Fundamentals of Craniofacial Malformations

Vol. 1, Disease and Diagnostics

Ulrich Meyer
Editor

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Dedicated to Ashley and Janine

Foreword

Surgery is an art; it is not pure science. And there is no other specialty where this is more evident than in craniofacial surgery. However even an artist would need at least a basic knowledge and a portfolio of techniques for his work.

With these textbooks a structured overview is given how craniofacial malformations develop, how they should be diagnosed, and which differential indications and treatment plans need to be taken into account before taking the patient to the operating theater.

I am really pleased to have this opportunity to pen some words at the beginning of these interesting books, which are comprehensive, well written, and illustrated by internationally recognized authors in their field.

The textbooks will serve as essential and valuable references for higher trainees and practicing clinicians in cranio-maxillofacial surgery, orthodontics, plastic and reconstructive surgery, and allied specialties.

On behalf of the patients who rely on our expertise, I wish this work as much success as possible.

Jürgen Hoffmann, MD, DMD, FEBOMFS
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Preface

Craniofacial malformations are severe diseases with a high structural and functional complexity. Patients, parents and relatives suffer not only from the disease and the medical treatments but also through the psychological and social impact of the disease. They search help in the medical society. Medical doctors from different disciplines, on the other hand, try hard to help such patients. Patients with severe craniofacial malformations like syndromic craniosynostosis or severe facial clefts have even nowadays a high burden of multiple surgical and non-surgical treatments, an altered psychological development and impaired social acceptance. "Beauty is skin deep, ugly to the bone" is a phrase that describes the effect of the anatomical situation of craniofacially malformed patients on the beholder. Therefore, engagement with craniofacial malformations needs a widespread approach.

Patients are in need to have improved conditions concerning the medical treatment options, and a better embedding in the society. Craniofacial malformation and facial disfigurement attract an increased attention nowadays not only in society but also in science. The fast growth of the craniofacial medicine discipline is mirrored by the high number of excellent research and clinical papers covering all aspects of these fields. Additionally, numerous high quality books are available describing in detail different aspects of the various disease entities. Despite the fact that such literature is already available, I decided, based on my experience in bone and soft tissue research and my focus and main clinical work in malformation surgery, to edit a comprehensive book on craniofacial malformations. There were three particular reasons for this decision:

1. First, during my experimental and clinical work on bone and cartilage healing, distraction osteogenesis, tissue engineering and stem cell use in Münster and Düsseldorf, which we have done for more than two decades in our interdisciplinary biomineralisation and tissue engineering research group with Hans-Peter Wiesmann, Jörg Handschel and Thomas Meyer and the transformation of our results in clinical practice, we observed that many specialists of the different fields, involved in approaching this area, had difficulties in overviewing the complexity of the field. At that time we edited in 2008 the book *Fundamentals of Tissue Engineering and Regenerative Medicine* in order to make a timely, comprehensive and

interdisciplinary publication of all major aspects of this field, which has the same complexity as craniofacial medicine. The success of this book format stimulated myself to edit now a comprehensive book on the complex field of craniofacial malformations.

2. Second, during the last decade an improvement and, at the same time, interdentation were seen between the various theoretical and medical subspecialities (biophysicists, material scientists, bioengineers, computer specialists, paediatricians, plastic surgeons, maxillofacial surgeons, neurosurgeons, dentists, speech therapists and all others). As the head is the most complex structure of the body, craniofacial medicine brings together basic researchers, mainly with a biological, biophysical or material science-oriented background, with the broad range of clinically oriented physicians and surgeons; I found that they differed in the "language" used. I therefore want to approach a level of communication and understanding between the disciplines by an overlap strategy of knowledge transfer in this book series. To reach this aim of a more interdisciplinary view on craniofacial medicine, I founded the open-access journal *Head and Face Medicine* to bring researchers and clinicians closer together. *Head and Face Medicine* was launched in 2005 as a multidisciplinary open-access journal that publishes basic and clinical research concerning all aspects of cranial, facial and oral conditions. The journal covered since the beginning all aspects of cranial, facial and oral diseases and their management. Designed as a multidisciplinary journal for clinicians and researchers involved in the diagnostic and therapeutic aspects of diseases which affect the human head and face, the journal developed to be wide-ranging, covering the development, aetiology, epidemiology and therapy of head and face diseases to the basic science that underlies these diseases. The aim of this book is also to contribute to an improved communication and understanding between various specialists in craniofacial malformation medicine.
3. Third, recent developments in basic and clinical approaches have shown to be promising to improve patient outcomes profoundly. As "Beauty is skin deep, ugly to the bone" describes the underlying situation of concerned patients, therapies structuring bone to the normal anatomy are of special relevance. Bone sculpturing has seen the most impressive advances in recent times through three newly developed treatment options: (a) the application of distraction osteogenesis in the craniofacial skeleton (introduced in craniofacial surgery by Joseph McCarthy), (b) the virtual planning and execution of orthognathic surgery procedures by patient-specific implants (PSI) and (c) the individual CAD/CAM-based planning, manufacturing and positioning of bone substitute materials on the skull. Whereas distraction osteogenesis has reached a level of clinical routine, individual bone sculpturing is in the beginning. My own experience with these therapeutic options and the possibility of the combined use of such strategies in one operation have shown to alter craniofacial malformation treatment profoundly.

I decided to present the demanding and complex aspects of craniofacial malformations by a structural homogeneity of *Fundamentals of Craniofacial Malformations* through an interdentation of chapters, written by specialists in their field. I have conceptualised the three book series according to a methodological approach (book one describes the genetics and biology of craniofacial malformations, their psychological and social impact as well as the diagnostic procedures; book two presents the complex treatment principles; book three is a surgical atlas, describing all relevant operative procedures in craniofacial malformation surgery). The various entities of craniofacial malformations (gene and chromosome alterations, craniosynostoses, clefts, branchial arch diseases, postural head deformations, dysgnathias, soft tissue malformations) are the main formal and structural guide within the book series. The series is therefore divided into the different aspects of these fields on a level that provides extended information for the specialists, but also is a usable access for non-specialists. The written description of this book is added by numerous tables, schematic illustrations and photos in order to give a better understanding of the information provided in this book. In order to be able to edit such a work I would like to thank my colleagues at the Cranio-Maxillofacial University Clinic of Münster, at the University Clinic for Maxillofacial and Plastic Surgery in Düsseldorf (Westdeutsche Kieferklinik), the Center for Jaw-, Face-, and Skull Malformations (Kieferklinik Münster) in Münster as well as the colleagues of the craniofacial team (Neurosurgery: Prof. Dr. U. Schick, Dr. B. Hoffmann; Children's Hospital: Dr. G. Hülskamp, PD Dr. O. Debus; Anesthesiology and Intensive Care Unit: Dr. N. Mertens) at the Clemenshospital Münster for their superior quality of work, advice and enthusiasm in research and clinical work on this issue. Their stimulating environment and a multitude of close collaborations and friendships were the basis of this book.

My time at the university clinic in Münster with the internationally recognised expertise of Professor Dr. Dr. Dr. hc. Ulrich Joos in surgical concepts of craniofacially malformed patients (especially cleft and craniosynostoses patients) and my experience of superior bone research (BMP) and microvascular surgery by Professor Dr. Dr. Norbert Kübler during my clinical work at the *Westdeutsche Kieferklinik* were the fundament to start this book project.

Of personal and special relevance is to give credit to the *Westdeutsche Kieferklink* (Fig. 1). The historically based experience of the *Westdeutsche Kieferklinik* with facially disfigured patients played a decisive role in my gratuity to craniofacially disfigured children. The *Westdeutsche Kieferklinik* had a special role in two aspects: it was the first clinic in inauguration of the German-based development of the double degree of dentistry and medicine (Professor Lindemann, PhD in 1926; first full Professorship in Germany in 1935) as a prerequisite for the specialisation for Craniofacial Surgery (*Mund-; Kiefer- und plastische Gesichtschirurgie)*. Second, it was the serving clinic for all facially injured soldiers at the west front during the First and Second World War. At the peak point, the clinic maintained at the end of the First World War 632 beds (spread over different hospital locations in Düsseldorf)

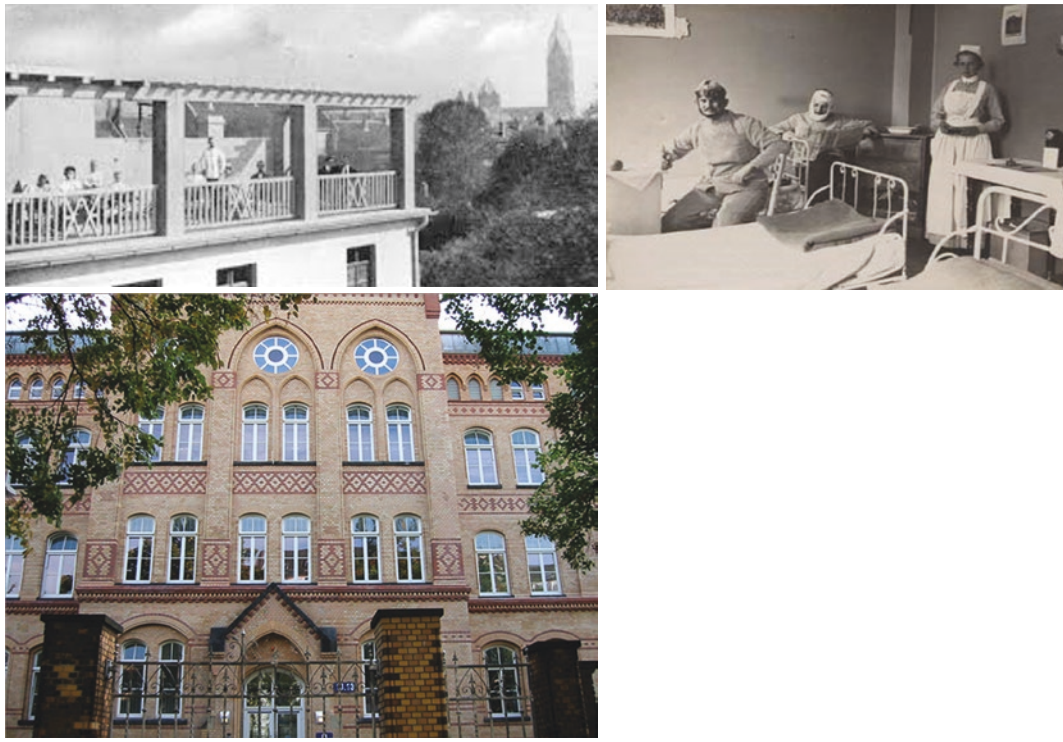

Fig. 1 The *Westdeutsche Kieferklinik* in Düsseldorf (bottom) was the germ cell for the German-based double degree development in craniofacial and facial plastic surgery (Mund-, Kiefer- und Plastische Gesichtschirurgie), developed from the necessity to interdisciplinarily treat the high number of facially injured patients (top right and left) of the west front during and after the First and Second World War. Source: Ulrich Meyer/Westdeutsche Kieferklinik

and was perhaps the largest institution in this field worldwide, ever; surpassing all other hospitals in the clinical challenge and expertise to restore facially disfigured patients. Therefore, such disfigured patients were and still are prominent themes and social issues in Düsseldorf.

I would like to thank the patients who have participated in the development of craniofacial surgery by carrying such treatments. The co-operation with the German parents initiative Apert group and their experience and recommendations improved the view on the situation of craniofacially diseased persons. As the recent detailed knowledge in craniofacial medicine far exceeds the content of a book, I have tried to find for the reader a compromise between a comprehensive and detailed description of all aspects of this field.

Fundamentals of Craniofacial Malformations is intended not only as a text for students but especially as a reference for research and clinical work. The content of the book covers therefore the most recent scientific, clinical and surgical aspects in the handling and treatment of patients having craniofacial malformations. A special aim of this book was to define the current state of craniofacial malformation medicine approaches which are applied in the various clinical medical specialities.

The expertise required to generate this book far exceeded that of its editor. No single expert, to date, is able to have detailed insight into all aspects of this fast growing and complex field. The content of the book represents the combined intellect and experience of more than one hundred researchers and clinicians, all of them leading specialists in their field. Their fundamental work has not only set the basis for the tremendous advances in this challenging field but has also given patients new and fascinating treatment options in clinical medicine. Finally, I believe that, especially today, it is also important to understand and reflect the current limitations of the field.

I hope this book will add further stimulus for all researchers and clinicians who are involved in investigating and treating patients with craniofacial malformations and will contribute to make patient outcomes better. It is important and my wish that this book series will not only give biologists, physicians and surgeons a deeper understanding of craniofacial malformations but may also help concerned patients on a long run to live their lives with less limitations.

Fundamentals of Craniofacial Malformations is about

- changing the face of the society towards disfigured persons
- changing the face of all beholders (who interact with such persons)
- changing the face of concerned person through medical and surgical treatments possible

Whereas the first two points seem to be of utmost importance, the third aspect represents the main concentration of this book series.

Nordrhein-Westfalen, Germany Ulrich Meyer

Acknowledgements

I would like to thank all the authors for their timely contribution of an overview of the various aspects of malformation biology, psychology and diagnostics. A special acknowledgement is given to the internationally leading research group of Dr. Chengji Zhou. His extraordinary contribution to the biology and genetics of orofacial clefts, as the most common craniofacial malformation, presents the recent knowledge and understanding of this complex and challenging disease.

Contents

About the Editor

Ulrich Meyer, DMD, MD, MSc, PhD started to work in the field of craniofacial surgery in the 1990s, at the Department of Craniofacial and Maxillofacial Surgery, University of Münster, Germany. He completed his specialization in oral and maxillofacial surgery in 1998 and was appointed senior physician at the Department of Oral and Maxillofacial Surgery, University of Münster in 2000, being promoted to chief senior physician in 2002. He performed pioneering research work in craniofacial distraction osteogenesis and tissue engineering. In 2004, he founded the interdisciplinary open-access journal Head and Face Medicine. In 2005, he was appointed university professor for life (Professorship for Plastic Facial and Craniofacial Surgery) and senior consultant and deputy clinic director at the West German Maxillary Clinic (Westdeutsche Kieferklinik), Department of Maxillofacial and Plastic Surgery, Heinrich Heine University, Düsseldorf. Based on his clinical and research expertise, he edited in 2008 the biomedical standard work *Fundamentals of Tissue Engineering and Regenerative Medicine*. In the course of his surgical work in Münster and Düsseldorf, Dr. Meyer developed a broad spectrum of surgical techniques. More recently, he founded the interdisciplinary Craniofacial Center (Kieferklinik Münster) in Münster, where his main focus is the treatment of patients suffering from craniofacial deformities or diseases, in very close cooperation with geneticists, biologists, pediatricians, neurosurgeons, ENT specialists, orthodontists, and others.

Part I

General Aspects

The Challenge of Craniofacial Malformation Medicine in Perspective

1

Ulrich Meyer

It is all about changing faces ….
….. in form and function.

Craniofacial malformations not only affect patients but also have a major impact on relatives, the medical doctors involved in the treatment of patients, individuals interacting with such persons, as well as the societal assessment. In order to understand craniofacial malformations in all aspects, it seems to be important to have an insight into the experience of concerned patients. Additionally, it is important to review the past, in order to understand the present and speculate on the future. These contemplation and perspective on craniofacial malformations may therefore be based and elaborated on an impressive essay of a patient with Crouzon syndrome (Ariel Henley, New York Times; 3. Nov. 2016):

> *I have never seen someone who looked like me on a mainstream television show. I have never seen someone who looked like me, playing anything but a villain in movies, or in an ad or on a billboard. I am invisible. That is, until I walk down the street. That is, until strangers stare just a little too long and rudely whisper, "look at her eyes."*

1.1 Craniofacial Malformations in Arts and Media

Facial disfigurement is seldom seen in arts and movies. Whereas people with facial disfigurement play some special roles in modern films, disfigured persons are even nowadays seldom displayed in arts. There has been scant representation of facial disfigurement in portraiture through the ages. Reasons include the frequent emphasis in art on beauty, symmetry and proportion, the need for the artist to sell his or her work, and the widespread tendency in commissioned work for the artist to flatter the sitter to indicate wealth, status, and power. Sitters with unusual faces often required artists to conceal or disguise any facial differences. Religion played an additional role in the portrayal of people with disfigurements. Some societies believed that evil and deviation were indelibly marked upon the face, a stereotype that continues sometimes today. Modern medicine can provide retrospective diagnosis of disfiguring ailments, requiring re-interpretation of portraits depicting those formerly labelled "freaks" (Figs. 1.1, 1.2, and 1.3).

Later on, as science and medicine progressed, a multitude of stories, paintings, and films dealt with the situation of malformed humans. Most movies are influenced by the social, cultural, and scientific background of individual persons at their time and reflect to a large extent the societal view on disfigured per-

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Fig. 1.1 William Roos (1808–1878). Portray of a blind person. By William Roos - National Museum Wales, Public Domain, https://commons.wikimedia.org/w/index.php?curid=11409877

Fig. 1.2 Quinten Massys (1466–1530). Portray of a person appearance, indicative for Paget's disease. https://commons.wikimedia.org/wiki/File:Quentin_Matsys_-_A_Grotesque_old_woman.jpg#/media/Datei:Quentin_Matsys_-_A_Grotesque_old_woman.jpg

Fig. 1.3 Portray of Emperor Karl V of Habsburg (family-based Habsburger long chin syndrome (mandibular prognathism), inbred by the common situation of marriages between relatives). Titian, Charles V, Holy Roman Emperor, Alte Pinakothek, München, KatalogNr. 632

sons. Some well-known examples in contemporary literature and movies present the theme of disfigured persons. The figure of Quasimodo (Fig. 1.4) in the novel from Victor Hugo [1] (1482) as well as Frankenstein [2] (written by Mary Shelley in 1818 (Fig. 1.5)) mirrors the desire to reflect on the situation of disfigured persons. In the twentieth century, medicine and art merged in the figure of Henry Tonks [3]. His drawings and pastels of soldiers with severe facial injuries in the First World War were concealed from public view as they were deemed bad for morale. Until very recently they had been quietly excluded from the history of that conflict but were displayed in an exhibition at the Royal College of Surgeons in 2014. In con-

Fig. 1.4 Quasimodo (painting by Antoine Wiertz). Von Antoine Joseph Wiertz - Quasimodo.jpg: photo by user:Szilas in the Wiertz Museum, Gemeinfrei, https://commons.wikimedia.org/w/index.php?curid=17816537

Fig. 1.5 Frankenstein. Von Theodor von Holst - https://archive.org/details/ghostseer01schiuoft, Gemeinfrei, https://commons.wikimedia.org/w/index.php?curid=1490898

Fig. 1.6 Rock group Maniac Street Preachers with the cover of their album, displaying a person with a facial port wine stain. The Quietus, John Doran, 30th April 2009

temporary arts, facial abstraction, destruction, and reassembly play a more complex role in the contemplation of facial beauty and disfigurement.

Even nowadays is the depiction of a disfigured face in the public a complex and controversial issue. In 2009, artist Jenny Saville portrayed a child with a facial port wine stain on a Manic Street Preachers album cover (Fig. 1.6). Fearful of negative public reaction, four major supermarket chains concealed the "offending" image. Since then, a debate about art, aesthetics, and disfigurement and three portraits of people living with facial difference were commissioned. One of these, Alastair Adams' powerful portrait of Marc Crank, is held by Girton College, University of Cambridge (Fig. 1.7) which is an offensive way to bring disfigurement to the public:

> *As a child, I was often asked why my eyes were shaped the way they were, so crooked and far apart.*
> *"I don't know," I would shrug. "I just came that way!" Sometimes this question bothered me, because I didn't understand why everyone felt the need to ask it. Most of the time, I just didn't know how to respond.*

Fig. 1.7 Alastair Christian Adams. Portray of a person with right-sided facial disfigurement. Alastair Christian Adams, https://wikioo.org/museumbycity.php?id=Girton+College+%28Cambridge%2C+United+Kingdom%29

1.2 Craniofacial Malformation and Societal Assessment

Despite indications that the possession of an aesthetically unattractive appearance may impair the social functioning of many, there has been little recognition from either society or psychologists of the problems encountered by those whose deviations from society's norms are primarily in terms of appearance, and not necessarily associated with a loss of body functioning. Different authors stated that of all the concerns within the field of physical disability and rehabilitation, for her the greatest was the large number of people with facial deviations who seem to be classified as "marginal" or "forgotten" people [4]. Yet the profound social significance of the face, taken together with society's prejudices toward those who have an atypical appearance, is indicative that an unattractive facial appearance could be a severe social handicap.

I was born with a craniofacial disease—Crouzon syndrome, a condition where the bones in the head do not grow. A condition that required too many surgeries and procedures to count, so I grew accustomed to being cut open, pulled apart, and put back together. Though I quickly learned that once something is taken apart, it's never quite the same."
To make matters more challenging, I never had anyone who had been through the same experiences to turn to for advice and support. I would search the internet for others like me, trying to find personal stories and tips and advice for how to get through it, but was never able to find anything. I felt alone. To get through it, I told myself I would grow up to be the stereotypical definition of beautiful. With each surgery I had, I assured myself I was getting one step closer to being able to walk down the street in peace. People would no longer stare at me in confusion and disgust, wondering why I looked the way I did. Instead, they would admire my beauty. I would finally be happy. Nobody told me happiness was an inside job.

1.3 The Concept of Beauty

The surgical creation of beauty faces by men is a matter of myth and dream throughout the history of medicine. The nature of beauty is one of the most enduring and controversial themes in Western philosophy and is—with the nature of art—one of the two fundamental issues in philosophical aesthetics.

Beauty has traditionally been counted among the ultimate values, with goodness, truth, and justice (Fig. 1.8). It is a primary theme among ancient Greek, Hellenistic, and medieval philosophers and was central to eighteenth- and nineteenth-century thought, as represented in treatments by a lot of philosophers as Shaftesbury, Hume, Kant, Schiller, Hegel, Schopenhauer, and Santayana. Perhaps the most familiar basic issue in the theory of beauty is whether beauty is subjective—located "in the eye of the beholder"—or whether it is an objective feature of beautiful things. A pure version of either of these positions seems implausible, and many attempts have been made to split the difference or incorporate insights of both subjectivist and objectivist accounts. Ancient and medieval accounts for the most part located beauty outside of anyone's particular experiences. Leonardo da Vinci was

Fig. 1.8 Nofretete, a symbol for facial beauty (With permission from The Nefertiti Bust; 1352–1332 BC; painted limestone; height: 50 cm; Neues Museum (Berlin, Germany))

one of the first artists and scientists, who did not only looked at the outside of the body. He investigated the inner structure. He was interested not only in the appearance but also in function (Fig. 1.9). Nevertheless, that beauty is subjective was also a common opinion from the time of the sophists. By the eighteenth century, Hume could write as follows, expressing one "species of philosophy":

Beauty is no quality in things themselves: It exists merely in the mind which contemplates them; and each mind perceives a different beauty.

One person may even perceive deformity, where another is sensible of beauty; and every individual ought to acquiesce in his own sentiment, without pretending to regulate those of others [5] (Hume 1757).

Beauty is easier to recognize than to define. It is widely agreed that beauty is an evolutionary adoption for ensuring the survival of the species, because physical attractiveness is rated as more vital in mate selection. Some argue that beauty is a myth and not reality and that the perception is learned and not developmental, and yet others argue that the perception of beauty is an innate developmental or biological ability. It includes a combination of qualities, starting from the grace of form to the charm of the colors that delight the sight and other senses. It does not have any norms; however individual or societal assessment of attractiveness is greatly influenced by cultural standards. Despite this centuries-old debate, that there does not appear to be a validated, widely used set of evidence-based rules or measurements that can influence clinical practice [6, 7] beatuy assessment seems to be concordant in individuals and the society towards distinct facial appearances. Understanding quantitative and objective features that constitute facial beauty or deformity is complex and confounded by multiple elements including society, culture, age, and ethnicity [8]. "Beauty is but skin deep, ugly to the bone. And when beauty fades away, ugly claims its own" is a sentence that still remains true in the eyes of a beholder.

> *The first time someone told me I was ugly, I was in the seventh grade. I didn't even realize I was "different" until I reached middle school. I always just assumed I was normal—I felt normal, but I quickly learned I did not look it.*
> *"Does it hurt to be disfigured?" Bullies would sometimes ask me.*
> *"Does it hurt to be an idiot?" I would sometimes respond.*
> *If you Google the word "disfigured," you get a definition "spoil the attractiveness of," derived from the Latin word "fingere," meaning "to shape." Many consider the word to be offensive; others do not. I was born with a facial disfigurement, and not only do I not find the term offensive, but I also do not believe myself to be unattractive.*

1.4 Craniofacial Malformations and Self-Assessment

The head is the most complex structure of the body. The skull, including the bones that enclose and protect the brain and sensory organs, also acts as a scaffold for the face to support the functions of feeding, breathing, and non-verbal communication in combination with connective tissue, musculature, vasculature, and associated innervation.

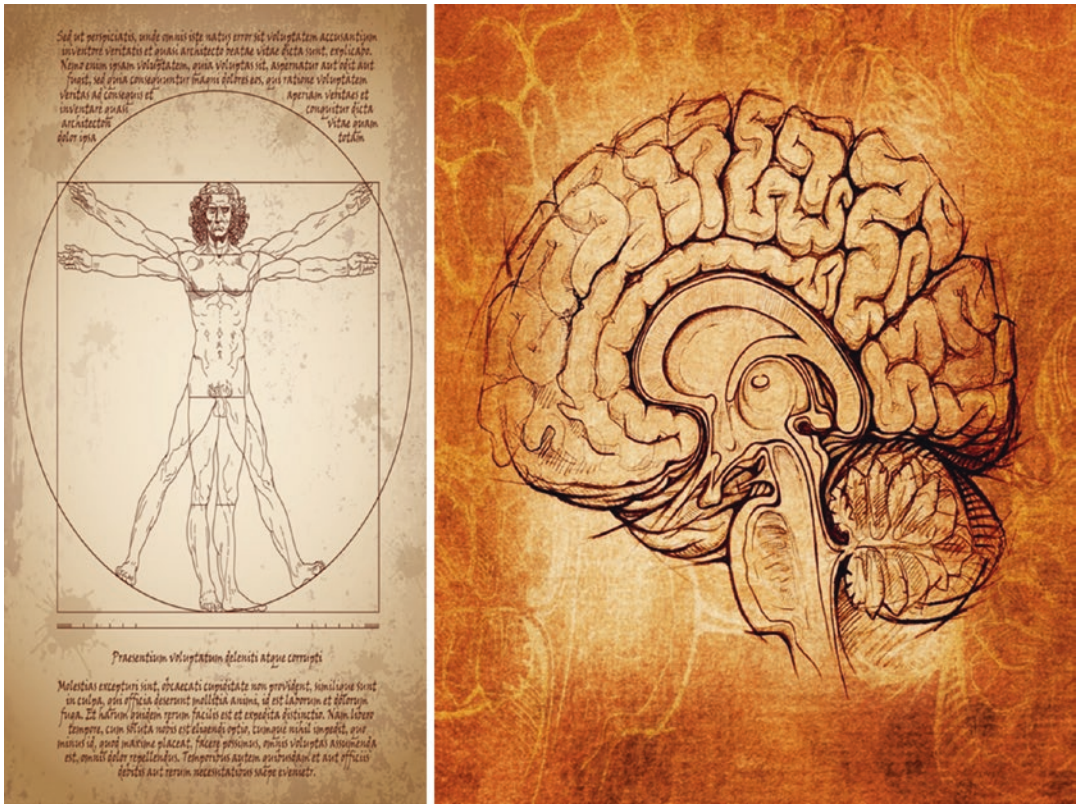

Fig. 1.9 Leonardo Da Vinci's drawings of the outer and inner structure and anatomy of humans. Luc Viatour / https://Lucnix.be

The face serves a dual role as both a biological organ and an organ of identity. Facial expressions are mainly used to display emotions to others in social interactions. The facial feedback, or information conveyed by facial expressions, is an essential factor used by individuals to infer others' personalities and intents from their faces.

Facial disfigurement significantly affects personal identity and access to social roles. "Self-concept" is an idea of the self that is constructed based on how one thinks about, evaluates, or perceives oneself as well as on the responses of others to the self [9]. As important as its physiological functions is the key role of the face in identity. Self-concept revolves around the face, as it is the primary means by which humans recognize and interact with each other and the primary mode of self-expression, emotional expression, and social interaction [10–14]. The intimate relationship between self-concept and appearance is also well documented, and the face is a major component of body image and self-worth. It affects how one is perceived and evaluated by others, guiding their impressions and behavior. Perhaps more so than in the general population, in people with facial disfigurement, appearance and self-concept are closely intertwined. Especially congenital facial disfigurement has profound psychosocial implications, including altered body image, reduced quality of life, and poor self-esteem [15, 16]. The most frequently reported difficulties relate to negative self-perception and impaired social interaction. As this negative self-perception in patients with congenital malformations starts early, it is deeply embedded in the socialization process of the concerned individual:

> *Because salvaging my physical health was so crucial, the emotional aspect of living with a facial disfigurement was overlooked by health professionals. While my mother and father did their best to offer support, there was only so much they could do.*

1.5 Parental Involvement

Parents will have expectations and hopes for their child even before it is born, with many prospective parents feeling slightly nervous about whether the child will be healthy. At the time of the birth, the two most common questions are about the sex (boy or girl) and the physical health and the absence of malformations. The reaction to having produced a child who is in some way disfigured begins at this point [17–19]. Researchers have conceptualized the pattern of the reactions that follow. They stated that parents of an abnormal child initially experience shock, a feeling of disbelief, and a desire to be left alone while coming to terms with the situation. These feelings are followed by a mourning reaction—a kind of grief for the perfect child the parents had hoped for. It was shown that the birth of a congenitally disfigured child is a shock to the family system. Anger and despair experienced by parents take the form of a bereavement reaction. Most parents experience a variety of emotions, including "grief, anxiety, confusion, depression, disappointment, disbelief, frustration, guilt, hurt, inadequacy, rejection, resentment, shock, stigmatization, and withdrawal." One problem of this initial reaction is that the initial repugnance experienced by parents following the birth of a child with a congenital defect is followed by overprotectiveness, with the child becoming reliant on a sheltered existence. It is obvious that parents initially worry about the survival of their child, later experiencing anxieties about the child's speech, dentition, and social development. Some parents do not only show less pride in their child but typically have many fears concerning the care and the costs of the care of their children.

> *I tried therapy, but therapists always seemed to ask the wrong questions and never seemed to understand what it was like to have my physical appearance change drastically time and time again. "It's like in 'Freaky Friday,'" I would tell them. "Except I never get my body back. I never get my face back." Despite their best efforts, they simply could not relate."*

1.6 Patient-Physician Communication

Patient-physician communication is difficult in multiple aspects. The early interaction is taken, when the child is newborn and there is no chance of verbal communication. Parents serve during this period and for a long time as a decider and communicator with physicians. Later on, as verbal communicative skills improve in children, it is difficult for both parties to find a level of emphatic and understandable interactions. Additionally, the surgeon as one mainstay in therapy has often a more technical approach to patients. As patients grow up and reach the stage of puberty, communication and patient-physician interactions become even more complicated. This complex situation seems to be the basis that patient often thinks that therapists cannot relate.

> *I did a lot of growing up during my time at the local children's hospital, where the bones of my head and face were routinely broken and restructured, rectifying the premature fusion of my skull. After surgeries, during my extended stays in the intensive care unit, I would tell myself that pain was not real. That it was imaginary and only in my mind. If I concentrated hard enough on the throbbing pain throughout my body, I could convince myself, if only for a moment, that I was numb. I would lie there, unable to sit up, my eyes swollen shut. Nurses and visitors bestowed words of comfort upon me, trying to ease my terror, but over time, I grew used to it. What was once horrific became normal.*

1.7 History of Facial Plastic, Reconstructive, and Cleft Surgery

Facial plastic and reconstructive surgery is the term that is used to describe the approach to generate functional and aesthetic results for concerned patients [20]. Facial disfigurement, as present in patients with craniofacial malformation, is of special concern for patients. While surgical correction of certain facial defects like cleft lip is often successful, reconstruction of severe

facial defects like syndromal craniosynostosis, branchial arch diseases, or complex facial clefts remains a challenge, as both functional and aesthetic deficits must be addressed to recreate the "normal" face. The extent of the anatomical disfigurement is dependent on the underlying disease (Fig. 1.10). Syndromal craniosynostoses, branchial arch diseases, and complex facial clefts have the most complex deviation from normal anatomy, whereas postural head deformations, non-syndromic craniosynostoses, and dysgnathias have a less complexity.

Functional deficits—particularly impaired verbal and emotional communication—are directly associated with these malformations. These functional deficits often affect mental well-being more negatively than the aesthetic impairments. In cases of extensive soft tissue or composite soft tissue and skeletal defects, conventional reconstruction remains largely unable to restore both facial and aesthetic functions, and patients are often left with life-long handicaps. Treatment of craniofacial malformations by physicians and surgeons is therefore a challenging field in medicine. It is even now to a large extent an unsolved clinical challenge, especially when severe disfigurement is present. The history of the surgery of craniofacial malformations can be exemplified and divided in the history of cleft repair (mostly a soft tissue facial plastic surgery) and repair of craniosynostoses (mostly a bone-based surgery including the skull; craniofacial surgery).

The earliest documented history of cleft lip is based on a combination of religion, superstition, invention, and charlatanism. While Greeks ignored their existence, Spartans and Romans would kill these children as they were considered to harbor evil spirits, since in the ancient times man was ignorant of embryology and morphogenesis. In ancient times, many congenital deformities, including the cleft lip and palate, were considered to be evidence of the presence of an evil spirit in the affected child. Facial deformities were most condemned, and the infants were "removed from the tribe or cultural unit and left to die in the surrounding wilderness," a practice that still prevails today in certain African tribes.

The first documented cleft lip surgery is from China in 390 BC. It took centuries, before a modern and scientific approach to reconstructive facial and cleft surgery started. Leading areas of reconstructive facial and cleft medicine in clinical use were then dentistry and orthopedics. Ambroise Paré (1510–1590) described in his work *Dix livres de la chirurgie* (Pare, 1575) [21] measures to reconstruct teeth, noses, and other parts of the body. A common method in the eigh-

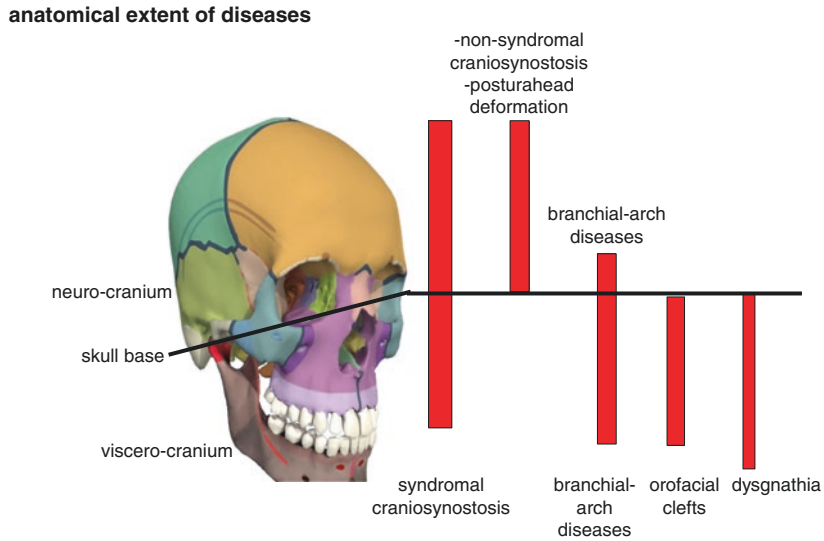

Fig. 1.10 Anatomical extent and severity of the various craniofacial malformations

teenth century to replace teeth was the homologous transplantation of teeth in humans [22]. Enlightenment into the biological and surgical mechanisms that accounted for the fate of surgical strategies was given by the fundamental biological work of Rudolf Virchow [23] (1821–1902, Fig. 1.11).

The knowledge of cleft lip and the surgical correction received a big boost during the period between the Renaissance and the nineteenth century with the publication of Pierre Franco's Petit Traité and Traité des Hernies in which he described the condition as "lievré fendu de nativité" (cleft lip present from birth). Germanicus Mirault [24] can be credited to be the originator of the triangular flap which was later modified by C.W. Tennison [25] in 1952 and Peter Randall [26] in 1959. In the late 1950s, Ralph Millard [27] gave further improvements. Since then a multitude of refinements were elaborated over time.

Fig. 1.11 Portray of Rudolf Virchow. http://ihm.nlm.nih.gov/images/B29494

1.8 The Evolution of Craniofacial Surgery

Except Hippocrates' first historical description of craniosynostosis in 100 BC, Sömmerring and Otto [28, 29] initially described in 1800 and in 1830 that premature cranial sutural fusion would result in deformity, and the etiology was thought to be based on either fetal or birth trauma. In 1851, Virchow [23] first introduced the term of "craniosynostosis" and formulated what is today known as Virchow's law: there is a cessation of growth that occurs in the direction perpendicular to that of the affected suture, while growth proceeds in a parallel direction. On the other hand, similar groups of the craniosynostosis had been reported and classified in terms of each characteristic problem over the years. In 1906, although brachycephalic craniosynostosis with syndactyly had already been reported toward the end of the nineteenth century, the French pediatrician Apert [30] is generally credited with describing the condition. In 1912, Crouzon [31], a neurologist, reported the condition that is named after him. Based on the concept, the abnormal calvarial growth due to a premature fusion of the sutures provided the basis for early operative treatment of craniosynostosis, with removing the offending suture in an attempt to release the constricted brain. In 1890, Lannelongue in Paris described bilateral strip craniectomies for the treatment of craniosynostosis, and Lane followed the intervention 2 years later in the USA [32, 33], but these surgeries had alarming outcomes with high morbidity and mortality. Faber and Towne reported in 1927 their success of a more extensive craniotomy [34]. Since then the development of anesthetic and blood management over the years has provided the opportunity for more difficult and advanced craniosynostosis surgery. As multidisciplinary teams developed all over the world, clinical geneticists became involved and studied inheritance patterns and syndromic features. The modern era of craniofacial surgery started in the 1960s with Tessier [35, 36] (Fig. 1.12), who

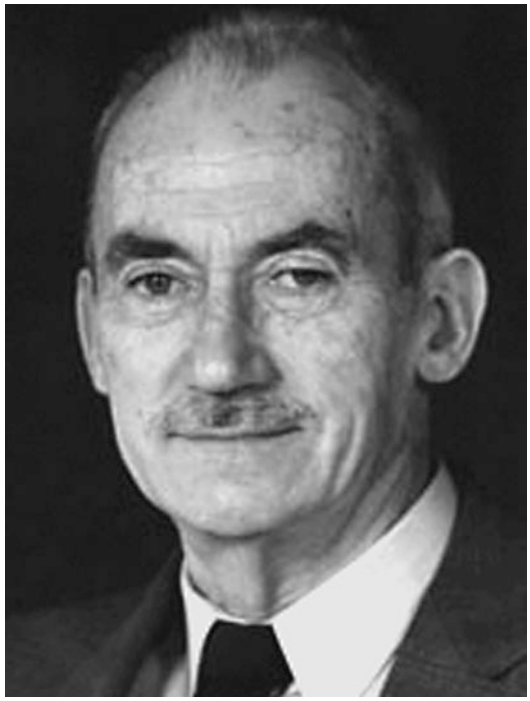

Fig. 1.12 Paul Tessier, the pioneer in craniofacial skull surgery. http://facethechallenge.org

first established a multidisciplinary craniofacial teams in Paris [37]. In 1967, he showed a procedure of fronto-orbital advancement with cranial vault remodeling, with reshaped removal bone pieces stabilizing back to the cranium.

Parallel to the surgical advancements, geneticists improved the understanding of the biological basis of craniofacial malformations. In 1993 the first genetic lesion, a specific missense mutation in the MSX2 gene, was identified by Melville et al. [38] in a large family with autosomal dominant craniosynostosis, known as Boston type. This discovery launched molecular diagnostics by identifying a key gene in calvarial development. And in the late 1990s, some craniofacial anomalies, like Crouzon or Pfeiffer syndromes, have been elucidated to be caused by a mutation of FGFR gene, but its phenotype does not correspond to one location by one, so that prenatal gene analysis may not lead a definite diagnosis. Furthermore, other responsible genes have been found out such as Saethre-Chotzen syndrome by TWIST.

1.9 Future Directions in Craniofacial Surgery and Medicine

Reconstruction of the craniofacial skeleton is extremely challenging even to the most experienced surgeon. Some of the critical factors that contribute to the complexity include the 3D anatomy, presence of vital structures adjacent to the affected part, uniqueness of each defect, and chances of infection. In any craniofacial reconstruction, restoration of aesthetics and function is the primary goal and calls for precise pre-surgical planning and execution of the plan.

Surgeons have adapted to this challenge by incorporation of enhanced visualization techniques. Such systems specifically focus on enhanced visualization tools—3D modeling or better termed as virtual reality gives the surgeon the ability for precise preoperative planning and to perform virtual osteotomies, define bone movements, and design patient specific implants preoperatively (Fig. 1.13). Advantages of virtual reality can be totally beneficial only when transferred to the clinical scenario, i.e., the operatory to achieve expected results. These virtual models can be imported into an intraoperative navigation system for precise placement of bone segments and artificial bone substitutes. Advances in manufacturing technology and material science have led to the possibility of turning such virtual model or design into reality as physical replica models, surgical guides, or cutting jigs or splints for intraoperative use and patient-specific implants. Development of computer-assisted design (CAD) and computer-assisted manufacturing (CAM) systems that adapt to the surgeons' needs has resulted in a gamut of the armamentarium for computer-assisted surgery.

Custom implants for the reconstruction of craniofacial defects have gained importance due to better performance over their generic counterparts. This is due to the precise adaptation to the region of implantation, reduced surgical times, and better cosmesis. Application of 3D modeling in craniofacial surgery is changing the way surgeons are planning surgeries and graphic designers are designing custom implants. Advances in

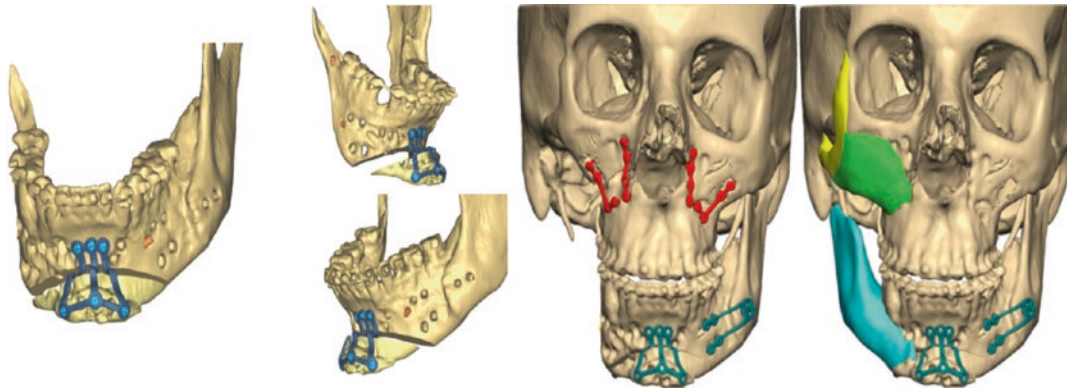

Fig. 1.13 Modern approach to computer-aided surgery. Fist, the chin plasty is simulated, and a patient-specific drill guide and osteosynthesis plates are fabricated. Based on the new dataset, CAD/CAM-based bone augmentation implants are planned (by mirroring the vertical facial axis). The facial reconstruction surgery is then performed with high precision in one operation (orthognathic surgery and bone augmentation)

manufacturing processes and ushering of additive manufacturing for direct production of implants have eliminated the constraints of shape, size and internal structure, and mechanical properties making it possible for the fabrication of implants that conform to the physical and mechanical requirements of the region of implantation.

Future improvements in craniofacial medicine can be based on advances in all medical specialties. Not only surgical proceedings but also innovative aspects of disease diagnostics and conservative treatments will help patient outcome. In the near future, medical genetics may play a prominent role. Studies of responsible gene location or clone hopefully reveal each minute pathology and prognosis and can be a foundation and application of gene therapy.

> *Even with the physically traumatic surgeries I was required to undergo, the physical aspect of my condition was nothing compared with the emotional toll of living with an appearance-altering condition. The everyday stares, comments, and subhuman treatment acted as a constant reminder of my painful medical history and my perceived shortcomings."*
> *"The Americans with Disabilities Act classifies facial disfigurement as a form of disability, recognizing the fact that individuals with facial disfigurements encounter discrimination and prejudice because of their appearance. I face discrimination and prejudice when I apply for jobs, when I'm on a date, when I walk down the street. The judgment is everywhere. Still, I refuse to live my life in seclusion, because other individuals are uncomfortable with my existence.*
> *People with Crouzon syndrome and other conditions that result in facial disfigurements are not represented in mainstream media. How can individuals with disfigurements and physical differences be expected to accept ourselves and love our differences, when we aren't even worthy of mainstream inclusion? Not only that, but there are people who would be angered if such inclusion were to exist. How are individuals with facial disfigurements supposed to be seen as equal when we still face discrimination in every area of our lives every single day?"*

1.10 The Societal Answer Toward Facially Disfigured Persons

To improve the situation of facially disfigured persons, the organization Changing Faces was founded in the UK to give respect to those, who are different. The organization worked with a wide range of health- and social care professionals for now more than 20 years, encouraging the integration of psychosocial rehabilitation into hospital and clinic settings for people who have disfigurements and their families. It is estimated that every year, over 540,000 people in the UK acquire a disfiguring condition to their face, hands, or body—from birth or other conditions.

Changing Faces advocates the development of health care that comprehensively and rou-

tinely addresses patients' physical and psychosocial needs as part of the patient care pathway. They are initiating campaigns for giving people with facial disfigurement a platform, in order to provide better and more information, increase awareness among health- and social care professionals, and improve both access to and quality of psycho-social therapies. They also influence and support the work of clinicians and professionals in the many specialties that care for people who have disfiguring conditions as well as those in primary care. An increasing number of health and social care professionals are now seeking to embed psychosocial interventions into their care as a crucial part of best practice as an essential component to successful rehabilitation. It is assumed that adequate psychosocial care not only decreases the distress experienced by patients; it also lowers costs in the longer term and helps to reduce health inequalities.

1.11 The Core

To fulfill the complex expectations of improving the situation of craniofacially malformed patients, it is therefore clear that a high level of challenges concerning scientific, technological, clinical, ethical, and also social issues needs to be met. Basic research and clinical treatments still require the evaluation and elaboration of fundamental medical and surgical processes and procedures in multiple fields but also address the individual and social aspect of the diseases.

> *Through this and other methods, I am working toward creating a world where those who are not disfigured recognize those who are as equals, where a person with Crouzon syndrome is given a role in a popular television show or is hired to appear in an ad or fashion campaign. In that world, I'd be able to go about my business or show up to a job interview without having my entire being called into question by strangers, and others with facial disfigurements would be truly viewed as a valuable addition to society. Because we are.*

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2 Prenatal Diagnosis of Fetal Cranial Anomalies

James D. Vargo, Ayesha Hasan, and Brian T. Andrews

2.1 Introduction

Congenital cranial anomalies are a rare group of heterogeneous malformations with many etiologies. With enhanced emphasis on prenatal screening and advances in radiographic imaging technology, early in utero diagnosis of cranial anomalies has become routine at many medical centers. Prenatal identification provides the expecting parents, members of the obstetric team, and other healthcare providers with important details that can help optimize care and medical decision-making. Ideally, once a cranial anomaly is identified in utero, the perinatologist (maternal-fetal medicine specialists) becomes the "clinical gatekeepers" for both mother and the developing infant [1]. Typically, a multi-disciplinary team approach is necessary to manage such conditions in utero through delivery and into the neonatal period. Surgical procedures are often required during infancy, and occasionally secondary procedures are necessary during adolescence and adulthood.

2.2 Embryology and Cranial Development

Cranial development begins during the third to fourth week of gestation. During this time, seven bones develop independently to form the cranial vault, ultimately providing protection to the brain and foundational support for the face (Fig. 2.1). The cranium is a complex structure that develops from both intramembranous (frontal, parietal) and endochondral (occipital, sphenoid, and ethmoid) ossification [2]. Between each individual cranial bone lies a cranial suture, composed of a fibrous joint or synarthrosis. The "major" cranial sutures include paired coronal, paired lambdoid, and unilateral, midline sagittal and metopic sutures. The junction of cranial sutures forms fontanelles or 'soft spots' commonly felt on the infant skull. In addition, there are several "minor" cranial sutures including bilateral squamosal, bilateral frontosphenoid sutures, zygomaticotemporal, and the occasional mendosal suture variant.

During early neonatal cranial development, the majority of cranial bone growth occurs at the cranial sutures. Cranial bone growth at the sutures

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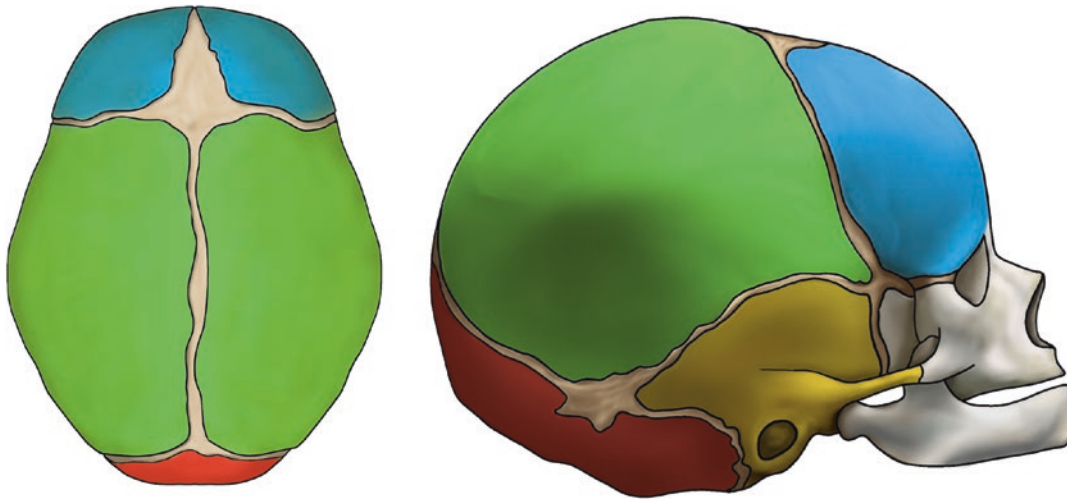

Fig. 2.1 Fetal cranial sutures. Blu, frontal bones; green, parietal bones; yellow, temporal bones; red, occipital Bone. ©James D. Vargo MD

results from brain growth and development which is rapid in the neonatal period. As the maturing brain develops, it creates stress on the cranial bones and sutures initiating a signaling cascade which results in new bone deposition. The majority of cranial bone growth is completed in the first few years of life (85% by age 3). All but the metopic cranial suture (fuses in first year of life) will remain patent or "open" into late adolescence or early adulthood.

2.3 Cranial Anomalies

2.3.1 Craniosynostosis

Craniosynostosis is a congenital anomaly of the skull causing premature fusion of one or more cranial sutures (Fig. 2.2). This pathologic process manifests in utero and can result in both detrimental neurocognitive effects and head shape deformities. It is identified in 1 in 1700–2500 live births and can affect either single or multiple cranial sutures [3–5]. Craniosynostosis exists mostly as an isolated non-syndromic entity but can also be involved in over 100 genetic syndromes.

Multiple theories exist as to the etiology of craniosynostosis, likely suggesting a complex interaction between many factors. The *Intrinsic*

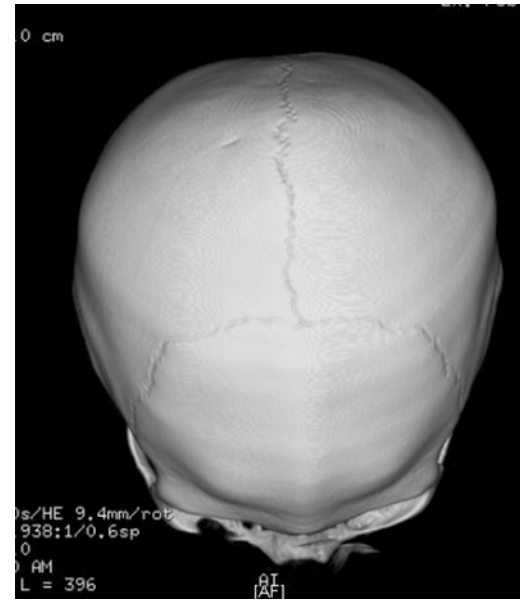

Fig. 2.2 3D computed tomography image of an infant with metopic craniosynostosis which resulted in trigonocephaly

Theory of craniosynostosis implicates derangements in the signaling pathway between the dura mater and the overlying cranial suture [6, 7]. This complex pathway has not been fully elucidated; however over 60 genes have been identified as part of the process [8]. Without

osteoinductive signaling from the dura, it is hypothesized that bony deposition halts, and the suture subsequently fuses. The first theory historically described is commonly referred to as the *Extrinsic Theory*, which suggests that forces outside of the normal signaling pathway limit sutural growth. This includes intrauterine compression, oligohydramnios, abnormal brain growth, or systemic diseases (Rickets, hypothyroidism). This theory has lost favor to the Intrinsic Theory but however may play a role in limited cases.

Limitation of cranial expansion, whether secondary to intrinsic or extrinsic processes, begins early during gestation. To accommodate the rapidly growing brain, the normal skull must also rapidly expand via the cranial sutures. When restricted by premature sutural fusion, growth must expand at the other sutures that remain patent to prevent elevation of intracranial pressure (ICP). Growth occurs primarily perpendicular to the expanding suture, which also means that when a suture is fused, growth is restricted perpendicular to that line. This idea was first described by Virchow and provides an explanation for the characteristic abnormal head shape patterns seen depending on which sutures are involved [9]. The sagittal suture is most commonly involved (55% of cases), followed by metopic (24%), unilateral coronal (15%), and lambdoid sutures (4%) [10]. The epidemiology of specific suture involvement appears to be changing, however, as recent studies have identified an increasing incidence of metopic craniosynostosis [11]. It is unclear whether this is secondary to improved practitioner awareness and diagnosis of more subtle phenotypes or if the true incidence is increasing.

Although abnormal head shape is frequently limited to an aesthetic abnormality, craniosynostosis can also be associated with significant developmental delay. The relationship between craniosynostosis and neurocognitive delay is complex, and it is unclear which process occurs first in this association. In most cases it is believed that premature fusion of the sutures limits the skull's ability to grow in concordance with the rapidly expanding brain. If the remaining patent sutures are unable to compensate for the decreased expansion, a craniocerebral disproportion occurs. This craniocerebral disproportion can lead to elevated ICPs, defined as borderline when >15 mmHg and pathologic when >20 mmHg. This can be compensated for to a degree by bulging fontanelles at the cranial vertex and bossing of non-affected cranial bones [12]. When this mechanism fails and ICPs are persistently elevated, neurologic dysfunction and developmental delays can occur.

Developmental delay and lower intelligence quotient (IQ) scores occur in subjects with craniosynostosis occuring at a higher frequency than the general population. This delay is more significant when multiple sutures are affected. The etiology of the delay is unclear and may be related to early brain growth restriction. It is possible that the cellular and molecular process causing early cranial suture fusion also negatively impacts central nervous system development. Evidence suggests that surgical repair of craniosynostosis results in overall improved intelligence, and those infants >12 months of age with delay can have improved developmental outcomes following surgery [13]. Prolonged elevation of ICPs can also cause papilledema and ultimately optic nerve atrophy, leading to irreversible visual impairment [7].

2.3.2 Deformational Plagiocephaly

Deformational plagiocephaly (DP) is a common head shape anomaly that is frequently confused with craniosynostosis. DP is distinguishable from craniosynostosis primarily in that the cranial sutures remain patent. Deformational plagiocephaly occurs secondary to external forces, and both brain growth and skull growth progress normally both prenatally and after birth. The most common cause of deformational plagiocephaly is supine infant positioning after birth. Its incidence has greatly increased since the 1992 "Back to Sleep" campaign from the American Academy of Pediatrics to decrease the risk of sudden infant death syndrome (SIDS) [14–18]. Although this has drastically decreased the risk of SIDS, DP

has increased by 600% and is now estimated to be 1 in 300 births [14].

Although most commonly caused by positioning after birth, intrauterine compression can also cause deformational plagiocephaly. This is most commonly seen with multiple gestations or abnormal intrauterine positioning. Regardless of the etiology, deformational plagiocephaly can almost always be managed non-operatively. Since the skull continues to grow normally, it is not associated with developmental delay.

2.3.3 Aplasia Cutis Congenita

Aplasia cutis congenita (ACC), or cutis aplasia, is a congenital anomaly of the skull which results in lack of formation of the skull, overlying soft tissue, and/or dura (Figs. 2.3 and 2.4). It is seen in 1 in 3000 births and most commonly affects the vertex of head [19]. There is no universally accepted theory for the etiology of ACC, although impaired cranial vascularity, teratogens, intrauterine trauma, and genetic factors have been implicated. Management of ACC is frequently conservative with local wound care, during which time the site will epithelialize. In certain cases, operative intervention may be required to address large defect size or recurrent bleeding from the site. For smaller size lesions, excision and primary closure may be possible. Larger lesions may require adjacent tissue transfer or skin grafting to achieve closure.

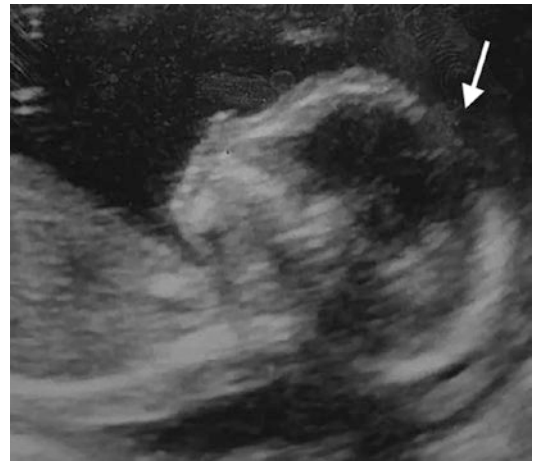

Fig. 2.3 Prenatal sagittal ultrasound image demonstrating aplasia cutis congenita

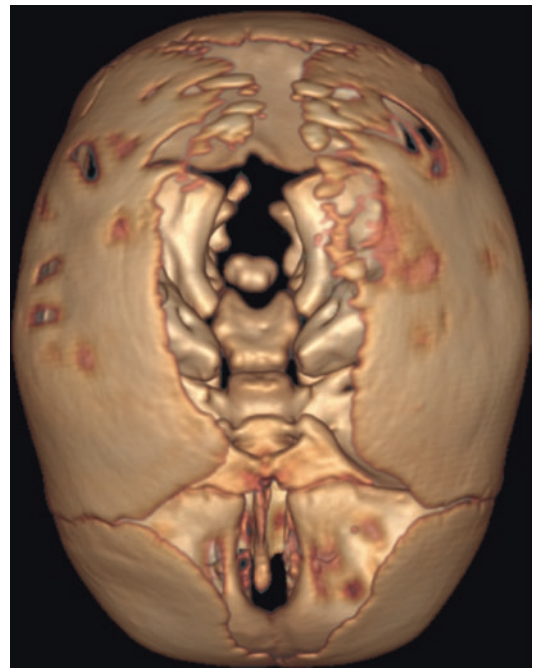

Fig. 2.4 3D Computed tomography of an infant skull with aplasia cutis congenita

2.3.4 Encephalocele

Encephaloceles are group of congenital defects resulting in herniation of the brain through the cranial vault. They most commonly occur posteriorly (75%); however, anterior (sincipital) encephaloceles are considerably more deforming [20]. When encephaloceles occur anteriorly, the tissue passes through the foramen cecum at the skeletal midline. The contents of the herniation and the presence of a persistent connection to the intracranial tissue determine the diagnosis. Most frequently, the foramen cecum fully closes, trapping a mass of skin material outside the skull, termed a dermoid cyst. If the foramen cecum remains patent, herniating neural tissue through the anterior skull base may contain the meninges alone in mild cases, meninges and brain in moderate cases, and the meninges, brain, and part of the ventricular system in severe cases.

The herniated neural tissue is commonly nonfunctional, and, as such, its removal at the time of surgical correction is inconsequential to future neurodevelopment.

2.4 Prenatal Evaluation and Management

The prenatal diagnosis of craniosynostosis most commonly occurs during an evaluation of other fetal anomalies, when a family history of craniosynostosis exists or, as in the case of syndromic craniosynostosis, with severe phenotypic features. When fetal craniosynostosis is suspected prenatally, the diagnostic work-up includes a referral for a detailed ultrasound, consultations with maternal-fetal-medicine specialists, genetic counselors, and prenatal genetic testing.

2.4.1 Two-Dimensional (2D) and Three-Dimensional Ultrasound

The diagnosis of craniosynostosis most commonly occurs postnatally, and the literature on prenatal diagnosis is limited to case reports and limited series of syndromic craniosynostosis. Given that observable findings often only present in the late second and third trimesters, a high degree of suspicion for craniosynostosis is required when an abnormal skull shape or growth parameters of the head are seen early in pregnancy. The most common way an abnormal skull shape is characterized is with a fetal cephalic index (CI). This is commonly performed by the sonographer during the imaging session and involves calculating the ratio of the biparietal diameter to the occipitofrontal diameter. A normal CI is between 75 and 85%, with dolichocephaly diagnosed when the CI is below 75% and brachycephaly above 85% [21].

The bones of the skull, which appear hyperechoic on ultrasound, can be seen as early as 9 weeks of gestation with suture formation at 16 weeks [22]. Cranial sutures can be individually interrogated by positioning the ultrasound transducer so that the acoustic beam is tangential to the bony surface of the skull and moving along the long axis of the suture from the metopic to the sagittal suture. By turning the transducer 90°, the coronal and lambdoid sutures can be examined [23].

Alternatively, the coronal suture can be seen on an axial view of the fetal head, and the metopic suture can be visualized from a frontal view of the face in an axial or sagittal slice. The loss of hypoechogenicity of the normal sutures is suspicious for premature closure. Fusion of one suture can cause widening of the non-fused sutures, which can be seen using 3D ultrasound [23].

Close examination of the fetal face, central nervous system, and limbs is also critical for differentiating isolated craniosynostosis with syndromic forms. Table 2.1 details a number of the most frequently encountered syndromic craniosynostoses and the associated ultrasound findings. While imaging of the fetal orbits and measurement of the interorbital distance is not standard on routine ultrasound examination, hypertelorism or hypotelorism can be present in cases of craniosynostosis when the anterior coronal or and frontal sutures are involved [24]. Proptosis, or protrusion of the globe of the eye out beyond the orbital rim, is often only diagnosed on MRI [24].

Fetuses with syndromic craniosynostosis are at a higher risk of also having midface hypoplasia, with concomitant complex abnormalities of the upper airway and tracheobronchial tree [24–26]. Tracheal cartilaginous sleeves (TCS) have been seen in children diagnosed with Apert, Crouzon, and Pfeiffer syndromes [24]. TCS is an airway malformation, in which the individual tracheal arches do not develop and a continuous fused tracheal cartilaginous piece exists in its place. Given the significant morbidity and mortality associated with TCS, early recognition and management with multidisciplinary care team are required [27].

Detailed examination of the structures of the fetal CNS should be performed, given that evidence of additional anomalies will inform how parents are counseled on the neurodevelopmental prognosis. Apert syndrome has been found to be

Table 2.1 Clinical findings of craniosynostosis syndromes

Syndrome	Cranial suture involvement	Genetic mutation	Inheritance pattern	Associated anomalies
Apert	Coronal, sagittal, lambdoid	FGFR-2	Autosomal dominant	Syndactyly (symmetrical, hands and/or feet) "Mitten-like hand" with broad thumb Broad great toe Hypertelorism Agenesis of corpus callosum Tracheal cartilaginous sleeve Fused cervical vertebrae
Crouzon	Coronal, sagittal	FGFR-2	Autosomal dominant	Normal limbs Exorbitism Beaked nose Protruded mandible with retrusion of the midface Tracheal cartilaginous sleeve Progressive hydrocephalus
Pfeiffer	Coronal, sagittal	FGFR-2	Autosomal dominant	Polysyndactyly Midface hypoplasia Broad radially deviated thumbs or medially deviated great toes Sometimes have Kleeblattschädel (cloverleaf) skull, but this is not specific for Pfeiffer Tracheal cartilaginous sleeve
Saethre-Chotzen	One or two coronal sutures	TWIST-1	Autosomal dominant	Polysyndactyly (hands and feet) Brachydactyly Prominent horizontal crura of the ears Ptosis
Jackson-Weiss	Coronal, sagittal	FGFR2	Autosomal dominant	Midface hypoplasia Enlarged great toe Syndactyly of second and third toes
Antley-Bixtler	Coronal	FGFR-2	Autosomal dominant or autosomal recessive	Radiohumeral synostosis multiple joint contractures Midface hypoplasia Femoral bowing Dysplastic ears Genitourinary anomalies
Muenke	Coronal	FGFR3	Autosomal dominant	Midface hypoplasia Thimble-shaped middle phalanges Carpal/tarsal fusion

associated with midline anomalies of the corpus callosum and septal leaflets, thalamic fusion, as well as over-convolution and overexpansion of the temporal lobe in the second trimester, when the fetal brain is still relatively smooth [24, 25, 28]. Pfeiffer syndrome is also associated with temporal lobe and amygdala dysplasia, ventricular dilation, and megalencephaly. Hand and foot syndactylies are among the limb abnormalities that are associated with certain craniosynostosis syndromes. At times the fingers are not separated in Apert syndrome, giving a "mitten-like" appearance, and broad, radially deviated thumb is often seen in Pfeiffer syndrome [24, 28].

2.4.2 Fetal Computed Tomography (CT)

While rarely used in the evaluation of fetal anomalies given the risk of radiation, CT with 3D reconstruction can also be a useful imaging tool for examining the patency of cranial sutures, as well as examining other skeletal anomalies

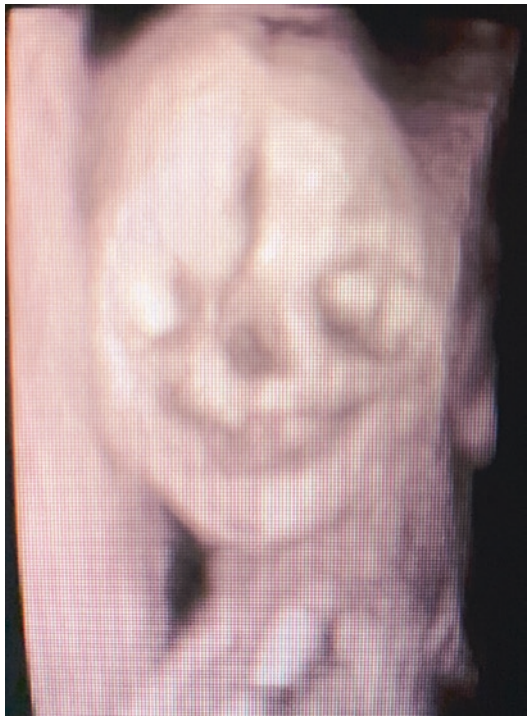

Fig. 2.5 Fetal 3D computed tomography image scan showing a patent metopic suture

(Fig. 2.5) [29]. In Gorincour's cohort of 198 CT scans performed in the prenatal period, fetal CT was found to be beneficial in confirming a case of premature coronal suture closure prenatally. However, as this paper mentions, fetal CT lacks validated reference values for measuring biometry, and established indications and technique for minimizing artifact do not currently exist. These limitations and the ubiquity of ultrasound and MRI make CT an unlikely imaging modality to be essential in the diagnosis of craniosynostosis.

2.4.3 Fetal Magnetic Resonance Imaging (MRI)

Secondary to ultrasound, MRI is the most common imaging modality used for the examination of fetal anomalies. Fetal MRI can be used in conjunction with 2D and 3D ultrasound to characterize cranial and brain anatomy, as well as associated anomalies that may provide valuable information for neonatal resuscitation [24, 28]. As found by a case series presented by Rubio et al., neonates with proptosis were better appreciated via MRI as compared to ultrasound. MRI can also assist in delineating the degree of possible fetal airway obstruction and secondary lung changes in cases of suspected tracheal cartilaginous sleeve [24, 25].

2.4.4 Prenatal Genetic Testing

Once the fetal anomalies have been identified and characterized, the parents should be counseled on the diagnosis and available testing for craniosynostosis syndromes. The genes that are most commonly associated with both syndromic and non-syndromic craniosynostoses include the genes encoding fibroblast growth factor receptors, FGFR-2 (32%) FGFR-3 (25%), as well as the transcription factors TWIST (19%) and MSX2 [23, 24, 28]. Fetal karyotyping and molecular testing can be performed either on biopsy specimen from chorionic villus sampling (CVS), performed between 10 and 14 weeks gestation, or on fetal amniocytes obtained via amniocentesis, which can be performed safely after 16 weeks [30]. After fetal karyotype and microarray analysis has confirmed no chromosomal deletions and or duplications are present, extracted DNA from CVS or amniocentesis can be sent to laboratories to test for specialized craniosynostosis panels. These large gene panels use next-generation sequencing to look for identified pathogenic variants of genes. The rarity of the additional 60 genetic mutations implicated in causing craniosynostosis limits their inclusion in these panels [11]. For patients with negative testing, exome or whole genome sequencing can be utilized to search for a causative mutation. Given the long turnaround time to obtain results, however, this may not be ideal for utilization in the prenatal setting [31].

While the majority of cases of craniosynostosis are inherited in an autosomal dominant fashion, severe types are often a result of de novo mutations. In these cases, parental testing should be performed prior to discussion of the potential risk of recurrence. Negative parental testing still leaves

a small (<1%) risk of recurrence given the possibility of gonadal mosaicism [23]. Medical genetics company has also started to develop noninvasive prenatal screening panels to assist in the diagnosis of craniosynostosis without incurring the risks of CVS or amniocentesis. Baylor Genetics and Natera, Inc., have both launched noninvasive prenatal screening panels, designed to screen for selected autosomal dominant and X-linked genes. Vistara, offered by Natera, Inc., and Preseek from Baylor Genetics include testing of the FGFR2 and FGFR3 gene regions and are marketed toward diagnosing syndromic craniosynostosis disorders including Antley-Bixler syndrome, Apert syndrome, Crouzon syndrome, Jackson-Weiss syndrome, Muenke syndrome, and Pfeiffer syndrome. These tests provide an attractive option for parents who want to avoid invasive testing. If the screening tests return positive, results should be followed up with confirmatory testing via chorionic villus sampling or amniocentesis. Furthermore, these companies focus on mutations in the FGFR2 and FGFR3 genes, and a negative screen may provide false reassurance if a pathogenic mutation exists on a different gene. Consultation with a genetic counselor should be sought prior to ordering genetic testing to educate patients about the advantages and limitations of each testing option, as well as calculating the risk of recurrence in future pregnancies.

2.4.5 Considerations for Delivery and Management

Depending on the severity and concomitant congenital anomalies, the diagnosis of craniosynostosis in the antenatal period may require the management of a multidisciplinary team to counsel the patient on fetal prognosis and care for the affected infant. While craniosynostosis itself is not an indication for cesarean delivery, it may increase the risk for unplanned cesarean delivery secondary to second-stage labor arrest if the noncompliant fetal skull is unable to pass through the vaginal canal [21]. Furthermore, non-cephalic presentations are more likely in cases of craniosynostosis.

Infants with isolated single-suture craniosynostosis generally have safe and successful delivery outcomes, and the diagnosis of craniosynostosis is made postnatally. Fetuses with multiple known anomalies associated with syndromic craniosynostosis should be delivered in a facility with a level III NICU. Given that these fetuses are at risk of airway obstructions, secondary to midface hypoplasia or lower airway obstruction, the ability to obtain an airway and provide adequate ventilation is a critical first step in their neonatal resuscitation [27].

2.5 Conclusion

Cranial anomalies are rarely encountered congenital malformations which may occur in isolated or syndromic forms. With improvements in prenatal imaging, these entities are able to be identified more readily in utero. Early identification of these anomalies allows the perinatologist to perform genetic testing if indicated, coordinate multidisciplinary care in syndromic cases, provide valuable information prior to delivery, and assist in family counseling.

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3 Signal Pathways from the Plasma Membrane to the Nucleus Regulating Craniofacial Pattern Formation

Thomas Meyer

3.1 Pattern Formation in Human Craniofacial Development

The cellular and developmental processes underlying facial development are complex and still not well understood. At early stages in human embryonic development, the facial primordia form around the stomodeum, which functions as the primitive mouth. The orofacial development is based on a series of coordinated pattern formations involving five facial primordia. The maxillofacial region develops from the fusion of the unpaired frontonasal prominence and the two paired lateral nasal and maxillary processes, whereas the lower jaw forms from two paired mandibular prominences. Both the maxillary and mandibular processes are derivatives of the first pair of the pharyngeal arch, which appear mainly as the expansion and migration of neural crest populations from the mesencephalic and rostral rhombencephalic neural folds. The frontonasal process develops to the forehead and the rostral boundary of the stomodeum including the nasal cavities. When the surface ectoderm in the inferolateral parts of the frontonasal prominence thickens, the nasal placodes are shaped and later develop to the nasal epithelium as part of the olfactory system. The maxillary processes merge with the lateral nasal prominence to constitute the lateral boundary of the stomodeum. In the sixth week of embryonic development, the two mandibular prominences fuse to form the lower jaw. The philtrum is formed by the medial nasal prominences and the medial extensions of the maxillary processes. During the seventh week, the fusion of the midfacial region is accomplished, and the remaining epithelial seams between the processes have disappeared.

Given the coordinated action of a variety of epithelial and mesenchymal cell populations in the morphogenesis of midfacial processes, any time delay or failure in the midfacial development will result in developmental defects [1, 2]. Likewise, abnormal fusion of the medial and lateral nasal as well as the maxillary prominences will lead to facial malformations with a varying degree of severity. Orofacial and palate clefts, hypoplasia of midfacial structures, and/or hypertelorism may occur as a consequence of asynchronic steps or retardations in midfacial morphogenesis. These pathologic features result from defects in the growth, expansion, and patterning of the various fusion processes during embryonic morphogenesis. Tightly controlled molecular steps in signal transduction networks are required to regulate the complex, three-dimensional signaling dynamics in orofacial tissue development during the early stages of human embryogenesis [3, 4].

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Spatial pattern formation in the midface is driven by cranial neural crest cells, which constitute a population of highly migratory precursor cells. These multipotent, proliferative cells follow well-defined ventral paths from the neural tube of the caudal forebrain, midbrain, and rostral hindbrain to the ventrolateral side of the face. The cranial neural crest cells, which play a key role in the craniofacial development, are involved in a cascade of defined steps ultimately resulting in the population of the mesenchyme in the surrounding tissues. Before migrating ventrally, the cranial neural crest cells acquire an anterior-posterior orientation through reception of signals from the microenvironment in their niche [2]. Along with migration, the cells undergo a process called delamination that involves epithelial-to-mesenchymal transition (EMT).

3.2 Signaling Pathways Regulating the Patterning and Growth of Facial Primordia

The signaling pathways underlying the morphogenesis of the craniofacial region have been partially uncovered by the identification of gene mutations that cause distinct clinical syndromes. Signal transduction pathways involved in craniofacial malformation are stimulated by the following extracellularly secreted ligands: fibroblast growth factors (FGFs), WNT, platelet-derived growth factor (PDGF), sonic hedgehog, transforming growth factor-β (TGFβ), bone morphogenetic proteins (BMPs), and cytokines. Pathogenic mutations in distal signaling components in these diverse pathways have been identified as genetic factors contributing to craniofacial malformations including midfacial abnormalities.

Missense mutations in the *TCOF1* gene encoding the nucleolar phosphoprotein treacle induce apoptotic cell death in neural crest cells and cause the autosomal dominant Treacher Collins syndrome, which is the most common human mandibulofacial dysostosis disorder [5–7]. The development of the first and second pharyngeal arch is affected by *TCOF1* mutations in a symmetrical manner. The majority of disease-associated mutations in the *TCOF1* gene are deletions leading to the formation of a premature termination codon and the subsequent shortening of the protein [8, 9]. In addition, mutations in genes encoding subunits of RNA polymerases I and III (*POLR1C* and *POLR1D*) have been identified in patients with Treacher Collins syndrome [10].

Mutations of the *ephrin-B1* gene, which encodes the ligand for Eph receptors, cause the X-linked craniofrontonasal syndrome [11]. The autosomal dominantly inherited Saethre-Chotzen syndrome, also known as acrocephalo-syndactyly type III, is caused by loss-of-function mutations in the *TWIST1* gene [12–14]. The *TWIST1* gene codes for a basic helix-loop-helix transcription factor putatively involved in transcriptional regulation of fibroblast growth factor receptor 2 (*FGFR2*). It is expressed during rat palatogenesis and odontogenesis [15]. Depending on the expression level of Twist1, cranial neural crest cells acquire ectomesenchyme potential during embryogenesis [16].

Mutations in the gene coding for fibroblast growth factor receptor 2 (*FGFR2*) have been identified in genetic syndromes associated with craniosynostosis, such as Crouzon syndrome, Pfeiffer syndrome, and Apert syndrome [17–20]. In humans, four genes encode FGFRs (FGFR1-4), which additionally undergo alternative splicing in their extracellular domain to produce a set of receptor variants with varying affinities for their ligands. Activation of FGFR serves as a posteriorizing factor during pattern formation of the neural plate and facilitates neural induction. In the majority of patients with Apert syndrome, gain-of-function mutations of FGFR2 with a serine-to-tryptophan substitution at amino acid residue 252 ($FGFR2^{S252W}$) or a proline-to-arginine exchange at position 253 ($FGFR2^{P253R}$) have been detected. Patients diagnosed with Apert syndrome usually have midfacial hypoplasia, which is often associated with a short cranial base and a reduced pharyngeal height, a narrow nasal cavity, and diminished nasopharyngeal space often leading to upper airway obstruction. In addition, severe bicoronal craniosynostosis is a character-

istic feature in patients with autosomal dominant Apert syndrome. It has been postulated that a higher number of precursor cells enter the osteogenic pathway in patients with Apert syndrome, leading to elevated subperiosteal bone matrix formation and premature calvaria ossification during fetal development [21].

3.3 WNT/β-Catenin and Sonic Hedgehog Signaling in Facial Development

Defects in the highly conserved WNT signaling have been found in human patients with craniofacial abnormalities as well as transgenic mouse models showing similar phenotypes [2, 22, 23]. The development of the endocranium and some facial bones derived from neural crest cells is under the control of the WNT/β-catenin pathway, and, therefore, it is not unexpected that essential components of this signal pathway contribute to the pattern formation in craniofacial tissue homeostasis [24].

Sonic hedgehog (SHH), which is one out of three vertebrate homologues of the *Drosophila melanogaster* protein hedgehog, functions as a developmental morphogen in humans and is involved in the formation of midline structures in the face [25, 26]. Mutations in the *SHH* gene disturb the hemisphere separation of the brain and result in a disorder termed holoprosencephaly. SHH is expressed during facial morphogenesis and is necessary for the normal formation of most of the head skeleton, as removing hedgehog signaling in murine cranial neural crest cells resulted in impaired cell proliferation and increased apoptosis in the brachial arches [26, 27].

3.4 The Role of SMAD Proteins in Craniofacial Development

Other important signal pathways in craniofacial development are induced by either transforming growth factor-β (TGFβ) or bone morphogenetic proteins (BMPs), which signal through SMAD transcription factors [28, 29]. The multifunctional cytokine TGFβ is an essential component required for palatogenesis, particularly during the late phase of palate development [30–32]. It has been well established that altered TGFβ signaling causes syndromic and nonsyndromic cleft palate. Smad-mediated signaling by TGFβ/BMP controls the homeobox gene patterning of spatial orientation within the first branchial arch. SMAD proteins executing TGFβ/BMP signaling have a critical role in mesoderm formation, where they contribute to left-right patterning and craniofacial development [33–37]. When TGFβ-activated kinase 1 (Tak1), an important regulator of Smad-independent TGFβ signaling, was inactivated in neural crest cells, the transgenic mice displayed palate clefting associated with micrognathia and malformed tongue, closely resembling human Pierre-Robin sequence clefting [38]. Missense mutations located in the R-SMAD-binding domain of the TGFβ repressor SKI have been identified in patients with Shprintzen-Goldberg syndrome, a rare, systemic connective tissue disorder characterized by skeletal and cardiovascular manifestations as well as craniosynostosis [39].

From a structural perspective, the SMAD and STAT (signal transducer and activator of transcription) signal pathways share similar design principles, namely, activation at the receptor complex, dimerization and nucleocytoplasmic shuttling, as well as transcriptional regulation. Both SMAD and STAT proteins are phosphorylated at their cognate transmembrane receptors upon ligand binding and function as transcription factors in the nucleus. In the following, the design principle of the STAT-mediated signal pathway will be discussed with a particular focus on the important role of STAT3 in early embryogenesis and craniofacial development.

3.5 Loss-of-Function STAT3 Mutations in Hyper-IgE Syndrome

Dominant negative mutations in the human gene encoding STAT3 cause hyperimmunoglobulin-E syndrome, also kown as Job's syndrome, a

multisystem disorder characterized mainly by immunological symptoms, such as staphylococcal infections, skin abscesses, eczema, recurrent sinopulmonary infections, and candidiasis [40–42]. In addition to eosinophilia and elevated serum levels of immunoglobulin E, patients with hyper-IgE syndrome display various nonimmunologic features, which are a characteristic facial appearance, retained primary teeth, pathologic bone fractures, scoliosis, joint hyperextensibility, midline anomalies, and craniosynostosis [43–45]. In 1972, Buckley et al. described the clinical features of two adolescent boys who had recurrent pyogenic infections associated with extreme hyperimmunoglobulinemia E, growth retardation, and coarse facies [46]. Six years later, Smithwick and colleagues first described the association of cranial synostosis with hyper-IgE syndrome in three immunodeficient boys with recurrent infections, of whom two had surgical corrections [47]. Höger et al. observed premature fusion of the sagittal and lambdoid suture leading to scaphocephaly and partial optic atrophy without any clinical signs of raised intracranial pressure in a 9-year-old boy with hyper-IgE syndrome [48].

Minegishi and co-workers reported the discovery that dominant-negative STAT3 mutations cause hyper-IgE syndrome. The authors found that 8 out of 15 unrelated non-familial hyper-IgE patients had heterozygous STAT3 mutations and that all these five different mutations were located in the DNA-binding domain [41]. Independently, Holland et al. demonstrated that all are STAT3 missense mutations or in-frame deletions were localized in the DNA-binding domain and SH2 (Src homology 2) domain. Later, pathogenic STAT3 mutations were identified also in the carboxy-terminal transactivation domain, although disease-associated genetic variants in this domain were less frequently observed [42].

Nieminen et al. showed that interleukin-11 signaling is essential for the normal development of teeth and craniofacial bones and that its function is to restrict tooth number and prevent suture inactivation [49]. Moreover, the authors demonstrated that the homozygous missense mutation Arg296Trp in the *IL11RA* gene, which codes for the α-subunit of the interleukin 11 receptor, rendered the mutant receptor complex unable to activate STAT3-mediated intracellular signaling. They concluded that deficient IL-11 signaling causes craniosynostosis, supernumerary teeth, and delayed tooth eruption through impaired STAT3 activation [49]. Donner and Williams demonstrated that a conserved STAT binding site provided a major contribution to the expression of a particular AP-2 gene, termed *Tcfap2*a, in the facial prominences and, furthermore, that STAT1 expression was detectable in extracts from E10.5 mouse heads [50].

3.6 Design Principles of STAT3 Signaling

STAT3 belongs to a family of evolutionary conserved transcription factors, which evolved at the boundary of primitive multicellular organisms [51]. The protein was first described in IL-6-stimulated hepatocytes as an acute phase response factor through interaction with promoter regions of acute phase response genes [52–55]. The domain architecture of STAT3 is structurally homologous to other STAT family members and contains a conserved amino-terminal domain, coiled-coil domain, DNA-binding domain, SH2 domain required for receptor recruitment and dimerization, linker domain, and carboxy-terminal transactivating domain [56]. In humans, seven different STAT proteins have been identified, i.e., STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6.

The STAT proteins are differentially activated by a variety of extracellular molecules, such as interleukins, interferons, growth factors, and hormones [57]. Under physiological conditions, the members of the STAT family execute different, non-redundant functions, such as cell differentiation, proliferation, apoptosis, immunity, and development. Glycoprotein 130 (gp130) is a receptor subunit capable of activating STAT3 through binding of extracellular cytokines of the interleukin-6 family. The receptor can be stimulated by IL-6, IL-11, IL-27, leukemia inhibitor factor (LIF), ciliary neurotrophic factor (CNTF),

oncostatin (OSM), or cardiotrophin-1, leading to the activation of STAT3. Notably, a biallelic mutation in the *IL6ST* gene encoding the gp130 co-receptor resulted in a loss of gp130 signaling and was associated with both immunodeficiency and craniosynostosis resembling features similar to the STAT3-deficient hyper-IgE syndrome [58].

Similar to the SMAD transcription factors, STAT signaling is a paradigm of a ligand-induced signal pathway which transmits signals directly from cell surface receptor to the transcriptional machinery in the nucleus, thereby connecting the extracellular environment to gene expression programs. The activation of the STAT pathway represents one of the best studied examples of direct signaling from the plasma membrane to the nucleus without the involvement of second messengers (Fig. 3.1). STATs interact directly with both membrane-bound receptors and genomic DNA, thereby integrating cellular processes at the membrane to alterations in gene expression. The basic model of STAT signaling depends on a cascade of essential tyrosine phosphorylation steps. Binding of the ligand to its cognate cell surface receptor triggers the dimerization of the transmembrane receptor subunits. Owing to conformational changes in the intracellular, carboxy-terminal receptor complex, the non-covalently attached Janus kinases (JAKs) are brought into close spatial proximity to each other, which allows their trans-phosphorylation on specific tyrosine residues. Subsequently, the activated JAKs phosphorylate specific tyrosine residues in the cytoplasmic receptor tails, thereby creating docking sites for cytoplasmic STAT proteins, which bind through their SH2 domain.

In the next step, the activated JAKs phosphorylate the receptor-associated STAT molecules on a conserved signature tyrosine residue near their carboxy-terminus, which in the case of STAT3 is the essential tyrosine residue Y705. Upon this posttranslational modification, the STAT proteins dissociate from the receptor complex and immediately dimerize via reciprocal phosphotyrosine (pY)-SH2 domain interactions between the two partner protomers. With the exception of STAT2, all human STAT proteins form homodimers and, in addition, heterodimers such as STAT1:STAT3 have been described.

In the nuclear compartment, the tyrosine-phosphorylated STAT dimers act as classical transcription factors after binding to specific regulatory sequences on genomic DNA to modulate the expression of their target genes. All members of the STAT family except for STAT2 bind to a palindromic consensus motif termed γ-interferon-activated sequence (GAS) (5´-TTCN$_3$GAA-´3). STAT2 is unable to bind to DNA by itself but instead associates with its partner STAT1 and interferon-regulatory factor 9 (IRF9) to form a ternary complex termed interferon-stimulated gene factor 3 (ISGF3) [59]. Phosphorylation at both a critical serine residue in position 727 and a tyrosine residue in position 705 is required for maximal transcriptional activation [60]. Tyrosine phosphorylation is a prerequisite for cooperative binding to GAS elements mediated by reciprocal amino-terminal interactions between two adjacent STAT3 dimers (Fig. 3.2), whereas phosphorylation of serine 727 is dispensable for DNA binding [62].

STAT proteins were first described to function as latent transcription factors which, upon stimulation of cells with cytokines, translocate to the nucleus and induce gene transcription exclusively. However, STAT1 and STAT3 were found to be present in the nucleus even in the absence of cytokine stimulation, regardless of tyrosine phosphorylation [63–66]. Some STAT family members, e.g., STAT1, STAT2, STAT3, and STAT6, promote gene expression also before exposure to extracellular stimuli and subsequent tyrosine phosphorylation, when bound as unphosphorylated molecules to promoter regions [67–72]. In contrast to tyrosine-phosphorylated STAT dimers (Fig. 3.3), which are actively imported into the nucleus via a Ran-mediated transport pathway, the nuclear import of unphosphorylated STAT proteins is facilitated by direct interactions with nucleoporins located in the nuclear pore complex [74]. This carrier-free translocation does not require metabolic energy and can be regarded as facilitated diffusion following a concentration gradient across the nuclear envelope. STAT1 and STAT3 are constantly shuttling between the cyto-

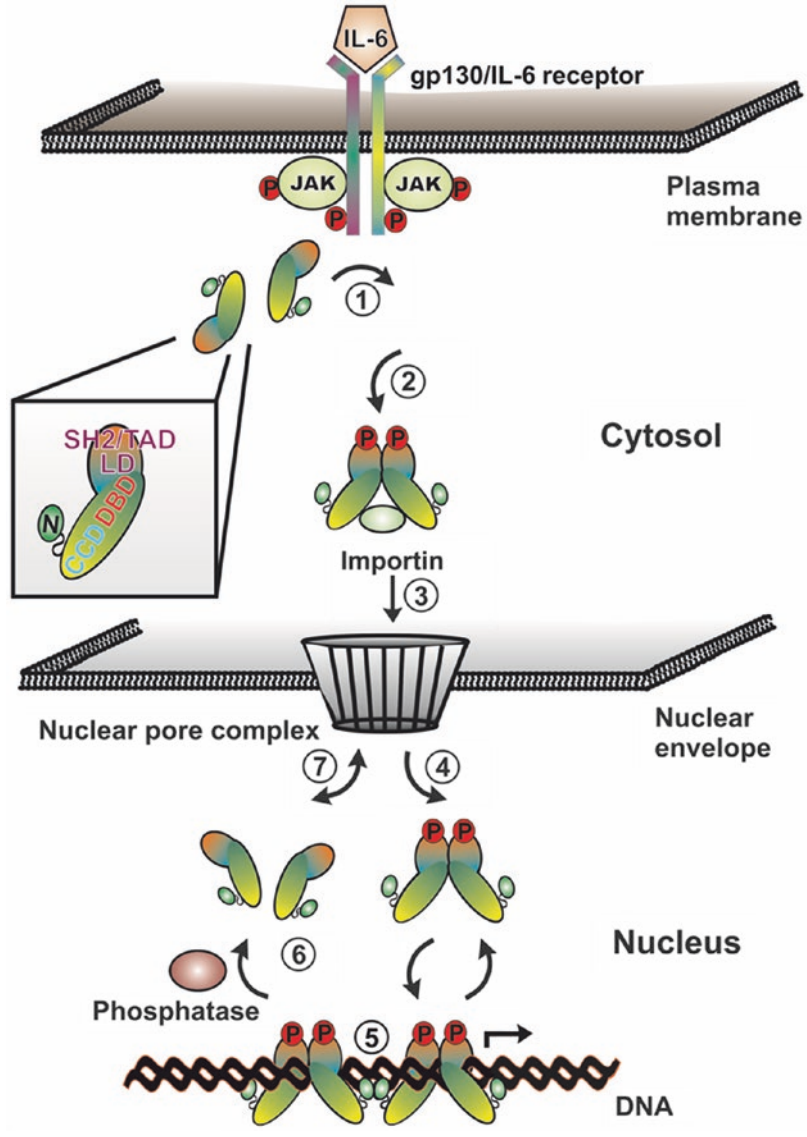

Fig. 3.1 Model of the interleukin-6-induced JAK/STAT3 signal pathway. The scheme depicts the nucleocytoplasmic translocation and activation-inactivation cycle of STAT3 transcription factor. Binding of the extracellular ligand interleukin-6 (IL-6) to its cell surface gp130/IL-6 receptor triggers JAK-induced tyrosine phosphorylation of the latent cytoplasmic transcription factor STAT3 (1). Dimerization of STAT3 occurs through reciprocal interactions between the tyrosine-phosphorylated Y705 residue on one and the SH2 (Src homology 2) domain on the partner molecule (2). Phosphorylated dimers are then translocated to the nucleus via binding to importins through nuclear core complexes (3). Nuclear STAT3 (4) then bind to γ-interferon-activated sequence (GAS) motifs in the promoter region of cytokine-inducible genes (5). After dissociation from DNA (6), STAT3 is susceptible to dephosphorylation by the nuclear phosphatases such as Tc45 (6) and, thereafter, exits the nucleus (7)

plasmic and nuclear compartment, irrespective of their activation status [65, 66, 75, 76]. The nuclear form of the T-cell protein tyrosine phosphatase (Tc-PTP) Tc45 and the two SH2 domain-containing phosphatases SHP1 and SHP2 are involved in the rapid dephosphorylation of STAT3 [77]. It was shown that binding to GAS elements protected the homologous STAT1 dimer

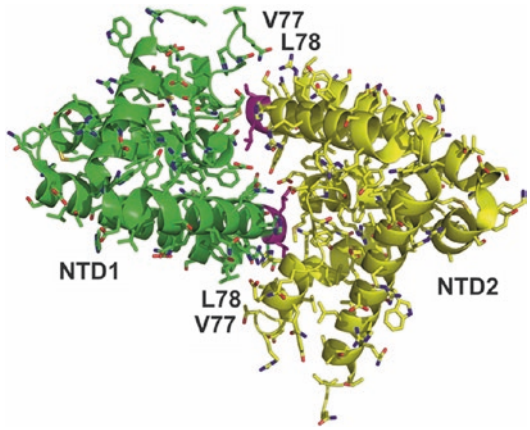

Fig. 3.2 Ribbon diagram of a dimer of the STAT3 amino-terminal domain. The figure was created using the program PyMOL (DeLano Scientific) and the Protein Data Bank (PDB) file 4ZIA [61]. Two amino acid residues (valine 77 and leucine 78) important for amino-terminal dimer formation are marked in magenta

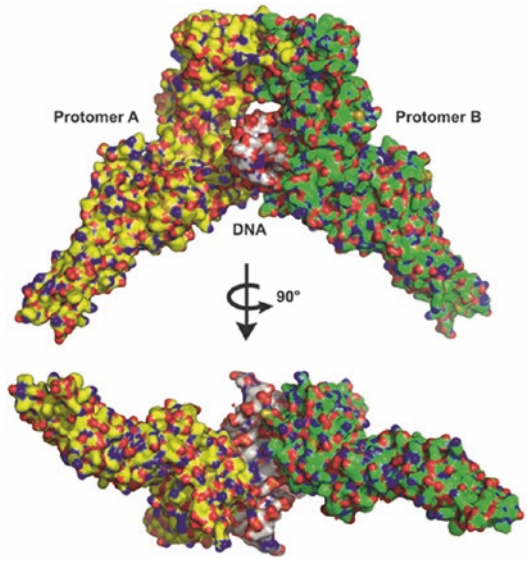

Fig. 3.3 Crystal structure of tyrosine-phosphorylated and lysine-acetylated STAT3 in a complex with DNA. The images show an orthogonal view of the molecular surface structure of DNA-bound STAT3 with the DNA axis going out of (top) or into the plane of the paper (bottom). The figure was created using data from the PDB archive for file 6QHD [73]

from dephosphorylation by the inactivating Tc45 phosphatase [78].

Corry et al. showed that bone morphometric parameters differed between mice with an osteocyte-specific knockout of STAT3 and those expressing the wild-type protein [79]. The osteocyte-specific STAT3 knockout resulted in decreased STAT3 protein expression in osteocytes and an overall lower bone mass with reduced osteoid surface of trabecular bone. STAT3 deficiency in osteocytes negatively affected biomechanical properties of cortical bones and suppressed mechanically induced bone formation [80].

Notably, Goel and co-workers found that activation of the osteopontin (*OPN)* gene was higher in IL-6-stimulated peripheral blood mononuclear cells from healthy controls (HCs) compared to cells from hyper-IgE syndrome patients with STAT3 loss-of-function mutations [81]. Activation of STAT3 is crucial for the IL-6-mediated regulation of TH17 cells, which are a source of significant production of the proinflammatory cytokine IL-17 [82]. Low TH17 cell numbers are frequently found in patients with hyper-IgE syndrome [83–90].

3.7 Nonclassical STAT3 Functions in Oxidative Respiration and Naïve Pluripotency

STAT3 was first discovered as an inducible nuclear transcription factor in acute phase response and was later shown to elicit also nonclassical functions in mitochondria by enhancing the activities of complex I and II of the electron transport chain. In mitochondria from STAT3-knockout mice, lower rates of oxygen consumption were measured when pyruvate or malate was used as a complex I and succinate as a complex II substrate, demonstrating that STAT3 expression upregulates mitochondrial respiration [91]. Previous studies have shown that mitochondrially located STAT3 interacts directly with the cell death regulator GRIM-19 (gene associated with retinoid-interferon-induced mortality 19) to inhibit STAT3-dependent gene expression [92, 93]. The transactivation domain of STAT3 and, in particular, the serine 727 residue is required to bind to the GRIM-19 inhibitor, as the serine-to-

alanine substitution mutant at position 727 ($S^{727}A$) has almost completely lost its capacity to bind to this component of the mitochondrial respiratory chain complex I [93].

Meier and co-workers demonstrated that cyclophilin D, a structural component of the mitochondrial permeability transition pore, interacts with STAT3 to reduce mitochondrial ROS production during oxidative stress [94]. The binding to cyclophilin D requires the amino-terminus of STAT3. Szczepanek and colleagues characterized the cytoprotective effects of mitochondrial STAT3 during ischemia using a transgenic mouse line with cardiomyocyte-specific overexpression of mitochondria-targeted STAT3 which harbors the DNA-binding mutation $E^{434}A$/$E^{435}A$, termed MLS-STAT3E [95, 96]. In mitochondria from MLS-STAT3E-expressing mice, the activities of the electron transport chain complex I (NADH-ubiquinone oxidoreductase) and complex II (succinate-ubiquinone oxidoreductase) were decreased compared with wild-type animals, whereas complex III (ubiquinol-cytochrome c oxidoreductase) and complex IV (cytochrome c oxidase) activities were unchanged. These observations underscore the hypothesis that STAT3 links gene activation in the nucleus to changes in energy metabolism and oxidative respiration.

Nichane et al. reported in a *Xenopus* model that cell cycle progression and neural crest specification are coordinated by STAT3 activity [97]. The authors reported that elevated STAT3 activity maintained cells in an undifferentiated state, whereas cell proliferation and neural crest differentiation were promoted by lower activity of STAT3. It was demonstrated that STAT3 directed self-renewal of pluripotent embryonic stem cells and induced pluripotent stem cells downstream of the LIF-receptor/gp130 axis [98, 99]. STAT3 cooperates with the homeoprotein NANOG, which is a key component of pluripotency named after the mythical Celtic land of youth (Tír na nÓg). NANOG amplifies STAT3 signaling by suppressing the expression of the STAT3-negative regulator SOCS3 (suppressor of cytokine signaling; [100]). The two transcription factors, STAT3 and NANOG, work synergistically together to upregulate genes associated with naïve pluripotency, such as Krüppel-like factor 4 (KLF4), which is a canonical Yamanaka factor required to induce pluripotent stem cells.

In summary, the LIF/IL-6-mediated transcription factor STAT3 integrates gene expression in the nucleus, oxidative respiration in the mitochondria, and maintenance of pluripotency [101]. These pleiotropic functions of STAT3 are essential for normal craniofacial development during the growth of the embryo, while its deficiency results in abnormal morphogenesis.

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4 The Developmental Interrelation Between the Nervous System and Craniofacial Complex as Evidenced from Craniofacial Malformations

Asher Ornoy

4.1 Introduction

The development of the craniofacial complex is a typical example of the intimate interaction between the developing nervous system and most developing organs in the embryo and fetus. The cranial neural crest cells, derived from the rostral part of the brain, are the most important contributors of tissue to the craniofacial complex. This close interaction between different parts of the brain and the face is a result of complex cellular and tissue interactions (i.e., epithelium mesenchyme interactions) as well as the effects of regulatory genes that originate from both organs—the brain and the face.

The craniofacial complex serves many functions: chewing and swallowing (nutrition), respiration, and protection for the brain and sense organs. Hence, its active development is most complex and of relatively short duration.

Due to the large number of malformations of the craniofacial complex, we chose to discuss briefly only the more common malformations with only partial description of the pertinent literature. Emphasis will be given on clinical parameters and on the interaction with brain development.

Many craniofacial anomalies have very complex etiologies, and the exact diagnosis is quite difficult. Hence, imaging techniques are often inadequate for the proper diagnosis of these anomalies, and genetic tools, including the most advanced methods in molecular biology, have to be used. Different medical disciplines must be available for consultation such as clinical genetics, pediatric neurology and neurosurgery, plastic and/or orofacial surgery, dentists, pathology, and proper imaging facilities. It is therefore advisable that the diagnosis and treatment of craniofacial malformations are performed in hospitals where all these facilities are available.

4.2 Development of the Craniofacial Complex

2a. General developmental processes: The craniofacial complex is composed of two main parts: the dorsal-rostral neuro-cranium that encapsulates the brain and the ventral-caudal viscero-cranium which is basically involved in nutrition and respiration and supports the mouth, pharynx, and upper larynx [1, 2]. Two tissues contribute to most of the craniofacial complex: the ectomesenchyme, mesenchyme that originates from the ectoderm, and the neu-

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ral crest cells which originate from the neural tube. Cephalic neural crest cells populate both the neurocranium and viscerocranium, while the ectomesenchymal tissue is found only in the neurocranium. The first and second pharyngeal arches and their constituents form most of the viscerocranium [3, 4]. Pharyngeal arch ectoderm and entoderm are involved in the formation of the oral and pharyngeal cavities. Cephalic and trunk neural crests (vagal neural crest cells) contribute to the circumpharyngeal region [4]. The two most important signaling regulators in these developmental processes are Sonic hedgehog (Shh) and Wnt [1, 2, 5, 6].

The development of the craniofacial complex involves complex ectodermal- mesenchymal interactions with various signaling pathways from the anterior visceral entoderm, anterior neural ridge and head mesoderm, and cranial neural crest cells [7]. The interaction of cranial neural crest with other tissues in the face is largely dominated by two groups of regulatory molecules: growth factors (i.e., FGF, TGFs, EGF) and retinoic acid superfamily [8]. Abnormalities of these regulatory systems might result in craniofacial malformations.

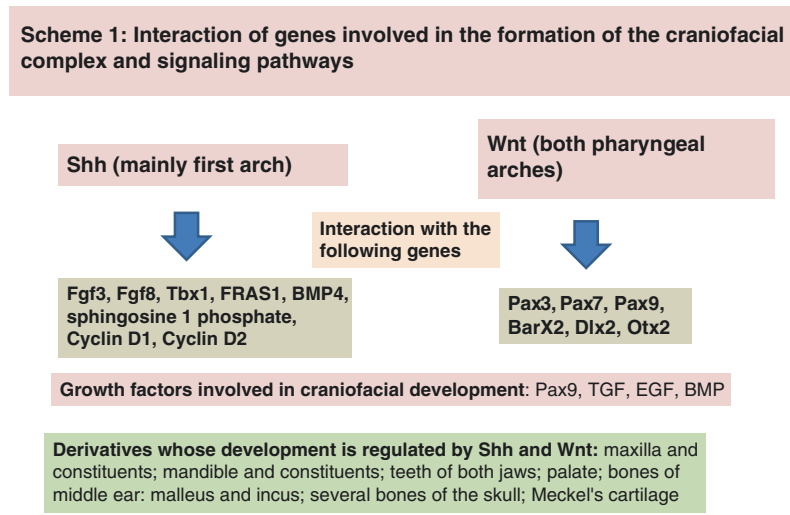
2b. Genes that control craniofacial development (Scheme 4.1): The high degree of conservation between chicken facial ontogeny and other model organisms including mammals enables us to translate the basic principles from chicks to mammals and thus better understand the role of neural crest cells in the formation of the craniofacial complex [1, 2].

Role of sonic hedgehog (Shh): It is accepted that Shh is the main molecule involved in the interaction between brain and facial development. The earliest *Shh* signals come from the prechordal plate, which then "turns on" the expression of *Shh* in the ventral diencephalon between 6 and 8 somite stages, which is the stage of neural crest cell formation [2, 5]. Interference with the expression of Shh may induce facial malformations, such as cleft lip and palate [2]. High levels of retinoic acid may inhibit Shh, often leading to various craniofacial malformations [5]. The non-Shh signaling cephalic neural crest cells are also involved in the formation of the prosencephalon and mesencephalon (forebrain and midbrain).

Role of Hox genes: The rostral neural crest cells that originate from the area of the diencephalon are Hox negative, i.e., no Hox genes are expressed, while the more caudal neural crest cells are Hox positive and are responsible for the formation of the hyoid bone [1]. Defects in the Hox-negative neural crest cells may lead to severe malformations of the brain, demonstrating the close developmental interrelationship between the brain and the craniofacial complex.

2c. Role of other genes: A variety of nervous system developmental genes are also involved

Scheme 4.1 Interaction of genes involved in the formation of the craniofacial complex and signaling pathways



in the formation of the craniofacial complex. Examples are Wnt genes (Wnt1, Wnt8b), Dlx2, and Otx2 ext [1]. Wnt signaling is of special importance as it also plays a crucial role in palatal development, and mutations in Wnt genes are associated with several craniofacial abnormalities including oral clefts [6]. There are plenty of clinical evidences in humans that demonstrate the close developmental associations between the brain and the face, as in many defined syndromes the brain and the face are affected in very specific ways [5]. Moreover, developmental deviations of the brain often induce typical facial dysmorphism which facilitate the diagnosis of the underlying brain malformation (i.e., Down syndrome).

Scheme 4.1 is a schematic description of some of the processes involved in craniofacial development.

2d. Morphological development of the face and lips: The major phases of face development occur during the second and third month post fertilization with important morphological changes that also take place thereafter, shaping the specific facial appearance [9, 10].

The main process is the fusion of five facial prominences that are derived from the frontal process of the early embryo: The upper middle part of the face (fronto-nasal prominence) is formed by the end of the third week and the beginning of the fourth week [11]. An ectodermal invagination establishes during the fourth week the primitive mouth. This invagination meets the most cranial part of the foregut, forming the oropharyngeal membrane that disintegrates during the fifth week. The neural crest-derived mesenchyme of the first pharyngeal arch forms the two maxillary processes and more caudally the mandibular processes [11]. A bilateral ectodermal invagination, the olfactory placode, starts to form during the fourth week and sinks into the mesoderm of the fronto-nasal prominence forming the two nasal pits and nasal sacs with two medial and two lateral nasal processes. The olfactory sacs deepen and enlarge to meet the oral cavity. In the meantime, the two maxillary processes and two mandibular processes develop in the first pharyngeal arch [9, 10].

Concomitant with the development of the nasal processes, there is enhanced growth of the left and right maxillary processes which fuse in the fifth–sixth week with the two lateral nasal processes forming during weeks 4–6 (days 24–37) the upper lip [11–14]. The exact timing of fusion of the different processes that form the face is crucial for its normal development [13] as any deviation in the sequence and timing of these processes may lead to abnormalities of the face, especially cleft lip with or without cleft palate (CL/CP).

Although Shh is one of the most important signaling pathways in the formation of the upper lip, Wnt signaling is complementary to Shh signaling in completing the process of normal lip fusion. If these signals do not operate in complete coordination, CL/CP may be the result.

During these early phases, the brain controls the timing and steps of facial development. Later, this control is lost, and the further development of the face and its specific shape is controlled by many local genes.

The lower jaw is formed by the fusion of the two mandibular processes in their ventral parts during the fifth week, forming the chin. Anomalies of the lower jaw are common, especially hypoplasia as occurs in the Pierre Robin sequence (syndrome) [15]. Often, there is a postnatal mandibular catch-up growth with increased age [16].

2e. Development of the palate: The palate enables eating and breathing at the same time, as by closing up the nasal airways, it prevents food from entrance to the lungs while swallowing. The palate develops from the relatively small primary palate, anterior to the incisive foramen and posterior to the four incisors and from the larger pair of palatine shelves of the secondary palate. These maxillary processes are composed of neural crest-derived mesenchyme of the first pharyngeal arch covered by oral epithelium. The palatine shelves merge in the midline around week 9–11 post fertilization and also fuse superiorly with the nasal septum [17]. Their growth and fusion are controlled by many genes and by complex epithelial mesenchymal interactions [17]. There are many chromosomal (Trisomy 18) genetic (gene mutations) and environmental (methotrexate

during pregnancy) causes that may interfere with the normal development of the palate, resulting in different degrees of cleft palate [14, 18].

Sonic hedgehog protein secreted by the oral epithelium is the most important signal protein for the outgrowth of the palatal shelves [7]. Wnt5a, Pax9, and Osr2 are involved in the elevation of the palatal shelves. Several genes are involved in the process of palatal fusion, especially interferon regulatory factor 6 (IRF6). In humans, the loss of function of IRF6 results in cleft palate [19]. An additional important signaling pathway is the FGF signaling [20].

Summary: The development of the craniofacial complex—the neuro-cranium and the viscerocranium—is complex and is dominated by the activity of many genes. There is a close association between its development and the development of the brain. Hence, abnormalities of brain development may result in craniofacial dysmorphism that may often be typical for a variety of clinical syndromes.

4.3 Craniofacial Dysmorphism

4.3.1 Dysmorphology

Dysmorphology is the study of structural birth defects of prenatal origin affecting the anatomy (morphology) of the individual. It is an important tool in clinical genetics focusing on standardizing the descriptive terminology used to define deviations from the normal structure. Many *birth defects* affecting the craniofacial complex have a significant and important effect on the appearance of the face, head, and neck (facial dysmorphic features), because they generally affect the different derivatives of the embryonic branchial arches [21].

Generally, the abnormal features of the face are continuous, being above or below 2 standard deviations from the mean and can be measured. Microotia (small auricles) or hypertelorism (increased distance between the pupils) are such examples [21, 22]. In addition, there are also discontinuous features causing facial dysmorphism, for example, pre-auricular ear pits that are not observed in the normal face.

If such deviations are present in isolation, they are considered as minor malformations and have very little clinical importance. However, if there is a combination of several "minor" dysmorphic features, they may be part of a wider clinical entity constituting a specific syndrome with significant clinical importance, for example, the distinct facial dysmorphic features in children with trisomy 21 (Down syndrome) or of trisomy 18 or the craniofacial features of other chromosomal abnormalities [21]. In the last decade, computer-based 3D face shape modeling is being used for a better delineation of facial dysmorphology [22, 23].

The overall prevalence of craniofacial malformations is high, but the total prevalence is largely unknown, as most studies report on the rate of individual craniofacial malformations. In the USA, for example, when Kirby reported in 2017 the rate of major congenital malformations, he only reported the prevalence of orofacial defects (cleft lip with or without cleft palate or isolated cleft palate) that was 17/10,000 birth (0.17%) [24]. In Europe, the EUROCAT registry does not include craniofacial anomalies as one group but includes several major craniofacial malformation each one as a separate diagnosis.

4.3.2 Malformations of the Craniofacial Complex

Since there are numerous different clinical entities with craniofacial dysmorphic features and malformations, it is impossible to discuss each one separately or use a specific classification. We will therefore discuss the more common malformations that constitute specific entities (i.e., oral clefts or craniofacial microsomia) or discuss several more common malformations of genetic, multifactorial, or teratogenic etiology. In many genetic syndromes with craniofacial dysmorphism, there are also malformations of dentition and/or occlusion. These dental anomalies will not be discussed.

4.3.3 Craniofacial Microsomia (CFM)

This is a spectrum of genetic and non-genetic craniofacial malformations characterized by a wide range of phenotypes differing in severity. Treatment depends on the degree and location of deformities of the facial structures and the presence of other congenital malformations. The characteristic facial malformations are microtia and mandibular hypoplasia (micrognathia), either isolated or combined, as found in over 50% of the cases. Less common dysmorphisms are orbital abnormalities and facial soft tissue abnormalities [25–27]. Caron et al. [25] studied 755 patients with craniofacial microsomia and found various malformations of first and second pharyngeal arch derivatives with unilateral or bilateral distribution and a high rate of extra-facial malformations. Birkfeld et al. [27] described a method of defining the typical facial dysmorphic features from facial photographs with about 90% precision compared to physical examination.

There are several types of classification systems of craniofacial microsomia. The commonly used classification is termed "OMENS" considering the orbit, mandible, ear, nerve, and soft tissue malformations. A more recent classification system—a modified version of OMENS—has since been published (OMENS PLUS), which is used when non-craniofacial structures are also involved [28, 29].

The mechanism behind CFM is thought to be related to the development of the first two pharyngeal arch structures. Any disruption of the complex interactions in craniofacial development, as well as abnormalities in facial blood supply, can lead to developmental abnormalities of this complex. Among the more common clinical-morphological presentations are mandibular and auricular malformations as well as abnormalities of masticatory muscles [25].

Central nervous system malformations are relatively common in children with craniofacial microsomia occurring in up to 18%. The more common anomalies are neural tube defects, corpus callosum hypoplasia or agenesis, intellectual disability, and various neurodevelopmental disorders [30]. Abnormalities of cranial nerves are found in slightly less than half. Other congenital malformations are also common, i.e., cardiovascular, oral clefts, and vertebral anomalies.

Treatment is generally surgical, correcting the facial asymmetries or cosmetic problems and the functional deficits, whenever they exist. There is still a debate whether surgery should be carried out early in life, often necessitating several surgical procedures during childhood and adolescence, or late when growth is almost over [25, 26, 28].

4.4 Oral Clefts

4.4.1 Cleft Lip With or Without Cleft Palate

This is apparently the most common craniofacial malformation, with a prevalence of 1/700 to 1/600 livebirth [18, 31, 32]. Cleft lip can be unilateral or bilateral, complete or incomplete, and accompanied or not with cleft palate [18]. This anomaly may be isolated or combined with other malformations as part of defined syndromes or genetic or chromosomal abnormalities. CL/P can be diagnosed during pregnancy by second trimester ultrasonographic evaluations [32]. Treatment is generally complicated and necessitates a team work [18, 32–35].

There are several known etiologies for CL/P, although in most cases the etiology is unknown. About one third of the CL/P cases also demonstrate other malformations since they are part of specific syndromes [32]. Many of the malformations have a genetic etiology (i.e., holoprosencephaly, trisomy 18) and defects in specific genes like FGFR2, BMP4, or van der Woude syndrome with a defect in gene IRF6 [32]. Teratogens are also frequent etiologic factors such as alcohol (heavy drinkers), some antiepileptic drugs, retinoids, methotrexate, and others (see below). Some maternal diseases such as maternal pregestational diabetes and folate deficiency are also associated with a higher prevalence of CL/P. Dental anomalies are generally observed in children with CL/P and therefore necessitate orthodontic and other treatments [34].

Treatment: Treatment is surgical, but due to the complication of the anomaly, it should involve a team as, in addition to the surgical correction of the cleft (lip and palate), there is a need to restore normal dentition, normal speech function, and general facial esthetics. Additionally, there are psychologic, psychosocial, and economic effects on the child and family. The cleft lip is generally corrected around 10–12 weeks of age unless there are contraindications to surgery. If cleft palate is present as well, it is corrected around 1 year of age. Orthodontic treatment and orthodontic devices are generally needed, being an essential part of treatment [34, 35].

4.4.2 Cleft Palate (Isolated Cleft Palate, CPO)

There are large differences in the prevalence of CPO between ethnic groups, ranging from 1.3/10,000 in Africa to the highest in Europe (14.3/10,000 in Finland) [36]. CPO is rarer than CL/P and is more common in females. About half of the cases are part of a syndrome (i.e., DiGeorge syndrome) or occur together with other malformations such as cardiac or renal. CPOs are unilateral, complete or incomplete; bilateral, complete or incomplete; or submucous. Since cleft palate may also affect speech, dentition, and swallowing, there is a need for long-term comprehensive care by a team of professionals, similar to that needed for the treatment of CL/P [34–37].

4.5 Craniofacial Anomalies Induced by Teratogens

Most teratogens that affect brain development may also induce craniofacial malformations as many of them also affect neural crest cells, especially cranial neural crest. The more commonly known human teratogens which cause specific syndromes and craniofacial malformations are folic acid antagonists, especially methotrexate (methotrexate embryopathy), retinoids (retinoid embryopathy), cyclophosphamide (cyclophosphamide embryopathy), mycophenolate mofetil (mycophenolate syndrome), valproic acid, and several other antiepileptic drugs (antiepileptic drug syndrome, i.e., valproate syndrome, phenytoin syndrome, carbamazepine syndrome ext), alcohol (fetal alcohol spectrum disorder (FASD)), and heavy smoking. Exposures that result in craniofacial malformations must generally occur in the first trimester of pregnancy, during major facial organogenesis. These craniofacial anomalies are discussed according to the responsible teratogenic agent.

4.5.1 Methotrexate Embryopathy

Methotrexate is a folic acid analog with an antifolate activity as it inhibits dihydrofolate reductase resulting in a decrease in tetrahydrofolate needed for various metabolic pathways and for gene expression [38]. Methotrexate is used today for the treatment of some types of cancer, to induce embryolethality in cases of extra-uterine pregnancies and in low doses for immunosuppression in autoimmune diseases. It is a well-established teratogen affecting most animals [38–40]. If administered during pregnancy, it may cause frontonasal dysplasia and a specific pattern of malformations – the methotrexate syndrome, also affecting the craniofacial complex [41]. The typical craniofacial malformations are hypoplasia of skull bones, “clover-leaf” skull with wide fontanelles and a large head, low-set ears, prominent eyes, wide nasal bridge, micrognathia, maxillary hypoplasia, and other facial dysmorphic features [38–40]. There are additional CNS malformations including anencephaly, hydrocephaly, and/or meningomyelocele. In addition, there are neurobehavioral disorders, including mental retardation.

4.5.2 Retinoid Embryopathy

Retinoids (13-cis retinoic acid and all-trans retinoic acid) are a group of drugs whose teratogenicity was suspected prior to their clinical use as a treatment for acne and for psoriasis and recently

for pro-myelocytic leukemia [41–46]. They are natural derivatives of vitamin A, and therefore low levels of 13-cis retinoic acid are normally present in the blood, but high levels are highly teratogenic affecting about 30% of the exposed fetuses and often causing the retinoid syndrome (embryopathy). Only systemic treatment with retinoids seems to be teratogenic as very little retinoid is absorbed following topical use, and topical use is not associated with retinoid embryopathy [43]. Retinoids may increase malformations of the heart, brain, ears, eyes, face, and limbs [43–48]. The typical craniofacial malformations are as follows: microotia and various auricular abnormalities, agenesis or stenosis of the external auditory canal that often leads to hearing impairment and deafness, damage to the middle or inner ear, and facial and palatine abnormalities. They may also affect the brain causing hydrocephalus and a variety of neurological, cognitive, and neurobehavioral disorders including mental retardation [47, 48].

4.5.3 Cyclophosphamide Embryopathy

This alkylating agent is used for chemotherapy and in small doses as an immunosuppressive agent. First trimester exposure has been associated with embryonic and fetal death, intrauterine growth restriction, and various craniofacial malformations including eye anomalies, cleft palate, micrognathia, low- set ears, microotia, hearing defects, craniosynostosis, and facial asymmetry, as well as malformations of the brain (hydrocephaly), limbs, and eyes [49–55]. Treatment in the second or third trimester of pregnancy has been associated with increased fetal death but no specific malformations. Low doses may be safe, but as there seems to be no data, treatment is as yet contraindicated [56].

4.5.4 Mycophenolate Mofetil

This immunosuppressive drug is used in organ transplantation or for the treatment of autoimmune diseases such as lupus and rheumatoid arthritis [57]. This is a relatively "newly recognized" teratogen, mainly affecting the craniofacial complex [57]. The main craniofacial anomalies are microotia, anomalies of the external ear and auricles, conductive hearing loss, cleft lip/palate, micrognathia, microphthalmia, cataracts, coloboma of the retina, and dental anomalies [58–60]. Often, there are also other malformations of the kidneys or heart and/or tracheo-esophageal atresia. Various brain anomalies have been reported as well, including meningocele, hydrocephaly, and agenesis of corpus callosum [57]. There is also a high risk for spontaneous abortions and intrauterine death. However, the accurate risk for malformation is not yet determined although it may be high [57, 61]. Hence, this drug is contraindicated in pregnancy.

4.5.5 Valproic Acid (VPA) and Other Antiepileptic Drugs

Antiepileptic drug syndrome (AEDS): Antiepileptic drugs are generally used to control seizure disorders, and some are also used as mood stabilizers in psychiatric disorders. As a group, many of these drugs, especially the "older" ones, are known teratogens inducing a variety of congenital malformations as well as neurodevelopmental problems. Of these drugs, VPA, which is an effective mood stabilizer and antiepileptic drug, seems to be the most teratogenic [62–65]. VPA, if taken during pregnancy, is known to cause neural tube defects (NTD) in 1–2% of the offspring as well as cardiac, skeletal, and limb defects and a specific craniofacial dysmorphism—the "fetal valproate syndrome." In addition, VPA may affect development, inducing speech and language delay, reduced cognitive abilities, and increased rate of autism spectrum disorder (ASD) [62, 64]. The typical craniofacial abnormalities include long and thin upper lip, shallow philtrum, epicanthal folds, mid-face hypoplasia with flat nasal bridge, small nose, and upturned angles of the mouth.

Facial dysmorphic features have also been described following maternal use of other anti-

epileptic drugs, especially phenytoin, phenobarbital, and carbamazepine [65–67]. For example, of 47 children prenatally exposed to carbamazepine, we found 6 with typical facial dysmorphism (carbamazepine syndrome) and developmental delay [67]. The typical facial dysmorphic features observed in the antiepileptic drug syndrome are hypertelorism, flat nasal bridge, low-set ears, reduced head size, and sometimes oral clefts. There are some minor differences in presentation among individual drugs [67, 68].

4.5.6 Alcohol (Ethanol) Embryopathy

Ethanol is a well-known teratogen affecting the brain and several other organs such as the heart and palate [69–72]. There seems to be a dose response regarding the extent and severity of damage. It is apparently the most important teratogen worldwide due to the habits of alcohol drinking [69]. The craniofacial abnormalities are generally found during infancy and childhood because with aging they become less distinctive [69]. These children may exhibit the "fetal alcohol spectrum disorder" (FASD). Maternal drinking of small amounts of alcohol may result in the fetal alcohol effects, with fewer clinical signs, that are more difficult to diagnose [69]. The alcohol-induced abnormalities include prenatal and postnatal growth deficiency, central nervous system dysfunction including mental retardation, hyperactivity, antisocial behavior, and increased tendency for substance abuse [69]. The abnormal facial features include small head size (microcephaly), short palpebral fissures, epicanthal folds, hypoplastic smooth philtrum, thin upper lip, flattened maxilla, short nose, and low-set ears [69]. Due to the variability of the clinical findings, it may be difficult to diagnose alcohol-induced abnormalities without a history of alcohol ingestion, and therefore several guidelines for the physical examination of children suspected to have FASD were published [71]. The specific craniofacial dysmorphic features in FASD are explained by the damage to neural crest cells induced by alcohol [73]. A major mechanism of alcohol-induced embryopathy is increased oxidative stress affecting the developing embryo and fetus, mainly due to the poor antioxidant capacity of neural crest cells and brain tissue.

Another well-established mechanism is related to the epigenetic effects of alcohol that may induce changes in the expression of various embryonic and fetal genes [74]. Generally, several studies demonstrated enrichment of H3K9ac, H3K27me2 and H3K27me3, and H3K9me2 and increased expression of histone acetyltransferases and methyltransferases [75].

4.5.7 Smoking and Oral Clefts

While the damaging effects of maternal smoking on the developing embryo and fetus are generally dose dependent, especially reduced neonatal weight and neurodevelopmental problems [76–78], there are inconsistent data regarding congenital malformations. It seems, however, that there is sufficient data to demonstrate that smoking early in pregnancy could increase the rate of CL/CP and of CPO. In a recent meta-analysis by Xuan et al. [77] analyzing 29 studies, the OR for CL/CP was 1.368 (95% CI 1.259–1.486) and for CP 1.241 (95% CI 1.117–1.378), both being of statistical significance ($P < 0.05$). Several studies also observed an increase in various cardiac malformations, especially if both parents smoked [79]. No increase in external facial dysmorphism was reported.

4.6 Craniosynostoses and Primary Abnormalities in the Shape of the Skull

This is a group of entities where the primary morphological manifestations (dysmorphism) are in the shape of the skull, often with secondary effects on the face. They are characterized by premature closure of one or more of the calvarial bone sutures. Most of these abnormalities are

isolated closures of specific sutures, but about 10% constitute specific syndromes (syndromic craniosynostoses). The general occurrence is about 1/2000 to 1/2500 liveborn infants [80]. These disorders may have imperative effects on the brain, generally interfering with its growth and development, and often also interfere with dentition or cause malocclusion [80–82]. They are divided into syndromic and non-syndromic craniosynostoses. We will discuss some of these more common syndromes.

4.6.1 Syndromic Craniosynostoses

These constitute specific defined syndromes with genetic backgrounds, as they are generally inherited as autosomal dominant diseases or result from de novo mutations [81, 82]. The more common mutations are in the following genes: genes of the FGF receptors (FGFR1, FGFR2, FGFR3, TWIST1 as in Apert, Pfeifer, Antley-Bixler, Crouzon, Muenke, and Saethre-Chotzen syndromes, respectively, all having a chromosomal dominant inheritance). Mutations in EFNB1 (cranio-fronto-nasal syndrome) are X-linked dominant. It is quite certain that more mutations will be found in the future [82].

In syndromic craniosynostoses there are often also extracranial malformations, especially of the heart, respiratory tract, and limbs [81, 82]. Sometimes, neurodevelopmental problems including mental retardation are integral features of the syndrome [82, 83]. A computerized program for the identification of the different craniosynostoses was developed by Shim et al. [84], but the accurate diagnosis is generally carried out by the identification of the affected gene [81, 82]. Prenatal diagnosis is possible by ultrasonography and the use of additional imaging techniques (MRI) and/or by genetic studies of fetal cells.

Treatment is aimed to avoid the possible deleterious effects on brain growth and correct the cosmetic and/or functional facial and skull deformities, including any dental and orthodontic problem or interference with mastication, swallowing, or respiration [85].

