Genetics of Ocular Diseases

H. V. Nema
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Foreword

It is a distinct honor for me to write the foreword for this important and timely book on the genetics of eye diseases that includes the work of several key leaders of eye genetics research. It is a very valuable presentation of the current knowledge that provides and outlines the future potential in the field of eye genetics in combating global blindness.

According to the World Report on Vision (October 2019), at least 2.2 billion people around the world have a vision impairment, of whom at least 1 billion have a vision impairment that could have been prevented or still need to be addressed. Furthermore, 90% of the global burden of eye diseases is shouldered by developing countries, where many treatable diseases often go undiagnosed. A comprehensive research strategy and international research collaborations between the developed and developing worlds are needed to address the prevention of global blindness. A wider collaboration of researchers is needed to advance high-quality science in many areas of vision research as well as improve the standard of care. A coordinated strategy for basic science and translational research, involving the tools of genetics, genetic counseling, and health services research, will help in reducing the global burden of eye diseases.

Indian researchers have played a key role in the development of our knowledge on eye genetics from the beginning of this discipline. Many independent research programs have been developed in India leading to many valuable findings in the field. In addition, by participating in numerous collaborative programs and working with researchers around the world, the Indian laboratories have generated a wealth of knowledge that is helping in the advancement of science. For example, Indian scientists have played key roles in the Global Eye Genetics Consortium (GEGC) that originated as a collaboration between the National Eye Institute (NEI) at the National Institutes of Health (NIH) in the USA and the National Institute of Sensory Organs at Tokyo Medical Center in Japan in 2014. India is one of the few countries in the world at this time that has established a GEGC-India team. In the past five years, the GEGC-India members have conducted several high-quality training programs and established dedicated research labs to advance research in eye genetics. It is a fast growing field of research globally as well as in India that has encouraged many scientists and clinicians to establish new programs in various corners of India. Many Indian eye hospitals have brought geneticists and genetic counselors to work

with the ophthalmologists to expand their services. I expect the field to expand significantly in the coming decade not only in India but in most parts of the world.

The field of eye genetics research is expected to grow significantly in India because not only many next-generation researchers are entering this field of research but also there are many unique populations across the country who are expected to provide useful information for many research developments on biomarkers and therapeutics. Several prominent international collaborative research programs are successfully being conducted in India, which are studying many patient populations with unique phenotypes and genotypes that until now have not been studied.

This book is very timely and expected to serve as a long-term resource for all those interested in working in this field. I am delighted to see that the book not only covers the genetic aspects of various common eye diseases, but it also covers the future applications through gene therapy and genetic counseling as separate chapters. The future programs include training and increased access to the new technologies to many research programs. Undoubtedly, the book will play a key role in providing the required background in training and research. It will further promote and provide guidance to many researchers and clinicians. I wish to congratulate and thank the authors and editors of the book for taking a lead role in expanding our knowledge in the area of eye genetics.

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Abstract

Retinitis pigmentosa (RP) is the most prevalent among the group of rare inherited retinal degenerative diseases (IRDs) leading to vision loss. RP is characterized by night blindness in the initial stages followed by progressive gradual decrease and loss of peripheral vision. The worldwide prevalence of RP is 1:4000 that varies in different geographic location. RP can be inherited as an autosomal dominant (adRP), autosomal recessive (arRP), X-linked (XLRP) or simplex trait; clinically present as isolated condition or syndromic (Bardet–Biedl syndrome, Alstrom syndrome, Ushers syndrome, Senior Loken syndrome, and Jouberts syndrome) with extra ocular abnormalities and partially overlapping genetic/clinical features. Inter and intra familial phenotypic variability, different mutation spectrum in RP explain the genetic heterogeneity in the disease. The overlapping genetic architecture and clinical heterogeneity pose challenge in diagnosis and molecular confirmation and differential diagnosis of the disease. In this book chapter, the current trends in molecular diagnosis of RP using next generation technologies like targeted panel, whole exome and genome sequencing. The implication of genetic diagnosis in checking the eligibility for treatment, future gene therapy trials, pre-implantation genetic diagnosis and thereby management. The other potential treatment strategies applicable in RP patients like optogenetics and cell based therapy that are under clinical trials.

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About the Editors

H.V. Nema post-graduated from GR Medical College, Gwalior. He did his fellowship from the University of Manchester. He is a former Professor and Head, Department of Ophthalmology, Institute of Medical Sciences, Banaras Hindu University, Varanasi. He has a distinguished academic career and is known for teaching, research, and publications. Dr Nema taught ophthalmology to the undergraduate and postgraduate students at Aligarh Muslim University, Aligarh, and Banaras Hindu University, Varanasi, for more than 30 years. He served as a consultant editor, an advisory editor to *Indian Journal of Ophthalmology, Afro-Asian Journal of Ophthalmology*, and *Indian Journal of Optometry*. He has more than 6 dozen publications in national and international journals and authored and edited books. Dr Nema has delivered guest lectures in many universities. He served as an honorary general secretary and a president of UP Ophthalmological Society and was conferred Lifetime Achievement Award.

Nitin Nema is a Professor of Ophthalmology at Sri Aurobindo Medical College and PG Institute, Indore. He is an experienced vitreoretinal surgeon. After his postgraduation from the Institute of Medical Sciences, Banaras Hindu University, he did his fellowship from Aravind Eye Hospital and PG Institute, Madurai. Additionally, he gained clinical and research experience from the University of Illinois at Chicago and University of Wisconsin, Madison, USA. He was awarded a fellowship by the All India Ophthalmological Society to work on a research project on uveitis at Sankara Nethralaya, Chennai. He was also awarded Dr Mohanlal Gold Medal for the best paper by the UP State Ophthalmological Society. He has published and presented many research papers and co-authored *Textbook of Ophthalmology*, *Anatomy of the Eye and Adnexa*, *Manual of Ophthalmology*, and *Diagnostic Procedures in Ophthalmology.*

Genes and Genetics in Ophthalmology

1

Chitra Kannabiran

1.1 Introduction

The knowledge of genes in the pathogenesis of eye diseases is an expanding area that has especially accelerated over the last two decades, ever since the human genome sequence became publicly available. The evolution of the field of genetics in general, and also in ophthalmology, may be traced to the study of disease phenotypes in large families with a disease that affected several members from different generations. The pattern in which the disease was seen in successive generations of a family gave a clue to its genetic character and also showed that manner in which it was inherited from one generation to the next. Though such studies clearly established a genetic transmission of the disease on the basis of a careful examination of all members of the families affected with the disease, the details of the gene itself or the chromosome in which it was located, were unknown. It was not until the latter part of the twentieth century, that molecular techniques became available to map the location of specific genes and later, to read the sequence of the genes to know the changes in the genetic code. The first gene for a human disease that was located on to a specific chromosome was that for Huntington's disease, a neurological disorder, first described by George Huntington in 1872. It is an autosomal dominant disease, usually adult-onset appearing after 30 years of age, with manifestations of loss of motor control leading to jerky movement, changes in personality, and a decline in cognitive function. The gene for Huntington's disease was first mapped in 1983 [1].

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In this study, the investigators showed by evaluating genetic markers on different chromosomes of patients from two large families with the disease, that the Huntington's disease gene was located on chromosome 4. This study was aided by the fact that the families studied were large and had several members available for the study, thus providing statistical support for the results of the mapping experiments.

1.2 Gene Mapping

Using the same type of approach, the gene for an eye disease known as retinitis pigmentosa (RP), was one of the first genes to be mapped for eye diseases, in 1984–1985. Retinitis pigmentosa is a form of blindness that develops in children and young adults due to a defect in the light-sensitive cells in the retina. Using the same mapping approach mentioned above, a few families with X-linked RP were studied, and the disease in these families was tagged to a marker on a specific part of the X chromosome, on the short arm of the chromosome [2]. This was the first report of the mapping of a gene for retinitis pigmentosa and one of the earliest genes mapped for any form of blindness. In the subsequent decade, the mapping and discovery of the gene for glaucoma, specifically primary open-angle glaucoma (POAG), was reported. The first such locus, designated as *GLC1A*, was mapped in a large American family with autosomal dominant inheritance of juvenile open-angle glaucoma (JOAG) to chromosome 1q [3]. JOAG differs from the common late-onset form of POAG in the onset before the age of 20 years, has a more aggressive course and has very high intraocular pressures. The gene for the *GLC1A* locus was identified as the myocilin gene a few years later [4]. In the same decade, genes for other forms of glaucoma such as primary congenital glaucoma were similarly mapped with genetic markers to specific chromosomal regions in large families with multiple members [5, 6].

Perhaps the earliest study to have mapped the genetic locus for an eye disease involves congenital cataract, in which the locus for the disease was linked to the same genetic locus as the blood group antigens known as the Duffy antigens, by Renwick and Lawler [7]. The Duffy blood group antigens were subsequently mapped onto chromosome 1 [8]. The family studied here had zonular, pulverulent cataracts which mapped to this locus, designated as CZP1 (cataract zonular pulverulent 1), was again followed up after several years and spanned eight generations, thus making it suitable to map the disease gene using linkage analysis. The gene on chromosome 1 was identified as the *GJA8* (gap junction protein 8) gene. It encodes a protein connexin 50, and a missense mutation in this gene was found to be associated with the cataract [9]. Gap junction proteins such as connexin 50, form channels within the lens to allow water and ions to pass through from one cell to another, and are thus important for lens homeostasis.

Numerous genes were identified over the next decade for severalother eye diseases including retinal blindness, glaucoma, corneal blindness, etc. using molecular

genetic methodsof gene mapping and sequencing. Another approach commonly used for identifying the genes associated with a disease, is the candidate gene approach, and selection of the gene for mutation screening is based on its functions or expression in the tissue that is affected. It is thus considered plausible that such candidate genes will have disease-associated mutations in a percentage of patients. Candidate gene approaches have also led to the discovery of genes for various disorders of the eye.

1.3 Human Genome Project

At the beginning of this century, a major landmark that shaped the course of human genetics was the Human Genome Project (HGP). This consisted of a megaproject led by teams of scientists from the USA and the UK, and other countries, and involved the process of finding the entire sequence of the human genome, which is made up of about three billion letters that constitute the genetic code. The availability of the entire genome sequence by the year 2003 meant that one knew the sequences of all the genes that are present in the genome. This knowledge catalyzed further discoveries in the genetics of eye diseases.

1.4 Next Generation Sequencing Technologies

There have been significant advances in technology for sequencing the genome in the current time, known as the next generation sequencing technologies (NGS), which are better and many times faster than the technology used by the HGP. We can now directly sequence the genome of individuals with a disease, and find the genes involved in the disease in question by looking for changes in the genome of the patient, as compared with the sequence of the normal genome. Using these methods, we now know many genes that are involved in eye diseases such as cataract, refractive error, macular degeneration, glaucoma, and so on. Genome sequences can be obtained in a matter of days and the procedure is being offered as a diagnostic test for patients with any eye disease, by several clinical laboratories across the world. The costs of these sequencing technologies have been coming down with improvements in capacity of the machines used for the purpose, thus leading to faster results. One can get tests that are based on finding out the sequence of the whole genome (whole genome sequencing or whole exome sequencing if one looks at a part of the genome that encodes proteins). In addition, the NGS technology can be used to test a set of specific genes that are relevant for a particular disease, an approach known as targeted NGS. Even in this method, hundreds of genes can be screened in parallel. This makes it possible to cover much larger ground than conventional genetic testing, in a fraction of the time. These next generation sequencing methods have also led to discovery of many new genes for eye diseases and thus increased our knowledge of the mechanisms of several eye diseases.

1.5 Gene Therapy

Genetic discovery has also led to possibilities of new treatments for genetic diseases that lead to blindness in infants and children. One such example is Leber congenital amaurosis (LCA), a disease affecting the retina and causing blindness at birth or very early childhood. LCA cannot be treated by any conventional form of treatment. One of the types of LCA involves mutations in a gene that encodes the retinal pigment epithelial 65 KDa protein (RPE65), a protein that is abundantly expressed in the retinal pigment epithelium. The RPE65 protein is absent or defective in patients with a mutation in this gene. Through the use of genetic testing methods mentioned above, one can carry out genetic testing and diagnosis of patients, to detect mutations in the *RPE65* gene. Advances in genetics over the last two decades have led to a new treatment for LCA patients with mutation in the *RPE65* gene. It is now possible to replace the mutant gene in these patients with a normal copy of the same gene. This process of gene therapy was developed after extensive research in animals such as mice and dogs that carried mutations in the RPE65 gene. Success in these animal models prompted the process of clinical trials for this gene therapy in human patients. The *RPE65* gene replacement therapy was a first of its kind among eye diseases, and was tried and tested in several patients with mutations in this gene.

Clinical trials of RPE65 gene replacement therapy were initiated in several centers in the USA, and the trials in phases I and II were designed to test safety and efficacy of the gene therapy. Follow-up of patients over time by evaluation of systemic and ocular adverse events and visual recovery indicated no serious adverse effects and treatment outcomes of increased visual field sensitivities and pupillary light responses in the treated eyes of patients. Essentially, the gene therapy with *RPE65* gene replacement was shown to be safe and effective and is under evaluation for further long-term effects on vision. The initial effects, however, peaked within a few weeks of injection, and in certain cases were retained up to 3 years after treatment [10]. Another disease affecting the retina, known as choroideremia, affects mostly males, and is due to a mutation in a particular gene, known as the *CHM* gene, on the X chromosome. The *CHM* gene encodes the Rab Escort Protein 1 (REP1). REP1 is required for adding lipid residues on the Rab small GTPases (RABs). Gene therapy for choroideremia has also been developed such that the mutant gene is replaced with a normal one through the use of the AAV2 vector. Patients administered with this gene therapy in clinical trials showed a mean gain of visual acuity and an increase in retinal sensitivity as compared with baseline [11]. Interestingly, there was a dose-dependent response to this treatment, since patients given a higher dose of the vector had better improvements in retinal sensitivity [12]. Overall, the *CHM* gene therapy has been shown to be safe to be administered into the eyes of affected patients, without any major adverse events noted. Despite these promising results in the therapy trials so far, there are still challenges to be overcome. Of major concern in these cases was the continuing degeneration of the retinal photoreceptors even after the gene therapy was carried out. Other areas to be tackled are in aiming for a better recovery of vision and more long-term benefits of the therapy.

1.6 Gene Editing

Another new tool with potential for therapeutic application that is now being investigated in the area of genetic diseases is a type of molecular scissor. This can be designed to clip out any mutation from a gene of interest and replace the clipped-out part of the gene with a normal copy, a process known as genome editing. Using this technique, researchers in different parts of the world are attempting to design therapies for various eye diseases that have no other treatments available by conventional methods [13]. Here again, investigations are in the process to develop a therapy for a form of LCA that is caused by a specific mutation in another gene known as *CEP290*. The CEP290 gene encodes a centrosomal protein that is a key component of the photoreceptor cells in the retina. There are a few mutations in the *CEP290* gene, which are very common among Caucasian patients with LCA. Thus, the therapy is envisaged to edit these common mutations in the *CEP290* gene in such patients. The results of these trials will provide exciting leads for further progress in treating various forms of blindness by gene therapy.

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References

1. Gusella JF, Wexler NS, Conneally PM, et al. A polymorphic DNA marker genetically linked to Huntington's disease. Nature. 1983;306:234–8.
2. Bhattacharya SS, Wright AF, Clayton JF, et al. Close genetic linkage between X-linked retinitis pigmentosa and a restriction fragment length polymorphism identified by recombinant DNA probe L1.28. Nature. 1984;309(5965):253–5.
3. Sheffield VC, Stone EM, Alward WL, et al. Genetic linkage of familial open angle glaucoma to chromosome 1q21-q31. Nat Genet. 1993;4:47–50.
4. Stone EM, Fingert JH, Alward WL, et al. Identification of a gene that causes primary open angle glaucoma. Science. 1997;275:668–70. https://doi.org/10.1126/science.275.5300.668.
5. Sarfarazi M, Akarsu AN, Hossain A, et al. Assignment of a locus (GLC3A) for primary congenital glaucoma (Buphthalmos) to 2p21 and evidence for genetic heterogeneity. Genomics. 1995;30(2):171–7. https://doi.org/10.1006/geno.1995.9888.
6. Akarsu AN, Turacli ME, Aktan SG, et al. A second locus (GLC3B) for primary congenital glaucoma (Buphthalmos) maps to the 1p36 region. Hum Mol Genet. 1996;5(8):1199–203. https://doi.org/10.1093/hmg/5.8.1199.
7. Renwick JH, Lawler SD. Probable linkage between a congenital cataract locus and the Duffy blood group locus. Ann Hum Genet. 1963;27:67–84. https://doi.org/10.1111/j.1469-1809.1963.tb00782.x.
8. Donahue R, Bias W, Renwick J, McKusick V. Probable assignment of the Duffy blood group locus to chromosome 1 in man. Proc Natl Acad Sci USA. 1968;61:949–55.
9. Shiels A, Mackay D, Ionides A, et al. A missense mutation in the human connexin50 gene (GJA8) underlies autosomal dominant "zonular pulverulent" cataract, on chromosome 1q. Am J Hum Genet. 1998;62(3):526–32. https://doi.org/10.1086/301762.
10. Pierce EA, Bennett J. The status of RPE65 gene therapy trials: safety and efficacy. Cold Spring Harb Perspect Med. 2015;5(9):a017285.

11. Kannabiran C, Mariappan I. Therapeutic avenues for hereditary forms of retinal blindness. J Genet. 2018;97(1):341–52.
12. McLaren RE, Groppe M, Barnard AR, et al. Retinal gene therapy in patients with choroideremia: initial findings from a phase ½ clinical trial. Lancet. 2014;383(9923):1129–37. https://doi.org/10.1016/S0140-6736(13)62117-0.
13. Gallego C, Gonsalves MA, Wijnholds J. Novel therapeutic approaches for the treatment of retinal degenerative diseases: focus on CRISPR/Cas-based gene editing. Front Neurosci. 2020;14:838.

Genetics in Corneal Dystrophy

2

Jayesh Vazirani and Mansi Rambhia

Roenouw [1]and Biber [2]introduced the term corneal dystrophy in 1890. Further Fuchs [3], Uhthoff [4], and Yoshida [5] continued to use the term "corneal dystrophy."Corneal dystrophy describes an inherited condition affecting cells, tissues, and organs, singly or in combination. These are inherited disorders that usually present bilaterally, symmetrically, and slowly progressive, and not related to systemic conditions and environmental factors [6].There are exceptions to the above statement, as in clinically unilateral dystrophy—PPCD (posterior polymorphous corneal dystrophy), systemic hypercholesterolemia in SCD (Schnyder corneal dystrophy), EBMD (epithelial basement membrane dystrophy), and central cloudy dystrophy of Francois (CCDF) are likely degenerative rather than hereditary conditions in most of the patients.

The IC3D publication in 2008 contained the traditional anatomic classification that categorized dystrophies according to the corneal layer that was chiefly affected [7, 8].The main limitation of this classification was absence of genetic mapping and genomic associations in corneal dystrophies. At present, genotyping revealed both genotypic heterogeneity (a single dystrophy associated with different genes) and phenotypic heterogeneity (one gene is associated with multiple distinct allelic dystrophy phenotypes).

2.1 Categorization of Corneal Dystrophy on Genetic Basis

Category 1: A well-defined corneal dystrophy,with a well identified mapped gene and the specific mutations are known.

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Category 2: A well-defined corneal dystrophy that has been mapped to one or more specific chromosomal loci, but the gene(s) yet to be identified.

Category 3: A well-defined corneal dystrophy, in which the disorder is yet to be mapped to a chromosomal locus.

Category 4: This category is reserved for a suspected, new, or previously documented corneal dystrophy, although the evidence for it, being a distinct entity is not yet convincing.

IC3D 2015 updated classification (C = Category) is an updated classification system that was proposed in 2015 with alterations of the ancient anatomic level classification to more accurately reflecting involvement of cellular layers. The revised classification removed the extensive table of gene loci and genes with specific mutations as this information is rapidly changing and can be obtained easily on the Internet. The revised classification system included representative histopathology and electron microscopy as well as confocal microscopy. Findings of anterior segment optical coherence tomography (OCT) were added when available.

IC3D 2015 updated classification [9]

Epithelial and subepithelial dystrophies

1. Epithelial basement membrane dystrophy (EBMD) majority degenerative, rarely C1
2. Epithelial recurrent erosion dystrophies (EREDs)—Franceschetti cornealdystrophy (FRCD) C3, Dystrophia Smolandiensis (DS) C3, andDystrophia Helsinglandica (DH) C3
3. Subepithelial mucinous corneal dystrophy (SMCD) C4
4. Meesmann corneal dystrophy (MECD) C1
5. Lisch epithelial corneal dystrophy (LECD) C2
6. Gelatinous drop-like corneal dystrophy (GDLD) C1

Epithelial–stromal TGFBI dystrophies

1. Reis–Bucklers corneal dystrophy (RBCD) C1
2. Thiel-Behnke corneal dystrophy (TBCD) C1
3. Lattice corneal dystrophy
   - Type 1 (LCD1) C1variants (III, IIIA, I/IIIA, IV) of lattice corneal dystrophy C1
4. Granular corneal dystrophy—type 1 (GCD1) C1
5. Granular corneal dystrophy—type 2 (GCD2) C1

Stromal dystrophies

1. Macular corneal dystrophy (MCD) C1
2. Schnyder corneal dystrophy (SCD) C1
3. Congenital stromal corneal dystrophy (CSCD) C1
4. Fleck corneal dystrophy (FCD) C1

5. Posterior amorphous corneal dystrophy (PACD) C1
6. Central cloudy dystrophy of Francois (CCDF) C4
7. Pre-Descemet corneal dystrophy-C1 or C4

Endothelial dystrophies

1. Fuchs endothelial corneal dystrophy—C1, C2, or C3
2. Posterior polymorphous corneal dystrophy (PPCD) C1 or C2
3. Congenital hereditary endothelial dystrophy (CHED) C1
4. X-linked endothelial corneal dystrophy (XECD) C2

Removed dystrophies
Grayson-Wilbrandt corneal dystrophy (GWCD) C4

2.2 Genetics in Corneal Dystrophy

Epithelial and Subepithelial Dystrophies

1. Epithelial basement membrane dystrophy (EBMD)
   Mendelian inheritance in man (MIM) # 121820
   Genetic locus-5q31
   Gene-transforming growth factor beta-induced-TGFB1 in 2 families.
   Inheritance-isolated familial cases have been reported, majority have no documented inheritance hence they are considered to be degenerative or secondary to trauma to eye.
2. Epithelial Recurrent Erosion Dystrophies (EREDs)
   MIM# 122400
   Genetic locus-unknown
   Gene-unknown
   Inheritance-autosomal dominant
3. Subepithelial mucinous corneal dystrophy (SMCD)
   MIM# 612867
   Genetic locus-unknown
   Gene-unknown
   Inheritance-autosomal dominant–most likely, but X-linked recessive inheritance not excluded
4. Meesmann Corneal Dystrophy (MECD)
   MIM #122100
   Genetic locus-Locus 12q13 (KRT3)
   Locus 17q12 (KRT12) Stocker–Holt variant
   Genes-Keratin K3 (KRT3)
   Keratin K12 (KRT12) Stocker–Holt variant
   Inheritance-autosomal dominant

5. Lisch Epithelial Corneal Dystrophy (LECD)
   MIM #300778
   Genetic locus-Xp22.3
   Gene-unknown
   Inheritance-X-chromosomal dominant
6. Gelatinous Drop-like Corneal Dystrophy (GDLD)
   MIM #204870
   Genetic locus-1p32
   Gene-Tumor-associated calcium signal transducer 2 (TACSTD2, previously M1S1)
   Inheritance-autosomal recessive

Epithelial–Stromal TGFBI Dystrophies

1. Reis–Bucklers Corneal Dystrophy
   MIM #608470
   Genetic locus-5q31
   Gene-Transforming growth factor beta-induced-TGFB1
   Inheritance-autosomal dominant
2. Thiel–Behnke Corneal Dystrophy (TBCD)
   MIM #602082
   Genetic locus-5q31
   Gene-Transforming growth factor beta-induced-TGFB1
   Inheritance-autosomal dominant
3. Lattice Corneal Dystrophy, type 1 (Classic) (LCD1) and Variants
   MIM #122200
   Genetic locus-5q31
   Gene-Transforming growth factor beta-induced-TGFB1
   Inheritance-autosomal dominant
4. Granular Corneal Dystrophy, type 1(Classic) (GCD1)
   MIM #121900
   Genetic locus-5q31
   Gene-Transforming growth factor beta-induced-TGFB1
   Inheritance-autosomal dominant
5. Granular Corneal Dystrophy, type 2 (GCD2)
   MIM #607541
   Genetic locus-5q31
   Gene-Transforming growth factor beta-induced-TGFB1
   Inheritance-autosomal dominant

Stromal Dystrophies

1. Macular Corneal Dystrophy (MCD)
   MIM #217800

Genetic locus-16q22
Gene-Carbohydrate sulfotransferase 6 gene—CHST6
Inheritance-autosomal recessive

2. Schnyder Corneal Dystrophy (SCD)
   MIM #21800
   Genetic locus-1p36
   Gene-UbiA prenyltransferase domain containing 1—UBIAD1
   Inheritance-autosomal dominant
3. Congenital Stromal Corneal Dystrophy (CSCD)
   MIM #610048
   Genetic locus-12q21.33
   Gene-Decorin, DCN
   Inheritance-autosomal dominant
4. Fleck Corneal Dystrophy (FCD)
   MIM #121850
   Genetic locus-2q34
   Gene-Phosphoinositide kinase, FYVE finger containing—PIKFYVE
   Inheritance-autosomal dominant
5. Posterior Amorphous Corneal Dystrophy (PACD)
   MIM #612868
   Genetic locus-12q21.33
   Gene-Deletion of keratocan (KERA), lumican (LUM), decorin (DCN), andepiphycan (EPYC)
   Inheritance-autosomal dominant
6. Central Cloudy Dystrophy of Francois
   MlM #217600
   Genetic locus/Gene-None
   Inheritance-unknown, autosomal dominant inheritance is occasionally reported
7. Pre-Descemet Corneal Dystrophy (PDCD)
   MIM: none
   Genetic locus-Isolated PDCD-Unknown
   PDCD associated with X-linked ichthyosis-Xp22.31
   Gene-Isolated PDCD-Unknown
   PDCD associated with X-linked ichthyosis-steroid sulfatase (STS)

Endothelial Dystrophies

1. Fuchs Endothelial Corneal Dystrophy (FECD)
   MIM #136800 (FECD1), MIM#610158 (FECD2), MIM #613267 (FECD3), MIM #613268 (FECD4), MIM #613269 (FECD5), MIM #613270 (FECD6), MIM #613271 (FECD7),MIM #615523 (FECD8)
   Genetic locus
   Early-Onset FECD
   1p34.3–p32 (FECD1)

Late-Onset FECD-Association has been reported to 13pter-q12.13 (FECD2),18q21.2-q21.3 (FECD3), 20p13-p12 (FECD4), 5q33.1-q35.2 (FECD5), 10p11.2 (FECD6), 9p24.1-p22.1 (FECD7), and15q25 (FECD8)

Gene-Early-onset FECD: collagen, type VIII, alpha-2, COL8A2

Inheritance-most commonly unknown, genetic basis of FECD is complex and heterogenous, suggesting incomplete penetrance and variable expressivity

2. Posterior Polymorphous Corneal Dystrophy (PPCD)
   MIM #122000 (PPCD1), MIM #609140 (PPCD2),MIM #609141 (PPCD3)
   Genetic locus-PPCD 1: 20p11.2-q11.2

   - PPCD 2: 1p34.3-p32.3
   - PPCD 3: 10p11.22
   - PPCD 4: 8q22.3-q24.12

   Gene-PPCD1: OVOL2 (ovo-like zinkfinger 2) [10]

   - PPCD2: collagen, type VIII, alpha-2 (COL8A2)
   - PPCD 3: zinc finger E box–binding homeobox 1 (ZEB1)
   - PPCD4: ectopic grainyhead-like transcription factor 2 (GRHL2)

Inheritance-autosomal dominant

3. Congenital Hereditary Endothelial Dystrophy (CHED)
   MIM #217700
   Genetic locus-20p13
   Gene-Solute carrier family 4, sodium borate transporter, member 11—(SLC4A11)

4. X-linked Endothelial Corneal Dystrophy (XECD)
   MIM: none
   Genetic locus-Xq25
   Gene-unknown
   Inheritance-X-chromosomal dominant

Newer Additions After IC3D (2015)

ERED (Epithelial recurrent erosion dystrophy)

In 2015, Swedish authors grouped 5 families and formed a large pedigree with autosomal dominant inheritance [11]. The whole genome sequencing resulted in identification of novel mutation in COL17A1 gene, that encodes collagen type XVII alpha 1 on chromosome 10.

The New Zealand authors screened four families with ERED in 2016 [12]and segregated two variants with ERED on chromosome 10. The COL17A1c.3156C > T variant was segregated in all four epithelial recurrent erosion dystrophy (ERED) families.

The 10q Thiel-Behnke corneal dystrophy is a misnomer. Infact, it is a variant in the COL17A1 gene on chromosome 10, both genotypically and phenotypically [13]. It demonstrates that the phenotype was misdiagnosed as Thiel-Behnke, when it was actually an ERED.

Future Horizons

The present genetic knowledge of corneal dystrophy is very limited and requires further investigations and integration. The knowledge of genetics is opening new prospects into gene therapy in the early stage of corneal dystrophies, and is an important scientific challenge for future.

References

1. Groenouw A. Knoetchenfoermige Hornhauttruebungen (Noduli corneae). Arch Augenheilkd. 1890;21:281–9.
2. Biber H. Ueber einige seltene Hornhauterkrankungen: die oberflaechlichegittrige Keratitis [Inaugural Dissertation]. A Diggelmann Zuerich. 1890.
3. Fuchs E. Dystrophia epithelialis corneae. Arch Ophthalmol. 1910;76:478–508.
4. Uthoff W. Ein Fall von doppelseitiger zentraler, punktfoermiger,supepithelialer knoetchen-foermiger Keratitis, Groenouw mit anatomischemBefunde. Klin Monatsbl Augenheilkd. 1915;54:377–83.
5. Yoshida Y. Ueber eine neue Art der Dystrophia corneae mit histologischemBefunde. Albrecht von Graefes Arch Clin Exp Ophthalmol. 1924;114:91–100.
6. Afshari NA, Bouchard CS, Colby KA, de Freitas D, Rootman DS, Tu EY, Weisenthal RW. Corneal dystrophies and ectasias. In: Weisenthal RW, editor. 2014–2015 Basic and Clinical Science Course,Section 8: External Disease and Cornea. San Francisco: AmericanAcademy of Ophthalmology; 2014. p. 253–87.
7. Waring GO III, Rodrigues MM, Laibson PR. Corneal dystrophies. I. Dystrophies of the epithelium, Bowman's layer and stroma. Surv Ophthalmol. 1978;23:71–122.
8. Waring GO III, Rodrigues MM, Laibson PR. Corneal dystrophies. II.Endothelial dystrophies. Surv Ophthalmol. 1978;23:147–68.
9. Weiss JS, Møller HU, Aldave AJ, Seitz B, Bredrup C, Kivelä T, Munier FL, Rapuano CJ, Nischal KK, Kim EK, Sutphin J, Busin M, Labbé A, Kenyon KR, Kinoshita S, Lisch W. IC3D classification of corneal dystrophies--edition 2. Cornea. 2015;34(2):117–59. https://doi.org/10.1097/ICO.0000000000000307. Erratum in: Cornea. 2015 Oct;34(10):e32. PMID: 25564336
10. Davidson AE, Liskova P, Evans CJ, Dudakova L, Nosková L, Pontikos N, Hartmannová H, Hodanová K, Strnecký V, Kozmik Z, Levis HJ, Idigo N, Sasai N, Maher GJ, Bellingham J, Veli N, Ebenezer ND, Cheetham ME, Daniels JT, Thaung CMH, Jirsova K, Plagnol V, Filipec M, Kmoch S, Tuft SJ, Hardcastle AJ. Autosomal-dominant corneal endothelial dystrophies CHED1 and PPCD1 are allelic disorders caused by non-coding mutations in the promoter OVOL2. Am J Hum Genet. 2016;98:1–15.
11. Jonsson F, Byström B, Davidson AE, Backman LJ, Kellgren TG, Tuft SJ, Koskela T, Rydén P, Sandgren O, Danielson P, Hardcastle AJ, Golovleva I. Mutations in collagen, type XVII, alpha 1 (COL17A1) cause epithelial recurrenterosion dystrophy (ERED). Hum Mut. 2015;36:463–73.
12. Oliver VF, van Bysterveldt KA, Cadzow M, Steger B, Romano V, Markie D, Hewitt AW, Mackey DA, Willoughby CE, Sherwin T, Crosier PS, McGhee CN. AL Vincent A COL17A1 splice-altering mutation is prevalent in inheritedrecurrent corneal erosions. Ophthalmology. 2016;123:709–22.

13. Lin BR, Le DJ, Chen Y, Wang Q, Chung DD, Frausto RF, Croasdale C, Yee RW, Hejtmancik FJ, Aldave AJ. Whole exome sequencing and segregation analysis confirms that a mutation in COL17A1 is the cause of epithelial recurrent erosion dystrophy in a large dominant pedigree previously mapped to chromosome 10q23-q24. PLoS One. 2016;11:e0157418. https://doi.org/10.1371/journal.phone.0157418.

Genetics of Keratoconus

3

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3.1 Introduction

Keratoconus is a corneal ectatic disorder characterized by bilateral asymmetrical progressive thinning, steepening, distortion, and protrusion of the cornea resulting in irregular astigmatism and impaired vision. It has a complex etiology involving the interplay of genetic and environmental factors contributing to the pathogenesis of the disease.

3.2 Epidemiology

The prevalence of keratoconus shows geographical variation due to various factors, one of which is the genetic variation between populations. The incidence of keratoconus varies between 1 in 500 and 1 in 2000 of the general population, with higher figures seen in Caucasian populations [1]. The Central India eye medical study estimated a prevalence of 2.3% in rural India based on corneal refractive power of 48D or more [2]. In concurrence with this were two studies conducted in the UK that compared the prevalence of keratoconus between Asian and white patients and reported a prevalence of 4.4–7.5 times greater for Asian subjects compared with white Caucasians. These variations could be explained by consanguinity contributing to a higher incidence of genetic disease [3, 4].

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3.3 Evidence of Role of Genetics/Heredity

The earliest evidence for genetic etiology of keratoconus was based on various studies including reports of monozygotic and dizygotic twins [5] and reports of familial aggregation [6, 7]. These were further strengthened by family-based linkage studies, studies on genetic mutations and genome-wide association studies. A questionnaire conducted on 218 patients with keratoconus and 183 age-matched controls revealed a positive family history in 10% of patients vs 0.5% of controls [8]. Overall, approximately 6–23.5% of patients with keratoconus have a positive family history [9]. Eighteen pairs of twins (13 monozygotic and 5 dizygotic) were evaluated in a study. All except one pair of dizygotic twins were concordant for keratoconus, the age of onset was earlier and severity was more in monozygotic twins, thereby supporting the genetic evidence. Several studies have reported a large number of cases of familial keratoconus with prevalence ranging between 5 and 27.9% for first-degree relatives which amounts to a 15–67 times higher incidence than in those with no relatives with keratoconus [10, 11]. The familial aggregation maybe under-reported as subclinical forms detectable by video-keratography/topography may not be reported. Corneal topography provided more information about the genetics of the disease by diagnosing keratoconus in family members who were suspects and had no obvious signs of the disease [12].

Among the cases with genetic linkage, sporadic, autosomal dominant mode with reduced penetrance and autosomal recessive mode have been described [13, 14]. Tynissma et al. studied 20 families in Finland with autosomal dominant keratoconus and showed a linkage to the locus 16q22.3–q23.1with a significant (>3) Logarithm to odds ratio (LOD) score. However, this finding could not be replicated and was specific to Finnish families.

3.4 Environmental Risk Factors

Certain environmental factors may be essential to act as a trigger for the onset of keratoconus in genetically predisposed individuals. These include eye rubbing [15], atopy [16, 17], contact lens wear and UV exposure [18]. These factors presumably act by causing oxidative damage to the cornea by reducing the amount of enzymes like aldehyde dehydrogenase (ALDH) and superoxide dismutase which are necessary to remove the reactive oxygen species.

3.5 Candidate Genes

Multiple genes have been implicated in the pathogenesis of keratoconus including VSX1, miR-184, DOCK9, SOD1, RAB3GAP1, *and* HGF [19].

A mutational analysis of five genes, the Visual System Homeobox 1 (VSX1), Secreted Protein Acidic and Rich in Cysteine (SPARC), Superoxide Dismutase 1 (SOD1), Lysyl Oxidase (LOX), and Tissue Inhibitor of Metalloproteinase 3 (TIMP3)

was carried out in 302 Italian patients with sporadic and familial keratoconus, which confirmed the possible pathogenic role of VSX1 in few patients [20].

VSX1is a gene located at the locus 20p11-q11 which is linked with posterior polymorphous corneal dystrophy(PPCD) [21]. Keratoconus and PPCD have been reported together in multiple case reports [22, 23]. Mutations within the VSX1 gene have been associated in few cases of keratoconus [24]. It is expressed by the keratocytes of injured corneas and is associated with fibroblastic transformation and also plays a role in craniofacial and ocular development. Although VSX1 is the most commonly studied gene in keratoconus, it accounts for only 2–3% cases. A large series with 302 Italian patients found VSX1 changes in 3.2% patients. This pointed toward the complex nature of the disease and the ethnic variation.

SOD1 gene located at 20p11 encodes the enzyme that metabolizes superoxide radicals. Since keratoconus corneas show high levels of oxidative stress and also because of the association of Trisomy 21 with keratoconus, SOD1 was studied as a potential candidate, however, conclusive mutations have not been demonstrated to prove its role in keratoconus presently [25].

Several other loci have been reported in families with keratoconus including Transforming growth factor (TGFβ1), Collagen gene COL4 (alteration in collagen structure being one of the theories in the pathogenesis of keratoconus), Fillagrin (FLG, associated with atopic dermatitis), Interleukin (IL-1, involved in keratocyte apoptosis), calpastatin (CAST) and TIMP-3. The 5q31 region was implicated in a family with autosomal dominant keratoconus by Rosenfeld et al. The same locus is also implicated in granular, lattice, and Reis–Bucklers corneal dystrophies due to mutations in the TGF β1 gene [26].

3.6 Genome-Wide Studies

They have the advantage of not relying on previously available knowledge about a gene and are unbiased studies but require a much higher level of statistical significance for reporting an association. Two subtypes have been used in keratoconus, the Linkage studies and the Genome-wide association studies (GWAS). Another approach, Identity by descent (IBD), has also been applied.

3.7 Linkage Studies in Families

Linkage studies are one of the methods to map the susceptible loci in keratoconus. In these studies, multiple families from different generations are recruited and both affected and unaffected members undergo genetic analysis. The likely chromosomal region that doesnot show an overlap is mapped. Penetrance can be analyzed by the number of individuals who have both the genotype and the phenotype for the disease. Using this approach, around 17 distinct genetic loci have been mapped, out of which 3 have been replicated independently (5q21.2, 14q11.2, 5q32). These studies

have the limitation of using multiple families with patients in small numbers to map the linkage.

The microRNA gene (miRNA) MIR184 is expressed in the cornea and lens epithelium. A mutation in the genomic region chr15q22-q25 of miRNA was reported in a family from Northern Ireland with hereditary keratoconus and anterior polar cataract. The mutant form, however, failed to compete with MIR-205 for the target sites [27]. A study conducted in 780 patients with keratoconus could identify the mutation in only 2 patients [28].

DOCK9 (Dedicator of cytokinesis 9) is another candidate gene for keratoconus and mutations of DOCK9 at the locus 13q32 were reported in an Ecuadorian family [29]. A mutation screening of eight candidate genes within this locus identified three genes that are expressed in the human cornea including DOCK9, the most functionally significant of these being DOCK9 [30]. However, a study conducted on 42 Polish patients with keratoconus and 50 controls which analyzed the role of five genes, found DOCK9 in only five patients and two controls suggesting that other genetic variants are involved in Polish patients.

Several other linkage studies have been conducted in small families. Based on these studies genetic loci have been mapped and associations have been studied in small populations [31–37].

Table 3.1 summarizes the linkage studies in keratoconus with chromosomal loci/Gene identified.

Table 3.1 Important linkage studies in Keratoconus with genes/loci identified

Gene	Locus	Reference	Association/Population studied/Mode of inheritance
VSX1	20p11-q11	[19]	Posterior polymorphous dystrophy
DOCK9	13q32	[29, 30]	Ecuadorian
MIR184	15q22-q25	[27, 28]	Cataract /Northern Irish/Autosomal dominant
ZEB1	10p11.22	[35]	Brittle cornea syndrome/Autosomal recessive
SOD1	20p11	[25]	Trisomy 21/Autosomal dominant
TGFß1	5q31.1	[26]	Corneal dystrophies
COL4A	2q36.3		
FLG	1q21		
CRB1	1q	[38]	Leber's congenital amaurosis
	1p36	[39]	Australian/Autosomal dominant
	2p24	[40]	European
	3p14-q13	[41]	Italian/Autosomal dominant
	5q14.3-q.21.1	[42]	Caucasian/Autosomal dominant
	14q24.3	[43]	Multiethnic
	15q22.33-24.2	[13]	Cataract/Northern Irish/Autosomal dominant
	16q22.3-23.1	[14]	Finnish/Autosomal dominant
	17p13	[44]	Leber's congenital amaurosis/Pakistani/Autosomal recessive
	20q12	[37]	Tasmania (Australia)/Autosomal dominant

3.8 Genome-Wide Association Studies

GWAS have been used for searching the genetic influence in the pathogenesis of complex and multifactorial diseases. In these studies, thousands of single nucleotide polymorphisms (SNPs) are tested in thousands of persons for association with a disease [45]. They follow a case–control cohort and the finding of an SNP implies a causative role.

Two significant GWAS have been carried out in keratoconus. Li et al. performed a comprehensive GWAS in 222 keratoconus patients and 3324 controls (USA). Their findings suggested that an SNP located near the RAB3GAP1 gene was a potential locus for keratoconus [46]. Another GWAS by Burdon et al. [47] was conducted in 97 patients and 216 controls (Australia and USA). The most significant association was found with an SNP located at the HGF (hepatocyte growth factor) gene which is associated with refractive errors [48].

Another recent GWAS identified mutations in Zinc Finger 469(ZNF469) and PR domain-containing protein 5 (PRDM5) genes associated with brittle cornea syndrome, which is an autosomal recessive inherited disorder alongwith progressive corneal thinning and keratoconus. ZNF469 regulates extracellular matrix synthesis [49].

3.9 Combination of GWAS and Linkage Studies

The GWAS of Li et al. pointed out a linkage signal observed in a family at chromosome 5q23.2. This led to an association with the LOXgene which is involved in cross-linking of collagen fibers in the corneal stroma [50].

3.10 Identity by Descent

This approach is used to detect a defect in genetically related individuals from the same population, not the same family. A high estimated keratoconus prevalence (1/200) was seen in a genetically isolated population in Tasmania. The genotyping of samples from this population suggested an association with the locus 20q12 [38].

3.11 Related Ocular and Systemic Diseases

Although in the vast majority of patients, keratoconus occurs as an isolated entity; its association with several systemic disorders has been reported including chromosomal disorders like Down's syndrome and Turner's syndrome [39]. The prevalence of keratoconus in Down's syndrome is significantly more than in the normal population, pointing toward a possible association with Trisomy 21. Corneal dystrophies including PPCDare associated with keratoconus. Mutations in VSX1 and zinc-finger E box binding homeobox 1 (ZEB1) have been previously

implicated in PPCD. Connective tissue disorders like Ehler-Danlos syndrome, osteogenesis imperfecta [40], craniosynostosis, and Marfan's syndrome [41] are also associated with keratoconus suggesting an underlying genetic mechanism involving collagen synthesis. Genetic association of keratoconus with various other disorders is also reported including cataract, Leber congenital amaurosis (due to AIPL1 and CRB1 mutations) [42], and retinal dystrophies. The onset is reported to be earlier and the severity of keratoconus more advanced in association with Leber's congenital amaurosis, probably indicating an underlying shared genetic pathway.

3.12 Recent Research

A recent study conducted on 129 Indian keratoconus patients and 20 controls observed that the enzymes matrixmetalloproteinase (MMP-9), Tumor necrosis factor (TNFα) and Interleukin (IL6) were upregulated in tears of patients compared to controls and treatment with cyclosporine A reduced their expression significantly [43].This supported the role of inflammation in keratoconus. The human MMP-9 gene has been mapped to chr20q11, pointing toward a genetic mechanism.

3.13 Future Research

Presently, several genetic loci have been implicated in keratoconus and no single gene-effect relationship has been established. Family-based studies have led to the identification of certain genes; however, the same has been replicated in other families in a very limited manner. Identification of specific genetic markers may provide a valuable tool in the clinical diagnosis of keratoconus. Genome-wide studies can help in unraveling the multigenetic nature of this disease. Cornea has been described as a powerful candidate for gene therapy due to its immune privilege and ex vivo stability, thereby making gene therapy in keratoconus an attractive and promising future prospect [44].

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References

1. Godefrooij DA, de Wit GA, Uiterwaal CS, Imhof SM, Wisse RP. Age-specific incidence and prevalence of keratoconus: a nationwide registration study. Am J Ophthalmol. 2017;175:169–72.
2. Jonas JB, Nangia V, Matin A, Kulkarni M, Bhojwani K. Prevalence and associations of keratoconus in rural maharashtra in central India: the central India eye and medical study. Am J Ophthalmol. 2009;148(5):760–5.

3. Georgiou T, Funnell CL, Cassels-Brown A, O'Connor R. Influence of ethnic origin on the incidence of keratoconus and associated atopic diseases in Asian and white patients. Eye (Lond). 2004;18:379–83.
4. Pearson AR, Soneji B, Sarvananthan N, Sandforth-Smith JH. Does ethnic origin influence the incidence or severity of keratoconus? Eye (Lond). 2000;14:625–8.
5. Tuft SJ, Hassan H, George S, Frazer DG, Willoughby CE, Liskova P. Keratoconus in 18 pairs of twins. Acta Ophthalmologica. 2012;90(6):e482–6.
6. Rabinowitz YS, Garbus J, McDonnell PJ. Computer assisted corneal topography in family members of keratoconus. Arch Ophthalmol. 1990;108:365–71.
7. Rabinowitz YS. The genetics of keratoconus. Ophthalmol Clin North Am. 2003;16:607–20.
8. Rabinowitz YS. Keratoconus. Surv Ophthalmol. 1998;42(4):297–319. 20.
9. Wheeler J, Hauser MA, Afshari NA, Allingham RR, Liu Y. The genetics of Keratoconus: a review. Reprod Syst Sex Disord. 2012;(Suppl 6):001.
10. Shneor E, Millodot M, Blumberg S, Ortenberg I, Behrman S, Gordon-Shaag A. Characteristics of 244 patients with keratoconus seen in an optometric contact lens practice. Clin Exp Optometry. 2013;96(2):219–24.
11. Wang Y, Rabinowitz YS, Rotter JI, et al. Genetic epidemiological study of keratoconus: evidence for major gene determination. Am J Med Genet. 2000;93(5):403–9.
12. Karimian F, Aramesh S, Rabei HM, Javadi MA, Rafati N. Topographic evaluation of relatives of patients with keratoconus. Cornea. 2008;27(8):874–8.
13. Hughes AE, Dash DP, Jackson AJ, et al. Familial keratoconus with cataract: linkage to the long arm of chromosome 15 and exclusion of candidate genes. Investig Ophthalmol Vis Sci. 2003;44(12):5063–6.
14. Tyynismaa H, Sistonen P, Tuupanen S, et al. A locus for autosomal dominant keratoconus: linkage to 16q22.3-q23.1 in Finnish families. Investig Ophthalmol Vis Sci. 2002;43(10):3160–4.
15. McMonnies CW. Abnormal rubbing and keratectasia. Eye Contact Lens. 2007;33(6):265–71.
16. Bawazeer AM, Hodge WG, Lorimer B. Atopy and keratoconus: a multivariate analysis. Br J Ophthalmol. 2000;84(8):834–6.
17. Wachtmeister L, Ingemansson SO. Atopy and HLA antigens in patients with keratoconus. Acta Ophthalmologica. 1982;60(1):113–22.
18. Newkirk KM, Chandler HL, Parent AE, Young DC, Colitz CM, Wilkie DA, Kusewitt DF. Ultraviolet radiation-induced corneal degeneration in 129 mice. Toxicologic Pathol. 2007;35(6):817–24.
19. Karolak JA, Polakowski P, Szaflik J, Szaflik JP, Gajecka M. Molecular screening of keratoconus susceptibility sequence variants in VSX1, TGFBI, DOCK9, STK24, and IPO5 genes in Polish patients and novel TGFBI variant identification. Ophthalmic Genet. 2016;37(1):37–43.
20. De Bonis P, Laborante A, Pizzicoli C, Stallone R, Barbano R, Longo C, Mazzilli E, et al. Mutational screening of VSX1, SPARC, SOD1, LOX, and TIMP3 in keratoconus. Mol Vis. 2011;17:2482–894.
21. Héon E, Mathers WD, Alward WL, et al. Linkage of posterior polymorphous corneal dystrophy to 20q11. Hum Mol Genet. 1995;4(3):485–8.
22. Driver PJ, Reed JW, Davis RM. Familial cases of keratoconus associated with posterior polymorphous dystrophy. Am J Ophthalmol. 1994;118:256–7.
23. Mazzotta C, Baiocchi S, Caporossi O, Buccoliero D, Casprini F, et al. Confocal microscopy identification of keratoconus associated with posterior polymorphous corneal dystrophy. J Cataract Refract Surg. 2008;34:318–21.
24. Paliwal P, Singh A, Tandon R, Titiyal JS, Sharma A. A novel VSX1 mutation identified in an individual with keratoconus in India. Mol Vis. 2009;15:2475–9.
25. Udar N, Atilano SR, Brown DJ, Holguin B, Small K, Nesburn AB, Kenney MC. SOD1: a candidate gene for keratoconus. Investig Ophthalmol Vis Sci. 2006;47(8):3345–51.
26. Klintworth GK. Advances in the molecular genetics of corneal dystrophies. Am J Ophthalmol. 1999;128:747–54.
27. Hughes AE, Bradley DT, Campbell M, et al. Mutation altering the miR-184 seed region causes familial keratoconus with cataract. Am J Hum Genet. 2011;89(5):628–33.

28. Willoughby CE, Lechner J, Bae JHA, et al. Mutational analysis of MIR184 in sporadic keratoconus and myopia. Investig Ophthalmol Vis Sci. 2013;54(8):5266–72.
29. Gajecka M, Radhakrishna U, Winters D, Nath SK, Rydzanicz M, et al. Localization of a gene for keratoconus to a 5.6-Mb interval on 13q32. Investig Ophthalmol Vis Sci. 2009;50:1531–9.
30. Czugala M, Karolak JA, Nowak DM, Polakowski P, Pitarque J, et al. Novel mutation and three other sequence variants segregating with phenotype at keratoconus 13q32 susceptibility locus. Eur J Hum Genet. 2012;20:389–97.
31. McMahon TT, Kim LS, Fishman GA, et al. CRB1 gene mutations are associated with keratoconus in patients with leber congenital amaurosis. Investig Ophthalmol Vis Sci. 2009;50(7):3185–7.
32. Burdon KP, Coster DJ, Charlesworth JC, et al. Apparent autosomal dominant keratoconus in a large Australian pedigree accounted for by digenic inheritance of two novel loci. Hum Genet. 2008;124(4):379–86.
33. Hutchings H, Ginisty H, Le Gallo M, et al. Identification of a new locus for isolated familial keratoconus at 2p24. J Med Genet. 2005;42(1):88–94.
34. Brancati F, Valente EM, Sarkozy A, et al. A locus for autosomal dominant keratoconus maps to human chromosome 3p14-q13. J Med Genet. 2004;41(3):188–92.
35. Tang YG, Rabinowitz YS, Taylor KD, et al. Genomewide linkage scan in a multigeneration Caucasian pedigree identifies a novel locus for keratoconus on chromosome 5q14.3-q21.1. Genet Med. 2005;7(6):397–405.
36. Liskova P, Hysi PG, Waseem N, et al. Evidence for keratoconus susceptibility locus on chromosome 14: a genome-wide linkage screen using single-nucleotide polymorphism markers. Arch Ophthalmol. 2010;128(9):1191–5.
37. Hameed A, Khaliq S, Ismail M, et al. A novel locus for Leber congenital amaurosis (LCA4) with anterior keratoconus mapping to chromosome 17p13. Investig Ophthalmol Vis Sci. 2000;41(3):629–33.
38. Fullerton J, Paprocki P, Foote S, Mackey DA, et al. Identity-by-descent approach to gene localization in eight individuals affected by keratoconus from north-west Tasmania, Australia. Hum Genet. 2002;110(5):462–70.
39. Vincent AL, Weiser BA, Cupryn M, et al. Computerized corneal topography in a paediatric population with down syndrome. Clin Exp Ophthalmol. 2005;33(1):47–52.
40. Beckh U, Schönherr U, Naumann GO. Autosomal dominant keratoconus as the chief ocular symptom in Lobstein osteogenesis imperfecta tarda. KlinMonbl Augenheilkd. 1995;206:268–72.
41. Bass HN, Sparkes RS, Crandall BF, Marcy SM. Congenital contractural arachnodactyly, keratoconus, and probable Marfan syndrome in the same pedigree. J Pediatr. 1981;98(4):591–3.
42. Elder MJ. Leber congenital amaurosis and its association with keratoconus and keratoglobus. J Pediatr Ophthalmol Strabismus. 1994;31(1):38–40.
43. Shetty R, Ghosh A, Lim RR, et al. Elevated expression of matrix metalloproteinase-9 and inflammatory cytokines in keratoconus patients is inhibited by cyclosporine A. Investig Ophthalmol Vis Sci. 2015;56(2):738–50.
44. Jun AS, Larkin DF. Prospects for gene therapy in corneal disease. Eye (Lond). 2003;17:906–11.
45. Manolio TA. Genomewide association studies and assessment of the risk of disease. N Engl J Med. 2010;363(2):166–76.
46. Li X, Bykhovskaya Y, Haritunians T, et al. A genome-wide association study identifies a potential novel gene locus for keratoconus, one of the commonest causes for corneal transplantation in developed countries. Hum Mol Genet. 2012;21(2):421–9.
47. Burdon KP, Macgregor S, Bykhovskaya Y, et al. Association of polymorphisms in the hepatocyte growth factor gene promoter with keratoconus. Investig Ophthalmol Vis Sci. 2011;52(11):8514–9.

48. Veerappan S, Pertile KK, Islam AF, et al. Role of the hepatocyte growth factor gene in refractive error. Ophthalmology. 2010;117(2):239–45.e452.
49. Lechner J, Porter LF, Rice A, et al. Enrichment of pathogenic alleles in the brittle cornea gene, ZNF469, in keratoconus. Hum Mol Genet. 2014;23(20):5527–35.
50. Li X, Rabinowitz YS, Tang YG, et al. Two-stage genome-wide linkage scan in keratoconus sib pair families. Investig Ophthalmol Vis Sci. 2006;47(9):3791–5.

Genetics of Dry Eye Disease

4

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4.1 Definition and Classification

The revised definition of dry eye disease (DED) states it to be a multifactorial disease of the ocular surface, involving loss of homeostasis of the tear film. It is characterized by presence of both symptoms and signs, ocular surface inflammation, tear film instability and hyperosmolarity as well as neurosensory abnormalities [1]. It is important to realize that dry eye is not one homogenous disease entity. A multitude of local and systemic conditions, acting in isolation or in combination, can cause DED. Therefore, the environmental and genetic risk factors for developing DED are multiple and diverse.

The spectrum of DED includes both evaporative and aqueous deficient forms, with a combined form predominating in most cases. A comprehensive enumeration of the causes of DED is beyond the scope of this chapter. However, the commonest causes of evaporative and aqueous deficiency DED are believed to be meibomian gland dysfunction (MGD) and Sjogren's syndrome, respectively.

4.2 Molecular Mechanisms and Genetic Basis

Approaches taken to understand the molecular mechanisms driving DED include studying genes or groups of genes that show altered sequences (genomics) or changed expression (transcriptomics) in correspondence with clinical presentation of DED. Studies in the fields of genomics and transcriptomics related to DED carried out in animal models as well as in humans have been summarized in a recent review [2]. We hereby summarize a few key findings from this review.

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Genomic studies have implicated multiple genes in the pathogenesis of MGD. These include genes related to meibomian gland development [e.g., ectodysplasin A (EDA), adaptor protein tumor necrosis factor receptor-associated factor 6 (TRAF6), and glucocorticoid receptors] as well as abnormal meibomian gland appearance [e.g., solute carrier family 27, Member 4 gene (SCF27A4), PR Domain Containing 1 gene (PRDM1)]. Gene expression studies have analyzed the effect of exposing mice or meibomian gland epithelial cells from mice with hormones such as estradiol-17β, progesterone, testosterone, and dihydrotestosterone (DHT) or drugs such as isotretinoin. A significant upregulation or downregulation of specific genes in response to such exposure gives clues to potential molecular pathways operative in MGD. Gene expression studies in humans have shown significant upregulation of genes such as S100-A8, S100-A9, SPRR2A, KRT10, IL-1β, and MMP-9 in subjects with MGD, compared to control [3, 4].

The role of inflammation in DED has also been elucidated to a great extent by genomic studies involving genes such as TNF receptor superfamily member 6 (Fas), Matrix metalloproteinase-9 (MMP-9), and Transforming growth factor-beta one (TGF-β1). Gene expression studies have identified genes such as EGFR, IL-6, IL-9, and NAMPT to be associated with DED in graft versus host disease [5].

Systemic autoimmune conditions such as rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, Stevens–Johnson syndrome, mucous membrane pemphigoid, etc., are known to be associated with DED. Multiple studies have attempted to determine risk alleles as well as protective alleles related to these conditions by focusing on the human leukocyte antigen (HLA) loci of the major histocompatibility antigen (MHC) complex [6–8].

4.3 Conclusions

A variety of local and systemic disease processes contribute to the development of DED. Studies have elaborated the genetic basis of many molecular mechanisms that cause clinical manifestations of DED. The role of processes such as structural and functional dysfunction of the meibomian gland, changes in tear film components, and inflammatory pathways operational at the ocular surface has been clarified to a great extent. Nonetheless, there are significant gaps in our knowledge. Future studies on the genetics of DED may help clarify aspects such as heritability, specific risk factors, and potential treatment pathways for this condition.

Financial Interest Nil

References

1. Craig JP, Nelson JD, Azar DT, et al. TFOS DEWS II report executive summary. Ocul Surf. 2017;15(4):802–12.
2. Lee L, Garrett Q, Flanagan J, Chakrabarti S, Papas E. Genetic factors and molecular mechanisms in dry eye disease. Ocul Surf. 2018;16(2):206–17.

3. Liu S, Richards SM, Lo K, Hatton M, Fay A, Sullivan DA. Changes in gene expression in human meibomian gland dysfunction. Investig Ophthalmol Vis Sci. 2011;52:2727–40.
4. Zhang L, Su Z, Zhang Z, Lin J, Li DQ, Pflugfelder SC. Effects of azithromycin on gene expression profiles of proinflammatory and anti-inflammatory mediators in the eyelid margin and conjunctiva of patients with meibomian gland disease. JAMA Ophthalmol. 2015;133:1117–23.
5. Cocho L, Fernandez I, Calonge M, et al. Gene expression-based predictive models of graft versus host disease-associated dry eye. Investig Ophthalmol Vis Sci. 2015;56:4570–81.
6. Kannabiran C, Ueta M, Sangwan V, et al. Association of human leukocyte antigen class 1 genes with Stevens Johnson syndrome with severe ocular complications in an Indian population. Sci Rep. 2017;7:15960.
7. Ueta M, Kannabiran C, Wakamatsu TH, et al. Trans-ethnic study confirmed independent associations of HLA-A*02:06 and HLA-B*44:03 with cold medicine-related Stevens-Johnson syndrome with severe ocular surface complications. Sci Rep. 2014;7:5981.
8. Cruz-Tapias P, Rojas-Villarraga A, Maier-Moore S, Anaya JM. HLA and Sjögren's syndrome susceptibility. A meta-analysis of worldwide studies. Autoimmun Rev. 2012;11(4):281–7.

