiligo based on expert based opinions and experience.[42–44] The repigmentation of TCI in vitiligo varies according to the anatomical location of the lesions. Most studies show favourable results mainly in the area of head and neck.[45] This is probably linked to the presence of melanocytes in remaining pigmented hair follicles. Besides, the influence of a reduced epidermal thickness might be of importance as this facilitates penetration of large molecules such as TCI into the skin. Moreover, the head and neck region is a UV exposed region of the body area and UV therapy is a therapy of vitiligo which could play a synergistic role. Most studies included repigmentation as their primary outcome. It can be discussed whether cessation of spreading of the depigmentation would have been a better primary outcome to measure the efficacy of TCI as repigmentation does not always occur in vitiligo after topical treatment alone. A correlation between the repigmentation rate and patients' age or duration of the disease has not been consistently demonstrated. According to some reports, the efficacy of TCI

in childhood vitiligo seems to be comparable to that in adult patients.[1, 7, 11, 45]

In summary, based on the available literature TCI can be recommended for treatment of depigmentations on the face and neck in both children and adult patients with vitiligo. TCI are also used for long-term control of the disease because of their efficacy and limited side effects compared to potent topical corticosteroids. Superior effect of the combinations with natural light of UV phototherapy has been demonstrated, although conclusive data related to the safety aspects are still required

References

1. Souza Leite RM, Craveiro Leite AA. Two therapeutic challenges: periocular and genital vitiligo in children successfully treated with pimecrolimus cream. *International journal of dermatology* 2007; 46: 986-9.
2. Mayoral FA, Vega JM, Stavisky H, *et al*. Retrospective analysis of pimecrolimus cream 1% for treatment of facial vitiligo. *Journal of drugs in dermatology : JDD*. 2007;6:517–21.
3. Allen A, Siegfried E, Silverman R, *et al*. Significant absorption of topical tacrolimus in 3 patients with Netherton syndrome. *Archives of dermatology*. 2001;137:747–50.
4. Grimes PE, Morris R, Avaniss-Aghajani E, *et al*. Topical tacrolimus therapy for vitiligo: therapeutic responses and skin messenger RNA expression of proinflammatory cytokines. *Journal of the American Academy of Dermatology*. 2004;51:52–61.
5. Kang HY, Choi YM. FK506 increases pigmentation and migration of human melanocytes. *The British journal of dermatology* 2006; 155: 1037-1040.
6. Lan CC, Chen GS, Chiou MH, *et al*. FK506 promotes melanocyte and melanoblast growth and creates a favourable milieu for cell migration via keratinocytes: possible mechanisms of how tacrolimus ointment induces repigmentation in patients with vitiligo. *The British journal of dermatology*. 2005;153:498–505.
7. Kanwar AJ, Dogra S, Parsad D. Topical tacrolimus for treatment of childhood vitiligo in Asians. *Clinical and experimental dermatology*. 2004;29:589–92.
8. Taher ZA, Lauzon G, Maguiness S, *et al*. Analysis of interleukin-10 levels in lesions of vitiligo following treatment with topical tacrolimus. *The British journal of dermatology*. 2009;161:654–9.
9. Udompataikul M, Boonsupthip P, Siriwattanagate R. Effectiveness of 0.1% topical tacrolimus in adult and children patients with vitiligo. *The Journal of dermatology*. 2011;38:536–40.
10. Lo YH, Cheng GS, Huang CC, *et al*. Efficacy and safety of topical tacrolimus for the treatment of face and neck vitiligo. *The Journal of dermatology*. 2010;37:125–9.
11. Lepe V, Moncada B, Castanedo-Cazares JP, *et al*. A double-blind randomized trial of 0.1% tacrolimus vs 0.05% clobetasol for the treatment of childhood vitiligo. *Archives of dermatology*. 2003;139:581–5.
12. Lotti T, Buggiani G, Troiano M, *et al*. Targeted and combination treatments for vitiligo. Comparative evaluation of different current modalities in 458 subjects. *Dermatologic therapy*. 2008;21(Suppl 1):S20–6.
13. Choi CW, Chang SE, Bak H, *et al*. Topical immunomodulators are effective for treatment of vitiligo. *The Journal of dermatology*. 2008;35:503–7.
14. Shim WH, Suh SW, Jwa SW, *et al*. A pilot study of 1% pimecrolimus cream for the treatment of childhood segmental vitiligo. *Annals of dermatology*. 2013;25:168–72.
15. Seirafi H, Farnaghi F, Firooz A, *et al*. Pimecrolimus cream in repigmentation of vitiligo. *Dermatology*. 2007;214:253–9.
16. Sendur N, Karaman G, Sanic N, *et al*. Topical pimecrolimus: a new horizon for vitiligo treatment? *The Journal of dermatological treatment*. 2006;17:338–42.
17. Farajzadeh S, Daraei Z, Esfandiarpour I, *et al*. The efficacy of pimecrolimus 1% cream combined with microdermabrasion in the treatment of nonsegmental childhood vitiligo: a randomized placebo-controlled study. *Pediatric dermatology*. 2009;26:286–91.
18. Dawid M, Veensalu M, Grassberger M, *et al*. Efficacy and safety of pimecrolimus cream