4.6.2 Specific Syndromes

1. Apert syndrome: This syndrome is characterized by premature closure of multiple calvarial sutures and syndactyly of the fingers [82, 86, 87]. The craniofacial dysmorphic features are often flat forehead, hypertelorism, retracted mid-face, and low-set auricles [82]. There may be severe malformations of other organs, especially the cardiovascular system. The differences in the severity of the facial dysmorphism are related to the specific mutated locus, whether S252W or P253R in the FGFR2 gene [87]. The cognitive abilities vary from normal intelligence to moderate-severe mental retardation.
2. Crouzon syndrome: The main anomaly is coronal suture synostosis, but there may be premature closure of several other sutures. The craniofacial manifestations are frontal bossing, maxillary hypoplasia, and micrognathia. Typical facial features are shallow orbits, ocular proptosis, and strabismus. In about one third, there is development of hydrocephalus and in over half hearing loss [88]. In the majority of cases, the mutation is in FGFR2 gene and in a minority in FGFR3 gene [82].
3. Pfeiffer syndrome: In addition to craniosynostosis of several cranial sutures, there are broad thumbs and big toes and often also partial syndactyly. Sometimes hydrocephalus, ankyloses of the elbows, and proptosis as well as mental retardation are also observed [82]. The mutation may be on FGFR1 or FGFR2, with complete penetrance but variability in the clinical expression. Proper prenatal imaging or molecular studies enable prenatal diagnosis [89].
4. Antley-Bixter syndrome: In addition to synostosis of cranial bones, there are typical synostoses in other joints of endochondral bones, especially in radio-humeral and radio-ulnar joints. Frontal bossing and mid-face hypoplasia are also prominent features. Cardiac and renal anomalies are also common [82]. Of the two possible affected genes FGFR2 and POR, those with POR mutations also have congenital adrenal hyperplasia and ambiguous genitalia

[90]. Prenatal diagnosis seems possible by CT and MRI of the developing fetus [91].

5. Saethre-Chotzen syndrome (acrocephalosyndactyly type III syndrome): The coronal suture is generally affected uni- or bilaterally. Additionally there are limb malformations and sometimes synostosis of additional cranial sutures. Low-set ears, hearing loss, ptosis, and hypertelorism are the more common facial malformations. Intelligence is generally normal [82, 92]. The affected genes are TWIST1 or FGFR2 [93].
6. Muenke Syndrome: Typically, there is coronal suture synostosis. The facial and extracranial malformations are quite similar to those of Saethre-Chotzen syndrome and include hearing loss, developmental delay, downslanting palpebral fissures, proptosis, and limb malformations [82, 94]. The common mutation is in FGFR3 gene [94].
7. Cranio-fronto-nasal syndrome: Both coronal sutures are affected by premature closure. There are typical mid-facial malformations including bifid nasal tip, CL/P (or at least high arched palate), frontal bossing, hypertelorism, and dental anomalies. Limb malformations are common [95]. The affected gene is EFNB1on chromosome Xq12. Inheritance is therefore sex linked dominant.

4.6.3 Non-syndromic Craniosynostosis

This type of craniosynostosis constitutes 80–90% of all cases of craniosynostosis [80]. There are multiple etiologies including genetic, environmental, and intrinsic bone abnormalities, but in many cases the etiology is unknown [80, 96, 97]. The sutures that close prematurely will dictate the shape of the head. As the growth of the bones perpendicular to the affected sutures is inhibited, the brain may expand in other directions causing distortion of the skull and face. In many cases, dentition is affected too. Treatment and prevention of complications are similar to that of the syndromic craniosynostoses. Prenatal diagnosis is possible in some cases depending on the intrauterine changes in calvarial shape or other anomalies observed by routine prenatal ultrasonographic screening. Optimal treatment is by a multidisciplinary team including neurosurgeons, plastic surgeons, dentists especially orthodontists, psychologists, social workers, and sometimes speech therapists [80, 96, 97]. Since these are all rare syndromes, we will not discuss them further.

4.7 Genetic Syndromes Affecting Extracranial Organs with Craniofacial Manifestations

In many genetic syndromes that primarily affect extra-cranial organs and systems, there are also craniofacial and dental malformations which might even be important diagnostic markers. The following is a list of the more common syndromes that also exhibit craniofacial manifestations [21]. These are most chromosomal trisomies, triploidy, most chromosomal deletions and microdeletions, Ataxia telangiectasia, Carpenter syndrome, cleido-cranial dysplasia (dysostosis), ectodermal dysplasias, Golge syndrome, Gorlin syndrome, Hallerman-Streiff syndrome, Miller syndrome, Moebius syndrome, Nager syndrome, neurofibromatosis 1, neurofibromatosis 2, Stickler syndrome, and velocardiofacial syndrome. This partial list emphasizes the importance of craniofacial dysmorphology in the diagnosis and treatment of many genetic disorders.

4.8 Anomalies of Dentition and Craniofacial Malformations

The development of the teeth is intimately connected with the development of the craniofacial complex. It involves reciprocal inductive interactions between epithelium and mesenchyme with cranial neural crest cells playing a crucial role as progenitors of the teeth [98, 99]. Shh and Wnt signaling is responsible for many of the

developmental phases of dentition in combination with bone morphogenetic proteins (BMP) and fibroblast growth factor (FGF) [98–101]. Shh signaling, in addition to its crucial role in craniofacial development, also plays an important role in the initiation of dental lamina formation and tooth number and continues into more advanced steps of the morphogenesis of individual teeth [101, 102]. Thus, disruption of the Shh signaling may cause abnormalities in teeth number, shape, and position as well as craniofacial and brain malformations [102].

Abnormalities of dentition and of the jaws can be isolated but are more often just one of the additional clinical signs of defined syndromes affecting the craniofacial complex and/or the brain [103–105]. Typical examples are the oral clefts which are generally also accompanied by orthodontic and dental anomalies [105]. However, as this review deals primarily with craniofacial malformations, the involvement of dentition will not be further discussed. It only stresses the importance of dentists in the multidisciplinary team that is mandatory for comprehensive treatment of these malformations.

4.9 Conclusions

Many cases of craniofacial abnormalities are of genetic or mixed genetic and environmental origin (multifactorial). Often they are part of a genetic syndrome with variable clinical manifestations. This review highlights the importance of craniofacial anomalies among the large group of congenital malformations. These anomalies impose not only a cosmetic problem, presenting craniofacial dysmorphic features, but may also interfere with important functions like chewing, swallowing respiration, speech, and abnormal dentition. The close relation between the development of the brain and the craniofacial complex induces various, but specific, craniofacial changes in a variety of brain disorders. Craniofacial malformations sometimes serve as a tool for the identification of the brain abnormalities. In addition, primary anomalies of the craniofacial complex might secondarily affect the brain, as occurs with the various craniosynostoses. As many craniofacial malformations can be visualized by imaging techniques in utero, or by genetic molecular studies, it is important to be aware of these possibilities and pay meticulous attention to possible craniofacial malformations during routine ultrasound examinations in pregnancy, especially in the second and third trimesters.

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5 Morphometrics, Optical 3D Imaging, and Monitoring of Craniofacial Development and Malformations

Helena Sophie Visse, Christoph Runte, Ulrich Meyer, and Dieter Dirksen

5.1 Introduction

The Nefertiti bust is one of the oldest preserved realistic three-dimensional images of a human head. It is assumed that the sculpture from limestone coated with stucco was made around 1345 BC in ancient Egypt [1]. Despite its old age, the artists have already created an almost perfectly symmetrical face at this time [2]. In the course of the centuries until today, humans have tried to create even more accurate images of their peers [3]. Whether in the form of sculpture or carving, the techniques have become more and more detailed. Thus, in the fifteenth century, Leonardo da Vinci invented the "dotting box": a box that could measure an inner object three-dimensionally with the help of rods that were pushed through the box's walls against the object. The technique was advanced by Sauvage (1785–1857) who, through his physionotype, was able to capture the face of a living model or bust three-dimensionally using a needle pattern solidified by wax (Fig. 5.1) [6]. The production of death masks was also part of history from the beginning of civilization until today. The three-dimensional impressions of the face, which mostly consist of plaster or wax, were formerly used as burial masks for burial gifts and more recently as memorabilia for relatives. With the beginning of digitalization, technologies for three-dimensional object acquisition have replaced the classic copying processes.

5.2 Cephalometry

The term cephalometry describes the measurement of bone and soft tissue cranial structures. The measurement is carried out using defined landmarks, which are marked on the two- or three-dimensionally captured object. Radiologic cephalometry was first introduced in the 1930s in the United States by Broadbent, as well as in Germany by Hofrath [7, 8]. The cephalometric analysis of these two orthodontists is until today one of the diagnostic basics in orthodontics and maxillofacial surgery. Additionally, two-dimensional photographs are in routine clinical use as diagnostic tools in orthodontics.

With the invention of three-dimensional radiological techniques such as computer tomographs and magnetic resonance scanners in the 1970s, three-dimensional measurement technology made its way into the field of medicine [9]. By reducing the radiation dose of modern computed tomography—especially the cone beam computed tomography—the radiological 3D image often replaces the conventional two-dimensional radiograph as

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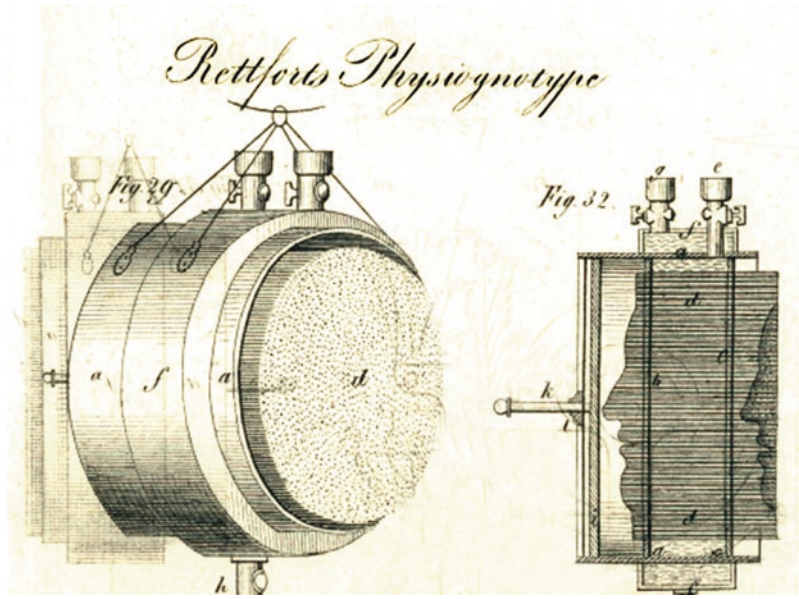

Fig. 5.1 Physionotype invented by Sauvage. A patent for this type of machine was granted to R. Rettfort in 1834 [4, 5]

standard diagnostics, because it allows combined hard and soft tissue examination [10, 11]. Whereas layered 3D investigations give information on both surface and inner structures, optical scans are able to gain a more precise representation of the skin.

5.3 Medical Applications

The application spectrum of 3D scanners in medicine is broad and ranges from orthopedics, plastic surgery, and dentistry to bionics. For cranial and facial 3D scans, mainly stationary systems, some of which are equipped with mirrors for all-round detection, have been used so far. However, mobile handheld scanners are becoming increasingly popular (Fig. 5.2) [12–16]. In addition, systems are beginning to emerge that use the sensors of a smartphone and do not require expensive additional hardware.

Digitization and improved technologies are opening increasing possibilities for medical diagnostics. Objects can be scanned, viewed, and measured in the computer. Dental or facial prostheses can be designed on a computer and manufactured with a 3D printer [17]. Sonography allows a three-dimensional imaging [18], and surgical results can be predicted in advance with dedicated software [19, 20].

5.4 Scanning Techniques

While tactile mechanical coordinate measuring machines are still an important tool for precise surface measurement in industrial applications, the optical measuring systems that have been emerging since the 1980s offer the advantage of contact-free operation. The techniques available today for the acquisition of object surfaces, in particular the craniofacial domain, are capable of delivering hundreds of thousands of 3D coordinates in fractions of a second [21].

5.4.1 Photogrammetry

This method forms the basis for many optical 3D scanning techniques. One or more cameras are used to capture the object from at least two different perspectives. Prior to the measurements, the system must be calibrated. This means that the imaging properties of the cameras and their relative positions must be determined in advance. By identifying corresponding pixels of object points, their 3D coordinates then can be calculated. In the case of two cameras, this method is similar to that of spatial vision in humans [21].

Fig. 5.2 Mobile handheld 3D scanner (Artec, 3D Systems, Rock Hill, South Carolina, USA)

5.4.2 Structure from Motion (SFM)

Structure from motion describes a special variant of a photogrammetric system that uses a single camera to capture a (video) sequence during a motion around the object. As with the system described above, the software identifies corresponding points and edges in the image sequence. The calibration is done along with the 3D coordinate calculation as so-called bundle adjustment [22].

This scanning method is increasingly available as software in smartphones. Its advantage is simplicity and the fact that no additional equipment other than camera and SFM software is required [23].

5.4.3 Structured Light

In this method, a light pattern is projected onto the object. Depending on the surface structure, the light pattern is deformed. Typically, a striped or a grid light image is employed for identification of object points, especially if the object does not provide sufficient texture. One or more cameras record the projected patterns, and photogrammetric techniques are used for surface reconstruction (Fig. 5.3).

The light projected onto the object may be either static or dynamic. The advantage of the *static method* is its speed. In contrast to sequential methods, only one frame is used. However, this benefit is at the expense of the achievable resolution. The limitation is determined by the distance that the projected elements of the pattern must maintain from each other in order to be identified by the image processing algorithms.

This problem is addressed by a *dynamic light* pattern, where a sequence of patterns is projected onto the object. Typically, rectangular fringe patterns with different spatial frequencies are employed. They allow the identification of object points through the recorded sequence of dark and light areas. The resolution can further be improved by additionally projecting a sequence of phase-shifted sinusoidal fringes. In addition, these methods reduce interference with other light sources [22].

A projector, one or more cameras, a computer, and software are needed. In combination, these tools form the basis for the fringe projection technique, which currently represents the gold standard for high-precision surface reconstruction [25].

5.4.4 Time-of-Flight Cameras

A time-of-flight system uses light pulses that illuminate the object and are in turn detected by a sensor. The system's sensor (rangefinder) measures the time it takes the light to travel to the object and back. The light propagation time is proportional to the distance to the object.

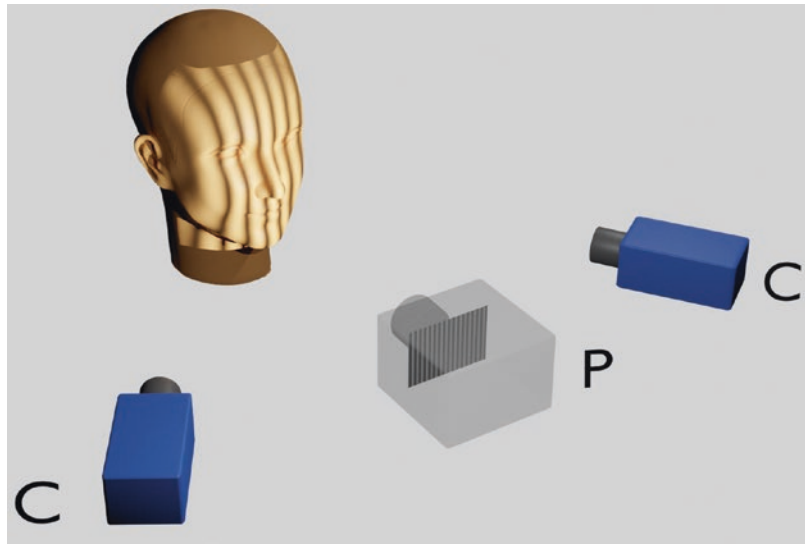

Fig. 5.3 3D scanner employing the fringe projection technique with projector (P) and two cameras (C). This technique has proven to be effective in capturing facial morphology [24]

Since the speed of light c is a known quantity (its inverse being 3.3 picoseconds/mm), the distance d between the light source and the object can be calculated as a function of time t. The path of the light corresponds to twice the distance to the object.

$$d = \frac{c \times t}{2}$$

The accuracy of this method essentially depends on the precision of the time measurement. Usual time-of-flight scanners can measure the distance of 10,000–100,000 points per second. This system requires an illumination unit that emits the light pulse, an optical system for detection, a sensor that measures the propagation time for each pixel, control electronics that coordinate the illumination and the sensor, and an interface that converts the readings into a distance.

The time-of-flight measurement is particularly advantageous because its principle is very simple, and it can evaluate data very efficiently at high speed. In addition, this system does not depend on patterns and therefore also works with highly reflective materials [21, 26].

5.4.5 Accuracy

The resolution achievable by the different techniques depends on various parameters. In the case of the fringe projection technique, these are the angle between camera(s) and projector, the size of the image field, and the resolution of the camera sensors as well as the optical properties of the surface under investigation. Bischoff et al. estimate for their system a resolution of 0.6 mm in the lateral direction and 0.2 mm in the axial direction in a measurement field of 600 mm × 450 mm when scanning human skin [27].

According to Amornvit and Sanohkan, the accuracy of facial 3D scans depends not only on the length and the pattern of scanning but also on the scanning method. In their study, the facial scanner that works with structured light [EinScan Pro 2X Plus (Shining 3D Tech. Co., Ltd. Hangzhou, China)] scored best. Laser scan [Planmeca ProMax 3D Mid (PM) (Planmeca USA, Inc., Hoffman Estates, IL, USA)] and structure from motion [iPhone X with Bellus3D app, including dot pattern projection (Apple Store, Cupertino, CA, USA)], on the other hand, showed a worse performance [16]. In another study the highest accuracy was achieved with a time of flight scanner (ca. 70 µm), followed by a stereophotogrammetry scanner with structured light (ca. 90 µm). A CT scan was used as reference [25]. For some scanners, setting landmarks beforehand can increase accuracy [28]. Mobile 3D handheld scanners showed comparable accuracy and reliability compared to conventional, stationary systems [14].

5.5 Clinical Application

Structured light scanners and stereophotogrammetry are equally suitable for the clinical application of optical three-dimensional scanning procedures in the facial area, as Zhao et al. concluded in their study [29]. Since the optical acquisition, in contrast to radiological imaging, does not lead to any ionizing radiation exposure, 3D scanning methods are very well suited for analysis of surface structures and follow-up checks. Growth in young children can be monitored over several years, and the three-dimensional images can be compared [30]. Particularly noteworthy is the advantage that optical images can be taken in a few milliseconds, which reduces motion artifacts. Thus, it is not a prerequisite to keep still for a longer period. This makes the technology particularly interesting for use with young children [31, 32].

This technique also enables the analysis of facial structures and facial expressions in the course of age [33] [34], as well as the comparison between healthy and affected patients [35] or the detection of facial defects after tumors or accidents [25]. 3D imaging by optical scanners is a standard method for monitoring the progress of molding helmet therapy in infants, and in most cases head orthoses are also made from a positive of the head produced by 3D printing [36].

Especially in the fields of maxillofacial surgery and orthodontics, where aesthetics and symmetry are particularly important, the three-dimensional recording of faces offers new possibilities. The benefits are mainly related to documentation, preoperative planning, and postoperative assessment [37]. For example, it is possible to compare the condition of the soft tissue after an osteotomy operation with the initial condition [38–40]. 3D data for intraoperative use, such as guided surgery or the insertion of individual implants, are usually generated from computed tomographic scans.

As a comprehensive classification of craniofacial malformations needs beneath the clinically driven embryologic classification and the laboratory-based genetic classification a precise documentation of the phenotype, standardized 3D scans allow the determination of the disease severity (Fig. 5.4). New devices enable clinicians to document the whole body by one scanning procedure, if wanted.

5.5.1 Augmented Reality

The term virtual reality describes a virtual scene that visually completely replaces the real environment. In augmented reality, on the other hand, images of the real world are overlaid with digital content [41].

In modern medicine 3D imaging systems [42] and software can also visualize in advance possible results for patients with high aesthetic standards or those who explicitly visit a surgeon or dentist for aesthetic corrections [43]. First systems are available on the market that can scan and display the desired results in real time [44], so that the patient gets an instant visual impression of the expected result. In dentistry patients can try on their future prostheses to assess the

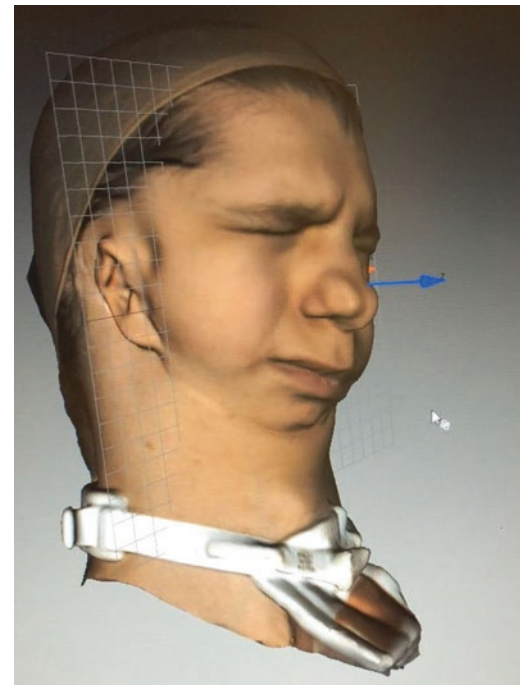

Fig. 5.4 Photorealistic 3D scan of a patient with Franceschetti syndrome. The underdeveloped maxillary and mandibular complex led to a tracheostomy since childhood

aesthetic outcome [45]. The patient can use the front camera of a smartphone or tablet like a mirror. The software then shows the predicted result on the screen [46]. However, a smartphone is not sufficient to consider the functional as well as the aesthetic aspects. This requires an optical intraoral scan [47].

Augmented reality has also found its way into the operating room: modern techniques enable the surgeon, for example, to project important structures directly onto the operating field or superimpose the location of perforations [48].

Wearable mixed reality devices like the HoloLens (Microsoft Corporation, Redmond, Washington, USA) provide a mix of virtual and augmented reality. They offer surgeons access to real-time, multimodal information without disrupting the surgical workflow [49]. These devices are worn as glasses and can display all kinds of necessary information [50].

5.6 Radiological Detection of Facial and Cranial Structures

Radiological systems such as computed tomography or magnetic resonance tomography do not capture point clouds from their recorded data. The data comprises a stack of two-dimensional slices that form a three-dimensional volume that can be represented by small volume elements (voxels).

The data can be visualized and evaluated by different volume rendering methods. A common approach includes the determination of iso surfaces of radiological densities and their representation as polygonal surfaces. Alternatively, direct volume rendering techniques may be applied, e.g., by mapping values for opacity and color to each voxel [51].

The main disadvantage of these techniques in respect to facial surface structure documentation is the voxel-based approach. Especially, when larger objects like a whole skull are under investigation, resolutions are quite poor, especially in the direction perpendicular to the used layer plane. Even modern MRI or CT machines go down to a layer resolution of up to 1 mm.

5.7 Symmetry Analysis

The ideal human face exhibits mirror symmetry in relation to the median sagittal plane, from which the real one deviates to a greater or lesser extent. This deviation is a significant factor in aesthetic perception and can, in extreme cases, reflect a pathological situation. For the quantification of this asymmetry, different measures based on the 3D analysis of the skull or face were proposed. These may include the analysis of areas, sections, or individual characteristic landmarks [52–55]. Two methods will be presented here as examples.

5.7.1 Cranial Vault Asymmetry Index (CVAI)

In order to determine the severity of axial head deformities, the Cranial Vault Asymmetry Index is commonly used in the current literature. This index is based on the two-dimensional evaluation of the cranial contour. The nasion and the two tragi are marked on the outline of the head—seen in caudal direction. The connecting line between the tragi defines the cranial width. The cranial length is, as shown in Fig. 5.5, a

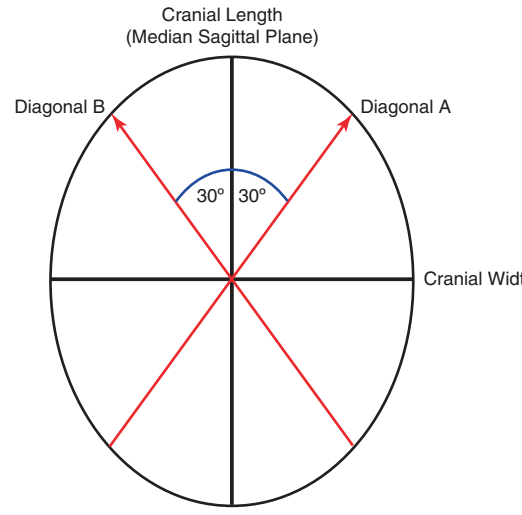

Fig. 5.5 View on the cranial contour from an axial direction. Diagonals in a 30° angle to the cranial length

straight line through the nasion point at a 90° angle to the cranial width. Starting at the intersection of these two lines at a 30° angle to the cranial length, two diagonals are drawn through the cranial contour (Fig. 5.5). The Cranial Vault Asymmetry Index is then the relative length difference between these two diagonals, based on the points of intersection with the outline of the head [53].

It is calculated as the difference between the length of the two diagonals multiplied by 100 and then divided by the length of the longer diagonal. The CVAI is given in percent. A CVAI >3.5% is considered asymmetric [53].

$$\text{CVAI} = \frac{\left(\text{Diagonal } A - \text{Diagonal } B\right) \times 100}{A} \quad \left(\text{if } A > B\right)$$

5.7.2 The 3D Asymmetry Index (3DAI)

One approach for 3D symmetry analysis is based on calculation of the mean distance between the original 3D surface and its mirrored and matched copy. It is a modification of a method proposed by Benz et al. in 2002 [56].

The original surface and its mirrored copy are matched (registered) employing the iterative closest point (ICP) algorithm [57], thus minimizing the distance between them. This process is repeated iteratively with refined mirror planes calculated from the centroids of corresponding points of the original surface and its mirrored copy (Fig. 5.6) [27, 58].

The final symmetry plane is the estimated median sagittal plane. The remaining asymmetries between the two surfaces can be visualized by a pseudo-color scale as seen in Fig. 5.6. An asymmetry index 3DAI may be defined as

$$\frac{d}{D} \times 1000$$

with d denoting the mean distance between the two surfaces and D being the diagonal of the bounding box that encloses the face [59].

5.7.3 Landmarks

Morphometric landmarks can be defined as anatomical points located on the facial surface. Landmarks occur either individually or in pairs. In a right-left comparison, commonly used in bilaterally symmetrical organisms, the individual points are on the median sagittal plane, and the pairwise points are about the same bilateral distance from it (Fig. 5.7) [61].

A comparison of two similar faces or a symmetry calculation can be performed by measuring the deviation of each individual point from the median sagittal plane and the difference in distance of the paired points from that plane [62].

In addition, standards are defined by reference values, giving the ratio or distance between certain points [63].

The analysis of the lateral cephalogram, which works with landmarks, has been part of standard diagnostics in orthodontics and combined dysgnathia surgery for almost 100 years [11]. This complex analysis, which was previously carried out manually, can now be carried out fully automatically by software that recognizes and analyzes landmarks [64]. Such an analysis can also be performed with facial images [65, 66]. The manual setting of

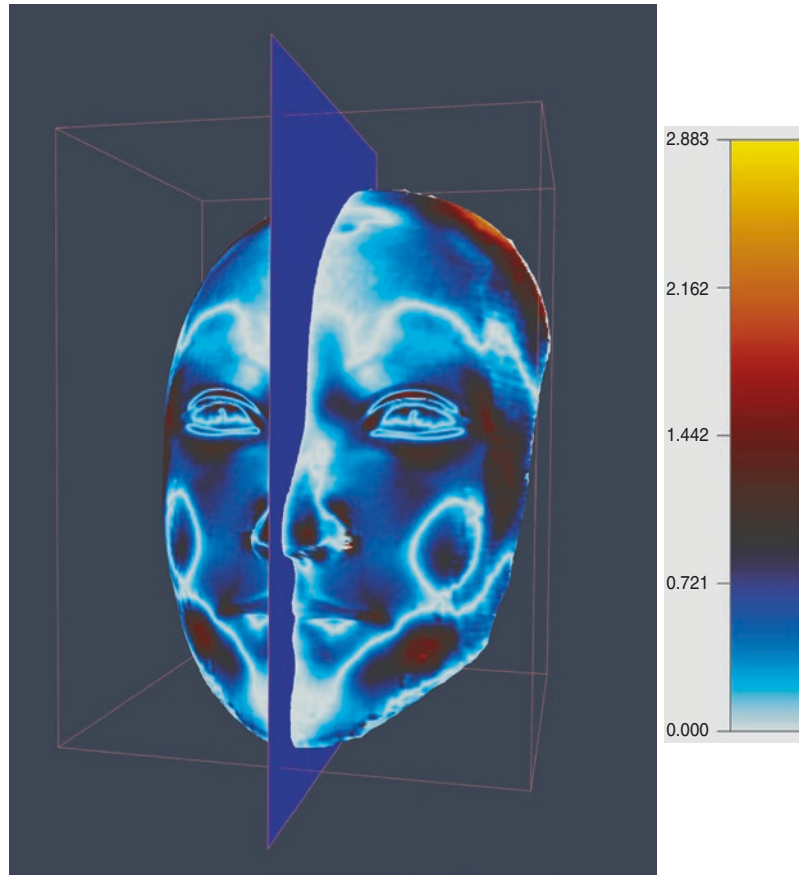

Fig. 5.6 Visualization of the asymmetrical areas of the face by superimposing the original face surface with the matched mirrored copy. Pseudo-color scale with distances in mm. The symmetry plane is the estimated median sagittal plane

landmarks on the patient can, however, improve the precision of anthropometric software [28].

Modern 3D surface scanners offer a reliable and accurate diagnosis of the soft tissue even without additional hard tissue information [67]. The analysis of the hard tissue should not be used to draw any direct conclusions about the soft tissue. Morphological differences between hard and soft tissue should always be considered separately [68].

5.8 Shape Analysis

Within this context, shapes are described by a fixed arrangement of landmarks. This means that the landmarks are ordered in a fixed sequence (in the example of Fig. 5.7 pairs of pixel coordinates). When quantifying the differences between two shapes, it must be taken into account that the measured coordinates of the landmarks may be provided in different scales and may be located differently in space, which is not relevant for the assessment of a shape. This means that the shapes must first be subjected to a transformation which compensates for these irrelevant differences. For example, in order to compare two different faces defined by characteristic landmarks such as in Fig. 5.7, one of the faces must be transformed to look as similar as possible in size and shape to the first face, regardless of its position in the coordinate system. Two common methods for this are the use of Bookstein coordinates and the Procrustes transformation.

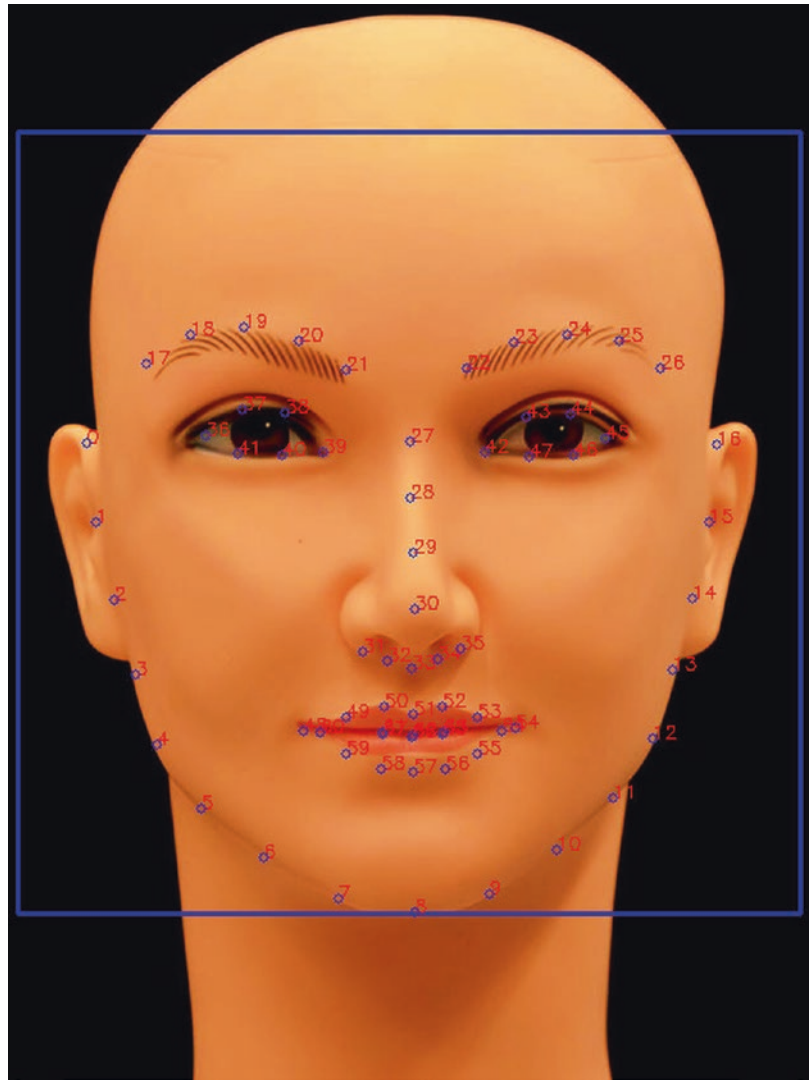

Fig. 5.7 Facial landmarks automatically localized using a machine learning approach [60]

5.8.1 Bookstein Coordinates

A simple approach to the problem is the transformation proposed by Bookstein. In this method, only the first two landmarks are aligned by translation, rotation, and scaling. The remaining landmarks are then adjusted accordingly. This then allows the calculation of (remaining) differences [69, 70]. To illustrate this approach, Fig. 5.8 shows two shapes that represent similar anatomical features with different scales and positions. In Fig. 5.9, the two shapes are transformed into Bookstein coordinates.

5.8.2 Procrustes Analysis

The term Procrustes refers to a bandit from Greek mythology who forced his victims into an iron bed, stretching or cutting off their limbs when they did not fit. By analogy, in the Procrustes transformation, two shapes are brought into maximum congruence through translation, rotation, and scaling. A prerequisite for the meaningful application of this procedure is that the two objects to be compared are similar [61]. Figure 5.10 shows the result of the Procrustes transformation of the two shapes displayed in Fig. 5.8. A simple mea-

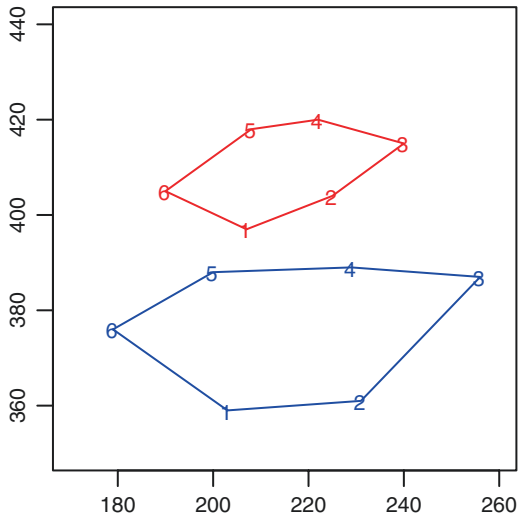

Fig. 5.8 Two shapes, each defined by a sequence of landmarks. The lower one is taken from Fig. 5.7 and represents the right eye, and the upper one is an arbitrary eye-like shape

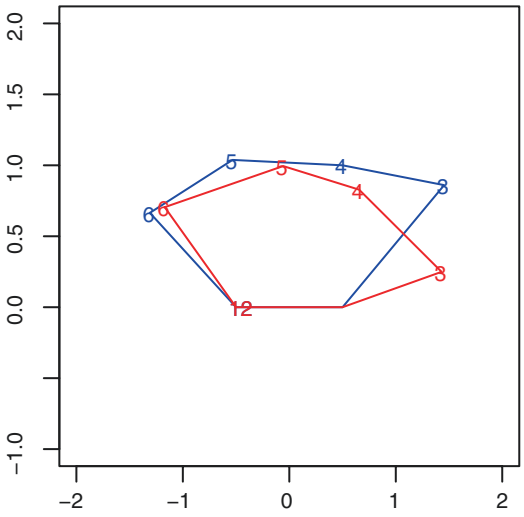

Fig. 5.9 The two shapes from Fig. 5.8 transformed into Bookstein coordinates. Only landmark one and two are aligned by transformation

sure to quantify the residual deviation between the shapes is the sum of the Euclidean distances between corresponding landmarks.

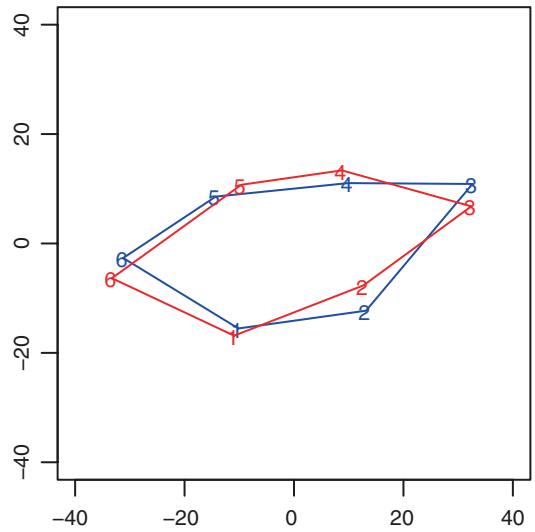

Fig. 5.10 Result of a Procrustes transformation of the two shapes from Fig. 5.8. All landmarks are brought into alignment as much as possible using a regression technique

5.8.3 Anthropometric Mask

The concept of landmarking was further developed by Claes et al. through the so-called anthropometric mask. It consists of about 10,000 quasi-landmarks, which are placed automatically over the face. By comparing the quasi-landmarks, e.g., pre- and post-surgery, differences in facial structures can be determined and displayed graphically [71].

5.9 Conclusion

Morphometrics and high-resolution optical 3D imaging systems are powerful tools for documenting facial structures and their changes in normal facial development and facial malformations. They can be used for a multitude of other applications. In combination with radiological layer systems (MRI, CT, CBCT), fundamental insights in healthy or diseased states of patients can be gained.

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6 Classification of Craniofacial Malformations

Ulrich Meyer

6.1 Introduction

Classification of craniofacial malformations is difficult to standardize. This is based on multiple aspects in disease development and disease manifestation. Additionally, the border between a disease and a norm variance is floating. Some aspects have to be recognized: on one hand, the determination of the disease and on the other hand the documentation of the disease outcome. Determination of a disease can be done on a genetic or a clinical level; documentation can be done on various imaging procedures (pictures, MRI, CT, CBCT, 3D scan, or sonographic images). In order to elaborate a comprehensive classification system for the broad range of craniofacial malformations, different issues have to be considered: the development of classification systems, current concepts of disease classification, genetics and pathogenesis of head malformation, and disease recognition and documentation. A new three-axis classification system is proposed that comprehensively includes all craniofacial malformations.

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6.2 The Development of Classification Systems

Disease classifications (taxonomies) are used ubiquitously in academic medicine, human genetics, the health industry, and economics. Much like any library's content catalogue, disease taxonomies strive to group together similar entities for ease of access and analysis [1]. Historically, changes in these groupings have reflected a progression toward etiologic, common-cause disease classifications [2–6]. The development of nosologies has closely paralleled the evolution of methods designed for the reconstruction of the evolutional process (Fig. 6.1). Approaches to species classifications were mostly subjective and made without any hint of the common-origin interpretation. They utilized only a small subset of all the visible morphological features of any given organism. Initially, many of these groupings were largely arbitrary—often guided by topographical or anatomical similarities. These early phylogenetic methods were followed by the use of maximum parsimony methods, explicitly minimizing the number of differences between proximal taxonomy leaves.

Disease taxonomy plays an important role in defining the diagnosis, treatment, and mechanisms of human diseases even now. The principle of the current clinical disease taxonomies, in particular the International Classification of Diseases (ICD) (Fig. 6.2), goes back to the

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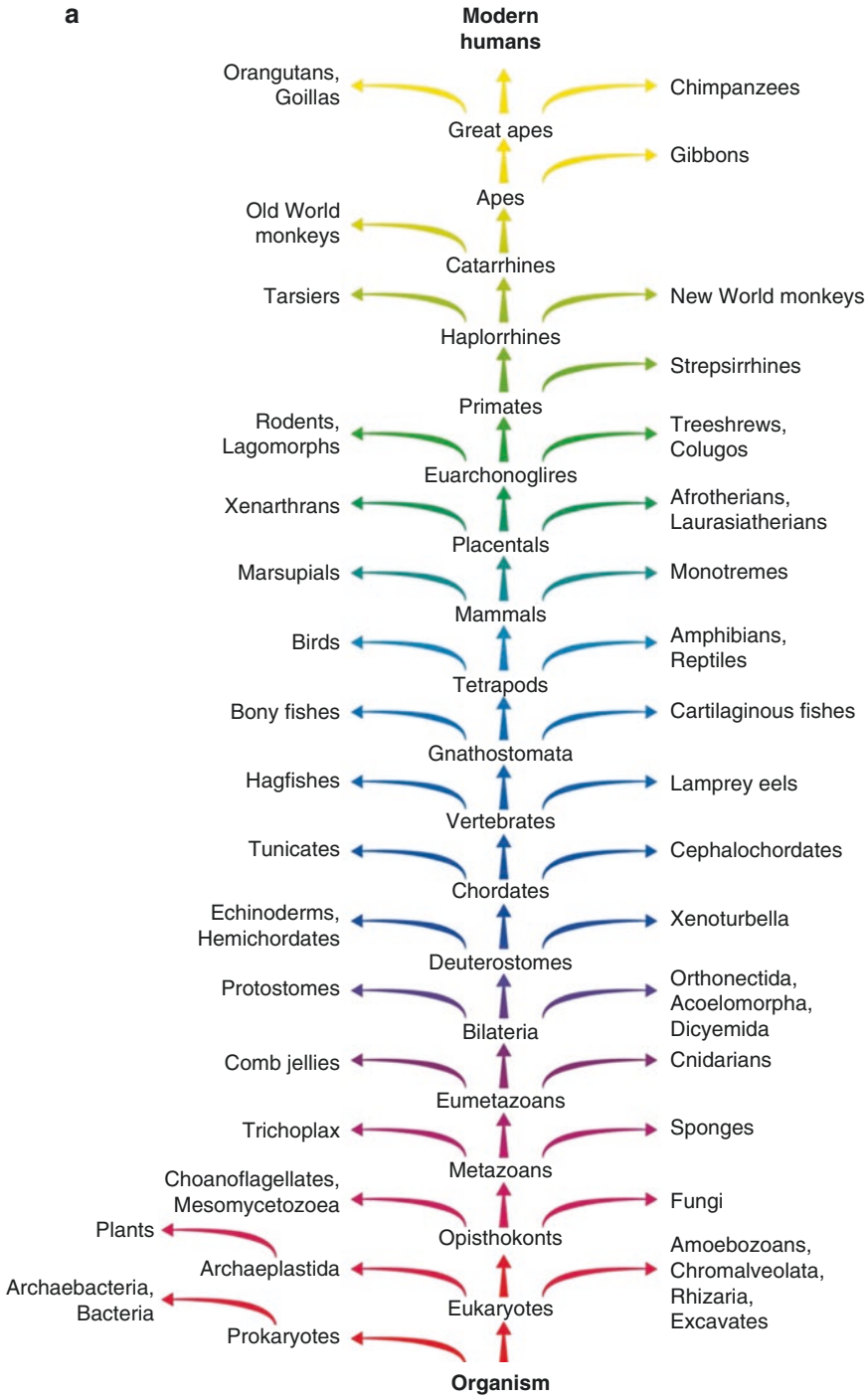


Fig. 6.1 (**a**) Taxonomy from simple organisms to the human species. (**b**) Taxonomy at a precise level. (With permission from Springer Nature: Nature, The global diversity of birds in space and time, Jetz, W., Thomas, G., Joy, J. et al., 2012). *Source: Reprinted from Peter Hermes Furian/Shutterstock.com with permission*

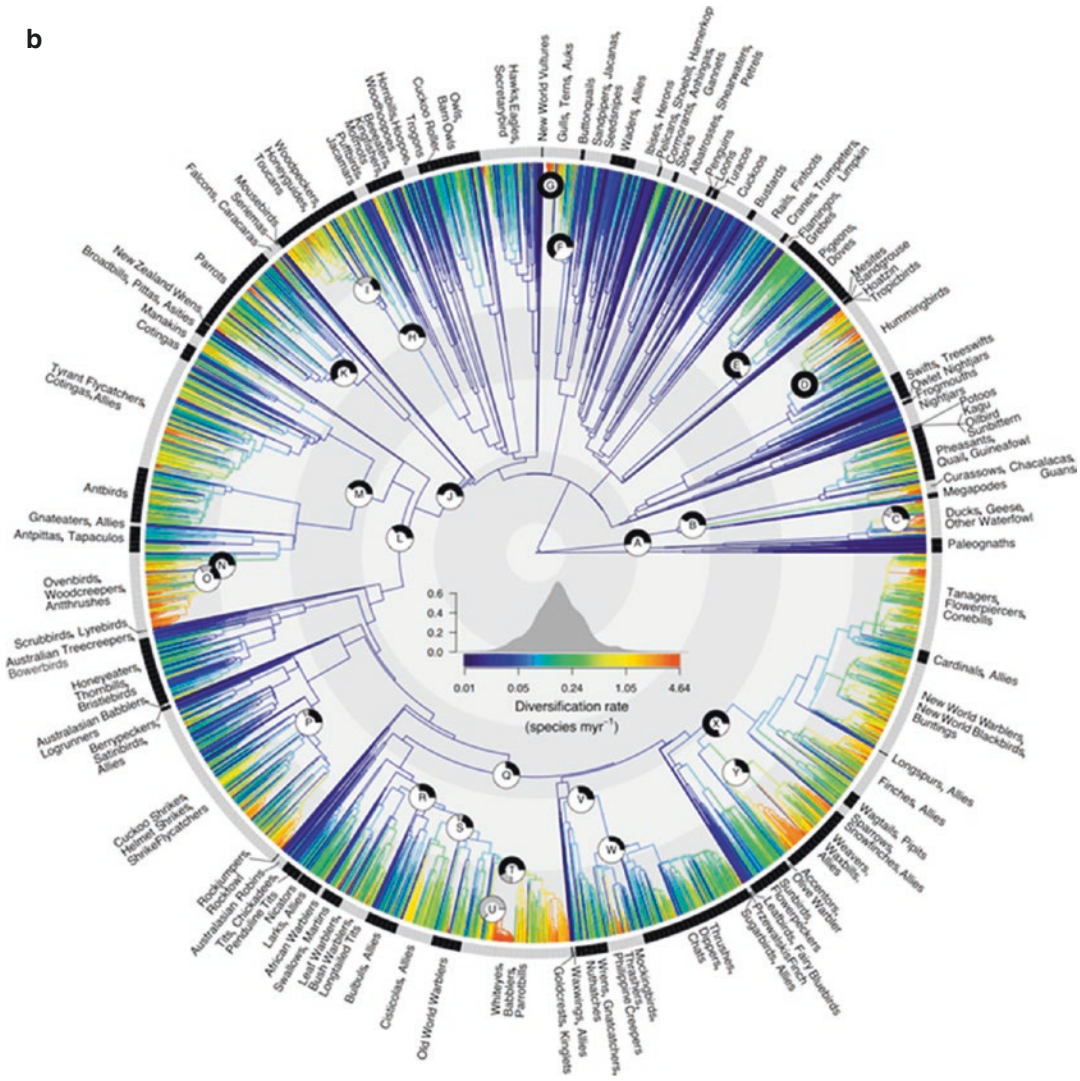


Fig. 6.1 (continued)

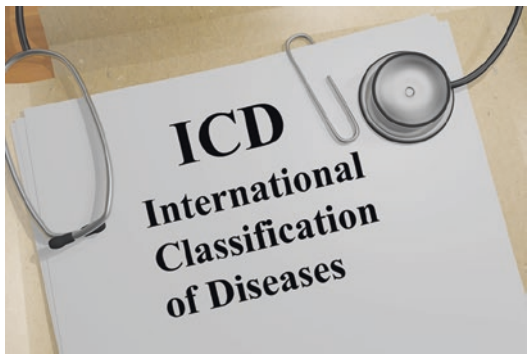


Fig. 6.2 International Classification of Diseases. *Source: Reprinted from hafakot/Shutterstock.com with permission*

work of William Farr in the nineteenth century and is primarily derived from the differentiation of clinical features (e.g., symptoms and micro-examination of diseased tissues and cells) [7]. Despite its extensive clinical use and elaboration for economical reasons, this classification system lacks the depth required for precision medicine with the limitations of its rigid hierarchical structure, and, moreover, it does not exploit the rapidly expanding molecular insights of disease phenotypes. Most recent arrivals to disease classification are statistical

tree-making methods, which infer taxonomies from very large datasets using explicit stochastic models of diverging organism traits during speciation [8].

6.3 Current Concepts of Disease Classification

Many diseases (e.g., cancer, chronic inflammatory diseases) in the current disease taxonomies have either high genetic heterogeneity [9, 10] or manifestation diversity [11–13], which give little basis for tailoring treatment to a patient's pathophysiology. This is also the situation in most craniofacial malformations.

Therefore, a deep understanding of diseases based on the advances in disease biology, bioinformatics, and multi-omics data may help in the reclassification of disease taxonomy [14]. In the past decade, efforts to reclassify diseases based on molecular insights have increased with studies related to molecular-based disease subtyping in different disease conditions. Given the molecular network mechanisms [15, 16], genetic pleiotropy [17], as well as complicated genotype-phenotype associations underlying diseases, the establishment of a molecular-based disease taxonomy with clear boundaries is essential but challenging.

In regard to the ICD classification, authors like Zhou et al. [18] found that although general correlations exist between disease closeness in ICD taxonomy and underlying molecular profiles, ICD still displays significant limitations with regard to the heterogeneity of molecular diversity and clear category boundaries. Recent studies show that a disease with a high molecular diversity tends to be classified into multiple disease categories, which indicates that there exist more disease subtypes for that disease. Despite the efforts made in data integration methods that utilized multiple types of data (e.g., ontological and omics data), the development of a molecular based disease taxonomy that links molecular networks and pathophenotypes still remains challenging [19–21].

6.4 Genetic Access to Normal and Disturbed Head Formation

In order to gain access to a more genetically based classification of craniofacial malformations, it is important to have insight into the genetic principles of normal and abnormal tissue development in the craniofacial region. Inborn or acquired craniofacial malformations are based on a disturbance or disbalance of the normal growth process. Therefore, it is essential to elaborate the classification on the kind of the disturbance. Disturbances can generally be based on genetic or non-genetic aspects. The size and growth of each of the facial bones (Fig. 6.3) are in part genetically predetermined, yet environmental influences play a role. The chromosomes and hundreds of genes are controlling the coordinated patterning, proliferation, and differentiation of tissues having multiple embryological origins. Malformations can be based on disturbances of a chromosomal, genetic, epigenetic, or external level (Fig. 6.4). They can be also complex with features from each level. The underlying cause of some malformations is well known; others are under investigation and not yet clarified or even not known at all [22].

Development of the craniofacial skeleton and the subsequent outcome of the whole hard and soft appearance is a highly orchestrated and complex three-dimensional morphogenetic process. These dynamic events are on one hand spatiotemporal and on the other hand time and tissue dependent (Fig. 6.5). Malformations can occur in all tissues. Complex ectodermal-mesodermal interacted tissues like teeth can be present as complex hyper-numerations of teeth.

The development of the face involves a coordinated complex series of embryonic events. From the moment of conception, the parental environment can influence the development of the fetus. Facial development occurs very early at a time when the mother is not always aware that she is pregnant. The developing fetus may be subject to adverse genetic or environmental stimuli (smoking, alcohol and drug intake, allergens, physical forces in the maternal body, others).

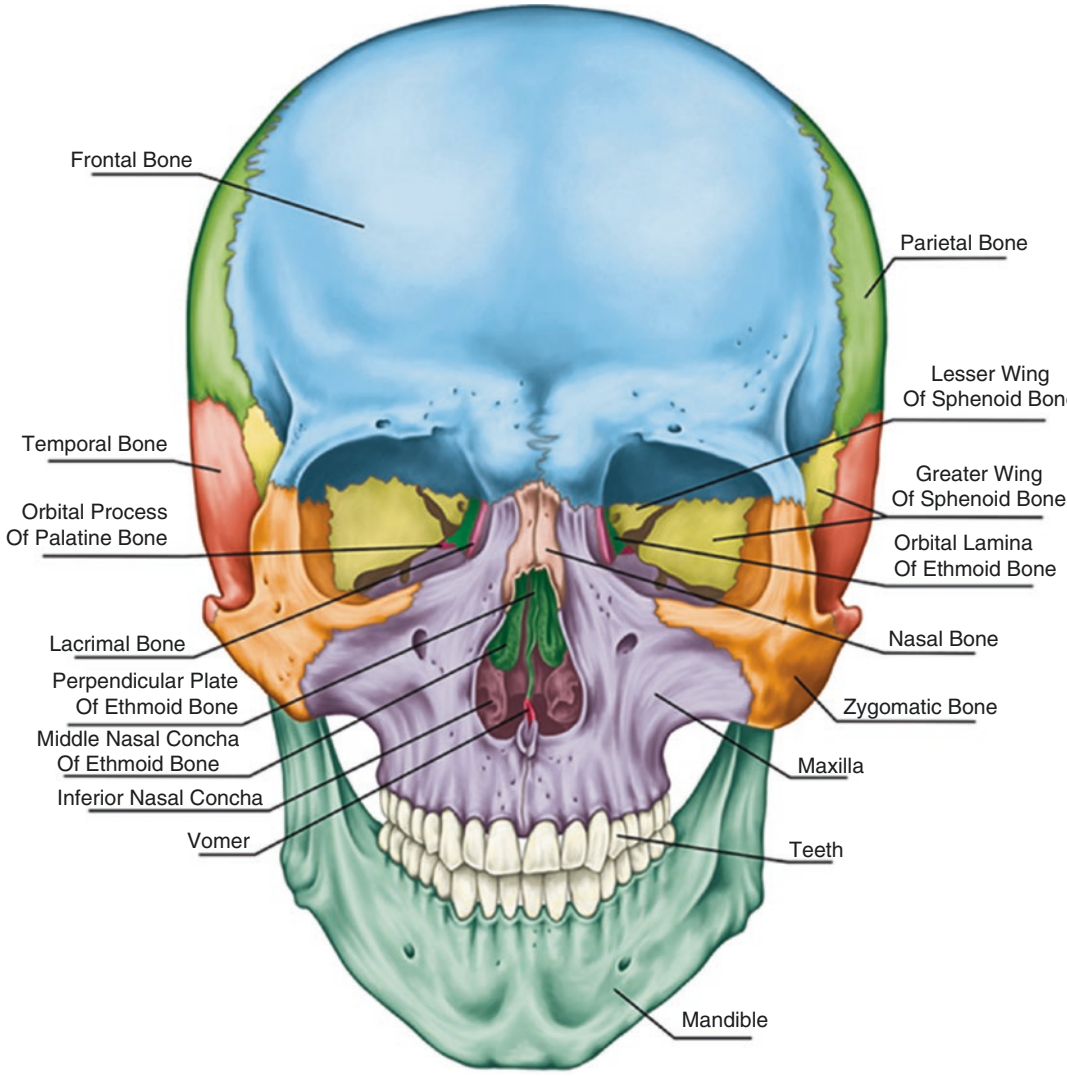


Fig. 6.3 The skull represents the most complex 3D bone configuration of the whole body. *Source: Reprinted from stihii/Shutterstock.com with permission*

Recognizable features of the human face develop around the fourth week of gestation (Fig. 6.5) and are closely related to cranial neural crest cells [23]. The facial processes fuse at different times; maxillary, 6 weeks; upper lip, 8 weeks; and palate, 12 weeks [24, 25] (Fig. 6.6). Molecular studies have shown that the growth, structure, and patternation of the facial primordia are controlled by a series of complex genetic interactions that involve defined genes, producing various cytokines such as fibroblast growth factors, sonic hedgehog proteins, bone morphogenetic proteins, homeobox genes *Barx1* and *Msx1*, the distal-less homeobox (*Dlx*) genes, and local retinoic acid gradients [26–32]. The fusion between the facial processes depends on a series of events involving cell migration, growth, adhesion, differentiation, and apoptosis (Fig. 6.7). Disruptions in the fusion of the facial processes may result in complete or partial clefts of the face, lip, and/or palate. All events are closely interacting and therefore specifically susceptible to dysregulation as evidenced by the high proportion of congenital defects that involve the skull and face. Whereas genetics play a pivotal role, most modifiable environmental factors have only subtle effects on

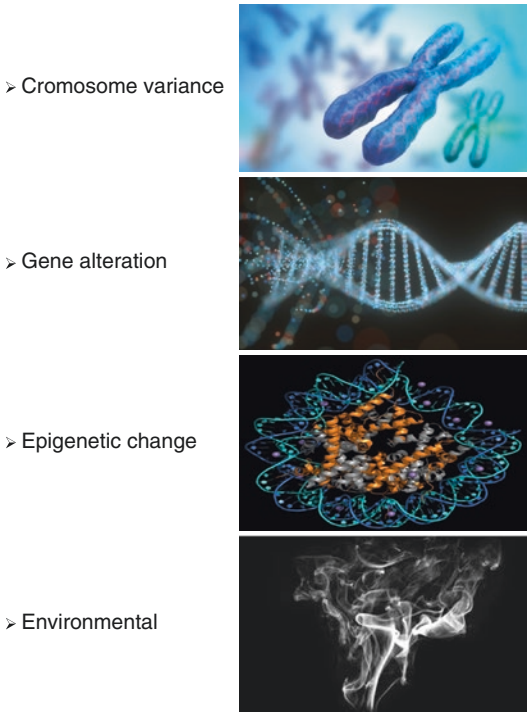


Fig. 6.4 Biological basis of craniofacial diseases. *Source: Reprinted from top-top vchal/Shutterstock.com, top-middle: ktsdesign/Shutterstock.com, middle-bottom: MoleculeQuest/Shutterstock.com, bottom-botoom: Olga Moonlight/Shutterstock.com with permission*

the face, except for strong environmental influences (fetal alcohol syndrome, virus infections).

Craniofacial malformations occur through the abnormal development (including cleft lip and/or palate, craniosynostosis, branchial arch diseases, conjoined twins, head deformations, others) during the gestational process. The whole gestational period is known to be very vulnerable. The resulting facial malformations comprise over one-third of all congenital birth defects, demonstrating the highest complexity of skull formation throughout the body. Whereas high-throughput sequencing has recently led to the identification of many new causative disease genes and functional studies have clarified their mechanisms of action, some defined chromosomal alterations or gene defect-related craniofacial diseases are long known.

Genetic studies of craniofacial Mendelian traits are well known to be involved in craniofacial development or genetic syndromes affecting the face. Down syndrome, Cri du chat syndrome, van der Woude syndrome, Prader-Willi syndrome, and Treacher Collins syndrome mostly present with facial abnormalities and have defined chromosomal or gene alterations. The altered facial appearance and the relation to normal facial development [33, 34] have been investigated intensely. In contrast to such defined chromosomal or gene-based craniofacial diseases, most malformations cannot be definitively related to singular genetic alterations.

Genome-wide association studies (GWAS) have therefore investigated the association between normal facial variation and millions of single nucleotide polymorphisms (SNPs). GWAS studies coupled with high-resolution three-dimensional imaging of the face (as a documentation system) have enabled the study of the spatial relationship of facial landmarks in great detail. Twin studies have historically been employed to explore the relative genetic and environment influence on facial shape (Fig. 6.8a) exploiting the genetic differences between monozygotic and dizygotic twins [35]. Twin studies suggest that 72–81% of the variation of height in boys and 65–86% in girls are due to genetic differences with the environment explaining 5–23% of the variation [36]. Similar levels of genetic-environmental contributions have been reported for some other facial features (Fig. 6.8b).

6.5 Pathogenetic Access to Normal and Disturbed Head Formation

The head with the highly complex three-dimensional structure of underlying bones is the scaffold for the facial connective tissues, the musculature, vasculature, and associated innervation. Collectively these tissues are derived from endoderm, mesoderm, ectoderm, and cranial neural crest cells (CNCCs) and their derivatives (Fig. 6.9). Signalling between these cellular components and the craniofacial mesenchyme (formed primarily by CNCCs with a mesodermal contribution) provides positional cues and regulates growth and differentiation [37]. During undisturbed embryogenesis, the first and second

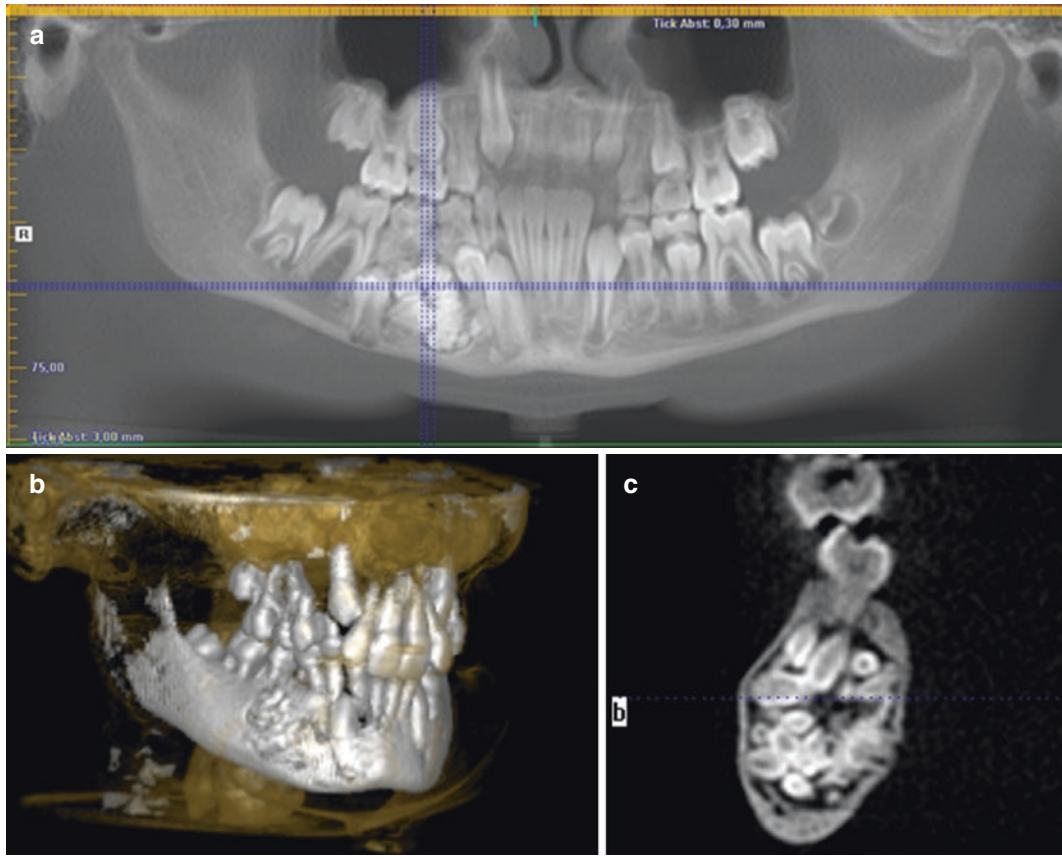


Fig. 6.5 (**a**) OPT, (**b**) CBCT and cross-sectional radiograph of dental malformations. The high number of complex teeth-Anlagen represents a temporal, spatial, and ectodermal-mesodermal tissue dys-development. (Source: Ulrich Meyer, informed consent of patient exists). (**c**) Cross-sectional radiograph of dental malformations. The high number of complex teeth-Anlagen represents a temporal, spatial, and ectodermal-mesodermal tissue dys-development. (Source: Ulrich Meyer, informed consent of patient exists)

branchial arches form facial prominences that develop into specific craniofacial and skeletal structures [38, 39]. Portions of the first branchial (or mandibular) arch develop into the skeletal, muscular, and neural elements of the mandible, whereas the dorsal edge of the first branchial (or hyomandibular) cleft forms the auditory meatus.

Manifestations of first and second branchial arch anomalies depend on which phase of neural crest cell development is disrupted (formation vs. differentiation, Fig. 6.10) [40]. For example, if neural crest cell formation is perturbed, such as few neural crest cells are produced or they fail to migrate to final destinations, this can result in phenotypes like branchial arch diseases or cleft palate. One of the characteristic disorders of this abnormal type is Treacher Collins syndrome (Fig. 6.11) [41]. Aberrant neural crest cell differentiation, on the other hand, results in premature suture mesenchyme ossification, which fuses the calvarial bones (craniosynostosis, Fig. 6.10) consequently restricting skull growth and impacting upon facial and brain growth, development, and maturation [42].

6.6 Aspects of Disease-Related Phenotype Documentation

Most craniofacial classification systems try to elaborate a disease-appearance relationship. Therefore, not only the investigation and standardization of the pathogenesis are important; a

standardized documentation and morphological nomenclature system is a pre-requisite for a good classification system.

The facial surface is readily visible and identifiable with a close relationship to the underlying cartilaginous and skeletal structures [43–47]. Differences in relative size, shape, and spatial arrangement (vertical, horizontal, and depth) between the various facial features (e.g., eyes, nose, lips, etc.) make each individual human face unique, although closely related individuals such as monozygotic twins have very similar facial structures. Standardized information on an individual's facial morphology (e.g., by the use of defined investigation settings) is important for classification systems (Fig. 6.12).

There are many imaging systems available to capture the external facial surface topography such as photography, lasers, photogrammetry, optical 3D scans, magnetic resonance imaging (MRI), computerized tomography (CT), and cone beam computerized tomography (CBCT). Many of these techniques have been evaluated in terms of facial coverage, speed of capture, processing time, accuracy, validity, and cost [47–50]. For an individual who can sit still with a neutral facial posture in natural head position, the speed of capture is not critical. Even with relatively long acquisition times for some photogrammetric, MRI, CT, and CBCT systems, facial landmark reliability of less than 0.5 mm can be achieved [51–53]. For infants and individuals with unpredictable facial or bodily movements, a faster acquisition time will be required although reliability of achieving the same facial posture will be significantly reduced.

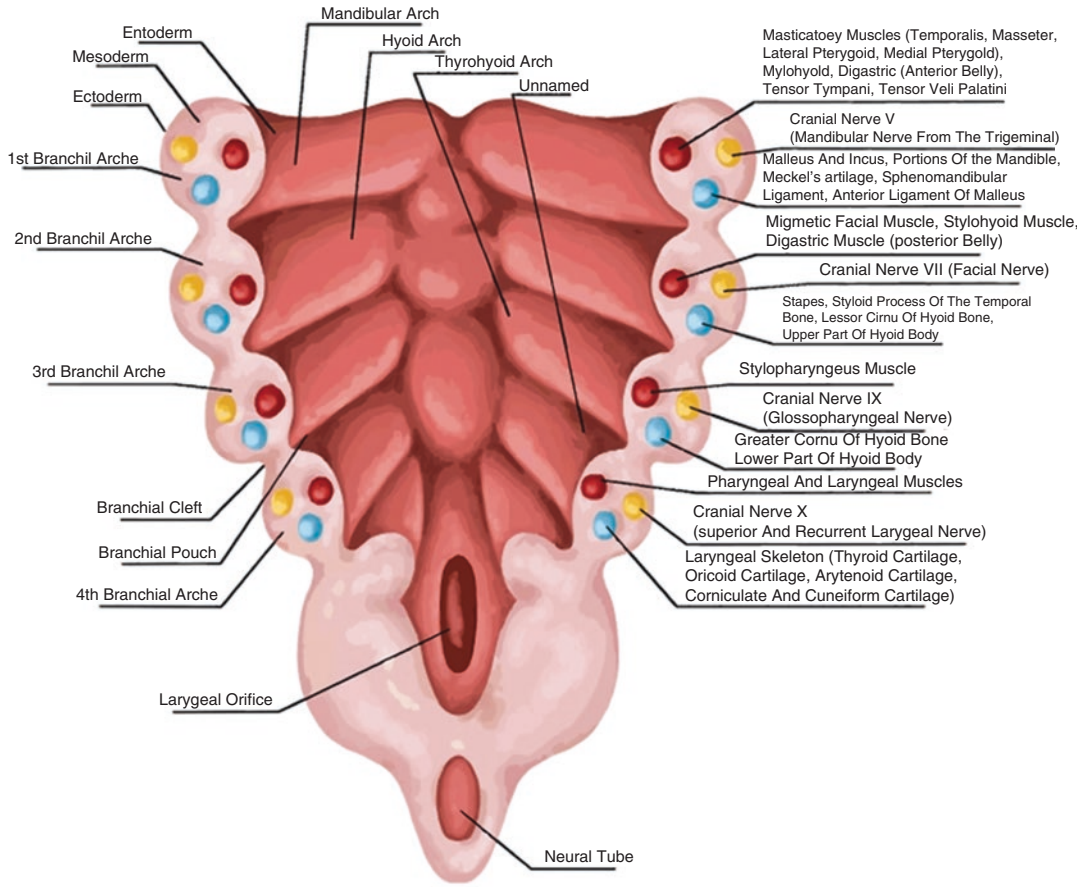


Fig. 6.6 Initial phase of facial development on the basis of the branchial arch system. *Source: Reprinted from top: stihii/Shutterstock.com, bottom: stihii/Shutterstock.com with permission*

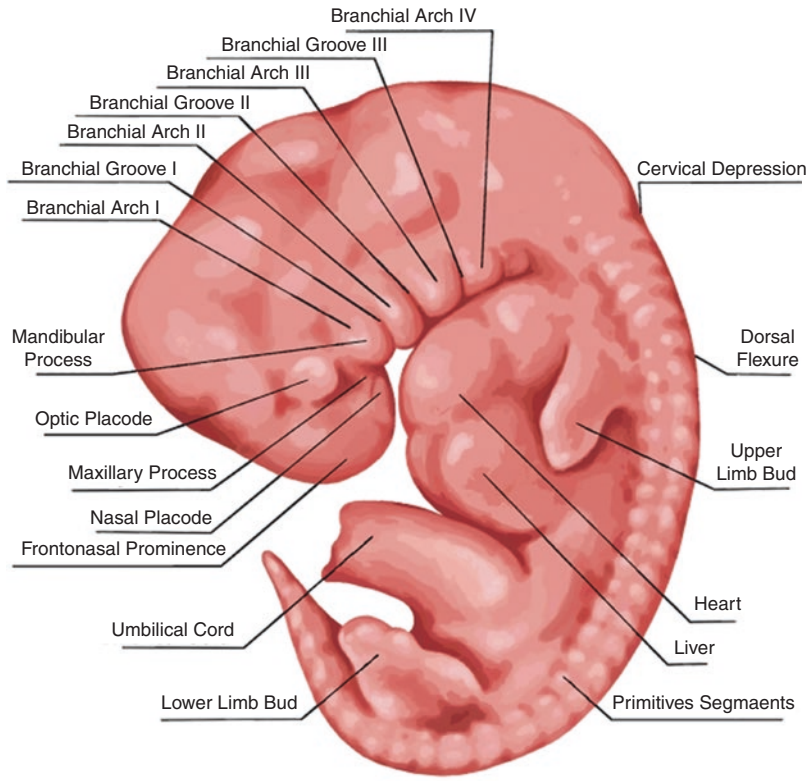


Fig. 6.6 (continued)

Standardized clinical facial charts/tables/measures are routinely used for newborns (e.g., head circumference, body length). Various specialties such as maxillofacial surgery and orthodontics use published norms for different treatment decisions. Phenotype analysis is used to identify individuals who fall within the normal range and identify any facial dysmorphologies, but these clinical charts are one descriptions of an alteration. They are of limited value, when details of a disease manifestation are important for classifications.

6.7 Craniofacial Classification Models

Classification models should ideally be aimed to disentangle parental biological contributions to heritable traits from environmental factors. Models should also try to incorporate the etiology of the disease and give respect to the situation that in a lot of craniofacial anomalies, affected and unaffected family members are present. Even nowadays, classification systems are often based on the group of persons who have developed such classification systems. For example, the geneticist might focus on discernible phenotypic differences and heritability, while the surgeon concentrates on appearance and function, and the developmental biologist centers on gene expression and tissue morphogenesis. Many systems have been developed to classify craniofacial malformation patterns to facilitate diagnosis, management, surgical treatment, and research. The use of imprecise terminology in facial appearance description as well as the growing appreciation of the spectrum of phenotypes that are encompassed by this term adds to the confusion. Yet, pediatricians, surgeons, speech pathologists, nutritionists, geneticists, and developmental biologists often classify craniofacial malformations differently. Much of this

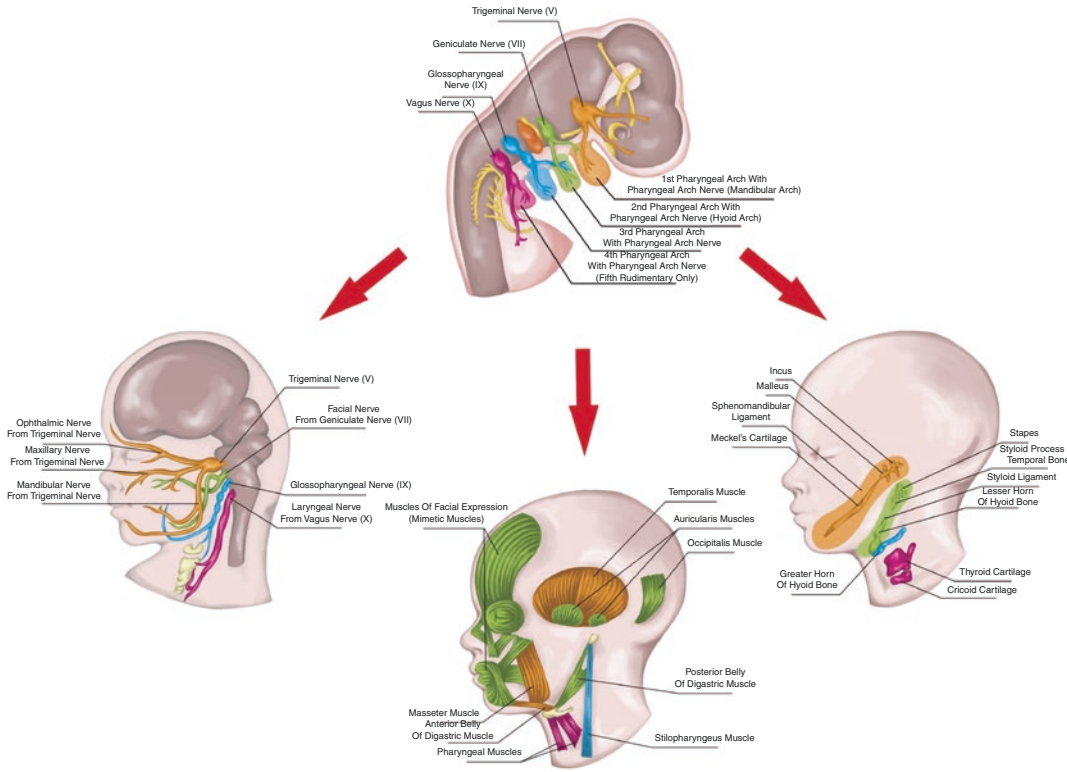


Fig. 6.7 Initial and late phase of facial development on the basis of the branchial arch system. *Source: Reprinted from stihii/Shutterstock.com with permission*

undocumented confusion resides also in the differing level of granularity with which each person considers the disorder. Craniofacial malformations are a "difficult-to-define group" of congenital anomalies named after the anatomical location of a given defect present at birth. According to working definitions, it could include any etiologic category (chromosomal, environmental, Mendelian, multifactorial, etc.), as well as any pathogenetic mechanism (malformation, deformation, disruption, dysplasia), or any clinical category (developmental field complex, isolated defect, sequence, syndrome, etc.) [54]

Orofacial clefting as the most common craniofacial malformation is a typical example of a classification problem, because it is a birth defect with wide different genetic and pathophysical patterns and a broad range of phenotypic variability. Studies using population-level data have detected significant associations between subclasses of cleft types and specific genomic regions. Such subclassification is also likely to be critical for identifying and understanding environmental contributions to clefting and resolving issues related to the optimal surgical approaches for CL repair. The benefits of describing cleft phenotypes with detail, accuracy, and reproducibility have been well-described [55].

As craniofacial malformations are relatively rare conditions that exist in a multitude of patterns and in varying degrees of severity, historically systems of classification either have been arbitrary or could not be standardized because of extreme or bizarre distortions. Additionally, there has been no unanimity of terminology or satisfactory standardization of the classification of the innumerable craniofacial syndromes. At present, there are over 700 craniofacial syndromes, with new syndromes being described and published at the rate of 25–50 per year [56, 57]. From a historical perspective, several of the craniofacial malformations are identified according to the

Fig. 6.8 (**a**) Facial appearance of monozygotic twins is representative of the strong influence of chromosomes and genes at conception. (**b**) Facial appearance of the racer family Schuhmacher. The resemblance of the faces is indicative for the strong genetic influence. *Source: Reprinted from a: Milan/Shutterstock.com with permission, b: Top left: emperornie/wikimedia.org, Bottom left: AngMoKio/wikimedia.org, Top right: SvenMandel/wikimedia.org*

names of the authors who first described them, such as the Goldenhar, Pierre Robin, Treacher Collins, and Pfeiffer syndromes [56–58]. Other malformations are identified by their descriptive appearance and have been given names such as hemifacial microsomia, retromandibulism, and hypertelorism, without regard to their various causes. Various classification systems are based on anatomic topography, with some authors dividing the face into various regions and others grouping the defects around the brain, sensory organs, or branchial arch system [58, 59]. Burian is credited with the first attempt to classify the whole range of craniofacial anomalies [59].

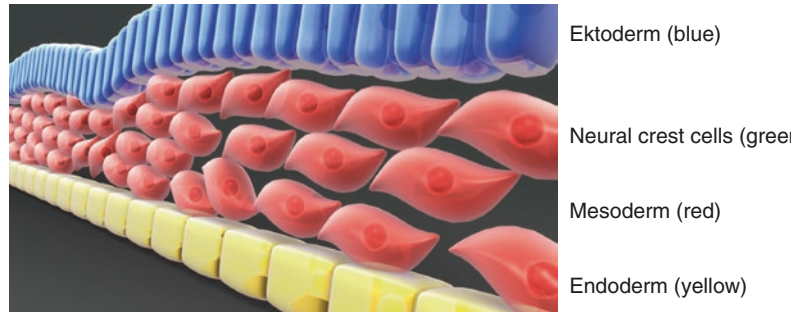
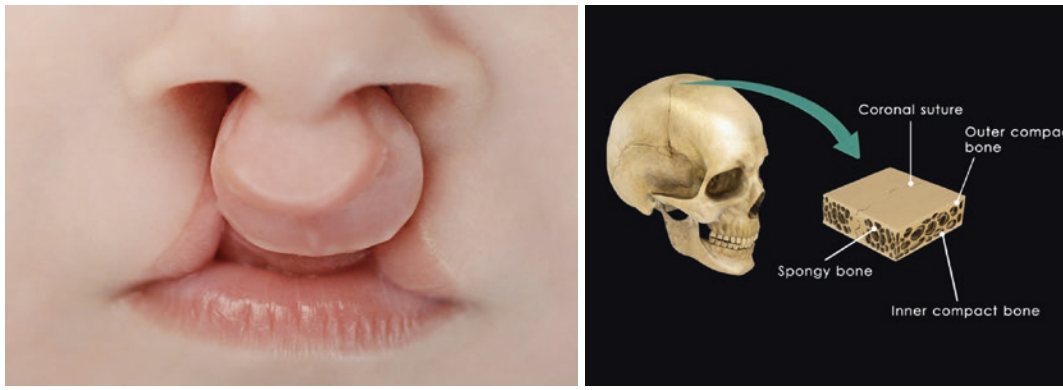


Fig. 6.9 Cranial neural crest cells (CNCCs) with their migration and differentiation have a great influence of facial development. *Source: Reprinted from sciencepics/Shutterstock.com with permission*

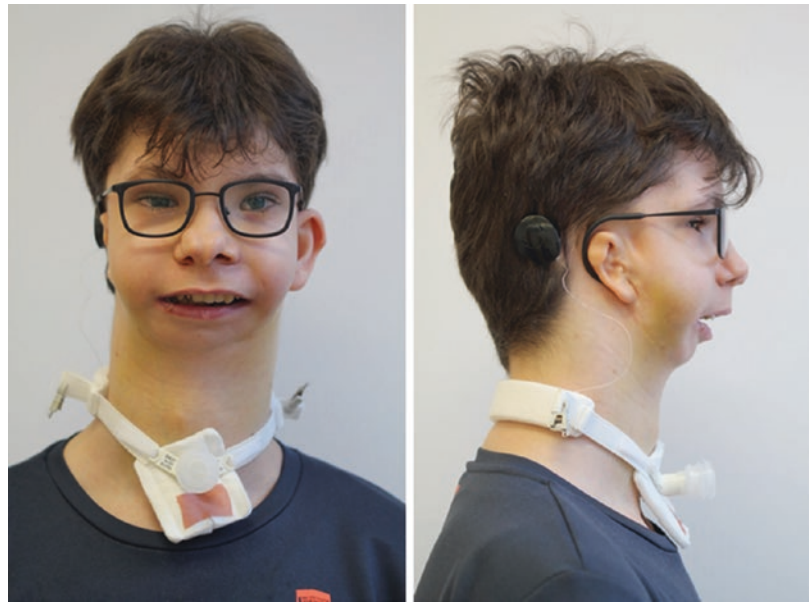


neural crest cell formation → cleft lip palate

neural crest cell differentiation → craniosynostosis

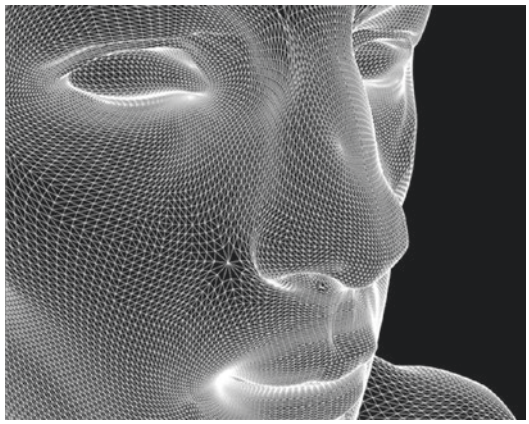
Fig. 6.10 Typical diseases of neural crest cell failure. *Soucre: Reprinted from left: malost/Shutterstock.com, right: sciencepics/shutterstock.com with permission*

Several subsequent classifications were attempted by such authors as Sanvenero-Rosselli [58], Burian [59], and other authors. Tessier, in 1976, was the first to present an orderly classification system for all the established craniofacial malformations [60, 61]. In order to simplify the nomenclature of the clefts, Tessier devised a system in which a number is assigned to the site of each malformation, based on its relationship to the sagittal midline. The classification system is purely descriptive, however, and not related to the embryologic development of the malformation or the underlying pathology. Nevertheless, this system has become widely accepted because of the ease of recording and simplicity of communication of the various malformations. It also has been found to correlate clinical appearance with practical surgical anatomy. Van der Meulen [62] introduced a more complete, general category of craniofacial malformations. He participated in a group of European plastic surgeons who proposed a redefinition of terms and a new classification in order to facilitate communication and attempt to avoid confusion among the craniofacial syndromes and embryologic pathophysiology. Their classification represented the collective experience of five craniofacial surgeons (van der Meulen, Mazzola, Vermey-Keers, Stricker, and Raphael) working in three different countries (Netherlands, France, and Italy). The van der Meulen et al. schema proposes that the "common denominator" for all the craniofacial malformations is a form of "dysplasia." Regardless of the cause, an arrest in skin, muscle,



Treacher Collins syndrome

Fig. 6.11 Facial appearance of a patient with Treacher Collins syndrome



Phenotype documentation

Fig. 6.12 STL surface representation enables a precise documentation of facial landmarks. *Source: Reprinted from Ryger/Shutterstock.com with permission*

or bone development manifests itself as a "focal fetal dysplasia." The ultimate appearance and severity of the dysplasia depend on the localization of the area(s) involved and the time the disturbance or developmental arrest occurs [62].

At present, there is no one classification that satisfactorily explains all of the various craniofacial malformations. Better classifications have evolved and are continuing to evolve through communication, standardization of terminology, and the advancement of the science of embryology. There is therefore the need for large clinical, genetic, and etiologic studies especially those that are multi-center or multi-national in nature. Additionally, the use of standardized and detailed phenotypic classification was recognized and urged [63–67]. At the moment, there is not an all-inclusive classification system present for craniofacial malformations. An improved classification systems should give correlation between the full phenotypic variability encompassed by the diagnosis of craniofacial malformations on the basis of morphological, developmental, and pathogenic properties and the genetic and pathogenetic attributes responsible for the heterogeneity of such diseases. Recent insights into the genetics of craniofacial malformations (for review see Richmond [22] or Ahmed [40]) enable a good insight between defined gene alterations and disease outcome. A new classification should enable researchers and clinicians to better appreciate the limitations and challenges associated with using disparate classification systems, and in the longer term, the resulting ontology should be of great utility for inter-center studies and population-level genetic investigations.

6.8 Proposed New Classification System

Important in craniofacial malformation classification is the underlying genetic state, the embryologic disease pattern, and the clinical outcome in respect to the resulting phenotype.

The proposed classification system is based on a comprehensive three-axis genetic, pathogenetic, and phenotype stratification approach (Fig. 6.13). It is elaborated (1) on the developmental steps during embryogenesis and (2) the underlying genetic disturbance and (3) the resulting phenotype. This primary classification (x-axis) system used in this book (Fig. 6.14) is based on the embryologic pathogenesis graduation as a leading and iterative arrangement. Figure 6.15 displays examples of the recently known genetic-clinical correlation of the diseases entities. As the book series *Fundamentals of Craniofacial Malformations* is conceptualized for basic researchers as well as for surgeons, all medical disciplines are incorporated in the stratification.

A stratified (axis related) classification approach as used in this book is often used in medicine. The phrase "axis of classification" means a way of classifying and studying diseases. When utilizing an axis of classification for morbid conditions, diseases are assigned to a system of categories based on established criteria. Such criteria may be based on the affected part of the body (anatomy/phenotype), the nature of a disease process (pathophysiology, embryology), or genetic etiology. The ICD 10-CM is a typical example of an axis-related system of classification (Fig. 6.16). Anatomy, for example, is the primary axis of classification of ICD-10-CM, as it was in ICD-9-CM. This is evident by the fact that most of the ICD-10-CM chapter titles reflect diseases of a particular body system, such as "Diseases of the Respiratory System," "Diseases of the Nervous System," etc. ICD-10-CM employs many other axes as well, such as etiology, as found in Chap. 1 ("Certain Infectious and Parasitic Diseases"). A combination of multiple and diverse axes are used in classifying some dis-

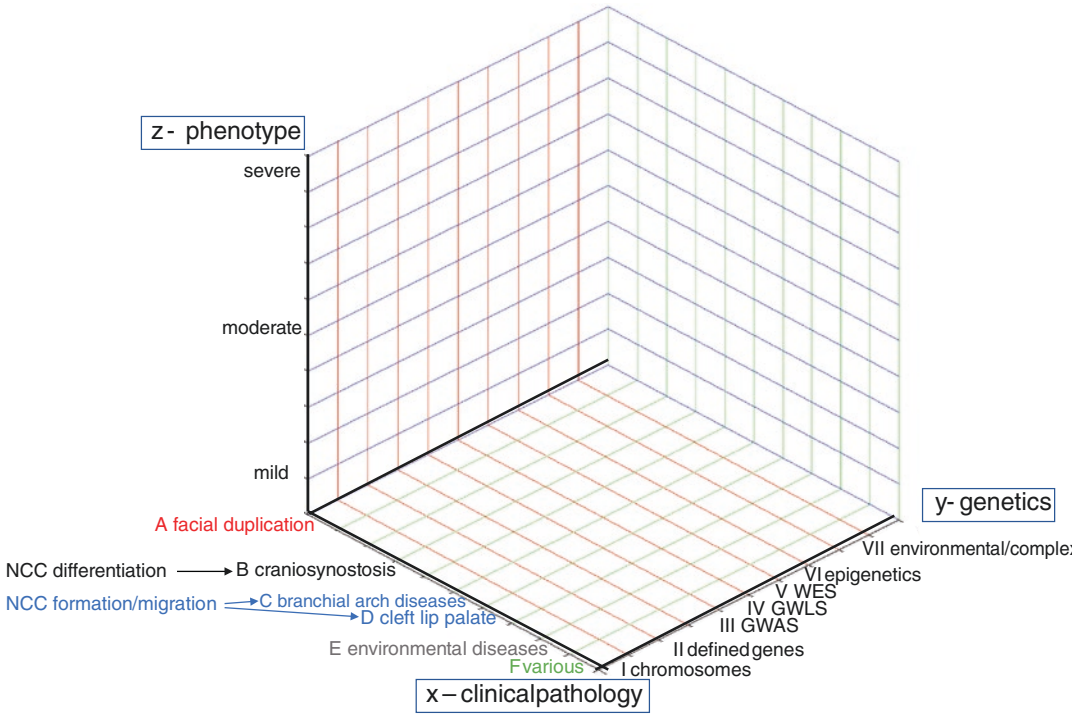


Fig. 6.13 Proposed three-axis classification scheme

- chromosomal disorder
- facial duplication
- craniosynostosis
- branchial arch diseases
- clefts
- postural head deformations
- others

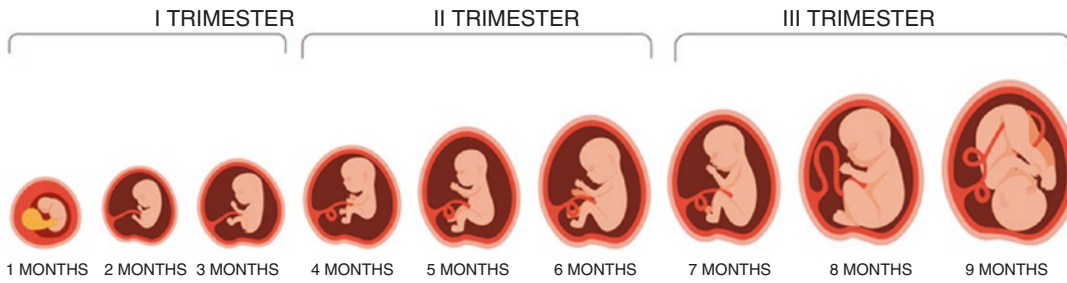


Fig. 6.14 Embryonic-pathogenetic classification model, used as a primary axis in this book. *Source: Reprinted from Shanvood/Shutterstock.com with permission*

Pathology	Disease	Chromosomes	Genetics defined genes
A conjoined tissues	Facial duplication Conjoined twins		
B craniosynostosis	ERF, TCF-12, ZIC-1 related craniosynostosis Apert Syndrome Crouzon syndrome Pfeiffer Syndrome		ERF, TCF12, ZIC1 (AD) FGFR2 FGFR2
D branchial arch dis.	Treacher-Collins-syndr. MFD with alopecia AFD Cincinatti type RCPS Burn- MC Keown syn. Cerebrocostmandibular syn. MFD Guion Almeida Nager Miller		TCOF1 (AD, AR possible) EDNRA (AD) alopecia POLR1A (AD) cincinatti EIF4A3 (AR) rcps TXNL4A (AR) bmc SNRPB (AD) cerebo EFTUD2 SF3B4 (AD, AR, spor.) DHODH (AR)
E clefts	van der Woude 2 Acromelic frontonasal dysostosis OpitzG/BBB		GRHL3 (AD) ZSWIM6 (AD) SPECC1L (AD)
G various/complex	Trisomie 21 Cri-du-chat ACS;IQME Catel-Manzke syn.	Chr.21 Chr. 5	

Fig. 6.15 Examples of chromosome and gene alteration-based craniofacial malformation The displayed list represents only a part of known genetic-disease relationships. *AD* autosomal dominant, *AR* autosomal recessive

eases within the same chapter. When designing a disease classification system, the primary axis reflects the most important statistical and clinical aspects of the disease. For example, for a diagnosis of heart failure, the first axis of classification is "type," and the second is "acuity." It is the variation and combination of these axes of classification that contribute to the tremendous increase in the number of codes available for assignment in ICD-10-CM as compared to ICD-9-CM.

Large-scale studies are now needed to identify the 3D correlations between genetic

- Etiology
- Manifestation or complication
- Specificity of anatomical site
- Chronicity (i.e. acute, subacute, chronic, unspecified vs.acute/subacute, chronic, unspecified, etc.)
- Degree (i.e., mild, moderate, severe, unspecified vs total/complete, partial/incomplete, etc.)
- Type (i.e., primary, secondary, unspecified, etc.)
- Laterality (i.e., R/L/unspecified or R/L/bilateral/unspecified)
- Episode of care (3-16 "extension" options, depending on code category)
- Trimester (i.e., 1, 2, 3,unspecified, etc.)
- Number of fetus (i.e. 1-5, other.)

Fig. 6.16 Axis (parameter) system of classification in the ICD10 stratification

influences, embryologic development, and the resulting phenotype. This is an inherent problem in seldom diseases like craniofacial malformations. One way to improve in future the approach to develop a modern classification system and to integrate such systems into a broader framework of craniofacial data, a specific ontology—the Ontology of Craniofacial Development and Malformation (OCDM)—was developed as part of a NIDCR-funded research network, FaceBase (https://www.facebase.org). The purpose of FaceBase is to provide diverse but standardized data to the craniofacial community and to facilitate collaboration among investigators to advance craniofacial research. The goal of the OCDM is to provide a unifying framework to represent and standardize the set of terms and relationships used to capture different forms of craniofacial data, including clinical data, within FaceBase and integrate data types to maximize their utility and accessibility [34]. This way of information technology has a great promise for future classification systems. The sheer volume of data collected in analyzing genetics, in documentation of phenotypes from 3D scans and omics data sets, generates massive and complex data sets. The size and heterogeneity of such data sets do not only pose new challenges to efficiently and effectively store data, but it is challenging to develop new algorithms to gain insight into the cause-and-effect correlations between genetics, embryological pathogenetics, and disease extent (phenotypic outcome).

It still remains in the future to develop an all-encompassing classification that will clarify (and include a correlation strength determination) the complex genetically driven morphopathogenesis of the resulting craniofacial malformation phenotype. By incorporation of limitations and challenges associated with using disparate classifications systems, it is hoped that future approaches will promote more discussion and cooperation in standardizing the classification of craniofacial malformations.

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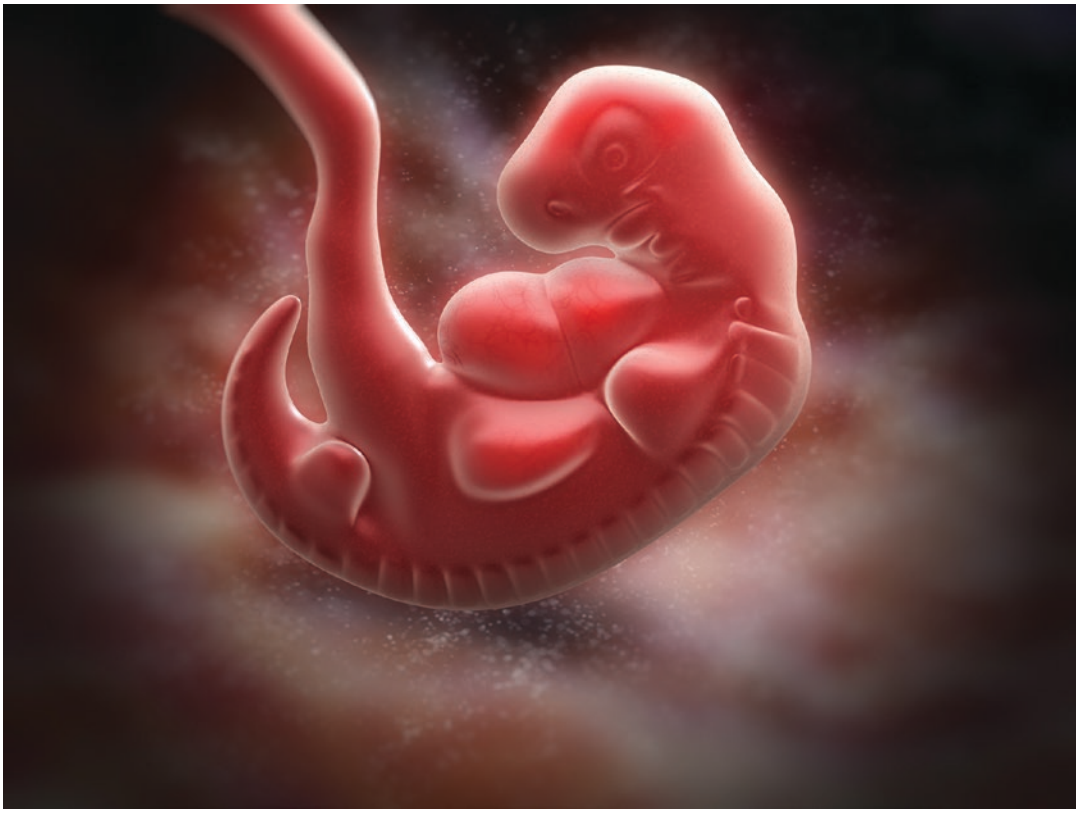
Part II

Biological Basis of Disease

Highlighted by an extraordinary and complex review on the biology of orofacial clefts by Dr. Chengji Zhou and colleagues.



7 The Biological Basis of Chromosomal and Single Gene Disorders

Shankargouda Patil, G. S. Vidya, and Khaled M. Alqahtani

7.1 DNA

DNA is a hereditary component of living organisms including humans. It is primarily located in the nucleus of a cell called the nuclear DNA, while a small portion of it may be present within the mitochondria referred to as the mitochondrial DNA. DNA stores its information in the form of a code comprising of four chemical bases, namely, adenine "A," cytosine "C," guanine "G," and thymine "T." The order or the sequence in which these bases pair up with each other forms the crux for the formation, development, and functioning of an organism. In humans, roughly about 3 billion bases take part in the creation of the double helix DNA structure [1–3] (Fig. 7.1).

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7.1.1 What Are Chromosomes?

Chromosomes are thread-like structures present within the nucleus of a cell. The tightly coiled DNA around the histone proteins forms the framework of a chromosome and stores the genetic information or code. In total, 23 pairs, i.e., 46 chromosomes, comprise the total DNA framework of a cell. Out of these, 22 pairs are autosomes, while 1 pair, i.e., X and Y, is the allosomes or sex chromosomes [4–6] (Fig. 7.2).

7.1.2 Then What Are Genes? Where Are They Located?

The basic physical and functional unit of hereditary is a gene. It is a DNA segment where the nucleotides are arranged in a specific sequence. Depending on the number of bases, a gene may vary in size. Their main functions are (1) to code protein and (2) to control the transmission and

Fig. 7.1 Schematic representation of double helix structure of DNA

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Fig. 7.2 Schematic representation of a chromosome present with the nucleus of a cell

Fig. 7.3 Schematic representation of a gene

expression of the traits procured through hereditary [6, 7] (Fig. 7.3).

7.2 Alleles

Variant form of a gene is called an allele. Diploid organisms, like humans, have two alleles, each inherited from both the parents individually. These alleles are responsible for the phenotype (outward appearance) of an organism. The genotype of a particular gene is represented by each pair of alleles. If the two alleles are identical at a specific locus, then the genotype is homozygous, and if they differ, then they are heterozygous in nature. Further, alleles can be either dominant or recessive in expression of the trait. In heterozygous genotype with one allele dominant and another recessive, the dominant phenotype is expressed. However, the phenotypic expression of the trait also depends on other factors such as the penetrance (frequency of expression of the trait in an individual) and expressivity (amount large enough of the trait to be expressed in an individual) [3, 6].

7.2.1 Derivation of Fundamental Laws of Inheritance

Based on the general information acquired, it can be summarized that the specific sequence of arrangement of the nucleotides forms our genetic makeup which is unique to each and every individual. This is the reason why we appear different from each other. Such observations and curiosity drive the quest for better perception of oneself and our surroundings.

This was what drew Gregor Johann Mendel, a scientist and an abbot, to conduct a series of experiments on pea plants and put forth the rules of inheritance which not only gained him recognition but also earned him the title of "Father of Modern Genetics." Mendel's laws of inheritance have not only answered the questions on expression and transmission of the inherited traits but also aided in the recognition of genetic disorders and the emergence and flourishment of geneticists [8, 9].

Inference drawn through his experiments provided answers to most of the abstract content around us. The very actuality of genes, presence of genes in pairs, existence of alleles, and gametic content are some of the observations protracted from his experiments. Further, he was able to deduce that the expression of the trait depends on the presence of an allele of either recessive/dominant phenotype.

The fundamental laws of inheritance can be broadly encapsulated as:

1. Law of segregation
2. Law of independent assortment
3. Law of dominance

These laws laid the very foundation of full-fledged research in the field of genetics in mankind. Sequencing of human genome has not only

provided us information on our genetic heritage but also helped us to understand the pattern of inheritance accompanied by unraveling the complex network of genetic events that contributes to a disease [3, 8, 9].

7.2.2 What Are Genetic Disorders?

In general, diseases which are a result of change in part or whole of the normal sequence of DNA are referred to as genetic disorders. Therefore, the mutations of a gene, i.e., any permanent alteration of the normal sequence of the DNA, e.g., thalassemia, either through inherited or environmental factors and even a combination of both are considered to be the prime culprits of genetic disorders [8, 9].

The ideology of classifying the genetic disorders is primarily dependent on the parameters such as the genes and the genetic-environmental interactions. Therefore, it is more of a gene-centric and factor-centric classification. These genetic disorders can be broadly classified into:

1. Single gene disorders
2. Chromosomal disorders
3. Multifactorial genetic disorders

Mitochondrial DNA present within the mitochondria of a cell can undergo mutations too, hence termed as mitochondrial disorders which also can be included under genetic disorders.

The estimated frequency of the genetic disorders in humans is 670 per 1000 which is quite a significant number of cases. Hence, the biological basis of these disorders forms the crucial link to understand their modes of inheritance, their penetrance, and expressivity of the inherited traits [10–13].

7.2.3 Single Gene Disorders

Single gene disorders are those disorders where the change in the sequence of the DNA is known to occur in a single gene. The inheritance pattern of these disorders is based on the Mendel's principles. Therefore, they are also referred to as Mendelian disorders/diseases [3, 8].

In general, we do come across numerous cases of complex disorders, for example, type II diabetes, which are more common and lifestyle oriented and their onset is manifested later in life. On the other hand, these single gene disorders are definitely not rare and are far more numerous than we generally assume. In contrast to the complex disorders, they manifest early in life, may present with severe conditions, and require support and care throughout life. Moreover, recent molecular studies have also provided evidence suggesting that these single gene disorders may also play a vital role in the etiopathogenesis of several complex/multifactorial disorders. Responsibility lies with us to have a thorough knowledge on the biological assembly of genes and their mutations to delineate its recognition and molecular makeup to aid in the early diagnosis, screening, and necessary counselling for the well-being of mankind [14–20].

7.2.4 Mode of Inheritance in Single Gene Disorder

Determining the probabilities of trait recurrence in the successive generation establishes the pattern of inheritance. In these disorders it is based on the Mendel's principles. More than one version of genes exists either due to mutations or polymorphism which are termed as alleles. The inheritance of the single gene disorders is either based on the location of the gene or depends on the number of copies of the genes required to express the phenotypic features of the disorder. Having said that, mutated allele expression in relation to the normal allele may either be dominant, recessive, or even co-dominant in nature [3, 10, 15]. Based on these features, the pattern of inheritance for the single gene disorders can be broadly listed as:

1. Autosomal dominant inheritance

   Dominant genotypic inheritance is present in a heterozygous individual where a single copy of mutant allele suffices for the manifestation of the disease. Presents with vertical mode of transmission, affecting both the gen-

ders equally. 50% risk of transmitting the mutant allele lies with the affected individual. E.g., achondroplasia (Fig. 7.4)

2. Autosomal recessive inheritance

   Recessive genotypic inheritance is seen in homozygous individual requiring two copies of mutant allele for the expression of the disease. Only one mutant allele leads to the failure of expression of the disease with the individual being a carrier. In case both the individuals are carries, the resultant offspring will have a 25% chance of inheritance and expression of the disease. E.g., cystic fibrosis (Fig. 7.5)

3. X-linked dominant inheritance

   In this mode of inheritance, the sex chromosome/allosomes are involved. X chromosome houses the mutant allele where even a single copy is responsible for the expression of the disease. The features are similar to the autosomal pattern of inheritance. However, the mutant allele will be transmitted to female offspring but not to the male offspring of the affected male. E.g., α-thalassemia (Fig. 7.6)

4. X-linked recessive inheritance

   Males are predominantly affected due to their hemizygyous state for most of the genes on X chromosome. In this mode of inheritance, the resultant male offspring will have 50% chance of inheritance of the disease, while female progeny will become a carrier. E.g., hemophilia (Fig. 7.7)

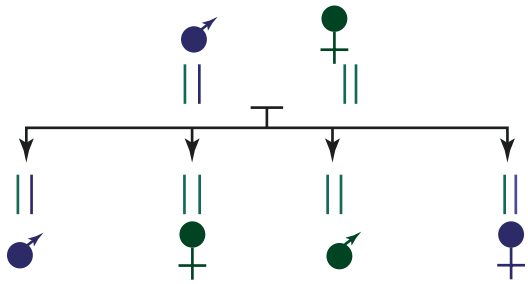

Fig. 7.4 Autosomal dominant mode of inheritance. Possible progeny of affected father with a dominant mutant gene and unaffected mother would either be an affected male or female offspring possessing one dominant mutant gene and normal gene or unaffected male and female offspring with normal genes. (Purple indicates dominant mutant gene; green indicates normal gene)

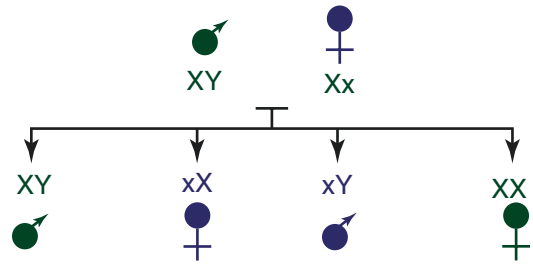

Fig. 7.6 X-linked dominant mode of inheritance. Possible progeny of unaffected father and affected mother with a dominant mutant gene would either be an affected female or male offspring possessing dominant mutant gene and normal gene or unaffected male or female possessing normal gene. (Purple indicates dominant mutant gene; green indicates normal gene)

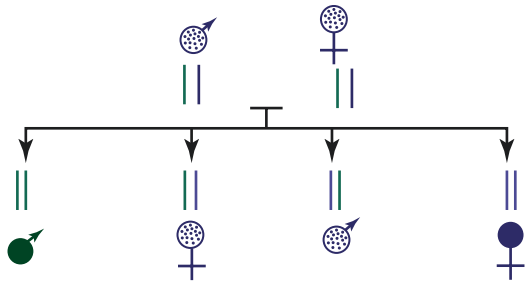

Fig. 7.5 Autosomal recessive mode of inheritance. Possible progeny of carrier father and carrier mother each with one recessive mutant gene and a normal gene would either be an affected female offspring possessing both the recessive mutant gene or unaffected male with normal genes or carrier female/male possessing one recessive and one normal gene. (Purple indicates recessive mutant gene; green indicates normal gene)

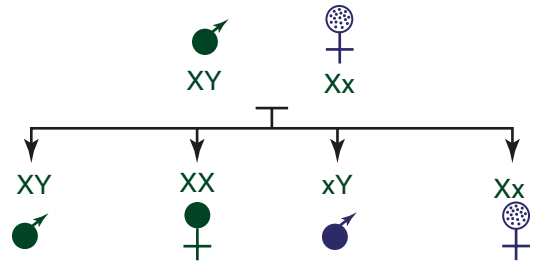

Fig. 7.7 X-linked recessive mode of inheritance. Possible progeny of unaffected father and carrier mother with a recessive mutant gene would either be an affected male offspring possessing recessive mutant gene and normal gene or unaffected male or female with normal genes or carrier female with one recessive mutant gene and a normal gene. (Purple indicates recessive mutant gene; green indicates normal gene)

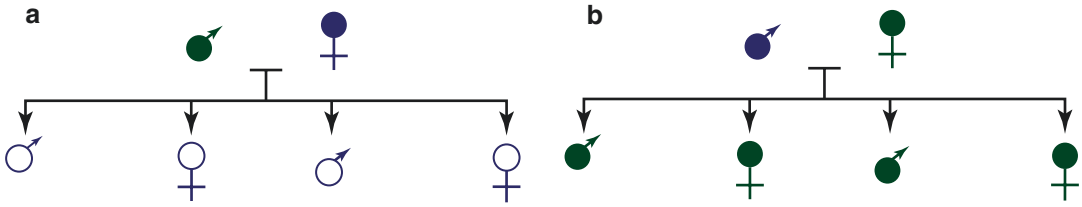

Fig. 7.8 Mitochondrial inheritance. (**a**) Possible progeny of unaffected father and affected mother would be affected children whose degree of penetrance depends upon the amount of mitochondria affected. (**b**) Possible progeny of affected father and unaffected mother would be unaffected children

5. Mitochondrial inheritance

   Both genders are equally affected with females being the only carriers. The expression of the disease occurs in every generation. E.g., Leber's hereditary optic neuropathy [10, 12, 14, 21] (Fig. 7.8)

7.2.5 Mutations in Single Gene Disorders

As we are already aware that the genes are responsible for the coding of proteins necessary for determining the structure and function of each cell in the body, their disruption due to various factors manifests significantly. Mutations are the essential element in single gene disorders. The form and the place in which they occur are diverse. The consequences of such a mutation results in a protein product that may be unable to perform its function or may perform its function but in a reduced capacity or even a new protein may be synthesized with damaging function or ultimately a protein may be entirely disabled due to mutation.

The above briefing strengthens that the prime etiology of the single gene disorders is mutations. Owing to the DNA chemical instability of the bases and replication errors are the factors responsible for the emergence of mutations. These mutations can either involve change in a single base pair or deletions of a few base pairs resulting in the disturbance in the function of the affected single gene. Molecular studies on abnormal hemoglobin and different forms of thalassemia were crucial in amassing our knowledge on different types of mutations.

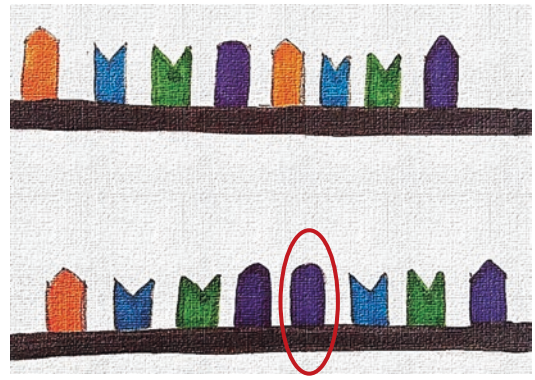

Fig. 7.9 Schematic representation of point mutation. Upper schematics represent normal sequence of base pairs, while the below represents mutated sequence of base pairs. Highlighted represents point mutation of the base pair

Point mutations are changes involving a single base pair of a gene (Fig. 7.9). They result in either of the following mutations. If an amino acid is replaced for another in a protein, it is termed as missense mutation. If an amino acid codon is replaced with a stop codon resulting in premature termination of the translation, it is termed as nonsense mutation. The specific sequence of codons that run from the start codon to the stop codon in mRNA is called a reading frame. Changes in the reading frame resulting in unrelated amino acid introduction into the protein are termed as frame shift mutations. Structural variation due to point mutations can be seen in alpha 1 antitrypsin deficiency, while nonsense and frame shift mutations are evident in factor VIII and IX deficiency.

Deletions of genes either partial or complete will subsequently lead to change in the gene number which is manifested in thalassemia and Lesch Nyhan syndrome. Inversions seen in δβ

thalassemia, initiation and termination codon mutations in α thalassemia, and RNA processing mutations are some examples of mutations that take part in the etiopathogenesis of single gene disorders [10, 12, 13, 15].

Accumulation of information on the molecular level of these disorders is essential to understand the variation in the penetrance and clinical phenotypic expression of them. Also, the reflection of the expression of these disorders may or may not be constricted to a particular organ/system of the body but may involve a group of organs or affect different systems of our body. Hence, we have tried to list some of the single gene disorders affecting the various systems of our body though their expression may be evident in other systems too (Table 7.1). The register of single gene disorders http://www.ncbi.nlm.nih.gov/omim continues given the number of genes and the sequences recorded [22]. However, some of the disorders with significant oral manifestations are listed in Table 7.2.

7.3 Chromosomal Disorders

Chromosomal disorders are those disorders where the chromosomes exhibit significant and evident changes in them. Each of the 46 chromosomes houses thousands of genes accommodating information required for the overall growth and development of our body. Any disturbance during the developmental stages of either the egg or the sperm formation or even during the fetus formation and development may lead to chromosomal abnormalities. However, the presentation of the different effects is dependent on the type of abnormalities. Therefore, any changes either affecting the number or structure of the chromosomes may hamper the normal functioning of the individual [3–7].

7.3.1 Numerical Abnormalities in Chromosomal Disorders

In humans, the somatic nucleated cells are diploid or 2N signifying the presence of 46 chromosomes, in contrast to the haploid/1N nature of the germ cells with 23 chromosomes. Therefore, any changes in the number either addition or deletion manifest as numerical abnormalities in chromosomal disorders. They are listed below:

1. Aneuploidy

   When the total number of chromosomes present is not an exact multiple of the haploid number, then it is termed as aneuploidy. E.g., 2N-1(45 chromosomes), 2N+1 (47 chromosomes). Nondisjunction, i.e., failure of normal separation of the chromosomes during cell division either during meiosis or mitosis, is considered as the most common mechanism responsible for aneuploidy. During mitosis, nondisjunction leads to two or more cell lines derived from the same zygote, termed as mosaicism, commonly observed in cancers. Anaphase lag is another form of nondisjunction resulting in one normal daughter cell, while the other is monosomic due to the missing chromosome (Table 7.3).
2. Polyploidy

   When the total number of chromosomes present is a multiple of the haploid number, then it is termed as polyploidy. E.g., 3N (69 chromosomes), 4N (92 chromosomes). It is commonly manifested in dividing cells and therefore may be the reason for spontaneous abortions [23].

7.3.2 Structural Abnormalities in Chromosomal Disorders

Structural abnormalities are those abnormalities within the structure of the chromosome as a result of breakage and incorrect rejoining of its segments. These abnormalities can be either balanced or unbalanced in nature. The presence of complete chromosomal set despite of rearrangement is termed as balanced while any additions or missing information is termed as unbalanced structural rearrangements. These abnormalities occur during gametogenesis and get transmitted to all the somatic cells leading to hereditary transmissible disorders or mutation of somatic cells (Fig. 7.10).

Table 7.1 Single gene disorders affecting the various systems of our body

Single gene disorders			
System affected	Disorder	Gene/locus affected	Pattern of inheritance
Blood and lymphatic system	Sickle cell anemia	HBB	AR
	Hemophilia B	F9	XLR
	Gaucher disease	GBA	AR
	Hemophilia A	F8	XLR
	Niemann-Pick disease	SPMD1 (type A and B) NPC1(C1 and D)	AR
	Porphyria	HMBS	AD
	Alpha-thalassemia	ATRX	XLD
Digestive system	Cystic fibrosis	CFTR	AR
	Diabetes, type II	PAX4 AKT	AD
	Glucose galactose malabsorption	SLC5A1	AR
	Zellweger syndrome	PEX	AR
	Wilson's disease	ATP7B	AR
ENT	Deafness	POU3F4	XLR
	Neurofibromatosis	NF2	AD
	Pendred syndrome	SLC26A4	AR
Eye	Macular dystrophy, vitelliform 2	BEST 1	AD
	Glaucoma	MYOC CYP1B1	AD AR
	Retinoblastoma	RB1	AD
	Gyrate atrophy of the choroid and retina	OAT	AR
Gland and hormones	Congenital adrenal hyperplasia	CYP21A2	AR
	Adrenoleukodystrophy	PEX1	AR
	Autoimmune polyglandular syndrome	AIRE	AD, AR
	Cockayne syndrome	ERCC	AR
	Diastrophic dysplasia	SLC26A2	AR
	Multiple endocrine neoplasia	MEN1 RET	AD
Heart and blood vessels	Ataxia telangiectasia	ATM	AR
	Long QT syndrome	KCNQ1 KCNH2 SCN5A	AD
	Von Hippel-Lindau syndrome	VHL	AD
Immune system	DiGeorge syndrome	TBX1	AD
	Familial Mediterranean fever	MEFV	AR
	Immunodeficiency with hyper-IgM	TNFSF5 AICDA	XLR AR
Muscle and bone	Charcot-Marie-Tooth syndrome	PMP22 MPZ	AD
	Myotonic dystrophy	DMPK CNBP	AD
	Marfan syndrome	FBN1	AD
	Fibrodysplasia ossificans progressiva	ACVR1	AD
	Achondroplasia	FGFR3	AD
	Duchenne muscular dystrophy	DMD	XLR
	Amyotrophic lateral sclerosis1	SOD1	AD, AR

(continued)

Table 7.1 (continued)

Single gene disorders			
System affected	Disorder	Gene/locus affected	Pattern of inheritance
Neonatal disease	Angelman syndrome	UBE3A	AD
	Fragile X syndrome	FMR1	XLD
	Prader-Willi syndrome	NDN SNRPN	AD
	Werner syndrome	RECQL2	AR
Skin and connective tissue	Menkes disease	ATP7A	XLR
	Ellis-van Creveld syndrome	EVC	AR
Respiratory system	Alpha-1-antitrypsin deficiency	SERPINA1	AR
Nutritional and metabolic system	Tay-Sachs disease	HEXA	AR
	Tangier disease	ABCA1	AR
	Refsum disease	PHYH	AR
	Phenylketonuria	PAH	AR
	Maple syrup urine disease	BCKDHA BCKDHB DBT	AR
	Hereditary hemochromatosis	HFE	AR
	Lesch Nyhan syndrome	HPRT1	XLR
Nervous system	Alzheimer disease	PSEN1	AD
	Huntington disease	HIT	AD
	Parkinson disease	SNCA PRKN	AD AR
	Friedreich's ataxia	FXN	AR
	Spinal muscular atrophy	DYNC1H1	AD
	Spinocerebellar ataxia	ATXN2	AD
Others	Rett syndrome	MECP2	XLD
	Alport syndrome	COL4A4	AR

7.3.2.1 Balanced Rearrangements

Balanced rearrangements may go undetected as full complement of DNA is still retained and fails to manifest as a disease. Complete absence or synthesis of nonfunctional protein due to breakage of chromosome or formation of a hybrid due to chromosomal segment fusion of two genes resulting in a new detrimental protein is the possible consequences required for the manifestation of the disease. Inversion and translocation of chromosomal regions are the two types of balanced rearrangements. Inversion involves the breakage of a single chromosome at two points and usually does not manifest as an abnormality. On the other hand, translocation literally means cross-over or exchange of chromosome fragments and is of two types, reciprocal and Robertsonian translocation. Exchange of genetic material due to single chromosomal breakage without the involvement of the centromere is termed as reciprocal translocation which can either be balanced or unbalanced in nature. Philadelphia chromosome, commonly seen in chronic myeloid leukemia, is a typical example for balanced type of reciprocal translocation. Robertsonian translocation occurs due to fusion manifesting as one large chromosome and other small one. They express normal phenotype but are usually accompanied with infertility.

7.3.2.2 Unbalanced Rearrangements

These include deletions, inversions, ring, and iso chromosome. Loss of genetic material either from the middle or terminal part of a chromosome is termed as deletion which is commonly seen in cri du chat syndrome. Rearrangement of a single chromosome due to breakage at two points is termed as inversion. Breakage of the terminal

Table 7.2 Single gene disorders with oral manifestations

Disorder	Gene/locus affected	Pattern of inheritance
Amelogenesis imperfecta	LAMB3 ENAM AMELX	AD AD XLD
Apert syndrome	FGFR2	AD
Basal cell nevus syndrome	PTCH2	AD
Beckwith Wiedemann syndrome	CDKN1C ICR1	AD AD
Cleidocranial dysplasia	RUNX2	AD
Cowden syndrome	PTEN	AD
Crouzon syndrome	FGFR2	AD
Dentinogenesis imperfecta	DSPP	AD
Clouston syndrome (ED II)	GJB6	AD
Ectodermal dysplasia I	EDA	XLR
Ehlers-Danlos syndrome	COL5A1 COL3A1	AD AD
Epidermolysis bullosa	KRT5 LAMB3 COL7A1	AD AR AD
Noonan syndrome	PTPN11	AD
Odontohypophosphatasia	ALPL	AD, AR
Osteogenesis imperfecta	COL1A1	AD
Pachyonychia congenita	KRT16	AD
Rubinstein-Taybi syndrome	CREBBP	AD
Stickler syndrome	COL2A1	AD
Treacher Collins syndrome	TCOF1	AD
Tooth agenesis	MSX1	AD
Tuberous sclerosis	TSC1	AD
Van der Woude syndrome	IRF6	AD

Table 7.3 Aneuploidy numerical abnormalities in chromosomal disorders

Chromosomal disorders	Aneuploidy numerical abnormalities
Downs syndrome	Trisomy 21
Mosaic variegated aneuploidy	Predominantly monosomies and trisomies
Pallister- Killian syndrome	Mosaicism for tetrasomy of chromosome 12p
Klinefelter syndrome	Sex chromosome trisomy, 47 XXY karyotype
Turner's syndrome	Monosomy (45, X0)

ends of a chromosome with deletion of the fragments that are broken followed by fusion of the ends is termed as ring chromosome, whose outcome is determined by the amount of genetic material lost. Transverse division of the centromere along the long axis of the chromosome results in iso chromosome, seen in few cases of Turner's syndrome [23–25].

7.4 Cancer: A Genetic Disease

Cancer, as we all are aware, is a pernicious epidemic and a leading cause of death worldwide. Irrespective of the vast research in this field, survival rates remain stunted and unaltered. We can speculate its complex behavior to be a reason for the same. Cancer is considered to be a multifactorial disease with combined genetic, metabolic, and environmental influences.

7.4.1 Genetic Factors in Cancer

Cancer may be due to inherited, somatic, or even a combination of these mutations. Mankind has strived to analyze the genetic basis of cancer as it forms the core for its emergence. To date, nearly 1000 known cancer-associated genes are identified composing of both oncogenes and tumor

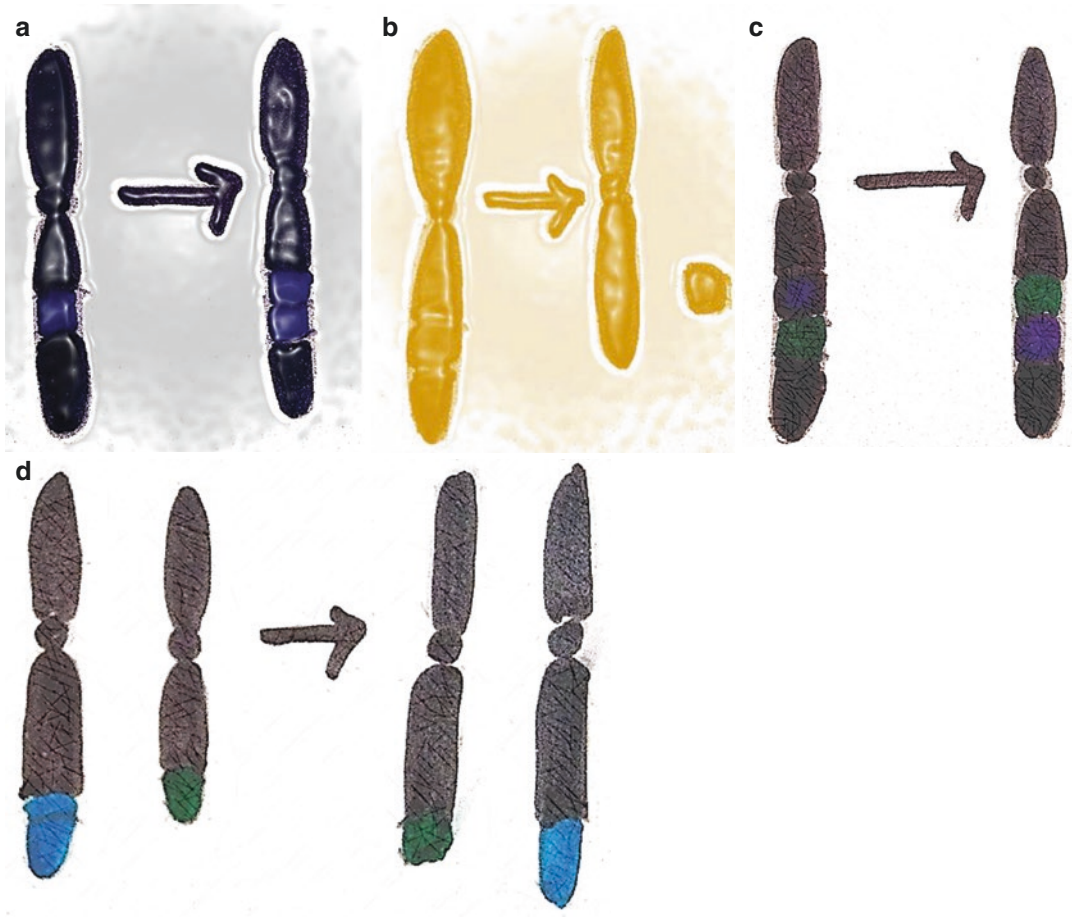

Fig. 7.10 Schematic representation of different types of mutations. (**a**) Duplication, (**b**) deletion, (**c**) inversion, (**d**) translocation

suppressor genes. Carcinogenesis is thought to be attained with two or more mutations in the above genes, which makes us realize that there may be more than a million cancer genotypes. Deciphering the cancer genetics, researchers have identified nearly more than million coding point mutations and non-coding mutations, more than ten thousand gene fusions and genome rearrangements, lakhs and millions of abnormal copy number segments, and varied expressions.

What's further more interesting is that in a study by Lee et al., they revealed that nearly 10–50 thousand of different single nucleotide variants in cancer cells were observed when compared to normal counterparts on whole genome sequencing [26, 27]. We have tried to list some of the cancers and their mode of inheritance in Table 7.4.