Genetics in Cataract: To Be or Not to Be

5

Rohit Shetty and Ann Sarah Koshy

"Our own genomes carry the story of evolution, written in DNA, the language of molecular genetics, and the narrative is unmistakable."

Kenneth R. Miller

With the advent of Human Genome Project in 2003, genetics in Medicine has progressed in leaps and bounds. Gene therapy in ophthalmology is even more pertinent as the eye is relatively immunoprivileged and has well-defined anatomy. It is also accessible to different routes of administration of gene therapy. Despite phenomenal progress in surgical treatment, age-related cataract still claims a spot as a clinically important cause of visual impairment in economically developed countries which hampers functionality and is also a leading cause of blindness [1]. The genetics of pediatric cataract is better characterized but heritability estimates for age-related cataract span from 35% to 48% for nuclear opacities and 24–58% for cortical opacities [2].

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5.1 Embryology

Let us first understand the development of the Lens in utero. It is an established fact that the eye develops from two of the three germ layers: the ectoderm and the mesoderm (Fig. 5.1). At the third week of gestation the Diencephalon gives two outpouchings which form the optic pit (Fig. 5.2) and the optic vesicle (Fig. 5.3) which is formed from neuroectoderm and develops into the optic nerve later. The neuroectoderm then stimulates the thickened surface ectoderm with transcription factors including PAX 6 gene (Fig. 5.9) to form the lens placode (Fig. 5.4) by 28 days of gestation [3].

The lens placode then invaginates inward and forms in a direction such that the bases of lens and optic vesicle cells are in apposition to each other at this stage. They are only separated by their basement membranes and a narrow space. It may be assumed that the basement membrane is a product of epithelial cells if it is formed as in the yolk sac [4].

The invagination later leads to the formation of the lens pit or cup (Fig. 5.5) that then goes on to form the lens vesicle (Fig. 5.6). This occurs by 33 days of gestation. Sox2 is a direct target of the transcription factor Six3 which also responds to inductive signals from the optic vesicle andis expressed on the lens placode [5]. In particular, Sox2 is upregulated by BMP4, an important signaling ligand from the optic cup to be implicated in lens induction [6].

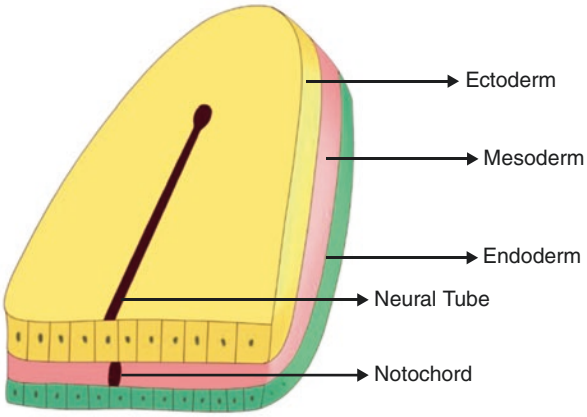


Fig. 5.1 The three germ layers seen in gastrulation: ectoderm, mesoderm, and endoderm. Figure 1 have been created by the mutimedia team under the authors' guidance. Courtesy:1–6: Dr. Pranesh Balasubramanium

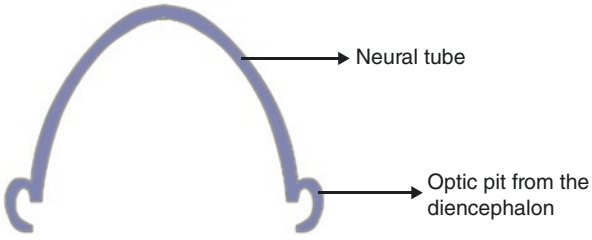


Fig. 5.2 Optic pit development from diencephalon of neuroectoderm. Figure 2 have been created by the mutimedia team under the authors' guidance. Courtesy:1–6: Dr. Pranesh Balasubramanium

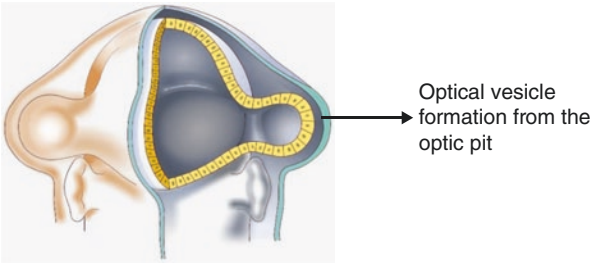


Fig. 5.3 Optic vesicle extending from the optic pit and releasing transcription factors optic vesicle formation from neuroectoderm. Figure 3 have been created by the mutimedia team under the authors' guidance. Courtesy:1–6: Dr. Pranesh Balasubramanium

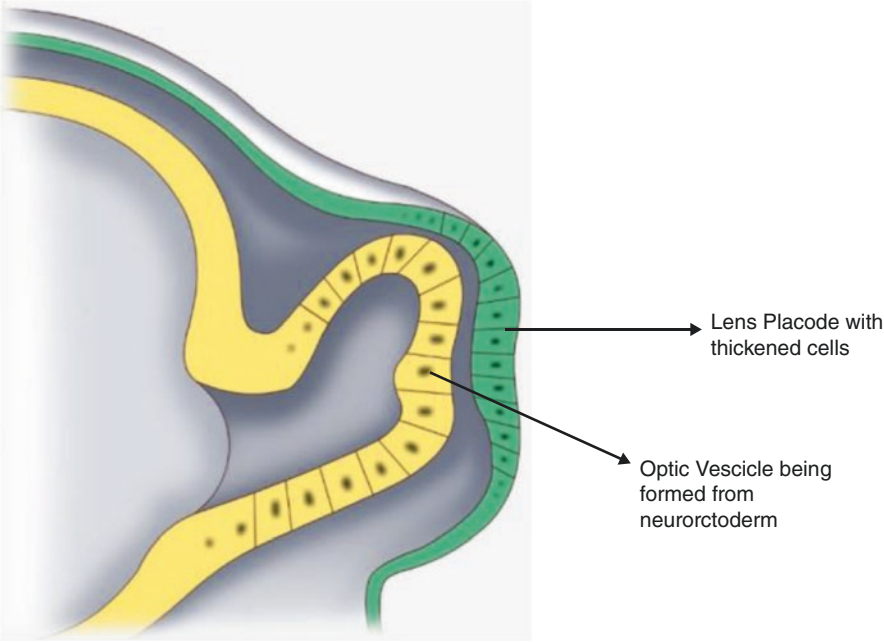


Fig. 5.4 Lens placode, i.e. surface ectoderm thickening after induction factors with Pax6 gene. Figure 4 have been created by the mutimedia team under the authors' guidance. Courtesy:1–6: Dr. Pranesh Balasubramanium

The posterior cells of the lens vesicle lens fibers then elongate and fill in the central forming the primary lens fibers and the nuclear bow formation occurs. Maf gene mutation may cause opaque flecks in the developmental stage here [7]. Themitotic cells from the anterior cells migrate to the equatorial region and for the secondary lens fibers. The region where these fibers join forms the anterior and posterior lens sutures which are y-shaped. There may be a role in the development of sutural cataract here as well.

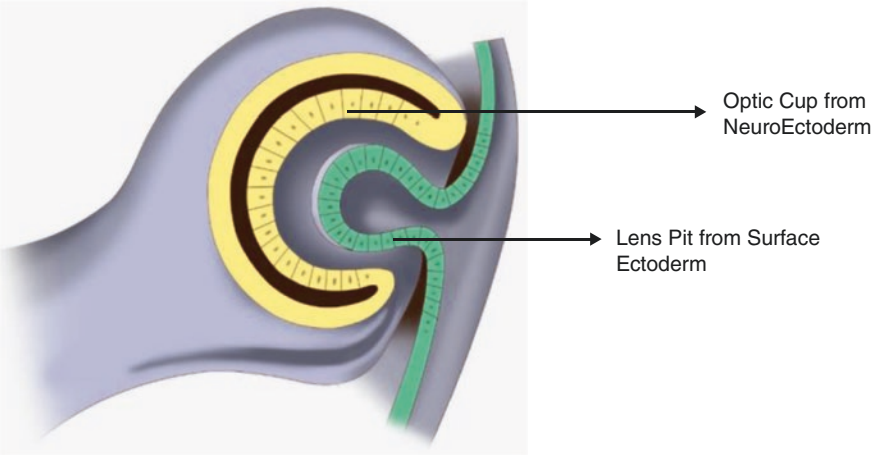
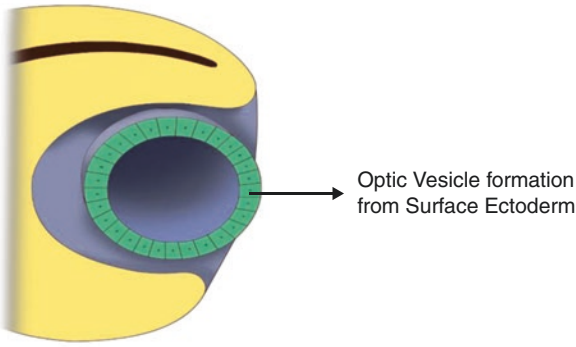


Fig. 5.5 Lens pit formation with invagination of the surface ectoderm. Figure 5 have been created by the mutimedia team under the authors' guidance. Courtesy:1–6: Dr. Pranesh Balasubramanium

Fig. 5.6 Lens vesicle formation with separation of the surface ectoderm. Figure 6 have been created by the mutimedia team under the authors' guidance. Courtesy:1–6: Dr. Pranesh Balasubramanium



5.2 Epidemiology

Congenital cataract is the foremost cause of reversible blindness in childhood. Around 70 million blind-person-years are caused by childhood blindness, and from these about ten million blind-person-years (14%) are due to childhood cataract [8]. India's burden ofvisually impaired children is around 280,000–320,000 [9] most of whom are found to have congenital cataract. Its occurrence, depending on the regional socioeconomic development, is of 1–6 cases per 10,000 live births in industrialized countries [10–12], and of 5–15 per 10,000 in the economically backward regions of the world [13]. Congenital cataract is found to be present at birth or during the first decade of life. Around 20,000–40,000 new cases of bilateral congenital cataract are diagnosed each year [14].

Inherited cataracts account for 8–25% of congenital cataracts [13], particularly for bilateral cataract. Rahi and Dezateux [15] reported that 27% of children with bilateral isolated congenital cataract had a genetic basis while this held true for 2% of unilateral cases. A variety of inheritance patterns are noted for transmission of cataract, the most frequent mode being autosomal dominant, this also has the highest penetrance, other modes including X-linked and autosomal-recessive transmission have been observed [16]. Inherited cataracts are clinically highly heterogeneous and demonstrate considerable inter and intrafamilial variability.

However, there is still a fundamental crux which still eludes us, which is the genetic basis of cataract. Varied studies have shown associations of a hereditary component. An inherited form of cataract (CAE1) that has been closely linked with the Duffy blood-group locus (Fy) became the first monogenic disease assigned to an autosome (chromosome 1) in humans. It was first identified in the 1960s [17]. So how can we identify newer gene loci?

5.3 Techniques for Genetic Screening

Genetic screening to identify genes associated with cataractcan be planned in different strategies like linkage analysis, genome-wide association studies, and candidate gene analysis (Fig. 5.7). Linkage analysis narrows down mutant genes causing inherited cataracts to a specific chromosomal region by comparing their inheritance patterns with those of known genetic markers. It is a reliable and strong tool to sort outand identify different genetic loci that are associated with the development human cataracts. Genome-wide association study (GWA study, or GWAS), also called whole genome association study (WGA study or WGAS), is an observational study of a genome-wide set of genetic variants in multiple individuals to see if any variant is associated with a trait. GWASs typically focus on associations between single-nucleotide polymorphisms (SNPs). Thanks to molecular advancement nowadays, most cataract loci are identified using microsatellite markers, although single-nucleotide polymorphisms (SNPs) are rapidly gaining favor. New analytical approaches using homozygosity mapping methods to recognize genomic regions that are identical by descentarefuturistic and promising tools which can be increasingly useful in studying rare autosomal-recessive cataracts from isolated populations. With the advent of massively parallel next-generation sequencing (NGS) as demonstrated in Fig. 5.8, the gene sequencing is becoming cost effective and also newer mutations along with unknown mutations can be screened.

5.4 Overview of Cataract Genetics

Eye starts developing at 22 days of gestation. Fibroblast growth factor (FGF) induces migration, differentiation, and may be held responsible for polarity of the lens [18]. Bone morphogenetic protein (BMP) interacts with FGF during lens

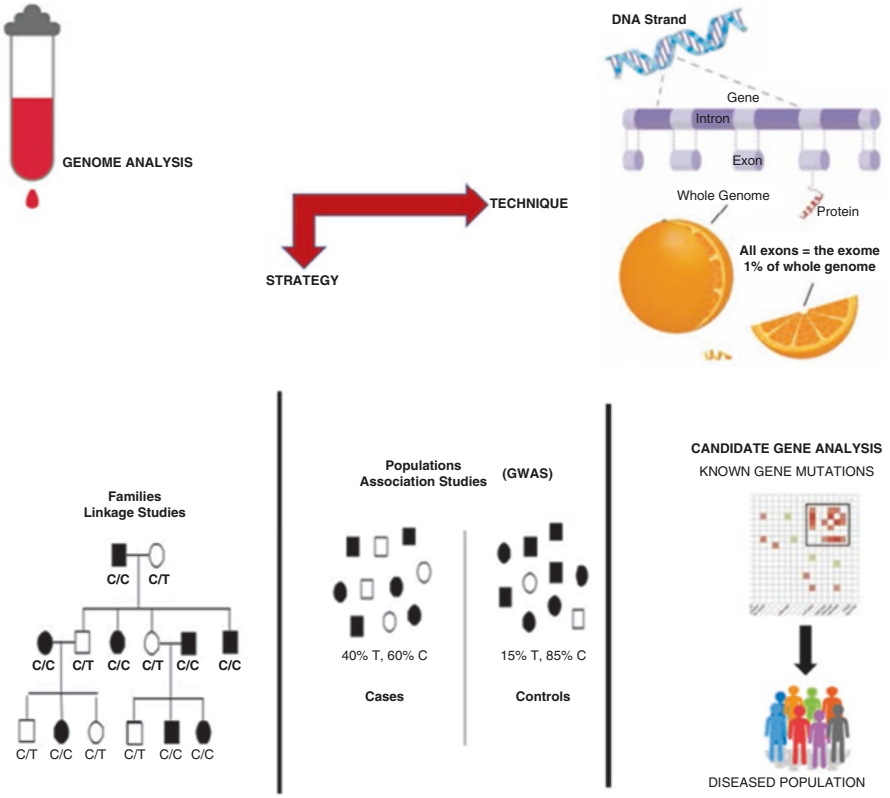


Fig. 5.7 Genetic analysis can be planned by different methods: (1) Family linkage analysis, (2) Genome wide association studies, and (3) Candidate gene analysis. Technique can be whole gene sequencing which is time consuming and not cost effective. The better alternative can be exon sequencing wherein the coding regions are sequenced. Illustration courtesy: Meghan Harrigan. Courtesy Meghan Harrigan. Figure 7 have been created by the mutimedia team under the authors' guidance. Courtesy:1–6: Dr. Pranesh Balasubramanium

induction as depicted in Fig. 5.9. The proteins encoded by genes Pax6, Pitx3, c-Maf, and Foxe3 are transcription factors which are vital for lens development [19]. The mutations are most commonly autosomal dominant, and absence of the function of a single copy has a profound effect on lens development. The timing of insult results in involving that part of lens developing during that period.

Autosomal-dominant cataract includes hyperferritinemia cataract syndrome, Volkmann-type congenital, Coppock-like, anterior polar, zonular with sutural, posterior polar, cerulean, crystalline aculeiform, zonular pulverulent and myotonic dystrophy 1-like cataracts. Autosomal-recessive cataract includes Warburg micro syndrome, Hallermann–Streiff syndrome, Martsolf syndrome, Smith–Lemli–Opitz syndrome, Rothmund–Thomson syndrome, Marinesco–Sjogren syndrome, Wilson's disease, and congenital cataract facial dysmorphism and neuropathy. X-linked recessive cataract includes Nance–Horan syndrome (NHS) and Norrie's disease [20, 21].

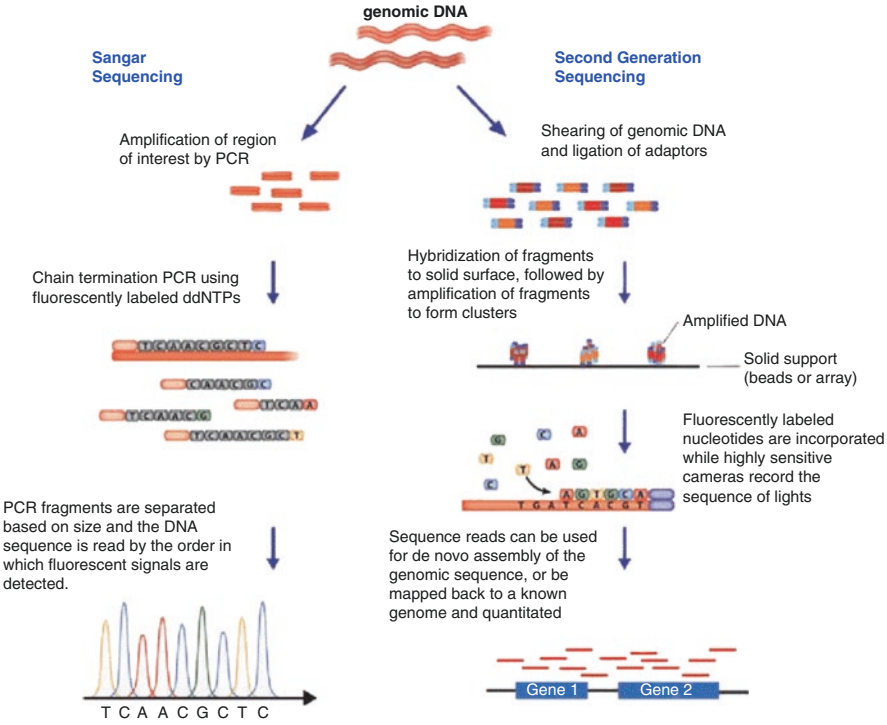


Fig. 5.8 Comparison between Sanger sequencing and next-generation sequencing (NGS) technologies. Sanger sequencing is limited to determining the order of one fragment of DNA per reaction, up to a maximum length of *700 bases. NGS platforms can sequence millions of DNA fragments in parallel (massively parallel) in one reaction, yielding enormous amounts of data. Figure 8 have been created by the mutimedia team under the authors' guidance. Courtesy:1–6: Dr. Pranesh Balasubramanium

Age-related cataract loci are much harder to decode than congenital cataracts. They are usually studied using a combination of model-based and model-free linkage analysis and association studies. Multiple generations of a single family are very difficult to study due to late age at onset. Also these cataracts commonly vary in severity and even appearance, and the mode of inheritance is complex.

5.5 Genes Underlying Isolated or Primary Inherited Cataract

According to Online Mendelian Inheritance in Man (OMIM), at least 42 loci have been identified for inherited forms of isolated orprimary cataract with minimal other ocular signs. They may be divided into four types based on subcellular localization and/or protein function, seen as cytoplasmic crystallins, membrane proteins, cytoskeletal proteins, and DNA/RNA-binding proteins.

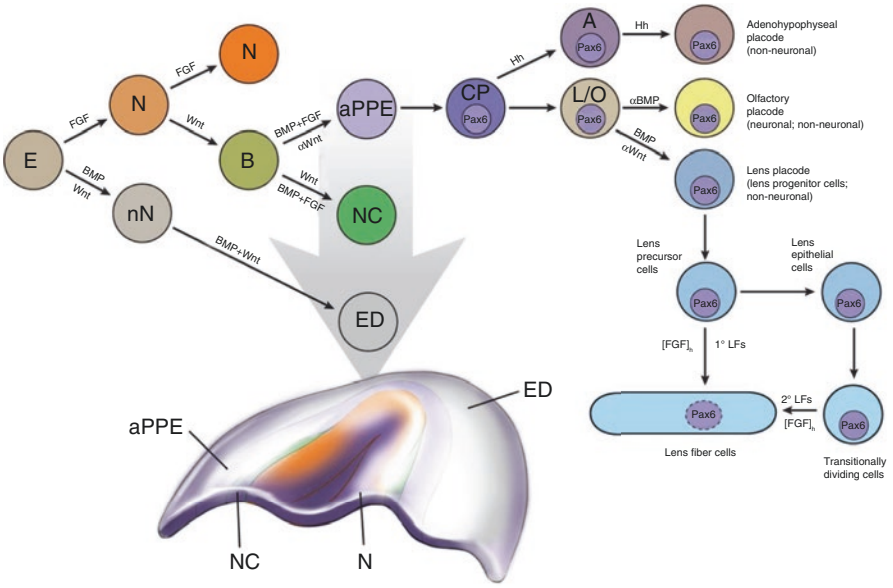


Fig. 5.9 Cell fate decisions during lens induction. Cell fate decisions and differentiation steps that occur prior to and during lens induction and differentiation. The signaling pathways involved (BMP, FGF, and Wnt) as well as the inhibition of specific pathways (indicated by αBMP and αWnt) are shown. Briefly, the induction process involves sequential partitioning of the anterior ectoderm (E) into neural ectoderm (N), non-neural ectoderm (nN), border ectoderm (B), anterior pre-placodal ectoderm (aPPE), neural crest (NC) cells, epidermis (ED), Pax6$^+$ common progenitors (CP), adenohypophyseal progenitors (A) and, finally, a common lens-olfactory progenitor (L/O). The inductive events culminate with the formation of Pax6$^+$ common progenitor cells within the area of the anterior pre-placodal ectoderm. The common pre-placodal progenitors are thus multipotent cells that ultimately produce distinct neuronal and non-neuronal cell types, as shown by the reiterative use of BMP signaling for non-neuronal lens formation and its transitional inhibition (αBMP) required for the formation of the neurogenic olfactory placode. Courtesy: Aleš Cvekl, Ruth Ashery-Padan. The cellular and molecular mechanisms of vertebrate lens development. Development 2014 141: 4432–4447

5.6 Cytoplasmic Cyrstallin Encoding Genes

Hundred different mutations in 12 crystallins genes segregating in over 100 families have been recognized. These make up about 40–50% of all autosomal-dominant cataracts that have been reported thus far. *CRYAA* and *CRYAB* encode α-crystallins, two parts of the small heat-shock protein (sHSP) family; they form large multimeric complexes (Mr ~500 kDa) with chaperone-like properties [22].

Mutations in *CRYAA* are variably related to nuclear-type opacities and microcornea. However, mutations in *CRYAB* are associated with several myopathies consistent with its large expression in muscle where its binds and stabilizes desmin. Mutations in beta and gamma crystallins cause misfolding of proteins which could enable cataractogenesis [23].

5.7 Membrane Proteins Encoding Genes

Transmembrane proteins account for transport, junctional, and kinase functions. *GJA3* and *GJA8* encode the gap-junction proteins *connexin-46* and *connexin-50*, respectively. These oligomerize to produce hexameric gap-junction channels that areinvolved in lens intercellular communication (e.g., ions, electrolytes). Mutations in *GJA3* and *GJA8* are commonly linked with nuclear and zonular-pulverulent opacities. They are responsible for about 20% of families with autosomal-dominant cataract. Functional expression studies demonstrate that mutant connexins exhibit failed targeting to the cell surface and/or alter channel properties which compromise intercellular communication [24].

MIP encodes the aquaporin-0 water channel which along with water transport, plays a critical role for lens integrity and transparency via important cell–cell adhesion. Mutations in MIP underlie autosomal-dominant cataract with varied morphologies largely believed to cause abnormal retention of the mutant protein within the endoplasmic reticulum (ER) [25, 26].

EPHA2 encodes a member of the ephrin receptor subfamily of protein-tyrosine kinases and through its interaction with SRC kinase has been implicated in lens cell migration. Mutations in EPHA2 have been linked with autosomal dominant and recessive forms of cataract consistent with deleterious gain-of-function and loss-of-function mechanisms, respectively. Mutations clustered in the cytoplasmic sterile-alpha-motif domain underlying autosomal-dominant cataract have been shown to destabilize the receptor and impair Akt-activated cell migration in vitro [27–29].

Mutations in AGK have also been linked with “syndromic” cataract including infantile mitochondrial disease and Sengers syndrome.

CHMP4B mutations are associated with posterior-polar cataract shown to have a role for endosome–lysosome pathway in lens homeostasis [30].

WFS1 encodes the transmembrane protein, wolframin, located predominantly in the ER. It playsa crucial role in regulating ER stress and calcium homeostasis. Typically, homozygous mutations in WFS1 underlie autosomal-recessive Wolfram syndrome 1, which entails diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (DIDMOAD) [31].

5.8 Cytoskeletal Protein Encoding Genes

Genes encoding key components of the lens cytoskeleton or proteins that have functional ties to the cytoskeleton are known to be fundamental in inherited cataract. *BFSP1 (CP115 or Filensin*) and *BFSP2 (CP49 or Phakanin*) encode intermediate filament-like proteins that combine with a-crystallin to form beaded-filament structures found only in lens fiber cells. Both autosomal dominant and recessive cataracts are demonstrated with mutations in BFSP1 and BFSP2. Mutations in the gene encoding ubiquitous intermediate filament, vimentin (VIM) has also been demonstrated with autosomal-dominant pulverulent cataract [32].

5.9 DNA- or RNA-Binding Proteins

Mutations in several transcription factor genes involved in eye development can present primarily as a cataract phenotype. HSF4 regulates transcription of sHSPs including lens CRYAB [33]. Mutations in HSF4 that underlie autosomal-dominant cataract lie within the alpha-helical DNA-binding domain.

PITX3 encodes a paired-like homeodomain transcription factor that plays a key role in the regulation of genes involved in lens development including MIP/AQP0 [42]. PITX3 can be responsible for both dominant and recessive forms of cataract with or without anterior segment dysgenesis and microphthalmia.

5.10 Genes Associated with Age-Related Cataract

Several studies using a candidate gene approach have reported coding and noncoding variations in some of the same genes underlying inherited cataract that arealso associated with age-related cataract. These genes include EPHA2 (1p), HSF4 (16q), GJA8 (1q), MIP (12q), LIM2 (19q), GJA3 (13q), and CRYAA (21q) [34–38]. It may be noted however, only the EPHA2 association has been replicated in different populations including with cortical cataract in Caucasians and Han Chinese, with any cataract in Caucasians and Asians from China and India and with cortical cataract and PSC in Indians [34, 39–42].

5.11 Conclusion

Inspite of the increasing genetic heterogeneity, genetic studies in cataract are scientifically and clinically relevant. They will provide a gene centric description of known Mendelian forms of inherited cataract. The morphological features of inherited cataract will aid in diagnosing syndromic cases. The present classification of cataract is predominantly phenotypically driven leading to various interobserver variations. There is also increasing evidence that several genes of inherited cataract can predispose to age-related cataract suggesting that there exists a molecular link between lens development and aging. As exome sequencing becomes cost effective, these evidences will eventually lead us toward personalized medicine with bespoke treatment.

References

1. Pascolini D, Mariotti SP. Global estimates of visual impairment: 2010. Br J Ophthalmol. 2012;96(5):614–8.
2. Sanfilippo PG, Hewitt AW, Hammond CJ, Mackey DA. The heritability of ocular traits. Surv Ophthalmol. 2010;55:561–83.
3. Smelser GK. Embryolohy and morphology of the lens. Investig Ophthalmol Visual Sci. 1965;4:398–410.

4. Pierce GB, Midgley AR, Sri Ram J. The epithelial origin of basement membranes. J Exper Med. 1963;117:339.
5. Kamachi Y, Uchikawa M, Collignon J, Lovell-Badge R, Kondoh H. Involvement of sox1, 2 and 3 in the early and subsequent molecular events of lens induction. Development. 1998;125:2521–32.
6. Furuta Y, Hogan BL. BMP4 is essential for lens induction in the mouse embryo. Genes Dev. 1998;12:3764–75.
7. Graw J. Eye development. Curr Topics Dev Biol. 2010;90:343–86. https://doi.org/10.1016/s0070-2153(10)90010-0.
8. Titiyal JS, Pal N, Murthy GV, Gupta SK, Tandon R, Vajpayee RB, et al. Causes and temporal trends of blindness and severe visual impairment in children in schools for the blind in North India. Br J Ophthalmol. 2003;87:941–5.
9. Shamanna BR, Dandona L, Rao GN. Economic burden of blindness in India. Indian J Ophthalmol. 1998;46:169–72.
10. Gilbert C, Foster A. Childhood blindness in the context of VISION 2020 – the right to sight. Bull World Health Organ. 2001;79(3):227–32.
11. Foster A. Worldwide blindness, increasing but avoidable. Semin Ophthalmol. 1993;8(3):166–70.
12. Rahi JS, Scripathi S, Gilbert C, Foster A. Childhood blindness in India: causes in 1318 blind school students in nine states. Eye (Lond). 1995;9(5):545–50.
13. Apple DJ, Ram J, Foster A, Peng Q. Elimination of cataract blindness: a global perspective entering the new millennium. Surv Ophthalmol. 2000;45(Suppl 1):S1–196.
14. Messina-Baas OM, Gonzalez-Huerta LM, Cuevas-Covarrubias SA. Two affected siblings with nuclear cataract associated with a novel missense mutation in the CRYGD gene. Mol Vis. 2006;12:995–1000.
15. Rahi JS, Dezateux C. National cross sectional study of detection of congenital and infantile cataract in the United Kingdom: role of childhood screening and surveillance. The British Congenital Cataract Interest Group. BMJ. 1999;318(7180):362–5.
16. Beby F, Morle L, Michon L, Bozon M, Edery P, Burillon C, et al. The genetics of hereditary cataract. J Fr Ophtalmol. 2003;26(4):400–8. French.
17. Hejtmancik JF. Congenital cataracts and their molecular genetics. Semin Cell Dev Biol. 2008;19:134–49.
18. Cvekl A, Duncan MK. Genetic and epigenetic mechanisms of gene regulation during lens development. Prog Retin Eye Res. 2007;26:555–97.
19. Santana A, Waiswo M. The genetic and molecular basis of congenital cataract. Arq Bras Oftalmol. 2011;74:136–42.
20. Huang B, He W. Molecular characteristics of inherited congenital cataracts. Eur J Med Genet. 2010;53:347–57.
21. Reddy MA, Francis PJ, Berry V, Bhattacharya SS, Moore AT. Molecular genetic basis of inherited cataract and associated phenotypes. Surv Ophthalmol. 2004;49:300–15.
22. Clark AR, Lubsen NH, Slingsby C. sHSP in the eye lens: crystallin mutations, cataract and proteostasis. Int J Biochem Cell Biol. 2012;44:1687–97.
23. Shiels A, Hejtmancik JF. Molecular genetics of cataract. Prog Mol Biol Transl Sci. 2015;134:203–18. https://doi.org/10.1016/bs.pmbts.2015.05.004.
24. Beyer EC, Ebihara L, Berthoud VM. Connexin mutants and cataracts. Front Pharmacol. 2013;4:43.
25. Shiels A. Focus on molecules: major intrinsic protein. Exp Eye Res. 2012;101:107–8.
26. Lo WK, Biswas SK, Brako L, Shiels A, Gu S, Jiang JX. Aquaporin-0 targets interlocking domains to control the integrity and transparency of the eye lens. Investig Ophthalmol Vis Sci. 2014;5:1202–12.
27. Park JE, Son AI, Hua R, Wang L, Zhang X, Zhou R. Human cataract mutations in EPHA2 SAM domain alter receptor stability and function. PLoS One. 2012;7:e36564.
28. Shi Y, De Maria A, Bennett T, Shiels A, Bassnett S. A role for epha2 in cell migration and refractive organization of the ocular lens. Investig Ophthalmol Vis Sci. 2012;53:551–9.

29. Cheng C, Ansari MM, Cooper JA, Gong X. EphA2 and Src regulate equatorial cell morphogenesis during lens development. Development. 2013;140:4237–45.
30. Shiels A, Bennett TM, Knopf HL, et al. CHMP4B, a novel gene for autosomal dominant cataracts linked to chromosome 20q. Am J Hum Genet. 2007;81:596–606.
31. Berry V, Gregory-Evans C, Emmett W, et al. Wolfram gene (WFS1) mutation causes autosomal dominant congenital nuclear cataract in humans. Eur J Hum Genet. 2013;21:1356–60.
32. Muller M, Bhattacharya SS, Moore T, et al. Dominant cataract formation in association with a vimentin assembly disrupting mutation. Hum Mol Genet. 2009;18:1052–7.
33. Somasundaram T, Bhat SP. Developmentally dictated expression of heat shock factors: exclusive expression of HSF4 in the postnatal lens and its specific interaction with alphaB-crystallin heat shock promoter. J Biol Chem. 2004;279:44497–503.
34. Shiels A, Bennett TM, Knopf HL, et al. The EPHA2 gene is associated with cataracts linked tochromosome 1p. Mol Vis. 2008;14:2042–55.
35. Shi Y, Shi X, Jin Y, et al. Mutation screening of HSF4 in 150 age-related cataract patients. Mol Vis. 2008;14:1850–5.
36. Zhou Z, Wang B, Hu S, Zhang C, Ma X, Qi Y. Genetic variations in GJA3, GJA8, LIM2, and agerelatedcataract in the Chinese population: a mutation screening study. Mol Vis. 2011;17:621–6.
37. Zhou Z, Wang B, Luo Y, et al. Major intrinsic protein (MIP) polymorphism is associated with agerelatedcataract in Chinese. Mol Vis. 2011;17:2292–6.
38. Validandi V, Reddy VS, Srinivas PN, et al. Temperature-dependent structural and functional properties of a mutant (F71L) alphaA-crystallin: molecular basis for early onset of age-relatedcataract. FEBS Lett. 2011;585:3884–9.
39. Jun G, Guo H, Klein BE, et al. EPHA2 is associated with age-related cortical cataract in mice andhumans. PLoS Genet. 2009;5:e1000584.
40. Tan W, Hou S, Jiang Z, Hu Z, Yang P, Ye J. Association of EPHA2 polymorphisms and age-relatedcortical cataract in a Han Chinese population. Mol Vis. 2011;17:1553–8.
41. Sundaresan P, Ravindran RD, Vashist P, et al. EPHA2 polymorphisms and age-related cataract in India. PLoS One. 2012;7:e33001.
42. Yang J, Luo J, Zhou P, Fan Q, Luo Y, Lu Y. Association of the ephreceptor tyrosinekinase-type A2(EPHA2) gene polymorphism rs3754334 with age-related cataract risk: a meta-analysis. PLoS One. 2013;8:e71003.

Genetics of Congenital Glaucoma

6

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6.1 Introduction

Derived from the Greek work glaukos (meaning bluish-green or gray color), glaucoma represents the leading cause of irreversible blindness worldwide [1]. It is characterized by the degeneration of retinal ganglion cells (RGCs) in a sequential manner leading to optic neuropathy with concomitant loss of visual field. Conventionally, vision loss starts peripherally trailed by the central fields and could progress to blindness, if left untreated. Individuals at any age group can be affected with glaucoma associated vision damage. Albeit it is an uncommon condition in the infancy and further stages of childhood, however, it is graver in nature impacting the visual development. The magnitude of glaucoma linked blindness as estimated by World Health Organization (WHO) is 4.5 million people. In Indian scenario, estimates showed that at least 12 million people are affected by glaucoma with approximately 1.2 million people had glaucoma associated blindness.

Vision impairment and blindness continued to be a public health issue across the globe, where estimates from India with respect to childhood blindness range from 0.5/1000 to 1.06/1000 [2]. Indeed, diminished vision or blindness at childhood age impacts on the growth, development, emotional conduct, and socioeconomic prospects, as approximately 75% of learning emanates through vision.

Glaucoma in the childhood can be divided as primary or secondary, where primary refers to the isolated developmental anomalies of anterior chamber filtration angle while secondary results in reduced outflow of aqueous humor as an independent subordinate mechanism along with other associated ocular or systemic

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abnormalities. Based on age of onset, primary glaucoma can be again divided into primary congenital glaucoma (PCG)—seen from birth to 3 years of age or early childhood and juvenile open-angle glaucoma (JOAG)—4 years to early adulthood. In primary congenital glaucoma, there exists a maldevelopment of trabecular meshwork structure solely ensuing to aqueous drainage impediment, thus increase in intraocular pressure (IOP). Genetics contributes one among the risk factors and has been evidenced by several studies with regard to various glaucoma subtypes. Indeed, eclectic nature of glaucoma with several associated genes and variants underlying the pathogenesis of different subtypes, making it a complex entity. Overall, vision deterioration affects the individual's quality of life. Appropriate timely recognition of disease by means of clinical and molecular diagnosis is of great importance to identify and treat avoidable blindness. This chapter is envisioned to provide an updated and comprehensive overview of clinical manifestations, epidemiology, pathophysiology, and genetic aspects of primary congenital glaucoma.

6.2 Primary Congenital Glaucoma (PCG)

6.2.1 Disease Overview and Definition

Primary congenital glaucoma (PCG) is a rare, genetically determined ocular disease, with abnormalities in the aqueous outflow pathways of the anterior chamber angle resulting in elevated intraocular pressure, in the absence of other ocular or systemic developmental anomalies. Earlier, several terms such as trabeculodysgenesis, goniodysgenesis, and primary infantile glaucoma have been used to describe this condition. International Classification of Childhood Glaucoma (2013) has replaced these variable terminologies with PCG. Based on age of onset, the variants of PCG included

- Newborn onset (0–1 month)
- Infantile onset (>1–24 months)
- Late onset or late recognized (>24 months)

Primary congenital glaucoma accounts for most of childhood glaucoma. It commonly manifests between the ages 3 and 9 months, and the new born onset PCG is the most severe form of presentation. Elevated IOP is often associated with the classic triad of photophobia, epiphora, and blepharospasm due to rapid expansion of the child's ocular coats causing Buphthalmos, corneal enlargement, Haab striae (breaks in Descemet membrane) with consequent corneal edema and opacification due to scarring. PCG, though accounts for less than 0.01% of all eye diseases, it causes 5% childhood blindness across the world. Vision loss is due to corneal scarring, optic nerve damage, and amblyopia. The prognosis of visual outcome is variable in children with PCG, those with milder disease or detected and treated earlier achieving vision 20/60 or better, whereas those with severe forms of the disease often manifest at birth cause blindness.

6.2.2 Epidemiology

Since primary congenital glaucoma is a relatively rare ocular disorder, large-scale epidemiologic studies are not available. Childhood glaucoma seems to affect more than 330,000 children worldwide, most with significant loss of vision and close to two-thirds blind [3]. In the USA and Europe, it has an incidence of about 1 in 10,000 to 68,000 live births. Reports from Sub Saharan Africa indicate that PCG accounts for 0.7–4% of all newly diagnosed glaucoma. PCG is ten times more common in ethnic and religious groups where consanguinity is very common [4]. The prevalence is higher, with more severe phenotypes reported in developing countries. In India, PCG is observed in 1 in 3300 live births and accounts for 4.2% of all childhood blindness [5]. In India, PCG typically presents with severe phenotype and near total corneal edema and scarring [6]. Highest known prevalence of PCG has been reported in Saudi Arabia (1 in 2500) and in Gypsies of Slovakia (1 in 1250) [7].

6.2.3 Clinical Presentation

Corneal epithelial edema that accompanies elevated IOP in PCG causes tearing, photophobia, and blepharospasm. Corneal edema also causes initially intermittent and then persistent haziness of the cornea, and precedes breaks in Descemet membrane, called *Haabs' striae*. Ocular enlargement, mainly at the corneo-scleral limbus under the influence of elevated IOP is characteristic of childhood glaucomas due to immature scleral collagen. As the cornea is continually stretched under the impact of persistently elevated IOP, breaks in Descemet's membrane occur, allowing influx of aqueous humor into the corneal stroma and epithelium, increasing corneal edema and haziness, with increase in symptoms of tearing and photophobia. Haabs striae are either single or multiple, appearing as parallel ridges on the posterior aspect of the cornea (referred to as the "rail-road track sign," presenting as curvilinear ridges concentric with the limbus and across the visual axis. Though corneal endothelium eventually heals over the breaks, and corneal edema resolves, it usually results in irregular astigmatism.

When IOP is uncontrolled, continued corneal enlargement and tears in Descemet membrane may lead to corneal scarring, erosions, and ulcerations. Continued enlargement of the globe is less common after the age of 3–4 years, though the posterior sclera remains elastic and progressive lengthening of the globe posteriorly with increased axial length and myopia is common. Increasing myopia is a sign of progressive glaucoma in young children.

Continued enlargement of the globe with uncontrolled glaucoma or high IOP is uncommon after the age of 3–4 years, though elasticity of posterior sclera can still cause progressive myopia. Pain and photophobia are uncommon in older children. Increased cupping of optic disks and visual field defects are more commonly seen with progressive glaucoma in older children.

6.2.4 Examination Under Anesthesia

Evaluation under anesthesia is generally performed to obtain details of clinical presentation when children with glaucoma present prior to the age of five and consist of measuring IOP, assessing cornea thickness and diameter, gonioscopy, ophthalmoscopy, axial length measurements, cycloplegic retinoscopy, and ultrasound biomicroscopy. In children with opaque corneas due to corneal edema or scarring, B Scan ultrasonography may also be indicated.

6.2.5 Pathophysiology of Primary Congenital Glaucoma

The normal development of the infant anterior chamber angle has been described by Anderson [8]. At about 5 months of gestation, the anterior surface of the iris meets the corneal endothelium to form the peripheral part of the anterior chamber angle. The cells destined to form the trabecular meshwork are posterior to the junction of the corneal endothelium and the iris. Ciliary muscles and ciliary processes enmeshed in loose connective tissue overlap the developing trabecular meshwork. The developing angle recess deepens and moves posteriorly due to differential tissue growth, exposing the trabecular meshwork to the anterior chamber angle by a process of posterior sliding of the iris, ciliary muscles and processes. The normal development of the anterior chamber of the eye is complete only by the first 6–12 months after birth. The various cells forming the anterior chamber angle structures are derived from neural crest by differentiating and migrating. Significant embryologic development of the anterior chamber angle occurs in the third trimester, although embryologic insults in first few weeks following fertilization can cause anterior chamber development anomalies and tissue dysgenesis. The anterior chamber angle of the eye, with peripheral iris and ciliary body in primary congenital glaucoma resembles that of the eye in the 7–8 months of gestation, due to a development arrest. The iris and ciliary body fail to recede posteriorly and resultant iris insertion and anterior aspect of ciliary body appear continuous with and overlap the posterior position of the trabecular meshwork. Anderson's observations have confirmed the findings of Maumenee's histologic studies which had shown an anterior insertion of ciliary body muscles. Longitudinal and circular muscles of the ciliary body insert into the trabecular meshwork rather than the scleral spur. Root of the iris was also observed to directly insert into the trabecular meshwork [9].

Anderson had also suggested that the apparent membrane described earlier by Barkan and Worst (Barkan's membrane) was composed of the thickened, compact trabecular beams in the area of the meshwork, which gives the appearance of a membrane at a relatively low magnification of gonioscopy and operating microscopes. Extensive histologic studies by both Anderson and Maumenee have failed to reveal the existence of a discrete Barkan's membrane in the infant anterior chamber angle. Clinical evidence supports the theory that the obstruction to aqueous outflow in primary congenital glaucoma is at the level of trabecular sheets. Incision of the trabecular sheets in goniotomy surgery, or opening of the Schlemm's canal

directly into the anterior chamber by trabeculotomy relieves the obstruction and normalizes IOP. In angle surgeries such as these, the iris falls posteriorly and relieves the compaction of the trabecular beams and permits inter-trabecular spaces to open [10].

6.3 Genetic Architecture of Primary Congenital Glaucoma (PCG)

Frequent occurrence of congenital glaucoma in the people belonging to specific ethnicities made investigators to ponder about the role of genetic elements that underlie disease phenotypes. As stated by Westerlund, among the first references for the familial incidence of congenital glaucoma was acknowledged by Grelios in 1836, where he observed the endemic occurrence of disease in the Jewish population of Algiers [11, 12]. Later, another report attributed to Junken in 1842 by describing a Swedish family in which seven brothers manifested congenital glaucoma phenotype while parents and two sisters were normally sighted which gives further allusion toward the existence of an autosomal recessive pattern of inheritance [12]. Primary congenital glaucoma (PCG) accounts for about half of the glaucoma cases in childhood and earlier noted observations by Grelios and Junken shed light on inherited nature of PCG disease. Contribution of genetics for PCG etiology is an established fact, though its sporadic form is mostly reported.

6.3.1 Pattern of Inheritance for PCG

As far as inheritance is concerned, autosomal recessive pattern in familial cases (10–40%) is mostly seen with variable penetrance (40–100%) [11, 13, 14]. Conceptually, in autosomal recessive condition, both parents carry mutation in heterozygous state (carriers) and without overt disease phenotype. Autosomal recessive inheritance is particularly observed in ethnic groups including Saudi Arabians [7], Turks [14], and Slovak Roms [15] where higher incidence has been reported. In several cases of familial PCG, consanguinity in parents reported at higher rate which further correlates with the higher PCG incidence in those families as compared to the secondary congenital glaucoma cases [16]. Further studies supporting the genetic basis for PCG explained by the higher concordance rate in monozygotic twins and discordance for dizygotic ones [13, 15].

Indeed, autosomal recessive pattern is mostly seen in familial cases; however, real scenario is bit complex, where bequest of PCG has been questioned. One of the reasons behind this is the variable penetrance in some families ranging from 90 to 100% to low penetrance in some with a smaller number of affected siblings observed than anticipated in native recessive form. Other is unequal gender distribution, where affected boys seen two times more frequent than the girls, thus supporting the multifactorial inheritance. This speculation again corroborated by the findings of Merin and Morin in which 64 families were studied and revealed the likely

polygenic or multifactorial heredity for congenital glaucoma [17]. Pedigrees with autosomal dominant pattern have also been observed [18]. Because of higher consanguinity rate and inbreeding practice in some ethnic groups, pseudo-dominance pattern has also been observed [12]. These discrepancies showed the existence of genetic heterogeneous nature of the disease [19–21] with variable penetrance and expressivity across diverse populations [22].

6.3.2 Genetic Loci for Primary Congenital Glaucoma

Substantial progress toward the understanding of PCG genetics has been enabled by the introduction of genetic linkage studies and positional cloning. These methods require the precise details of the disease phenotype and larger families who inherit disease as Mendelian traits. For the identification of loci associated with glaucoma, a specific nomenclature was made by the Human Genome Organization (HUGO) [23, 24]. In general, "GLC" stands for gene loci in glaucoma; "1, 2 and 3 numbers denote the respective primary glaucoma subtypes (open-angle, closed-angle and congenital/infantile glaucoma)"; while "A, B, C and D indicates the order of the mapped genes." Herein, we will be focusing on the GLC3 loci referring to PCG disease.

6.3.3 PCG Related GLC3 Loci

Efforts have been made to discover the PCG associated loci and starting from 1995 till 2008, four genetic loci have been identified for PCG which includes GLC3A (2p21), GLC3B (1p3), GLC3C (14q24.3), and GLC3D (14q24). In parallel, research groups also focused to determine the potential candidate genes in these loci. Later, fifth locus GLC3E (9p21) was also defined related to PCG. Table 6.1 depicts the overview of the identified PCG associated loci and candidate genes so far.

Table 6.1 Primary congenital glaucoma (PCG) associated genetic loci and genes

Locus	Chr position	Phenotype MIM No.	Gene MIM No.	Inh.	GenBank Accession No.
GLC3A	2p21	231300	CYP1B1 (601771)	AR	NM_000104.3
GLC3B	1p36	600975	–	AR	–
GLC3C	14q24.3	613085	–	AR	–
GLC3D	14q24.2-24.3	613086	LTBP2 (602091)	AR	NM_000428.2
GLC3E	9p21	617272	TEK/TIE2 (600221)	AD	NM_000459.4

Chr Chromosome, *Inh.* Inheritance, *CYP1B1* Cytochrome P4501B1(see Sect. 6.4), *LTBP2* Latent-transforming growth factor beta binding protein 2 (see Sect. 6.6), *TEK* Tunica interna endothelial cell kinase (see Sect. 6.7; Angiopoietin-1 receptor), *AR* Autosomal Recessive, *AD* Autosomal Dominant

GLC3A The very first genetic locus GLC3A linked for PCG mapped at chromosomal location 2p21 identified through linkage analysis of the families affected with PCG [25]. This study involved 17 Turkish families having bilateral PCG within 6 months after birth and lacking other abnormalities. Furthermore, study revealed homogeneity to the associated markers at region 2p21-22 by 85% of the families representing it as a main PCG-linked locus. As the name indicates GLC3 "A" meant for first congenital glaucoma related locus as per the HUGO nomenclature. Later, association of GLC3A locus with PCG phenotype was also confirmed in large families belonging to Saudi Arabia and Gypsies of Slovakia [7, 26]. Consequently, all the research findings and evidences accumulated through different ethnic studies confirmed the speculation regarding linkage of GLC3A with PCG, which possibly accounts for 85–90% of familial cases.

GLC3B Subsequently, a second locus was mapped on chromosome 1p36 in a study involving eight families (seven Turkish and one Canadian) with PCG phenotype. Since, no association was identified at primary 2p21 locus in these families; further treads were taken to explore another new locus for PCG, which finally led to the identification of this second locus GLC3B. However out of eight families, four families did not link to this region on chromosome 1, suggesting the presence of another locus for recessive form of PCG [27].

GLC3C Some years later after the identification of two PCG loci, Stoilov and Sarfarazi studied a five-generation consanguineous family unlinked to GLC3A and GLC3B loci to unravel additional PCG associated genetic locus through genome-wide screening of 235 polymorphic markers. This work demonstrated the presence of third locus GLC3C on 14q24.3 chromosomal region [28].

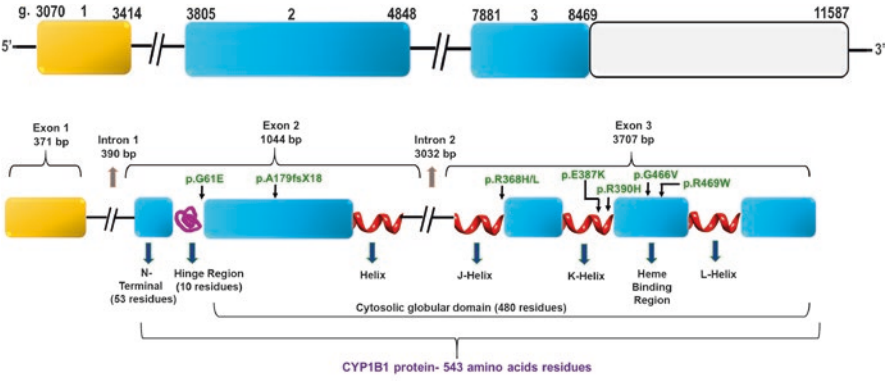
GLC3D Firasat et al. reported another locus GLC3D mapped to chromosome 14q24 for recessive PCG in two consanguineous families from Pakistan which yet again added to the genetic repertoire for PCG [29].

6.3.4 Candidate Genes Identification for the PCG-Linked Loci

Probing for potential disease-causing genes, one can think of basic approaches. Firstly, either expression or function of a presumed gene substantiates with the disease phenotype and pathology for its candidature; or secondly, positional cloning as an alternative choice where position of candidate gene narrowed down amidst the genetic locus under study, which is actually challenging. However, first method often hindered by the inadequate information in terms of gene function, expression and its causal mechanism toward the development of disease phenotype. Using conventional molecular and genetic methods (linkage analysis, positional cloning, etc.), so far three candidate genes linked with PCG pathogenesis have been discovered (see Table 6.1) which will be discussed in consecutive sections.

6.4 Cytochrome P4501B1 (*CYP1B1*)—"GLC3A"

Identification of PCG loci made a pathway for the researchers to further hunt for the definite disease related genes. Ensuing earlier studies, Stoilov and colleagues in 1997 identified the first candidate gene responsible for PCG pathogenesis [30] using positional cloning approach (see Fig. 6.1). This gene was cytochrome P4501B1 (*CYP1B1*) which mapped to the candidate region of GLC3A (2p21). Three different mutations in affected families were detected on sequencing of the gene. These mutations were also segregated in five families with PCG phenotype which were earlier related to locus GLC3A. Subsequently, many other reports from different ethnicities including Saudi Arabia, Turkey, United Kingdom, Canada, Slovaks, and Japanese showed the association of *CYP1B1* mutations in PCG families [7, 31–34].



EXON 2 Mutations

p.Q19*	p.V84F	p.M132R	p.L181P	p.S239R	p.I297N
p.L24R	p.Q86K	p.M132T	p.F190L	p.D242A	p.N319S
p.L26R	p.L89P	p.A133T	p.F190L	p.D242N	p.V320L
p.L27Q	p.G90D	p.F134S	p.D192Y	p.M244L	p.F328L
p.G36D	p.V95A	p.Q144R	p.D192V	p.Q248*	p.G329D
p.Q37*	p.V96G	p.Q144P	p.P193L	p.F261L	p.G329V
p.Q42*	p.L97P	p.F156C	p.V198I	p.E262*	p.A330T
p.P52L	p.A106D	p.Q159*	p.A202D	p.H279D	p.S331I
p.G53S	p.L107V	p.R163C	p.N203S	p.H279L	p.S336Y
p.A56V	p.Q110*	p.Q164*	p.C209R	p.H279P	p.A338P
p.W57C	p.A115P	p.G168D	p.G211D	p.C280*	p.Q340H
p.W57X	p.D116Y	p.E173K	p.S215I	p.E281*	p.W341*
p.L59*fs	p.R117Q	p.E173*	p.D218H	p.S282N	268delSNF
p.I60M	p.R117P	p.R175P	p.E229K	p.L283F	p.I194X
p.G61E	p.R117W	p.L177R	p.F231I	p.A287T	376inA
p.L77P	p.P118S	p.L177P	p.G232R	p.A288P	4339delG
p.Y81N	p.F123L	p.V178E	p.V235A	p.R290C	4340delG
p.Y81*	p.S131R	p.L181Q	p.A237E	p.D291G	

EXON 3 Mutations

p.P350T	p.E387K	p.R444*
p.R355*	p.A388T	p.F445C
p.L360L	p.R390C	p.F445I
p.V363D	p.R390H	p.F445S
p.V364M	p.R390S	p.S464P
p.G365E	p.I399S	p.G466D
p.G365W	p.P400S	p.G466S
p.R368C	p.H401D	p.G466V
p.R368H	p.N423Y	p.R469W
p.R368L	p.D430E	p.C470Y
p.C371F	p.W434*	p.I471S
p.D374N	p.P437A	p.S476P
p.D374E	p.P437L	p.Q479R
p.L378Q	p.D441G	p.L480P
p.P379L	p.P442R	p.L487P
p.Y380C	p.P442L	p.N498D
p.A383T	p.A443G	p.E499G
p.L385F	p.R444Q	p.N519S
8037dup10	8182delG	p.R523K

Fig. 6.1 Schematic representation for *CYP1B1* gene, protein structure, and spectrum of reported mutations. Predominant mutations across various ethnic groups marked in green

6.4.1 CYP1B1 Protein and Expression

CYP1B1 encodes cytochrome P450 1B1 protein (cytochrome P450, family 1, subfamily B, polypeptide 1) belonging to CYP450 superfamily which are heme-binding monooxygenases [30, 35, 36]. These CYP450 have absorption at 450 nm and consist of a heme group [37]. They are reported to be localized on membrane of endoplasmic reticulum or mitochondrial inner membrane [38]. CYP1B1 is involved in metabolism of substrates including endogenous (such as estradiol, steroids, retinals, arachidonic acid) and exogenous ones, cell signaling, catalyzes NADPH-dependent monooxygenation of various xenobiotics [36, 38–41]. Besides, it is also capable to metabolize all-trans-retinol (vitamin A) into all-trans-retinal and all-trans-retinoic acid [42, 43]. Among the CYP450 superfamily, it was the first instance where mutations in human *CYP1B1* associated with primary developmental defect [30, 44]. Importance of CYP1B1 to metabolize retinoic acid (RA) which is crucial for ocular development also been suggested to have implications in PCG pathology [45, 46]. Additionally, arachidonate which is a cytochrome-P450-dependent metabolite has been reported for inhibiting corneal Na^+-K^+-ATPase having a vital role in regulating the transparency of cornea and aqueous secretion. Noteworthy, concordance of this research finding is with the key diagnostic phenotypes seen for PCG patients such as corneal clouding and increased intraocular pressure [47].

Concerning the expression pattern, Sutter et al. reported the human CYP1B1 protein expression in different non-ocular tissue samples [39]. Lacking the knowledge of protein expression in ocular tissues, it was tough to construe the role of CYP1B1 in the congenital glaucoma pathogenesis. Later, immunohistochemical and immunolocalization studies reported the CYP1B1 expression in various tissues of eyes which includes iris, corneal epithelium, retina, ciliary body, and trabecular meshwork (TM) in addition to nucleus of kidney tubule cells, breast secretory cells [48–51]. However, no expression of CYP1B1 in the TM was also suggested along with its significant association in the development of human fetal eyes which is supported by the higher expression of CYP1B1 in fetal ocular tissues than adult ones [52, 53]. Overall, these observations suggest that CYP1B1 might have significance in the ocular tissue development and their maturation [51, 54–56]. On the contrary, in case of ocular tissues of the mouse, scenario is reverse which was demonstrated by Choudhary and colleagues. Immunohistochemical analysis of CYP1B1 protein in mouse eye revealed increased expression with ascending age suggesting the different expression patterns in humans and mouse [56].

6.4.2 Structural Components of CYP1B1

CYP1B1 gene consists of three exonic regions and two introns, of which first exon is non-coding. With ORF of 1629 bp, 543 amino acid long CYP1B1 protein encoded by exon 2 and 3 [57] (see Fig. 6.1). This protein comprises a membrane-bound hydrophobic region at amino terminal, proline rich hinge region of ten residues, and a 480 amino acid globular domain at cytosolic site. Proline residues in peptide chain

aids in flexibility to the structure and mutations inducted in this region affect proper folding along with property of heme-binding by other CYP450 molecules [58]. It is noteworthy, highly conserved C-terminal regions of CYP450 family consist of conserved core structures (CCS) or domains which are responsible for binding of heme group by these molecules. This heme-binding region is vital and truncating mutations at this essential region might abolish it, perhaps resulting into functional null alleles [48]. Moreover, enzymatic activity and stability could also be impaired because of *CYP1B1* mutations besides affecting the protein localization to the mitochondrial membrane as well [59, 60]. Further studies are on the way to understand the functional importance of *CYP1B1* mutations in PCG disease pathogenesis.

6.4.3 *CYP1B1* Mutational Landscape of PCG

Cytochrome P4501B1 (*CYP1B1*) gene mutations have been acknowledged as the prime molecular cause for primary congenital glaucoma. A wide landscape of PCG associated mutations in the *CYP1B1* gene been known with existing clinical and genetic heterogeneity. So far, more than 150 distinct mutations (including missense, nonsense, and indels) have been reported in the *CYP1B1* gene across various countries with varied prevalence [61]. It is 20% for Japanese PCG cases, 44% for Indians, 33.3% among Indonesians, and 50% in case of Brazilians [61]. For the patients belonging to Morocco, it is about 47.7%, 14.9% for the USA, and about 75.9% for Saudi Arabians. Predominant *CYP1B1* mutations have been observed in the PCG patients belonging to Saudi Arabia and Gypsies due to practice of consanguineous marriages and inbreeding. Moreover, mutations in this gene constitute about 87% familial and 27% of sporadic cases [12, 61]. Mostly, missense mutations are reported, either at hinge or cytosolic region having the conserved core structures of the CYP1B1 protein, thus affecting protein function. Comprehensive list of mutations across the *CYP1B1* gene exons has been reviewed by Li et al. [62]. Schematic representation of *CYP1B1* gene and protein structure marked with the reported mutations (including mostly missense) associated with PCG is summarized in Fig. 6.1. Though, this is not a complete list, worldwide mutation list can be viewed from various databases including LOVD (Leiden Open Variation Database) and HGMD (Human Gene Mutation Database).