1% in adult patients with vitiligo: results of a randomized, double-blind, vehicle-controlled study. *Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG*. 2006;4:942–6.
19. Kose O, Arca E, Kurumlu Z. Mometasone cream versus pimecrolimus cream for the treatment of childhood localized vitiligo. *The Journal of dermatological treatment*. 2010;21:133–9.
20. Kathuria S, Khaitan BK, Ramam M, *et al*. Segmental vitiligo: a randomized controlled trial to evaluate efficacy and safety of 0.1% tacrolimus ointment vs 0.05% fluticasone propionate cream. *Indian journal of dermatology, venereology and leprology*. 2012;78:68–73.
21. Radakovic S, Breier-Maly J, Konschitzky R, *et al*. Response of vitiligo to once- vs. twice-daily topical tacrolimus: a controlled prospective, randomized, observer-blinded trial. *Journal of the European Academy of Dermatology and Venereology : JEADV*. 2009;23:951–3.
22. Ho N, Pope E, Weinstein M, *et al*. A double-blind, randomized, placebo-controlled trial of topical tacrolimus 0.1% vs. clobetasol propionate 0.05% in childhood vitiligo. *The British journal of dermatology*. 2011;165:626–32.
23. Stinco G, Piccirillo F, Forcione M, *et al*. An open randomized study to compare narrow band UVB, topical pimecrolimus and topical tacrolimus in the treatment of vitiligo. *European journal of dermatology : EJD*. 2009;19:588–93.
24. Esfandiarpour I, Ekhlasi A, Farajzadeh S, *et al*. The efficacy of pimecrolimus 1% cream plus narrow-band ultraviolet B in the treatment of vitiligo: a double-blind, placebo-controlled clinical trial. *The Journal of dermatological treatment*. 2009;20:14–8.
25. Cavalie M, Ezzedine K, Fontas E, *et al*. Maintenance therapy of adult vitiligo with 0.1% tacrolimus ointment: a randomized, double blind, placebo-controlled study. *The Journal of investigative dermatology*. 2015;135:970–4.
26. van Geel N, Speeckaert R, Mollet I, *et al*. In vivo vitiligo induction and therapy model: double-blind, randomized clinical trial. *Pigment cell & melanoma research*. 2012;25:57–65.
27. Ostovari N, Passeron T, Lacour JP, *et al*. Lack of efficacy of tacrolimus in the treatment of vitiligo in the absence of UV-B exposure. *Archives of dermatology*. 2006;142:252–3.
28. Dayal S, Sahu P, Gupta N. Treatment of Childhood Vitiligo Using Tacrolimus Ointment with Narrowband Ultraviolet B Phototherapy. *Pediatric dermatology*. 2016;33:646–51.
29. Kawalek AZ, Spencer JM, Phelps RG. Combined excimer laser and topical tacrolimus for the treatment of vitiligo: a pilot study. *Dermatologic surgery : official publication for American Society for Dermatologic Surgery* [*et al*.]. 2004;30:130–5.
30. Passeron T, Ostovari N, Zakaria W, *et al*. Topical tacrolimus and the 308-nm excimer laser: a synergistic combination for the treatment of vitiligo. *Archives of dermatology*. 2004;140:1065–9.
31. Mehrabi D, Pandya AG. A randomized, placebo-controlled, double-blind trial comparing narrowband UV-B Plus 0.1% tacrolimus ointment with narrowband UV-B plus placebo in the treatment of generalized vitiligo. *Archives of dermatology*. 2006;142:927–9.
32. Hui-Lan Y, Xiao-Yan H, Jian-Yong F, *et al*. Combination of 308-nm excimer laser with topical pimecrolimus for the treatment of childhood vitiligo. *Pediatric dermatology*. 2009;26:354–6.
33. Li R, Qiao M, Wang X, *et al*. Effect of narrow band ultraviolet B phototherapy as monotherapy or combination therapy for vitiligo: a meta-analysis. *Photodermatology, photoimmunology & photomedicine*. 2017;33:22–31.
34. Bae JM, Hong BY, Lee JH, *et al*. The efficacy of 308-nm excimer laser/light (EL) and topical agent combination therapy versus EL monotherapy for vitiligo: A systematic review and meta-analysis of randomized controlled