7.4.2 Other Contributing Factors in Cancer

Apart from inheritance of mutated genes, several other factors influence the microenvironment for the development of cancer. Our environment and lifestyle contribute tremendously in our well-being. Influence of habits such as tobacco, both smokeless and smoking forms, and alcohol is the major culprits in cancer. Tobacco is known to be associated with carcinoma of lung, mouth, esophagus, and larynx. It is observed that cytochrome P450 (CYP) gene polymorphism either alone or in combination of certain deficiencies may predispose a smoker to cancer [28]. It is an established fact that most of

Table 7.4 List of cancers with genes affected

Cancer	Gene/locus affected	Pattern of inheritance
Lung cancer	CYP2A6 EGFR CASP8	AD
MEN IIB	RET	AD
Thyroid cancer	NKX2-1	AD
Colorectal cancer	TLR2 PLA2G2A ODC1 MSH6 CyclinD1	AD
Leiomyomatosis and renal cell cancer	FH	AD
Gardner syndrome	APC	AD
Basal cell nevus syndrome	PTCH1 PTCH2	AD
Breast cancer	RAD54L CASP8 BARD1 BRCA2 CDH1	AD
Adrenocortical carcinoma	TP53	AD
Prostate cancer	CDH1 BRCA2	AD
Muir-Torre syndrome	MSH2 MLH1	AD
Head and neck squamous cell carcinoma	TNFRSF10B	AR
Gastrointestinal stromal tumor	SDHB SDHC KIT	AD
Cutaneous telangiectasia and cancer syndrome, familial	ATR	AD
Palmoplantar carcinoma, multiple self-healing	NLRP1	AD
Basal cell carcinoma	TP53	AD
Nasopharyngeal carcinoma	MST1R	AD
Wilms tumor	POU6F2 WT1 BRCA2	AD
Pheochromocytoma	SDHD KIF1B SDHB	AD
Peutz-Jeghers syndrome	STK11	AD

the HPV-associated oral cancer patients with a habit of chewing tobacco present with p53 alterations such as point mutations, overexpression, or even degradation [27]. Thence, exposure to tobacco leads to DNA single-strand breaks, polymorphisms, genetic mutations, chromosome aberrations, micronuclei, reactive oxygen species-induced oxidative stress, etc. Further, literature supports the role of alcohol consumption combined with polymorphism of ADH1B (alcohol dehydrogenase) and ALDH2 (aldehyde dehydrogenase) in cancer. Therefore, cancer is an amalgamation of genetic changes either inherited or acquired due to the environmental influences or a combination of both [29–33].

7.5 Conclusion

The repertoire of chromosomal and single gene disorders strives upon the importance of assimilation of the biological basis as it is the core for the recognition and diagnosis and forms the basis for counselling in these disorders. Having a thorough knowledge of the facts and figures at the molecular level aids in correlation of the clinical presentation with that of identification of the disorders; otherwise we might end up looking for a needle in a haystack.

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8 Fundamental Mechanisms of Orofacial Clefts

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8.1 Introduction

Orofacial cleft (OFC) is a congenital disorder referring to any cleft involving the mouth and possibly the nose, face, or combined. It is among the most common birth defects worldwide, occurring at an average incidence of 1:700 births globally with significant geographical and ethnic variance [1]. The high incidence is reflective of the complex and sensitive nature of human craniofacial development through the first 10–12 weeks of gestation [2]. While OFCs are associated with several congenital syndromes, nonsyndromic patients account for approximately 70% of all cases [1]. OFCs may be further classified into various subtypes including cleft lip

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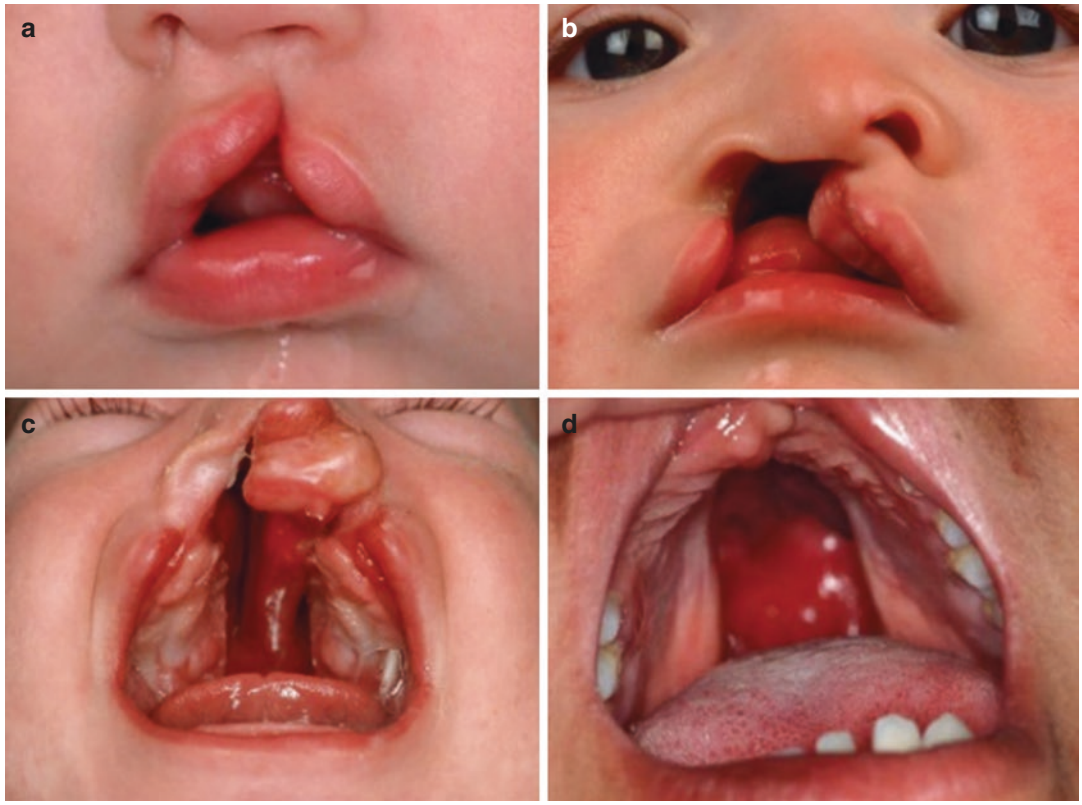

Fig. 8.1 Pediatric patients with various OFC subtypes. (**a**) Cleft lip only (it can be left or right or both sides). (**b**) Unilateral cleft lip with cleft palate (it can be left or right). (**c**) Bilateral cleft lip and cleft palate. (**d**) Cleft palate only. (From Stoll et al. [3], BMC Medical Genetics. Copyright@2004, Springer Nature)

only (CLO, Fig. 8.1a), cleft lip with or without cleft palate (CL/P, shown with cleft palate, Fig. 8.1b), bilateral cleft lip and palate (BCLP, Fig. 8.1c), and cleft palate only (CPO, Fig. 8.1d). Some studies may also differentiate between CL/P and cleft lip with cleft palate (CLP). Others describe submucous cleft palate (SMCP), a condition where the palatal cleft is obscured by an intact mucous membrane [4]. Oro-orbital cleft has been described in the context of parasitic infection [5]. At present, it is generally accepted that OFCs can be etiologically stratified into one of two major subtypes: CL/P or CPO. However, there is increasing evidence that CLO may have etiologies that are distinct from CL/P [6].

Orofacial morphogenesis involves tightly regulated cellular processes including cell migration, proliferation, differentiation, transition, and apoptosis. These processes are reviewed in greater by Ji et al. [7]. Craniofacial development begins with the epithelial-mesenchymal transition (EMT) of cranial neural crest cells (CNCCs) that stream from the cephalic end of the neural tube into the frontonasal process (FNP) and first branchial arch (BA1) [8–10]. CNCCs differentiate into structural craniofacial tissues including the bone, cartilage, and connective tissues [7, 11]. While the mandibular structures are derived from the posterior portion of BA1, the maxillary structures develop from the anterior portion of BA1. The two major developmental processes implicated in OFCs include midfacial morphogenesis, involving upper lip and primary palate development, and palatogenesis, which involves development of the secondary palate [7].

Despite some differences between species, the cellular and molecular mechanisms underlying these processes are generally conserved between

humans and mammalian models such as mice [12]. Midfacial morphogenesis occurs during the fourth–seventh week of development in humans (Carnegie stages 10–18) or embryonic days (E) 8.5–12.5 in mice [13]. At stage 15 (E10.5 in mice), three paired tissue primordia known as the medial nasal prominence (MNP), lateral nasal prominence (LNP), and maxillary prominence (MxP) grow together during robust mesenchymal expansion. By stage 16 (E11 in mice), the distal portions of the three prominences merge to form the lambdoidal (λ) junction and form an epithelial seam [14–17]. This undergoes subsequent degradation, resulting in the fusion of mesenchyme among the three prominences [18]. Merging also takes place in the nasolacrimal groove between the LNPs and MxPs, as well as between the MNP pair [19]. Unlike the mesenchymal fusion at the λ-junction, this merging involves a proliferation of mesenchymal cells to generate a smooth exterior of the midfacial region. Morphogenesis of the upper lip is completed by stage 19 (E12.5 in mice), along with expansion of the MNP into the oral cavity to form the primary palate [20]. Preventing the appropriate fusion or merging of the prominences may result in cleft lip with or without cleft palate.

The secondary palate is derived from paired structures, called palatal shelves, that are formed by expansion of the MxP into the oral cavity at the end of the sixth week (E11.5 in mice) [21]. The palatal shelves grow in a vertical orientation during the seventh week and shift to a horizontal orientation to elevate over the tongue during the eighth week. Fusion of the palatal shelves occurs during the ninth week. In mice, vertical growth of the palatal shelves occurs from E11.5 to E14, followed by rapid elevation and horizontal orientation by E14.5, palatal epithelial fusion by E15.5, and completed palatogenesis by E16.5 [7]. Palatal growth, elevation, and fusion involve several coordinated processes that facilitate the formation and dissolution of the medial epithelial seam (MES) between the shelves [22]. These processes include EMT, extracellular matrix (ECM) dynamics, cell migration and apoptosis, and developmental signaling through primary cilia [7]. Disruption of any of these processes may be sufficient to cause cleft palate.

The morphogenetic processes described above are spatiotemporally coordinated by molecular signaling pathways and transcription factors [23]. For example, mutations affecting Wnt signaling pathways, which interact with various aspects of neural crest development and morphogenesis, are associated with OFCs and other diseases [9, 24]. In humans, genetic mutations that affect the function or regulation of these proteins have been linked to both syndromic and nonsyndromic OFCs. For some of the affected genes, the mechanistic roles of these mutations in human orofacial development have been elucidated in mouse models [25]. Further, it is increasingly understood that environmental factors are major contributors to OFC etiology. Exposure to chemicals through behavioral, occupational, or domestic means is known to increase the risk for OFCs, while nutrients such as retinoids and folates are necessary for cellular processes and may protect against environmental insults [26–29]. Genetic variants in both mother and fetus modulate the risks imposed by these environmental factors, making gene-environment (GxE) interactions an important focus of OFC research [30]. Demographic variables are major drivers for OFCs, and they may influence etiology through a combination of associated genetic and environmental factors [1]. Environmental risk factors can perturb the tightly coordinated expression of genes required for orofacial development. This sensitive balance is now understood to involve epigenetic modifications and microRNAs that modulate gene expression [31].

This chapter summarizes key findings in the literature involving fundamental mechanisms for OFCs. We first describe human genetics and animal studies in the etiology of syndromic and nonsyndromic OFCs, including the role of gene mutations that affect developmental signaling and transcription factors. We then describe how environmental factors contribute to OFC etiology including prenatal nutrition, chemical exposure, demographic influence, and maternal health. Finally, we describe the emerging roles of epigenetic mechanisms and microRNAs in orofacial development and OFCs.

8.2 Genetics and Signaling Mechanisms of Orofacial Clefts

It has long been recognized that OFCs, like many other traits and diseases, may be subject to inheritable risk factors, and studies to understand the underlying genetics have advanced significantly in recent years with rapidly improving technology. A history of the evolution of genetic studies of human OFC causes has been reviewed in detail elsewhere [32]. In the genomic era, modern sequencing technologies have vastly improved our ability to determine molecular contributions to the appearance of clefts, and currently over 350 candidate genes have been identified which may contribute to OFC risk. Clefts may be a component of a broader syndrome, but more commonly observed are nonsyndromic OFCs. Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is the most common type of cleft present at birth. It is often difficult to determine the underlying causes of nonsyndromic OFCs. Many genomic variants, despite genetic association with OFCs, may be minor missense or noncoding mutations that can be insufficient to disrupt orofacial fusion processes on their own. Many different contributing risk factors can play a role in any given case or patient, often requiring large association studies to determine linkage between genomic variants and phenotype. Often different studies may present conflicting results for the same genes or even variants, potentially reflecting differences in genetic backgrounds or environmental influences on patient cohorts, experimental techniques, or statistical methods, further confusing analysis. On the other hand, many syndromic conditions are caused by more detrimental truncating mutations or genomic deletions, which may be detectable in individual patients. There are hundreds of syndromes which may be typified by the presence of a cleft. However, many of these are both genotypically and phenotypically heterogeneous, and OFC inclusion can be infrequent in many cases. Efforts to determine the underlying causes of the specific forms of a given syndrome which can include an OFC may be difficult, especially when dealing with conditions that are particularly rare or those which have been attributed to many different causal genes. The following discussion will address some of the major studies in recent years to determine the genetic influences on nonsyndromic OFCs and some of the more notable OFC-associated syndromes for which a genetic component has been identified. We also describe some of the developmental signaling pathways that are adversely affected by these mutations. Although beyond the scope of this discussion, a more detailed overview of the underlying mechanisms is presented in a review by Reynolds et al. [23].

8.2.1 Genetics of Nonsyndromic Human Orofacial Clefts and Mechanistic Animal Model Studies

Many studies seeking to associate OFC risk with genetic loci use a targeted approach to genotype or sequence candidate genes or genomic regions in patients and controls. Such studies may determine whether a particular variant is present at a higher frequency in cleft patients. These types of studies are useful to study genes for which suggestive evidence of linkage has previously been identified or for those which may be involved in cellular processes that have been implicated in midfacial development using animal models. Such linkage may not always be available, and genome-wide association studies (GWAS) allow for an unbiased analysis of genetic risk factors. These allow the identification of novel genomic regions which may be associated with OFCs. As these types of study assess co-segregation of genomic markers, they are commonly accompanied by targeted studies to specifically address candidate loci identified during the first phase. The following discussion will cover several of the landmark NSCL/P GWAS and key loci identified with significant association and related animal model studies that have strengthened evidence for their involvement.

A number of the early genome scans for NSCL/P association were unable to establish

genome-wide significance of association for key markers, and conflicting results have been unable to determine a role for several candidate genes, such as transforming growth factor alpha (*TGFA*), a ligand for epidermal growth factor (EGF) signaling and one of the most extensively studied genes in NSCL/P [33]. One such scan performed by Prescott et al. (2000) was the first major GWAS for OFCs. While no significant loci were reported by this group, suggestive evidence for linkage was reported for a few regions, which has been able to inform future studies. In 2004, a GWAS and meta-analysis analyzed data from 13 genome scans, generating robust evidence at that time for NSCL/P association with several genomic regions. Strong evidence for association was found at chromosome region 9q21, which houses several candidate OFC genes. A follow-up genotyping study was performed to further assess linkage with variants of key genes at this locus, and positive associations were reported with SNPs in four of these genes, patched 1 (*PTCH1*), receptor tyrosine kinase (RTK)-like orphan receptor 2 (*ROR2*), transforming growth factor beta (TGF-β) receptor 1 (*TGFBR1*), and forkhead box E1 (*FOXE1*) [34].

Studies using animal models have helped to clarify the roles of several of these genes in lip and palate formation. *PTCH1* encodes a core member and negative regulator of the sonic hedgehog (*SHH*) pathway, and Hedgehog (Hh) signaling contributes to several aspects of craniofacial formation, including midfacial expansion and patterning the palatal shelves. SHH is a key mediator of epithelial to mesenchyme interaction, and conditional deactivation of *Ptch1* in mouse neural crest results in constitutive pathway activation in the palatal mesenchyme. This prevents fusion between the nasal processes, resulting in cleft lip and primary palate [35]. While this model is lethal prior to secondary palatogenesis, constitutive activation of *Shh* in *K14*-expressing epithelium also results in complete secondary cleft palate, further demonstrating the importance of factors such as PTCH1 in modulating hedgehog signaling during lip and palate development [36]. The Wnt pathway, which includes canonical and noncanonical modes of signaling, regulates several processes important for orofacial development including transcription, cell adhesion, epigenetic modifications, and crosstalk with other signaling pathways [24]. *ROR2* encodes a Wnt receptor that typically transduces noncanonical signals to control planar cell polarity and calcium signaling pathways. *Ror2*-null mice exhibit cleft palate, as do those lacking a functional copy of its ligand, *Wnt5a.* Compound heterozygous mouse embryos for mutant alleles of *Wnt5a* and *Ror2* also have cleft palate, which is not the case with embryos heterozygous for either gene individually. This strengthens the evidence of this important signaling interaction in forming the palate, and it is a likely mechanism by which variants may contribute to the formation of clefts in human patients [37, 38]. The murine homolog of *TGFBR1* (previously known as *ALK5*), a type I receptor for TGF-β signaling, has demonstrated to be indispensable in both tissue lineages of the developing facial prominences. Epithelium-specific *Tgfbr1* deletion results in a posterior palatal cleft, while *Tgfbr1* deletion in neural crest is much more severe, disrupting convergence of the two MNPs to cause a midline frontonasal cleft with full secondary cleft palate [39].

Significant linkage with *FOXE1* was demonstrated again in a 2009 GWAS, and several studies have further strengthened the evidence for its role in NSCL/P [40–44]. This scan implicated several new loci including interferon regulatory factor 6 (*IRF6*), another key orofacial transcription factor linked in several targeted studies [45–48]. IRF6 is an epithelial transcription factor that is important for keratinocyte differentiation in the oral primordia [49]. *Irf6* also mediates epithelial apoptosis, acting downstream TGF-β signaling. Conditional epithelial *Tgfbr2* mutant mice display a submucous cleft in the anterior palate due to failed apoptosis, which can be rescued with IRF6 supplementation. These embryos also have a full posterior cleft, which is not rescued by IRF6 [50]. Additionally, *Irf6*-null mice have severe defects including a cleft palate in which the palatal shelves fail to elevate entirely, possibly reflecting other roles for *Irf6* in communication between tissue lineages [51].

Several GWAS have demonstrated a strong NSCL/P association with genomic region 8q24.21, which lies in a gene desert [52–54]. It is not clear how this locus contributes to lip and palate formation, but it may be that variants affect expression of the nearby transcription factor c-MYC (*MYC*), which plays key roles throughout development [55]. One of these studies also reported NSCL/P association with *MAFB* and *ABCA4* [52].

Two important genes with strong evidence for NSCL/P association, ventral anterior homeobox 1 (*VAX1*) and noggin (*NOG*), were first identified in another 2010 GWAS [56]. VAX1 is a transcription factor with roles in craniofacial development, and *NOG* encodes a bone morphogenic protein (BMP) regulator. Both have been implicated in subsequent scans [12, 57, 58], and several targeted studies have further strengthened the evidence for association of *VAX1* SNPs with NSCL/P [59–61]. *Vax1*-null mice do not have a cleft, so its role may not be conserved across species, or it may not directly contribute to the appearance of cleft lip or palate [62]. However, Noggin (*NOG*), which specifically binds key craniofacial BMP ligands BMP2, BMP4, and BMP7, is required for palatogenesis in mouse, and *Nog*-null embryos show increased BMP activity and proliferation resulting in a fully clefted secondary palate with anomalous maxillary-mandibular fusion [63].

A 2017 GWAS and meta-analysis of OFC data from a Chinese population identified several novel loci and strengthened evidence for several known genes with NSCL/P association. These authors also performed a network analysis demonstrating likely interactions linking several important craniofacial transcription factors and signaling pathways implicated in the study [12]. The muscle segment homeobox (MSX) transcription factor genes are regulated by BMP signaling, and *MSX1* had been a strong OFC candidate linked with NSCL/P in multiple targeted association studies prior to its identification in this GWAS, firmly establishing its role in the presentation nonsyndromic clefts [64–67]. *Msx1*-null mouse embryos also have cleft palate, and in addition to BMP, *Foxe1* regulates *Msx1* in the mouse palatal shelf. Mice lacking *Msx1* have dysregulated palatal *Bmp2* and *Shh* expression, which is rescued by ectopic BMP4, illustrating an important regulatory loop between *Msx1* and *Bmp4* [68, 69]. *Msx1* and its homolog *Msx2* are downstream target genes of Lrp6-mediated Wnt/β-catenin signaling that plays a crucial role in upper lip and primary palatal formation and fusion [70].

Another novel gene identified in this study, transcription factor AP-2 alpha (*TFAP2A*), is a key NSCL/P candidate thought to regulate craniofacial *IRF6*, which was previously linked in targeted studies [60, 71, 72]. Interaction among multiple key morphogenetic signaling pathway genes was demonstrated by Yu et al. [12], including several fibroblast growth factor (FGF) pathway components (*FGF10*, *FGFR1*, and antagonists *SPRY1/2*), Hh (*PTCH1*), BMP (*NOG*), and Wnt (*WNT9B*) pathways [12], the roles for several of which have been demonstrated in animal models. *Tfap2a* mutant mice have fully penetrant BCLP, and its role in craniofacial formation, at least in part, includes modulating expression of *Fgf8* in the developing nasal processes, which is also thought to play key roles in the developing lip and palate [73]. *Fgf10*, which is expressed on the oral side of the palatal shelf and its progenitor lateral side of the MxP, signals to the epithelium to help pattern the oronasal axis of the palatal shelf. Consistent with its expression in palatal tissues, *Fgf10*-null mouse embryos have cleft palate [74].

Both *Fgfr1* and *Fgfr2* are important receptors in the developing mouse palate. Conditional mutants lacking *Fgfr1* in neural crest lineage cells have severe phenotypes including midline nasal and lip cleft and both primary and secondary palatal cleft [75]. When using *Twist*-Cre driver to target *Fgfr1*, palatal defects are less severe, and CPO penetrance is only 16%, and when targeting *Fgfr2*, no cleft is observed. However, in *Fgfr1/2* double conditional mutants, cleft palate penetrance is 100%, reflecting a partial redundancy or capacity for compensation between the two receptors in patterning the palatal shelves [76].

The Sprouty genes encode FGF inhibitors, and a previous targeted sequencing study had previously shown a possible NSCL/P link with one *SPRY2* variant. More recently, gene-gene interactions between *SPRY1* and *SPRY2* as well as between *SPRY2* and *SPRY4* were shown to contribute to the risk of OFC [77, 78]. A 1 Mb genomic deletion including *Spry2* causes highly penetrant CPO and occasional CLP in homozygous mutants. This can be rescued with transgenic *Spry2*, revealing it as the primary gene responsible for OFC in this mouse line [79]. Targeted deletion of *Spry2* revealed that cleft palate occurs due to excess mesenchymal proliferation in the palatal shelf, resulting in elevation failure. In contrast, ossification and epithelial fusion processes are unaffected [80]. Additionally, ectopic activation of *Spry1* in neural crest lineage cells also causes cleft palate due to insufficient FGF signaling, demonstrating the requirement for appropriate levels of FGF activity during palatal shelf morphogenesis [81]. Introduction of a *Spry2* overexpression vector in chick embryos resulted in truncated facial prominences, with osteogenesis similarly unaffected, further emphasizing the role FGF signaling plays in craniofacial tissue growth, rather than differentiation [82].

Among other promising genes identified is *ARHGAP29*, with which significant association was found in three NSCL/P genome-wide studies [12, 41, 83]. A targeted SNP genotyping association study further strengthened the evidence of NSCL/P linkage and revealed gene-gene interactions between *ARHGAP29* and *TP63*, *PBX1*, *PBX2*, *WNT3*, and *WNT9B* [84]. *ARHGAP29* encodes a Rho GTPase activating protein, and mice carrying a loss-of-function *Arhgap29* allele have abnormal oral epithelial adhesions between the palatal shelves and tongue or mandible. These experiments also revealed that *Arhgap29* may act downstream of *Irf6* in oral periderm [85].

WNT9B is another interesting OFC candidate gene clustered at 17q21 with *WNT3*, a gene that also has polymorphisms and haplotypes that have been linked with NSCL/P or nonsyndromic cleft palate only (NSCPO) in multiple targeted genotyping studies [44, 86–89]. One study, which explored SNPs in both genes, also found association between a *WNT9B* variant and NSCL/P, while another demonstrated haplotype association as well as an epistatic interaction between *WNT9B* and *MSX1* [44, 90]. Additionally, the murine homolog was determined to be the gene mutated at the *Clf1* locus in an important CL/P model, the A/WySn mouse line, which has spontaneous clefts [91]. Incompletely penetrant CL/P is similarly observed in *Wnt9b* mutant embryos, associated with reduced FGF signaling and cell proliferation in the palatal shelves, causing elevation and convergence to delay or fail altogether [92]. A study found that the pre-B-cell leukemia homeobox (PBX) transcription factors may regulate *Wnt3-Wnt9b* in the midfacial ectoderm, which in turn regulates tumor protein p63 (*Tp63*) [93]. *TP63* encodes an epithelial transcription factor and canonical Wnt target, and *TP63* variants have also been recently been implicated in NSCL/P, including in both GWAS and exome-sequencing studies [41, 94]. A subsequent genotyping study examined the possible contribution of variants in the genes within this regulatory network in human OFC patients and found *PBX1* and *PBX2* association with NSCL/P. This group further demonstrated that gene-gene interactions between *PBX1* and *WNT9B*, as well as between *IRF6* and each of *PBX1*, *PBX2*, and *TP63,* contributed to the etiology of NSCL/P. This strengthened the evidence that a regulatory network involving PBX-regulated Wnt that activates *Tp63* and *Irf6* in the developing midfacial ectoderm is conserved across Mammalia [95].

8.2.2 Syndromic Human Orofacial Clefts and Mechanistic Animal Model Studies

Many important developmental mechanisms are conserved across different systems of the body, and so it is common for deleterious mutations that disrupt morphogenetic processes to affect many different aspects of embryogenesis. Craniofacial defects such as OFCs are commonly accompanied by altered development of other organs and systems. This often includes neural,

genital, and skeletal development but, depending on the cellular process affected by a particular mutation, can include nearly every system in the human body. Many syndromes have multiple causal factors or are caused by complex mutations that complicate attempts to determine the mechanism by which lip or palate fusion is affected. However, in many cases, modern sequencing technologies have allowed researchers to identify the factors that contribute to the presentation of craniofacial phenotypes in OFC-inclusive syndromes.

8.2.2.1 Van der Woude Syndrome

The most common form of syndromic OFC, Van der Woude syndrome (VWS), is characterized by CL/P, may include lip pits, and is often indistinguishable from NSCL/P. *IRF6* mutations most commonly cause VWS as well as dominant popliteal pterygium syndrome (PPS), a condition that affects the skin and genitals and may also include CL/P [96]. More recently, several genes that interact with *IRF6* have been found to account for some of the VWS cases where no *IRF6* mutation could be found. These include grainyhead-like 3 (*GRHL3*), which encodes an ectodermal IRF6 transcriptional target and may be a key effector of its function during craniofacial development [97, 98]. *GRHL3* was also implicated in NSCPO in two separate GWAS [41, 99]. *Grhl3*-null mice have altered oral periderm development and exhibit low penetrant CPO, and compound mutant analysis revealed an epistatic relationship between *Grhl3* and *Irf6* [98]. Two more genes, nonmetastatic expressed 1 (*NME1*) and *NME2*, encode proteins that regulate cell adhesion and cytoskeletal dynamics. NME1 and NME2 interact with cytosolic IRF6, and this interaction may also be key for craniofacial formation. VWS-causing mutations in *IRF6* were shown to disrupt its ability to interact with NME. Further, a missense variant in *NME1* was recently identified in one VWS patient, while a missense *NME2* mutation was identified in another with NSCL/P, strengthening evidence of this interaction's importance in oral periderm development [100].

8.2.2.2 Pierre Robin Sequence

Several characteristic craniofacial phenotypes that are commonly observed together include micrognathia, glossoptosis, cleft palate, and upper airway obstruction. Referred to as the Pierre Robin sequence (PRS), these defects are often, but not always, observed as part of a broader syndrome. Long recognized to frequently accompany each other, common underlying pathologies between these defects may contribute to their appearance, and several models for an underlying mechanism have been proposed. The dominant models suggest that micrognathia alters positioning of mandible and tongue to disrupt other aspects of oral development. As such, the palatal clefts do not appear to be rooted in defective activity within the developing palatal shelves (PS) themselves, but secondary to hypoplastic mandible and tongue development that affects their movement and prevents elevation or fusion. In a similar manner, the micrognathia results in a high posterior tongue positioning that blocks the upper airway. This may occur as a result of genetic mutations that affect craniofacial development, as with several syndromes which include PRS. It is also possible that intrauterine mandible compression or a delay in neuromuscular development, required for the tongue to stimulate mandible growth, may alter tongue and jaw development to block the PS and airway [101].

Several genes that code for ECM components and ECM-interacting proteins have also been associated with syndromes. Many syndromes typified by OFCs, including PRS, are associated with altered ECM activity and mutations in ECM component genes. Two conditions with significant overlap in phenotypic and genotypic spectra are the related Stickler and Marshall syndromes. Both frequently include PRS and are caused by mutations in several collagen genes including *COL2A1*, *COL11A1*, *COL11A2*, *COL9A1*, *COL9A2*, as well as lysyl oxidase-like 3 (*LOXL3*), which encodes a collagen crosslinking enzyme. However, only *COL2A1*, *COL11A1*, and *LOXL3* mutations have been found in Marshall-Stickler patients with OFCs [102, 103]. TARP syndrome (talipes equinovarus, atrial septal defect, Robin sequence, and persistent left superior vena cava)

is an X-linked disorder defined by the inclusion of PRS caused by mutations in the gene encoding RNA-binding motif protein 10 (*RBM10*) [104]. Campomelic dysplasia is another disorder which commonly includes PRS that affects development of the genital and skeletal systems. It is caused by mutations in a key chondrogenesis gene, SRY-related HMG box 9 (*SOX9*) [105, 106], and *SOX9* variants have also been associated with isolated PRS [107, 108]. Conditional inactivation in cranial neural crest or haploinsufficiency of mouse *Sox9* results in a hypoplastic craniofacial skeleton missing cartilage and endochondral bones, along with a cleft secondary palate [109, 110]. *BMPR1B* encodes a BMP signaling receptor and important craniofacial regulator, and causal mutations in *BMPR1B* were recently reported in two unrelated families with isolated PRS [111].

8.2.2.3 Chromosome 22. 22q11.2 Deletion Syndrome

Also known as velo-cardio-facial syndrome, chromosome 22q11.2 deletion syndrome (22q11.2DS) involves perturbed development of neural crest-derived tissues, including craniofacial mesenchymal cells of the palate [112]. T-box transcription factor 1 (*TBX1*) lies in this region of chromosome 22, and its deletion is believed to contribute to some of the palate phenotypes. These may include a high-arched palate or bifid uvula on the milder end of the spectrum or a complete cleft secondary palate in more severe cases [113, 114]. DiGeorge syndrome is a related disorder with overlapping characteristic phenotypes and is caused by mutations at the same region but without complete deletion of 22q11.2 [112]. A similar disorder, known as Opitz G/BBB syndrome, can be either X-linked or autosomal dominant. X-linked Opitz G/BBB is caused by mutations in *MID1* and *SPECCL1*, but the autosomal dominant form is also caused by a genomic deletion at 22q11.2 [115].

A 1.5-Mb region at human chromosome 22q11.2 is highly conserved in mouse on chromosome 16. Deleting the homologous region in mice generates a model that mimics the phenotypes of 22q11.2DS patients, including cardiovascular and craniofacial defects associated with the functions of the *TBX1* gene [116–118]. Studies using *Tbx1* knockout mice provided further evidence for its role as the gene responsible for the palatal clefts observed in the genomic deletion model. *Tbx1*-null embryos have cleft secondary palate with defective shelf elongation and diminished hyaluronic acid levels, which are thought to be critical for generating the force necessary for palatal shelf movement and elevation [119, 120]. These embryos also have reduced *Fgf8* expression in the palatal shelf, suggesting TBX1 may be necessary for its activation during secondary palatogenesis. TBX1 also helps control keratinocyte growth and differentiation, and mutants also exhibit abnormal oral epithelial adhesions associated with excessive proliferation [119, 121].

8.2.2.4 Craniofacial Ciliopathies

Primary cilia consist of three major parts including the axoneme, basal bodies, and a specialized ciliary membrane [122]. They play a critical role in coordinating a number of signaling pathways during craniofacial development, including Hh, platelet-derived growth factor (PDGF), polycystin, and Wnt [123]. Primary cilia help control aspects of development in many systems, including limb and craniofacial formation, and several syndromes associated with cilia dysfunction, or ciliopathies, can include OFC. Ellis-van Creveld syndrome is a ciliopathy that affects bone growth as well as cardiac and craniofacial development, often including dental abnormalities and CL/P. It is caused by mutations in two genes clustered at 4p16 that code for cilia proteins involved in Hh signaling, *EVC* and *EVC2* [124]. Another group of conditions which usually include clefts is orofaciodigital syndrome (OFDS), with over 15 different types described. Most forms are extremely rare, each owing to unique underlying genetics of which many are known to affect cilia formation or function. Type I, also known as Papillon-Leage-Psaume syndrome, is the most common. It affects the skin and hair in addition to abnormal facial and digital morphology and usually includes CL/P or CPO. OFDS type 1 is caused by mutations in the eponymous *OFD1*, an X-linked

gene encoding a centrosomal protein [125]. Other genes linked with cleft-associated OFD forms include *TCTN3* (type IV, or Mohr-Majewski syndrome), *DDX59* (type V, or Thurston syndrome), *CPLANE1* and *TMEM216* (type VI, or Varadi-Papp syndrome, a Joubert-related disorder), *TBC1D32* and *SCLT1* (type IX), and *C2CD3* (type XIV), all of which encode cilial or centrosomal proteins [126–131]. In addition to OFD6, *TMEM216* is associated with another ciliopathy which may include CL/P, Meckel-Gruber syndrome. Mutations at several centrosome/cilia genes have been identified in Meckel-Gruber patients, with *RPGRIP1L*, *TCTN2*, *TMEM67*, as well as *TMEM216* having confirmed association with OFCs [131–134].

Using mouse models, studies investigating the roles that cilia play during development have shown that the intraflagellar transport complex is essential to craniofacial morphogenesis. The intraflagellar transport complex exists at the basal body and axoneme of cilia and mediates bidirectional movement of cellular cargos along axonemal microtubules [135]. Intraflagellar transport protein 88 (*Ift88*) is one of the main components of the intraflagellar transport complex, and its deletion in cranial neural crest cells causes BCLP in mouse, while deletion specifically in the palatal mesenchyme leads to isolated CPO [136]. Similarly, the loss of another two intraflagellar transport genes *Kif3a* and *Ttc21b* also contributes to craniofacial defects, including CPO when *Ttc21b* is disabled in the neural crest and BCLP when *Kif3a* is targeted. However, embryos display BCLP when either gene is ablated in the surface ectoderm [137].

Hh signaling takes place within the cilium and is intimately linked with cilial function. While several of the previously discussed ciliopathy-associated genes affect Hh signaling, animal studies further confirmed that coordination between primary cilia and Shh signaling plays an essential role in craniofacial development. In the *Ift88*-deficient mouse, which exhibited defective ciliogenesis and OFCs, *Shh* was also disrupted [136]. Deficiency of the intestinal cell kinase (*Ick*) gene causes an abnormally elongated primary cilia in mouse, which results in diminished SHH-mediated signaling with reduced palatal shelf proliferation and CPO. This can be rescued with a Shh-activating smoothened agonist [138].

8.2.2.5 Hedgehog Signaling

Besides coordinating with the primary cilia, Hh signaling also independently influences craniofacial development based on evidence derived from human and animal studies. One function of Hh signaling involves coordination of midfacial expansion, and it may contribute to specific phenotypes along with OFC mainly affecting the midline craniofacial structures [139, 140]. Altered hedgehog signaling is associated with holoprosencephaly, in which the developing forebrain does not form two separate hemispheres. Holoprosencephaly may be accompanied by CL/P, and a number of causal genes and loci encoding core Hh pathway components have been identified in OFC-inclusive holoprosencephaly patients, including *SHH*, *PTCH1*, and *GLI2* [141–143]. Hh deficiency in mouse contributes to similar phenotypes, and both targeted *Shh* gene disruption and exposure to the Hh pathway antagonist cyclopamine result in cleft lip and cleft palate as well as holoprosencephaly [144, 145]. *PTCH1* mutations also cause nevoid basal cell carcinoma syndrome (also known as Gorlin-Goltz syndrome, not to be confused with Goltz-Gorlin syndrome or focal dermal hypoplasia, which may also include CL/P), which frequently includes OFCs [146]. Additionally, mutations altering the genes encoding the important forebrain patterning transcription factor Sine oculis homeobox 3 (*SIX3*), as well as the Nodal/TGF-β modulating protein, transforming growth-interacting factor (*TGIF1*), may also cause holoprosencephaly including CL/P [147–150]. *Gli2* is a dominant transcription factor and Hh signaling effector, and its deficiency in mouse causes a severe phenotype of holoprosencephaly including forebrain and face malformations [151]. Notably in these studies, the variation in phenotypes of holoprosencephaly co-occurring with the orofacial clefts, in both human and animal studies, indicates the interaction between environment and genetics profoundly affects developmental patterning. A more comprehensive list

of syndromes that may include OFC phenotypes can be found in a recent review [23].

8.3 Essential Nutrients and Orofacial Clefts

8.3.1 Dietary Folate

Folate, also known as vitamin B_9, is a water-soluble nutrient that humans must obtain through the diet. In mice, loss-of-function mutations in the folate receptor gene (*Folr*$^{-/-}$), which is involved in cellular import of folate, resulted in OFCs and neural tube defects (NTDs) in embryos [152, 153]. This provided experimental evidence that folate is required for normal mammalian orofacial development. As the primary source of methyl groups (-CH_3) for biochemical reactions, folate is necessary for DNA synthesis and repair, amino acid synthesis, polyamine synthesis, post-translational modifications to biomolecules (e.g., histone methylation), and the methylation of both DNA and RNA molecules [154–157]. While important for maintaining adult health, folate is particularly critical for development because of its role in cellular proliferation and differentiation. It is important for the conversion of methionine into S-adenosylmethionine (SAM), the methyl donor for several reactions [157]. Folate metabolism additionally prevents the toxic accumulation of homocysteine (hyperhomocysteinemia), a condition which may lead to OFCs in the children of afflicted mothers [158].

Folate deficiency during pregnancy is strongly associated with NTDs such as spina bifida, and prenatal supplementation with folic acid (synthetic folate) is protective against these birth defects. In 1998, the United States Food and Drug Administration implemented mandatory folic acid fortification of grains (minimum 0.14 mg per 100 g grain) to protect against NTDs [159]. There is evidence that folic acid fortification also protects against OFCs. In California, a cross-sectional study found that both CL/P (prevalence ratio (PR) = 0.91, 95% confidence interval (CI): 0.82, 1.00) and CPO (PR = 0.81, 95% CI: 0.70, 0.93) began declining in the post-folic acid fortification period [29]. In terms of voluntary prenatal folic acid supplementation, studies of aggregate data have reached different conclusions. Meta-analysis by Johnson and Little [160] failed to identify a strong relationship between OFCs and folic acid supplementation alone, whereas a more recent meta-analysis by Jahanbin et al. [161] determined that supplementation was protective [160, 161]. However, analyses of aggregate data have been hampered by extensive heterogeneity between studies [160].

Several variables may factor into the effectiveness of folic acid supplementation in the prevention of OFCs. The timing of supplementation, relative to conception and orofacial development, is important since folic acid appears to be most protective when taken periconceptionally and/or during the first trimester [161, 162]. It has also been suggested that large dosages (≥6 mg) may be necessary for a protective effect, which was more pronounced for CL/P compared to CPO [163]. For comparison, a dose-response model indicated that supplementation with 5 mg folic acid would prevent 85% of NTD cases [164]. Another important consideration is variation between populations. As an example, Chile implemented mandatory folic acid fortification of wheat flour in 2000, but unlike California, there was no decrease in OFC prevalence [165, 166]. It has been hypothesized that variations in genes involved in folate metabolism can at least partly explain such population differences [167]. Finally, folate metabolism and complete downstream utilization of folic acid require additional vitamins, including pyridoxine (vitamin B_6) and cobalamin (vitamin B_{12}). This may at least partly explain the results of Johnson and Little [160], who found that multivitamins were protective against OFCs versus folic acid alone [160].

8.3.2 Dietary Retinoids

Retinoids are a class of fat-soluble compounds that include retinol (vitamin A) along with its precursor and derivative metabolites. Like folate, retinoids are an essential nutrient required for human development and homeostasis [168]. Both

excess and deficiency of retinoids can cause orofacial cleft [169]. They serve as precursors for retinoic acid (RA), a signaling molecule that can alter gene expression to regulate orofacial development and other processes. Retinoids may be obtained from animal products as retinol or retinyl esters, or from plant products as carotenoids [170]. Retinol is the primary circulating retinoid in animals, while retinyl esters are stored primarily in the liver [168, 171]. During human development, retinol is acquired through the maternal diet and is exchanged at the placenta from mother to fetus [172]. Upon cellular uptake, retinol undergoes oxidation to become RA, the bioactive retinoid that binds its receptors in the nucleus to control gene expression [173, 174]. The cellular abundance of RA is balanced through retinaldehyde dehydrogenases (RALDH) and cytochrome P450s (CYP26). While RALDH enzymes oxidize retinaldehyde into RA, CYP26 enzymes facilitate elimination of RA through the covalent addition of oxygen [168]. In mice, loss-of-function mutation in both *Raldh2* and *Raldh3* resulted in failure of frontonasal fusion and other craniofacial defects [175]. Another loss-of-function mutation in *Cyp26b1* prevented palatal shelf elevation, possibly due to failed tongue depression [176]. A tight balance of enzymatic activities and RA abundance is therefore necessary for appropriate cellular function and orofacial development.

Evidence from both laboratory and human population studies demonstrated that excess maternal retinoid exposure increases the risk of OFCs in offspring. Maternal consumption of vitamin A at teratogenic levels has been reported to induce cleft palate in various mammalian species [177–180]. In a human population study conducted in the northeastern United States, overconsumption of vitamin A during gestation (in excess of 10,000 IU or approximately 3000μg) was associated with cleft lip and other birth defects [181]. Notably, birth defects were associated with excess consumption during or before development of the affected organs. These data support that overconsumption of vitamin A may be teratogenic toward orofacial development.

In contrast to these studies, others have demonstrated a protective role for vitamin A in orofacial development. Studies in Scandinavian populations found protective effects of vitamin A derived from diet or multivitamin supplementation [26, 182]. A common source of vitamin A in this region is liver and related products such as cod liver oil, which are enriched with vitamins A, C, E, and omega-3 fatty acids. Consistently, a study of a Chinese population found similar protective effects of cod liver oil, indicating that this effect may be found across populations [183]. Another study in China found that increased newborn serum levels of retinol binding protein (RBP4), a carrier protein that correlated with retinol levels, was lower in children with CL/P [184]. Notably, supplementation with multivitamins or liver products provides vitamin A in the context of additional nutrients. It is unclear whether the benefits derived from supplementation are due to effects on circulating retinoid abundance or other factors such as antioxidant effects.

The guidance on whether to supplement with vitamin A during pregnancy varies. In Norway, pregnant women have reportedly been advised to consume cod liver oil supplements for its protective effects [26]. However, the United Kingdom National Health Service recommends against cod liver oil consumption for pregnant women [185]. The World Health Organization recommends against vitamin A supplementation except in cases where the expecting mother is unable to maintain a well-balanced diet during pregnancy [186].

8.4 Pharmaceuticals and Orofacial Clefts

8.4.1 Pharmaceutical Retinoids

Retinoids, in addition to their role as nutrients, are the active ingredient in certain medications such as cancer therapeutics and skin creams for acne [187]. The prenatal use of retinoid medications such as isotretinoin (13-cis-retinoic acid) is associated with cleft palate among several other birth defects [188]. Retinoid pharmaceuticals

have been utilized in the laboratory to chemically induce cleft palate or to study the effect of retinoids on palatogenesis. Sufficient maternal dosage of tretinoin, or all-trans retinoic acid (ATRA), can induce cleft palate in mouse embryos at a high frequency [189].

8.4.2 Glucocorticoids

Glucocorticoids (GCs) are among the earliest pharmaceuticals to be associated with OFCs. Endogenous GCs like cortisol and cortisone are steroid hormones that regulate several biological processes including the immune and stress responses, energy metabolism, wound healing, and others [190]. Some synthetic GCs, such as dexamethasone, can be hundreds of times more potent than their endogenous counterparts [191]. The activities of endogenous and exogenous GCs are dependent on their binding with the glucocorticoid receptor, a transcription factor that can modulate the expression of thousands of genes [190]. Physiologically normal levels of endogenous GCs are necessary for palatogenesis, but elevated levels during this process can result in OFCs [192, 193].

In mice, cortisone exposure is known to cause hypoplasia of the palatal shelves during development. Early observations demonstrated a decreased mesenchymal proliferation and secretion of extracellular matrix components, preventing contact between shelves [194]. Later studies have shown that GCs may prevent cell proliferation by modulating TGF-β signaling to interfere with the expression of cell cycle genes [195, 196]. GCs may also adversely affect palatogenesis by interfering with Wnt, FGF, and BMP signaling [197–199].

In cases where a pregnant woman must take GCs for her own health, the risk of fetal cleft must be considered. One literature review concluded that the use of synthetic but non-fluorinated GCs like prednisone and prednisolone increased the risk of cleft palate during first trimester use but is otherwise safe during later stages of pregnancy [200]. Several epidemiological studies conducted in Hungary [201], Spain [202], Sweden [203, 204], Australia [205], and the United States [206] have concluded that oral, systemic, and topical use of GCs during pregnancy may increase the risk of OFCs. Maternal GC use during pregnancy may have therapeutic benefits for the fetus, such as in cases of congenital adrenal hyperplasia. In these cases, GC treatment may ameliorate the associated hyperplastic phenotype during development; however, at least one case report of OFC associated with dexamethasone treatment has been reported [207]. Not all studies agree that GC use during pregnancy increases the risk for OFCs, though. A prevalence study in Denmark concluded that there was no association between GC use and OFCs [208].

8.4.3 Anti-epileptic Drugs

The use of several anti-epileptic drugs (AEDs) during pregnancy is hazardous for orofacial development, although the risk depends on the specific drug used. In mouse studies, maternal administration of carbamazepine, ethosuximide, phenobarbital, phenytoin, primidone, and valproate was associated with cleft palate, while clonazepam was not [209, 210]. The most potent of these was phenytoin, a drug long known for its ability to increase the risk of CL/P in human populations [211, 212]. The effect of phenytoin on orofacial development was originally believed to involve its antagonism toward folic acid supplementation or its sharing of a common receptor with GCs [213, 214]. However, because of its inhibition of delayed rectifier K^+ channels, the underlying mechanism is currently thought to involve hypoxia caused by altered heartbeat and limited oxygen delivery to orofacial tissues [215]. A comparative study on AED safety during pregnancy found that the risk for OFCs was more elevated for valproate compared to phenytoin [216]. This study suggested that older AEDs carried higher birth defect risk compared to the more recent generation of drugs such as lamotrigine or levetiracetam, although a risk is still present in the later drugs [217].

8.5 Domestic and Occupational Exposures and Orofacial Clefts

8.5.1 Solvents

Exposure to organic solvents has been linked with OFCs, although this appears to be dependent upon solvent class and other factors. Solvents are often encountered in occupational or industrial settings, but exposure may also occur outside these scenarios. While the solvent itself may cause OFC, the teratogenic effects may be due to a toxic secondary metabolite. Methanol, for example, is suspected to induce OFCs in mouse embryos through the actions of its toxic metabolite, formaldehyde [218].

A study in China found that exposure to solvents was associated with an increased risk for both CL/P (odd ratio (OR) = 6.07, 95% CI: 1.49, 24.76) and CPO (OR = 10.65, 95% CI: 2.54, 44.67) [183]. Recently, a meta-analysis of studies examining maternal occupational exposure and incidence of OFC found that glycol ethers are associated with both OFCs (OR = 1.95, 95% CI: 1.38, 2.75) and NTDs (OR = 1.93, 95% CI: 1.17, 3.18) [28]. When stratified for OFC subtype and infant gender, a study using registry data from the Northern Netherlands (Eurocat) associated CL/P with exposure to various solvents (alkanes, alcohols, and esters) in male infants [219]. A case-control study in a French population identified an association between OFCs and exposure to oxygenated, chlorinated, and petroleum solvents, with positive correlation between level of exposure and OFC risk among the oxygenated solvents [220]. However, not all studies have identified an OFC risk in the context of solvent exposure. Exposure to benzene, toluene, ethylbenzene, and xylene (BTEX) solvents could not be associated with OFCs in a study of a Texan population, while another study using data from the National Birth Defects Prevention Study failed to associate OFCs with exposure to any solvent [221, 222].

8.5.2 Pesticides

Pesticides represent a broad category of insecticides, herbicides, fungicides, and other biocides that span a large range of chemical classes. Exposure to pesticides in general, as well as specific chemicals, has been associated with increased OFC risk. Meta-analysis of 19 studies examining an association between occupational pesticide exposure and OFCs indicated a small yet elevated risk (OR = 1.37, 95% CI = 1.04, 1.81), while paternal or residential exposures were not significantly associated [223]. Residential proximity to agricultural areas may increase the risk for pesticide exposure. A population study focused on California's San Joaquin Valley, an agricultural region with high pesticide usage, found an association between CL/P and early pregnancy exposure to 2,6-dinitroaniline herbicides (trifluralin) and dithiocarbamates-methyl isothiocyanate (maneb) [224]. Trifluralin affects microtubule function and cell cycle control in plant cells, while maneb is a fungicide that contains manganese and is associated with parkinsonism [225, 226]. Exposure to pesticides was found to increase the risk of CL/P (OR = 5.97, 95% CI: 2.10, 16.98) and CPO (OR = 3.48, 95% CI: 1.06, 11.46). Insecticide use was also associated with OFCs in a population sample of the Democratic Republic of the Congo (OR = 130.306, 95% CI: 13.19, 1286.95) [27]. Azole biocides can induce cleft palate in mice through inhibition of CYP51, a sterol 14α-demethylase that is necessary for steroid biosynthesis [227]. Notably, loss of *Cyp51* function in mice produces a host of phenotypes mimicking Antley-Bixler syndrome, a condition that may present with cleft palate [228].

8.5.3 Dioxins

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a persistent organic pollutant and well-known chemical inducer of cleft palates in mammals [229–231]. As an industrial contaminant, TCDD

is often regarded as the most toxic compound made by humans and has no utility outside of chemical exposure research [232–234]. TCDD is perhaps best known as a contaminant in the synthesis of chlorophenoxyacetic acid herbicides, such as those used as defoliants in Agent Orange during the Vietnam War. Along with other dioxins, TCDD may also be generated during fossil fuel combustion, waste incineration, metal production, and paper bleaching [31, 235–242].

Mouse studies have shown that TCDD is a potent disruptor of palatogenesis, although it has no effect on lip development. This teratogenic effect of TCDD is fully dependent on the aryl hydrocarbon receptor (AHR), a promiscuous receptor that binds several different classes of endogenous and exogenous compounds [231]. Following activation, AHR functions as a transcription factor to increase expression of phase I and II metabolic enzymes [243, 244]. It can also directly or indirectly modulate expression of genes involved in development, such as those related to EGF, TGF-β, BMP, and RA signaling [245–248]. In mice, AHR activation by TCDD may indirectly affect the expression developmental genes such as *Oct4* through DNA methylation [249]. Histological analysis of embryonic mouse palates found that TCDD exposure caused a postfusional split following convergence of the palatal shelves [250]. One possible mechanism for this defect involves the interaction of AHR with Slug, a transcription factor involved in EMT [251]. AHR activation in palatal tissues also disrupts the cell cycle, cell proliferation, osteogenesis, and myogenesis [250–252]. The ability of TCDD and AHR to disrupt palatogenesis is described in greater detail in two companion reviews [31, 253].

8.5.4 Polychlorinated Biphenyls and Polychlorinated Dibenzofurans

The AHR binds to several classes of persistent organic pollutants, including polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs). While PCDFs are dioxin-like compounds, PCB species may or may not be dioxin-like [254]. These compounds can induce cleft palate in rodents, likely through activation of the AHR [255–259]. As industrial fluids, PCBs have been used for electrical insulation, heat transfer, hydraulics, and lubrication [253, 260]. They have also been found in plasticizers, carbonless copy paper, and fire-retardant mixtures. PCB manufacturing has been discontinued in the United States since the late 1970s due to health concerns, but they still present an environmental hazard due to their continued use and resistance to degradation. PCDFs are usually generated as combustion products of chlorinated organic compounds, like PCBs, and have no industrial or commercial application [261]. Massive human population exposure to PCBs and PCDFs has previously occurred due to food contamination. In 1968 during the Yusho incident in Japan, rice oil was contaminated with PCBs, dioxins, and their heat degradation products (including PCDFs). *In utero* exposure to this contaminated rice oil has caused abnormal skin pigmentation, but not OFCs [262].

8.5.5 Metals

Developmental exposure to toxic metals, such as cadmium, arsenic, lead, and nickel, is known to have a range of teratogenic effects in animal models including OFCs [263–268]. Specific mechanisms may be involved for different metals, but common mechanisms have been hypothesized to include competitive binding with metalloenzymes or generation of ROS [269–273]. There is increasing evidence that metal exposure is linked to OFCs in human populations. In the Democratic Republic of the Congo, consumption of metal-contaminated Kapolowe fish was strongly associated with OFCs [27]. A study in China found that maternal exposure to heavy metals was associated with increased risk of both CL/P (OR = 7.00, 95% CI: 3.07, 15.99) and CPO (OR = 6.48, 95% CI: 2.50, 16.81) [183]. Increased concentration of arsenic, cadmium, lead, nickel, and uranium in umbilical cord samples is associated with an increased risk for OFCs

[265, 274]. Placental levels of barium, but not aluminum, are also positively associated with OFCs [275]. Maternal occupational exposure to arsenic is associated with CPO, while maternal dietary exposure is linked with CL/P [276].

8.5.6 Air Pollutants

Exposure to air pollution is known to cause adverse birth outcomes including preterm birth, low birth weight, and decreased fetal growth [277]. Common pollutants such as carbon monoxide, ozone, sulfur dioxide (SO_2), nitrogen oxides (NO_X), polycyclic aromatic hydrocarbons (PAHs), and particulate matter of coarse diameter ($\leq$10μM; PM_{10}) or fine diameter ($\leq$2.5μM; $PM_{2.5}$) may each act through their own teratogenic mechanisms. Developmental exposure to either indoor or outdoor air pollution can increase the risk for OFCs. In China, living near a factory during pregnancy was found to increase the risk for OFCs (OR = 1.90, 95% CI: 1.60, 2.25) [278]. In the Democratic Republic of the Congo, indoor air pollution from use of charcoal was also associated with increased OFC risk (OR = 6.54, 95% CI: 1.23, 34.48) [27]. Some population level studies have examined the effects of specific air pollutants. One study examined umbilical cord levels of PAHs but did not find a positive correlation between elevated PAH levels and OFCs [279]. A study in Brisbane, using data from 1998 to 2004, found that elevated ambient SO_2 was associated with CL/P (OR = 1.27, 95% CI: 1.01, 1.62) [280]. Using data from the Taiwanese Birth Registry from 2001 to 2003, a study found an increased association between CL/P and elevated ozone levels at the first gestational month (OR = 1.20 95% CI: 1.02, 1.39) as well as the second (OR = 1.25; 95% CI: 1.03, 1.52). Ozone may be teratogenic by creating oxidative stress through the generation of superoxide, hydrogen peroxide, and hydroxyl radicals [281]. A study in the United States examined the association of OFCs and air pollutants at different periods of maternal exposure [282]. At 3 months preconception, elevated SO_2 was associated with increased CL/P (OR = 1.93, 95% CI: 1.16, 3.21), PM_{10} was associated with CPO (OR = 1.72, 95% CI: 1.12, 2.66), and CO was associated with CPO (OR = 2.24, 95% CI: 1.21, 4.16). At 3–8 weeks gestation, CPO was linked with higher levels of CO (OR = 2.74, 95% CI: 1.62, 4.62), NO_X (OR = 3.64, 95% CI: 1.73, 7.66), and $PM_{2.5}$ (OR = 1.74, 95% CI: 1.15, 2.64). Another study in the United States linked elevated $PM_{2.5}$ with CPO (OR = 1.43, 95% CI: 1.11, 1.86) [283].

8.6 Parental Behavior, Metabolic Status, and Orofacial Clefts

8.6.1 Tobacco Smoking

Once a matter of controversy, a causal link between maternal exposure to tobacco smoke during pregnancy and OFCs has been firmly established based on evidence from individual and aggregate studies [284, 285]. This association has been corroborated in studies of populations across the world [286–293]. It is estimated that modified behavior associated with smoking could prevent up to 6.1% of OFC cases in the US population [294]. While initially focused on maternal active smoking, studies have increasingly found that environmental tobacco smoke (ETS) exposures (otherwise known as maternal passive smoking or secondhand smoke) are an important contributor to OFC etiology [295, 296]. In some cases, ETS was found to carry a higher risk of OFC compared to maternal active smoking, but this difference may be relatively small [297, 298]. In the Japanese population, the population-attributable factor (PAF) of CL/P was estimated at 10.8% for maternal passive smoking versus 9.9% for active smoking. It is not yet clear whether paternal smoking increases the risk for OFCs due to effects on sperm or maternal exposure to ETS. However, there is some evidence that smoking dosage is positively correlated with increased OFC risk [295, 299]. Because nicotine is a major component of tobacco smoke, one study attempted to make a correlation between maternal levels of the nicotine metabolite coti-

nine and OFCs [300]. Such a correlation was not established, and as the authors suggested, future studies should consider large-scale prospective analyses that account for the rapid rate of cotinine metabolism.

Tobacco smoke consists of thousands of chemicals including many combustion products [301]. Aside from nicotine, these include carbon monoxide, dioxins, PAHs, heavy metals, pesticides, and many others. Because so many teratogens are present in tobacco smoke, it is possible that the causation for OFCs is multifactorial. However, exposure to the individual chemicals listed above is sufficient to induce OFCs. Nicotine can induce hypoxia by impairing uterine vascular function, while carbon monoxide may also increase OFC risk through hypoxic effects [302–305]. Heavy metals like cadmium can cause oxidative stress and possibly affect redox-sensitive signaling pathways [265].

Population genetics studies have shown that the smoking-related risk for OFCs can be modified by variants of genes involved in developmental signaling and detoxification. Developmental GxE interactions appear to specifically affect fusion events in orofacial development. Polymorphisms in *TGFA*, which encodes an EGF signaling ligand expressed in the medial edge epithelium during fusion, increased the GxE risk for OFCs in population samples from Maryland, California, Washington D.C., Taiwan, Singapore, South Korea, Iran, and India [290, 306–310]. Meta-analysis identified an increased risk for CPO specifically [311]. Variation of *TGFB3*, which is required for fusion of the MES, has been associated with GxE risk in samples from Maryland, Washington D.C., Iowa, and China [308, 312–314]. *TGFB3* polymorphism is also associated with SMCP in a German population [4]. Variation of *BMP4*, which is necessary for appropriate fusion of medial and lateral nasal processes, is associated with increased GxE risk for CL/P in China [313].

Polymorphisms in phase I and II metabolism also contribute to OFC risk in the context of maternal smoke exposure. Fetal variants of phase I enzymes appear to convey protective effects. A *CYP1A1* polymorphism, which may contribute to its mRNA stability, is protective against CL/P in samples from Danish, Iowan, and French populations [315, 316]. A polymorphism of the microsomal epoxide hydrolase gene *EPHX1*, believed to increase enzymatic activity, is protective against OFCs in a sample of the Iowan population [316]. Among phase II enzymes, polymorphisms mostly confer increased risk for OFCs. The best studied in the context of orofacial development are N-acetyltransferases (NATs) and glutathione-S-transferases (GSTs). In a study sampling the Californian population, two fetal polymorphisms of *NAT1* were found to increase the GxE risk for CL/P by approximately fourfold when mothers smoked during early pregnancy [317]. Notably, NAT1 may have an endogenous role in folate metabolism, providing a mechanism by which smoking could interfere with the folate cycle [318, 319]. Loss-of-function mutations in GST genes, *GSTM1* and *GSTT1*, also contribute to the GxE risk for OFCs attributed to maternal smoking [320, 321]. Fetal polymorphisms in *GSTA4* and *GSTP1* may also increase this risk [316, 322].

8.6.2 Electronic Nicotine Delivery Systems

A recent alternative to tobacco smoking is the use of electronic nicotine delivery systems (ENDS) or "e-liquid" vaporizers. Although marketed as a safer alternative to smoking, there are concerns about its effects on human health and development due to its use among girls and women of reproductive age [323–325]. The components of e-liquid vapor may include nicotine, nitrosamines, aldehydes, carbon monoxide, heavy metals, and free radicals, many of which are suspected etiological agents of OFCs [253, 326, 327]. A study using *Xenopus* found that aerosols from some e-liquid vapors could induce median oral cleft [328]. Further, exposing O9-1 neural crest cells to these aerosols could perturb regulatory networks associated with craniofacial development. Further study is needed to determine the health risks of ENDS usage for orofacial development.

8.6.3 Alcohol Consumption

Consumption of alcohol during pregnancy can lead to various symptoms of fetal alcohol spectrum disorder. There is limited evidence that drinking also increases the risk for OFCs, although the evidence is comparatively less than for tobacco smoking. Population studies in China, Democratic Republic of Congo, and Mexico have associated an unspecified frequency of drinking during pregnancy with OFCs [27, 287, 292]. A study in Brazil found that drinking during pregnancy increased the risk of CL/P vs CPO (relative risk (RR) = 1.54, 95% CI: 1.07, 2.38) [329]. Analysis of data pooled from six studies found that multiple binges of ≥5 drinks during ≥3 sessions increased the risk for CLO (OR = 1.95, 95% CI: 1.23, 3.11) but not other subtypes [330]. It is unclear whether alcohol consumption poses a greater risk for upper lip development compared with palatogenesis. A more recent meta-analysis failed to make any association between drinking and OFCs [331].

Only a few studies examining the relationship between OFCs and drinking have accounted for GxE interactions. A study sampling the Iowan population found that alcohol consumption (≥4 drinks/month) was associated with CL/P, particularly among infants with *MSX1* polymorphisms [314]. A population study in Norway identified an increased risk of OFCs among mothers that drank ≥5 drinks per session during the first trimester of pregnancy, but only among mothers or offspring with a polymorphism in an alcohol dehydrogenase (*ADH1C*) [332]. A case-parent triad study suggested an interaction between drinking and a parent-of-origin effect of *ARHGEF10* polymorphisms [333]. It is possible that fetal polymorphism of phase I enzyme *CYP2E1*, which is involved in the metabolism of ethanol and other organic solvents, can also modify the risk of OFCs [30].

It is not immediately clear how exactly alcohol consumption might increase the risk for OFCs, but oxidative stress, deficiency of RA and folate, and epigenetic changes may be involved [334–338]. It is possible that acetaldehyde, a metabolic product of ethanol, drives the teratogenic effects. Acetaldehyde may indirectly mediate teratogenesis through competitive binding with ALDH1A2 (retinaldehyde dehydrogenase 2), preventing synthesis of RA [335]. This hypothesis has been corroborated in a study using the *Xenopus* model [339]. Supplementation with folic acid in mouse embryos and co-exposure with RA or folic acid during larval zebrafish development are protective against the teratogenic effects of ethanol [334, 336].

8.6.4 Metabolic Disease

Diabetes and obesity are major health concerns, especially in Western countries where unhealthy diets and sedentary behavior are common [340, 341]. However, developing countries that are adopting a Western lifestyle are also facing increased incidence of these metabolic diseases [342]. Studies have linked maternal metabolic health status associated with weight and diabetes to OFCs. In these studies, pregestational diabetes mellitus (PGDM) refers to a diagnosis of type I or II diabetes before pregnancy, while gestation diabetes mellitus (GDM) refers to insulin deficiency and resistance induced by pregnancy. Notably, data in birth defect studies may be confounded since some cases of GDM are actually a latent diagnosis of type 2 diabetes [343, 344]. In 2008, a US study using data from the National Birth Defects Prevention Study (1997–2003) linked PGDM with increased risk of isolated CL/P (OR = 2.92, 95% CI: 1.45, 5.87) and GDM with isolated CL/P (OR = 1.45, 95% CI: 1.03, 2.04) and CPO (OR = 1.54, 95% CI: 1.01, 2.37) [345]. In a Brazilian study, diabetes in general was associated with a higher incidence of OFC compared to the general population (OR = 4.5, 95% CI: 3.5, 5.8) [346]. Maternal hyperglycemia is believed to affect developmental processes through oxidative stress, hypoxia, epigenetic changes, and altered control over gene expression [347–349].

Maternal obesity has been linked with NTDs and cardiac defects, and it likely increases the risk of OFCs as well [350–352]. Analysis of data from a Swedish population identified an increased

risk of isolated OFCs when maternal body mass index (BMI, kg weight/m^2 height) was greater than 29 (OR = 1.20, 05% CI: 1.00, 1.44), although the risk was greater when other defects were included [350]. A prospective study in Kolkata, India, concluded that newborn infants of obese women had a high incidence of cleft lip and cleft palate [353]. Three meta-analyses found that obesity increased the risk of CL/P (Stothard et al. [354], OR = 1.20, 95% CI: 1.03 to 1.40; Blanco et al. [355], OR = 1.13, 95% CI 1.04 to 1.23; Izedonmwen et al. [356], OR = 1.16, 95% CI: 1, 1.34), although only two found an association with CPO (Stothard et al. [354], OR = 1.23, 95% CI: 1.03, 1.47; Blanco et al. [355], OR = 1.22, 95% CI: 1.09, 1.35) [354–356]. A case-control study in Brazil also linked obesity with OFCs (OR = 2.28, 95% CO: 1.17, 4.41) [297]. A study of US and northern European populations suggested that maternal underweight status (BMI < 18.5) may increase the risk of OFCs to a lesser degree [357].

8.7 Geography, Ethnic Influences, and Orofacial Clefts

While the global average prevalence of OFCs is approximately 1 in every 700 live births (1:700), the average prevalence rate can vary considerably across countries and regions [1]. According to the United States Centers for Disease Control and Prevention, the incidence of OFCs in the United States are 1:1563 for CLP, 1:2807 for CL/P, and 1:1687 for CPO [358, 359]. Prevalence rates (PR) for many countries outside of the United States have been aggregated by the WHO Global Registry and Database on Craniofacial Anomalies based on a registry meeting on craniofacial anomalies in December 2001 [1]. The highest PR (per every 10,000 births) for CL/P is 22.94 in Bolivia, while the lowest was 3.37 in Israel. For CPO, the highest was 14.31 in Finland, while Cuba had the lowest at 1.35. In Europe, countries in the highest global PR quartile for CL/P (>10 per 10,000 births) included Norway and Finland, along with regions in Denmark, Germany, and the Netherlands. European OFC prevalence was generally higher in northern and lower in southern countries [360]. In Asia, PRs in the highest quartile had also been observed in the Philippines, Japan, and regions of China, although CPO prevalence was reportedly lower in the latter two countries [1, 361, 362]. In South America, the highest global CL/P PR quartile was observed in several countries including Bolivia, Brazil, Argentina, and Paraguay, while Mexico and the Canadian province Alberta represented regions of high CL/P in North America [1, 360]. The CL/P PRs of Oceana and Australasia were relatively high at 18.5 and 20.1, respectively [363]. In African regions including north and sub-Saharan, OFC PRs ranged between 3.8 and 5.4 [363]. The PR in the Middle East was roughly twice that of African regions at 10.5.

Stratified analyses of population samples demonstrate that ethnicity is a major driver of PR variation in OFCs between regions, although environmental factors may still contribute to these geographical differences [1]. A general observation is that African ethnicity presents the lowest risk for OFCs, Caucasian ancestry presents an intermediate risk, and Mongolian ethnicity presents the greatest risk. It has been proposed that the increased risk for OFCs in the Bolivian population is due to the Mongolian background of Indigenous peoples in the region. Notably, data in the Bolivian registry is primarily derived from La Paz, which lies at a 4 km elevation. This may generate hypoxic conditions during pregnancy that could contribute to increased OFC risk [302]. The WHO report compared the Bolivian data to that of a Tibetan population of Mongolian ethnicity, who also live at a similar altitude and experience high OFC prevalence [1]. It is unclear whether the ethnicity, elevation, or an interaction of these factors contributed to the increased risk. In North America, populations of Indigenous peoples, Indigenous people of mixed race, or mestizo ancestry also had an increased OFC prevalence in British Columbia, California, and Mexico [1, 364, 365]. A similar trend was also observed in South American countries like Bolivia, Chile, and Argentina [360]. Venezuela and Santo Domingo had lower frequencies of

OFCs, possibly due to a larger proportion of the population having African ancestry [1, 366].

Population-based estimates of birth defects in the United States from 2010 to 2014 found large variations in OFC prevalence between several ethnic groups [358]. The group with the highest prevalence for all orofacial cleft variations was non-Hispanic American Indian/Alaskan native (CLP: PR = 15.21, 95% CI: 12.46, 18.38). In contrast, the group with the lowest prevalence for all cleft types was non-Hispanic Black (CLP: PR = 6.55, 95% CI: 5.91, 7.23). For CLP, similar prevalence rates were observed for whites (PR = 10.68, 95% CI: 10.25, 11.12), Hispanics (PR = 10.59, 95% CI: 10.12, 11.08), and non-Hispanic Asian/Pacific Islander (PR = 9.35, 95% CI: 8.15, 10.66). A review of the 2008 Nationwide Inpatient Sample (NIS) database compared the relative risks for CL/P between Caucasians and African-American, Hispanic, and Asian populations [367]. The relative risk for CL/P among African-Americans was significantly lower (RR: 0.4, 95% CI: 0.3, 0.5), while that of Hispanics (RR: 1.2, 95% CI: 0.8, 1.3) was statistically similar and Asians were at slightly elevated relative risk (RR: 1.7, 95% CI: 1.3, 2.0). In Hawaii, a population study found that the rate for CL/P was higher for Far East Asians, Pacific Islanders, and Filipinos compared to whites [368]. Further, the rate for CPO was higher in Far East Asians and Pacific Islanders relative to whites, whereas whites had a greater rate compared to Filipinos. These observations are generally consistent with ethnic and geographical trends across the world.

8.8 Pathogens and Orofacial Clefts

It has been long suspected that maternal infection during pregnancy is an etiological factor of OFCs, and there is increasing evidence that this may be true. Viral, bacterial, and parasitic infections may act through common or unique mechanisms of teratogenesis. Several viruses have been suspected etiological agents of OFCs including varicella, rubella, rubeola, Epstein-Barr virus, herpesvirus, cytomegalovirus, common cold, and influenza [253, 369–371, 372–376]. Bacteria that may be associated with increased OFC risk include mycoplasma (*Mycoplasma pneumoniae*), chlamydia (*Chlamydia trachomatis*), and syphilis (*Treponema pallidum pallidum*) [377–379].

Analysis of data from the Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA) set found an increased risk for CL/P when mothers had influenza (prevalence odds ratio (POR) = 3.2, 95% CI: 2.0, 5.3). Use of antipyretic drugs (as well as folic acid supplementation) was protective, which suggested a febrile mechanism of teratogenesis [369]. Another study using the HCCSCA data found an association between CL/P and maternal cold, influenza, orofacial herpes, and gastroenteritis during months 2–4 of pregnancy [371]. Additional analysis of those data suggested that orofacial herpes did not directly increase OFC risk but was rather a symptom of an immune response that included fever [370]. This study also linked CPO with influenza, sinusitis, and bronchitis. Several case-control studies conducted using data from Chinese populations have also linked colds, influenza, and fevers with OFCs [183, 278, 292]. However, cold without fever was associated with increased risk for CL/P (OR = 6.06, 95% CI: 4.05, 9.09) and CPO (OR = 6.65, 95% CI: 4.08, 10.84), suggesting that viral infections may cause OFCs through a non-febrile mechanism. Currently, cytomegalovirus (CMV) is the only virus understood to cause OFCs through specific molecular mechanisms. Animal and cell culture studies suggest that CMV induces OFCs by perturbing TGF-β and Hh signaling through an NFκB-dependent mechanism [380, 381].

The single-celled organism, *Toxoplasma gondii,* is an apicomplexan parasite that can cause toxoplasmosis. During pregnancy, this condition is associated with craniofacial birth defects such as microcephaly and hydrocephalus [382]. There is also evidence that toxoplasmosis during pregnancy can lead to OFCs [383–385]. A 2017 case

study from a hospital in Mexico City reported an infant with oblique oro-orbital cleft attributed to congenital toxoplasmosis [5]. In latent infections, *T. gondii* likely poses no hazard to the fetus since it is generally present as a cystic, non-migratory bradyzoite. In fact, a study in 2001 found that 15% of women of childbearing age in the United States show signs of toxoplasmosis [386]. Risk for congenital toxoplasmosis is greatly elevated when pregnant women accidentally ingest oocytes. The oocyte transforms into the migratory tachyzoite stage, which is the only form that can cross the placenta and infect the fetus. Because cats are the definitive hosts for *T. gondii*, it is recommended that pregnant women avoid any exposure to used cat litter to avoid congenital toxoplasmosis.

8.9 Epigenetic Mechanisms of Orofacial Clefts

During craniofacial development, gene expression is tightly controlled through several mechanisms. Among these are covalent modifications to the genome (including DNA and histone proteins) that are conserved during cell proliferation. Such epigenetic modifications are crucial to the normal development of orofacial structures, and their disruption has been associated with OFCs. Epigenetic mechanisms can prevent or allow transcriptional machinery to access the gene promoters or enhancers, thereby permitting spatiotemporal control over gene expression. In the context of orofacial development, the best described epigenetic mechanisms include DNA methylation and histone modification. Environmental exposures are believed to affect orofacial development at least partly through epigenetic mechanisms. In fact, maternal smoking during pregnancy is known to alter DNA methylation of genes previously associated with OFCs [387]. Here we describe DNA methylation and histone modifications during orofacial development, as well as how their disruption may contribute toward OFC risk.

8.9.1 DNA Methylation

In mammals, cytosine (C) residues may be methylated at the 5C position to yield 5-methylcytosine. These modifications are carried out by DNA methyltransferases (DNMTs) that catalyze the transfer of a methyl group from the donor molecule, SAM [388]. Consequently, DNA methylation is strongly associated with folate metabolism. Methylation occurs at specific sequence motifs involving an adjacent guanine (G) residue. This ensures that both DNA strands are methylated at the same position and the modification is conserved during replication. The best studied motif is 5′-CpG-3′, which are enriched in regions known as CpG islands [389]. Another motif is 5′-CCWGG-3′, where W is either adenine or thymine [390]. While gene promoter elements may be enriched with CpG islands, enhancers are known to harbor both CpG and CCWGG motifs. Methylation may prevent the binding of transcriptional machinery through spatial hindrance [391]. Methylation can also recruit CpG-binding proteins, which themselves recruit histone deacetylases that modify chromatin to an inaccessible conformation.

In the embryonic mouse palate, DNA methylation at CpG islands is elevated at E14.5 compared to E13.5 or E18.5 [392]. This increased elevation coincides with an important step of palatogenesis, just prior to formation of the MES. Between E12–14, a microarray study found that 73% of detected genes had some extent of methylation, but only ~30% occurred at CpG islands, while over 70% occurred within gene bodies [393]. Decreased expression of *Sox4*, a gene which may integrate developmental signaling pathways involved in palatal fusion, was correlated with altered methylation patterns in a CpG-poor promoter region [394]. *Sox4* expression is restricted to the epithelial rugae at E14, corroborating a role in the regulation of palatal fusion.

The role of DNA methylation during orofacial development has been studied using a chemical inhibitor of DNMTs called 5′-aza-2′-deoxycytidine (decitabine).This compound is registered with the United States Food and Drug Administration and European Commission as a

cytostatic agent for the treatment of hematological malignancies [395]. When administered to pregnant mice, decitabine causes cleft palate, hind limb phocomelia, and other abnormalities in embryos [396–398]. In mouse embryos exposed to decitabine, analysis of first branchial arch cells at E9.5 revealed altered expression of morphogenetic factors, decreased cell proliferation, and increased apoptosis [399]. Some differentially expressed genes associated with cleft palate, such as *Axin2*, *Efna5*, and *Hic2*, were hypomethylated [400]. However, many others were not differentially expressed. In contrast, several endogenous retroviral elements were hypomethylated following decitabine exposure. The authors suggested that decitabine altered the expression of certain genes indirectly by permitting viral expression and a subsequent interferon-mediated response.

Exposure to the teratogens ATRA and TCDD may also cause OFCs at least partly through altered DNA methylation. Several studies have been performed to characterize changes in global methylation patterns in E14.5 mouse palatal shelves induced by maternal ATRA exposure [401–403]. These studies identified hypermethylation of a CCWGG motif in *Hdac4*, a histone deacetylase that regulates osteogenesis. They also demonstrated that ATRA induced differential methylation of genes involved in Wnt (*Tcf7l2*), TGF-β (*Smad3*), and PDGF signaling (*Pdgfrb*). Mouse studies have also demonstrated a role for altered DNA methylation in TCDD-induced cleft palate. Exposure before or during palatogenesis causes hypomethylation and increased expression of a DNA methyltransferase, *Dnmt3a*. Further, genes encoding CpG-binding proteins also had elevated expression following TCDD exposure [404, 405]. These animal studies have collectively demonstrated that DNA methylation may contribute to OFC etiology.

In the past decade, epigenome-wide association studies (EWAS) have provided evidence of altered DNA methylation in patients with OFCs. Tissue samples, primarily derived from blood, revealed altered methylation status of genes encoding growth factors and regulators of Wnt, BMP, Ephrin, and RA signaling, as well as transcription factors, extracellular matrix proteins, and histone modifiers [279, 406–409]. Some of these studies have suggested that specific changes in DNA methylation might contribute to distinct OFC subtypes such as CL/P, CLO, and CPO [408, 410]. There is also some evidence that folate metabolism affects human DNA methylation status. In a study on newborn blood spot samples collected from American individuals with OFC, prior to mandated folic acid fortification in 1998, Gonseth et al. [279] found that genes previously associated with OFCs trended toward hypomethylation. Although DNA methylation status in human populations is still an emerging area of research, present data suggests that epialleles may be important contributors to OFC etiology.

8.9.2 Histone Modifications

In the cell nucleus, DNA is organized by wrapping around histone proteins to form chromatin. Histone proteins can be post-translationally modified at specific amino acid residues to make DNA accessible or inaccessible to transcriptional machinery and may also recruit protein complexes that remodel chromatin [411, 412]. Many studies have focused on histone methylation and acetylation, although several other modifications may also be important [413, 414]. Methylation is known to occur on both arginine (R) and lysine (K) residues, while acetylation occurs on K residues. These modifications are catalyzed by specific enzymes whose mutation or inhibition is associated with OFCs. Dysfunction of these enzymes can affect several developmental processes and consequently often lead to syndromic effects.

Kabuki syndrome is a type of neurocristopathy that involves loss-of-function mutations in either of two enzymes that modify H3K27 residues: the X-linked demethylase *KDM6A* and the methyltransferase *KMT2D*. Patients with *KDM6A* mutations may or may not present with cleft palate, and the ability of this enzyme to affect craniofacial development has been demonstrated in zebrafish and mouse studies [415–418]. There is evidence that KDM6A affects orofacial development inde-

pendently of its demethylase activity, as male mice can compensate for mutations with a Y-linked homolog that lacks this function. In contrast to those with *Kdm6a* mutation, those with nullified *Kmt2d* exhibit fully penetrant cleft palate [416]. This observation appears to be consistent between humans and mice. A zebrafish study found that the developmental effects of *kmt2d* mutation can be rescued by small molecule inhibitors of MAPK signaling, demonstrating a connection between *kmt2d* and this pathway [419, 420].

Mutation of another demethylase, *PHF8*, can result in cleft palate associated with Siderius X-linked disability syndrome [421–424]. This enzyme has H4K20 and H3K9 demethylase activities and is known to interact with *Rara* in mice to regulate neuron differentiation [425]. Because the catalytic domain is a 2OG oxygenase, it is hypothesized that its functionality is compromised under hypoxic conditions [424]. Phf8 can regulate *msx1* expression in zebrafish, possibly contributing to its role in neural crest cell induction and survival [426–428].

Mutation in the methyltransferase gene *WHSC1* results in Wolf-Hirschhorn syndrome, and *Whsc1* is expressed in both epithelial and mesenchymal tissue during palatogenesis in mice [429]. It is believed to regulate cell proliferation, and its expression is diminished in response to ATRA exposures. Zebrafish studies have demonstrated that two H3K4 and H3K9 methyltransferases, *prdm3* (*MECOM*) and *prdm16*, are involved in craniofacial development and regulate expression of homeotic genes *dlx2a* and *barx1* [430]. In mice, conditional knockout of *Prdm3* (*Sox2-Cre*) is embryonic lethal, while *Prdm16* is required for palatogenesis [431]. An arginine methyltransferase, *Prmt1*, regulates *Msx1* expression and Bmp signaling in murine craniofacial development [432].

Two histone deacetylases, HDAC3 and HDAC4, control important cellular processes in neural crest cells. Murine HDAC3 regulates cell proliferation and apoptosis, and is required to maintain the balance of *Msx1*, *Msx2*, and *Bmp4* expression during orofacial development [433]. HDAC4 controls endochondral ossification through its interactions with *Mef2c*, and its knockdown in zebrafish causes malformations of the ethmoid plate [434, 435].

8.10 MicroRNAs and Orofacial Clefts

Gene expression can be regulated through several different mechanisms. Non-coding RNAs (ncRNA), which include a diverse range of transcripts that are generally understood to lack the capacity for translation into peptides, can modulate gene expression through a variety of mechanisms [436]. The most abundant and best understood of these are the microRNAs (miRNAs), a class of small RNA molecules (18–25 bases) that bind transcripts and prevent their translation into proteins [437]. miRNAs can target specific transcripts at 3′-UTR seed sequences through base pair complementarity and recruit the RNA-induced silencing complex (RISC) [438]. While perfect complementarity can result in cleavage of the target, imperfect complementarity can destabilize mRNA through poly-A deadenylation or prevent translation by causing steric hindrance at the ribosome. In humans, roughly 60% of genes are regulated post-transcriptionally by miRNAs [439]. Misexpression and dysfunction of miRNAs are known to be associated with or directly involved in OFC etiology [440].

Several processing steps are required to generate mature miRNA transcripts [441]. They are initially transcribed as long primary miRNAs and are subsequently shortened into pre-miRNAs by the microprocessing complex, which includes the Drosha protein [438]. Pre-miRNAs are exported from the nucleus and further processed by Dicer into their mature forms, a duplex consisting of a -5p and -3p strand. Either of these strands may be loaded onto an Argonaute (AGO) protein, a component of the RISC complex. miRNA biogenesis can be globally disrupted by targeting Dicer, the cytosolic enzyme that generates mature miRNAs. In mouse embryos, conditional knockout of Dicer in either the palatal mesenchyme (*Wnt1-Cre*) or epithelium (*Pitx2-Cre*) generated cleft palate, although it was incompletely penetrant in the

latter mutants [440, 442, 443]. These studies demonstrated an important role for miRNAs in orofacial development, especially in palatogenesis.

Because miRNAs function through base complementarity, their targets can be identified by computational prediction. These interactions can be validated in cell reporter studies and anti-correlated expression analysis. In mice, studies have identified over a hundred miRNAs that are differentially expressed during orofacial development [444]. Many of these are predicted to target several genes involved in critical processes of orofacial development including EMT, migration, apoptosis, and others. The expression of miRNAs that regulate such processes may also be epigenetically controlled through DNA methylation during palatogenesis [445].

miRNAs can regulate these processes at least partly through their targeting of developmental signaling pathways; conversely, developmental pathways can regulate miRNA expression. The first miRNA associated with OFCs was miR-140, whose knockdown caused cleft palate in developing zebrafish [446]. Initially found to target PDGF signaling (*pdgfra*), it is now understood to target BMP signaling in human palatal mesenchyme cells and FGF signaling (*FGF9*) in both human and mouse palatal mesenchyme cells [447, 448]. miRNAs from the miR-17-92 cluster are regulated by BMP signaling/Ap-2α, and they commonly antagonize TGF-β signaling in cancers and murine palatal mesenchyme cells [449, 450]. In developing mouse palates, this cluster had decreased expression during E12–14. However, complete double knockout of the miR-17-92 cluster along with its paralog cluster, miR-106a-25, resulted in fully penetrant cleft lip and palate in mice. miR-17-92 was additionally found to target FGF signaling (*Fgf10*) and the transcription factors *Tbx1*, *Tbx3*, and *Shox2* [450]. miR-4680-3p and miR-374a-5p were predicted to target Wnt signaling (*WNT5A*), while the latter was predicted to target EGF signaling (*ERBB2*) and folate metabolism (*MTHFD1*) [451]. These targets were subsequently validated in cultured human palatal mesenchyme cells. A more comprehensive list of validated miRNA targets is provided in a recent review [31].

Human miRNA association studies indicate that polymorphisms within miRNAs, or their seed sequences in the 3′-UTR of target genes, can affect orofacial development. Most studies have been conducted in Asian populations. Samples from Han Chinese and Thai populations have confirmed that miR-140 targets PDGF signaling through PDGFRA, and polymorphisms in either miR-140 or its seed region on *PDGFRA* are associated with CPO [448, 452, 453]. Polymorphism in the miR-3649 seed region of a critical neural crest regulator, *MSX1*, is associated with CL/P in Chinese individuals [454]. Regulation by miRNAs may also underlie the etiologies of specific OFC subtypes. Polymorphisms in the 3'-UTR of *FGF5* and *FGF2*, which, respectively, contain seed sequences for miR-145 and miR-469, are associated with CLO (as well as CPO and CL/P, respectively) in a sample of the Chinese population [455]. A similar observation has been made for a 3′-UTR polymorphism of *FOXE1*, which lies in the seed sequence for miR-423-3p [456].

8.11 Conclusions and Perspectives

As one of the most common birth defects worldwide, OFCs have been a major focus of developmental research for decades. The field is beginning to elucidate the diverse etiologies and mechanisms underlying this congenital disorder. It is currently understood that OFCs are the result of genetic, environmental, or GxE interactions affecting craniofacial development. In cases of genetic mutation or inheritance, they may occur as part of a syndrome alongside other phenotypes. However, they most commonly present as isolated or nonsyndromic CL/P or CPO. To date, polymorphisms associated with OFCs have been detected in hundreds of genes. These adversely affect transcription factors that regulate cell identity and fate, developmental signaling proteins that coordinate morphogenesis, and ECM proteins that define tissue properties. Environmental factors such as nutrients and contaminants can modify the risk for OFCs; while some factors like folate are protective, others increase the risk such

as industrial pollution and contaminants. Some environmental factors modify the risk for OFCs by themselves, or they do so through interaction with allelic variants. Maternal health status, including factors such as smoking, drinking, body mass, or pathogenic infection, are all risk factors for OFCs. Parental demographics and ethnicity also play a major role in geographical OFC prevalence. Finally, epigenetic mechanisms and miRNAs are emerging as key etiological factors.

Although OFCs can be treated through reconstructive surgery and/or various therapies, the most effective approach would be to prevent OFCs whenever possible to minimize suffering and medical expenses. Understanding the developmental, genetic, and environmental factors involved in OFC etiology is of paramount importance for this approach. Advances in biomedical technology such as microscopy, gene expression platforms, and animal models have enabled a greatly improved understanding of orofacial development. Single-cell RNA sequencing, for example, is an emerging approach that may allow a better understanding of cellular contributions to the development of complex orofacial structures [457]. In combination with mutant animal models, it will serve as a powerful tool for investigating the role of specific genes and pathways during midfacial morphogenesis and palatogenesis. To understand the role of environmental factors, additional meta-analyses of population data will be necessary to yield critical insights into OFC trends. Elucidating the toxicological mechanisms underlying pollutant exposures may also help identify therapeutic interventions that protect orofacial development. A worthwhile consideration is whether folates are truly protective against OFCs and whether large doses are necessary and/or safe for achieving such an effect. Even though our understanding of OFCs has greatly improved in recent decades, additional studies will be necessary to better understand their etiologies and provide further guidance on how to prevent these birth defects.

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9 Biological Basis of Craniosynostosis

Christian Freudlsperger and Michael Engel

9.1 Introduction

The development of the human skull is composed of the ventral viscerocranium, i.e., the facial skull, and the dorsal neurocranium, which encapsulates the developing brain. The neurocranium has dual developmental origin including mesoderm and neural crest cells [1] and consists of the fontal bone, pars petrosa of the parietal bone, temporal bone, and occipital bone. As the occipital and the pars petrosa of the temporal bone are built during chondral osteogenesis, the frontal and parietal bone are formed by dermal osteogenesis. Cranial sutures are formed at the sites of approximation of these bones. The six major skull sutures are the metopic and the sagittal suture, the two coronal sutures, and the two lambdoid sutures. They act as interosseous ligaments and are the primary site of bone growth. The tension on the cranial sutures caused by the expanding brain acts as the adequate stimulus for bone remodeling and therefore coordinates brain growth with bone growth. This process relies on the production of sufficient new bone cells to be released into the bone fronts, while cells within the suture remain undifferentiated. To function as bone growth sites, sutures need to remain patent [2]. Premature obliteration of cranial sutures (i.e., craniosynostosis) by fusion of bone fronts suppresses bone growth at this side, enhances growth at patent sutures, and therefore leads to abnormal morphogenesis and craniofacial deformity affecting the growth of viscerocranium, neurocranium, and brain tissue itself [3]. Shaping growth of the human skull mainly takes place from the first months after conception to the end of the first year of life. As premature osseous obliteration of cranial sutures mainly takes place intrauterine, craniosynostosis becomes visible shortly after birth. The extent of the craniofacial deformity depends on the affected suture and the moment of intrauterine obliteration: The earlier this obliteration occurs, and the more sutures affected, the greater the deformity. Specific isolated craniosynostosis typically results in pathognomonic deformities, e.g., scaphocephaly due to synostosis of the sagittal suture. However, the biological basis of craniosynostosis is multifactorial and understanding the biological basis starts by recognizing the heterogeneity of their pathogenesis. Craniosynostosis can be divided into primary (with genetic origin) and secondary forms, which are extremely rare. The classification of primary intrauterine craniosynostosis discriminates syndromic and non-syndromic forms, whereas the latter account for approximately 70% of cases [4–6]. Monogenetic mutations are responsible for intrauterine craniosynostosis (85% of cases), and some patients though present chromosomal disorders, i.e., numeric and structural aberrations.

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9.2 Syndromic Forms of Primary Craniosynostoses

Over 150 syndromes associated with intrauterine craniosynostoses have been described [7] and are associated with additional congenital anomalies and/or developmental delays, e.g., growth and developmental retardation. However, surgical therapy of craniosynostoses is based on the deformity itself and is not affected by the categorization of a specific syndrome. Nevertheless, these connotations allow precise conclusions concerning inheritance and prognosis.

The first human craniosynostosis gene was discovered by E. W. Jabs and colleagues in Boston-type craniosynostosis and described a mutation in the human *MSX2* gene in an affected family in 1993 [8]. Since then, mutations of the fibroblast growth factor receptors have been associated with eight syndromic forms of primary craniosynostoses (Crouzon, Apert, Pfeiffer, Jackson-Weiss, Muenke, FGFR2-related isolated coronal synostosis, Crouzon syndrome with acanthosis nigricans, and Beare-Stevenson syndrome). The most common will be discussed in the following section.

9.2.1 Crouzon Syndrome

Crouzon syndrome is a rare disease described by Louis Edouard Octave Crouzon in 1912 and is estimated to occur in 1 case per 60,000 live birth in the United States [9]. It is transmitted in an autosomal dominant manner with variable penetrance and characteristic features including craniosynostosis, maxillary hypoplasia, and exophthalmos. Mandibular prognathism, ocular hypertelorism, and nasal deformity are further malformations associated with Crouzon. It can also cause hearing loss and airway challenges due to deformities in the nasal cavity and nasopharyngeal airway [10] interfering with normal neuropsychological development [11]. Affected sutures are variable leading to brachycephaly, scaphocephaly, trigonocephaly, and cloverleaf skull. Due to abnormal cranial vault formation, patients may present with hydrocephalus resulting in elevated intracranial pressures with papilledema, compression of optic nerves, and Chiari malformation [12, 13]. Crouzon syndrome is caused by gain-of-function mutations of the fibroblast growth factor receptor 2 (FGFR2) (10q25-q26) [14]. The proteins of the fibroblast growth factor (FGF) family are involved in a wide variety of processes including mitogenesis and morphological effects and are critical during embryogenesis. The FGFR1-3 belongs to the tyrosine kinase superfamily. They are activated by binding to FGF isoforms resulting in dimerization and autophosphorylation, finally affecting multiple downstream targets including canonical Wnt, Src, and STAT signaling as well as protein kinase C, RAS-MAPK, PI3K-AKT, and PLCγ pathways [15, 16]. The abovementioned gain-of-function mutations result in increased affinity of FGFR to FGF, decreased specificity, or enhanced intrinsic receptor activity [17]. Although detailed molecular mechanisms leading to craniosynostosis are not clear, translational research shows that these mutations result in enhanced bone mineralization and FGFR signaling plays an important role in osteoblast differentiation [17].

A distinct type of this disorder is Crouzon syndrome with acanthosis nigricans caused by a specific mutation in the FGFR3 gene (Ala391Glu). Patients present additional characteristic dermatological findings, choanal atresia, short vertebral bodies, as well as broad, short metacarpals and phalanges [13, 18].

9.2.2 Apert Syndrome

Eugene Charles Apert, a French pediatrician, first described this autosomal dominant disorder in 1906.

It is an uncommon disease with an estimated prevalence of 1 case per 65,000 live birth and instantly recognizable on the basis of the syndactyly of both the hands and feet [19]. Further characteristic clinical features include brachycephaly, delayed closure of fontanels, dysmorphic features like flattened asymmetrical face, downslanting palpebral fissures, hypertelorism, shallow orbits, exorbitism, strabismus, and markedly

depressed nasal bridge, and cleft palate [13]. In addition, structural brain abnormalities (e.g., ventriculomegaly or malformation of corpus callosum) are associated with Apert syndrome with delayed psychomotor development and mild to moderate intellectual disability [11]. Further findings are otitis media and conductive hearing loss associated with malformed and/or fused middle ear ossicles, dehiscence of semicircular canals, and cochlear malformations [13]. As well as in Crouzon syndrome, *FGFR2* mutations have been identified to be causative in Apert syndrome [20]. Two heterozygous gain-of-function substitutions, Ser252Trp and Pro253Arg, in exon 7 of the FGFR2 gene are responsible for over 98% of Apert syndrome cases [13]. They are transmitted in an autosomal dominant manner and show complete penetrance with variable expressivity. Most mutations occur de novo and are mainly of paternal origin with age effect [13].

9.2.3 Pfeiffer Syndrome

Rudolf Arthur Pfeiffer, a German pediatrician, first described this autosomal dominant disorder with complete penetrance and significant variability in 1964 [21]. It is uncommon and affects one patient per 100,000 newborn. Premature fusion of coronal, lambdoid, and (occasionally) sagittal suture leads to a characteristic wide skull shape with flat occiput, high forehead, midfacial hypoplasia, hypertelorism, and proptosis. It is associated with broad thumbs and big toes and variable partial syndactyly on both hands and feet [22]. Delayed psychomotor development, abnormal viscera, ankylosed elbows, exorbitism, and hydrocephaly caused by aqueductal stenosis are rare findings in patients with Pfeiffer syndrome [22]. Tracheal cartilaginous sleeve, a severe airway anomaly with missing distinct tracheal rings, has been reported in this entity [23]. Pfeiffer syndrome is divided into three major subtypes according to severity (Table 9.1). Type II and III phenotypes have an increased risk for early death due to neurological complications [22]. Mutations in *FGFR1* (Pro252Arg) and *FGFR2* are causative in Type I, whereas Types II and III are caused by mutations in *FGFR2* [22, 24–26]. About 21% of patients clinically diagnosed with Pfeiffer syndrome lack FGFR1/2 mutations [13].

Table 9.1 Major subtypes of Pfeiffer syndrome

Pfeiffer type I (classic phenotype)	• Brachycephaly, midface hypoplasia, hand and feet abnormalities • Normal neurological and intellectual development • Good outcome
Pfeiffer type II	• Cloverleaf skull, extreme proptosis, hand and feet abnormalities, elbow ankylosis or synostosis • Developmental delay and neurological complications: limited brain growth due to skull shape, visual impairment due to proptosis
Pfeiffer type III	• Similar to type II, but without cloverleaf skull

9.2.4 Jackson-Weiss Syndrome

Charles Jackson, Lester Weiss, and colleagues first described this unusual syndrome within a large Amish kindred in 1976 [27]. Jackson-Weiss syndrome is similar to the condition described by Pfeiffer but lacks thumb abnormality. It is of autosomal dominant inheritance with varying phenotypic expression.

Mutations in FGFR2 have been described to be causative in this rare condition [28, 29].

9.2.5 Muenke Syndrome

Maximilian Muenke and colleagues defined this distinct disorder on a molecular level in 1997 [30]. They identified a common mutation (Pro250Arg) located between the second and third immunoglobulin-like domains of the FGFR3 protein, which is related to the FGFR1 (Pro252Arg) mutation in Pfeiffer and FGFR2 (Pro253Arg) mutation in Apert syndromes. They reported inter- and intrafamilial variability whose main characteristics include bilateral or unilateral coronal synostosis and specific bone anomalies of the hands and feet in some affected individuals. Interestingly, some mutation carriers did not

show any signs of craniosynostosis, having only macrocephaly or even normal head size. Midface hypoplasia, ptosis, sensorineural hearing loss, and downslanting palpebral fissures are very rare findings in patients with Muenke syndrome [30].

9.2.6 Saethre-Chotzen Syndrome

First described by Saethre and Chozen in the early 1930s, Saethre-Chotzen syndrome is characterized by unilateral or bilateral synostosis of the coronal suture, limb anomalies, i.e., syndactyly of digits two and three of the hand [31, 32]. Patients present with facial asymmetry in case of unilateral synostosis, characteristic appearance of the ear (small pinna with a prominent superior and/or inferior crus), strabismus, and ptosis [33]. Other dysmorphic features such as parietal foramina, radioulnar synostosis, maxillary hypoplasia, ocular hypertelorism, increased intracranial pressure, short stature, and congenital heart malformations are rare, but have been reported [33]. Cognitive developmental is usually normal. It is important to know that individuals with SCS with no evidence of craniosynostosis have been described. The locus for Saethre-Chotzen syndrome maps to chromosome 7p21-p22, and loss-of-function mutations in TWIST1 have been reported to be causative in Saethre-Chotzen syndrome. TWIST1 encodes an important transcription factor for mesodermal patterning of the calvaria, is expressed in the osteoprogenitor cells of cranial sutures, and is important in osteoblast differentiation. Mutations in TWIST1 result in disruption of the RUNX2 pathway affecting the transcription of FGFR [17, 34, 35].

9.2.7 Craniofrontonasal Syndrome

Craniofrontonasal syndrome, an X-chromosomal-dominant disorder with paradoxically greater severity in heterozygous females than in hemizygous males, is caused by mutations in EFNB1, whose gene product ephrin-B1 plays a role in cell adhesion [36]. It is thought that, in heterozygous females, patchwork loss of ephrin-B1 disturbs tissue boundary formation at the developing coronal suture, whereas in males deficient in ephrin-B1, an alternative mechanism maintains the normal boundary [36].

Although most of syndromic craniosynostoses show dominant inheritance, approximately half of patients present with de novo mutations. In recent years, causative mutations in over 50 genes have been identified and multiple novel gene/disease associations in syndromic craniosynostosis have been detected by use of next-generation sequencing [37, 38].

9.3 Non-syndromic Forms of Primary Craniosynostoses

Non-syndromic craniosynostoses are a group of isolated malformations resulting in premature intrauterine suture fusion: sagittal, coronal, metopic, and lambdoid sutures in decreasing order of frequency. They built a genetically heterogeneous and largely unexplored group, as diagnostic success rates are low in trigonocephaly and scaphocephaly (<1%) and slightly higher in unicoronal (13%), multisuture (15%), or bicoronal (60%) synostosis [17]. Non-syndromic forms of craniosynostosis are sporadic in more than 95% [39]. As Mendelian patterns of inheritance are uncommon, and the disease likely arises from a combination of polygenic influences and epigenetic factors, a large cohort of patients is needed to study these complex traits and to identify genetic risk factors. However, recent progress in the genetics of non-syndromic craniosynostosis has been made and reviewed by Timberlake and Persing [38]. By using a trio-based whole-exome sequencing approach including both parents and patients with non-syndromic craniosynostosis, several causal genes and pathways have been identified (see Table 9.2). These recent findings implicate mutations in similar pathways (Wnt, BMP and Ras/ERK) as frequent causes of syndromic and non-syndromic craniosynostoses [38].

Table 9.2 Genes in which mutations confer high risk of non-syndromic craniosynostosis recurrence in subsequent offspring, Timberlake and Persing [38]

Gene	Mechanism	Type
SMAD6	SMAD6 is an inhibitor of BMP signaling. Loss-of-function mutations lead to augmented SMAD signaling	Sagittal, metopic, combined sagittal and metopic
TWIST1	TWIST1 is a basic helix-loop-helix transcription factor downstream of several developmental signaling pathways. Loss-of-function mutations lead to transcriptional dysregulation	Coronal, sagittal
TCF12	TCF12 is a basic helix-loop-helix transcription factor that heterodimerizes with TWIST1. Loss-of-function mutations phenocopy TWIST1 mutations	Coronal, sagittal
ERF	ERF shuttles phosphorylated ERK from the nucleus, thus regulating RAS/MAPK/ERK signaling. Loss-of-function mutations in ERF lead to augmented ERK signaling	Metopic, sagittal, multisuture
MSX2	MSX2 is a transcription factor downstream of BMP signaling. Mutations at a recurrent codon (p.148) lead to increased DNA-binding affinity and increased transcription at target sites	Coronal, sagittal
FGFR3	A recurrent gain-of-function mutation (p.P250R) in FGFR3 leads to augmented FGF signaling	Coronal

9.4 Secondary Craniosynostoses

Secondary craniosynostoses represent an extremely rare form with wide etiologic variety including teratogenic, metabolic, and hematologic disorders as well as developmental disorders, e.g., microcephaly or holoprosencephaly. They uncover at a later point of time of around 12–24 months postpartum.

Metabolic disorder-based forms, such as hypophosphatemic rickets, can be associated with loss-of-function PHEX mutations affecting FGF23 expression [40] and prolonged therapy with phosphate-binding antacids and vitamin D deficiency caused by malnutrition [41]. Hyperparathyroidism and lysosomal storage disease are further associated conditions. Teratogenic disorders include congenital infections and radiation exposure to teratogenic chemicals, e.g., alcohol or retinoic acid. Hematologic disorder-based forms can be caused by polycythemia vera, thalassemia, or sickle cell anemia.

9.5 Conclusion

As the biological basis of craniosynostosis is multifactorial and heterogeneous, affected families need to be advised properly in specialized centers with extensive experience in both craniofacial surgery and human genetics. This is particularly true for multisuture and inherited forms of craniosynostoses. However, we advise center-based treatment even in single-suture craniosynostoses to continue recent research progress.

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Biological Basis of Branchial Arch Diseases

10

Ulrich Meyer

10.1 Introduction

Because tissue and organ structures of the head and neck migrate during fetal development, an understanding of embryologic development helps determine the origin and nature of congenital lesions [1]. Disorders of the frontonasal prominence (FNP) and the first and second branchial arches (BAs) are generally thought to result from a combination of inadequate migration and formation of facial tissues. Branchial arch disease is the term [2, 3] that describes the pathogenetic basis of a specific subset of craniofacial anomalies, also termed facial dysostoses, which can be subdivided into mandibulofacial dysostosis, which present with craniofacial defects only, and acrofacial dysostosis, which encompasses both craniofacial and limb anomalies. Knowledge of the genetic basis of human disease and its effect on embryologic development has greatly expanded in recent years. These malformations have etiologic and pathogenic similarities, specifically their unique deficiencies in global processes including ribosome biogenesis, DNA damage repair, and pre-mRNA splicing, all of which affect neural crest cell development and result in similar tissue-specific defects.

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10.2 Pathogenesis of Facial Development

Head and face development begins during early embryogenesis with formation of the frontonasal prominence and the pharyngeal arches, which are transient medial and lateral outgrowths of cranial tissue [4–6] (Fig. 10.1). These craniofacial structures develop into nerves, muscles, cartilage, bone, and connective tissue, including the body's primary sense organs and necessary for undisturbed feeding, respiration, and facial expression. The human head and face are anatomically complex structures that form during embryogenesis, from the FNP and the PAs (pharyngeal arches). The FNP gives rise to the forehead and the nose, while the paired PAs give rise to the lower face (the jaw), the neck, and part of the upper thorax (Fig. 10.2). Pharyngeal arch development is dependent upon a multipotent, migratory population of neural crest cells, which generate most of the bone and cartilage of the head and face [7–10]. Since the discovery of the neural crest, the special ability of these cells to function as a source of species-specific pattern has been clearly recognized during the last decades. Initially, this observation arose in association with chimeric transplant experiments among differentially pigmented amphibians, where the neural crest origin for melanocytes had been duly noted. Shortly thereafter, the role of cranial neural crest cells in transmitting species-specific information on size

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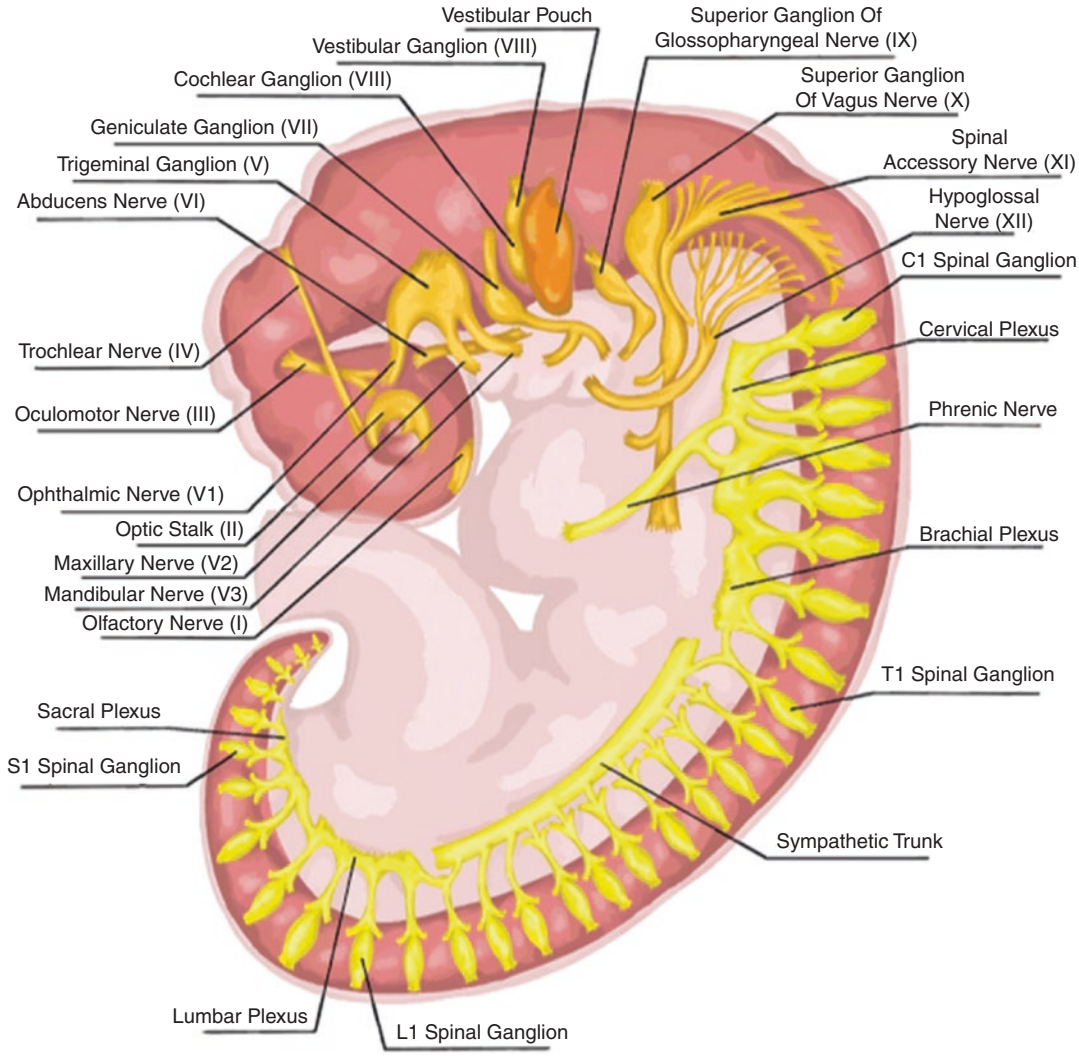

Fig. 10.1 Embryologic body development with corresponding nerve distribution. *Source: Reprinted from stihii/Shutterstock.com with permission*

and shape to the pharyngeal arch skeleton as well as in regulating the timing of its differentiation became readily apparent [11–13].

The basic structure of the frontonasal prominence and the arches is similar in higher species (Fig. 10.3). The head and neck originate from six embryonic structures called the pharyngeal apparati, which resemble the branchial apparatus in fish [14]. Each pharyngeal apparatus comprises a pouch, an arch, a groove, and a membrane. In the fourth week of gestation, neural crest cells migrate from the neural tube to begin the development of the pharyngeal arch ectomesenchyme [12, 13]. Each arch has three layers (endoderm, mesenchyme from ectomesenchyme and mesoderm, and ectoderm), which produce the four primordial components: muscle, artery, nerve, and cartilage. Internally, all these structures are lined with endoderm, forming the pharyngeal pouches (Fig. 10.4). Concerning branchial arch diseases, the third layer between the ectoderm and endoderm epithelia is of importance [14–16]. This layer is composed of neural crest cells (NCCs) in the frontonasal prominence, whereas in the pharyngeal arches the mesenchymal core is composed of NCC and mesoderm.

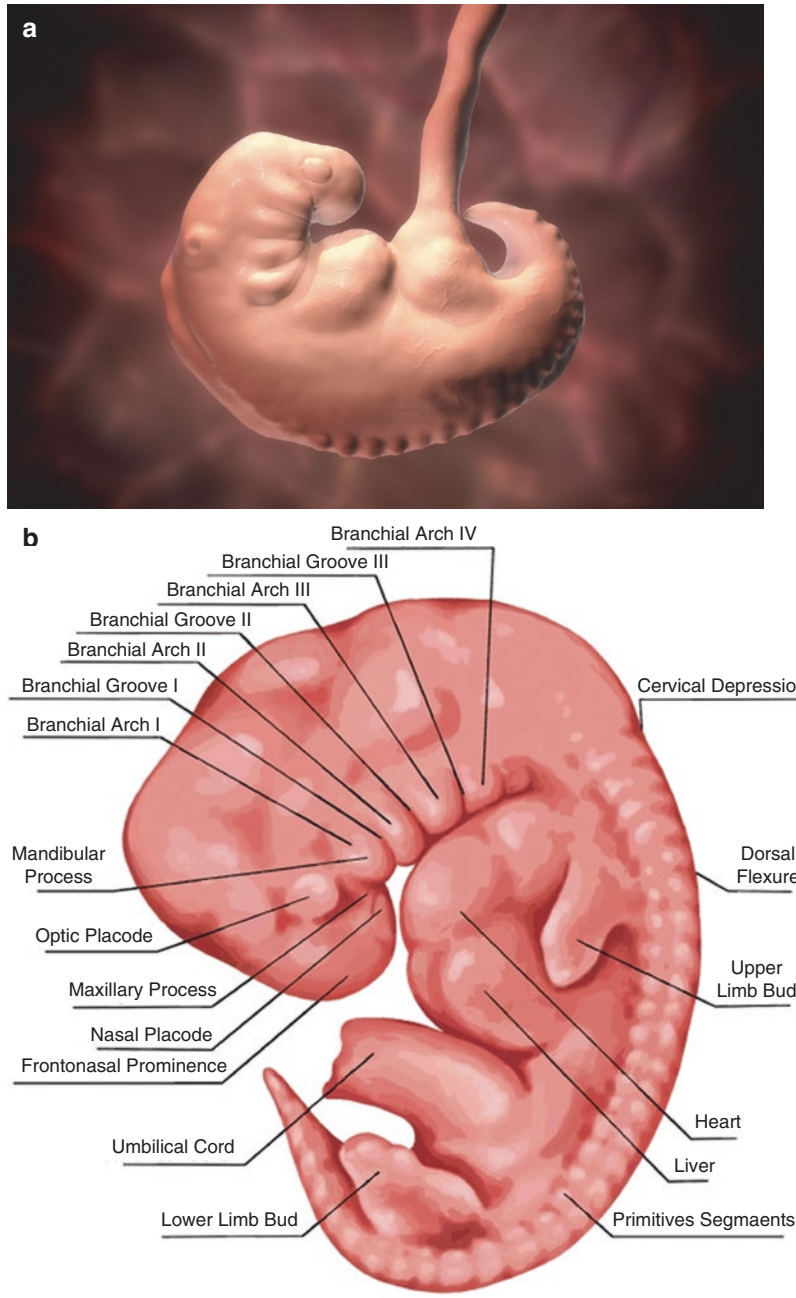

Fig. 10.2 Body and face development. (**a**) Schematic drawing of embryo with umbilical cord. (**b**) The anatomical distribution of the frontonasal prominence, the branchial arches, and the body and limb processes. *Source: Reprinted from a: Kateryna Kon/Shutterstock.com, b: stihii/Shutterstock.com*

The number of PAs is species dependent and may vary from 4 to 9. For example, in mammals there are 5 pairs of PAs (Fig. 10.5), numbered 1, 2, 3, 4, and 6 (as the fifth PA disappears almost as soon as it forms), while zebrafish possesses 7 PAs. However, in each case they develop sequentially in a cranial to caudal manner and are separated by a cleft and pouch which appose each other. The first PA which is also called the mandibular arch appears first,

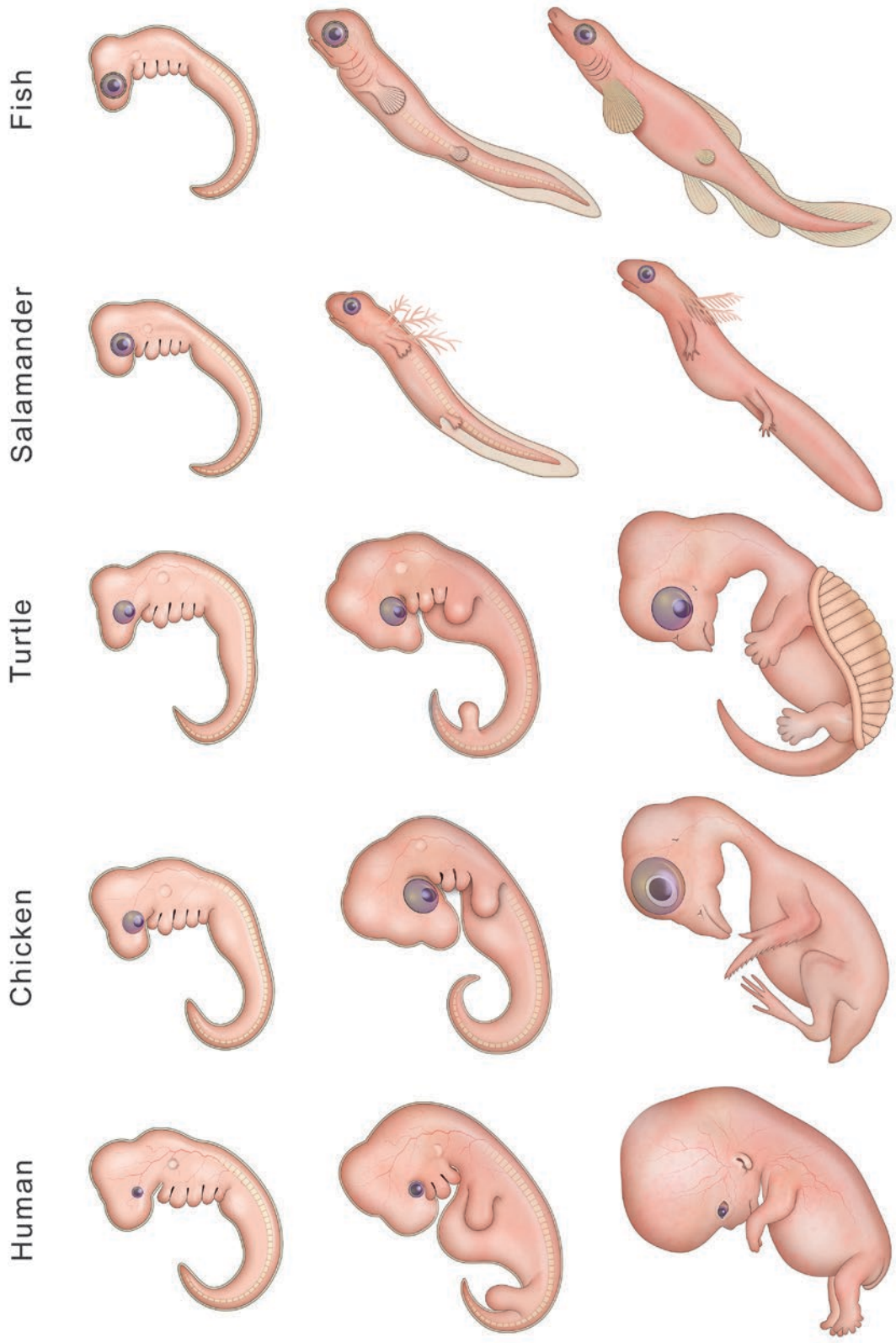

Fig. 10.3 The embryologic development is highly conserved in higher species. *Source: Reprinted from Aldona Griskevicience/Shutterstock.com with permission*

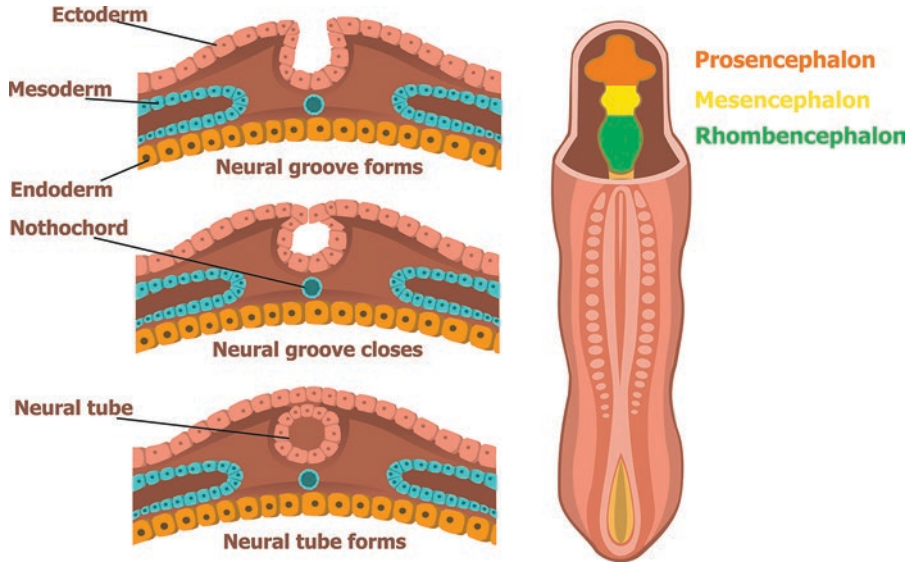

Fig. 10.4 Appearance of the neural groove and somites with the brain *Anlage*. *Source: Reprinted from Systemoff/ Shutterstock.com with permission*

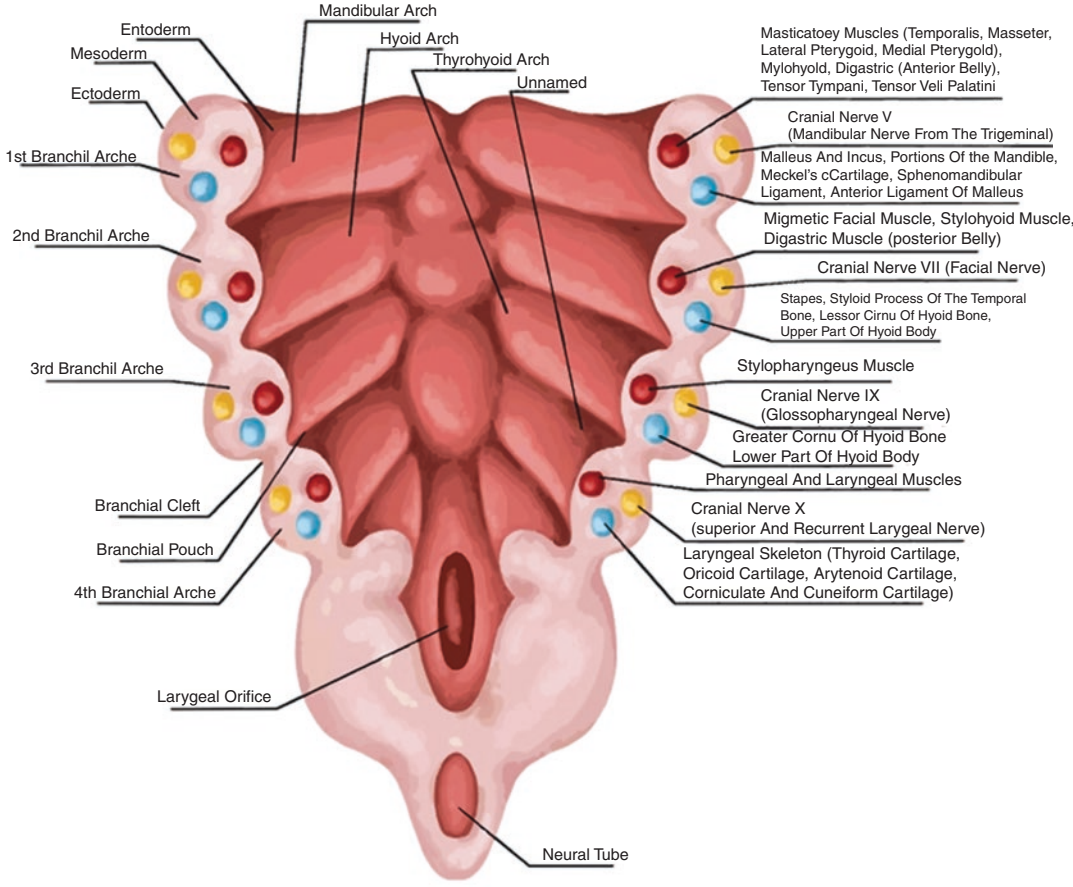

Fig. 10.5 Distribution of bones, nerves, and muscles according to the branchial arches. *Source: Reprinted from stihii/ Shutterstock.com with permission*

followed by the second PA or the hyoid arch, then the others one by one.

Concerning the primary tissue origin, it is important that the **endoderm** gives rise to viscera including the thymus, thyroid, and parathyroid glands which comprise part of the endocrine system. The **mesoderm, in contrast,** gives rise to endothelial cells and myoblasts, the progenitors of the vasculature and musculature, respectively. The **ectoderm c**an be subdivided into a lateral domain of surface ectoderm and a medial domain of neural ectoderm which gives rise to the skin and nervous system, respectively. Furthermore, the dorsal neural ectoderm that forms a boundary with the surface ectoderm also generates **NCCs** that migrate into the FNP, contributing to bones and connective tissue of the face and skull. The NCCs that colonize the PAs give rise to the bones of the jaw, the three small bones of the middle ear (malleus, incus, and stapes), as well as the cartilages of the neck. They also contribute to the formation of the teeth, as NCCs give rise to the dentin-secreting odontoblasts and pulp. In contrast, the enamel is produced by oral ectoderm-derived ameloblasts. Similar to the teeth, the peripheral nervous system is also of dual cellular origin. NCCs generate sensory neurons and glia that integrate with surface ectoderm-derived cranial sensory placode generated neurons. NCCs also form mural cells, the pericytes, and smooth muscle cells that surround the endothelial cells within the great blood vessels of the head, and parts of the neck and upper thorax (for review see Frisdal and Taylor [4]). Therefore, different anatomical structures in various spatial locations in the head and neck region originate from defined arches (Fig. 10.6).