Variability in the expression and penetrance of *CYP1B1* gene mutations has been observed. To exemplify, PCG in newborns is more likely to be associated with *CYP1B1* mutations, while there might be reduced severity in the disease phenotype by the same mutation at childhood stage [24]. Factors such as disease severity, bilaterality, presence of family history, and consanguinity increase the possibility for the identification of mutations linked to disease pathogenicity.

Concerning the distribution of *CYP1B1* mutations in Indian population, published literature showed the varied frequency of predominant mutations among different ethnic backgrounds due to heterogeneity in Indian population. Several mutations including both novel and reported ones have been revealed through various research groups from India (see Table 6.2).

Table 6.2 Overview of the PCG associated *CYP1B1* mutation studies from Indian population

Study details	Identified mutations	Additional information
Identification of Novel Mutations Causing Familial Primary Congenital Glaucoma in Indian Pedigrees Panicker et al. [63] Recruited 5 consanguineous PCG families—22 members Identified five different mutations in eight patients	*Novel mutations* Frameshift Hom mutation: 376insA (Ter@223) Compound Het Missense: P193L & E229K *Reported Mutations* Hom Missense mutations: G61E & R368H	• **376insA:** Premature Stop codon leading to Truncation of ORF Ter@223—eliminated all CYP1B1 domains resulting in functional null allele (natural functional CYP1B1 knockout). Patient with this mutation were blind • **P193L & E229K:** Daughter (Proband) and Mother—two affected generations with varying severity of disease
Mutation spectrum of the CYP1B1 gene in Indian primary congenital glaucoma patients Reddy et al. [64] Recruited 64 unrelated cases of PCG from different ethnic groups of India Identified 16 pathogenic mutations (including 7 novel) in 24 cases	*Novel mutations* Frameshift mutations: c.100_122del23 (H34Afs*182; Del of 23bp) c.1063_1064delCG (R355Sfs*19; Del of 2bp) Missense mutations: A115P, M132R. Q144P, S239R, G466D	• **G466D:** residue is a part of the "signature sequence" (NH2-FXXGXXXCXG-COOH), present in all heme-binding cytochromes; Poor visual prognosis seen in the proband • Observed lower *CYP1B1* mutation frequency than Saudi Arabian, Gypsies, and Turkish populations—genetic heterogeneity and ethnic diversities **Genotype–phenotype correlation**—Variable prognosis observed • **Q144P:** Proband had better prognosis with normal IOP and improved visual acuity • **R368H:** Most prevalent; relatively better visual prognosis for hom and het allele; Probands-compound het for R368H either with G61E/R390H-intervened at later ages
Molecular genetic analysis of a consanguineous south Indian family with congenital glaucoma: relevance of genetic testing and counseling Ramprasad et al. [65] Recruited consanguineous South Indian Family with congenital glaucoma	*Novel mutation* Truncation hom mutation: Q110*	• **Q110*:** Present in the presumed active site of the protein which is adjacent to the critical hinge region (near N-terminal). • **Truncation of crucial protein motifs:** I-Helix, K-Helix, Meander, Heme binding regions

(continued)

Table 6.2 (continued)

Study details	Identified mutations	Additional information
Mutation spectrum of CYP1B1 in North Indian congenital glaucoma patients Tanwar et al. [66] Recruited 50 patients with congenital glaucoma and 50 controls Screened for 6 prevalent mutations: Ter@223, G61E, R390C, R368H, P193L, and E229K	*Novel Mutations* Missense mutations: L24R, F190L & G329D *Reported Mutations* Ter@223, R390H & R368H	Percentage of cases identified with mutations: • **Ter@223**—18% • R390H in 16% • R368H in 8% • **Ter@223:** Most prevalent mutation in this study • **R368H:** Most prevalent in south India • Variable frequency seen—due to the heterogeneous Indian population
Identification of four novel cytochrome P4501B1 mutations (p.I94X, p.H279D, p.Q340H, and p.K433K) in primary congenital glaucoma patients Tanwar et al. [67] Recruited 23 unrelated PCG patients and 50 controls	*Novel mutations* I94X (Hom), H279D (Het), Q340H (Het), K433K *Reported mutations* Pathogenic mutations E229K, R355X, R368H, R390C, R390H *6 Reported SNPs*: rs2617266, rs10012, rs1056827, rs1056836, rs1056837, rs1800440	• **I94X (Hom)—**lacks all functional domains of CYP1B1 protein • **R355X—**produces 354 amino acids truncated protein resulting into loss of the heme-binding domain (functionally inactive protein) • **Truncating mutations:** I94X and R355X associated with most severe disease phenotype
Candidate *Gene Analysis Identifies Mutations in CYP1B1 and LTBP2 in Indian Families with Primary Congenital Glaucoma* Yang... P. Sundaresan. [68] Recruited eight Indian PCG families—WES	*Novel mutation* Missense mutation (Hom): G466V *Reported mutations* Missense mutations (Hom): R469W, P400S, R368H	This study further expands the mutation landscape of PCG in the Indian population

Het Heterozygous, *Hom* Homozygous, *CD Ratio* cup-to-disk ratio, *WES* Whole-exome sequencing

6.4.4 Predominant PCG Associated *CYP1B1* Mutations in Different Ethnic Populations

While looking at the overall mutations distribution of *CYP1B1* gene, the missense mutation G61E predominates followed by R368H/L, R390H, E387K, and R469W. This sequence further trailed by 8037dup10, 4340delG, 4490G>A, R390C/S, and 4339delG mutations [62].

Asians Literature reviewed in a study by Li et al. showed that mutations, namely V364M, L385F, and R390H are the most prevalent one. However, for Indian PCG cases R368H mutation has been identified as predominant, yet no clinical association was seen for this mutation with regard to disease severity [64, 69, 70]. However, mutations P129L, E229K, R368H, R390C, and G61E found to be recurrent with

cumulative frequency of 30% in Indian PCG families. Furthermore, haplotype analysis in different populations revealed that in case of PCG carrying *CYP1B1* mutations, "C-G-G-T-A" being the most prevalent haplotype, whereas the most common haplotype for control is "G-T-C-C-A" [71].

Caucasians Nine mutations, viz. R368H, 8037dup10, R390H, 7901del13, 4340delG, G61E, E387K, E229K, and R390C/S appeared to be most prevalent in Caucasians [62].

Gypsies Observations made by Li et al. showed that mutation E387K was the most predominant (79.63%) among all *CYP1B1* mutations for Gypsies [62].

Middle East Countries Mutation G61E is the most common or founder *CYP1B1* mutation (45.52%) for Middle Easterners followed by R390H (8.71%), R469W (8.21%), and 4339delG (5.72%) [62].

Brazil 4340delG mutation is the most prevalent one and patients harboring this variation had disease in both eyes, age onset within first month with increased IOP (25–55 mmHg) [72].

6.4.5 Genotype (*CYP1B1* Mutations)–Phenotype (PCG) Correlations: A Complex Scenario

With a mounting number of PCG-linked *CYP1B1* mutations, attempts are on the way to comprehend the correlations of these potential mutations with disease phenotype which could furnish profound insights into pathogenetic mechanism. Thus far, few studies provide the evidences for genotype–phenotype associations. A good exemplar has been demonstrated in a study by Stoilov and colleagues, where 21 Brazilian PCG patients out of 52 (20.2%) identified with predominant 4340delG *CYP1B1* mutation. Clinical assessment of twelve subjects (with 4340delG mutation) revealed severe disease phenotype. They were bilateral with age of onset within first month of life and elevated intra ocular pressure between 25 and 55 mmHg [72], together with poor surgical response observed in comparison to subjects lacking this mutation.

Even though, G61E is the founder mutation for Middle Easterners, noteworthy, 44.2% of Saudi patients harboring G61E did not display disease phenotype on presentation, demonstrating incomplete penetrance [52]. In case of Iranian patients with G61E mutation, onset age was seen within 3 months of life bilaterally along with elevated IOP [73].

Moving toward further understandings underlying the variable gene–phenotype correlations, a study has reported four affected American PCG subjects of a family who were compound heterozygous for E387K and 268delSNF mutations [74]. Among the four subjects, only two displayed severe phenotypes with early onset and IOP range of 25 mmHg for right and 28 mmHg for left eye together with

corneal edema. On the contrary, other two siblings carrying the same mutation did not manifest any symptoms of glaucoma until their mid-teenage age. Another patient from third generation had severe glaucomatous symptoms including corneal edema with increased IOP (28–30 mmHg) from the time of birth. This patient harbors compound heterozygosity for 8037_8046dupTCATGCCACC and 268delSNF mutations.

With reference to the perspectives from Indian population, a study was attempted by Panicker et al. using larger number of PCG patients to understand the correlations between severity of PCG disease phenotype and six *CYP1B1* mutations (viz. Ins376A, P193L, E229K, R390C, G61E, and R368H) [70]. Clinical parameters considered in this study included IOP, cup-to-disk ratio (CD ratio), corneal diameter, corneal clarity, and visual acuity. This study revealed that the genotype perhaps could be an indicator for disease prognosis prediction. Patients harboring ins376A frameshift (100% of cases) and R390C (83.3%) homozygous mutation revealed the most severe phenotypes with very poor prognosis which could be explained by lacking of CYP1B1 protein domains due to functional null allele [63]. To add more, patients presented with severe phenotype in at least one eye for the respective mutations in percentages were 80% for E229K mutation, 72% with R368H, 66.7% for G61E mutation, and 62.5% with P193L. Variety of different mutations results into diverse pathological clinical phenotypes ranging from least or mild effect to extremes. Notably, correlating the genotype–phenotype for the patient data sets is the most significant step which could be helpful to understand the disease prognosis in a better way and hence abet in genetic counseling as well to the affected families.

6.5 Genes for GLC3B and GLC3C Loci

Reported to be GC-rich region and mapped to chromosome 1p36, PCG-linked GLC3B locus harbors malignancies associated various tumor suppressor genes, but none of them reported to be linked with PCG [52]. Likewise, genes including Neurexin 3A, Maleylacetoacetate Isomerase, Nuclear Receptor ERRB2, KIAA0759, Glutathione Transferase Zeta 1, Serine Palmitoyl Transferase Subunit II, and Alk B protein Homolog covered in the GLC3C locus region, however, neither of them been characterized for PCG. Therefore, hitherto no candidate gene has been recognized for both of these PCG-linked loci.

6.6 Latent Transforming Growth Factor (TGF)-Beta Binding Protein 2 (LTBP2): Locus "GLC3D"

Initial linkage studies defined fourth PCG associated locus (1.3 Mb proximal to GLC3C locus) on chromosome 14q24.2-24.3 (GLC3D) in consanguineous families of Pakistani origin [29]. Subsequently, in 2009, Ali et al. studied four Pakistani consanguineous families and patients belonging to the European Gypsies ethnicity with PCG phenotype [75]. After careful analysis, literature review, and probable eye

related function, they prioritized the target genes and finally arrived at the second most gene *LTBP2* implicated for PCG. Various mutations in *LTBP2* (latent transforming growth factor beta binding protein 2 gene, MIM 602091) have been reported in Pakistani patients and Gypsies following the autosomal recessive inheritance pattern.

Being the largest member of latent TGF-beta family, *LTBP2* gene consists of 36 exons and encodes a multi-domain extracellular matrix (ECM) protein of 1821 amino acid residues (see Fig. 6.2). With the probable role in cell adhesion and tissue repair, LTBP2 protein reported to associate with microfibrils containing fibrillin-1. The distribution and role of transforming growth factor-β1 and LTBP (latent form binding protein) in the anterior segment of eyes with pseudoexfoliation syndrome has been already studied [76]. Moreover, expression of LTBP2 has also been identified in trabecular meshwork, ciliary body and its processes [75]. Consequently, *LTBP2* gene mutations could be the plausible reason underlying PCG associated ocular congenital abnormalities, thereby might lead to increase in intraocular pressure (IOP) which is an important risk factor.

Various research groups documented *LTBP2* gene mutations in different population [68, 75, 77–80]. Details for the *LTBP2* mutations reported from different ethnic groups are given in Table 6.3. Ali et al. reported c.C895T (p.R299X) mutation in one of the Pakistani family and in Gypsies [75]. Their study suggested p.R299X as the founder mutation in the Gypsies which accounts for about 40% of PCG patients and more than half of the *CYP1B1* negative cases. Therefore, this mutation is considered for molecular diagnosis. It was also suggested that *LTBP2* null mutations consequently might modify structural and elasticity of trabecular meshwork, thus producing PCG disease phenotype. Homozygosity for the same mutation p.R299X in Roma/Gypsy PCG patients has been correlated with severe clinical phenotype and poor prognosis [78].

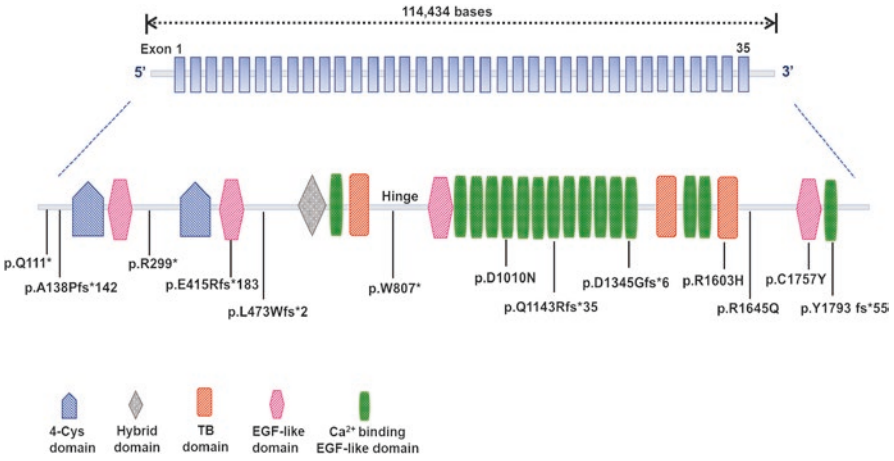


Fig. 6.2 Representative image of *LTBP2* gene, protein domains with reported mutations

Table 6.3 Pathogenic *LTBP2* mutations identified in PCG patients

Nucleotide change	Protein change	Ethnicity	Reference
c.331C>T	p.Q111*	Pakistan	Ali et al. [75]
c.412delG	p.A138Pfs*142	Pakistan	
c.895C>T	p.R299*	Gypsy	
c.1243_1256del14	p.E415Rfs*183	Pakistan	
c.5376delC	p.C1793Afs*55	Iran	Narooie-Nejad et al. [77]
c.1417delC	p.L473Wfs*2	Iran	
c.4808G>A	p.R1603H	Iran	
c.5376delC	p.Y1793 fs*55	Iran	
c.895C>T	p.R299*	Roma/Gypsy	Azmanov et al. [78]
c.4031_4032insA	p.D1345Gfs*6	Pakistan	Micheal et al. [79]
c.4934G>A	p.R1645Q	Pakistan	
c.2421G>A	p.W807*	South India	Yang et al. [68]
c.3028G>A	p.D1010N	Pakistan	Rauf et al. [80]
c.3427delC	p.Q1143Rfs*35	Pakistan	
c.5270G>A	p.C1757Y	Pakistan	

Genetic mutations in the *LTBP2* gene were also reported by research groups from India. In 2013, Mohanty et al. detected a single nucleotide polymorphism rs3742793 in the intronic region. There were no pathogenic mutations identified in north Indian patients [81]. In another study on Indian PCG patients, whole-exome sequencing (WES) identified a single *LTBP2* mutation (W807X) and suggested this mutation as a founder mutation, hence could be considered for molecular diagnosis in the Indian population [68]. Furthermore, in some rare cases which include other ocular anomalies such as megalocornea, ectopia lentis, microspherophakia, primary open-angle glaucoma, pseudoexfoliation syndrome, primary angle-closure glaucoma, and Weill–Marchesani syndrome, homozygous *LTBP2* mutations were reported by different investigators [82–86].

6.7 Tunica Interna Endothelial Cell Kinase (TEK): "GLC3E" Fifth Locus for PCG

Fifth locus "GLC3E" mapped at chromosome 9p21 has been identified for PCG containing tunica interna endothelial cell kinase gene (TEK/TIE2) which encodes tyrosine kinase receptor to which its ligand angiopoietin-1 (ANGPT-1) binds and regulates the vascular system stability. Representative illustration for *TEK* (Tie2) gene and encoded protein domains is given in Fig. 6.3. Located predominantly in vascular endothelial cells [87] including Schlemm's canal (SC) in the anterior chamber angle of eye, TEK tyrosine kinase receptors mediate their work by auto- and transphosphorylation [88]. In a study by Thomson and colleagues, involvement of angiopoietin/TIE2 as a crucial signaling pathway for the development of SC in mice has been demonstrated [89]. Deletion experiments of TEK and angiopoietin ligands showed developmental loss of SC, IOP elevation, loss of retinal ganglion, and glaucoma in the mice.

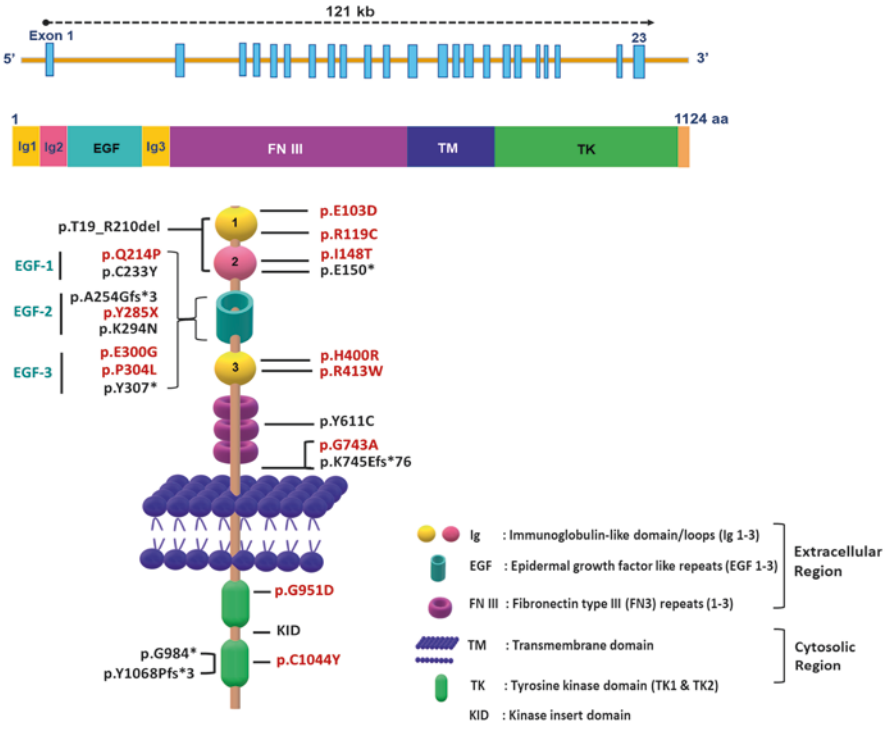


Fig. 6.3 Schematic representation for *TEK* (Tie2), protein domains with reported mutations marked across various domains. Marked reported PCG associated mutations (Black-Souma et al. [88]; Red-Kabra et al. [91] from Indian PCG patients; Green-Young et al. [92])

In 2016, Souma et al. published the results of exome sequencing on PCG families with unidentified etiology and revealed heterozygous *TEK* mutations in 10 patients out of 189 following autosomal dominant inheritance pattern with variable expressivity [88]. Out of ten cases, seven were without family history of glaucoma. Cellular mechanisms behind these TEK loss of function (Lof) mutations resulting in haploinsufficiency include no normal production of protein, aggregate formation of protein, greater proteosomal degradation, reduced ligand stimulation, thus affecting signaling. Collectively, their results demonstrated abridged TEK signaling affecting the aqueous humor outflow as a mechanism underlying or predisposing toward PCG disease development.

Followed by another study in 2017, the aforementioned research group identified three *ANGPT1* mutations out of 284 PCG cases and demonstrated the importance of ANGPT/TEK axis in the pathogenesis of glaucoma with severely hypomorphic Schlemm's canal resulting into increase in IOP, thus emphasizing the functional role of this pathway in the eye's anterior part [90]. A study from India also reported TEK mutations [91] and observed the interaction between TEK and CYP1B1 suggesting potential digenic inheritance.

Recently, Young et al. investigated eight families with PCG and reported new LoF TEK variants in heterozygous state [92]. In one of the family, four affected

subjects had an extra *SVEP1* gene variant (p.R997C) along with a *TEK* variant (p.A841V). SVEP1 (MIM 611691) is Sushi, Von Willebrand factor type A, epidermal growth factor, and pentraxin domain containing-1 gene, essential for the formation of lymphatic vessel in zebrafish and mice [93, 94]. In the study by Young et al. authors hypothesized SVEP1 as a modifier of TEK expression. Furthermore, decreased TEK signaling and consequent increased penetrance and severity of disease was stated [92]. Details of *TEK* receptor and *ANGPT1* gene mutations reported from different ethnicities are listed in Table 6.4.

Table 6.4 List of *TEK* and *ANGPT1* gene mutations identified in PCG patients and overview of digenic inherited gene mutations

Nucleotide change	Protein change	Ethnicity	Reference
TEK mutations			
c.448G>T	p.E150*	European (American)	Souma et al. [88]
c.760+2T>C	p.A254Gfs*3	European (Australian)	
c.921C>A	p.Y307*	Latino	
c.2232dupG	p.K745Efs*76	European (Australian)	
c.2950G>T	p.G984*	European (Italian)	
c.3300+2delT	p.Y1068Pfs*3	European (Australian)	
c.698G>A	p.C233Y	European (American)	
c.882G>C	p.K294N	African (American)	
c.1832A>G	p.Y611C	European (American)	
c.53_628del (exon 2-4)	p.T19_R210del	Romani	
TEK mutations from South Indian cohort			
c.309A>C	p.E103D	Indian	Kabra et al. [91]
c.355C>T	p.R119C		
c.443T>C	p.I148T		
c.641A>C	p.Q214P		
c.855T>A	p.Y285X		
c.899A>G	p.E300G		
c.911C>T	p.P304L		
c.1199A>G	p.H400R		
c.1237C>T	p.R413W		
c.2228G>C	p.G743A		
c.2852G>A	p.G951D		
c.3131G>A	p.C1044Y		
		European	
c.563T>G	p.V188G	European (Portugal)	Young et al. [92]
c.2712C>G	p.Y904*	European (Australia)	
c.3103G>C	p.G1035R	European (Australia)	
c.407G>T	p.G136V	South Asian (Iran)	
c.731C>G	p.P244R	South Asian (Iran)	
c.578A>G	p.Y193C	European (America)	
c.1624+5G>A	p.Val497_Ile541del	European (America)	
c.2522C>T (*TEK*) c.2989C>T (*SVEP1*)	p.A841V p.R997C	European (America)	

(continued)

Table 6.4 (continued)

Nucleotide change	Protein change	Ethnicity	Reference
ANGPT1 mutations			
c.706C>T	p.Q236*	European (Australian)	Thomson et al. [90]
c.1480C>T	p.R494*	European (American)	
c.746A>G	p.K249R	African (Australian)	
Evidence for digenic inheritance (TEK and CYP1B1 mutations co-occurrence)			
TEK mutations	*CYP1B1* mutations		
p.E103D	p.A115P	Indian	Kabra et al. [91]
p.I148T	p.R368H		
p.Q214P	p.E229K		
p.G743A			

p.E150*, p.Y307*, p.G984*, p.A254Gfs*3, p.K745Efs*76—undergo either nonsense-mediated decay (NMD) mediated loss of transcript or TEK protein truncation. p.Q236*—Nonsense-mediated decay of transcript; p.K249R—study could not identify defects in variant protein with respect to oligomerization, secretion, ability of receptor binding; p.K249R might be a population-specific benign variant.
Reference sequences: TEK—NM_000459.4 and NP_000450.2., SVEP1—NM_153366.4. and NP_699197.3., ANGPT1—NM_001146.3. and NP_001137.2.

6.8 Digenic Inheritance in PCG Associated Genes

For complex genetic diseases, digenic inheritance (DI) is defined as the simplest form of oligogenic inheritance. In humans, the foremost literature on DI was reported in case of retinitis pigmentosa (RP) in 1994 [95]. Following this, several studies reported DI in many disease phenotypes. Digenic mode of inheritance for primary congenital glaucoma (PCG) has been suggested and furnished by evidences, where genes interact with each other and might explain the phenotype in patients. The initial studies on DI for glaucoma was provided by Vincent et al. which suggested *CYP1B1* as a probable modifier gene for the expression of *MYOC* and their possible interaction by a mutual pathway [96]. Later, in 2005, a study from south Indian cohort reported implication of *MYOC* gene for PCG [97]. The study involves 72 PCG patients, out of which only 12 had heterozygous *CYP1B1* mutations, suggesting the partaking of other gene(s). On screening, a patient was identified as duple heterozygous for *CYP1B1* (c.G1103A; R368H) and *MYOC* (c.G144T; Q48H), implying digenic inheritance of these two unlinked genes for PCG. Three additional PCG patients were identified with same *MYOC* mutation but no *CYP1B1* mutation.

Yet another study by Chakrabarti and group identified two cases with two heterozygous variants in *FOXC1* and *CYP1B1* genes, however their role in disease pathology has to be known and overall, this study suggested limited involvement of *FOXC1* in PCG pathogenesis [98]. Additionally, interaction of *CYP1B1* with tyrosinase (*Tyr*) gene has also been reported. In mice studies, with both *CYP1B1* and *Tyr* knocked out showed severe defects in eye anterior angle drainage structures

compared to mice lacking *CYP1B1* alone [99]. They identified that deficiency of Tyr increases the extent of dysgenesis, possibility of this pathway in PCG. However, no association of *Tyr* was suggested for PCG with being a non-modifier for *CYP1B1* in humans [100].

One more important exemplar for the existence of digenic interaction between *CYP1B1* with *TEK* (an angiopoietin receptor) has been noted [91]. Involvement of *TEK* mutations in PCG pathogenesis has already been explained in aforementioned segment (see Sect. 6.7). Autosomal recessive inheritance pattern for PCG is well established, though autosomal dominant pattern has also been reported in case of *TEK* gene mutations in a study of Souma et al. [88]. To ascertain the second mutation in autosomal recessive PCG cohort harboring heterozygous *TEK* mutations and for evaluating the co-occurrence of two mutations, a study using targeted sequencing strategy was conducted in south Indian patients [91]. Twelve heterozygous *TEK* gene mutations were identified. Intriguingly, four mutations: p.E103D, p.I148T, p.Q214P, and p.G743A co-occurred with three *CYP1B1* gene heterozygous mutations: p.A115P, p.E229K, and p.R368H (see Table 6.4). However, parents were asymptomatic with either of these mutations, which implies a potential digenic inheritance. *CYP1B1* variants: p.E229K and p.R368H were reported to display altered estradiol and retinoid metabolism [101]. These interactions were also ascertained by various methodologies together with co-transfection and pull-down assays using HEK293 cell lines. Experiments clearly demonstrated the perturbed interactions in presence of disease associated alleles, reduced TEK responsiveness of ligand stimulation, thus affecting signaling process. Altogether, these evidences help at least in part toward understanding the variable expression, clinical and genetic heterogenous nature of disease entity, and CYP1B1 interactions with MYOC and TEK further bestow support to digenic mode of inheritance pattern for PCG, yet much more remains to be explored.

6.9 Developmental Anomalies and Glaucoma

Glaucoma at early age may also arise secondarily to developmental anomalies of the anterior angle structures of eye owing to abnormal differentiation of neural crest cells and sometimes associated with other systemic features as well. These conditions include Axenfeld–Rieger Syndrome (ARS), Peters' anomaly, Aniridia, and so on with variable phenotypic expression.

6.9.1 Axenfeld–Rieger Syndrome

Axenfeld–Rieger syndrome (ARS; OMIM 180500) is a rare developmental disorder with both ocular and systemic manifestations. There is a congenital defect of the ocular anterior segment (trabecular meshwork) and increased IOP resulting into glaucomatous condition in approximately 50% of ARS patients. Non-ocular features include hypodontia or microdontia, maxillary hypoplasia, sensory hearing loss, skeletal limb anomalies, and congenital heart defects. Through large pedigree

studies with autosomal dominant inheritance, linkage analysis reported association of causal mutations in two genes: paired-like homeodomain transcription factor 2 (*PITX2*) and forkhead box C1 (*FOXC1*) transcription factor gene.

PITX2 (Paired-Like Homeodomain Transcription Factor 2) Different mutation types have been reported in *PITX2* gene, crucial for tissue morphogenesis and embryonic development [102, 103]. Noteworthy, being a negative regular of FOXC1, interaction of PITX2 and FOXC1 is important for ocular development pathway [104, 105].

FOXC1 (Forkhead Box Transcription Factor C1) FOXC1 regulates developmental processes, cell migration in many human tissues [106]. Human *FOXC1* mutations manifest range of phenotypic features of ARS and iris hypoplasia [107, 108]. Also, about half of the ocular dysgenesis patients have glaucoma who harbor either *PITX2* or *FOXC1* mutations [108]. Patients with anterior segment dysgenesis with *FOXC1* causal mutations may also have heart defects besides hearing defects [109].

6.9.2 Peters' Anomaly

It is a congenital anomaly allied with glaucoma and other phenotypes comprising central corneal opacity, absence of stroma in the posterior corneal region, etc. Genetic mutations in *PITX2*, *FOXC1,* and *PAX6* genes were reported related to Peters' anomaly.

6.9.3 Aniridia

Rare developmental disease, characterized by anomalous development of iris [110] and inherits either as autosomal dominant or sporadic form. *PAX6* (Paired Box 6) gene missense mutations have been linked to aniridia with autosomal dominant form, while deletions with sporadic form of inheritance [111]. It has been observed that *PAX6* mutations in the patients with developmental anomalies of eye also manifest early onset form of glaucoma [110].

6.10 Evidence of Mitochondrial Mutations in PCG

Several human diseases including ocular disorders have been associated with mitochondrial DNA (mt-DNA) mutations. With regard to glaucoma, due to high energy demand, there exist a high number of mitochondria in the optic nerve head for the RGCs survival [112]. Being the major site of oxidative stress mechanism, mitochondrial DNA is susceptible to damage, hence occurrence of mt-DNA mutations. Increased oxidative stress condition in glaucoma affects eyes anterior segment structures including TM leading to increase in IOP which is considered as a key risk factor for PCG as well [113].

Association of mt-DNA mutations in the pathogenesis of different glaucoma subtypes (POAG, PACG, pseudoexfoliation glaucoma; PEG and PCG) has been studied by various research groups [113–116]. As already known, reported genetic markers underlying PCG resolves only a fraction of cases, therefore based on the aforementioned hypothesis and known genetics, it is necessary to look for other factors (including mt-DNA mutations) for PCG pathogenesis. In a study by Tanwar and colleagues, 35 PCG and 40 control samples were evaluated for possible mt-DNA mutations [116]. Their study reported about 22.85% of PCG cases with potential pathogenic mt-DNA variations which is higher while compared to control group, thus proposing the plausible TM dysgenesis mechanism induced by mt-mutations.

6.11 Genetics Research and Testing Toward Molecular Diagnosis

Thus far, we understood the PCG disease, its clinical presentation, incidence, inheritance pattern, and ample number of evidences furnishing the involvement of genetics. Incidence of PCG differs among different ethnicities, with higher prevalence in the communities practicing consanguineous marriages. In addition, both sporadic and familial cases of PCG with the established inheritance pattern have been nicely reviewed.

With the aid of various cutting-edge research methodologies and advances in technologies, molecular diagnosis helped to identify the disease associated mutations and further characterization to gain insights into disease mechanisms. Initial studies used linkage analysis and direct DNA sequencing for identification of pathologic markers and potential pathogenic mutations. In the past years, advent of high throughput next generation technologies enables to screen large number of samples across the target genes panel or through whole genome/exome sequencing in less time and cost-effective manner, specifically where expected gene mutation is absent. For instance, one of our research studies used whole-exome sequencing (WES) strategy to detect causative mutations in eight Indian PCG families (see Table 6.2) [68]. In this study, two novel mutations were identified out of five identified mutations (four *CYP1B1* gene homozygous missense mutations; one nonsense mutation in *LTBP2* gene).

Well-designed molecular genetics researches aimed at early and reliable diagnosis for ophthalmic diseases have shown advances and bolster the genetic testing and risk assessment for the patient, their family members, and future generations. This helps to underpin the genetic markers, genetic etiology, and genotype–phenotype correlations underlying the respective disease pathology. Additionally, it allows the researchers and clinicians to know the carrier status of causal mutation in the family which could allow prepare the family and the clinician for proper medical or surgical interventions priorly for better prognosis, thus can halt or slow down the disease progression and prevent the vision loss. For glaucoma (specifically for early onset glaucoma), through collection of pedigree, family history information along with genetic testing, patients at risk can be identified and monitored for better treatment plan to maintain the valuable eyesight. On the whole, ascertaining the molecular

diagnosis abets to elucidate the pattern of inheritance for further determination of risks to the family.

6.12 Genetic Counseling

Ensuing to genetic testing, step toward counseling to the patient and family members about risk of disease constitutes an important element. Deciphering the plausible role of identified mutation with clinical phenotype makes a way to understand the genetic architecture in the particular family. It allows to know the risks for the sibling based on the inheritance pattern followed. For example, in case of PCG, it is known that *CYP1B1*or *LTBP2* gene mutations follow autosomal recessive inheritance. Proband's parents will be obligate heterozygotes (i.e., carriers having one copy of a recessive allele) and are asymptomatic [117]. In case of sibling, there is 25% possibility of getting affected, 50% chance as asymptomatic carrier, and a chance of 25% as unaffected and non-carrier. Further, progeny of *CYP1B1* or *LTBP2* linked PCG will also be the obligate heterozygotes (or carriers) for the respective mutation. Also, sibling of proband's parents will be carriers for the mutation with 50% risk. Specifically, for ethnic groups and communities where consanguineous marriages are practiced (e.g., Gypsies, Saudi Arabians, south India), genetic testing and counseling helps to bring awareness among the people about disease inheritance, associated pathogenic genetic markers and their role in disease pathology for the management of PCG patients.

6.13 Understanding Glaucoma Through *In Vitro* and Animal Models

An eclectic collection of study models including *in vitro* and animal models have been developed to understand pathophysiology of glaucoma subtypes [118]. With respect to PCG, animal models such as rabbit, rat, mouse, etc., have been studied by various research groups. For instance, CYP1B1 knockout mice models were developed to explore PCG associated abnormalities in the drainage angle tissues of eye. Various *in vitro* models have also emerged facilitating glaucoma research [119]. Because of complex intricacies linked to glaucoma, efforts are underway to develop an ideal model to mimic the disease pathology and thus understand the disease mechanisms.

6.14 Summary

Being the most common cause of childhood blindness, primary congenital glaucoma is a devastating disorder with developmental anomalies of anterior segment of the eye. Consanguinity increases the prevalence of PCG in certain ethnic groups. As an inherited blinding disease of infancy, genetics research aided in the identification of PCG-linked loci and candidate genes. A number of pathogenic

mutations have been identified in the past decades which account for a small fraction only, yet much more have to be deciphered. Genetic testing offers early-detection of individuals at risk for implementing disease surveillance, better planning of the treatment measures, and further risk assessment for the family. Understanding the disease genetic background, interacting pathways, and pathomechanisms of the identified relevant mutations will aid to develop potential therapeutic targets and therapies in near future that might slow down the disease progression, thus saving the vision.

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References

1. Sarfarazi M, Stoilov I, Schenkman JB. Genetics and biochemistry of primary congenital glaucoma. Ophthalmol Clin North Am. 2003;16(4):543–54., vi. https://doi.org/10.1016/s0896-1549(03)00062-2.
2. Gudlavalleti VSM. Magnitude and temporal trends in Avoidable Blindness in Children (ABC) in India. Indian J Pediatr. 2017;84(12):924–9. https://doi.org/10.1007/s12098-017-2405-2.
3. Gilbert C, Foster A. Childhood blindness in the context of VISION 2020--the right to sight. Bull World Health Organ. 2001;79:227–32.
4. Papadopoulos M, Cable N, Rahi J, et al. The British Infantile and Childhood Glaucoma (BIG) eye study. Investig Ophthalmol Vis Sci. 2007;48:4100–6.
5. Senthil S, Badakere S, Ganesh J, et al. Profile of childhood glaucoma at a tertiary center in South India. Indian J Ophthalmol. 2019;67:358–65.
6. Dandona L, Williams JD, Williams BC, Rao GN. Population-based assessment of childhood blindness in southern India. Arch Ophthalmol. 1998;116:545–6.
7. Bejjani BA, Lewis RA, Tomey KF, et al. Mutations in CYPIBl, the gene for cytochrome P4501B1, are the predominant cause of primary congenital glaucoma in Saudi Arabia. Am J Hum Genet. 1998;62:325–33.
8. Anderson DR. The development of the trabecular meshwork and its abnormality in primary infantile glaucoma. Trans Am Ophthalmol Soc. 1981;79:458–85.
9. Maumenee AE. The pathogenesis of congenital glaucoma: a new theory. Trans Am Ophthalmol Soc. 1958;56:507–70.
10. Hoskins HD, et al. Developmental glaucomas: therapy. In: New Orleans Academy of ophthalmology symposium on glaucomas. St Louis: Mosby; 1981.
11. Westerlund E. Clinical and genetic studies on the primary glaucoma diseases. Copenhagen: NYT Norsdisk Forlag, Arnold Busck; 1947.
12. Sarfarazi M, Stoilov I. Molecular genetics of primary congenital glaucoma. Eye. 2000;14:422–8. https://doi.org/10.1038/eye.2000.126.
13. François J. Congenital glaucoma and its inheritance. Ophthalmologica. 1980;181(2):61–73. https://doi.org/10.1159/000309028. PMID: 7219964.
14. Turacli ME, Aktan SG, Sayli BS, Akarsu AN. Therapeutical and genetical aspects of congenital glaucomas. Int Ophthalmol. 1992;16:359–62.
15. Genčík A. Epidemiology and genetics of primary congenital glaucoma in Slovakia. Description of a form of primary congenital glaucoma in gypsies with autosomal-recessive inheritance and complete penetrance. Dev Ophthalmol. 1989;16:76–115.

16. Liu B, Huang W, He M, Zheng Y. An investigation on the causes of blindness and low vision of students in blind school in Guangzhou. Yan Ke Xue Bao. 2007;23(2):117–20.
17. Merin S, Morin D. Heredity of congenital glaucoma. Br J Ophthalmol. 1972;56:414–7.
18. Mandal AK, Chakrabarti D. Update on congenital glaucoma. Indian J Ophthalmol. 2011;59(Suppl1):S148–57. https://doi.org/10.4103/0301-4738.73683.
19. Jay MR, Rice NSC. Genetic implications of congenital glaucoma. Metab Ophthalmol. 1978;2:257–8. 20.
20. Demenias F, Bonaiti E, Briard ML, Feingold L, Frezal J. Congenital glaucoma: genetic models. Hum Genet. 1979;46:305–17.
21. Gencik A, Gencikova A, Gerinec A. Genetic heterogeneity of congenital glaucoma. Clin Genet. 1980;17:241–8.
22. Cascella R, Strafella C, Germani C, Novelli G, Ricci F, Zampatti S, Giardina E. The genetics and the genomics of primary congenital glaucoma. Biomed Res Int. 2015;2015:321291. https://doi.org/10.1155/2015/321291.
23. Bruford EA, Lush MJ, Wright MW, Sneddon TP, Povey S, Birney E. The HGNC Database in 2008: a resource for the human genome. Nucleic Acids Res. 2008;36(Database issue):D445–8. https://doi.org/10.1093/nar/gkm881.
24. Khan AO. Genetics of primary glaucoma. Curr Opinion Ophthalmol. 2011;22(5):347–55. https://doi.org/10.1097/ICU.0b013e32834922d2. PMID: 21730848.
25. Sarfarazi M, Akarsu AN, Hossain A, Turacli ME, Aktan SG, Barsoum-Homsy M, et al. Assignment of a locus (GLC3A) for primary congenital glaucoma (Buphthalmos) to 2p21 and evidence for genetic heterogeneity. Genomics. 1995;30:171–7.
26. Plásilová M, Feráková E, Kádasi L, et al. Linkage of autosomal recessive primary congenital glaucoma to the GLC3A locus in Roms (Gypsies) from Slovakia. Hum Hered. 1998;48(1):30–3. https://doi.org/10.1159/000022778.
27. Akarsu AN, Turacli ME, Aktan SG, Barsoum-Homsy M, Chevrette L, Sayli BS, et al. A second locus (GLC3B) for primary congenital glaucoma (Buphthalmos) maps to the 1p36 region. Hum Mol Genet. 1996;5:1199–203.
28. Stoilov IR, Sarfarazi M. Third genetic locus (GLC3C) for primary congenital glaucoma (PCG) maps to chromosome 14q24.3. Investig Ophthalmol Vis Sci. 2002;43:E3015.
29. Firasat S, Riazuddin SA, Hejtmancik JF, Riazuddin S. Primary congenital glaucoma localizes to chromosome 14q24.2-24.3 in two consanguineous Pakistani families. Mol Vis. 2008;14:1659–65.
30. Stoilov I, Akarsu AN, Sarfarazi M. Identification of three different truncating mutations in the cytochrome P4501B1 (CYPIB1) gene as the principal cause of primary congenital glaucoma (buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. Hum Mol Genet. 1997;6:641–7.
31. Stoilov I, Akarsu AN, Alozie I, et al. Sequence analysis and homology modelling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. Am J Hum Genet. 1998;62:573–84.
32. Kakiuchi T, Isashiki Y, Nakao K, Sonoda S, Kimura K, Ohba N. A novel truncating mutation of cytochrome P4501Bl (CYPIBl) gene in primary infantile glaucoma. Am J Ophthalmol. 1999;128:370–2.
33. Plasilova M, Stoilov I, Sarfarazi M, Kadasi L, Ferakova E, Ferak V. Identification of single ancestral CYP1B1 mutation in Slovak gypsies (Roms) affected with primary congenital glaucoma. J Med Genet. 1999;36:290–4.
34. Bejjani BA, Stockton DW, Lewis RA, et al. Multiple CYP1B1 mutations and incomplete penetrance in an inbred population segregating primary congenital glaucoma suggest frequent de novo events and a dominant modifier locus. Hum Mol Genet. 2000;9(3):367–74. https://doi.org/10.1093/hmg/9.3.367.
35. Moore DB, Tomkins O, Ben-Zion I. A review of primary congenital glaucoma in the developing world. Surv Ophthalmol. 2013;58(3):278–85. https://doi.org/10.1016/j.survophthal.2012.11.003.

36. Faiq MA, Dada R, Sharma R, Saluja D, Dada T. CYP1B1: a unique gene with unique characteristics. Curr Drug Metab. 2014;15(9):893–914. https://doi.org/10.2174/1389200216666150206130058.
37. Hasler JA, Estabrook R, Murray M, Pikuleva I. Pharmacogenetics of cytochromes P450. Mole Aspects Med. 1999;20:25–137.
38. Stoilov I, Jansson I, Sarfarazi M, Schenkman JB. Role of cytochrome P-450 in development. Drug Metabol Drug Interact. 2001;18:33–55.
39. Sutter TR, Tang YM, Hayes CL, et al. Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. J Biol Chem. 1994;269:13092–9.
40. Choudhary D, Jansson I, Stoilov I, Sarfarazi M, Schenkman JB. Metabolism of retinoids and arachidonic acid by human and mouse cytochrome P450 1b1. Drug Metab Dispos. 2004;32:840–7.
41. Alsubait A, Aldossary W, Rashid M, Algamdi A, Alrfaei BM. CYP1B1 gene: implications in glaucoma and cancer. J Cancer. 2020;11(16):4652–61. https://doi.org/10.7150/jca.42669.
42. Chen H, Howald WN, Juchau MR. Biosynthesis of all-trans-retinoic acid from all-trans-retinol: catalysis of all-trans-retinol oxidation by human p-450 cytochromes. Drug Metab Dispos. 2000;28:315–22.
43. Zhang QY, Dunbar D, Kaminsky L. Human cytochrome P-450 metabolism of retinals to retinoic acids. Drug Metab Dispos. 2000;28:292–7.
44. Nelson DR, Zeldin DC, Hoffman SM, Maltais LJ, Wain HM, Nebert DW. Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. Pharmacogenetics. 2004;14:1–18.
45. Cvekl A, Wang WL. Retinoic acid signaling in mammalian eye development. Exp Eye Res. 2009;89:280–91. https://doi.org/10.1016/j.exer.2009.04.012.
46. Duester G. Keeping an eye on retinoic acid signaling during eye development. Chemico Biol Interact. 2009;178:178–81. https://doi.org/10.1016/j.cbi.2008.09.004.
47. Schwartzman ML, Balazy M, Masferrer J, Abraham NG, McGiff JC, Murphy RC. 12(R) hydroxyicosatetraenoic acid: a cytochrome-P450-dependent arachidonate metabolite that inhibits Na+,K+-ATPase in the cornea. Proc Natl Acad Sci U S A. 1987;84(22):8125–9. https://doi.org/10.1073/pnas.84.22.8125.
48. Sarfarazi M. Recent advances in molecular genetics of glaucomas. Hum Mol Genet. 1997;6:1667–77.
49. Muskhelishvili L, Thompson PA, Kusewitt DF, Wang C, Kadlubar FF. In situ hybridization and immunohistochemical analysis of cytochrome P450 1B1 expression in human normal tissues. J Histochem Cytochem. 2001;49:229–36.
50. Tomarev S, Wistow G, Raymond V, Dubois S, Malyukova I. Gene expression profile of the human trabecular meshwork: NEIBank sequence tag analysis. Investig Ophthalmol Vis Sci. 2003;44:2588–96.
51. Doshi M, Marcus C, Bejjani BA, Edward DP. Immunolocalization of CYP1B1 in normal, human, fetal and adult eyes. Exp Eye Res. 2006;82:24–32.
52. Kaur K, Mandal AK, Chakrabarti S. Primary congenital glaucoma and the involvement of CYP1B1. Middle East Afr J Ophthalmol. 2011;18(1):7–16. https://doi.org/10.4103/0974-9233.75878.
53. Wang HW, Sun P, Chen Y, et al. Research progress on human genes involved in the pathogenesis of glaucoma (Review). Mol Med Rep. 2018;18(1):656–74. https://doi.org/10.3892/mmr.2018.9071.
54. Hakkola J, Pasanen M, Pelkonen O, et al. Expression of CYP1B1 in human adult and fetal tissues and differential inducibility of CYP1B1 and CYP1A1 by Ah receptor ligands in human placenta and cultured cells. Carcinogenesis. 1997;18:391–7.
55. Stoilov I, Rezaie T, Jansson I, Schenkman JB, Sarfarazi M. Expression of cytochrome P4501b1 (Cyp1b1) during early murine development. Mol Vis. 2004;10:629–36.

56. Choudhary D, Jansson I, Rezaul K, Han DK, Sarfarazi M, Schenkman JB. Cyp1b1 protein in the mouse eye during development: an immunohistochemical study. Drug Metab Dispos. 2007;35:987–94.
57. Sharafieh R, Child AH, Sarfarazi M. Molecular genetics of primary congenital glaucoma. In: Genetic diseases of the eye. Chapter 17. 2nd ed. Oxford: Oxford University Press; 2012. https://oxfordmedicine.com/view/10.1093/med/9780195326147.001.0001/med-9780195326147.
58. Ray K, Mukhopadhyay A, Acharya M. Recent advances in molecular genetics of glaucoma. Mol Cell Biochem. 2003;253:223–31. https://doi.org/10.1023/A:1026059800470.
59. Chavarria-Soley G, Sticht H, Aklillu E, et al. Mutations in CYP1B1 cause primary congenital glaucoma by reduction of either activity or abundance of the enzyme. Hum Mutat. 2008;29:1147–53.
60. Campos-Mollo E, López-Garrido MP, Blanco-Marchite C, et al. CYP1B1 mutations in Spanish patients with primary congenital glaucoma: phenotypic and functional variability. Mol Vis. 2009;15:417–31.
61. Ling C, Zhang D, Zhang J, Sun H, Du Q, Li X. Updates on the molecular genetics of primary congenital glaucoma (Review). Exp Ther Med. 2020;20:968–77. https://doi.org/10.3892/etm.2020.8767.
62. Li N, Zhou Y, Du L, Wei M, Chen X. Overview of Cytochrome P450 1B1 gene mutations in patients with primary congenital glaucoma. Exp Eye Res. 2011;93(5):572–9. https://doi.org/10.1016/j.exer.2011.07.009.
63. Panicker SG, Reddy ABM, Mandal AK, et al. Identification of novel mutations causing familial primary congenital glaucoma in Indian pedigrees. Investig Ophthalmol Vis Sci. 2002;43:1358–66.
64. Reddy AB, Kaur K, Mandal AK, et al. Mutation spectrum of the CYP1B1 gene in Indian primary congenital glaucoma patients. Mol Vis. 2004;10:696–702.
65. Ramprasad VL, George RJ, Sripriya S, Nirmaladevi J, Vijaya L, Kumaramanickavel G. Molecular genetic analysis of a consanguineous south Indian family with congenital glaucoma: relevance of genetic testing and counseling. Ophthalmic Genet. 2007;28(1):17–24. https://doi.org/10.1080/13816810701199449.
66. Tanwar M, Dada T, Sihota R, Das TK, Yadav U, Dada R. Mutation spectrum of CYP1B1 in North Indian congenital glaucoma patients. Mol Vis. 2009;15:1200–9.
67. Tanwar M, Dada T, Sihota R, Dada R. Identification of four novel cytochrome P4501B1 mutations (p.I94X, p.H279D, p.Q340H, and p.K433K) in primary congenital glaucoma patients. Mol Vis. 2009;15:2926–37.
68. Yang Y, Zhang L, Li S, Zhu X, Sundaresan P. Candidate gene analysis identifies mutations in CYP1B1 and LTBP2 in Indian families with primary congenital glaucoma. Genet Test Mol Biomarkers. 2017;21(4):252–8. https://doi.org/10.1089/gtmb.2016.0203. Epub 2017 Feb 27.
69. Reddy AB, Panicker SG, Mandal AK, Hasnain SE, Balasubramanian D. Identification of R368H as a predominant CYP1B1 allele causing primary congenital glaucoma in Indian patients. Investig Ophthalmol Vis Sci. 2003;44:4200–3.
70. Panicker SG, Mandal AK, Reddy AB, Gothwal VK, Hasnain SE. Correlation between genotype and phenotype in Indian primary congenital glaucoma patients. Investig Ophthalmol Vis Sci. 2004;45:1149–56.
71. Chakrabarti S, Kaur K, Kaur I, et al. Globally, CYP1B1 mutations in primary congenital glaucoma are strongly structured by geographic and haplotype backgrounds. Investig Ophthalmol Vis Sci. 2006;47(1):43–7. https://doi.org/10.1167/iovs.05-0912.
72. Stoilov IR, Costa VP, Vasconcellos JP, et al. Molecular genetics of primary congenital glaucoma in Brazil. Investig Ophthalmol Vis Sci. 2002;43:1820–7.
73. Chitsazian F, Tusi BK, Elahi E, et al. CYP1B1 mutation profile of Iranian primary congenital glaucoma patients and associated haplotypes. J Mol Diagn. 2007;9:382–93.
74. Sena DF, Finzi S, Rodgers K, Del Bono E, Haines JL, Wiggs JL. Founder mutations of CYP1B1 gene in patients with congenital glaucoma from the United States and Brazil. J Med Genet. 2004;41:e6.

75. Ali M, McKibbin M, Booth A, et al. Null mutations in LTBP2 cause primary congenital glaucoma. Am J Hum Genet. 2009;84(5):664–71. https://doi.org/10.1016/j.ajhg.2009.03.017.
76. Schlötzer-Schrehardt U, Zenkel M, Küchle M, Sakai LY, Naumann GO. Role of transforming growth factor-beta1 and its latent form binding protein in pseudoexfoliation syndrome. Exp Eye Res. 2001;73(6):765–80. https://doi.org/10.1006/exer.2001.1084.
77. Narooie-Nejad M, Paylakhi SH, Shojaee S, et al. Loss of function mutations in the gene encoding latent transforming growth factor beta binding protein 2, LTBP2, cause primary congenital glaucoma. Hum Mol Genet. 2009;18(20):3969–77. https://doi.org/10.1093/hmg/ddp338.
78. Azmanov DN, Dimitrova S, Florez L, et al. LTBP2 and CYP1B1 mutations and associated ocular phenotypes in the Roma/Gypsy founder population. Eur J Hum Genet. 2011;19:326–33.
79. Micheal S, Siddiqui SN, Zafar SN, Iqbal A, Khan MI, den Hollander AI. Identification of novel variants in LTBP2 and PXDN using whole-exome sequencing in developmental and congenital glaucoma. PLoS One. 2016;11:e0159259.
80. Rauf B, Irum B, Khan SY, et al. Novel mutations in *LTBP2* identified in familial cases of primary congenital glaucoma. Mol Vis. 2020;26:14–25.
81. Mohanty K, Tanwar M, Dada R, Dada T. Screening of the LTBP2 gene in a north Indian population with primary congenital glaucoma. Mol Vis. 2013;19:78–84.
82. Désir J, Sznajer Y, Depasse F, et al. LTBP2 null mutations in an autosomal recessive ocular syndrome with megalocornea, spherophakia, and secondary glaucoma. Eur J Hum Genet. 2010;18(7):761–7. https://doi.org/10.1038/ejhg.2010.11.
83. Kumar A, Duvvari MR, Prabhakaran VC, Shetty JS, Murthy GJ, Blanton SH. A homozygous mutation in LTBP2 causes isolated microspherophakia. Hum Genet. 2010;128(4):365–71. https://doi.org/10.1007/s00439-010-0858-8.
84. Haji-Seyed-Javadi R, Jelodari-Mamaghani S, Paylakhi SH, et al. LTBP2 mutations cause Weill-Marchesani and Weill-Marchesani-like syndrome and affect disruptions in the extracellular matrix. Hum Mutat. 2012;33(8):1182–7. https://doi.org/10.1002/humu.22105.
85. Jelodari-Mamaghani S, Haji-Seyed-Javadi R, Suri F, et al. Contribution of the latent transforming growth factor-β binding protein 2 gene to etiology of primary open angle glaucoma and pseudoexfoliation syndrome. Mol Vis. 2013;19:333–47.
86. Safari I, Akbarian S, Yazdani S, Elahi E. A possible role for LTBP2 in the etiology of primary angle closure glaucoma. J Ophthalmic Vis Res. 2015;10(2):123–9. https://doi.org/10.4103/2008-322X.163783.
87. Davis S, Aldrich TH, Jones PF, et al. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell. 1996.; 27;87(7):1161–9. https://doi.org/10.1016/s0092-8674(00)81812-7.
88. Souma T, Tompson SW, Thomson BR, et al. Angiopoietin receptor TEK mutations underlie primary congenital glaucoma with variable expresssivity. J Clin Investig. 2016;126:2575–87.
89. Thomson BR, Heinen S, Jeansson M, et al. A lymphatic defect causes ocular hypertension and glaucoma in mice. J Clin Investig. 2014;124(10):4320–4. https://doi.org/10.1172/JCI77162.
90. Thomson BR, Souma T, Tompson SW, et al. Angiopoietin-1 is required for Schlemm's canal development in mice and humans. J Clin Investig. 2017;127(12):4421–36.
91. Kabra M, Zhang W, Rathi S, et al. Angiopoietin receptor TEK interacts with CYP1B1 in primary congenital glaucoma. Hum Genet. 2017;136(8):941–9.
92. Young TL, Whisenhunt KN, Jin J, et al. SVEP1 as a genetic modifier of TEK-related primary congenital glaucoma. Investig Ophthalmol Vis Sci. 2020;61(12):6. https://doi.org/10.1167/iovs.61.12.6.
93. Karpanen T, Padberg Y, van de Pavert SA, et al. An evolutionarily conserved role for polydom/Svep1 during lymphatic vessel formation. Circ Res. 2017;120:1263–75.
94. Morooka N, Futaki S, Sato-Nishiuchi R, et al. Polydom is an extracellular matrix protein involved in lymphatic vessel remodeling. Circ Res. 2017;120:1276–88.

95. Kajiwara K, Berson EL, Dryja TP. Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. Science. 1994;264(5165):1604–8. https://doi.org/10.1126/science.8202715.
96. Vincent AL, Billingsley G, Buys Y, et al. Digenic inheritance of early-onset glaucoma: CYP1B1, a potential modifier gene. Am J Hum Genet. 2002;70(2):448–60. https://doi.org/10.1086/338709.
97. Kaur K, Reddy AB, Mukhopadhyay A, et al. Myocilin gene implicated in primary congenital glaucoma. Clin Genet. 2005;67(4):335–40. https://doi.org/10.1111/j.1399-0004.2005.00411.x.
98. Chakrabarti S, Kaur K, Rao KN, et al. The transcription factor gene FOXC1 exhibits a limited role in primary congenital glaucoma. Investig Ophthalmol Vis Sci. 2009;50(1):75–83. https://doi.org/10.1167/iovs.08-2253.
99. Libby RT, Smith RS, Savinova OV, et al. Modification of ocular defects in mouse developmental glaucoma models by tyrosinase. Science. 2003;299(5612):1578–81. https://doi.org/10.1126/science.1080095.
100. Bidinost C, Hernandez N, Edward DP, et al. Of mice and men: tyrosinase modification of congenital glaucoma in mice but not in humans. Investig Ophthalmol Vis Sci. 2006;47(4):1486–90. https://doi.org/10.1167/iovs.05-0763.
101. Banerjee A, Chakraborty S, Chakraborty A, Chakrabarti S, Ray K. Functional and structural analyses of CYP1B1 variants linked to congenital and adult-onset glaucoma to investigate the molecular basis of these diseases. PLoS One. 2016;11(5):e0156252. https://doi.org/10.1371/journal.pone.0156252.
102. Gage PJ, Camper SA. Pituitary homeobox 2, a novel member of the bicoid-related family of homeobox genes, is a potential regulator of anterior structure formation. Hum Mol Genet. 1997;6(3):457–64.
103. Ephrussi A, Johnston DS. Seeing is believing: the bicoid morphogen gradient matures. Cell. 2004;116(2):143–52.
104. Berry FB, Lines MA, Oas JM, et al. Functional interactions between FOXC1 and PITX2 underlie the sensitivity to FOXC1 gene dose in Axenfeld-Rieger syndrome and anterior segment dysgenesis. Hum Mol Genet. 2006;15(6):905–19. https://doi.org/10.1093/hmg/ddl008.
105. Acharya M, Huang L, Fleisch VC, Allison WT, Walter MA. A complex regulatory network of transcription factors critical for ocular development and disease. Hum Mol Genet. 2011;20(8):1610–24.
106. Jackson BC, Carpenter C, Nebert DW, Vasiliou V. Update of human and mouse forkhead box (FOX) gene families. Hum Genomics. 2010;4(5):345–52.
107. Hjalt TA, Semina EV. Current molecular understanding of Axenfeld-Rieger syndrome. Expert Rev Mol Med. 2005;7(25):1–17.
108. Reis LM, Tyler RC, Volkmann Kloss BA, et al. PITX2 and FOXC1 spectrum of mutations in ocular syndromes. Eur J Hum Genet. 2012;20(12):1224–33. https://doi.org/10.1038/ejhg.2012.80.
109. Du RF, Huang H, Fan LL, Li XP, Xia K, Xiang R. A novel mutation of FOXC1 (R127L) in an Axenfeld-Rieger syndrome family with glaucoma and multiple congenital heart diseases. Ophthalmic Genet. 2014;10:1–5.
110. Lee HJ, Colby KA. A review of the clinical and genetic aspects of aniridia. Semin Ophthalmol. 2013;28(5-6):306–12.
111. Lewis CJ, Hedberg-Buenz A, DeLuca AP, Stone EM, Alward WLM, Fingert JH. Primary congenital and developmental glaucomas. Hum Mol Genet. 2017;26(R1):R28–36. https://doi.org/10.1093/hmg/ddx205.
112. Barron MJ, Griffiths P, Turnbull DM, Bates D, Nichols P. The distributions of mitochondria and sodium channels reflect the specific energy requirements and conduction properties of the human optic nerve head. Br J Ophthalmol. 2004;88(2):286–90.
113. Abu-Amero KK, Morales J, Bosley TM. Mitochondrial abnormalities in patients with primary open angle glaucoma. Investig Ophthalmol Vis Sci. 2006;47(6):2533–41.