trials (RCTs). *Journal of the American Academy of Dermatology*. 2016;74:907–15.
35. Li L, Liang Y, Hong J, *et al*. The effectiveness of topical therapy combined with 308-nm excimer laser on vitiligo compared to excimer laser monotherapy in pediatric patients. *Pediatric dermatology*. 2019;36:e53–e5.
36. Fleischer AB Jr, Ling M, Eichenfield L, *et al*. Tacrolimus ointment for the treatment of atopic dermatitis is not associated with an increase in cutaneous infections. *Journal of the American Academy of Dermatology*. 2002;47:562–70.
37. Luger T, Boguniewicz M, Carr W, *et al*. Pimecrolimus in atopic dermatitis: consensus on safety and the need to allow use in infants. *Pediatr Allergy Immunol*. 2015;26:306–15.
38. De D, Kanwar AJ. Tacrolimus-induced hyperpigmentation in a patch of vitiligo. *Skinmed*. 2008;7:93–4.
39. Bakos L, Bakos RM. Focal acne during topical tacrolimus therapy for vitiligo. *Archives of dermatology*. 2007;143:1223–4.
40. Gan EY, Taieb A. Unwanted lentigines after topical tacrolimus for vitiligo. *The Australasian journal of dermatology*. 2017;58: e259–e60.
41. Margolis DJ, Abuabara K, Hoffstad OJ, *et al*. Association Between Malignancy and Topical Use of Pimecrolimus. *JAMA dermatology*. 2015;151:594–9.
42. Lommerts A. Vitiligo: een update. *Nederlands Tijdschrift voor Dermatologie en Venereologie*. 2016;26:149–54.
43. Oiso N, Suzuki T, Wataya-Kaneda M, *et al*. Guidelines for the diagnosis and treatment of vitiligo in Japan. *The Journal of dermatology*. 2013;40:344–54.
44. Taieb A, Alomar A, Bohm M, *et al*. Guidelines for the management of vitiligo: the European Dermatology Forum consensus. *The British journal of dermatology*. 2013;168:5–19.
45. Boone B, Ongenae K, Van Geel N, *et al*. Topical pimecrolimus in the treatment of vitiligo. *Eur J Dermatol*. 2007;17(1):55–61.
46. Silverberg NB, Lin P, Travis L, *et al*. Tacrolimus ointment promotes repigmentation of vitiligo in children: a review of 57 cases. *Journal of the American Academy of Dermatology*. 2004;51:760–6.

4. A section on Tissue Grafting in chapter 36, "Surgical Therapies" had been missed out and the text has been included below.

Tissue grafting for Vitiligo

Contributed by Kanika Sahni, Assistant Professor, and Dr. Somesh Gupta, Professor, Department of Dermatology & Venereology, All India Institute of Medical Sciences, New Delhi, India

Tissue grafting procedures involve transplantation of skin and/or hair follicle grafts directly from donor site to recipient site. This may involve minor modifications to the tissue but no chemically induced modification.

Tissue grafting procedures may be subclassified into:

1. Pure epidermal grafts: These have the advantage of minimum scarring or postinflammatory changes at donor site and excellent texture match at recipient site
   (a) Suction blister grafting
   (b) Ultra-thin STSG
2. Dermo-epidermal grafts: As these grafts involve damage to superficial dermis they are often associated with scarring at donor sites and poorer textural match at recipient site
   (a) Mini punch grafting
   (b) Split thickness skin grafts
   (c) Mesh grafting
   (d) Smash grafting
3. Hair follicle grafting: This technique is particularly useful for hair bearing areas with leukotrichia including scalp, eyebrows and eyelashes.