In addition to their species-specific pattern, structures likewise possess many more "species-

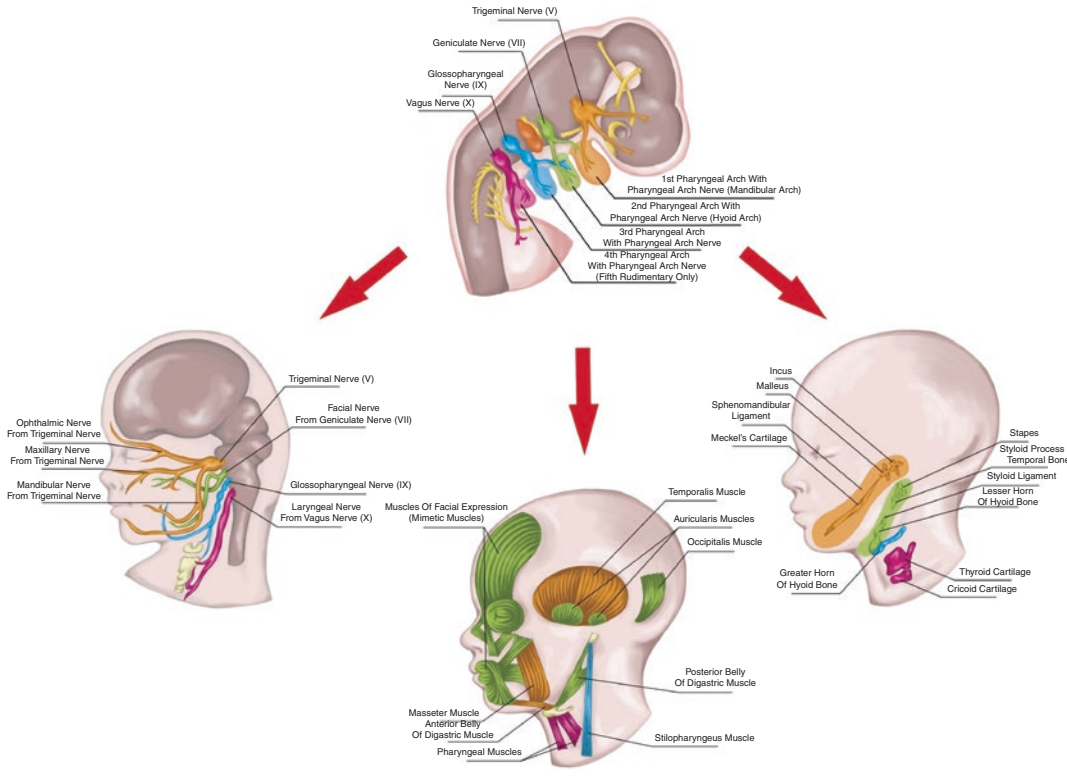

Fig. 10.6 Development of the cranial, midface, lower face, and neck structures. *Source: Reprinted from stihii/Shutterstock.com with permission*

generic" aspects of pattern. These include their axial orientation (e.g., dorsal-ventral, medial-lateral, proximal-distal, oral-aboral), anatomical identity (e.g., upper versus lower jaw, eye versus ear), and tissue type (e.g., cartilage, bone, muscle, tendon, nerve). For the most part, epithelia in the craniofacial complex appear to supply the cues required for the establishment of generic pattern and express the factors necessary to maintain outgrowth of individual components. For example, signaling by ectodermal epithelium around the frontonasal process (i.e., the primordium that gives rise to the mid and upper face) is essential for proper expansion and orientation of skeletal elements along the dorsoventral, mediolateral, and proximodistal axes [17]. The development of the facial region as a segmented structure of a series of reiterated structures (the arches on the exterior surface, the pouches on the interior, and a mesenchymal core) is controlled by several genes (Fig. 10.7). Between all of them, it is well known that *Hox* genes are important regulators in the spatial identity along the anterior-posterior axis of the developing vertebrate embryo. Each of the distinct segmented regions has a unique pattern of *Hox* expression, which conveys crucial positional information to the cells and tissues within it. In the context of

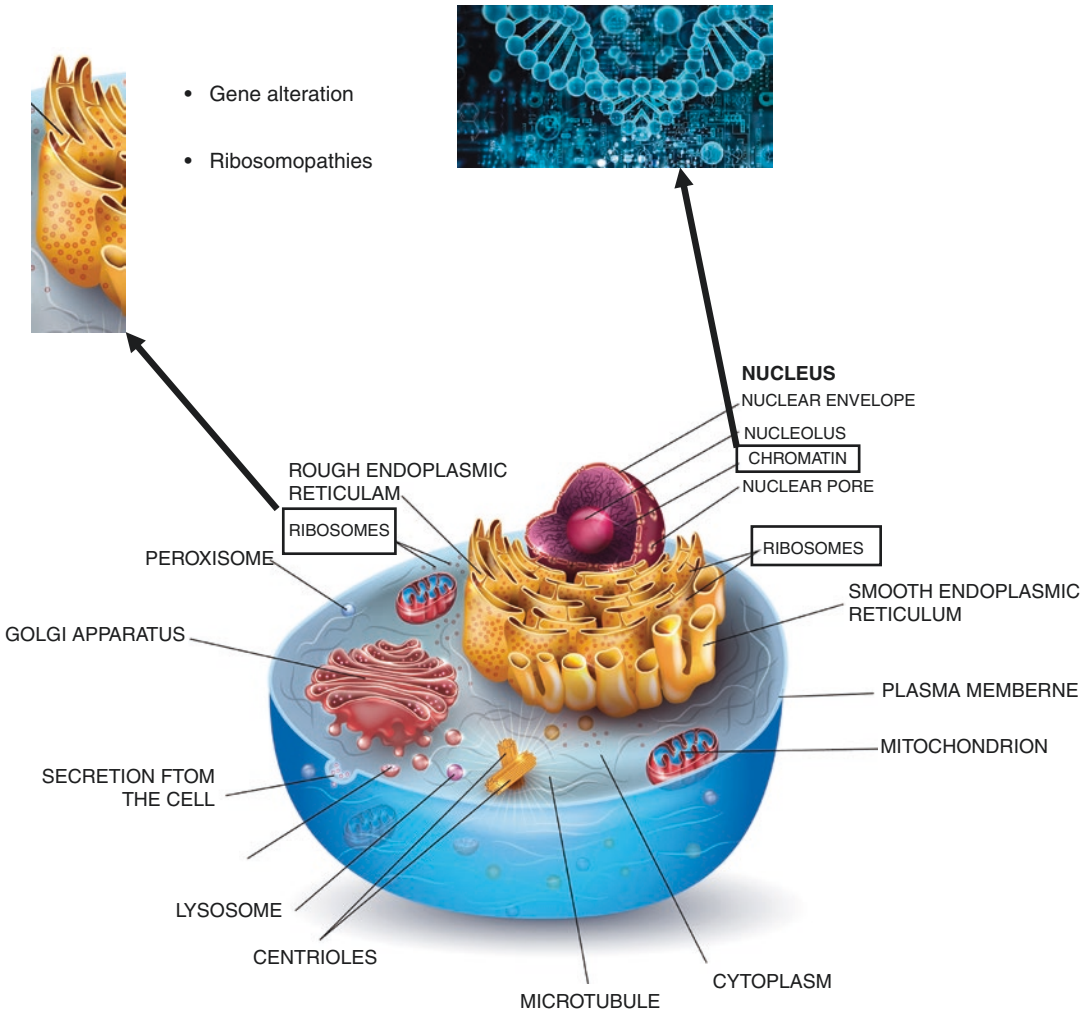

Fig. 10.7 Ribosomopathies as well as alterations in genes (HOX genes, collagen genes) are important for the cell-based origin of branchial arch diseases. *Source: Reprinted from Tefi/Shutterstock.com with permission*

pharyngeal organ development, molecular data suggest that HOXA3 is responsible for specifying organ identity within the third pharyngeal pouch, and in its absence, thymus and parathyroid organogenesis fails to proceed normally. *Dlx* genes help to establish the pattern and polarity of both neural crest cell-derived facial bones and the first branchial arch. Similar to the relationship of RA to *Hox* genes, endothelin signaling serves to regulate *Dlx* gene expression as an upstream regulator. Studies involving various *Dlx* gene deletions in mice suggest that regulation of the formation of the lower jaw is by *Dlx* transcription factor activity.

Fibroblast growth factor (Fgf) is another signaling regulator that plays a role in pharyngeal segmentation and lateral migration of endodermal cells. This process helps to facilitate the formation of pharyngeal pouches through evagination of the endoderm tissue toward the ectoderm. This process of endodermal tissue migration is referred to as "outpocketing" and is crucial for branchial arch segmentation through the approximation of endoderm and ectoderm.

10.3 Tissue and Organs Involved in Branchial Arch Diseases

Nearly all tissues in the head and neck region can be affected by the mis-development of branchial arches. Some syndromes have a unilateral involvement, whereas others present with bilateral involvement. Clinical manifestations are present in different regions of the skull and face. Additionally, phenotypic variability is common in these disease entities. Whereas some individuals have subtle facial involvement (e.g., slight facial asymmetry), others have severe involvement of multiple tissues and organs. The clinical extent of these malformations includes the skull base, the midfacial region, the mandible, and the neck (Fig. 10.8). Involved tissues and organs comprise bones of the neurocranium and the viscerocranium, the eye, the oral region, the jaws, and cranial nerves. Table 10.1 indicates the alteration of normal anatomy. The involvement of the alteration concerning branchial arch diseases is tissue and location specific [14].

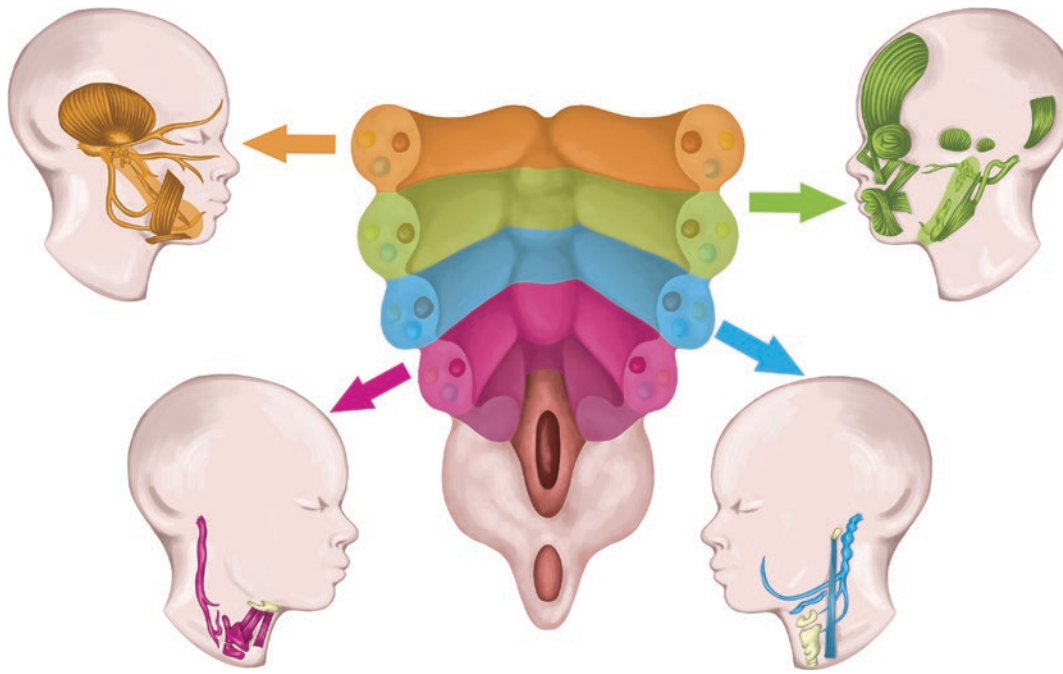

Fig. 10.8 Branchial arches and the corresponding facial structures. *Source: Reprinted from stihii/Shutterstock.com with permission*

Table 10.1 The tissues and organs that are typically misdeveloped in branchial arch diseases

Skull
• Asymmetry of the skull base
• Deformed external auditory canal
• Deformed middle ear
• Deformed internal ear
Jaw
• Midface hypoplasia (underdevelopment of the midface, usually asymmetric)
• Ankylosis (limited opening of the mouth)
• Malocclusion
Eye
• Epibulbar dermoid
• Vertical displacement of the orbit
• Microphthalmia/anophthalmia (rare)
• Coloboma of the upper eye lid and/or iris
Oral region
• Macrostomia (lateral oral clefting). Unilateral macrostomia is the most common form of facial clefting associated with CFM, though all types of clefts can be observed
• Cleft lip and/or palate
Skeleton
• Malformed and/or fused cervical vertebrae are common, though anomalies can be noted throughout the spine
• Hemivertebrae are also common
Cranial nerves
• Facial palsy (unilateral or bilateral involvement of either part or all branches of cranial nerve VII)
• Sensorineural hearing loss
• Asymmetric palatal elevation
• Impairment of extraocular movements

10.3.1 Anatomical Involvement

10.3.1.1 Bones

Included in branchial arch diseases are bones of the skull base and the midfacial and lower facial region (nasal bones, zygomatic bone, jaws) (Fig. 10.9). The bones of the skull (petrosal bone and part of the zygoma) are altered. The jaw is composed of the maxilla and dentary bones, which define the upper and lower jaw, respectively. These two bones articulate to facilitate mastication, respiration, and vocalization. The jaw is formed during embryogenesis primarily from the first PA, which is composed of two paired processes known as the maxillary and mandibular prominences. The maxillary process gives rise to the upper jaw and the palate, while the mandibular process gives rise to the lower jaw. Concerning the type of ossification, two types of ossification, intramembranous and endochondral, are present. During intramembranous ossification, NCCs differentiate directly into functional osteoblasts, which induce an osteoid matrix that becomes a center of ossification. The maxilla and dentary bones undergo direct ossification of NCCs and are therefore classified as membrane or dermal bones.

In endochondral ossification, NCCs initially form a cartilage template, which is subsequently replaced by osteoblasts. Two such cartilages derived from the first PA are Meckel's cartilage, in the mandibular prominence, and palatopterygoquadrate, in the maxillary prominence. The malleus which is derived from Meckel's cartilage, together with the anterior ligament of the malleus and the sphenomandibular ligament, collectively contributes to the temporomandibular joint. The palatopterygoquadrate gives rise to the alisphenoid, a bone that is part of the orbital wall, and it also gives rise to the incus. Reichert's cartilage of the second PA forms the third middle ear bone known as the stapes.

10.3.1.2 Muscles

Muscles of the face head origin from different PAs. Concerning their function, some muscles of the face control facial expression, others control mastication, and others control the movement of the eyes and lips. All of them are derived primarily from the mesodermal cores of the corresponding PA (Fig. 10.10). In adults, the muscles of the face can be categorized by their function. For example, the muscles that have a role in mastication are derived from the first PA mesoderm, while the muscles that govern facial expression are derived from the second PA mesoderm.

10.3.1.3 Nerves

Among the 12 pairs of cranial nerves (Fig. 10.11) (olfactory n. (I), optic n. (II), oculomotor n. (III), trochlear n. (IV), trigeminal n. (V), abducens n. (VI), facial n. (VII), vestibulocochlear n. (VIII), glossopharyngeal n. (IX), vagus n. (X), accessory

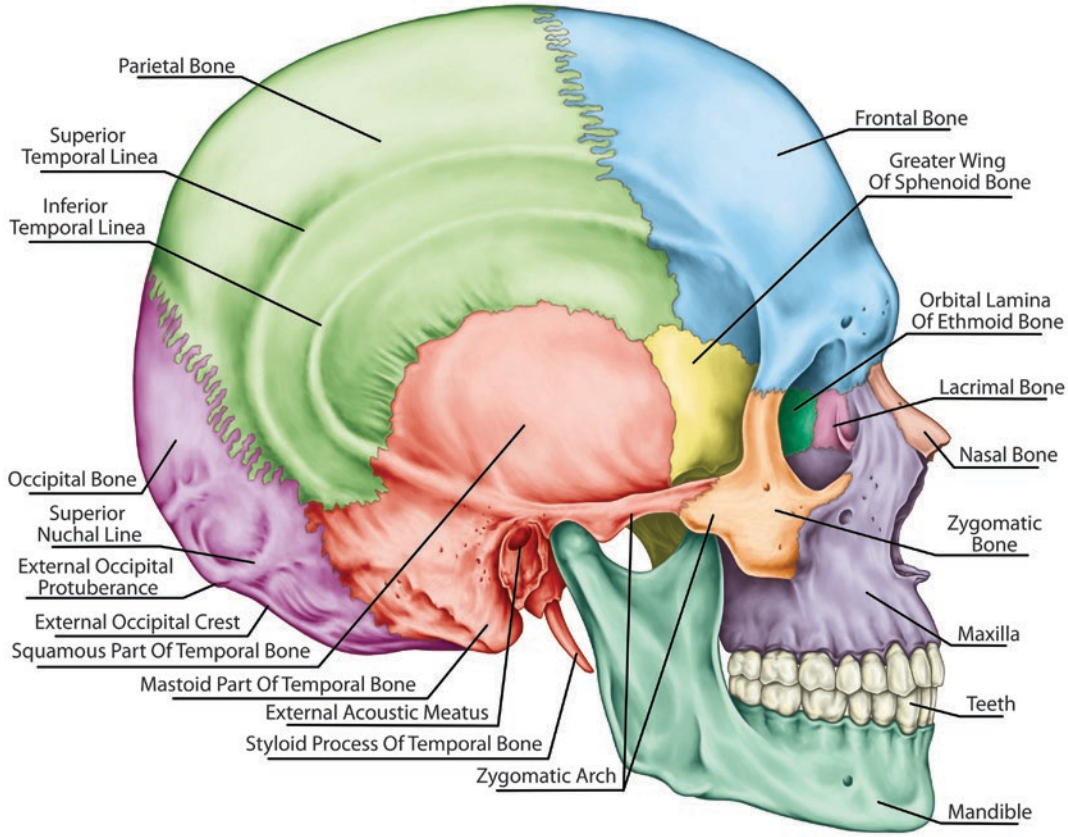

Fig. 10.9 The bones of the human skull. *Source: Reprinted from stihii/Shutterstock.com with permission*

n. (XI), and hypoglossal n. (XII)), 4 invade the PA to innervate muscles derived from the mesoderm core of the corresponding PA. The **trigeminal** (V), **facial** (VII), **glossopharyngeal** (IX), and **vagus** (X) invade the first, second, third, and fourth PA, respectively. These nerves are involved in branchial arch diseases to various extents.

10.3.2 Sensory Organs (Eyes, Ears, Tongue)

10.3.2.1 Ears

The ears are important for balance and hearing and it is derived in part from the otic vesicle which forms dorsal to the second and third PAs. The pharyngeal pouch and cleft that separate the second and third PAs are crucial for the formation of the external and middle ear. The pouch (endoderm) gives rise to the tubotympanic recess, the epithelium of the tympanic cavity, and the Eustachian tube that links the nasopharynx to the middle ear. In addition, the first pharyngeal pouch also gives rise to the tympanus, which defines the boundary of the middle ear. In contrast, the pharyngeal cleft (ectoderm) gives rise to the external auditory canal. The vestibulocochlear nerve (VIII) is derived from the otic placode and innervates the developing inner ear.

10.3.2.2 Eyes

Malformations of the orbit and eyes are based on an abnormal morphogenesis of the frontonasal and maxillary process (derived from forebrain neural crest) with abnormal development of the first and second branchial arches (derived from hindbrain neural crest).

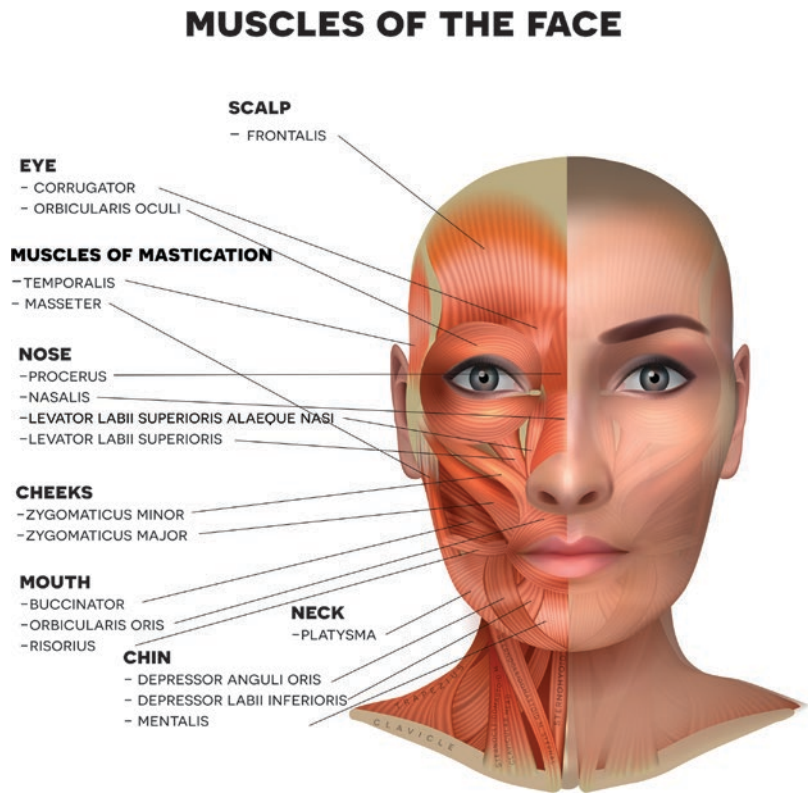

Fig. 10.10 Location of masticatory muscles (innervated by the trigeminal nerve, CN. V) and the facial expression muscles (innervated by the facial nerve, CN. VII). *Source: Reprinted from Tefi/Shutterstock.com with permission*

10.3.2.3 Tongue

The tongue is a particular muscle that manipulates food during mastication, perceives taste, and facilitates phonetic articulation. Its musculature derives from the mesoderm of three different PAs: the first PA mesoderm forms the body of the tongue, the second PA mesoderm is responsible for the formation of the midtongue, and the third PA mesoderm forms the root. The neuronal component of the tongue derives from NCCs; therefore, neural innervation of the tongue has a diversity concerning the location. Innervation of the anterior 2/3 of the tongue comes from the lingual (mandibular division of the trigeminal nerve V3) and chorda tympani (branch of the facial VII) nerves. The posterior 1/3 of the tongue is innervated by the glossopharyngeal nerve (IX).

10.4 Branchial Arch Syndromes

Branchial arch syndromes are clinically and etiologically heterogeneous anomalies of the craniofacial tissues. Most of them are based on NCC alterations, and others have different underlying causes like vascular disturbances or collagen synthesis alterations. These sets of malformations generally arise as a consequence of the abnormal development of the arches. Historically, they are subdivided into two subtypings: mandibulofacial dysostosis and acrofacial dysostosis. The most important syndromes (Table 10.2) are Treacher Collins syndrome, hemifacial microsomia/oculo-auriculo-vertebral dysplasia (OAV complex) with subtype Goldenhar syndrome, auriculocondylar syndrome, Stickler syndrome, DiGeorge syndrome, Pierre Robin syndrome, and acrofacial dysostosis with subtypes as follows:

- Cincinnati type
- Nager syndrome
- Miller syndrome

As the different syndromes have similar phenotypic outcomes, an overview is given on the various disease entities, with special respect to the general disease, epidemiology, underlying biological basis (genetics), and the subsequent clinical outcomes.

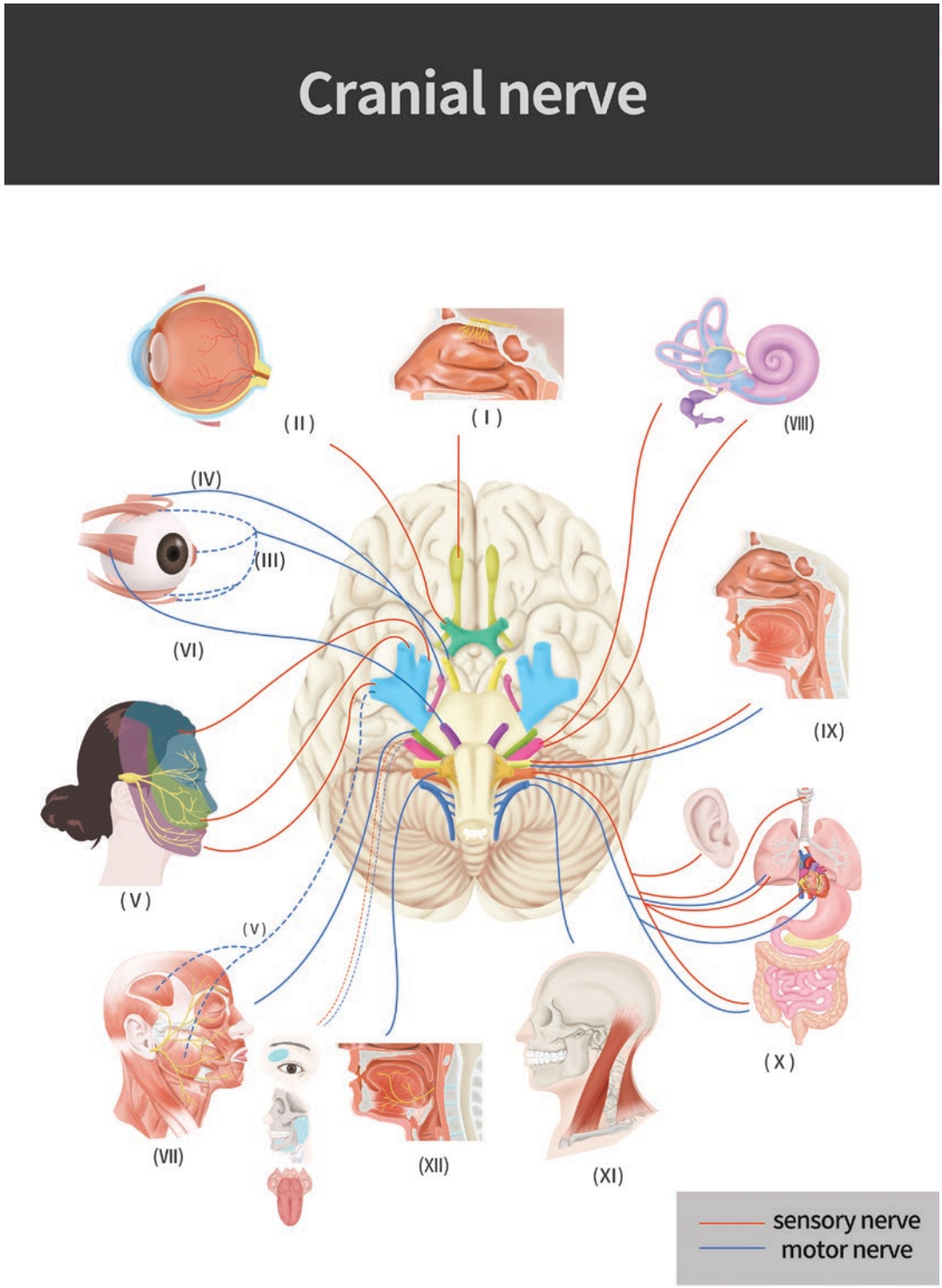

Fig. 10.11 Cranial nerves and their function (involved in branchial arch diseases are CN V, VII, IX, and X). *Source: Reprinted from Chu Kyung Min/Shutterstock.com with permission*

10.4.1 Treacher Collins syndrome

10.4.1.1 General

Treacher Collins syndrome (TCS) is a disorder characterized by deformities of the ears, eyes, cheekbones, and chin (Fig. 10.12). The degree to which a person is affected, however, may vary from mild to severe [18, 19]. Complications may include breathing problems, visual problems, cleft palate, and hearing loss. Those affected patients generally have an average intelligence. Franceschetti syndrome is synonymously used withTCS syndrome.

Table 10.2 An overview of the various branchial arch syndromes

• Treacher Collins syndrome
• Hemifacial microsomia/oculo-auriculo-vertebral dysplasia (OAV complex)
• Auriculocondylar syndrome
• Stickler syndrome
• Di George syndrome
• Pierre Robin syndrome
• Acrofacial dysostosis with subtypes
• Cincinnati type
• Nager syndrome
• Miller syndrome

10.4.1.2 Epidemiology

TCS occurs in about 1 in 50,000 people. The condition has been first described by Thompson in 1846. The syndrome is named after Edward Treacher Collins, an English surgeon and ophthalmologist, who described its essential traits in 1900. The first extensive review of the condition was published by Franceschetti and Klein in 1949, who first used the term "mandibulofacial dysostosis" and also identified its hereditary nature.

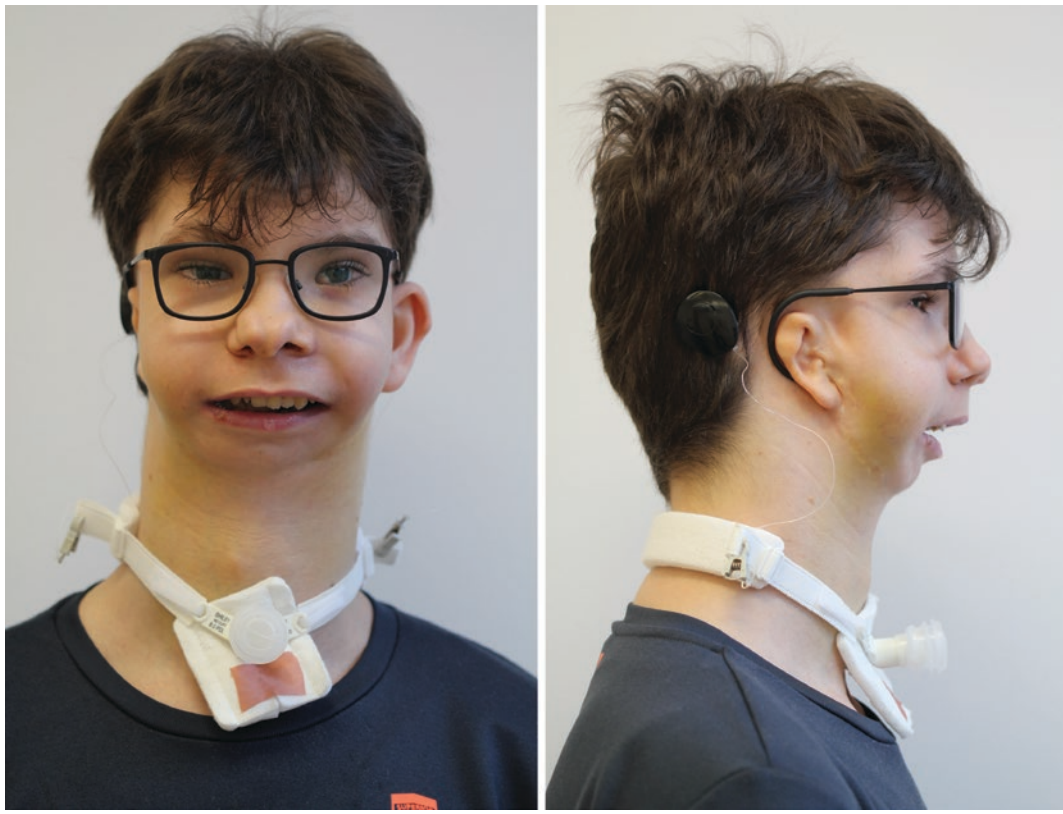

Fig. 10.12 Clinical appearance of a patient with Treacher Collins syndrome, displaying all features of the disease

Genetic basis of branchial arch diesases

Disease	Genes
TCS -	TCOF1, POLR1C/D,
Stickler-	COLL11A1/2, COLL2A1
PRS-	SOX9, GAD1, PVRL1, KCNJ2
ACF-Cincinatti	POLR1A
Nager	SF3B4
ACD-syndrome	Mutation 1p21.1-q23.3
DiGeorge	Deletion 22q11.2

Fig. 10.13 Gene involvement in branchial arch diseases. *Source: Reprinted from Pop Tika/Shutterstock.com with permission*

10.4.1.3 Genetics

A lot of genetic alterations in craniofacial diseases are well known (Fig. 10.13). TCS, for example, is usually autosomal dominant [20–23]. More than half the time it occurs as a result of a new mutation rather than being inherited from a person's parents. Forty percent of patients with TCS have a family history of the disease, and 60% of cases are seen sporadically. The occurrence of TCS is gene based presented with variable penetrance and phenotypic expression. TCS mostly arises as the result of mutations in the *TCOF1* gene [24–27]. Other involved genes may include *POLR1C* and *POLR1D*. *TCOF1* gene mutations are the most common cause of the disorder, accounting for 81 to 93% of all cases. The majority of mutations are small deletions or insertions, though splice site and missense mutations also have been identified [28–38]. *TCOF1* is found on the fifth chromosome in the 5q32 region. It codes for a nucleolar protein called treacle that is thought to be involved in ribosome assembly [39–46].

POLR1C and *POLR1D* gene mutations cause an additional 2% of cases. *POLR1C* is found on chromosome 6 at position 6q21.2 and codes for a protein subunit of RNA polymerase I.

POLR1D is found on chromosome 13 at position 13q12.2 and codes for a protein subunit of RNA polymerase III. Both of these polymerases are similar to the TCOF1 influence, important for ribosome biogenesis [47–53].

In individuals without an identified mutation in one of these genes, the genetic cause of the condition is unknown [54].

10.4.1.4 Clinical Manifestation

Symptoms in people with Treacher Collins syndrome vary. Some individuals are so mildly affected that they remain undiagnosed, while others have moderate to severe facial involvement and life-threatening airway compromise. Although facial deformity is often associated with developmental delay and intellectual disability, more than 95% of people affected with TCS have normal intelligence. The psychological and social problems associated with facial deformity can affect the quality of life in individuals with TCS.

- Skull
- Although an abnormally shaped skull is not distinctive for Treacher Collins syndrome, brachycephaly with bitemporal narrowing is sometimes observed.
- Midface/Jaws
- Facial bone hypoplasia, involving the mandible and zygomatic complex in >75% of patients, is an extremely common feature of TCS. Underdevelopment of the zygomatic bone gives the cheeks a sunken appearance. The maxilla may also be hypoplastic but sometimes can be seen as overprojecting. The nose may be broad or protruding. Choanal atresia or stenosis as a narrowing or absence of the choanae is sometimes present. The internal opening of the nasal passages may also be observed. Underdevelopment of the pharynx as a fate of the disease may narrow the airway.

- Eyes
- Other characteristic abnormalities include downward slanting of the palpebral fissures with notching of the lower eyelids and a scarceness of lid lashes medial to the defect.
- Ear
- Auricular anomalies include absent external ear canal, middle ear malformations, and pinna deformities. The external ear is sometimes small, rotated, malformed, or absent entirely in people with TCS. Symmetric, bilateral narrowing or absence of the external ear canals is also described. In most cases, the bones of the middle ear and the middle ear cavity are deformed. Inner ear malformations are rarely described. As a result of these abnormalities, a majority of the individuals with TCS have conductive hearing loss. The hearing loss is generally bilateral with a conductive loss of about 50–70 dB. Even in cases with normal auricles and open external auditory canals, the ossicular chain is often malformed.
- Oral Cavity
- Cleft palate is a common co-occurrence and may be severe. This can be accompanied by the tongue being retracted. The small mandible often results in a poor occlusion of the teeth or in more severe cases, trouble breathing or swallowing. Dental anomalies are seen in 60% of affected people, including tooth agenesis (33%), discoloration (enamel opacities) (20%), malplacement of the maxillary first molars (13%), and wide spacing of the teeth.
- Body Involvement
- Limb anomalies do not occur in TCS, which helps differentiate it from other syndromes that manifest with similar facial features.

10.4.2 Hemifacial Microsomia/ Oculo-auriculo-vertebral Dysplasia with Subtype Goldenhar Syndrome

10.4.2.1 General

Hemifacial microsomia (HFM) is a common facial birth defect involving the first and second BA structures and ranks second in prevalence only behind facial clefting [55]. Males are affected more frequently than females. About 45% of patients have affected relatives, and 5–10% of them have affected siblings [56]. The phenotype is highly variable. There may be cardiac, vertebral, and central nervous system defects, in addition to craniofacial anomalies. Ear deformities occur along a spectrum from the size and shape of the external auricle to anotia. When epibulbar dermoids and vertebral anomalies are seen along with other findings of HFM, the syndrome is called Goldenhar syndrome [57]. Goldenhar [58] first described the triad of epibulbar choristomas, preauricular skin appendages, and pretragal blind-ending fistulas in association with mandibular facial dysplasia.

A variety of terms have been proposed that serve to indicate and sub-differentiate the spectrum of anomalies. Additional names of these variants include Goldenhar-Gorlin syndrome, first arch syndrome, first and second BA syndrome, lateral facial dysplasia, unilateral craniofacial microsomia, otomandibular dysostosis, unilateral mandibulofacial dysostosis, unilateral intrauterine facial necrosis, auriculobranchiogenic dysplasia, and facioauriculovertebral malformation complex. The terms and systems of classification have been reviewed multiple times [59–63]. Later patients with associated vertebral anomalies were given the classification of OAV dysplasia [58]. The combination of OAV features and microtia is termed the "OAV complex." When the features of the OAV complex are predominantly unilateral and lack vertebral anomalies and epibulbar dermoids, the condition has been called HFM. This pattern is thought to represent a variant of the expanded OAV complex [64]. Intellectual disability is not typically seen in people with HFM.

10.4.2.2 Epidemiology

Hemifacial microsomia has an incidence in the range of 1:3500 to 1:4500; it is the second most common birth defect of the face, after cleft lip and cleft palate [56]. HFM shares many similarities with Treacher Collins syndrome.

10.4.2.3 Genetics

The condition develops in the fetus at approximately 4 weeks of gestational age, when some form of vascular problem such as blood clotting leads to insufficient blood supply to the face. This

can be caused by physical trauma, though there is some evidence of it being hereditary [64, 65]. This restricts the developmental ability of that area of the face. Currently there are no definitive reasons for the development of the condition.

10.4.2.4 Clinical Manifestation

The clinical presentation of HFM is quite variable [66]. The severity may depend on the extent of the area with an insufficient blood supply in utero, and the gestational age of the fetus at which this occurs. In severe cases, multiple parts of the face may be affected.

- Skull
- Although an abnormally shaped skull is not distinctive, malformation of the external and internal ear complex leads to an asymmetric and deformed skull base at the site of involvement.
- Midface/Jaws
- In most patients, the maxilla is tilted and the mandible is deformed to different extents (Fig. 10.14). Figueroa and Pruzansky classified HFM patients, according to the mandible, into three different types [59]:

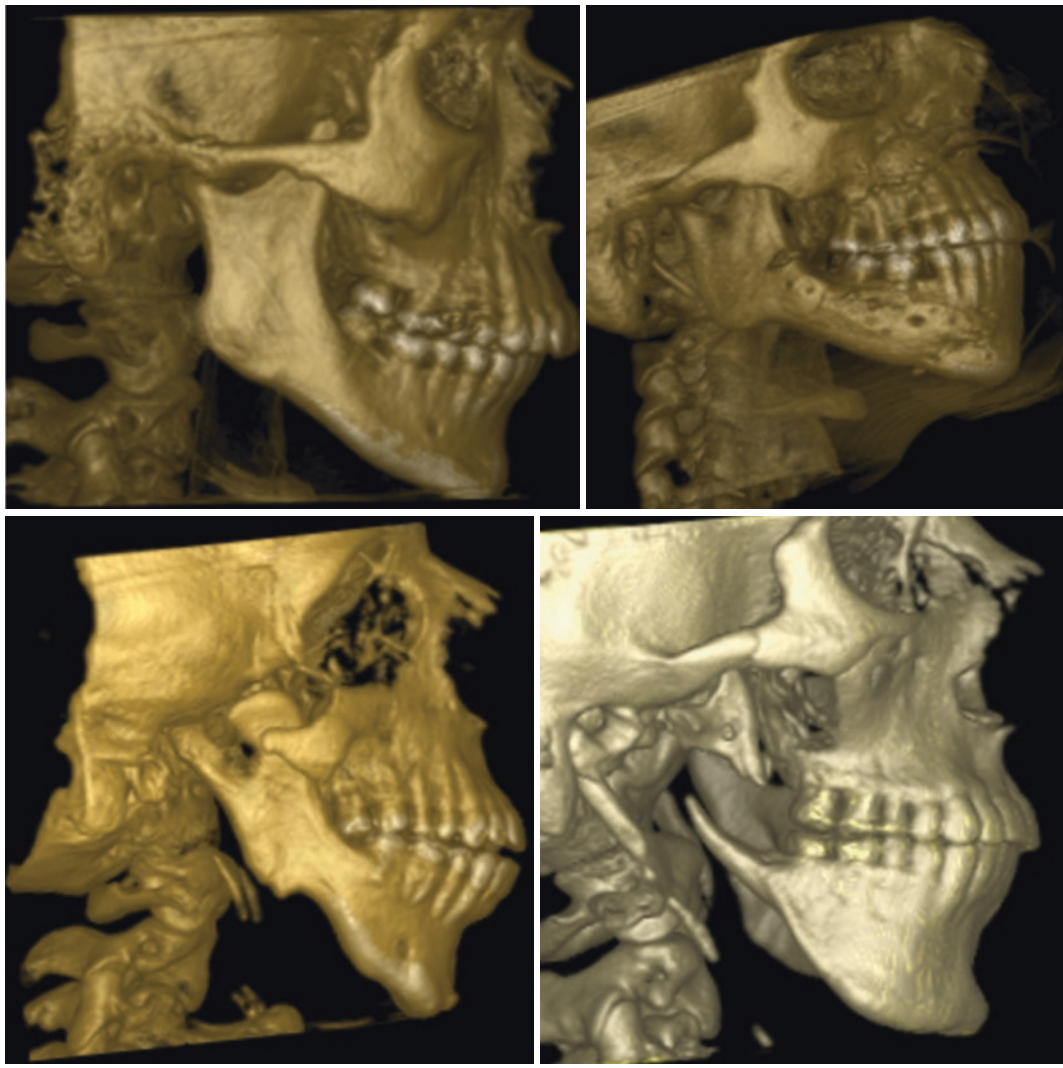

Fig. 10.14 Cone beam computed tomographs of the skull of patients with branchial arch diseases, demonstrating various involvements of the maxilla and mandible (from mild to severe)

  - Type I: Mild hypoplasia of the ramus, and the body of the mandible is slightly affected.
  - Type II: The condyle and ramus are small; the head of the condyle is flattened; the glenoid fossa is absent; the condyle is hinged on a flat, often convex, infratemporal surface; and the coronoid may be absent.
  - Type III: The ramus is reduced to a thin lamina of bone or is completely absent. There is no evidence of a TMJ.
- Eyes
- In severe cases, the orbital frames are located in a tilted fashion; they are placed not perpendicular to the vertical facial axis.
- Ear
- In some people, the only physical manifestation may be a small and underdeveloped external ear. Some people with HFM may have sensorineural hearing loss and decreased visual acuity or even blindness.
- Oral Cavity
- The small mandible can result in a laterotrusion of the mandible with poor occlusion of the teeth or in more severe cases, trouble breathing or swallowing.
- Body Involvement
- Goldenhar syndrome as a particularly severe form of HFM presents to some extent extracranial anomalies. Some of the internal organs (especially the heart, kidneys, and lungs) may be underdeveloped or in some cases even absent altogether. The affected organs are typically on the same side as the affected facial features, but bilateral involvement occurs in approximately 10% of cases. Deformities of the vertebral column such as scoliosis may also be observed in Goldenhar syndrome.

10.4.3 Auriculocondylar Syndrome

10.4.3.1 General

The ACS, first described by Uuspää in 1978, is now recognized as a distinct autosomal dominant disorder. The features seen in ACS have previously been ascribed the names "Cosman ear" and the "question mark ear" [67, 68].

10.4.3.2 Epidemiology

Due to the sporadic appearance of this syndrome, epidemiologic data are inconsistent.

10.4.3.3 Genetics

Inter- and intrafamilial variability is marked, and some obligate carriers are nonpenetrant [67]. A genome-wide search of two families with ACS revealed evidence of linkage to 1p21.1-q23.3 in one of the families and nonlinkage in the other [69]. These findings suggest evidence for genetic heterogeneity and the existence of at least two loci responsible for this syndrome.

10.4.3.4 Clinical Manifestation

Prominent malformed ears, with auricular clefts, mandibular condyle aplasia or hypoplasia, and a number of other auricular and oral abnormalities characterize ACS [70]. In its most severe form, there are severe micrognathia and a characteristically round facial appearance with prominent cheeks. A characteristic auricular cleft malformation is seen in ACS, which consists of a protuberant cupped pinna with a cleft or notching between the lobule and the helix. The cleft may be subtle or severe enough to detach the lobule from the helix. The anomalies can be unilateral or bilateral and are typically asymmetric. Some individuals have low-set and posteriorly rotated ears. Pre- and postauricular tags may be present. Hearing and middle ear functions are generally normal; however, sensorineural hearing loss has been reported.

Complete mandibular condyle agenesis, hypoplasia, or more subtle clinical and radiographic anomalies may be present. These findings include micrognathia, short mandibular rami, small coronoid processes, poorly formed TMJs, small condylar necks with anterior placement of the condylar articulations, and increased distances between the EACs and the posterior glenoid fossa. In some first-degree relatives of patients with ACS, the auricular malformations may be seen associated with macrognathia (type III malocclusion). Additional anomalies, somewhat specific to

ACS, include a prominent bony ridge along the lateral aspect of the mandible.

10.4.4 Stickler Syndrome

10.4.4.1 General

Stickler et al. [71] first described this autosomal dominant syndrome, also called hereditary progressive arthro-ophthalmopathy, characterized by ocular and orofacial changes, arthritic changes, and deafness. The clinical picture is highly variable and sometimes confusing, with phenotypic features varying from dwarfism/marfanoid habitus to phenotypically healthy individuals. This variability can lead to diagnostic difficulties [72].

10.4.4.2 Epidemiology

In the USA, the estimated prevalence of Stickler syndrome is about 1 in 10,000 people, but it can affect as few as 1 in 1,000,000 in other areas of the world.

10.4.4.3 Genetics

The syndrome is thought to arise from a mutation of several collagen genes during fetal development. It is a sex-independent autosomal dominant trait, meaning a person with the syndrome has a 50% chance of passing it on to each child. There are three variants of Stickler syndrome identified, each associated with a collagen biosynthesis gene [73–75]. Mutations in the COL11A1, COL11A2, and COL2A1 genes cause Stickler syndrome. These genes are involved in the production of type II and type XI collagen. Mutations in any of these genes disrupt the production, processing, or assembly of type II or type XI collagen.

Other, as yet unknown, genes may also cause Stickler syndrome because not all individuals with the condition have mutations in one of the three identified genes.

10.4.4.4 Clinical Manifestation

A characteristic feature of Stickler syndrome is a flattened facial appearance to different extents. The phenotypic appearance is caused by underdeveloped bones in the middle of the face, including the cheekbones and the bridge of the nose. Despite the genotypic heterogeneity, the systemic features are similar for the different types. Diagnostic criteria have been proposed for type 1, comprising most patients with Stickler syndrome, which include molecular or family history data and characteristic ocular, orofacial, auditory, and musculoskeletal findings [76–78].

- Skull
- The skull is seldom involved in Stickler syndrome.
- Midface/Jaws
- The typical phenotypic facial features are caused by underdeveloped bones in the midface, including the cheekbones and the bridge of the nose. This leads to a flattened facial appearance.
- Eyes
- The most serious manifestations of the syndrome are ocular aspects, including retinal detachment, high nonprogressive myopia, and vitreoretinal degeneration. These features may lead to eventual blindness. Less common ophthalmologic features include perivascular pigmented lattice degeneration and cataracts.
- Ears
- Patients with Stickler syndrome may have congenital sensorineural, congenital conductive, or acquired conductive hearing loss. Defects of the auditory ossicles can be seen with associated congenital conductive hearing loss. Forty percent of patients show some evidence of sensorineural hearing loss, which in many patients may be clinically occult. If there is an association with CP and a high arched palate, an increased incidence of serious otitis media is found, which may lead to conductive hearing loss.
- Oral Cavity
- Some patients present with an additional cleft palate; often findings are high arched palates.
- Body Involvement
- Body involvement shows a high variability in expression. Enlarged joints, epiphyseal changes, and mild platyspondyly are typical of the disorder. Mild ligamentous laxity is seen early in life that occasionally leads to

generalized ligamentous stiffness. Osteoarthritis typically develops in the third or fourth decade. Mild spondyloepiphyseal dysplasia is often apparent radiologically. Occasional findings include slender extremities and long fingers.

10.4.5 DiGeorge Syndrome

10.4.5.1 General

DiGeorge first reported the association of the absence of the thymus with aplasia of the parathyroid glands. These observations were appreciated with variable anomalies of the cardiovascular system and craniofacial syndromes. DiGeorge syndrome, also known as 22q11.2 deletion syndrome [79], is a syndrome caused by the deletion of a small segment of chromosome 22 [80]. While the symptoms can vary, they often include congenital heart problems, specific facial features, frequent infections, developmental delay, learning problems, and cleft palate. Associated conditions include kidney problems, hearing loss, and autoimmune disorders such as rheumatoid arthritis or Graves' disease.

10.4.5.2 Epidemiology

DiGeorge syndrome is estimated to affect between 1 in 2000 and 1 in 4000 live births [81, 82]. This estimate is based on major birth defects and may be an underestimate, because some individuals with the deletion have few symptoms and may not have been formally diagnosed. It is one of the most common causes of intellectual disability due to a genetic deletion syndrome [83].

10.4.5.3 Genetics

DiGeorge syndrome is inherited in an autosomal dominant pattern [84]. It is typically due to the deletion of 30 to 40 genes in the middle of chromosome 22 at a location known as *22q11.2*. This syndrome is characterized by incomplete penetrance. Therefore, there is a marked variability in clinical expression between the different patients. This often makes early diagnosis difficult. Although there has been debate about the distinct etiologic nature of DiGeorge syndrome and velocardiofacial syndrome (VCFS), there is considerable phenotypic and genotypic overlap. A 1.5- to 3.0-Mb hemizygous deletion of chromosome 22q11.2 causes VCFS [85]. This monoallelic microdeletion is considered the most common human deletion syndrome. DiGeorge syndrome has been shown to share a genetic defect with VCFS in 45–85% of cases in different series [86]. About 90% of cases occur due to a new mutation during early development, while 10% are inherited from a person's parents. It is autosomal dominant, meaning that only one affected chromosome is needed for the condition to occur [87]. Diagnosis is suspected based on the symptoms and confirmed by genetic testing [88].

10.4.5.4 Clinical Manifestation

- Skull
- Some patients present with microcephaly accompanied by a small skull.
- Midface/Jaws
- Skeletal anomalies are not uncommon and responsible for the altered facial appearance [89]. Characteristic facial features (present in the majority of Caucasian individuals) may include hypertelorism.
- Ears
- Some patients present with minor auricular anomalies. Additionally, they may present with conductive and sensorineural hearing loss.
- Oral Cavity
- Palatal abnormalities (50%), particularly velopharyngeal incompetence, submucosal cleft palate, and cleft palate, are often present. VCFS is the most frequent clefting syndrome, accounting for approximately 8.1% of children with palatal clefts seen in some centers [90].
- Body Involvement
- VCFS consists of CP, cardiac anomalies, typical facies, and learning disabilities. In a recent study, cortical areas of reduced gyration were observed, further substantiating the pattern of cerebral alterations presented with the syndrome. Almost all individuals with 22q11 deletion syndrome have behavior and/or learning problems, with >40% meeting the

criteria for either autism spectrum disorder, attention-deficit/hyperactivity disorder, or both. More than half of patients, in some series, meet the criteria for mental retardation. Less frequent features include microcephaly, short stature, slender hands and digits, minor auricular anomalies, and inguinal hernia. Cardiac anomalies have been described in 82% of patients, including isolated ventricular septal defect and tetralogy of Fallot.

Two emergent clinical situations may arise in children with VCFS on the basis of the variable associated defects of the third and fourth BAs. The first is tetany, which can be sudden and fatal, due to hypocalcemia relating to aplasia of the parathyroids. Although the absence of parathyroid gland function is rare, parathyroid dysfunction is present in approximately half of patients with VCFS. The second emergent situation is related to infections from deficiencies with the T-cell-mediated response of the immune system due to an absent or hypoplastic thymus. Immunologic evaluation is critical in affected children to identify those that may require either lymphocyte or thymus transplantation. Both of these situations require special care of patients who may require cardiac surgery.

10.4.6 Pierre Robin Syndrome/ Sequence (PRS)

10.4.6.1 General

The first publication of PRS was in 1923 by a French physician [91], describing neonates with unusually small mandibles (micrognathia), posterior displacement or retraction of the tongue (glossoptosis), and upper airway obstruction. Because incomplete closure of the roof of the mouth (CP) is present in most patients, Robin later added CP deformity as an associated feature [92–96]. Two of the main features (micrognathia and glossoptosis) cause breathing problems due to obstruction of the upper airway. A wide, U-shaped cleft palate is commonly also present.

10.4.6.2 Epidemiology

The prevalence of PRS is estimated to be 1 in 8,500 to 14,000 people.

10.4.6.3 Genetics

PRS is not merely a syndrome, but rather it is a sequence—a series of specific developmental malformations which can be attributed to a single cause. PRS may be caused by a genetic disorder. In the case of PRS which is due to a genetic disorder, a hereditary basis has been postulated, but it usually occurs due to a de novo mutation. Specifically, mutations at chromosome 2 (possibly at the GAD1 gene), chromosome 4, chromosome 11 (possibly at the PVRL1 gene), or chromosome 17 (possibly at the SOX9 gene or the KCNJ2 gene) have all been implicated in PRS [94]. Some evidence suggests that genetic dysregulation of the SOX9 gene (which encodes the SOX-9 transcription factor) and/or the KCNJ2 gene (which encodes the Kir2.1 inward-rectifier potassium channel) impairs the development of certain facial structures, which can lead to PRS [95, 96]. PRS may occur in isolation, but it is often part of an underlying disorder or syndrome [97]. Disorders associated with PRS include Stickler syndrome, DiGeorge syndrome, fetal alcohol syndrome, Treacher Collins syndrome, and Patau syndrome [98].

10.4.6.4 Clinical Manifestation

- Skull
- If a singular disease, no involvement.
- Midface/Jaws
- Studies have documented that there is also associated bimaxillary retrognathia, with reduced sagittal length of not only the mandible but also the maxilla. Although the possibility that the mandible may grow forward and partially or fully catch up during the first years of life has been discussed in the literature, recent studies have suggested that no significant catch-up growth of the mandible in PRS occurs in the first 22 months of life. The differential growth shown in these studies does not improve the size of the pharyngeal airway but does improve the relative size of the oropharynx, which can have a positive effect on breathing difficulties.

- Eyes
- If a singular disease, no involvement.
- Ears
- If a singular disease, no involvement.
- Oral Cavity
- The most prominent feature is the micrognathic state, often accompanied by a cleft palate of various extents.
- Body Involvement
- If a singular disease, no involvement.

10.4.7 Acrofacial Dysostosis

Acrofacial dysostosis describes a congenital syndrome which presents with craniofacial defects similar to those observed in mandibulofacial dysostosis (see Sect. 3.3.2.) but with the addition of limb defects.

10.4.7.1 Nager Syndrome

General

Nager syndrome is the most frequent and well-studied type of acrofacial dysostosis [99, 100]. In addition to overlapping craniofacial phenotypes with hemifacial microsomia and TCS, including downward slanting of the palpebral fissures, Nager syndrome was identified as an acrofacial dysostosis condition due to the presence of pre-axial limb defects, most commonly hypoplasia or absence of the thumbs [101, 102]. The similar phenotypes observed in Nager syndrome in comparison to other facial dysostoses, plus the small number of reported cases (n~100), make the diagnosis and identification of common mutations in Nager syndrome challenging.

Epidemiology

It is a very rare syndrome. Detailed epidemiological data are unprecise.

Genetics

Despite these limitations, recent studies identified mutations in SF3B4 in about 60% of Nager syndrome cases. Similar to TCS, Nager syndrome is rare and is primarily associated with de novo mutations, although both autosomal dominant and autosomal recessive mutations have also been reported.

Clinical Manifestation

The clinical manifestations are often similar to hemifacial microsomia (see Sect. 3.3.2.) with additional limb defects.

10.4.7.2 Miller Syndrome

General

Miller syndrome, also termed post-acrofacial dysostosis (POADS), Genee-Wiedemann, and Wildervanck-Smith syndromes, is classified as an acrofacial dysostosis disorder [103–107]. Miller syndrome was the first Mendelian syndrome whose molecular basis was identified via whole-exome sequencing.

Epidemiology

It is a very rare syndrome. Detailed epidemiological data are unprecise.

Genetics

The syndrome was found to correlate with autosomal recessive or compound heterozygous mutations in dihydroorotate dehydrogenase [108, 109].

Clinical Manifestation

Similar to TCS and Nager syndromes, Miller syndrome is characterized by craniofacial abnormalities such as downward slanting of the palpebral fissures, coloboma of the lower eyelid, hypoplasia of the zygomatic complex, micrognathia, and microtia, which can lead to conductive hearing loss [110, 111]. Signifying Miller syndrome as a form of acrofacial dysostosis is the presence of post-axial limb defects, which contrasts with the pre-axial defects presented by Nager syndrome.

10.4.7.3 Cincinnati Type

General

Acrofacial dysostosis (ACF)-Cincinnati type is diagnosed in individuals with variable phenotypes ranging from mild mandibulofacial dysostosis to more severe acrofacial dysostosis [112].

Epidemiology

It is a very rare syndrome. Detailed epidemiological data are unprecise.

Genetics

Patients were found to carry a heterozygous mutation in *POLR1A*, which encodes the largest subunit of RNA polymerase I, which is responsible for transcribing rRNA [112].

Clinical Manifestation

Most patients present with variable craniofacial phenotypes similar to those observed in TCS, including hypoplasia of the zygomatic arches, maxilla, and mandible; severe micrognathia; down-slating palpebral fissures; coloboma or inferiorly displaced orbits; bilateral anotia; and conductive hearing loss. Additionally, similar to other acrofacial dysostoses, some patients present with limb anomalies, including short bowed femurs; delayed epiphyseal ossification; flared metaphysis and dysplastic acetabula; or short and broad fingers and toes.

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11 The Biological Basis of Craniofacially Conjoined Twins

Ulrich Meyer

11.1 Introduction

Conjoined twins (CT) are rare and present a unique challenge to all physicians involved in the treatment of such patients. The presence of conjoined twins can be seen through ancient cave drawings, carved figurines, and ceramics of human conjoined twins. It can be concluded that these malformations existed long before the human race finished descending from its ancestors [1]. Scientists were speculating on the underlying biological basis, as possible etiopathogenetic causes were hampered by a lack of early (molecular) embryological knowledge, especially regarding the processes of (in)complete twinning. However, throughout multiple centuries, the etiopathogenesis of conjoined twins has crystallized into two currently conjectured theories: partial fission [2] versus secondary fusion [3]. The kind of twin formation is mostly classified according to the site of the main connection (Table 11.1): thorax (thoracopagus), abdomen (omphalopagus), sacrum (pygopagus), pelvis (ischiopagus), skull (craniopagus), face (cephalopagus), lateral (parapagus), or back (rachipagus). The most frequent type of conjoined twins is thoracopagus (32.7%), with joining at or near the sternal wall and contained viscera, and the rarest type is diprosopus (0.4). Conjoined twinning occurs in 1/100 of monozygotic twins, 1/50,000 gestations, and 1/250,000 live births [4]. It is the consequence of a division event at the primitive streak stage of the human embryonic development, about 13–14 days after fertilization, in monochorionic monoamniotic gestations [5] (Figs. 11.1 and 11.2). There seems to be no association with maternal age, race, parity, or heredity and the risk of recurrence is negligible [6].

The risk factors for conjoined twinning are not yet fully understood. An increase in the incidence of monozygotic twinning occurs in pregnancies after induced ovulation with exogenous gonadotrophins. It also has been reported in pregnancies that occurred within 6 months of stopping oral contraceptives. It has been hypothesized that in these situations, there is an abnormal uterine environment that leads to abnormalities of zygote division, but the mechanism remains unknown [2].

11.2 Epidemiology of Conjoined Twins

Conjoined twins (CT) are a very rare developmental accident of uncertain etiology. The prevalence has been previously estimated to be 1 in 50,000 to 1 in 400,000 births. The process by which monozygotic twins do not fully separate

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Table 11.1 Classification of twinning

(A) Symmetrical twinning
Craniopagus: Joined by the skull, share meninges but rarely the brain surface, and do not include the face and trunk
Cephalopagus: There are two faces and are joined from the top of the head to the umbilicus
Thoracopagus: Are joined face to face from the upper thorax to the upper part of the abdomen and always involve the heart
Omphalopagus: The fusion includes the umbilicus region frequently at the lower thorax, but never the heart
Ischiopagus: The union usually includes the lower abdomen and duplicated fused pelvic bones, and external genitalia and anus are always involved
Parapagus: Are laterally joined, regularly share the pelvis. Varieties of parapagus conjoined twins are parapagus dithoracic (separated thoraces), parapagus dicephalus (one trunk two separate heads), and parapagus diprosopus (one trunk, one head, and two faces)
Pygopagus: Are dorsally fused sharing the perineal and sacrococcygeal areas, have only one anus but two rectums
Rachipagus: Dorsally fused, the defect may involve the dorsolumbar vertebral column and rarely the cervical vertebrae and the occipital bone
(B) Non-symmetrical twinning
Parasitic twinning: One main fetus and a rudimentary second embryological structure
Fetus in Fetu: Fetus in fetu (or foetus in foetu) is a developmental abnormality in which a mass of tissue resembling a fetus forms inside the body

but form CT is not well understood. A worldwide multicenter study, using the International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR) structure, was conducted and included the largest sample of CT ever studied [4]. A total of 383 carefully reviewed sets of CT obtained from 26,138,837 births reported by 21 Clearinghouse Surveillance Programs (SP) were included in the analysis. Total prevalence was 1.47 per 100,000 births (95% CI: 1.32–1.62). Salient findings including an evident variation in prevalence among SPs; a marked variation in the type of pregnancy outcome; a similarity in the proportion of CT types among programs; a significant female predominance in CT, particularly of the thoracopagus type, and a significant male predominance in parapagus and parasitic types; significant differences in prevalence by ethnicity; and an apparent increasing prevalence trend in South American countries. Conjoined twins rarely survive early infancy—approximately 30% dies in utero, 40–60% are stillborn, and 35% survives 1 day [7, 8].

11.3 History of Conjoined Twins (Siamese Twins)

From a historical perspective, ancient cave drawings, ceramics of human conjoined twins and sculptured (Fig. 11.3), as well as their demonstration in arts concerning conjoined twins in animals are indicative of the reflection of humans concerning these malformations [9, 10]. In early ages, the birth of a conjoined twin was seen as an inauspicious sign of impending disaster [8]. The early speculation on the biological basis of conjoined twins started late as in the eighteenth and early twentieth century, the beginning of descriptive teratology [11]. *Chang and Eng Bunker* (1811–1874), *Thai* brothers born in Siam, now *Thailand*, traveled widely for many years and became famous as "The Siamese Twins" (Fig. 11.4). Chang and Eng were joined at the torso by a band of flesh, cartilage, and their fused livers. In modern times, they could have been easily separated. Due to the brothers' fame and the rarity of the condition, the term "Siamese twins" came to be used as a *synonym* for conjoined twins.

Many embryological theories are extrapolated by reasoning backward from late phenotypical stages to early embryological development [12, 13]. Teratology as a defined, modern science has existed for about 60 years; however, human interest in congenital malformations and their possible causes reaches back over many millennia [14]. If "teratology" is defined as the scientific study of the causes, mechanisms, and manifestations of congenital malformations, the words "scientific," "causes," and "mechanisms" carry contextual meanings that are strongly influenced by the time period in which they are applied. People of a given era interpret their observations based on the contemporary state of knowledge or understanding of the physical world, contemporary philosophical ideologies, and, importantly, the religious beliefs of the period. The recent state of scientific knowledge leads to a better insight into embryological pathways, but it must be stated,

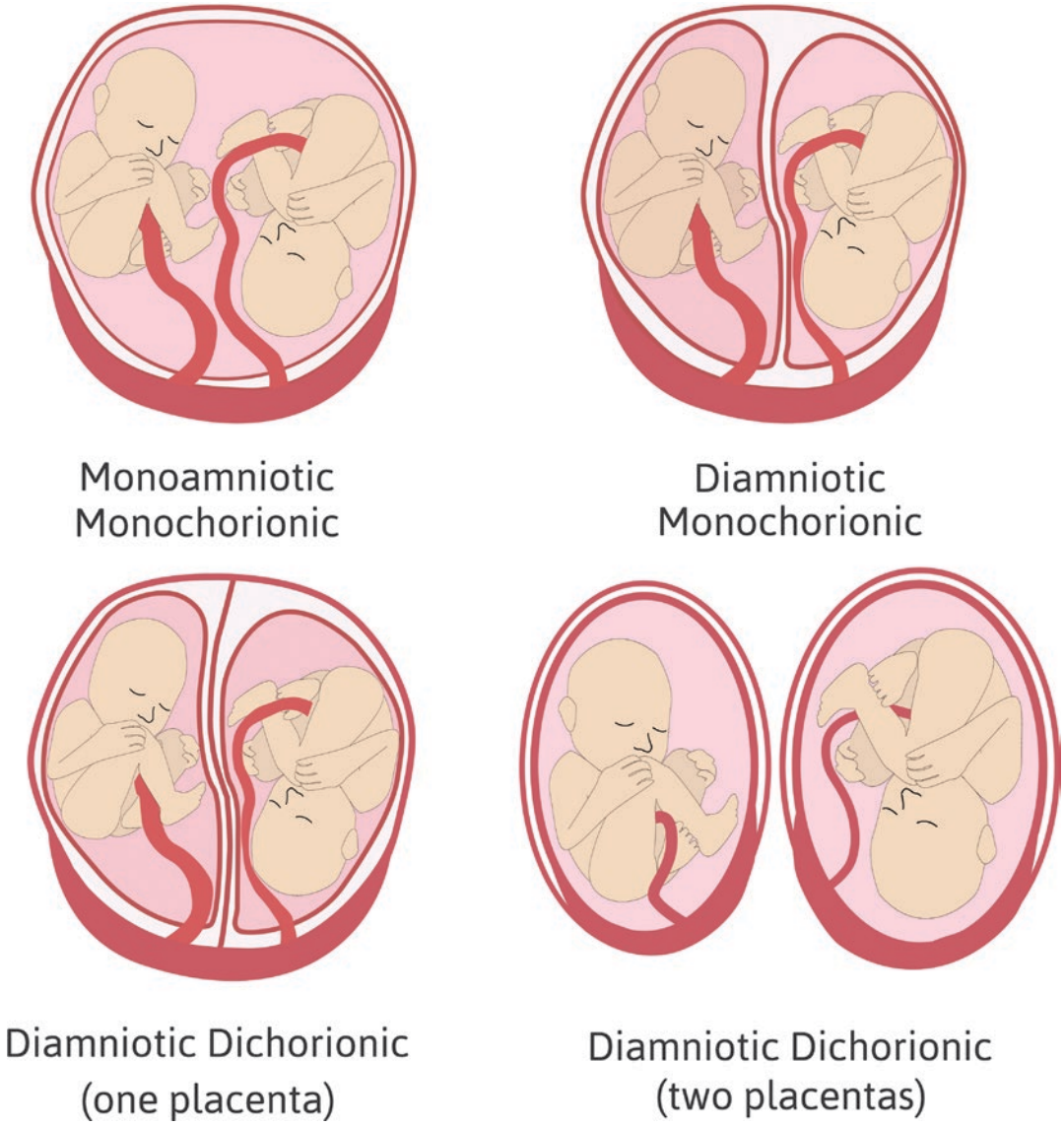

Fig. 11.1 Possibilities of twin development concerning amnion and chorion configuration. *Source: Reprinted from Betty Ray/Shutterstock.com with permission*

that even now, the biological basis of conjoined twinning remains not clarified.

11.4 Types of Conjoined Twins

The first discrimination in conjoined twins is the fact that some are symmetrical and others are not. The latter are characterized by gross underdevelopment of one of the twin members, presenting as "parasites" (also labeled "heteropagi" [15]) or fetus in fetu. It is important to note that the biology of parasitic twins differs from symmetric twins and is of possibly heterogeneous nature. Fetus in fetu (or foetus in foetu) is a *developmental abnormality* in which a mass of tissue resembling a *fetus* forms inside the body. There are two theories of origin concerning "fetus in fetu." One theory is that the mass begins as a normal fetus but becomes enveloped inside its *twin*. The other theory is that the mass is a highly developed *teratoma*. "Fetus in fetu" is estimated to occur in 1 in

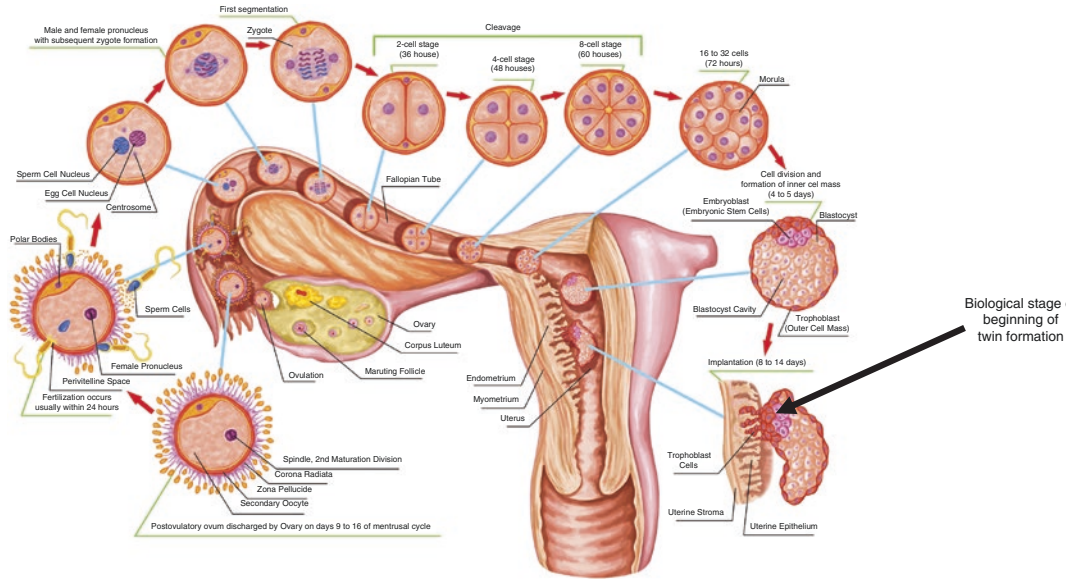

Fig. 11.2 Time frame and developmental stage of fetal development, at the critical time of conjoined twinning formation. *Source: Reprinted from stihii/Shutterstock.com with permission*

Fig. 11.3 Ancient sculpture of conjoined twins https://upload.wikimedia.org/wikipedia/commons/a/ad/Conjoinedtwinslarcomuseum.jpg. *Source: Reprinted from Conjoinedtwinslarcomuseum/wikimedia.org with permission*

Fig. 11.4 The twins Chang and Eng Bunker from Siam (now Thailand) were well known all over the world. They are the basis that conjoined twins became synonymous with the label Siamese twins http://www.lib.unc.edu/ncc/gallery/twins.html. *Source: Reprinted from Catherine Munro/wikipedia.org with permission*

500,000 live births. A fetus in fetu can be considered alive, but only in the sense that its component tissues have not yet died or been eliminated. Thus, the life of a fetus in fetu is akin to that of a tumor in that its cells remain viable by way of normal metabolic activity.

Beneath the most commonly used classification according to the anatomical attachment sites (thorax (thoracopagus), abdomen (omphalopagus), sacrum (pygopagus), pelvis (ischiopagus), skull (craniopagus), face (cephalopagus), or back (rachipagus)), other classifications divide

symmetric conjoined twins according to their orientation of attachment into four general conjunction groups: ventral, lateral, caudal, and dorsal conjunction. In these four groups, 11 more or less well-defined entities can be discriminated [3]. However, many conjunction types show overlapping lateroventral, laterocaudal, and intermediate conjunction patterns, ultimately creating a divergent variability and heterogeneous phenotypical spectrum of conjunction, indicating a continuum between the different types of twins [16].

11.5 Types of Twinning in the Craniofacial Region

In the craniofacial region three different sub-terms (Fig. 11.5) are used as various tissues and organ fusions are seen in the head and neck region. When the skull is mainly involved, they are termed craniopagus, whereas if the face is mainly involved they are termed cephalopagus or facial duplication (diprosopus). If the twinning is asymmetric, they are labeled parasites. There is no precise margin in between these groups. Conjoined twins are known to result from aberrant embryogenesis. Diprosopus, or partial facial duplication, is a very rare congenital abnormality, even in the group of conjoined twins. Diprosopus, a Greek term meaning duplication of face, is conceptualized as a craniofacial duplication with normal trunk and limbs. The earliest description of diprosopus is credited to Ambroise Pare of the sixteenth century. Whereas the underlying biological basis of most craniofacial malformations is well understood, the etiology and pathogenesis of (conjoined) twinning like cranio-facial duplication is rare and enigmatic. This disease entity has about 35 reported cases in the literature [17–20]. It is a rare form of conjoined twins with a reported incidence of 1 case in 180,000 to 15 million births. Advanced maternal age, polyhydramnios, and consanguineous marriage are considered high-risk factors for diprosopus. This extremely rare sub-form of craniofacial malformation gives insight and speculation on this disease development. Partial facial duplication may be symmetric or not and may involve the nose, the maxilla, the mandible, the palate, the tongue, and the mouth. Craniopagus parasiticus (CP) is a rare type of malformation of conjoined twins, with one degenerated or underdeveloped parasite twin united at the cranium with the other fully developed twin. Only a handful of cases have been documented in the literature to date. The incidence of this rare deformity is approximately 4–6 out of every 10,000,000 live births.

Fig. 11.5 Types of craniofacial twinning: craniopagus, cephalopagus, and parasites Left: http://www.lamazmorradelogrotesco.com/2010/10/anomalias-extranas-craniopagus.html Middle: http://www.beloit.edu/~nurember/book/images/Miscellaneous/ Right: http://thehumanmarvels.com/28/the-two-headed-boy-of-bengal/parasitic-twins. *Source: Reprinted from Left: SK Hasan Ali/ Shutterstock.com Middle: unknown artist/ beloit.edu Right: unknown artist/ thehumanmarvels.com with permission*

11.6 Biology of Conjoined Twinning

Whereas the underlying biological basis of most craniofacial malformations is well understood, the etiology and pathogenesis of conjoined twinning remains enigmatic. Normal human pregnancy will lead to a single offspring. Therefore, craniofacial twinning has to be considered a congenital anomaly [21].

Conjoined twins develop from monoamniotic monochorionic pregnancies. Monoamniotic twin pregnancies are necessarily monochorionic and are defined by the development of two fetuses in a single amniotic cavity (Fig. 11.1). This pregnancy is the result of a division of the egg between the eighth and 13th day after fertilization. They are identical *twins* that share the same *amniotic sac* within their mother's *uterus*. Monoamniotic twins are always identical, are always *monochorionic,* and are usually termed monoamniotic-monochorionic ("MoMo" or "Mono Mono") twins. They share the *placenta*, but have two separate *umbilical cords*. Monoamniotic twins develop when an embryo does not split until after formation of the amniotic sac, at about 9–13 days after *fertilization*. Monoamniotic triplets or other monoamniotic multiples are possible, but extremely rare. Other obscure possibilities include multiple sets where monoamniotic twins are part of a larger gestation such as triplets, quadruplets, or more.

Regarding the mechanism of conjoined twinning, there are currently two postulates: partial fission and secondary fusion. The fission theory suggests that all types of monozygotic twins and conjoined twins are entities in a single etiopathogenetic continuum [2]. In contrast to the fission theory, the fusion theory—predominantly embraced in current research papers—suggests that conjoined twins result from two, initially separate monozygotic embryos, which coalesce and become secondarily and homologously fused [22]. This fusion theory was espoused by Spencer [3] and is a widely accepted theory, cited in a lot of papers on this topic. Spencer proposed that conjoined twins originate when the inner cell mass divides (implying an early fission) during the first week after fertilization into two separate monozygotic embryonic primordia staying close enough together to share either the amniotic cavity or the yolk sac. When these embryos continue their rapid growth, they might come in contact with one another and become reunited to result either in ventrally, laterally, caudally, or dorsally conjoined twins.

The etiopathogenesis of conjoined twins remains a matter of ongoing debate and is currently cited as partial fission or secondary fusion, but it appears both the fission and fusion theories cannot be applied to the full range of conjunction possibilities and thus remain a matter of persistent inconclusiveness. In addition to the fission and fusion theories, a third conjecture to explain conjoined twins may be the initial "crowding and thereby duplication of morphogenetic potent primordia" [23–25]. Whereas the underlying biological mechanism is not fully understood, and as different mechanisms may lead to conjoined twinning, the time frame of the initiation of this developmental disorder is known (Fig. 11.6).

Boer et al. [5] (2019) rejected in an actual review paper both the fusion and the fission theories as causative explanations. The authors proposed that initial duplication of axially located morphogenetic potent primordia in one inner cell mass of the blastocyst (Fig. 11.7) is the initiating factor in the genesis of non-dorsally conjoined twins. Moreover, they mentioned that such a mechanism seems to be responsible for separate twinning, in which they assumed that the initial reciprocal distance between the axial primordia seems to be large enough to prevent mutual developmental interference from occurring.

11.7 Biology of Facial Duplication

Diprosopus as an extremely rare form of craniofacial malformation presents with duplication of face which may be partial or complete. There are different classifications of this rare form of malformation.

In 1982, Barr [26] classified duplication into three main forms:

I. Duplication of the eyes and nose with or without maxillary duplication by itself or with mandible duplication.
II. Duplication of the nose with or without maxillary duplication.

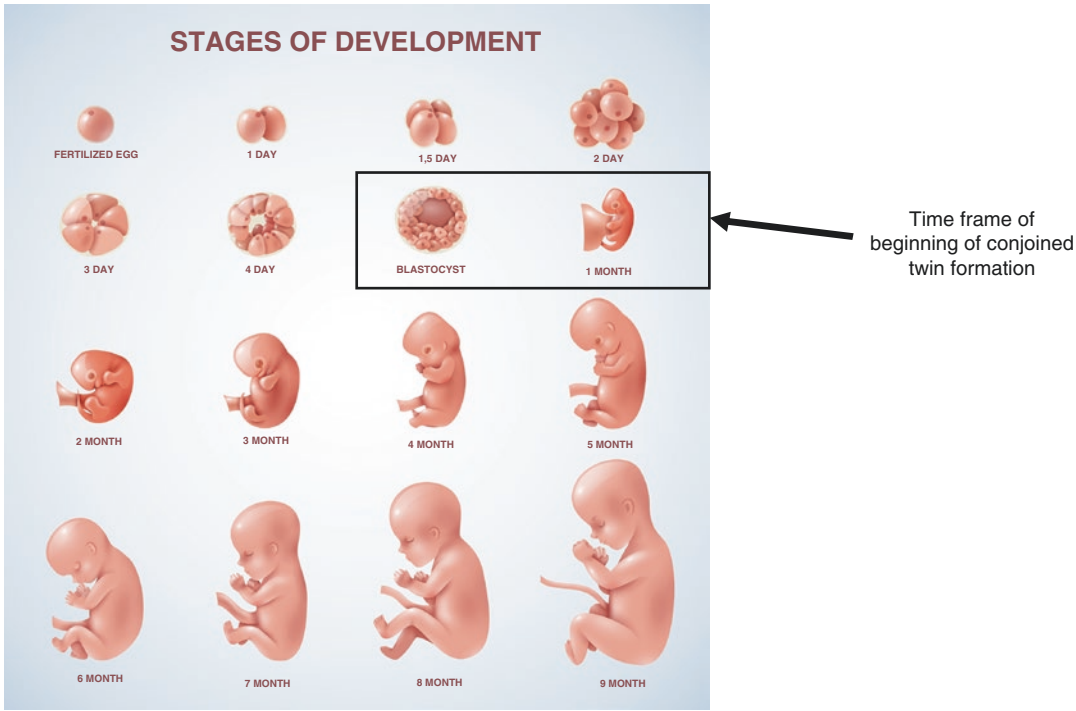

Fig. 11.6 The time frame of the development of conjoined twinning during pregnancy is well known. *Source: Reprinted from Macrovector/Shutterstock.com with permission*

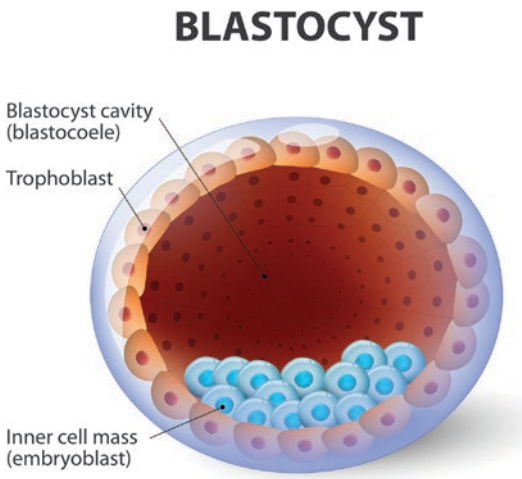

Fig. 11.7 Histology of normal blastocyst. *Source: Reprinted from Designua/Shutterstock.com with permission*

III. Duplication of the maxilla with or without mandible or pituitary duplication.

He further described pituitary duplication in isolation but was uncertain regarding the existence of isolated mandibular duplication.

Gorlin [27] created later (1990) a classification scheme with an emphasis on oral duplication:

I. A single mouth with duplication of the maxillary arch.
II. A supernumerary mouth laterally placed with a rudimentary mandible.
III. A single mouth with replication of mandibular segments.
IV. Diprosopus with or without anencephaly.

In the rare case of the development of facial duplication (diprosopus), different possible mechanisms have been proposed [18, 20]. One possible mechanism is the cranial bifurcation of the notochord during neurulation (Fig. 11.8). The bifurcation causes two vertebral axes and neural plates to develop alongside each other. Another proposal is an increase in the expression of the protein sonic hedgehog, which is essential for craniofacial patterning during development [28]. The exact etiology of the condition is unknown. Various mechanisms have been proposed, but the

most accepted one is due to the abnormality of sonic hedgehog genes and protein (Shh). Shh protein and corresponding genes are responsible for signaling and patterning of craniofacial structure. It also organizes the embryonic cells to specific areas, which later develops into specialized organs. In the brain, absence of Shh protein leads to holoprosencephaly and failure to move optic disc leads to cyclopia. If the activity of protein is increased, it leads to duplication of organs leading to diprosopus. Few authors also feel that the anomaly is due to the fusion of the parallel notochord in close proximation or fission of single notochord because of the abnormality of Dix homeobox gene. But till date, no genetic abnormality has been recorded with diprosopus.

11.8 Early Diagnostics of Conjoined Twins

Prenatal diagnosis using ultrasonography, computed tomography (CT) scan, and magnetic resonance imaging (MRI) is possible. Most conjoined twins are detected in the pre-natal period by ultrasound [29]. Ultrasound has revolutionized the management of multiple pregnancies and their complications (Fig. 11.9). Increasing

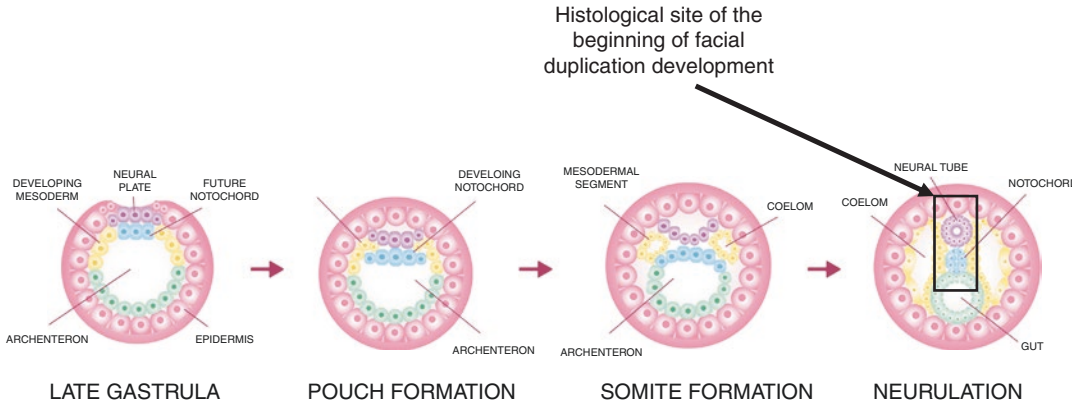

Fig. 11.8 Histology of facial development. Disturbance of the normal notochord formation during neurulation through cranial bifurcation is a proposed mechanism of facial duplication. *Source: Reprinted from Betty Ray/Shutterstock.com with permission*

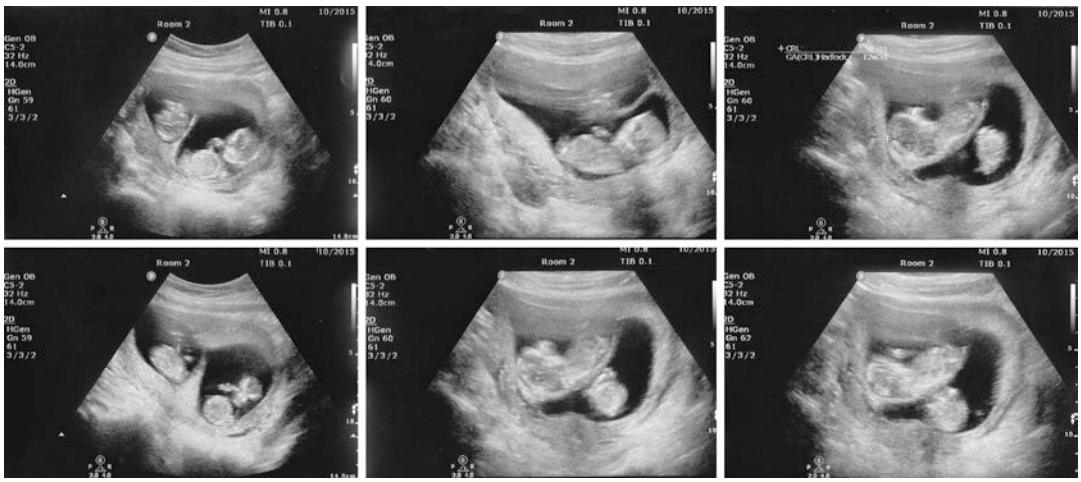

Fig. 11.9 Ultrasound investigations are the primary mode of twin pregnancy analysis. Modern 3D ultrasound machines enable a precise documentation of facial structures. *Source: Reprinted from Semmick Photo/Shutterstock.com with permission*

frequency of twin pregnancies mandates therefore familiarity of all clinicians with the relevant pathologies and evidence-based surveillance and management protocols for their care. Such twins can often at this stage be classified according to the most prominent site of connection: the thorax (thoracopagus), abdomen (omphalopagus), sacrum (pygopagus), pelvis (ischiopagus), skull (craniopagus), face (cephalopagus), or back (rachipagus). The first trimester (11–13 w + 6 days) ultrasound is the best method for diagnosing conjoined twins early in pregnancy. Given the high risk of preterm delivery in twins, accurate first trimester dating is important in later management of the pregnancy. After dating and determination of the diagnosis of multiple pregnancy, the most important additional information to determine is the precise number of fetuses and the chorionicity (number of placentae) and amnionicity (number of amniotic sacs) of the pregnancy. While the majority (>80%) of twin pregnancies are dichorionic, monochorionic pregnancies are associated with worse perinatal outcomes, are affected by several conditions specific to twins sharing a placental circulation, and require significantly more antenatal surveillance. As the rarest complication of monochorionic pregnancy is conjoined twinning, a condition resulting from very late splitting of the blastocyst and occurring in only 1% of monochorionic twin pregnancies, careful ultrasound diagnosis with high resolution devices is of special relevance [29–33]. The diagnosis remains often easy, even if some of the congenital abnormalities cannot be seen at these gestational stages. Increased nuchal translucency is common, even in fetuses with two independent hearts and no cardiac congenital abnormalities in the embryopathological study. The early diagnosis of this condition is mandatory to allow an early information of parents. Advances in ultrasound mean in consequence that conjoined twins are most commonly identified in the first trimester when many parents will opt for termination of pregnancy in view of the high risk of morbidity and mortality in an ongoing pregnancy. In families choosing to continue pregnancies, around 25% would be expected to survive to discharge and almost all with significant morbidity. The prognosis is ultimately determined by the degree and site of the junction between the twins, and therefore detailed ultrasound studies are necessary to fully explore the nature of the connections between the twin pair. The most common site of union is at the thorax with the twins facing each other, and bowels, liver, and hearts may be shared. Mapping blood vessels and structures can help plan postnatal surgery.

For more detail, an MRI investigation is useful even in the prenatal period. In the postnatal period it is important to get insight into the anatomical extent of twinning. Additionally, when separation is planned, planning of the surgical strategy is aided by accurate preoperative imaging. The area of fusion largely determines the imaging modalities used. Thoracic conjunction is most common and requires cardiac assessment. Magnetic resonance imaging and computed tomography provide excellent anatomic and bone detail, demonstrating organ position, shared structures, and limited vascular anatomy in the craniofacial region. Contrast material radiography allows evaluation of the gastrointestinal and urogenital tracts, and a shared liver requires assessment of anatomy, vascularization, and biliary drainage. Angiography helps define specific vascular supply, which is useful in determining the distribution of shared structures between the twins at surgery. Each set of conjoined twins is unique. An imaging strategy to accurately define anatomic fusion, vascular anomalies, and other associated abnormalities is important for prognostic information and surgical planning.

However, new cases should be critically evaluated not only with radiological imaging but also by genetic diagnostics [34]. Surgery to separate conjoined twins may range from very easy to very difficult depending on the point of attachment and the internal parts that are shared. Most cases of separation are extremely risky and life threatening. In many cases, the surgery

results in the death of one or both of the twins, particularly if they are joined at the head or share a vital organ.

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12 Biological Basis of Craniofacial Soft Tissue Malformations

Kai Wermker

12.1 Introduction

Soft tissue malformations in the head and neck region are a heterogeneous group of pathologic findings involving different tissues like skin, vessels and muscles. The malformations can involve only one defined part of craniofacial tissue, e.g. congenital nevi which only involves the skin, or they can affect multiple tissues, e.g. vascular malformations like haemangioma that can be found in superficial soft tissues (like the skin) and deeper tissues like muscle, fascia or even bone. The malformations are mainly hereditary, but some are also discussed developing spontaneously. This chapter focuses on the aetiology and epidemiology of these malformations and their biological basis, including some aspects concerning diagnostics.

A rough classification can be made by differentiating the so-called phakomatoses, nevoid skin malformations and vascular malformations not related to phakomatoses.

12.2 Phakomatoses

Several neuro-oculo-cutaneous syndromes are grouped together as so-called phakomatoses, affecting in various degrees the craniofacial soft tissues and sometimes also the hard tissues. The neurocutaneous disorders are linked to structures derived from the embryogenic ectoderm, whereas to various amounts also mesodermal and endodermal tissues also may be involved. Often the central nervous system, the skin and the eyes are involved, classifying them as multisystem disorders. Genetic and acquired phakomatoses can occur in the craniofacial area, and the clinical extent and severity vary considerably.

Due to the involvement of multiple organs and systems, diagnosis in patients has to consider various disciplines and aspects. In a multidisciplinary approach, clinical examination should be performed in the fields of paediatrics, neurology, ophthalmology, dermatology, dentistry and oral and maxillofacial surgery, and internal medicine. Complete imaging of the cranium, head and neck region, thorax and abdomen should be performed using MRI (magnetic resonance imaging) and/or CT (computed tomography) scans. If a phakomatosis is suspected or even confirmed, the patient and his family should be referred to human geneticist.

The following syndromes and disorders with affection and involvement of soft tissues of the head and neck are attributed to this group: the nevoid basal cell carcinoma syndrome (NBCCS, Gorlin-Goltz syndrome), neurofibromatosis (types I (NF1, Recklinghausen disease) and II), Sturge-Weber syndrome, von Hippel-Lindau disease, ataxia telangiectasia, incontinentia pigmenti,

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tuberous sclerosis and Wyburn-Mason syndrome. In the following, disorders with relevant pathology in the craniofacial region are described in more detail.

12.2.1 Nevoid Basal Cell Carcinoma Syndrome (NBCCS, Gorlin-Goltz Syndrome)

The nevoid basal cell carcinoma syndrome (NBCCS) was first described in 1960 by Gorlin and Goltz and is therefore synonymously known as Gorlin-Goltz syndrome. Its prevalence is 1 in 56,000 to 164,000 with higher frequency in Australia. NBCCS is inherited in an autosomal dominant fashion (70–80% of all NBCCS cases), but 20–30% of new Gorlin-Goltz patients are caused by spontaneous de novo mutations. Underlying genetic mechanisms are mutations in the PTCH1 (patched) gene on chromosome 9q. PTCH1 as tumour suppressor gene encodes for a transmembrane receptor protein of the sonic hedgehog family. This protein is involved in cell regulation and cell growth, and its (homozygous) inactivation increases the risk of tumorigenesis. The highest risk of tumour development is given for cutaneous malignancies, especially basal cell carcinoma (BCC)—leading to the name of this syndrome. According to the "two-hit" theory for tumour suppressor genes, NBCCS patients with one (inherited) defect in the PTCH1 gene have the disorder, and a second mutation (e.g. caused by ultraviolet light (UV) through sun exposure or ionizing radiation like X-rays) leads to full expressivity and development of a neoplasm. Depending on the affected tissue, different tumour entities are possible [1–4].

Leading clinical and radiographic symptoms and signs of NBCCS are multiple non-melanoma skin cancers (NMSC, especially basal cell carcinoma (BCC; see Fig. 12.1), details below), palmar and plantar pits, a distinct facial appearance (characterized by macrocephaly with frontal and temporal edge configuration, broadened root of the nose and hypertelorism, and prognathism of the mandible with the appearance of progenia and dental Angle class III), development of often multiple odontogenic keratocysts (formerly also known as keratocystic odontogenic tumour; see Fig. 12.2) in the lower and upper jaw in three of four NBCCS patients, intracranial pathologies like early calcification of the falx cerebri, bridging of the sella turcica and in up to 10% formation of medulloblastoma, skeletal disfigurements like bifid ribs and scoliosis and occurrence of other tumours (bilateral ovarian fibromas, cardiac fibromas) [5–8].

Table 12.1 gives an overview of major and minor criteria of NBCCS. NBCCS is confirmed if a patient shows two major criteria or one major and two minor criteria [9].

Skin tumours in NBCCS are by far most often BCCs. The number and extent of BCC increase

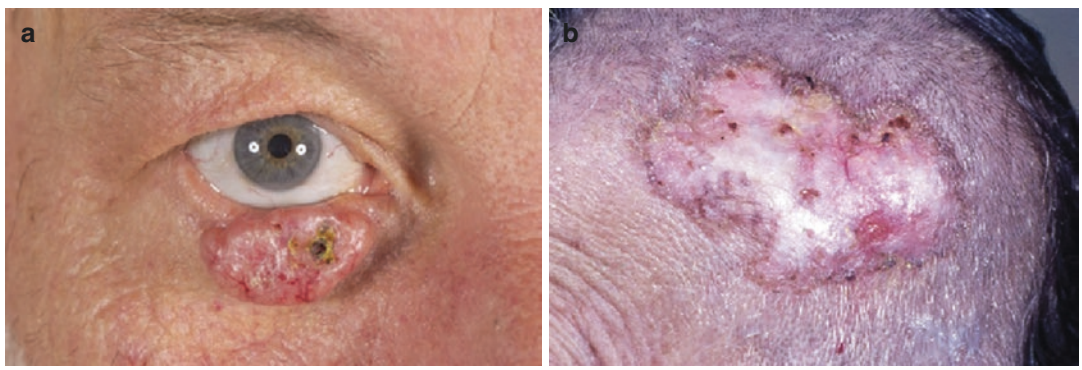


Fig. 12.1 (**a**, **b**) Different types of facial basal cell carcinoma (BCC). Nodular type of basal cell carcinoma (BCC), localization at the lower eyelid (**a**) and sclerodermiform type of BCC (**b**), localized at the lateral forehead and temporal

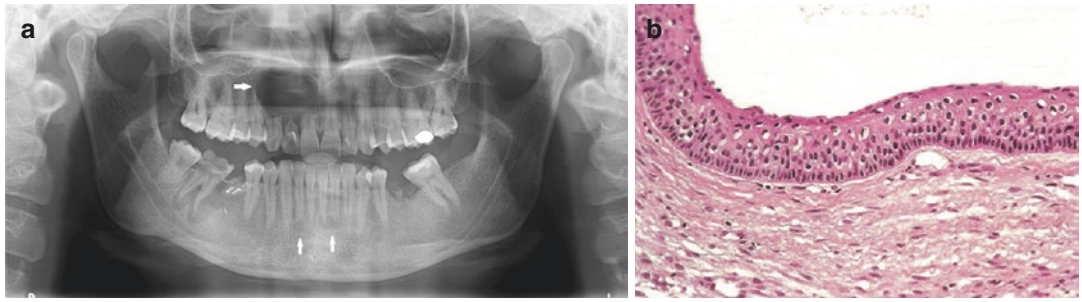


Fig. 12.2 (**a**, **b**) Keratocystic odontogenic tumours (KOT, keratocysts). X-ray (orthopantomogram) of a 26-year-old woman with three keratocystic lesions of the jaw (**a**, white arrows): one major keratocyst of the upper right jaw and two minor lesions in the anterior mandible. (**b**) Histologic aspects of KOT/keratocysts (HE, 100x)

Table 12.1 Diagnostic criteria in nevoid basal cell carcinoma syndrome (NBCCS, Gorlin-Goltz syndrome)

Region/ organ	Major criteria	Minor criteria
Skin	More than 2 BCCs or 1 BCC in patients <20 years; 3 or more palmar or plantar pits	
Craniofacial/ jaws	Odontogenic keratocysts	Macrocephaly; congenital malformations like CLAP, frontal bossing, eye anomaly (cataract, coloboma, etc.); hypertelorism
Intracranial	Ectopic calcification or calcification of the falx cerebri in patients <20 years	Bridging of the Sella turcica
Skeleton	Rib deformity (bifid, fused or splayed)	Other skeletal deformities: Sprengel deformity, pectus excavatum, poly- or syndactyly; vertebral anomalies
Other tumours		Ovarian fibroma, cardiac fibroma
Genetics	Family history (first-degree relative with NBCCS)	

NBCCS nevoid basal cell carcinoma syndrome, *BCC* basal cell carcinoma, *CLP* cleft lip, alveolus and palate

typically with age, but even younger patients can show BCC. BCC risk is increased in areas with high UV exposure like the face, but in Gorlin-Goltz syndrome patients NMSC also can occur in regions with very low sun exposure (e.g. palmar and plantar). BCC is a local aggressive form of skin cancer, leading to destruction of local tissues if not treated properly, but usually metastasization does not occur. The more aggressive entities of NMSC also found in NBCCS patients sometimes are metatypical BCC and basosquamous carcinoma (BSC), both of which are able to disseminate and the latter one showing histologic and biologic characteristics of cSCC (Fig. 12.3).

12.2.2 Neurofibromatosis (NF)

Three types of disorders, which are characterized by the development of benign tumours in the nervous system, are denominated as neurofibromatosis types 1 and 2 and schwannomatosis [10–12].

Neurofibromatosis type 1 (NF-1, syn.: Recklinghausen disease) is the most common NF type with a prevalence of approximately 1–3500 in Western countries. NF1 is characterized by the development of nerve sheath tumours—neurofibromas—along the nerves and in the skin in all parts of the body. It is caused by microdeletion and mutations in the NF-1 gene on chromosome 17q11.2, encoding for neurofibromin which is part of the RAS oncogene pathway. Through disturbance of cell signalling, tumour development is enhanced especially in nervous tissue. Inheritance is autosomal dominant, but up to 50% of all NF-1 cases can be attributed to spontaneous mutations. NF-1 is a progressive disease with first signs and symptoms starting in early childhood and aggra-

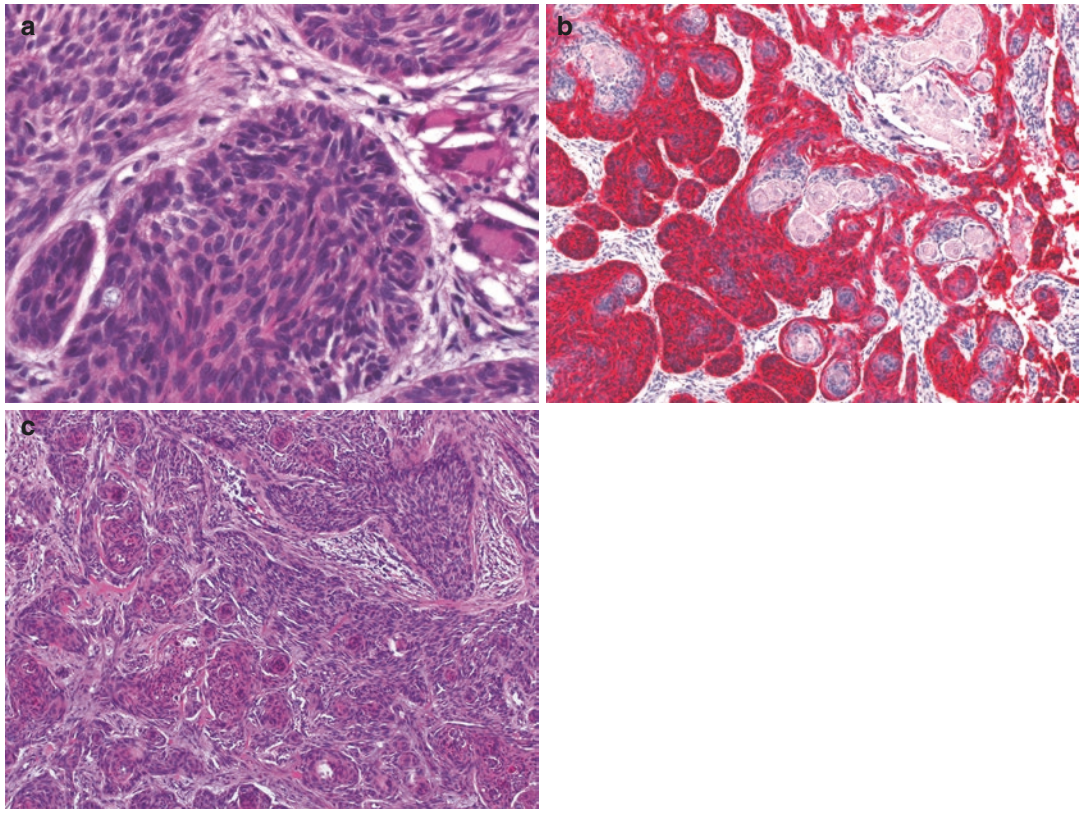


Fig. 12.3 (**a**, **b**, **c**) Histologic characteristics of basosquamous carcinoma (BSC). In conventional histology (**a**, HE 400×), BSC shows nuclear pleomorphism, with many mitotic cells showing atypical mitosis, and a loss of palisades, which are usually typical for BCC (basal cell carcinoma). Immunohistochemical staining with BerEP4 (**b**, BerEP4 100x) illustrates irregular mixture of BCC typical areas (red) and dedifferentiated areas with more similarities to squamous cell carcinoma (SCC). Conventional histology of the same tumour (**c**, HE 100x) depicts the aforementioned BSC characteristics

vates in later age and adolescence. Nevertheless, if no complications (e.g. severe nerve compression symptoms) or malignant tumours (e.g. switch from benign neurofibroma to malignant nerve sheath tumour/malignant schwannoma) occur, life expectancy is normal. A chief complaint of NF-1 patients is disfigurement (see clinical signs and symptoms below). Due to a penetrance that varies tremendously (from only few mild symptoms without any impact on normal life up to severe rapidly progressive course), prognosis is difficult and depends on the individual situation [13, 14].

Pathognomonic symptoms are so-called "café au lait" macules that can be found even in newborn and grow during life—often increasing in size and number during hormonal changes (puberty, gravidity). The lesions are flat and of brown colour with smooth or irregular borders. Furthermore, benign neurofibroma of the skin is typical. These nodules vary in size and number and can grow up to large tumours (progredient with time), leading to disfigurement and functional problems or even damage of the adjacent tissue and organs. The neurofibromas can be subdivided into plexiform types (which encase or infiltrate the nerves and blood vessel and reach into deeper tissues), solitary neurofibromas (located at deep nerve trunks) and schwannomas (benign nerve sheath tumour). The latter can turn into malignant tumours with a lifetime risk of 8–12%. The neurofibromas can occur at every

site and of course also in the craniofacial region (in the skin and also intraorally in the mucosa), and deeper tissues can be involved [15, 16]. Figure 12.4 shows a patient with NF-1, who developed multiple extended neurofibromas of the upper jaw, left midface and orbit—requiring partial maxillectomy and midfacial tumour resection including exenteration of the orbit—rehabilitated with a facial prosthesis. Another skin anomaly is axillar or inguinal freckling. In the ophthalmologic field, the so-called Lisch nodules (accumulation of dendritic melanocytes in the iris) and gliomas at the optic nerve or chiasma opticum can occur. Bony symptoms can involve the extremities (bowing and increased fracture risk with compromised bone healing and increased risk for pseudarthrosis, unilateral accelerated growth), scoliosis and also craniofacial bones (sphenoid dysplasia, defects in the area of the maboid suture). Furthermore, in some NF-1 patients noticeable problems in the neurobehavioural and musculoskeletal field were described: motor deficits, attention-deficit/hyperactivity disorder, autism, epilepsy and muscle weakness [17].

NF-1 is secured with the following clinical diagnostic criteria: at least six café au lait macules, at least two neurofibromas or at least two Lisch nodules [18].

Neurofibromatosis type 2 (NF-2) is characterized by the development of acoustic neuromas (vestibular schwannoma, VS), which are benign tumours of the nerve sheath of the eighth cranial nerve (N. vestibularis). In a relevant proportion of NF-2 patients, this kind of intracerebral tumour occurs bilaterally, and furthermore, also other

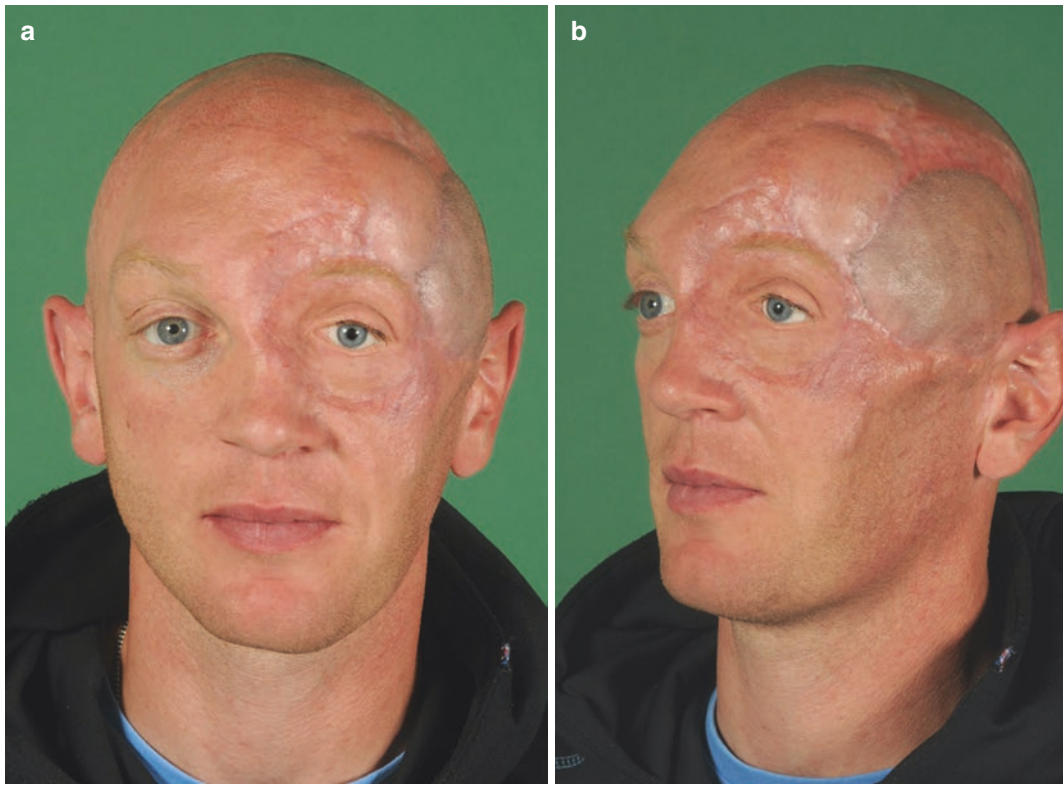


Fig. 12.4 (**a**, **b**) Patient with neurofibromatosis type 1 (NF-1, Recklinghausen disease). Adult patient who suffered from multiple neurofibromas of the left face and orbit, leading to multiple resective and reconstructive operations including exenteration of the left orbit due to compression syndromes and growth intracranially. (**a**) Remarkable is the asymmetry also in the midface due to subcutaneous and deeper neurofibroma growth. Rehabilitation was achieved by multiple steps of reconstructive flap surgery and insertion of craniofacial orbital implants to retain the facial prosthesis (**b**)

benign brain and spinal neoplasias were observed. Diagnosis is secured bilateral VS, unilateral VS and NF-2 family history or two other benign intracranial tumours. Clinical symptoms are often correlated to localization and extent of the VS and include hearing loss, headache, dizziness and imbalance and—more often as sequelae of neurosurgery than spontaneously—facial paralysis. Typical craniofacial disorders or other clinical signs are not obvious in contrast to NF-1. An early onset form (Wishart phenotype) in patients younger than 20 years of age with multiple cerebral and spinal involvement and rapid growth can be distinguished from a mild form (Feiling-Gardner phenotype) characterized by later onset after 20 years of age and single and slow tumour progression. NF-2 is a rare (incidence is 1 to 60,000) disorder, caused partly by autosomal dominant inheritance and partly by spontaneous mutation. The relevant gene locus is on chromosome 22q12.2, and in pathogenesis the proteins merlin and schwannomin as structural parts of the neuronal actin cytoskeleton (especially in the nerve sheath) play a crucial role [19–21].

Schwannomatosis is extremely rare (estimated incidence is 1–1,700,000) and classic typical signs (hallmarks) of neurofibromatosis are missing. This subtype of NF leads to multiple cutaneous peripheral, intracerebral and spinal schwannomas and consecutive neurological problems. Chief complaints of these patients are pain syndromes, caused by the schwannomas, and nerve compression syndromes. Sometimes numbness, tingling and weakness also occur. In contrast to NF-1 and NF-2 with their proven genetic background, in schwannomatosis a mutation in the tumour suppressor gene SMARCB1 is found in many cases, but not all, leading to an assumption that probably another gene may be affected. Located also on chromosome 22, SMARCB1 is involved in cell cycle regulation, differentiation and growth [11, 12, 22].

12.2.3 Sturge-Weber Syndrome (SWS)

With a prevalence of 1 in 50,000 births, the SWS belongs to the rare diseases. SWS is not inherited, it is caused by sporadic somatic mosaic mutations in the GNAQ gene, leading to errors in meso- and ectodermal tissues and impairment in blood vessel and neuronal network development especially in the head region. In the craniofacial area, facial angioma—being obvious as port-wine stain—is a pathognomonic sign. Typically this birthmark of reddish colour—reaching from light pink to deep purple—is located unilateral in the forehead and upper eyelid (area of the *N. ophthalmicus*, the first branch of the trigeminal nerve (N V1)) or can even extend up to over the whole face. Histologically, an increase and multiplication of capillaries is evident, giving the figure of a capillary haemangioma. This overabundance of capillaries can occur also intracranial as ipsilateral leptomeningeal angioma (cerebral malformations in the pia mater) or cerebral blood flow anomalies in SWS. This can in consequence presuppose calcifications and decline of cortical neuronal cells. Other symptoms are glaucoma, seizures and intellectual disability [23, 24].

SWS can be differentiated into three types. Type 1 is the most common and characterized by facial and leptomeningeal angiomas, glaucoma or choroidal disorders (dilated bulbar vessels). In Type 2 facial haemangioma with port-wine stains is found and sometimes glaucoma, but intracranial tissues are not affected. Type 3 can only be diagnosed by imaging (CT or MRI) because solely intracranial leptomeningeal affection is present, facial signs are absent and glaucoma is rare [25–28].

Clinically, up to 90% of all SWS patients develop seizures, 40–60% headache and delayed cognitive development, 30–70% glaucoma and nearly the half hemianopsia and hemiparesis. Ophthalmic and neurological symptoms can aggravate with age.

12.2.4 von Hippel-Lindau Disease (VHL)

Caused by a mutation on chromosome 3p25.3 in the so-called von Hippel-Lindau tumour suppressor gene, VHL is a phakomatosis with high penetrance involving multiple organs. Both germline mutations with autosomal dominant inheritance

and sporadic somatic mutations were described. VHL genes are related to HIF1-alpha (HIF = hypoxia-inducible factor) and also in part to HIF1-beta. Both are involved in the regulation of intracellular oxygen level, and disturbances are discussed to be related to development of benign and malignant tumours, even head and neck cancer. For confirmation of VHL disease in patients with VHL history in their relatives, one typical VHL tumour is required; in sporadical cases two tumours are requested to confirm VHL. Incidence of VHL is 1 in 36,000 births [29–32].

Typically patients show at least one or two of the following tumours: hemangioblastoma, pheochromocytoma or renal cell carcinoma; furthermore, angiomatosis, pancreatic cysts (pancreatic serous cystadenoma), endolymphatic sac tumour and bilateral papillary cystadenomas of the epididymis (in men) or broad ligament of the uterus (in women) were described [33–35].

Clinical symptoms are often unspecific like dizziness, headache and hypertonia or can mimic ophthalmological (disturbances in visual perception) or neurological (walking problems, imbalance) diseases. In the craniofacial region, no clinical characteristic symptoms are described, but it is discussed that VHL patients may be at higher risk also for head and neck tumours [36, 37].

12.2.5 Ataxia Telangiectasia (AT)

With a prevalence of 1 in 40,000 to 300,000, ataxia telangiectasia (AT, syn.: Louis-Bar syndrome) is a rare complex of symptoms inherited in an autosomal recessive fashion. It is caused by mutations of the ATM gene (ATM serine/threonine kinase or ataxia–telangiectasia mutated, located on chromosome 11q22.3), resulting in deficiencies in DNA repair and DNA damage response and therefore genomic instability [38, 39].

Due to this pathophysiology, the clinical signs and symptoms affect multiple organs and tissues (multisystem disease). In most cases early onset is seen in childhood when the child begins to walk, showing ataxia and later on oculomotor anomalies. Swallowing problems and speech problems aggravate daily life for AT patients. In the head and face region, scleral telangiectasia (dilated blood vessels), later on telangiectasia and sometimes granulomas in the skin, increased the rate of respiratory infections (sinusitis, middle ear infection, bronchitis and pneumonia), and in adolescence vitiligo in the skin and skin cancers increase in frequency. In general, due to the pathophysiology, AT patients suffer from impaired immune system and have a higher risk to develop cancer, especially lymphomas and leukaemia [40, 41].

Furthermore, delayed development (growth and puberty), diabetes in adolescent AT patients and premature changes (signs of early aging) in hair and skin can be observed. Immune and DNA repair deficiency lead to increased immune-related complications and cancer and furthermore increased radiosensitivity [38, 39].

12.2.6 Incontinentia Pigmenti (IP)

Incontinentia pigmenti (IP) is a rare disorder with high penetrance and is prevalent more often in females than males (ratio 20:1, birth prevalence 0.6–0.7 per 1,000,000) due to X-linked dominant inheritance. Its characteristics are erythema and red blisters of the skin, healing with less elastic skin and grey or brown patches that fade during time. Further signs in the craniofacial region are woolly hair or progressive alopecia (in adolescence) and dental anomalies (hypodontia and in rare cases anodontia, microdontia or abnormally shaped teeth). Eye abnormalities, lined or pitted fingernails and toenails, delayed somatic and/or intellectual development and neurological problems (e.g. seizures) can also be found in IP patients. If males suffer from IP, their prognosis is significantly worse and they often don't survive childhood [42–44].

The genetic background of IP is a mutation in the IKBKG gene, located on chromosome Xq28 and encoding for the NEMO protein. Mutation leads to reduced cell protection against TNF-alpha-induced apoptosis [45, 46].

12.2.7 Tuberous Sclerosis Complex (TSC)

Tuberous sclerosis complex (TSC) is a multisystem disease with a total population prevalence of 7–12 cases per 100,000 (live-birth prevalence 10–16 per 100,000). It is inherited in an autosomal dominant fashion and causes growth of benign tumours in different organs like the brain, skin, kidney, heart, liver, eyes and lung [47].

Symptoms and clinical severity are variable and correlate to prognosis, which is good for most patients having a normal life expectancy. Clinical symptoms occur in the neurological field (seizures and intracranial tumours like astrocytoma, cortical tubers and subependymal nodules), in neuropsychiatry (intellectual and developmental retardation, behavioural abnormalities like attention-deficit/hyperactivity disorders, psychiatric diseases like autism or depressions) and in urology (kidney tumours like hamartomas or angiomyolipomas, which can cause haematuria and can lead to life-threatening bleeding in the case of more than 4 cm in diameter and even minor traumas). In the lungs lymphangioleiomyomatosis can occur and myocardial tumours (rhabdomyomas) can cause cardiac arrhythmia with fainting. In ophthalmic examinations coloboma, retinal astrocytic hamartoma and papilledema related to increased intracranial pressure and hydrocephalus are sometimes found [48].

Nearly all TSC patients (up to 96%) develop skin abnormalities that involve nearly almost the head, face and neck. Typical are the so-called ash leaf spots (hypomelanotic macules) and facial angiofibromas, presenting as reddish spots or knobs/nodules distributed often bilaterally in a "butterfly "fashion on the nose and cheeks. Besides ungual fibromas, in the neck and cephalic area, fibromas, plaques and the so-called shagreen patches (thick leathery and often pigmented skin lesions, whose amount rises with age and located also in the trunk and sometimes the scalp) are depicted in TSC patients in the maxillofacial area [49, 50]. Intraorally fibromas at gingiva, cheek mucosa or tongue and dental enamel pits can be found, especially in adult patients.

In 2012 a Consensus Conference classified in more detail the abovementioned symptoms and TSC signs in the different organs and tissues into major and minor diagnostic criteria [51–53].

Despite its autosomal dominant inheritance, in TSC expressivity is variable and penetrance incomplete; and up to two-third of all TSC cases are assumed to be caused by spontaneous mutations. Two gene loci could be identified: TSC1, located on chromosome 9 q3, encoding for the protein hamartin, and TSC2 on chromosome 16 p13.3, encoding for tuberin. Both are tumour suppressor genes, therefore a second mutation has to occur for tumour development, explaining the variety of TSC symptoms and severity. Hamartin and tuberin are both involved in the control of cell cycle and cell division via mTOR signalling in the growth factor signalling pathway, giving the option of targeted therapy using mTOR inhibitors [54].

12.2.8 Wyburn-Mason Syndrome (WMS)

The Wyburn-Mason syndrome (**syn.: Bonnet-Dechaume-Blanc syndrome**) is a very rare non-hereditary congenital disorder with less than 100 documented cases to date (2019). The syndrome is characterized by arteriovenous malformations (AVMs) in the retina and often in cerebral tissues, and in most cases it is unilateral. The exact cause or a specific genetic background is not known and AVMs begin to develop around the seventh week of gestation during maturation of retinal mesenchymal cells. The syndrome is often diagnosed later in childhood and clinical symptoms and severity can differ significantly. The main symptoms are headache, impairment of vision like hemianopsia, retro-bulbar pain and exophthalmus in later stages [55–57]. In the maxillofacial region, nevi and angiomas of the skin, faint skin discoloration or even high-flow AVMs can occur [58, 59].

12.3 Nevoid and Other Skin Malformations

Nevi of the skin are solely restricted to the epidermis and dermis and deeper tissues are not affected. In general the term "nevus" is used for a skin lesion that is characterized by increased number (neoplasia) or size (hyperplasia) of melanocytes and/or increased amount of melanin (the protein responsible for skin colour and pigmentation), i.e. hypermelanosis, or decreased melanin (hypomelanosis, hypopigmentation).

Congenital nevi (congenital melanocytic nevus with or without hypertrichosis ("animal coat nevus"), nevus of Ito, nevus of Ota) can be differentiated from usually acquired nevi (melanocytic nevus, atypical dysplastic nevus, blue nevus, Spitz nevus, etc.). Another subdivision can be made between epidermal nevi (verrucous, eccrine and apocrine nevus, nevus sebaceous, nevus comedonicus, junctional melanocytic nevus), dermal nevi (intradermal melanocytic nevus, connective tissue nevi like collagenoma and elastoma), nevi involving the epidermis and dermis (e.g. compound type of melanocytic nevus), mucosal nevi (e.g. pigmented lesions in the oral cavity) and vascular nevi (e.g. nevus flammeus). The latter ones are described in Sect. 12.3 "Vascular Malformations". In the following, some of the mentioned nevi are described in more detail.

12.3.1 Congenital Skin Nevi

The most common congenital type of skin nevi is the **congenital melanocytic nevus (CMN)** that can occur in every part of the body and therefore also in the head and face. CMN can vary in form (irregular forms, but usually clearly circumscripted), colour (reaching from light brown and red to dark brown, often irregular within one CMN) and size. Hairiness is variable and sometime is more prominent with thicker and larger hairs after puberty. Figures 12.5 and 12.6 demon-

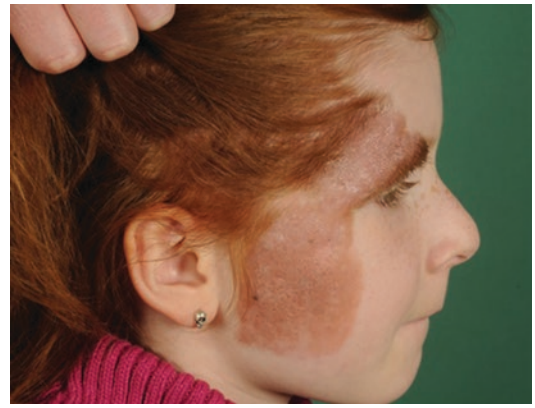


Fig. 12.5 Congenital melanocytic nevus (CMN). A 6-year-old girl with a huge congenital melanocytic nevus (CMN) of the right face, involving multiple facial aesthetic subunits

Fig. 12.6 Congenital melanocytic nevus (CMN). Congenital melanocytic nevus (CMN) in a 17-year-old woman. The CMN is located medially in the forehead and shows areas with degrees of pigmentation

strate two clinical examples of facial CMNs. CMNs up to 2 cm in diameter are classified as small, CMNs between 2 and 20 cm in diameter are medium sized, and if a CMN extends to more than 20 cm in diameter it is classified as large or "giant CMN" [60–62]. CMNs are derived from the neuroectodermal melanocytes during embryogenesis in the first 3 months of pregnancy. In some CMN mutations, KRAS or NRAS genes were found, but mostly the genetic background is unknown [63, 64]. CMNs usually increase in size proportionally to general growth, but in some cases especially during puberty and maturity, thickening was observed [61].

Histologically CMNs are similar to acquired melanocytic nevi, but in contrast to them melanocytes and nevus tissue can be found much deeper in the dermis, and it is even possible that the nevus reaches deep to the subcutaneous layer [65, 66].

Of clinical relevance are the aspects concerning CMNs. On the one hand, depending on size, configuration and localization—especially in the craniofacial region—CMNs can be a relevant burden for affected patients concerning aesthetic impairment and also consecutive psychosocial aspects (stigmatization) [67]. On the other hand, especially in large CMNs a recognizable risk for development of malignant melanoma is discussed. Unfortunately, concerning the risk of malignant transformation, data is inconsistent and published range reaches from 2% to 42%, making evidence-based therapeutic approaches difficult [61, 65, 68].

If a patient shows CMN and additional melanocytic tumours in the central nervous system or leptomeningeal, a rare disorder, so-called neurocutaneous melanosis, should be considered. Clinical signs are headache, vomiting, papilledema (signs of increased intracranial pressure), epilepsy and sometimes even palsy of cranial nerves. With a 3-year life expectancy of only 50% after onset of symptoms, the prognosis is bad [69, 70].

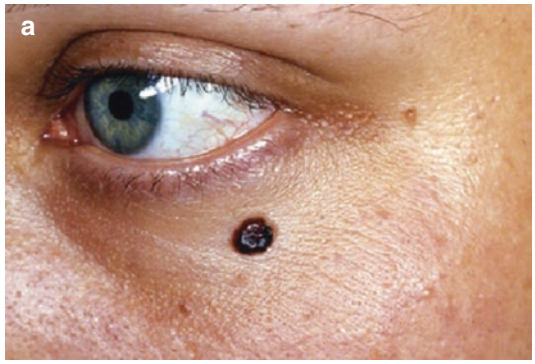
Whereas the nevus of Ito is localized in the deltoid region and shoulder, the **nevus of Ota** (histologically also characterized by excessive amount of melanocytes in the epidermis) is restricted to the appearance in the face and eye in the area of N. V1 and N. V2 (*N. ophthalmicus* and *N. infraorbitalis*). Females are five times more affected than males; it is rare in Caucasians and in approximately 60–65% of patients, a brown macula in the sclera occurs (associated with increased risk for glaucoma) [71, 72].

12.3.2 Acquired Skin Nevi

Usually these nevi are described in detail in dermatology books and papers, but at this point a short overview is given without claim on completeness. The most common form is the **melanocytic nevus (MN)**, also known as nevocytic nevus or nevus-cell nevus. This lesion is benign and, caused by a defect in the first 3 months of pregnancy, can occur from birth on. Usually most adults develop up to 30–40 MNs and UV light increases the incidence of MNs. Figure 12.7 shows a typical MN. Depending on the involvement of different skin layers, three types are differentiated: junctional MN are located in the epidermis at the junction to the dermis, the intradermal MN is solely within the dermis, and a classical birthmark and the compound MN involves both the epidermis and dermis [63, 73–75].

UV exposure and other risk factors (ionizing radiation, immunosuppression, other carcinogenes) increase the risk of development into a **dysplastic nevus (DN)** or even into **malignant melanoma (MM)**. DN shows clear signs of cellular and histologic architectural dysplasia, is often greater in size than MN and has more irregular concerning borders and colouring. If patients show more than 100 MNs and increased rate of DNs, **dysplastic nevus syndrome (DNS)** should be considered. DNS is inherited autosomal dominant, the relevant gene locus is the CDKN2A gene on chromosome 9p21.3, and patients are at high risk to develop MM arising from DN lesions, especially superficial spreading melanoma [76–79]. Figure 12.8 gives clinical examples for MMs of the face.