114. Abu-Amero KK, Morales J, Osman MN, Bosley TM. Nuclear and mitochondrial analysis of patients with primary angle closure glaucoma. Investig Ophthalmol Vis Sci. 2007;48(12):5591–6.
115. Abu-Amero KK, Bosley TM, Morales J. Analysis of nuclear and mitochondrial genes in patients with pseudoexfoliation glaucoma. Mol Vis. 2008;14:29–36.
116. Tanwar M, Dada T, Sihota R, Dada R. Mitochondrial DNA analysis in primary congenital glaucoma. Mol Vis. 2010;16:518–33.
117. Abu-Amero KK, Edward DP. Primary congenital glaucoma. 2004 Sep 30 [Updated 2017 Aug 17]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2020.
118. Bouhenni RA, Dunmire J, Sewell A, Edward DP. Animal models of glaucoma. J Biomed Biotechnol. 2012;2012:692609. https://doi.org/10.1155/2012/692609.
119. Aires ID, Ambrósio AF, Santiago AR. Modeling human glaucoma: lessons from the in vitro models. Ophthalmic Res. 2017;57(2):77–86. https://doi.org/10.1159/000448480.

Genetics in Glaucoma

7

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7.1 Introduction

Glaucoma is a condition of progressive optic neuropathy with intraocular pressure (IOP) as a modifiable risk factor. As per the Global data for visual impairment (2002), Glaucoma was the second leading cause of blindness [1]. The knowledge of its pathogenesis can aid in the development of new therapeutic approaches and thereby reduction in blindness due to glaucoma.

The current management strategies are targeted towards reducing the secretion of aqueous, increasing the outflow facility or creating alternate drainage pathway. The evolving research on genetics of glaucoma and next generation sequencing is opening new insights into its pathogenesis and thereby new targets for the management.

7.2 Genes Involved in the Development of the Eye

The development of eye from the surface ectoderm, mesoderm and neural crest involves complex interactions of various growth factors with their receptors, signalling factors, transcription factors and the structural components that form the anatomy of the eye. The genes coding for these proteins can be broadly classified as:

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1. Structural genes form the cytoskeletal components and are responsible for the structural and biochemical functions
2. Regulatory genes regulate the expression of genes which code for proteins like transcription factors and signalling factors
3. Cell specific genes express cell specific proteins

In particular interest are the genes that contribute to the development of the anterior segment of the eye, i.e. the genes coding for the transcription factors like PITX2, PITX3, PAX6, FOXC1, FOXC2 and FOXC3 which have been frequently associated with anterior segment dysgenesis [2]. The abnormalities in the expression of these genes or abnormalities in the interaction between multiple gene products due to mutation can lead to structural and functional defects in the eye. For example, in transgenic mice, multiple factors like the cell signalling molecule, bone morphogenetic proteins and related signalling factors and their interaction have shown to be associated in the development of the anterior segment of the eye [3].

New molecules involved in the development of ocular structures are being identified. In a recent study, serine proteinase PRSS56 has been shown to play a role in the development and maintenance of ocular drainage tissues [4].

7.3 Discovering Candidate Genes for Glaucoma

It has been known that many types of glaucoma including primary open angle glaucoma, congenital glaucoma and developmental glaucoma run in families. Von Graefe identified multiple families with glaucoma occurring in many generations [5]. In addition, it has been observed that there is a higher concordance of glaucoma between mono zygotic than dizygotic twins. All these factors pointed to the fact that glaucoma is inherited at least in some proportion.

It is interesting to note that the elevation in IOP, reduced outflow facility and IOP rise in response to steroid administration have shown hereditary tendencies. Studies are being conducted in analysing a particular trait like IOP, CCT, etc. Such traits that influence the disease course and are known to have genetic component are called endophenotypes. The loci identified by genetic studies influencing a particular trait are called Quantitative trait loci (QTL).

Significant among such studies are the Beaver Dam study and Salisbury Eye Study which showed that elevated IOP is influenced by genes in seven loc on chromosomes 2, 5, 6, 7, 12, 15 and 19 and by environmental factors and that several optic disc parameters including vertical cup to disc ratio are heritable even more than the IOP [6, 7].

Another study, the Blue Mountain Eye Study showed that at least in 18% of patients with glaucoma, the IOP variance is genetically influenced [8]. A family study showed that chromosome 10q22 is associated with IOP in addition to a study of an affected sibling pair which showed that chromosome 5q22 and 14q22 also are associated with glaucoma [9, 10]. But the pattern of inheritance is elusive as they did not follow the laws of Mendelian Inheritance.

The genes involved in any disease can be identified and analysed by various methods like:

1. Linkage analysis: Analysing the pattern of inheritance of the identified gene in subsequent generations in families with the phenotype under study.
2. Association analysis: Analysing the contribution of a genetic variance or an environmental factor between case and control in a large cohort in causing the phenotype or a particular trait like IOP.
3. Genome Wide Association Studies (GWAS): A recently developed method which rapidly scans the genome of large study group for markers or variants of diseases, especially useful in analysing the diseases with low penetrance like POAG.

These approaches can also be used to study the pharmacogenetics (How an individual's body responds to a particular drug based on their genomic sequence?) and pharmacogenomics (How a drug responds to an individual based on his genomic sequence?) which will aid in individualised therapeutic approaches based on the genomic sequence of each individual in contrast to one treatment to all patients with same phenotype.

7.4 How Genes Cause a Disorder?

From the concept of single gene causing a rare disease, now we know that the genetic involvement is complex and multifactorial as follows:

1. Polygenic traits: Defects in multiple genes contribute to one disease. For example, Axenfeld–Rieger syndrome appears to be caused by at least three different genes located on chromosomes 4, 6 and 13 showing the genetic heterogeneity [11–13].
2. Single gene plus environmental factors: In exfoliative glaucoma which is associated with multiple polymorphisms of the LOXL1 gene, the genetic expression seems to be influenced by an environmental factor.
3. Single gene can be associated with multiple disorders, e.g. Rieger syndrome and iris hypoplasia can arise from mutations in the same gene on 4q25 (PITX2) and primary congenital glaucoma and iridogoniodysgenesis can be caused by mutations in the FKHL7 gene on 6p25 [14–16].
4. Incomplete penetrance: It is seen in cases where the gene is not expressed as phenotype in all individuals with the gene. For example, if an allele is present in 10 individuals and 7 express in their phenotype, the allele is said to have 70% penetrance as shown in Fig. 7.1.
5. Co dominant inheritance: Different genotypic combination of an allele causes a phenotype with characteristics of all the alleles in varying proportions and no allele is completely suppressed as shown in Fig. 7.2.

Fig. 7.1 Diagrammatic representation of incomplete penetrance—the allele is not translated to phenotype in all the individuals bearing the allele

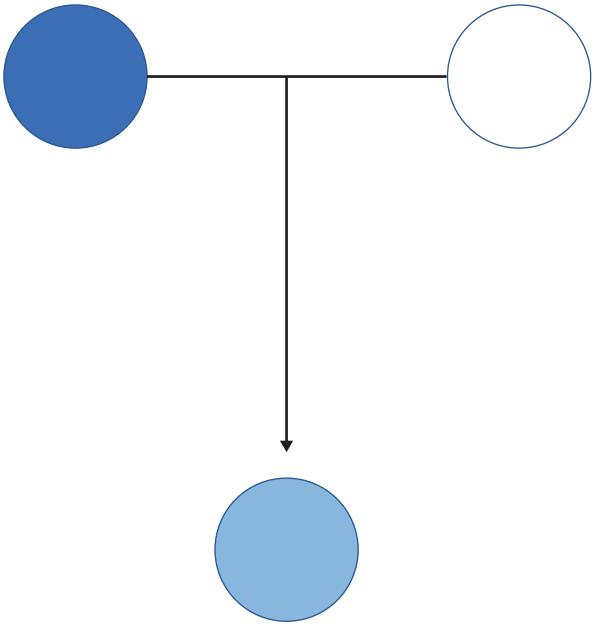

Fig. 7.2 Co-dominant inheritance—the offspring of two different alleles expresses a phenotype with characteristics of both the alleles

6. Imprinting effects: An epigenetic mechanism in which an allele of one parent is expressed and the other allele from other parent is imprinted possibly by post translational modifications of DNA like DNA methylation without altering the genetic sequence as shown in Fig. 7.3.
7. Mitochondrial inheritance

The pattern of inheritance seen in glaucoma varies with the type of glaucoma and is shown in Fig. 7.4.

7.5 Identification of Genes—Significance

The Human Genome Project has shown the possibility of decoding the entire sequence of the genome. A part of the Human Genome Project is the HapMap Project which catalogues the variances in the genome among individuals of diverse population. It has been identified that certain sequences of DNA variation are shared

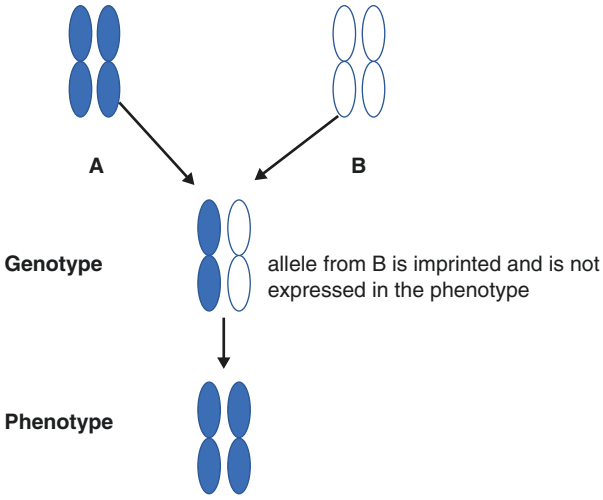

Fig. 7.3 Genomic Imprinting—one allele is imprinted by post translational modification in the offspring on a parent specific pattern

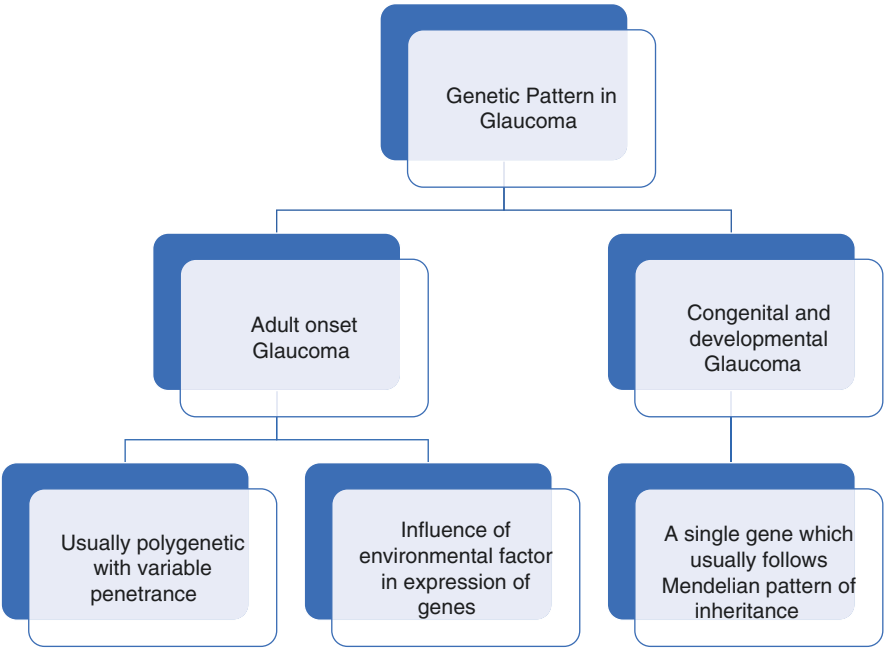

Fig. 7.4 The pattern of genetic inheritance in glaucoma follows the Mendelian pattern of inheritance in congenital and developmental glaucoma while the adult onset glaucoma exhibits genetic heterogeneity and environmental influence

among individuals of a population. They are called Single Nucleotide Polymorphisms (SNPs) and can be used as genetic markers. This can pave way to

1. Identify glaucoma even before its manifestation by the genetic analysis of the individuals with high risk.
2. To insert reparative genetic sequence through vectors like viruses in an individual with the faulty genes.
3. Selective embryo selection in couple with high risk genes thereby negating the possibility of inheriting the gene associated with glaucoma.

7.6 Genetic Nomenclature

The genes identified are given designation in a defined format by the HUGO (The Human Genome Organisation). It is usually in the following pattern: the first three letters denote the name of the disease—GLC indicates Glaucoma followed by a number 1,2 or 3 and an alphabet. (1—open angle, alphabet—identification of the gene.) For example: GLC1A, A refers to myocilin.

7.7 Primary Congenital Glaucoma (PCG)

It is a rare disease with the incidence of 1:10000 of which most of the cases are sporadic [17]. Around 10% cases are familial, and the inheritance is autosomal recessive. The gene frequently associated with congenital glaucoma is CYP1B1 and is related to the development of the eye. Although the exact mechanism is unknown, mice with mutant CYP1B1 gene had defective anterior chamber angle, increase in basal lamina and poorly developed Schlemm canal. This gene has been mapped in many linkage analyses of families with PCG. It encodes a protein of the cytochrome P450 family and is actively involved in the detoxification, xenobiotics, etc., which is highly expressed in foetal eyes. Its presence has been documented in corneal epithelium, keratocytes and iris stromal cells [18].

The identification of this gene led to search of factors that modify the expression of it. This search resulted in the identification of Tyrosinase gene which acts as a modifier gene, mutation of which lead to exacerbation of the defects in an individual with mutant CYP1b1. It was also interesting to note that administration of L-DOPA to these mutants circumvented the phenotypic defects showing that Tyrosinase pathway is involved in the development of anterior segment [19]. The locus for the gene CYP1B1 was mapped to the short arm of chromosome 2 in 2p21 [20]. In addition to this gene, further studies conducted on families with PCG have identified a second locus related to congenital glaucoma in the chromosome 1p36 [21]. The exact role of this locus remains to be analysed.

Extensive studies on CYP1B1 in Indian patients have shown various mutations—missense and termination mutations predominantly,although few deletions and frame shift mutations have also been observed [22]. The mutation that is observed consistently in an analysis of over 140 Indian families was Arg368His [23]

along with Pro129Leu, Glu229Lys, Arg390Cys, Gly61Glu and 367insA. Arg368His mutation is seen more frequently in South Indian population when compared to North Indian population, while the mutations Leu24Arg, Phe190Leu and Gly329Asp were observed in North Indian population [24].

LTBP2 (Latent Transforming Growth Factor Beta Binding Protein 2) Gene in chromosome 14q24 has been shown to be associated with PCG recently and is designated as GLC3C. This locus has been studied in Iranian population [25], Pakistani population and European gypsies [26]. It is intriguing to note that the specific mutation Arg299Stop in LTBP2 was found in both Pakistanis and European gypsies. It suggests that both these population have common ancestry as indicated by anthropological and genetic evidence.

7.8 Juvenile Onset Open Angle Glaucoma (JOAG)

It is characterised by early onset, high IOP and usually seen in myopes. The gene primarily associated with JOAG is TIGR (Trabecular Meshwork Induced Glucocorticoid Response gene),which is now referred as myocilin found in the chromosome 1q21-31. Around 10–20% of the JOAG patients have defective myocilin gene. At least five loci have been associated with JOAG including mutations like myocilin [27].

7.9 Primary Open Angle Glaucoma (POAG)

POAG is known to occur in more frequency with a positive family history and positive family history is an important risk factor for the development of primary open angle glaucoma as concluded by many studies including Barbados eye study and Baltimore eye studies. The screening of first-degree relatives of POAG in Indian population too has given similar results [28].

The genetic pattern of inheritance of this common type of glaucoma is complex and is associated with genetic heterogeneity. POAG exhibits variability in age of onset and is characterised by its low penetrance. The development of POAG is influenced by polygenic interactions and environmental factors and hence the inheritance pattern of POAG cannot be studied effectively by linkage analysis.

The gene that has been consistently shown to be involved in the pathogenesis of the POAG is MYOC gene identified in trabecular meshwork (earlier known as TIGR [29]). Mutations like Gly367Arg in MYOC gene cause reduced outflow through the trabecular meshwork (TM) due to aggregation of the mutant myocilin in the endoplasmic reticulum of meshwork cells [30, 31]. This causes defective secretion of the myocilin in the TM and thereby defective outflow through the TM. There have been various studies which have analysed the different mutations and polymorphisms in MYOC gene. The other mutations identified to be associated with POAG are OPTN gene coding for the protein Optineurin, WDR36,Neurotrophin 4 (NTF4),ankyrin repeat,TANK binding kinase 1(TBK1) and SOCS box-containing 10 [32].

In addition, genetic polymorphisms also play a role in POAG. Although they are not causative, the genetic polymorphism in ADRB1 and ADRB2 (the genes coding for β adrenergic receptors in trabecular meshwork and ciliary body) seems to play a role in the development of POAG and NTG in the Japanese population.

Given the heterogenous nature of genetic role in POAG, multiple genes have been identified by the Genome Wide Association studies(GWAS) which include Caveolin (CAV1/CAV2) [33], CDKN2B antisense RNA, TMCO1, ATOH 7, SIX1/SIX6, GAS7, chromosome 8q22, ABCA1, AFAP1, GMDS, PMM2, FNDC3B, TFGBR3, TXNRD2, ATXN2 and LRP12/ZFPM2 genes or actual loss of DNA(TBK1 and GALC). These are found in normal individuals but are found in high frequency in patients with POAG. Optineurin (GLC1E) gene in chromosome 10p15-14 plays a limited role specifically in familial and normal tension POAG. The genes associated with POAG are listed in Table 7.1. It is

Table 7.1 List of genes associated with POAG

Locus	Chromosome	Gene	Details of the study	Reference
GLC1A	1q31	MYOC	JOAG and adult onset POAG	Stone et al. [34]
GLC1B	2 cen-q13		Linkage analysis done in 6 families	Stoilova et al. [35]
GLC1C	3q21-24		A large family from North America	Wirtz et al. [36]
GLC1D	8q23		A family with POAG without mutations in 2 ce-q13 and 3q21-24	Trifan et al. [37]
GLC1E	10p15-14	OPTN	A British family with normal tension glaucoma	Sarfarazi et al. [38] and Rezaie et al. [39]
GLC1F	7q35-36	ASB10	A single family from Oregon, USA	Wirtz et al. [40] and Pasutto et al. [41]
GLC1G	5q22.1	WDR 36	Mapping of gene involved in T cell activation in seven families	Monemi et al. [42]
GLC1H	2p15-16	Yet to be identified	Seven families of POAG	Suriyapperuma et al. [43]
GLC1I	15q11-q13	Yet to be identified	Mapped in early adult onset POAG	Allingham et al. [44] and Wiggs et al. [45]
GLC1J	9q22	AD	In JOAG	Wiggs et al. [46]
GLC1K	20p12	AD	In JOAG	Wiggs et al. [46]
GLC1L	3p21-22	Yet to be identified	POAG in a Tasmanian family	Baird et al. [47]
GLC1M	5q22-31	Yet to be identified	JOAG in Filipino family studied in five generations	Pang et al. [48]
GLC1N	15q22-24	Yet to be identified	JOAG	Wang et al. [49]
GLC1O	19q13	NTF4	Normal tension and high tension POAG	Pasutto et al. [50]
GLC1P	12q14	AD	Normal tension glaucoma	Fingert et al. [51]

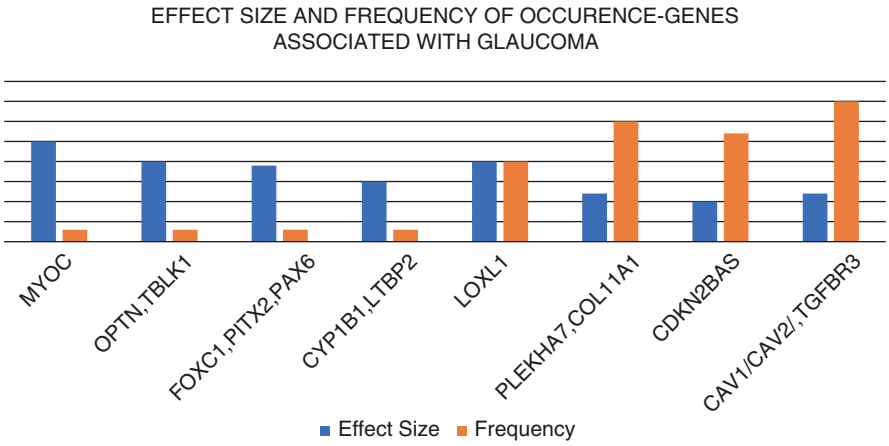

Fig. 7.5 Frequency and effect size of few genes associated with glaucoma. The genes with high effect size tend to occur rarely and the genes with low effect are seen more frequently

interesting to note that the genes with Mendelian form of inheritance (high effect) occur less frequently when compared to the genes with low effect size as shown in Fig. 7.5.

In South India, a study conducted to assess the MYOC mutations in Indian Population (107 subjects with POAG and 90 Normal subjects of a relatively unexplored ethnicity) found it in about 2% of the participants with POAG. Additionally, the study also analysed the type of mutations which showed Gly367 Arg and Thr 377Met was seen only in the POAG patients and not in normal controls. These mutations led to charged or bulky protein, defect in oligomer formation and poor secondary structure formation. Also, the non-sense mutation gln368stop frequently seen in Western Population was characteristically absent in Indian population [52].

Majority of the myocilin mutations are missense mutations (>80%) with few patients of frame shift and non-sense mutation (<5%) seen commonly in the sequence coding for olfactomedin like domain found in third exon of myocilin gene. These mutations have been documented in database (www.myocilin.com). The other common mutations seen in Indian populations are listed in Table 7.2.

Another possible mechanism for the aggregation of myocilin in primary open angle glaucoma could be due to variations in splicing of the protein structure. The mutations in the myocilin genomic region could result in synonymous codon changes or changes in the intron regions that may not change the amino acid sequence but may possibly cause variations in intron–exon splicing. The study done to analyse the possibility of polymorphism in the intronic region of the myocilin gene, showed that g.14072G>A polymorphism and g.1293C/T heterozygous polymorphism were present instead of the expected g.1293C/-polymorphism. Also,two new SNPs (g.1295G>T and g.1299T>G) and two previously reported SNPs (g. 1284G>T and g.1286G>T) were also identified [53].

Table 7.2 Common mutations in myocilin observed in Indian population

Reference	MYOC mutations identified	Method used
Mukhopadhyay et al. (2002)	Gln48His; Pro370Leu	Sequencing
Kanagavalli et al. (2003)	Gly367Arg, Thr377Met	Single Stranded Conformation Polymorphism—SSCP
SriPriya et al. (2004)	Gln48His	Sequencing
Chakrabarti et al. (2005)	Gln48His	PCR-RFLP and sequencing
Bhattacharjee et al. (2007)	Gln48His, Thr256Met, Thr353Ile, Pro370Leu, Gln368Stop, Gln399Asp, Ala427Thr	Sequencing
Kumar et al. (2007)	Gln48His	SSCP, PCR-RFLP and sequencing
Rose et al. (2007, 2011)	Ser331Thr, Pro370Leu, Gln48His, Thr353Ile/Asn480Lys	Sequencing and SSCP
Banerjee et al. (2012)	D273fsX344, Gln368Stop, Pro370Leu, Gly399Asp, Ala427Thr, Thr256Met, Ser331Leu	Sequencing

Table 7.3 Loci identified with the associated pathway in pathogenesis of POAG

Pathway	Loci associated
Cell division	CDKN2BAS, GAS7
Autophagy	CAV1,ABCA1GMDS,PMM2
Development	SIX6,FOXC1
Mitochondria	TXNRD2
TGF beta	CDKN2BAS, FNDC3B, TGFBR3
Lipids membranes	CAV1, ABCA1
Vascular tone	CAV1
Extracellular matrix	AFAP 1
CSF pressure	8q22

Another study on POAG was done for the clinical characterisation of a large POAG Pedigree (84 members of the identified family) along with genetic analysis of the participants for the genes myocilin, optineurin and TBK1. Interestingly, the participants did not harbour any of the three genes commonly associated with POAG [54]. This raises the need for further studies to identify responsible genes in different populations.

The genes identified in association with POAG contributing to various pathways of cell biology. Analysing their role in the pathways may shed more light on the pathogenesis of POAG. Some loci identified with the associated pathway in the pathogenesis of POAG are shown in Table 7.3.

Table 7.4 Identified Loci for physical traits of POAG

Factors	Qualitative trait loci
Intra ocular pressure	TMCO1,ABCA1
Central corneal thickness	Collagen Pathway
Optic disc size	ATOH7,CDC7
Vertical cup to disc ratio	CDKN2BAS, SIX1,SIX6

The International Glaucoma Genetic Consortium which strives to identify loci (QTL) related to individual physical trait and thereby the genes related to POAG through meta-analyses of many GWASes has identified qualitative trait loci of various features of POAG and are shown in Table 7.4.

7.10 Primary Angle Closure Glaucoma (PACG)

The incidence of primary angle closure glaucoma is high in certain regions of the world (East Asia) suggesting that PACG could be inherited at least in some proportion. It has been found that there is a sixfold higher chance of developing PACG in individuals with familial history. A study conducted with large number of subjects with family history of angle closure glaucoma or angle closure suspect to analyse the risk of developing angle closure glaucoma has shown that the risk is higher with history of PACG in the family when compared to an angle closure suspect [55]. This further emphasises the heritable nature of the traits. The physical traits like shallow anterior chamber trait was also observed to be running in families and is shown to be heritable in 70% of the individuals.

It has been known that CYP1B1 is associated with angle closure glaucoma in addition to primary open angle glaucoma and primary congenital glaucoma. A number of newer loci associated with PACG identified in GWAS conducted in 2012 and in 2016 with meta-analysis of GWAS are SNP-rs11024102 in PLEKHA7, rs1015213 in the intergenic sequence found between PCMTD1 and ST18 on chromosome 8q, rs3753841 in COL11A1, EPDR1, GLIS3, DPM2-FAM102, ChAT and FERMT2 [56, 57].

With multiple studies identifying an array of loci, the need for more studies to understand the role of these loci in the pathogenesis cannot be over emphasised. In a study from South India to detect three SNPs associated with PACG, only one was identified in the study population which highlights the need for studies with larger sample size to confirm the role of the other SNPs—PLEKHA7 and COL11A1 in the pathogenesis of PACG [58].

In case of nanophthalmos, wherein the affected individuals are susceptible to angle closure glaucoma, a locus NNO1 has been mapped to chromosome 11 in a family with high penetrance. The detailed analysis of this locus may shed further light in the pathogenesis of this condition.

Table 7.5 Pathways and the loci associated with PACG

Pathway	Loci associated
Collagen pathway	COL1A1
Cell adhesion	PLEKHA7,EPDR1, DPM2-FAM102A
Developmental gene—ACD	ABCC5
Cholinergic system	ChAT
Unknown function	ST18-PCMTD1

Like POAG, the loci identified with various pathways associated with PACG are shown in Table 7.5.

7.11 Developmental Glaucoma

Developmental glaucoma arise due to the defect in the morphogenesis of the anterior segment. The genes involved in the developmental glaucoma are those that are involved in the embryogenesis of the anterior segment of the eye as discussed earlier *vide supra* in the genes in development of the eye, i.e. the genes coding for transcription factors PITX2, PITX3 and FOXC1.

7.12 Pigmentary Glaucoma

Pigmentary glaucoma is known to follow autosomal dominant inheritance, commonly found in young myopic males. Like most types of glaucoma, this type also shows heterogenous genetic involvement. One locus has been identified in the chromosome 7q35-36. In mouse models, locus coding for the glycoprotein transmembrane protein Gpnmb has been associated with pigmentary dispersion. Further studies are needed to confirm the definite role of this locus.

7.13 Exfoliative Glaucoma

The exfoliative glaucoma involves deposition of microfibrillary material due to abnormal metabolism of extracellular material. The SNPs consistently associated with exfoliative glaucoma are found in the LOXL1 gene on the chromosome 15q24 coding for the Lysyl oxidase like 1 protein. This protein is involved in the formation of elastin. Multiple polymorphisms of this gene have been associated with exfoliative glaucoma like SNPs—rs2165241, rs1048661,rs3825942. All these polymorphisms have been detected in the first exon of LOXL1 gene.

It is interesting to note that population with the same genetic sequence of LOXL1 gene residing in different parts of the world have shown variations in their risk of developing exfoliative glaucoma. This shows that additional genetic or environmental factors may exist, which influence the expression of LOXL1 gene. Hence,

in-depth analysis of various factors can shed further light on the pathogenesis of exfoliative glaucoma.

Loci identified by the GWAS on exfoliative glaucoma different population, rs3825942 risk allele was found to be protective for South African population in contrast, the same allele was associated with risk of XFS in Caucasians. A rare allele p.Y407F of LOXL1 found in Japanese population has shown to be protective against XFS. This necessitates further analysis to identify how the individual susceptibility to the risk allele leads to the development of XFS.

7.14 Calcium Voltage-Gated Channel Subunit Alpha1 A (CACNA1A)

This locus identified in GWAS of XFS codes for the alpha 1A subunit of P/Q voltage dependent calcium channel and is seen to be distributed in different ocular tissues [59]. It is known that high calcium concentration is seen in the XFS fibrils and that calcium is needed for aggregation of fibrils. Whether altered calcium transport and its accumulation form the background for fibrillary deposition in XFS is to be studied. This raises the scope for newer therapy target for XFG. The various loci identified by the GWAS on exfoliative glaucoma are shown in Table 7.6.

The result of Genome Wide Association Study (GWAS) is based on the phenotype of the cases and controls participated in the study and the locus identified by a study involving larger sample size points to a greater association(relative risk attributable to the locus) with the disease or trait under study. If the participants under study exhibit a more defined phenotype, it aids in the deeper understanding of the function of the locus under study. For example, if we can study a large cohort of cases with exfoliative glaucoma associated with iris atrophy only or associated with lens subluxation only, the effects of that particular locus in the developing iris atrophy or lens subluxation can be studied in detail.

Table 7.6 Loci identified by the GWAS on exfoliative glaucoma [59]

Name of the locus	SNP/ Chromosome	Function
Proteasome maturation protein (Pomp)	rs7329408 on the Chr 13	Ubiquitin—proteasome synthesis
Transmembrane protein 136 (TMEM136)	rs11827818 on Chr 11	Unknown
1-acylglycerol-3-phosphate O-acyltransferase 1 I(AGPAT1)	rs3130283 on Chr 6	Located in MHC, it is involved in the synthesis of glycerolipids
RNA binding motif single stranded interacting protein 3 (RBMS3)	rs 12490863 on Chr 3	Shown to inhibit cell proliferation, angiogenesis and induce apoptosis and has tumour suppression properties
Semaphorin 6A	rs10072088 on Chr 5	Transmembrane protein

7.15 Limitations of GWAS

GWAS is a method of approach in analysing the genetic markers of many diseases in recent days. It is important that we realise it is not without limitations like:

1. It identifies only the signal, i.e. the region around the gene responsible and not the exact exon and hence can be taken as surrogate genetic marker only
2. Regulatory genetic sequences are often missed in the study.

Hence, the technology that can study the locus specifically can throw more light on the genetic pathways.

7.16 Genetics in Glaucoma—A Step in Future

7.16.1 Whole Exon/Genome Sequencing

The whole genome sequencing can bring out massive information on the functioning of each locus opening up a whole new therapeutic approach to our existing armamentarium. The high cost is the limiting factor at present but it may be more affordable in the future.

7.16.2 Comparative RNA Sequencing of Tissues

This technology analyses the RNA from the ocular tissues of the cases and control to identify the expression of the candidate gene or locus.

7.16.3 Gene Therapy

This therapeutic approach is based on altering the genetic sequence by replacing it or suppressing the candidate gene through the vectors like recombinant adeno-associated virus-rAAV, lentiviral vectors, etc.

The defective phenotype could also be modified by delivering the protein which is structurally and functionally designed to carry out the specific function. The ideal therapy would be cell specific without causing any toxicity or without eliciting unfavourable immune reaction and be able to address the symptoms in a single dose.

Eye being one of the accessible, immunologically unique and highly compartmentalised organ, it facilitates easy drug delivery and monitoring of the effects of the intervention. Also, the other eye can be ideal control for the intervention under study. Gene therapy has given promising results in retinal dystrophies like Leber's congenital amaurosis.

In glaucoma, retinal ganglion cells, trabecular meshwork and optic nerve head could be potential targets for the gene therapy. For example, BDNF (Brain Derived

Neurotrophic Factor) has neuroprotective properties and is being studied to reduce the retinal ganglion cell loss in glaucoma [60]. Also studies to increase the outflow in the trabecular meshwork through a specifically designed gene are being undertaken [61].

The knowledge of genetic role in glaucoma holds promises in greater understanding of the genesis of the disease, its molecular mechanisms and eventually leads to novel therapeutic approaches.

References

1. Resnikoff S, Pascolini D, Etyaolin D, et al. Global data on visual impairment in the year 2002. Bull World Health Organ. 2004;82:844–51.
2. Sowden JC. Molecular and developmental mechanisms of anterior segment dysgenesis. Eye (Lond). 2007;21(10):1310–8.
3. Wordinger RJ, Clark AF. Bone morphogenetic proteins and their receptors in the eye. Exp Biol Med (Maywood). 2007;232(8):979–92.
4. Labelle-Dumais C, Pyatla G, Paylakhi S, et al. Loss of PRSS56 function leads to ocular angle defects and increased susceptibility to high intraocular pressure. Dis Model Mech. 2020;13(5):dmm 042853. Published 2020 May 29.
5. von Graefe A. Pathology and treatment of glaucoma. Royal London Ophthalmic Hospital Rep J Ophthalmic Med Surg. 1871l Lon;7:108.
6. Duggal P, Klein AP, Lee KE, et al. A genetic contribution to intraocular pressure: the Beaver dam eye study. Investig Ophthalmol Vis Sci. 2005;46(2):555–60.
7. Klein BE, Klein R, Lee KE. Heritability of risk factors for primary open-angle glaucoma: the Beaver Dam Eye Study. Investig Ophthalmol Vis Sci. 2004;45:59.
8. Viswanathan AC, Hitchings RA, Indar A, et al. Commingling analysis of intraocular pressure and glaucoma in an older Australian population. Ann Hum Genet. 2004;68(Pt 5):489–97.
9. Charlesworth JC, Dyer TD, Stankovich JM, et al. Linkage to 10q22 for maximum intraocular pressure and 1p32 for maximum cup-to-disc ratio in an extended primary open-angle glaucoma pedigree. Investig Ophthalmol Vis Sci. 2005;46(10):3723–9.
10. Rotimi CN, Chen G, Adeyemo AA, et al. Genome wide scan and fine mapping of quantitative trait loci for intraocular pressure on 5q and 14q in West Africans. Investig Ophthalmol Vis Sci. 2006;47(8):3262–7.
11. Semina EV, Reiter R, Leysens NJ, et al. Cloning and characterization of a novel bicoid-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. Nat Genet. 1996;14:392–9.
12. Mirzayans F, Gould DB, Heon E, et al. Axenfeld-Rieger syndrome resulting from mutation of the FKHL7 gene on chromosome 6p25. Eur J Hum Genet. 2000;8(1):71–4.
13. Phillips JC, del Bono EA, Haines JL, et al. A second locus for Rieger syndrome maps to chromosome 13q14. Am J Hum Genet. 1996;59(3):613–9.
14. Héon E, Sheth BP, Kalenak JW, et al. Linkage of autosomal dominant iris hypoplasia to the region of the Rieger syndrome locus. Hum Mol Gene. 1995;4:1435–9.
15. Nishimura D, Swiderski R, Alward W, et al. The fork-head transcription factor gene FKHL7 is responsible for glaucoma phenotypes which map to 6p25. Nat Genet. 1998;19:140–7.
16. Mears AJ, Jordan T, Mirzayans F, et al. Mutations of the fork-head/winged-helix gene, FKHL7, in patients with Axenfeld-Rieger anomaly. Am J Hum Genet. 1998;63:1316–28.
17. Ho CL, Walton DS. Primary congenital glaucoma: 2004 update. J Pediatr Ophthalmol Strabismus. 2004;41(5):271–88.
18. Zhao Y, Sorenson CM, Sheibani N. Cytochrome P4501B1 and primary congenital glaucoma. J Ophthalmic Vis Res. 2015;10:60–7.
19. Libby RT, Smith RS, Savinova OV, et al. Modification of ocular defects in mouse developmental glaucoma models by tyrosinase. Science. 2003;299:1578–81.

20. Sarfarazi M, Akarsu AN, Hossain A, Turacli ME, Aktan SG, Barsoum-Homsy M, et al. Assignment of a locus (GLC3A) for primary congenital glaucoma (Buphthalmos) to 2p21 and evidence for genetic heterogeneity. Genomics. 1995;30(2):171–7.
21. Akarsu AN, Turacli ME, Aktan SG, et al. A second locus (GLC3B) for primary congenital glaucoma (Buphthalmos) maps to the 1p36 region. Hum Mol Genet. 1996;5(8):1199–203.
22. Panicker SG, Reddy ABM, Mandal AK, Ahmed N, Nagarajaram HA, Hasnain SE, Balasubramanian D. Identification of novel mutations causing familial primary congenital glaucoma in Indian pedigrees. Investig Ophthalmol Vis Sci. 2002;43(5):1358–66.
23. Reddy ABM, Panicker SG, Mandal AK, Hasnain SE, Balasubramanian D. Identification of R368H as a predominant CYP1B1 allele causing primary congenital glaucoma in Indian patients. Investig Ophthalmol Vis Sci. 2003;44:4200–3.
24. Reddy ABM, Kaur K, Mandal AK, Panicker SG, Thomas R, Hasnain SE, et al. Mutation spectrum of the CYP1B1 gene in Indian primary congenital glaucoma patients. Mol Vis. 2004;10:696–702.
25. Narooie-Nejad M, Paylakhi SH, Shojaee S, Fazlali Z, Rezaei Kanavi M, Nilforushan N, et al. Loss of function mutations in the gene encoding latent transforming growth factor beta binding protein 2, LTBP2, cause primary congenital glaucoma. Hum Mol Genet. 2009;18(20):3969–77.
26. Ali M, McKibbin M, Booth A, Parry DA, Jain P, Riazuddin SA, et al. Null mutations in LTBP2 cause primary congenital glaucoma. Am J Hum Genet. 2009;84(5):664–71.
27. Sud A, Del Bono EA, Haines JL, Wiggs JL. Fine mapping of the GLC1K juvenile primary open-angle glaucoma locus and exclusion of candidate genes. Mol Vis. 2008;14:1319–26.
28. Rajendrababu S, Gupta N, Vijayakumar B, Kumaragurupari R, Krishnadas SR. Screening first degree relatives of persons with primary open angle glaucoma in India. J Curr Glaucoma Pract. 2014;8(3):107–12.
29. Polansky JR, Fauss DJ, Chen P, et al. Cellular pharmacology and molecular biology of the trabecular meshwork inducible glucocorticoid response gene product. Ophthalmologica. 1997;211(3):126–39.
30. Kanagavalli J, Pandaranayaka E, Krishnadas SR, et al. In vitro and in vivo study on the secretion of the Gly367Arg mutant myocilin protein. Mol Vision. 2007;13:1161–8.
31. Kanagavalli J, Pandaranayaka E, Krishnadas SR, Krishnaswamy S, Sundaresan P. A review of genetic and structural understanding of the role of myocilin in primary open angle glaucoma. Indian J Ophthalmol. 2004;52(4):271–80.
32. Abu-Amero K, Kondkar AA, Chalam KV. An updated review on the genetics of primary open angle glaucoma. Int J Mol Sci. 2015;16(12):28886–911.
33. Wiggs JL, Kang JH, Yaspan BL, et al. Common variants near CAV1 and CAV2 are associated with primary open- angle glaucoma in Caucasians from USA. Hum Mol Genet. 2011;20:4707–13.
34. Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, et al. Identification of a gene that causes primary open angle glaucoma. Science. 1997;275:668–70.
35. Stoilova D, Child A, Trifan OC, Crick RP, Coakes RL, Sarfarazi M. Localization of a locus (GLC1B) for adult-onset primary open angle glaucoma to the 2cen-q13 region. Genomics. 1996;36(1):142–50.
36. Wirtz MK, Samples JR, Kramer PL, Rust K, Topinka JR, Yount J, et al. Mapping a gene for adult-tonset primary open-angle glaucoma to chromosome 3q. Am J Hum Genet. 1997;60:296–304.
37. Trifan OC, Traboulsi EI, Stoilova D, Alozie I, Nguyen R, Raja S, et al. A third locus (GLC1D) for adult-onset primary open-angle glaucoma maps to the 8q23 region. Am J Ophthalmol. 1998;126(1):17–28.
38. Sarfarazi M, Child A, Stoilova D, Brice G, Desai T, Trifan OC, et al. Localization of the fourth locus (GLC1E) for adult-onset primary open-angle glaucoma to the 10p15-p14 region. Am J Hum Genet. 1998;62(3):641–52.
39. Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, et al. Adult-onset primary open-angle glaucoma caused by mutations in optineurin. Science. 2002;295:1077–9.
40. Wirtz MK, Samples JR, Rust K, Lie J, Nordling L, Schilling K, et al. GLC1F, a new primary openangle glaucoma locus, maps to 7q35-q36. Arch Ophthalmol. 1999;117:237–41.

41. Pasutto F, Keller KE, Weisschuh N, Sticht H, Samples JR, Yang YF, et al. Variants in ASB10 are associated with open-angle glaucoma. Hum Mol Genet. 2012;21(6):1336–49.
42. Monemi S, Spaeth G, DaSilva A, Popinchalk S, Ilitchev E, Liebmann J, et al. Identification of a novel adult-onset primary open-angle glaucoma (POAG) gene on 5q22.1. Hum Mol Genet. 2005;14(6):725–33.
43. Suriyapperuma SP, Child A, Desai T, Brice G, Kerr A, Crick RP, et al. A new locus (GLC1H) for adult-onset primary open-angle glaucoma maps to the 2p15-p16 region. Arch Ophthalmol. 2007;125:86–92.
44. Allingham RR, Wiggs JL, Hauser ER, Larocque-Abramson KR, Santiago-Turla C, Broomer B, et al. Early adult-onset POAG linked to 15q11-13 using ordered subset analysis. Investig Ophthalmol Vis Sci. 2005;46(6):2002–5.
45. Wiggs JL, Allingham RR, Hossain A, Kern J, Auguste J, DelBono EA, et al. Genome-wide scan for adult onset primary open angle glaucoma. Hum Mol Genet. 2000;9(7):1109–17.
46. Wiggs JL, Lynch S, Ynagi G, Maselli M, Auguste J, Del Bono EA, et al. A genome-wide scan identifies novel early-onset primary open-angle glaucoma loci on 9q22 and 20p12. Am J Hum Genet. 2004;74(6):1314–20.
47. Baird PN, Foote SJ, Mackey DA, Craig J, Speed TP, Bureau A. Evidence for a novel glaucoma locus at chromosome 3p21-22. Hum Genet. 2005;117(217):249–57.
48. Pang CP, Fan BJ, Canlas O, Wang DY, Dubois S, Tam PO, et al. A genome-wide scan maps a novel juvenile-onset primary open angle glaucoma locus to chromosome 5q. Mol Vis. 2006;12:85–92.
49. Wang DY, Fan BJ, Chua JK, Tam PO, Leung CK, Lam DS, et al. A genome-wide scan maps a novel juvenile-onset primary open-angle glaucoma locus to 15q. Investig Ophthalmol Vis Sci. 2006;47(12):5315–21.
50. Pasutto F, Matsumoto T, Mardin CY, Sticht H, Brandsttool JH, Michels-Rautenstrauss K, et al. Heterozygous NTF4 mutations impairing neurotrophin-4 signalling in patients with primary open-angle glaucoma. Am J Hum Genet. 2009;85(4):447–56.
51. Fingert JH, Robin AL, Stone JL, Roos BR, Davis LK, Scheetz TE, et al. Copy number variations on chromosome 12q14 in patients with normal tension glaucoma. Hum Mol Genet. 2011;20(12):2482–9.
52. Kanagavalli J, Pandaranayaka E, Krishnadas SR, et al. Evaluation and understanding of myocilin mutations in Indian primary open angle glaucoma patients. Mol Vision. 2003;9:606–14.
53. Pandaranayaka PJ, Prasanthi N, Kannabiran N, et al. Polymorphisms in an intronic region of the myocilin gene associated with primary open-angle glaucoma--a possible role for alternate splicing. Mol Vis. 2010;16:2891–902. Published 2010 Dec 29.
54. Kader MA, Namburi P, Ramugade S, et al. Clinical and genetic characterization of a large primary open angle glaucoma pedigree. Ophthalmic Genet. 2017;38(3):222–5.
55. Kavitha S, Zebardast N, Palaniswamy K, et al. Family history is a strong risk factor for prevalent angle closure in a South Indian population. Ophthalmology. 2014;121(11):2091–7.
56. Vithana EN, Khor CC, Qiao C, et al. Genome-wide association analyses identify three new susceptibility loci for primary angle closure glaucoma. Nat Genet. 2012;44(10):1142–6.
57. Khor CC, Do T, Jia H, et al. Genome-wide association study identifies five new susceptibility loci for primary angle closure glaucoma. Nat Genet. 2016;48(5):556–62.
58. Duvesh R, Verma A, Venkatesh R, et al. Association study in a South Indian population supports rs1015213 as a risk factor for primary angle closure. Investig Ophthalmol Vis Sci. 2013;54:5624–8.
59. Prof. Tin Aung, Singapore National Eye Centre, National University of Singapore- Proceedings of All India Ophthalmology Conference 2020.
60. Osborne A, Khatib TZ, Songra L, et al. Neuroprotection of retinal ganglion cells by a novel gene therapy construct that achieves sustained enhancement of brain-derived neurotrophic factor/tropomyosin-related kinase receptor-B signalling. Cell Death Dis. 2018;9(10):1007.
61. Konda SM, Kiland JA, Mohr M, et al. Schlemmath Dis. of retinal ganglion asurement, catheterization and substance delivery in live monkeys. Investig Ophthalmol Vis Sci July. 2019;60:1788.

Genetics of Retinoblastoma

8

Pradeep Sagar and Mahesh Shanmugam

8.1 Introduction

Retinoblastoma is the most common malignant intraocular tumor in children [1]. Heritable retinoblastoma is a genetic disease with predisposition to retinoblastoma and second non-ocular tumors. The incidence of retinoblastoma is 1 in 16,000–18,000 live births [2]. The genetics of retinoblastoma is well studied and it is a prototype model for understanding the genetics of other cancers. This chapter will focus on the genes involved in retinoblastoma, development of retinoblastoma from genetic perspective, correlation between the genotype and the phenotype, indications and advantages of genetic testing.

8.2 Genes Involved in Retinoblastoma

8.2.1 RB1 Gene

RB1 gene is located on human chromosome 13 at locus 13q14.1–13q14.2. The RB transcript is encoded in 27 exons. Deletion of exons 13—17 is associated with the development of tumors like osteosarcoma, breast cancer, and retinoblastoma. RB1 gene is a tumor suppressor gene and encodes retinoblastoma protein (Rb) [3].

Rb protein is composed of 928 amino acids and has 3 domains: the central domain named "pocket," the amino terminal domain and carboxy terminal domain [4]. Other sequences in the Rb protein include insertion loops and linker sequences.

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The linker sequences contain cyclin-dependent kinase (CDK)-dependent phosphorylation sites. This is noteworthy as, Rb protein is inactivated by phosphorylation [5, 6].

Function of Rb protein is still debated. Earlier it was believed that various stimuli are channeled through regulation of Rb by CDK dependent Rb phosphorylation, which controls the activity of E2F transcription factors. But the recent data suggests that Rb is a multifunctional protein and acts in response to multiple stimuli and results in various outcomes. Rb protein acts as a tumor suppressor by its role in cell cycle control, heterochromatin, and chromosome stability and regulation of apoptosis [7].

8.2.2 Cell Cycle Control

Rb protein is involved in cell cycle control by various mechanisms which includes regulation of E2F transcription factors and CDK inhibition [7].

Regulation of E2F transcription factors: Rb is in hypophosphorylated active form in G1 phase and associates with E2F transcription factors and differentiation related polypeptide (DP) heterodimers and inhibits transcription of cell cycle genes. In S phase, Rb is inactivated by phosphorylation and it no longer binds to E2F transcription factors. The E2F promotes expression of cell cycle genes and allows progression of cell cycle [7, 8, 9].

CDK inhibition: p27 is an inhibitor of CDK. Stabilization of p27 favors CDK inhibition. Two models of p27 stabilization by Rb protein are described:

1. Rb protein binds to S phase kinase-associated protein 2 (SKP2) through its carboxy terminal domain. One of the functions of SKP2 is to recognize and facilitate degradation of p27 by ubiquitin-proteasome system. But inhibition of SKP2 by Rb, allows stabilization of p27 which inhibits CDK and results in cell cycle arrest [10].
2. Rb interacts with SKP2 and anaphase-promoting complex (APC) simultaneously. This targets SKP2 for degradation via ubiquitin-proteasome system. So P27 is stabilized and inhibits CDK [11].

8.2.3 Heterochromatin and Chromosome Stability

Rbprotein plays a role in heterochromatin and chromosomal stability by various mechanisms:

1. Loss of Rb leads to uncontrolled activity of E2F transcription factors. This leads to overexpression of mitosis arrest deficient 2 (MAD2) gene. This results in fusion of chromosome arms (lagging chromosome) and double-stranded deoxyribonucleic acid (DNA) breaks and mis segregation [12, 13]. Dysregulation of E2F may also lead to low nucleotide pool and replication stress. This may lead to abnormal firing of replication origins and aneuploidy [14].

2. Rb is also recognized as a regulator of heterochromatin domain that surrounds the centromere and plays a role in chromosome structure and the attachment to spindle microtubule. Deficiency of Rb protein leads to tangled chromosome at metaphase, mis segregation, and aneuploidy [7].

8.2.4 Regulation of Apoptosis

Recent studies have shown that Rb has pro-apoptotic activity by its interaction with E2F1. E2F1, compared to other E2Fs is identified to have an additional unique binding site on Rb [15]. The association between Rb and E2F1 is known to persist even when Rb is phosphorylated [16]. This complex containing phosphorylated Rb and E2F1 is demonstrated to have pro-apoptotic activity [17, 18].

8.2.5 MYCN

The MYCN gene is located on human chromosome 2 at locus2p24.3. MYCN gene is expressed in developing embryos and encodes a transcription factor of MYC family. Amplification of MYCN oncogene is seen in neuroblastoma [19]. High-level amplification of MYCN gene is noted in 1.5% of unilateral sporadic retinoblastoma.

8.3 Genetic Alterations and Development of Retinoblastoma

RB1 is a tumor suppressor gene. Both the copies of the tumor suppressor gene should be downregulated to promote development of tumor. Retinoblastoma develops from cells with cancer-predisposing pathogenic variants in both the copies of RB1 gene (Knudson two hit hypothesis) [20].

8.3.1 The First Hit

The first hit (mutation in one allele) is usually due to structural alterations in genome which are usually small or large scale chromosomal deletions and nonsense mutations [21]. Retinoblastoma can be sporadic or heritable. Heritable retinoblastoma is due to germline mutation and is an autosomal dominant susceptibility for retinoblastoma [22]. In heritable retinoblastoma with family history, one inactivated/pathogenic copy of RB1 is inherited from one of the parents and is present in all the cells of the body. In heritable retinoblastoma without family history, mutation of first RB allele arise de novo and can occur in ova or sperm during gametogenesis or can occur during conception in one cell stage [23, 24]. De novo mutations occur more commonly in sperms than ova owing to more rapid cell division in spermatogenesis. About 85% of new germline mutations are noted to occur in paternally

derived allele [25]. All the cells will have one pathogenic/inactivated RB allele. If the de novo mutation occurs after one cell stage in the embryo, a fraction of cells in the body will harbor the mutated allele. This is termed mosaicism [24]. In sporadic retinoblastoma, the inactivation of RB gene occurs in the somatic cell (retinal progenitor cell).

8.3.2 The Second Hit

The second hit can be due to mutation in the other copy of RB1 gene or due to loss of heterozygosity (LOH) [26, 27]. Human somatic cells contain two copies of gene, one derived from each parent. If the copies derived from each parent contain different bases, it is termed heterozygous. If a cell has only one mutated allele, it is heterozygous. As one copy is normal, the cell will not have potential for tumor development. Loss of this heterozygosity is known to account for 72.9% of second hit in retinoblastoma [28]. Loss of heterozygosity can be due to:

1. Chromosomal nondisjunction: Failure of separation of sister chromatids (normal allele) during mitoses can lead to loss of normal allele in one daughter cell. Then the mutated allele can duplicate and result in LOH.
2. Uniparental disomy: Both the copies of chromosome are derived from one parent.
3. Mitotic recombination: Exchange of genes between sister chromatids of the two allele

Retinoblastoma has high penetrance. The second hit usually occurs in 90% of the cases who inherit one mutated allele (germline mutation). In heritable retinoblastoma with one affected parent, 50% of the offspring can inherit the mutated allele and as the chance of second hit is 90%, the risk of retinoblastoma in offspring is 45% [21].

8.3.3 Mutation 3 and Genomic Instability

Inactivation of both the copies of RB1 gene permits the cell to undergo uncontrolled replication. This would result in the development of retinoma, a benign form of retinoblastoma [29]. Additional genetic alterations are required for the development of retinoblastoma. Gain of 1q31, 6p22, 2p24-25, 13q32-34, and loss of 16p22 are reported in retinoblastoma tumor cells [30, 31]. Amplification of MYCN gene at 2p24-25 is known to promote progression of retinoblastoma [31]. Transcription factor E2F3 is implicated in some of these chromosomal abnormalities. E2F3 is overexpressed in retinoblastoma and it is involved in the regulation of genes involved in DNA replication such as histone H2A9, DNA polymerase, and H2 folate reductase [27, 32]. $P16^{INK4A}$is identified to be involved in progression of retinoblastoma. $P16^{INK4A}$ is overexpressed in the early stages of uncontrolled proliferation of cells and inhibits subsequent proliferation. Failure in activation or inactivation of $P16^{INK4A}$

allows the cells to escape this inhibition and facilitates uncontrolled proliferation. RB1 gene is required for stability of genome and loss of RB1 leads to genomic instability [29].

8.3.4 Epigenetics in Retinoblastoma

Epigenetics refers to alterations in the genome that are heritable and affects the function of gene without any change in the DNA sequence. The epigenetic mechanisms involved in retinoblastoma include methylation of DNA, histone modification, and regulation of microRNA (miRNA) [33]. Hypermethylation of RB1 promoter CpG island leads to inactivation of RB1 [34]. O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme. Hypermethylation of MGMT is associated with advanced retinoblastoma [21]. Hypermethylation of $P16^{INK4A}$ promoter leads to downregulation of $P16^{INK4A}$. In a study by Indovina P et al., downregulation of $P16^{INK4A}$ was detected in 55% of patients with retinoblastoma [35]. miRNA are noncoding RNA molecules which are involved in silencing mRNA by base pairing with complementary sequences in mRNA. Dysregulation of miRNA pathway is also implicated in retinoblastoma [33, 36].

8.4 Genotype–Phenotype Correlation

American Joint Committee on Cancer (AJCC) included factor "H" in the tumor node metastases (TNM) staging of retinoblastoma, in view of significant role of hereditability in prognosis of retinoblastoma [37].

HX—Individual with unknown or insufficient evidence of germline RB1 pathogenic variant.

H0—Individual who did not inherit a known familial germline RB1 pathogenic variant confirmed by molecular genetic testing.

(H0*. Individual with retinoblastoma or retinoma with no germline RB1 pathogenic variant identified on molecular genetic testing; residual risk of mosaicism is <1%.)

H1. Individual with bilateral retinoblastoma, trilateral retinoblastoma, retinoblastoma with positive family history, or identification of a germline RB1 pathogenic variant.

1. Absence of germline RB1 pathogenic variant

It is usually termed sporadic retinoblastoma. The mean age at diagnosis is usually later (24 months) compared to heritable retinoblastoma (12 months) [38]. It usually presents with unilateral, unifocal retinoblastoma. Intraocular seeding may mimic multifocal tumor. It does not carry the risk of second non-ocular tumors. The siblings and offsprings are not at increased risk of retinoblastoma.

2. Mosaicism

In individuals with mosaicism, a fraction of cells in the body harbor the pathogenic RB1 variant. It can present with multifocal and bilateral tumors. In such cases, the development of pathogenic variant is a postzygotic event after one cell stage of embryogenesis [39]. So the parents and siblings do not have increased risk of retinoblastoma. If the germ cells (sperm or ova) carry the pathogenic variant, the risk of retinoblastoma is higher in offspring. Genetic testing of leukocyte DNA in peripheral blood may not detect the presence of pathogenic variants.

3. Presence of germline RB1 pathogenic variant

It is usually termed as heritable retinoblastoma. All the cells of the body will have one copy of RB1 pathogenic variant. The mean age at diagnosis is earlier (12 months). It usually presents with multifocal, bilateral retinoblastoma. But 15% of cases with unilateral retinoblastoma can have a germline mutation [40]. It is associated with a higher risk of second non-ocular tumors. The risk of second non-ocular tumor was noted to be 51% in cases exposed to radiation and 27% in cases that were not exposed to radiation at 50 years [41]. Offsprings have an increased risk of retinoblastoma. The risk in siblings is higher if one of the parents harbors the pathogenic variant. If the RB1 mutation/inactivation occurs during embryogenesis in one cell stage, the siblings will not have an increased risk of retinoblastoma.

Rarely, a person with a pathogenic variant of RB1 may not develop retinoblastoma. This is called "low penetrance retinoblastoma" and it runs in a few families. Members of affected family can have unilateral retinoblastoma or retinoma. This could be due to missense mutations in RB1 gene which can lead to partially inactive Rb protein or reduced expression of Rb protein [42].

4. 13q deletion syndrome

Abnormalities of chromosome 13 such as 13q deletions and translocation can be seen in 5–6% of cases of retinoblastoma. Cases with 13q deletion syndrome have systemic and ocular abnormalities. The ocular associations include congenital cataract, coloboma, and microphthalmos. The systemic associations include severe mental retardation, motor impairment, and dysmorphic facies characterized by high and broad forehead, anteverted ear lobes, and prominent philtrum [43].

5. MYCN amplification

It accounts for around 1.5% of cases of retinoblastoma. Pathogenic RB variant is absent in the tumor. It presents with unilateral retinoblastoma [44].

8.5 Genetic Testing

Genetic tests used in retinoblastoma

1. Sequence analysis

Sequence analysis can detect sequence variants in gene. The sequence variants can be pathogenic (reported in literature), likely pathogenic (not reported in literature), of unknown significance, likely benign (not reported in literature), and benign (reported in literature). Sequence analysis can detect approximately three-fourth of RB1 germline mutations. Large deletions can be missed on sequence analysis. Next generation sequencing is the most efficient way [21, 22].

2. Gene targeted deletion/duplication analysis

It detects intragenic duplications and deletions ranging from one exon to the entire gene. It can detect 16 to 20% of RB1 germline mutations.

3. Chromosome microarray analysis
4. Karyotyping and fluorescent in situ hybridization (FISH)

Large chromosomal deletions like 13q deletion syndrome can be detected. In patients with systemic features consistent with 13q deletion syndrome, karyotyping can confirm the diagnosis and obviates the need for expensive genetic tests [21].

5. Methylation analysis

In 15% of unilateral sporadic retinoblastoma, methylation of RB1 promoter would be responsible for epigenetic inactivation of RB1. In such cases, analysis of tumor cells may not show pathogenic variants in RB1 gene. Analysis of RB1 promoter methylation may be required in such cases to identify inactive RB1 gene [22, 45].

8.5.1 Indications of Genetic Testing

1. Any child with diagnosis of retinoblastoma

In bilateral retinoblastoma, identification of the pathogenic variant in the RB1 gene, helps in screening of siblings and parents for that specific pathogenic variant.