Suction blister epidermal grafting (SBEG)

This technique involves the use of negative pressure to create blisters at donor site followed by utilization of the roofs of these suction blisters as a graft to cover the dermabraded recipient site. This technique leads to creation of a split at the dermoepidermal junction, being the weakest among all layers of skin, thus providing a purely epidermal graft which follows the concept of "recipient dominance". This implies that the graft

takes up the colour and texture of the recipient site in contrast to thicker dermoepidermal grafts which follow the concept of "donor dominance". Thus these grafts tend to provide better cosmetic outcome especially on cosmetically important sites like lips, areolae etc. Another advantage is the absence of scarring or textural change at the donor site, which allows repeated harvesting from same donor site.

The use of this technique was first described by Falabella for treating depigmented and granulating areas such as vitiligo and chronic wounds.1 The technique has undergone various modifications and the currently widely used method of creating blisters was pioneered by one of the authors (SG).2,3

Technique

Blister induction

The blisters are usually created on a hidden area of skin, either the lateral upper thigh or upper inner arm. We recommend to use the area on the lateral thigh just below the trochanter of the femur as the tense skin and underlying bone provide a good surface for rapid induction of blisters. The patient is made to lie down in lateral position and the donor area is cleansed and anesthetized by a field block using a mixture of bupivacaine (0.5%) and lignocaine (2%) in a 1:3 ratio in order to provide long lasting anesthetic effect. The site is then lubricated with white petroleum jelly, which forms an impermeable layer between suction syringe and the skin surface.

Depending on the size of recipient area, 10-ml syringes are prepared by removing the plunger and attaching the three-way cannula to the hub of syringe. The three-way cannula is placed vertically to allow the syringe to communicate with the air at one end of cannula only. One by one these syringes are placed on the skin surface with the broad end in good contact with the skin. The assistant then attaches a 50-ml syringe to the communicating end of the three-way cannula and pulls the plunger up to a volume of 25 to 30 ml to achieve a vacuum of −300 to −400mm Hg. The surgeon then rotates the three-way cannula lock to a horizontal position in order to cut off the communication of 10-ml syringe with the external air. This allows the maintenance of the vacuum hence created and results in a dome-shaped elevation of the donor skin visible inside the lower end of the syringe. Remaining syringes are applied in a similar manner half to one centimetre apart.

The patient is asked to lie down in the same position for around 2 to 3 hours which is the usual suction blister induction time. Once the blisters are formed, the suction is released by rotating the lock of the 3-way cannula and the syringes are removed.

Harvesting of grafts

Once the blisters have formed, with the help of a pair of curved scissors, the roofs of the blisters are cut all along the margins of the blister except one edge and transferred onto sterile glass slides (with antibiotic cream smeared on it) with the dermal side of blister facing up. After this the remaining attachment of the blister is also cut and the grafts are spread with the help of a blunt forceps to remove any wrinkles and strands of fibrin. The grafts are kept moist by placing in a sterile tray containing normal saline. These pure epidermal grafts are then transferred onto the dermabraded recipient site with the dermal side facing down. It is very important to place the grafts with the correct side facing down, as an upside down placed graft would lead to failure of repigmentation. The donor site is dressed with sterile non-adherent chlorhexidine gauze (Bactigras®) and a layer of dry gauze kept in place with micropore tape. The patient is asked to keep the area dry and remove the dressing after 3 days.

Preparation of recipient site

The vitiligo patch to be treated is cleaned, draped and anesthetized using 1% lignocaine buffered with sodium bicarbonate. This is followed by dermabrasion using either a manual or a motorised dermabrader upto the level of dermoepidermal junction which is recognized by the appearance of tiny pin point bleeding points. The dermabraded sites are covered with a saline-soaked gauze to assist in hemostasis. Then the suction blister epidermal grafts are transferred

onto the dermabraded sites by placing the slide upside down and gently removing the slide while leaving the grafts (dermal side down) on the denuded surface. The grafts are then gently spread to their maximum size by using a fine forceps in order to cover the largest area. Successive grafts may be placed adjoining each other or 0.5-1 cm apart as pigmentation from each graft is expected to spread around 0.5-1cm beyond the graft margin.