Other acquired nevi are benign and less often. The blue nevus—named by its colour—shows



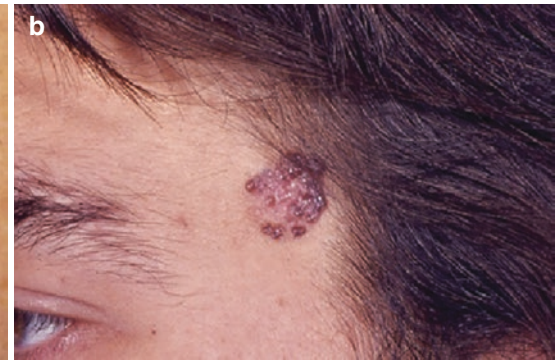
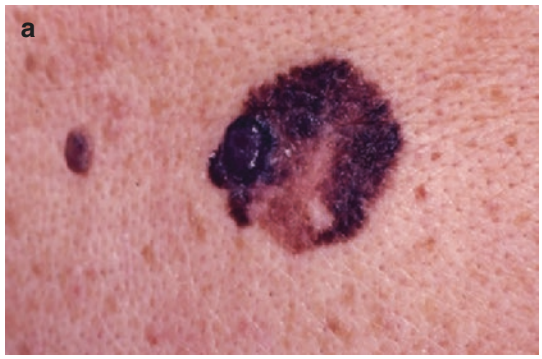
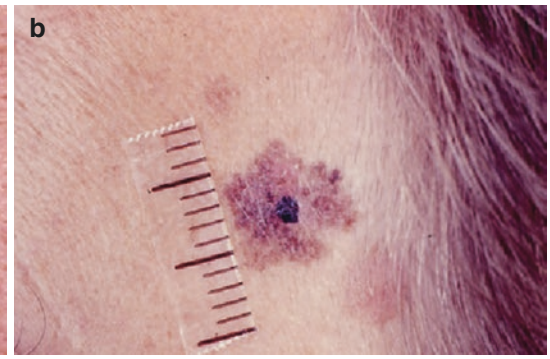


Fig. 12.7 (**a**, **b**) Different kinds of facial melanocytic nevi (MN). Simple melanocytic nevus of the infraorbital region (**a**) and extended nevus, showing an irregular configuration (**b**) that must be differentiated from malignant melanoma





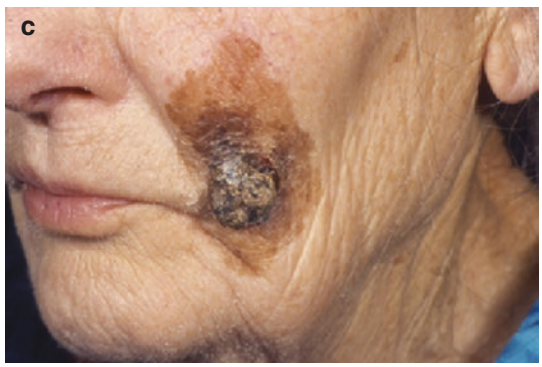


Fig. 12.8 (**a**, **b**, **c**) Malignant melanomas (MM) of the face. Clinical aspect of malignant melanomas (MM) of the face. (**a**) depicts the irregular configuration, unclear borders, different degrees of pigmentation, signs of regression and beginning nodular growth. (**b**) shows a superficial spreading MM and (**c**) extended MM of the left nasolabial region and cheek, arising in a lentigo maligna (so-called lentigo maligna melanoma, LMM)

melanocytes deeper in the skin. The Spitz nevus is mostly found in children and is a subtype of a dermal nevus. The verrucous nevus, nevus sebaceous, eccrine and apocrine nevus and nevus comedonicus are derived histologically from the cells they are named after [80].

12.3.3 Other Skin-Related Syndromes or Malformations

A further syndrome related to craniofacial lesions is the **Gardner's syndrome (GS)**. This subtype of familial colorectal polyposis is inherited in an

autosomal-dominant fashion (mutation in the APC gene, chromosome 5q21). Besides colorectal polyps and adenomas with increased risk for colorectal cancer, in the craniofacial area GS is characterized by dental abnormalities (hyperodontia), osteomas (skull and jaws) and odontomas (jaws), dermoid tumours, epidermoid cysts and fibromas. Incidence is estimated to be 1 in 14,000 with equal distribution between male and female [81–83].

Within the group of teratoma, **dermoid cysts (DC)** can play a role in the craniofacial area. They may be present at birth and in childhood, develop from embryonal tissues and contain mature tissue of the skin and skin organs like sweat gland and hair. They are benign and can grow during age and a frequent localization in childhood is periorbital. Usually clinical signs are restricted to aesthetic impairments, depending on their size [84]. In contrast, **epidermoid cysts (EC)** develop as epidermal inclusion cysts and can occur in every age. They often contain keratin and are layered by a thin epithelium. Located in the upper skin, in contrast to DC never in deep tissues, they can show superinfection and rupturing, causing pain and inflammation. They are benign and their leading clinical sign is a bump in the skin [85].

12.4 Vascular Anomalies

Vascular anomalies can be divided into vascular tumours, which are characterized by overgrowth of structural nearly normal vessels and increased proliferation of the endothelium and vessels, and vascular malformations, which are caused by errors in the development of vessels (non-proliferative). In both groups, the blood vessels and/or lymph vessels can be affected and vascular anomalies can occur in every part of the body, and therefore also in the craniofacial region, and can be linked to syndromes [86, 87].

12.4.1 Vascular Tumours (VT)

Vascular tumours (VT) are usually benign and a relevant proportion can occur from birth on and can be found in 1–2% of the population. A tumour assessed as borderline (local aggressive behaviour, but no metastasization) is the (Kaposiform) hemangioendothelioma, with infiltration even into deep tissues like muscle, fascia or bone. Malignant vascular tumours are angiosarcomas, hemangiopericytomas, lymphangiosarcomas and epithelioid hemangioendotheliomas. These malignant neoplasias show classical signs of cancer like local tissue destruction and infiltration and dissemination into locoregional lymph nodes and into distant organs. All these borderline and malignant vascular tumours are not described within this chapter, because they don't belong to malformation in the classical meaning and they should be addressed as rare head and neck cancers. The benign tumours which can be observed in a relevant proportion in newborns and children have more in common with malformations themselves and are described shortly as overview in the following.

Infantile hemangioma (IH) is the most frequent benign vascular tumour and females and newborns with lower birth weight are at higher risk. 60% of all IHs appear in the head and neck, and they mostly appear during the first weeks after birth and show their growth initially within the first year of life. After that time, growth adapts to normal growth rate or below, and involution and even disappearance can be observed. Until the tenth year of life, involution rates of up to 70% were reported. In the pathomechanism of IH hypoxia is discussed as an inducing factor [88, 89].

In contrast to IH, **congenital hemangioma (CH)** is present at birth fully expressed in its maximum size due to its development during pregnancy. Predominant sites are the lower extremity (40–50%) and the head and neck (ca. 40%) and two major forms are discriminated: rapidly involuting CHs and non-involuting CHs. The first ones are often red plaques and ulcerate, and after involution atrophic tissues (skin, subcutaneous layer) can occur. The latter ones are reddish to purple plaques, similar to vascular malformations (see below), and usually do not ulcerate and grow similar to the general growth of the child [90–92].

The third common vascular tumour to mention is the **pyogenic granuloma (PG)**, which in more than 60% of cases occurs in the craniofacial region at the skin or mucosa. The tumour is benign and non-inherited and shows a rapid growth up to a size of approximately 5–7 mm in diameter. Histologically the tumour shows increased number or size of blood vessels, sometimes with a larger central vessel. Its appearance is a red papule that becomes pedunculated in some cases, and bleeding, ulceration and crusting can be noted. Figure 12.9 shows a clinical case of a craniofacial PG. As possible cause trauma, hormonal influence and development as side effect of medications are discussed [93, 94].

12.4.2 Vascular Malformations (VM)

Vascular malformations (VM) are derived from the blood vessels or lymph vessels and are present at birth. The underlying mechanism is an impairment of vessel development, and these malformations usually tend to grow similar to general child growth, but in rare cases also a faster growth is possible. Vascular malformations don't show spontaneous regredience and they are subdivided into high-flow (arteriovenous malformation and fistula, arterial malformations) and low-flow (capillary, venous and lymphatic malformations) types. If two different types of malformations and vessels compose the VM, they are referred as combined-complex VM [95–97].

Fig. 12.9 Clinical aspect of a pyogenic granuloma (PG). A 3-year-old boy with a pyogenic granuloma of the medial lower eyelid of unknown cause (may be a minor bagatelle trauma)

Arteriovenous fistula (AVF) described a direct shunt and communication between a vein and an artery. These high-flow VM can be caused by trauma (penetration from artery to vein or vice versa), inflammation and necrosis of adjacent vessels (e.g. vasculitis) and rupture of aneurysm; they can be congenital or they can also be surgically made, e.g. with the intention to use them for haemodialysis. Undetected AVF can lead to massive life-threating bleeding in case of even minor trauma or other reasons when being harmed. They can be located in either soft or hard tissues of every area (craniofacial, intracranial) and can increase in size, causing compression syndromes or disfigurement [98].

Arteriovenous malformations (AVMs) are usually congenital, characterized by an abnormal connection between the arteries and veins and can occur everywhere—also in the craniofacial area and especially intracranial area. Incidence is estimated between 1 and 1.5 per 100,000/year in Western countries. Partially genetic background is known with mutations in genes of the RAS family, e.g. mutation in the PTEN gene affecting endothelial growth. In the majority of cases, the genetic mechanism is unknown. Supported by non-physiologic blood circulation and pressure within the AVMs, permanent growth is possible. Depending on their localization, a broad variety from no clinical symptoms up to severe pain, compression syndromes or life-threating bleeding can occur. It is feared intracranial AVMs are a cause of intracranial haemorrhage [58, 99, 100].

Capillary malformations (CapM) are characterized by increased number and sometimes size of capillaries and are the most common type of congenital VMs. Clinically they present themselves as reddish (from light red up to purple is possible) macula or lesion and they are present from birth on, showing growth usually similar to general growth. These lesions are also called "port-wine strains" and "nevus flammeus" and plenty of them occur in the head and neck. Association to syndromes is possible (e.g. Sturge-Weber syndrome or Klippel-Trenaunay-Weber syndrome), and genetically they are caused by somatic mutations in the GNAQ gene [101, 102].

Venous malformations (VenM) are comparable to CapMs with the difference being that they consist of venous vessels and they therefore differ in colour from the latter ones (violet to dark blue). They are compressible and can reach—especially in the head and neck—into deeper soft tissue, e.g. salivary gland [103].

Lymphatic malformations (LM)—commonly known also as **lymphangioma**—derive from abnormal developed lymph vessel and are congenital. During early embryological development these lymph vessels fail to connect in a normal physiologic manner to the lymph drainage system, resulting in the development of lymphatic cysts. Depending on the size of these cysts, microcystic LM (cysts smaller than 2 cm^3) are differentiated from macrocystic LM (cysts greater than 2 cm^3, e.g. cystic hygroma, often located in the craniofacial area). Mixed type of micro- and macrocystic LM is also possible. Growth is usually slow, they stay benign, and association is sometimes present to syndromes like the Noonan syndrome or trisomy 13, 18 or 21 (Down syndrome). The cause and detailed genetic background are unknown. Depending on localization and size, they cause disfigurement and can stigmatize patients; only in rare and extended cases that signs of compression of adjacent tissue and organs like pain or respiratory impairment may occur [104–106].

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Biological Basis of Positional Head Deformations

13

Christian Linz, Felix Kunz, and Tilmann Schweitzer

13.1 Growth Pattern and Pathogenesis

At birth, cranial sutures are physiologically not fused [1], which allows for some movement between the bony skull segments and enables a certain degree of physiological reversible deformation of the skull, for example, when passing through the birth canal [2]. This skull alteration usually resolves itself within a few days [2, 3]. What is more, the patent sutures allow for expansion of the brain parenchyma, which leads to an increase in volume of the skull, as reflected in its percentile growth [4]. The increase in brain size serves as the critical force behind cranial growth and causes the skull to nearly double in size within the first 6 months of life. The necessary intramembranous bone enlargement takes place mainly by ossification at the bone margins or at so-called osteogenic fronts of patent cranial sutures. These sutures contain fibrous tissue and represent not only articulations but also the distinct sites at which osteogenesis takes place after the proliferation and differentiation of osteoprogenitor cells [1].

The initially rapid growth rate of the brain parenchyma then declines. After 2–4 years, the brain reaches 75% and after 6–8 years 90% of its final volume, respectively, and reaches its final size at the age of 12. In the following years, the bony skull continues to display a minor increase in size, which is mainly due to a thickening of the skull bones. However, almost all skull sutures remain patent for some years thereafter: With the exception of the metopic suture, which fuses by the second year of life, other large sutures—such as the sagittal, coronal, and lambdoid sutures—do not fuse physiologically before the third decade of life [1]. The fact that cranial sutures remain patent is regulated by mechanical forces as well as by factors that stimulate or inhibit bone growth [5]. Different signaling pathways affect the transcription factor RUNX2 [6], which is decisive in regulating osteoblast activity [1].

This physiology of the cranial sutures results in an easily moldable skull in the first weeks and months of life. During this period, external gravitational forces that act persistently on the same region of the skull may cause a deformity of the neuro- or viscerocranium. This is highly relevant since positioning of the baby—resulting in corresponding external forces—represents such a mechanism, which may then lead to an abnor-

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mal skull shape [7]. Supine positioning of the baby—as recommended in order to reduce the risk of sudden infant death syndrome—can thus lead either to posterior flattening of the entire occiput (positional/deformational brachycephaly (DB)) or to one-sided occipital flattening (positional/deformational plagiocephaly (DP)) if the baby has a preferred side. As mentioned above, both types of head deformities arise due to lasting external molding forces, which alter cranial growth and display criteria, which partly overlap. However, a clear distinction between DB and DP should be made [8].

13.2 Positional/Deformational Brachycephaly (DB)

The prolonged influence of an external force on the entire occiput might cause DB, which is defined as bilateral and therefore symmetric flattening of the occipital region, resulting in a reduced length of the entire skull (Fig. 13.1) [7, 8]. This reduction in length leads to an increase of the cephalic index (CI), which is defined by the ratio of the maximum width to the maximum length of the head [10, 11]. While a brachycephalic head shape was defined by a CI > 92–93% in earlier reports, recent publications define a cut-off at a CI > 94% [10, 12, 13]. This new cutoff point reflects the general observation that the physiological width–length ratio of infants' skulls has changed in recent decades. While a CI of 77% was considered normal in the 1970s, recent publications report that a normal CI lies in the range of 80–85% [9, 14–16]. Further possible characteristics of brachycephaly include a compensatory widening of the occipital region [7, 17] and of the fronto-lateral or temporo-occipital skull [17]. These changes lead to the characteristic appearance of a trapezoidal head shape in vertex view.

In this context, it should be noted that purely symmetric occipital flattening occurs very rarely since an asymmetric, unilateral occipital aspect is very often detectable. As DB is defined as a symmetric deformity, a skull with such components of asymmetry must be classified as representative of a case of deformational/positional plagiocephaly (DP) [17].

13.3 Positional/Deformational Plagiocephaly (DP)

The prolonged influence of a unilateral external force on the occiput might cause DP [18–20], which is defined by a one-sided occipital flattening of the head, resulting in an asymmetric head shape (Fig. 13.1). Currently, DP is defined as a difference of more than or equal to 0.3 cm in both diagonal diameters of the head, measured on the horizontal plane [21]. In addition to this unilateral deficit, an ipsilateral anterior shift of the ear and a compensatory bulging of the ipsilateral forehead are further characteristics of DP [17, 22–25]. In some cases, facial asymmetry is also possible, which often involves an excess of fatty tissue and—in more severe cases—some bony hyperplasia in the area of the zygoma [17]. The extent to which positional asymmetries affect the development of mandible/maxilla, of dental occlusion, and of potentially resulting malocclusions has not been studied to a satisfactory degree; however, several studies have indicated an association between DP and lateral crossbites, particularly on the contralateral side of the posterior skull flattening [26, 27].

While the above-mentioned CI is a suitable parameter for describing the symmetric head deformity found in DB, there are many ways to classify the asymmetric head deformities caused by DP, with cranial vault asymmetry (CVA) and the cranial vault asymmetry index (CVAI) serving as the most common parameters [21, 28, 29]. As mentioned above, a brachycephalic aspect exists in many DPs and goes hand in hand with an altered CI; however, it is not used for classifying DP as it does not take the asymmetric element into account. For detailed information on existing classifications, see the chapter "Diagnosis".

13.4 Development of DP/DB

Intrauterine restraints might lead to a skull deformity [30–32]. Childbirth is also associated with several possible forces on the malleable skull as

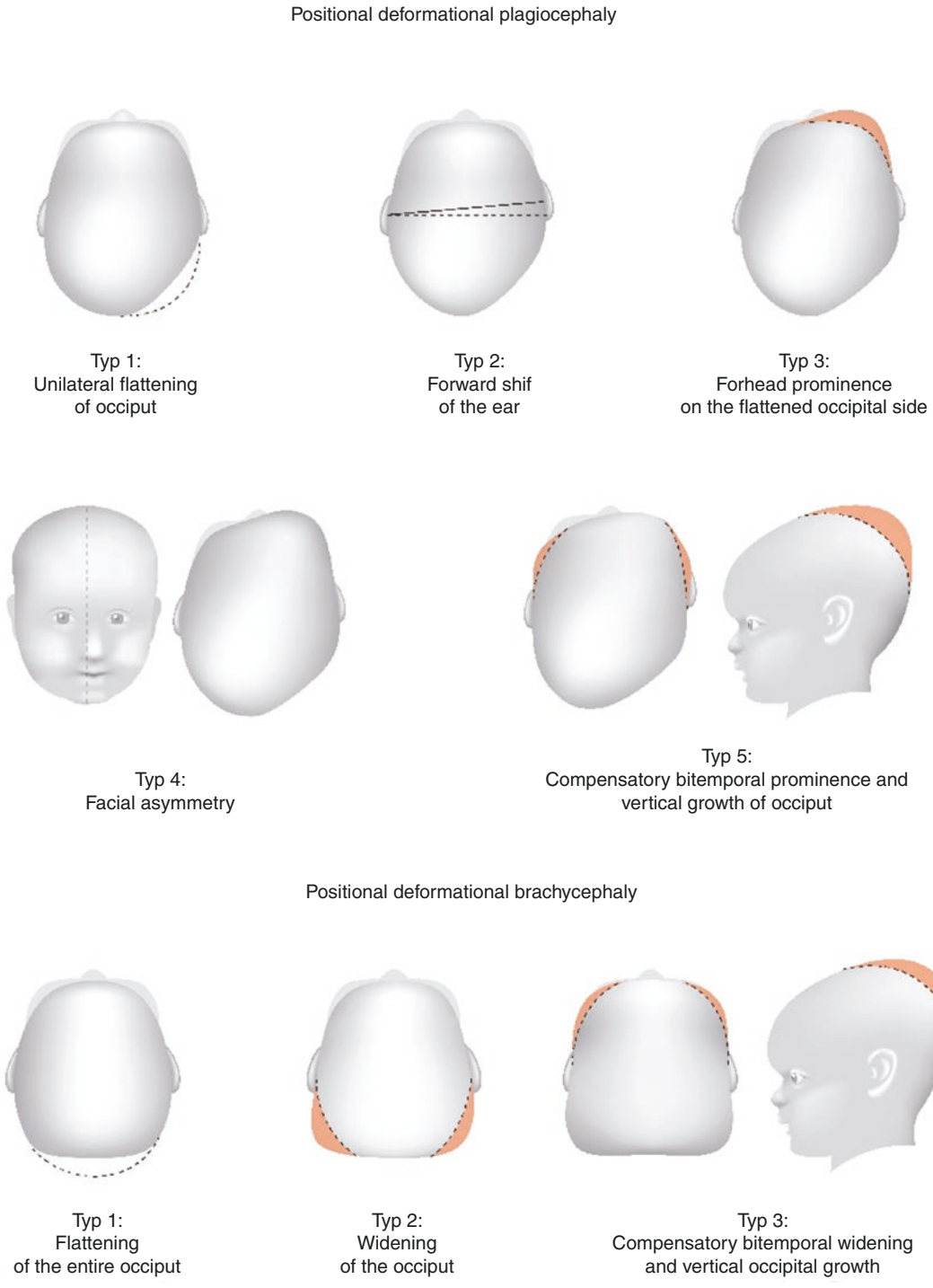

Fig. 13.1 Positional plagiocephaly and brachycephaly as described by Argenta [7, 9]

passage through the birth canal or the use of obstetrical techniques (e.g., forceps, suction cup) might cause skull deformations that are visible directly after delivery [2, 32, 33]. Many of these deformations resolve spontaneously and rapidly within several days or weeks [2, 3, 34]. It is important to differentiate these skull deformations from DP, the diagnosis of which should not be determined before 6 weeks of life. However, birth deformations may persist and evolve into DP [35], and some intrauterine and birth-related conditions are thus also important risk factors for developing DP (see also the Sect. 13.5 "Risk Factors").

The 1992 recommendation of putting infants to sleep in the supine position (the so-called "Back to Sleep" campaign) led to a drastic reduction of sudden infant death syndrome (SIDS), the most common cause of infant mortality in industrialized nations [36, 37], and this reasonable recommendation should be therefore continued to be followed [37, 38]. However, while the "Back to Sleep" campaign has reduced the incidence of SIDS by a power of ten, it has simultaneously led to a roughly tenfold increase in DP [39].

While the incidence of isolated DB is low, DP is the most common head deformity in otherwise healthy infants. The incidence of DP reported in the literature ranges from around 0.3% up to 50%, a very wide range that can be explained by varying cohorts, differing time points of investigation, and varying classifications [8, 40–42]. According to Ahluwalia et al., nearly one in four children is affected by some degree of deformational skull abnormalities [43]. The incidence of DP is age dependent, with a peak of prevalence within the first 6 months of life. Increasing incidence can be observed between the sixth week and the fourth month of life [40, 44]. In the following months and up to the 24th month of life, the incidence decreases to 3.3% [3, 44]. However, one prospective epidemiologic study described moderate to severe asymmetries in 1% of investigated children of age 5.5 years [40]. A study on adolescents (ages 14–17 years), all born after the recommendation to place babies on their back had been made ("Back to Sleep" campaign, 1992), described a persisting skull deformity in 2.1% of the cohort [45]. In addition to the aforementioned differences in the incidence of positional deformities, the described rate of spontaneous improvement varies among studies [27, 44, 46]. This finding may again be explained by differences in age, data collection, and methods among studies, which would also explain why a few studies assume improvement without treatment, whereas most existing studies recommend stage-related therapy [47, 48].

13.5 Risk Factors

A variety of risk factors are involved in the pathogenesis of positional head deformities, though there is poor concordance regarding clear risk factors in the literature [49]. To provide a better understanding of the risk factors, we subdivide prepartum, peripartum, and postpartum factors.

13.5.1 Prepartum Factors (Including Preexisting Determinants)

The incidence of DP/DB nearly doubles in male babies, who are usually bigger than girl babies [3, 18, 31, 32, 42]. This increased incidence becomes relevant due to the consecutively reduced intrauterine space [8, 31]. The same condition is apparent in multiple births and is reported as a further risk factor [50, 51]. In the same context, forced abnormal intrauterine positions are also mentioned as predisposing factors [3, 50, 52, 53]. Positional head deformities are also more common in children of primiparae [44, 50, 54]. A younger age of the mother and a lower educational status have been reported as potential sociodemographic risk factors [44, 50, 54–57].

13.5.2 Peripartum Factors

Known risk factors for the development of an abnormal skull shape include younger gestational age, the associated decreased mobility of the preterm baby, and the resulting earlier exposure of

the very malleable skull to external positional forces [5, 50, 52, 55, 57].

Furthermore, a higher birth weight and large head circumference also lead to an increased rate of a deformed head shape [3, 7, 52, 58, 59]. These abnormal head shapes can also result from difficult deliveries and the usage of a ventouse cup or forceps [44, 50, 53, 54].

13.5.3 Postpartum Factors

A positional preference for one side represents an important risk factor [5, 14, 50–52, 54–57, 59]. In this context, mobility restrictions of the cervical spine—caused, for example, by bleeding into the sternocleidomastoid muscle or by torticollis—are predisposing conditions for developing DP [3, 14, 35, 50, 52, 58, 60]. While torticollis is present in only 0.1–2% of children with a symmetrical head shape, its incidence increases to up to 20% in children with DP [3]. At least 8% of children younger than 16 weeks have a preferred sleeping side, often the cause of a developing DP. One contributing factor to DP is formed by unilateral stimuli, such as a baby's unchanged feeding position [46, 56, 57, 59]. Bottle-feeding without changing position is therefore associated with an increased risk of DP in contrast to breastfeeding with a changing position, which has a protective effect [18, 46]. Another protective effect is achieved by the so-called tummy time—that is, putting the baby in the prone position while awake and under observation [61, 62].

The supine position—which is recommended in the valuable guideline that prevents SIDS—is also one of the main risk factors discussed in developing positional head deformities [7, 14, 32, 42, 44, 50, 51, 63]. The use of car seats, swings, carriers, bouncy seats, and rockers is associated with an increased risk of skull deformities, as is parents' smoking [7, 54].

Every developmental delay that is accompanied by reduced activity also correlates with an increased risk of deformational skull deformity [50, 52, 54, 59].

Ultimately, the pathogenesis, the underlying mechanisms of skull deformation, as well as disease-promoting factors and their influence have not yet been fully explained [8, 18].

13.6 Impairment of Neurocognitive Development

Several reports on developmental delays in the context of deformational skull abnormalities exist [64–66]. However, comparing of infants with and without skull deformity, it should be noted that most children score within the average range of the test norms [67].

A few reports of differences in motor development have been reported for infants within the first months of life exist. However these differences between affected and non-affected infants decrease at the age of 3, when differences in cognition and language become apparent. Infants with mild DP/DB display minimal—if any—differences, whereas infants with more severe forms demonstrate statistically significant differences.

These motor developmental delays can also be reflected in deferred vocal and language development since speech is based on a fine coordination between laryngeal and supra-laryngeal mechanisms, including auditory feedback. These mechanisms require very rapid neuro-physiological control, which leaves no time for compensatory regulation in the case of dysfunction. However, in examinations, it has not yet been possible to demonstrate an association between delays in early prespeech babbling articulatory skills or early language production on the one hand and positional skull deformities on the other hand [68–70].

However, a significant relationship between preterm birth on the one hand and neonatal complications, mortality, and developmental delay on the other hand is known to exist [71]. Consequently, a preexisting developmental delay is a possible reason for deferred mobilization causing extended time for the influence of external forces on the infant's skull. This fact highlights the difficulty of differentiating between cause and consequence in the question of a possible association between developmental delays and deformational head abnormalities.

In general, it should be noted that many studies exhibit methodological problems, such as the use of non-homogenous groups, a lack of standardized testing or of control groups, or the insufficient consideration of influencing variables, such as socioeconomic status, the parents' IQ, or individual support.

We regard a developmental delay as a risk factor for positional skull deformations [72]. The fact that slight deficits might either precede or follow DP/DB highlights the need for close monitoring of affected infants since an association of any kind with a developmental risk is possible.

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Part III

Psychological Aspects of Disease

14 Aspects of Facial Esthetics and Disfigurement

Christoph Runte and Dieter Dirksen

14.1 Introduction

Why do human faces look the way they do? And how do we judge attractiveness? Still we do not have final answers to these and related questions [1, 2], but recently, science has at least found some clues.

Through the centuries, there have been many attempts to find out how beauty in general and beauty of the human face in particular are determined. Beauty has been equated with truth [3], usefulness [4], and good [5]. The French novelist Marie-Henri Beyle, better known as Stendhal, noted beauty was nothing other than the "promise of happiness" [6], which means that we are attracted to objects or persons which might help us to get happy, and this attraction is what we call beauty, but this only shifts the problem to the definition of happiness. If happiness is to live in the company of trustworthy [7], healthy [8], and guiding [9] people, faces are believed to reveal those qualities [10]. Evolutionary biology would explain beauty, or, strictly speaking, attractiveness, mainly with regard to mate selection as a product of selection by survival and sexual selection. Another evolutionary aspect of beauty and disfigurement is its influence on parental care [11, 12]. There have been attempts to describe beauty as a consequence of the adherence to objective morphologic parameters, e.g., bilateral symmetry and certain proportions. Other authors countered that the beauty of a person or an object might be judged differently by different persons, which leads to the conclusion that beauty is "not judged objectively but according to the beholders estimation" (Theocritus [13]). In his *Critique of Judgment*, Immanuel Kant [14] defined beauty as something causing pleasure without any interest and understanding. Kant then continued that if beauty was caused by the pleasing perception without personal interest, it should be pleasing to every beholder. Therefore, beauty as a universally pleasing perception would be mistaken as a logically deducible quality of the object. However, later philosophers dismissed Kant's idea of pleasure without interest.

After all these attempts, still there seems to be no simple explanation or geometrical formula to define beauty in detail, and, as many people agree in their esthetic judgment on who is attractive and who is not, beauty seems not only to depend on a beholders' free and unconditioned estimation.

An individual, intuitive judgment of beauty in general seems to be determined by the process of visual perception, sociocultural conditions, and individual experience. Chelsea Wald [2] recently summed up four fundamental open questions on beauty, starting with the problem of its definition: "What is the point of human beauty?"

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Beauty and disfigurement are often used as opposites, as we did in the chapter title. However, disfigurement and beauty are not mutually exclusive. If beauty is at least partially judged by the beholder, its opposite would also be subject to the beholder's estimation. Disfigurement is as difficult to define as it is true for beauty. If we use disfigurement as the opposite of the given definitions of beauty, disfigurement could be defined as equal to falsity, futility, and bad. It could be described as the "promise of bad luck" or the violation of proportional rules. As an opposite of Kant's definition, disfigurement would be a perception that is unpleasant without interest and understanding. None of these definitions is truly convincing.

14.2 Biological Aspects of Facial Beauty and Disfigurement

As it has been said before, beauty may be seen as an aspect of perception ("in the eye of the beholder") or as a result of certain morphologic properties. We will therefore discuss these aspects of perception and of morphogenesis in more detail.

14.2.1 Visual Perception and its Influence on the Esthetic Judgment

All humans share fundamental physiologic processes of visual perception and recognition. Visual perception and recognition enable us to experience the world around us, beginning with the faces of our parents. It is necessary to understand these processes because they are the reason why our visual perception is selective and also why our judgment of the beauty of a face is not free and according to our individual assessment.

The first step of visual perception is light entering the eye bulb through the lens and being projected to the retina. Here, the photon energy is transformed into chemical energy. Illuminated rhodopsins activate G-proteins, which then start the signal transduction. At this stage the signal represents an excitation at a specific point of the two-dimensional retina with information on location, brightness (rods and cones), and color (cones). Passing through the layer of retina bipolar cells, the signals of a number of photoreceptors from a receptive field are collected by a third neuron (retinal ganglion cell). There are different types of retinal ganglion cells; on-cells will transmit a signal with a higher firing rate if the input signal from center of the receptive field is more intense than in the peripheral areas. Off-cells would show a reverse reaction. The ganglion cell axons follow the optical nerve with the information from the median part of the retina crossing to the contralateral hemisphere (chiasma opticum) while the information from the lateral part remains on the same side.

The next signal transformation step (located in the corpus geniculatum laterale) is an extraction of edges or outlines with a high contrast. The visual information is then projected primarily to the occipital visual cortex. The signal transformation to outlines is illustrated in Fig. 14.1. Understanding of this fundamental process of visual perception was founded in the late 1950s by David Hubel and Torsten Wiesel with their experiments on the cat's striate cortex [15]. Larsson et al. [16] confirmed the account of structures with high contrast to pattern recognition. However, the ability to recognize faces with only a few outlines from a sketch or even from randomly distributed elements has been well known for centuries. In his treatise on painting, Leonardo da Vinci [17] claimed that "by looking attentively at old and smeared walls or stones and veined marble of various colors, you may fancy that you see in them several compositions, […and] strange countenances […]. By these confused lines the inventive genius is excited to new exertions."

Efferences from the corpus geniculatum laterale also reach the superior collicles, which are important for eye movements. They send signals to nuclei of the cranial nerves (esp. oculomotor, trochlear, and abducens nerve) and the pulvinar (central medial, posterior inferior, and middle inferior pulvinar nuclei [18]). If a visual stimulus is presented, the following eye movements are

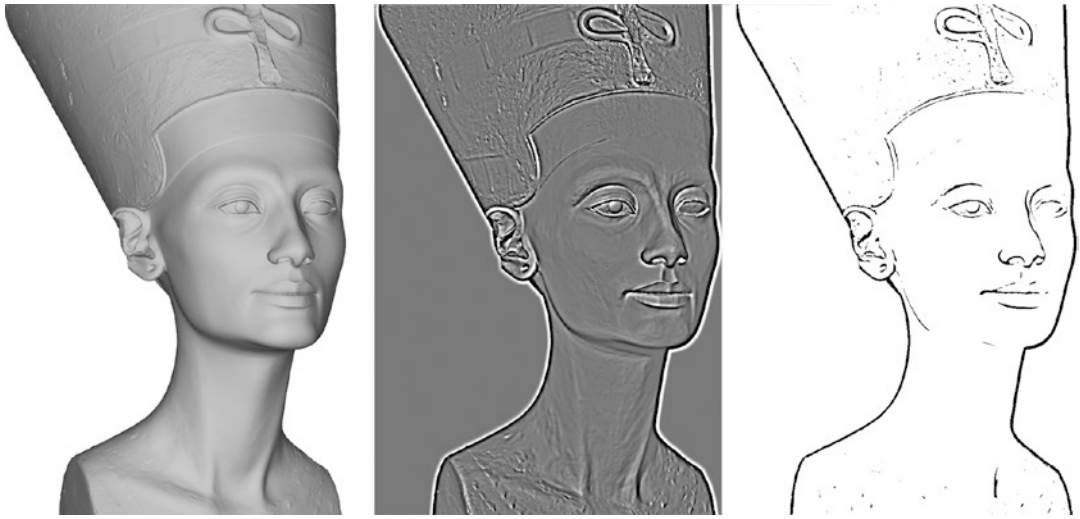

Fig. 14.1 Steps of visual perception simulated by digital image processing – original picture (left), the signal high-pass filtered and posterized (middle) and reduced to areas below a brightness threshold (right). By these simple procedures, the image is reduced to lines indicating the contours, but recognition of the object is still possible. Data is correspondingly reduced from 389 KB (.tif-format, LZW-compressed file from left image) to 90 KB (same format, right). With similar procedures, visual recognition is focused on contour lines. These lines are not restricted to physical boundaries of the object. Lines of contrast can also be caused by color or material changes and by lighting and reflection at curvatures and edges, e.g., at the eyelids, nose, ears, and philtrum. The pictures of Nefertiti were generated using the 3D model "bust of Nefertiti at the Neues Museum, Berlin" (https://www.myminifactory.com/object/3d-print-bust-of-nefertiti-at-the-egyptian-museum-berlin-2951)

directed in a way to "track" the areas of high brightness contrast. This "tracking" was examined and described by Alfred Yarbus [19], who also identified a special area of interest within the face, called the "Yarbus triangle." This triangle is defined by the eyes and mouth (Fig. 14.2). Consequently, Karl Popper and John Eccles [20] compared the process of visual perception to the painting of a picture, not to the taking of a photograph. Subsequent studies showed that there is also a dominance of the left "field of gaze" [21] if we are looking at a face. There was no preference of one side of the field of gaze when looking at other, nearly symmetrical figures. Thus, the right side of the face might have more impact on the esthetic judgment than the left one. Interestingly, Meyer-Marcotty et al. [22] found that subjects affected by cleft lip and palate focus their attention on the upper lip and nose if they look at the faces of healthy persons.

Visual stimuli showing regular geometry [23], reflection symmetry [24], familiar shapes, or matching expectations [25, 26] are supposed to

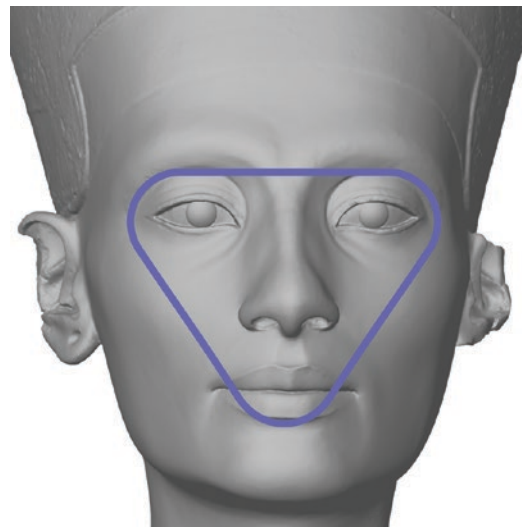

Fig. 14.2 The Yarbus triangle is a region of the face where structures with high contrast are more frequently focused than other regions. In his original publication from 1967, Yarbus [19] used pictures from faces and also one from a bust of Nefertiti (in profile) and simultaneously recorded the eye movements. With his technique, he could superimpose eye movement tracing with the visual stimuli

draw the viewer's attention to themselves, especially if they show clearly visible outlines within the Yarbus triangle.

In recent studies it was shown that perception of faces is different from perception of other familiar objects in view. A fast and intense neuronal response to visual stimuli called the N170 effect is selective for faces in certain areas of the brain [27]. The source of this neuronal response to the presentation of faces is located primarily in the right fusiform and infratemporal gyri, especially in regions called inferior occipital gyrus face area (OFA) and fusiform face area (FFA, [28]). Damage or intracranial stimulation of the FFA is associated with the disability to recognize faces (prosopagnosia, [29]). However, these areas are only part of a distributed neural network responsible for face recognition [30]. Prosopagnosia can also be caused by injuries of the temporal lobe, e.g., after surgical treatment of epilepsia. Recent studies indicate that the ventral anterior temporal lobe plays an important role in person-specific face perception [31]. However, the question how the different parts of our brain influence our esthetic judgment in detail remains largely unanswered.

Our knowledge on visual perception suggests that the fundament for our esthetic judgment is focused on facial structures, especially those within the Yarbus triangle, showing clear outlines of high contrast. As a consequence, these structures will be of importance in the definition of beautiful properties, e.g., using facial proportions.

However, the determination of an objects' physical shape is only the start of a process of recognition and assessment. Eleanor Rosch [32, 33] tested older concepts of Gestalt psychology experimentally and found that there are "ideal types" of perceptual stimuli, e.g., colors. These prototypes represent the "clearest cases, best examples" of a category ("a concept designatable by words"). A perceptual stimulus is therefore evaluated by comparison to the prototype of its category [1]. Whether a visual stimulus will cause pleasure or disgust will therefore largely depend on its categorization. For example, one person might react with disgust to the view of an oyster, another one with pleasure. The difference cannot be explained by the shape or texture of the oyster itself, but by the presentation and the personal experience one (or her/his social environment) has with oysters and if it is reasonable to categorize the oyster as delicacy. Tattoos and piercings have been held in very different degrees of regard in different times and cultures. Examples from the orofacial region are "decorative crowns" and "dental grills" among certain ethnic groups and cultural scenes [34]. The polished metallic surfaces of crowns or amalgam fillings found their own esthetic reverence in dental professionals in the past. However, this assessment was not always shared by patients. The more natural look of composite resins and ceramic veneers changed the view on metallic restorations. Within one category, perceptual stimuli are compared to the prototype. It has been assumed that the closer the stimulus fits to the prototype, the more pleasing it will be. On the other hand, typical stimuli are assumed to be more difficult to remember [35].

Classifying human beings into categories, especially by a look at their face, is problematic. Racism, nationalism, and class consciousness are only a few and extreme examples for the classification connected to discrimination. At least, conscious or unconscious classification will have a great influence on our esthetic judgment. Prototype theory assumes that objects or subjects will cause pleasure if they are "prototypical" for their category [1].

When a visual stimulus has been matched to the category "face." studies of Oosterhoff and Todorov [36] suggested that it will be evaluated basically on the two dimensions, "valence" and "dominance." Thus, the beholder gathers information from facial cues whether the person approaching is harmless or harmful and physically capable of implementing his intentions. These cues can be found mostly in the eye and mouth region, e.g., if the eyebrows are lifted, the resulting signal is trustworthiness.

14.2.2 Evolutionary Biology, Beauty, and Averageness

Facial morphogenesis, the influence of facial properties on mate selection and parental care, and the inheritance of genes regulating them are

connected by the complex mechanisms of evolutionary biology. A current publication by Xiong et al. [37] listed a number of gene loci associated with different facial proportions within the Yarbus triangle. Although there still are many open questions on human facial morphogenesis and how it has evolved, a simple example may illustrate some of the possible ways of facial development over generations. Stalk-eyed flies exhibit bizarre head morphology [38]. Arthropod body plan is very different to the human one, but evolutionary mechanisms are the same. Although it has been assumed that a wider interocular distance would be beneficial in stereoscopic viewing (Fig. 14.3), this argument is not convincing, as there are also drawbacks like the necessity to calculate an increased parallaxis for spatial perception and possible handicaps in flight. However, there is evidence for a connection between a large eyespan and "good genes" as only flies in good condition develop wide eyespans. If a wider eyespan is a signal for "good genes," the idea of female flies preferring male ones with a wider interocular distance would be convincing (Fig. 14.4). A third mechanism is caused by an X-chromosomal meiotic drive that can be suppressed by genetic factors associated with male fly wide eyespan (Fig. 14.5).

Evolutional biology would suggest that an advantageous feature would automatically become the average by elimination of other variants.

However, a connection between beauty and averageness has been postulated even before Darwin's publications. In his *Critique of Judgment*, Kant [14] described how he thought our perception of beauty was influenced by the average image of our visual experiences: He described how our imagination recalls form and view of a subject and is capable of "adding" all views of a certain kind, e.g., of a man. And to his belief, somebody who has seen a thousand men can estimate the average stature by superimposing a lot of or even all of the thousand images in his mind. The outlines of the average man would then also represent the stature of a beautiful man.

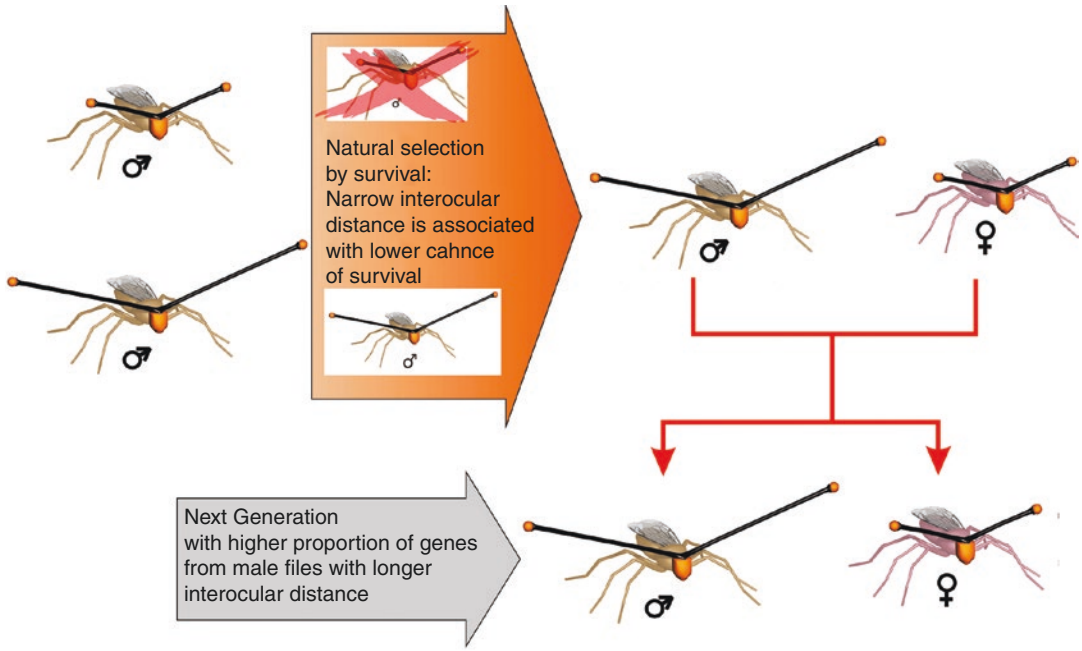

Fig. 14.3 Stalk-eyed flies exhibit unusual facial properties with exaggerated eyespan (schematic representation). This was assumed to be a result of functional adaptation caused by an improved stereoscopic perception advantageous for survival, but the more convincing explanation is that only flies in good condition (with "good genes") exhibit a wide eyespan. Thus, a wide eyespan is not the reason for but the result of an advantage in survival

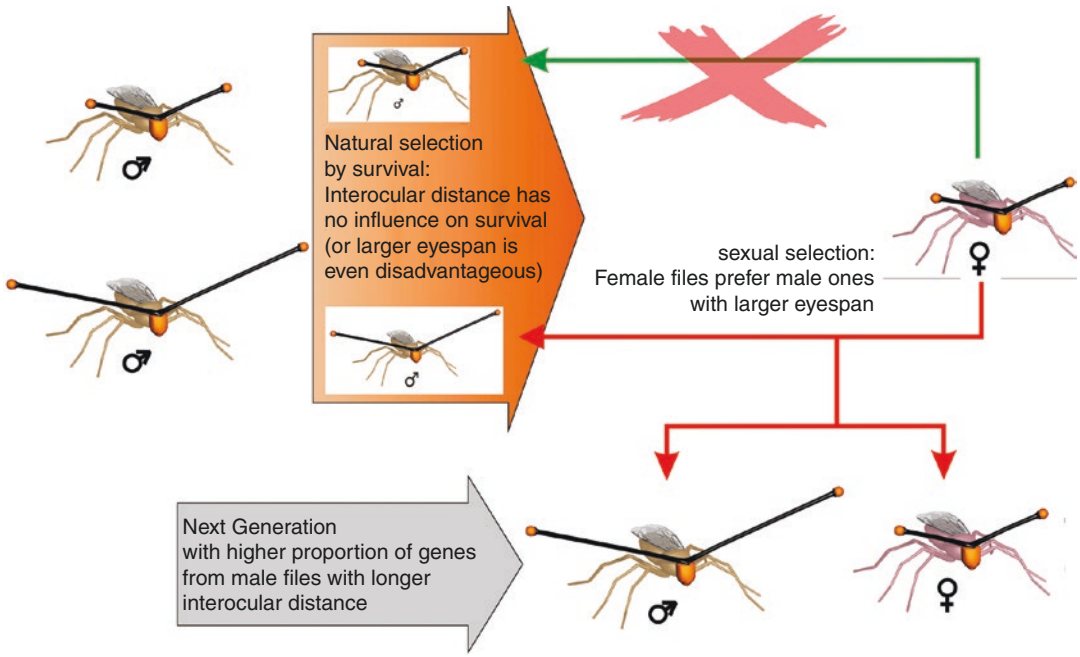

Fig. 14.4 Another mechanism is sexual selection. In this example, female flies preferably choose male flies with wider eyespan as mates

Superimposition of images became possible with the introduction of photography in the nineteenth century. In 1883, Francis Galton [39] published his *Inquiries into Human Faculty and Its Development*. He observed that "All composites are better looking than their components, because the averaged portrait of many persons is free from the irregularities that variously blemish the looks of each of them."

Evolutionary biology can explain a connection between facial attractiveness and averageness, following two basic concepts: On the one hand, average shapes could simply be the features of the most successful subjects (Fig. 14.6). On the other hand, subjects with attractive facial features should have a higher reproductive success (Fig. 14.7). However, being attracted to certain facial shapes that are signs of success (sign for "good genes") would be an advantageous strategy in mate choice, and these properties would prevail over the generations.

In addition to "survival of the fittest" and "reproduction of the most attractive," there are even more evolutionary mechanisms to be taken into consideration. If a small population gets isolated, e.g., on a distant island, and an above-average number of individuals bearing certain "founder mutation" passes through this evolutionary "bottleneck," the following generations will show a high percentage of this mutation and also a reduced genetic variation (Fig. 14.8), even though this mutation might be indifferent to survival or reproduction.

Beauty may be determined by our visual experience of our own local social and cultural environment. The faces we see share some common properties, characteristic for our ethnic group. We get used to those properties and check whether they can also be found within a stranger's face. Sociocultural factors influence our judgment of those faces, e.g., watching a movie like *Beauty and the Beast* might influence a child's judgment on beauty and ugliness. If certain properties of the face are associated with a higher social status or cultural or religious value, it may lead to a higher reproductive success. If there is no or only limited viability drawback, even a small reproductive advantage connected to

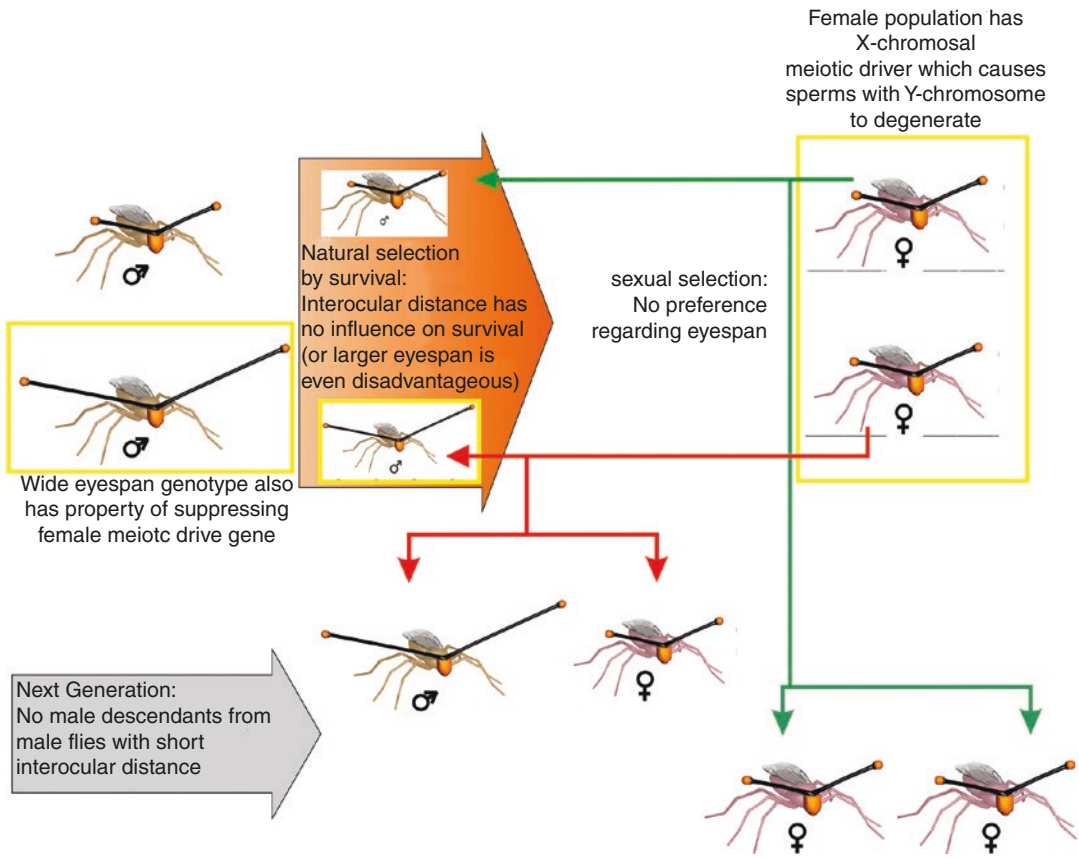

Fig. 14.5 In stalk-eyed flies, a meiotic drive gene has been identified. This meiotic drift causes sperms with Y-chromosome to degenerate. In this model, male flies with wide eyespan have the potential to suppress the meiotic drift gene, those with a narrow interocular distance have not. Real stalk-eyed flies in addition exhibit sexual preference for large eyespan

a facial feature might be sufficient to elicit a higher frequency. This mechanism is called "cultural selection" and has, e.g., been discussed as another possible reason (apart from the founder effect) associated with a higher prevalence of albinism in Native American people [40].

Superimposition of images to find an average face from a defined population was performed, e.g., by Perrett et al. [41], using vectorized outlines from portrait photographs. However, they connected the faces to degrees of attractiveness judged by a number of observers, and with these data they were able to identify facial properties of more attractive subjects. They could, by exaggerating the difference between the average face of all subjects and the average of the most attractive faces, create a "super-beauty."

In contrast to beauty, facial disfigurement frequently causes a feeling of disgust [42, 43]. To explain avoidant behavior with unhealthy or poisonous dishes or drinks with evolutionary biology is suggesting itself. Evolution biology would likewise provide an obvious explanation for the behavior of keeping a healthy distance to persons who exhibit signs of a contagious disease. However, any threatening exposition or phenotypic trait can be to the detriment of the organism by reducing the probability to survive or to reproduce, indifferent or even an advantage for an organism by surviving and by this

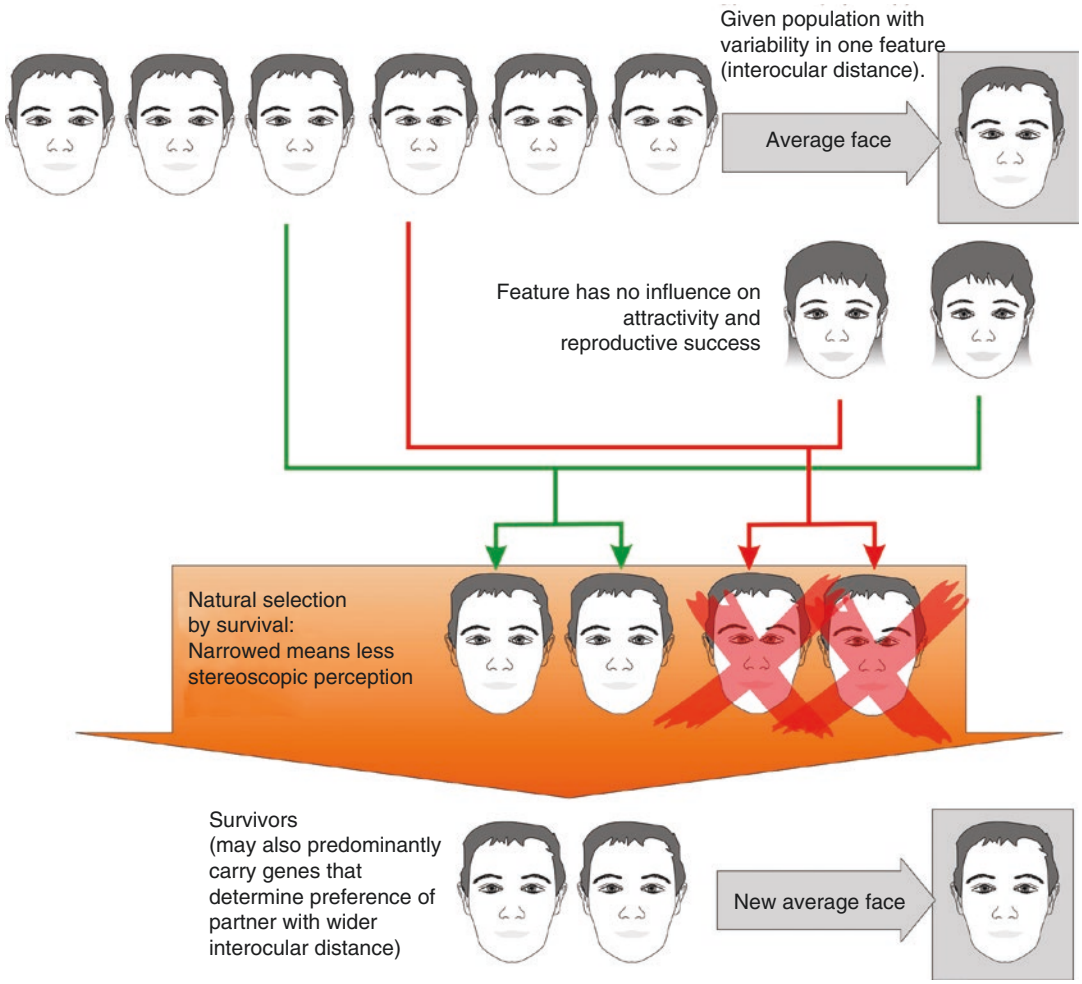

Fig. 14.6 Averageness as a result of evolution by "survival of the fittest." In this example, a narrowed interocular distance would be a drawback in spatial perception. Another important factor for survival would be the induction of parental care by cuteness

proving the higher qualification to survive, i.e., the "good genes." In fact, even an obvious facial disfigurement has been discussed as beneficial from evolutionary biology's perspective: Acne has been hypothesized as an evolutionary mechanism of protection while being at an immature age [44].

As a conclusion, it can be summarized that evolutionary biology gives good and reasonable explanations for many phenomena, but instead of simple chains of causation, it offers several mechanisms for the selection of facial properties, beauty, and disfigurement. Some traits of the human face may not have evolved by a process of adaptation but as a genetic by-product of another process. As long as not all factors and their interactions are understood, attempts to explain aspects of beauty of the human face by a simple chain of causation would be speculative.

14.3 Beauty and Facial Symmetry

In mathematical terms, symmetry is the property of an object to be invariant to certain transformations: reflection, rotation, scaling, and transla-

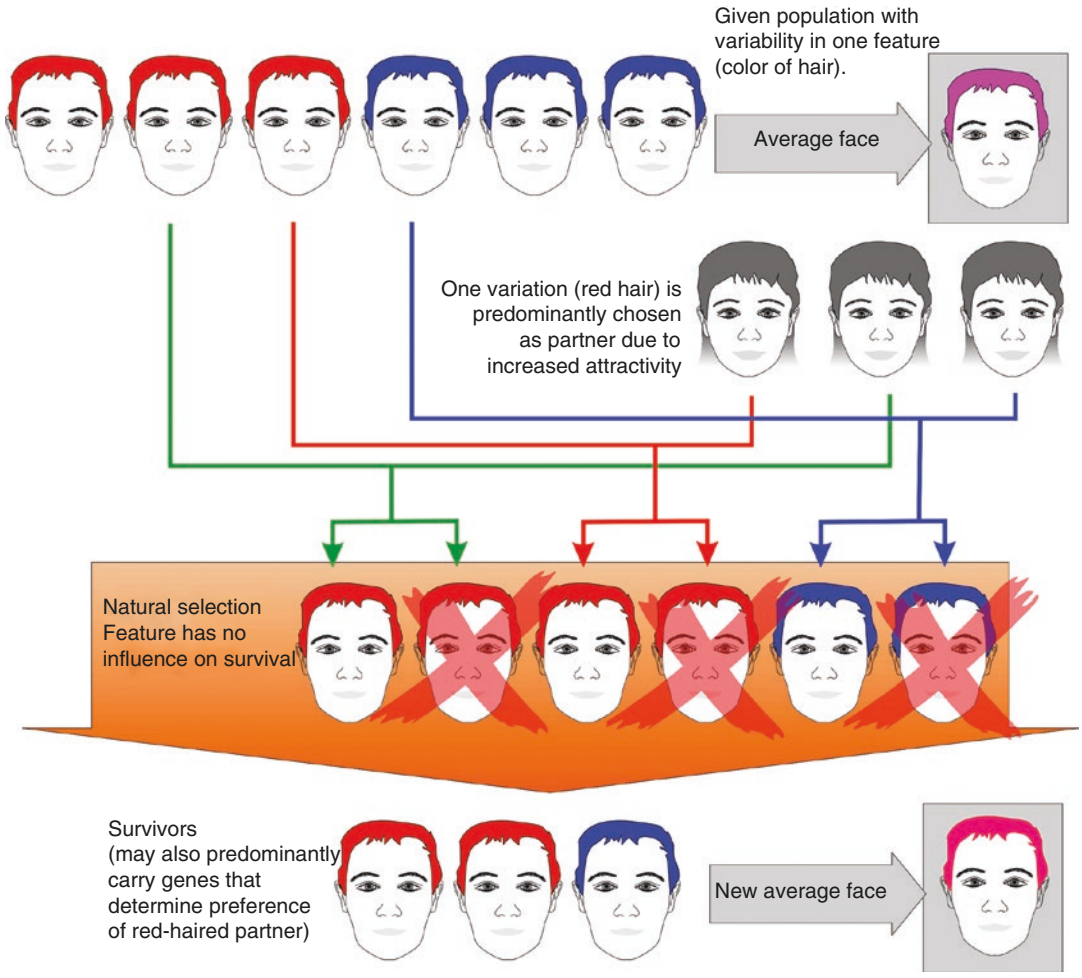

Fig. 14.7 Averageness as a result of evolution by "reproductive success of the most attractive." In this example, red would be the most frequent color of hair (or nearly the "average" color) after a few generations. This mechanism would include genetically determined preferences in mate choice as well as preferences by sociocultural conventions

tion. The human face and the human body plan except for the internal organs are mirror symmetric. This is also true for most species of the animal kingdom. Radial symmetry is found, e.g., in jellyfish and starfish or in plants. In early animal life, bilateral symmetry of the body plan was probably advantageous for locomotion and was genetically fixed for the whole body plan at an early stage of evolution. As a consequence, the detection of bilaterally or radially symmetrical structures was advantageous because it was a reliable strategy to detect plants and animals in the environment. This capability was necessary to find predators, mates, or food. Visual perception of bilateral and radial symmetry was studied, e.g., by Martinovic et al. [45] and Jennings and Kingdom [46].

In addition, translational transformation invariance, i.e., periodic structures, may occur in sights with regularly or irregularly repeating similar elements, like a host of flowers, a shoal of fish, a site with many mushrooms, or a tree full of fruit (Fig. 14.9). Regular translation in two dimensions leads to characteristic areal patterns, e.g., fish scales. Realizing scaling symmetry might have been advantageous in pattern recognition and spa-

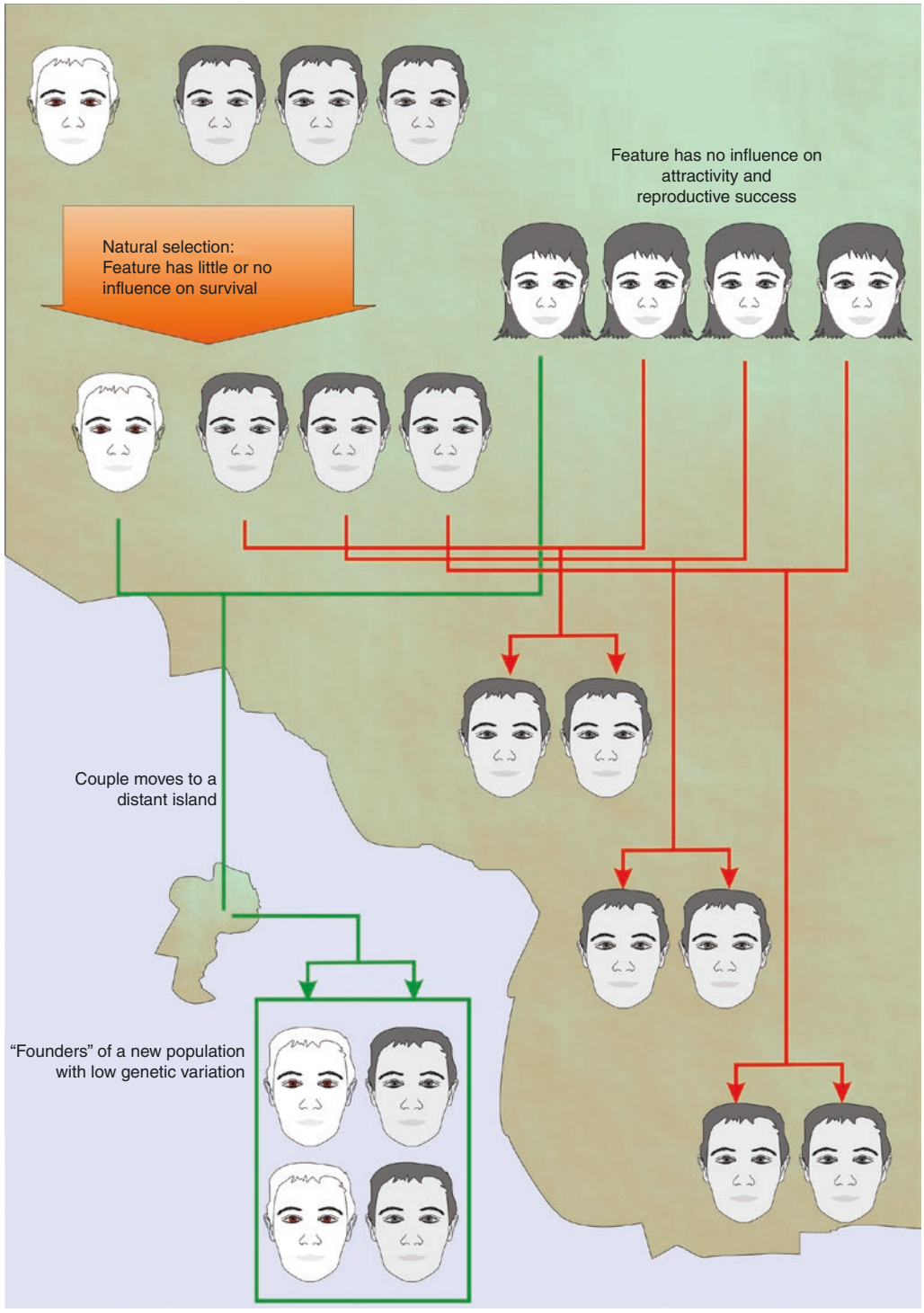

Fig. 14.8 "Founder effect" caused by isolation of a small group. Following generations lead to a population with a higher percentage of special properties and a reduced genetic variation. This mechanism has been suggested to be the reason for the high prevalence of oculocutaneous albinism in Hopi, Navajo, Zuni, Kuna, and other Native American people [40]

Fig. 14.9 Banana plant with repetitive elements exhibiting combinations of translational and high-order rotational symmetry on leafs and fruit

tial orientation, e.g., in a forest with trees of approximately the same diameter. Architecture has frequently used all four kinds of symmetry: Facades, cupolas, colonnades, and the repetition of similar arches in different seizes (e.g., the Pont du Gard) exemplify the high esthetic importance of all mentioned kinds of symmetry.

Perception of symmetry is easier with structures of high brightness contrast and independent from the recognition of the subject. In Salvador Dali's painting "Metamorphosis of Narcissus" from 1937, the painter showed two different objects with remarkably high translational invariance.

Dali also depicted scaled "faces within faces," e.g., in his paintings "Slave Market with the Disappearing Bust of Voltaire" from 1940 and "The Face of War" from 1941, the latter also exemplifying self-similarity.

Deviation from symmetry is often assumed to be correlated to reduced attractiveness. However, the correlation is not strong [47]. Unsurprisingly, even small deviations are easily detected in the structures rich in contrast located at the edges of the Yarbus triangle, eyes and mouth [48].

It can be concluded that different types of symmetry attract our attention and influence our esthetic judgment. However, the whole natural human face only shows bilateral symmetry. The eyes are also roughly mirror symmetrical for themselves and the iris and pupils show rotational symmetry. If the teeth are exposed in a smiling face, the teeth and interdental spaces show some kind of transformation and scaling symmetry. However, at a closer look the differences between the shapes of the lateral incisors and canines become visible.

14.4 Beauty and Facial Proportions

As it has been stated before, structures rich in contrast are extracted from all visual information and therefore probably have more impact on the process of perception. In ancient Egypt, artisans used strings imbibed with red paint to draw a rectangular grid before starting with the sketch and the final painting. As the grids were made up of square elements, at least major proportions were usually ratios of integral numbers.

One early attempt to determine beauty by facial proportions was the Canon of Polykleitos. Although the text was lost during the centuries, it had great influence beyond its time. The roman architect Vitruvius argued that a building could only be suitable if it was well proportioned following the example of the well-proportioned human body as it had been observed and described by ancient sculptors (Vitruvius Pollio, first century BC [49]). In this context he mentioned the vertical division of the face in three equal parts: "One third of the length of the face is from the chin to the nasal orifices: From the nasal orifices to the place, where the nose ends between the eyebrows, an equal distance; and from here to the beginning of the hair, where the forehead ends, one third as well" (authors translation from German edition; Fig. 14.10). The height of the face was said to be one tenth of the whole body height, the whole head one eighth. This would mean the part above the trichion would be one quarter of the facial height.

In medieval times, artists like Villard de Honnecourt also depicted the Vitruvian vertical trisection of the human face. However, as the given proportions are put into fractions of small integer numbers, the accuracy will be limited.

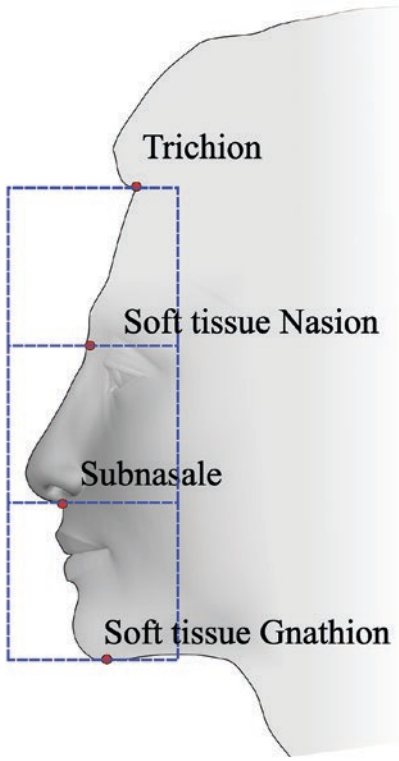

Fig. 14.10 Vitruvian vertical trisection of the face

Still in the early eighteenth century, Dionysius of Fourna instructed painters in his manual of iconography:

> Start by making the first measurement, which you divide into three sections: the forehead for the first, the nose for the second, and the chin for the third. Draw the hair out of your first measurement at the length of one nose. Divide the space between chin and nose into another three parts; two for the chin and one for the mouth […]. As big as one eye is, the other one is also and just as much is one away from the other (Authors' translation from German edition, Schaefer [50]).

During the Renaissance (ca. fifteenth and sixteenth century), in Europe ancient knowledge of geometry and facial proportions was rediscovered by artists and mathematicians like Piero della Francesca (1415–1492), Luca Pacioli (ca. 1447–1517), Leonardo da Vinci (1452–1519), and Albrecht Dürer (1471–1528). Both Dürer and Leonardo were not satisfied with the traditional information but published their own measurement results. Furthermore, they developed and built mechanical and optical instruments to improve measuring techniques and perspective. Dürer [51] still took over the Vitruvian division of the face by three. His sketch of a head was also based on a division of the whole body height by eight. In vertical direction, the proportion of the face to the whole head was 23/30 (i.e., ≈76.67%). Only if the head was considered as too big in comparison to the face, he reduced the upmost part but kept all other proportions. However, in contrast to Vitruvius and Dürer, other authors measured this proportion as one sixth of the facial height, thus dividing the height of the whole head by seven with the lower six seventh (i.e., ≈85.71%) making up the face from the chin to the trichion (e.g., Schadow 1834 [52]; Fig. 14.11). As the top of the head is often hidden by hair, a crown, or a hat or showing low contrast to the background, this structure is obviously not of much importance for the esthetic perception. Only if there are unfamiliar visual elements in this position, e.g., an extraordinary haircut, the top of the head arouses more interest (Yarbus described one situation, where an observer "spent considerable time examining the amusing tuft of hair on the child's head" ([14], p. 192). We would therefore suspect that the vertical proportion is not as important for the esthetic judgment as the area of the facial surface to the area of the whole head.

In horizontal direction, Dürer divided the face into ten equal parts. The outmost 1/10 was subdivided by 2. In the outmost 1/20, he drew the ears and short cut hair (Fig. 14.12). All in all, his division by ten reflects the older division by five, with each eye and the nose measuring one fifth.

From his construction of the head, Dürer went on by studying the esthetic effects if he scaled and bended the grid.

14.4.1 The Golden Proportion

If a line combined from two lines a and b has the total length $a + b$ and the ratio a/b equals $(a + b)/a$, then their proportion a/b is called the golden ratio, golden proportion, or golden section. There are only two possible solutions for a/b to the

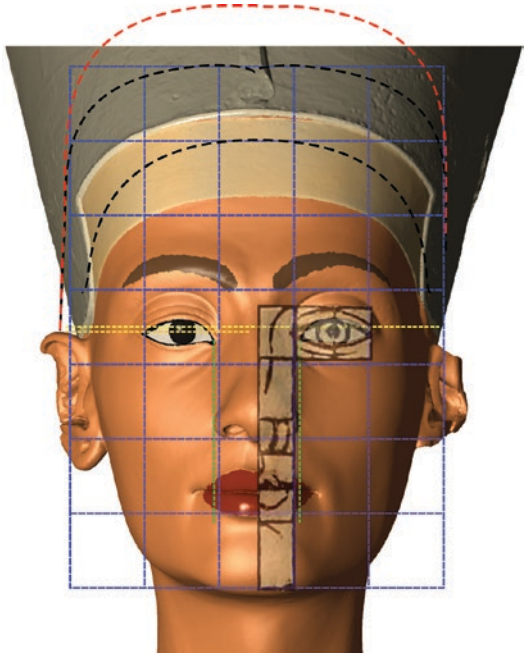

Fig. 14.11 Division of the face by seven squares in vertical and five in horizontal direction. In this example, the trichion and the top of the head (dashed black lines) had to be added because of Nefertiti's crown. In our picture, the right eye is slightly lower than its predicted position, and the inter-endocanthal distance and nasal width (green dotted lines) are slightly longer than the width of the eyes. The red dotted line depicts the position of the top of the head according to Dürer's proposal. He also used equal distances between the sides of the nose and the corners of the mouth (green dotted lines). The superimposed sketch from a detail of Dürer's proportional study (depicting a male figure) of the head was scaled to the pupil position of Nefertiti's bust in vertical direction and to the bipupillar distance in horizontal direction (which means that Dürer's sketch had to be stretched slightly in vertical direction). The comparison shows the narrow mouth, long nose, and short upper lip using Dürer's proposals. However, in his own portraits including his famous self-portrait from the year 1500, he obviously violated some of his rules

equation: 1.61803… and 0.61803…, both are irrational numbers and have interesting mathematical properties. The golden proportion can easily be constructed by using a right triangle with one leg half the length of the second one. Making a circle from the shorter leg to the hypotenuse and another from the point of intersection to the longer leg will cut the long leg in the golden section. The perimeter of a circle can be divided

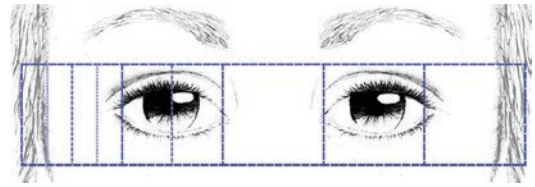

Fig. 14.12 Horizontal division of the face at the level of the eyes by five squares. The lateral borders usually are difficult to outline precisely as the perspective and hairstyle influence the appearance (artwork by Nina Runte). Dürer used a division by ten and subdivided the outmost section into 2/20. The outline of the face extended to the inner border of the last 1/20, hair and ears were placed within this last 1/20. Figures drawn by this rule have very tight fitting ears

into two parts in the golden proportion by the golden angle (222.49224…°; Fig. 14.13).

The golden proportion can be found in several two- and three-dimensional regular geometrical objects, e.g., the pentacle and Dürer's truncated triangular trapezohedron (Fig. 14.14). Approximations of the golden proportion are, e.g., 5/8 = 0.625, 8/13 = 0.61538…, 13/21 = 0.61905…, 21/34 = 0.61765…, and 34/55 = 0.61818. Each of these approximations is composed of two consecutive numbers from the Fibonacci sequence. Fibonacci numbers and the golden proportion often appear in nature, especially in the plant world (Fig. 14.15).

The golden proportion was supposed to be of special esthetic value. In 1876, Gustav T. Fechner [53] published an experiment, where test persons had to select the most pleasing rectangle from ten samples with different edge length ratios. His results indicated there was a preference for rectangles near the golden proportion (Fig. 14.16). However, Fechner's results could be reproduced in some studies, in others this attempt failed. Therefore, Friedenberg [54] called the confirmation of the golden proportion an "ephemeral" finding. In his studies, he found no preference for the golden proportion in triangles.

Nonetheless, some authors proposed the golden proportion as the clue to facial beauty (Danilas and Panagopoulos [55]: "Optimum aesthetic results may be accomplished with the application of the Golden Ratio in facial and body-contouring procedures").

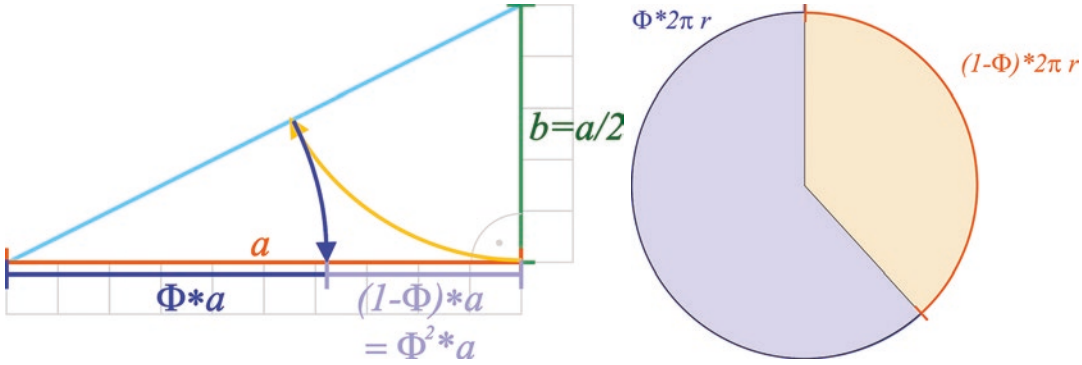

Fig. 14.13 The golden ratio derived geometrically (left) and the circle divided by the golden angle. *a* and *b* are the two legs of a right-angled triangle with $a = 2*b$. Φ is the golden ratio, *r* the radius of a given circle divided by the golden angle

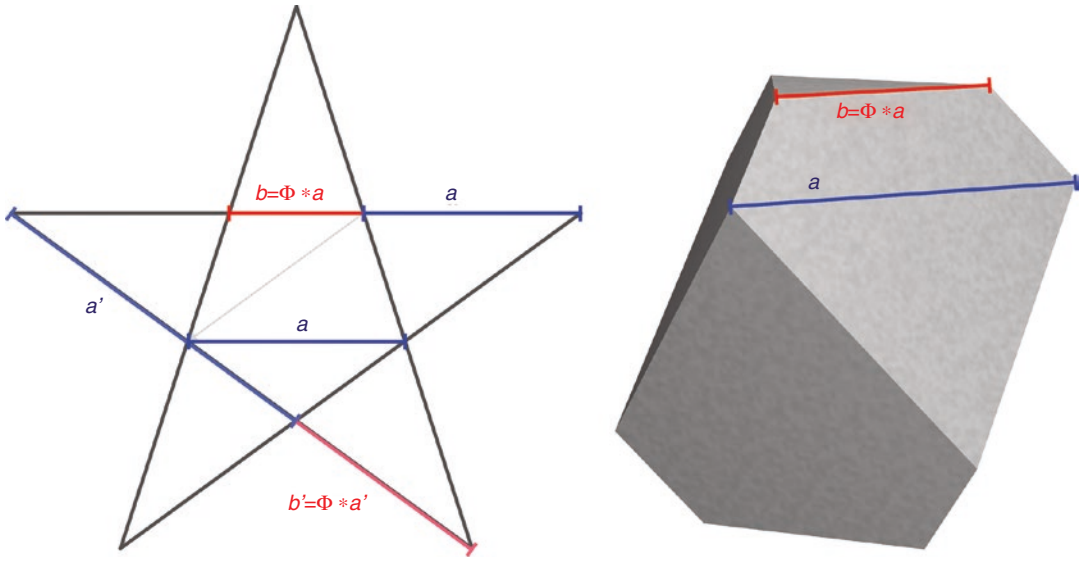

Fig. 14.14 The golden proportion within the pentagram and in Dürer's truncated triangular trapezohedron from the famous chalcography "Melencolia I"

Vertical and horizontal divisions of the head and face are depicted in Figs. 14.17 and 14.18, using the suggestions of Danilas and Panagopoulos [55] and Kois [56]. However, there are open questions about the use of the golden section for facial proportions: Do certain facial proportions really follow the golden ratio precisely? Is it reasonable to think of a proportion with favorable mathematical properties in facial structures? Does our understanding of the process of visual perception support the idea of a facial proportion that will please automatically? If this would be the case – why do other facial structures of esthetic importance (like the ratio of the Yarbus triangle, width/height of the eyes and lips) so obviously fail to match the golden ratio?

Alam et al. [57] could not verify the golden ratio in the height/width ratio and vertical division of the face in a Malaysian population with three ethnic groups. Only 17.1% of the subjects ($n = 286$ randomly selected persons) had a facial height/width ratio (facial index) between 1.6 and 1.699; the majority of the subjects had a shorter face (facial index <1.6). In addition, there was no correlation between the facial index and the facial evaluation score. Similar results were found for the Afro-

Caribbean population examined by Mantelakis et al. [58]. The only proportion matching the golden ratio in their study was the distance intereye line—soft menton/intereye line—stomion. Yet it must be noticed that using the soft tissue nasion instead of the intereye line and the subnasal point instead of the interalar line would have increased the vertical ratio in favor of the nasal length: In Mantelakis' study, the median was 1.523 for the best graded and 1.265 for the least well-graded photographs (male subjects). This should not be taken as a proof for the value of the golden proportion, as the proportion of the best graded faces is closer to 2/3 than it is to the golden proportion.

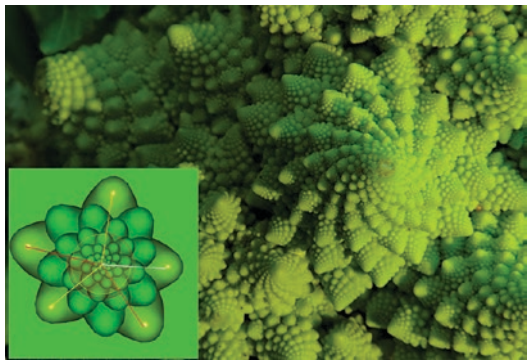

Fig. 14.15 Romanesco cabbage shows phyllotaxis using the golden angle. The inset on the left side shows the golden angles between the five most external branches. Fibonacci spirals and self-similarity at different scales are also properties with possible esthetic implications

The central incisor width was also suspected to be part of the sequence of golden proportions in horizontal direction. Abdullah's measurements [59] among 120 male and 109 female subjects showed a mean proportion between the inner canthal distance and twice the width of a single central maxillary incisor was 0.6181 for male subjects and 0.6222 for females, respectively. His results were confirmed by Arun Kumar et al. [60].

Kois [56] proposed the golden ratio not only between facial landmarks, but also between the perceived widths of the upper incisors, canines and bicuspid teeth for an esthetic outcome of dental treatment (Fig. 14.19). However, his proposals were only confirmed for long teeth by Rosenstiel [61]. Other authors did not verify the golden ratio in tooth proportions [62, 63].

We described several common landmarks used for proportional studies. If we only take the top of the head, trichion, nasion, tip of the nose, subnasale, stomion, pogonion and gnathion, and the points of intersection in the median sagittal plane with the intereye line and the interalar line, respectively, this makes in total ten points. The number of possible proportions between three points out of these ten can be calculated using the formula for combinations without repetition.

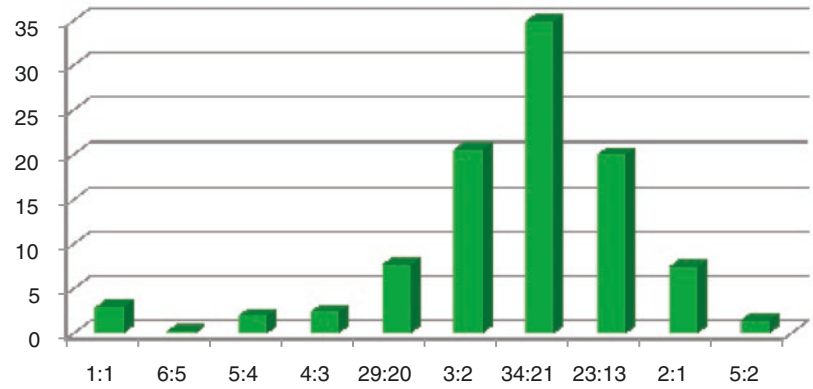

Fig. 14.16 Preference of the golden proportion (approximated as 34/21) from ten given rectangles (data from Fechner's experiment). Rectangle proportions are given on the x-axis; the y-axis shows the percentage of subjects choosing the respective ratio. Fechner used white rectangular pieces of paperboard with the same area (64 square centimeters) on a black background. His test persons had to choose the most pleasing rectangle. In cases where they were indecisive between two or three rectangles, all were chosen and rated in their proportion (1/2 or 1/3, respectively)

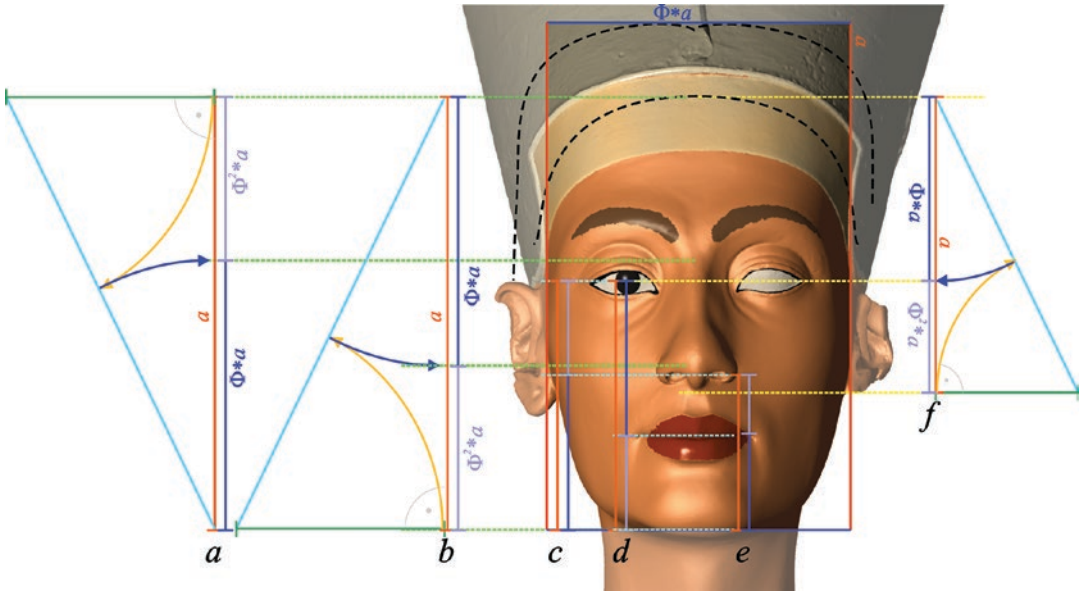

Fig. 14.17 Vertical division of the face by the golden ratio (Φ): several proportions of the face (but not all of them) approximate the golden ratio of 0.618034…. Most proportions are taken from Danilas and Panagopulos (2004, [55]): The frontal view of the head is said to show the golden proportion in height to width, which in this example leads to a slightly more slender proportion than Nefertiti's head. The classical proportion of 7/5 is slightly wider than the golden proportion and Nefertiti's head. The eyes should be at the center of the height of the head. In this example (from left to right), the soft tissue nasion divides the height of the face roughly in the golden proportion (*a*), and the tip of the nose is a landmark dividing the height of the face in the golden ratio as well (*b*) but in opposite direction. Both divisions are contradicting the Vitruvian division by three, as the height of the nose is much less than one third of the facial height. The vertical distance from the soft tissue gnathion to the bipupillar line is divided approximately in the golden ratio by the base of the nose (*c*) and by the inter-lips line (or the corners of the mouth) in the opposite direction (*d*). The mouth (stomion) divides the lower part of the face from the nasal base to the soft tissue gnathion near the golden proportion (*e*). This is an obvious difference to Dürer's proportions, as he used a ratio of 1/3 for the upper lip. And finally, the bipupillar line divides the distance soft tissue subnasal point to trichion roughly in the golden ratio (*f*). Other proportions, e.g., the height/width ratio of a triangle between the pupils and the mid-point of the lips, are approximately 12/11

In this case, 120 vertical combinations of three points are available (with the restriction to 2 lines with 1 common point). In addition, facial width measured between the two most external points, exocanthal and endocanthal points, pupils, alae, and corners of the mouth can be used as landmarks for horizontal or inclined measurements, adding another 12 points or 6 points on each half of the face. Counting out symmetrically identical points, a number of 16 points is available for proportional measurements; this makes 560 proportions in vertical, horizontal, and inclined directions. Obviously, there are enough possibilities to find any given proportion between 1/1 and 1/10 with a sufficient tolerance and measurement error. It is not our intention to abnegate any meaning of given proportions, but it seems sensible to judge them with less enthusiasm and more caution than it is frequently seen. Especially it should be questioned whether there is a true connection between a mere number (even if it is a number with special mathematical properties, easy to calculate, or called the "golden" one) and its appearance in the distances between three landmarks of the beautiful face or if this appearance is only coincidental or even a result of wishful thinking and choosing the appropriate landmarks.

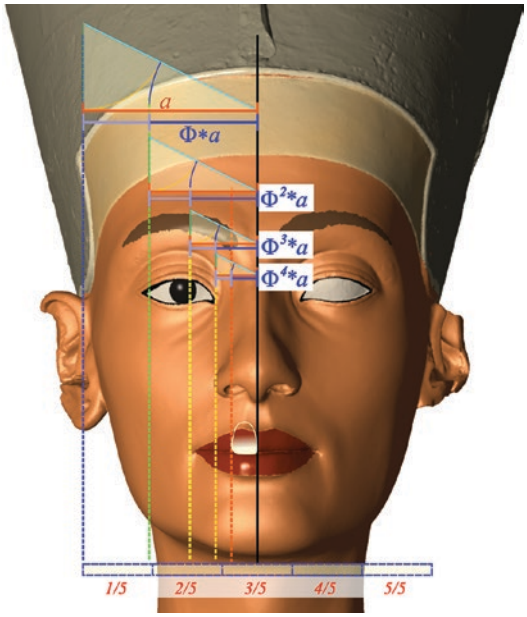

Fig. 14.18 Repeated horizontal division of the face by the golden ratio: In comparison to the division by five in equal parts (scale given below), using the golden ratio the way it was proposed by Kois [56] leads to slightly wider distance (+3%) of the exocanthal points (3/5 = 0.6 and $\Phi \approx 0.618034$). The same is true for the nasal width and the endocanthal distance (1/5 = 0.2 and $\Phi^3 \approx 0.236068$, i.e., +18%). Nefertiti's pupil does not fit to the position calculated by the nasal width in the golden proportion. The right central incisor was added according to Abdullah's measurement [59]

14.5 Beauty and Skin Color and Texture

Skin discoloration and unusual texture are frequent symptoms of diseases, some of them contagious and some even fatal. The healthy appearance of facial skin has been shown to be correlated to attractiveness [64, 65]. Several theories explain the evolution of skin pigmentation in humans based on the fact that pigmentation decreases with distance to the equator [66]. However, this does not necessarily affect attractiveness, as Langlois et al. [13] found a high cross-ethnic agreement in attractiveness judgments.

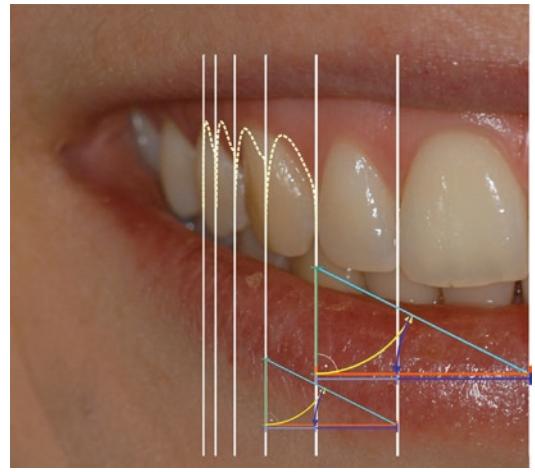

Fig. 14.19 Repeated horizontal division of the anterior teeth by the golden ratio in comparison to a natural smile: In this case and in the given perspective, only the incisors are well within the predicted ratio, but the visible surfaces of the canine and the following teeth are differently proportioned, e.g., the canine close to 90% of the lateral incisor. Dotted outlines indicate the predicted gingival shape within the golden ratio

14.6 Variation, Divergence, and Disfigurement

As we have discussed, beauty is difficult to define. The same is true for disfigurement. Disfigurements may originate from mechanical injuries, burns, congenital facial disorders, skin disorders including paraneoplastic and neoplastic ones, conditions after tumor surgery or radiation, and systemic diseases. They range from discrete to severely disfiguring. A variation from average parameters that is not relevant in the whole visual perception or presented in a special context may not be perceived as unpleasant but beautiful in its own way. Discolored faces are unremarkably in the context of the Holi festival. If a threat by a contagious disease is conclusively ruled out to the beholder's belief, an obvious skin discoloration like vitiligo [67] may not be disfiguring. Today, vitiligo is no longer an impediment for even becoming a famous model (e.g., Winnie

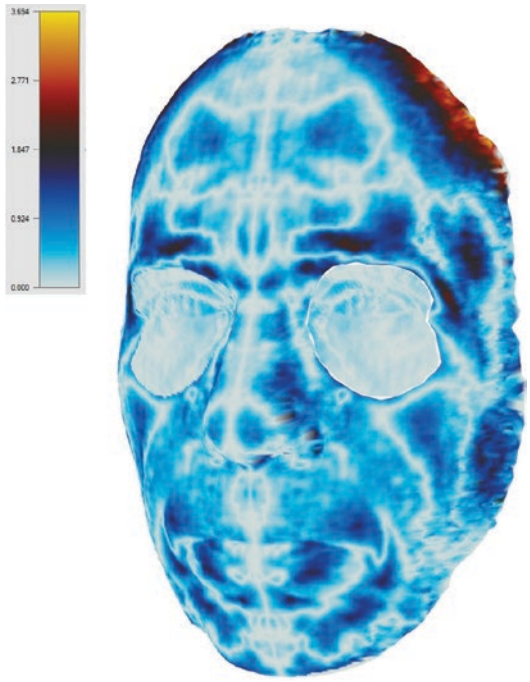

Fig. 14.20 Computerized analysis of a facial scan after orbital exenteration. Colors represent distances between the original and the mirrored surface. The orbital region from the healthy side has been mirrored to the affected one in order to construct a facial prosthesis. Therefore, symmetry is nearly ideal in this region (color scale in upper left corner)

Harlow). The concept categories and prototypes might be used as an explanation: If a new category with its own prototype is learned from the social environment or a given explanation, a formerly disgusting stimulus might become indifferent or pleasing (and vice versa).

Intra- and cross ethnic variations of facial proportions and skin color do not affect attractiveness substantially, as it has been shown by Langlois [13]. There are obvious variances in facial dimensions between trisomy 21 and euploid subjects; however, there is a large overlap [68].

In 1987, Higgins published his "self-discrepancy theory" [69]. According to this theory, the self-concept implies perceptions of actual, ideal, and ought self. Higgins explains how disfigurement leads to a discrepancy between the actual and the ideal self. As discrepancies are related to specific emotional reactions, depression might be a consequence of disfigurement [69, 70]. Psycho-social support may be necessary to help affected persons [71]. The serious consequences of a "lost face" and the feelings of affected persons have been described to the general public by Cole [72]. The reduction or elimination of prejudices against visual stimuli should be a social and political obligation. However, if a person's health, self-esteem, and social position are threatened by visible features, medical treatment has also to be taken into consideration, as disfigured faces cause disgust in the eye of the beholder [42]. Of course, the treatment decisions are among the most challenging ethic tasks in medicine. The threat of a pathologic background to the patient, the risks of therapy, and the relevance of the disfigurement have to be weighed up. The risk of body dysmorphic disorder also has to be ruled out [73].

The greatest need of treatment is found in the facial regions of attention, i.e., the edges of the Yarbus triangle. Not surprisingly, Stone and Potton [43] found the most negative emotional reactions to faces with disfigurements within the Yarbus triangle. Skin texture and structure variations with high contrast are distinctive and therefore should be corrected if possible, as they will also attract attention. Particularly, children are in need of care, as their development might be severely disturbed by disfigurements [74].

Hypertelorism is a severe consequence of craniosynostosis in Apert and Crouzon syndrome. It also gives the affected children characteristic facial appearance. Treatment includes craniofacial surgery to correct the facial appearance [75]. Another severe disfigurement is the orbital exenteration [76]. Treatment options include surgical reconstruction as well as facial prostheses. The latter can be constructed symmetrically using facial scans and CAD-CAM technology (Fig. 14.20). By reestablishing facial symmetry on the affected side, it is possible to approximate the patients' actual face to his "ought self" to a certain extent. However, the function of the eye cannot be regained today and facial prostheses

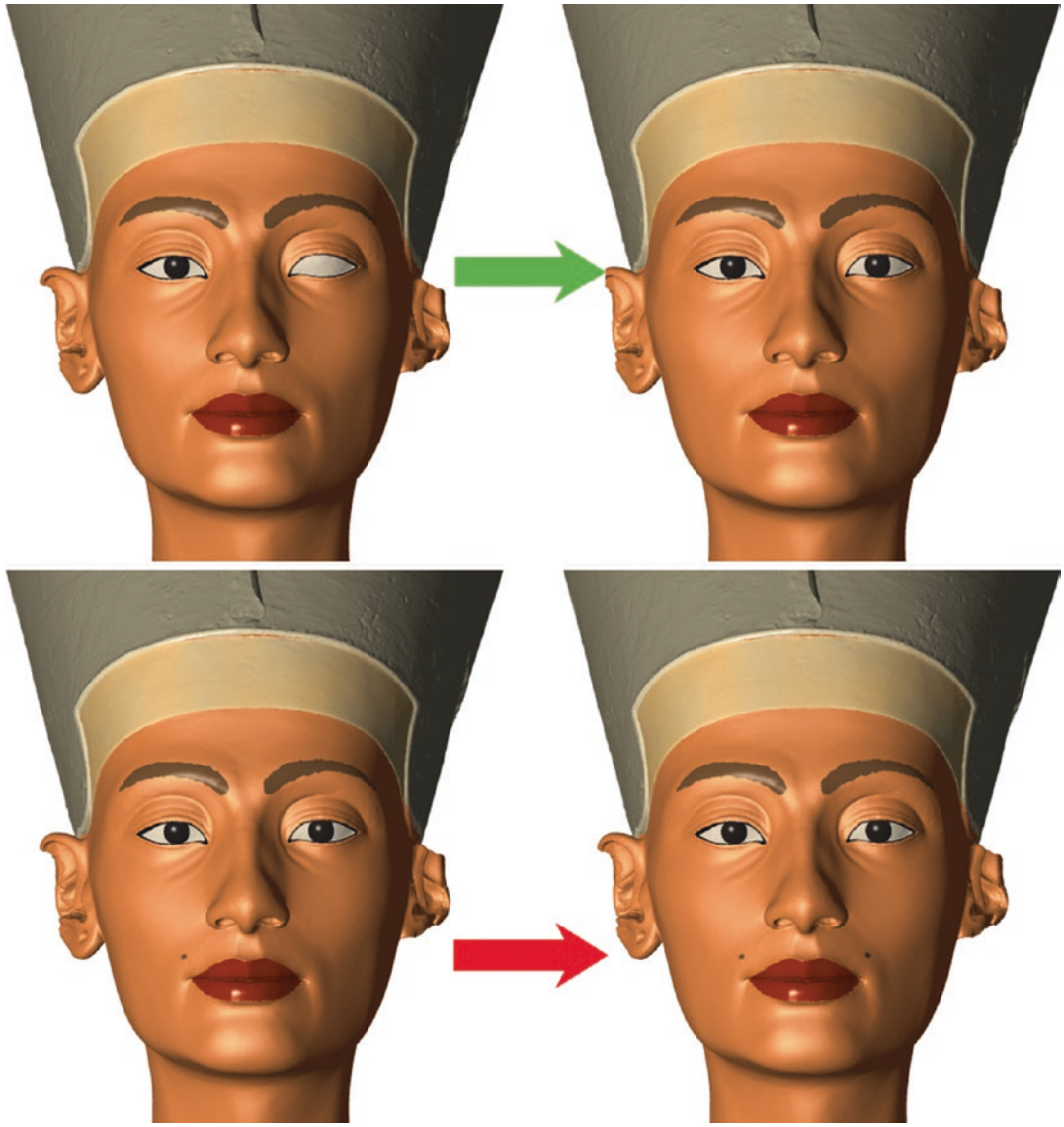

Fig. 14.21 Effects of symmetrization: Addition of Nefertiti's left eye reduces disfigurement (comparable to leukoma treatment [83, 84]), whereas a symmetrization of a nevus, which is disfiguring for itself, even increases disfigurement [85]. In contrast, adding leukoma to the right eye would even increase disfigurement as well as removing the single nevus on the right side would increase beauty. This exemplifies that categorizing a pattern as disfiguring might be more important than its symmetrical or asymmetrical appearance. Both patterns, the white, opaque eye and the dark spot on the facial surface, might indicate a contagious disease or genetic unfitness and therefore be explained by processes of evolutionary biology mentioned above

usually cannot mimic eye and lid movements, although prototypes of moving facial prostheses have been described [77].

The approximation of the ideal type of face, the average face, or the face of the patients' self-concept, respectively, is the goal for treatment. Proportions and symmetry are just helpful instruments to get there. For example, regaining symmetry by adding a blemish or nevus symmetrically on the healthy side will not at all reduce disfig-

urement (Fig. 14.21). Symmetry is not a sufficient condition for beauty as well as slight asymmetry is not for disfigurement.

Less disfiguring features of the periorbital region like receding eyelids or an upward shift of the upper eye frame by fat atrophy can be corrected with the help of proportion analysis (e.g., Benslimane et al. [78]; Fig. 14.22).

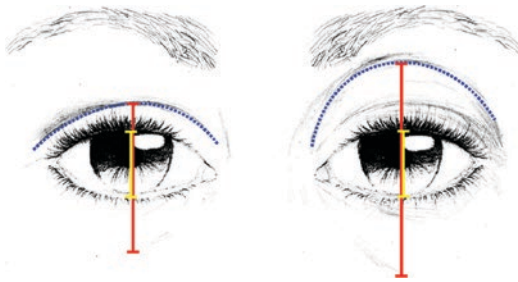

Fig. 14.22 Benslimane et al. [78] described a "frame ratio" defined by two distances: first the distance (red line) from the shadow of tear trough and lid-cheek junction to the most superior peripheral shadow (dotted blue line) of the upper lid and second the inter-lid distance at the vertical mid-pupillary line. With aging, especially the upper margin is influenced by fat atrophy leading to an upward shift. Furthermore, the upper margin is also usually more clearly visible because of a stronger curved surface resulting in a higher contrast. Benslimane claims that a lower frame ratio is related to a higher females' gaze attractiveness. This example shows frame ratios of 2.26 (left) and 3.23 (right; artwork by Nina Runte)

At the lower edge of the Yarbus triangle, the white line of anterior teeth, exposed while smiling, has a highlighted position. Unesthetic malocclusions will draw more attention to the oral region and significantly deteriorate facial attractiveness [79]. The beauty of a smile with reference to teeth can be summarized as the absence of discoloration, unbroken completeness (although the esthetic evaluation of a median diastema depends on the cultural background and may have changed in time [80, 81]), mirror symmetry, and balanced alignment and proportions. Dental treatment can have a positive influence on perceived disfigurement. Especially the upper anterior teeth show a high contrast to the oral cavity behind if the person is smiling with slightly opened mouth. Discolored or missing teeth as well as an unusual gingival display or gingival height discrepancies and asymmetries can be perceived as disfiguring (Figs. 14.23–14.25). From the laypersons' perspective [82], the highest level of agreement was found in variations concerning the overbite (in this context meaning the display of lower gingiva or intermaxillary inter-incisor space), the gingival display (i.e., the so-called

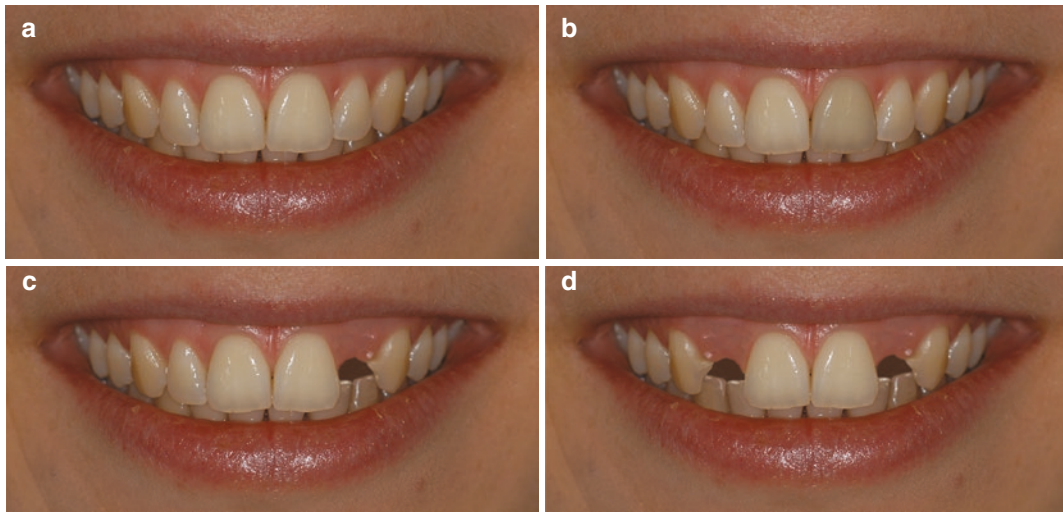

Fig. 14.23 Constructed variations of the esthetic smile: (**a**) original photograph, (**b**) discoloration indicating endodontic disease, (**c**) asymmetrical loss of lateral incisor, and (**d**) symmetrical loss of lateral incisors

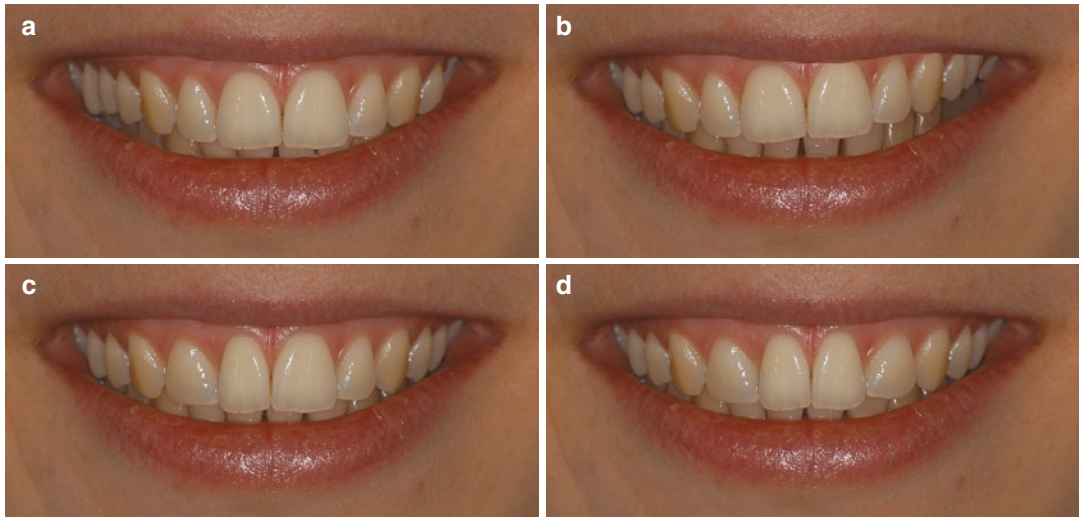

Fig. 14.24 Constructed variations of the esthetic smile in symmetry and proportion: (**a**) asymmetry by lateral shift, (**b**) asymmetry by inclination of the occlusal plane, (**c**) asymmetrical disproportion in lateral and central incisor width, and (**d**) symmetrical disproportion in lateral and central incisor width

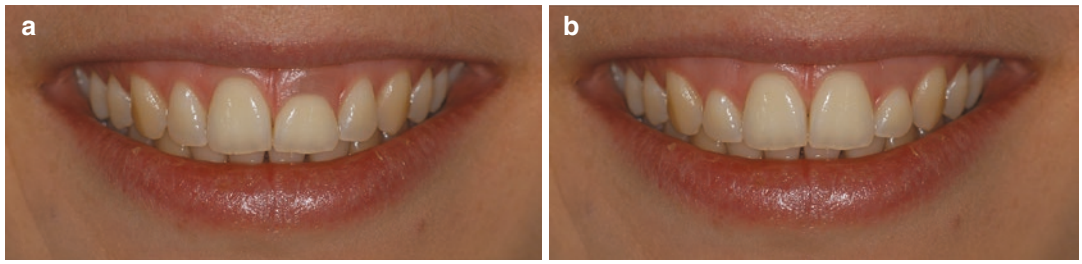

Fig. 14.25 Constructed variations of the esthetic smile in gingival height display: (**a**) asymmetric step in gingival display and (**b**) symmetrical step in gingival display as it was used similarly and identified as disfiguring by Ker et al. [82]

gummy smile), the width of the buccal corridor, and maxillary lateral incisor gingival height discrepancy (a step between the lateral incisor gingiva and the central incisor gingiva). Interrater agreement reliability was only poor in the judgment of midline discrepancies. Though dentists pay attention to midline asymmetries, a close look at Michelangelo's paintings in the Sistine Chapel, especially the Delphic Sibyl, will reveal that a symmetric mesiodens with no approximate contact in the midline does not necessarily affect the esthetic appearance of the face.

14.7 Conclusions

Although for centuries authors have tried to explain beauty, there are still many open questions. Our esthetic judgment is on the one hand not free but determined by the mechanisms of perception and influenced by experiences. On the other hand, people differ significantly in their esthetic judgment. However, violation of fundamental properties of the face like symmetry and average proportions leads to disfigurement and

may have serious psycho-social consequences. Treatment in these cases is essential for a successful rehabilitation.

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15 Psychosocial Adjustment of Patients with Congenital Craniofacial Malformations

Thomas Meyer

15.1 Psychological Factors Affecting Surgical Decision-Making

In children and adolescents with congenital orofacial malformations, the risk of self- and parent-perceived stigmatization and social discrimination is an important determinant in the decision to undertake reconstructive and orthognathic surgery. The indication for restorative intervention to correct for orofacial malformation and single-suture, nonsyndromic craniosynostosis in paediatric patients is based on the expected postoperative anatomical outcome as well as the improvement in quality of life and overall mental health [1, 2]. In a significant number of patients with visible craniofacial differences, advances in plastic and orthognathic surgery have placed these individuals at a lower risk of experiencing social stigmatization, most likely by activating an arsenal of pre-existing effective coping strategies that can help foster a positive self-image and better health-related well-being [3]. Vulnerability to social stigmatization is particularly high in early adolescence and puberty when physical attractiveness and outward appearance become important aspects of forming interpersonal interactions in peer relationships [4, 5].

Disfiguring conditions in patients with congenital craniofacial anomalies may result in various age-dependent psychosocial problems such as elevated anxiety, appearance-related social avoidance, and poorer quality of life [6]. However, trajectories of psychosocial functioning from childhood to early and later adulthood have not been well studied, and the long-term effects of restorative interventions need to be addressed [7, 8]. Particularly, the moderating effects of surgical corrections on the development of resilience and social functioning require further research efforts.

Given the complex relationships between the degree of orofacial disfigurement, the experienced pre-operative distress, and the expected postoperative achievement in mental well-being, the surgeon has to meet the psychological needs of a patient before considering a surgical treatment approach [9, 10]. The process of decision-making on surgery requires a comprehensive understanding of the patient's and his/her proxy's ability to cope with the social meaning of the disfigurement, the level of family support, and the age-dependent developmental stage [11]. The surgeon needs to understand the feelings of the patient and parents, which often differ with respect to the impact of the facial disfigurement on psychological vulnerability.

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15.2 Psychological Adjustments to Orofacial Disfigurement

Although the majority of infants and children with craniofacial malformations develop in a typical manner without major psychological problems, a significant number of affected children experience internalizing and externalizing problems and develop behavioural disorders, such as fearful shyness, depressive symptoms, and somaticizing disorders [12–14]. The available literature on psychological adjustments to congenital craniofacial abnormalities shows inconsistent results due to the lack of consensus on psychological constructs and diagnostic criteria as well to the heterogeneity of clinical phenotypes and disease entities [15–19]. The age-dependent development of social interaction skills may be impaired in socially inhibited children with severe craniofacial birth defects. Low self-esteem, reduced quality of life, and altered mental and emotional adjustment including the risk of social inhibition have been described in patients with craniofacial conditions [20].

Although numerous studies have suggested that children and adolescents with non-intellectually impairing craniofacial malformation may have an elevated risk of some types of psychosocial adjustment problems, it must be noted that most of these children develop normally and do not experience significant problems at a clinical level [21, 22]. The individual diagnosis and severity of the craniofacial anomaly may elicit specific patterns of psychosocial adjustment [23]. The majority of subjects with craniofacial conditions have learned to accept their visible facial difference and find ways to develop internal strength as a significant source of comfort and meaning in their lives [24]. Numerous publications studying psychological adjustments in children and adults with nonsyndromic orofacial malformations found comparable results to unaffected reference groups, while other papers reported significant variations in psychometrically assessed key psychological domains, such as mental well-being, health-related quality of life, and social functioning among patients with congenital craniofacial deformity [20].

15.3 Clinical Studies on Psychosocial Adjustments

In a cohort of 724 children and adolescents with congenital craniofacial anomalies aged 2–18 years from an urban hospital, who completed Child Behavior Checklists, most subjects did not report experiencing psychosocial adjustment problems, but may still be at an elevated risk of internalizing problems [22]. In adolescents with a cleft aged between 11 and 16 years from 145 families, Berger and Dalton found no significant psychosocial adjustment difficulties above that of the normal population [25]. In a later publication, these authors reported that psychosocial adjustment in adolescents was predicted by both their former social experiences and maternal well-being [26]. Using a cross-sectional postal questionnaire design, they demonstrated that dissatisfaction with appearance, speech problems, and the use of avoidant coping strategies were also important negative predictors for psychosocial adjustment [26]. In boys with clefts of the lip and/or palate aged 7–12 years, there was evidence from magnetic resonance imaging which suggested that aberrant development of the ventral frontal cortex was correlated with social dysfunction, but not with psychometrically assessed measures of self-concept [27].

Using the Strengths and Difficulties Questionnaire (SDQ), Brand and co-workers demonstrated that study participants with and without cleft lip and/or palate did not significantly differ with respect to emotional problems, conduct problems, or hyperactivity [28]. However, difficulties in interactional competence as measured by the PIELCQ questionnaire were more frequently observed in 32 children and adolescents from the group with clefts as compared to the 34 controls. Furthermore, the authors reported irregular sleep patterns to be associated with psychosocial strain rather than the presence of the cleft lip and palate deformity [28].

In a small sample of 25 adults with cleft lip and palate, Gassling and co-workers found no evidence of an abnormal habitual emotion regulation, as compared to an equal-sized control group of unaffected volunteers [29]. Scores from the Emotion Regulation Questionnaire (ERQ)

and the Ambivalence over Emotional Expressiveness Questionnaire G 18 (AEQ-G18) questionnaire showed no differences between patients and subjects from the control group. Likewise, using the Facially Expressed Emotion Labeling (FEEL) test, the authors reported that facial emotion encoding was similar between the two groups. In a Chinese sample of 94 patients with cleft lip and palate aged between 10 and 40 years, patients suffered from significantly lower general and social self-esteem as compared to 116 healthy controls with no dentofacial deformities [30]. In a recently published paper from the Whole of Life Survey in adults born with cleft lip and/or palate from the United Kingdom, the authors demonstrated that affected adults are at a risk of emotional distress from an early age that may persist to adulthood [31].

Interestingly, scientists from the Erasmus University Medical Center, Rotterdam, underlined the significance of patient satisfaction with facial appearance for social functioning rather than the objective severity of the deformity [32, 33]. Van der Elzen et al. showed that while social anxiety and distress did not significantly differ between adult patients with facial disfigurement and a reference group without facial deformities, the patient's own subjective appearance was a predictor of social functioning. The authors suggested that less frequent interpersonal behaviour was observed in those affected subjects who avoided stress caused by stigmatization. Van den Elzen and co-workers stated that disfigured patients used more often what they classified as immature defence styles, suggesting that low self-esteem may result in less frequent utilization of mature defence styles [32, 33].

15.4 Quality of Life in Patients with Craniofacial Abnormalities

Patients with major craniofacial malformations resulting in severe forms of deformity may be at a particularly high risk of experiencing sociopsychological stress, enduring low quality of life and/or developing psychopathological comorbidity [34]. Schliephake and colleagues from the University of Göttingen reported that, in their sample of 170 consecutive paediatric patients with orofacial clefts aged between 8 and 12 years, the quality of life was superior as compared to that of an age- and sex-matched control group of unaffected schoolchildren [35]. The quality of family functions affecting quality of life was lower in parents with cleft lip and/or palate children as compared to a control group, particularly when their children reach adolescence [36]. While the level of satisfaction with facial appearance is often reduced in congenital and acquired facially disfigured adults, any attempts to improve satisfaction with facial appearance either by surgery or by enhancement of self-esteem should probably improve long-term psychological functioning [37, 38].

15.5 Anxiety and Depression in Subjects with Congenital Orofacial Malformation

A recently published meta-analysis identified 11 studies reporting on psychosocial symptoms in adolescents with a visible difference as compared to unaffected peers [39]. The authors found that adolescents with a visible difference had experienced more symptoms of anxiety, but not depressive mood. Fear of negative evaluation by others, the perceived social support, and self-esteem are important predictors for anxiety in adult patients with congenital craniofacial conditions [40]. Interestingly, periods of depressive mood were more common in a cohort of 28 Scandinavian patients with Apert syndrome (acrocephalosyndactyly type 1), but patients did not differ with respect to a generally positive attitude towards life from a matched control group [41]. In a Norwegian study of 196 adolescents with a visible cleft, Feragen and colleagues found that affected boys, when compared to 1832 controls, reported significantly more positive perceptions of friendships and fewer depressive symptoms than the comparison group [42]. Besides sleep irregularities, patients with orofacial clefts were considered to have elevated levels of anxiety and depression and

to be at a higher risk of development of chronic pain states [43, 44]. The authors interpreted these results in the context of perceptions of social acceptance and emotional resilience.

15.6 Problems in Social Interactions Related to Craniofacial Abnormalities

Psychometric assessment using well-validated questionnaires, such as the self- and patient-reported outcome measure CLEFT-Q or the Craniofacial Experiences Questionnaire (CFEQ), provides valuable clinical information regarding the need for future surgical interventions [45–49, 19]. In addition, these instruments can be used to study associations between speech problems, the degree of unhappiness with facial differences, and health-related quality of life [50].

Negative self-perception of physical appearance is often found in young patients with congenital craniofacial anomalies, and concerns about their appearance are at a peak in adolescence [51]. Usually children with facial malformation first notice their difference at a mean age of 3 years [52]. An overall negative view of the self and difficulties with social interaction are particularly prevalent in preadolescents and adolescents [3, 53, 54]. The transition from childhood to early adulthood is the time when a subject feels increased strain to conform to social constructs of beauty standards and physical attractiveness. There is a significant societal pressure for these age groups to conform to cultural standards for both feminine and masculine beauty ideals. Damiano and colleagues analysed data from telephone interviews with mothers of children with nonsyndromic oral clefts and found that speech and aesthetic concerns became more important as their children got closer to adolescence, probably because the psychosocial burden related to the acceptance by peers generally becomes more critical in preadolescence [55].

There are well-established links between physical attractiveness and the likelihood of social acceptability, and personal achievement may be disadvantageous for subjects with orofacial malfunctions. Individuals with orofacial disfigurement have not only an abnormal facial appearance, but often additionally speech problems with atypical consonant production, abnormal nasal resonance and nasal airflow, termed hypernasality [56]. The imperfect physical appearance and the phonation disorder may result in subtle changes in the normal patterns of verbal and non-verbal communication. Patients with orofacial abnormalities often experience unfavourable social responses including teasing, bullying, and unwanted questioning, which they interpret as a form of not being fully accepted [57–59]. While the number of operations was not related to the overall psychological functioning, adult patients with a higher degree of residual facial deformity displayed more dissatisfaction with their facial appearance and usually had more frequent experiences of discrimination [8].

15.7 Gender Effects Related to Coping Strategies in Congenital Disfigurement

Although the view is widely held that females will have more trouble with orofacial disfigurement, this assumption may not be true since boys and young men are especially vulnerable to bullying when afflicted by facial disfigurement, which makes them feel physically weaker and less attractive to girls [11]. In 170 consecutive patients with nonsyndromal orofacial clefts, Kramer and co-worker found that, although gender was not significantly associated with family functioning, boys experienced a lower quality of life than girls, as measured using the Impact on Family Scale and the KINDL questionnaires [35]. In a sample of 74 children with craniofacial abnormalities, Shapiro and colleagues demonstrated that concerns about peer relationships were particularly prominent for boys, whereas girls reported the quality of their peer relationships as being comparable to non-affected peers [60, 61]. The authors demonstrated that self- and proxy ratings of child

satisfaction were uncorrelated and that dissatisfaction with the appearance of their faces was significantly associated with negative psychosocial outcomes in girls but not boys [60, 61]. Health-related quality of life in adolescents with oral cleft, as measured through the Short-Form Health Survey (SF-36) questionnaire, showed females in the three domains Bodily Pain, Vitality, and Mental Health statistically lower than males [62]. One study reported that parents of females with orofacial clefts expressed more concerns about their daughter's appearance than parents of males, whereas, in contrast, parents of boys were more concerned about vocational problems in their offspring [63]. Nidey and colleagues demonstrated that perceived social support was reported to be higher among parents of male affected children compared with female affected children. In contrast, parental psychosocial functioning was unrelated to the cleft type [64].

Shapiro and colleagues demonstrated that more complex diagnoses of craniofacial difference were associated with increased parenting stress and that there was a positive association between parental flexibility with respect to gender views and child-reported parent-child relationship quality [65]. Caregivers with more flexible gender attitudes were seen more supportive by their daughters but not their sons [65]. A longitudinal study in 47 children with craniofacial anomalies showed that parenting stress in early infancy predicted psychosocial adjustment in later toddlerhood, suggesting that dysfunctional patterns in parent-child interactions persist in some families with a child with a craniofacial anomaly [66].

15.8 Parenting Stress in Caregivers of Children with Congenital Orofacial Conditions

Maris et al. tested for insecure mother-child attachments in infants with orofacial clefts versus non-affected controls using the Strange Situation procedure, and they found that children with a palate cleft were less likely to be classified as having a stable attachment at 12 months, whereas at 24 months, no significant group differences in the attachment classification were observed [67]. In children aged between 5 and 6 years, the self-reported KINDL scores were higher in all dimensions than the proxy-rated estimation by their parents, demonstrating that self-rated quality of life in the children is superior to that which their caregivers estimated. These findings suggest that patients with craniofacial differences more frequently than their parents have developed the ability to implement a variety of effective coping strategies in order to counteract social stigma [3]. They consider themselves to be well adapted to their condition, having achieved positive self-esteem and developed stable interactional competence. There may also be a shift among various coping strategies in the mothers of affected children, as mothers of 13- to 18-year-old patients with nonsyndromic clefts reported greater use of a problem-solving coping strategy when compared with mothers of 8- to 12-year-old, younger patients [68].

One study suggested a reciprocal relationship between parenting stress and child adjustment [66]. Mothers of newborns and toddlers with craniofacial anomalies may be at an increased risk of experiencing clinically relevant depression and anxiety symptoms [69]. Perceived social support mediates the relationship between maternal psychological distress and their quality of life [69]. In 287 parents of children with oral clefts, fathers had a higher self-esteem and lower concern of being negatively judged by others than mothers [64]. However, fathers also reported a lower perception of communicating their problems to others than the mothers did.

15.9 Psychological Problems in Subjects with Craniosynostosis

In the existing literature, there are some reports on altered psychological development in children with single-suture craniosynostosis [16]. As compared to non-affected controls, patients with complex congenital malfunctions and impaired

neuropsychological development usually displayed fewer psychosocial conditions. Although the quality of life in adult patients with craniosynostosis is usually regarded to be lower than that of non-affected subjects, Lloyd et al. reported that adult syndromic patients with similar cognitive capacity perceive their self-rated quality of life as being even better than that experienced in a normative control population with no facial difference [70]. Fischer et al. showed that adult patients with Crouzon syndrome were less often married or had a partner when compared to a matched control group [71]. In their cohort of 31 patients and 285 controls, the authors reported that patients with Crouzon syndrome had fewer children of their own or experience of a sexual relationship while, in addition, they had a lower level of education. Stavropoulos and co-workers, who classified coping strategies in subjects with Crouzon syndrome, named this adaptation "lowering the expectations of finding a love partner" [72].

Although syndromic facial deformity is often associated with neurodevelopmental delay, patients affected with some syndromes showed normal development and intelligence. Cognition and intelligence are usually unaffected in subjects with Treacher Collins syndrome, a rare autosomal dominant disorder of the craniofacial development resulting in dysostosis mandibulofacialis and frequently also conductive hearing loss. Subjects with Treacher Collins syndrome showed no intellectual disability [73]. In a sample of six adolescents with Treacher Collins syndrome, ranging in age from 12 to 18 years, Beaune et al. found good psychosocial adjustment indicative of resilient adaptive strategies to balance the challenge of facial difference and social stigma [74]. A longitudinal study of parenting stress revealed that mothers of infants with a single-suture craniosynostosis reported stable and higher stress levels than fathers [75]. A Swedish group from the University of Göteborg reported data from a small cohort of 66 patients aged 3 years, who were operated on for nonsyndromal single-suture craniosynostosis. These patients did not differ from 180 randomly selected controls of the same age with respect to parental estimation of psychological development [76]. In a sample of 179 school-aged children with single-suture craniosynostosis from the United States, modest differences in language and memory were observed when compared to 183 controls [77]. From these and other studies, neurodevelopmental screening in infants with single-suture craniosynostosis may be justified [78, 79].

15.10 Summary and Outlook

Congenital craniofacial deformities may be associated with the risk of psychological problems, including social inhibition, low self-concept, externalizing problems, anxiety, and depression. However, having a facial difference can also foster greater resilience and shape more effective and mature coping styles. To enhance the aesthetic results, speech, self-image, and social competence in subjects with congenital craniofacial malformation, it is essential to identify the important determinants which modulate, both negatively and positively, their health-related quality of life. Given the complexity of measuring psychological adjustments and quality of life in paediatric and adult populations, currently available psychometric measures should be tested longitudinally and compared with each other. A particular focus should be given to long-term effects of surgical interventions on psychological parameters, which requires further research efforts. In addition, the development of comprehensive, valid, and reliable psychological instruments would be a valuable addition to both patient care and clinical research studying the impact of surgical and non-surgical treatments for patients with congenital craniofacial malformations.