In unilateral retinoblastoma, 15% of the cases can be due to germline mutation in RB1 gene [40]. Identification of germline mutation would prompt evaluation of siblings, close observation to look for recurrence, tumor in fellow eye and second non-ocular tumors. Absence of germline mutation would reassure the sporadic nature of the tumor.

2. Siblings of germline retinoblastoma

Identification of germline mutation in siblings, prompts close monitoring for the development of tumor and ensures early treatment. Absence of germline mutation ensures that the child does not have an increased risk of retinoblastoma.

3. Parents of germline retinoblastoma

Identification of germline mutation in one of the parents confirms increased risk of retinoblastoma in the siblings (50%). The absence of pathogenic variant in the parents suggests either de novo mutation during gametogenesis or during embryogenesis and the risk of retinoblastoma in siblings would be less (2%) [22]. So genetic testing of parents before planning the subsequent pregnancy can be helpful in predicting the risk in the offspring. In some instances, the absence of pathogenic variants in parents can be due to parental mosaicism. Next generation sequencing can detect parental mosaicism in >4% of families [46].

4. Amniocentesis: Genetic testing at second trimester

With advancements in management of retinoblastoma, retinoblastoma survivors are opting for family. The offsprings of RB survivors and offsprings of parents with germline mutations have a 50% risk of developing retinoblastoma. So amniocentesis and genetic testing of the fetal DNA in second trimester of pregnancy can be used to establish the molecular diagnosis. If the fetal DNA is free of the pathogenic variant in RB1 gene, it assures that the fetus does not have risk of retinoblastoma [47, 48]. If the pathogenic variant is identified, the parents can be given an option of discontinuing the pregnancy. If the parents decide to continue the pregnancy, fetal ultrasonography (USG) and magnetic resonance imaging (MRI) can be used to monitor the eyes for the development of retinoblastoma. If retinoblastoma is detected, premature delivery and early treatment of tumor can be planned when they are amenable to local therapy. Only moderately large tumors can be picked up on USG and MRI [49]. So even if the tumors are not visible on USG, elective delivery at 36 weeks can be considered to identify tiny vision-threatening tumors.

5. Embryo selection

Parents with germline mutation can opt for invitro fertilization (IVF). Preimplantation genetic testing can be considered for the embryos generated by IVF. The embryos free of pathogenic variants in RB1 gene can be implanted in the

uterus, thereby assuring that the offspring will not have increased risk of retinoblastoma [50].

8.5.2 How to Conduct Genetic Testing?

Pre-test counselling is performed to communicate with the patient regarding the purpose, cost, and benefits of genetic testing.

Bilateral retinoblastoma and unilateral retinoblastoma with family history: Sequence analysis or gene targeted duplication/deletion analysis is performed on peripheral blood leukocyte DNA. In 3% of cases with bilateral retinoblastoma, the pathogenic variant may not be detected due to mosaicism [24].

Unilateral retinoblastoma with no family history (if the tumor tissue is not available): Sequence analysis or gene targeted duplication/deletion analysis is performed on peripheral blood leukocyte DNA.

Unilateral retinoblastoma with no family history (if the tumor tissue is available): Sequence analysis or gene targeted duplication/deletion analysis is performed on tumor DNA. The peripheral blood leukocyte DNA is screened for the pathogenic variant detected in tumor. If the pathogenic variant is not detected in the tumor, methylation analysis of the promoter is performed. If hypermethylation of the RB1 promoter is not identified, tumor DNA is tested for MYCN amplification [22].

Post-testing counselling is performed to communicate the results of the test and its implications on further screening and management.

8.5.3 Conclusion

Understanding the genetic mechanisms involved in development, inheritance, and prognosis of retinoblastoma helps in proper management of children with retinoblastoma and screening of children at risk of retinoblastoma. Antenatal molecular diagnosis and embryo selection can help in ensuring the birth of healthy children and also breaks the chain of transmission.

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References

1. Dimaras H, Corson TW, Cobrinik D, White A, Zhao J, Munier FL, et al. Retinoblastoma. Nat Rev Dis Primers. 2015;1:1502.
2. Kivelä T. The epidemiological challenge of the most frequent eye cancer: retinoblastoma, an issue of birth and death. Br J Ophthalmol. 2009;93:1129–31.
3. Hong FD, Huang HJ, To H, Young LJ, Oro A, Bookstein R, et al. Structure of the human retinoblastoma gene. Proc Natl Acad Sci U S A. 1989;86:5502–6.
4. Lee JO, Russo AA, Pavletich NP. Structure of the retinoblastoma tumour-suppressor pocket domain bound to a peptide from HPV E7. Nature. 1998;391:859–65.

5. Burke JR, Deshong AJ, Pelton JG, Rubin SM. Phosphorylation-induced conformational changes in the retinoblastoma protein inhibit E2F transactivation domain binding. J Biol Chem. 2010;285:16286–93.
6. Burke JR, Hura GL, Rubin SM. Structures of inactive retinoblastoma protein reveal multiple mechanisms for cell cycle control. Genes Dev. 2012;26:1156–66.
7. Dick FA, Rubin SM. Molecular mechanisms underlying RB protein function. Nat Rev Mol Cell Biol. 2013;14:297–306.
8. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell. 1995;81:323–30.
9. Classon M, Harlow E. The retinoblastoma tumour suppressor in development and cancer. Nat Rev Cancer. 2002;2:910–7.
10. Ji P, Jiang H, Rekhtman K, Bloom J, Ichetovkin M, Pagano M, et al. An Rb–Skp2–p27 pathway mediates acute cell cycle inhibition by Rb and is retained in a partial-penetrance Rb mutant. Mol Cell. 2004;16:47–58.
11. Binne UK, Classon MK, Dick FA, Wei W, Rape M, Kaelin WG Jr, et al. Retinoblastoma protein and anaphase-promoting complex physically interact and functionally cooperate during cell-cycle exit. Nat Cell Biol. 2007;9:225–32.
12. Schvartzman JM, Duijf PH, Sotillo R, Coker C, Benezra R. Mad2 is a critical mediator of the chromosome instability observed upon Rb and p53 pathway inhibition. Cancer Cell. 2011;19:701–14.
13. Kabeche L, Compton DA. Checkpoint-independent stabilization of kinetochore–microtubule attachments by Mad2 in human cells. Curr Biol. 2012;22:638–44.
14. Bester AC, Roniger M, Oren YS, Im MM, Sarni D, Chaoat M, et al. Nucleotide deficiency promotes genomic instability in early stages of cancer development. Cell. 2011;145:435–46.
15. Dick FA, Dyson N. pRB contains an E2F1-specific binding domain that allows E2F1-induced apoptosis to be regulated separately from other E2F activities. Mol Cell. 2003;12:639–49.
16. Cecchini MJ, Dick FA. The biochemical basis of CDK phosphorylation-independent regulation of E2F1 by the retinoblastoma protein. Biochem J. 2011;434:297–308.
17. Ianari A, Natale T, Calo E, Ferretti E, Alesse E, Screpanti I, et al. Proapoptotic function of the retinoblastoma tumor suppressor protein. Cancer Cell. 2009;15:184–94.
18. Carnevale J, Palander O, Seifried LA, Dick FA. DNA damage signals through differentially modified E2F1 molecules to induce apoptosis. Mol Cell Biol. 2012;32:900–12.
19. Wei JS, Song YK, Durinck S, Chen QR, Cheuk AT, Tsang P, et al. The MYCN oncogene is a direct target of miR-34a. Oncogene. 2008;27:5204–13.
20. Knudson AG Jr. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A. 1971;68:820–3.
21. Athavale V, Khetan V. Knudson to embryo selection: A story of the genetics of retinoblastoma. Taiwan J Ophthalmol. 2018;8:196–204.
22. Lohmann DR, Gallie B. Retinoblastoma. Gene Rev. 2000.
23. Valverde JR, Alonso J, Palacios I, Pestaña A. RB1 gene mutation up-date, a meta-analysis based on 932 reported mutations available in a searchable database. BMC Genet. 2005;6:53.
24. Soliman SE, Racher H, Zhang C, MacDonald H, Gallie BL. Genetics and molecular diagnostics in retinoblastoma – an update. Asia Pac J Ophthalmol (Phila). 2017;6:197–207.
25. Dryja TP, Morrow JF, Rapaport JM. Quantification of the paternal allele bias for new germline mutations in the retinoblastoma gene. Hum Genet. 1997;100:446–9.
26. Cavenee WK, Dryja TP, Phillips RA, Benedict WF, Godbout R, Gallie BL, et al. Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature. 1983;305:779–84.
27. Jagadeesan M, Khetan V, Mallipatna A. Genetic perspective of retinoblastoma: from present to future. Indian J Ophthalmol. 2016;64:332–6.
28. Ramprasad VL, Madhavan J, Murugan S, Sujatha J, Suresh S, Sharma T, et al. Retinoblastoma in India: microsatellite analysis and its application in genetic counseling. Mol Diagn Ther. 2007;11:63–70.
29. Dimaras H, Khetan V, Halliday W, Orlic M, Prigoda NL, Piovesan B, et al. Loss of RB1 induces non-proliferative retinoma: increasing genomic instability correlates with progression to retinoblastoma. Hum Mol Genet. 2008;17:1363–72.
30. Chen D, Gallie BL, Squire JA. Minimal regions of chromosomal imbalance in retinoblastoma detected by comparative genomic hybridization. Cancer Genet Cytogenet. 2001;129:57–63.

31. Bowles E, Corson TW, Bayani J, Squire JA, Wong N, Lai PB, et al. Profiling genomic copy number changes in retinoblastoma beyond loss of RB1. Genes Chromosomes Cancer. 2007;46:118–29.
32. Fry CJ, Slansky JE, Farnham PJ. Position-dependent transcriptional regulation of the murine dihydrofolate reductase promoter by the E2F transactivation domain. Mol Cell Biol. 1997;17:1966–76.
33. Benavente CA, Dyer MA. Genetics and epigenetics of human retinoblastoma. Ann Rev Pathol. 2015;10:547–62.
34. Ohtani-Fujita N, Fujita T, Aoike A, Osifchin NE, Robbins PD, Sakai T, et al. CpG methylation inactivates the promoter activity of the human retinoblastoma tumor-suppressor gene. Oncogene. 1993;8:1063–7.
35. Indovina P, Acquaviva A, De Falco G, Rizzo V, Onnis A, Luzzi A, et al. Downregulation and aberrant promoter methylation of p16INK4A: a possible novel heritable susceptibility marker to retinoblastoma. J Cell Physiol. 2010;223:143–50.
36. Thériault BL, Dimaras H, Gallie BL, Corson TW. The genomic landscape of retinoblastoma: A review. Clin Exp Ophthalmol. 2014;42:33–52.
37. Mallipatna A, Gallie BL, Chévez-Barrios P, Lumbroso-Le Rouic L, Chantada GL, Doz F, et al. Retinoblastoma. In: Amin MB, Edge SB, Greene FL, editors. AJCC cancer staging manual. 8th ed. New York: Springer; 2017. p. 819–31.
38. Shields JA, Shields CL. Intraocular tumors: an atlas and textbook. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2008.
39. Munier FL, Thonney F, Girardet A, Balmer A, Claustre M, Pellestor F, et al. Evidence of somatic and germinal mosaicism in pseudo-low-penetrant hereditary retinoblastoma, by constitutional and single-sperm mutation analysis. Am J Hum Genet. 1998;63:1903–8.
40. Lohmann DR, Gerick M, Brandt B, Oelschlager U, Lorenz B, et al. Constitutional RB1-gene mutations in patients with isolated unilateral retinoblastoma. Am J Hum Genet. 1997;61:282–94.
41. Abramson DH, Ellsworth RM, Zimmerman LE. Nonocular cancer in retinoblastoma survivors. Trans Sect Ophthalmol Am Acad Ophthalmol Otolaryngol. 1976;81:454–7.
42. Harbour JW. Molecular basis of low-penetrance retinoblastoma. Arch Ophthalmol. 2001;119:1699–704.
43. Baud O, Cormier-Daire V, Lyonnet S, Desjardins L, Turleau C, Doz F, et al. Dysmorphic phenotype and neurological impairment in 22 retinoblastoma patients with constitutional cytogenetic 13q deletion. Clin Genet. 1999;55:478–82.
44. Rushlow DE, Mol BM, Kennett JY, Yee S, Pajovic S, Thériault BL, et al. Characterisation of retinoblastomas without RB1 mutations: genomic, gene expression, and clinical studies. Lancet Oncol. 2013;14:327–34.
45. Zeschnigk M, Böhringer S, Price EA, Onadim Z, Masshöfer L, Lohmann DR. A novel real-time PCR assay for quantitative analysis of methylated alleles (QAMA): analysis of the retinoblastoma locus. Nucl Acids Res. 2004;32:e125.
46. Chen Z, Moran K, Richards-Yutz J, Toorens E, Gerhart D, Ganguly T, et al. Enhanced sensitivity for detection of low-level germline mosaic RB1 mutations in sporadic retinoblastoma cases using deep semiconductor sequencing. Hum Mutat. 2014;35:384–91.
47. Shah PK, Sripriya S, Narendran V, Pandian AJ. Prenatal genetic diagnosis of retinoblastoma and report of RB1 gene mutation from India. Ophthalmic Genet. 2016;37:430–3.
48. Soliman SE, Dimaras H, Khetan V, Gardiner JA, Chan HS, Héon E, et al. Prenatal versus postnatal screening for familial retinoblastoma. Ophthalmology. 2016;123:2610–7.
49. Paquette LB, Miller D, Jackson HA, Lee T, Randolph L, Murphree AL, et al. In utero detection of retinoblastoma with fetal magnetic resonance and ultrasound: Initial experience. AJP Rep. 2012;2:55–62.
50. Xu K, Rosenwaks Z, Beaverson K, Cholst I, Veeck L, Abramson DH, et al. Preimplantation genetic diagnosis for retinoblastoma: the first reported liveborn. Am J Ophthalmol. 2004;137:18–23.

9 Molecular Mechanisms in the Pathogenesis of Retinopathy of Prematurity (ROP)

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Abbreviations

AGTR1	Angiotensin II receptor type 1
Ang2	Angiopoietin-2
ANGPT2	Angiopoietin 2 gene
BDNF	Brain-derived neurotrophic factor
BH4	Tetrahydrobiopterin
BRB	Blood–retinal barrier
BW	Birth weight
C3	Complement C3
CETP	Cholesteryl Ester Transfer Protein
CFB	Complement Factor B
CFH	Complement Factor H
CXCR4	C-X-C Motif Chemokine Receptor 4
CYP	Cytochrome P450
DNA	Deoxyribonucleic acid
EEQs	Epoxyeicostetraenoic acid
EPA	Eicosapentaenoic acid
EPAS1	Endothelial PAS domain-containing protein 1
EPO	Erythropoietin
EPOR	Erythropoietin receptor
FBLN5	Fibulin 5
FEVR	Familial exudative vitreoretinopathy

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FLT1	fms-related tyrosine kinase-1
FZD	Frizzled
FZD4	Frizzled 4
GA	Gestational age
GP1BA	Glycoprotein Ib-alpha
GPX	Glutathione peroxidase
H2AFX	H2A histone family member X
HIF-1	Hypoxia-induced growth factor-1
IFN	Interferons
IGF-1	Insulin-like growth factors
IHH	Indian Hedgehog Signaling Molecule
IL-1ra	Interleukin-1 receptor antagonist
IL6	Interleukin 6
IL8	Interleukin 8
JAK	Janus kinases
KDR	Kinase insert domain receptor
Keap1	Kelch-like ECH-associated protein 1
LRP5	Low-density lipoprotein receptor-related protein 5
MAPK	Mitogen-activated protein kinase
MCP	Monocyte chemoattractant protein
MCP-1	Monocyte chemotactic protein 1
MIP-1a	Macrophage Inflammatory Proteins
miRNA	Micro RNA
MMP2	Matrix metallopeptidase 2
MMP9	Matrix metallopeptidase 9
MMTV	Mouse mammary tumor virus
mRNA	Messenger RNAs
NADPH	Nicotinamide adenine dinucleotide phosphate
NDP	Norrie Disease Protein
NOS	Nitric oxide synthase
Nrf2	Nuclear factor erythroid 2-like 2
OIR	Oxidative stress-induced retinopathy
p62	p62/SQSTM1
PDGF	Platelet-derived growth factor
PI3K	Phosphoinisitol-3-kinase
PIGF	Placental growth factor
PMN	Polymorphonuclear neutrophils
Prx	Peroxiredoxins
PUFAs	Polyunsaturated fatty acids
RANTES	Regulated upon Activation, Normal T Cell Expressed and Presumably secreted
RNA	Ribonucleic Acid
ROI	Reactive Oxygen Intermediates
ROP	Retinopathy of prematurity

ROS	Reactive oxygen species
SDF-1	Stromal cell-derived factor-1
SNP	Single nucleotide polymorphism
STAT	Signal transducer and activator of transcription proteins
TBX5	*T-Box Transcription Factor 5*
TGFβ1	Transforming growth factor beta-1
TNFα	Tumor necrosis factor alpha
TSPAN12	Tetraspanin-12
UTR	Untranslated Region
VEGF	Vascular endothelial growth factor
VEGFR1	Vascular endothelial growth factor receptor 1
VEGFR2	Vascular endothelial growth factor receptor 2
VHL	Von Hippel—Lindau protein

9.1 Introduction

Advancements in neonatal care modalities have led to increased preterm births worldwide. However, babies born prematurely with low gestational age (GA) and birth weight (BW) are increasingly susceptible to the risk of several prematurity-related complications and other diseases. In India, incidence of ROP is approximately 18.4% while the incidence of severe ROP is 6.4% in infants with GA <34 weeks and/or BW <1750 g [1]. Retinopathy of prematurity is one such vision-threatening disease associated with neovascularization in immature retinas of premature infants [1]. In phase-I of the disease, retinal vascularization slow or ceases after preterm birth due to hyperoxia. Subsequently in phase-II retina becomes hypoxic due to increased metabolic demand and poor vascularization which further stimulates vasoproliferation resulting in fibrovascular retinal detachment [2]. The transition from phase-I to phase-II of the disease is very complex and involves a lot of molecules and their interactions, e.g., genes, RNA, proteins, and lipids. A large number of factors including maternal and infant factors, prenatal and perinatal factors, comorbidities of prematurity, their treatment and genetics may contribute to the development and progression of ROP [3] by modifying signal transduction pathways. These modifications can result in transition from normal physiological pathways to pathological conditions. Even in the absence of risk factors, some infants tend to develop severe ROP. Since, preterm infants have variable ROP susceptibility, progression and response to treatment, studies focusing on understanding the causative factors and their interactions that can result in pathophysiological mechanisms leading to ROP are highly warranted. The goal of this book chapter is to provide a brief overview about involvement of various molecules and mechanisms in the pathophysiology of ROP. The individual molecules and mechanisms beginning from genetics to miRNA, oxidative stress, lipids and proteins will be discussed in the consecutive sections.

9.2 Role of Genetics in Pathophysiology of ROP

ROP is a complex self-restricting condition that progresses to severe stages in only a subset of premature infants who require timely intervention and treatment while in other diseases regress spontaneously without any treatment. Severe ROP is characterized by the formation of abnormal blood vessels in vitreous causing vitreoretinal traction/pull and retinal detachment eventually leading to irreversible blindness.

Several studies have been performed on the oxygen stress-induced retinopathy (OIR) mouse model and ROP patients to understand its pathogenesis and management, however, it still remains unclear. Besides known clinical and demographical risk factors, there are substantial evidence that support a genetic basis of ROP [3, 4]. The genetic factors associated with the disease could explain why only a set of premature infant's progress to severe stages of ROP despite timely intervention while in others it regresses. In past, several studies have reported the varied incidence rate [5] and frequency of ROP across different countries and among different ethnic groups [6–9]. Even the male infants are shown to be at increased risk of ROP as compared to female infants [10]. The studies pertaining to heritability and concordance estimates among monozygotic and dizygotic twin pair have also suggested for the role of genes/ genetic pathways in the ROP development [11]. All these studies suggest for the role of several internal disease progressing factors, mainly genetic variants including polymorphisms and mutations in the development of ROP [12]. This raises a need for screening the genetic variants/genes involved in the retinal vascular formation and abnormal angiogenesis in the pathophysiology of ROP. Only a few studies have found genes/variants associated with different stages of ROP, however, their exact role in disease pathogenesis needs to be elucidated [12, 13].

The clinical manifestations and phenotypic changes of ROP strongly correlate with familial exudative vitreoretinopathy (FEVR) hinting for a shared involvement of common genetic factors among them. FEVR is a congenital vitreoretinal disorder with three different modes of inheritance patterns; autosomal dominant [14], autosomal recessive [15], and X-linked recessive [16]. Four candidate genes involved in the Wntβ-catenin signalling pathways (Fig. 9.1) have been identified for FEVR, including *FZD4*, *LRP5*, *NDP* and *TSPAN12*. These genes play a crucial role in the fetal vasculature development and retinal maturation. Interestingly, these genes are candidates for other retinal vascular diseases also, like Norrie and Coat's disease. In the proceeding paragraphs, we will discuss about these candidate genes and other genes in association with ROP pathogenesis.

Frizzled 4 (*FZD4*) Gene Human Frizzled-4 gene is a member of the frizzled gene family, that codes for seven transmembrane domain proteins that act as receptors for the Wnt family of signalling proteins. The Wnt signalling is very important for cell fate determination and polarity, etc. [17]. Wnt ligand binding and activation are facilitated by G-protein-coupled frizzled receptor. So far, ten FZD receptors and 20 Wnt ligand isoforms have been identified in Homo sapiens [18]. FZD4 expression was detected in retina, being required for its proper development and function via

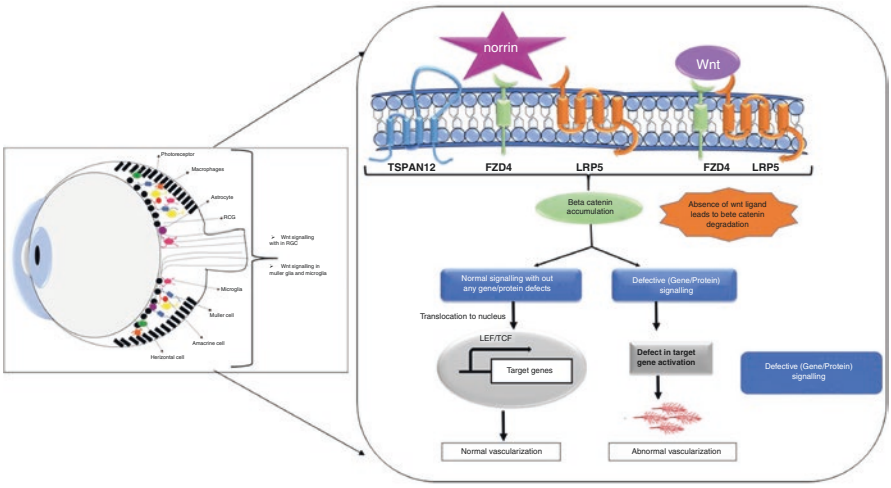

Fig. 9.1 Wnt signalling in retinal development and maturation (Adapted and modified from [11, 51]). Ligands of norrin and Wnt binds to FZD4 receptor and LRP5 co-receptor. Once bound, Wnt ligand beta-catenin gets deposited in the cytoplasm and then translocate to nucleus. There it activates the transcription of Wnt target genes by interacting with a member of Tcf/Lef family. A norrin-LRP5-FZD4 complex is formed by their binding to norrin that further regulates the downstream effects on norrin-beta-catenin signalling. Any gene abnormalities/defects in Wnt and norrin targeting genes could lead to abnormal vascularization in developmental stages of ROP

canonical Wnt/b-catenin pathway. It plays an important role in vascularization of retina by regulating the expression of its downstream target genes [19, 20]. The muller glial cells in retina produce the non-Wnt ligand that binds to vascular endothelial cells by FZD receptor. The FZD signalling was lost in endothelial cells of knockout mice causing abnormal vascular growth that leads to retinal neuron loss and interrupts the integrity of blood–retinal barrier (BRB) [21].

Mutations in *FZD4* gene have been studied in different populations for its association with ROP. Only a few variations have been reported till date that are associated with advanced stages of ROP. The variants p.(I256V), p.(A370G), p.(K203N), p.(H69Y), p.(R127H), and p.(Y211H) are found to be associated with advanced stages of ROP [22–24]. Another three heterozygous variants [p.(G424E), p.(P33S) and p.(P168S)] and a compound heterozygous variant p.(P33S)/p.(P168S) have been shown to be associated with all stages of ROP [25, 26]. Our *in-house* Indian study validated and confirmed similar results where these known variants [p.(P33S), p.(P168S), p.(P33S)/p.(P168S) along with another novel heterozygous variant p.(I360V) were found to be associated with all stages of ROP [27].

NDP **Gene** *NDP* gene encodes for 133 amino acids containing norrin protein that plays role in chemical signalling pathways and affects the way cells and tissue develop. Defects in the gene lead to Norrie disease. Norrie disease is a state of rare congenital blindness following X-linked recessive pattern. It is also known as retinal

dysplasia. Norrie is a specific ligand for the FZD4 receptor, it directly activates the Wnt/b-catenin pathway. In retina, norrin is expressed in muller glial cells [21] and retinal neurons during development stages [28]. This signalling pathway plays a pivotal role in the vascularization of retina in its developmental process. Few studies done on ROP found genetic variations in 5′ and 3′ UTRs of *NDP* exon1. In Norrie disease, variations in the gene lead to deafness and mental retardation along with bilateral retinal malformation, but in case of ROP, it shows only abnormal vascular development and retinal detachment.

To date, only two variations in exon region of *NDP* gene (R121W and L108P) have been reported for their association with ROP [29]. By a direct sequencing method, C597A variation was detected in prematurely born Kuwaiti infants with severe ROP [30]. While in patients who had regressed ROP, a 14-bp deletion was found in the 5′ UTR of *NDP* gene. The heterozygous 14 bp deletion was also found in the unaffected premature control infants [31] suggesting for a protective role for this variant.

Several studies worldwide have shown that mutations/polymorphisms in *NDP* gene are associated with the different stages of ROP disease. In a study performed on 100 ROP patients from different ethnic groups, only two patients with advanced stage of disease showed mutations in the NDP gene. In a patient, a 12-bp CT repeat insertion was found in exon 1 while in another a 14-bp deletion in 5′UTR of same exon was found [32]. The same 14-bp deletion in 5′UTR was also present in 9.6% of regressed ROP patients [31]. In an Indian report along with similar 14 bp deletion, 2 more novel variants (IVS1 + 16A > G, C.522 T > C in 3′ UTR) have been reported in 3.84% of ROP patients [27]. Another two variants in 5′UTR of exon 1 of the gene, i.e., c.597 C > A was found to be present in 83% and 237A > G was found to be present in 5.9% of severe ROP patients [30].

LRP5 **Gene** *LRP5* is a FZD4 co-receptor, the gene belongs to the low-density lipoprotein family and composed of 1615 amino acids with 23 exons. It consists of four domains, six YWTD repeats that form a beta-propeller structure and an epidermal growth factor-like repeat. Loss-of-function mutations in the *LRP5* gene have been reported in several diseases like FEVR, Osteoporosis-Pseudoglioma Syndrome. The expression of *LRP5* gene has been shown in the retina, pancreas, bone, and heart [33, 34].

However, there are only few studies concerning *LRP5* screening in ROP patients. In a Japanese ROP cohort, a 3-bp (CTG) heterozygous insertion within the coding sequence of gene was found. The insertion of three bases leads to elongation of the leucine repeat in the signal sequence that eventually accounts for the pathogenic features associated with the disease. The study proposed that this variation could cause defects in translation process during protein processing thereby affecting retinal vascularization [35].

TSPAN12 *TSPAN12* gene belongs to the tetraspanin superfamily, encodes for 305 amino acid proteins involved in the norrin signalling and retinal neovascu-

larization. This gene is positioned on chromosome 7q31.31 and contains eight exons and encodes a protein consisting of four transmembrane domains and two extracellular loops [36]. Its gene expression was seen in the endothelial cells of retinal blood vessels. It plays an important role in norrin-beta catenin signalling pathways [37]. Mutations in this gene are responsible for 3–10% of FEVR cases [38].

Very few studies have focused on the association of *TSPAN12* gene with ROP. Our in-house study found an association of p. (L119R) variation in this gene with risk of threshold ROP [27]. In another study, c.954G > A mutation was reported in the *TSPAN12* gene and predicted to cause alterations in protein structure and stability which can further contribute to the pathogenesis of ROP [39]. In a recent study on Malaysian premature infants, two more variants in *TSPAN12* gene viz. c.765G > T (p.P255P) and c.*39C > T (3′UTR) were found but their association with ROP needs to be studied. These were suggested to be common polymorphisms of the Malaysian population [40]. Genetic modifications in *TSPAN12* gene could lead to interference in molecular mechanisms of membrane association activities like cell proliferation and other signalling mechanisms [41] that can alter signal transduction pathways leading to ROP.

Besides the above four FEVR candidate genes, the contribution of other genes was also investigated that can influence the susceptibility to ROP.

Other Candidate Genes Associated with ROP Genetics and hereditary factors control various signalling molecules and pathways that are implicated in the pathogenesis of ROP through known biochemical, molecular, and genetic associations. Vascular endothelial growth factor (VEGF), Insulin-like growth factors (IGF-1), and inflammatory mediators are some of these molecules. Any change in the genes coding these biochemicals involved in different physiological pathways can alter the normal mechanisms hence can lead to diseases state.

VEGF is the most important triggering factor for angiogenesis in the retina. It plays a very critical role in the development of ROP besides being a neuronal survival factor. The intravitreal treatment against VEGF, can revert severe ROP progress [42]. Previous studies have found several SNPs in human *VEGF* gene including rs2010963 (−634G > C and + 405 G > C) which is most commonly associated with ROP [43–48]. In a British study done on preterm babies, the G allele at rs2010963 was found increasingly common among babies with ROP [43]. The same was validated in an Egyptian ROP cohort too [44].

In addition to VEGF, another growth factor, i.e., hypoxia-induced growth factor-1 (*HIF-1*) regulates the cell's response to diminished oxygen levels as sufficient and uniform oxygen supply is required for appropriate tissue maintenance and homeostasis [49]. Owing to its functional profile, HIF-1 alpha could be an important gene that might be involved in ROP pathogenesis. Under hypoxic stress conditions, *HIF-1* is known to get activated further regulating several genes that play a crucial role in retinal angiogenesis, like *VEGF*, *VEGFR1*, *PDGF*, *SDF-1* and *ANG2* [50].

Besides the genes controlling various growth factors, changes in the gene involved in other cellular pathways including inflammation and complement system can also modify an individual's susceptibility to ROP.

Association of Inflammatory and Complement System Genes with ROP Two independent studies have shown an association of ROP with alternative complement system pathways genes and association of several other genetic variations in *IHH, AGTR1, TBX5, CETP, GP1BA, EPAS1, BDNF* and *other* genes with ROP [52, 53].

A study done on Indian preterm infants with and without ROP revealed a significant association of genes with ROP including *CFH, CFB, CXCR4, FBLN5, CFH, CFB, FBLN5, CETP*, *CXCR4, AGTR1, ANGPT2, C3, H2AFX, IHH, MMP2, TGFβ1, CETP, VEGF* and *TSPAN12*. Association of inflammatory markers (IL6, IL8, IL-1ra, MMP2, MMP9, MCP, IFN gamma) in the vitreous and tear samples was also found [54]. Only a few previously known common variations were replicated in this study but the associated SNPs in these studies suggested that inflammation and other associated genetic defects may expand the risk of ROP by directly recruiting proangiogenic factors or by modifying other genes. Novel genetic interactions identified in this study revealed the potential involvement of immune regulation pathways in abnormal neovascularization of the retina. The expression and regulation of all these genes are controlled at various levels and are under the control of other small molecules in the cell like transcription factors and small non-coding RNAs. One of the important types of small RNAs that control gene expression is microRNAs (miRNA). By targeting and inhibiting specific growth and developmental pathways/processes miRNAs can also influence the process of angiogenesis.

9.3 Role of MicroRNAs in the Pathophysiology of ROP

Besides other cellular components involved in the process of gene regulation, small microRNAs (miRNA) also regulate gene expression through interaction with messenger RNAs (mRNA). miRNAs being small non-coding class of RNAs can regulate the expression of genes that are directly involved in the development of retina and other similar processes hence indirectly affecting the vasculature development in retina of the eye. MiRNA mostly regulates gene expression by its sequence-specific binding to 3′ untranslated region (3′-UTR) of specific mRNA targets. This binding can result in degradation, deadenylation or reduced translational activity of the target mRNA. Thus, they play an important role in posttranslational gene regulation [55] and in cellular processes like angiogenesis, cell growth, embryonic development, cell proliferation and differentiation and apoptosis [56, 57]. miRNAs also play a central regulatory role in vascular angiogenesis [58]. By controlling gene expression for cell differentiation, miRNAs perform a vital role in retina throughout the developmental process as well as in disease conditions [59–61]. There are some common miRNA molecules that help to maintain both structure and function of retina [62]. The miRNA profiling of human endothelial cells revealed many

miRNAs that included miR-126, miR-210, miR-221/222, miR-17-92 and miR-23-27-24 clusters. These miRNAs target angiogenesis-related genes and so, are also known as "angiomiRs" [63]. The miRNA-126 inhibits neovascularization in oxygen-induced retinopathy (OIR) by regulating growth factors comprising VEGF, HIF-1α and IGF-2 [64]. This indicates that miRNAs have an important role in ROP pathogenesis. Any type of reduction, dysfunction and dysregulation of miRNA can result in altered expression of their target genes that may result in pathophysiological conditions. Plasma miRNA levels were also compared between preterm infants with and without ROP. The results revealed four miRNAs to be significantly dysregulated of which miR-23a and miR-200b-3p were upregulated while miR-27b-3p were downregulated in ROP [62]. These significantly dysregulated miRNAs could either target antiangiogenic or pro-angiogenic genes and thus confirms their role in causing pathological angiogenesis during ROP development.

In preterm infants, retinal vascular development is insufficient that causes hypoxia which in turn precipitates the production of various proteins/growth factors by upregulation/downregulation of multiple pathways resulting in new and abnormal blood vessel growth. Hence, these proteins can also act as the new targets for managing the disease.

9.4 Major Proteins Involved in the Pathophysiology of ROP

In humans and other large mammals, both in diseased and in normal conditions, the vasculature is formed from two main physiological processes namely vasculogenesis and angiogenesis. While the former occurs during embryogenesis and comprises the formation of new blood vessels directly from the hematopoietic precursor cells, the latter refers to the formation of blood vessels from the pre-existing ones and takes place throughout the life of an organism. Both the processes require many growth factors and other molecules that are involved in directing the precursor cells to differentiate and form mature vessels. During the fetal development, levels of these growth factors are maintained at an optimal level by placental supply from the mother. The sudden loss of maternal–fetal interaction in preterm infants with low birth weight, the concentration of these growth factors also decreases leading to retarded/incomplete vascular development in retina at birth making them very sensitive to hyperoxia environment at preterm birth. This can lead to altered regulation of growth factors and hence pathological retinal vasculature development. The major proteins and growth factors involved in the pathogenesis of ROP are HIF-1, VEGF, IGF-1, PIGF and erythropoietin.

Hypoxia-Inducible Factor-1 (HIF-1) The low oxygen tension (hypoxia) occurs in avascular retina when preterm infants are removed from supplementary oxygen to room air. For cell survival under hypoxic state, a large number of genes involved in angiogenesis, metabolism and cell proliferation get activated [65]. HIF-1 is one of the most important transcriptional mediators in response to hypoxic conditions and master regulator of physiological and pathological angiogenesis. The binding of

HIF-1 to DNA at hypoxia-responsive element enables the transcription of angiogenic genes including growth factor like VEGF [66]. In normal conditions, HIF-1 is hydroxylated by enzyme prolyl hydroxylases which facilitates its binding to the von Hippel—Lindau protein (VHL) further resulting in its eventual degradation by ubiquitination. Under hypoxic conditions, the hydroxylases become less efficient leading to accumulation of HIF-1 and thereby increased VEGF expression [67].

Vascular Endothelial Growth Factor Another protein family known to play a key role in angiogenesis is vascular growth factors. The family includes placental growth factor (PlGF) and other vascular endothelial growth factors (VEGF) [68]. The three common human isoforms of VEGF (VEGF121, VEGF165 and VEGF189) are generated by various combinations of eight exons in this gene. It has two receptors, i.e., fms-related tyrosine kinase-1 (FLT-1 or VEGFR-1) and the kinase insert domain-containing receptors (KDR or FLK-1 or VEGFR-2) that are specific for each isoform [69]. Both the receptors are present in all embryonic tissues, however, with variable expression levels at different gestational ages [70]. HIF-1α binds to the promoter region of flt-1 gene and hence regulates gene expression in hypoxia conditions while the *KDR* gene is not regulated by HIF-1α. During early gestational age, both the genes are highly expressed while the expression reduces significantly with advanced gestational age. The KDR is required for vasculogenesis and hematopoiesis and therefore its loss of function during embryogenesis can be lethal [71]. This receptor is involved in the induction of proliferation, migration, differentiation and maturation of vascular endothelial cells [72, 73]. VEGF is very important during the development phase-I of ROP, its expression being downregulated by hyperoxia whereas the expression is again increased in phase-II under hypoxia which further induces VGEF mRNA and protein expression resulting into neovascularization [74].

9.5 Insulin-Like Growth Factor-1

A potent growth factor that is maternally derived and mediates many other signalling pathways is required for normal retinal development and also been implicated in the ROP pathogenesis [75]. Patients with genetic defects in Insulin-like growth factor-1 (IGF-1) production show a reduced retinal vascularization that could not be restored even after the supplementation of VEGF [76]. The levels of IGF-1 at birth are very low but show a rapid increase in preterm infants who do not develop ROP. The low levels of IGF-1 arrest vasculogenesis resulting in avascular retina that eventually creates hypoxia and accumulation of VEGF in the vitreous. The normal levels of IGF-1 and higher expression of VEGF results into neovascularization in the retina [75]. Increased expression of IGF-1 observed during phase-II of ROP activates Akt signaling pathway along with VEGF and blocks the apoptosis of epithelial cells hence promoting neogenesis. HIF-1 expression that acts through P13k/Akt and MAPK pathways is induced by IGF-1 thereby contributing to neovascularization [77].

9.6 Placental Growth Factor

Placental growth factor (PIGF) also belongs to vascular growth factor family and it plays vital roles in cellular developmental processes along with proliferation and migration of endothelial cells [78]. It increases the activity and expression of VEGF by acting as cofactor. A heterodimer formed from between VEGF and PlGF causes angiogenesis by FLT-1 receptor activation [79, 80], though, the exact involvement of PlGF in this process is still not clear. Some studies showed that PlGF binding to FLT-1 receptor increases the levels of circulating VEGF thereby activating a variety of small less characterized signaling molecules leading to angiogenesis. A reduced PlGF level was observed during hypoxia while it increased during hyperoxia [81]. The dysregulation of PlGF in retina during ROP suggests its contribution to VEGF induced angiogenesis [82].

9.7 Erythropoietin

Erythropoietin (EPO) is hypoxia-induced angiogenic factor that performs the neuroprotective functions in neonates. EPO inhibits apoptosis in endothelial cells and neurons and its receptors are shown to present in the developing retina [83]. EPO is overexpressed in retina under hypoxia [84]. The binding of EPO on erythropoietin receptor (EPOR) activates JAK/STAT pathway. The activated receptor can further form "tissue protective factor" by binding with common beta receptors which are known to have protective effect in stroke and inflammation models [85]. The levels of EPO and VEGF were significantly higher in the vitreous of infants with ROP as compared to those without ROP [86]. The anti-apoptotic and angiogenic action of EPO is important in phase-I ROP while it can worsen the ROP condition in phase-II by overactivating STAT3 in endothelial cells by interaction of activated EPOR and VEGFR2 stimulating abnormal angiogenesis [85].

Our understanding about the involvement of proteins like HIF-1, VEGF, IGF-1 and EPO in pathogenesis of ROP can help to find out new treatment modalities for the disease. The structure and functioning of these proteins/growth factors can be altered further by changing the microenvironment of the cell. Redox imbalance/oxidative stress is also one such alteration caused by increased reactive oxygen and nitrogen species. Increased reactive species can damage the structure of macromolecules of the cell including proteins. The modified proteins can have a detrimental effect on growth and development of preterm infants.

9.8 Role of Oxidative Stress in ROP Pathophysiology

Besides several other intrinsic and extrinsic risk factors, oxidative stress imbalance too plays an important role to initiate the pathological events leading to cardiovascular, renovascular and neurovascular complications including ROP. Under balanced oxidative potential conditions, reactive oxygen species (ROS) are involved in normal physiological functions including inflammation and autophagy. However,

any deviation from the normal function can result in pathological inflammation and autophagy which can further cause damage to retina (Fig. 9.2).

Oxidative stress is a result of an imbalance in generation and degeneration of ROS. ROS also known as reactive oxygen intermediates (ROI) are generated as by-products of physiological metabolic activity of the cells. ROS are also formed in response to endogenous sources like mitochondrial chain reaction, respiratory burst, inflammatory disorders, oxygenases, chronic infections, ischemia-reperfusion injury and exogenous factors like pollutants, ultraviolet radiation, alcohol and cigarette smoking. Several enzymes such as superoxide dismutase, glutathione peroxidase and catalase actively protect the cells from the pathogenic effects of ROS. ROS have beneficial/supportive effects on different biological processes including clearance of invading pathogens, wound healing and tissue repair processes. Imbalance in the generation of ROS can disrupt the retinal homeostasis and thereby leading to cell death.

The key ROS molecules that contribute to oxidative stress are hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), hydroxyl anions (OH^-), hypochlorous acid (HOCl) and superoxide (O^{2-}). Enzymes such as NADPH oxidase/Xanthine oxidase, endothelial nitric acid synthase or the enzymes of the electron transport chain reduce the molecular oxygen to yield superoxide molecules. Superoxide dismutase (SOD) enzyme rapidly converts superoxide molecules into hydrogen peroxides. It also forms the highly reactive intermediate peroxynitrite ($ONOO^-$) by reacting with nitric oxide (NO), which can be protonated to peroxynitrous acid to generate the hydroxyl radical (OH.). At the site of inflammation, the enzyme myeloperoxidase (MPO), expressed by phagocytic neutrophils leads to the formation of highly reactive hypochlorous acid from H_2O_2. H_2O_2 is then disintegrated into toxic hydroxyl anions (OH^-) or scavenged to water and molecular oxygen by antioxidant enzymes such as catalase, glutathione peroxidase (GPX), or peroxiredoxins (Prx).

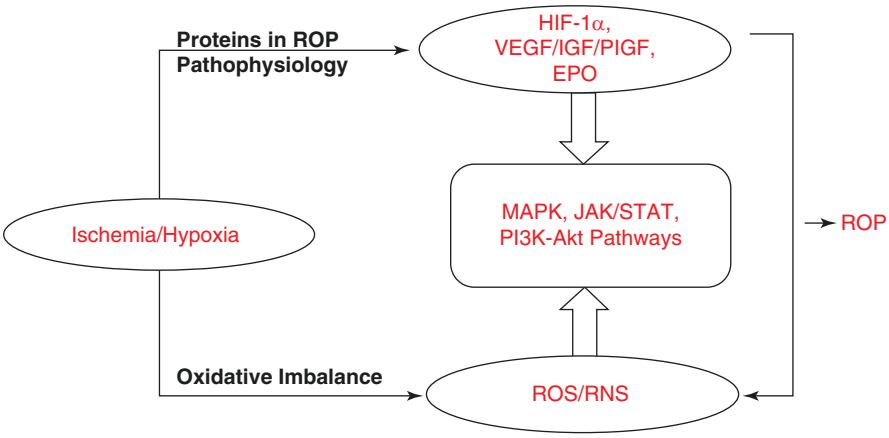

Fig. 9.2 Representative image showing pathways involved in ROP progression

The retina of the eye is extremely vulnerable to oxidative insults by the ROS molecules. Retina is highly susceptible to photooxidation being constantly exposed to incoming light and high on oxygen consumption [87] and likely to further generate ROS molecules. Also, the high lipid constituent in the retina due to abundant PUFAs in the photoreceptors layer of retina makes it prone to lipid peroxidation. During pathological disorders such as retinopathy of prematurity, the balance between the generation of ROS and the capability of cells to scavenge these ROS by endogenous antioxidant is disrupted that activates several signalling pathways affecting lipids, proteins and DNA present inside the cell and consequently causes cell death.

9.9 Retinopathy of Prematurity and ROS

Retinal ischemia or hypoxia is a characteristic feature of ROP. ROP being a biphasic ocular disease is characterized by vascular deformities induced by two alternate phases (hyperoxia followed by hypoxia). The first phase is characterized by state of hyperoxia that subsequently leads to the obliteration of developing retinal vessels. In the second phase, high metabolic demand of the relatively avascular retina inflicts a hypoxic injury to the retinal tissues. This relative hypoxic condition of the retina produces the abnormal proliferation of blood vessels and thereby neovascularization [88]. In both perinatal and neonatal periods, new born infants are exposed to oxidative stress due to low efficacy of enzymatic and nonenzymatic antioxidants like catalase, glutathione peroxidases, superoxide dismutase and vitamin E (responsible for maintaining ROS level). These two phases of ROP pathogenesis result in overproduction of ROS that activates the enzyme NADPH oxidase. NADPH oxidases can present in seven different homologs (NOX1–NOX5 and Duox1–2), which have variable expression as well as activation mechanisms. Major homologs of NADPH oxidase like NOX1, NOX2 and NOX4 are strongly associated with inflammation-activated blood vessel damage. Enhanced NOX2 expression can lead to increase in VEGF expression, retinal ROS generation and vascular permeability. The increased NADPH oxidase also activates the JAK/STAT signalling pathway which eventually leads to intravitreal neovascularization [89]. Inhibition of JAK/STAT signalling pathway and NADPH oxidase reduces the level of caspase-3, which checks the neovascularization and apoptosis in ROP pathogenesis. Independent studies have shown that NADPH oxidase-derived ROS are important for ischemia-induced neovascularization as it increases VEGF and retinal neovascularization.

Oxides of nitrogen also referred to as reactive oxygen species (RNS) such as NO-, NO, ONO and NO_2 are also a major contributor to the ROP progression. Increased concentration of nitric oxide synthase (NOS) significantly contributes to the increase in NO production under the hypoxic environment [90]. NOS is a group of enzymes that acts on L-arginine to produce NO. NOS in mammals have three different isoforms, namely neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). Among these isoforms, eNOS is the major source of NO in

the endothelial cells responsible for the maintenance of blood vessels and angiogenesis. Deficiency of tetrahydrobiopterin (BH4) that plays important role in maintaining integrity of eNOS can lead to uncoupling of eNOS and finally resulting in enhanced superoxide radicals. Increased apoptosis of retinal endothelial cells and synthesis of nitric oxides are known to contribute to the increase in cleaved caspase-3 and tyrosine nitration of phosphoinisitol-3-kinase (PI3K). Increased NOS production further causes the activation of MAPK signalling pathway and decrease in Akt Phosphorylation. The use of inhibitors like N-acetyl cysteine and epicatechin are shown to block tyrosine nitration and decrease the ROP severity induced by oxides of nitrogen. Deletion of NOS genes or application of NOS inhibitors (NG-nitro-l-arginine) has also been shown to decrease the progression of ROP in mice models, depicting their crucial role in ROP pathogenesis [91].

9.10 ROS, Inflammation and ROP

Besides activation of various signalling pathways in ROP, ROS generation is also a key characteristic for the progression of many inflammatory diseases. Inflammation is a major feature of the immune system for the repair of damaged tissue of the body. Role of inflammation is poorly investigated in the case of ROP progression though it plays an important role in blood vessel proliferation under both normal and diseased conditions [86]. Moreover, a series of epidemiological studies since last one decade have supported the hypothesis that inflammation in the eyes of premature infants is a key modulator in the progression and development of ROP [92]. Studies suggested that a gradual increase in ROP progression might be due to prenatal and postnatal inflammation [93]. Small inflammatory protein molecules such as cytokines and chemokines released by immune system plays important role in ROP progression. Moreover, ROS generated by the polymorphonuclear neutrophils (PMNs) causes tissue injury and endothelial dysfunction at the site of inflammation. The vascular endothelial layer plays a significant role in transporting the inflammatory proteins from blood to tissue. Oxidative stress under inflammatory conditions promotes the opening of gap junctions present in the endothelial cell and transport of inflammatory proteins across the barrier.

Cytokines such as TNFα, IL-1β, and IL-6 are key inflammatory markers that contribute to tissue damage or infection [94]. Retinal microglial cells secrete IL-1β and TNFα when conditioned to relative hypoxia. Moreover, IL-1β is linked to retinal microvascular degeneration [95]. While cytokines IL-4 and IL-10 being anti-inflammatory, protects the developing retinal and brain cells from inflammation [96]. Certain experimental studies in oxygen-induced retinopathy mice models suggested that IL-10 is involved in promoting pathological angiogenesis, leading to inhibition of TNFα and MIP-1a in microglial cells [97]. New born infants with high expression levels of IL-10 genes tend to have less chances of severe ROP [98].

Chemokines are primarily involved in regulation of movements of microglial cells to the site of inflammation. Chemokines such as monocyte chemotactic protein 1 (MCP-1), IL-8 and RANTES are shown to be involved in ROP

physiology. Inflammation and neovascularization in the eye are regulated by IL-8 in case of tissue damage [99]. An enhanced level of IL-8 homologue is observed during neovascularization in rat model of ROP [100]. The role of RANTES, a chemotactic chemokine, in ROP progression is not clear but a low concentration of RANTES was observed in serum as well as in vitreous humor of patients with vasoproliferative severe ROP [101]. MCP-1 is majorly expressed in the microglia, astrocytes and neurons and neuro–retinal junction. MCP-1 causes disruption in blood–brain barrier and many other neurodegenerative diseases. The concentration of MCP-1 is seen to be higher in case of ROP infants when compared to the premature born normal infants [102]. It is also observed that premature infants who receive higher doses of oxygen, tend to have high level of MCP-1 concentration in the serum [103].

ROS is required at nominal concentration for maintaining the homeostasis of cellular components while the higher ROS levels are significantly involved in pathological changes. If these ROS concentrations are not checked by the antioxidants, it will eventually lead to inflammation related to tissue damage and injury. ROS being the important contributor of inflammation, much more have to be explored about how these ROS functions physiologically under ROP pathogenesis and contribute to inflammation and tissue injury.

9.11 ROS, Autophagy, and ROP

Apart from the inflammation, increased oxidative stress also plays an important role in autophagy. Autophagy is removal and recycling of damaged cellular components in response to their exposure to conditions like oxidative and nutritional stress as well as pathogenic infections. The major pathway involved in the regulation of oxidative stress and autophagy is through p62/Nrf2/Keap1 pathway [104] (Fig. 9.3). Mitochondria act as the main cellular component which regulates autophagy under the oxidative and nitrosative stress. Although the role of autophagy is not well understood in ROP progression but certain lipid and protein molecules involved in astrocyte survival are also linked to autophagy. Soluble epoxide hydrolase (sEH) promotes astrocyte survival in ROP [105] and are also involved in the regulation of inflammation and autophagy [106]. Nutritional supplements such as long-chain polyunsaturated fatty acids (PUFAs) are structural components of retina and endothelial layer. Soluble epoxide hydrolase is involved in the conversion of these PUFAs into hydroxyl alcohols which regulate angiogenesis and neovascularization. The link between PUFAs (particularly docosahexaenoic acid, DHA) and ROP is important as the sEH in retinal layer can metabolize PUFAs into fatty acid mediators, affecting the cellular viability and angiogenesis. Soluble epoxide hydrolase is required for the maintenance of mitochondrial integrity by preventing mitochondrial pathway-dependent apoptosis and retinal astrocyte survival in ROP [106]. The role of autophagy under two different phases of ROP development will give clear understanding between different pathways involved in the angiogenesis, autophagy and apoptosis. While the total metabolome profile of ROP retina and vitreous has

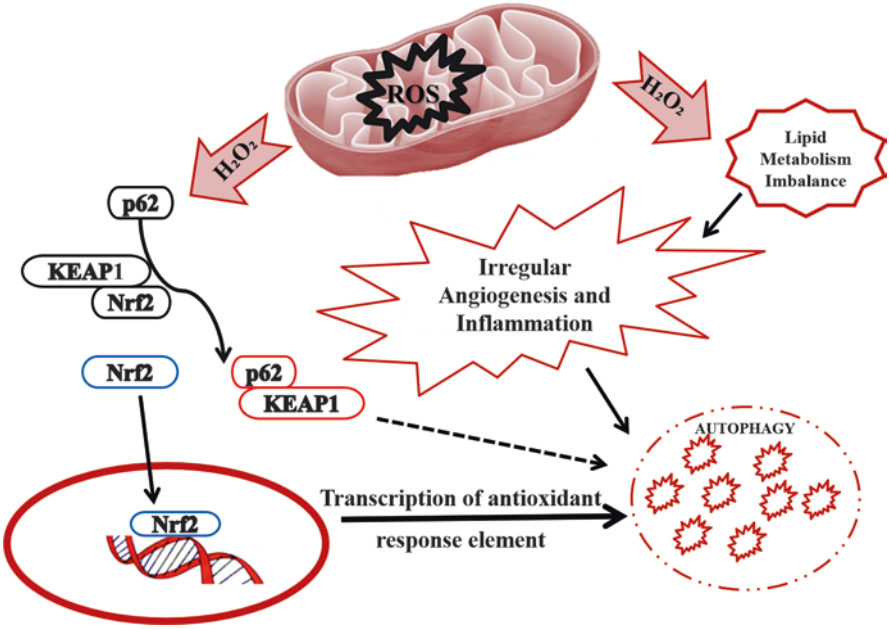

Fig. 9.3 Representative image showing ROS generation in mitochondria leading to imbalance in lipid metabolism, angiogenesis, inflammation, and autophagy by p62/Nrf2/Keap1 Pathway [1]. p62, a protein that is bound to ubiquitylated protein aggregates undergoes phosphorylation, thereby requisitioning Keap1 and leading to its detachment from Nrf2. While Nrf2 is not involved in ubiquitin-mediated proteasome degradation system, it is translocated to the nucleus, where it binds to antioxidant-responsive elements (AREs) located in the promoter regions of antioxidant genes and activates their transcription leading to autophagy

not been explored, certain PUFAs are found to play important role in microglial cells survival under autophagy and oxidative stress.

9.12 Conclusion

ROP is a developmental disease modulated by several intrinsic and extrinsic factors. All extrinsic risk factors could act as stimuli or trigger for various developmental pathways. The activation and deactivation of those pathways further depend on the expression of genes that regulate them. The gene expression in turn depends upon micro/macromolecules of the cell like free radicals, RNA, proteins, lipids and other molecules involved in various physiological pathways. The altered expression of genes further modifies the expression of its downstream target genes and molecules in the cell leading to diseases like ROP. The identification and association of genetic variants and other cellular molecules with ROP may be useful to predict the risk of ROP progression among premature infants and hence, can be helpful in providing genetic counselling to the parents and for the development of new therapies. Several omics studies performed using genomic-, transcriptomic- and proteomic-based

approaches have provided evidence for genetic and molecular defects in ROP progression. However, still there is a huge knowledge gap in the understanding of molecular mechanism that leads to neovascularization in retina of eye leading to ROP. The screening of such hidden genetic and molecular markers could make the disease pathogenesis more comprehensible and therefore enhanced understanding of the pathophysiology of ROP could lead to better understanding of therapeutic options for the affected infants.

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References

1. Balakrishnan U, Shaik S, Manian N, Muthukumar M, Thomas M, Amboiram P, et al. Screening based on incidence of severe retinopathy of prematurity in a tertiary care center in India: are Indian infants different? Int J Contemp Pediatr. 2016;3(3):847–53. https://doi.org/10.18203/2349-3291.ijcp20162263.
2. Hartnett ME. Pathophysiology and mechanisms of severe retinopathy of prematurity. Opthalmology. 2015;122:200–10. https://doi.org/10.1016/j.ophtha.2014.07.050.
3. Kim SJ, Port AD, Swan R, Campbell JP, Chan RVP, Chiang MF. Retinopathy of prematurity: a review of risk factors and their clinical significance. Surv Opthalmol. 2018;63:618–37. https://doi.org/10.1016/j.survophthal.2018.04.002.
4. Swan R, Kim SJ, Campbell JP, Chan RVP, Sonmez K, Taylor KD, et al. The genetics of retinopathy of prematurity: a model for neovascular retinal disease. Ophthalmol Retina. 2018;2:949–62. https://doi.org/10.1016/j.oret.2018.01.016.
5. Saunders RA, Donahue ML, Christmann LM, Pakalnis AV, Tung B, Hardy RJ, et al. Racial variation in retinopathy of prematurity. The Cryotherapy for Retinopathy of Prematurity Cooperative Group. Arch Ophthalmol. 1997;115:604–8. https://doi.org/10.1001/archopht.1997.01100150606005.
6. Hussain N, Clive J, Bhandari V. Current incidence of retinopathy of prematurity, 1989–1997. Pediatrics. 1999;104:e26. https://doi.org/10.1542/peds.104.3.e26.
7. Tommiska V, Heinonen K, Ikonen S, Kero P, Pokela ML, Renlund M, et al. A national short-term follow-Up study of extremely low birth weight infants born in Finland in 1996-1997. Pediatrics. 2001;107:E2. https://doi.org/10.1542/peds.107.1.e2.
8. Larsson E, Carle-Petrelius B, Cernerud G, Wallin A, Holmström G. Incidence of ROP in two consecutive Swedish population based studies. Br J Ophthalmol. 2002;86:1122–6. https://doi.org/10.1136/bjo.86.10.1122.
9. Bowe T, Nyamai L, Ademola-Popoola D, Amphornphruet A, Anzures R, Cernichiaro-Espinosa LA, et al. The current state of retinopathy of prematurity in India, Kenya, Mexico, Nigeria, Philippines, Romania, Thailand, and Venezuela. Digit J Ophthalmol. 2019;25(4):49–58. https://doi.org/10.5693/djo.01.2019.08.002.
10. Darlow BA, Hutchinson JL, Henderson-Smart DJ, Donoghue DA, Simpson JM, Evans NJ. Prenatal risk factors for severe retinopathy of prematurity among very preterm infants of the Australian and New Zealand neonatal network. Pediatrics. 2005;115:990–6. https://doi.org/10.1542/peds.2004-1309.
11. Shastry BS. Genetic susceptibility to advanced retinopathy of prematurity (ROP). J Biomed Sci. 2010;17(1):69. https://doi.org/10.1186/1423-0127-17-69.
12. Holmstrom G, Wijngaarden PV, Coster DJ, Williams KA. Genetic susceptibility to retinopathy of prematurity: the evidence from clinical and experimental animal studies. Br J Ophthalmol. 2007;91(12):1704–8. https://doi.org/10.1136/bjo.2007.117283.