The area is dressed with sterile non-adherent chlorhexidine gauze (Bactigras®) followed by a layer of dry gauze which is kept in place with elastic adhesive bandage (Dynaplast®). Patient is asked to keep the area dry and prescribed oral antibiotic and analgesic medications for a week after which the dressing is removed. The grafts are visible as dry pieces of skin and may fall off soon after, however a period of 1 week is sufficient for the melanocytes to transfer from the graft onto the recipient site. The patient is then asked to initiate phototherapy and the spread of pigmentation occurs over the next 3-4 months (Fig. 1). Often, the grafts come out as thin, dry membrane, leaving behind faint pigmentation which, in due course, darkens and covers the entire area. Epidermal grafts act as melanocyte-keratinocyte carrier.

Modifications of technique

A number of other methods for induction of blistering have been described including the use of suction cups attached to a respiratory suction apparatus, Chinese cupping technique and few other tech-

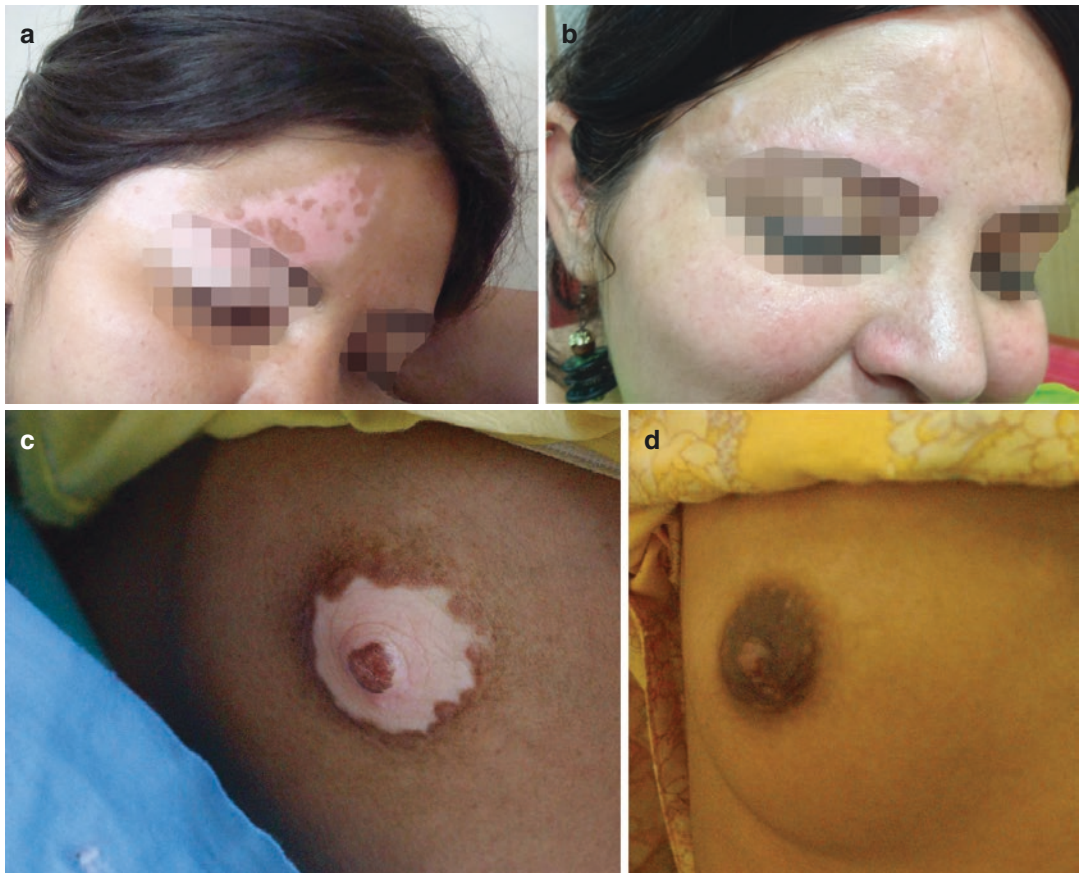


Fig. 1 (**a**) before suction blister grafting in a patient with mixed vitiligo. (**b**) after grafting. (**c**) Acrofacial vitiligo on nipple and areola. (**d**) complete repigmentation after suction blister grafting

niques.4,5,6 Various factors have been shown to affect the blister induction time such as the strength of negative pressure applied, the size of the suction cup/ syringe and the temperature with a higher temperature of 40°C hastening the blistering process.7,8,9 This has also been confirmed in a recent in vitro study on optical coherence tomography in induced suction blisters.10 The size of partially formed blisters may be expanded by inserting saline into the blister cavity by introducing a needle into the cavity through its floor.11Another modification is the use of transparency sheets instead of glass slides to transfer the grafts; however as glass slides are readily available we recommend their use for this purpose.