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16 Cognitive State, Behaviour and Self-Assessment of Patients with Syndromic Craniosynostosis

Lennart Paul Sarbock and Ulrich Meyer

16.1 Introduction

Craniosynostoses are congenital disorders characterized by the early fusion of the skull's sutures. In rare cases these early fusions affect more than one area. Patients sometimes show body involvement. The condition can be simple, non-syndromic, complex or syndromic [1]. The most severely diseased patients are syndromic, of which Apert, Crouzon, Muenke and Saethre–Chotzen are the most common. Additionally, there is a wide range of possible outcomes even within a specific disease [1]. The kind and extent of the disease, as well as therapeutic outcomes, have a major influence on their life. Environment factors such as socio-economic status, schools and patient's and parental view play also a crucial role (Table 16.1, full details of patient-related factors). Patients with syndromic and complex craniosynostosis are known to have a lower health-related quality of life (HRQoL) [2], while patients with isolated craniosynostosis score within the normal range for quality of life and behavioural problems [3]. Reasons for the lower

Table 16.1 Influential factors of cognitive state, behaviour and self-assessment

Patient-related factors
Disease
Syndromic
Non-syndromic
Organ involvement
Viscerocranium
Neurocranium
Limbs
Body functioning
Phonetics, speech
Obstructive sleep apnoea
Motoric skills
Hearing
Vision
Smell
Cognitive functionality
Mastication
Surgery
Time of surgery
Kind of surgery
Number of operations
Post-traumatic stress disorder
Functional success of operation
Aesthetic result
Environmental-related factor behaviour
Self-assessment
Psychosocial aspects
Quality of life (QoL)
IQ
School performance
Formal education
Socio-economic environment
Physician–parental–patient interaction

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HRQoL are problems concerning physical functioning, bodily pain and mental health [4]. Commonly reported health-related problems in syndromic craniosynostosis are hearing and visual disorders, masticatory impairments, sleep apnoea and hand and foot anomalies [5]. The prevalence and severity of these problems vary per syndrome. It is unknown to what extent they influence the HRQoL and parents' perceived quality of life. The impairments imply the need for multidisciplinary care, with a varied staff of specialists, including craniomaxillofacial surgeons; plastic surgeons; neurosurgeons; geneticists; dentists; neurologists; speech and language pathologists; ear, nose and throat doctors; orthopaedists; social workers; and others [6]. The aim of this chapter is to outline which areas are most crucial when treating patients with syndromic craniosynostosis, based on adjusting their surroundings to their functional deficits to allow them to fully develop the maximum amount of cognitive skills within their limitations. The understanding of language and learning disorders observed in patients with syndromic craniosynostosis, relating to the several factors investigated, allows a better therapeutic approach and contributes to the understanding of neuropsycholinguistic disorders, addressing the parallelism between biological aspects (neuronal connectivity and brain circuits as a whole) and environmental aspects (adequate stimulation by healthy affective and challenging cognitive interactions) [7].

16.2 Genetics and Classification of Patients with Craniosynostosis

16.2.1 Classification of Syndromic Patients

An autosomal dominant mode of inheritance is suggested by the equal sex distribution of affected children in those families [8]. While the aetiology remains not fully clear, the causative factor in syndromic craniosynostosis is a dominant mutation in one or some of three fibroblast growth factor receptor genes: FGFR1, FGFR2 and FGFR3. Mutations of FGFR2 are most common with 93%, FGFR3 mutations occur in about 5%, and FGFR3 mutations occur in 2% of cases reported by the *American Journal of Medical Genetics* in 1998 [9]. Most cases are sporadic but there are reports in which affected females have given birth to affected children [8]. The gender of syndromic patients is evenly distributed [8] and their parents tend to be older than 35 years [10].

General classification of patients with syndromic craniosynostosis. Groups a–c show an increased risk of intellectual disability. For group d only a tendency towards intellectual disability has been shown so far [1]:

(a) **Apert syndrome.**
(b) **Crouzon or Pfeiffer syndrome.**
(c) **Muenke syndrome.**
(d) **Saethre–Chotzen syndrome.**
(e) **Complex craniosynostosis.**
(f) **Syndromic trigonocephaly.**
(g) **Frontal plagiocephaly.**

16.2.2 Classification of Non-syndromic Patients

There is a large amount of research on the cognitive functions and behaviour of children with non-syndromic craniosynostosis [1]. Children with non-syndromic craniosynostosis are of normal intelligence during their school-age years [11, 12]. Some show a tendency to isolate themselves and achieve slightly lower expressive language scores. However, the results of these studies vary greatly; some researchers report hardly any cognitive and/or behavioural problems in these children [1], while others report percentages up to 100%.

Non-syndromic craniosynostosis by classification [1] according to the concerned suture is:

(a) **Sagittal suture synostosis (scaphocephaly).**
(b) **Metopic suture synostosis (trigonocephaly).**
(c) **Coronal suture synostosis, unilateral (frontal plagiocephaly).**
(d) **Coronal suture synostosis, bilateral (frontal brachycephaly).**
(e) **Lambdoid suture synostosis (pachycephaly).**

16.3 Phenotype of Syndromic Craniosynostosis: Affected Parts of the Body and Organs

While non-syndromic patients usually exhibit a premature fusion of a single suture, syndromic patients are affected not only in the skull area (neurocranium) but also in the viscerocranium, some of them have also whole-body involvement. As the most common disease, Apert syndrome shows affections also in the extremities (hands and feet). The involvement of all these structures influences the life of patients, and therefore the most important are mentioned.

Midface: Skeletal hypoplasia of the midface and skull base changes functionality of the nose and eyes. Eyes can be protruding to various degrees and therefore may become dry if they cannot be shut fully. Correction of the affected vision is usually needed but there are cases of it being outside current possibilities.

CNS and brain morphologies: There are several studies showing the affected areas of the CNS, reporting congenital abnormalities of the CNS in 46.4% of patients [7]. According to the *American Journal of Medical Genetics* [13], these include malformations of the corpus callosum, the limbic structures or both. Other frequent findings include megalencephaly, gyral abnormalities, encephalocele, pyramidal tract abnormalities, hypoplasia of cerebral white matter and heterotopic grey matter. Progressive hydrocephalus seems to be uncommon and has frequently been confused with nonprogressive ventriculomegaly in the past [13]. The foramen magnum is smaller in patients with craniosynostosis syndromes compared to controls and is already smaller at birth. In addition to the timing of intra-occipital synchondrosis closure, other factors may influence foramen magnum size [14]. Multiple CNS and cervical spine (c-spine) abnormalities are common in Apert syndrome. The significance of these abnormalities remains largely unknown [15]. It was shown that hydrocephalus occurred more frequently in children with complex craniosynostosis syndromes [16].

Further anatomy changes: They are usually seen in the mid-ear, which, in combination with frequent otitis media with effusion, can cause permanent damage to hearing or even loss of hearing.

Extremities (hands, elbows, feet) frequently show syndactyly in Apert patients; not all areas have to be affected at the same time. Changes to the oral cavity, lip and tongue include clefts, anodontia, size and or form discrepancies of the maxilla and mandible.

16.4 Phenotype-Based Functionality Influenced in Patients with Syndromic Craniosynostosis

A range of factors influence the psychosocial development of patients. Intellectual deficiencies of children with craniosynostosis may be overestimated in the society, since impairments in phonetics, speech and articulation and motoric skills, hearing and vision lead to a deficiency in interpersonal communications and may be assessed by others as intellectual deficiencies.

16.4.1 Phonetics, Speech and Articulation

Syndromic and non-syndromic patients are both at an elevated risk of specific language/speech problems that are not necessarily the result of a lowered IQ [17]. Impaired speech and/or articulation must not be mistaken with overall intelligence. Causes for speech problems can be hearing deficits, oral anomalies, learning disabilities or impaired social interaction [17]. In one study, abnormalities in language abilities were observed in 66.67% of patients (based on school achievement tests) [7]. Verbal scale IQ was consistently lower than performance IQ in all of these children [8]. In another study, normal speech and language development occurs in one in 1.7 patients with non-syndromic craniosynostosis. The authors warrant that speech therapy for such abnormal development is needed in one in

3.4 of patients—a prevalence two to five times higher compared with the general paediatric population [18].

16.4.2 Obstructive Sleep Apnoea

Patients with syndromic craniosynostosis are at an increased risk to suffer from obstructive sleep apnoea syndrome (OSAS). OSAS in children is defined as a 'disorder of breathing during sleep characterized by prolonged partial upper airway obstruction and/or intermittent complete obstruction that disrupts normal ventilation during sleep and normal sleep patterns' [19].

It results in a variety of effects such as hypercapnia, hypoxemia and abnormal sleep architecture. Depending on the severity of the syndrome, vital dysfunctions to even fatal pulmonary heart disease may occur. Airway obstructions are more apparent during active sleep. There is a significant correlation between severity of upper airway obstruction and increased intracranial pressure in active sleep. Intracranial hypertension is also frequent in this group [20]. The impact of OSAS on the quality of life (QoL) in children has been largely underestimated [21]. Between 40% and 68% of children with syndromic craniosynostosis will have OSAS but the means of making this diagnosis vary between studies [22].

16.4.3 Motoric Skills

As part of the phenotype of the craniosynostosis syndromes, deformities of the extremities are frequently seen, varying from very mild with hardly any functional consequences to very complex with very severe functional limitations [1]. Deformities of the hands and feet are symmetrical; brachydactyly and osseous or cutaneous syndactyly ranging from total to partial fusion, but at least involving the second, third and fourth digits, are present. Synonychia is present in some degree. The distal phalanges of the thumb and the great toe are often broad and malformed [23, 24]. Patients with (still) unoperated hands frequently show paronychia. Limitations in performing tasks may lead to impatience and frustration, which in turn results in a difficult learning curve. However, patients show increased performance IQs (than verbal IQs), which contradicts the initial hypothesis of poorer visuo-motor skills related to hand deformities [8, 10].

16.4.4 Hearing

Unlike patients with non-syndromic craniosynostosis, patients with syndromic craniosynostosis tend to have hearing impairments [25–27]. Hearing loss can be an additional cause for developmental delay in children who already have an increased risk of such delay [1]. One longitudinal study describes how, over time, persisting otitis media with effusion (OME) led to permanent sequelae such as atelectasis, perforation and cholesteatoma in patients with Crouzon syndrome [28]. Ear and hearing impairment rates increased from 37% in infancy to 62% in older patients [29], confirming the high prevalence of otologic diseases in such patients. Middle ear disorders were responsible for the hearing impairment also in patients with mixed hearing loss due to secondary inner ear damage. Audiologic follow-ups are recommended.

16.4.5 Vision

Common abnormalities include orbital hypertelorism, telecanthus, abnormal slant of the palpebral fissures due to superior displacement of the medial canthi, ptosis, epiphora, proptosis and nasolacrimal apparatus abnormality, such as duct obstruction and punctal anomalies. Many of these manifestations are disfiguring and can threaten vision as a result of corneal exposure and globe luxation. Maintaining and restoring ocular and visual health are important parts of the overall care of a patient with isolated and syndromic craniosynostosis [30]. Additionally, high intracranial pressure over a longer period of time will destroy the optical nerve and may lead

to blindness. Eyes can be protruding to various degrees and therefore may become dry if they cannot be shut fully. Correction of the affected vision is usually needed, but there are cases of it being outside current possibilities. This impairs the capabilities of learning social interactions and results in a lowered QoL.

16.4.6 Smell

A potential loss of this sense will lead to the patients missing out on potentially important environmental information. There is some clinical evidence that olfactory function is not significantly altered on the biological basis of craniosynostosis, but iatrogenically induced; however, there aren't many studies. A loss of smell may be the result of fronto-basis operations like fronto-orbito-nasal advancements or Le Fort III distraction.

16.4.7 Brain Functionality

Cohen and his colleagues [23] assumed that mental delays were due to the increased intracranial pressure and elevated cerebrospinal fluid pressure that are found in patients with syndromic craniosynostosis. However, they also emphasised that the pathogenesis of this raised intracranial pressure and of the variable hydrocephalus, often but not necessarily associated with it, is largely unknown [8, 10]. Premature fusion of skull sutures presumably restricts skull growth and predisposes to elevated intracranial pressure. In another study [31], 20% of patients had raised intracranial pressure and demonstrated a significant restriction of skull growth. However, mental retardation is related not only to raised intracranial tension but also to important structural changes in the brain [32].

Mental retardation may also relate to the development of hydrocephalus, given the progressive destruction of axons and secondary myelin loss that may accompany this condition. Such a process may be operant in some instances, but a primary contribution of hydrocephalus to mental retardation in craniosynostosis appears minimal [33]. Hydrocephalus in the complex craniosynostosis syndromes occurs not as a late manifestation of uncorrected synostosis but, in most cases, secondary to intrinsic abnormalities in the embryologic development of the brain, presumably related to the defective formation of the cranium [16]. The incidence of hydrocephalus and mental retardation in craniosynostosis is lower than reported previously [16]. Early hydrocephalus can result in an uneven growth of intelligence during childhood. The cognitive deficit is neither due to the hydrocephalic condition itself or its treatment, but rather the development of brain anomalies and symptoms to which the hydrocephalic child is prone [34]. Malformations of the corpus callosum and size of the ventricles seem to play no role in the final IQ, whereas anomalies of the septum pellucidum seem to have a significant effect, with the proportion of patients with an IQ over 70 increasing more than twofold in patients with a normal septum compared with patients with septal anomalies [35].

16.5 Cognitive Aspects: Potential Risk Factors to Cognitive Aspects and Behaviour

16.5.1 IQ

Intelligence varies greatly per syndrome, but also within every syndrome [2]. Syndromic patients have a high to very high risk of a lower IQ. Due to impaired speech, a potential hearing loss, an altered sense of smell and/or an impaired vision, their learning abilities are strongly influenced and need the highest attention on a regular basis to adjust to their individual needs. A predictable outcome is subject to change if treatments occur timely. In the studies that have been conducted so far, it is important to note that an average IQ was found among most patients. More modern research indicates a higher perceived QoL, a potential motivation for future parents, because findings are contrary to the his-

torical impression that has regarded syndromic craniosynostosis as synonymous with intellectual disability [11, 36]. Reduced IQ and behavioural problems are negatively correlated [1]. Various studies [7, 8, 11, 16, 17, 37] show a high variety of IQ measurements. The wide intellectual variability is contributed to by overall adaptive functioning. More detailed evaluation of the array of cognitive skills, beyond intelligence, that contribute to the overall adaptive functioning would help elucidate whether the current sample of children displayed cognitive deficits in areas that may not be detectable by means of intelligence testing alone [11].

16.5.2 Socio-Economic Status

Family environment is an important factor involved in intellectual achievement. Its quality influences the mental development: 12.5% of patients who were institutionalized or in difficult family situation have an IQ >70 compared to 39% of those who live in a normal family. All the institutionalized patients in this series were children who were abandoned at birth, and therefore, mental retardation appears to be the consequence rather than the cause of institutionalization [8, 35]. Several authors [38–40] have stressed the 'irreversible tragedy of the institutionalization' [39] of these children. Frequently, children with Apert syndrome are misdiagnosed as mentally retarded solely based on their appearance and are withdrawn from regular schooling where they may well have coped. Children with syndromic craniosynostosis should be kept in a normal family environment and actively stimulated with the aid of psychologists [35].

16.5.3 School

Acceptance within the school may vary vastly from case to case. Isolation due to avoidance of conflicts or uncomfortable situations can be frequently observed. Therefore, learning is influenced and may result in lowered IQ.

16.5.4 Gender

For all genders, the beginning of puberty marks a change in perceived situations. Patients might start developing a reluctance to be treated. The development of the child's self-perception and self-confidence is mainly influenced by their parents [1]. Aesthetics are perceived as a greater issue for female patients. Surgical outcomes must be communicated to avoid mismatched expectations.

16.5.5 Success of the Operations

The kind of clinical outcome is dependent on the quality of conservative and surgical therapies. The outcome will improve in centres with an extensive experience with these patients. A wide range of possible complications from the very first operation and all treatments throughout the years until the patient's adulthood may influence the outcome. Beneath the objective results of operative procedures, communication between patients, parents and physicians is always key. Often the physicians are more content about the surgical outcome than the patients might be, if not explained properly beforehand. Young children with congenital facial deformities usually rate their appearance more favourably than do their parents and strangers, but that these self-ratings of appearance and self-esteem sharply decrease in adolescence [41].

16.5.6 Timing of Surgeries

The timing of surgery for craniosynostosis is still controversial [42]. The age at operation appeared to be the main factor associated with changes in mental development in one study [35]. The final IQ was greater than 70 in 50% of patients operated on before 1 year of age versus only 7.1% in patients operated on later in life [35]. Self-esteem improved significantly after surgery. The mean increase was 29% (range 2–49%) which is highly significant [8].

16.5.7 Post-Traumatic Stress Disorder and the Amount of Operations

Surgery for craniosynostosis implies a relevant strain on the child and the parents. Pain, complications of each operation or specific complications unrelated to craniosynostosis, such as prematurity, meningitis or trauma, will stress the child [43].

16.6 Behaviour of Patients with Syndromic Craniosynostosis

Patients with craniosynostosis, syndromic or non-syndromic, often face social discrimination. Their facial appearance is typically considered to be less attractive and is often stereotypically considered as less capable, less intelligent and less honest. Their facial appearance interferes with personal life, employability and social interaction. Many investigations have shown that disfiguring conditions can lead to various psychosocial problems such as high level of social anxiety and social avoidance and poorer quality of life. There is a growing body of literature pointing to an increased prevalence of learning difficulties, attention-deficit/hyperactivity disorder [34] and social and behavioural dysfunction in school-aged children with syndromic and non-syndromic craniosynostosis as they mature [1]. Higher levels of behavioural and emotional problems are related to lower levels of intellectual functioning [34].

Non-syndromic or syndromic craniosynostosis patients with an IQ lower than 85 have a strongly increased risk of behavioural problems, similar to all other children without craniosynostosis [1]. Children with Apert syndrome often present with clinical features of hyperactivity [8] and other signs of attention-deficit disorder. It is important to note though this could be part of the syndrome or related to an extraneous variable such as sleep disorder or head shape.

16.7 Self-Assessment

16.7.1 Psychosocial Aspects

Both the genetic changes and their outcomes but also the medical treatments, operations and factors involved around the various treatments influence psychosocial aspects. Furthermore, severe craniosynostosis is not only influencing the patient's life but also their families, friends, schools and workplaces [1]. The following stages can be differentiated:

1. **Age until the first operation**: Uncertainty about the diagnosis and expected development, coping of the patient and parents, the abnormal appearance, education and having to deal with contrasting explanations in the hospital. This time period can however serve as a framework for early intervention [1].
2. **Age until first school**: Choosing a school often is difficult. The availability of trained social workers varies widely.
3. **Age until puberty**: Influenced by the success of operations, differing greatly among gender.
4. **Age after reaching adulthood**: Influenced by unoperated areas or the aftercare of treatments.

16.7.2 Quality of Life (QoL)

QoL is an important tool in measuring health-related outcomes in clinical medicine. The overall quality of life is lower in patients with syndromic and complex craniosynostosis [2, 4]. However, more recent studies indicate counterintuitive findings: adult syndromic patients with similar cognitive capacity perceive their QoL as being above that experienced in a normative non-syndromic population with no correlation to the degree of facial difference [44], and both the highest-functioning Apert patients and the Crouzon patients presented a satisfactory quality of life, demonstrating that these syndromic patients had acquired the necessary repertoire to manage the adverse daily situations of their lives [45].

16.7.3 Patient's View and Parental View

Parents of a child with syndromic craniosynostosis suffer from different obstacles: many referrals, unnecessary or inaccurate or incorrect diagnostics and receiving incomplete or incorrect information. As the child and the parents are the persons who have to cope with the main stressors and strains associated with their child's disorder and its treatment, their opinions should ideally be part of any outcome evaluation. This topic has not been adequately addressed in the medical literature [46]. An important factor to keep in mind in these parent–patient settings is the possibility of a significant disruption in the mother–infant attachment and bonding process in the group of malformed infants. Early corrections may help to minimize any disruption in the mother–infant bonding process [37].

16.7.4 Suggestions on how to Approach Treatment

Coordinated care is necessary, given the complexity of the medical, surgical and psychosocial factors. Early intervention can lead to fewer operations. Care in a team setting is essential because outcomes are measured throughout the child's growth and development. Until recently however, there was no consensus on the parameters of care [30].

Regular screening on obstructive sleep apnoea, ophthalmologic disorders and hearing deficits to allow a timely intervention according to the individual needs to allow full cognitive development and coping mechanism for the patients and their families is important. There should be a regular control of the wishes of the patients and parents. Objectively showcasing the therapies of choice and their potential success is of special relevance. One personal/care coordinator for the families to approach who handles and supervises the multidisciplinary treatment should be the leader and should be easily accessible for the patients. The interdisciplinary treatment team must be established as an integral part of the social network of patients. Regular consultations alongside the therapies are advisable. If operations are necessary, it is of special relevance that an appropriate postoperative pain management is delivered. Assessment of cognitive functioning, behaviour and psychosocial functioning is considered an essential aspect of follow-up care for children with craniosynostosis, both non-syndromic and syndromic [47]. Future research directions should ideally centre on examining interrelationships between cognitive, genetic, neurologic and morphologic factors so that predictors and correlates of cognitive outcomes can be explored in these disorders [11, 36].

16.8 Recommendations on Assessing the Patients' Quality of Life

Based on existing questionnaires on patients with facial disfigurements, we developed a modified questionnaire. This questionnaire offers an approach of acquiring comprehensive medical, social and psychological assessment of patients and relatives (parents).

It is based on established scientific QoL tools, but subsequently adjusted by a multidisciplinary working group involved in treating patients with craniosynostosis and related professional disciplines and involved groups such as the self-aid group in Germany with the intention of standardizing the approach on how to evaluate and manage patients with severe craniosynostosis—a view similarly shared by other authors [47].

It must be noted that all data should be interpreted with caution, as the number of participants in studies is generally low. This is the weakest point of all studies, but difficult to overcome, since craniosynostoses are rare diseases. Therefore, it is difficult to recruit a larger number of patients and create homogeneous groups for comparison. In general, the groups are heterogeneous, since also surgical procedures and results differ in between studies [48].

Quality of Life Questionnaire of Patients with Syndromic Craniosynostosis

Please answer every question. There are no correct or incorrect options.

Please choose the most appropriate option available to you.

Please fill out both parent parts. (Additional questionnaires are attached.)

This questionnaire is based on internationally approved questionnaires (CHQ, OPQOL-35 for adults and kids), but modified for patients with syndromic craniosynostosis.

Objective Evaluation of the Anamnesis and Therapies

General information	Parent I	Parent II	Kid
Gender	m/f/d	m/f/d	m/f/d
Date of birth			
Occupation			
Highest degree of education (year)			
Time of diagnosis (and which syndrome)			
Time(s) of operation(s) Amount of operations			Additional information may be added separately (results, doctor statements, etc.)

Affected body parts		If parent is affected (I/II)	Kid
Maxillo-facial	Please fill in	∘ Healthy	∘ Healthy
Ear–nose–throat		∘ Healthy	∘ Healthy
Eyes		∘ Healthy	∘ Healthy
Neurology		∘ Healthy	∘ Healthy
Orthopaedics		∘ Healthy	∘ Healthy
Psychology		∘ Healthy	∘ Healthy

		If parent is affected (I/II)		Kid	
Current therapies		Journey	X-rays	Journey	X-rays
Surgery	Please fill in	No/km	No/amount	No/km	No/amount
Orthodontics		No/km	No/amount	No/km	No/amount
Logopaedics		No/km	No/amount	No/km	No/amount
Physiotherapy		No/km	No/amount	No/km	No/amount
Ergotherapy		No/km	No/amount	No/km	No/amount
Additional med. Cond.		No/km	No/amount	No/km	No/amount

Planned therapies		If parent is affected (I/II)	Kid
∘ Yes	∘ No	∘ Yes	∘ No
∘ Please fill in		∘ Please fill in	

Subjective Evaluation of the Medical Condition and its Aspects

First, parents fill in the parent's part. (2. A).

Second, kids fill in the kid's part. (2. B)

– Ask your kid(s) if they are older than 10 years.
– Guess the most appropriate answer for you kid(s) if they are younger than 10 years.

A—Parent I/Parent II

Parent's estimation of the bodily situation of their kid(s)				
○ Very good	○ Good	○ Neutral	○ Bad	○ Very bad

Parent's estimation of the emotional situation of their kid(s)				
○ Very good	○ Good	○ Neutral	○ Bad	○ Very bad

☹ I do not agree ☹ I slightly disagree 😐 I don't know ☺ I slightly agree 😀 I fully agree					
Keeping in mind all of what's positive and negative in your life, How would you describe your life generally?	☹	☹	😐	☺	😀
I am content with my life.	☹	☹	😐	☺	😀
I am happy most of the time.	☹	☹	😐	☺	😀
I am happy about what's to come in the future.	☹	☹	😐	☺	😀
Many things seem difficult in my life.	☹	☹	😐	☺	😀
I have a lot of energy.	☹	☹	😐	☺	😀
Pain is influencing my life.	☹	☹	😐	☺	😀
Due to my medical condition I need help at home.	☹	☹	😐	☺	😀
I am healthy enough to be independent.	☹	☹	😐	☺	😀
Family and friends are of great help.	☹	☹	😐	☺	😀
I yearn for more joy or social contacts.	☹	☹	😐	☺	😀
I can share my life with somebody.	☹	☹	😐	☺	😀
I have kids which are important to me.	☹	☹	😐	☺	😀
I have a strong connection with my kid(s).	☹	☹	😐	☺	😀
I have more, non-syndromic kids.	☹	☹	😐	☺	😀
The medical condition of my kid(s) is a burden for its siblings.	☹	☹	😐	☺	😀
I cannot devote my time evenly among my kids.	☹	☹	😐	☺	😀
The birth of my kid was a traumatic experience in my life.	☹	☹	😐	☺	😀
I have experienced fear and sorrow for my kid.	☹	☹	😐	☺	😀
I have learned to cope with the medical situation of my kid(s).	☹	☹	😐	☺	😀
I have control over my life.	☹	☹	😐	☺	😀
Do you have psychological counselling?	☹				😀
Was the psychological counselling adequate?	☹	☹	😐	☺	😀
As parents we are able to distribute the responsibilities evenly.	☹	☹	😐	☺	😀
I can work for my own financial freedom.	☹	☹	😐	☺	😀
I have enough money to pay all bills.	☹	☹	😐	☺	😀
State insurance sufficiently covers all needed therapies.	☹	☹	😐	☺	😀
The choice of kid's school was simple.	☹	☹	😐	☺	😀

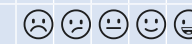
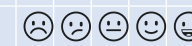
I am content about the educational programme of my kid's school.	☹	☹	😐	🙂	😀
The medical condition of my kid caused him/her to have developmental issues.	☹	☹	😐	🙂	😀
The developmental issues of my kid were easier to handle than expected.	☹	☹	😐	🙂	😀
My kid is well integrated into his/her school.	☹	☹	😐	🙂	😀
My kid's behaviour is making it harder for him/her to have friends.	☹	☹	😐	🙂	😀
I take life for what it is and make the most of it.	☹	☹	😐	🙂	😀
There is more joy in my life than in others.	☹	☹	😐	🙂	😀
I try to see the positive in all things.	☹	☹	😐	🙂	😀
If there's something I cannot do, I seek alternatives.	☹	☹	😐	🙂	😀
I have social activities and/or hobbies which I enjoy.	☹	☹	😐	🙂	😀
Due to my kid's operations, I am absent at work frequently to look after him/her.	☹	☹	😐	🙂	😀
My responsibilities prevent me from pursuing more of my interest.	☹	☹	😐	🙂	😀
The operations of my kid are taking a toll on me.	☹	☹	😐	🙂	😀
Cultural/religious events are of great importance in my life.	☹	☹	😐	🙂	😀
The medical situation of my kid(s) is affecting my emotions.	☹	☹	😐	🙂	😀
The medical situation of my kid(s) is consuming much of my time.	☹	☹	😐	🙂	😀
The medical situation of my kid(s) is preventing me from doing the things I want to.	☹	☹	😐	🙂	😀
Due to the medical situation of my kid(s), I get to spend less time with my friends.	☹	☹	😐	🙂	😀
Due to the medical situation of my kid, financial issues arise.	☹	☹	😐	🙂	😀
Medical staff is taking good care and advising me well on the situation of my kid.	☹	☹	😐	🙂	😀
Self-aid groups help me…	☹	☹	😐	🙂	😀
I feel supported with the medical situation of my kid(s).	☹	☹	😐	🙂	😀
The medical caretakers of my kid(s) are well connected.	☹	☹	😐	🙂	😀
The medical staff are handling my kid well.	☹	☹	😐	🙂	😀
Finding specialists was easy.	☹	☹	😐	🙂	😀
I feel well advised about the medical condition of my kid(s).	☹	☹	😐	🙂	😀
I wish to have more written information about the medical condition of my kid(s).	☹	☹	😐	🙂	😀
The medical condition of my kid is mostly described in a negative way.	☹	☹	😐	🙂	😀
Please feel free to make any additions or extra remarks to any of the topics mentioned or not mentioned.					

B—Kid

– Ask your kid(s) if they are older than 10 years.
– Guess the most appropriate answer for you kid(s) if they are younger than 10 years.

Score the extent of the specific symptoms.

Head	☹ 😕 😐 🙂 😀
Midface	☹ 😕 😐 🙂 😀
Teeth alignment	☹ 😕 😐 🙂 😀
Breathing/sleep	☹ 😕 😐 🙂 😀
General looks	☹ 😕 😐 🙂 😀
Hands	☹ 😕 😐 🙂 😀
Feet	☹ 😕 😐 🙂 😀
Additional symptoms (please fill in):	☹ 😕 😐 🙂 😀
☹ I do not agree 😕 I slightly disagree 😐 I don't know 🙂 I slightly agree 😀 I fully agree	
I am sleeping well.	☹ 😕 😐 🙂 😀
Eating and drinking is easy for me.	☹ 😕 😐 🙂 😀
There is nothing affecting my taste.	☹ 😕 😐 🙂 😀
Speaking is easy for me.	☹ 😕 😐 🙂 😀
I have no hearing issues.	☹ 😕 😐 🙂 😀
My sight is good.	☹ 😕 😐 🙂 😀
My flow of saliva is of no issue.	☹ 😕 😐 🙂 😀
I can smell well.	☹ 😕 😐 🙂 😀
I have to clean my face often (tears, runny nose, etc.).	☹ 😕 😐 🙂 😀
Writing of manual tasks is easy for me.	☹ 😕 😐 🙂 😀
I can tolerate my limitations well.	☹ 😕 😐 🙂 😀
I am fine with the way I look.	☹ 😕 😐 🙂 😀
I am often in pain.	☹ 😕 😐 🙂 😀
Heavy work is easy for me.	☹ 😕 😐 🙂 😀
I can move freely and well.	☹ 😕 😐 🙂 😀
Pain affected my sleep during the last week.	☹ 😕 😐 🙂 😀
I have to take medication regularly.	☹ 😕 😐 🙂 😀
Each operation is affecting me greatly.	☹ 😕 😐 🙂 😀
All of my operations went well.	☹ 😕 😐 🙂 😀
My therapists make me feel safe.	☹ 😕 😐 🙂 😀
I feel limited in my education.	☹ 😕 😐 🙂 😀
I get along well with my classmates/colleagues.	☹ 😕 😐 🙂 😀
Classmates/colleagues are isolating me.	☹ 😕 😐 🙂 😀
My different appearance is accepted well.	☹ 😕 😐 🙂 😀
I am content about my life.	☹ 😕 😐 🙂 😀
I am happy most of the time.	☹ 😕 😐 🙂 😀
I am looking forward to the future.	☹ 😕 😐 🙂 😀

Much in life seems to be difficult for me.	
I have a lot of energy.	
Pain is influencing my life.	
Due to my medical situation I need help at home.	
Getting to my therapists takes a lot of time.	
I have had a lot of negative experience with specialized medical staff.	
Friends and family are of great help.	
I wish to have more social contacts.	
There is somebody I can share my life with.	
I have social activities or hobbies which I enjoy.	
I take life for what it is and make the most of it.	
I have more luck in life than others.	
I try to see the positive in life.	
If there is something I cannot do, I am looking for alternatives.	

How many times were you in pain during the last week?	None	Seldom	Sometimes	Often	Constant
How long did the pain last?	Seconds	Minutes	Hours	Days	The whole week
How is your pain at different times?	None	Seldom	Sometimes	Often	Constant

Free Text/Doctor Statements

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Psychological Impact of Facial Disfigurement

17

Jörg Handschel

Man as "homo sociologicus"—this definition by Dahrendorf [1] brings the high importance of social community for human existence to the point like probably no other before. Man is a social being and therefore needs a certain social embedding in order to be able to live his life successfully. How this social embedding looks like in concrete terms, however, can vary considerably [2]. In order for this embedding and social interaction to succeed, rules and norms are formed in societies. People orient themselves by these rules, values and norms when they cast their actions in the form of roles or when they carry out their behaviour according to certain regularities, in interaction and relationship structures, then social order is established and continued. But this harmonious picture of social order is only one side of society. Expectations and norms are always violated; rules are disregarded, openly questioned or abolished. People and their behaviour do not always correspond to what is expected (both statistically and normatively expected). People deviate from the rule, from the norm and from custom [3]. Normality (from Latin norma = rule, guideline) is merely a statistical measure. The average expression of a characteristic of the majority of a population is called normal. A person is considered normal if his appearance and behaviour corresponds to that of the majority. The measure of the normal lies outside of itself. It is assigned to him by collective conditions. So it is obvious that people with a different appearance are outside this norm and may experience corresponding reactions from their environment or at least feel themselves to be abnormal, not belonging. Especially changes in the head and neck area are predestined to attract the attention of others. These changes can be innate or acquired. The latter often results from trauma or the consequences of surgery after tumour operations (Head and Neck Cancer). The psychological effects are often reflected in reduced self-esteem, increased dissatisfaction with one's own appearance and increased fear of negative reactions from fellow human beings [4, 5]. No significant differences were found between patients with congenital and acquired disfigurements [5]. However, not every patient is equally affected by their altered appearance. If people acquire a facial change in the course of their lives (e.g. through trauma), it has been shown that women in particular—in contrast to men—and younger people (younger than 50 years), in contrast to older people, suffer more from the disfigurements [6]. However, not only the subjective assessment of affected women and men, younger and older people differs, but also uninvolved third parties assess the same changes as being worse for women than for men and worse for

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younger people than for older people [7]. From these results it can be concluded that an attractive appearance that corresponds to the norm must have a high significance for social life and well-being, especially among younger women. Several studies have described that people's appearance influences their success—in the partnership, but especially in professional life. According to this, superiors and human resources managers tend to pay attractive employees a higher salary and create more lucrative positions [8]. Psychologists explain this phenomenon with the so-called "what-is-beautiful-is-good" stereotype. Good-looking people are subconsciously assigned positive characteristics such as resilience, diligence and trust. However, disfigurement not only affects the patient himself but can also be very stressful for the partner and significantly affect the quality of his life [9]. Considering the enormous importance of a normal appearance for many people, it is hardly surprising that patients are willing to take high risks to regain a normal appearance. Young adults with craniosynostoses usually want to undergo radical surgery even if the risk of serious complications is significant. And they do so for the only reason—to look "normal" again [10].

This also applies to the much larger group of patients with dysgnathia requiring orthognathic surgery. These patients obviously benefit psychologically from corrective surgery [11]. If we consider patients with various pronounced malpositions of the jaws (dentofacial deformity class II compared with class III), the benefit of surgical correction seems to be comparable in terms of aesthetics, function and psychosocial impact [12]. The evaluation of the treatment success by the patient is certainly subjectively influenced. Patients who feel better informed and have the impression that they have a good system of communication with the orthodontist or surgeon are regularly more satisfied with the treatment outcome [13].

The significance of the face for the human being becomes particularly clear when its correction—which by objective standards is quite successful—plunges the patient concerned into an identity crisis [14]. After dysgnathia surgery, for example, patients may need time and, if necessary, professional psychological help to get used to their new face, despite objective improvement of the facial profile. These patients are rationally aware that the dysgnathia treatment has been successful, but they have difficulty identifying with their new face.

The face is therefore of existential importance for the person. With its shape, facial expression and linguistic expressiveness, it not only represents the individual personality, but the reactions of others to its face also influence the social identity of the person. As a mirror of identity, the human face is a mediator of essential aspects of interpersonal coexistence [15]. Anyone dealing with facial deformities/diseases and disfigurements must also consider the psychological significance of the face for the patient and also face the consequences associated with the surgical treatment.

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Part IV

Diagnostic Issues

18 Early Clinical Investigations and Management of Syndromes Affecting Craniofacial and Dental Structures

Theodosia Bartzela

18.1 Introduction

Craniofacial malformations account for almost 30% of all congenital anomalies and have a broad phenotypic diversity and range of severity [1]. Early diagnosis is of vital importance not only for a favorable functional and developmental outcome but also because, in many instances, these conditions are associated with life-threatening complications like respiratory or feeding impairment. Data from 18 EUROCAT registries, from 10 different countries, observed that prenatal diagnosis has a significant impact on the prevalence of perinatal mortality [2]. Nevertheless, the affected craniofacial structures isolated or as part of a syndrome might not be identified early, especially in patients without a known family history.

In many patients, the problem is not apparent or present at the time of examination. Some anomalies are not detected before the fourth or fifth year of life, due to developmental variations or mild phenotypic expression [3]. Besides the general and genetic diagnosis, most dental phenotypes can only be identified after the first years of life. All reasons mentioned above delay a proper diagnosis and, therefore, the evaluation of prognostic factors and treatment management. Moreover, the treatment of these patients is often complicated because of insufficient medical knowledge and phenotypic variability in the craniofacial area.

The interdisciplinary team, including molecular and developmental geneticists, and different medical and dental specialists, are called to initiate early diagnosis and optimal therapeutic strategies.

Despite shortcomings in the literature, the primary goal of this chapter is to provide an update of clinical phenotypes, and genotype-phenotype correlations for early diagnosis, and management of syndromes affecting the craniofacial and dental structures. Furthermore, it aims to promote interaction and a consensus basis of the interdisciplinary team that is involved in the treatment of these patients for a customized, intergraded, long-term treatment planning.

In this chapter, six syndromes have been selected. The selection criteria were based on:

1. Available supportive information in the literature and online databases such as OMIM (Online Mendelian Inheritance in Man).
2. Estimated prevalence of 1:100,000 or more.

The following categories of syndromes with craniofacial anomalies have been included:

1. Syndromes involving orofacial clefts.
2. Syndromes involving branchial arches.

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For each syndrome is provided a section with general characteristics and etiopathogenesis, craniofacial and oral phenotypes, and management recommendations.

Furthermore, in Tables 18.1 and 18.2 (syndromes involving orofacial clefts and syndromes involving branchial arches, respectively) are listed synonyms and aliases, OMIM and Orpha numbers, genes, loci, inheritance patterns, and prevalence of the disorders.

18.2 Syndromes Involving Orofacial Clefts

Orofacial cleft (OFC) is the most common craniofacial deformity. The overall incidence of OFC is ranging about 1.7 per 1,000 live births, but it varies depending on ethnicity, geographical characteristics, and socioeconomic background [4]. The recurrent risk is not associated with the severity of the cleft among offsprings and siblings [5].

Table 18.1 Syndromes involving orofacial clefts

Syndrome	Synonyms	OMIM	Orpha	Gene	Locus	Inheritance	Prevalence/100,000
22q11.2 deletion	CATCH22 Cayler cardiofacial syndrome Conotruncal anomaly face syndrome (CTAF) DiGeorge sequence DiGeorge syndrome Microdeletion 22q11.2 Monosomy 22q11 Sedlackova syndrome Shprintzen syndrome Takao syndrome Velocardiofacial Syndrome	192430 188400	567	*TBX1*	22q11.2	Autosomal dominant	25–50
Pierre Robin sequence	Glossoptosis, micrognathia, and cleft palate Pierre Robin Malformation Pierre Robin Sequence Pierre Robin Syndrome Pierre Robin Anomalad Robin sequence Robin syndrome	261800	718	*SOX9*	17q24.3-q25.1	New mutation (autosomal dominant)	7.1–11.8
Kabuki 1	Kabuki makeup syndrome Niikawa-Kuroki syndrome	147920	2322	*KMT2D*	12q13.12	Autosomal dominant	3.2 (Japan)
Kabuki 2		300867	2322	*KDM6A*	Xp11.3	X-linked dominant	
Van der Woude 1	Cleft lip and/or palate with mucous cysts of lower lip Lip-pit syndrome	119300	888	*IRF6*	1q32.2	Autosomal dominant	1–3
Van der Woude 2		606713	888	*GRHL3*	1p36	Autosomal dominant	1–3

Table 18.2 Syndromes involving branchial arches

Syndrome	Synonyms	OMIM	Orpha	Gene	Locus	Inheritance	Prevalence/100,000
Hemifacial microsomia	Goldenhar syndrome Oculo-auriculo-vertebral (OAV) spectrum or dysplasia or complex otomandibular dysostosis Facio-auriculo-vertebral sequence	164210 141400	374	Heterogenous	14q32 and others	Mainly sporadic, some autosomal dominant	1.8–3.9
Treacher Collins 1	Treacher Collins syndrome Treacher Collins-Franceschetti syndrome Franceschetti-Zwahlen-Klein syndrome Mandibulofacial dysostosis	154500	861	*TCOF1*	5q32	Autosomal dominant	2.0
Treacher Collins 2		613717		*POLR1D*	13q12	Autosomal dominant	
Treacher Collins 3	Treacher Collins type autosomal recessive	248390		*POLR1C*	6p21	Autosomal recessive	

OMIM Online Mendelian Inheritance in Man

Genetics plays a major role. More than 50 genes may contribute to the pathogenesis of non-syndromic CL/P (NSCLP) [6] and more than 260 for the syndromic [7]. Variants in interferon regulatory factor 6 (*IRF6)* have contributed to non-syndromic cleft lip and palate (CL/P) [8] but also in syndromic clefts like the van der Woude syndrome [9]. Furthermore, differential environmental factors trigger the etiopathogenesis of nsCL/P [10], as it has been concluded from monozygotic (MZ) twin studies [10]. Even though the majority of OFC (almost 70%) are isolated or non-syndromic (nsCL/P) [11], there are types of OFC like the cleft palate only (CPO) that are more often associated to a syndrome (50%) [12]. The severity of cleft seems to play a role in the association with a syndrome or a malformation. Thus, patients with bilateral CL/P (BCLP) have a more frequent association with congenital abnormalities and syndromes in comparison to patients with unilateral CL/P (UCLP) [13]. In the "London Dysmorphology" Database, almost 500 syndromes in association with OFC have been reported [12].

Evaluation of patients with unoperated clefts has shown that these patients have normal craniofacial growth potential. Palatal scar tissue formed after the surgeries is the reason for maxillofacial growth discrepancies, which mainly depends on the surgeon's skills and the techniques used and not on the timing of the surgical procedures [14].

The selection of the most common syndromes of OFC presented in this section is included in Table 18.1.

18.2.1 22q11.2 Deletion Syndrome (DS)

18.2.1.1 General Features and Etiopathogenesis

The 22q11.2 DS has an incidence of 2–5 in 10,000 live births. Initially, it was described as DiGeorge syndrome (1965), and the main manifestations were thymic aplasia, hypoparathyroidism, and congenital heart disease. Later on, anomalies of organs derived from the third and fourth pharyngeal arches were added in the clinical phenotypes [15]. Shprintzen (1978) [16] described first the entity named velocardiofacial syndrome (VCFS) with cardinal characteristics as cleft palate, heart defects, typical facial features, and learning disabilities [17].

The name CATCH22 syndrome is an acronym derived from the typical characteristics of the syndrome (C, cardiac malformations; A, abnormal faces; T, thymus hypoplasia; C, cleft palate; H, hypocalcemia; 22, the involved chromosome). The term CATCH22 is not used any longer. The reason is that it emphasized only the congenital physical malformations and not the cognitive and behavioral anomalies [18], which are cardinal characteristics.

(The synonyms and aliases of the syndromes are presented in Table 18.1)

Almost 90% of the cases are de novo deletions, and only 7% inherit the disorder in an autosomal dominant mode [19]. The 22q11.2 DS is caused by a 1.5–3.0 Mb hemizygous deletion of chromosome 22q11.2. Haploinsufficiency of the *TBX1* (T-box transcription factor 1) gene or point mutations are related to the medical disorders of the syndrome [20]. Moreover, the gene *CRKL* (CRK like proto-oncogene, adaptor protein) contributes to the clinical phenotypes, acting on the same genetic pathway with *TBX1* [20]. The severe associated cardiovascular malformations determine the mortality rate (up to 4%) of the affected individuals [19]. 22q11.2 DS is considered the most common microdeletion syndrome [21] and the most common syndrome associated with schizophrenia.

The major medical conditions described are immunodeficiency (low T-cell counts, humoral immunity, and often dysgenesis or aplasia of the thymus) and allergies (77%) [19] followed by heart [22], gastrointestinal (65%) [19], and skeletal malformations (scoliosis (50%) and cervical spine (46%)) [19], low muscle tone, and seizures often related to hypocalcemia (up to 21%) [23]. Malformation and functional problems of the parathyroid glands (hypoparathyroidism), which often remain undiagnosed [24], disorders of the urinary system (renal anomalies (16%)) [19], and neurological morbidities [19] have been observed.

The aforementioned clinical manifestations were also similar to phenotypes observed in fetuses in cases of uncontrolled gestational diabetes or maternal exposure to teratogens [15].

Disturbances in cognitive development, attention difficulties, autism spectrum (ASD), or psychotic disorders, and verbal IQ decline over time also belong to the broad phenotypic spectrum [25]. Almost 6% of patients develop malignancies during their lifetime (thyroid carcinoma, leukemia, etc.) [19].

Many of the medical problems observed are drug induced, related to the treatment of the condition, such as the corticosteroids, which increase the osteoclastic activity, resulting in decreased bone mineral density and therefore increased risk of bone fractures.

In the "22q and You Center" in Children's Hospital of Philadelphia, the majority of the referrals of children with 22q11.2 DS were made from clinical geneticists (63.8%), followed by cardiologists (almost 20%) and plastic surgeons (5%) [19].

The birth weight of the affected individuals was average [26], but a growth delay of 6–9 months has been registered later on. Even though a catch-up growth period follows, the majority of patients are in height and weight in the lower percentiles of the growth charts [27].

18.2.1.2 Craniofacial Features

Microcephaly was described in almost 30% of the boys and 25% of the girls before the first year of life in a cohort of 1421 patients [19]. Almost 75% of the affected individuals have associated CL/P. Additional craniofacial characteristics are hypotonic muscles, long and asymmetric faces [28–30], hypoplastic zygomatic bones, rounded nasal tip, narrow and thin nasal base, and/or hypertelorism and hooded eyelids or other ocular and ear abnormalities [31].

Abnormal development of the skull due to severe craniosynostosis has been diagnosed in a patient with 22q11.2 DS at the 23rd gestational week [32]. The prevalence of 22q11.2 DS and associated craniosynostosis is almost 1% [33].

Lateral cephalographic measurements in patients with 22q11.2 DS showed mandibular micrognathia [34] and/or retrognathia [35, 36], retruded chin, steep mandibular plane angle, increased anterior facial height [37], malformed or short cranial base, large cranial base angle [37], retroclined lower incisors, and increased interincisal angle [37].

Skeletal class II and anterior open bite malocclusion are additional craniofacial findings [38, 39].

18.2.1.3 Oral and Dental Features

Many patients with 22q11.2 DS have velopharyngeal insufficiency and asymmetric development of the pharynx and larynx [30]. Almost 10% of these patients have submucous cleft palate only (CPO), a short velum, and alternated palatal motion [21]. Because of these anatomical characteristics, the patients have respiratory difficulties; hypernasal speech, which deteriorates with age [33]; and dysphagia associated often (80%) with tracheal aspiration, a potentially life-threatening condition [40].

These patients have a small mouth; protruded, incompetent lips with lowered tonicity; and short philtrum [30].

The prevalence of agenesis of permanent teeth was reported in almost 20% of the affected individuals [39]. Most commonly missing teeth are the mandibular incisor, the maxillary second premolars, and the maxillary lateral incisors [39]. Solitary median maxillary or mandibular central incisors have been reported in isolated cases [37].

Patients with 22q11.2 DS had a delayed tooth eruption of 0.4 years only, in comparison to the general Finnish population. Nevertheless, the variability range (range, −1.2 to 2.7 years) of patients' dental and chronological age was rather broad [41]. In isolated cases, delayed tooth eruption and development in the permanent dentition have been noted [42].

Enamel hypomineralization and hypoplasia [24] have been reported, mainly in the permanent dentition [43]. Even though it has been suspected that enamel aberrations are related to hypoparathyroidism [44] and hypocalcemia [24], the clinical findings could not support this hypothesis [43]. The incisors of *([Tbx1(cKO)]* mice showed a complete lack of enamel due to low amelogenin expression and reduced proliferation, differentia-

tion, and mineralization of ameloblasts [45]. The expression of the *Tbx1* gene implicated in human DiGeorge syndrome determines the ameloblast lineage [46], explaining possibly the enamel hypoplasia and the tooth agenesis phenotypes often observed in these patients [46].

Patients with 22q11.2 DS have increased caries risk due to reduced salivary buffer capacity and salivary flow [30].

18.2.1.4 Management

Abnormal facial features including CL/P or cardiovascular (in 95% of DS patients), urogenital, respiratory, skeletal, and central nervous system malformations [26] detected in the prenatal examination are of importance for planning the delivery in a tertiary care hospital, where the appropriate neonatal management can be provided. Further, the prenatal diagnosis will reduce the patients' mortality rate and improve the treatment outcome and parents' emotional response [15].

At birth, early clinical signs such as congenital heart abnormalities or hypocalcemia may assist in early diagnosis of the syndrome [39].

Patients with associated heart disorders are diagnosed with the syndrome significantly earlier (median age, 2.6 months) in comparison to those without heart anomalies (median age, 3.1 years) [19]. Besides the cardiovascular defects that may initiate a diagnostic examination, other clinical signs are the typical asymmetric crying facies, with ear or nose abnormalities [19]. Clinical phenotypes inconsistent with the 22q11.2 DS may point to a secondary associated syndrome [21].

The broad phenotypic spectrum makes the diagnosis challenging and may result in a years-long search for the complete range of the associated anomalies [15].

Some patients (five already reported) have been diagnosed only during their pregnancy after a prenatal diagnosis of carrying affected fetuses [19]. Furthermore, routine microdeletion testing in all patients with Cl/P has been proposed and not only to clinically suspected cases [31].

The treatment includes management of the velopharyngeal insufficiency (VPD, 52%); the CL/P [19]; the heart and psychiatric disorders; speech pathology; ear, ophthalmological, and urological defects; developmental delay; allergies, etc. An ophthalmologic examination should be considered early due to extensive ophthalmologic congenital anomalies observed in these patients [19].

Patients with an autosomal dominant condition have a 50% chance of having an affected child, and the phenotypic severity cannot be determined [47].

Hematologists and oncologists are also involved in the interdisciplinary team because of the associated malignancies that have been reported [19].

Genetic evaluation and counseling should be repeated from the transition to adolescence and adulthood for the detection of additional associated anomalies [47]. Hence, if feasible, these patients should be treated or evaluated in interdisciplinary care centers. Taking also into consideration the cognitive decline that is observed in these individuals [48], early educational support [48] and individualized treatment care [49] should be provided.

Good oral hygiene and early and regular contact with the dental team may promote a good oral status. Dental treatment with general anesthesia is required in cases of limited compliance.

18.2.2 Pierre Robin Sequence

18.2.2.1 General Features and Etiopathogenesis

Pierre Robin (PR) syndrome was described in 1923 by the surgeon Pierre Robin. The term used today is Pierre Robin sequence (PRS) [50, 51], pointing out to the sequential order of the cardinal features. Hence, micrognathia, the primary characteristic [52], leads to glossoptosis and obstruction of the upper airway (UAO) [52]. Therefore, vital functions as breathing and feeding are severely affected [53]. Most of the patients with PRS (50–100%) have obstructive sleep apnea (OSA) in different degree of severity [54].

Non-syndromic PRS (nsPRS) arise through de novo mutations and only 10% of the affected individuals through autosomal dominant

inheritance. The leading cause of PRS is the embryologic developmental defect of the mandible, which is associated with a mutation in the *SOX9* gene. The transcription factor Sox9 is essential for the chondrocyte differentiation pathway [55]. Other structures related to *SOX9* gene mutation besides the hypoplastic mandible are the stapes, the hyoid, styloid, and the thyroid [56, 57].

Two *BMPR1B* (bone morphogenetic protein receptor type 1B) mutations have been detected in two unrelated families [58]. *BMPR1B* is also involved in endochondral bone formation during embryogenesis.

The etiopathogenic mechanism, though, has not yet been elucidated [59]. The European Surveillance of Congenital Anomalies (EUROCAT) found an association between the PRS and the methadone exposure during the gestational period. Nevertheless, as it is retrospective data, many confounders could not be evaluated [60].

Pierre Robin sequence (PRS) can be diagnosed as isolated (nsPRS) or associated with multiple congenital anomalies (PRS-plus) [61] or other syndromes (sPRS). PRS-plus has a variable phenotypic expression and unsolved pathogenesis [59]. The most common associated anomalies described in patients with PRS-plus were the dysmorphic facial features, hypoplastic thyroid, and malformations of the musculoskeletal system [61].

More than 50% of patients have besides PRS an additional syndrome (sPRS). The most commonly associated syndromes are 22q11.2 DS [62] and Stickler syndrome [61]. Other less common associated syndromes are Treacher Collins syndrome (TCS), craniofacial microsomia [62], van der Woude, and Möbius syndrome [61].

PRS is associated with a 10% mortality rate, and the affected individuals are mainly sPRS or with severe respiratory distress or neurological abnormalities [53].

18.2.2.2 Craniofacial Features

Dysmorphic facial features, malformed eyes and ears, hearing loss, microcephaly or macrocephaly, and hypoplasia of temporomandibular joint (TMJ) and zygomatic bones have been described in patients with PRS [61]. Many of these findings, though, may be attributed to generalized growth delay. The most common associated abnormalities in PRS-plus patients are choanal stenosis or atresia [59].

A series of lateral and anteroposterior cephalograms were evaluated in infants affected by PRS [63]. The method applied was neither practical nor precise due to difficulty in standardizing and reproducing the infants' head position [52]. Thus, until now, the term micrognathia used by the clinicians is rather a subjective clinical evaluation [52]. The jaw index is used for the assessment of the micrognathia in infants, but further validation in a population basis of this tool is needed [64]. In a multicenter study, CT scans of newborns with PRS (33 days) were compared with scans of healthy controls. The patients with PRS had reduced mandibular ramus and body length. At 4 months of age, the children with PRS were treated with mandibular distraction osteogenesis (MDO). The comparison data showed that the post-treatment mandibular bodies were longer, and the mandibular rami were shorter in comparison to the control non-affected group [65]. The facial growth of children younger than 8 years of age with nsPRS was evaluated with the use of 3D images. The transverse and vertical facial dimensions were comparable to the non-affected control group, but the sagittal midfacial and mandibular dimensions were reduced [65]. Lateral cephalograms, in children of PRS, before orthodontic treatment (age, 11.8 years) showed bimaxillary retrognathism, vertical growth pattern [66], and a greater Wits appraisal in comparison to the healthy controls [66]. The nasolabial and the mentolabial angles of PRS group were comparable to the controls [67]. The hypoplastic mandibles of the PRS patients showed no catch-up growth during adolescence [66–69]. Even though in a recently published systematic review only a few studies reported mandibular catch-up growth comparable to the non-affected individuals [70], the vast majority of clinicians from 101 European centers observed a catch-up growth in their nsPRS patients [71]. This contradiction is due to the fact that many of these children have favorable growth potential.

Furthermore, the treatment outcome of patients with sPRS is more deficient in comparison to the nsPRS [72].

18.2.2.3 Oral and Dental Features

The abnormal high-up position of the tongue (glossoptosis) prevents the fusion of the palatal shelves, causing cleft palate only (CPO), in almost 75–100% of patients [51, 73, 74]. Glossoptosis is associated with respiratory obstruction, feeding problems, and vasovagal syncope [50]. So far, it has not defined if the severity of micrognathia is related to the extent of glossoptosis [75]. Patients with mild glossoptosis have breathing disorders only during sleeping [52].

The increased feeding time recorded in these children was related to the low neuromuscular tongue activity [76]. Furthermore, swallowing dysfunction was also associated with the structural anomalies of the hyoid bone, as it was observed in computed tomography scans in newborn patients with PRS [77]. In almost 13% of these individuals, ankyloglossia was an associated oral anomaly [51].

Taurodontism was the most prevalent dental phenotype, seen in 92.73% of the ns-PRS patients, 40.91% in nsCL/P, and 44.55% in control non-affected individuals [78].

Tooth agenesis (TA) (excluding the third molars) in the mandible of PRS children is ranging up to almost 50%, but in the maxilla it is not a common finding [79]. The symmetric agenesis of the mandibular second premolars is the most frequent tooth agenesis pattern observed [79, 80].

The maxillary dental arches of Finnish children with nsPRS, from 0.2 to 6 years old, were comparable with those of children with cleft palate only (CPO), before the surgical palatal closure [81]. The mandibular arch widths of nsPRS children were significantly smaller (< 0.01) than the CPO children at 6 years [81]. The surgical procedures for the correction of the CL/P have a permanent effect on the maxillary arch development of the individuals with nsPRS [82]. The underdeveloped dental arches of children with PRS may be attributed to the associated hypodontia, to postural and poor neuromuscular activity of the tongue, and to intrinsic growth factors [83].

18.2.2.4 Management

Up today, there is no main genetic diagnostic criterion for the PRS [61].

The micrognathia can be diagnosed even during the prenatal period with the help of ultrasonography or immediately after birth [59]. The treatment management of these children is focusing on regulating vital functions like breathing and feeding difficulties. Even though the management of these patients is challenging, there is no consensus on treatment guidelines. Almost 50% of patients with PRS need temporal feeding support [73]. Management of patients with PRS varies depending on the severity of upper airway obstruction (UAO) and the center's protocol [84]. In mild forms of micrognathia, prone and lateral positioning may be effective for facilitating the respiratory distress. Another type of airway adjunct is the nasopharyngeal airway (NPA) or the continuous positive airway pressure (CPAP) [84].

In a cohort of patients with PRS, the management with the pre-epiglottic baton plate, together with Manual Orofacial Therapy (MOT) and special feeding methods, was adequate to address the UAO and the feeding difficulties [85]. The need for tracheostomy in this cohort was limited to only 4% [86]. Almost 10% of children with PRS need respiratory support during childhood [73]. The respiratory problems remain in one out of four (1/4) children until 18 years of age [87]. The nonsurgical treatment interventions, though, have raised concerns on the effectiveness of respiratory distress management. It has been speculated that inadequate respiratory management is associated with neurocognitive and growth deficits of these children [88].

Mandibular distraction osteogenesis (MDO) is the treatment applied in selected patients for the management of micrognathia and relief of respiratory distress [89]. The airway volume in patients with nsPRS treated with MDO was increased to almost 400% [65]. The activation of MDO ends when the mandibular sagittal discrepancy is slightly overcorrected [90]. Iatrogenic complications related to MDO are scar tissue formation, damages on the developing tooth buds, or fractures of the stabilization pins [91].

In patients with associated CL/P, the surgical reconstruction of the primary palate, in the first and second year of life [66], is depending on the child's clinical conditions and weight.

Nocturnal polysomnography (PSG) is the screening tool in sleep medicine for the diagnosis of OSA [92]. Early diagnosis of OSA may help to prevent neurocognitive impairment and developmental growth disturbances, pulmonary hypertension, etc. [92].

The vast majority of the published studies on treatment modalities for OSA on children with PRS [84] are retrospective and non-comparative. Only one cross-over clinical trial compares the pre-epiglottic with the conventional plate [93], indicating the safety and efficacy of the pre-epiglottic plate. There is need for standard nomenclature [94] and evidence-based diagnostic and treatment consensus for these patients [71]. The treatment outcome evaluation of children with PRS should be based on growth, feeding, and respiratory factors [95]. Infants with PRS should be examined longitudinally for the diagnosis of any associated malformation or a syndrome [96]. Late testing for isolated cases is recommended, mainly if the genetic evaluation is undertaken beyond infancy. Nevertheless, even if a new genetic diagnosis confirmed the coexistence of an additional syndrome besides the PRS, only 1/3 of the practitioners alter their initial treatment plan [94].

Clinical whole-exome sequencing has led to the diagnosis in up to one-third of those individuals who had not received a diagnosis with other methods. Genetic counseling should be offered to all families, even in the event of sporadic cases.

18.2.3 Kabuki Syndrome

18.2.3.1 General Features and Etiopathogenesis

Kabuki syndrome (KS) was initially described in Japanese children [97], and its prevalence in the Japanese population is 3.2/100.000. The name was taken from the similarity of the affected individuals with the makeup worn by the performers in the "Kabuki" traditional Japanese theater. The phenotype of the syndrome has late onset; therefore, it is often underdiagnosed, especially in the non-Japanese population [98]. The differential diagnosis of KS with CHARGE syndrome, a sporadic, autosomal dominant malformation, is challenging, especially at young ages [99]. Males are affected more often than females [100].

The pathogenic mechanism of KS is still unknown [99]. In zebrafish models, knockdown of *kmt2d, kdm6a, kdm6al* genes was associated to brain defects [101]. Moreover, knockdown in kmt2d and kdm6a in morphants was related to severe abnormalities in the craniofacial structures [101]. Extended defects were observed from the third to the seventh branchial arches and Meckel's and ceratohyal cartilage [101]. Furthermore, the kdm6al morphants exhibited severe defects in the heart [101] and the kdm6a and kdm6al morphants in body length and spine [102], denoting the overlapping function of the genes mentioned above and their role in craniofacial structures and body axis [101]. The major structural defects of the morphants were observed in the viscerocranium, which derived from the neural crest cells [101]. A recently published study gave evidence that the KS is a neurocristopathy because it is linked to defects of the neural crest (NC) development.

An international team of experts determined consensus diagnostic criteria for KS [103], which are infantile hypotonia, developmental and/or cognitive delay, and either pathogenic mutation in *KMT2D* or *KDM6A* or the development during the lifetime of one of the prominent facial characteristics of the syndrome. These prominent facial features are the long palpebral fissures and/or the browed and arched eyebrows and/or eversion of the lower eyelid [103]. Additionally, the diagnostic criteria should be followed by at least two of the secondary characteristics, which are broad and sparse or notching eyebrows, flat nasal tip with short columella, ear deformities, and/or prominent finger pad tips [103].

These individuals have, in many cases, early pubertal onset [104]. Almost 30% of patients after puberty are overweight [105]. The growth retar-

dation may be attributed to the dysfunction of either hormonal factors or peripheral organs, such as the kidneys [105]. Brachydactyly or clinodactyly of the fifth finger, prominent finger fat pads, brachymesophalangy, or prominent fingertips, alter dermatoglyphics, or pachyonychia in all toes are often encountered [98]. Musculoskeletal abnormalities, including the vertebra and the ribs, scoliosis (35%), or spina bifida occulta (19%) [101], peroneal atrophy, muscle hypotonia, or neuropathy, and seizures [98] are additional clinical features. The interrupted clavicles have been speculated as an additional clinical feature of the KS phenotype [106]. Heart defects (septal, atrial, and ventricular) [107, 108], gastrointestinal, urogenital abnormalities [101] frequent ear infections, hearing loss, and compromised humoral immunity [109, 110] belong to the phenotypic spectrum [98]. Generalized joint hypermobility and dislocation of the hip and knee joints and facial skin laxity are the associated anomalies related to the connective tissue [98]. Hypoglycemia is a more common finding in KS2 than the KS1 patients [111].

18.2.3.2 Craniofacial Features

Craniofacial manifestations in patients with KS are microcephaly, plagiocephaly, low posterior hairline, interrupted and arched eyebrows, long palpebral fissures, and palpebral eversion of the lower lid, long eyelashes, strabismus, nystagmus, broad nasal bridge and a wide and flat tip of the nose, short columella, and large protruding or cupped earlobes, and preauricular pits [100].

Skeletal malocclusions most commonly observed are anterior open bite [112, 113], posterior crossbite [112], and Angle class III [114].

18.2.3.3 Oral and Dental Features

Almost half of the KS patients have CPO, and those without an associated cleft have a high arched palate [115], micrognathia, and/or abnormal tongue movement [116]. Patients also had uvula bifida, lip pits, and nodules [115]. Recently, it has been proved that *KMT2D* and *KDM6A gene expression* play a pivotal role in early tooth developmental stages [115].

Therefore, most of the patients with KS have hypodontia [117], and the most common missing teeth are the incisors and/or premolars [118]. Furthermore, supernumerary teeth and widely spaced teeth have been reported [97, 112, 119]. Shape-size tooth marked malformations are microdontia [117], peg-shaped and screwdriver-shaped incisors [120], talon cusp in the maxillary primary incisor [121], and shortened tooth roots [115]. Retention of primary [120] and permanent teeth [119] and ectopic upper molars [122] are complicating the tooth eruption sequence. Dental caries is a common finding in these children [115].

18.2.3.4 Management

Growth parameters and the stature growth prognosis were calculated in 91 patients with KS1, the largest cohort until now [105], but still not powerful enough for growth assessment. Normative growth curves for males and females were created. Almost 40% of the participated patients had postnatal growth deviation (up to 2 SD) from the control, non-affected French children. Treatment with growth hormone (GH) did not increase the predictive height of the patients, but obesity can be prevented by dietary monitoring [105]. An echocardiogram is recommended as soon as the syndrome is diagnosed [123]. Patients with focal epilepsy episodes may not respond promptly to medication [124]. The hypoglycemia has to be evaluated after birth for the prevention of structural and functional neurological complications [111]. The immunodeficiency observed in a high prevalence of patients with KS should be evaluated for the prevention of chronic conditions and autoimmune diseases [125]. The orthodontic treatment with fixed appliance of patients with KS has limitations because of the increased risk of external apical root resorption and the lack of patients' compliance [126]. The desmethyl-Dabrafenib (dmDf) (a BRAF inhibitor involved in the cell proliferation) ameliorated the phenotypic craniofacial and neurological anomalies in zebrafish models. These experimental observations may have a clinical application [127].

18.2.4 Van der Woude Syndrome

18.2.4.1 General Features and Etiopathogenesis

Van der Woude syndrome (VWS) accounts for 2% of all CLP patients, and it is the most common syndrome associated with OFCs [128]. It was first reported by Demarquay (1845) and described by van der Woude in 1954 [129]. Besides the cardinal sign, the paramedian lip pits, or eminences of the lower lip vermillion, CL/P is observed in about 70–80% of these patients [130]. Males and females are evenly distributed.

VWS is an autosomal dominant condition with high penetrance and variable expressivity. Heterozygous mutations of *IRF6* on chromosome 1q32.2. are associated with VWS1. These mutations are seen in almost 70% of the affected individuals [131]. It has been shown, in a Brazilian family, that an upstream disrupting gene function and not the gene mutation was the causative factor [131]. *IRF6* regulates the embryonic development of lip and palate, the tooth morphogenesis, and the proliferation of the epidermal cells, participating in skin development [132]. *IRF6* can also suppress cell proliferation in the early and late stages of cancer [133]. Common mutations of *IRF6* are contributing to non-syndromic OFCs [8, 9], while structural variants are the causative factor of VWS1 and popliteal pterygium syndrome (PPS) (OMIM #119500) (popliteal web syndrome or facio-genito-popliteal) [134].

PPS, besides the standard features of VWS, is characterized by popliteal pterygia, syndactyly, skin pyramidal folds at the toes and fingers, genital malformations, and in some cases, fusion of the maxilla and mandible and the upper and lower eyelids. PPS accounts for 0.2% of all patients with CL/P. It has been speculated that the VWS and PPS belong to the phenotypic spectrum of the same entity [135]. Phenotypic and molecular analysis in a three-generation family member with VWS and PPS showed that patients with minor signs of the VWS syndrome could have offsprings with the most severe phenotypic symptoms of PPS [135]. Heterozygous mutations in grainyhead-like 3 (*GRHL3)* on chromosome 1p36.11 cause VWS2 (OMIM #606713) [136]. The interaction of *GRHL3* with other genes associated with the development of nsCL/P has also been confirmed recently [137].

Patients with VWS have developmental language delay and, in some cases, mild cognitive impairment [138–140].

In magnetic resonance imaging (MRI), structural changes were observed in the anterior cerebrum of adults with VWS. Moreover, the intelligence score was declined, and men were more affected than women [140].

Additional malformations of the VWS spectrum are congenital heart anomalies, thumb hypoplasia, syndactyly, and club foot. Sensorineural hearing loss [141] and otitis media have also been registered mainly in patients with associated CL/P.

The importance of detailed phenotyping for the identification of minor clinical signs of the syndrome has been underlined, for the differential diagnosis of VWS, PPS, and the nsCLP [135]. VWS has been reported in concurrence with Pierre Robin sequence (PRS) [142] or Turner syndrome (TS). The associated TS was diagnosed only when the delayed onset of pubertal development was noticed [143].

18.2.4.2 Craniofacial Features

OCF is an associating finding in patients with VWS, observed in 21–100% of the affected individuals [144, 145] with varying degrees of severity [141, 144]. In a younger group of VWS patients (range: 5.7–6.7 years of age), the craniofacial morphology was similar to the nsCLP patients [146], and the lower pharyngeal airway was slightly smaller [146]. The midfacial developmental deficiency was observed in a group of VWS/PPS patients in comparison to patients with nsCLP [134]. ANB angle and Wits appraisal measurements were significantly smaller in VWS individuals in comparison to the non-affected controls [147, 148].

18.2.4.3 Oral and Dental Features

More than 80% of patients with VWS have labial pits, mostly bilateral on the vermillion of the lower lip, in about 0.5 cm from the facial midline [130, 149]. The pits are superficial lip imprints or

connected with channels with constant or intermittent, watery, or salivary secretion [130, 141]. The pit depth can be up to 25 mm (mean = 15.7 mm) [150]. In 64% of patients, the pits are the only clinical manifestations [145]. Additionally, conical elevations of the lower lip [151], ankyloglossia [141], and bifida uvula are among the common oral findings of VWS.

Submucous cleft palate has been reported in 23% and CPO in 9% of individuals with CL/P [152]. If the labial pits are not observed, might also the submucous cleft remain undiagnosed [152].

Hypodontia and dental hypoplasia are also considered cardinal features in VWS patients [141, 151]. The prevalence of tooth agenesis is higher than in the non-syndromic cleft patients, and it is associated with the severity of the cleft [148]. VWS patients with unilateral or bilateral cleft lip and palate (UCLP and BCLP) have a higher incidence of tooth agenesis (66% and 75%, respectively) than patients with cleft palate only (CPO) or submucous cleft palate (50%) [148]. The most commonly missing teeth are the maxillary second premolars, followed by the maxillary lateral incisors [153].

TA and OFC in combined phenotypes have been associated with rare variants of *IRF6* in syndromic and non-syndromic forms, as it has been presented in a systematic review [154].

A possible association with taurodontism was reported in a small group of patients (6 out of 13) [155]. In conditional knockout of *Irf6* (*Irf6-cKO*) mice, dental aberrations, e.g., enamel translucency, taurodontism, peg-shaped molars, c-shaped roots, and hypo- and hyperdontia, have been observed [132].

VWS patients show underdevelopment of the maxillary length and height (N-ANS) [147, 148].

18.2.4.4 Management

A classification system for the labial pits evaluates the involvement of the white skin roll of the lip and the depth of the pit, indicating the degree of difficulty of the surgical procedure. Labial pits up to 6 mm in depth are considered shallow [156]. The surgical treatment of the pits is aiming to improve aesthetics and/or prevent or treat chronic infections [156]. The optimal aesthetic outcome is challenging and not feasible in all instances [156]. The excision can be associated with recurrence or mucoceles [157].

In the case of an associated CL/P, the treatment protocol of VWS is like the patients with CL/P. Patients with VWS have increased risk for oronasal fistulas [134] after the primary cleft repair and increased need for pharyngeal flap surgery, in comparison with nsCLP patients [134].

When records of patients with VWS were reviewed from birth until 10 years of age, wound healing or major surgical complications requiring additional surgical repair have been noted [158]. Recently published data supported the role of IRF6 in cutaneous wound healing, granulation tissue formation, keratinocyte or immune cell function, and inflammatory cytokine level regulation [159]. There is a link between *IRF6* and chronic wound conditions and different types of cancer such as melanoma [160] and oral and breast carcinomas [161]. Therefore, their association needs further investigation [159] for patients' risk factor evaluation.

Treatment outcome evaluation, as reported in patients' records of a period of 30 years of children with VWS ($n = 21$) and PPS ($n = 7$), showed surgical complications and poor treatment outcomes. Consequently, patients with VWS and PPS and their family members should be informed about the complexities and limitations of the treatment [134].

18.3 Syndromes Involving Branchial Arches

Branchial arch syndromes affect the first and second branchial arch derivatives [162], including skeletal, muscular, ligamental, and neural facial structures. The first branchial arch is innervated by the trigeminal nerve (V) and the second branchial arch by the facial nerve (VII). Therefore, besides the facial expression and appearance, vital functions are affected, such as breathing, feeding, but also hearing [162].

Genetic and environmental triggers are implicated in the pathogenesis of these syndromes during the fifth and eighth gestational week (TCS) [163].

The most frequent syndromes described in this group are the craniofacial microsomia (CFM) and Treacher Collins syndrome (TCS) (Table 18.2).

18.3.1 Craniofacial Microsomia (Hemifacial Microsomia)

18.3.1.1 General Features and Etiopathogenesis

Craniofacial microsomia (CFM) occurs in approximately 1 out of 3,500 live births. It is the second most common craniofacial condition after CL/P [164]. CFM encompasses a broad spectrum of phenotypic severity. Unilateral or bilateral hypoplasia or, in some cases, aplasia of the craniofacial structures is detected [165]. Bilateral involvement has been observed in almost one-third of patients with CFM (29%) [165]. However, in three-dimensional (3D) computed tomography (CT) scans of patients with CFM, a compensatory remodeling on the unaffected side was observed, and the mandible was rotated toward the affected side [166]. Therefore, CFM replaced the previously used term "hemifacial macrosomia" (HFM) [167], because both sites are involved. Nevertheless, the one side is more affected than the other, and asymmetry was observed even in patients with bilateral involvement [168].

The right side is affected more often than the left side and males more often than females (3:2) [169]. Contrarily, in a group of 154 patients, this predominance in sex and sidedness was not verified [170].

Neonatal symptoms described are jaundice (9% versus 4% in the control group) or feeding difficulties (5% and 1% in the control group) [171].

The most severe forms of CFM, besides the craniofacial structures, involve extra-cranial anomalies [165]. In a big cohort of 981 patients, almost half of the included patients (47%) had associated malformations in the circulatory, urogenital, gastrointestinal, skeletal (vertebral column, ribs), and central nervous systems [165].

The aliases of CFM are Goldenhar syndrome, oculo-auriculo-vertebral (OAV) dysplasia, OAV spectrum (OAVS), facio-auriculo-vertebral (FAV) sequence, and otomandibular dysostosis (OTM) (Table 18.2).

In a 20-year database of patients' medical charts, it was observed that the term "Goldenhar" had been used inconsistently, referring mainly to patients with severe symptoms of CFM [172].

Positive family history has been reported in almost 30% of the examined patients [168]. Although an autosomal dominant (AD) mode of inheritance has been identified [173], the phenotypic discordances in monozygotic twins make the pathogenic mechanism poorly understood [174]. Patients with AD inheritance have malformations limited to the craniofacial structures, with bilateral involvement [173].

The OAVS and CFM have been genetically differentiated [175]. In a family with AD inheritance of OAVS, a 14q23.1 duplication of 1.34 MB was detected [175]. The orthodenticle homeobox 2 (*OTX2)* is in this region and is involved in the embryologic development of the inner ear, eye, optic nerve, pituitary gland, mid- and forebrain, cardiac, and thymic malformations [176]. Therefore, the *OTX2* has been proposed as a good candidate gene for OAVS.

So far, only 11 patients with CFM/OAVS have been identified with deletions in the 22q11.2. Even if these patients share some phenotypic features (preauricular tags, facial asymmetry, CL/P, and congenital heart anomalies), a broad spectrum of clinical manifestations is observed [177].

A de novo missense mutation in *MYT1* (myelin transcription factor 1) gene has been identified in a patient with the severe phenotypic expression of OAVS [178]. As *MYT1* is involved in the retinoic acid (RA) pathway, other genes also involved in the RA pathways are good candidates for the elucidation of the pathogenic mechanism of the OAVS [178].

Many candidate genes such as *ROBO1, GBX2, NRP2, EDNRB, EPAS1, KLF12, ARID3B, GBX2,* and *FGF3* are contributing in the neural crest cell (NCC) development and the vasculogenesis

[179]. Therefore, further investigations are required to confirm and clarify how these genes act on the development of the craniofacial anomalies and syndromes [179].

Besides the genetic component, other factors, such as poorly controlled gestational diabetes [180] or maternal exposure to teratogens, can result in these phenotypes [181]. Different pathogenic models for CFM have been described. Teratogenic agents, e.g., thalidomide, destroy the immature blood vessel network of the fetus, leading to cell death or disruption of the gene signaling pathway [182]. Another causal mechanism is the induced hemorrhage close to the Meckel's cartilage, which may disrupt the chondrogenesis [183]. Additionally, hemorrhage of the stapedial artery induced by vasoactive drugs (e.g., epinephrine, phenylephrine, etc.) can cause hypoxia or pressure in the area around the condyle [183] leading to a similar phenotype.

18.3.1.2 Craniofacial Features

The minor phenotyping expression of the syndrome is microtia without any other associated anomalies [184]. The CLOCK (Craniofacial microsomia: Longitudinal Outcomes in Children pre-Kindergarten) multicenter study comprised 108 patients with CFM and 84 non-affected control individuals. In almost 95% of patients with CFM, microtia was reported as the cardinal manifestation [171]. Ear involvement can be uni- or bilateral, with absent or malpositioned and deformed structures, associated with hearing impairment [168]. Mandibular hypoplasia was observed in 59% of patients [171]. The primary anatomic abnormalities are seen in the condyle, the mandibular angle, and the body in descending order [185], followed by secondary deformation of the maxilla, nose, orbit, zygomatic bone, cranium, eye, or neck [168].

In a phenotypic description of 51 index patients, 90% (46 patients) had mandibular hypoplasia, and 17 of them had an associated facial nerve palsy (FNP) [168].

In a small group of patients (9 patients), with unilateral involvement of CFM, clinical examination and computed tomography (CT) scan were performed for the evaluation of the soft tissue. The masseter was the most affected structure. In the most severe phenotypes, the masseter can be utterly absent, but in mild cases, it was much thinner than in the contralateral side. The volume and electromyographic findings of the masseter [186] were correlated with the degree of skeletal asymmetry [187]. However, the degree of bone atrophy was not related to muscular hypoplasia [186].

The temporal and pterygoid muscles and the parotid glands were also affected [188]. The area around the parotid gland, on the affected side, showed on the CT images increased density of fat tissue [188].

Nevertheless, the affected mandibular side had a growth rate similar to the control non-affected group [189]. As the patient grows, the skeletal and soft-tissue deformity is deteriorating progressively, because no "catch-up" growth occurs on the affected side. The mandibular asymmetry is correlated to the mandibular swift, independently of the temporomandibular joint and the ramus [169]. The smaller mandibular body and ramus at the affected side were associated with an inclination of the occlusal plane [190]. However, in less severe cases (type II), in a 3D representation, some growth modifications were observed, mainly in the condyle and less in the mandibular body [191]. There was no growth modification in patients with the absence or severe deformity of the ramus (Pruzansky classification III) [191]. Thus, the growth impairment is varying according to condylar cartilage deformity [192]. The most severe growth impairment was observed mainly in the glenoid fossa and mastoid process [193].

Nevertheless, the maxillary deficiency was not associated with the mandibular hypoplasia [194]. Additionally, no sinus involvement was observed [194]. In the case of associated cerebral palsy, smile dysfunction, incomplete eye closure, and difficulty in eyebrow elevation and facial expression were seen.

The cephalometric measurements showed retrognathic mandible, convex facial profile, and steep gonial angle [195]. Almost 10% of patients with CFM have associated CL/P. A multicenter cohort of 755 patients with CFM

showed that patients with bilateral involvement had a more severe phenotype and were associated with CL/P [196]. The prevalence of OSA in these patients is up to 10 times higher than the non-affected population [197]. Orbit displacement or smaller size [184] and other ocular anomalies have been reported in 29% of the observed patients (15 out of 51) {Beleza-Meireles, 2015 #3207}. Brain abnormalities were associated with intellectual disability and were present in 10% of patients [168].

18.3.1.3 Oral and Dental Features

All permanent and primary molars and permanent mandibular canines, on both sides, were smaller in comparison to the non-affected controls. Nevertheless, the front teeth had normal dimensions [195]. These findings confirm that CFM is rather a bilateral condition and that the dental lamina is also involved [195].

The prevalence of tooth agenesis (TA) is approximately 25%, and the most affected teeth are the mandibular second premolars and second molars [198, 199]. Tooth development is delayed in patients with CFM, but asymmetric tooth development between the right and the left side has been observed only in the most severe cases [198]. In a recently published systematic review, the lack of knowledge on dental phenotypes of patients with CFM has been emphasized [200].

CFM patients often have mild tongue deformities, which, most of the time, remain undiagnosed [201].

18.3.1.4 Management

In 85% of patients, a suspected diagnosis for CFM was made at birth, in 2% prenatally, 20% at a postnatal diagnosis, and 1% remained undiagnosed [171]. By prenatal ultrasonography at 24th gestational week or birth, marked anatomical asymmetries can be identified [202].

The need for neonatal intensive care of these children is double as the controls (20% vs. 11% of the controls), and the average stay in the intensive care unit was ranging from 1 to 45 days (mean, 9.6 days) vs. 1–8 days in the controls (mean, 3 days) [171]. CFM patients are receiving extensive clinical care in infancy, and 28% of them have been operated as soon as they had received their first diagnosis (mean age, 4 ± 4 months). More than half (59%) of these children are treated in a craniofacial unit [171].

Different classification systems have been used to indicate the severity of the malformation or the features involved, facilitating the communication among clinicians (OMENS, SAT, Pruzansky, and the Pruzansky modification by Kaban) [170]. These systems are based on two-dimensional (2D) imaging [166]. They are rather precise when the ramus and mandibular body are involved and unreliable when the head of the condyle and the glenoid fossa are implicated [191]. The classification systems are not widely accepted, making the research and epidemiological data interpretation difficult [184].

An accurate diagnosis of the 2D- in combination with 3D-facial images has been recommended for precise and long-term treatment planning [203].

Even though serial photographic images have captured the facial expression in a cohort of 39 children with CFM, the final diagnosis of an underlying nerve palsy was possible only by a clinical investigation [167].

Cone-beam computed tomography is an accurate tool for the identification and quantification of craniofacial and vertebral skeletal anomalies [204].

Treatment involves orthodontic and surgical interventions for the correction of facial asymmetry. Orthopedic treatment with a hybrid functional appliance (type Frankel I) [205] aims to promote the overall facial symmetry and growth by normalizing the mandibular position and stretching the deficient soft tissue and muscles. Orthodontic treatment with fixed orthodontic appliances [206] is correcting the canting occlusal plane, dental midline deviation, and arch asymmetry, resulting in functional and aesthetic occlusion.

Although MDO is a selected treatment for children with CFM of varying severity, in the long term, it has been associated with an increased relapse rate. Contrarily, treatment advocated in the late mixed dentition or later demonstrated more stable results [207].

A hybrid treatment that combines MDO and a functional appliance ascertains a balanced occlusion in patients with CFM [208].

The timing of surgical intervention for the correction of facial asymmetry is still controversial. In the mild expression of CFM (I and IIA), MDO or osteotomies without bone grafts are adequate to improve the facial asymmetry. In more severe types (IIB and III), though, the ramus/condyle or even the temporomandibular joint construction is required [209].

Customized computer-guided surgery has been used for conservative and well-defined incision and predicted treatment outcome [210].

The costochondral grafts (CCG) have been used for the reconstruction of the temporomandibular joint, especially for children, because of their osteogenic potential [211].

Mandibular canal variations should be carefully evaluated before surgical interventions, especially in the most severe cases (Pruzansky-Kaban types IIb and type III) [212].

The identification of the etiopathogenic area [191] and differentiation of the CFM from conditions related to trauma, local infection, or iatrogenic factors are of importance for treatment planning. Early and accurate diagnosis of patients with facial structural asymmetries and/or signs of poor neurodevelopment is of pivotal importance for the treatment outcome. Differential diagnosis from the TCS and auriculo-condylar syndrome (ACS) should be considered [175]. Patients with CFM have been labeled as having increased risk for developing medulloblastoma [213]. The reason is that the *OTX2* is the most likely implicated gene in the CFM and in the oncogenic development of medulloblastoma [213]. Therefore, risk evaluation for these patients should be considered.

18.3.2 Treacher Collins

18.3.2.1 General Features and Etiopathogenesis

Treacher Collins syndrome (TCS) is also called mandibulofacial dysostosis. TCS is a neurochristopathy (NCP) because the developmental defects are derived from aberrations of the cranial neural crest cells [214]. There are three types of TCS (TSC1, TSC2, TSC3), which are sharing a typical craniofacial phenotype, the bilateral otomandibular malformation. Patients with mild phenotypes can remain undiagnosed, and only molecular genetic testing can provide the type of TSC. TSC1 is an AD disorder, with a high degree of penetrance and a broad phenotypic spectrum [215], which resulted in most of the cases in a loss-of-function mutation in the treacle ribosome biogenesis factor 1 (*TCOF1*) gene on 5q32-q33.1. *TCOS1* gene is responsible for almost 90% of the TCS cases and has a regulatory role in the development of the craniofacial skeleton [216]. More than 200 mutations in *TCOF1* have been associated with TCS1 [1], and up to 60% of the affected cases are sporadic, explained by de novo mutations. *TCOF1* encodes the nuclear phosphoprotein named treacle. Treacle is involved in the ribosome biogenesis linked to cell growth and proliferation [217]. Therefore, the disease belongs also to the ribosomopathies. Less common pathogenic genes are the RNA polymerase I subunit D (*POLR1D)* at 13q12.2 with AD mode of inheritance implicating in TCS2, and RNA polymerase I subunit D (*POLR1C)* gene at 6p21.1 with autosomal recessive pattern (AR) reported in association with TCS3. Nevertheless, about 10% of patients with TSC are carrying undiscovered pathogenic variants [163].

Besides the malformations observed in the craniofacial and dental structures, other less common abnormalities involved are in the heart (12%), the brain, the kidney, and the limbs [218].

The cognitive development of these patients is normal, but the height and weight are below the average range [218].

18.3.2.2 Craniofacial Features

TCS is characterized by hypoplasia of malar bones (in 99%) and condyles, as well as by retruded chin and maxillary and mandibular retrognathism (in 87%). Ear defects such as atresia of the external auditory canal and microtia in

almost 70% of patients are associated with conductive deafness (in 91%) [218]. The cardinal findings of individuals with TSC (mean age: 20.2 ± 4.7), identified in lateral cephalometric measurements, were the hyperdivergent growth pattern and Cl ll skeletal classification [219]. Other cephalometric variables were the reduced anterior and posterior cranial base length, cranial base angle, maxillary length, and anterior and posterior facial heights [218]. The gonial and mandibular plane angles were increased, and a clockwise rotation and retroposition of the mandible were observed in patients from 3 to 22 years of age [220]. Pharyngeal dimensions in 3D images were significantly reduced in comparison to the controls [219]. Anteroposterior cephalograms of non-operated TCS patients showed normal intraorbital measurements but reduced lateral orbital wall lengths [218]. The zygomatic and the bitemporal width measurements were decreased in comparison to the controls [218].

Additional clinical features are deep antegonial notching, similar to juvenile rheumatoid arthritis or to cases with condylar growth disturbances [218], coloboma or hypoplasia of the lower eyelid (in 65%), facial asymmetry (in 53%), CL/P (22%), and choanal stenosis or atresia (14%) [221]. The complete absence of the zygomatic arch and cleft palate only (CPO) (28%) belongs to the most severe phenotypic spectrum [215].

Obstructive sleep apnea (OSA) syndrome (OSAS) is a frequent diagnostic finding of patients with TCS, observed in all ages [222].

Severe malocclusions, such as skeletal open bite, and decreased maxillary width, including dental crowding [223], have been reported in 94% of these children [222].

18.3.2.3 Oral and Dental Features

More than half of the affected individuals have 1–8 dental anomalies. Tooth agenesis (TA) is observed most commonly in mandibular second premolars, maxillary second premolars, lateral incisors, and canines [224]. Supernumerary, impacted, or malpositioned teeth are among the common dental findings [224].

TCS patients are diagnosed with different degree of salivary gland hypofunction [225]. These patients have increased caries risk due to the deficient salivary gland secretion, mouth breathing, enamel hypoplasia, dental crowding, and soft diet due to mastication problems [224].

18.3.2.4 Management

Prenatally, the abnormal cranial features of TFC patients can be detected in the most severe phenotypes only [216]. In patients with unknown gene variants or underlying diagnosis, CT scans and clinical phenotypes are initial tools for obtaining a diagnosis [163]. In some case reports, an intensive clinical investigation or coincidental clinical findings later on in life contributed to the final diagnosis.

Two sisters (2 and 4 years of age) received a molecular genetic diagnosis of the recessive form of TCS with a mutation in *POLR1C*. The molecular test was initiated only after a clinical diagnosis on consecutive hearing deficiency [226]. A patient searched for a medical examination after complaints of pain and swelling in the left submandibular area [227]. Clinical investigation revealed a sialolith in this location and multiple craniofacial anomalies, such as malar hypoplasia, retrognathia, flat nasal tip, etc. Salivary gland scintigraphy showed agenesis of both parotid glands [227].

Ultrasound examination for implicated salivary glands, combined with a caries prevention program, is required in cases of a confirmed diagnosis of TCS [225].

Management of patients with TCS needs a multidisciplinary team care approach, since birth, focusing on respiratory distress and OSA, feeding, and swallowing difficulties [228]. In a systematic review based on the treatment of TCS patients, tracheostomy for airway obstruction has been performed in less than 41% of the reported cases [222]. After the third month of life, hearing, vision, and later on articulation problems should be considered. Standard craniofacial procedures for bony and soft-tissue reconstruction of the orbit, ear, zygoma, and mandible should be addressed. The surgical procedure for bone

conduction hearing device is taking place when the patient is about six years of age when the cranial bone is at least 4 mm thick.

In cases with severe condylar dysfunction, the surgical reconstruction of the TMJ should be performed before the MDO [229].

The orthodontic/orthognathic treatment of patients with TSC is focusing on the malocclusions and the functional and aesthetical problems.

Often the orthognathic surgery includes Le Fort I and sagittal split ramus osteotomies and many times genioplasty as well. Three-dimensional virtual reality is a useful tool for pediatric surgical interventions, reducing the required surgical time [230]. The nasal aesthetic outcome is somewhat satisfactory, but functional considerations like snoring and phonation remain an issue for these patients [231]. The hypoplastic zygomas are reconstructed with bone grafts from calvarial bone, rib cartilage, or implants [222]. In other cases, the vascularized bone flap procedure is used [228].

Possible prevention for the TCS would be the blockage of the apoptotic mechanism of neural crest cell by an inhibitor of p53 tumor suppressor protein [232].

The proteasome inhibitor, bortezomib, can decrease the clinical manifestations of the TCS, but after risk-benefit assessment, it has not been approved by the FDA for TCS patients. Protease inhibitors are associated with an increased risk of tumorigenesis.

Some of the protease inhibitors are approved for the treatment of life-threatening diseases, such as the multiple myeloma or viral infections related to craniofacial anomalies, such as the Zika virus disease, or other viral infections such as HIV, hepatitis C, etc. Therefore, it has been suggested that the potential risk should be critically evaluated and the exact timing and duration of a possible intervention in patients with TSC should be further investigated [233].

Prevention with antioxidants during pregnancy may alleviate the clinical phenotype of the developing fetus. Their function may differentially suppress the neuroepithelial apoptosis, facilitating the development of the craniofacial structures [1].

18.4 Discussion

This chapter presents an overview of early clinical phenotypes and genetic-phenotypic correlations aiming to promote communication and interaction basis of the specialties involved in the multidisciplinary team for the treatment of patients with syndromes affecting the craniofacial and dental structures. Further, this chapter is providing an update for diagnostic and management recommendations for these individuals.

The broad phenotypic spectrum, even among family members with the same casual variants, and the late expression or identification of some phenotypes together with the developmental variability among individuals make an early or a precise diagnosis challenging.

Early fetal diagnosis is of importance for improved genetic counseling, delivery planning in a specialized hospital, postnatal management, and medical decision making. Additionally, an early diagnosis has a significant impact on the postnatal mortality rate, the treatment outcome, and the emotional preparation of the parents [15]. Early clinical phenotyping and genetic evaluation or facial analysis technology will ultimately set the diagnosis.

The role of the team is crucial for the evaluation of the prognostic factors, prevention and medical intervention for the proper function, and craniofacial development. Nevertheless, there are syndromes, such as the 22q11DS, that the patients develop physical or psychiatric comorbidities and learning disabilities later on in life [47]. Therefore, the clinical phenotypes should be reevaluated in different developmental stages of these patients.

A thorough cardiac evaluation enhances the possibility for early diagnosis of a congenital condition such as the 22q11.2 DS and reduces the mortality rate [19]. Moreover, detection of one malformation should enhance a suspicion of more associated anomalies, and further investigation is required.

The etiopathogenesis should be critically evaluated in patients with facial asymmetries. In patients with neurological involvement, not only

the morphologic corrections but also rehabilitation of facial expression should be encountered for a better aesthetic outcome. The 3D stereophotogrammetry is a tool for facial growth evaluation with application in children with facial deformities [234].

Controlling environmental factors to reduce the exposure to teratogenic agents and prevent the development of malformations is challenging. Clinical investigations, together with epidemiological data, are needed to identify the role of epigenetic factors in these conditions, the role of chance, the bias, and the variability of the clinical phenotypes. Antioxidants or other agents [1], since early pregnancy, may improve or even prevent the craniofacial malformations. Further research, though, is required to elucidate clinical recommendations [174].

Detailed clinical phenotyping of facial characteristics may contribute to the identification of genetic variants that cause congenital anomalies [235], advancing precision medicine, and translational research. Nevertheless, "deep phenotyping" conditions with dental involvement are only possible when all permanent teeth have been erupted by 12–14 years of age [236].

The goal of the team is to overcome existing clinical challenges that hamper a satisfying functional and aesthetic outcome, improving the patients' but also their families' satisfaction and above all their quality of life.

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Genomic Aspects for the Diagnosis of Craniofacial Disorders

19

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19.1 Introduction

A deeper understanding of craniofacial diseases is based on the increased knowledge in disease biology and genetics. Advanced technologies in chromosomal and genetic analysis as well as the recent possibilities in bioinformatics and multi-omics data help to get a deeper insight in genotype-phenotype relation. In the past decade, efforts to classify diseases were based on molecular insights increased with studies related to molecular-based disease subtyping in different disease conditions [1]. The sheer volume of data collected in analysing genetics and in documentation of phenotypes from 3D scans and omics data generates massive and complex data sets. The size and heterogeneity of such data sets do not only pose new challenges to efficiently and effectively store data but are also challenging to develop new algorithms to gain insight into the cause-and-effect correlations between genetics, embryological pathogenetics and disease extent (phenotypic outcome).

19.1.1 Technical Approaches

Today, genetics play an important role in medical practice. With the knowledge of genetics, it is possible to provide the final precise diagnosis to many different diseases. Understanding the reason of the disease helps to make it tangible, generate a better treatment plan, and maybe even a cure. Technically, the human genome can be determined on various levels, starting at a chromosomal level up to changes in the base sequence. Many genetic diseases appear phenotypically different and defining the underlying cause in the DNA for each disease was often complicated in the past. However, genetic measures were profoundly inspired by Paul Berg [2], Frederick Sanger [3], and Walter Gilbert [4] as they introduced DNA sequencing. Further studies led to Sanger's "chain-termination" sequencing technology [5] and later to detecting the human genome [6]. In the last two decades, two new techniques of measurement were introduced, that are important in today's genetics. DNA microarrays can genotype millions of specific positions in each human genome, while "next-generation sequencing" (NGS) can even generate billions of sequences in a few hours.

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Approximately half the cases with suspected syndromic diagnosis no underlying cause can be detected despite the use of exome sequence and genome sequence. Both techniques present limitations: structural modifications, methylation processes, repeats and mosaics may be partly or fully detectable. In addition, non-coding sections can hardly be interpreted.

The knowledge of clinically driven genetics and their inherent limitations in paediatrics is therefore important for the evaluation of craniofacial disorders. This rapid development of molecular diagnostics helps practitioners and patients nowadays to get a deeper insight into the diagnostic approach towards craniofacial disorders. In order to gain insight into the genetic-disorder relationship, it is on one hand important to know the definition of subsets of craniofacial anomalies and on the other hand to be aware of technical approaches in genetic testing.

19.1.2 Definition

19.1.2.1 Syndromes and Sequences

A syndrome is defined by a set of symptoms and is correlated independently. It etymologically means 'concurrence' and consequently means medical signs that appear together. A clinical example might be Treacher Collins syndrome. Infants with Treacher Collins present with hypoplasia of the viscerocranium, cleft palate, malformation of the ears, pharyngeal hypoplasia and several other symptoms [7].

A sequence needs to be differentiated from this as it shows a set of symptoms that depend on one primary defect affecting other structures consecutively. A clinical example might be Pierre Robin sequence (PRS). Patients with PRS present with the triad of micrognathia, glossoptosis and resulting airway obstruction. Pierre Robin himself declared the drop of the base of the tongue as a disturbance of the nasopharyngeal airway [8]. The sequence is also often accompanied by cleft palates. It is commonly assumed that the micrognathia causes a dislocation of the tongue to an upper and posterior direction medially between the two parts of the developing palates during pregnancy. This irregular development results in a U-shaped cleft [9].

19.1.2.2 Pathogenesis of Anomalies

In general, anomalies mean the departure of a common phenotype. There are four underlying mechanisms of pathogenesis that lead to structural craniofacial anomalies (Fig. 19.1) [10]:

(i). Deformations.
(ii). Malformations.
(iii). Disruptions.
(iv). Dysplasias.

Deformations

Deformation means that a part of the head or face has a different shape or position because of distorting mechanical influence (e.g. turricephaly). This may cause a loss of symmetry or abnormal position [11].

Malformations

Malformation is defined as an alteration of the primary developmental program (e.g. pharyngeal arch) that leads to a congenital morphological anomaly. This may cause further structural or physiological failures [12].

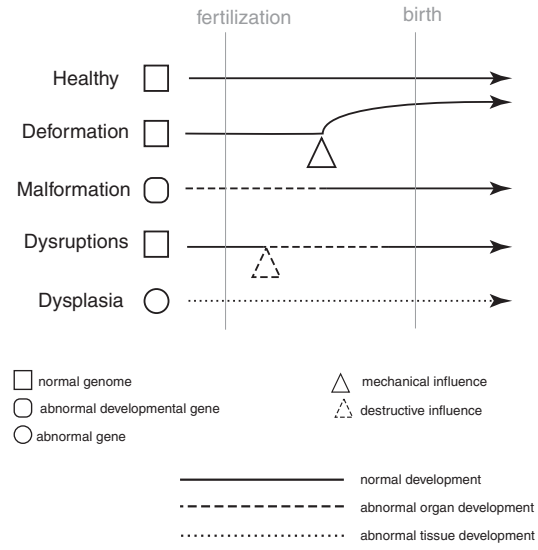

Fig. 19.1 Pathogenesis of anomalies. Based on the graphic by Hennekam et al. [10]

Disruptions
Disruption is a breakdown of a normal, healthy body structure that leads to a congenital morphological anomaly.

Dysplasias
Dysplasia means abnormal tissue architecture (e.g. skeletal dysplasias) [13].

19.2 Genomic Testing

Prenatal tests like the early preimplantation genetic diagnosis or amniocentesis give information about the presence of genetic diseases in an early state of pregnancy [14]. However, many pregnant women decide to non-invasive prenatal testing (NIPT) that analyses small pieces of embryonic genome to detect chromosomal aberrations including trisomy 21, 13 and 18 [15]. Furthermore, it is postnatally also possible to screen for germ line mutations by tests based on blood or saliva samples. Nowadays, many different biological materials can be used to test for genetically caused anomalies. Examination material, more precisely genomic DNA, can be extracted from lymphocytes or fibroblasts postnatally and from chorionic villi prenatally.

There are many different kinds of genomic testing. However, new genomic testing techniques have not replaced older ones but have expanded traditional diagnostic possibilities. Figure 19.2 provides an overview chart on genomic testing methods.

19.2.1 Cytogenetics

Cytogenetics mean the science of chromosomes, their number and structure.

19.2.1.1 Chromosomal Analysis

The chromosomal analysis evaluates chromosomes by light microscopy. Karyotype gives information about the number and structure of chromosomes. A karyogram shows the size,

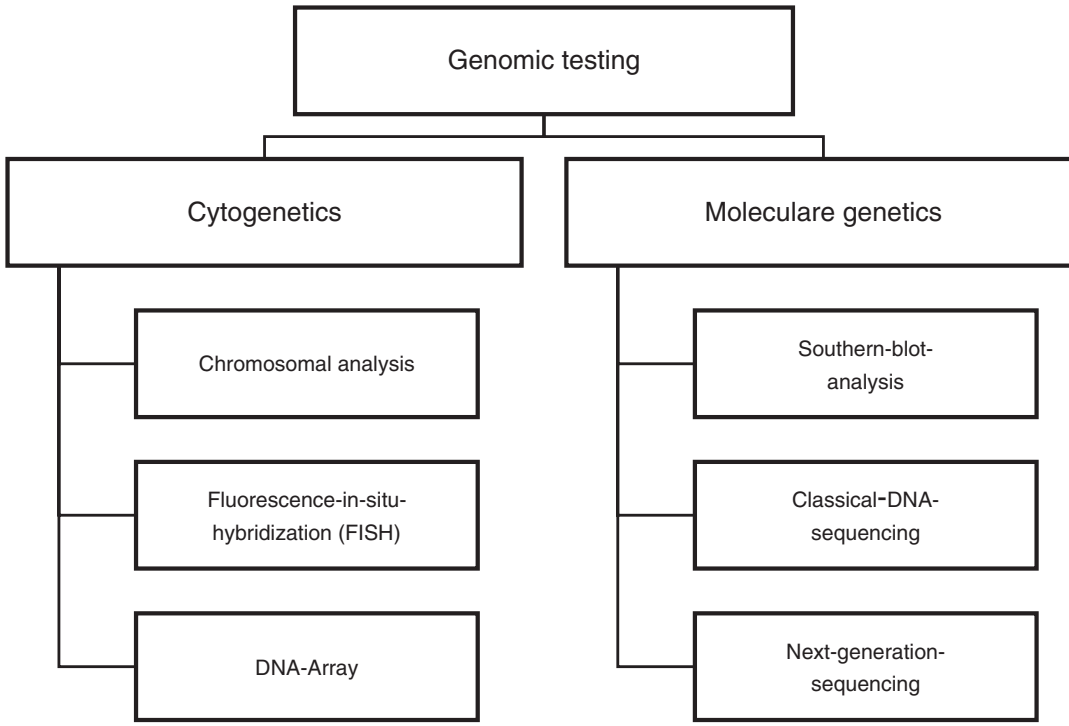

Fig. 19.2 Overview chart on genomic testing methods

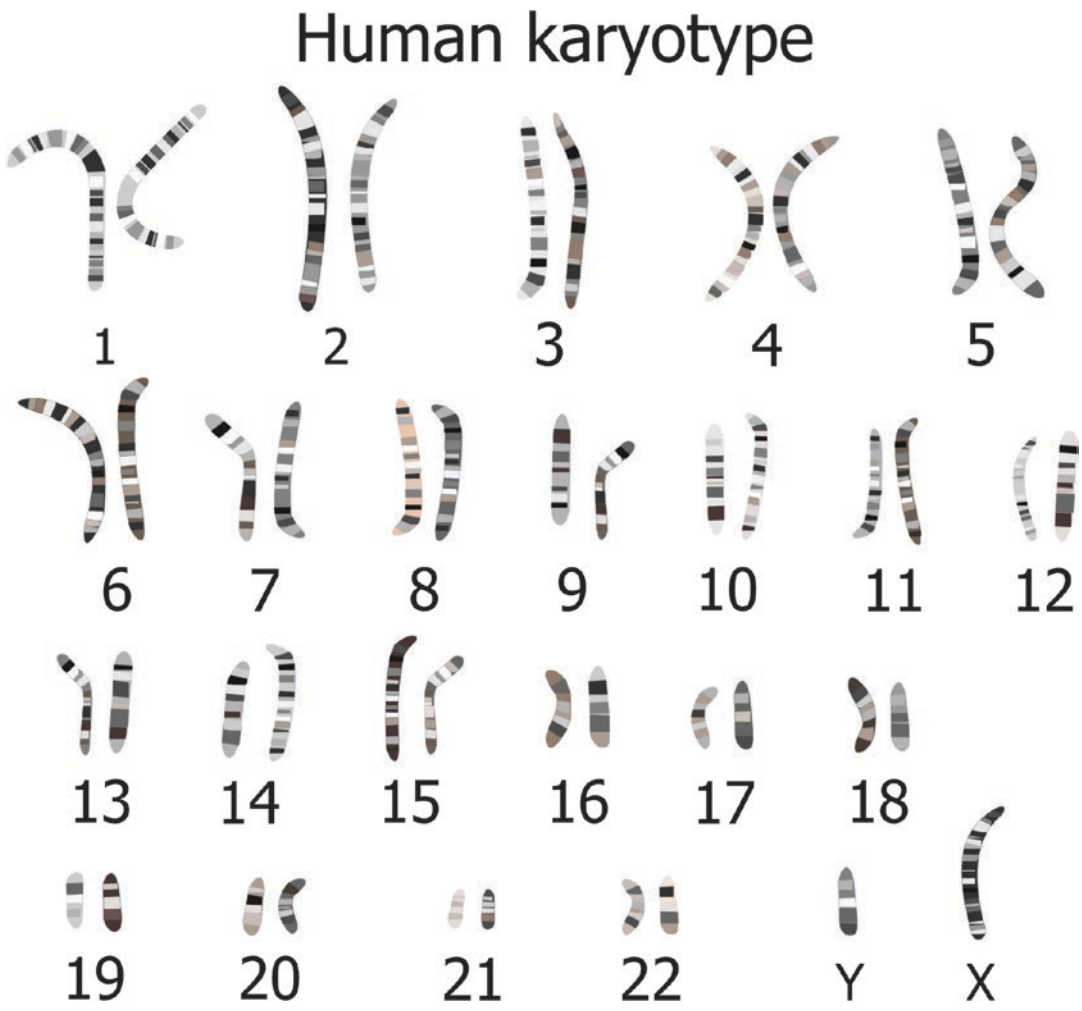

Fig. 19.3 Human karyotype

shape and banding of an individual by the use of cytogenetic technique. This analysis with limited resolution was the first technique to reveal the genome (Fig. 19.3).

The classical cytogenetic technique is able to detect chromosomal aberrations, including numerical and structural changes. Nondisjunctions, incorrect distributions and structural aberrations can be displayed. Numerical changes can be due to nondisjunction, which means the missing separation of homologous chromosomes during meiosis I and of the sister chromatids during meiosis II or mitosis. Risk factors include increased maternal age as well as ionizing radiation. Incorrect distribution might affect gonosomes (e.g. Turner syndrome, monosomy 45,X0; Fig. 19.4) or autosomes (e.g. Down syndrome, trisomy 21; Fig. 19.5).

0.5% of all infants show chromosomal aberrations, and more than half of spontaneous aborts are caused by numerical chromosomal aberrations [16]. Somatic chromosomal aberrations also play an important role in tumorigenesis as it is in detail investigated in translocation t(9;22), also known as the Philadelphia chromosome, that leads to chronic myeloid leukaemia [17].

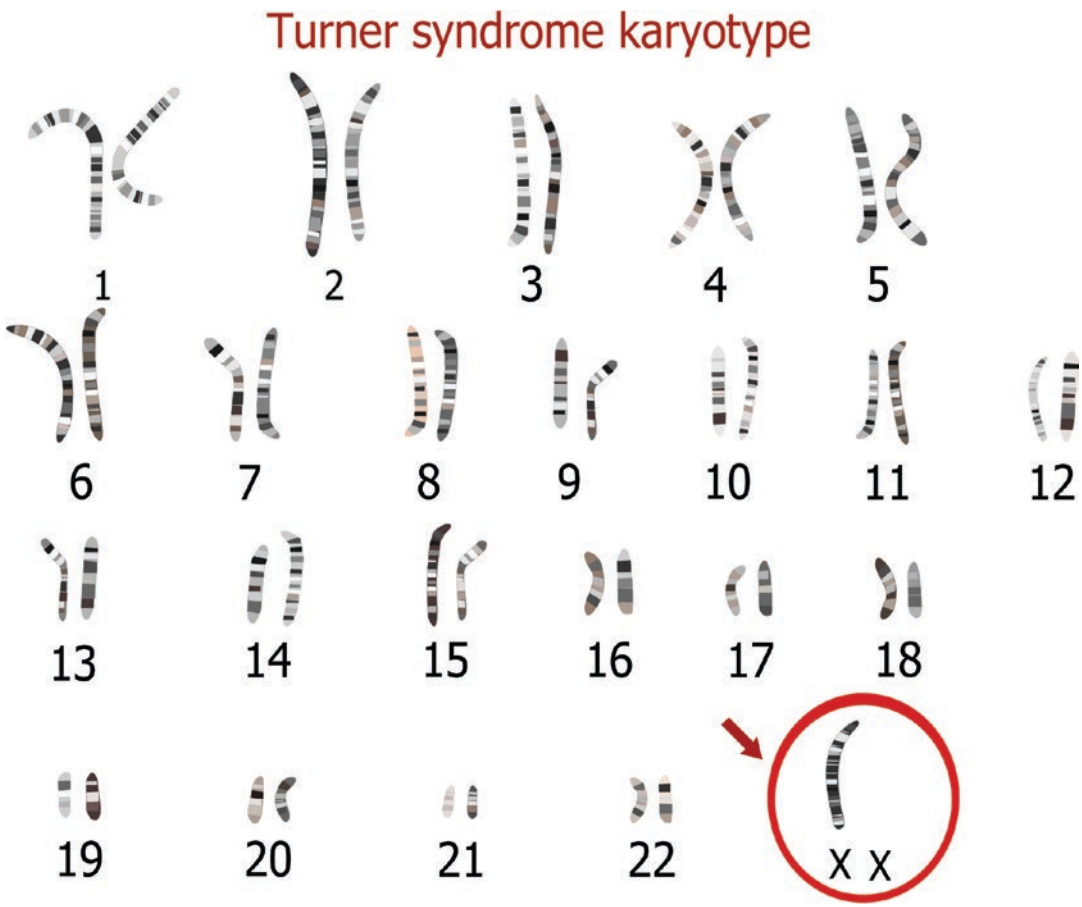

Fig. 19.4 Karyotype with monosomy 45,X0 (Turner syndrome)

19.2.1.2 Fluorescence in Situ Hybridization (FISH)

Further developments lead to fluorescence in situ hybridization (FISH) in the late 1980 that provides deeper insights and more detailed examination of the individual chromosome. FISH combines cytogenetic and molecular genetic approaches and offers the opportunity to display chromosomes and chromosomal sections in colour by fluorescence microscopy. Fluorescently labelled DNA probes join single-stranded DNA (hybridization) directly on the patient's sample (in situ). With this technique, diagnosticians are able to detect specific chromosomal sections and reveal microdeletions that could not be recognized by classical chromosomal analysis [18].

Locus-specific FISH analysis can detect with high resolution but is limited to single chromosomal sections and therefore cannot provide genome-wide examination.

19.2.1.3 DNA Microarray

Today, DNA microarrays combine the advantages of both the chromosomal analysis and FISH. DNA microarrays are able to examine the whole genome on many areas of the genome at once with a resolution of a few thousand nucleotides. Hereby, unbalanced chromosomal changes, including small copy number variants, can be detected. This reveals all numerous and unbalanced structural chromosomal aberrations, such as microdeletion syndromes (Fig. 19.6).

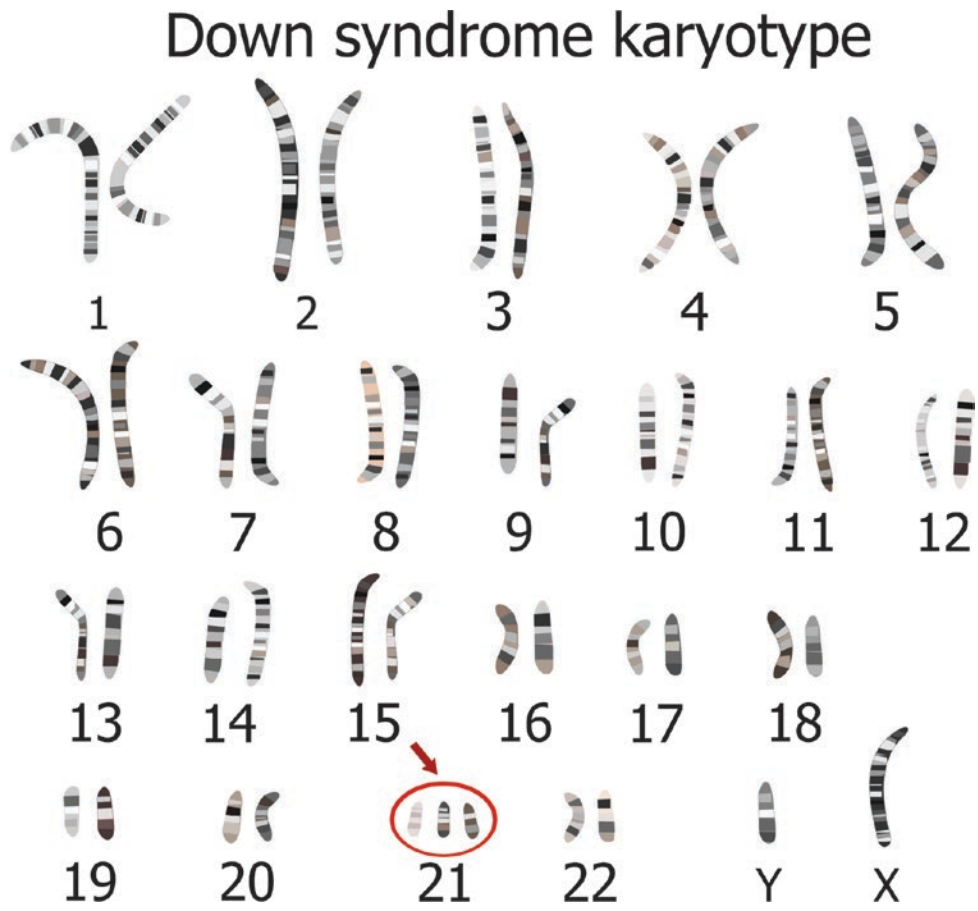

Fig. 19.5 Karyotype with trisomy 21 (Down syndrome)

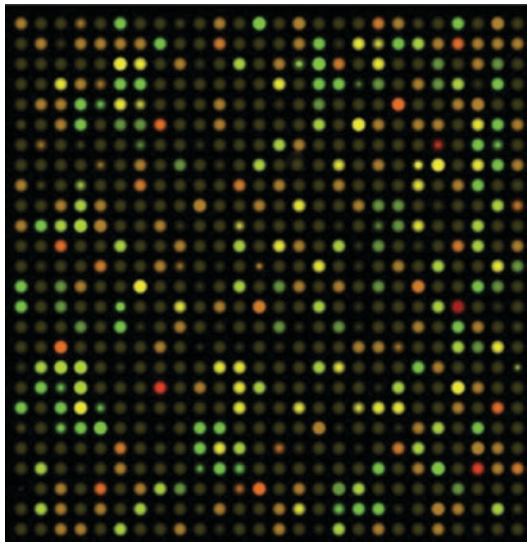

Fig. 19.6 DNA microarray

Modern SNP arrays are also able to give quantitative information about copy numbers [19]. Copy number variation means structural differences of the genome due to a gain (duplication) or loss (deletion) of chromosomal material. In conclusion, the number of gene copies in a sample differs to the number in a reference genome. Duplications and deletions can affect the phenotype of a patient in a highly wide range depending, among others, on the length of the DNA section and its gene content [20]. Modern SNP arrays are also able to detect uniparental disomy, which means that both homologous chromosomes originate from the same parent. DNA microarrays are not able to detect balanced chromosomal aberrations, including balanced translocations.

The diagnostic yield of a karyotype or DNA microarray analysis used to screen individuals with craniofacial malformations varies by type and category. For example, in some studies, the diagnostic yield for isolated nonsyndromic single-suture craniosynostosis cases is very low or near zero. Yet in syndromic craniosynostosis, the yield ranges from 6.7% to 28%. The vast majority (85%) of craniosynostosis due to chromosomal aberrations affects the midline (metopic and sagittal) sutures. Karyotype and DNA microarray studies in individuals with oral clefts also have varying degrees of diagnostic yield depending on whether the clefts were detected prenatally or postnatally. Maarse et al. summarized a comprehensive review of prenatal and postnatal chromosomal and microarray studies [21]. Of 407 foetuses with oral clefts, cleft lip and palate had the highest prevalence of associated anomalies (54%, range 39.1–66%). There were 23 cases of cleft lip without cleft palate, and three of these had associated anomalies, while only one had a chromosomal defect. Studies that grouped both cleft lip and cleft palate had a lower prevalence of associated anomalies (29.9%, range 17.2–57.1%). The prevalence of chromosomal defects in cleft cases with associated anomalies was 50% (74/146), while it was 0.9% in cases with clefts that were formerly presumed to be isolated. Of 28,953 postnatally assessed infants, almost all chromosomal abnormalities were found in association with additional anomalies. Cleft palate was the category most frequently associated with other anomalies (45.9%, range 22.2–78.3%). The prevalence of associated anomalies in cleft lip cases was approximately 10%. One study of isolated cleft lip cases found a chromosomal defect in 1.8% (2/110) of cases (both having a 22q11.2 deletion). Overall, the diagnostic yield of screening for chromosomal defects in cases of cleft lip with or without cleft palate was 9.5% (range 0.5–12.6%) [21].

19.2.2 Molecular Genetics

Molecular genetics contains all diagnostic approaches that examine alterations of genetic information in extracted RNA or DNA. Molecular genetics made giant leaps due to the development of polymerase chain reaction (PCR) and is increasingly expanding classical analyses.

19.2.2.1 Southern Blot Analysis

Southern blot analysis provides information about the length of a specific DNA section. Specific restriction enzymes cut the genomic DNA into pieces that will be separated electrophoretically afterwards. After transferring onto a nylon membrane (blotting), the technique is able to detect restriction fragment length polymorphism (RFLP) (Fig. 19.7).

The approach used to be very effective in detection of point mutations before PCR was established but is still the method of choice in detection of massive repeat expansions occurring in trinucleotide diseases.

19.2.2.2 Sanger Sequencing

Sanger sequencing is the gold standard and detects mutations reliably. Polymerase chain reaction (PCR) is able to amplify small DNA sections that can then be sequenced. The technique determines the sequence of nucleic acids (order of nucleotides in DNA) (Fig. 19.8).

Most recognizable craniofacial syndromes are monogenic Mendelian disorders, but even within the same condition, there is often allelic heterogeneity with most individuals having different mutations in the same gene. One exception to this are the recognizable craniosynostosis syndromes involving the FGFR genes (*FGFR1*, *FGFR2*,

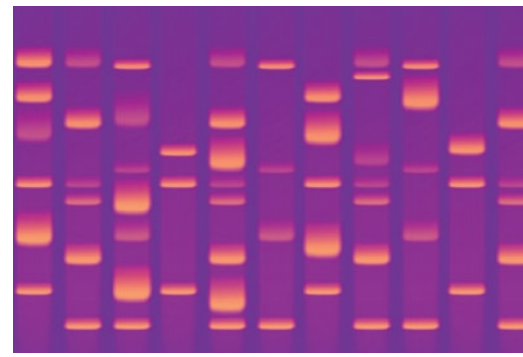

Fig. 19.7 A DNA Southern blot chart

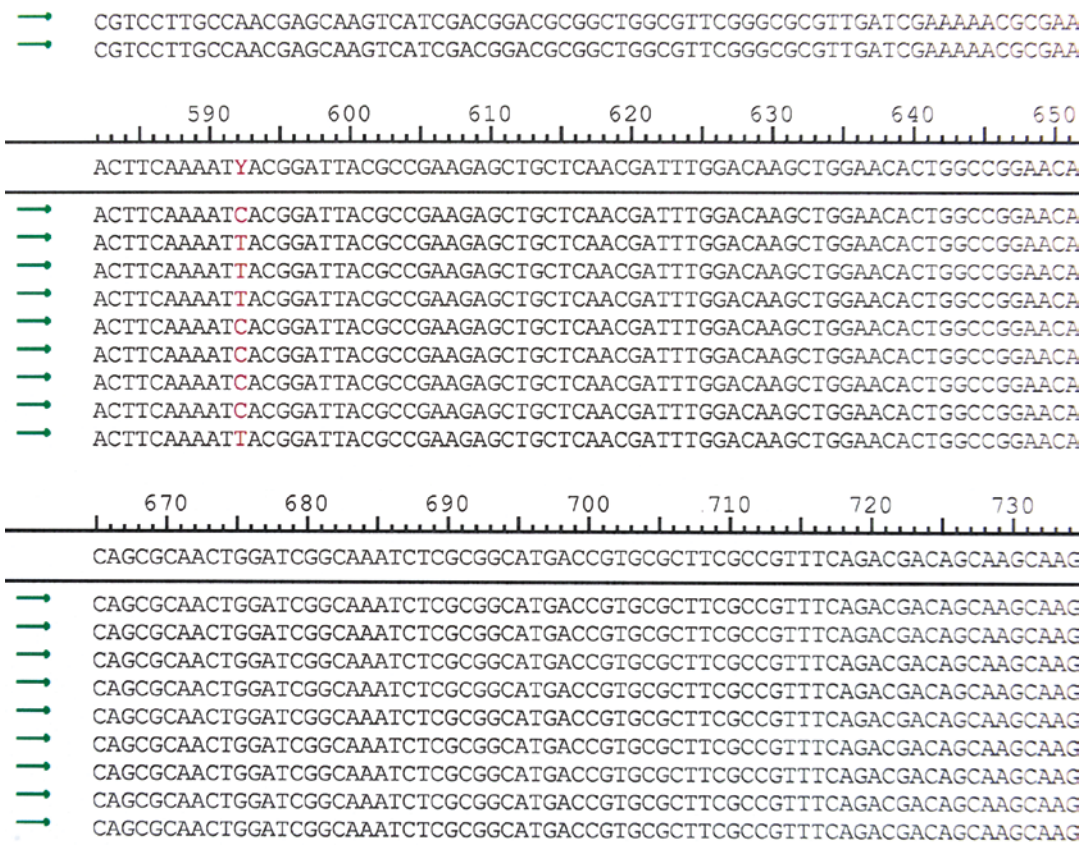

Fig. 19.8 Sanger sequencing. In this case determining a point mutation

FGFR3). In craniofacial medicine, there are a growing number of clinically indistinguishable or overlapping phenotypes that may be caused by mutations in different genes (locus heterogeneity). Examples include rasopathies, cohesinopathies, mandibulofacial dysostoses and Stickler syndrome [19]. When using a major malformation as the only search criterion, the number of genes involved can range from just a few to more than a hundred. For many genetically heterogeneous craniofacial disorders, the full complement of causal genes is yet to be established.

A craniofacial condition such as Treacher Collins syndrome, which had initially been reported to be monogenic and autosomal dominant, has subsequently been found to be multigenic with autosomal dominant (*TCOF1*, *POLR1D*) and recessive forms (*POLR1C*). Stickler syndrome is another rare condition with a growing number of genes related to an overlapping phenotype with autosomal dominant (*COL2A1*, *COL11A1*, *COL11A2*, *VCAN*) and autosomal recessive inheritance (*COL9A1*, *COL9A2*, *COL9A3*, *LOXL3*) [25].

As of today, Sanger sequencing remains the gold standard molecular diagnostic tool used to screen DNA for unknown point mutations in defined genes; this may change as the confidence and quality of newer technologies improve. Up until recently, some larger genes had remained inaccessible to clinical testing because the older methods were too burdensome on laboratory staff, or the condition was too rare for a test to be commercially viable.

19.2.2.3 Next-Generation Sequencing

Next-generation sequencing (NGS) contains all new approaches of high-throughput sequencing. The underlying idea is the massive parallel

sequencing of millions of DNA sections in a single sequencing run. This results in the theoretical opportunity to detect nearly all genetic alterations (like minor insertions or major translocations and even aneuploidy) by a single test. The use of this next-generation sequencing (NGS) technologies to interrogate the exome sequence (ES) or genome sequence (GS) may circumvent some of the difficulties of older technologies.

Today, this technique is work and cost intensive. Since the importance of introns is still not clarified, the sole sequencing of exomes as the protein coding area of the genome represents a good alternative. However, in 2010, next-generation sequencing was used to reveal the underlying gene for the Miller syndrome, a pathology with micrognathia, cleft lip and palate and other anomalies [22].

19.3 Management

Determining genetic causality for a particular disease and establishing a molecular diagnosis in clinical practice can be challenging. In recent years, exome and genome sequencing have increased the rate of gene discovery for single-gene disorders among patients with suspected, but previously undiagnosed, genetic disorders. Although exome and genome sequencing are becoming more readily available, the value of molecular diagnosis should be viewed from a clinical perspective as similar to other diagnostic tests. The decision to proceed with molecular testing must integrate many factors specific to clinical status of the affected individual, such as probability of diagnostic yield and the patient's/family's personal preference.

19.3.1 Clinical Evaluation

To evaluate craniofacial disorders, it is obligatory to get detailed information about risk factors. First of all, potential prenatal exposures have to be retrieved and checked on their teratogenic potential [23]. Among other things, this is important to calm parents who are afraid of exposures of drugs that are not teratogenic. Other common risk factors for malformations are maternal diseases like diabetes or alcoholism. Furthermore, a pedigree analysis can reveal genetic diseases due to its penetrance, anticipation and expressivity. Besides the genetic diagnosis, standard paediatric assessments like growth measurements should be mentioned. The results of this analysis lead to differential diagnoses.