13. Bizzarro MJ, Hussain N, Jonsson B, Feng R, Ment LR, Gruenet JR, et al. Genetic susceptibility to retinopathy of prematurity. Pediatrics. 2006;118(5):1858–63. https://doi.org/10.1542/peds.2006-1088.
14. Gow J, Oliver GL. Familial exudative vitreoretinopathy. An expanded view. Arch Ophthalmol. 1971;86(2):150–5. https://doi.org/10.1001/archopht.1971.01000010152007.
15. Shastry BS, Trese MT. Familial exudative vitreoretinopathy: further evidence for genetic heterogeneity. Am J Med Genet. 1997;69(2):217–8. https://doi.org/10.1002/(sici)1096-8628(19970317)69:2<217::aid-ajmg19>3.0.co;2-o.
16. Plager DA, Orgel IK, Ellis FD, Hartzer M, Trese MT, Shastry BS. X-linked recessive familial exudative vitreoretinopathy. Am J Ophthalmol. 1992;114(2):145–8. https://doi.org/10.1016/s0002-9394(14)73977-7.
17. Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. Genes Dev. 1997;11(24):3286–305. https://doi.org/10.1101/gad.11.24.3286.
18. Kirikoshi H, Sagara N, Koike J, Tanaka K, Sekihara H, Hirai M, et al. Molecular cloning and characterization of human Frizzled-4 on chromosome 11q14-q21. Biochem Biophys Res Commun. 1999;264(3):955–61. https://doi.org/10.1006/bbrc.1999.1612.
19. Robitaille J, MacDonald ML, Kaykas A, Sheldahl LC, Zeisler J, Dubé MP, et al. Mutant frizzled-4 disrupts retinal angiogenesis in familial exudative vitreoretinopathy. Nat Genet. 2002;32(2):326–30. https://doi.org/10.1038/ng957.
20. Xu Q, Wang Y, Dabdoub A, Smallwood PM, Williams J, Woods C, et al. Vascular development in the retina and inner ear: control by Norrin and Frizzled-4, a high-affinity ligand-receptor pair. Cell. 2004;116(6):883–95. https://doi.org/10.1016/s0092-8674(04)00216-8.
21. Ye X, Wang Y, Cahill H, Yu M, Badea TC, Smallwood PM, et al. Norrin, frizzled-4, and Lrp5 signaling in endothelial cells controls a genetic program for retinal vascularization [published correction appears in Cell. 2010 Apr 2;141(1):191]. Cell. 2009;139(2):285–98. https://doi.org/10.1016/j.cell.2009.07.047.
22. MacDonald ML, Goldberg YP, Macfarlane J, Samuels ME, Trese MT, Shastry BS. Genetic variants of frizzled-4 gene in familial exudative vitreoretinopathy and advanced retinopathy of prematurity. Clin Genet. 2005;67(4):363–6. https://doi.org/10.1111/j.1399-0004.2005.00408.x.
23. Ells A, Guernsey DL, Wallace K, Zheng B, Vincer M, Allen A, et al. Severe retinopathy of prematurity associated with FZD4 mutations. Ophthalmic Genet. 2010;31(1):37–43. https://doi.org/10.3109/13816810903479834.
24. Kondo H, Kusaka S, Yoshinaga A, Uchio E, Tawara A, Tahira T. Genetic variants of FZD4 and LRP5 genes in patients with advanced retinopathy of prematurity. Mol Vis. 2013;19:476–85.
25. Drenser KA, Dailey W, Vinekar A, Dalal K, Capone A Jr, Trese MT. Clinical presentation and genetic correlation of patients with mutations affecting the FZD4 gene. Arch Ophthalmol. 2009;127(12):1649–54. https://doi.org/10.1001/archophthalmol.2009.322.
26. Dailey WA, Gryc W, Garg PG, Drenser KA. Frizzled-4 variations associated with retinopathy and intrauterine growth retardation: a potential marker for prematurity and retinopathy. Ophthalmology. 2015;122(9):1917–23. https://doi.org/10.1016/j.ophtha.2015.05.036.
27. Rathi S, Jalali S, Musada GR, Patnaik S, Balakrishnan D, Hussain A, et al. Mutation spectrum of NDP, FZD4 and TSPAN12 genes in Indian patients with retinopathy of prematurity. Br J Ophthalmol. 2018;102(2):276–81. https://doi.org/10.1136/bjophthalmol-2017-310958.
28. Berger W, van de Pol D, Bächner D, Oerlemans F, Winkens H, Hameister H, et al. An animal model for Norrie disease (ND): gene targeting of the mouse ND gene. Hum Mol Genet. 1996;5(1):51–9. https://doi.org/10.1093/hmg/5.1.51.
29. Shastry BS, Pendergast SD, Hartzer MK, Liu X, Trese MT. Identification of missense mutations in the Norrie disease gene associated with advanced retinopathy of prematurity. Arch Ophthalmol. 1997;115(5):651–5. https://doi.org/10.1001/archopht.1997.01100150653015.
30. Haider MZ, Devarajan LV, Al-Essa M, Kumar H. A C597-- > A polymorphism in the Norrie disease gene is associated with advanced retinopathy of prematurity in premature Kuwaiti infants. J Biomed Sci. 2002;9(4):365–70. https://doi.org/10.1007/BF02256593.

31. Dickinson JL, Sale MM, Passmore A, Wheatley CM, Burdon KP, Craig JE, et al. Mutations in the NDP gene: contribution to Norrie disease, familial exudative vitreoretinopathy and retinopathy of prematurity. Clin Exp Ophthalmol. 2006;34(7):682–8. https://doi.org/10.1111/j.1442-9071.2006.01314.x.
32. Hiraoka M, Berinstein DM, Trese MT, Shastry BS. Insertion and deletion mutations in the dinucleotide repeat region of the Norrie disease gene in patients with advanced retinopathy of prematurity. J Hum Genet. 2001;46(4):178–81. https://doi.org/10.1007/s100380170085.
33. Kim DH, Inagaki Y, Suzuki T, Ioka RX, Yoshioka SZ, Magoori K, et al. A new low density lipoprotein receptor related protein, LRP5, is expressed in hepatocytes and adrenal cortex, and recognizes apolipoprotein E. J Biochem. 1998;124(6):1072–6. https://doi.org/10.1093/oxfordjournals.jbchem.a022223.
34. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, et al. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell. 2001;107(4):513–23. https://doi.org/10.1016/s0092-8674(01)00571-2.
35. Hiraoka M, Takahashi H, Orimo H, Hiraoka M, Ogata T, Azuma N, et al. Genetic screening of Wnt signaling factors in advanced retinopathy of prematurity. Mol Vis. 2010;16:2572–7.
36. Nikopoulos K, Gilissen C, Hoischen A, Nouhuys CE, Boonstra FN, Blokland EAW, et al. Next-generation sequencing of a 40 Mb linkage interval reveals TSPAN12 mutations in patients with familial exudative vitreoretinopathy. Am J Hum Genet. 2010;86(2):240–7. https://doi.org/10.1016/j.ajhg.2009.12.016.
37. Junge HJ, Yang S, Burton JB, Paes K, Shu X, French DM, et al. TSPAN12 regulates retinal vascular development by promoting Norrin- but not Wnt-induced FZD4/beta-catenin signaling. Cell. 2009;139(2):299–311. https://doi.org/10.1016/j.cell.2009.07.048.
38. Hemler ME. Tetraspanin functions and associated microdomains. Nat Rev Mol Cell Biol. 2005;6(10):801–11. https://doi.org/10.1038/nrm1736.
39. Zhang T, Sun X, Han J, Han M. Genetic variants of TSPAN12 gene in patients with retinopathy of prematurity. J Cell Biochem. 2019;120(9):14544–51. https://doi.org/10.1002/jcb.28715.
40. Mohd Khair SZN, Ismail AS, Embong Z, Mohamed Yusoff AA. Detection of *FZD4*, *LRP5* and *TSPAN12* genes variants in malay premature babies with retinopathy of prematurity. J Ophthalmic Vision Res. 2019;14(2):171–8. https://doi.org/10.4103/jovr.jovr_210_17.
41. Garcia-España A, Chung PJ, Sarkar IN, Stiner E, Sun TT, Desalle R. Appearance of new tetraspanin genes during vertebrate evolution. Genomics. 2008;91(4):326–34. https://doi.org/10.1016/j.ygeno.2007.12.005.
42. Mintz-Hittner HA, Kennedy KA, Chuang AZ, BEAT-ROP Cooperative Group. Efficacy of intravitreal bevacizumab for stage 3+ retinopathy of prematurity. N Engl J Med. 2011;364(7):603–15. https://doi.org/10.1056/NEJMoa1007374.
43. Cooke RW, Drury JA, Mountford R, Clark D. Genetic polymorphisms and retinopathy of prematurity. Investig Ophthalmol Vis Sci. 2004;45(6):1712–5. https://doi.org/10.1167/iovs.03-1303.
44. Ali AA, Hussien NF, Samy RM, Husseiny KA. Polymorphisms of Vascular Endothelial Growth Factor and Retinopathy of Prematurity. J Pediatr Ophthalmol Strabismus. 2015;52(4):245–53. https://doi.org/10.3928/01913913-20150506-02.
45. Vannay A, Dunai G, Bányász I, Szabó M, Vámos R, Treszl A, et al. Association of genetic polymorphisms of vascular endothelial growth factor and risk for proliferative retinopathy of prematurity. Pediatr Res. 2005;57(3):396–8. https://doi.org/10.1203/01.PDR.0000153867.80238.E0.
46. Kaya M, Çokakli M, Berk AT, Yaman A, Yesilirmak D, Kumral A, et al. Associations of VEGF/VEGF-receptor and HGF/c-Met promoter polymorphisms with progression/regression of retinopathy of prematurity. Curr Eye Res. 2013;38(1):137–42. https://doi.org/10.3109/02713683.2012.731550.
47. Shastry BS, Qu X. Lack of association of the VEGF gene promoter (−634 G-- > C and − 460 C-- > T) polymorphism and the risk of advanced retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmol. 2007;245(5):741–3. https://doi.org/10.1007/s00417-006-0480-6.

48. Kusuda T, Hikino S, Ohga S, Kinjo T, Ochiai M, Takahata Y, et al. Genetic variation of vascular endothelial growth factor pathway does not correlate with the severity of retinopathy of prematurity. J Perinatol. 2011;31(4):246–50. https://doi.org/10.1038/jp.2010.111.
49. Semenza GL. Oxygen sensing, homeostasis, and disease [published correction appears in N Engl J Med. 2011 Sep 8;365(10):968]. N Engl J Med. 2011;365(6):537–47. https://doi.org/10.1056/NEJMra1011165.
50. Campochiaro PA. Molecular pathogenesis of retinal and choroidal vascular diseases. Prog Retin Eye Res. 2015;49:67–81. https://doi.org/10.1016/j.preteyeres.2015.06.002.
51. Garcia AL, Udeh A, Kalahasty K, Hackam AS. A growing field: the regulation of axonal regeneration by Wnt signaling. Neural Regen Res. 2018;13(1):43–52. https://doi.org/10.4103/1673-5374.224359.
52. Mohamed S, Schaa K, Cooper ME, Ahrens E, Alvarado A, Colaizy T, et al. Genetic contributions to the development of retinopathy of prematurity. Pediatr Res. 2009;65(2):193–7. https://doi.org/10.1203/PDR.0b013e31818d1dbd.
53. Hartnett ME, Morrison MA, Smith S, Yanovitch TL, Young TL, Colaizy T, et al. Genetic variants associated with severe retinopathy of prematurity in extremely low birth weight infants. Investig Ophthalmol Vis SciI. 2014;55(10):6194–203. https://doi.org/10.1167/iovs.14-14841.
54. Rathi S, Jalali S, Patnaik S, Shahulhameed S, Musada GR, Balakrishnan D, et al. Abnormal complement activation and inflammation in the pathogenesis of retinopathy of prematurity. Front Immunol. 2017;8:1868. https://doi.org/10.3389/fimmu.2017.01868.
55. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation [published correction appears in Nat Rev. Genet. 2004 Aug;5(8):631]. Nat Rev Genet. 2004;5(7):522–31. https://doi.org/10.1038/nrg1379.
56. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008;18:997–1006. https://doi.org/10.1038/cr.2008.282.
57. Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. Circ Res. 2007;101(1):59–68. https://doi.org/10.1161/CIRCRESAHA.107.153916.
58. Shen WF, Hu YL, Uttarwar L, Passegue E, Largman C. MicroRNA-126 regulates HOXA9 by binding to the homeobox. Mol Cell Biol. 2008;28(14):4609–19. https://doi.org/10.1128/MCB.01652-07.
59. Reh TA, Hindges R. MicroRNAs in retinal development. Ann Rev Vision Sci. 2018;4:25–44. https://doi.org/10.1146/annurev-vision-091517-034357.
60. Karali M, Banfi S. Non-coding RNAs in retinal development and function. Hum Genet. 2019;138:957–71. https://doi.org/10.1007/s00439-018-1931-y.
61. Zuzic M, Rojo Arias JE, Wohl SG, Busskamp V. Retinal miRNA functions in health and disease. Genes. 2019;10:377. https://doi.org/10.3390/genes10050377.
62. Metin T, Dinç E, Görür A, Erdoğan S, Ertekin S, Sarı AA, et al. Evaluation of the plasma microRNA levels in stage 3 premature retinopathy with plus disease: preliminary study. Eye. 2018;32:415–20. https://doi.org/10.1038/eye.2017.193.
63. Wang S, Olson EN. AngiomiRs-Key regulators of angiogenesis. Curr Opinion Genet. 2009;19(3):205–11. https://doi.org/10.1016/j.gde.2009.04.002.
64. Bai Y, Bai X, Wang Z, Zhang X, Ruan C, Miao JC. MicroRNA-126 inhibits ischemia-induced retinal neovascularization via regulating angiogenic growth factors. Exp Mol Pathol. 2011;91(1):471–7. https://doi.org/10.1016/j.yexmp.2011.04.016.
65. Zimna A, Kurpisz M. Hypoxia-inducible factor-1 in physiological and pathophysiological angiogenesis: applications and therapies. Biomed Res Int. 2015;2015:549412. https://doi.org/10.1155/2015/549412.
66. Hartnett ME, Penn JS. Mechanisms and management of retinopathy of prematurity. N Engl J Med. 2012;367(26):2515–26. https://doi.org/10.1056/NEJMra1208129.
67. Ozaki H, Yu AY, Della N, Ozaki K, Luna JD, Yamada H, et al. Hypoxia inducible factor-1alpha is increased in ischemic retina: temporal and spatial correlation with VEGF expression. Investig Ophthalmol Vis Sci. 1999;40(1):182–9.

68. Olofsson B, Korpelainen E, Pepper MS, Mandriota SJ, Aase K, Kumar V, et al. Vascular endothelial growth factor B (VEGF-B) binds to VEGF receptor-1 and regulates plasminogen activator activity in endothelial cells. Proc Natl Acad Sci U S A. 1998;95(20):11709–14. https://doi.org/10.1073/pnas.95.20.11709.
69. Cavallaro G, Filippi L, Bagnoli P, Marca GL, Cristofori G, Raffaeli G, et al. The pathophysiology of retinopathy of prematurity: an update of previous and recent knowledge. Acta Opthalmologica. 2013;91(1):2–20. https://doi.org/10.1111/aos.12049.
70. Peters KG, De Vries C, Williams LT. Vascular endothelial growth factor receptor expression during embryogenesis and tissue repair suggests a role in endothelial differentiation and blood vessel growth. Proc Natl Acad Sci U S A. 1993;90(19):8915–9. https://doi.org/10.1073/pnas.90.19.8915.
71. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature. 1995;376(6535):62–6. https://doi.org/10.1038/376062a0.
72. Terman BI, Dougher-Vermazen M, Carrion ME, Dimitrov D, Armellino DC, Gospodarowicz D, et al. Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem Biophys Res Commun. 1992;187(3):1579–86. https://doi.org/10.1016/0006-291x(92)90483-2.
73. Quinn TP, Peters KG, de Vries C, Ferrare N, Williams LT. Fetal liver kinase 1 is a receptor for vascular endothelial growth factor and is selectively expressed in vascular endothelium. Proc Natl Acad Sci U S A. 1993;90:7533–7.
74. Pierce EA, Avery RL, Foley ED, Aiello LP, Smith LE. Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. Proc Natl Acad Sci U S A. 1995;92(3):905–9. https://doi.org/10.1073/pnas.92.3.905.
75. Hellstrom A, Perruzzi C, Ju M, Engström E, Hard AL, Liu JL, et al. Low IGF-I suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. Proc Natl Acad Sci. 2001;98(10):5804–8. https://doi.org/10.1073/pnas.101113998.
76. Hellström A, Carlsson B, Niklasson A, Segnestam K, Boguszewski M, Lacerda L, et al. IGF-I is critical for normal vascularization of the human retina. J Clin Endocrinol Metab. 2002;87(7):3413–6. https://doi.org/10.1210/jcem.87.7.8629.
77. Fukuda R, Hirota K, Fan F, Jung YD, Ellis LM, Semenza GL. Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. J Biol Chem. 2002;277(41):38205–11. https://doi.org/10.1074/jbc.M203781200.
78. Rakic JM, Lambert V, Devy L, Luttun A, Carmeliet P, Claes C, et al. Placental growth factor, a member of the VEGF family, contributes to the development of choroidal neovascularization. Investig Ophthalmol Vis SciI. 2003;44(7):3186–93. https://doi.org/10.1167/iovs.02-1092.
79. Michels S, Schmidt-Erfurth U, Rosenfeld PJ. Promising new treatments for neovascular age-related macular degeneration. Expert Opin Investig Drugs. 2006;15(7):779–93. https://doi.org/10.1517/13543784.15.7.779.
80. Cao Y. Positive and negative modulation of angiogenesis by VEGFR1 ligands. Sci Signal. 2009;2(59):re1. https://doi.org/10.1126/scisignal.259re1.
81. Simpson DAC, Murphy GM, Bhaduri T, Gardiner TA, Archer DB, Stitt AW. Expression of the VEGF gene family during retinal vaso-obliteration and hypoxia. Biochem Biophys Res Commun. 1999;262(2):333–40. https://doi.org/10.1006/bbrc.1999.1201.
82. Carmeliet P, Moons L, Luttun A, Vincenti V, Compernolle V, Mol MD, et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nat Med. 2001;7:575–83. https://doi.org/10.1038/87904.
83. Juul SE, Anderson DK, Li Y, Christensen RD. Erythropoietinand erythropoietin receptor in the developing human centralnervous system. Pediatr Res. 1998;43:40–9.

84. Wang GL, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. Proc Natl Acad Sci U S A. 1993;90(9):4304–8. https://doi.org/10.1073/pnas.90.9.4304.
85. Yang Z, Wang H, Jiang Y, Hartnett ME. VEGFA activates erythropoietin receptor and enhances VEGFR2-mediated pathological angiogenesis. Am J Pathol. 2014;184(4):1230–9. https://doi.org/10.1016/j.ajpath.2013.12.023.
86. Sato T, Kusaka S, Shimojo H, Fujikado T. Vitreous levels of erythropoietin and vascular endothelial growth factor in eyes with retinopathy of prematurity. Ophthalmology. 2009;116(9):1599–603. https://doi.org/10.1016/j.ophtha.2008.12.023. Epub 2009 Apr 15.
87. Sickel W. Electrical and metabolic manifestations of receptor and higher-order neuron activity in vertebrate retina. In: Arden GB, editor. The visual system. Advances in experimental medicine and biology, vol. 24. Boston: Springer; 1972. p. 101–8. https://doi.org/10.1007/978-1-4684-8231-7_11.
88. Rivera JC, Sapieha P, Joyal JS, Duhamel F, Shao Z, Sitaras N, et al. Understanding retinopathy of prematurity: update on pathogenesis. Neonatology. 2011;100:343–53. https://doi.org/10.1159/000330174.
89. Hartnett ME. The effects of oxygen stresses on the development of features of severe retinopathy of prematurity: knowledge from the 50/10 OIR model. Documenta ophthalmologica. Adv Ophthalmol. 2010;120(1):25–39. https://doi.org/10.1007/s10633-009-9181-x.
90. Kaur C, Sivakumar V, Foulds WS, Luu CD, Ling E. Cellular and vascular changes in the retina of neonatal rats after an acute exposure to hypoxia. Investig Ophthalmol Vis SciI. 2009;50(11):5364–74. https://doi.org/10.1167/iovs.09-3552.
91. Brooks SE, Gu X, Samuel S, Marcus DM, Bartoli M, Huang PL, et al. Caldwell; reduced severity of oxygen-induced retinopathy in eNOS-deficient mice. Invest Ophthalmol Vis Sci. 2001;42(1):222–8.
92. Holmström G, Broberger U, Thomassen P. Neonatal risk factors for retinopathy of prematurity--a population-based study. Acta Ophthalmol Scand. 1998;76(2):204–7. https://doi.org/10.1034/j.1600-0420.1998.760216.x.
93. Lee J, Dammann O. Perinatal infection, inflammation, and retinopathy of prematurity. Semin Fetal Neonatal Med. 2012;17(1):26–9. https://doi.org/10.1016/j.siny.2011.08.007.
94. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340(6):448–54. https://doi.org/10.1056/NEJM199902113400607.
95. Rivera JC, Sitaras N, Noueihed B, Hamel D, Madaan A, Zhou T, et al. Microglia and interleukin-1beta in ischemic retinopathy elicit microvascular degeneration through neuronal semaphorin-3A. Arterioscler Thromb Vasc Biol. 2013;33:1881–91. https://doi.org/10.1161/ATVBAHA.113.301331.
96. Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest. 2000;117(4):1162–72. https://doi.org/10.1378/chest.117.4.1162.
97. Kremlev SG, Palmer C. Interleukin-10 inhibits endotoxin-induced pro-inflammatory cytokines in microglial cell cultures. J Neuroimmunol. 2005;162:71–80. https://doi.org/10.1016/j.jneuroim.2005.01.010.
98. Dordelmann M, Kerk J, Dressler F, Brinkhaus MJ, Bartels DB, Dammann CE, et al. Interleukin-10 high producer allele and ultrasound defined periventricular white matter abnormalities in preterm infants: a preliminary study. Neuropediatrics. 2006;37:130–6. https://doi.org/10.1203/PDR.0b013e3181b3b0fa.
99. Silveira RC, Filho FJB, Procianoy RS. Assessment of the contribution of cytokine plasma levels to detect retinopathy of prematurity in very low birth weight infants. Investig Ophthalmol Vis Sci. 2011;52:1297–301. https://doi.org/10.1167/iovs.10-6279.
100. Powers MR, Davies MH, Eubanks JP. Increased expression of chemokine KC, an interleukin-8 homologue, in a model of oxygen-induced retinopathy. Curr Eye Res. 2005;30(4):299–307. https://doi.org/10.1080/02713680590923276.
101. Hellgren G, Willett K, Engstrom E, Thorsen P, Hougaard DM, Jacobsson B, et al. Proliferative retinopathy is associated with impaired increase in BDNF and RANTES expression levels after preterm birth. Neonatology. 2010;98:409–18. https://doi.org/10.1159/000317779.

102. Yao Y, Tsirka SE. Monocyte chemoattractant protein-1 and the blood-brain barrier. Cell Mol Life Sci. 2014;71:683–97. https://doi.org/10.1007/s00018-013-1459-1.
103. Natarajan G, Shankaran S, McDonald SA, Das A, Stoll BJ, Higgins RD, et al. Circulating beta chemokine and MMP 9 as markers of oxidative injury in extremely low birth weight infants. Pediatr Res. 2010;67(1):77–82. https://doi.org/10.1203/pdr.0b013e3181c0b16c.
104. Filomeni G, Zio D, Cocconi F. Oxidative stress and autophagy: the clash between damage and metabolic needs. Cell Death Differ. 2015;22(3):377–88. https://doi.org/10.1038/cdd.2014.150.
105. Jiong H, Sofia-Iris B, Janina W, Sven Z, Jihong L, Rüdiger P, et al. Soluble epoxide hydrolase promotes astrocyte survival in retinopathy of prematurity. J Clin Investig. 2019;129(12):5204–18. https://doi.org/10.1172/JCI123835.
106. Cristina L, Jose A, Veronica G, Bruce D, Vicente A, Joan C, et al. Inhibition of soluble epoxide hydrolase modulates inflammation and autophagy in obese adipose tissue and liver: Role for omega-3 epoxides. Proc Natl Acad Sci U S A. 2015;112(2):536–41. https://doi.org/10.1073/pnas.1422590112.

Genetics in Age-Related Macular Degeneration

10

Giridhar Anantharaman and Aarti Jain

10.1 Introduction

Age-related macular degeneration (AMD) is a late-onset, progressive multifactorial disease characterized by accumulation of extracellular deposits called drusen in the macula, localized inflammation, and ultimately neurodegeneration affecting mainly the central vision.

10.2 Epidemiology

AMD is the third largest cause of vision loss, accounting for 8.7% of all cases of blindness worldwide [1]. It is the most common cause of vision loss in developed countries [2–4]. Owing to the increase in life expectancy globally, its prevalence is expected to increase. It is estimated that by 2040, 288 million population will be affected by AMD worldwide [5].

10.3 Etiology

The exact etiology of AMD is not known, but the synergistic role of various environmental factors and genetics has been proposed. The environmental risk factors include age, race, smoking, dietary nutrients, and hypertension [6, 7]. Advanced age and smoking are the strongest risk factors [8, 9]. In the Framingham Study, the

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prevalence of AMD was 11% for those aged between 65 and 74 years and 28% for those between 75 and 85 years [10]. In the Beaver Dam Eye Study, approximately 30% of individuals aged 75 years or over had early AMD [11].

10.4 Genetics

Over the past decades, extensive research has been performed to determine the genetic component of AMD. Identification of genetic risk factors will aid in understanding the pathophysiology underlying AMD better and eventually developing appropriate and effective therapies for its prevention and treatment.

AMD is a complex polygenic disease in which a number of genetic variants, each contributing a small-to-moderate amount of increased risk, add to disease in addition to environmental factors [12].

Till now, multiple risk alleles associated with AMD have been identified. Risk alleles or polymorphisms are genetic variants associated with increased risk of a disease whereas protective alleles are associated with decreased risk of the disease. Risk alleles are not necessarily "abnormal" as they are generally present in at least 1% population. Individuals having one or more risk alleles are prone to developing the disease but may not always manifest the disease [13].

The earliest evidence of the role of genetics in AMD is provided by familial aggregation studies and twin studies.

1. ***Familial aggregation studies***: These studies compared the rates of AMD in relatives of cases and controls. Seddon et al. evaluated first-degree relatives (mostly siblings) of 119 AMD cases and 72 controls. The authors found the prevalence of AMD to be significantly higher in the relatives of the cases than in the relatives of controls (23.7% vs. 11.6%) [14]. The risk of developing the disease is three times higher in individuals having a family member with AMD than in individuals without a first-degree relative with AMD [14, 15].
2. ***Twin studies***: These studies compared the disease concordance rates among-monozygotic and dizygotic twins. The heritability of early and advanced AMD was estimated to be 46% and 71%, respectively, implying that 46%–71% of AMD variation could be elucidated by genetic factors [16].
3. ***Linkage studies***: Genetic linkage studies identify the regions of the genome that contain genes that predispose to the disease. Various linkage studies revealed that the most replicated linkage findings have been on chromosomes 1q25-31 and 10q26 [17–19]. Their importance was later confirmed with the finding of common genetic variants at these two loci—the complement factor H (*CFH*) gene on chromosome 1 and the age-related maculopathy susceptibility 2/HtrA serine peptidase (*ARMS2*/*HTRA1*) genes on chromosome 10.
4. ***Association studies***: Technological developments have made the analysis of whole genome possible resulting in acceleration in the discovery of new genetic associations with AMD. Genome-wide association studies (GWASs) analyze a genome-wide set of genetic variants, particularly single-nucleotide polymorphisms (SNPs), for associations with the disease of interest. GWASs are better at

detecting low-penetrance common genetic variants [with a minor allele frequency (MAF) >5%] associated with complex genetic diseases.

The first GWAS for AMD was conducted in 2005. It was also the first successful GWAS for a complex genetic disease. A subsequent GWAS identified 52 gene variants distributed across 34 locus regions (Table 10.1). Of these 34 locus regions,

Table 10.1 Thirty-four loci with their gene variants for AMD [20]

Lead variant	Chromosome	Locus name	OR	*P* value
rs10922109	1	*CFH*	0.38	9.6×10^{-618}
rs62247658	3	*ADAMTS9-AS2*	1.14	1.8×10^{-14}
rs140647181	3	*COL8A1*	1.59	1.4×10^{-11}
rs10033900	4	*CFI*	1.15	5.4×10^{-17}
rs62358361	5	*C9*	1.80	1.3×10^{-14}
rs116503776	6	*C2-CFB-SKIV2L*	0.57	1.2×10^{-103}
rs943080	6	*VEGFA*	0.88	1.1×10^{-14}
rs79037040	8	*TNFRSF10A*	0.90	4.5×10^{-11}
rs1626340	9	*TGFBR1*	0.88	3.8×10^{-10}
rs3750846	10	*ARMS2-HTRA1*	2.81	6.5×10^{-735}
rs9564692	13	*B3GALTL*	0.89	3.3×10^{-10}
rs61985136	14	*RAD51B*	0.90	1.6×10^{-10}
rs2043085	15	*LIPC*	0.87	4.3×10^{-15}
rs5817082	16	*CETP*	0.84	3.6×10^{-19}
rs2230199	19	*C3*	1.43	3.8×10^{-69}
rs429358	19	*APOE*	0.70	2.4×10^{-42}
rs5754227	22	*SYN3-TIMP3*	0.77	1.1×10^{-24}
rs8135665	22	*SLC16A8*	1.14	5.5×10^{-11}
New (reported with genome-wide significance for the first time)				
rs11884770	2	*COL4A3*	0.90	2.9×10^{-8}
rs114092250	5	*PRLR-SPEF2*	0.70	2.1×10^{-8}
rs7803454	7	*PILRB-PILRA*	1.13	4.8×10^{-9}
rs1142	7	*KMT2E-SRPK2*	1.11	1.4×10^{-9}
rs71507014	9	*TRPM3*	1.10	3.0×10^{-8}
rs10781182	9	*MIR6130-RORB*	1.11	2.6×10^{-9}
rs2740488	9	*ABCA1*	0.90	1.2×10^{-8}
rs12357257	10	*ARHGAP21*	1.11	4.4×10^{-8}
rs3138141	12	*RDH5-CD63*	1.16	4.3×10^{-9}
rs61941274	12	*ACAD10*	1.51	1.1×10^{-9}
rs72802342	16	*CTRB2-CTRB1*	0.79	5.0×10^{-12}
rs11080055	17	*TMEM97-VTN*	0.91	1.0×10^{-8}
rs6565597	17	*NPLOC4-TSPAN10*	1.13	1.5×10^{-11}
rs67538026	19	*CNN2*	0.90	2.6×10^{-8}
rs142450006	20	*MMP9*	0.85	2.4×10^{-10}
rs201459901	20	*C20orf85*	0.76	3.1×10^{-16}

Each locus with the genome-wide significance *P* value ($P < 5 \times 10^{-8}$) and effect size (odds ratio) for the variant have been shown

Table 10.2 Seven rare variants out of 54 variants for AMD [20]

Lead variant	Chromosome	Locus name
p.Arg1210Cys	1	*CFH*
p.Gly119Arg	4	*CFI*
p.Pro167Ser	5	*C9*
p.Lys155Gln	19	*C3*
rs148553336	1	*CFH*
rs191281603	1	*CFH*
rs35292876	1	*CFH*

significantly associated 16 loci were identified for the first time. It was found that 45 of 52 associated variants were common (MAF >5%), having odds ratios (ORs) from 1.1 to 2.9 whereas 7 of 52 were rare variants (MAF 0.01% and 1%) with ORs between 1.5 and 47.6. All seven rare variants were located in or near the complement genes [20]. Table 10.2 shows the seven rare variants.

The two most important loci with large effect sizes and relatively high frequencies are *CFH* and *ARMS2* [18, 20–22].

10.5 Pathogenesis of AMD

Various biological pathways involved in the pathogenesis of AMD are the following [23]:

- Immune and complement system
- Lipid transport
- Extracellular matrix (ECM) remodeling
- Angiogenesis
- Cell survival and homeostasis, including DNA repair, apoptosis, and stress

10.5.1 Immune and Complement System

The complement system is a vital component of the innate immune system that participates in the elimination of pathogens and also protects highly metabolic tissues in the body such as the retinal pigmented epithelium(RPE) from reactive oxygen species. Of the three complement pathways, the alternate pathway is mainly implicated in AMD. The alternate pathway works through proteins C3 and C5. CFH is a soluble complement regulator essential for controlling the alternative pathway. Faulty recognition of host cell surfaces by CFH due to mutations and polymorphisms has been associated with complement-mediated tissue damage and disease, including AMD. *CFH*, *C2/CFB*, *C3*, *CFI*, and *C5* are the complement factors associated with AMD. C2, C3, and C5 complement proteins make up the drusen and are proinflammatory [24, 25].

The Compliment polymorphisms that increase the AMD risk are the following:

- C3: rs11569536, rs2230199, rs104728, 6R102G, L314P
- C2: rs9332739, rs547154, rs116503776
- CFH: Y402H, which accounts for significantly earlier age of exudative disease onset (7 years earlier) and increases the risk of AMD progression [26].

Complement genes contribute approximately 57% of known variants to disease risk. *CFH* risk variants appear to slightly favor progression toward geographic atrophy (GA) [26].

10.5.2 Lipid Transport

Lipoproteins secreted by RPE are a major source of peroxidizable lipids. The ECM is implicated in the retention of lipoproteins in Bruch's membrane. Any alteration in the RPE and ECM can lead to the formation of drusen. Various genes and genetic variants related to the lipid transport pathway are as follows:

1. Apolipoprotein E (*ApoE*): This gene is located on chromosome 19. It is involved in lipid and cholesterol transport and metabolism. There are three isoforms of *ApoE*: epsilon 2 (E2), epsilon 3 (E3), and epsilon 4 (E4) [27, 28]. The E2 isoform increases the risk of AMD by 1.5-fold [28]. The E4 confers a protective effect with a two to threefold decrease in AMD development [27]. A combination of *ApoE* and *CFH-Y402H* increases the risk for AMD and Alzheimer's disease [29].
2. LIPC and CETP: These are expressed in the subretinal space and participate in rapid cholesterol transfer from the RPE.
3. Scavenger receptor class B, member 1 (*SCARB1*) gene
4. Solute carrier family 16, member 8 (*SLC16A8*)

10.5.3 Extracellular Matrix Remodeling

1. Age-related maculopathy susceptibility 2*(ARMS2)*: Previously termed *LOC387715*, ARMS2 is located on chromosome 10. It is a common and most important gene along with *CFH*. ARMS2 alleles in a single copy could have a 53% population-attributable risk for late AMD [30]. The precise function of *ARMS2* in the pathogenesis of AMD is not clearly known. Results of a recent study indicate that *ARMS2* is a constituent of the ECM [31]. ARMS2 binds to COL1A1 and COL4A2. COL1A1 is a constituent of type I collagen, which is a main component in Bruch's membrane [32]. Type I collagen shows a potent angiogenic function and is capable of inducing the expression of further angio-

genic genes [33]. Type IV collagen is a main component of the basement membrane of the choriocapillaris [34]. ARMS2 also interacts with ECM proteins, such as fibulin-6, which has been recognized as a risk factor in AMD. There is evidence indicating that ARMS2 confers a greater risk for wet AMD compared with GA [35]. When ARMS combines with CFH and C3, the attributable risk for AMD increases to 76% [36].

2. Tissue inhibitors of matrix metalloproteinases (TIMPs): Excess TIMP3 reduces the permeability of Bruch's membrane, leading to atrophy of RPE and photoreceptors [37]. It is also associated with Sorsby fundus dystrophy. A rare missense mutation in TIMP3 (C1113G) has been found to be associated with earlier age of disease onset (average age 65 years) and bilateral choroidal neovascularization [38].
3. Fibulin 5: It has a function in the assembly and stabilization of ECM complexes.
4. Hemicentin-1: It is a member of the immunoglobulin superfamily. It interacts with ARMS2 in the causation of AMD [39].
5. Fibulin 3: It is an epidermal growth factor consisting of fibulin-like extracellular matrix protein 1 (EFEMP1). EFEMP1 plays a role in Doyne honeycomb dystrophy and drusen formation [40].

Less common genetic factors: [41]

1. SerpinG1
2. ADAMTS9
3. Filamin interacting protein 1
4. FRK/Col10A1
5. Collagen type 8, α1

10.5.4 Angiogenesis

Vascular endothelial growth factor (VEGF) is a platelet-derived growth factor that-promotes angiogenesis and inhibits apoptosis. VEGF-A (VEGF-165) is a potent angiogenic agent that acts through receptor VEGFR-2. It is produced by endothelium, RPE, astrocytes, Muller, and ganglion cells. VEGF polymorphisms dysregulate gene expression, leading to disease manifestation. An imbalance between angiogenic and anti-angiogenic factors leads to the formation of new blood vessels. VEGF SNPs rs3025000, rs833068, rs844069, and rs699946 show good outcome responses for treatment [42].

10.5.5 Survival and Homeostasis, Including DNA Repair, Apoptosis, and Stress

Various rare genes that can increase the risk for AMD are *IER3-DDR1* immediate early response 3, *RAD51*, *RAD51B*, *MYRIP*, insulin-like growth factor-1 (*IGFR1*), *REST-C4/F14-PolR2BIGFBP7*, TNFRSF10A chemokine (C-C motif) ligand 2

Table 10.3 Various pathways and the respective genes involved in pathogenesis of AMD

	Biological pathways	Genes and genetic variants
1.	Complement system	*CFH*, *C2/CFB*, *C3*, *CFI*, *C5*
2.	Lipid transport	Apolipoprotein E, *LIPC*, *CETP*, *SCARB1*, *SLC16A8*
3.	ECM remodeling	*ARMS2*, *TIMP*, Fibulin 3 and 5, hemicentin 1, *SerpinG*, *ADAMTS9*, Filamin interacting protein 1, *FRK/Col10A1*
4.	Angiogenesis	*VEGF*
5.	Cell survival and homeostasis	IER3-DDR1 immediate early response 3, *RAD51*, *RAD51B*, *MYRIP*, *IGFR1*, *REST-C4/F14-PolR2BIGFBP7*, *TNFRSF10A CCL2*, *ERCC6*, *FSCN2*

(*CCL2*), *ERCC6*, and *FSCN2* [41]. Recently, mosaic loss of chromosome Y has been found to be associated with the risk of AMD, particularly in individuals between 65 and 75 years of age [43].

The summary of various biological pathways with their respective genes and variants has been shown in Table 10.3.

10.6 Genetic Testing

As AMD is a complex genetic disease with multiple genes and genetic variants implicated in the disease process, the knowledge of a single risk allele is of limited value in assessing its risk. Instead, a genetic risk score, calculated from known genetic and environmental risk factors, is clinically effective in advising patients about individual risk [44]. Moreover, currently, there is no evidence showing benefit in visual outcome when altering the management of genetically higher risk progression patients compared with individuals of lower genetic susceptibility. Also, genetic testing in patients with neovascular AMD does not provide clinically relevant information with regard to anti-VEGF treatment response. The American Academy of Ophthalmology Task Force specifically advises against testing for complex genetic diseases such as AMD and recommends avoiding direct-to-consumer genetic testing [45].

10.7 Conclusion

AMD is a complex disease with both genetic and environmental risk factors playing a role. Approximately 50% of the heritability is explained by the known genetic risk variants, the most important being *CFH* and *ARMS2*. Unfortunately, the exact mechanism through which *CFH* and *ARMS2* increase the risk of AMD is still not clear. Further work is needed to unravel the remaining heritable component of AMD, which can aid in a better understanding of the disease pathogenesis and lead to the planning of newer strategies for its prevention and treatment. Currently, there is no convincing evidence of the benefit of genetic testing in the routine clinical care of patients with AMD. However, genetic testing is more useful as a research strategy than in clinical management.

References

1. Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, et al. Global data on visual impairment in the year 2002. Bull World Health Organ. 2004;82(11):844–51.
2. Klein R, Klein BE, Cruickshanks KJ. The prevalence of age-related maculopathy by geographic region and ethnicity. Prog Retin Eye Res. 1999;18:371–89.
3. Klaver CC, Assink JJ, van Leeuwen R, et al. Incidence and progression rates of age-related maculopathy: the Rotterdam study. Investig Ophthalmol Vis Sci. 2001;42:2237–41.
4. Kawasaki R, Yasuda M, Song SJ, et al. The prevalence of age-related macular degeneration in Asians: a systematic review and meta-analysis. Ophthalmology. 2010;117:921–7.
5. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Global Health. 2014;2(2):e106–16.
6. Clemons TE, Milton RC, Klein R, Seddon JM, Ferris FL 3rd, Age- related Eye Disease Study Research Group. Risk factors for the incidence of advanced age-related macular degeneration in the Age-Related Eye Disease Study (AREDS): AREDS report no. 19. Ophthalmology. 2005;112:533–9.
7. Charbel Issa P, Chong NV, Scholl HP. The significance of the complement system for the pathogenesis of age-related macular degeneration—current evidence and translation into clinical application. Graefes Arch Clin Exp Ophthalmol. 2011;249:163–74.
8. Edwards AO, Lee SJ, Fridley BL, Tosakulwong N. Density of common complex ocular traits in the aging eye: analysis of secondary traits in genome-wide association studies. PLoS One. 2008;3(6):e2510.
9. Thornton J, Edwards R, Mitchell P, Harrison RA, Buchan I, Kelly SP. Smoking and age-related macular degeneration: a review of association. Eye (Lond). 2005;19(9):935–44.
10. Leibowitz HM, Krueger DE, Maunder LR, et al. The Framingham Eye Study monograph: an ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults, 1973–1975. Surv Ophthalmol. 1980;24:335–610.
11. Klein R, Klein BE, Jensen SC, et al. The five-year incidence and progression of age-related maculopathy: the Beaver Dam Eye Study. Ophthalmology. 1997;104:7–21.
12. Seddon JM, Sobrin L. Epidemiology of age-related macular degeneration. In: Albert D, Miller J, Azar D, et al., editors. Albert & Jakobiec's principles and practice of ophthalmology. Philadelphia: W.B. Saunders; 2007. p. 413–22.
13. Schwartz SG, Hampton BM, Kovach JL, Brantley MA Jr. Genetics and age-related macular degeneration: a practical review for the clinician. Clin Ophthalmol. 2016;10:1229–35.
14. Seddon JM, Ajani UA, Mitchell BD. Familial aggregation of age-related maculopathy. Am J Ophthalmol. 1997;123(2):199–206.
15. Klaver CC, Wolfs RC, Assink JJ, et al. Genetic risk of age-related maculopathy. Population-based familial aggregation study. Arch Ophthalmol. 1998;116:1646–51.
16. Seddon JM, Cote J, Page WF, Aggen SH, Neale MC. The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences. Arch Ophthalmol. 2005;123:321–7.
17. Iyengar SK, Song D, Klein BE, et al. Dissection of genome wide-scan data in extended families reveals a major locus and oligogenic susceptibility for age-related macular degeneration. Am J Hum Genet. 2004;74:20–39.
18. Jakobsdottir J, Conley YP, Weeks DE, et al. Susceptibility genes for age-related maculopathy on chromosome 10q26. Am J Hum Genet. 2005;77:389–407.
19. Weeks DE, Conley YP, Tsai HJ, et al. Age-related maculopathy: an expanded genome-wide scan with evidence of susceptibility loci within the 1q31 and 17q25 regions. Am J Ophthalmol. 2001;132:682–92.

20. Fritsche LG, Ig W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet. 2016;48(2):134–43.
21. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. Science. 2005;308(5720):419–21.
22. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science. 2005;308(5720):421–4.
23. Fritsche LG, Fariss RN, Stambolian D, et al. Age-related macular degeneration: genetics and biology coming together. Annu Rev Genomics Hum Genet. 2014;15:151–71.
24. Hageman GS, Luthert PJ, Chong VNH, et al. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. Prog Retin Eye Res. 2001;20:705–32.
25. Mullins RF, Russell SR, Anderson DH, et al. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J. 2000;14:835–46.
26. Baird PN, Islam FM, Richardson AJ, et al. Analysis of the Y402H variant of the complement factor H gene in age- related macular degeneration. Investig Ophthalmol Vis Sci. 2006;47:4194–8.
27. Zannis VI. Genetic polymorphism in human apolipoprotein E. Methods Enzymol. 1986;128:823–51.
28. Klaver CC, Kliffen M, van Duijn CM, et al. Genetic association of apolipoprotein E with age-related macular degeneration. Am J Hum Genet. 1998;63:200–6.
29. Zetterberg M, Landgren S, Andersson ME, et al. Association of complement factor H Y402H gene polymorphism with Alzheimer's disease. Am J Med Genet B Neuropsychiatr Genet. 2008;147B:720–6.
30. Klein R, Myers CE, Meuer SM, et al. Risk alleles in CFH and ARMS2 and the long-term natural history of age-related macular degeneration: the Beaver Dam Eye Study. JAMA Ophthalmol. 2013;131:383–92.
31. Kortvely E, Hauck SM, Duetsch G, Gloeckner CJ, Kremmer E, Alge-Priglinger CS, Deeg CA, Ueffing M. ARMS2 Is a constituent of the extracellular matrix providing a link between familial and sporadic age-related macular degenerations. Investig Ophthalmol Vis Sci. 2010;51(1):79–88.
32. Guymer R, Luthert P, Bird A. Changes in Bruch's membrane and related structures with age. Prog Retin Eye Res. 1999;18:59–90.
33. Imai H, Honda S, Kondo N, Ishibashi K, Tsukahara Y, Negi A. The upregulation of angiogenic gene expression in cultured retinal pigment epithelial cells grown on type I collagen. Curr Eye Res. 2007;32:903–10.
34. Marshall GE, Konstas AG, Reid GG, Edwards JG, Lee WR. Type IV collagen and laminin in Bruch's membrane and basal linear deposit in the human macula. Br J Ophthalmol. 1992;76:607–14.
35. Sobrin L, Reynolds R, Yu Y, et al. ARMS2/HTRA1 locus can confer differential susceptibility to the advanced subtypes of age-related macular degeneration. Am J Ophthalmol. 2011;151(2):345–52.
36. Spencer KL, Olson LM, Anderson BM, et al. C3 R102G polymorphism increases risk of age-related macular degeneration. Hum Mol Gen. 2008;17:1821–4.
37. Kamei M, Hollyfield JG. TIMP-3 in Bruch's membrane: changes during aging and in age related macular degeneration. Investig Ophthalmol Vis Sci. 1999;40:2367–75.
38. Warwick A, Gibson J, Sood R, Lotery A. A rare penetrant TIMP3 mutation confers relatively late onset choroidal neovascularization. Eye (Lond). 2016;30:488–91.
39. Wong TY, Chakravarthy U, Klein R, et al. The natural history and prognosis of neovascular age-related macular degeneration: a systematic review of the literature and meta-analysis. Ophthalmology. 2008;115:116–26.

40. Marmorstein LY, McLaughlin PJ, Peachey NS, et al. Formation and progression of sub-retinal pigment epithelium deposits in Efemp1 mutation knock-in mice: a model for the early pathogenic course of macular degeneration. Hum Mol Gen. 2007;16:2423–32.
41. Fritsche LG, Chen W, Schu M, Yaspan BL, Yu Y, et al. Seven new loci associated with age-related macular degeneration. Nat Genet. 2013;45:433–9.
42. Fauser S, Lambrou GN. Genetic predictive biomarkers of anti-VEGF treatment response in patients with neovascular age-related macular degeneration. Surv Ophthalmol. 2015;60:138–52.
43. Grassmann F, Kiel C, Hollander AI, Weeks DE, Lotery A, Cipriani LA. Y chromosome mosaicism is associated with age-related macular degeneration. Eur J Hum Genet. 2019;27:36–41.
44. Cooke Bailey JN, Hoffman JD, Sardell RJ, Scott WK, Pericak-Vance MA, Haines JL. The application of genetic risk scores in age-related macular degeneration: a review. J Clin Med. 2016;5(3):31.
45. Csaky KG, Schachat AP, Kaiser PK, Small KW, Heier JS. The use of genetic testing in the management of patients with age-related macular degeneration: American Society of Retina Specialists Genetics Task Force Special Report. J Vitreoretin Dis. 2017;1(1):75–8.

Genetics of Rhegmatogenous Retinal Detachment

11

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Abbreviations

GRT	Giant retinal tear
GWAS	Genome-wide association study
LD	Lattice degeneration
RD	Retinal detachment
RRD	Rhegmatogenous retinal detachment
SNPs	Single nucleotide polymorphisms

11.1 Introduction

A rhegmatogenous retinal detachment (RRD) results from a full thickness break in the neurosensory retina leading to the separation of the neurosensory retina from the underlying retinal pigmented epithelium. While descriptions of this disease entity have been around since the nineteenth century, true understanding of the condition in vivo became possible only after invention of the ophthalmoscope in 1851 [1]. It was Jules Gonin in 1904 who described three cases of RRD which paved the way for research and development of techniques to treat this condition [2]. Since then, our understanding of the etiology and management of this blinding condition has substantially improved.

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Rhegmatogenous retinal detachments are more often found in men (57–60%) when compared to women [3]. Ethnicity too has proven to play an important role. For instance, the incidence of RRD in patients of Chinese ethnicity was found to be three times that of Indian ethnicity (11.6 per 100,000 versus 3.9 per 100,000, respectively) [4]. The African and West Indian origin population have an extremely low incidence of RRD [3]. These racial variations suggest a disparity in the genetic profile, which could be an underlying risk factor. Several inherent risk factors for RRD such as myopia, lattice degeneration, and posterior vitreous detachment are already well described in literature. This chapter describes the various genetic abnormalities associated with RRD and also sheds light on the genetics of non-syndromic retinal detachments.

11.2 Genetics of Conditions Associated with Rhegmatogenous Retinal Detachment

Several conditions which are thought to be risk factors of the development of retinal detachment exhibit a genetic predisposition. These conditions are described below.

1. ***Lattice degeneration*** (LD) is a degeneration of the peripheral retina characterized by oval or linear patches of retinal atrophy. Prevalence of LD ranges between 6 and 9.5% [5]. It is the most common vitreoretinal degeneration that predisposes to RRD [6]. The role of heredity in the etiology of LD has been reported in literature [6–9]. Both autosomal dominant [7, 8] and autosomal recessive modes [9] have been described. Mukarami et al. [10] investigated 100 patients of LD without RRD and concluded that it had a complex inheritance pattern, with a threefold higher prevalence of LD in first-degree relatives. In 2012, Meguro et al. published a genome-wide association study (GWAS) suggesting an association of LD with COL4A4 in a Japanese population [11].
2. ***Retinal dialysis*** accounts for approximately 6% of all RRD cases [12]. It is largely believed to be traumatic [13] with existing literature suggesting that a genetic predisposition is unlikely [14]. However, the high prevalence of bilateralism in dialysis is suggestive of a genetic etiology.
3. ***Giant retinal tears*** (GRT) account for approximately 1.3% of all RRDs. Trauma is a common cause of GRT, however, many of the hereditary vitreoretinopathies also have a high rate of GRTs [15].
4. ***Myopia:*** The genetics of myopia is a very vast and complex topic. Two main characteristics, the axial length and corneal curvature have shown to have high heritability with one study demonstrating this to be 0.95 for the former and 0.67 for the latter [16]. In addition, family aggregation studies have demonstrated a higher prevalence of myopia in children with myopic parents compared to those without [17]. Siblings of myopes also have increased risk of developing myopia [18]. Over 50 genetic loci have been described that are associated with the development of myopia. These have multiple patterns of inheritance [19].

11.3 Genetics of Syndromic Rhegmatogenous Retinal Detachment

1. ***Stickler Syndrome*** is the most common cause of inherited RRD. It falls under the spectrum of Type II/XI collagenopathies. Stickler syndrome is characterized by five clinical features which include: (1) mid-face hypoplasia and small chin; (2) a bifid uvula and submucous or frank cleft palate; (3) congenital abnormalities of the vitreous; (4) cataract; and (5) a high rate of retinal detachment [20, 21]. In addition, myopia, characteristic lamellar cortical cataract, and angle anomalies may exist. The main clinical characteristic feature is the vitreous abnormality. Four subtypes have been described; STL1, STL2, STL3, and STL4; however, only STL1, STL2, and STL4 present with ocular features [15].
   (a) Type 1—Most common; highly penetrant autosomal dominant; mutations in COL2A1 on chromosome 12q13; skeletal, ocular, and auditory features; congenital non-progressive myopia; high risk of developing a GRT; membranous vitreous in the retrolenticular area extending to the periphery.
   (b) Type 2—Autosomal dominant; mutations in COL11A1 on 1p21; beaded congenital vitreous anomaly; arthropathy and cleft palate.
   (c) Type 4—Autosomal recessive, mutations in COL9A1 and COL9A2.

2. Marshall syndrome: Heterozygous splice site mutations in COL11A1 mutations [22] with myopia, early cataracts, vitreous liquefaction, and retinal breaks; short stature, hypoplastic nasal bones, and round faces.
3. Wagner's syndrome [23]: Optically empty vitreous with pre-retinal condensations to the periphery; progressive chorioretinal degeneration; pseudostrabismus; cataract; anterior segment dysgenesis and RD is reported in up to 75% of patients. Linked to gene coding chondroitin sulfate proteoglycan-2 (CSPG2), also known as versican on chromosome 5.39.
4. Erosive vitreoretinopathy: Described in 1994 by Stone; allelic to Wagner's syndrome; marked vitreous syneresis, RD and diffuse rod-cone dystrophy; high myopia absent; progressive "erosion" of the retinal pigment epithelium (RPE) resulting in visualization of choroidal vessels [24].
5. Snowflake vitreoretinal degeneration: Described by Schepens in 1974 [25] autosomal dominant; mutation in the KCNJ13 gene which codes for the Kir7.1 K+ channel [26], fibrillar vitreous degeneration; absent optic nerve cup; parapapillary sheathing with radial perivascular degeneration; 20% develop RD [27].
6. Autosomal Dominant Vitreoretinochoriodopathy: Autosomal dominant condition caused by mutations in VMD2on 11q13 [28] annular ring of chorioretinal-hypopigmentation anterior to the vortex veins to the ora serrata [29]. This pigmentation is believed to confer protection to development of RRD. To date, one case with associated RD has been reported [30].
7. Knobloch Syndrome: Recessive disorder caused by mutations in COL18A1; encodes type XVIII collagen; characterized by high myopia; vitreoretinal changes; occipital encephaloceles; RRD [31], rates of RRD are reported to be greater than 50% [20].

Table 11.1 Genes associated with syndromic forms of rhegmatogenous retinal detachment[a]

Disease	Gene	Locus	Function/end product
STL1	*COL2A1*	12q13.11	Type II collagen, a-1 chain
STL2	*COL11A1*	1p21.1	Type XI collagen, a-1 chain
STL4	*COL9A1*	6q13	Type IX collagen, a-1 chain
Marshall	*COL11A1*	1p21.1	Type XI collagen, a-1 chain
Wagner	*CSPG2/VCAN*	5q14.2-q14.3	Versican
Knobloch	*COL18A1*	21q22.3	Type XVIII collagen, alpha-1 chain
SVD	*KCNJ13*	2q37.1	Kir7.1 K^+ channel
XLRS	*RS1*	Xp22.13	Retinoschisin, protein, cell adhesion
MFS	*FBN1*	15q21.1	Fibrillin
ADVIRC	*VMD2*	11q12.3	Anion channel

STL Stickler syndrome, *SVD* Snowflake vitreoretinal degeneration, *XLRS* X-linked retinoschisis, *MFS* Marfan syndrome, *ADVIRC* Autosomal dominant vitreoretinochoroidopathy
[a]Adapted from Johnston T, Chandra A, Hewitt AW. Current Understanding of the Genetic Architecture of Rhegmatogenous Retinal Detachment. Ophthalmic Genetics. 2016

8. Marfan's Syndrome: Autosomal dominant disorder; mutations in the fibrillin-1 (FBN1) gene; rate of RRD varies from 5 to 15% [32], multiple musculoskeletal manifestations, ectopia lentis. Table 11.1 lists the various genes that have been implicated in the development of syndromic RRD.

11.4 Genetics of Non-syndromicr hegmatogenous Retinal Detachment

While there is paucity of research on non-syndromic RRD, a genetic predisposition was first proposed nearly five decades ago [33]. Mitry et al. suggested that the sibling recurrence risk for RRD was 2.1, and the parent-offspring risk as 2.9 [34]. In fact, a positive first degree relative family history of RRD ranges between 1% and 8.2%. Go et al. [35] and Richards et al. [36] both reported a mutation in COL2A1 in persons without classical features of Stickler's syndrome. Edwards et al. have also described a family with autosomal dominant RRD having the pathogenic C192A mutation in exon 2 of COL2A1 [37].

In 2013, Kirin et al. using the GWAS found the strongest associations of non-syndromic RRD with single nucleotide polymorphisms (SNPs) at the CERS2, SS18, TSTA3, TIAM1, and LDB2 loci. The CERS2 gene has been implicated in the apoptosis of photoreceptors. The other genes play a role in molecular adhesion and in maintaining the cytoskeletal framework. Their observations suggest a polygenic etiology of RRD [38]. Magliyah et al. in 2019 described the occurrence of recessive LRPAP1 gene, which confers a high risk of childhood onset RRD [39]. Table 11.2 lists the various genes that have been implicated in the development of idiopathic RRD.

A number of genetic associations with RRD exist. However, the precise genetic risk of non-syndromic RRD is yet to be well defined. As our understanding improves, it could facilitate screening programs for prevention and early detection, and with a potential to reduce the morbidity of rhegmatogenous retinal detachment.

Table 11.2 Genes associated with idiopathic rhegmatogenous retinal detachment[a]

Gene	Locus	Function/end product
COL2A1	12q13.11	Type II collagen, a-1 chain
CERS2	1q21.3	Ceramide synthase
SS18	18q11.2	Modify Integrins
TSTA3	8q24.3	Modify Integrins
TIAM1	21q22.11	Cell migration
LDB2	4p15.32	Cytoskeletal reorganization
LRPAP1	4p16.3	Molecular chaperone for LDL receptor-related proteins

[a]Adapted from Johnston T, Chandra A, Hewitt AW. Current Understanding of the Genetic Architecture of Rhegmatogenous Retinal Detachment. Ophthalmic Genetics. 2016

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References

1. Helmholtz HV. Helmholtz's treatise on physiological optics. 3rd ed. New York: Dover Publication; 1962.
2. Gonin J. La pathogenie du décollement spontané de la rétine. Ann Ocul. 1904;132:30.
3. Mowatt L, Shun-Shin G, Price N. Ethnic differences in the demand incidence of retinal detachments in two districts in the West Midlands. Eye. 2003; https://doi.org/10.1038/sj.eye.6700245.
4. Wong TY, Tielsch JM, Schein OD. Racial difference in the incidence of retinal detachment in Singapore. Arch Ophthalmol. 1999; https://doi.org/10.1001/archopht.117.3.379.
5. Byer NE. Clinical study of lattice degeneration of the retina. Trans Am Acad Ophthalmol Otolaryngol. 69(6):1065–81.
6. Byer NE. Lattice degeneration of the retina. Surv Ophthalmol. 1979; https://doi.org/10.1016/0039-6257(79)90048-1.
7. Everett WG. Study of a family with lattice degeneration and retinal detachment. Am J Ophthalmol. 1968; https://doi.org/10.1016/0002-9394(68)93592-7.
8. Delaney WV, Podedworny W, Havener WH. Inherited retinal detachment. Arch Ophthalmol. 1963; https://doi.org/10.1001/archopht.1963.00960040050010.
9. Lewkonia I, Davies MS, Salmon JD. Lattice degeneration in a family: with retinal detachment and cataract. Br J Ophthalmol. 1973; https://doi.org/10.1136/bjo.57.8.566.
10. Murakami F, Ohba N. Genetics of lattice degeneration of the retina. Ophthalmologica. 1982; https://doi.org/10.1159/000309235.
11. Meguro A, Ideta H, Ota M, Ito N, Ideta R, Yonemoto J, et al. Common variants in the COL4A4 gene confer susceptibility to lattice degeneration of the retina. PLoS One. 2012;7(6):e39300.
12. Mitry D, Singh J, Yorston D, Siddiqui MA, Wright A, Fleck BW, et al. The predisposing pathology and clinical characteristics in the Scottish retinal detachment study. Ophthalmology. 2011;118(7):1429–34.
13. Ross WH. Traumatic retinal dialyses. Arch Ophthalmol. 1981; https://doi.org/10.1001/archopht.1981.03930020245005.
14. Ross WH. Retinal dialysis: lack of evidence for a genetic cause. Can J Ophthalmol. 1991;26(6):309–12.
15. Snead MP, McNinch AM, Poulson AV, Bearcroft P, Silverman B, Gomersall P, et al. Stickler syndrome, ocular-only variants and a key diagnostic role for the ophthalmologist. Eye. 2011;25(11):1389–400.
16. Klein AP, Suktitipat B, Duggal P, Lee KE, Klein R, Bailey-Wilson JE, et al. Heritability analysis of spherical equivalent, axial length, corneal curvature, and anterior chamber depth in the Beaver Dam Eye Study. Arch Ophthalmol. 2009;127(5):649–55.