The preparation of the recipient site may be done in several other ways, using CO2 laser dermabrasion, Er:YaG laser dermabrasion, suction blister induction on donor site, liquid nitrogen cryofreezing, and phototoxic induction of blisters.4612,13,14,15

Modifications are also required depending on the location of vitiligo patches. Stay sutures or tissue glue are recommended to hold the dressing in place following surgery for lip vitiligo. Patients are asked to take liquid diet with straw for a week until the dressing is in place in order to avoid soiling of the dressing. For lesions on fingers and toes, results may be improved by good immobilization of the operated area using a plaster of Paris slab.

Results

The dressings are removed after one week and this time is sufficient for the melanocytes to take up in the recipient skin from the SBEG. Thus, even if the grafts are lost at the time of removal of dressing, it is not a cause of concern as transfer of melanocytes has already occurred. Repigmentation is noticed within a few weeks and if followed by phototherapy to the site, there is significant spread of pigment around the graft margins which results in repigmentation of the intergraft areas also.

In a large study on SBEG by Gupta *et al*., results were found to be significantly better in segmental/focal vitiligo where 91% patients achieved successful repigmentation) compared to 53% patients of generalized vitiligo. Another factor predicting better response included age younger than 20 years. No significant difference was found in results based on body site treated or the period of stability if it was more than 1 year.16 SBEG has been found to be inferior to both non-cultured epidermal suspension (NCES) transplantation and STSG in two different comparative trials while results were comparable to punch grafting and cultured melanocyte transplantation in another study.17,18,19

Complications

Most adverse events of the procedure are mild. Patients often report discomfort and pain during blister induction which may be lessened by infiltrating the area with long acting local anesthetics such as a combination of bupivacaine and lignocaine prior to the procedure. The donor site heals without textural change or scarring in most patients, however, hyperpigmentation may persist for few months with gradual lightening. Less common complications at donor site include Koebner phenomenon and secondary infection. At the recipient site, secondary infection, scarring, keloid formation and hyperpigmentation have been reported infrequently. Unlike, STSG or punch grafting, there is no stuck-on appearance, wrinkling or cobblestoning and the technique offers excellent cosmetic outcome even on facial and mucosal lesions.

Mini punch grafting (Minigrafting)

This is one of the oldest and simplest techniques described for the treatment of vitiligo, however, it is often associated with risk of variegate repigmentation and cobblestone appearance. Early studies reported the use of larger sized punches (2-3mm) which were associated with a higher risk of cobblestone appearance, hence the current recommendation is to use smaller sized punches (1-1.5 mm).

Technique

It is ideal to choose a donor site on a hidden area where the skin has nearly similar dermal thickness as the recipient site. For facial lesions, it is ideal to harvest punches from the retroauricular skin and for other areas, any other hidden site like inner arms or thighs may be used. After cleaning

and draping, the donor and recipient sites are anesthetized using lignocaine with adrenaline. After this, punches of size 1-1.5 mm are harvested first from the recipient site in order to create chambers where punch grafts will be placed. These are created first along the margin of the vitiligo patch followed by other parts of the patch at a distance of 0.5-1cm from each other. After this, 0.8-1mm sized punch grafts are harvested from the donor site using disposable biopsy punches and placed in a petridish containing normal saline. Once the chambers at recipient site have stopped bleeding, the grafts are placed with the dermal side down in them in such a manner that the upper surface of the graft lies at the level of the surrounding skin in order to prevent cobblestoning. The area is dressed using non-adherent antibiotic coated sterile gauze followed by a layer of gauze and bandaged. The dressing is removed at day 8 followed by phototherapy to aid in pigment spread. Modifications of the procedure include use of motorized punches and use of tissue glue to fix the grafts in place.20

The technique is simple to perform and can be used easily in limited resource settings. It is also considered to be one of the treatments of choice for vitiligo on tips of fingers and toes and to treat small residual patches remaining after prior surgery. Risk of cobblestoning can be minimized by careful technique and smaller sized punches. The technique is also useful while assessing patient's suitability for surgery in patients with doubtful stability, in the form of test grafting where perigraft spread of pigment is considered to be indicative of good prognosis following surgery.