Besides this standard examination, experienced practitioners are able to detect common syndromes or sequences based on typical patterns of morphological anomalies [24]. However, orphan diseases or minor variants of certain disorders might remain unrecognized. In these cases, molecular diagnostics offer a powerful instrument to detect the underlying cause of craniofacial disorders.

19.3.1.1 Genetic Test

Molecular genetic tests are of increasing importance in all medical professions. However, there is a great discrepancy between technical abilities and sensible use of these instruments. The practitioner has to evaluate the right diagnostic methods in order to gain the best information and to cause least costs. Detection of an underlying pathogenic DNA variant is only one aspect in the diagnosis of craniofacial disorders. The clinical question arises which information will be gathered after testing, how this information will help the patient and how will it affect the patient.

19.3.1.2 Future Directions

Large-scale studies are needed and are recently on the way to identify the complex correlations between genetic influences, embryological development and the resulting phenotype. This is especially important for seldom diseases like craniofacial malformations. One way to improve in future the approach to gain a deeper insight into disease biology of rare diseases is to integrate genetic and molecular data as well as phenotypic appearances into a broad network of craniofacial data. A specific ontology—the Ontology of Craniofacial Development and Malformation (OCDM)—was developed years

ago as part of a NIDCR-funded research network, FaceBase (https://www.facebase.org). FaceBase provides diverse but standardized data to the craniofacial community in order to facilitate collaboration among investigators to advance craniofacial research. By a standardized set of terms and relationships in craniofacial information (including molecular, genetic and clinical data), FaceBase tries to integrate data types to maximize their utility and accessibility [25]. This way of information technology has a great promise for future advances in diagnostics and personal targeted therapies.

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Neurosurgical Investigation of Craniofacial Malformations

20

Bernd Hoffmann, Ulrich Meyer, and Uta Schick

20.1 Introduction

Neurosurgical investigations of craniofacial malformations face the whole range between patients presenting with positional plagiocephaly up to syndromic craniosynostosis. As both disease entities implicate a complete different therapeutic approach, familiarity with the diagnosis of craniofacial head deformations is of special relevance. Neonatal cranial head shape anomalies are common. The biological basis is based on a multitude of factors. Despite recent advances in the awareness of such malformations, the incidence of some cranial anomalies has been reported to increase, making awareness, early differential diagnosis, and individually oriented treatment important. Cranial head shape anomalies are commonly identified in the first months of life by primary care providers, who mostly refer these infants to a center that specializes in craniofacial disorders.

Deformational plagiocephaly (DP), also termed positional plagiocephaly, and craniosynostosis (CS) are two of the most common cranial anomalies encountered in craniofacial clinics. Deformational plagiocephaly is the most common neonatal head shape anomaly affecting 13–48% of infants less than 1 year of age. This condition is thought to be based on prolonged supine positioning. As a result, the skull develops an oblique, parallelogram shape that varies in the severity of the calvarial vault asymmetry.

The term craniosynostosis describes the premature fusion of one, multiple, or all of the cranial sutures, resulting in characteristic deformities of the cerebral cranium, in many cases also associated with deformities of the facial skeleton. CS is a much less common disorder (incidence of 1 in 1800 to 3000) and results from early fusion of fibrous cranial sutures which serve as growth centers separating immature, growing cranial bones. The sagittal suture is affected in 55%, coronal suture in 25%, metopic suture in 15%, and lambdoid suture in 5% of the cases (Fig. 20.1). Premature ossification and union of individual cranial bones, according to Virchow's rule, results in abnormal cranial growth parallel to the fused suture(s). CS requires surgical correction and often cranial vault expansion/remodeling to restore the "normal" infant head shape and aesthetics. The most important neurosurgical issues concern increased intracranial pressure (ICP), caused by different pathophysiological mechanisms as well as by hydrocephalus, amaurosis following increased ICP and papilledema,

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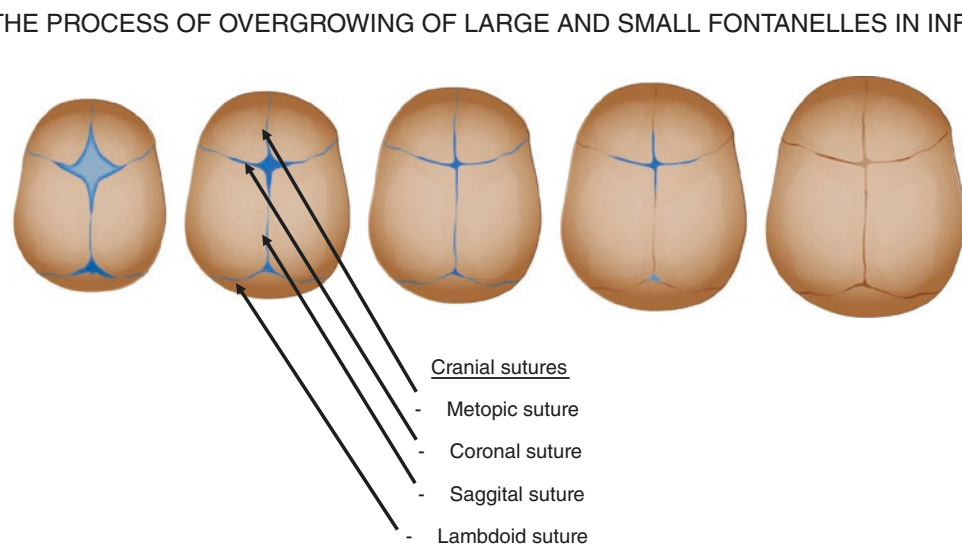

Fig. 20.1 Locations and time-dependent ossification of cranial sutures. (Reprinted from Artemida-spy/Shutterstock.com with permission)

impairment of cognitive functions, and Chiari malformation with tonsillar herniation, brain stem compression, and syringomyelia, compromising the function of the spinal cord. In this chapter, we describe pathophysiological and clinical findings in syndromic and nonsyndromic patients, which are important to reflect for an interdisciplinary approach of therapy in a setting of neurosurgical, neuropediatric, and craniomaxillo-facial specialists.

20.2 Biology of Craniosynostosis

A craniosynostosis is a developmental anomaly which occurs as a consequence of abnormal and nonphysiological sutural fusion. In newborns, the membranous bones of the cranial vault are separated by intervening sutures. The presence of such sutures enables the infant's skull to pass more easily through the birth canal and allows the compensatory growth of the skull during the brain growth. When one or more sutures are prematurely closed, the compensatory growth starts perpendicular to the patent sutures since the brain still grows and expands in the direction of lower resistance, resulting in an abnormally shaped skull.

20.3 Classification of Craniosynostosis

Classification of craniosynostotic patients can be done on the underlying biology of the disease as well as on the extent of the disease. Some classification models of craniosynostosis are used depending on the underlying mechanism, presence of other disorders, or number of fused sutures (Fig. 20.1). For example, if a craniosynostosis develops due to a primary defect of the ossification process, it is called primary craniosynostosis. Secondary craniosynostosis is the result of known systemic diseases with hematologic or metabolic dysfunction, such as rickets and hypothyroidism. Secondary craniosynostosis can also develop in newborns with microcephaly due to a failure of brain growth or following shunt placement in children with hydrocephalus. In addition, craniosynostosis can be classified into syndromic, e.g., as part of Apert, Crouzon, or Pfeiffer syndrome, and more commonly encountered, nonsyndromic craniosynostosis, where it develops as an isolated disorder. Simple craniosynostosis is a term used when only one suture fuses prematurely, while complex craniosynostosis is used to describe a premature fusion of multiple sutures.

20.4 Diagnostics

The diagnosis of a typical craniosynostosis is usually clinical by inspection and palpation of fontanels and sutures, and it is commonly diagnosed in the first year of life (Fig. 20.2). In uncertain cases, ultrasound investigations will bring additional information. The clinical assessment determines the following: (a) whether a craniosynostosis is present, (b) which and how many sutures are involved, and (c) whether there are signs suggesting an associated syndrome. Initially, a careful medical history also of parents should be obtained. Systematic evaluation includes primary phenotypical evaluation and may be added by imaging, clinical, and laboratory tests. The clinical examination is at this stage the most important part. The clinical investigation should focus on the search of typical signs of syndromes, especially possible congenital anomalies (e.g., a broad, radially deviated thumb in Pfeiffer syndrome or syndactyly in Apert syndrome), dysmorphic features of the face (hyper- or hypotelorism, hypoplasia of the midface, asymmetry, the position, shape, and size of the ears), or alterations of a normal skull shape. Measurement of the head circumference for calculating the cephalic index (the ratio of maximum breadth to maximum length of the skull) is a reliable method to get insight into skull deformities. Any sutural ridging, prominent blood vessels on the scalp, and the size, shape, and tension of the fontanels should also be assessed. Morphological evaluation of the skull should be performed for overall shape and size and palpation of the anterior and posterior fontanels (Fig. 20.2) with attention to size, shape, and fullness with the infant in both the upright and supine positions. In addition to looking at the infant from the front and sides, it is important to observe the skull shape from above, particularly to note any asymmetries in ear position and any flattening of the skull posteriorly, as well as from behind so the levelness of the skull base can be assessed. Deformational plagiocephaly (positional deformation) is the most common differential diagnosis and may confuse the investigator. Positional deformation is different from craniosynostosis in that the parallel quadrangular

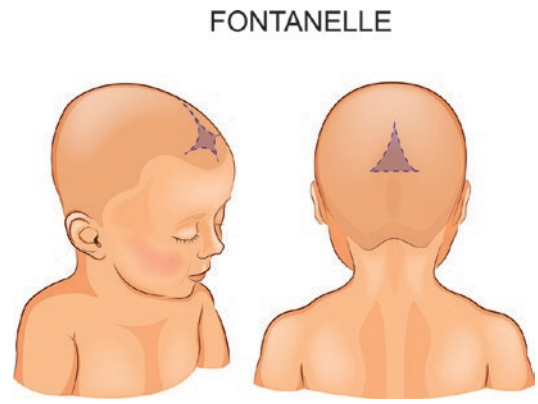

Fig. 20.2 Palpation of anterior and posterior fontanels is one important clinical investigation to evaluate craniofacial disorder. (Reprinted from Artemida-spy/Shutterstock.com with permission)

shape results from a positional effect instead of trapezoid or more complex geometric shape and compensatory contralateral bulging in craniosynostosis (Fig. 20.3). Occipital flattening is a relatively common feature in oriental neonates and should not be confused with bilateral lambdoid synostosis. Sometimes, multiple synostotic patients present with a symmetric, normal-looking appearance. In this case, the skull is small for their age.

Beneath the clinical investigations, imaging procedures should be initiated in uncertain cases. The ultrasound diagnosis of craniosynostosis per se is made when there is a loss of hypoechogenicity in a segment of the normal skull sutures, which is usually associated with an enlargement of other orthogonal sutures. In a population at risk for this condition, the positive predictive value of direct examination of the sutures may be high, while in low-risk pregnancies, it may be minimal, and further investigation may be necessary (3D ultrasound, CT scan, or MRI). The most important consideration in the prenatal assessment of craniosynostosis is the distinction between isolated, multisuture, and syndromic craniosynostosis. Therefore, it is extremely relevant to detail fetal anatomy as a whole. Special attention should be paid to fetal hands and feet, long bone growth, central nervous system, and the heart. The evaluation of

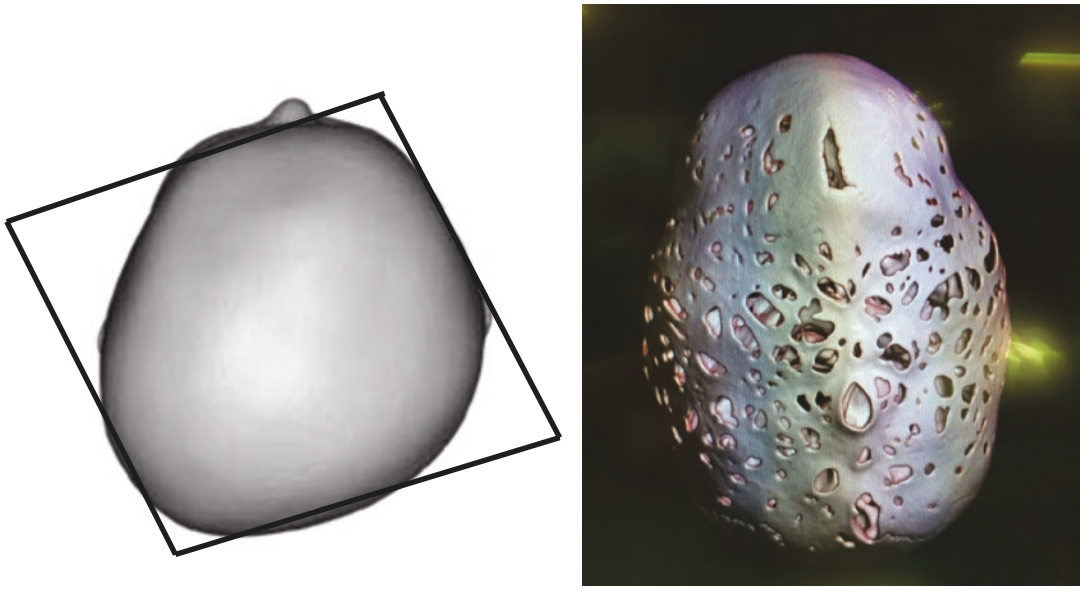

Fig. 20.3 View of a parallel phenotype in deformational plagiocephaly, in contrast to a complex phenotype in craniosynostosis (pansynostosis)

fetal head, face, and sutures can be complemented by 3D ultrasound. MRI can be considered complementary to ultrasound and seems to have negative predictive value when synostosis is suspected on ultrasonography. Cranial sutures cannot be visualized directly by MRI, but indirect signs such as skull deformities can be seen as temporal indentations ("notch at the level of coronal sutures") or thickening of the calvarium in the region of the suture. 3D CT is a third imaging procedure that allows direct examination of cranial sutures and bony anatomy of the fetal face and skull base. The disadvantage of such technique is the presence of fetal irradiation, although the theoretical exposure to radiation is very similar to that of conventional fetal radiological examination.

As special comorbidities are important for the fate of patients with craniosynostosis, these issues are of special neurosurgical concern. Special attention to these factors (intracranial pressure, brain development, orbital pathologies, as well as Arnold-Chiari malformations and syringomyelia) is of vital relevance.

20.5 Intracranial Pressure in Craniosynostosis

Dependent on the number of involved sutures and dependent on concomitant pathologies such as upper airway obstruction, increased intracranial pressure is a frequent problem in patients with craniosynostosis [1–4]. In severe cases of pansynostosis, the copper beaten skull (Figs. 20.4 and 20.5) is classically a reliable radiological indicator for diagnosis and a well-known sign since decades. In cases of single-suture synostosis, increased ICP can be observed in 15–20%, whereas more than 50% are concerned in multisuture affection [5–9]. Furthermore there is a higher risk for syndromic patients, depending on the type of syndrome [8, 10]. In the past, the main idea behind this was the impact of a decreased intracranial volume (ICV), restricting the growth of the brain [11]. Today, we know from CAT and MRT findings that most of the patients have a normal ICV [12], so we have to suppose that ICV is only one factor for increased ICP, boosted by additional factors like compromised dynamics of

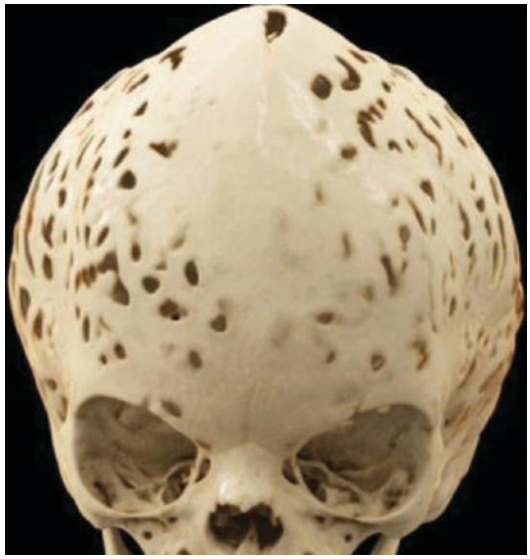

Fig. 20.4 Typical copper beaten skull in a case of Crouzon syndrome

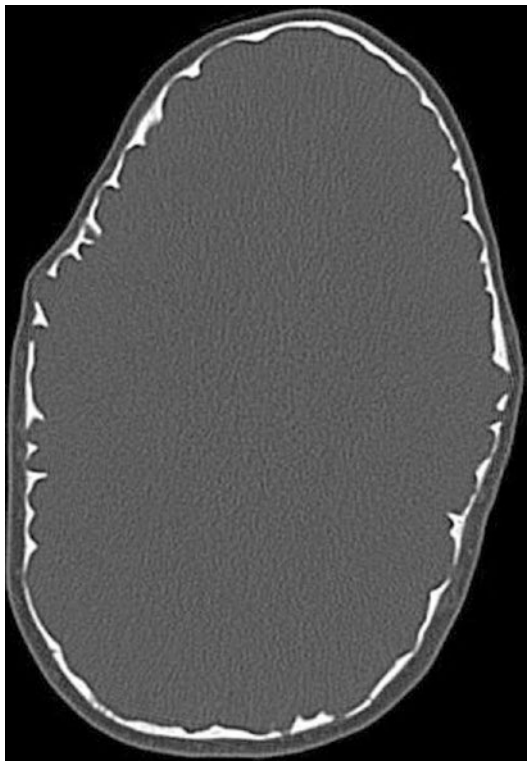

Fig. 20.5 The same patient, CAT scan, bone window with axial slices and evidence of spiculae

cerebrospinal fluid (CSF) with dilatation of the subarachnoid spaces [1] or hydrocephalus, reduced CSF absorption, restricted venous outflow [10], and upper airway obstruction [8, 9]. The last one is a frequent problem in syndromic patients such as Crouzon syndrome and is likely to raise CO_2 levels resulting in an increased cerebral blood volume with a decreased intracranial compliance [10]. Nevertheless, a decreased ICV can be affirmed in patients with pansynostosis [13–16]; that is why timing of surgery in these cases is highly influenced by confirming diagnosis of increased ICP and exploration of its causes. At least it is widely accepted today that the most relevant pathophysiological mechanism for ICP increase in patients with craniosynostosis is impairment of CSF absorption [5].

Dynamic hydrocephalus (Figs. 20.6 and 20.7), caused by malabsorption or obstruction of the CSF outflow, is clearly related to a nonsyndromic or syndromic etiology [14, 17–19]. Whereas a progressive course with indication for shunt surgery can be observed in 0.28% of the nonsyndromic cases, the frequency in syndromic cases raises up to 12.1% [5, 17]. The pathophysiological cause for the higher incidence in syndromic cases is still controversial: some authors proclaim an increased CSF outflow resistance due to constriction of the posterior fossa [18, 20–22]. This hypothesis is contradicted by the missing success to restore sufficient CSF circulation after decompression of the posterior fossa [18, 20]. We even observed one case with deterioration of CSF drainage after decompression of the posterior fossa. Another hypothesis emphasizes a venous outflow restriction by a stenosis of the jugular foramen [17, 18, 22, 23]. Actually, a combination of both mechanisms is under discussion [3]. In cases of scaphocephaly with premature synostosis of the sagittal suture, direct compression of the superior sagittal sinus is discussed as well as impairment of the arachnoid granulations resulting in CSF malabsorption [7]. Principally, the diagnosis of hydrocephalus is reliable by sonography in cases of scaphocephaly or MRT in

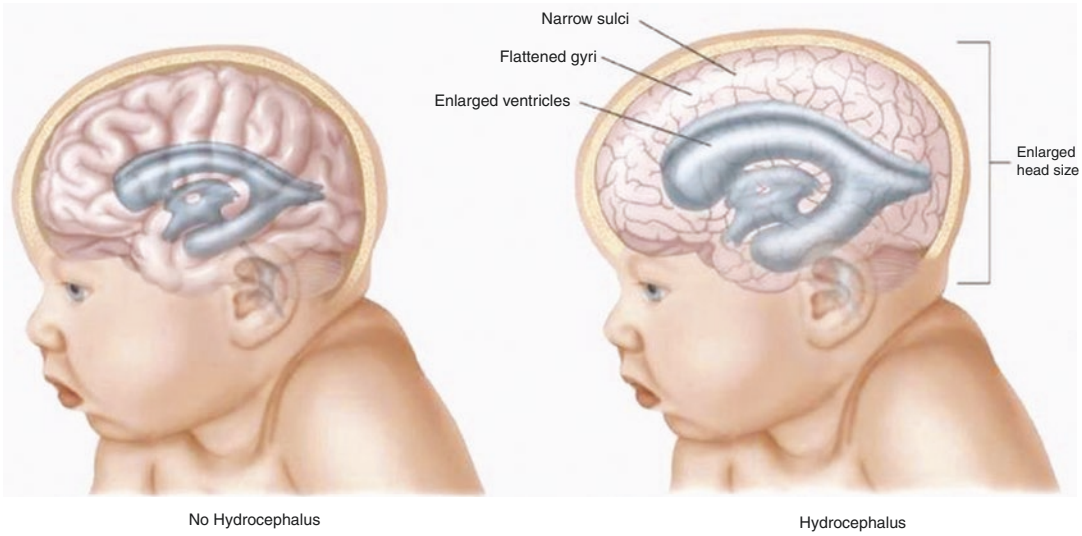

Fig. 20.6 Schematic drawing of hydrocephalus. (Reprinted from CDC/wikipedia.org with permission)

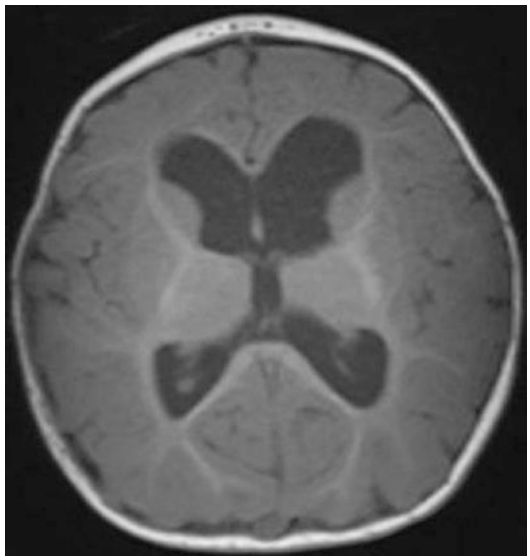

Fig. 20.7 Massive ventricular enlargement in a case of Pfeiffer syndrome with pansynostosis

complex patients, but it might be tricky to reduce it only to evidence of ventricular enlargement: it is a known mechanism that ventricular enlargement is limited by the restriction resulting from the rigidity of the synostotic skull, what has to be considered especially in cases of pansynostosis [3].

Clinical signs indicating an increased ICP are headache, dizziness, nausea and vomiting, bulging fontanel, and impaired consciousness [11]. Nevertheless, none of these symptoms is really specific. So we have to know that headache appears only in 19% of the cases of craniosynostosis with increased ICP, detected by intracranial pressure monitoring, and nausea in 12% of the cases [6, 10, 24]. One of the most threatening symptoms is the development of papilledema [24], leading to optic atrophy and amaurosis. That means that a fundoscopic examination by an experienced ophthalmologist is vital for early diagnosis and therapy. Following this, diagnosis of increased ICP in craniosynostosis has to be confirmed by a careful assessment of clinical as well as sonographic or CAT and MRT findings. In this way, normalization of ICP by shunt application (Figs. 20.8 and 20.9) is the most important neurosurgical objective of surgery.

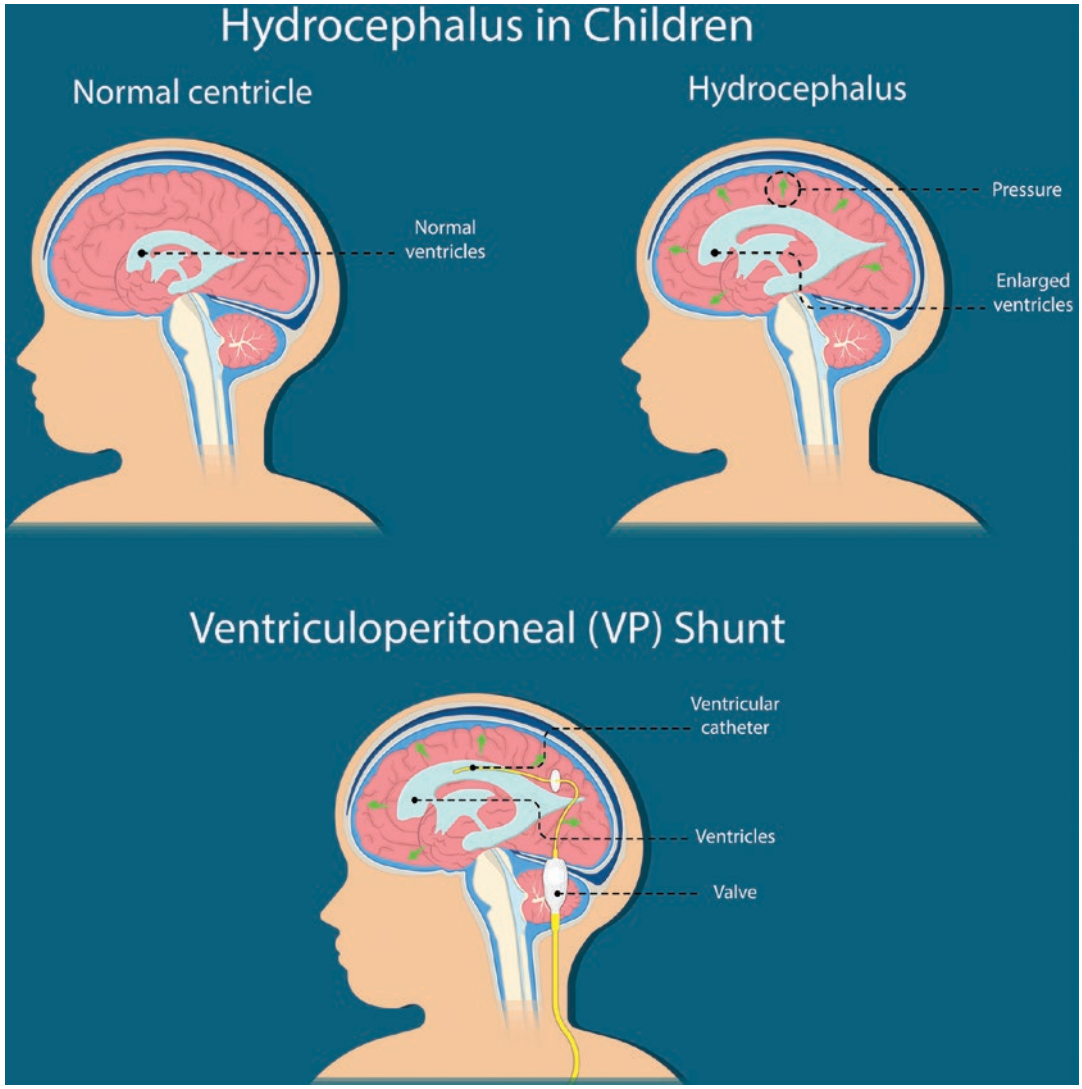

Fig. 20.8 Shunt application as an urgent measure to treat hydrocephalus. (Reprinted from rumruay/Shutterstock.com with permission)

20.6 Brain Development and Cognitive Dysfunction

Our growing knowledge about genetic mutations and more sophisticated genome testing revealed mutations of FGFR, TWIST, MSX2, or EFNB1 gene in cases of craniosynostosis. Resulting malformations of the brain are depending on the specific syndrome with distinct variations [25]. So we can observe in cases of Apert syndrome absence of olfactory bulbs and tracts, incomplete development of the hippocampus, dysgenesis of the corpus callosum [26], as well as abnormalities of the septum pellucidum [25]. In Crouzon syndrome, agenesis and hypoplasia of the corpus callosum are typical findings [25].

Brain malformation, additionally compromised by hydrocephalus and increased ICP, therefore may result in cognitive dysfunction [27]. Intelligence quotient showed to be lower (83.1) in children with syndromic craniosynostosis, even single-suture synostosis seems to corre-

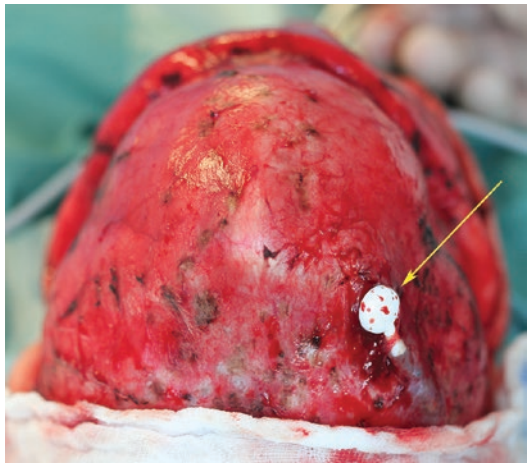

Fig. 20.9 Crouzon syndrome with pansynostosis. Ventricular shunt (yellow arrow) had been implanted prior to reconstructive surgery

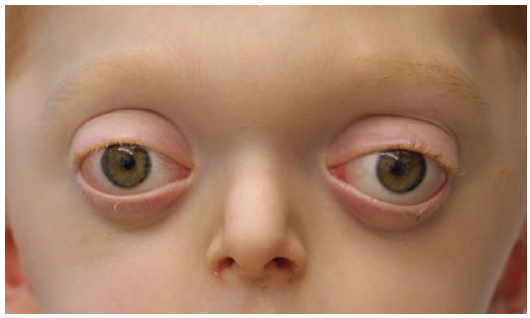

Fig. 20.10 Patient with Crouzon syndrome, hypoplasia of the orbits with bulging bulbs

late with a mild but significant deficit [28, 29]. That means that each patient should undergo an explicit examination by an experienced neuropediatrist, not only at the beginning of diagnostics and planning of surgery, but also in the follow-up for the decision about appropriate therapeutic support to care for the rehabilitation potential after surgery.

The benefit of surgery concerning cognitive development still is inconclusive; nevertheless, we have some evidence that surgery helps to improve neurocognitive and behavioral functions [30].

20.7 Orbital Pathologies

Clinically important malformations also concern the orbits in cases of multisuture synostosis or pansynostosis resulting from a shortening of the anterior skull base due to premature fusion of the basal sutures [31–35]. In consequence, orbital volume is reduced uni- or bilaterally, as well the angle between midline and orbital axis widens. This causes typical symptoms such as exophthalmos (Fig. 20.10) and hypertelorism. It is important to understand that bulging bulbs are not only an aesthetical problem. Depending on the mismatch between the bony orbit and orbital viscerae, closing of the lids becomes incomplete. This situation threatens the cornea. In extreme cases luxation of the ocular bulbs is possible compromising the optic nerve by traction (Fig. 20.11). Beside the orbital roof, the orbital floor is mostly shortened as well. The disease inherent midface hypoplasia worsens the problem. In this way, orbital hypoplasia is a demanding threat with imminent hazards for visual function up to amaurosis. The most common syndromes for this are Crouzon syndrome, Pfeiffer syndrome, and Apert syndrome (FGFR2 syndromes). Neurosurgical investigation in this pattern has to include, in cooperation with the maxillofacial surgeon, high-resolution CAT scan of the cranium, orbits, and midface for exact pre-surgery design of fronto-orbito-nasal advancement with decompression of the orbits [36–38].

20.8 Chiari Malformation and Syringomyelia

Chiari malformation is typically defined by a tonsillar herniation through the foramen magnum [22, 35]. The herniation varies from minimal extension just below the foramen magnum level up to extreme findings and extension down to the laminae of vertebra 2 or 3 resulting in a compression of the medulla oblongata and the upper cervical spinal cord [39]. In extreme cases, there may also occur a compression of the vertebral

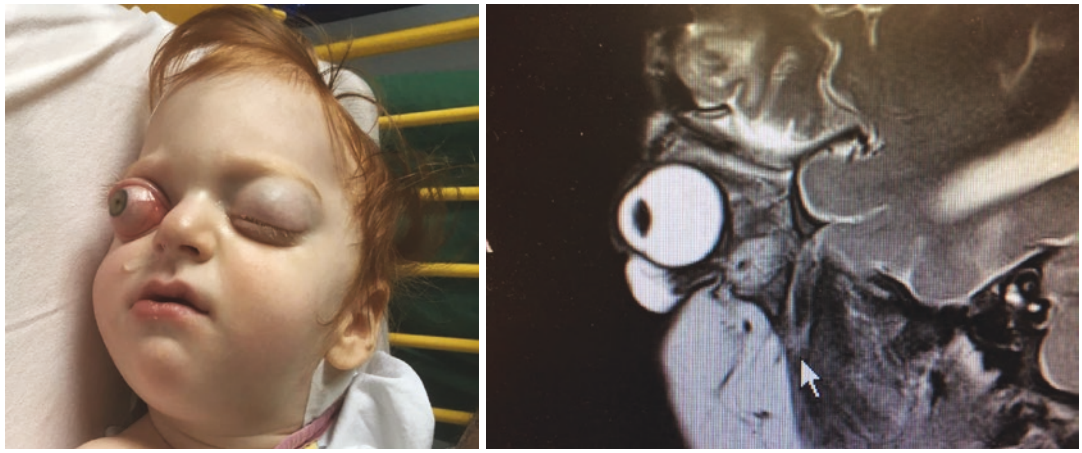

Fig. 20.11 Crouzon patient with prolapse of the right bulb. Tension-related affection of the optical nerve may lead to blindness. MRI of the peribulbar anatomy of a Crouzon patient. A prolapse of the bulb is possible due to the small volume of the orbit, pronounced by the short dimension of the orbital floor through the midfacial hypoplasia

arteries with compromised vascularization of the posterior fossa [2, 39]. Chiari malformation can be observed in nonsyndromic as well as in syndromic patients. The incidence in syndromic patients is as high as 50% in Pfeiffer syndrome [20], 70% in Crouzon syndrome [21, 40], and nearly 100% in kleeblattschädel deformity [20]. Surprisingly, patients with Apert syndrome are affected only in 1.9% of the cases [21]. One theory explains that in Apert syndrome the synostosis of the coronal suture starts before the synostosis of the sagittal and lambdoid sutures (median 5 months), whereas in Crouzon syndrome the synostosis starts the other way around. As far as we know, the synostosis of the lambdoid suture seems to be an important pathophysiological factor to influence the development of Chiari syndrome [40, 41].

An important factor, influencing decisions about timing and procedures of surgery, is the development of and coincidence with internal hydrocephalus due to obstruction of the CSF outflow from the fourth ventricle [5, 17]. At least 88% of the syndromic patients with hydrocephalus are showing Chiari malformation [17]. Especially patients with pansynostosis, associated with Chiari malformation and hydrocephalus, need early ventricular shunting. This may even be favorable to prepare the patient for fronto-orbito-nasal advancement surgery by relaxation of the brain to minimize retraction during preparation of the supraorbital bandeau.

Chiari malformation as well is associated with syringomyelia (Figs. 20.12 and 20.13) in almost one-third of the cases [40]. Etiologically, this may result from obliteration of the cerebellomedullary cistern and occlusion of the obex as well as by developmental anomalies of the central canal of the spinal cord. Therefore, MRT of the complete neuroaxis is obligatory in all of the multisuture and syndromic cases.

Clinical symptoms of cerebellar herniation and syringomyelia widely vary: depending on the severity of herniation, there are asymptomatic cases as well as significant symptoms such as dyspnea, dysphagia, and ataxia. Typical symptoms for syringomyelia are atrophy of upper limb muscles by compression of the motoneurons at syrinx level, spasticity of the legs, impaired temperature sensation, spinal

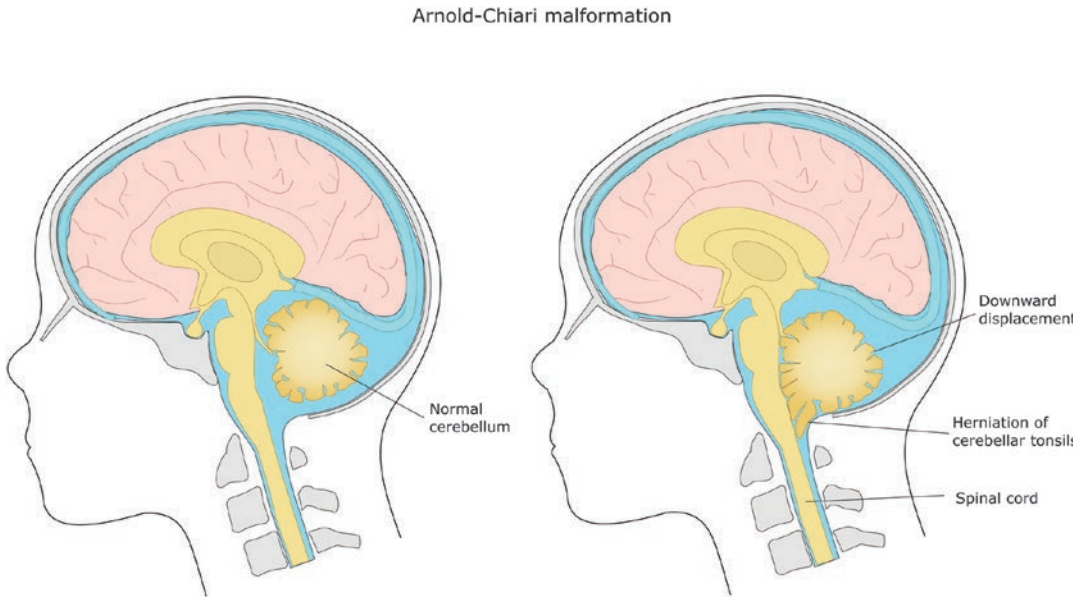

Fig. 20.12 Schematic drawing of Arnold-Chiari malformation. (Reprinted from ellepigrafica/alamy.com with permission)

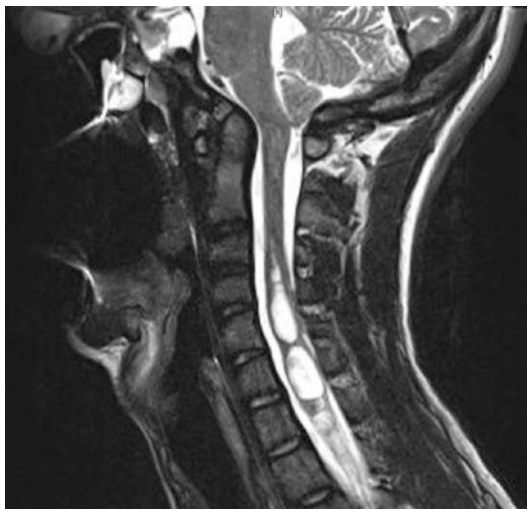

Fig. 20.13 Patient with Crouzon syndrome after fronto-orbito-nasal advancement. Mild Chiari malformation with tonsillar herniation and significant syringomyelia

ataxia, and disorders of the vegetative functions. A typical symptom in children is the appearance of scoliosis of the cervical spine. All of the symptoms are difficult to evaluate in small children, so it needs neuropediatric expertise to detect the clinical state for further decisions on therapy [20, 22].

There is general agreement on timing of surgery in cases of craniosynostosis with Chiari malformation and hydrocephalus: these cases should be treated by ventricular shunting prior to correction of craniosynostosis. A controversial discussion remains for the best timing of craniocervical decompression or the appropriate timing and technique of surgery for syringomyelia [40–42]. Some authors recommend craniocervical decompression at the time of craniosynostosis correction [41], but there were also observations of improvement of tonsillar herniation after craniosynostosis surgery. Other groups even propose craniocervical decompression prior to craniosynostosis repair [40].

So all kinds of surgical techniques of craniosynostosis imply significant stress for the small patients including anesthesia, artificial ventilation, bleeding with blood transfusions, and other interventions. Craniocervical decompression is far away to be a small intervention as well, we prefer surgery on craniosynostosis first, followed by surgery of Chiari syndrome later depending on the clinical course of the patient and follow-up MRT controls. This includes the coincidence with syringomyelia, because not all of the cases improve after surgery and need extended decompression of the syrinx by drainage into the spinal subarachnoid space.

20.9 Summary

Neurosurgical investigation of patients with craniofacial malformations needs a reliable interdisciplinarity between neurosurgeons, neuropediatric specialists, as well as ophthalmologists and cranio-maxillo-facial surgeons. Diagnostics must not only precisely define the diagnosis and its expression in an individual case but must also respect pathophysiological mechanisms to develop a therapeutic concept for the patient including an appropriate timing for surgical interventions. If cases of nonsyndromic single-suture synostosis of the sagittal suture with scaphocephaly may be sufficiently diagnosed by anamnesis, clinical examination, and sonography, complex cases of syndromic pansynostosis require an interdisciplinary diagnostic concept including molecular genetics, developmental state, and CAT and MRT scans.

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Radiological Investigations of Craniofacial Malformations

21

Christoph Mönninghoff

Abbreviations

2D	two-dimensional
3D	three-dimensional
4D	four-dimensional
CBCT	cone beam computed tomography
CT	computed tomography
DVT	digital volume tomography
MDCT	multidetector computed tomography
MPR	multiplanar reconstruction
MRI	magnetic resonance imaging
OAVS	oculo-auriculo-vertebral spectrum
TCS	Treacher Collins syndrome
US	ultrasound, ultrasonography
VRT	volume rendering technique

21.1 Radiological Imaging Modalities for Craniofacial Malformations

Imaging of craniofacial malformations is crucial for the precise pre- and postnatal diagnosis, surgical therapy planning, therapy monitoring, and for the exclusion of intracranial pathology and other complications associated with these malformations. Interdisciplinary diagnosis and treatment of these craniofacial developmental disorders by pediatricians, neurosurgeons, maxillofacial surgeons, and radiologists is based not only on clinical examination but also on US, conventional X-rays, CT, and MRI. The optimal use of these different imaging modalities for the visualization of craniofacial bone structures and soft tissues requires knowledge of the normal anatomy and the diagnostic advantages and disadvantages of the different methods.

21.1.1 Cranial Ultrasonography

A basic principle of pediatric radiology is to keep exposure to X-rays for children as low as possible. Ultrasound (US) is always the first study of choice in the fetus and nearly always the first in neonates [1, 2]. This imaging technique requires no ionizing radiation, is noninvasive, inexpensive, and mostly available. US examinations can be performed repeatedly pre- and postnatal without sedation [3, 4]. In recent years, high-quality US examinations

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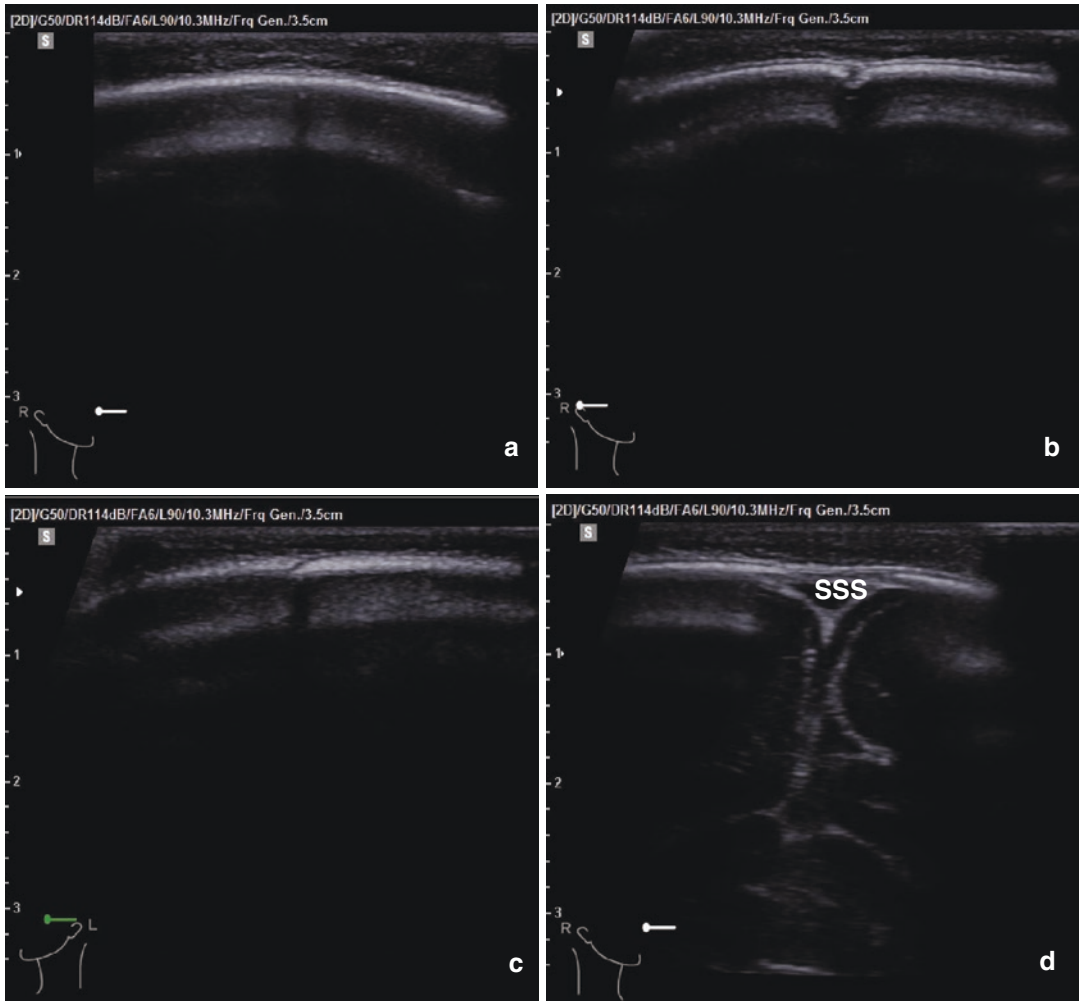

Fig. 21.1 Ultrasonographic image of a closed metopic suture (**a**) of a 3-month-old girl. The outer layer of the cortical bone of the skull is already closed. A transversal sonogram of the lambdoid suture reveals it as an open hypoechoic gap between the hyperechoic frontal bones (**b** right, **c** left side). The sagittal suture (**d**) is still patent revealing the superior sagittal sinus (SSS) and underlying interhemispherical fissure between the frontal lobes. Courtesy of Dr. Ulrike Materna, Clemenshospital Muenster, Germany

markedly improved by the introduction of high-frequency transducers, high-bandwidth tissue harmonic imaging techniques, and the use of multiple acoustic windows with competitive results for cranial exams in newborns compared to MRI [5] (Fig. 21.1). After the first months of life, the closing fontanels and sutures limit the applicability for cerebral and spinal imaging of infants, making MRI the imaging method of choice [1]. A well-trained sonographer can verify the results of US examinations by the targeted use of multiple transducers functioning at variable frequencies, a combination of vector, curved, and linear array transducers used with adjusted frequencies (between 8 and 17 MHz). All sutures and regions of the brain can be analyzed via the anterior and posterior fontanels and the temporal, mastoid, and occipital synchondroses. Changes in echogenicity can be monitored in real time and at several examination time points [6]. Doppler techniques reveal peak systolic velocities, end-diastolic velocities, and resistive indices of larger physiological and pathological vessels. It is a helpful imaging technique to differentiate vascular malformations, e.g., arteriovenous malformations, hemangioblastomas, and lymphangiomas [7, 8].

21.1.2 Magnetic Resonance Imaging (MRI)

Diagnostic neuroimaging performed with MRI is often dependent on sedation to acquire diagnostic images in children. In the hospital setting, permanent monitoring and MR-compatible life-support equipment has to be available for pediatric patients [9]. In general, MRI is the imaging study of choice in children older than 4 months [6]. Cerebrospinal and soft tissue anatomy can be well depicted in high anatomical resolution with detailed soft tissue contrast. The use and dosage of gadolinium has to be determined, and imaging protocols have to be adapted to compensate for the smaller size of the pediatric brain, the different water content, and the varying status of myelinization. Besides multiplanar anatomical imaging of the craniofacial region, specific MR sequences allow for vascular, microstructural, and metabolic imaging. MRI with tailored imaging protocols can add valuable diagnostic information and greatly improve the medical care of children with neurological and craniofacial disorders.

21.1.3 Computed Tomography (CT)

In the pediatric population, the use of CT carries a significantly increased risk of malignancy in later life. Hence, the exposure of children should be limited to the diagnostic minimum necessary to prevent radiation-associated diseases, e.g., radiation cataract after repeated CTs of the eye lens. The perception of this problem has led to the definition of low-dose protocols including iterative reconstructions. Especially children with craniofacial malformations may benefit from these low-dose CTs, if repetitive CT examinations are unavoidable for posttreatment monitoring [10–15]. Multidetector CT (MDCT) technology has significantly accelerated acquisition times. Fewer than 1.5% of pediatric patients now require sedation with this imaging modality [16]. With adapted protocols for midface structures, radiation dose can be reduced by 89% compared with conventional craniofacial CT scans with adequate diagnostic quality [17]. Two- and three-dimensional CT still play a prominent role in the diagnostic and preoperative imaging of craniofacial malformations involving bony structures. For radiation protection reasons, it is only used at the end of the first year of life and as late as possible in children with diagnosed complicated types of craniosynostosis [18]. With modern multidetector CT scanners, 3D scans of the skull can be acquired in diagnostic image quality with approximately 0.2–2 mSv effective doses [19–21]. In combination with model-based iterative reconstruction algorithms, 3D CT scans of the head with 0.008 mSv are reported without reduced image quality [22]. In phantom studies, ultralow-dose CT protocols of the head have been acquired with 0.02 mSv dose equal to the exposure to radiation of a plain skull radiography ranging from 0.01 to 0.04 mSv [11]. Current state-of-the-art multidetector CT (MDCT), also known as medical CT, has an important role in the diagnosis and management of craniofacial injuries and pathology. Micro-computed tomography (micro-CT) has accelerated craniofacial biology research by allowing higher-resolution scanning of teeth beyond the capabilities of MDCT and CBCT [23].

21.1.4 Digital Volume Tomography (DVT)

Digital volume tomography (DVT), based on 3D cone beam CT (CBCT) and the principles of rotational tomography, was introduced in 1998 in preoperative dental and craniofacial imaging [24]. This imaging technique produces similar 3D images to CT with faster image acquisition but at a radiation dose comparable with panoramic radiography and at lower cost [25]. For high-contrast structures of the midface, DVT can be considered as the gold standard for imaging the oral and maxillofacial area and as an alternative imaging modality to CT for midface structures [25–27].

21.1.5 Plain Radiography

Digital X-ray detectors use X-ray-sensitive plates to directly capture data during the patient examination, immediately transferring it to a computer system without the use of an intermediate cassette [28]. In pediatric patients, plain radiogra-

phy, including computed radiography (CR) and digital radiography (DR), is only used for specific diagnostic questions, e.g., for nondepressed linear skull fractures or single-suture craniosynostosis [29–31]. The latter may depict as a linear sclerotic line, which correlates with a bony ridge on CT images. Digital radiography causes much lower radiation exposure than CT, but spatial resolution and detailed depiction of bone structures is also very limited in plain radiography. Therefore, plain radiographs are usually postponed to the date of surgery or the end of the first year [30].

21.2 Craniosynostosis

Craniosynostosis is based on a premature fusion of one or more cranial sutures that exist along adjacent cranial bones, namely, the frontal, parietal, temporal, and occipital bones [18]. If left untreated, premature fusion may cause skull deformities, facial asymmetry combined with pathologically increased intracranial pressure, deafness, visual impairment, and cognitive decline [18, 32–37]. The incidence is about 1–2000 live births [38]. Craniosynostosis can be subdivided into two categories. Primary craniosynostoses are the premature fusions of one or more cranial sutures based on a developmental defect during embryogenesis. Secondary craniosynostoses include the premature ossification and fusion of the skull sutures due to other causes such as teratogens, intrauterine cranial compression, extrauterine positional deformity, or insufficient cerebral growth. Craniosynostoses may occur sporadically in single individuals as nonsyndromic craniosynostosis in 85% of all cases or as a manifestation of syndromes in combination with other developmental anomalies (syndromic craniosynostosis) in 15% of cases [39, 40]. The cranial vault may be variably deformed, depending on the fused sutures with compensatory growth of the skull in the regions that are not restricted by prematurely closed sutures. Single-suture synostosis occurs as scaphocephaly from premature sagittal synostosis along the sagittal suture, as trigonocephaly caused by premature metopic synostosis along the metopic suture, and as plagiocephaly secondary to unilateral premature coronal or lambdoid suture synostosis [41].

After clinical examination, US is the first-line imaging modality for neonates and infants younger than 8–12 months with suspected craniosynostosis [1, 42, 43]. Compared to CT, cranial US is an effective and reliable technique for the diagnosis of closed sutures with 100% sensitivity and 86–100% specificity before the age of 12 months [3] (Fig. 21.1). In children with abnormal or asymmetric skull shape, e.g., from deformational plagiocephaly, US has proven to be an effective screening tool for craniosynostosis [2, 43, 44]. Two- and three-dimensional (2D/3D) US are successfully applied for skull deformities even in the prenatal period [4, 45, 46].

In children older than 12 months, MRI becomes increasingly important to assess sutures and underlying cerebral pathologies as fontanels and sutures get closed and sound windows for ultrasonography shrink. A black bone MR sequence performed as a 3D low flip angle gradient-echo MRI sequence may have the potential to replace CT for the diagnosis and monitoring of craniosynostosis [47]. MR imaging is the imaging modality of choice in infants with congenital midface masses and craniofacial syndromes [48]. Despite radiation exposure, cranial CT with 3D reconstructions of the calvarium is requested by many surgeons in order to plan individualized reconstructive operations (Fig. 21.2). Two-dimensional reformatted images of high-resolution CT scans in axial, coronal, and sagittal orientation, which are obtained at a slice thickness of 3 mm or less with a bone algorithm, are suitable to assess osseous midface and calvarial deformities easily. Anomalies of the external and middle ear resulting from the abnormal formation of the skull base are best depicted on 1 mm CT sections acquired with bone algorithm through the petrous pyramids supplemented by coronal reformations to reveal possible asymmetries [18]. Both CT and MR imaging are required to comprehensively assess skull, brain, and soft tissue disorders of midface anomalies and craniofacial syndromes [48].

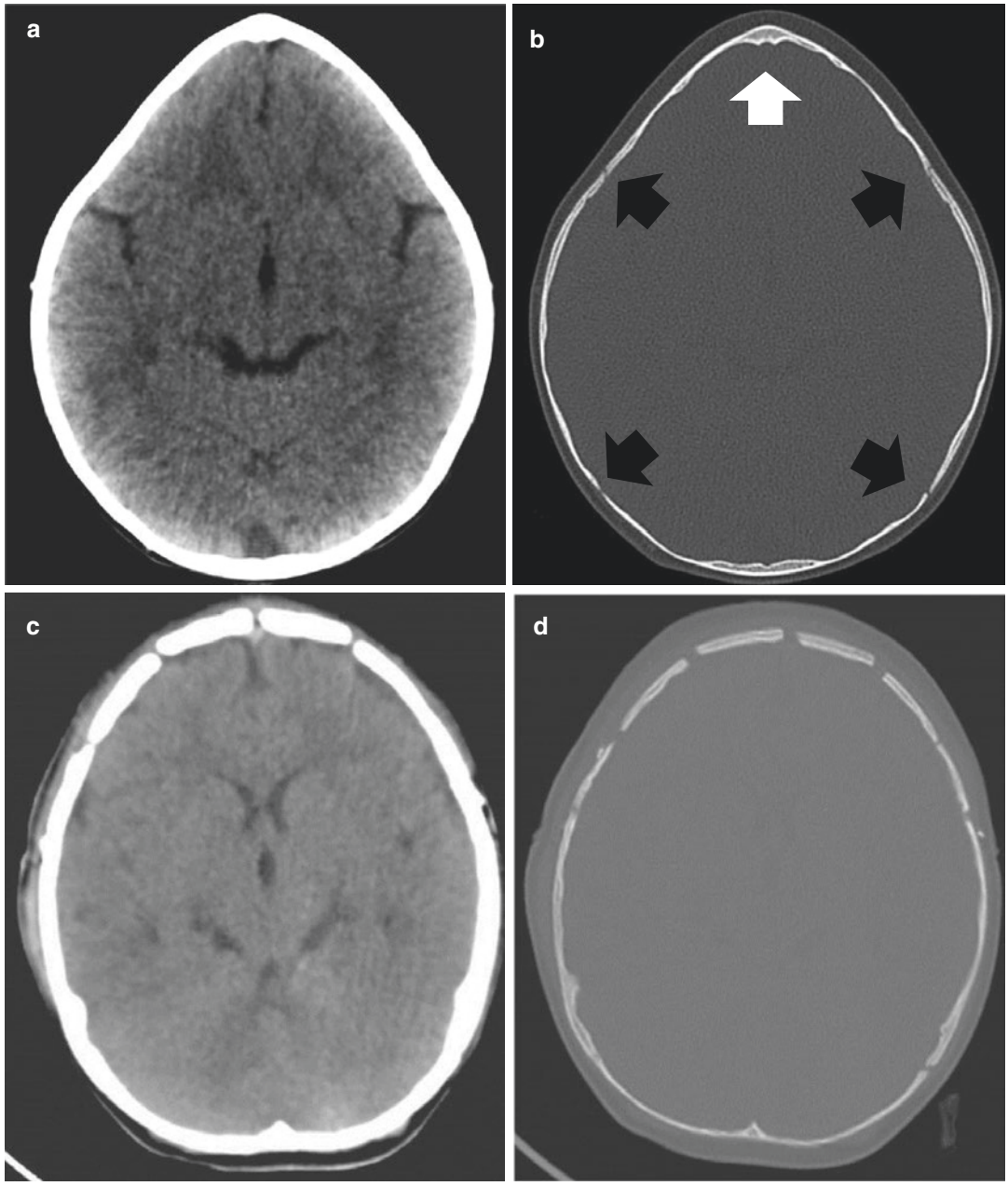

Fig. 21.2 Transversal CT images of a 10-month-old boy with metopic craniosynostosis (white Arrow) in brain window (**a**, **c**) and in bone window (**b**, **c**). The coronal and lambdoid sutures (**b**, black arrows) are regularly open, whereas the prematurely fused metopic suture (white arrow) forms palpable ectocranial ridge with trigonocephaly and parieto-occipital bossing due to the constricted growth of the frontal bone. After neurosurgical correction with opened metopic and coronal sutures (**c**, **d**) a normal oval head form was reconstructed. Courtesy of Dr. Claudia Moeller-Hartmann, University Hospital Essen, Germany

21.2.1 Nonsyndromic Craniosynostosis

In 85% of cases, craniosynostosis is not accompanied by other developmental disorders (nonsyndromic) [49]. Recent analysis of exome sequence data from nonsyndromic craniosynostosis has underlined the impact of genetic mutations in one-quarter of sporadic cases with detected mutations in two genes, TCF12 and ERF [50, 51]. Depending on which suture is affected, nonsyndromic craniosynostosis occurs as sagittal, coronal, metopic, lambdoid, or multisuture synostosis. Ultrasonographic key features of craniosynostosis are the loss of the hypoechoic fibrous gap between the hyperechoic bony plates, an irregular sclerosed inner sutural margin, the loss of a beveled edge, and asymmetric fontanels [52]. Isolated single-suture craniosynostosis or positional plagiocephaly is diagnosed clinically and may be confirmed by digital X-ray images of the skull. Cross-sectional imaging studies are not indicated to assess single, nonsyndromic sutures. Especially, CT scanning should be indicated carefully for the assessment of single-suture craniosynostosis taking into account that there is a quantifiable risk of developing cancer in further lifetime [30]. If inevitable, low-dose CT images (20–30 mAs) with three-dimensional reformations depict prematurely closed sutures as sclerotic bony bridges with reduced serration linearly along the affected suture. The complete absence or pathological shortening of a suture on plain radiographs of the skull and on "black bone" MRI indicates craniosynostosis [18, 40, 53].

Sagittal synostosis results in scaphocephaly with occipital protrusion and ridging of the fused sagittal suture with frontal bossing. Also clinocephaly with flattened calvarium and tall and narrow skull deformities (leptocephaly) can develop from sagittal synostosis [18, 54]. Coronal synostosis causes a growth disturbance of the skull in anterior-posterior direction along the coronal suture with compensatory expansion of the skull in parietal direction. Anterior plagiocephaly is a result of unicoronal synostosis with diminished anterior cranial fossa and contralateral frontal bossing and elevated roof and lateral wall of the ipsilateral orbit ("harlequin appearance") [18]. The bicoronal craniosynostosis leads to a shortening of the skull (brachycephaly) and is often associated with upper and midface hypoplasia and craniofacial deformities in syndromic cases (Fig. 21.3).

Metopic synostosis, which is in one-third of cases syndromic, results in a too small anterior cranial fossa with a triangular pointed forehead (Fig. 21.2). It needs to be distinguished from metopic ridge, which is a physiological variant of the closed metopic suture in 4% of children between 0 and 18 months of age without trigonocephaly or other symptoms [55].

Lambdoid synostosis may occur uni- or bilaterally. Unilateral lambdoid synostosis results in posterior plagiocephaly (oblique deformity of the posterior cranium), which is more often caused by positional deformation (deformational or positional plagiocephaly) than by premature fusion of this cranial suture. The first is based on an asymmetric occipital flattening of the skull after preferred head positioning on one side during sleep. It can be treated conservatively, whereas the latter needs surgical correction. If the lambdoid suture synostosis occurs bilaterally, a tower-like deformation of the skull (turricephaly, oxycephaly, or acrocephaly) results.

Multisuture craniosynostosis is mostly syndromic with variable patterns, depending on the affected sutures. If coronal, lambdoid, and sagittal sutures simultaneously merge prematurely, the resulting pansynostosis leads to oxycephaly or a cloverleaf deformity of the skull (severe proptosis combined with dilated bitemporal regions described with the German word "kleeblattschädel") [18].

21.2.2 Syndromic Craniosynostosis

More than 180 different syndromes are associated with craniosynostosis [38]. Roughly 15% of all craniosynostoses occur with other developmental anomalies of the body [49]. The so-called syndromic craniosynostoses comprise several diagnoses and underlying genetic mutations,

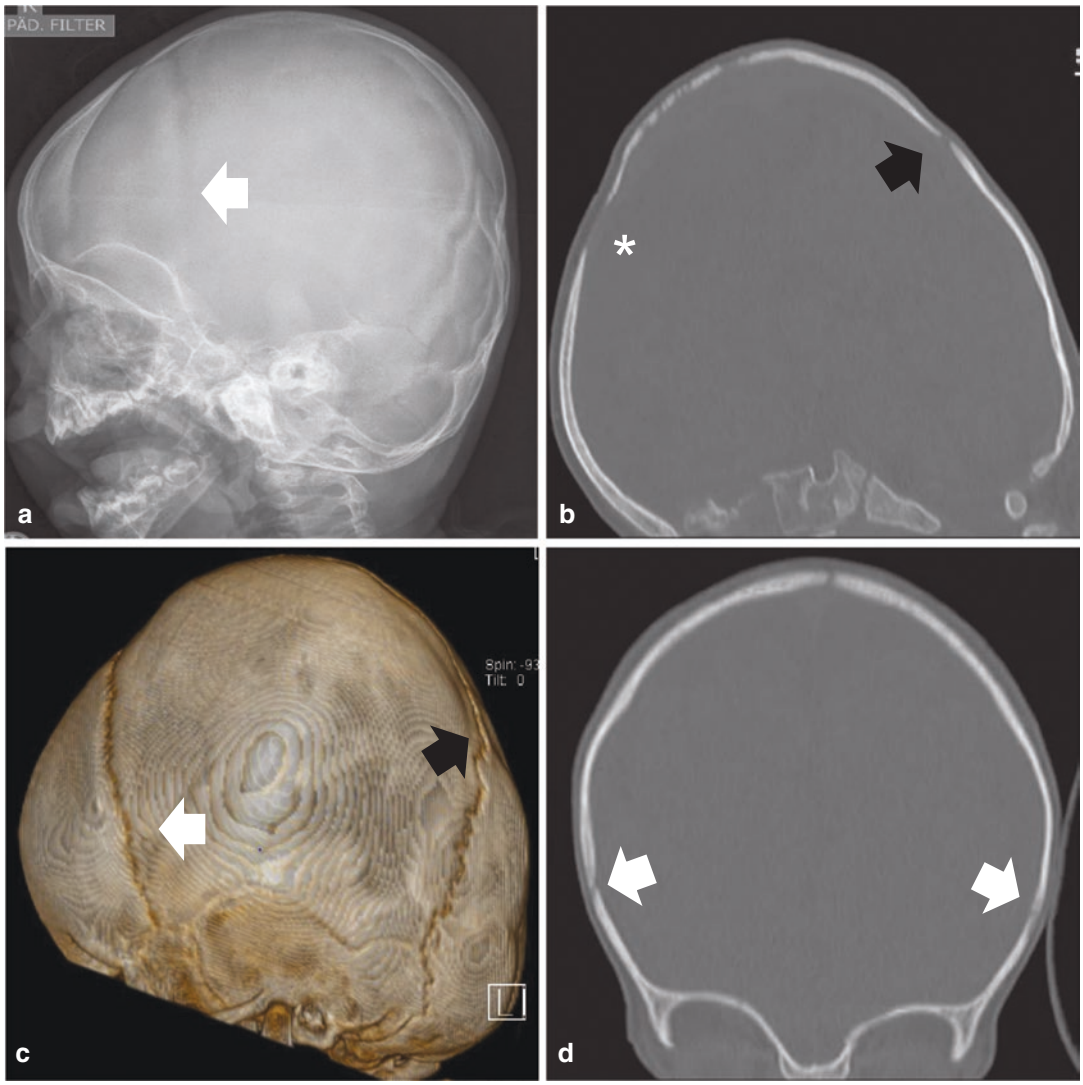

Fig. 21.3 9-Month-old boy with coronal craniosynostosis. Lateral plain skull radiography (**a**), lateral (**b**) and coronal (**d**) 2D CT images in bone window, and lateral 3D CT-image (VRT) (**c**) show premature bilateral fusion of coronal sutures (white arrows) with resulting turricephaly. The anterior (asterix) and posterior fontanel (black arrows) are not yet closed, typical for this age group. Courtesy of Dr. Claudia Moeller-Hartmann, University Hospital Essen, Germany

which cause developmental malformations of the skull, the face, and the central nervous system. Syndromic craniosynostosis is usually combined with developmental delay [34]. Gene mutations encoding fibroblast growth factor receptors 1, 2, and 3 (FGFR1, FGFR2, FGFR3), TWIST, and MSX2 (muscle segment homeobox 2) have been identified in syndromic craniosynostosis [56, 57]. The most frequent syndromic craniosynostoses are Apert (FGFR2), Crouzon (FGFR2), Pfeiffer (FGFR1 and FGFR2), Muenke (FGFR3), and Saethre-Chotzen (TWIST) [34]. The severity of the anomalies varies from mild suture involvement to severe pansynostosis with a spectrum of extracraniofacial dysmorphic manifestations [34]. The affection of the central nervous system in these syndromes often affects intelligence, prognosis, and outcome of these patients. Hence,

the detection of intracranial deformities, skull deformities, and cerebral lesions is mostly based on CT and MR imaging.

21.2.3 Craniofacial Syndromes

Craniofacial syndromes include developmental disorders of the face and skull associated with anomalies of the central nervous system, which often have a negative influence on mental development, prognosis, and outcome. Diagnostic is based on clinical examination including anomalies of the central nervous system, extremities, and a positive family history for syndromal changes [48]. The detection of intracranial lesions and alterations of the intracranial vasculature is based on MRI with MR angiography, whereas CT allows detailed anatomical examinations of skull base and midface deformations for surgical planning [58].

Common imaging findings of craniofacial anomalies are multiple craniosynostosis, an enlarged anterior fontanel, a reduced skull base, dysplastic calvarial bones, ventriculomegaly probably based on abnormal intracranial venous drainage, and anomalies of the external and middle ear system. Less frequently craniofacial syndromes are associated with herniation of the cerebellar tonsils, Chiari I malformation, agenesis or hypogenesis of the corpus callosum and/or septum pellucidum, dysmorphic changes of the cerebral cortex, and periventricular nodular heterotopia [59].

Craniofacial Syndromes

- Apert syndrome
- Carpenter syndrome
- Crouzon syndrome
- Coffin-Lowry syndrome
- Jackson-Weiss syndrome
- Fibular aplasia syndrome
- Lowry syndrome
- Noack syndrome
- Pfeiffer syndrome
- Roberts syndrome
- Saethre-Chotzen syndrome
- Treacher Collins syndrome

Alphabetic list modified from [48].

21.2.3.1 Apert Syndrome

Apert syndrome or acrocephalosyndactyly type 1 is an autosomal dominant syndrome with incomplete penetrance in 5.5 of one million neonates [60] (Fig. 21.4). A defect on the fibroblast growth factor receptor 2 (*FGFR2*) gene located on chromosome 10q26 has been found responsible for the syndrome [60]. The typical phenotypic appearance of this mostly sporadic abnormality comprises the triad of craniosynostosis, symmetric syndactyly of the hands and feet, and maxillary hypoplasia. Other typical features of the syndrome include turribrachycephaly due to coronal synostosis, hypoplastic midface with downturned mouth and shallow orbits with proptosis, cleft palate, and kleeblattschädel deformity besides hypertelorism [61]. Optional intellectual retardation may be associated with gyral abnormalities, megalocephaly, and ventriculomegaly [60]. Abnormal intracranial venous drainage is discussed as a factor in the development of ventriculomegaly. The fifth and sixth vertebrae are fused in up to 71% of Apert syndrome patients [60].

The naso- and oropharyngeal region may be affected by hypoplasia of the posterior choanae, mostly as choanal stenosis, rather than choanal atresia. Transversal CT scans are the cross-sectional imaging method of choice to depict gradual narrowing of the nasal cavity from front to back, midface hypoplasia, and narrowing of the pyriform aperture in anatomical detail. Uncorrected, these midface deformities may be responsible for obstructive sleep apnea, respiratory distress, cor pulmonale, and even sudden death [48].

21.2.3.2 Crouzon Syndrome

Crouzon syndrome is an autosomal dominant disorder, which affects the first branchial arch as precursor of the maxilla and mandible but also manifests itself in non-cranial localizations [62] (Figs. 21.5 and 21.6). Malformations affect the whole cranio-orbito-zygomatic region [63]. Like Apert syndrome, it is based on a defect of the fibroblast growth factor receptor 2 (*FGFR2*) gene located on chromosome 10q26 [64]. This finding underlines the fact that a single genetic mutation on the same gene can cause different phenotypic

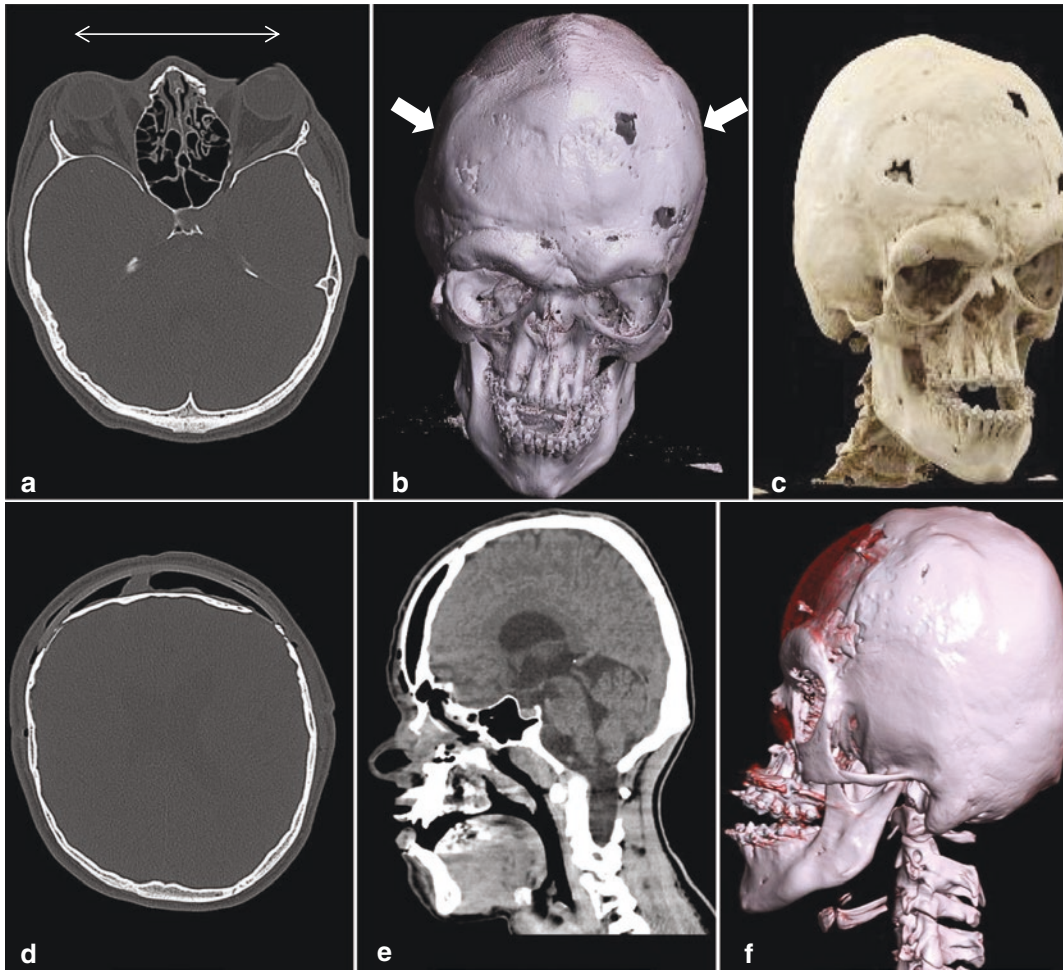

Fig. 21.4 CT-images of a 16-year-old boy with sporadic Apert syndrome. On transversal CT images (**a**) shallow distended orbits with wide set eyes are noticeable. Coronal synostosis (**b**, white arrows, volume rendering technique (VRT)) causes characteristic brachycephaly associated with a retruded midface and a down-turned mouth. The sagittal suture is normally formed, while an initially widened metopic suture is fused in time (**c**, CT-based cinematic reconstruction). The flattened forehead was reconstructed by implantation of a patient-specific cranioplasty manufactured with computer-assisted design (CAD) (**d**, transversal CT image, bone window; **e**, lateral CT image, brain window). The jaw deformity was corrected with fixed braces, which cause CT artifacts (**f**, lateral VRT image). Courtesy of Prof. Dr. Johannes Wessling, Clemenshospital Muenster, Germany

manifestations in patients with craniofacial syndromes [65]. Main features of Crouzon syndrome are craniosynostosis, maxillary hypoplasia in all three planes, mandibular asymmetry, bifid uvula, shallow orbits with proptosis, and cleft palate [66–68]. Intraoccipital synchondroses close earlier in Crouzon patients and premature fusion of sutures starts at 10 months of age with posterior intraoccipital synchondroses and lambdoid sutures, followed by occipitomastoid synchondroses at about 2 years and anterior intraoccipital synchondroses at approximately 2.80 years. Spheno-occipital and petro-occipital synchondroses fuse last, at approximately 3 years of age [69]. The reduced foramen magnum is associated with anomalies of the craniocervical venous drainage with possible hydrocephalus [70, 71] (Fig. 21.5). Shortening of the anterior skull base and posterior fossa linked with spheno-occipital synchondrosis leads to a compensatory widening of the anterior

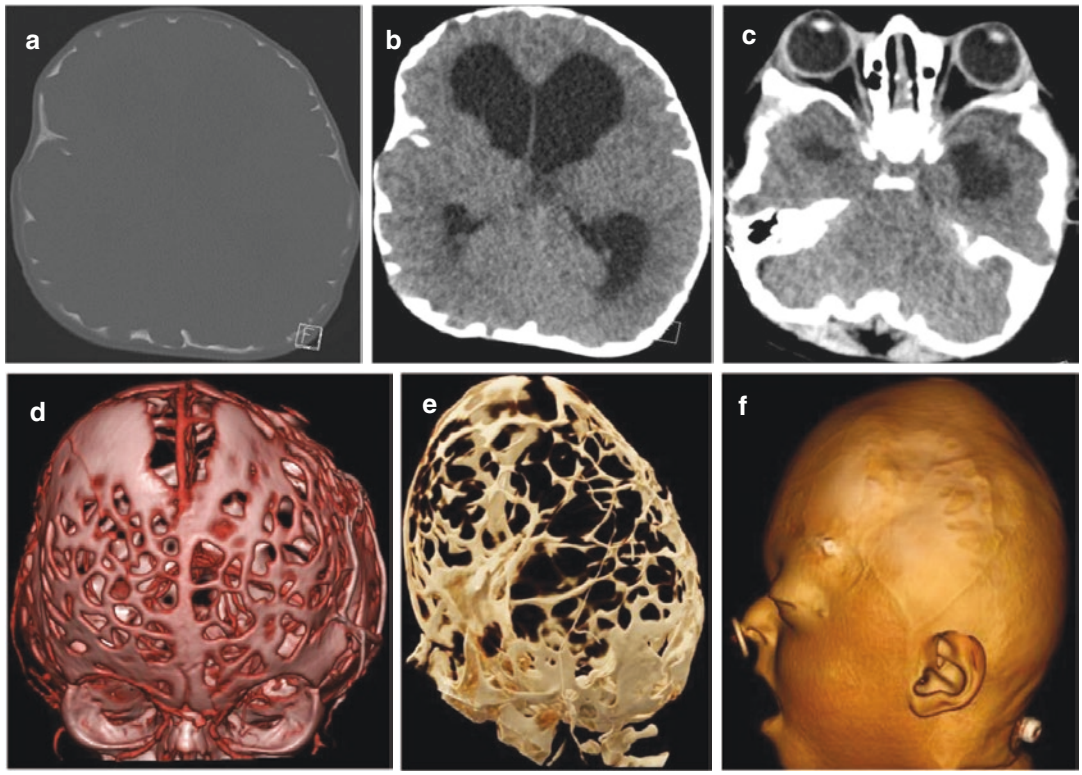

Fig. 21.5 Presurgical low-dose CT of a 2-month-old boy with Crouzon syndrome reveals a "copper beaten" thinning of the calvarium (**a**) on axial images in bone window. Compensated hydrocephalus, elapsed external cerebrospinal fluid spaces (**b**), and orbital proptosis (**c**) shown on axial CT images in brain window are consequences of craniosynostosis and chronically increased intracranial pressure. 3D volume rendering technique (VRT) images of head CT emphasize the small orbits and turribrachycephaly in a frontal (**d**, conventional VRT) and lateral view (**e**, cinematic VRT). VRT reconstruction of the CT data also allows spatial imaging of the skin surface of the head with its appendages and the proptosis prior to reconstructive surgery (**f**). Courtesy of Prof. Dr. Johannes Wessling, Clemenshospital Muenster, Germany

skull base [72, 73, 69]. Cerebellar tonsil herniation into the smaller foramen magnum (Chiari I malformation) was found in 71.4% of Crouzon cases and is associated with premature lambdoid suture synostosis [74, 75]. Thin-layer T2-weighted MR images in sagittal orientation are best suited to assess not only cerebellar herniation but also cervical spine fusion anomalies of C2 to C5 [62]. Presurgical low-dose CT or DVT with 2D and 3D reconstructions are often needed to assess craniosynostosis and craniocervical bone malformations (Fig. 21.6).

21.2.3.3 Pfeiffer Syndrome

Pfeiffer syndrome (acrocephalosyndactyly type 5) is strongly associated with mutations of the fibroblast growth factor receptor 1 (FGFR1) gene on chromosome 8p11 and the fibroblast growth factor receptor 2 (FGFR2) gene on chromosome 10q26 and others [76, 77]. Phenotypic characteristics are craniosynostosis, polydactyly, soft tissue syndactyly of second, third digits, malformed enlarged thumb and great toe, and stenosis or atresia of the external auditory canal combined with normal intelligence. Three phenotypic types are differentiated: Classic Pfeiffer (type 1) is inherited with autosomal dominant transmission and mostly does not influence the intelligence and lifespan of affected individuals. Type 1 is associated with mutations in *FGFR1* and *FGFR2* gene. Phenotypic characteristics are brachycephaly, midface hypoplasia, and finger and toe abnormalities (Fig. 21.7). Pfeiffer syndrome

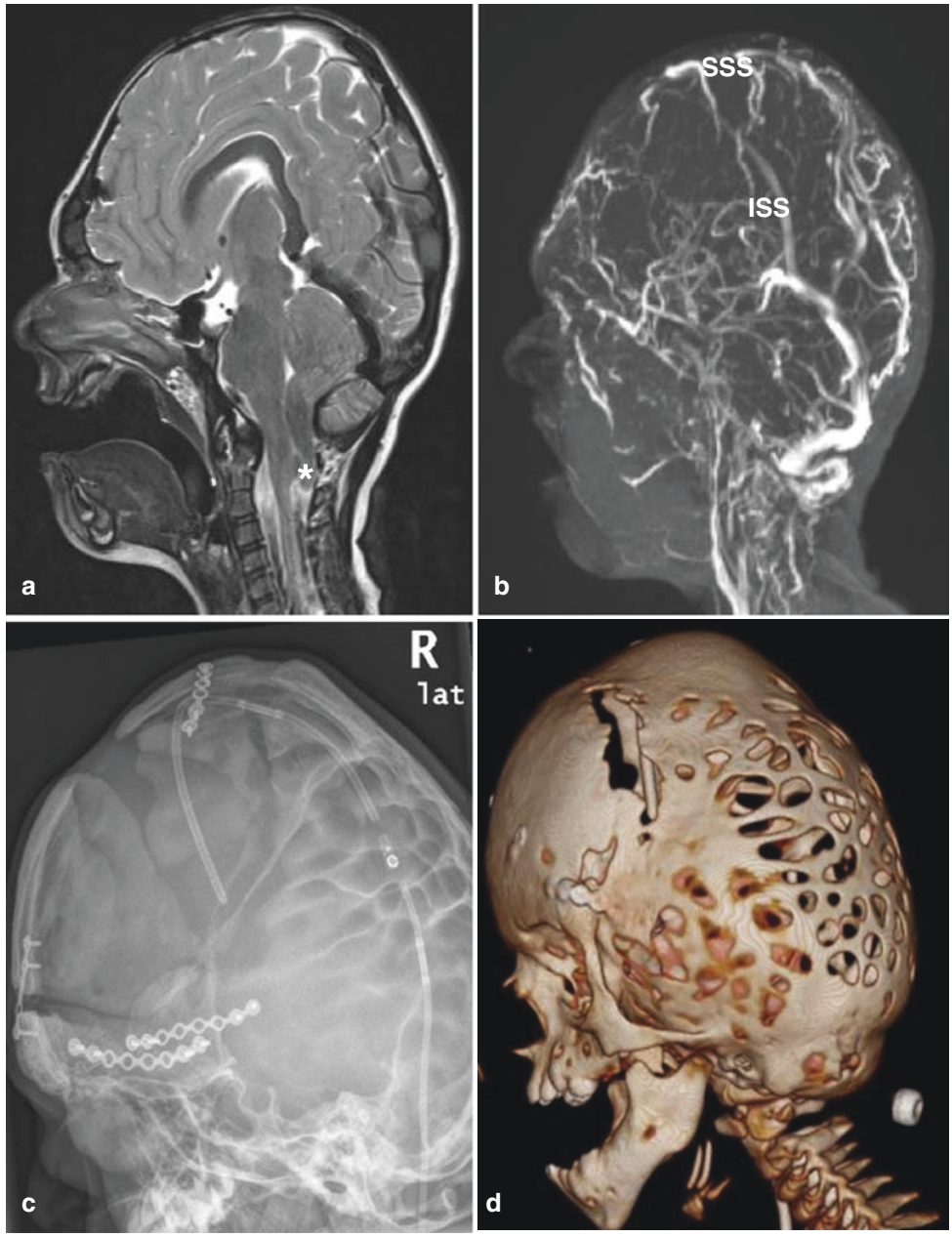

Fig. 21.6 Crouzon syndrome in a 5-year-old boy. Sagittal T2-weighted TSE MR images (**a**) shows a vertically oriented brainstem, Chiari I malformation (*) with typical herniation of the cerebellar tonsils into the foramen magnum, a small posterior fossa, and turribrachycephaly. Phase contrast MR angiography (**b**) of the cerebral veins reveals abnormal venous drainage with abnormally reduced flow in the superior sagittal sinus (SSS) and vertical orientation of the inferior sagittal sinus (ISS). In the posterior fossa and foramen magnum multiple collateral veins indicate an altered intracranial venous drainage normally provided by larger transverse and sigmoid sinuses. Lateral plain skull radiography (**c**) at the age of 6 years depicts the gyral pattern of the calvarium after surgical correction with opening of the prematurely fused coronal sutures and after implantation of a ventriculoperitoneal shunt. 3D VRT CT images (**d**) at the age of 1 year already display the "copper beaten" deformation of the calvarium due to chronically elevated intracranial pressure. Courtesy of Prof. Dr. Johannes Wessling, Clemenshospital Muenster, Germany

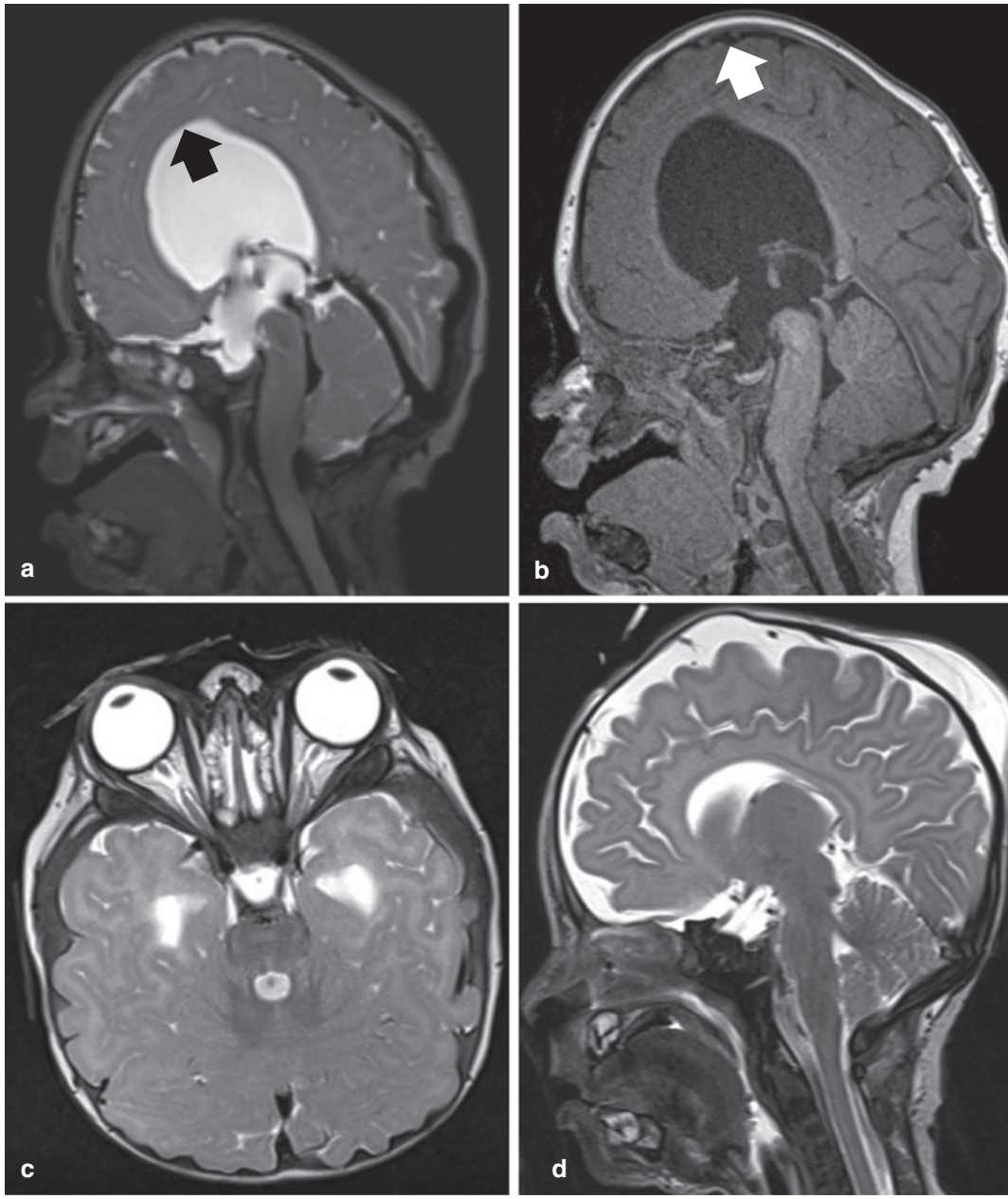

Fig. 21.7 Pfeiffer syndrome is a craniofacial syndrome with turribrachycephaly secondary to bilateral coronal synostosis. Lateral T2- (**a**) and T1-weighted (**b**) MR images of a 7-month-old girl reveal the tower-like skull deformity with an abnormally high, broad forehead and a "beak-shaped" nose. The frontal lobe and the thin anterior corpus callosum (black arrow) are cranially displaced according to the growth tendency towards the late closed anterior fontanel (white arrow). Kinking of the brain stem, a steep tentorium, hydrocephalus, and downward displacement of the cerebellar tonsils through the foramen magnum (Chiari type 1 malformation) in a small posterior fossa are intracranial consequences of prematurely closed cranial sutures. On transversal T2-weighted turbo spin-echo MR images (**c**) severe ocular proptosis, ocular hypertelorism, and midface hypoplasia become obvious. The temporal horns of both lateral ventricles are enlarged (hydrocephalus) due to altered intracranial flow of the cerebrospinal fluid. After surgical reconstruction of the coronal craniosynostosis, the skull deformity and cerebral deformations were visibly corrected (**d**, sagittal T2w MR image). Courtesy of Prof. Dr. Johannes Wessling, Clemenshospital Muenster, Germany

type 2 and type 3 are associated with mutations in FGFR2. Type 2 occurs sporadically and is characterized by a cloverleaf skull (kleeblattschädel) combined with extreme proptosis, elbow ankylosis or synostosis, finger and toe abnormalities, developmental delay, neurological complications, and early death. Hallmarks of the sporadic type 3 are craniosynostosis and severe proptosis but without cloverleaf skull with poor prognosis. The incidence of all types of Pfeiffer syndrome is approximately 1/100,000 [78].

In addition to molecular genetic testing, prenatal US and MRI can detect characteristic signs of Pfeiffer syndrome like craniosynostosis, hypertelorism associated with proptosis, and broad thumbs [4, 79–81]. Plain X-ray images of the hands and feet are suitable to reveal syndactyly, broad and deviated thumbs and great toes, and partial syndactyly of the hands and feet combined with joint fusion and ankylosis of small and large joints [82]. In analogy to other syndromes with multiple craniosynostosis, pre- and postnatal US is suitable to detect premature fusion of sutures [83, 84].

21.3 Branchial Arch Diseases

Disorders of the first and second branchial arches are generally caused by an inadequate migration and formation of facial mesenchyma during embryologic development [85]. Stickler syndrome, Treacher Collins syndrome, auriculocondylar syndrome, Pierre Robin sequence, and velocardiofacial syndrome are part of a growing list of developmental craniofacial disorders, which are better understood due to deeper insights into their genetic and embryologic background [86].

List of branchial arch diseases:
- Auriculocondylar syndrome
- Goldenhar syndrome
- Pierre Robin sequence
- Stickler syndrome
- Treacher Collins syndrome
- Velocardiofacial syndrome

21.3.1 Treacher Collins Syndrome (TCS)

Treacher Collins syndrome (TCS), also known as Franceschetti-Zwahlen-Klein syndrome, describes a rare autosomal dominant genetic abnormality, which results in mandibulofacial dysostosis based on bilateral, relatively symmetric malformations of the first and second branchial arches [85–87]. It derives from loss-of-function mutations in the gene *TCOF1* on chromosome 5, which encodes the nucleolar phosphoprotein, "Treacle," which is important in pre-ribosomal processing and ribosomal biogenesis [88]. Two other genes named *POLR1C* and *POLR1D* have been identified as rare causes of the syndrome [89]. Dysmorphic structures derive from the first and second pharyngeal pouch, groove, and arch accompanied by conductive hearing defects. The incidence is estimated at approximately 1 in 50,000 live births, with 60% of cases being sporadic [86, 90]. Absent limb abnormalities in TCS help to distinguish this branchial arch disease from other syndromes with comparable facial manifestations.

Prenatal 3D and 4D sonography is able to detect polyhydramnios, microcephaly, facial and ear abnormalities with microphthalmos and micrognathia, and abnormal fetal swallowing in TCS [91, 92]. Radiographic features best examined with cross-sectional CT and MR imaging studies comprise developmental disorders of the head and neck (Fig. 21.8).

Retro- or micrognathia, macrostomia, hypo- or aplasia of the coronoid and condylar processes of the mandible, emphasized bowing of the lower border of the mandible and concave formation of the horizontal ramus of the mandible may occur as pathognomonic signs. Cleft palate, aplasia of the parotid glands, and hypo- or aplasia of the zygomatic arch are further associated phenotypic findings.

The otic region is characterized by microtia and aplasia of the external auditory meatus, the middle ear ossicles are hypo- or even aplastic, pinna deformities, and hypoplasticity of the middle ear cavity.

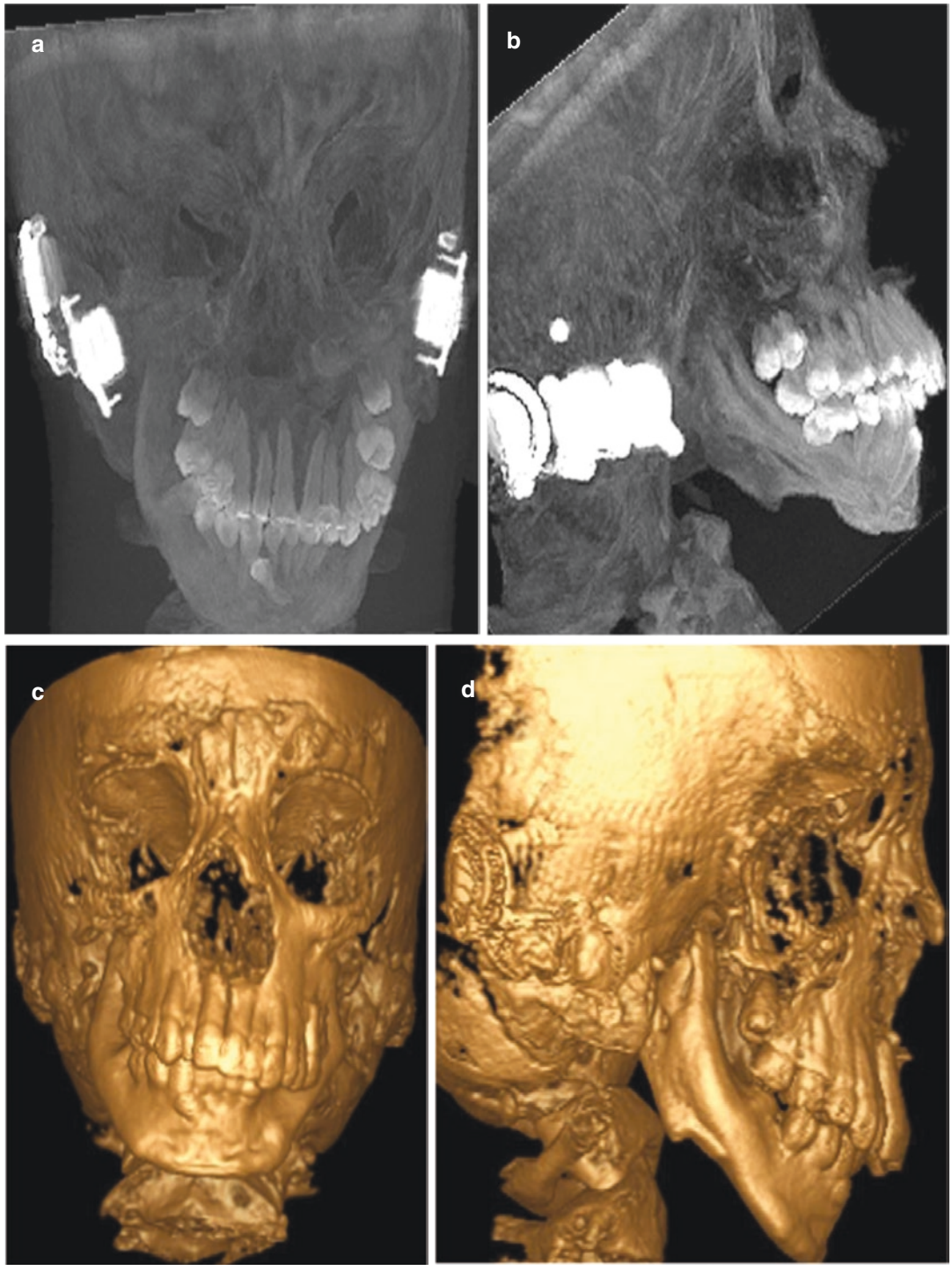

Fig. 21.8 The autosomal dominant Treacher Collins-Franceschetti syndrome (mandibulofacial dysostosis) affects midface structures mostly bilaterally that originate from the second and third branchial arches. DVT images in frontal (**a**) and lateral view (**b**) as well as VRTs (**c**, **d**) show absent zygomatic arches, a narrow and overprojected maxilla, a retruded chin, and a hypoplastic mandibula. Bilateral cochlear implants are necessary in this patient due to hearing loss caused by malformations of the middle ear, lack of the external auditory channel, and pinna deformity

In the nasal region, a broad or protruding nose can be accompanied by obliteration of the nasofrontal angle with narrow nares, choanal shortening, hypoplastic alar cartilages, and hypoplastic paranasal sinuses.

Ocular deformities include downward slanting palpebral fissures, absence or notching of the lower eyelids, notching of the iris and choroid, as well as colobomas [48].

21.3.2 Goldenhar Syndrome

Goldenhar syndrome, also known as oculo-auriculo-vertebral spectrum (OAVS), facio-auriculo-vertebral dysplasia, or Goldenhar-Gorlin syndrome, is a mostly sporadic congenital anomaly affecting primarily aural, ocular, oral, and mandibular development, with vertebral anomalies, and epibulbar dermoids [93, 94]. The incidence is 1 in 3000–5000 newborns with a small male predominance (M:F = 3:2). It is based on a developmental defect of the first and second branchial arches and can be considered as a variant of hemifacial microsomia [94]. Key features of Goldenhar syndrome are hemifacial microsomia and facial asymmetry that can best be assessed by maxillofacial CBCT [94, 95] (Fig. 21.9). Most of these abnormalities can be detected on prenatal US [96–101]. In particular, the CBCT with a large field of view (18 × 16 cm) proved useful to visualize vertebral fusion abnor-

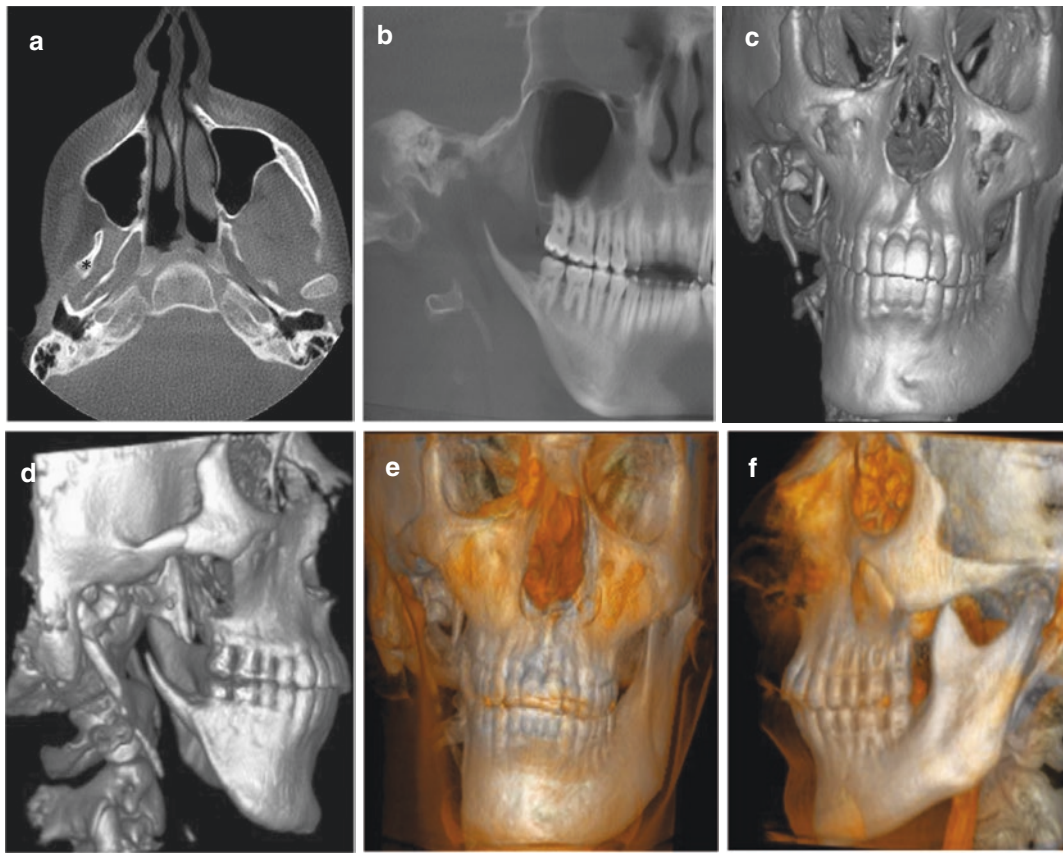

Fig. 21.9 Goldenhar syndrome is a rare condition characterized by facial asymmetry, deformation (microtia) or absence of the ear (anotia), ocular dermoid cysts, and spinal abnormalities. Transversal digital volume tomography (DVT) (**a**) based on cone beam computed tomography (CBCT) reveals a spur-like hypoplasticity of the right mandibular ramus and an abnormal condylar process (*) in an adolescent male patient. An orthopantomographic view of the DVT (**b**) emphasizes the deformation of the mandibula on the right side with unimpaired teeth of the maxilla and mandibula. DVT allows 3D reconstructions, e.g., in frontal view (**c**, **e**), right lateral view (**d**), left lateral view (**f**) with and without transparent soft tissue overlay for maxillofacial surgery planning

malities and posterior arch deficiencies and to assess asymmetries between the affected and nonaffected sides for maxillofacial treatment planning [95]. Asymmetric mandible hypoplasia, facial clefts, nasal hypoplasia, and asymmetric skull deformities of Goldenhar syndrome can also be assessed by high-resolution multislice CT with multiplanar reconstructions [102]. Low-dose CT can be the imaging key for obstructive sleep apnea in children with Goldenhar syndrome, which can be caused by nasal hypoplasia, inverted teeth, maxillary clefting, and tonsillar hyperplasia [103]. Computed tomography of the temporal bone structures has proved helpful in imaging external and inner ear abnormalities in one-third of patients, who require complex hearing loss therapy [104]. Multidetector CT and CBCT provide exact spatial information about the abnormal variations in the facial skeleton and help to discriminate between the different craniofacial syndromes [102, 105]. After clinical inspection, MR imaging is the method of choice for the evaluation of common soft tissue disorders like preauricular appendages, ear anomalies like microtia, ocular anomalies like unilateral microphthalmia or unilateral anophthalmia, and epibulbar dermoids in Goldenhar syndrome. Diagnostic of multi-organ involvement, e.g., gastrointestinal, cardiovascular, and genital tract abnormalities, is based on the MRI, whereas CT is the favorite cross-sectional imaging procedure for respiratory tract anomalies [106, 107].

21.4 Soft Tissue Disorders and Midface Anomalies

Duplex ultrasonography (US) is the first-line imaging modality for the evaluation of superficial palpable masses of the head and neck in pediatric patients. This interactive diagnostic tool allows a quick and cost-effective image acquisition, providing information on size, shape, location, echogenicity, and vascularity of the mass [108]. High-resolution MRI and CT are supplementary imaging tools for adequate description of the extent of congenital soft tissue abnormalities of the midface, to visualize possible connections to the neurocranium, and to plan individualized surgical corrections. Multidetector row CT has improved the ability to depict detailed bone structures of the midface in extremely short scan time combined with the ability to produce high-quality multiplanar reformations (MPR) and 3D reconstructions based on virtually isotropic images. High-resolution CT scans through the midface are usually acquired in transversal sections and intervals of 3 mm or less perpendicular to the hard palate. The thinnest possible slice thickness combined with a bone reconstruction algorithm guarantees the best imaging results for small midface structures. Coronal reformations are easily acquired from high-resolution MDCT scans. If highly resolved CT images of the midface are needed, real coronal scans obtained from sedated children or infants in prone position are the better alternative [48]. Three-dimensional CT reconstructions, e.g., VRT images, can be post-processed with emphasis on different visual impressions. Although not needed for radiological diagnostics, the resulting 3D images can provide the surgeon with a quick overview of the symmetry of midface structures. Dual-source CT (DSCT) use two X-ray sources and two detectors at the same time. Third-generation DSCT demonstrates an optimal compromise between dose and image quality for the imaging of midface structures if performed with 100 kv, tin prefiltration to constrict the energy spectrum in combination with iterative reconstruction [109]. The effective dose of Sn100 kV/150 mAs (volume CT dose index, 1.22 mGy) for midface structures, especially the parasinus region, is comparable with that of conventional radiography and superior to CBCT with regard to higher image quality at even lower radiation exposure [109].

21.4.1 Lymphangiomas

Lymphangiomas are benign congenital abnormalities of the lymphatic vasculature, which form variably sized cystic formations preferably in the craniocervical region [110, 111]. Approximately 75% of lymphangiomas occur in the cervical region [110]. Depending on the size and location, lymphangiomas can cause airway obstruction, movement disabilities, and esthetic problems. As

part of a rare multisystem congenital disorder named generalized lymphatic anomaly, these lymphatic vascular malformations can involve several organs with poor prognosis [112]. Lymphangiomas are subdivided into three types depending on the size of the lymphatic cavities: capillary or microcystic lymphangiomas, cavernous or macrocystic lymphangiomas, and cystic lymphangiomas [110, 113]. The incidence of these vascular malformations is approximately one in 6000–16,000 live births with equal gender distribution [114]. Most lymphangiomas are sporadic, but they can also be part of syndromes like lymphangiomyomatosis, Turner syndrome, Noonan syndrome, and trisomies 13, 18, and 21 [115].

Ultrasonography commonly depicts lymphangiomas as anechogenic or hypoechogenic cystic masses with internal septa of variable thickness [108, 116]. The cystic parts may also appear hyperechoic after internal hemorrhage, superinfection, and if an elevated lipid content is present [108, 117]. Doppler US sometimes reveals arterial or venous vessels in the septa [115]. In the case of large lymphoma manifestations, the use of MRI and sometimes even CT is justified in order to assess the penetration of deep cervical and thoracic tissue layers and organs by the pathologically dilated lymph vessels [112, 118]. Magnetic resonance imaging is the cross-sectional imaging modality of choice to visualize the T2 hyperintense and T1 hypointense liquid content within the thin-walled cysts in fine detail [119] (Fig. 21.10). On CT images, lymphangiomas appear as hypodense, liquid cysts with thin hyperdense septa. After hemorrhage, the liquid content can be hyperdense on CT and hyperintense on T1-weighted images on MR images [118, 120].

21.4.2 Hemangiomas

Hemangiomas are benign congenital tumors of vascular origin lined by endothelial cells that can occur literally anywhere [115] (Fig. 21.11).

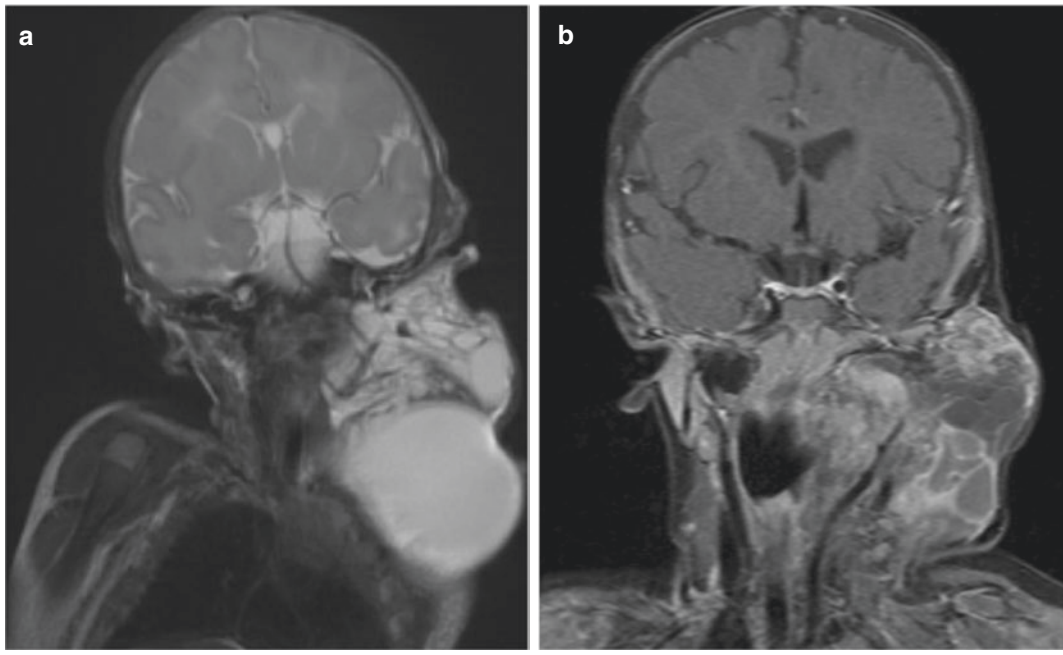

Fig. 21.10 Cervical lymphangioma of the left side of a 6-month-old boy on T2-weighted coronal MR images (**a**). The liquid isointense cystic parts of different size are bordered by thin hypointense cyst walls. The size of the vascular malformation and its extension into the deep muscles of the neck causes limited mobility of the neck and head to the affected side, which can manifest itself as scoliosis if left untreated. Six months after punctation of the largest cyst a T1w image after administration of Gadolinium (**b**) still shows a recurrent mass effect. Courtesy of Dr. Bernd Schweiger, University Hospital Essen, Germany

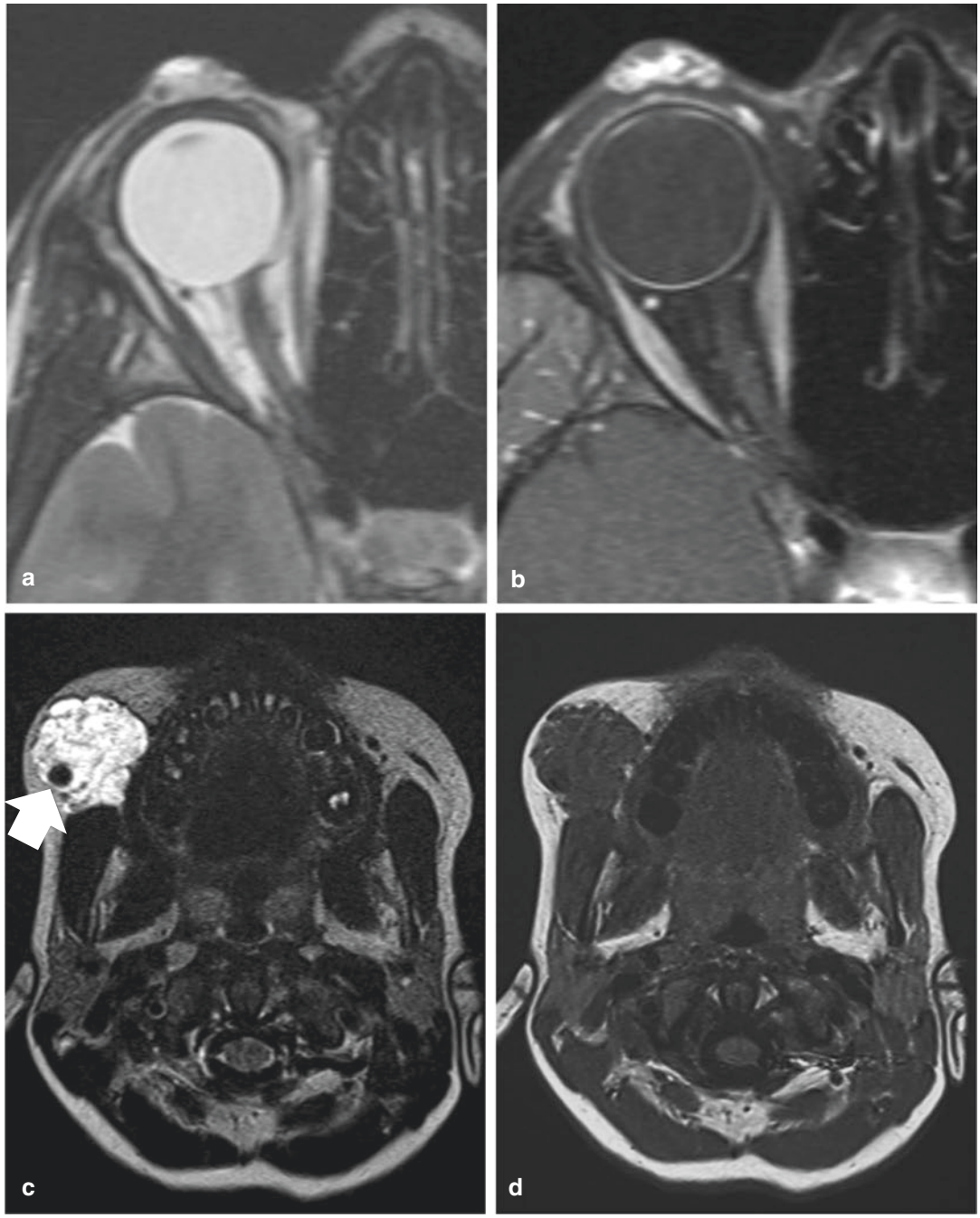

Fig. 21.11 Hemangioma of the upper right eyelid on transversal T2w MR images (**a**) of a 2-year-old girl shows an inhomogeneous hyperintense vascular tumor protruding in the preseptal skin. On axial T1w images with fat suppression the vessel walls of the vascular tumor enhance brightly after administration of Gadolinium (**b**). A 6-year-old boy with a right buccal hemangioma (**c**) presents with a focal calcification (white arrow) within the hyperintense vascular tumor in the subcutaneous fat (**d**) without infiltration of the right maxilla and the masseter muscle. Courtesy of Dr. Claudia Moeller-Hartmann, University Hospital Essen, Germany

Infantile and congenital hemangiomas are indistinguishable on imaging but have different clinical and histological characteristics. Congenital hemangiomas are completely formed lesions at birth without significant growth, whereas the infantile type is characteristically small or absent at birth. During the first year of life, they proliferate with progressive growth, followed by a stationary period, and finally, a progressive involution during the early childhood. Up to 50% involute by 5 years. There is a prevalence of 1–2% in neonates, and by 1 year of age, there is a prevalence of 12%. Females, Caucasians, and premature neonates are disproportionately affected [121]. Duplex US shows hemangiomas as cutaneous or subcutaneous soft tissue masses with dilated and irregular internal vascularity with simultaneous arterial and venous flow within the masses including high-velocity arterial waveforms and low-resistance venous waveforms [7, 122]. Most hemangiomas do not require therapy, but the beta-blocker propranolol is safely used in the management of infantile hemangiomas in different locations [123]. Magnetic resonance imaging is essential for identification, characterization, and delineation of hemangiomas in retro-orbital, intraosseous, intraparenchymal locations of organs and even in intracranial locations [7, 124–127]. For intraosseous manifestations, CT imaging can be beneficial to evaluate the stability of affected bones, e.g., vertebrae [128–133].

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22 Maxillofacial Investigation in Craniofacially Malformed Patients

Rita Depprich

Craniofacial malformations (CM) occur when there is perturbation due to genetic anomalies, environmental influences, or both [1]. CM comprise diverse diagnoses, implying a wide range of morbidity and disability; therefore, a thorough clinical investigation is indispensable. The complexity of CM requires interdisciplinary collaboration between geneticists, pediatricians, neurosurgeons, ENTs, maxillofacial surgeons, ophthalmologists, dentists, speech therapists, psychologists, etc., for diagnosis and therapy.

Oral clefts (OC) of different extents are the most common (Fig. 22.1) CM, Asian population shows a higher incidence compared to Caucasian ethnicity, and African ethnicity shows the lowest incidence [2]. OC are known to negatively affect feeding, hearing, and speech. Early symptoms commonly include feeding difficulties, nasal regurgitation, malnutrition, and hearing loss, whereas later in life, speech problems and orthodontic problems often require treatment [2].

Malformations of the human brain (e.g., encephaloceles) are often associated with various facial anomalies. Craniosynostosis (CS) primarily affects the brain and the oculo-orbital region; CS can occur isolated or can be part of multiple congenital abnormality syndromes (e.g., Apert and Crouzon; Fig. 22.2) [3–5]. Mandibulofacial dysostosis can be isolated or consist of micrognathia with a variety of congenital anomalies (e.g., Treacher Collins syndrome (Fig. 22.3), Nager syndrome, etc.) [6–8]. The Pierre Robin sequence, a combination of micrognathia, glossoptosis, and cleft palate (airway obstruction), is often associated with various syndromes (e.g., Stickler syndrome, velocardiofacial syndrome, van der Woude syndrome, etc.) (Table 22.1) [9, 10].

To diagnose and assess the sometimes complex medical problems of CM, it is important to take the patient's history, carry out physical examination, and request appropriate investigation (e.g., hematological evaluation, abdominal ultrasound, spine X-ray, cranial CT scan audiogram, etc.). Maxillofacial and craniofacial assessment covers the whole region of the skull (neurocranium and viscerocranium) with special respect to the jaws, the oral cavity, and the occlusion.

22.1 Medical History

Details regarding the presenting complaints and medical and family history should be obtained from the patient and/or the parents. If there is suspicion of a congenital CM, taking the family history is particularly important. Parents, grandparents, siblings, and cousins should be examined, and a human genetic investigation should be carried out if necessary. A complete family tree should be constructed showing all members

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Fig. 22.1 Phenotypes of different clefts

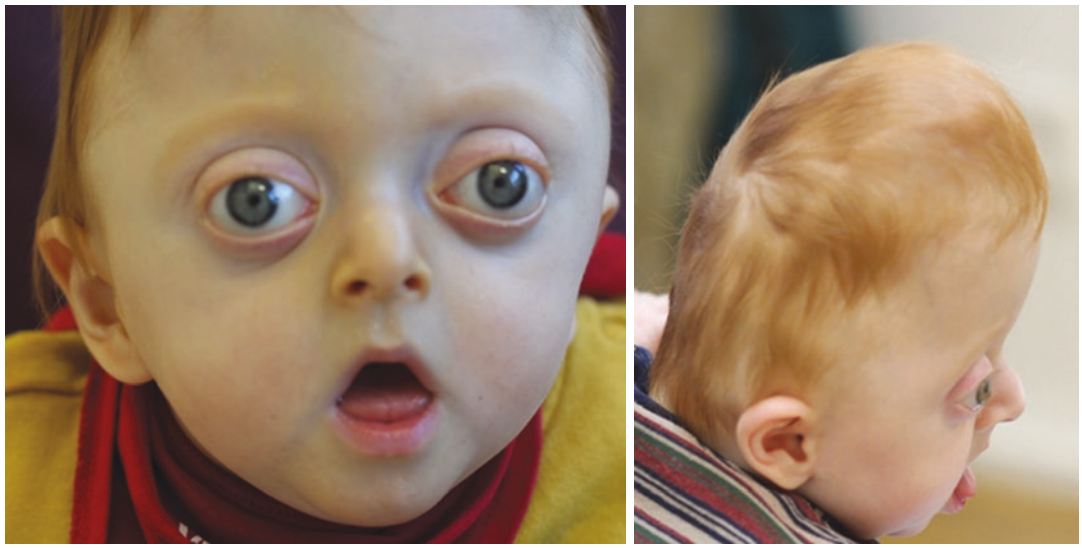


Fig. 22.2 Typical appearance of Crouzon syndrome

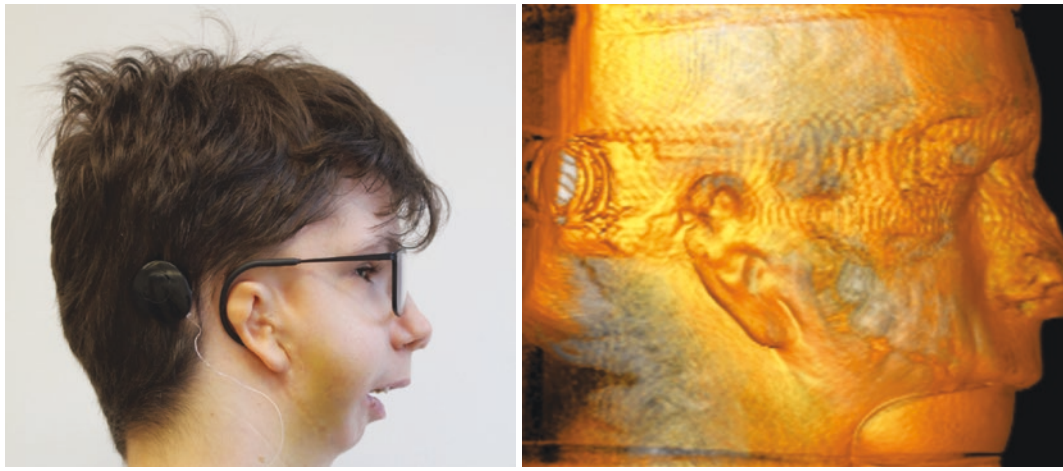


Fig. 22.3 Clinical picture and CBCT of a Treacher Collins syndrome patient

Table 22.1 Important syndromes with Robin sequence

Syndrome	Symptoms	Frequency	Inheritance/gene
22q11.2 deletion syndrome (DiGeorge syndrome/ velocardiofacial syndrome) [48]	Cardiac anomaly, polyhydramnios, polydactyly, cleft palate	1 in 4000	AD, 22q11.2
Stickler syndrome [49]	Cleft palate, micrognathia, midfacial hypoplasia, hearing loss, vitreoretinal degeneration, joint hypermobility, premature osteoarthritis	1 in 7500 to 9000	AD/AR, COL2A1, COL11A1, COL11A2, COL9A1, COL9A2, COL9A3
van der Woude syndrome [50]	Cleft lip and/or palate, lip pits	1 in 35,000 to 1 in 100,000	AD, IRF6
Treacher Collins syndrome (mandibulofacial dysostosis) [33]	Micrognathia, malar hypoplasia, eye abnormalities, cleft palate, ear deformation, hearing loss	1 in 50,000	AD, TCOF1, POLR1D; AR, POLR1C
Nager acrofacial dysostosis [60]	Malar hypoplasia, micrognathia, cleft palate, ear deformation, hearing loss, abnormalities of upper extremities, malformed or absent thumbs, clinodactyly, syndactyly	Very rare	AD/AR, SF3B4

affected. Consanguinity and ethnic background could be crucial and also be reviewed. Furthermore, parents should be asked for details concerning pregnancy, maternal drug history during pregnancy, delivery, newborn period, and early childhood. All relevant information is documented in the medical record.

22.2 Clinical Examination

The clinical examination of patients with CM should not be limited to the head and neck but should include a comprehensive and systematic physical examination since several systems of the body are often affected (Table 22.2). It is important to be aware that not the full house of all classical symptoms of a specific disease is present in every patient. The existence of more than one malformation or a malformation in combination with a minor anomaly may be clues to a specific diagnosis. In some rare cases, manifestations of more than one syndrome are found (overlap syndrome/associational syndrome) [11], making it difficult to assign the right diagnosis. All findings (height, body weight, head circumference, etc.) are noted meticulously in the medical record and plotted on adequate growth curves, if appropriate.

Table 22.2 Components of physical examination [51]

System	Feature examined
General	Height, body proportions, general appearance
Skin and hair	Pigmentation, texture, hair distribution (hirsutism)
Head size and shape	Asymmetry, sutural synostosis, microcephaly, macrocephaly
Eyes	Shape, size, placement, hypo−/hypertelorism, enophthalmos/exophthalmos, morphology of iris (coloboma)
Ears	Shape, size, location, morphology, tags, pits
Nose	Shape, asymmetry, configuration of the root, columella, nares
Mouth	Palate, uvula, tongue, mucosa, dentition
Lips	Vermilion, philtrum, pits, symmetry
Chin	Size, position
Neck	Webbing, masses, sinuses, pits
Chest	Shape, heart auscultation, symmetry
Abdomen	Shape, masses, scars, umbilicus, hepatosplenomegaly
Extremities	Size, shape, symmetry, configuration of the hands, feet, nails, creases, syndactyly, clinodactyly, mobility of joints
Back	Curvature of the spine
Neurological	Developmental status, cranial nerves, motor tone and strength, reflexes, gait, cerebellar function

Standardized photo documentation is recommended after obtaining patient consent.

Some CM are associated with general growth disturbances. The Silver-Russell syndrome or the Kabuki syndrome, for example, shows short stature postnatal dwarfism respectively, while the Beckwith-Wiedemann syndrome is the most common overgrowth syndrome [12]. In the latter, lateralized overgrowth and embryonic tumors are also typical symptoms [13] (Table 22.3).

Ectodermal dysplasias are a group of clinically heterogeneous heritable malformations, characterized by abnormalities of two or more ectodermal-derived structures such as hair, skin, teeth, and epidermal appendages [14, 15]. The EEC syndrome (ectrodactyly, ectodermal dysplasia, and orofacial clefts) is associated with numerous developmental disorders characterized by the triad of ectrodactyly, ectodermal dysplasia, and orofacial clefts [13]. The Costello syndrome is characterized by craniofacial, musculoskeletal, neurological, cardiologic, ocular, and genital abnormalities [16, 17]. Cardinal manifestations are thick, loose skin on the dorsal aspects of the hands and feet and deep palmar and plantar creases. Hyperpigmentation and rugosities are symptoms of Crouzon syndrome (craniofacial dysostosis) with acanthosis nigricans [18].