17. Hui J, Peck L, Howland HC. Correlations between familial refractive error and children's non-cycloplegic refractions. Vision Res. 1995; https://doi.org/10.1016/0042-6989(94)00224-A.
18. Farbrother JE, Kirov G, Owen MJ, Guggenheim JA. Family aggregation of high myopia: estimation of the sibling recurrence risk ratio. Investig Ophthalmol Vis Sci. 2004; https://doi.org/10.1167/iovs.03-1155.
19. Young TL. Molecular genetics of human myopia: an update. Optom Vis Sci. 2009; https://doi.org/10.1097/OPX.0b013e3181940655.
20. Edwards AO. Clinical features of the congenital vitreoretinopathies. Eye. 2008; https://doi.org/10.1038/eye.2008.38.
21. Richards AJ, Scott JD, Snead MP. Molecular genetics of rhegmatogenous retinal detachment. Eye. 2002; https://doi.org/10.1038/sj.eye.6700195.
22. Annunen S, Körkkö J, Czarny M, Warman ML, Brunner HG, Kääriäinen H, et al. Splicing mutations of 54-bp exons in the COL11A1 gene cause marshall syndrome, but other mutations cause overlapping marshall/stickler phenotypes. Am J Human Genetics. 1999;65(4):974–83.
23. Meredith SP, Richards AJ, Flanagan DW, Scott JD, Poulson AV, Snead MP. Clinical characterisation and molecular analysis of Wagner syndrome. Br J Ophthalmol. 2007;91(5):655–9. https://doi.org/10.1136/bjo.2006.104406.
24. Brown DM, Graemiger RA, Hergersberg M, Schinzel A, Messmer EP, Niemeyer G, et al. Genetic linkage of wagner disease and erosive vitreoretinopathy to chromosome 5q13-14. Arch Ophthalmol. 1995; https://doi.org/10.1001/archopht.1995.01100050139045.
25. Hirose T, Lee KY, Schepens CL. Snowflake degeneration in hereditary vitreoretinal degeneration. Am J Ophthalmol. 1974; https://doi.org/10.1016/0002-9394(74)90665-5.
26. Hejtmancik JF, Jiao X, Li A, Sergeev YV, Ding X, Sharma AK, et al. Mutations in KCNJ13 cause autosomal-dominant snowflake vitreoretinal degeneration. Am J Human Genetics. 2008;82(1):174–80.
27. Lee MM, Ritter R, Hirose T, Vu CD, Edwards AO. Snowflake vitreoretinal degeneration: follow-up of the original family. Ophthalmology. 2003;110(12):2418–26.
28. Yardley J, Leroy BP, Hart-Holden N, Lafaut BA, Loeys B, Messiaen LM, et al. Mutations of VMD2 splicing regulators cause nanophthalmos and Autosomal Dominant Vitreoretinochoroidopathy (ADVIRC). Investig Ophthalmol Vis Sci. 2004;45(10):3683.
29. Kaufman SJ, Goldberg MF, Orth DH, Fishman GA, Tessler H, Mizuno K. Autosomal dominant vitreoretinochoroidopathy. Arch Ophthalmol. 1982; https://doi.org/10.1001/archopht.1982.01030030274008.
30. Traboulsi EI, Payne JW. Autosomal dominant vitreoretinochoroidopathy: report of the third family. Arch Ophthalmol. 1993; https://doi.org/10.1001/archopht.1993.01090020048021.
31. Menzel O, Bekkeheien RC, Reymond A, Fukai N, Boye E, Kosztolanyi G, et al. Knobloch syndrome: Novel mutations inCOL18A1, evidence for genetic heterogeneity, and a functionally impaired polymorphism in endostatin. Hum Mutat. 2004;23(1):77–84.
32. Sharma T, Gopal L, Shanmugam MP, Bhende PS, Agrawal R, Shetty NS, et al. Retinal detachment in Marfan syndrome: clinical characteristics and surgical outcome. Retina. 2002; https://doi.org/10.1097/00006982-200208000-00005.
33. Francois J. The role of heredity in retinal detachment. Int Ophthalmol Clin. 1968; https://doi.org/10.1097/00004397-196800840-00010.
34. Mitry D, Williams L, Charteris DG, Fleck BW, Wright AF, Campbell H. Population-based estimate of the sibling recurrence risk ratio for rhegmatogenous retinal detachment. Investig Ophthalmol Vis Sci. 2011; https://doi.org/10.1167/iovs.10-6375.
35. Go SL, Maugeri A, Mulder JJS, Van Driel MA, Cremers FPM, Hoyng CB. Autosomal dominant rhegmatogenous retinal detachment associated with an Arg453Ter mutation in the COL2A1 gene. Investig Ophthalmol Vis Sci. 2003; https://doi.org/10.1167/iovs.02-0736.
36. Richards AJ, Meredith S, Poulson A, Bearcroft P, Crossland G, Baguley DM, et al. A novel mutation of COL2A1 resulting in dominantly inherited rhegmatogenous retinal detachment. Investig Ophthalmol Vis Sci. 2005; https://doi.org/10.1167/iovs.04-1017.

37. Edwards TL, Burt BO, Black GC, Perveen R, Kearns LS, Staffieri SE, et al. Familial retinal detachment associated with COL2A1 exon 2 and FZD4 mutations. Clin Exp Ophthalmol. 2012;40(5):476–83.
38. Kirin M, Chandra A, Charteris DG, Hayward C, Campbell S, Celap I, et al. Genome-wide association study identifies genetic risk underlying primary rhegmatogenous retinal detachment. Human Mol Genetics. 2013;22(15):3174–85.
39. Magliyah MS, Alsulaiman SM, Nowilaty SR, Alkuraya FS, Schatz P. Rhegmatogenous retinal detachment in nonsyndromic high myopia associated with recessive mutations in LRPAP1. Ophthalmology Retina. 2019; https://doi.org/10.1016/j.oret.2019.08.005.

An Overview on the Genetic Etiology, Testing, and Therapeutic Options for Retinitis Pigmentosa

12

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12.1 Introduction

Retinitis pigmentosa (RP) is an inherited retinal degenerative disease leading to loss of cone and rod photoreceptors [1]. The term "retinitis" is considered as a misnomer as dystrophy or degeneration of photoreceptors rather than inflammation, defines the exact pathophysiology of the condition. The first clinical manifestation in patients is the inability to see in night or dim light (nyctalopia), followed by gradual narrowing of the visual fields. Tunnel vision (poor peripheral vision) or even complete loss of vision (complete blindness) may occur depending on the progression and severity of the disease [2].

RP is shown to have an average global prevalence of 1:4000 irrespective of age groups [1]. This differs among different populations where in Korea it is 1 in 9000 (for all ages) and 1 in 6000 (aged over 40 years) [3]. In a Danish population study, it was recorded to be 1:3943 [4] and the Beijing eye study reported RP in 1:1000 Chinese above 40 years [5]. So far India recorded the highest prevalent rate with about 1 in 750 in adults (rural Central India) [6], 1 in 930 (urban), and 1 in 372 of rural subjects in the south Indian population above 40 years of age [7].

12.2 Etiology of RP

RP is genetic in origin and inherited as an autosomal dominant (10–20%), autosomal recessive (20%) or X-linked recessive (10%), pseudo-dominance in certain XLRP variants [8–10]; detection of more than one genetic cause in the same family has also been reported [11, 12]. The remaining cases with little or no family history

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of the patients are termed to be sporadic. In the autosomal dominant inheritance, the disease severity is mildest and the patients express the disease symptoms at age of 50 years as against that of autosomal recessive forms with an age of onset during the first decade (some mild forms have also been reported). X-linked RP is highly associated with myopia [2].

Histopathological sections from enucleated eyes of an autosomal recessive RP patient showed shortened and disorganized rod, cone outer segments loss with a reduced number of photoreceptors. In ten patients with autosomal dominant RP, shortened inner segments and poorly organized, shortened/absent outer segments were observed. In three cases, inclusion bodies and/or perinuclear cytoplasmic membranous swirls were also seen [13].

The pathophysiology of RP is defined by heterogeneous genetic architecture, with varying mutation spectrum globally. There are 116 genes mapped for isolated, non-syndromic RP which are shown to be involved in processes such as visual cycle, phototransduction pathway, ciliogenesis, ciliary function, and transcription, etc.

12.2.1 Genes Involved in Phototransduction Cascade

The cascade, triggered by excitation of opsin by the photon thus generating electrical signal is transmitted to the visual cortex via the optic nerve, resulting in image perception. The pathway is largely similar in both rod and cone cells [1]; Mutations in *RHO* cause autosomal dominant RP and rarely in autosomal recessive cases, intrafamilial variation due to genetic modifiers or environmental factors have also been reported. Figure 12.1 illustrates the process in phototransduction cascade and the genes involved.

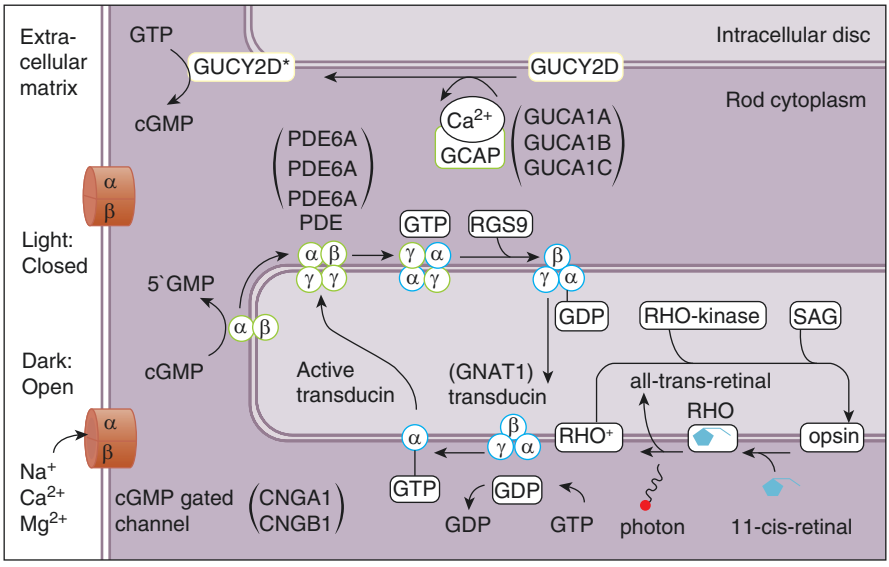

Fig. 12.1 Genes and phototransduction cascade [1]

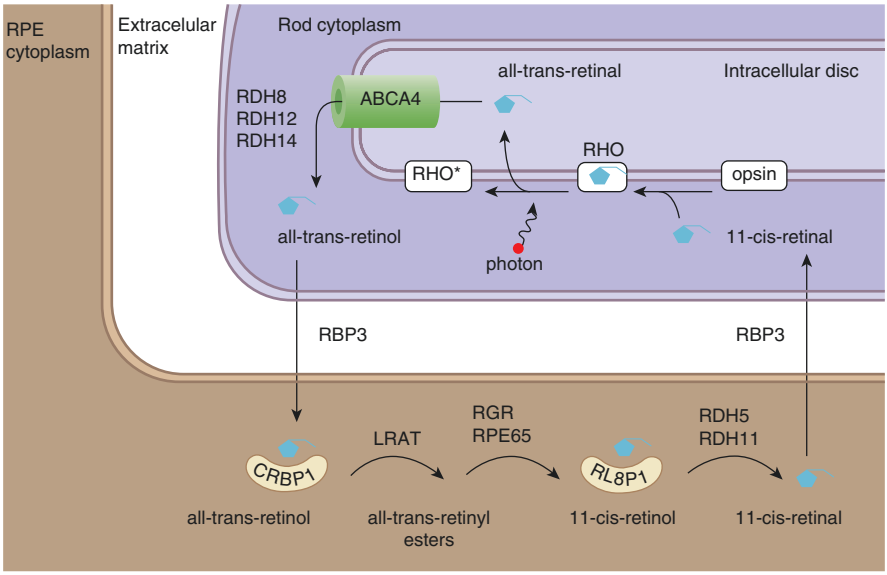

Fig. 12.2 Genes involved in visual cycle [1]

12.2.2 Genes Involved in Visual Cycle (Fig. 12.2)

ABCA4 gene encodes ATP binding cassette subfamily A protein. It transports the activated all-trans retinal to disc outer segment. Retinal dehydrogenase (coded by genes *RDH8, RDH12*, and *RDH14*) reduces all-trans-retinal to all-trans retinol. *RBP3* gene encoding IRBP (Interphotoreceptor Retinoid-Binding Protein) binds to all-trans retinol after it is transported to the subretinal space. In the cytoplasm, *LRAT, RPE65, RDH5*, and *RDH11 genes* de-isomerize all-trans retinol bound to cellular retinol-binding protein 1 (*CRBP1*). *CRAL1BP* transports the de-isomerized retinol into the photoreceptor matrix, where it binds to another opsin molecule, thereby starting the cycle afresh. Mutations in genes involved in the visual cycle generally follow an autosomal recessive inheritance pattern [1, 14].

12.2.3 Genes Involved in Ciliary Transport

The outer segment of the photoreceptor cells lacks biosynthetic machinery. The components are partially pre-assembled in the inner segment and transported to the outer segment via sensory and connecting cilia. Cilia assembly and maintenance are facilitated by IFT proteins (managed by *IFT27* and *IFT140* genes). The BBSome complex involved in ciliary transport consists of eight subunits encoded by *BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, BBS9,* and *BBS18* genes. Mutations in these genes have been found to cause Bardet–Biedl syndrome. However, mutations in *BBS1, BBS2, BBS3,* and *BBS9* have also been reported in non-syndromic RP cases [1].

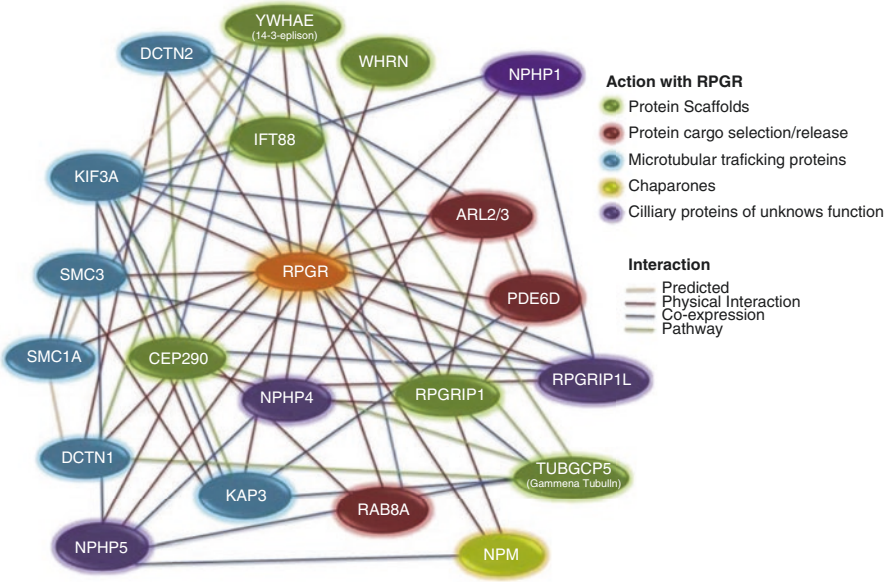

Fig. 12.3 RPGR interaction network [15]

In the ciliary tip, the localization of motor units is mediated by proteins encoded by genes *ARL3* and *RP2*. MKS (Meckel–Gruber) and NPHP (Nephronophthisis) genes assemble cilia transition zone and control their gating function. Reports suggest that the proteins encoded by these genes are associated with ciliopathies as they interact with BBsome complex and *RPGR* (Fig. 12.3). *RPGR* mutations are responsible for 70–80% of X-linked recessive RP and 10–20% of all RP cases. *RPGR* is connected to the cilium by RPGRIP1 protein and localized by *SPATA7* [1].

12.2.4 Genes Involved in Structural Processes

PRPH2 gene is involved in the formation of the outer segment disc rim. Altered *PRPH2* protein results in the absence of outer segment disc rim, loss of disc stability, and disc shedding. *RP1* encodes a photoreceptor protein involved in outer segment disc morphogenesis and orientation. It shows synergistic interaction with *RP1L1*.

The interphotoreceptor matrix plays an important role in retinal metabolism and transport. Its principal components are hyaluronic acid, proteoglycans, collagen, and elastin. *IMPG2, RBP3,* and *EYS* are genes associated with non-syndromic RP which bind to the hyaluronic acid network. *RP1* also has hyaluronic acid-binding motifs [1, 16].

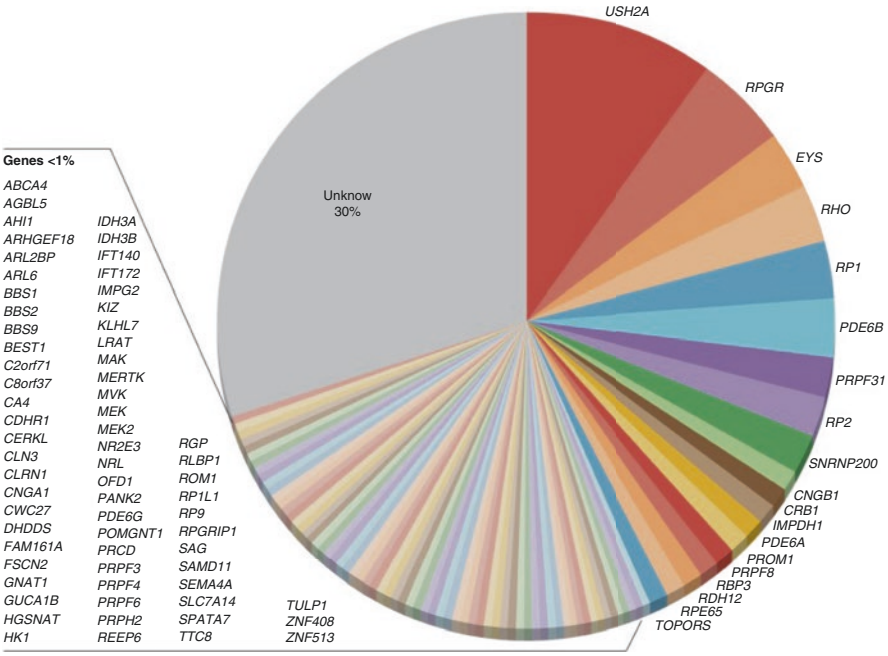

Fig. 12.4 Estimated relative contribution of genes to non-syndromic RP [1]

12.3 Genetic Heterogeneity of RP

Genetic heterogeneity in RP is evident from the broad clinical heterogeneity observed in RP which is defined by the phenotypic variability (both within and between the families) like age of onset, disease progression [17, 18] and fundus picture defining the retinal feature and the outcome [19, 20]. The candidate genes codes for protein essential for the normal functioning of the photoreceptor cells. Candidate gene mutations alter or even completely disrupt the pathway or the underlying cell structure and functions, leading to ciliary transport dysfunction, light damage, endoplasmic reticulum stress, apoptosis, and ultimately photoreceptor damage. In addition, the overlapping spectrum of candidate genes for various inheritance patterns adds to the heterogeneity of the disease (Fig. 12.4).

12.3.1 Mutation Spectrum of RP

The frequency and spectrum of mutations vary for different populations in different genes. There are more than 150 unique mutations reported in RP genes, which also adds to both the genetic and clinical heterogeneity of the disease [21]. The proteins

encoded by RP genes are not only retina specific but also some are ubiquitous [22]. Genetic overlap is seen between RP and various other retinal dystrophies (Fig. 12.5). Mutations in *RPE65, BEST1, NRL, NR2E3, RHO, and RP1* genes are observed in both autosomal dominant and recessive RP [23]. In addition, mutations in several genes, like *ABCA4* [24], *PROM1* [25], *PRPH2* [26],*C8orf37* [27], and *PRPF31* can lead to both RP and macular degeneration [28]. This highly heterogenetic nature of RP imposes a challenge in the clinical diagnosis as well as molecular diagnosis. *EYS* mutations (23.5%) are more prevalent and rhodopsin mutations (2.0%) are least prevalent in the Japanese population [29, 30]. *RPGR* (16%), *EYS* (13%), *PRPF31* (10%), and *USH2A* (9%) mutations are observed in the German cohort. In the Ashkenazi Jewish population, the most common RP mutations were identified in *MAK* (39%) and *DHDDS* (33%) genes [31].

In autosomal recessive RP families of Indian origin, homozygosity mapping has identified the causative gene in approximately 15% (5/34 and 4/26) of the families studied, indicating that still newer causative genes have to be identified for autosomal recessive RP. There are only a few reports on the distribution of common mutations in codons 345 and 347 in *RHO* gene(gene for adRP) observed in other ethnic

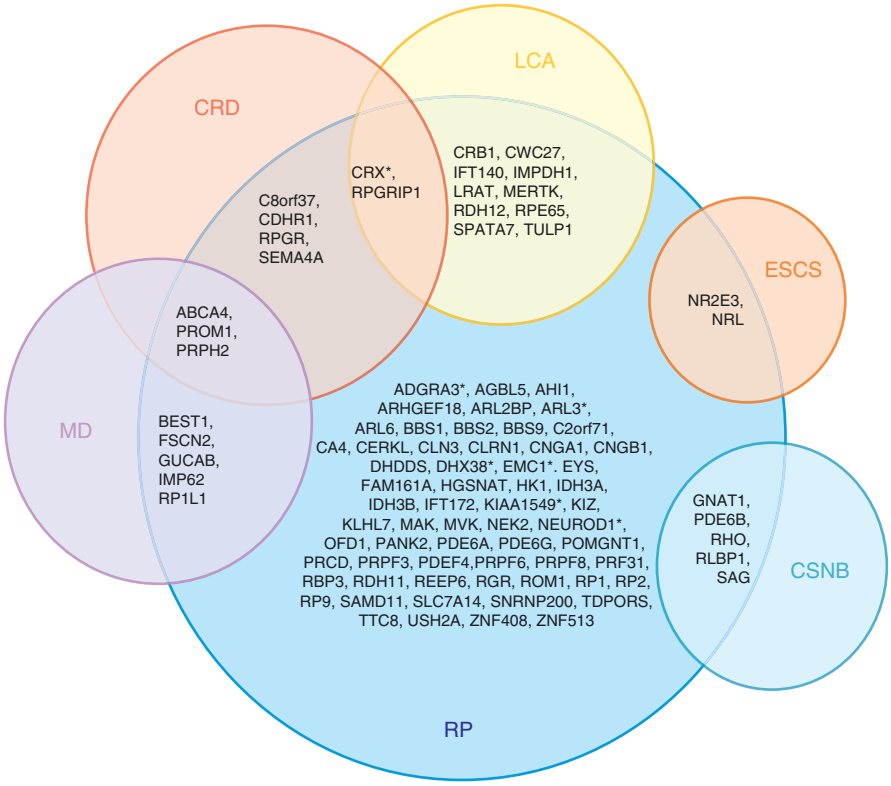

Fig. 12.5 The genetic overlap among inherited retinal dystrophies [1]

populations [33, 34]. Analyzing RP families with various inheritance patterns by Allele-Specific PCR (10 autosomal dominant, 32 autosomal recessive, 2 X-Linked families, 27 sporadic cases, and 7 inconclusive inheritance), Dikshit et al. reported that codon 345 mutations in the *RHO* gene is less frequent in the Indian cohort. Mutation screening of *PRPF31*, *RHO*, *IMPDH1*, and *RP1* genes in isolated (48 cases) and autosomal dominant RP (53 cases) showed a low frequency of mutations in *RHO* and *PRPF31 genes* [34]. Genome-wide screening for regions of homozygosity in RP patients from India has identified homozygous disease segregating mutations in genes *TULP1 (tubby like protein 1)*, *NR2E3 (nuclear receptor subfamily 2, group E, member 3)* and *MFRP (membrane frizzled-related protein)* [35]. Novel mutations in the *EYS* gene were identified by whole-exome sequencing in RP and sporadic cases from India [36]. Genetic testing in 171 cases diagnosed with retinitis pigmentosa at Medical Research Foundation, Chennai, India (unpublished data from the authors' lab) had shown a varied mutation spectrum with frequent mutations in *USH2A (8%), CERKL (7%), ABCA4 (6%), EYS (5%)*, *RDH12, PROM1, CRB1 (4%), BEST1 and MERTK (3%)* genes.

Mutations in *USH2A* have been reported in both non syndromic autosomal recessive RP and Usher syndrome. *USH2A* gene mutations are reported in 12% of [37] non-syndromic RP and in a certain population, in which mutation hot spots [38] are also reported. There was also an appreciable difference in the distribution of the mutant alleles within the protein domains where mutation in the LE-8 domain was most prevalent in RP and FN3-15 domain mutations in USH2 groups study, respectively. In 12 patients of Indian origin, novel *CERKL* gene variant in exon 8 (c.1045_1046delAT) was identified accounting for ≈7% of the RP cases [39].

RPE65 is another gene of specific interest for screening. *RPE65* mutations frequently manifest as LCA and occasionally as early-onset hereditary retinal disease or specifically retinitis pigmentosa [40] and rarely present as fundus albipunctatus (FA) [41, 42] or cone rod dystrophy [43]. Heterozygous dominant mutations in *RPE65*genehave been associated in both retinitis pigmentosa and choroideremia patients [44]. The prevalence of mutations varies between RP and LCA (2% in recessive RP to 16% in LCA) [40]. Unpublished data from our lab showed that 8% of LCA cases [45] and 2% of RP cases were tested positive fo*r RPE65* gene mutations.

12.4 Current Trends in Molecular Diagnostics of RP

The tremendous heterogeneity of the disease is evident from the broad spectrum of clinical features in inherited retinal dystrophies and the genetic heterogeneity indicates the need for an effective, precise genetic diagnostic approach that eventually supports/confirms/suggest consideration to re-valuate the clinical diagnosis of RP. This makes genetic testing to be too expensive, time-consuming, and labor-intensive [14, 47]. Molecular diagnostics in RP is indispensable in the clinical care of patients in genetic counseling, to rule out differential diagnosis, and in prenatal/preimplantation genetic diagnosis. With extreme genetic heterogeneity and multiple

inheritance patterns in RP, it becomes very difficult to provide genetic counseling and explain risk prediction to the successive generation without genetic testing. The association of genes such as *RHO, RPE65 and RP1* gene mutations observed in both autosomal dominant and recessive forms of RP is correlated with phenotypic variability and difference in age of onset of the disease condition. Molecular diagnostics in RP is much more challenging due to the increasing number of RP-associated genes and loci. Difference in mutation spectrums among different ethnic groups/geographical locations demand the need for an effective and reliable genetic testing. For example, the most frequent RP variant p.(Pro347Leu) in the *RHO* gene accounted for only 3.6–5% of adRP and ~ 0.5–1% of unrelated patients emphasizing that screening the target genes or more frequent mutation is not adequate for identifying the genetic cause in heterogenous diseases like RP [48]. Technological advances in genomics like next-generation sequencing (NGS)-based whole exome or genome sequencing facilitates ongoing discovery of new genes and loci associated with RP. NGS-based molecular diagnostic test hence increases the mutation detection rate for all inherited retinal diseases. In a single, one-generation, non-syndromic RP, whole-exome sequencing helped in identifying *DHDDS* (dehydrodolicholdiphosphate synthase) as the candidate gene that was also confirmed *in vivo* using zebra fish models [49].

Early-onset retinitis pigmentosa and LCA have both clinical and genetic overlaps with age of onset being the major differentiating feature. Leber congenital amaurosis (LCA) is congenital and most severe, manifesting total vision loss with an absent electroretinogram (ERG). The other childhood dystrophies feature visual loss in early infancy, i.e., before 5 years with minimally preserved ERG [50]. These include early-onset retinitis pigmentosa (EORP), juvenile retinitis pigmentosa [51], and severe Early Childhood Onset Retinal Dystrophy (SECORD) [52]. NGS-based genetic testing (either by clinical exome or whole exome) could possibly aid in differential diagnosis in these diseases and also in confirmative/supportive diagnosis of associated syndromes.

Unpublished data from our lab on genetic testing in childhood retinal dystrophy cases has shown a re-diagnosis of non-syndromic to syndromic forms of RP in ~8% of the cohort. These syndromes initially presented as LCA/early-onset retinal disease, and these ocular component manifests prior to the onset of other systemic features, thus highlighting the effectiveness and also the need for genetic testing in these cases. Systemic reevaluation in these patients depending on the molecular diagnosis has helped us in defining the cases as Senior–Loken syndrome (*IQCB1*), Jalili syndrome (*CNNM4*), Alstrom syndrome (*ALMS1*), and Thiamine responsive megaloblastic anemia syndrome (*SLC19A2*). We observe that there is a genetic overlap between non-syndromic and syndromic IRDs due to a high degree of heterogeneity that helps in identifying these syndromes in a proportion of these cases for early treatment and management.

The considerable variations due to the variable effects of mutations and genetic modifiers are observed within the RP subtypes. *RHO* mutations have been reported in both pericentral and sectoral RP [53, 54]. *RHO, USH2A, PDE6B, TOPORS, CERKL, NR2E3, RDS and HGSNAT* have also been shown to be associated with

pericentral RP [54, 55]. Jan et al. reported two families with pericentral RP having *RHO* mutation A164V with a favorable prognosis and I179F causing extreme variable expressivity [56].

Apart from the number of genes, the non-Mendelian inheritance patterns like digenic or triallelic inheritance, variable expressitivity, incomplete penetrance add to the complexity in the molecular diagnosis of RP. Several deep intronic variants resulting in cryptic splice site/exon inclusion/frameshift leading to aberrant truncation of the protein like in *CEP290* [57] and *USH2A* are being reported in RP patients. Whole-genome sequencing has proven more promising in identifying such a deep intronic variation. Although the pathogenicity of such variants remains elusive, iPSC technologies along with next-generation and Sanger sequencing has demonstrated the pathogenicity of deep intronic variants in non-syndromic *USH2A*-associated RP patients [58]. In highly heterogeneous diseases like retinitis pigmentosa (RP), where the associated genes and mutations are high or unknown, NGS with its low cost and high throughput makes it a more feasible approach [59]. Messer et al. assessed the attitude of RP patients and family members toward predictive testing and prenatal testing in adRP which demonstrated that a comprehensive genetic counseling and risk predictions after molecular diagnostics of RP has proven to assist the patients in undertaking informed decisions on predictive testing in 73% of the siblings, prenatal testing in 67% of the patients, and preimplantation genetics in seven families [60–62].

12.5 Genetic Modifiers in RP

Genetic modifiers are also shown to attribute to the extensive clinical variability observed in RP patients. *RPGR* gene mutations involving exons 1 to 14 along with *NPHP5* (I393N) variant are associated with more severity in XLRP patients *ROBO1*, *ROBO1-AS*, *ROBO2-AS*, and ubiquitin-specific peptidase 25 [*USP25*] were also identified as genetic modifiers in *RPGR*-associated RP [63]. Such observations are of special attention with the emerging gene-based therapies for retinal degenerations.

NR2E3 gene mutations are associated with Goldmann–Favre syndrome (GFS), enhanced S-cone syndrome (ESCS), and 1% of adRP. The nuclear hormone receptor gene, *NR2E3*, is a part of many potential gene networks involved in retinal homeostasis. The OCU400 (*NR2E3*-AAV) is thus under preclinical trials for the treatment of RP [64].

12.6 Genetic Testing: A Way Forward Toward Better Diagnosis and Screening

RP can be distinguished as non-syndromic RP and syndromic RP [65], both being caused by rare mutations which are inherited in Mendelian patterns. Reports on the non-Mendelian form of inheritance such as mitochondrial, digenic or *de novo*

mutations complicate the diagnosis in a minor proportion of cases [66]. A precise genotype–phenotype correlation has been difficult to establish, as this disease exhibits immense phenotype variability and genetic heterogeneity. The development of high-throughput technologies like NGS allows screening of a large number of genes among specific diseases or a group of related disorders [65].

Compared to conventional methods, a higher detection rate of pathogenic variants through targeted NGS has proved to be the most effective approach for the screening of known retinal dystrophy genes [67]. Targeted panel screening has shown a detection rate in ~60% of the patients [68]. A custom-based panel, designed based on the mutation spectrum in Spanish RP families rendered a mutation detection rate of 27% [69]. Using targeted NGS panels the diagnostic yield in different RP studies ranges from 36% to 82% [70, 71]. But there are still a large number of RP patients without genetic diagnosis, which could be possibly explained by variants within non-coding regions and hypomorphic variants in unknown RP-associated genes, or patients with disease entities like RP (e.g., autoimmune retinopathy or autoimmune disease) [72].

The most comprehensive targeted NGS retinal panel includes over 200 genes with retinitis pigmentosa genes being the largest category. These panels cover exon/intron boundaries and some known pathogenic deep intronic variants. Although management of retinal dystrophies was not impacted by genetic test results, there are other benefits such as in gene therapy trials for *RPE65*-associated retinal dystrophy [73], identifying the syndromic forms of retinitis pigmentosa in which the patients are at risk for co-morbidities.

Apart from the heterogeneity poised by the underlying genetic and clinical features, the types of identified variants reported as per the standard guidelines for interpreting the sequence variants adds difficulty in interpretation of the genetic test results.

Molecular testing has assisted in diagnosis, but there are few barriers which affect the selection of disease causative gene like indeterminate inheritance patterns in families, generation of a large volume of data giving rise to various bioinformatics challenges, more than one gene involved and the identification of "variants of unknown significance"(VUS) [74]. The quality of NGS platforms and bioinformatic pipelines along with specific clinical features attributed to various subtypes of RP may also alter the diagnostic yield in genetic testing for RP patients. The genetic counselors/clinicians may have to suggest additional testing in either the patient or family members, to confirm the pathogenicity of detected variants.

With emerging NGS-based technologies, the genetic analysis of many Mendelian disorders has been evolving by overcoming limitations of the older genotyping methods, allowing sequencing of the whole genome at a lower cost which has significantly increased the efficacy of molecular diagnosis in RP [75].

The emerging NGS-based exome/targeted panel sequencing [76, 77] thus provides an option for detailed genetic diagnosis, and thus improves/supports/confirms the clinical diagnosis, evaluates the inheritance pattern, and disease perception in

the future for the afflicted families. However, NGS-based methods are to be cautiously chosen considering the gene coverage. *RPGR* gene is one such example, where open reading frame 15 (ORF15) is not completely covered due to its highly repetitive sequences and purine-rich 3′ end of the gene. Approximately, 60% of disease-causing *RPGR* mutations were detected in this region [78–83]. Similarly for certain rare genetic diseases with one candidate gene at low coverage and many large deletions/duplications NGS can give only a 60% mutation detection rate.

12.7 Preimplantation Genetic Diagnosis and Its Implications

Introduced in 1990, prenatal diagnosis and preimplantation genetic diagnosis (PGD) are crucial methods for reducing the risk of giving birth to an affected baby. In order to avoid the transmission of genetic disorders, by PGD, one or a few cells are extracted from an in-vitro fertilization (IVF)-derived embryo; and genetic analysis is performed in those cells. Only the embryos that are negative for the specific genetic mutation are selected for implantation [84–87]. Thus, in PGD, the risk of having a child with a genetic disorder is eliminated, thereby preventing the trauma of terminating an affected embryo during pregnancy. Single-cell DNA testing through polymerase chain reaction and restriction enzyme analysis has helped in the detection of the presence of an *ABCA4* mutation carrier or affected embryos, before transfer. This has resulted in an infant without Stargardt disease [88]. Reports on successful PGDs in families with retinal diseases such as albinism, retinitis pigmentosa, retinoblastoma, blue cone monochromatism, achromatopsia, aniridia, Leber congenital amaurosis, Norrie disease, Usher syndrome type 1F, microphthalmia with coloboma, and X-linked retinoschisis are also available [89, 90].

Recently, mutated allele revealed by sequencing with aneuploidy and linkage analyses (MARSALA) is being used, which involves a one-step next-generation sequencing (NGS) procedure combining PGD, preimplantation genetic screening (PGS), and linkage analysis, thereby increasing success rates of clinical pregnancy and live birth [91, 92]. In an X-linked recessive RP family with RPGR mutation, MARSALA was performed by NGS in three biopsied blastocysts to confirm the unaffected status of the embryo before transferring into the uterus [93].

PGD is extremely accurate, with a misdiagnosis rate of less than 1% [94, 95]. These misdiagnoses may occur due to causes, such as embryonic/chromosomal mosaicism [96]. Multiple displacements have become a suitable approach for PGT-M due to low error rate and improved genome coverage. The capture sequencing and linkage analysis of SNPs located near the gene of interest provide a convenient and efficient way for PGT-M experiment design. Combining different NGS-based genetic detection methods along the successive reproductive stages can provide comprehensive information for genetic counseling and clinical decision in genetic diseases. Multiple displacement amplification (MDA) and capture sequencing were applied in a *USH2A* mutation family, to identify the embryo without paternal rare variant for implantation [97].

12.8 Evolving Treatment for RP

The initial treatment trails for RP aimed at providing management or relief for patients through cataract extraction in RP cases [98] and carbonic anhydrase inhibition in patients with macular edema [99]. Administration of vitamin A was the foremost clinical trial conducted for the treatment of RP. Oral vitamin A as retinylpalmitate alone or combined with a diet rich in omega-3 had shown a statistically significant difference in ERG amplitudes among the participants [100]. But the visual acuity loss or the benefit on the progression of the visual field were not reported and there is a high risk of hypervitaminosis A in these patients on a long-term administration [101, 102]. Other pharmacological agents considered as potential therapeutic drugs were Lutein and Valproic acid. Although both were promising, certain limitations in a clinical study concerning safety and efficacy need to be performed to take the molecules further [103, 104].

Antioxidant cocktails including α-tocopherol, ascorbic acid, and α-lipoic acid have been shown to reduce visual cone degeneration in dominant models of RP where the reactive oxygen released by rod degeneration leads to a subsequent reduction in cone density due to oxidative damage [105]. Neurotrophic factors like glial cell-derived neurotrophic factor (GDNF), ciliaryneurotrophic factor (CNTF) [106, 107], transferrin [108], and growth factors like basic fibroblast growth factor (bFGF) have been shown to protect photoreceptor degeneration. However, further trials with more subjects are indispensable to show the safety of treatment with these compounds [109, 110].

Unlike pharmacological therapies discussed above, molecular therapies involve molecular diagnostics to determine the eligibility of patients for clinical trials or gene-specific treatments. Currently, the most advanced therapy in retinal diseases involves gene replacement therapy/augmentation therapy targeting either the photoreceptors or RPE cells. Although numerous clinical trials are in progress, treatment for retinitis pigmentosa 20 (RP20), i.e., autosomal recessive *RPE65* mutation-associated gene therapy by using Luxturna developed by Spark Therapeutics became the first FDA-approved drug [111]. An update on the current various developments towards treatment for retinitis pigmentosa is available (https://www.fightingblindness.org/research/retinitispigmentosa-research-advances-3).

12.8.1 Ongoing Gene Therapy Trials

Several other gene augmentation therapies currently in clinical trials include *MERTK, MYO7A, ABCA4, PDE6B, RLBP1,* and *RPGR* [112–114]; but this treatment option is not available for subjects with advanced stage of degeneration because it needs surviving PR or RPE cells to take up the vector with the gene for expression. In dominant conditions, due to *RHO* or *PRPF31* mutations, the

treatment strategies must involve suppression of the wild type and mutant allele and suppression-resistant replacement of the wild-type gene.

Novel molecular agents are being used to perform gene suppressions such as antisense oligonucleotides, RNAi, CRISPR, ribozymes, etc. which along with targeting proteins like zinc finger proteins (ZFP), aptamers and antibodies have proven to be competitive therapy options [115–118]. With progress in such gene-dependent treatments, it is imperative that the specific genetic cause of disease in patients with any IRD is determined.

12.8.2 Potential Treatment Strategy Underway in End-Stage RP Patients

Though treatment options for RP are not wide, extensive research is being carried out worldwide to restore vision in affected patients. Artificial eye implants have been in the pipeline since the early 1950s. Through extensive studies [16, 119, 120], artificial vision retinal implants are shown to be a feasible option for patients as they are safe and reliable [121].

In patients with end-stage RP/other retinal degenerations, restoration of vision through gene-based augmentation therapy or personalized iPSC-based gene correction becomes impossible. Retinal implants and a neuromodulation method called optogenetics are suggested in such patients. Optogenetics confer vision restoration by artificially stimulating retinal activity in retinal cells. This is done by targeting light sensors genetically over a retinal cell and activating the cells when the current flows across its cell membrane. The PIONEER optogenetic study is the first human optogenetic trial started recently in 2018. The trial involves injecting AAV-mediated ChrimsonR channel rhodopsin targeting retinal ganglion cells. The result of the trial is still underway [122]. The retinal prosthetic implant on the other hand is a device that performs image acquisition by using its light-sensitive microphotodies that convert the images to little current which are then directed to the intact middle and inner retinal cells. In 2013, the first device to receive FDA approval was The Argus II epiretinal prosthesis (Second Sight Medical Products Inc., Sylmar, CA, USA). Following Argus II, epiretinal prostheses IRIS and EPI-IRET3, Subretinal prostheses (ASR, IMS/AMS, PRIMA, and BSI) are under clinical/preclinical trials [123]. Thus, retinal implants or optogenetics are a boon to end-stage RP patients whose vision cannot be restored through gene therapies.

12.8.3 Cell-Based Therapy

Another promising approach for treating RP especially relevant to dominantly inherited retinal degenerations is Stem cell-derived retinal cell transplantation. The transplantation of human embryonic stem cell (ESC)-derived photoreceptors

precursors into the subretinal space of the CRX mouse model had shown to result in the expression of differentiated PR cell markers and restored light response [124]. As ESCs had ethical concerns, for transplantation and treatment of retinal degenerative disease, adult fibroblast-derived iPSCs were used as a viable source for the production of retinal precursors.

The use of graft techniques and different cell types has been carried out with varying degrees of success. Maclaren et al. showed that postnatal, postmitotic photoreceptor precursor cells were the most efficient as they formed outer segments (OS) and established synaptic connections with host cells, thereby rescuing light-sensitive vision [125]. However, precursor cells are those that have undergone their final mitotic divisions and are a non-expandable, limited population. As an alternative, pluripotent stem cells, especially the iPSCs act as a source of an unlimited number of cells [126].

Using autologous patient-derived induced pluripotent stem cells (iPSC) for transplantation is another potential source of treatment. Gene editing is done using CRISPR-Cas9 to obtain healthy cells without mutations. This has led to the development of proof of concept where personalized iPSC-based gene correction by CRISPR-Cas9 mechanism was demonstrated in *RPGR* gene mutant of XLRP patient and *USH2A* gene mutation in Usher syndrome patient [127, 128]. A study by Alexander G. Bassuk et al. aims to repair an *RPGR* point mutation caused by X-linked RP [127]. The iPSC manipulation and transplantation has proven to be much cheaper and robust than creating an animal model making it a most reliable option for the treatment of RP patients. Currently, clinical trials for subretinal transplantation of hESC-derived retinal pigment epithelium have begun for Stargarts disease—an RP variant and it is reported to be promising [129].

12.8.4 *In-Vivo* Gene Editing

In-vivo gene editing can be done with zinc finger nucleases (ZFN), CRISPR/Cas9 systems (RNA-based approach), and transcription activator-like effector nucleases (TALENs) (protein-based approach). Non-homologous end joining (NHEJ) or homology-directed repair (HDR) was used for repairing the double-stranded breaks. However, there is also CRISPR/Cas9 system is not economically feasible to test as personalized medicine on individuals. An overview on the basics of gene editing and its applications towards treatment for various monogenic diseases including retinitis pigmentosa is discussed by Dasgupta et al. [130].

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References

1. Verbakel SK, van Huet RAC, Boon CJF, den Hollander AI, RWJ C, Klaver CCW, et al. Non-syndromic retinitis pigmentosa. Prog Retin Eye Res. 2018;66:157–86.
2. O'Neal TB LE. Retinitis Pigmentosa: StatPearls [Internet]; 2020.
3. Na KHKH, Kim KH, Han S, Kim P, Hann HJ, Ahn HS. Prevalence, age at diagnosis, mortality, and cause of death in retinitis pigmentosa in Korea-a nationwide population-based study. Am J Ophthalmol. 2017;176:157–65.
4. Haim M. Epidemiology of retinitis pigmentosa in Denmark. Acta Ophthalmol Scand Suppl. 2002;233:1–34.
5. Xu L, Hu L, Ma K, Li J, Jonas JB. Prevalence of retinitis pigmentosa inurban and rural adult Chinese: the Beijing eye study. Eur J Ophthalmol. 2006;16(6):865–6.
6. Nangia V, Jonas JB, Khare A, Sinha A. Prevalence of retinitis pigmentosa in India: the Central India Eye and Medical Study. Acta Ophthalmol. 2012;90(8):e649–50.
7. Sen P, Bhargava A, George R, Ve Ramesh S, Hemamalini A, Prema R, et al. Prevalence of retinitis pigmentosa in South Indian population aged above 40 years. Ophthalmic Epidemiol. 2008;15(4):279–81.
8. Birtel J, Gliem M, Mangold E, Muller PL, Holz FG, Neuhaus C, et al. Next-generation sequencing identifies unexpectedgenotype-phenotype correlations inpatients with retinitis pigmentosa. PLoS One. 2018;13(12):e0207958.
9. Churchill JDBS, Sullivan LS, Lewis RA, Wheaton DK, Birch DG, Branham KE, Heckenlively JR, Daiger SP. Mutations in the X-linked retinitis pigmentosa genes RPGR and RP2 found in 8.5% of families with a provisional diagnosis of autosomaldominant retinitis pigmentosa. Investig Ophthalmol Vis Sci. 2013;54:1411–6.
10. Jones KD, Wheaton DK, Bowne SJ, Sullivan LS, Birch DG, Chen R, et al. Next-generation sequencing to solve complex inherited retinal dystrophy: a case series of multiple genes contributing to disease in extended families. Mol Vis. 2017;23:470–81.
11. Chen X, Sheng X, Liu Y, Li Z, Sun X, Jiang C, et al. Distinct mutations with different inheritance mode caused similar retinal dystrophies in one family: a demonstration of the importance of genetic annotations in complicated pedigrees. J Transl Med. 2018;16(1):145.
12. Mendez-Vidal C, Bravo-Gil N, Gonzalez-Del Pozo M, Vela-Boza A, Dopazo J, Borrego S, et al. Novel RP1 mutations and a recurrent BBS1 variant explain theco-existence of two distinct retinal phenotypes in the same pedigree. BMC Genet. 2014;15:143.
13. Ben-Arie-Weintrob Y, Berson EL, Dryja TP. Histopathologic-genotypic correlations in retinitis pigmentosa and allied diseases. Ophthalmic Genet. 2005;26(2):91–100.
14. Ferrari S, Di Iorio E, Barbaro V, Ponzin D, Sorrentino FS, Parmeggiani F. Retinitis pigmentosa: genes and disease mechanisms. Curr Genomics. 2011;12(4):238–49.
15. Megaw RD, Soares DC, Wright AF. RPGR: its role in photoreceptorphysiology, human disease, and future therapies. Exp Eye Res. 2015;138:32–41.
16. Stingl K, Bartz-Schmidt KU, Besch D, Chee CK, Cottriall CL, Gekeler F, et al. Subretinal visual implant alpha IMS--clinical trial interim report. Vision Res. 2015;111(Pt B):149–60.
17. Hull S, Arno G, Plagnol V, Chamney S, Russell-Eggitt I, Thompson D, et al. The phenotypic variability of retinal dystrophies associated with mutations inCRX, with report of a novel maculardystrophy phenotype. Investig Ophthalmol Vis Sci. 2014;55(10):6934–44.
18. Yuan Z, Li B, Xu M, Chang EY, Li H, Yang L, et al. The phenotypicvariability of HK1-associated retinal dystrophy. Sci Rep. 2017;7(1):7051.
19. El Shamieh SB-SE, Lancelot ME, Antonio A, Démontant V, Condroyer C, Letexier M, Saraiva JP, Mohand-Saïd S, Sahel JA, Audo I, Zeitz C. Targeted next generation sequencing identifies novel mutations in RP1 as a relatively common cause of autosomal recessive rod-cone dystrophy. Biomed Res Int. 2015;2015:485624.

20. Avila-Fernandez A, Corton M, Nishiguchi KM, Munoz-Sanz N, Benavides-Mori B, Blanco-Kelly F, et al. Identification of an RP1 prevalent founder mutationand related phenotype in Spanish patients with early-onset autosomal recessive retinitis. Ophthalmology. 2012;119(12):2616–21.
21. Li S, Datta S, Brabbit E, Love Z, Woytowicz V, Flattery K, Capri J, Yao K, Wu S, Imboden M, Upadhyay A, Arumugham R, Thoreson WB, DeAngelis MM, Haider NB. Nr2e3 is a genetic modifier thatrescues retinal degeneration and promotes homeostasis in multiple models of retinitis pigmentosa. Gene Ther. 2020:2.
22. Singh HP, Jalali S, Narayanan R, Kannabiran C. Genetic analysis of Indian families with autosomal recessive retinitis pigmentosa by homozygosity screening. Investig Ophthalmol Vis Sci. 2009;50(9):4065–71.
23. Huang L, Zhang Q, Huang X, Qu C, Ma S, Mao Y, et al. Mutation screening ingenes known to be responsible for Retinitis Pigmentosa in 98 Small Han Chinese families. Sci Rep. 2017;7(1):1948.
24. Sun H, Smallwood PM, Nathans J. Biochemical defects in ABCR protein variants associated with human retinopathies. Nat Genet. 2000;26(2):242–6.
25. Yang Z, Chen Y, Lillo C, Chien J, Yu Z, Michaelides M, et al. Mutantprominin 1 found in patients with macular degeneration disrupts photoreceptor disk morphogenesis in mice. J Clin Investig. 2008;118(8):2908–16.
26. Ali RR, Sarra GM, Stephens C, Alwis MD, Bainbridge JW, Munro PM, et al. Restoration of photoreceptor ultrastructure and function in retinal degenerationslow mice by gene therapy. Nat Genet. 2000;25(3):306–10.
27. van Huet RA, Estrada-Cuzcano A, Banin E, Rotenstreich Y, Hipp S, Kohl S, et al. Clinical characteristics of rod and cone photoreceptor dystrophies inpatients with mutations in the C8orf37 gene. Investig Ophthalmol Vis Sci. 2013;54(7):4683–90.
28. Lu F, Huang L, Lei C, Sha G, Zheng H, Liu X, et al. A novel PRPF31mutation in a large Chinese family with autosomal dominant retinitis pigmentosa and macular degeneration. PLoS One. 2013;8(11):e78274.
29. Arai YMA, Hirami Y, Ishigami C, Kosugi S, Mandai M, Kurimoto Y, Takahashi M. Retinitis pigmentosa with EYS mutations is the most prevalent inherited retinal dystrophyin Japanese populations. J Ophthalmol. 2015;2015:819760.
30. Oishi M, Oishi A, Gotoh N, Ogino K, Higasa K, Iida K, et al. Comprehensive molecular diagnosis of a large cohort of Japanese retinitis pigmentosa and Ushersyndrome patients by next-generation sequencing. Investig Ophthalmol Vis Sci. 2014;55(11):7369–75.
31. Kimchi A, Khateb S, Wen R, Guan Z, Obolensky A, Beryozkin A, et al. Nonsyndromic Retinitis Pigmentosa in the Ashkenazi Jewish Population: Genetic and Clinical Aspects. Ophthalmology. 2018;125(5):725–34.
32. Guo H, Qin Y, Meng Q, Zhang H, Jin H, Chen Y. Linkage analysis and mutation screening of the rhodopsin gene in a Chinese Bai family with autosomal dominant retinitis pigmentosa. J Human Genetics. 2010;55(9):571–6.
33. Dikshit M, Agarwal R. Mutation analysis of codons 345 and 347 of rhodopsingene in Indian retinitis pigmentosa patients. J Genetics. 2001;80(2):111–6.
34. Gandra M, Anandula V, Authiappan V, Sundaramurthy S, Raman R, Bhattacharya S, et al. Retinitis pigmentosa: mutation analysis of RHO, PRPF31, RP1, and IMPDH1 genes in patients from India. Mol Vis. 2008;14:1105–13.
35. Kannabiran C, Singh H, Sahini N, Jalali S, Mohan G. Mutations in TULP1,NR2E3, and MFRP genes in Indian families with autosomal recessive retinitis pigmentosa. Mol Vis. 2012;18:1165–74.
36. Di Y, Huang L, Sundaresan P, Li S, Kim R, BallavSaikia B, et al. Whole-exome sequencing analysis identifies mutations in the EYS gene in retinitis pigmentosa in the Indian population. Sci Rep. 2016;6:19432.
37. Seyedahmadi BJ, Rivolta C, Keene JA, Berson EL, Dryja TP. Comprehensive screening of the USH2A gene in Usher syndrome typeII and non-syndromic recessive retinitis pigmentosa. Exp Eye Res. 2004;79(2):167–73.

38. Zhu T, Chen DF, Wang L, Wu S, Wei X, Li H, et al. USH2A variants in Chinese patients with Usher syndrometype II and non-syndromic retinitispigmentosa. Br J Ophthalmol. 2021;105:694–703.
39. Sen P, Maitra P, Natarajan S, Sripriya S, Mathavan S, Bhende M, et al. CERKL mutation causing retinitis pigmentosa(RP) in Indian population - a genotype and phenotype correlation study. Ophthalmic Genet. 2020;41(6):570–8.
40. Morimura H, Fishman GA, Grover SA, Fulton AB, Berson EL, Dryja TP. Mutations in the RPE65 gene in patients with autosomal recessive retinitis pigmentosa or leber congenital amaurosis. Proc Natl Acad Sci U S A. 1998;95(6):3088–93.
41. Schatz P, Preising M, Lorenz B, Sander B, Larsen M, Rosenberg T. Fundusal bipunctatus associated with compound heterozygous mutations in RPE65. Ophthalmology. 2011;118(5):888–94.
42. Hull S, Holder GE, Robson AG, Mukherjee R, Michaelides M, Webster AR, Moore AT. Preserved visual function in retinal dystrophy due to hypomorphic RPE65 mutations. Br J Ophthalmol. 2016;100:1499–505.
43. Jakobsson C, Othman IS, Munier FL, Schorderet DF, Abouzeid H. Cone-rod dystrophy caused by a novel homozygous RPE65 mutation in Leber congenital amaurosis. Klin Monbl Augenheilkd. 2014;231(4):405–10.
44. Bowne SJ, Humphries MM, Sullivan LS, Kenna PF, Tam LC, Kiang AS, et al. A dominant mutation in RPE65 identified by whole-exome sequencing causes retinitis pigmentosa with choroidal involvement. Eur J Hum Genet. 2011;19(10):1074–81.
45. Srikrupa NN, Sen P, Arokiasamy T, Meenakshi S, Bhende M, Kapur S, Soumittra N. Genetic profile and mutation spectrum of Leber congenital amaurosis in a larger Indian cohort using high throughput targeted re-sequencing. Clin Genet. 2018;93(2):329–39.
46. Seong MW, Kim SY, Yu YS, Hwang JM, Kim JY, Park SS. Molecular characterization of Leber congenital amaurosis in Koreans. Mol Vis. 2008;14:1429–36.
47. Wang DY, Chan WM, Tam PO, Baum L, Lam DS, Chong KK, et al. Gene mutations in retinitis pigmentosa and their clinical implications. Clin Chim Acta. 2005;351(1-2):5–16.
48. Yeung KY, Baum L, Chan WM, Lam DS, Kwok AK, Pang CP. Molecular diagnostics for retinitis pigmentosa. Clin Chim Acta. 2001;313(1-2):209–15.
49. Zuchner S, Dallman J, Wen R, Beecham G, Naj A, Farooq A, Kohli MA, Whitehead PL, Hulme W, Konidari I, Edwards YJK, Cai G, Peter I, Seo D, Buxbaum JD, Haines JL, Blanton S, Young J, Alfonso E, Vance JM, Lam BL, Vance MAP. Whole-exome sequencing links a variant in DHDDS to retinitis pigmentosa. Am J Human Genet. 2011;88:201–6.
50. Kumaran N, Moore AT, Weleber RG, Michaelides M. Leber congenitalamaurosis/early-onset severe retinal dystrophy: clinical features, molecular genetics and therapeutic interventions. Br J Ophthalmol. 2017;101(9):1147–54.
51. Foxman SG, Heckenlively JR, Bateman JB, Wirtschafter JD. Classification ofcongenital and early onset retinitis pigmentosa. Arch Ophthalmol. 1985;103(10):1502–6.
52. Lorenz B, Gyurus P, Preising M, Bremser D, Gu S, Andrassi M, et al. Early-onset severe rod-cone dystrophy in young children with RPE65 mutations. Investig Ophthalmol Vis Sci. 2000;41(9):2735–42.
53. Selmer KK, Grondahl J, Riise R, Brandal K, Braaten O, Bragadottir R, et al. Autosomal dominant pericentral retinal dystrophy caused by a novel missensemutation in the TOPORS gene. Acta Ophthalmol. 2010;88(3):323–8.
54. Coussa RG, Basali D, Maeda A, DeBenedictis M, Traboulsi EI. Sectorretinitis pigmentosa: report of ten cases and a review of the literature. Mol Vis. 2019;25:869–89.
55. Comander J, Weigel-DiFranco C, Maher M, Place E, Wan A, Harper S, et al. The genetic basis of pericentral retinitis pigmentosa-a form of mild retinitis pigmentosa. Genes (Basel). 2017;8(10):256.
56. Grondahl J, Riise R, Heiberg A, Leren T, Christoffersen T, Bragadottir R. Autosomal dominant retinitis pigmentosa in Norway: a 20-year clinical follow-upstudy with molecular genetic analysis. Two novel rhodopsin mutations: 1003delGand I179F. Acta Ophthalmol Scand. 2007;85(3):287–97.

57. Den Hollander AIKR, Yzer S, Lopez I, Arends ML, Voesenek KE, Zonneveld MN, Strom TM, Meitinger T, Brunner HG, Hoyng CB, van den Born LI, Rohrschneider K, Cremers FP. Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. Am J Hum Genet. 2006;79(3):556–61.
58. Tucker BA, Mullins RF, Streb LM, Anfinson K, Eyestone ME, Kaalberg E, Riker MJ, Drack AV, Braun TA, Stone EM. Patient-specific iPSC-derived photoreceptor precursor cells as a means to investigate retinitis pigmentosa. Elife. 2013;2:e00824.
59. Vaché CBT, le Berre P, García-García G, Baux D, Larrieu L, Abadie C Et al. Usher syndrome type 2 caused by activation of an USH2A pseudoexon: implications for diagnosis and therapy. Hum Mutat. 2012;33(1):104–8.
60. Sara J, Bowne LSS, Koboldt DC, Ding L, Fulton R, Abbott RM Et al. Identification of disease-causing mutations in Autosomal Dominant Retinitis Pigmentosa (adRP) using next-generation dna sequencing. Investig Ophthalmol Visual Sci. 2011;52(1):494–503.
61. Ridnõi KŠM, Vaidla E, Pajusalu S, Kelder L, Reimand T, Õunap K. Aprenatally diagnosed case of Meckel-Gruber syndrome with novel compoundheterozygous pathogenic variants in the TXNDC15 gene. Mol Genet Genomic Med. 2019;7(5):e614.
62. Mezer E, Babul-Hirji R, Wise R, Chipman M, DaSilva L, Rowell M, et. al. Attitudes regarding predictive testing for retinitis pigmentosa. Ophthalmic Genet. 2007;28(1):9–15.
63. Fahim ATBS, Sullivan LS. Allelic heterogeneity and genetic modifier locicontribute to clinical variation in males with X-linked retinitis pigmentosa dueto RPGR mutations. PLoS One. 2011;6:e23021.
64. Sujun Li SD, Brabbit E, Love Z, Woytowicz V, Flattery K, Capri J, Yao K, Wu S, Imboden M, Upadhyay A, Arumugham R, Thoreson WB, DeAngelis MM, Haider NB. Nr2e3 is a genetic modifier that rescues retinal degeneration and promotes homeostasisin multiple models of retinitis pigmentosa. Gene Ther. 2020;28(5):223–41.
65. Strafella C, Caputo V, Pagliaroli G, Iozzo N, Campoli G, Carboni S, et al. NGS analysis for molecular diagnosis of Retinitis Pigmentosa (RP): detection of a novel variant in PRPH2 gene. Genes (Basel). 2019;10(10):792.
66. Neveling K, Collin RW, Gilissen C, van Huet RA, Visser L, Kwint MP, et al. Next-generation genetic testing for retinitis pigmentosa. Hum Mutat. 2012;33(6):963–72.
67. Wang X, Wang H, Sun V, Tuan HF, Keser V, Wang K, et al. Comprehensive molecular diagnosis of 179 Leber congenital amaurosis and juvenile retinitispigmentosa patients by targeted next generation sequencing. J Med Genet. 2013;50(10):674–88.
68. Costa KA, Salles MV, Whitebirch C, Chiang J, Sallum JMF. Gene panel sequencing in Brazilian patients with retinitis pigmentosa. Int J RetinaVitreous. 2017;3:33.
69. Fernandez-San Jose P, Corton M, Blanco-Kelly F, Avila-Fernandez A, Lopez-Martinez MA, Sanchez-Navarro I, et al. Targeted next-generation sequencing improves the diagnosis of autosomal dominant retinitis pigmentosa in Spanish patients. Investig Ophthalmol Vis Sci. 2015;56(4):2173–82.
70. Simpson DA, Clark GR, Alexander S, Silvestri G, Willoughby CE. Molecular diagnosis for heterogeneous genetic diseases with targeted high-throughput DNA sequencing applied to retinitis pigmentosa. J Med Genet. 2011;48(3):145–51.
71. Salvo J, Lyubasyuk V, Xu M, Wang H, Wang F. NguyenD, et al. Next-generation sequencing and novel variant determination in a cohort of 92 familialexudative vitreoretinopathy patients. Investig Ophthalmol Vis Sci. 2015;56(3):1937–46.
72. Birtel J, Gliem M, Oishi A, Muller PL, Herrmann P, Holz FG, et al. Genetic testing in patients with retinitis pigmentosa: features of unsolved cases. Clin Exp Ophthalmol. 2019;47(6):779–86.
73. Sahel JA, Marazova K, Audo I. Clinical characteristics and currenttherapies for inherited retinal degenerations. Cold Spring Harb Perspect Med. 2014;5(2):a017111.
74. Hoffman-Andrews L. The known unknown: the challenges of genetic variants of uncertain significance in clinical practice. J Law Biosci. 2017;4(3):648–57.
75. Cavalleri GL, Delanty N. Opportunities and challenges for genome sequencing in the clinic. Adv Protein Chem Struct Biol. 2012;89:65–83.