Results

In a review on minipunch grafting, there is significant difference in size of punches used for surgery, with most earlier reports using 3-4mm punches with the trend now being more in favor of smaller (1-2mm) sized punches (Fig. 2). Onset of repigmentation occurs as soon as 14 days after transplantation and pigment spread beyond margin of grafts has varied from 1-15mm in different studies.21 A study comparing the results of MPG with transplantation of extracted follicles, no significant difference in repigmentation was observed between the two groups. 22

Hair follicle grafting

This may be considered as a modification of minipunch grafting. This technique is suitable for hair bearing sites like scalp, eyebrows and hair bearing areas on the body. This technique involves harvesting of follicular unit grafts from the scalp by the technique of follicular unit extraction (FUE) or strip harvesting followed by implantation onto slits or punches created on the hair bearing vitiligo patches. This technique may also be used on hairless sites, by removing the hair bulb before transplanting the extracted follicular unit. Recently body hair transplantation has also been described for the treatment of vitiligo.23

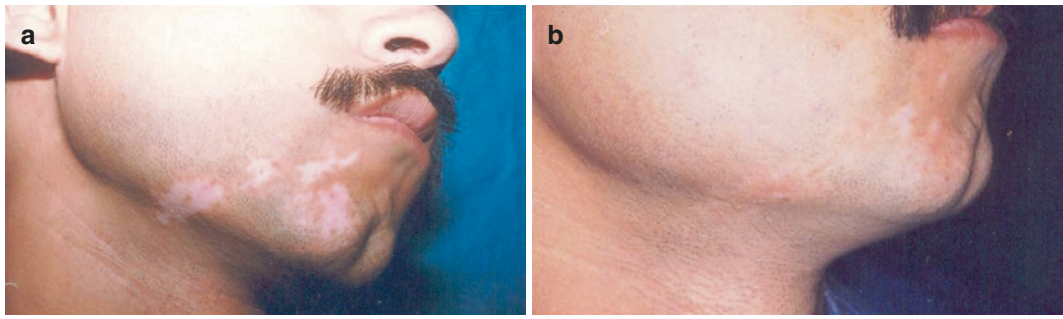


Fig. 2 (**a**) before suction blister grafting in a patient with mixed vitiligo. (**b**) after grafting

Thin and ultra-thin split thickness skin grafting (STSG)

Split thickness skin grafting involves the harvesting of a thin sheet of epidermis from a covered part of the body followed by transplantation onto dermabraded vitiligo patches. The grafts may be harvested either manually using a Humby's knife or Silver's knife or using motorized dermatomes like Davol's dermatome or Zimmer's dermatome.

Technique:

The donor site is chosen over the thigh, buttocks or upper arms and is first cleaned, draped and anesthetized using a field block using lignocaine. Alternatively topical anesthesia has been used in some patients providing adequate pain relief without the need for injectable anesthetics. A thin slice of skin is harvested using either a Humby's knife or alternatively motorized dermatomes like Davol's or Zimmer's dermatome may be used to harvest ultra thin skin grafts. The motorized dermatomes have the advantage of harvesting larger sized nearly pure epidermal grafts which provide better textural match.

The recipient site is dermabraded and the STSG is placed on it with the dermal side down followed by dressing with non adherent gauze dressing.

A modification of the technique which can allow covering of larger surface area is "mesh grafting" in which either manually (by creating slits with a blade) or using a specialized apparatus (Ampligreffe®), the graft is meshed to expand it in a ratio of 1:1, 1:2, 1:4, or 1:6. This allows for larger surface area to be covered.24,25

Results

A study on STSG in 32 patches of stable vitiligo found that 100% repigmentation was achieved in 22 patches and 90-95% repigmentation in 10 patches.26 In a recent study in 40 patients with stable vitiligo, a combination of UTSG followed by NBUVB therapy yielded more than 90% repigmentation in 83% of patients.27

Complications

Due to slightly uneven thickness of harvested graft with varying amounts of dermal tissue, there may be stuck-on appearance cosmetic result may be inferior to that with purely epidermal grafts or with cellular grafts. This is less likely to occur with grafts harvested using motorized dermatome. A thicker graft is also likely to heal with more prominent scarring at the donor site.

Index

M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5

J

K

L