Table 22.3 CM associated with growth disturbances

Short stature [52–55]	Clinical appearance	Inheritance, genes
Kabuki syndrome	Short stature, congenital mental retardation, skeletal abnormalities, microcephaly, cleft palate, flat broadened tip of the nose, arched eyebrows, long eyelashes, long palpebral fissures with eversion of lateral parts of the lower lids, and large protruding or cupped earlobes	AD, KMT2D (MLL2); X-linked dominant, *KDM6A*
Cornelia de Lange syndrome	Short stature, moderate to severe intellectual disability, abnormalities of bones of upper extremities, low-set ears, teeth abnormalities, cleft, hirsutism, microcephaly, hearing loss	AD, NIPBL, RAD21, SMC3; X-linked dominant, HDAC8, SMC1A
Silver-Russell syndrome	Low birth weight, short stature, small triangular face, clinodactyly, relative macrocephaly, ear anomalies, skeletal asymmetry	Sporadic, chromosomes 7 and 11
Overgrowth [48, 59, 60]		
Weaver syndrome	Tall stature with or without macrocephaly, usually mild intellectual disability, characteristic facial features: Broad forehead; hypertelorism; large, low-set ears; micrognathia	AD, EZH2
Beckwith-Wiedemann syndrome	Macrosomia, macroglossia, omphalocele/umbilical hernia, hemihypertrophy, micrognathia, microcephaly	AD, 11p15.5 aberration
Sotos syndrome (cerebral gigantism)	Prenatal and postnatal overgrowth, triangular facies, marked prognathism, macrocephaly, intellectual disability	AD, NSD1

22.3 Oral Clefts

Cleft lip and palate is the most common craniofacial anomaly with an overall incidence of about 1 in 700 live births [19]. They occur with a broad spectrum of variations (complete/incomplete, unilateral/bilateral) either isolated or in combination with other developmental disorders. Nonsyndromic cleft lip and palate derives from two separate genetic entities. Oral clefts which originate from the primary palate usually affect the lip, alveolar process, and hard palate anterior to the incisive foramen. Such clefts are therefore always paramedian clefts and described as unilateral or bilateral cleft lip, with or without cleft palate. Clefts of the secondary palate are located dorsally to the incisive foramen. They can occur simultaneously with clefting of the lip and primary palate [20]. Submucous cleft palate is the most common cleft of the posterior palate and typically presents as the triad of bifid uvula, diastasis of the palatal musculature, and midline notching of the posterior hard palate [21, 22]. As the occult variant of submucous cleft palate is often not noticeable until velopharyngeal insufficiency occurs, careful intraoral examination is recommended. Clinical findings are noted in the medical record. To facilitate the documentation of the findings, classification systems and pictograms are widely used [23]. The Veau classification system distinguishes four different types of cleft: (1) clefts of the soft palate only, (2) clefts of the soft and hard palate, (3) clefts from the soft palate to the alveolus usually involving the lip, and (4) complete bilateral clefts (Fig. 22.4). While y-pictograms (e.g., Kernahan and Stark) were mainly used for this in the past [24], the trend is now towards digital documentation based on anatomical y-pictogram [25].

22.4 Craniofacial/Hemifacial Microsomia

Malformations of the craniofacial skeleton are typical features of CM. Craniofacial microsomia (hemifacial macrosomia) represents mostly congenital malformations resulting from the variable dysmorphogenesis of craniofacial structures either derived from or intimately related to the first and second brachial arches [26]. Malformations of the head size and shape due to premature fusion of cranial sutures (craniosynostosis) are also typical representatives of CM. Malformations of the craniofacial skeleton can occur symmetrically or asymmetrically and can be mainly congenital or less commonly acquired (e.g., tumors, atrophies, neurological paralysis, hypertrophies).

Craniofacial/hemifacial microsomia is one of the most common sporadically occurring congenital malformations of the head and neck, second only to cleft lip/cleft palate. A broad spectrum of phenotypes exists, as the external/middle ear, mandible, and contiguous bones of the facial skeleton along with their overlying musculature, cranial nerves, and connective tissue can be affected. Despite this heterogeneity, 89–100% of the patients present with mandibular hypoplasia, the most prevalent anomaly of craniofacial microsomia [27]. Hemifacial microsomia is generally asymmetric, although bilateral hypoplasia has been noted in 5–30% of cases [26, 28]. The Goldenhar syndrome, for example, is a sporadically occurring oculo-auriculo-vertebral dysplasia and defined by hemifacial hypoplasia, epibulbar lipodermoids, and vertebral anomalies, including fused vertebrae and/or hemivertebrae [29]. Due to the vastly heterogeneous phenotypic spectrum, categorization of hemifacial microsomia is difficult. The OMENS classification system developed by Vento et al. in 1991 [30] and later modified by Horgan et al. in 1995 (OMENS-Plus) [31] is a very simple and reproducible scheme. Dysmorphic severity of *o*rbital asymmetry, *m*andibular hypoplasia, *e*ar deformity, *n*erve dysfunction, and *s*oft-tissue deficiency ranges on a scale from 0 to 3. Scoring is done on the basis of physical examination, conventional radiographs, and photographs. OMENS-Plus additionally considers extra-craniofacial anomalies (e.g., central nervous system, cardiac, pulmonary, renal, gastrointestinal, and vertebral deformities) [31] (see Fig. 22.5). The Pruzansky-Kaban clas-

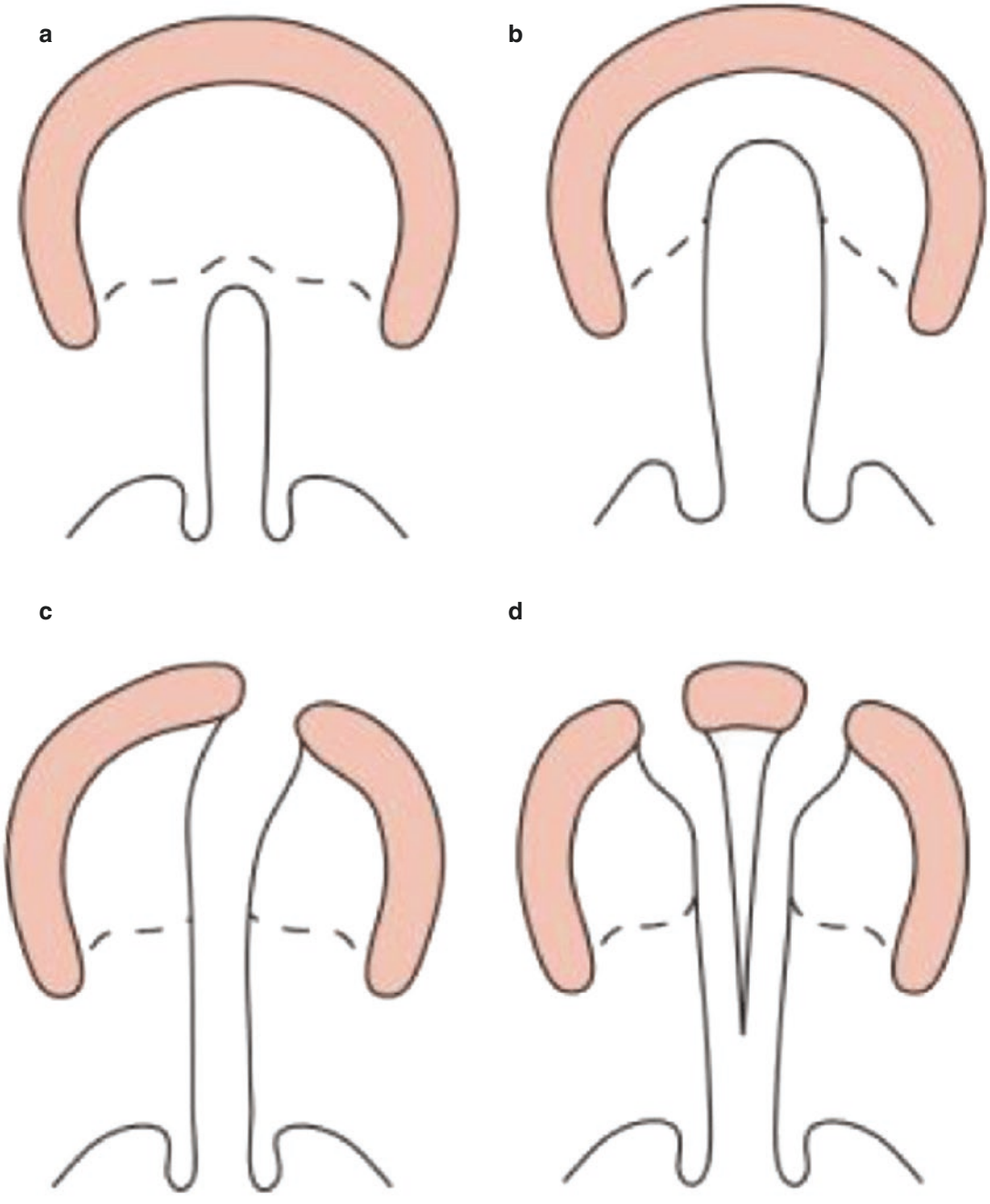


Fig. 22.4 Veau classification system of cleft lip and palate

Modified O.M.E.N.S. (+) Classification of Hemifacial Microsomia[1]

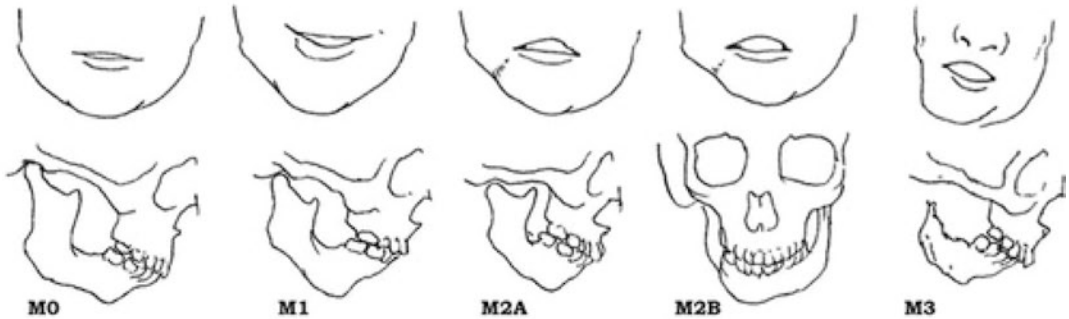
(check all that apply)

Side (Each side evaluated separately in cases of bifacial microsomia)
__ *R* __ *L*

Orbit[2]

__ ***O0*** Normal orbital size and position __ ***O1*** Abnormal orbital size __ ***O2↓*** Inferior orbital displacement __ ***O2↑*** Superior orbital displacement __ ***O3*** Abnormal orbital size and position

Mandible



__ ***M0*** Normal mandible __ ***M1*** Small mandible and glenoid fossa with short ramus __ ***M2A*** Abnormally shaped and short ramus[3] (Glenoid fossa in acceptable position with reference to contralateral TMJ) __ ***M2B*** Abnormally shaped and short ramus (Glenoid fossa is inferiorly, medially and anteriorly displaced with a severely hypoplastic condyle) __ ***M3*** Absence of ramus and glenoid fossa (no TMJ)

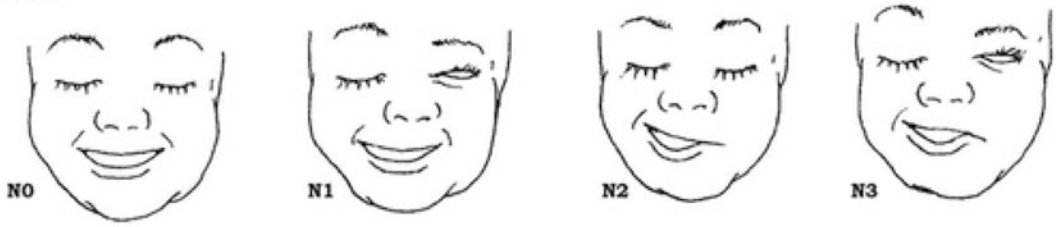
Ear

__ ***E0*** Normal auricle __ ***E1*** Mild hypoplasia and cupping with presence of all structures __ ***E2*** Absence of external canal with variable hypoplasia of concha __ ***E3*** Malpositioned lobule with absent auricle; lobular remnant typically inferiorly and anteriorly displaced

1.As modified from Vento AR, LaBrie RA, Mulliken JB. The O.M.E.N.S. classification of hemifacial microsomia Cleft Palate-Craniofacial Journal. 28(1):68-76.
2. Orbital position determined as follows: Midsagittal plane defined as vertical line between crista galli and anterior nasal spine. Horizontal line is then drawn perpendicular to midsagittal plane, tangent to supraorbital rims.
3. Evaluated with submental vertex radiographs or CT.

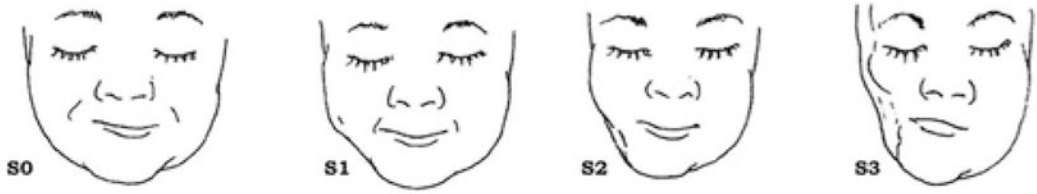
Fig. 22.5 OMENS-classification system developed by Vento et al. in a 1991 [30], and later modified by Horgan et al. in 1995 (OMENS-plus) [31]

Nerve



__ ***N0*** No facial nerve involvement __ ***N1*** Temporal and or Zygomatic branch involvement
__ ***N2*** Buccal and/or Mandibular and/or Cervical branch involvement __ ***N3*** All branches affected

Soft Tissue



__ ***S0*** No soft tissue deficiency __ ***S1*** Minimal soft tissue deficiency
__ ***S2*** Moderate soft tissue deficiency (between S1 and S3) __ ***S3*** Severe soft tissue deficiency

Macrostomia (Tessier # 7 Cleft)

__ ***C0*** No cleft __ ***C1*** Cleft terminates medial to anterior border of masseter __ ***C2*** Cleft terminates lateral to anterior border of masseter

Miscellaneous

O.M.E.N.S. (+)[4] { __ *Yes* / __ *No*

__ ***Goldenhars*** (Hemifacial Microsomia with epibulbar lipodermoids and fused/hemivertebrae)

Notes:

4. Horgan, J.E., Padwa, B.L., LaBrie, R.A., et al. OMENS-plus: analysis of craniofacial and extracraniofacial anomalies in hemifacial microsomia. *Cleft Palate Craniofac. J.* 32:405, 1995.

Fig. 22.5 (continued)

sification system is used to determine the degree of mandibular hypoplasia and therefore preferably employed for treatment planning (e.g., distraction osteogenesis, orthognathic surgery, or costochondral graft) [28].

22.5 Craniosynostoses

Malformations of the head size and shape due to premature fusion of cranial sutures (craniosynostosis) emerge as the growth of the skull and brain is impaired in a direction perpendicular to the fused sutures, giving rise to craniofacial abnormalities of varying degrees (Table 22.4). Craniosynostosis can involve a single suture or multiple sutures and be symmetrically or asymmetrically, and the involvement of specific sutures gives the patient a characteristic head shape at birth. The most common is the premature fusion of the coronal suture [32, 33]. Involvement to the skull base leads to significant growth impairment of the neurocranium and viscerocranium, resulting in abnormal shape of the head with midface hypoplasia and protrusio bulbi. Craniosynostosis can be a part of a genetic syndrome (e.g., Apert, Crouzon, Pfeiffer, Saethre-Chotzen) (Table 22.5), but more often it is an isolated defect—approx. 85% of all craniosynostosis cases are nonsyndromic [18, 32, 34].

Table 22.4 Craniosynostoses [33]

Scaphocephaly	Trigonocephaly	Plagiocephaly	Brachycephaly	Oxycephaly
Synostosis of the sagittal suture	Synostosis of the metopic suture	Unilateral synostosis of the coronal suture	Bilateral synostosis of coronal sutures	Synostosis of coronal and sagittal sutures
Narrowed and elongated head, with projection of the frontal and occipital areas	Triangular shape of the forehead, always associated with hypotelorism	Flattened forehead on the affected side, elevation of the orbit and distortion of the root of the nose	Vertically recessed forehead, retrusion of the supraorbital rim and root of the nose; bulging of the upper part of the forehead and of temporal fossae	Recessed and backward tilted forehead in continuity with the nasal dorsum; recessed supraorbital rim, exophthalmos

Table 22.5 Clinical and genetic features of important craniosynostotic syndromes [18, 33, 56–58]

Syndrome	Involved sutures	Clinical appearance	Inheritance, genes
Crouzon syndrome (dysostosis craniofacialis)	Bicoronal synostosis, but other sutures can be involved	Midfacial underdevelopment, micrognathia, exorbitism, hypertelorism	AD, 10q26
Pfeiffer syndrome (acrocephalosyndactyly syndrome type V)	Bicoronal synostosis with brachysyndactyly; three clinical subtypes based on the severity of phenotype	Midface retrusion, hypertelorism, varus deviation, soft-tissue syndactyly of the hands (usually second and third digits) and feet, short and broad thumbs and great toes	AD, 8p11, 10q26
Saethre-Chotzen syndrome (acrocephalosyndactyly syndrome type III)	Premature fusion of the coronal suture with finger and/or toe abnormalities (musculoskeletal abnormalities)	Midface hypoplasia, hypertelorism, ptosis, ear anomalies, brachydactyly, clinodactyly, cutaneous syndactyly (second and third fingers and second and third toes and, less frequently, from the second to the fourth fingers), cervical spine anomalies	AD, 7p21
Apert syndrome (acrocephalosyndactyly syndrome type I)	Bicoronal synostosis with syndactyly	Facial retrusion, anterior open bite, cleft palate, symmetric second to fourth digit syndactyly in the hands and feet, hypertelorism, more frequent abnormalities of the CNS than in other syndromic craniosynostoses	AD, 10q25-10q26

Patients with craniosynostosis present with altered head shapes, visual deficits, and/or neurological impairment. The latter can occur due to the craniosynostosis, or it can be an associated cerebral development disorder. Most craniosynostoses are recognizable at birth (e.g., Apert syndrome), but the examiner must be aware that deformity of the head at birth is not always due to a premature fusion of cranial sutures. Some deformities are related to fetal position, forceps delivery, or resting position of the head and will disappear during the first year of life. In these cases, roentgenograms show normal sutures, a normal curve on the cranial vault, and normal shape of the orbits [33]. In contrast, patients with progressive craniosynostosis (e.g., Crouzon) demonstrate no skull shape abnormalities at birth but midface manifestations typically observed among patients with syndromic craniosynostosis. With time, these patients progressively develop holocalvarial craniosynostosis and ultimately symptoms of increased intracranial pressure [34] (signs of increased intracranial hypertension; see Table 22.6).

Increased intracranial hypertension is more frequent in craniosynostoses affecting several sutures and also more frequent in older children. Chronic increased intracranial hypertension may cause brain atrophy with cerebral dysfunction. Since the development of intracranial hypertension is a very slow process, due to the growth of the brain in the inadequate cranium, clinical symptoms are often missing. Therefore, diagnosis of increased intracranial hypertension can be difficult, and ICP monitoring by an extradural sensor may be appropriate. Papilledema due to increased intracranial hypertension may lead to irreversible severe visual impairment by optic atrophy, if untreated.

Table 22.6 Signs of increased intracranial pressure [34]

Symptoms of increased intracranial pressure	Headaches, nausea/vomiting, irritability, decreased mental status, visual disturbances, increased sleepiness, bulging fontanelles
Radiological signs	Thumbprinting or a beaten-copper appearance of the skull, slit-like ventricles, sulcal effacement, and optic nerve involvement
Ophthalmologic signs	Papilledema or optic nerve atrophy

The evaluation of the oral cavity as well as the whole masticatory system is based on the knowledge of the functional anatomy. The bony components of the skull base and jaws, especially the TMJ containing mandible, as well as muscles and ligaments play a central role. The knowledge of the anatomy is elaborated, since these structures are regularly disturbed in craniofacial malformations.

22.6 Skeletal Components

The maxilla forms a crucial aspect of the upper viscerofacial skeleton. The two bones fuse during organogenesis at the intermaxillary suture during development forming the upper jaw. Maxillary development during gestation forms the palate of the oral cavity and also supports the alveolar ridges that hold the upper teeth in place. The lower viscerocranium, on the other hand, is formed through the mandible, a U-shaped bone, which supports the lower teeth and also forms part of the TMJ. The mandibular condyle and the squamous portion of the temporal bone at the base of the cranium articulate with one another.

22.7 Temporomandibular Joint (TMJ) [35]

The TMJ is formed from the temporal bone of the cranium, specifically the glenoid fossa and articular tubercle and the condyle of the mandible, with a fibrocartilaginous disc lying in between. It is classified as a ginglymoarthrodial joint and can perform a range of gliding and hinge type movements. The disc which lies in between is composed of dense fibrous tissue and is predominantly avascular and lacking nerves.

22.8 Muscles

There are various muscles that contribute to occlusion of the teeth including the muscles of mastication and other accessory muscles. The temporalis, masseter, and medial and lateral pterygoids are the muscles of mastication, and these contribute to the elevation, depression, protrusion, and retraction of the mandible. The anterior and posterior bellies of the digastric are also involved in the depression of the mandible and elevation of the hyoid bone and are therefore relevant to the masticatory system.

22.9 Ligaments

There are some ligaments associated with the TMJ, and these limit and restrict border movements by acting as passive restraining devices. These ligaments do not contribute to joint function, rather exert a protective role. The key ligaments relevant to the TMJ are:

- The temporomandibular ligament.
- The medial and lateral discal ligaments.
- The sphenomandibular ligament.
- The stylomandibular ligament.

22.10 Jaw Deformities (Dysgnathia)

Various forms of mandibular and maxillary deformities (dysgnathia) can either be congenital or acquired. They are usually characterized by an inharmonious appearance and by functional impairment of the masticatory system and possibly impaired speech production. Dysgnathias with occlusion problems appear in nearly all of craniofacial malformations. Underdevelopment of the midface/maxilla is generally present in syndromic craniosynostoses as well as in CLP patients. Underdevelopment of the mandible is a key feature of branchial arch diseases.

The evaluation and control of the jaw positions is central to the maxillofacial investigations. The focus in craniofacial patients is on the jaw to skull base position as well as on the occlusion. The maxillofacial surgeon is the key discipline in coordination of growth control and treatment. In early years of growth of these children as well as during puberty and adulthood, close cooperation with orthodontists is mandatory. The whole masticatory system is involved in the function of biting and chewing. Additionally, the jaw positions as well as the occlusion are central for food intake, breathing, and esthetics. The masticatory system also involves the periodontium, the TMJ (and other skeletal components), and the neuromusculature; therefore, the tooth contacts should not be looked at in isolation, but in relation to the overall masticatory system. The examiner should look for the various causes of skeletal dysgnathia as early tooth loss, sucking habits, mouth breathing, poor nutrition, hormonal disorders (e.g., acromegaly), and others. Particular attention must be paid to the size, position, and function of the tongue. Tongue malposition and deviant swallowing pattern are common causes of dysgnathia and speech problems [36]. The open bite and its frequent recurrences can be mostly attributed to tongue malfunction [37]. Clinical examination evaluates face form, bilateral symmetry, vertical proportion, lip and chin position, temporomandibular joints, and occlusion. Correction of the bite is of outer important for patients, since this will influence function (eating, biting, swallowing, articulation, and esthetics).

It is important to investigate and differentiate between two aspects:

(i). The presence of dysgnathic positions of jaws (related to the skull base).
(ii). An altered occlusion (related to the interdental cuspidation).
  (a) Aberration of jaws are seen in the size as well as in the position of the jaw. They can affect all cardinal directions (anteroposterior (prognathism/retrognathism), transverse (laterognathia), vertically) symmetrically or asymmetrically with disturbed positional relationship of the jaws to each other or to the skull base (see Table 22.7). Jaw deformities

Table 22.7 Symmetrical maxillary and mandibular anomalies [38]

Bilateral mandibular hypoplasia	
Mandibular retrognathism	Skeletal too far posterior positioned mandible, maxilla in normal position
Mandibular micrognathism	Complete mandible abnormally small/underdeveloped
Mandibular hypo−/retroalveolism	Mandibular alveolar ridge abnormally small/underdeveloped, normal skeletal relation of the mandible and maxilla
Retrogenia/microgenia	Chin abnormally small/underdeveloped, normal skeletal relation of the mandible and maxilla
Bilateral maxillary hypoplasia	
Maxillary retrognathism	Skeletal too far posterior positioned maxilla, mandible in normal position
Maxillary micrognathism	Complete maxilla abnormally small/underdeveloped
Maxillary hypo−/retroalveolism	Maxillary alveolar ridge abnormally small/underdeveloped, normal skeletal relation of the mandible and maxilla
Bilateral mandibular hyperplasia	
Mandibular prognathism	Skeletal too far anterior positioned mandible, maxilla in normal position
Mandibular macrognathism	Complete mandible abnormally large
Mandibular proalveolism	Mandibular alveolar ridge abnormally large, normal skeletal relation of the mandible and maxilla
Progenia	Chin abnormally large, normal skeletal relation of the mandible and maxilla
Bilateral maxillary hyperplasia	
Maxillary prognathism	Skeletal too far anterior positioned maxilla, mandible in normal position
Maxillary proalveolism	Maxillary alveolar ridge abnormally large, normal skeletal relation of the mandible and maxilla
Midface protrusion	Protrusion of complete midface (maxilla and cheekbones)
Vertical maxillary hyperplasia	Elongated middle third of the face

can affect the size, position, orientation, shape, symmetry, and completeness [38]. Mandibular and maxillary malformations that do not respond to orthopedic growth modification or are too severe to camouflage by orthodontic treatment need to be treated by orthognathic surgery. As mandibular prognathism (e.g., "the Hapsburg jaw") or mandibular retrognathism is frequently consistent within a family, patients should be compared to other family members [39, 40].

(b) Occlusion is the relationship between the maxillary (upper) and mandibular (lower) teeth when they approach each other, as occurs during chewing or at rest. Occlusion can be divided in static and dynamic. Static occlusion refers to contact between teeth when the jaw is closed and stationary, while dynamic occlusion refers to occlusal contacts made when the jaw is moving [41].

Angle's classification describes and classifies the occlusion based on the relationship of the permanent first molars when the patient is in centric occlusion (see Table 22.8). Class I is considered normal occlusion, class II disto-occlusion, and class III mesio-occlusion [42]. It is important for dentists, orthodontists, and maxillofacial surgeons to evaluate the functional bite position. Three terms are used to define the static and dynamic occlusion.

Intercuspal position (ICP) is defined at the position where the maxillary and mandibular teeth fit together in maximum interdigitation. This position is usually the most easily recorded and is almost always the occlusion the patient closes into when they are asked to "bite together." This is the occlusion that the patient is accustomed to, hence sometimes termed the habitual bite [43].

Centric relation (CR) describes the reproducible jaw relationship (between the mandible and maxilla) and is independent of tooth contact. This is the position in which the mandibular condyles

Table 22.8 Angle classification of malocclusion [42]

Classes	Molar and canine relationship
Class I	Buccal groove of the mandibular first permanent molar occludes with the mesiobuccal cusp of the maxillary first molar Cusp of maxillary canine occludes with the distal half of the mandibular canine and the mesial half of the first premolar
Class II	The buccal groove of the mandibular first permanent molar occludes posterior to the mesiobuccal cusp of the maxillary first molar Cusp of maxillary canine occludes anterior to the embrasure between the mandibular canine and the first premolar
Division 1	The molar and canine relationship is that of class II The maxillary central incisors are proclined or normally inclined and the overjet is increased
Division 2	The molar and canine relationship is that of class II The maxillary central incisors are retroclined
Class III	The buccal groove of the mandibular first permanent molar occludes anterior to the mesiobuccal cusp of the maxillary first molar Cusp of maxillary canine occludes posterior to the embrasure between the mandibular canine and the first premolar

are positioned stress-less in the fossae in a relaxed anterosuperior position against the posterior slope of the articular eminence. This position is not influenced by muscle memory, but rather by the ligament which suspends the condyles within the fossa.

Retruded contact position (RCP) describes the position of the mandible is in this retruded position, when the first teeth are in contact and when the mandible opens and closes on an arc of curvature around an imaginary axis drawn through the center of the head of both condyles. This imaginary axis is termed the terminal hinge axis. RCP can be reproduced within 0.08 mm of accuracy due to the nonelastic TMJ capsule and restriction by the capsular ligaments.

Centric occlusion (CO), in contrast, is a confusing term and is often incorrectly used synonymously with RCP. Both CO and RCP are used to define a position where the condyles are in CR; however, RCP describes the initial tooth contact on closure, and this may be an interference contact [44].

Occlusal and bite-related investigations should examine:

- TMJ opening.
- Jaw relations.
- Intercuspal position (ICP).
- Retruded contact position (RCP).
- RCP-ICP slide.
- Lateral excursions.
- Protrusion.

There are some standards in the evaluation of patients concerning the occlusion and jaw position. En face and profile photographs, cephalometric radiographs, and diagnostic casts are the mainstay in orthodontic and maxillofacial investigation. Cephalometric assessment is crucial for the diagnosis of dysgnathia [45]. Cranial reference planes (e.g., Frankfort horizontal plane, sella-nasion line), several angles (SNA, SNB), and measurements (e.g., WITS) are used to determine whether there is a skeletal or dentoalveolar malformation. Nowadays, the cephalometric evaluation is carried out by computer programs, and if there is appropriate imaging (e.g., CT or cone beam CT), a 3D analysis of the bone and soft tissue can also be carried out [46, 47].

The overall assessment should be recorded properly. Investigations should be iterative at different time intervals to control the state and the dynamic of growth and growth-related disturbances in order to plan proper therapies.

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23 Communication Disorders Secondary to Clefts and Other Craniofacial Malformations

Ann W. Kummer

23.1 Introduction

A *malformation* is a birth defect that is caused by a genetic etiology which results in *dysplasia* (abnormal growth or development) of a tissue or organ. In contrast, a *deformation* is a birth defect that is the result of mechanical factors or teratogens. Most craniofacial anomalies, including cleft lip and most cases of cleft palate, are malformations rather than deformations [1–3]. Because of their genetic etiology, malformations can recur in subsequent offspring.

Malformations of the head, face, and neck can involve the primary organs of communication, which are the brain, ears, oral cavity, velopharyngeal mechanism, and vocal folds. Therefore, children with craniofacial malformations, particularly those with craniofacial syndromes where there are multiple malformations, are at a particular risk for communication disorders.

When abnormal structure interferes with normal articulation placement and articulatory movements, the speech of the individual will be characterized by obligatory distortions and/or compensatory errors [4]. *Obligatory distortions* occur when the articulation placement is correct, but an abnormality of the structure results in a distortion of speech. Common obligatory distortions due to dental/occlusal anomalies include lateral distortion of sibilant sounds (s, z, sh, zh, ch, j). In contrast, *compensatory errors* are the result of incorrect articulatory placement in response to abnormal structure. An example of a compensatory error is the substitution of a palatal-dorsal placement for a lingual-alveolar sound when dental malocclusion affects the tongue tip placement. Making a differential diagnosis of this is important because obligatory distortions can be corrected by correcting the structure. Speech therapy is not indicated. In contrast, compensatory speech errors usually require speech therapy for correction, but therapy will not be effective until after correction of the structure [4].

The following is a discussion of how clefts of the lip and palate, and various other craniofacial malformations, affect speech, resonance, voice, hearing, language, and cognition. The effects on feeding and swallowing are covered in another chapter.

23.2 Cleft Lip and Palate

Orofacial clefts can be of the lip only, the palate only, or of both. Clefts of the lip and palate vary in length and in width, depending on the degree

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of fusion of the individual parts. In addition, the structures surrounding the cleft are often hypoplastic, in that the tissues (e.g., bone, muscles, and nerves) are underdeveloped in their formation.

The *primary palate* consists of the structures that are anterior to the incisive foramen and fuse around 7 weeks of gestation [5]. These structures include the lip and alveolar ridge. Clefts of the primary palate can be incomplete or complete and also unilateral or bilateral (Fig. 23.1a–d). A complete cleft of the primary palate is one that extends through the entire lip, nostril sill, and alveolar ridge to the incisive foramen. An incomplete cleft of the primary palate is one that does not extend all the way to the incisive foramen.

The *secondary palate* consists of the structures that are posterior to the incisive foramen and fuse around 9 weeks of gestation [5]. These structures include the hard palate (excluding the alveolar ridge), the velum, and the uvula. Clefts of the secondary palate are always in midline, following the medial palatine suture. They can also be incomplete or complete (Fig. 23.2a, b). A complete cleft of the secondary palate extends through the uvula, velum, and hard palate all the way to the incisive foramen. An incomplete cleft of the primary palate does not extend to the inci-

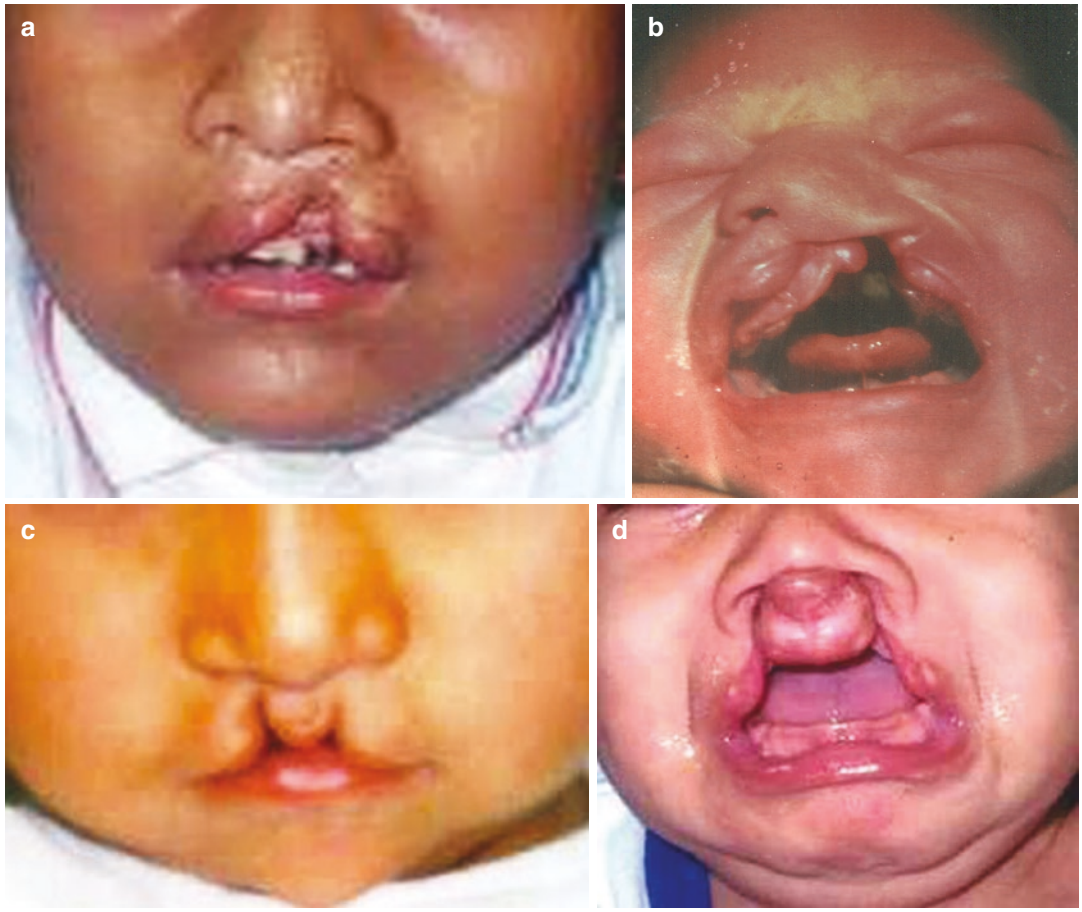


Fig. 23.1 Clefts of the primary palate. (**a**) Left unilateral incomplete cleft of the primary palate. Note the asymmetry of the nasal ala. (**b**) Left unilateral complete cleft of the primary palate. Note the asymmetry of the nasal ala. (**c**) Bilateral incomplete cleft of the primary palate. Note the flattened nasal tip. (**d**) Bilateral complete cleft of the primary palate. Note the lack of a columella to raise the nasal tip

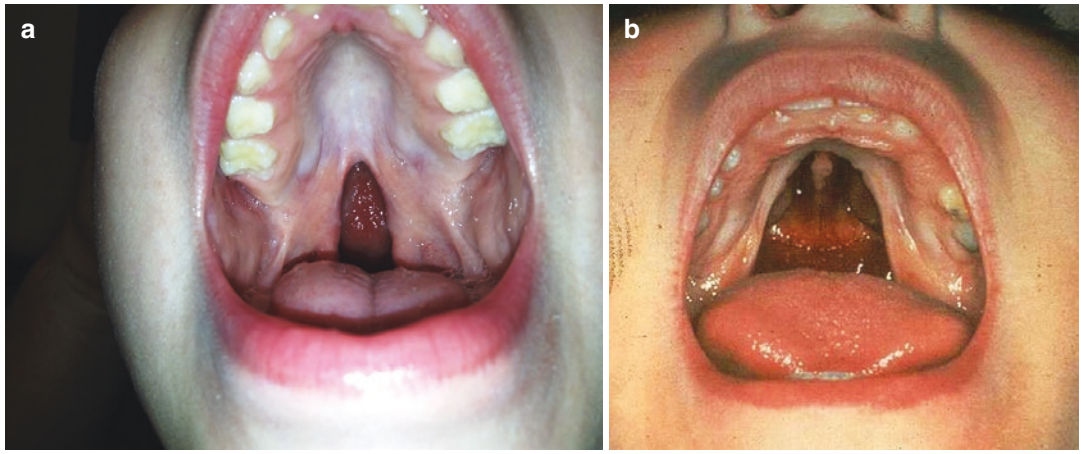


Fig. 23.2 Clefts of the secondary palate. (**a**) Incomplete cleft palate. (**b**) Complete cleft palate

sive foramen. Clefts of the secondary palate can also be submucous, therefore affecting primarily the nasal surface. Evidence of the cleft can often be seen on the oral surface (Fig. 23.3a, b) but sometimes can only be seen on the nasal surface through nasopharyngoscopy (Fig. 23.3c).

Both clefts of the primary palate and clefts of the secondary palate can affect the development of communication skills, but in different ways. In addition, clefts are frequently accompanied by additional craniofacial anomalies, which can further affect communication skills [6–8]. (See Table 23.1.)

23.3 Cleft of the Primary Palate (Cleft Lip)

A cleft of the lip only (incomplete cleft of the primary palate) has virtually no effect on speech. Some children with simple cleft lips have difficulties achieving bilabial closure at rest and during the production of bilabial sounds (p, b, m) due to a shortened upper lip as the lip scar matures. However, they can easily compensate for this by producing these sounds with a labiodental placement.

A cleft that extends through the alveolar ridge (complete cleft of the primary palate) can affect eruption of the teeth, causing supernumerary teeth or ectopic teeth [9]. These abnormally placed teeth can interfere with tongue tip movement during speech and can also divert the airstream for certain sounds, causing a lateral distortion [4, 9–11].

Malocclusion of the jaws is common in children with cleft lip and/or cleft palate. A complete cleft of the primary palate (particularly if the cleft is bilateral and is also associated with a cleft palate) can ultimately result in Class III malocclusion of the jaws. Class III malocclusion can cause the tongue tip to rest in front of the alveolar ridge and even in front of the maxillary teeth (Fig. 23.4a) [4, 11–13]. In contrast, micrognathia (as is seen with Pierre Robin sequence) can result in a Class II malocclusion. In this case, the tongue tip may rest under the arch of the palate (Fig. 23.4b).

Whenever malocclusion disrupts the tongue tip to alveolar ridge relationship, there is usually a significant effect on articulation of all lingual-alveolar sounds (t, d, n, l, s, z, sh, zh, ch, j). To a lesser extent, this abnormal jaw relationship can also affect labiodental sounds (f, v) and bilabial sounds (p, b, m). The difficulty in producing these sounds normally due to abnormal structure can result in obligatory distortions and/or compensatory errors.

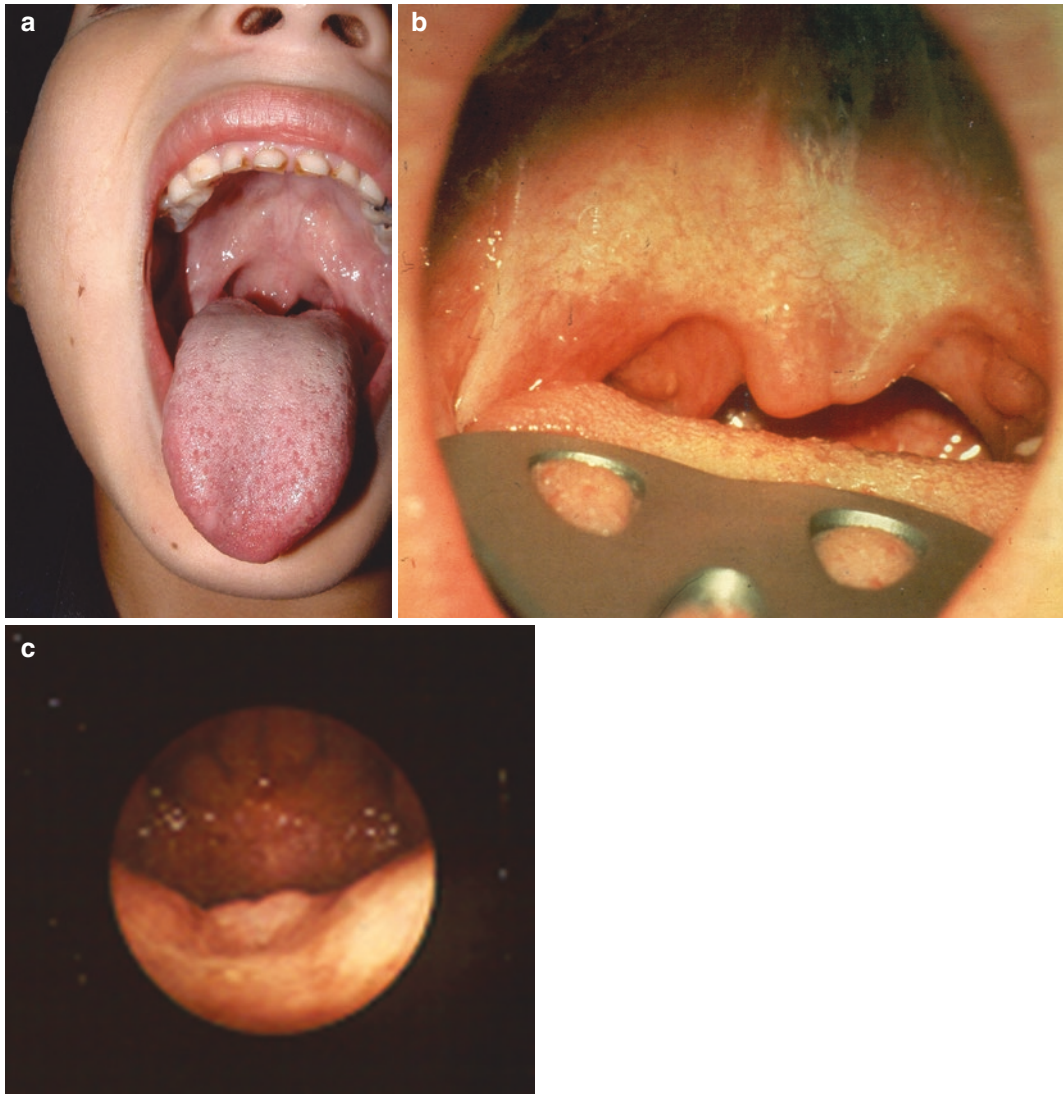


Fig. 23.3 Submucous clefts. (**a**). A submucous cleft which can be viewed on the oral surface of the velum. The tenting of the velum is caused by abnormal insertion of the levator veli palatini muscle into the hard palate rather than in midline of the velum. (**b**) Submucous cleft palate characterized by a hypoplastic uvula and zona pellucida (thin zone) in the velum. (**c**) Nasopharyngoscopy view of a submucous cleft. The concavity of the nasal surface of the velum is due to dysplasia of the nasal surface of the velum, including the musculus uvulae muscles

23.4 Cleft of the Secondary Palate (Cleft Palate)

The biggest concern with cleft palate is the possibility that the palate repair alone will not be sufficient to result normal velopharyngeal closure. In fact, approximately 20–30% of children with cleft palate will demonstrate some degree of velopharyngeal insufficiency (VPI), despite the palatoplasty. This is because the inherent dysplasia of the velar tissue results in a velum that is not of sufficient length to close firmly against the posterior pharyngeal wall during oral speech production (Fig. 23.5a–c) [14, 15].

Velopharyngeal insufficiency can result in significant issues with both speech and resonance.

Table 23.1 Syndromes and conditions commonly associated with cleft lip (with or without cleft palate) and cleft palate only

Cleft lip (with or without cleft palate)	Cleft palate only
Amniotic bands	CHARGE syndrome
CHARGE syndrome	Fetal hydantoin syndrome
Fetal alcohol syndrome	Hemifacial microsomia
Hemifacial microsomia	Kabuki syndrome
Opitz syndrome	Stickler syndrome
Orofaciodigital syndrome type I (OFD I)	Van der Woude syndrome
Popliteal pterygium syndrome	Velocardiofacial syndrome
Trisomy 13	
Van der Woude syndrome	
Wolf-Hirschhorn syndrome	

The specific speech characteristics are dependent on the size of the velopharyngeal opening [16, 17]. (See Table 23.2.)

During oral speech production, VPI can cause hypernasality, which is a resonance disorder caused by an inappropriate leak of sound into the nasal cavity during production of oral speech. Hypernasality affects all vowels and voiced consonants. In many cases, oral consonants will seem to be substituted by nasal sounds (m, n, ng). For example, a /b/ sound will be perceived to be an /m/ sound. When there is hypernasality, there is also low volume because much of the sound is absorbed by the tissues of the pharynx and nasal cavity [14, 15].

In addition to hypernasality, VPI can cause nasal emission, which is a leak of airflow into the nasal cavity during consonant production [14, 15]. If the velopharyngeal opening is relatively small, nasal emission will be very audible on consonants and therefore distracting to the listener. If the opening is relatively large however, the nasal emission will be inaudible, partly because the hypernasality masks the noise. However, the reduction of oral airflow due to a large leak into the nasal cavity has a significant effect on speech sound production. It causes the consonants to be very weak in intensity and pressure. As a result, the child may develop compensatory articulation productions in the pharynx because that is where there is airflow. In addition, a significant leak of airflow causes the child to have to take more frequent breaths during speech to replace the airflow for phonation and consonant production.

Finally, children with cleft palate are at increased risk for fluctuating conductive hearing loss. This occurs because with cleft palate, there is often congenital dysplasia of the tensor veli palatini muscle, which is responsible for opening the Eustachian tube during swallowing. Inadequate Eustachian tube function leads to chronic middle ear effusion, which causes the conductive hearing loss.

23.5 Malformations of the Nose Secondary to Cleft Lip and Palate

Clefts of the lip and palate, particularly if they are complete clefts, will also affect the nose (note Fig. 23.1a–d) [5]. Typically, nasal anomalies cause obstructive breathing and reduce normal nasal resonance during speech [18–20].

Clefts of the primary palate often cause external anomalies of the nose (i.e., asymmetry and reduced projection of the nasal tip), which affect aesthetics. Anomalies of the inside of the nose, such as a narrowing of the pyriform aperture, a deviated septum, or choanal stenosis or atresia, affect function, causing obstructive breathing and hyponasality or nasal cul-de-sac resonance [15].

Clefts of the secondary palate (particularly if associated with a bilateral cleft of the primary palate) often result in maxillary retrusion (Fig. 23.6). Although the maxilla continues to grow with age, it typically remains about 30% smaller than normal due to the inherent hypoplasia of the bony structures and the restrictive effects of surgical correction [21]. Because the maxilla serves as the floor of the nose, a retrusive maxilla causes the nasal cavity to also be small. Reduced nasal cavity size can affect nasal breathing and also restrict normal nasal resonance during speech, causing hyponasality.

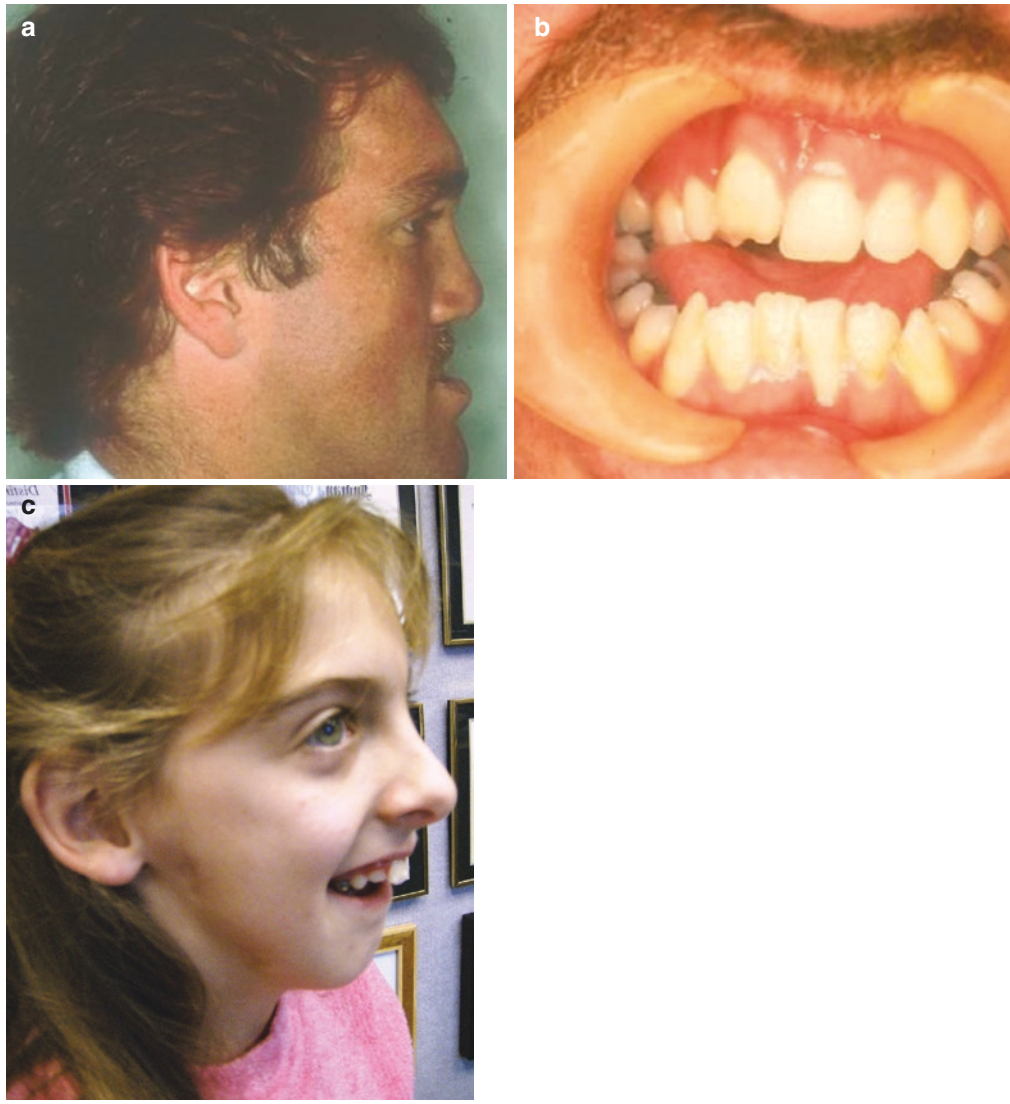


Fig. 23.4 Malocclusion of the jaws. (**a**) Maxillary retrusion causing a Class III malocclusion. (**b**) This is the same patient as in Fig. **a**. This shows a Class III malocclusion with anterior open bite. As a result of the malocclusion, the tongue tip is anterior to the alveolar ridge. (**c**) Class II malocclusion due to micrognathia. As a result of the malocclusion, the tongue tip is posterior to the alveolar ridge and under the palatal arch

23.6 Other Craniofacial Malformations

23.6.1 Malformations of the Cranium and Brain

There are hundreds of craniofacial syndromes caused by a genetic etiology [1, 2]. Many of these syndromes affect the development of the brain, which can affect the development of speech, language, and cognition for normal communication.

Malformations of the cranium are often associated with malformations of the brain. One example is *microcephaly*, where the baby's head is smaller than normal, usually causing the brain to also be small and undeveloped. This condition can cause mild to severe developmental delays

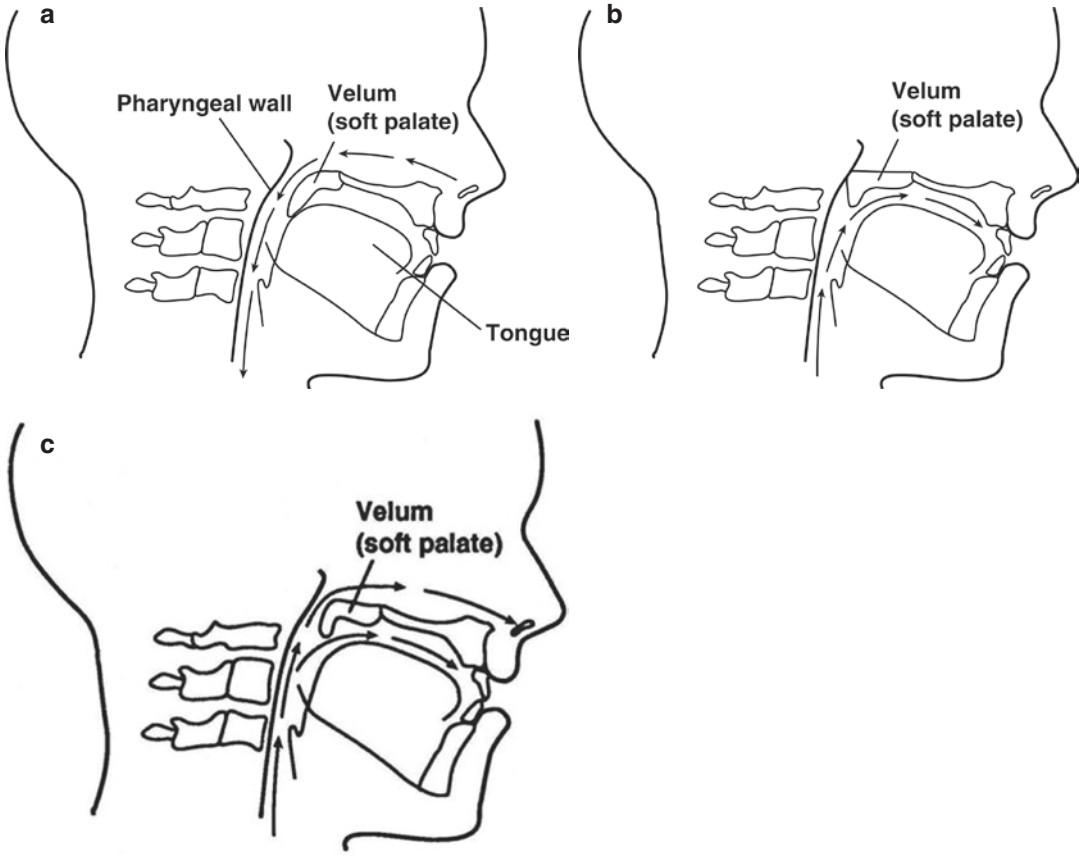
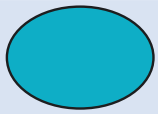
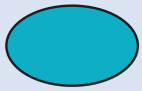


Fig. 23.5 Normal velopharyngeal function and velopharyngeal insufficiency. (**a**) Position of the velum during normal nasal breathing. (**b**) Position of the velum during normal velopharyngeal closure. (**c**) Velopharyngeal insufficiency caused by a short velum

Table 23.2 Speech characteristics expected based on the relative size of the velopharyngeal (VP) opening

Size of VP opening	Expected speech characteristics
	Hypernasality, *inaudible* nasal emission, weak consonants, short utterance length, low volume, compensatory errors
	Hypernasality, *audible* nasal emission, weak consonants, may have compensatory errors
	Audible nasal emission and possibly mild hypernasality
	Normal resonance, but inconsistent nasal rustle (aka nasal turbulence)

and disabilities, including in the areas of speech, language, and cognition.

Another example of cranial malformation is *craniosynostosis*, which is a condition that occurs due to premature closure of one or more of the sutures in the skull [3]. Craniosynostosis causes abnormal skull growth and can affect the growth of the brain. There are several coronal craniosynostosis syndromes, including Crouzon syndrome (Fig. 23.7a), Apert syndrome (Fig. 23.7b), Pfeiffer syndrome, and Saethre-Chotzen syndrome. Some of these syndromes include an increased risk for cognitive and language disabilities, partly due to the genetic phenotype and

also due to increased intracranial pressure. In contrast, sagittal craniosynostosis is rarely associated with increased intracranial pressure or abnormal brain growth and development.

Finally, Chiari malformations are sometimes associated with communication disorders. A *Chiari malformation* occurs when part of the skull is abnormally small or misshapen, causing part of the cerebellum to be pushed down into the foramen magnum and spinal canal. This can also cause compression of the **vagus nerve (CN X), affecting the** pharyngeal branches (which innervate the velopharyngeal valve), and also the superior laryngeal nerve and the recurrent laryngeal nerve (which innervate the vocal folds)**.** As such, vagus nerve compression can cause hypernasality and also voice disorders characterized by hoarseness, breathiness, and even aphonia.

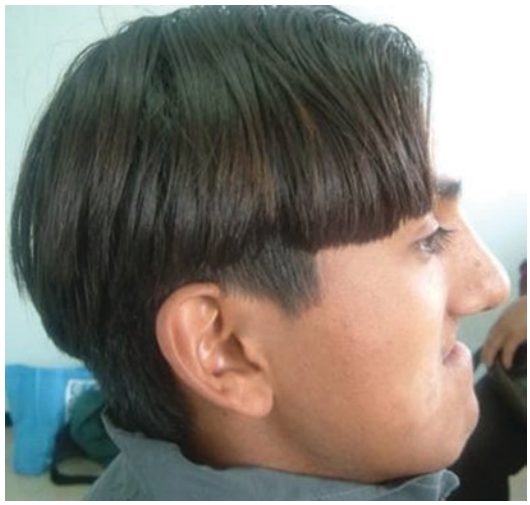


Fig. 23.6 Maxillary retrusion. This is common in patients with cleft lip and palate due to the inherent dysplasia of the bones and possible restriction of maxillary growth due to the palate repair

Brain abnormalities are associated with some syndromes, despite the absence of abnormalities of the cranium. One such example is 22q11.2 deletion syndromes (also called velocardiofacial syndrome), which is considered a neurodevelopmental disorder [22, 23]. In addition to language and cognitive disorders, affected children often demonstrate apraxia of speech (a neuromotor speech disorder) [24].

23.6.2 Malformations of the Ear

Congenital ear malformations and hearing loss can be unilateral or bilateral. They can occur in isolation or as part of certain syndromes, such as CHARGE syndrome, hemifacial microsomia (also known as Goldenhar syndrome, brachial arch syndrome, facio-auriculo-vertebral syndrome, oculo-auriculo-vertebral spectrum, or lat-

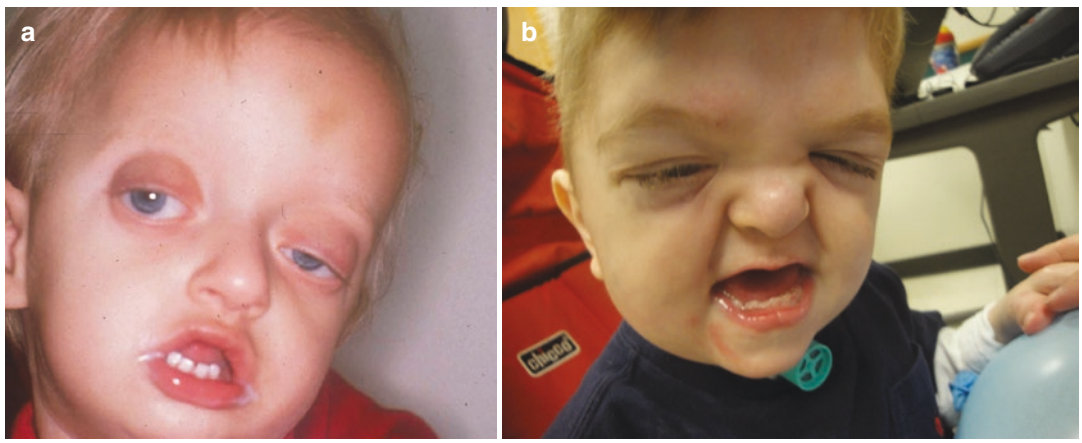


Fig. 23.7 Coronal craniosynostosis causing a wide forehead, hypertelorism, and a flattened midface. This can result in upper airway obstruction, which causes obstructive sleep apnea and hyponasality. It can also result in a Class III malocclusion which causes an anterior tongue position relative to the alveolar ridge, thus affecting speech production. (**a**) Crouzon syndrome. (**b**) Apert syndrome

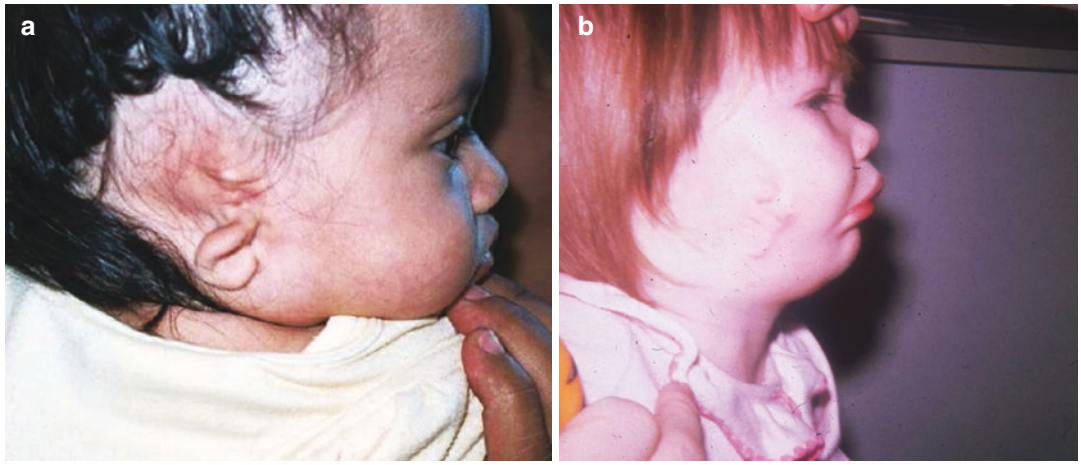


Fig. 23.8 Ear malformations. (**a**) Microtia secondary to hemifacial microsomia. (**b**) Anotia secondary to hemifacial microsomia

eral facial dysplasia), Stickler syndrome, and Treacher Collins syndrome [18, 25].

Malformations of the external ear (the pinna and the external auditory canal) include *microtia* (underdeveloped outer ear) (Fig. 23.8a), *anotia* (missing external ear) (Fig. 23.8b), and *aural atresia* (closure of the external auditory canal). Congenital middle ear malformations include dysplasia of the tympanic membrane and ossicles. Both external and middle ear malformations cause conductive hearing loss. Conductive hearing loss can be treated with surgery and/or bone conduction hearing aids.

Congenital inner ear malformations can include as single or complex malformations a dysplasia of the bony labyrinth, defects of the membranous structures of the inner ear or a pathology of the inner ear hair cells. Inner ear malformations are particularly concerning in that they cause sensorineural hearing loss or deafness. Some cases may be managed by a hearing aid or aids, others need cochlear implantation, and still others need an auditory brainstem implantation (ABI).

Of course, difficulty hearing affects the ability to understand the speech of others. In a developing child, hearing loss, particularly a loss that is severe or sensorineural, causes difficulties in learning speech production and developing language skills. Early intervention, which involves hearing treatment and speech/language therapy, is critical in mitigating these effects [26].

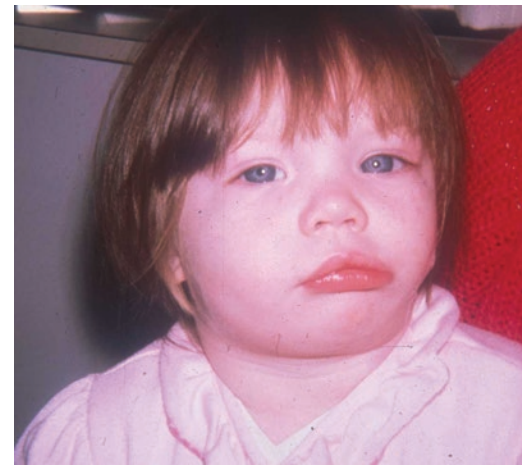


Fig. 23.9 Macrostomia. This is caused by a cleft at the left corner of the mouth secondary to hemifacial microsomia

23.6.3 Malformations of the Mouth

Congenital abnormalities of the size and shape of the mouth can occur, especially with some syndromes [3, 18]. *Macrostomia* refers to an excessively large opening of the oral aperture. This is particularly common with hemifacial microsomia, where one corner of the mouth can extend into the cheek, making the mouth opening on that particular side large and distorted in appearance (Fig. 23.9). Macrostomia does not cause speech

or resonance problems. In contrast, *microstomia* refers to a small mouth opening. If the microstomia is severe, it can affect articulation and cause oral cul-de-sac resonance, which makes the speech low in volume and sounding like "mumbling." [15].

23.6.4 Malformations of the Tongue

Lingual anomalies are associated with certain syndromes. *Macroglossia*, a condition in which the tongue is abnormally large, is one of the main characteristics of Beckwith-Wiedemann syndrome (Fig. 23.10) [18, 27]. Because the tongue is too large to fit in the oral cavity, it protrudes past the alveolar ridge, causing an open-mouth posture. The biggest concern initially is the effect it can have on the airway. As dentition develops, an anterior open bite may occur because the tongue is in the area where the teeth are erupting.

Macroglossia can affect speech by interfering with the production of bilabial sounds (p, b, m), labiodental sounds (f, v), and lingual-alveolar sounds (t, d, n, l, s, z, sh, ch, j). Its anterior position can result in either a frontal or lateral distortion of sibilants. Macroglossia can also contribute to the use of palatal-dorsal articulation as a compensation, especially if the tongue tip rests anterior to the alveolar ridge. Macroglossia also affects resonance in that it leaves little space in the oral cavity for normal oral resonance of the sound [18].

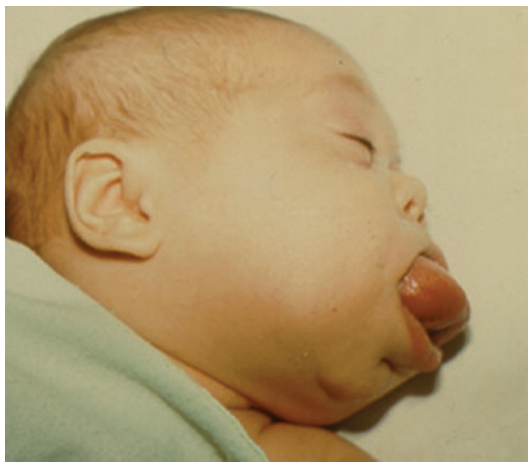


Fig. 23.10 Macroglossia. This is a common characteristic of Beckwith-Wiedemann syndrome

In contrast to macroglossia, *microglossia* is a relatively small tongue for the oral cavity space. It usually has no detrimental effect on speech, unless the tongue is not able to reach the alveolar ridge for articulation.

Ankyloglossia is a congenital condition that occurs in two clinical presentations. It can be seen as a very short lingual frenulum or as an broad anterior attachment (Fig. 23.11a, b) [18]. The diagnostic criteria for ankyloglossia include the inability to elevate the tongue tip to the alveolar ridge with the mouth open or the inability to protrude the tongue tip past the mandibular incisors (or mandibular gingiva) [18]. Typically, the tip of the tongue is heart shaped in that it has a midline indentation when protruding the tongue (Fig. 23.11c).

Ankyloglossia can affect the infant's ability to latch on to the nipple for feeding, although it tends to correct itself as the child grows. Later in life, it can affect the person's ability to move a bolus in the mouth, particularly if the bolus is in the buccal sulcus. Infrequently, a short lingual frenulum can pull the gingiva away from bottom teeth between the mandibular incisors, causing dental issues. However, contrary to common belief, there is no clear evidence that ankyloglossia affects speech [28, 29]. This is because very little tongue tip excursion is needed for normal speech production [29]. In English, the most the tongue tip needs to elevate to the alveolar ridge (without the mouth widely open) is for the /l/ sound. If that is not possible, the /l/ can be produced with the tongue tip down and dorsum up. The farthest that the tongue needs to protrude is against the back of the maxillary incisors for "th" (/θ/ and /ð/) sounds. It does not need to protrude beyond the mandibular incisors. It has been suggested the lingual trill sound in other languages (as in Spanish) may be affected by ankyloglossia, although this remains to be proven. Because ankyloglossia rarely causes problems with speech, frenulectomy is usually not indicated for speech purposes.

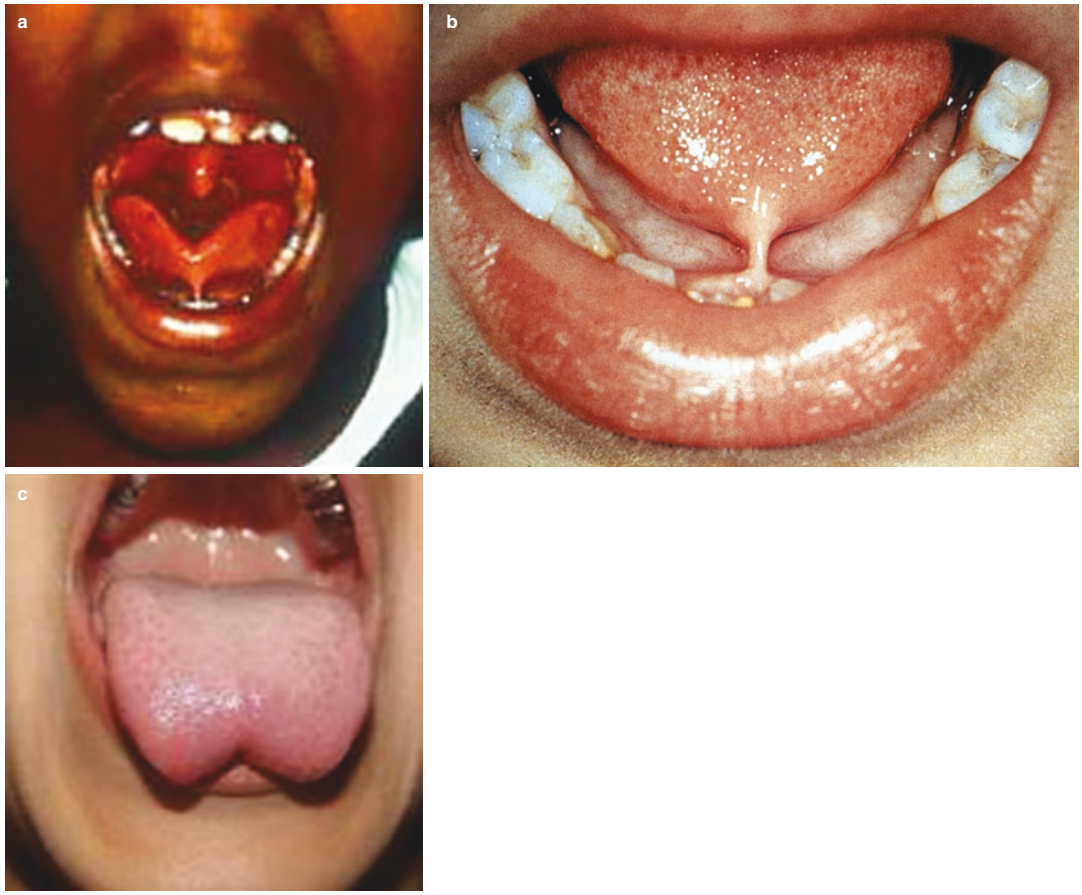


Fig. 23.11 Ankyloglossia. (**a**) A short lingual frenulum. (**b**) An anterior attachment of the lingual frenulum. (**c**) Restriction of the midline with protrusion, resulting in a heart shape

Finally, a lobulated tongue is a characteristic of orofaciodigital syndrome type I (OFD I) (Fig. 23.12). Although the tongue is unusual in that there are multiple lobes, the movement is not affected. As such, this malformation does not affect speech [18].

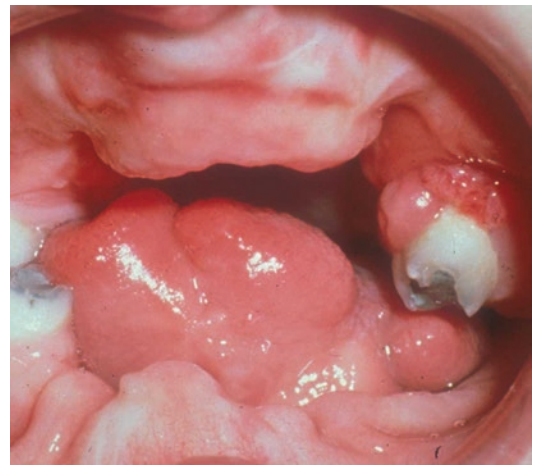


Fig. 23.12 Lobulated tongue. This is a characteristic of oro-facial-digital I (OFD I) syndrome

23.6.5 Malformations of the Larynx

Congenital laryngeal anomalies (i.e., laryngomalacia, laryngeal web, and vocal fold paralysis) are common in children born with craniofacial syndromes [18, 30]. *Laryngomalacia* is a congenital softening of the tissues of the larynx above the vocal folds. It is the most common cause of noisy breathing in infancy. For most infants, this

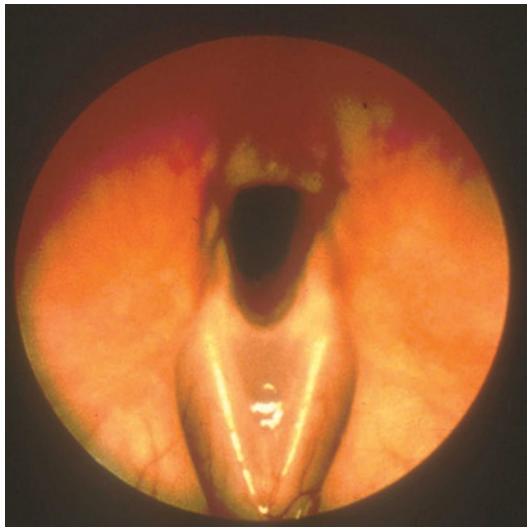


Fig. 23.13 Laryngeal web. This is a phenotypic characteristic of 22q11.2 deletion syndrome (AKA velocardiofacial syndrome)

resolves without treatment by 18–20 months of age. Therefore, it does not affect phonation or speech. A *laryngeal web* is characterized by tissue between the vocal folds near the anterior commissure (Fig. 23.13). It is a common phenotypic feature of 22q11.2 deletion syndrome (also called velocardiofacial syndrome and DiGeorge syndrome). A laryngeal web can cause shortness of breath, a weak cry, *stridor* (a high-pitched, wheezing sound), and poor feeding. It can also cause a high-pitched voice, hoarseness, and aphonia. Surgical treatment is needed to break the web to eliminate airway obstruction.

Finally, *congenital vocal fold paralysis* is a condition which causes a lack of movement of one or both vocal folds. It can be caused by a Chiari I malformation, which affects the function of the *vagus nerve* or recurrent laryngeal nerve trauma. The symptoms include inspiratory stridor, a weak cry, and aspiration during feeding. In addition, it can cause a voice disorder characterized by severe breathiness during phonation.

23.6.6 Multi-Malformation Conditions

A s*yndrome* is a pattern of multiple anomalies that are pathogenically related and therefore have a common known or suspected cause. Children with multiple craniofacial malformations are often diagnosed with a recognized syndrome, particularly children with clefts. In fact, it has been estimated that there are over 400 distinct syndromes that are associated with facial clefts [1, 2].

Syndromes are much more common in patients with cleft palate only than in those with clefts involving the lip. In fact, the London Dysmorphology Database lists 485 syndromes, excluding chromosome disorders, in which cleft palate only can be a feature [31].

Some patients have multiple anomalies, but the combination of anomalies does not fit with a known syndrome. This is called an *association*, which is defined as a nonrandom occurrence of multiple anomalies in many individuals, in which the genetic etiology has not yet been identified. Once the genetic etiology of an association is established, it will "graduate" to a syndrome.

Finally, multiple anomalies can occur as a result of a sequence. A s*equence* is a condition where there are several malformations that occur in sequence as a result of a single genetically caused malformation. An example of this is Pierre Robin sequence, where *micrognathia* (an underdeveloped mandible) prevents the tongue from descending during fetal development. The position of the tongue then prevents fusion of the hard and soft palate. As a result, the infant is born with not only micrognathia, but also *glossoptosis* (abnormal placement of the tongue posteriorly and superiorly in the pharynx), and a wide, bell-shaped cleft palate. Initial issues with Pierre Robin sequence are breathing and then feeding. As the child develops speech, velopharyngeal insufficiency is often apparent due to a short velum.

Patients with multi-malformation conditions are at greater risk for a communication disorder than those with single anomalies (Fig. 23.14a, b). This is because each individual malformation can affect an aspect of communication, causing more complex issues and a worse prognosis. (See Table 23.3 for common craniofacial syndromes and their effect on communication.)

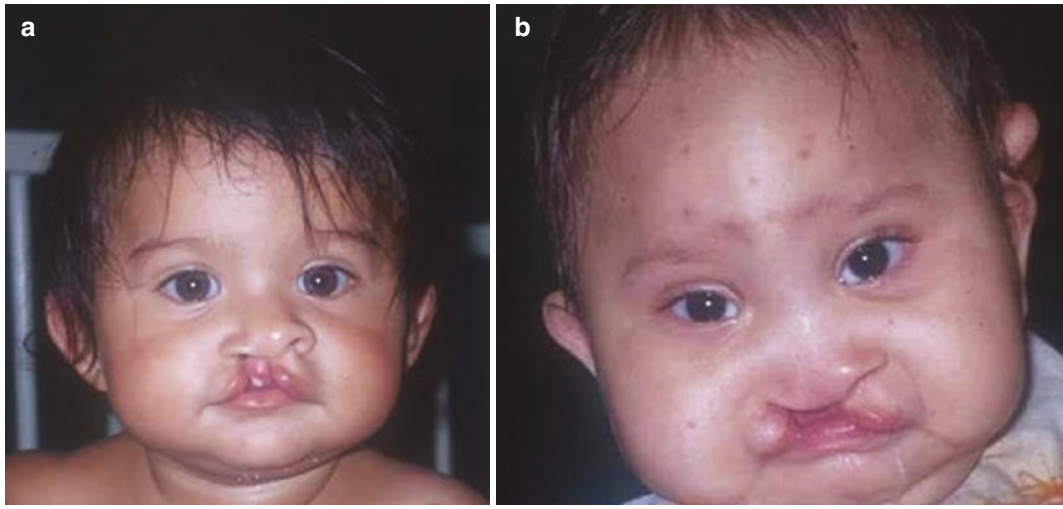


Fig. 23.14 Right unilateral complete cleft lip and palate. (**a**) Nonsyndromic unilateral complete cleft lip and palate. (**b**) Unilateral complete cleft lip and palate with multiple additional craniofacial malformations. Note the frontal bossing, mongoloid slant of the eyes, hypertelorism, flattened nasal bridge, malformed ears, and micrognathia. In addition to the risk for speech and resonance disorders due to the cleft lip and palate, this child is also at risk for cognitive disability, language disorder, hearing loss, and hyponasality (due to upper airway obstruction). All figures are courtesy of the Cleft Palate and Craniofacial Center at Cincinnati Children's Hospital Medical Center and are also reprinted with permission from the following textbook: Kummer, AW. (2020). *Cleft Palate and Craniofacial Conditions: A Comprehensive Guide to Clinical Management,* fourth edition. Burlington, MA: Jones & Bartlett Learning

Table 23.3 Common craniofacial syndromes and possible associated issues that can affect communication

Syndrome	Communication concerns related to…
Apert syndrome	**Cleft palate, class III malocclusion, upper airway obstruction, and cognitive impairment**
Beckwith-Wiedemann syndrome	**Macroglossia and dental malocclusion**
CHARGE syndrome	**Hearing loss, cleft palate, and cognitive impairment**
Crouzon syndrome	**Cleft palate, class III malocclusion, and upper airway obstruction**
Down syndrome	**Cognitive impairment and large or hypotonic tongue**
Ectrodactyly-ectodermal dysplasia-cleft syndrome	**Vocal fold dehydration, cleft palate, and hearing loss**
Fetal alcohol syndrome	**Cognitive impairment and neurological dysfunction**
Fetal hydantoin syndrome	**Cognitive impairment and neurological dysfunction**
Hemifacial microsomia	**Velopharyngeal insufficiency or incompetence and hearing loss**
Kabuki syndrome	**Cognitive impairment and cleft palate**
Moebius syndrome	**Facial and bilabial paralysis**
Neurofibromatosis type 1 (NF1)	**Velopharyngeal incompetence from brainstem tumors**
Opitz syndrome	**Tracheostomy, cleft palate, learning disabilities, and cognitive impairment**
Orofaciodigital syndrome type I	**Cleft palate, cognitive impairment, and developmental disabilities**
Pfeiffer syndrome	**Cognitive impairment or hearing loss**
Popliteal pterygium syndrome	**Jaw restriction, learning disabilities, and cognitive impairment**
Stickler syndrome	**Cleft palate and hearing loss**
Saethre-Chotzen syndrome	**Cleft palate and cognitive impairment**
Treacher Collins syndrome	**Hearing loss and micrognathia**
Van der Woude syndrome	**Cleft palate**
Wolf-Hirschhorn syndrome	**Developmental disabilities, cognitive impairment, and hearing loss**

23.7 Management of Communication Disorders Secondary to Craniofacial Malformations

The management of communication disorders for children with craniofacial malformations may require several professionals. As such, the team approach to management is essential for best outcomes [32].

For hearing issues, the otolaryngologist and audiologist must work together to diagnose the type of hearing loss and the cause so that appropriate treatment can be recommended. Treatment may include medical treatment, surgery, and/or hearing aids. The child may also benefit from aural habilitation therapy done by either an audiologist or speech-language pathologist.

For language, learning and cognitive issues, the speech-language pathologist, neuropsychologist, and teachers typically work together for comprehensive management of these disorders. Treatment may include individual therapy, group therapy, and/or special assistance in school.

For speech disorders due to dental or occlusal interference, the dental professionals and speech-language pathologist must work together. Speech therapy is often delayed until correction of the dentition or occlusion is complete.

For children with velopharyngeal insufficiency, the speech-language pathologist must diagnose the problem. Additional tests (e.g., videofluoroscopy and nasopharyngoscopy) may be necessary for surgical planning. Treatment may then include surgical correction of the VPI and then speech therapy to correct compensatory productions that developed as a result of the VPI.

23.8 Summary

Patients with multiple craniofacial malformations typically demonstrate significant issues with various aspects of communication, including speech, resonance, voice, hearing, language, and cognition. These functional areas may require medical, surgical, dental, audiological, and speech-language pathology services. Not only do these patients require treatment from a variety of professionals, but the treatment occurs over a very long period of time, usually from infancy into adulthood. As such, most families prefer a coordinated team approach over multiple individual appointments [33]. Therefore, because of the complexity of needs, the number of professionals needed, the length of time for treatment to be completed, and the preferences of the families, team care is essential for the best treatment outcomes for patients with craniofacial malformations.

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24 Feeding and Breathing Aspects in Infants with Craniofacial Malformations

Valentin Kerkfeld

24.1 Introduction

Many infants with craniofacial malformations suffer from various difficulties regarding feeding and breathing. The pathogenesis of craniofacial malformations is closely correlated with feeding and breathing issues due to the anatomic differentiations in the craniomandibular segment. To satisfy the demand for nutrition can be difficult due to anatomic or physiological differences. Infants with craniofacial malformations may suffer from feeding and breathing problems depending on the type and extent of the malformation. The diagnosis and understanding of the individual clinical picture of the infant helps to solve problems in the daily routines.

Craniofacial malformations often lead to pediatric dysphagia. Therefore, infants occur with disturbed food intake accompanied with poor weight gain and aspiration-caused infections of the upper and lower airways [1–3]. It is important to note that dysphagia affects many developmental aspects like motoric development, the acquisition of linguistic competences, and social interactions [4]. Besides, craniofacial malformations frequently result in neurological developmental disorders. The basic relationship between craniofacial malformations and neurological developmental disorders has been known since decades [5]. However, the pathogenesis of disrupted feeding as well as the interactions between oropharyngeal anatomy and neural networks has not been understood in detail yet. The clinical characteristics for dysphagia in relation to craniofacial malformations are well examined [6–8]. To determine the feeding issues in infants with craniofacial malformations, the physiological mechanisms of feeding and sucking need to be understood.

Many infants with craniofacial malformations appear to have a disordered breathing while sleeping. The interdependence between feeding and breathing aspects becomes clear by the fact that infants occurring with primary respiratory issues very frequently show feeding difficulties. In these cases, infants often interrupt sucking in order to breathe via the opened mouth. However, this behavior might be misunderstood as a primary feeding problem. Therefore, precise knowledge of the genesis as well as the diagnosis is of particular importance.

24.2 Feeding

24.2.1 General Aspects

Feeding is a very important and fundamental behavior in all mammals. Therefore, it is no wonder that disturbances of this behavior lead to far-reaching consequences. Parents need support

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for raising their children with craniofacial malformations in every stage of development. Feeding is a major psychological factor in bonding between a newborn and their parents, but also a physical, because of the importance for a right nutrition to gain weight. Parents need to be advised about the appropriate ways of feeding an affected newborn [9].

24.2.1.1 Breast- and Bottle-Feeding

Breastfeeding strengthens the bond between mother and child. Breast milk delivers adequate, high-caloric nutrition and provides immune competence for the newborn. At an early age, the milk inherits the abilities to protect as an example the gastrointestinal tract and lungs against diarrhea pathogens and respiratory diseases. In the long term, breast milk decreases the risk of developing allergies, autoimmune diseases, and obesity.

For breastfeeding in general, it is recommended to start feeding the newborn on a regular basis but with short durations to protect the nipple from soreness.

If bottle-fed, infants need food intake six times a day in the first month, and later on five times a day. The usual milk intake should be about 80 g, with no difference between healthy infants and infants with craniofacial malformations. Infants with craniofacial anomalies often show signs of dysphagia like burping and spitting. However, burping and spitting in a moderate extent is considered a normal behavior and it does not indicate diseases [10].

24.2.1.2 Physiological Feeding Development

Two main actions are important for a sufficient feeding process. First, the infant has to gain milk out of the mother's breast (sucking); afterwards, the bolus has to be swallowed (deglutition).

Breastfeeding is a natural congenital reflex. The glands around the mother's nipple help the newborn to find the nipple. Coordinated movements of many different oral structures lead to an expression of milk out of the breast. The act of sucking is the alternative application of positive and negative pressures [11].

At the age of 6 months, infants are physiologically able to be spoon-fed [2].

24.2.1.3 Physiological Mechanism of Sucking

The upper and lower lips are forming, with the help of a ring muscle (m. orbiculares oris), the anterior seal between the nipple and the oral cavity. In combination with the cheek muscles (m. buccinator), the mouth stabilizes the interaction with the nipple.

A complete cycle of sucking can be described as follows:

1. The mother's teat contains the nipple and a big part of the areola. The infant pulls the teat with adjacent breast tissue (ducts) into his/her mouth. The soft palate is relaxed and the nasopharynx is opened up for a steady airflow while breathing.
2. The infant closes its mouth by raising the mandible. Thereby, the base of the teat is pressed.
3. The tongue compresses its multiple muscles in a rhythmic wave, beginning at the tip of the tongue to the posterior part. Due to the applied negative pressure, milk drains from the ducts through the milk canals into the mouth of the infant.
4. When the wave rhythmic action of the tongue reaches the soft palate, mm. levatores veli palatini contract and elevate the velum. As a consequence, the nasopharynx is functionally sealed against the oropharynx and milk is not able to flow into the nasal cavity. When the oropharynx is filled up with milk, the swallowing mechanism starts and initiates the esophageal phase.
5. At the end of the cycle, the posterior tongue depresses and causes thereby a negative pressure. By opening its mouth, the infant allows new bolus of milk to drain into the oral cavity. The cycle starts all over again [12].

The tongue has many important functions and interacts with other oral structures to obtain milk extraction:

(i) The posterior part of the tongue provides the posterior seal of the oral cavity in combination with the soft palate.

(ii) The anterior part of the tongue condenses the nipple in combination with the hard palate.
(iii) Moving the tongue downwards generates a negative pressure in the oral cavity.
(iv) The tongue allows the bolus to drain from anterior to posterior into the oropharynx by the negative pressure and gravity [13].

The described rhythmic licking action of the tongue is accompanied by a pronounced opening and closing of the jaw to reinforce the negative pressure. All these processes lead to a successful sucking of milk.

24.2.1.4 Physiological Mechanism of Swallowing

The act of swallowing (Fig. 24.1) can be divided into four parts that are actively and/or automatically coordinated [11]:

1. The oral preparatory phase collects the food or liquid and prepares the bolus.
2. The tongue builds a slide for the bolus during the oral phase to proceed it from the anterior part of the mouth into the oropharynx.
3. The pharyngeal phase is defined by the closure of the nasopharynx by the soft palate and the closure of the trachea by the epiglottis.
4. As part of the pharyngeal phase, the esophageal sphincter relaxes and the epiglottis folds to initiate swallowing. The bolus enters the esophagus.
5. As swallowing continues, esophagus fills up with bolus and the oropharynx closes.
6. Once the upper pharyngeal muscles are constricted actively, the automated peristalsis occurs going aboral. The mechanisms of the esophageal phase are similar to those of the gastrointestinal passage: a peristalsis wave slides the bolus autonomously to the stomach.

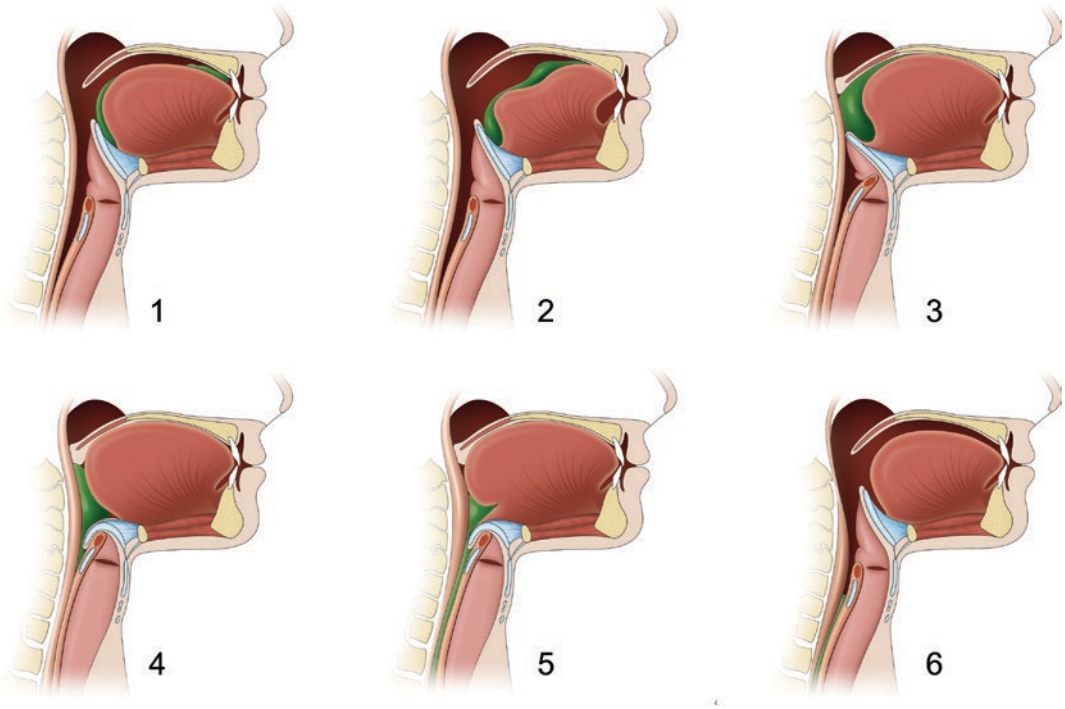

Fig. 24.1 Swallowing process. (Reprinted from Aldona Griskeviciene/Shutterstock.com with permission)

24.2.2 Pathological Difficulties

Infants with craniofacial abnormalities (CLP, branchial arch diseases, syndromic craniosynostoses, others) suffer from many problems regarding feeding. As mentioned before, lips should seal the nipple while the palate grants stability and delivers the foundation for a functional compression of the nipple with the collaboration of the tongue. Furthermore, it is necessary that infants are able to breathe during feeding. Deficient lips and/or palates or a compromised airway (Fig. 24.2) have different occurrences. Their effects for feeding and sucking need to be declared individually [14, 15].

24.2.2.1 Clefts

Cleft Lip

Infants with a cleft lip are not able to form a labial seal; therefore, there is less negative pressure built up while sucking. However, in general, the disadvantage is not incisive in the clinical picture of isolated cleft lips, because the gap is often sealed by the breast or the bottle [16].

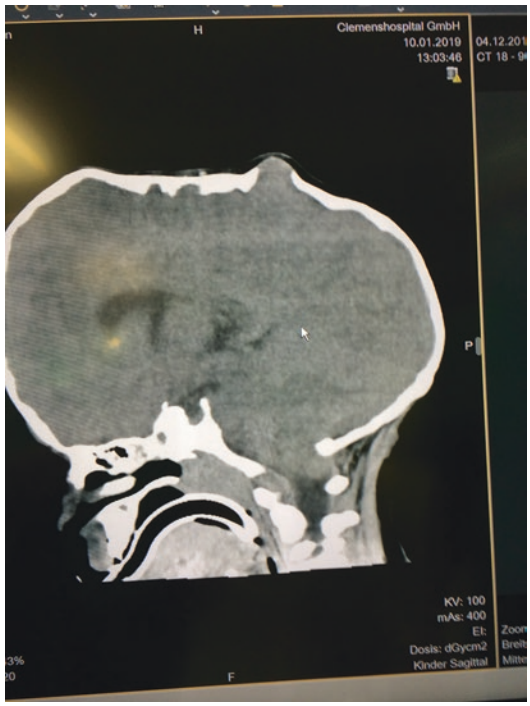

Fig. 24.2 CBCT in a median view

Cleft Lip and Alveolar Process

Due to combined insufficiencies of the lips and alveolar process, the act of sucking is much more difficult. The cleft lip decreases the buildup of negative pressure. In addition, a gap in the osseous structures of the alveolar process prevents a sufficient compression of the nipple. As a consequence, the infant tries to compensate the physical disabilities by a more distinctive elevation of the mandible. Due to excessive work during the act of sucking, the infants are quickly exhausted. Most of the infants with cleft lip and alveolar process have to be bottle-fed, in addition to the breast, to guarantee an adequate uptake of calories per day.

Cleft Palate

The clinical patterns of cleft palates can be divided into isolated cleft hard palates, submucous cleft palates, or clefts including the soft palate.

In patients with cleft hard palates, the compression and stability of the nipple is restricted. However, the sealing of the nipple is not affected. Like the clinical picture of cleft lip and alveolus, the infant has to afford an increased amount of physical work to gain milk and suffers from fatigue.

In patients with submucous cleft palates, the velopharyngeal valve is incomplete. The submucous gap as well leads to reduced negative pressure while sucking, but in most patients, breastfeeding does not seem to be limited by the malformation.

Cleft soft palates are associated with the clinical picture of a remaining gap between the oral and nasal cavity. During the act of sucking, a steady airflow fills the oral cavity with gas. Swallowing milk in combination with air leads to an early satiation, even though the caloric uptake is not sufficient. The swallowing of air can lead to massive burping and even emesis. The remaining gap between the two cavities can also cause a regurgitation of the milk through the nose. Milk in the infant's nose inhibits physiological

breathing; in consequence, the infant is not able to take a breath during the act of swallowing. Most of the time, the tongue of the infants, in reflection, is able to close minor clefts of the soft palate while being fed. Depending on the extent of the soft palate cleft, breastfeeding is possible most of the time. However, children with major clefts of the soft palate cannot be breastfed [15].

Cleft Lip and Palate

All the deficiencies and their effects on feeding and sucking convene in the combined pathology of cleft lip and palate (Fig. 24.3). The difficulties mentioned occur at the same time, and therefore, breastfeeding infants with cleft lip and palate is most likely not possible [17].

In conclusion, the feeding limitations lead to fatigue and a limited caloric intake. The impact of the limitations always depends on the clinical picture and the extension and type of the cleft.

24.2.2.2 Oropharyngeal Dysphagia

Oral dysphagia arises commonly from diseases and malformations of the mouth or throat, such as clefts or craniofacial syndromes. Cricopharyngeal dysphagia cannot be discriminated clinically from oropharyngeal dysphagia and is therefore assigned in the same category.

Infants with oropharyngeal dysphagia tend to have a leak of saliva or bolus outside of the mouth and are most likely not skilled in initiating or terminating the act of swallowing. In addition, the accumulation of liquids in the pharynx leads to choking, coughing, and postnasal regurgitation.

Fig. 24.3 Clefts

Affected infants often show symptoms of apnea and cyanosis.

In children, a slurred speech as well as speech defects can reveal an insufficient closure of the soft palate or deficiencies of the pharyngeal muscles. Even after a successful operation of the cleft a speaking defect can remain, but logopedics assist before and after [18, 19].

24.2.2.3 Syndromes with Craniofacial Anomalies

Many infants with syndromes suffer from feeding issues. Some syndromic groups display special features of feeding difficulties.

Pierre Robin Sequence (CLP Group)

Infants with Pierre Robin sequence suffer from micrognathia, which causes glossoptosis accompanied by obstruction of the airway. In addition, many infants appear with cleft palate. All named characteristics have to be differentiated separately in the context of breastfeeding difficulties.

Micrognathia leads to an incongruity between the upper jaw and the mandible. Therefore, the infant is not able to compress the nipple appropriately. This drawback is accompanied by a limited stability of the nipple. A sufficient milk transfer from the anterior part of the tongue to the oropharynx is mostly not possible due to the glossoptosis. In addition, the cleft palate leads to the difficulties mentioned above, such as an insufficient lip seal and a reduced compression of the nipple. Furthermore, the upper airway is not only obstructed due to the nasal regurgitation but also by the retracted tongue [20].

All these limitations lead to a frustrating feeding process with an early fatigue accompanied by breathing difficulties leading to apneas.

Goldenhar Syndrome (Branchial Arch Disease Group)

The hypoplasia of the craniofacial skeleton and the masticatory muscles leads to feeding issues [21]. The prevalence of feeding difficulties in Goldenhar infants is between 42% [22] and 83% [23]. Especially the sucking efficiency is decreased by a restricted excursion of the

mandible arch because of the hypoplasia. Additionally, the weakness of the N. facialis limits the cheek and lip movements, which play an important role in the physiological mechanism of sucking. Malformations of the tongue may disrupt a normal sucking behavior as well. Besides the clinical picture of the craniofacial malformations, patients with Goldenhar syndrome as well suffer from gastrointestinal malformations and heart failures that might aggravate the feeding issues as well [2]. Feeding difficulties in Goldenhar infants can only be treated with a nasogastric tube that ensures continuous feeding [24–26].

Apert Syndrome (Syndromic Craniosynostosis Group)

Infants with Apert syndrome suffer from multisuture craniosynostosis and a retrusion of the midface. The clinical picture typically appears with syndactyly of the second to fourth digits as well. Nearly all infants show a coronal craniosynostosis and a majority also present synostosis of the sagittal and lambdoidal sutures. The midface is both retruded and hypoplastic. Some also show cleft palates.

Feeding difficulties are often observed in infants with Apert syndrome. Besides the cleft-related problems previously described, a constriction of the posterior nasal aperture or the nasal meatus may lead to an interrupted sucking by often breathing through an opened mouth. This behavior can be mistaken for primary feeding issues.

24.2.3 Social-Emotional Interactions

Besides the technical aspects of feeding infants with craniofacial malformations, the social-emotional interactions between parents and infant need to be given attention. Infants with clefts often show a decreased readiness to be fed. In comparison to not affected infants, they show overall fewer positive emotions before and during the feeding, even though there is no difference in the behavior of the mothers.

It is necessary to pay attention on both aspects, the physiological (feeding technique) and the psychological (social interactions). Prenatal medical advices can help parents to learn about the technical aspects of feeding and the individual anatomic issues, so that they can probably focus on the social demands and the enjoyment with their newborn [17, 27]. Severely impaired feeding often leads parents to a feeling of frustration and anxiety.

24.2.4 Management

Feeding and swallowing issues occurring congenitally and remaining throughout the whole life are very common problems in relation to craniofacial malformations. Dysphagia can be divided into oral and pharyngeal pathologies. Most clinical cases mentioned before are oral pathologies caused by craniofacial anomalies. Craniofacial anomalies are very individual and lead to different types and extents of feeding issues. Pharyngeal pathologies can compound the feeding issues. Some disturbances are even caused neurologically during pregnancy and lead to neurological disorders with altered hindbrain. These malfunctions can also provide craniofacial malformations and/or altered development of cranial nerves in addition to the previously described pathogenesis of the syndromes.

To find the best support for feeding an affected infant, it is necessary to obtain a full medical history, a good clinical examination, including objective physiological skills, and a critical investigation of the individual feeding process. To achieve objective outcomes of the swallowing, instrument-based diagnostics can be used. The fiber-optic endoscopic evaluation of swallowing reveals the function of the laryngeal structures. A barium swallow study is able to reveal even more phases of swallowing: the oral, the pharyngeal, and the esophageal phase.

In a differential diagnosis approach, besides the craniofacial malformation due to syndromes, pediatric dysphagias on the basis of disrupted hindbrain patterning have to be kept in mind.

24.2.4.1 Medical History

A detailed medical history delivers many important details about the individual feeding issue. Therefore, parents need to describe the issues from their point of view regarding the overall problems, such as the regularities and amount of time necessary to feed the infant and the individual amount of milk swallowed. The mother's milk and/or the nutritional supplements and its composition the infant receives have to be examined, to differentiate the uptake and number of calories and also the structure of the liquid, regarding the viscosity. It has to be identified how the infant is regularly fed, if there is only breast- or bottle-feeding, or a combination of both.

24.2.4.2 Observation

In addition to the observation of the act of swallowing, it is important to look wisely at the phase of resting and especially pay attention on the interaction between the feeder and the infant. Important subjects are the posture of the infant, the airway (stridor, phases of apnea, position of the chest), swallowing of saliva, drooling, and coughing.

A comparison of the resting phase and the act of swallowing helps to understand the individual issues of the infant. It is important to examine how the physiological parameters change and to pay attention on the oral, pharyngeal, and esophageal phase of swallowing. Potential obstacles have to be spotted and eliminated.

24.2.4.3 Examination

Clinical Examination

First of all, vital parameters (pO_2, heart rate, breathing rate) and the oral status need to be examined. Some minor obstacles can be identified and eliminated directly. Depending on the situation, it can be helpful to decrease the air intake during the act of swallowing to correct the nasal regurgitation. A simple modification of the position of the infant during feeding can decrease the unwanted airflow. Variances in the viscosities of the bottle liquid or the amount of the milk/liquid flow and an individual support during milk intake can enhance the situation.

Instrumental Examination

Instrumental examination can show hidden obstacles or consolidate present diagnoses. The fiber-optic endoscopic evaluation and the modified barium swallow study can both detect the swallowing pattern and the individual restrictions. The evaluation of the anatomic and physiological circumstances of the swallowing might detect possibilities to advance or modify the feeding process. Modifications can be different postures, other viscosities of the exposed liquids, and a variation of the dosage forms (e.g., breastfeeding, bottle-feeding, cup-feeding, spoon-feeding).

Both clinical methods mentioned above have advantages and disadvantages; in some cases, the endoscopic evaluation, in other cases the barium swallow method, or sometimes a combination of both techniques can be clinically indicated.

Fiber-Optic Endoscopic Evaluation of Swallowing

The endoscopic technique (Fig. 24.4) obtains a direct view on laryngeal structures and helps to determine the laryngeal function. It is always preferable to the barium swallow, because in this direct technique there is no application of radioactive technology. In addition, the advantage of the endoscopic method is the practicability. There is no radiology department needed to execute the examination [28, 29].

Modified Barium Swallow Study

Video: https://www.shutterstock.com/de/video/clip-19,831,825-x-rays-esophagus-contrast-barium

The barium swallow study (Fig. 24.5) offers the way to examine the oropharyngeal swallowing pattern. This technique convinces by its clear view that might be masked in endoscopic methods by the bolus. Globus sensations, cricopharyngeal malfunctions, and unspecific discomforts can be evaluated better. Another advantage is the high acceptance rate in infants that sometimes do not tolerate the endoscopic procedure. A disadvantage is the application of radioactive emission and the necessity to cooperate with a specialized radiology department [30].

Fig. 24.4 Fiber-optic investigation. (Reprinted from: a katz/ Shutterstock.com with permission)

24.2.5 Treatment

Some exercises can be adopted to improve the feeding procedure. First of all, the posture of both the feeder and the infant should be stabilized in an upright position.

In cases of cleft lips, the breast and/or nipple should seal the oral cavity to the outside; sometimes the mother can manage a closure by supporting the breast/nipple in a specific angle to the infant's mouth. The mandible can be stabilized as well so that a sufficient closure of the mouth can be granted. Most children with cleft palates do not benefit from synthetic obturators.

24.2.6 Conclusion

There is a high variance on feeding and sucking abilities in infants with craniofacial malformations. Besides the overall medical diagnosis, individual physiological and anatomic structures can be manifested in different extents. The innate demand on nutrition can differ to the infant's competence to ingest a certain amount of milk. The feeding issues can be determined by observation and mechanical techniques for an individual support of the feeding procedure. The overarching goal is to provide an adequate nutritional uptake and to guarantee a seasonable physiological development of the child.

24.3 Breathing

24.3.1 General Aspects

Many infants with craniofacial malformations appear to have a disordered breathing while sleeping to a various extent. Sleep disordered breathing can be divided into obstructive sleep apnea syndrome (OSAS), central sleep apnea, and sleep-related hypoventilation. There are many factors that influence breathing and lead to sleep disordered breathing. Midface hypoplasia comes along with constrictions of the posterior nasal aperture or the nasal meatus. For example, infants with Pierre Robin sequence show glossoptosis that obstructs the oropharynx. In conclusion, all disturbances reduce the patency of the upper airway and may lead to breathing difficulties.

24.3.1.1 Obstructive Sleep Apnea Syndrome

Most infants with craniofacial malformations suffer from a diagnosed obstructive sleep apnea syndrome (OSAS). The prevalence is 67% [31].

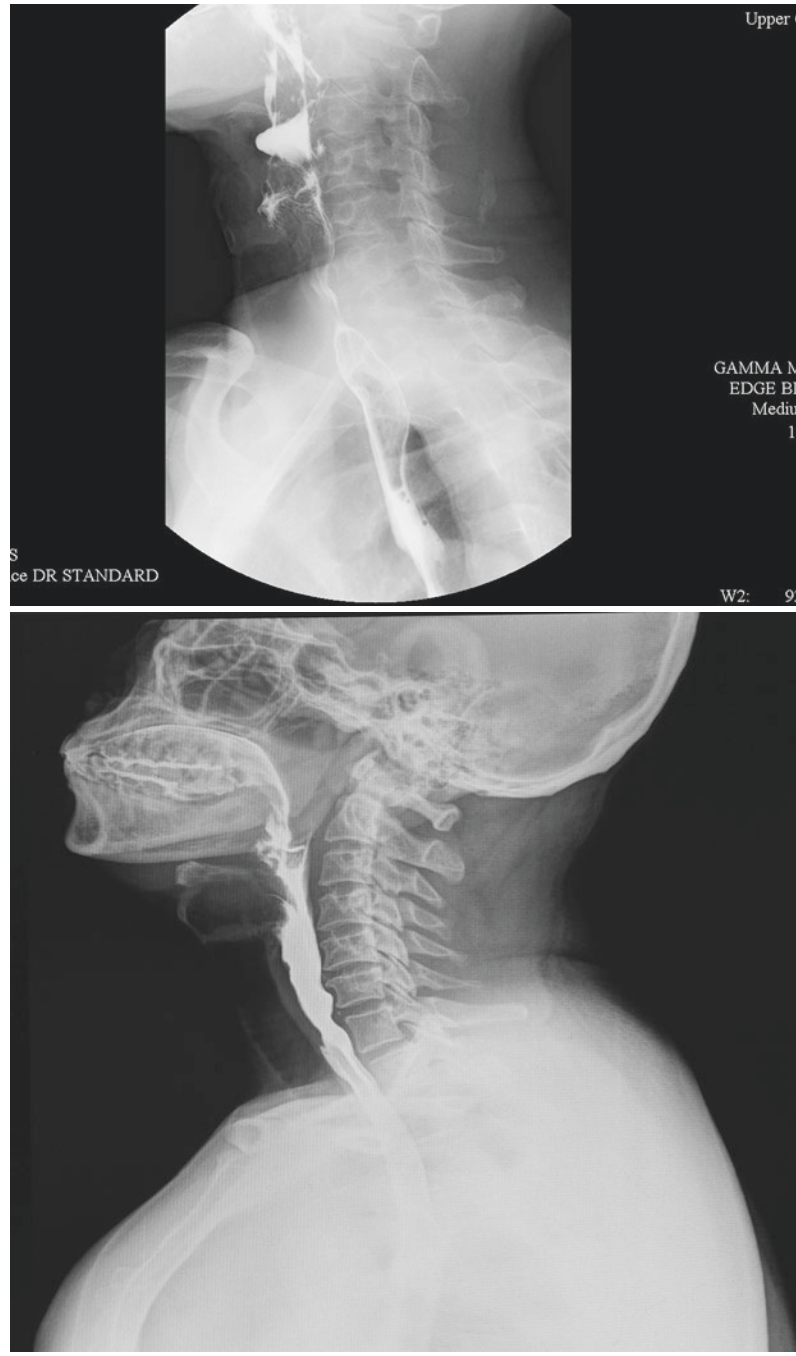

Fig. 24.5 Barium swallow. (Reprinted from top: April stock/Shutterstock.com, bottom: whitetherock photo/Shutterstock.com with permission)

The infants tend to have a partial or temporarily complete obstruction of the upper airway that affects the breathing as well as the sleeping pattern [32]. In addition, the OSAS causes many diseases such as cardiovascular and neurological disorders [32–35]. Obstructive sleep apnea is characterized by partial or complete interruption of airflow resulting in a temporarily decreasing pO_2 [36]. The extent of the malfunction depends on the patency of the upper airways, which is often affected in children with craniofacial malformations [37]. Hypoplasia of the midface,

especially micrognathia, and glossoptosis are well-known risk factors for breathing issues. Besides, the high incidence of tonsillar and adenoidal encroachment is also a form of restriction for breathing. However, the etiology and pathogenesis are determined by many factors.

24.3.2 Pathological Difficulties

24.3.2.1 Craniosynostosis

Many syndromes are accompanied by craniosynostoses such as Pfeiffer, Apert, Crouzon, Muenke, Saethre-Chotzen, and Carpenter. The prevalence of those syndrome-affected infants is 68% [38] to 87% [39]. Etiologically a mutation of the FGFR gene causes the premature fusion of sutures. Breathing restrictions tend to be very variable due to the different manifestations of the malformations.

OSAS in Patients with Craniosynostosis

As mentioned before, OSAS is often caused by hypoplasia of the midface. Therefore, patients mostly breathe by mouth and tend to snore, which may result in sleep apnea symptoms [40, 41].

In addition, craniosynostoses can provoke an increased intracranial pressure due to cranial disproportions, pathological venous drain, and an increased amount of brain liquor (hydrocephalus). However, intracranial pressure is also affected by an increased pCO_2 that occurs due to obstructive sleep apnea and its effects on the blood pressure. In consequence, the cerebral perfusion is different [42–45]. It is recommended to screen children with syndromes on a regular basis every year by polysomnography to detect OSAS [46].

Infants with craniosynostosis and OSAS benefit from a nasopharyngeal airway that circumvents the obstruction.

Central Apneas in Patients with Craniosynostosis

Infants with craniosynostosis also suffer from central apneas. The pathogenesis is not well known, but pressure on the respiratory center is discussed. Etiologically a Chiari malformation can cause this appearance [47]; therefore, patients with Crouzon or Pfeiffer syndrome often occur with this malformation [48]. However, Chiari malformations are rarely seen in patients with Apert syndrome [49]. In the end, breathing issues lead to developmental disturbances, minor quality of life, and behavior problems [50].

24.3.2.2 Clefts

Many syndromes are accompanied by clefts such as Down, Pierre Robin, and Treacher Collins. However, 70% of patients occur with isolated clefts without other comorbidities [51].

There is a higher incidence in children with clefts to suffer from OSAS. Pharyngeal airways are smaller and the craniofacial relation differs to those of healthy infants. Cleft palates affect the oropharyngeal muscles that aggravate the speech and the act of swallowing and influence the patency of the airway [52]. Sixty-nine percent of infants with isolated lip and cleft palates suffer from sleep apnea [53]. Facial dimensions such as the length of the mandible and the height of the face are important aspects for the extent of obstructive sleep apnea [54].

Surgical treatment aims to improve the velopharyngeal function and to restrict the unwanted nasal airway [55]. In surgery, the intent is to reduce the space between the soft palate and the posterior pharynx [56]. The surgical treatment itself is able to induce obstructive sleep apnea as a complication that might result to the use of CPAP [57].

24.3.2.3 Syndromes with Craniofacial Anomalies

Many syndromes with craniofacial anomalies result in breathing issues.

Pierre Robin Sequence

Patients with Pierre Robin sequence occur with the triad of micrognathia, glossoptosis, and resulting airway obstruction. Pierre Robin himself declared the drop of the base of the tongue as a disturbance of the nasopharyngeal airway [58]. The sequence is also often accompanied by cleft

palates. It is commonly assumed that the micrognathia causes a dislocation of the tongue to an upper and posterior direction medially between the two parts of the developing palates during pregnancy. This irregular development results in a U-shaped cleft [59].

For a long time, practitioners thought of glossoptosis to be responsible for obstructive sleep apnea in infants with Pierre Robin sequence. However, endoscopic procedures of the nasopharynx revealed a multifactorial genesis: some patients show that the base of the tongue presses the soft palate against the posterior pharynx, while some suffer from the lateral pharynges coming close to each other and others occur with a combination of both, resulting in a circumferential constriction of the pharynx [60]. In addition, maxillary hypoplasia plays another important part in the genesis of OSAS [61]. Eighty-five percent of infants with Pierre Robin sequence occur with OSAS [62].

Children with Pierre Robin sequence should be screened every year to detect OSAS. This is very important due to the high incidence of sleep apnea in patients with Pierre Robin sequence. In addition, usual symptoms are often veiled, such as snoring which is not represented in every case or inadequate motions of the chest and abdomen during sleep that might be misunderstood by parents.

Achondroplasia

Patients with achondroplasia occur with macrocephaly and hypoplasia of the midface [63]. Because of the malformation, 54% of affected children have OSAS [64].

Down Syndrome

Patients with Down syndrome occur with hypoplasia of the midface and are associated to clefts and obesity [65]. Therefore, a majority of the affected children (80%) have OSAS [66].

Treacher Collins Syndrome

Infants suffering from Treacher Collins syndrome often show a minor patency of airways resulting in breathing issues. Many patients need to be tracheostomized to ensure a steady airflow to grant a sufficient oxygenation. Surgical interventions on the cleft palates might affect the airways and aggravate the breathing issues.

Infants with Treacher Collins occur with hypoplasia of the viscerocranium, cleft palates, malformation of the ears, pharyngeal hypoplasia, and various other symptoms [67]. Fifty-four percent of children with Treacher Collins syndrome suffer from obstructive sleep apnea [68]. Studies supported by endoscopic procedure of the nasopharynx show multifactorial genesis by many different anatomic variations between the nasal septum and the trachea. However, most obstructive malformations can be found in the oropharynx. The diversity of obstructions of the upper airway leads to the recommendation to use an endoscopic technique for diagnosis [69].

Goldenhar Syndrome

Many children suffering from Goldenhar syndrome are at risk of OSAS. The prevalence lays between 7% and 67% [70, 71]. Primarily, the hypoplasia of the mandible causes the breathing issue similar to the pathogenesis of OSAS in children with Pierre Robin sequence and Treacher Collins syndrome [70, 72]. The hypoplasia causes minor patency in the oropharynx and therefore hinders a physiological airflow.

24.3.3 Management

The treatment of breathing issues, especially OSAS, contains CPAP, tracheostomy (Fig. 24.6), and surgical intervention. In many cases, surgical treatment in even more than just one part of the nasopharyngeal airway becomes necessary. However, it is important to determine which intervention suits the infant. Some may benefit more from CPAP, while others profit from tracheotomy [73].

All infants with severe craniofacial malformations should be supervised by overnight polysomnography in order to detect (hidden) OSAS [74]. Pulsoximetry is a number-two choice due to its lower sensitivity [75]. To evaluate the individual extent of apnea and hypopnea phases, the apnea-hypopnea index counts the episodes of those events per hour.

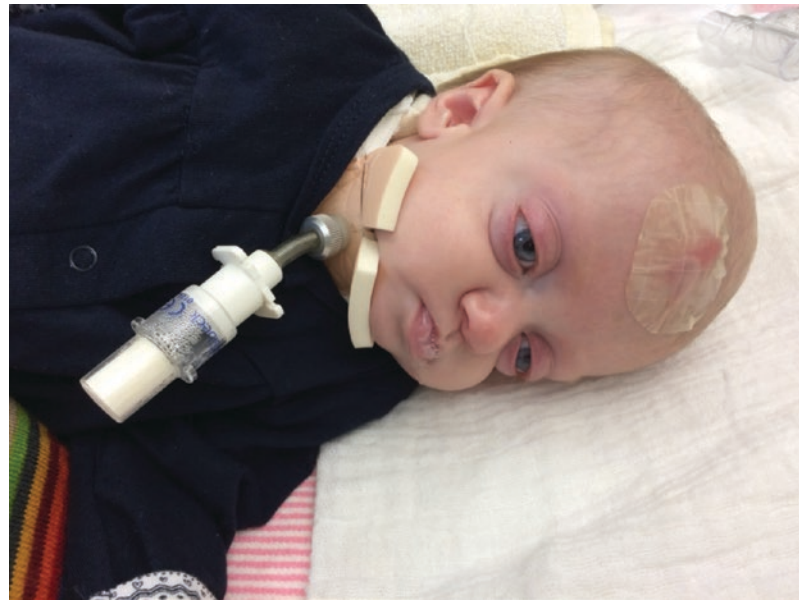

Fig. 24.6 Tracheostomy

Fig. 24.7 Continuous positive airway pressure (CPAP). (Reprinted from JPC-PROD/ Shutterstock.com with permission)

24.3.4 Treatment

There are many ways to handle OSAS, and the correct treatment needs to be detected individually for every infant. Many practitioners recommend prone position in non-severe extents of sleep-related breathing difficulties. Besides, there are other nonsurgical treatment approaches like nasopharyngeal airway tubes or continuous positive airway pressure (CPAP) (Fig. 24.7). In addition, another nonsurgical treatment is the use of Tübingen palatal plate (TPP) (Fig. 24.8) in more severe cases. TPP is an intraoral orthodontic palatal appliance with a posterior extension that leads the tongue in an anterior direction. This ensures patency of the upper airway and enhances breathing. internal treatments may contain in distinct cases weight-loss and anti-inflammatory drugs, while other infants benefit from an orthodontic intervention with removable appliances for a rapid maxillary expansion. Depending on the situation, a surgical operation can be helpful

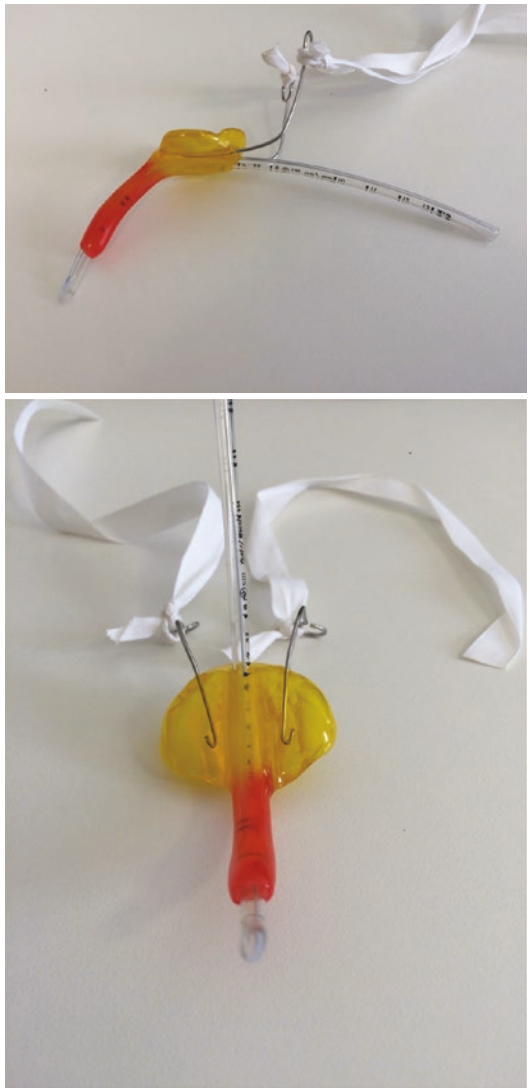

Fig. 24.8 Tübingen palatal plate (TPP)

to distract the mandible or to eliminate obstructions in the upper airway. Those patients suffer from hypoplasia of the mandible. Therefore, a surgical distraction of the mandible can cure or attenuate the breathing issues.

24.3.5 Conclusion

Every infant with craniofacial malformation should be screened for sleep-related breathing disorders due to the high prevalence in malformed children. The most common breathing disorder is the OSAS, while central apneas should not be disregarded especially in population of infants with craniosynostosis. Besides facial and nasopharyngeal malformations like hypoplasia of the midface and glossoptosis, deficient upper airways due to oropharyngeal dysfunctions can cause sleep apneas. Infants benefit from an abdominal position, nasopharyngeal airway bypassing the obstruction, surgical procedures, and as well CPAP in order to suffer less from sleep apneas. In infants with therapy-refractory sleep apneas, tracheotomy might be evaluated as an ultima ratio.

24.4 Case Report

The strong interrelation between breathing and feeding becomes obvious by a closer inspection of an infant with a syndromic disease. Therefore, a case report is presented in the following.

24.4.1 Initial Situation

An infant with Pfeiffer syndrome appears in a desolate state (Fig. 24.9). Overall, he/she is not able to swallow adequately and struggles for air. Because of the feeding and breathing issues, it is dystrophic and underdeveloped. Besides, the infant occurs with craniosynostosis and hydrocephalus accompanied by eye proptosis (Fig. 24.10). However, it lacks the common appearance of syndactyly that is observed in other patients affected by Pfeiffer syndrome.

24.4.2 Treatment

24.4.2.1 Breathing Management

First of all, proper breathing needed to be ensured. Initially, an endoscopic examination (Fig. 24.11) was performed due to detect constriction of the upper airway. A nasal intubation followed (Fig. 24.12). However, this procedure was not able to grant breathing sufficiently. Therefore, a modified Tübingen palatal plate with an endotra-

Fig. 24.9 Infant with Pfeiffer syndrome in a desolate state. The dystrophic thighs are a result of the malnutrition

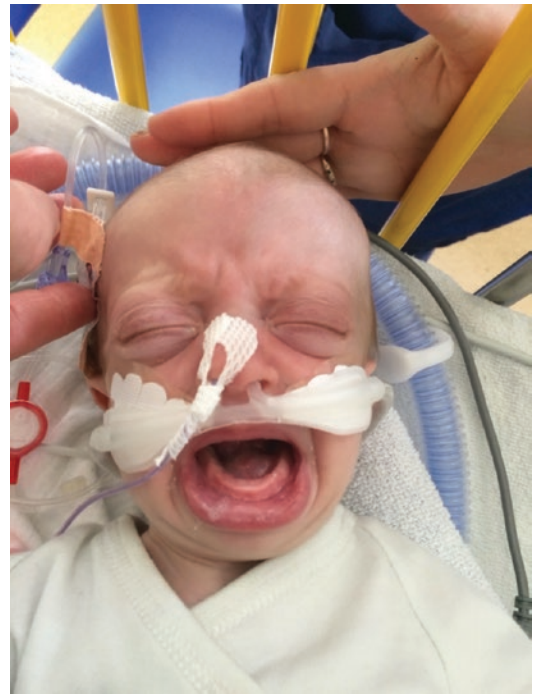

Fig. 24.10 Appearance of craniosynostosis, hydrocephalus, and eye proptosis

Fig. 24.11 Nasal endoscopic examination of the upper airway

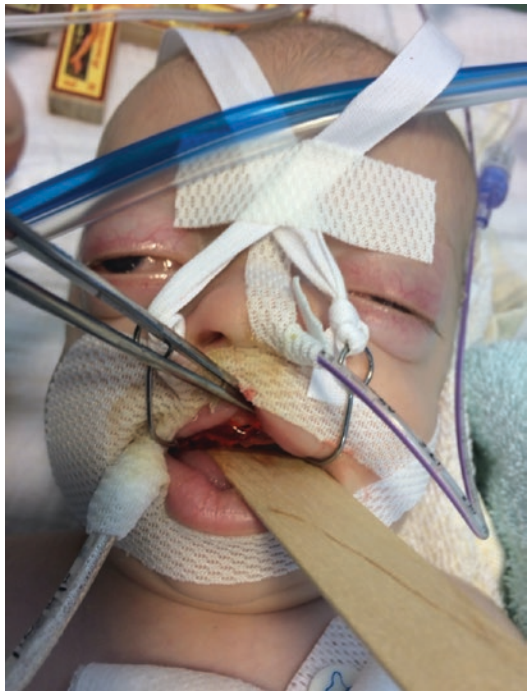

Fig. 24.12 Nasally intubated infant

cheal tube (Fig. 24.8) was manufactured and applied. A patency of the upper airway could not be restored. Thus, after all, a tracheostomy (Fig. 24.6) was accomplished in order to ensure breathing properly.

24.4.2.2 Outlook

Following treatment involves a surgical solution to the craniosynostosis that is carried out in several steps. Surgical intervention in the midface can reduce exophthalmos and midface hypoplasia.

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