76. Choi M, Scholl UI, Ji W, Liu T, Tikhonova IR, Zumbo P, et al. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. ProcNatl Acad Sci U S A. 2009;106(45):19096–101.
77. Dan H, Huang X, Xing Y, Shen Y. Application of targeted panel sequencing and whole exome sequencing for 76 Chinese families with retinitis pigmentosa. Mol Genet Genomic Med. 2020;8(3):e1131.
78. Bassuk AG, Sujirakul T, Tsang SH, Mahajan VB. A novel RPGR mutationmasquerading as Stargardt disease. Br J Ophthalmol. 2014;98(5):709–11.
79. Branham K, Othman M, Brumm M, Karoukis AJ, Atmaca-Sonmez P, Yashar BM, et al. Mutations in RPGR and RP2 account for 15% of males with simplex retinaldegenerative disease. Investig Ophthalmol Vis Sci. 2012;53(13):8232–7.
80. Saudi MG. Comprehensive gene panels provide advantages over clinicalexome sequencing for Mendelian diseases. Genome Biol. 2015;16:134.
81. O'Sullivan J, Mullaney BG, Bhaskar SS, Dickerson JE, Hall G, O'Grady A, et al. A paradigm shift in the delivery of services for diagnosis of inheritedretinal disease. J Med Genet. 2012;49(5):322–6.
82. Efthymiou S, Manole A, Houlden H. Next-generation sequencing in neuromuscular diseases. Curr Opin Neurol. 2016;29(5):527–36.
83. Gilissen C, Hoischen A, Brunner HG, Veltman JA. Disease gene identification strategies for exome sequencing. Eur J Hum Genet. 2012;20(5):490–7.
84. Poulton ALS, Hui L, Halliday JL. Prenatal and preimplantation genetic diagnosis for single gene disorders: a population-based study from 1977 to 2016. Prenat Diagn. 2018;38(12):904–10.
85. Xu K, Rosenwaks Z, Beaverson K, Cholst I, Veeck L, Abramson DH. Preimplantation genetic diagnosis for retinoblastoma: the first reported liveborn. Am J Ophthalmol. 2004;137(1):18–23.
86. Lledo B, Ten J, Rodriguez-Arnedo D, Llacer J, Bernabeu R. Preimplantation genetic diagnosis of X-linked retinoschisis. Reprod Biomed Online. 2008;16(6):886–92.
87. Chang J, Boulet SL, Jeng G, Flowers L, Kissin DM. Outcomes of in vitrofertilization with preimplantation genetic diagnosis: an analysis of the United States assisted reproductive technology surveillance data, 2011–2012. Fertil Steril. 2016;105(2):394–400.
88. Sohrab MA, Allikmets R, Guarnaccia MM, Smith RT. Preimplantation genetic diagnosis for stargardt disease. Am J Ophthalmol. 2010;149(4):651–5e2.
89. Yahalom C, Macarov M, Lazer-Derbeko G, Altarescu G, Imbar T, Hyman JH, Eldar-Geva T, Blumenfeld A. Preimplantation genetic diagnosis as a strategy to prevent having a child born with anheritable eye disease. Ophthalmic Genet. 2018;39(4):450–6.
90. Xu K, Rosenwaks Z, Beaverson K, Cholst I, Veeck L, Abramson DH. Preimplantation genetic diagnosis for retinoblastoma: the first reportedliveborn. Am J Ophthalmol. 2004;137(1):18–23.
91. Yan L, Huang L, Xu L, Huang J, Ma F, Zhu X, et al. Live births after simultaneous avoidance of monogenic diseases and chromosome abnormality by next-generation sequencing with linkage analyses. Proc Natl Acad Sci U S A. 2015;112(52):15964–9.
92. Ren Y, Zhi X, Zhu X, Huang J, Lian Y, Li R, et al. Clinical applications of MARSALA for preimplantation genetic diagnosis of spinal muscular atrophy. J Genet Genomics. 2016;43(9):541–7.
93. Huang X, Liu Y, Yu X, Huang Q, Lin C, Zeng J, et al. The clinical application of preimplantation genetic diagnosis for X-linked retinitispigmentosa. J Assist Reprod Genet. 2019;36(5):989–94.
94. Braude P, Pickering S, Flinter F, Ogilvie CM. Preimplantation genetic diagnosis. Nat Rev Genet. 2002;3(12):941–53.
95. Harton GL, De Rycke M, Fiorentino F, Moutou C, Sen Gupta S, Traeger-Synodinos J, et al. ESHRE PGD consortium best practice guidelines foramplification-based PGD. Hum Reprod. 2011;26(1):33–40.

96. Baruch S, Kaufman D, Hudson KL. Genetic testing of embryos: practices and perspectives of US in vitro fertilization clinics. Fertil Steril. 2008;89(5):1053–8.
97. Luo HCC, Yang Y, Zhang Y, Yuan Y, Wang W, Wu R, Peng Z, Han Y, Jiang L, Yao R, An X, Zhang W, Le Y, Xiang J, Yi N, Huang H, Li W, Zhang Y, Sun J. Preimplantation genetic testing for a family with usher syndrome through targeted sequencing and haplotype analysis. BMC Med Genomics. 2019;12(1):157.
98. Newsome DA, Stark WJ, Maumenee IH. Cataract extraction and intraocular lens implantation in patients with retinitis pigmentos a or Usher's syndrome. Arch Ophthalmol. 1986;104:852–4.
99. Cox SN, Hay E, Bird AC. Treatment of chronic macular edema with acetazolamide. Arch Ophthalmol. 1988;106:1190–5.
100. Berson EL, Rosner B, Sandberg MA, Weigel-Di Franco C, Willett WC. ω-3 intakeand visual acuity in patients with retinitis pigmentosa receiving vitamin A. Arch Ophthalmol. 2012;130(6):707–11.
101. Massof RWFD. Supplemental vitamin A retards loss of ERG amplitude inretinitis pigmentosa. Arch Ophthalmol. 1993;111(6):751–4.
102. Schwartz SGWX, Chavis P, Kuriyan AE, Abariga SA. Vitamin A and fishoils for preventing the progression of retinitis pigmentosa. Cochrane Database Syst Rev. 2020;6(6):CD008428.
103. Semba RDDG. Are lutein and zeaxanthin conditionally essential nutrientsfor eye health? Med Hypotheses. 2003;61(4):465–72.
104. Clemson CM, Tzekov R, Krebs M, Checchi JM, Bigelow C, Kaushal S. Therapeutic potential of valproic acid for retinitis pigmentosa. Br J Ophthalmol. 2011;95(1):89–93.
105. Komeima K, Rogers BS, Campochiaro PA. Antioxidants slow photoreceptor cell deathin mouse models of retinitis pigmentosa. J Cell Physiol. 2007;213:809–15.
106. Talcott KE, Ratnam K, Sundquist SM, Lucero AS, Lujan BJ, Tao W, Porco TC, Roorda A, Duncan JL. Longitudinal study of cone photoreceptors during retinaldegeneration and in response to ciliary neurotrophic factor treatment. Investig Ophthalmol Vis Sci. 2011;52(5):2219–26.
107. Del Rio P, Irmler M, Arango-Gonzalez B, Favor J, Bobe C, Bartsch U, Vecino E, Beckers J, Hauck SM, Ueffing M. GDNF-induced osteopontin from Muller glial cellspromotes photoreceptor survival in the Pde6brd1 mouse model of retinaldegeneration. Glia. 2011;59(5):821–32.
108. Bigot K, Gondouin P, Bénard R, Montagne P, Youale J, Piazza M, Picard E, Bordet T, Behar-Cohen F. Transferrin non-viral gene therapy for treatment of retinal degeneration. Pharmaceutics. 2020;12:836.
109. Huang XF. Current pharmacological concepts in the treatment of the retinitis pigmentosa. Adv Exp Med Biol. 2018;1074:439–45.
110. Uteza Y, Rouillot JS, Kobetz A, Marchant D, Pecqueur S, Arnaud E, Prats H, Honiger J, Dufier JL, Abitbol M, et al. Intravitreous transplantation of encapsulatedfibroblasts secreting the human fibroblast growth factor 2 delays photoreceptorcell degeneration in Royal College of Surgeons rats. Proc Natl Acad Sci U S A. 1999;96(6):3126–31.
111. Russell S, Bennett J, Wellman JA, Chung DC, Yu ZF, Tillman A, Maguire AM. Efficacy and safety of voretigeneneparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. Lancet. 2017;390(10097):849–60.
112. Ghazi NG, Abboud EB, Nowilaty SR, Alkuraya H, Alhommadi A, Cai H, Hou R, Deng WT, Boye SL, Almaghamsi A, Al Saikhan F, Al-Dhibi H, Birch D, Chung C, Colak D, La Vail MM, Vollrath D, Erger K, Wang W, Conlon T, Zhang K, Hauswirth W, Alkuraya FS. Treatment of retinitis pigmentosa due to MERTK mutations by ocular subretinal injection of adeno-associated virus gene vector: results of a phase I trial. Hum Genet. 2016;135(3):327–43.
113. Garafalo AV, Cideciyan AV, Héon E, Sheplock R, Pearson A, WeiYang Yu C, Sumaroka A, Aguirre GD, Jacobson SG. Progress in treating inherited retinal diseases: early subretinal gene therapy clinical trials and candidates for future initiatives. Prog Retin Eye Res. 2020;77:100827.

114. Vázquez-Domínguez I, Garanto A, Collin RWJ. Molecular therapies for inherited retinal diseases-current standing, opportunities and challenges. Genes(Basel). 2019;10(9):654.
115. Mussolino C, Sanges D, Marrocco E, Bonetti C, Di Vicino U, Marigo V, et al. Zinc-finger based transcriptional repression of rhodopsin in a model of dominantretinitis pigmentosa. EMBO Mol Med. 2011;3:118–28.
116. Millington-Ward S, Chadderton N, O¢Reilly M, Palfi A, Goldmann T, Kilty C, et al. Suppression and replacement gene therapy for autosomal dominant disease in amurine model of dominant retinitis pigmentosa. Mol Ther. 2011;2011(19):642–9.
117. Cideciyan AV, Jacobson SG, Drack AV, Ho AC, Charng J, Garafalo AV, Roman AJ, Sumaroka A, Han IC, Hochstedler MD, Pfeifer WL, Sohn EH, Taiel M, Schwartz MR, Biasutto P, Wit W, Cheetham ME, Adamson P, Rodman DM, Platenburg G, Tome MD, Balikova I, Nerinckx F, Zaeytijd J, Van Cauwenbergh C, Leroy BP, Russell SR. Effect of an intra vitreal antisense oligonucleotide on vision in Lebercongenital amaurosis due to a photoreceptor cilium defect. Nat Med. 2019;25(2):225–8.
118. Yu WMS, Chaitankar V, Hiriyanna S, Kim JW, Brooks M, Ataeijannati Y, SunX DL, Li T, Swaroop A, Wu Z. Nrl knockdown by AAV-delivered CRISPR/Cas9 prevents retinal degeneration in mice. Nat Commun. 2017;8:14716.
119. Zrenner E, Bartz-Schmidt KU, Benav H, Besch D, Bruckmann A, Gabel VP. etal. Subretinal electronic chips allow blind patients to read letters and combinethem to words. Proc Biol Sci. 2011;278(1711):1489–97.
120. Ho AC, Humayun MS, Dorn JD, da Cruz L, Dagnelie G, Handa J, et al. Long-term results from an epiretinal prosthesis to restore sight to the blind. Ophthalmology. 2015;122(8):1547–54.
121. Mills JO, Jalil A, Stanga PE. Electronic retinal implants and artificial vision: journey and present. Eye (Lond). 2017;31(10):1383–98.
122. Simunovic MP, Shen W, Lin JY, Protti DA, Lisowski L, Gillies MC. Optogenetic approaches to vision restoration. Exp Eye Res. 2019;178:15–26.
123. Bloch E, Luo Y, da Cruz L. Advances in retinal prosthesis systems. Ther Adv Ophthal Mol. 2019;11:2515841418817501.
124. Lamba DA, Gust J, Reh TA. Transplantation of human embryonic stem cell-derived photoreceptors restores some visual function in Crx-deficient mice. Cell Stem Cell. 2009;4(1):73–9.
125. MacLaren RE, Pearson RA, MacNeil A, Douglas RH, Salt TE, Akimoto M, et al. Retinal repair by transplantation of photoreceptor precursors. Nature. 2006;444(7116):203–7.
126. Zheng A, Li Y, Tsang SH. Personalized therapeutic strategies for patientswith retinitis pigmentosa. Expert Opin Biol Ther. 2015;15(3):391–402.
127. Bassuk AG, Zheng A, Li Y, Tsang SH, Mahajan VB. Precision medicine: genetic repair of retinitis pigmentosa in patient-derived stem cells. Sci Rep. 2016;6:19969.
128. Fuster-García C, García-García G, González-Romero E, Jaijo T, Sequedo MD, Ayuso C, Vázquez-Manrique RP, Millán JM, Aller E. USH2A gene editing using the CRISPR system. Mol Ther Nucleic Acids. 2017;8:529–41.
129. Schwartz SD, Regillo CD, Lam BL, Eliott D, Rosenfeld PJ, Gregori NZ, et al. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two openlabel phase 1/2 studies. Lancet. 2015;385(9967):506–16.
130. Ishani Dasgupta, Terence R. Flotte, and Allison M. Keeler. Human Gene Therapy. Mar 2021.275–293. http://doi.org/10.1089/hum.2021.013

Genetics in High Myopia

13

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Abbreviations

AD	Autosomal dominant
AR	Autosomal recessive
ATOM	Atropine for the treatment of childhood myopia
CCT	Central corneal thickness
CREAM	Consortium for Refractive Error and Myopia
GWAS	Genome-wide association study
GxE	Gene–environment interaction
MR	Mendelian Randomization
NGS	Next-generation sequencing
OCT	Optical Coherence Tomography
OMIM	Online Mendelian Inheritance in Man
Ortho-k	Orthokeratology
SNP	Single nucleotide polymorphism
XL	X-linked recessive

13.1 Introduction

Myopia is a type of refractive error, usually occurring due to axial length elongation, causing the light rays to focus in front of the retina making distant objects appear blurred and near objects clearer, hence known as "short-sightedness" or "near sightedness". It is defined as a refractive error of ≥ -2 diopters (D). Its

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prevalence has increased significantly in the last two decades making it one of the leading ocular disorders leading to visual impairment worldwide [1].

Myopia can be classified as non-syndromic myopia (if it occurs alone) and syndromic myopia (if it is associated with another ocular or systemic disease or as physiologic myopia (usually low-grade myopia) and pathological myopia (usually associated with degenerative retinal changes). Pathological myopia is defined as a refractive error ≥ -6D or an axial length >26 mm. Although the refractory symptoms of myopia can be completely or partially relieved through spectacles, contact lenses, or refractive surgery, the risk of complications like retinal detachment, glaucoma, and myopic macular degeneration is always there, which increases with an increasing axial length and can potentially lead to blindness [2].

Myopia has a heterogeneous aetiology including both environmental and genetic factors [1]. Till date, over 100 genes and more than 20 chromosomal loci have been associated with myopia or related traits via linkage analysis, candidate gene analysis, genome-wide association study (GWAS), and next-generation sequencing (NGS) [3]. The current knowledge about the roles of loci and genes in myopia is limited making the mechanism of myopia quite complex.

13.2 Genetic Characteristics of Myopia

Prevalence and Heritability of Myopia The prevalence of myopia in children in India is relatively low compared to other Asian countries, though it has increased dramatically in last few years. A prevalence of 4.1% among rural children (aged 7–15 years) has been reported from southern India and 7.4% among urban children (aged 5–15 years) in a study from north India [4].

Population-based epidemiological studies have shown that genetic factors contribute to the onset and progression of myopia significantly. Parental myopia history plays a major role [5]. Inheritance of myopia has been found to be over 90% in large twin studies [6].

Syndromic myopia (myopia associated with another ocular or systemic disorder) is usually monogenic and can occur with a wide range of clinical presentations. While investigating these syndromes, we come to know about development of myopia. Many heritable syndromes result in extreme axial elongation, due to developmental connective tissue abnormalities (e.g. Marfan syndrome, OMIM #154700; Stickler syndrome, OMIM #108300 #604841, #614134, #614284; and Ehlers–Danlos syndrome, OMIM #225400, #601776). Similarly, inherited retinal dystrophies lead to myopia due to defects in photoreceptors, e.g. X-linked retinitis pigmentosa (mutations in *RPGR*-gene) and congenital stationary night blindness [7]. Several genes of syndromic myopia were found to be associated with other ocular traits like central corneal thickness (*ADAMTS2*, *COL4A3*, *COL5A1*, *FBN1*) [8] and Fuchs' dystrophy (*TCF4*) [9]. However, recently an over representation for syndromic myopia genes in GWAS studies was noted suggesting their important role in the development of myopia [10].

Myopia-Related Phenotypic Traits Epidemiologic studies show that refractive development is a dynamic process and refractive changes continue to occur throughout life at varying rates. During the neonatal period, the distribution of refractive error is more towards hyperopia. After the early period of rapid eye growth, the refractive changes slow down, but quite often lead to the development of myopia. Corneal curvature appears to remain relatively stable after the age of 6 years and therefore does not seem to play a major role in juvenile- and adult-onset myopia [11]. On the other hand, corneal thickness is highly heritable and is associated with the occurrence of refractive errors [12]. Myopia is largely the result of increase in axial length which increases predominantly during the school going years, and tends to stabilize during adulthood [11].

Interplay of Genetic and Environmental Factors Gene–gene as well as gene–environment interactions have been observed in the pathogenesis of myopia. Although myopia is heritable within specific cohorts, dramatic changes in the environment of human population have led to gradual changes in myopia prevalence as well [13, 14]. Myopia usually exhibits apparent familial aggregation [15], but genetic factors alone are insufficient to explain the rapid increase in the myopia prevalence. Epidemiological studies show that outdoor activity reduces the risk of myopia associated with near work, while more time spent in studying and a higher socioeconomic status increase the prevalence [16].

There are two areas where the role of environment can be confirmed by genetic studies. First is the gene–environment studies which can highlight the places of interactions. Second is the observational studies which can establish association but not the causation, though in some circumstances, genetic data can strengthen the possibility for an environmental risk factor causally (or not) influencing the risk of myopia (Mendelian randomization).

Gene–environment (GxE) studies on myopia have primarily focused on education. A North American study examined GxE for myopia and the matrix metalloproteinases genes (*MMP1-MMP10*): a subset of SNPs was only associated with refraction in the lower education levels [13, 17]. A subsequent Singapore cohort study found variants in *DNAH9*, *GJD2,* and *ZMAT4*, which had a larger influence on myopia in a high education subset [18]. Subsequent efforts to examine GxE considered the aggregate effects of many single nucleotide polymorphisms (SNPs) together. A European study found that a genetic risk score comprising of 26 genetic variants was strongly associated with myopia and a university level education [19]. A study examining GxE in children considered near work and outdoor time in association with 39 SNPs and did not find strong evidence for an interaction with near work [19, 20]. A Consortium for Refractive Error and Myopia (CREAM) study was finally able to identify additional myopia risk loci through a GxE approach [21].

Mendelian randomization (MR) determines whether a risk factor is causally associated with a disease. The basis of MR is the fact that germline genotypes are randomly assigned at meiosis, to allow for a “natural” randomized controlled trial.

Since the assigned genotypes are independent of non-genetic confounding and remain unmodified by disease processes, MR offers a better causality assessment than observational studies [13, 22].

World Society of Pediatric Ophthalmology and Strabismus consensus statements guidelines state that outdoor activity has been shown to reduce myopia progression and one should ensure at least 1 h of outdoor activity per day [23]. This will not only keep myopia epidemic at bay but also will promote fitness and take care of Vitamin D deficiency. Recent evidence suggests that prolonged near work at closer distances (<33 cm) may be more damaging. Furthermore, taking frequent breaks during near activities and following 20–20–20 rule help reduce eyestrain which maybe contributing to further progression of myopia.

Genetic Transmission Traits of Myopia High myopia is often transmitted through families in Mendelian patterns, including autosomal dominant (AD), autosomal recessive (AR), and X-linked recessive (XL) inheritance. Based on linkage analysis, 18 myopia and high myopia loci have been discovered and documented in the Online Mendelian Inheritance in Man database (OMIM), including nine high myopia loci in AD inheritance (*MYP2*, *MYP3*, *MYP5*, *MYP11*, *MYP12*, *MYP15*, *MYP16*, *MYP17*, and *MYP19*), one high myopia locus in AR inheritance (*MYP18*), two XL recessive high myopia loci (*MYP1*and *MYP13*), and six loci (MYP6, MYP7, MYP8, MYP9, MYP10, and MYP14) associated with complex myopia [3]. The identification of these 18 loci in the human genome not only points to the role of genetic factors in myopia but also provides guides for further screening of genes.

Genetic Studies for Myopia In recent years, a large number of genome-wide association studies (GWAS) and follow-up association studies have been done. Numerous variants have been repeatedly found to be associated with myopia-related phenotypic traits, like refractive error, axial length, and macular thickness, or have been linked with high myopia. The CREAM published findings from GWAS separately, and later combined studies in a GWAS meta-analysis, identifying 161 common variants for refractive error but could explain only about 8% of the phenotypic variance of this trait [20]. Parent–offspring heritability estimated in studies is generally low, but sibling heritability is usually high, suggesting the dominating influence of environmental factors over genetics in determining refractive errors [24].

In a large meta-analysis of GWASs on 12,531 Europeans and 8,216 Asians, in addition to the *ZC3H11B* gene, eight other genes (*RSPO1, C3orf26, LAMA2, GJD2, MIP, ALPPL2, CD55,* and *ZNRF3)* were found to be associated with axial length [25]. Lin et al. revealed associations between myopia and polymorphisms within sclera-related *TGFB2* [26].

In candidate gene studies, the focus is on genes with suspected biological, physiological, or functional relevance to high myopia. Sometimes though such studies are limited by their reliance on existing knowledge. Particularly notable are genes encoding extracellular matrix-related proteins [*COL1A1*, *COL2A*1 [27, 28] and *MMP1*, *MMP2*, *MMP3*, *MMP9*, *MMP10* [29]]. For *PAX6* and *TGFB1*candidates,

the results were replicated in multiple independent high myopia studies and validated in a large GWAS meta-analysis in 2018, respectively [13, 30]. In few cases, the candidates were subsequently implicated in GWAS of other ocular traits: *TGFβ2* and *LUM* for central corneal thickness (CCT), a glaucoma and keratoconus endophenotype, *PAX6* with optic disc area [31] and *HGF* [32].

Considering the significant hereditary basis in myopia, understanding the underlying genetics would be the only option in demystifying the pathogenesis of the disease. Currently, this knowledge is very limited due to some inherent challenges in the field of genetic research. Some of them are outlined below:

1. A number of suspicious genes do not adequately correlate with the disease patterns in families which arouses the possibility of multiple genetic influences involved in pathological myopia.
2. There is no consensus on the pathogenicity of the identified abnormalities. There can be involvement of single or different combinations of multiple sites in the pathogenesis such as sclera, pigment epithelium, ciliary body, etc. [33].
3. The "missing heritability" problem—SNPs (single nucleotide polymorphisms) cannot fully explain the inheritance in all cases, possibly due to a greatnumber of variants with small effects which yet remain undiscovered [34].
4. Survival analysis of population-based samples may not be adequate to detect variants for rare myopia subtypes like the Mendelian forms of high myopia. For such cases, sequencing of highly ascertained pedigrees could offer better information.

In general, genetic factors influence the pathogenesis of high myopia more than low myopia. It is easy to find genetic basis of early onset high myopia compared to late onset lower myopia because the previous is more likely to be monogenetic while the latter is more genetically complex [35].

13.3 Epigenetics

Epigenetics is important due to the known effects of environmental factors on refractive error and myopia but this field is still developing and some of its characteristics make itfurther difficult to unravel. Epigenetic features can be influenced by environment and are time dependent and tissue specific. This complicates studies since refractive errors develop during childhood and young adolescence and obtaining retinal and scleral tissue would be unethical.

13.4 Management of High Myopia

13.4.1 Prevention of Progression

Myopia progression can be halted by inducing changes in the structure and focusing abilities of the eye. Currently, three types of treatment show promise:

- Atropine eye drops
- Orthokeratology ("ortho-k")
- Multifocal contact lenses and eyeglasses

Atropine Eye Drops These have now been used for control of myopia with effective results. ATOM 1 and ATOM 2 (Atropine for the treatment of childhood myopia) studies have shown that use of low-dose atropine in children prevents myopia progression [36]. The antimuscarinic action of Atropine helps in preventing axial elongation of the globe. Recent ATOM study shows that 0.01% atropine prevents the progression of myopia close to 50% [37]. Higher doses of atropine seem to have greater effect; however, rebound progression is greater. Atropine 0.01% appears the most reasonable approach to retard myopia progression with the least side effects. Atropine once started should be used at least for 2 years as maximum effect appears in the second year.

Usually it is prescribed for children (more than 5 years of age) who have progressive myopia (>0.50 D per 6 months) with exclusions being children with anisometropia, astigmatism more than 1.50 D, syndromic children with myopia, retinopathy of prematurity kids with myopia, and myopic shift seen in children after paediatric cataract surgery. They are started on low dose 0.01% (once daily at bedtime) atropine eye drops with monitoring of axial length and side effects on each visit.

Side effects include discomfort, increased sensitivity to light, blurring of near vision, and need for bifocals or progressive glasses for near [37, 38]. These side effects are higher with increased dose of the eye drops. A recently noted rare side effect is a convergence excess consecutive esotropia in children operated for intermittent exotropia [39]. A similar phenomenon has been reported by Lyu *et al*. wherein they have reported increased esodeviation following cycloplegia with 0.5% tropicamide and 0.5% phenylephrine mixed eye drops in patients of hyperopia and esotropia [40]. The prescribing clinician must be aware of these side effects and the treatment must be stopped whenever these are observed [41]. The mechanism of atropine controlling myopia has not been fully explained yet. Atropine is a competitive antagonist of muscarinic acetylcholine receptor types M1 to M5. The density and distribution of these receptors varyin differently pigmented eyes [42]. For each receptor (mAChRs), there is a significant gene polymorphism [43].

Orthokeratology It is the use of specially designed gas permeable contact lenses worn during sleep to transiently correct the refractive error so that glasses and contact lenses are not needed during waking hours. They are also shown useful in controlling progression of myopia apart from alleviating the need to wear glasses. Evidence shows that children who undergo several years of orthokeratology may develop lesser myopia, compared to children who wear eyeglasses or contact lenses during the peak years of myopia progression. This modality is also not free from

side effects especially complications related to use of contact lens such as foreign body sensation, watering, infection, allergic conjunctivitis, etc. [44, 45].

Multifocal Contact Lenses and Eyeglasses Multifocal lenses and glasses appear to be more effective than bifocal glasses and as effective as orthokeratology. Soft multifocal contact lenses can retard the development of myopia and elongation of the eye. Stronger treatment effect is seen in children who are younger and whose myopia is progressing more quickly. Contact lenses offer benefits beyond vision correction like cosmesis and comfort of outdoor sports activity. These factors also make children feel better about their interactions with peers [46].

13.4.2 Genetic Counselling

Genetic counselling can help individuals, couples, and families to understand and adapt to the medical, psychological, familial, and reproductive implications of the genetic aspect of the development and progression of myopia. This process includes:

- Interpretation of family histories to evaluate the probability of occurrence or recurrence of disease.
- Education about the natural history, inheritance pattern, diagnosis, management, and prevention of the condition.
- Counselling to promote informed choices.
- Encouragement for adjustment to the disorder in an affected family member and/or to the risk of its recurrence [47].

13.5 Conclusion

Genetics and environment both play a significant role in the development and progression of myopia in children. A conjunction of functional studies and use of different technologies will enhance our understanding of the factors associated with myopia, leading to improvements in the prediction of its onset, prevention, and treatment.

Encouraging a healthy working distance, good posture, and lighting while reading and increased outdoor activity may help retard myopia progression.

Spectacles and contact lenses do not contribute much in prevention of myopia. Orthokeratology should not be considered as a first-line strategy due to its high risk of infectious keratitis and relatively poor patient compliance. Low-dose atropine (0.01%) has been found to be effective and safe due to the lowest rebound effect and negligible side effects.

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References

1. Morgan IG, Ohno-Matsui K, Saw SM. Myopia. Lancet. 2012;379(9827):1739–48.
2. Saw SM, Gazzard G, Shih-Yen EC, Chua WH. Myopia and associated pathological complications. Ophthalmic Physiol Opt. 2005;25(5):381–91.
3. Li J, Zhang Q. Insight into the molecular genetics of myopia. Mol Vis. 2017;31(23):1048–80.
4. Singh NK, James RM, Yadav A, Kumar R, Asthana S, Labani S. Prevalence of myopia and associated risk factors in school children in North India. Optom Vis Sci. 2019;96(3):200–5.
5. Lam DS, Fan DS, Lam RF, Rao SK, Chong KS, Lau JT, Lai RY, Cheung EY. The effect of parental history of myopia on children's eye size and growth: results of a longitudinal study. Investig Ophthalmol Vis Sci. 2008;49(3):873–6.
6. Hammond CJ, Snieder H, Gilbert CE, Spector TD. Genes and environment in refractive error: the twin eye study. Investig Ophthalmol Vis Sci. 2001;42(6):1232–6.
7. Hendriks M, Verhoeven VJM, Buitendijk GHS, Polling JR, Meester-Smoor MA, Hofman A, RD5000 Consortium, Kamermans M, van den Ingeborgh Born L, Klaver CCW. Development of refractive errors-what can we learn from inherited retinal dystrophies? Am J Ophthalmol. 2017;182:81–9.
8. Iglesias AI, Mishra A, Vitart V, et al. Cross-ancestry genome-wide association analysis of corneal thickness strengthens link between complex and Mendelian eye diseases. Nat Commun. 2018;9:1864.
9. Baratz KH, Tosakulwong N, Ryu E, Brown WL, et al. E2-2 protein and Fuchs's corneal dystrophy. N Engl J Med. 2010;363:1016–24.
10. Flitcroft DI, Loughman J, Wildsoet CF, Williams C, Guggenheim JA, CREAM Consortium. Novel myopia genes and pathways identified from syndromic forms of Myopia. Investig Ophthalmol Vis Sci. 2018;59(1):338–48.
11. Zadnik K, Mutti DO, Mitchell GL, Jones LA, Burr D, Moeschberger ML. Normal eye growth in emmetropic schoolchildren. Optom Vis Sci. 2004;81(11):819–28.
12. Dimasi DP, Burdon KP, Craig JE. The genetics of central corneal thickness. Br J Ophthalmol. 2010;94(8):971–6.
13. Tedja MS, Haarman AEG, Meester-Smoor MA, Virginie JM, Klaver VCCW, MacGregor S. The genetics of myopia. Updates Myopia Clin Perspect. 2020:95–132.
14. Williams KM, Bertelsen G, Cumberland P, et al. European Eye Epidemiology (E(3)) Consortium. Increasing prevalence of myopia in Europe and the impact of education. Ophthalmology. 2015;122:1489–97.
15. Fotouhi A, Etemadi A, Hashemi H, Zeraati H, Bailey-Wilson JE, Mohammad K. Familial aggregation of myopia in the Tehran eye study: estimation of the sibling and parent offspring recurrence risk ratios. Br J Ophthalmol. 2007;91(11):1440–4.
16. Mountjoy E, Davies NM, Plotnikov D, Smith GD, Rodriguez S, Williams CE, Guggenheim JA, Atan D. Education and myopia: assessing the direction of causality by Mendelian randomisation. BMJ. 2018;361:k2022.
17. Wojciechowski R, Bailey-Wilson JE, Stambolian D. Association of matrix metalloproteinase gene polymorphisms with refractive error in Amish and Ashkenazi families. Investig Ophthalmol Vis Sci. 2010;51:4989–95.
18. Fan Q, Wojciechowski R, Kamran Ikram M, et al. Education influences the association between genetic variants and refractive error: a meta-analysis of five Singapore studies. Hum Mol Genet. 2014;23:546–54.
19. Verhoeven VJM, Buitendijk GH, Consortium for Refractive Error and Myopia (CREAM), Rivadeneira F, et al. Education influences the role of genetics in myopia. Eur J Epidemiol. 2013;28:973–80.
20. Fan Q, Guo X, Tideman J, et al. Childhood gene-environment interactions and age-dependent effects of genetic variants associated with refractive error and myopia: the CREAM Consortium. Sci Rep. 2016;6:25853.

21. Fan Q, Verhoeven V, Wojciechowski R, et al. Meta-analysis of gene-environment-wide association scans accounting for education level identifies additional loci for refractive error. Nat Commun. 2016;7:11008.
22. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. Int J Epidemiol. 2004;33:30–42.
23. WSPOS Myopia Consensus Statementwspos.org/world-society-of-paediatric-ophthalmology--strabismus-consensus-statements; 2016. http://www.wspos.org/wspos-myopia-consensus-statement/. Last accessed 31 March 2020.
24. Guggenheim JA, Kirov G, Hodson SA. The heritability of high myopia: a reanalysis of Goldschmidt's data. J Med Genet. 2000;37(3):227–31.
25. Cheng CY, Schache M, Ikram MK, Young TL, Guggenheim JA, Vitart V, et al. Nine loci for ocular axial length identified through genome-wide association studies, including shared loci with refractive error. Am J Hum Genet. 2013;93(2):264–77.
26. Lin HJ, Wan L, Tsai Y, Tsai YY, Fan SS, Tsai CH, Tsai FJ. The TGFbeta1 gene codon 10 polymorphism contributes to the genetic predisposition to high myopia. Mol Vis. 2006;12:698–703.
27. Mutti DO, Cooper ME, O'Brien S, et al. Candidate gene and locus analysis of myopia. Mol Vis. 2007;13:1012–9.
28. Metlapally R, Li Y, Tran-Viet KN, et al. COL1A1 and COL2A1 genes and myopia susceptibility: evidence of association and suggestive linkage to the COL2A1 locus. Investig Ophthalmol Vis Sci. 2009;50:4080–6.
29. Hall NF, Gale CR, Ye S, Martyn CN. Myopia and polymorphisms in genes for matrix metalloproteinases. Investig Ophthalmol Vis Sci. 2009;50:2632–6.
30. Tedja MS, Wojciechowski R, Hysi PG, et al. Genome-wide association meta-analysis highlights light-induced signaling as a driver for refractive error. Nat Genet. 2018;50:834–48.
31. Springelkamp H, Iglesias AI, Mishra A, et al. New insights into the genetics of primary open-angle glaucoma based on meta-analyses of intraocular pressure and optic disc characteristics. Hum Mol Genet. 2017;26:438–53.
32. Burdon KP, Macgregor S, Bykhovskaya Y, et al. Association of polymorphisms in the hepatocyte growth factor gene promoter with keratoconus. Invest Ophthalmol Vis Sci. 2011;52:8514–9.
33. Jin ZB, Wu J, Huang XF, Feng CY, Cai XB, Mao JY, et al. Trio-based exome sequencing arrests de novo mutations in early-onset high myopia. Proc Natl Acad Sci. 2017;114(16):4219–24.
34. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461(7265):747–53.
35. Ohno-Matsui K, Ishibashi T. Pathological myopia. Ryan's retina. 6th edn. p. 4295–4331.
36. Chia A, Chua WH, Cheung YB, Wong WL, Lingham A, Fong A, et al. Atropine for the treatment of childhood myopia: Safety and efficacy of 0.5%, 0.1%, and 0.01% doses (Atropine for the treatment of myopia 2). Ophthalmology. 2012;119:347–54.
37. Sathyan S. The use of atropine in childhood myopia: experience in Indian eyes. Kerala J Ophthalmol. 2019;31:24–7.
38. Galvis V, Tello A, Parra MM, Merayo-Lloves J, Larrea J, Julian Rodriguez C, Camacho PA. Topical atropine in the control of myopia. Med Hypothesis Discov Innov Ophthalmol. 2016;5(3):78–88.
39. Kothari M, Modak M, Khan H, Jahan S, Solanki M, Rathod V. Convergence excess consecutive esotropia associated with 0.01% atropine eye drops usage in patients operated for intermittent exotropia. Indian J Ophthalmol. 2020;68(4):653–6.
40. Lyu IJ, Park KA, Oh SY. Increase in esodeviation under cycloplegia with 0.5% tropicamide and 0.5% phenylephrine mixed eye drops in patients with hyperopia and esotropia. BMC Ophthalmol. 2017;17(1):247.
41. Gaur N, Sharma P. Commentary: a drop a day, keeps myopia away? Indian J Ophthalmol. 2020;68(4):656–7.
42. Salazar M, Shimada K, Patil PN. Iris pigmentation and atropine mydriasis. J Pharmacol Exp Ther. 1976;197:79–88.

43. Lin HJ, Wan L, Tsai Y, Chen WC, Tsai FJ, et al. Muscarinic acetylcholine receptor 1 gene polymorphisms associated with high myopia. Mol Vis. 2009;15:1774–80.
44. Lee YC, Wang JH, Chiu CJ. Effect of Orthokeratology on myopia progression: twelve-year results of a retrospective cohort study. BMC Ophthalmol. 2017;17(1):243.
45. VanderVeen DK, Kraker RT, Pineles SL, Hutchinson AK, Wilson LB, Galvin JA, Lambert SR. Use of Orthokeratology for the prevention of myopic progression in children: a report by the American Academy of Ophthalmology. Ophthalmology. 2019;126(4):623–36.
46. Tyler J, Wagner H. Myopia treatments: how to choose and when to use? Effective management in children and young adults may require different interventions. Learn which works best for your case. Rev Optometry. 2019:46–53.
47. National Society of Genetic Counselors' Definition Task Force, Resta R, Biesecker BB, Bennett RL, Blum S, Hahn SE, Strecker MN, Williams JL. A new definition of genetic counseling: National Society of Genetic Counselors' task force report. J Genet Couns. 2006;15(2):77–83.

Genetics in Strabismus

14

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14.1 Introduction

Strabismus is a disorder of the alignment of the visual axes of the eyes. When the amount of misalignment is the same in all directions of gaze it is classified as comitant. If the amount of misalignment varies in different directions of gaze it is classified as incomitant. It is a common disorder in children, occurring in 3–4% of the population [1–5]. Strabismus can result in visual impairment, absence of binocular vision and stereopsis, multiple surgeries, psychosocial problems affecting self-image, social interaction, education and employability of affected individuals [6, 7].

14.2 Etiology

The etiopathogenesis of strabismus is not clearly understood, although various hypotheses have been put forth to explain the cause of strabismus [8–12]. Comitant strabismus has been shown to have multifactorial etiology including genetic and environmental factors [2]. The rarer incomitant strabismus seems to follow mendelian inheritance [2]. Disruption of the following structures may underlie the final common pathway in the development of strabismus—extraocular muscles, orbital connective tissues, cranial nerves, fusion centres, and the visual cortex [13].

It is difficult to identify the genetic factors in a multifactorial disease. But the identification of such factors will help to better understand the etiopathogenesis of the disease. Those at risk can be identified early. Impairment due to strabismus, such as amblyopia and absence of binocular vision and stereopsis can be prevented or treated better resulting in better visual outcomes [14]. It could result in new techniques for prevention or treatment.

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14.3 Comitant Strabismus

Strabismus is one of the earliest recorded genetic disorders. Hippocrates was the first to suggest that strabismus was passed on from parent to offspring 2500 years ago [15]. Further observations of strabismus occurring in families, twins, the different prevalence rates of strabismus in various ethnic populations and the difference in the rate of prevalence of different types of strabismus in different populations indicate a genetic basis of the disease. However, various environmental factors have also been associated with strabismus. Factors significantly related to strabismus include advanced maternal age, smoking during pregnancy, premature birth, low birth-weight, retinopathy of prematurity and refractive error [16] (Fig. 14.1).

The genetics of strabismus are complex as comitant strabismus does not follow a Mendelian pattern of inheritance. Instead it has a multifactorial pattern of inheritance which includes interaction of multiple genes and environmental factors [17]. To explain multifactorial etiology a Gaussian distribution of many factors is assumed in the general population. The disease occurs when a certain threshold is attained. In an at risk population, which includes the first order relatives of an affected individual, the Gaussian curve is shifted to the right—the threshold is attained earlier. The actual risk depends on how much the Gaussian curve of the at risk population is shifted with regard to that of the normal population (Fig. 14.2) [18].

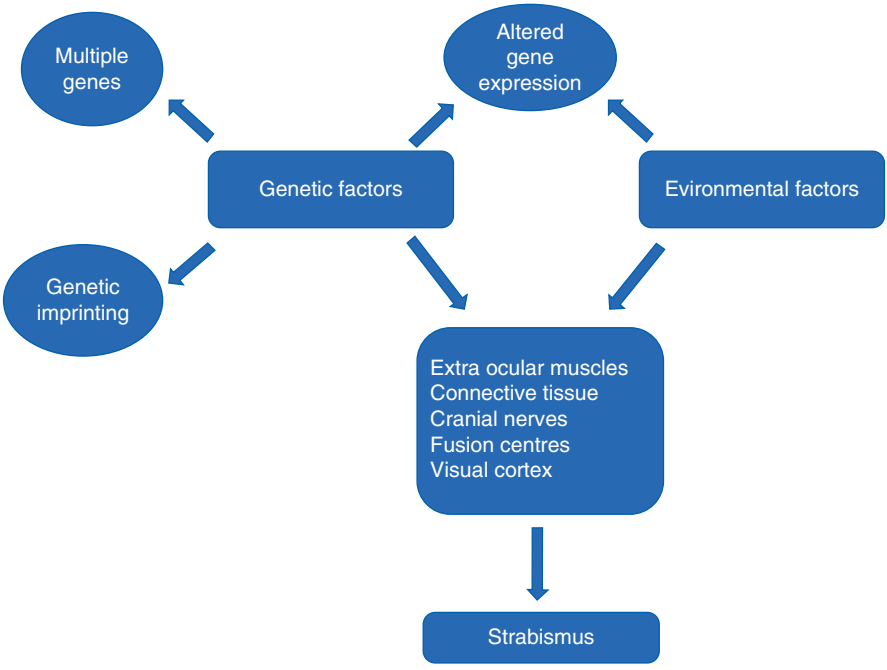

Fig. 14.1 Showing the contribution of genetic and environmental factors in the etiopathogenesis of strabismus

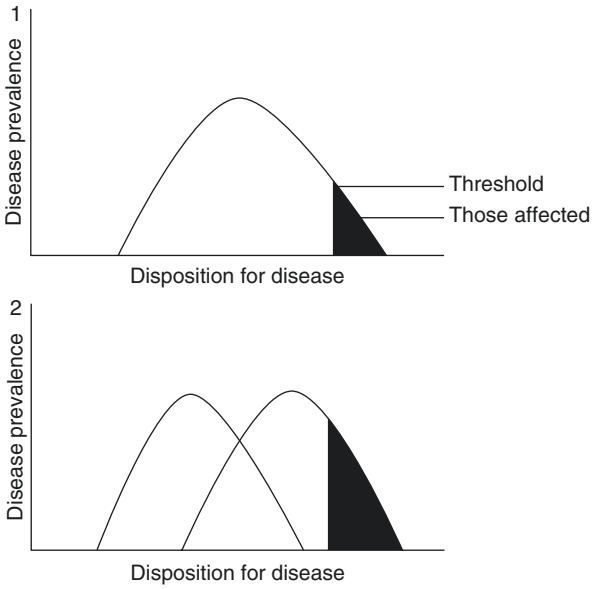

Fig. 14.2 Showing multifactorial inheritance (after Carter, 1973) [19]. Gaussian distribution of various factors contributing to disease in the general population. The distribution is shifted to the right in populations at risk with a larger number of individuals reaching the threshold and manifesting the disease

14.4 Ethnic Variations

The prevalence of comitant strabismus is different in different ethnic groups as is the type of strabismus. This supports the heritability of comitant strabismus. It is 2% to 4% among the white population [20–22] compared to 0.6% among African [23] and Asian [24, 25] populations. Esotropia is more frequent than exotropia among the white population of the United States and Europe [21] whereas exotropia is more common in the Asian and black populations of the United States and Africa [24, 25]. In Hawaii, esotropia is more frequent in white populations, exotropia is more in Asian populations, and the two forms are mostly equal in the mixed ethnic population [24].

14.5 Family Studies

There were many early studies on strabismus occurring in families. But the heritability and genetic risk could not be clearly identified due to variations in inheritance mode, heritability, type of strabismus and the influence of environmental risk factors [16]. Nevertheless, the rates were much greater than those in general population (5% approximately), supporting a genetic component to strabismus risk [13].

Various surveys that were conducted between 1910 and 1950 found that heritability ranged from 20% to 50% in families that had esotropia [26]. Schlossman and Priestley found that 47.5% of 158 people with strabismus, 48.9% of 139 esotropes, and 36.8% of 19 exotropes were from families in which two or more additional members were affected. The real number could be more since subtle alignment

deviations could be unnoticed [27]. The highest incidence of strabismus in families is reported as 65% [28]. A study observed that 18% of 34 babies born to one parent with esotropia manifested constant or intermittent esotropia at 6 months [29]. Family history was significant for intermittent or constant exotropia and accommodative esotropia when compared to infantile esotropia ($P < 0.0001$ and $P = 0.0267$, respectively, Fisher's exact test) [30].

Strabismus occurring in siblings was observed by a study by Chew et al. They found that having an affected sibling increased the risk of developing esotropia (OR 2.6; 95%CI, 1.2–3.2). However, similar increase in risk was not observed in exotropia [31]. Schlossman and Priestley found that if the type of strabismus was the same in the siblings, the post-operative result was similar. This could be an indicator for improving the management of strabismus in siblings [27].

The concordance of the type of strabismus was varied across multiple studies. One study reported that concordance was 80% for strabismus manifesting in the same family [16]. Another study found a concordance of 54% in 39 families [32]. Families which manifested a mixture of esotropia and exotropia were also reported. This can be explained by the presence of single gene with variable expressivity or by the presence of two common genes. For example, it has been observed that there is increased prevalence of monofixation (7.8%) among parents of children with infantile esotropia [33] suggesting variable expressivity of a single gene.

14.6 Twin Studies

Twin studies are necessary to confirm and quantify the relative genetic contribution as familial clustering can be attributed to genetic factors as well as an unrecognized common environmental factor. Studies of strabismus in twins found a higher concordance among monozygotic twins than dizygotic twins, implying the predominance of a genetic factor [16]. Matsuo et al.'s study of strabismus in twins found that the type of strabismus of 67.3% of 49 pairs of twins was concordant, and the rate of concordance was more in monozygosity (82.4%) than in multizygosity (47.6%) [34]. Another study observed that there was concordance only in the direction of the strabismus; the associated features of strabismus were disconcordant in the majority of the twins [35].

The genetic risk of esotropia and exotropia appear to be very different. In the Podgor study, the odds ratio was 330 for exotropia in cases of multiple birth where one of the twins was affected, whereas the odds ratio was extremely low at 2.2 for single births, implying a strong impact of multiple birth environmenton on the risk of exotropia [36]. A study among 1462 twins reported that heritability was significant for esotropia. The genetic heritability of eso-deviation was 64% while no genetic heritability was found for exo-deviation [37]. A Chinese twin study found that there was greater concordance for exotropia (75%) when compared to esotropia (65.7%) [38]. It can be attributed to the influence of multiple birth environment on esotropia as well as ethnicity in the contribution to esotropia. Thus, esotropia is

more commonly associated with heritable factors while exotropia has a stronger environmental contribution.

14.7 Linkage Analysis

Linkage analysis helps to identify specific locations in the human genome that are linked to a particular trait. Parikh et al. [39] were the first to successfully identify a locus for comitant strabismus using linkage analysis. This study found a significant association of familial comitant strabismus with a region on the p arm of chromosome 7(7p21.3–15.3), designated STBMS1 which was linked to esotropia. Rice et al. [40] studied 12 families with comitant esotropia for the occurrence of STBMS1. A significant linkage was found in only one family, implying the limited role of STBMS1 in the genetic factor of strabismus. The STBMS1 locus has 33 genes. Further studies are needed to find which of these have a role in the etiopathogenesis of strabismus.

While STBMS1 has been found to be associated with strabismus mainly in European populations, various other loci have been identified in different ethnicities. In Japan, Matsuo et al. [41] studied 55 families with comitant strabismus in at least two family members. Linkage analysis identified loci at 4q28.3 and 7q31.2. Genes in this locus have been found to encode proteins such as neuronal cell adhesion molecule expressed in the brain. This supports theories that state that the cause of strabismus is a neurological deficit that affects the development of binocular fusion and stereopsis.

Recent research has found that two genes from the above loci, *MGST2* and *WNT2,* are associated with comitant strabismus in Japanese population [42]. In Saudi Arabia, linkage analysis was done on a family in which strabismus was present in three of ten children and a first cousin [43]. They identified a locus at 16p13.12–p12.3. Each of these four individuals had a different type of strabismus which may indicate variable expressivity of a genotype.

14.8 Altered Gene Expression

Various studies have indicated that strabismus may be caused by variation in expression of gene rather than a particular defect in a gene [17]. This alteration in gene expression is most often caused by environmental factors like in utero stress due to hypoxia [17]. The alteration in gene expression can be quantified by measuring the RNA transcript or the protein coded by the gene.

Altick et al. [44] studied RNA isolated from the distal segments of horizontal rectus muscles either obtained during surgery for strabismus or from age-matched deceased organ donors. A total of 604 genes were found to be differently expressed in strabismic muscles belonging to one of three categories including upregulated genes, down regulated genes, genes associated with energy metabolism. These

results imply that strabismus may occur due to changes in metabolism, decreased contractility, and increased fibrosis in extraocular muscles.

Liu et al. [45] found decreased expression of genes coding for the glucagon precursor, pituitary adenylate cyclase-activating polypeptide, and cAMP dependent protein kinase inhibitor in discordant monozygotic twins compared to normal orthophoric children.

Proteomics and gene expression analysis which compared strabismic and normal extra ocular muscles found significant differences in the composition of extraocular muscles of patients with strabismus with respect to important motor proteins, elements of the ECM, and connective tissue [46]. Recent studies have found that the noncoding RNA segments might also contribute to the development of strabismus [47].

14.9 Gene Imprinting

Some genes are epigenetically modified during gametogenesis resulting in only the paternal or maternal copy being expressed after fertilization. Conditions classically associated with genetic imprinting like Angelman syndrome, and Prader Willi syndrome are associated with strabismus. Recent studies have detected a series of significant imprinted loci, including the 7q31.2 locus [48].

14.10 Genome Wide Association Studies

Genome wide association studies identified a locus within the first intron of the *WRB* (tryptophan rich basic protein) gene on chromosome 21 with accommodative esotropia. The gene shows maternal imprinting [49]. *NPLOC4–TSPAN10–PDE6G* is another locus on chromosome 17q25.3 identified by GWAS as contributing to susceptibility to strabismus [50].

14.11 Gene Analysis

So far, more than 233 genes are associated with strabismus. The analyses indicate that the strabismus genes consist of multiple subsets, which is consistent with the heterogenous phenotype of strabismus. The analyses and literature review show three anatomical regions and one signaling pathway as being associated with the strabismus genes: retina, cerebellum, and amygdala; and the Ras-MAPK signaling pathway [51].

14.12 Incomitant Strabismus

Incomitant strabismus comprises about 5% of all cases of strabismus [2]. These are rare forms of complex strabismus that can be inherited as mendelian traits. The genetic basis of several types of incomitant strabismus have been identified [2] (Table 14.1).

Table 14.1 Strabismus syndromes with defined genetic basis

Disorder	Gene	Inheritance
Isolated DRS	CHN1 MAFB	AD AD
Duane radial ray (Okihiro) syndrome	SALL4	AD
Holt-Oram syndrome	SALL4	AD
Acro-renal-ocular syndrome	SALL4	AD
Townes-Brocks syndrome	SALL1	AD
HOXA1-related syndromes Bosley-Salih-Alorainy syndrome Athabascan brain stem dysgenesis syndrome	HOXA1	AR
HoxB1	HOXB1	AR
CFEOM1	KIF21A Rarely TUBB3	AD
CFEOM2	PHOX2A	AR
CFEOM3	TUBB3 Rarely KIF21A	AD
HGPPS	ROBO3	AR

AD autosomal dominant, *AR* autosomal recessive, *DRS* Duane retraction syndrome, *CFEOM* congenital fibrosis of extraocular muscles, *HGPPS* horizontal gaze palsy with progressive scoliosis

14.13 Isoated DRS

Isolated DRS is a mostly sporadic condition. Hereditary forms of DRS comprise 5–10% of the cases [52]. Bilateral DRS with an autosomal dominant pattern of inheritance has been described by many investigators [53]. The DURS2 locus on chromosome 2 and subsequently, heterozygous mutations in CHN1 have been identified in families with DRS inherited as a dominant condition [52, 54]. The *CHN1* gene encodes the protein α2-chimerin. This protein is thought to play a role in the primary development of abducens and oculomotor nerves [53]. CHN1 hyperactivation has been found in patients with supraduction deficits even in the absence of DRS [53]. DRS has also been associated with abnormalities in chromosomes 10 and 22 [53]. Park et al. [55] studied 401 individuals with DRS. They found a pathogenic variant in MAFB in four probands. It has been observed that there is greater incidence of bilateral involvement and more vertical movement abnormalities in the hereditary form even though the phenotype is similar to sporadic DRS [53].

14.14 Syndromic DRS

There are characteristic systemic features associated with DRS in about 30% of cases [53]. Duane radial ray (Okihiro) syndrome, Holt-Oram syndrome and acro-renal-ocular syndrome are associated with SALL4 mutations [56]. Townes-Brocks syndrome is an autosomal dominant syndrome associated with SALL1 mutations [57]. It is characterized by abnormalities of the ear, limb, anus and kidney. It has also been rarely associated with DRS.

Abnormalities of chromosome eight are associated with DRS. CPAH has been identified as a possible etiology of simplex DRS as implicated by a reciprocal

balanced translocation in chromosome 8q13 [58]. Chromosome 8q12 duplications [59] are associated with DRS with sensorineural deafness, developmental delay, cardiac defects, and hypotonia.

The association of Duane retraction syndrome, congenitally fused cervical vertebrae (Klippel-Feil deformity) and hearing loss is referred to as Wildervanck syndrome (also known as cervico-oculo-acoustic syndrome). It is more common in females. Genetic evaluation of an affected male showed a microdeletionon chromosome X involving Fibroblast Growth Factor Homologous Factor 13 (FGFHF13) [60].

Chromosomal microarray analysis has identified duplications at multiple loci in DRS patients—Yp11.2, Yq11.222– q11.223, Xp11.21, Xq13.2 and deletions in Yp11.2 and Yp11.31–p11.2. The role of these abnormalities in the development of DRS is not known [61].

Three patients of Duchenne muscular dystrophy(DMD) have been reported to have DRS. Mutated *dystrophin* has been found in extraocular muscles as well as in the central nervous system in DMD [62]. Goldenhar syndrome is found to occur in upto 3% of DRS patients. Deletions in chromosome 22q11.2 have been found in Goldenhar syndrome [53]. DRS has also been linked to many conditions, like Marfan syndrome [53]. But a specific genetic association is yet to be found.

14.15 HOXA1 Mutations

Horizontal gaze palsy, sensorineural hearing loss, facial palsy, developmental delay and hypoventilation have been found to be associated with recessive, homozygous mutations of HOXA1 [63]. There is significant overlap with Type 3 DRS as well as Moebius syndrome. HOXB1 mutations have been identified in a German-American population. Homozygous HOXB1 mutation has been reported with comitant strabismus, hearing impairment, and bilateral facial palsy. Affected individuals manifested facial palsy andesotropia. But they did not meet the criteria for Moebius syndrome, as abduction of both eyes was full. The mutation is thought to cause loss of function of HOXB1 [64].

14.16 CFEOM

Three phenotypically and genetically distinct types of CFEOM have been reported. Inheritance of CFEOM type 1 is autosomal dominant. It is associated with mutations in the gene KIF21A on chromosome 12. KIF21A encodes a kinesin motor protein [65]. Rarely patients with CFEOM1 have mutations in the TUBB3 gene also [66]. Mutations in the KIF21A gene are very rarely associated with clinical findings similar to CFEOM3 [53]. CFEOM type 2 is associated with homozygous mutations in PHOX2A [67]. CFEOM type 3 is associated with heterozygous missense mutations of the TUBB3 gene which encodes a β tubulin isotype which is present in neuronal microtubules [66]. Some specific TUBB3 mutations can cause abnormalities of other cranial and spinal nerves, abnormal development of the corpus

callosum and basal ganglia [66]. The TUBB3 E410K syndrome results from a specific TUBB3 mutation resulting in an E410K amino acid substitution [68]. The TUBB3 E410K syndrome clinically overlaps atypical Moebius syndrome. One mutation-negative pedigree clinically resembles CFEOM3. It is associated with a translocation which implicates a locus on chromosome 13q12(FEOM4) [53].

It was noted that patients with *TUBB3* mutations required more robust surgery for the horizontal strabismus. They also were more susceptible to ocular surface issues. It was observed that patients with *KIF21A* needed stronger procedures to improve their upgaze limitation [69].

14.17 Horizontal Gaze Palsy

It has been associated with mutation of ROBO3 in families with consanguinity characterized by autosomal recessive pattern of inheritance [53]. These patients presented with torticollis and plagiocephaly followed by scoliosis [70]. These ocular and molecular cues can help in close monitoring of these children to identify the onset of scoliosis and initiate prompt treatment.

14.18 Moebius Syndrome

It has been associated with prenatal exposure to misoprotol [53]. But no clear genetic basis exists. The atypical forms have been associated with some mutations. But there are no mutation reported in typical patients [53].

14.19 Conclusion

Genetics has been observed to play a major role in the various forms of strabismus. But the most frequent forms of strabismus are not caused by isolated genetic mutations. This makes it difficult to identify the underlying cause of strabismus. But further research must continue as identification of the causative factors will help us understand the etiology and pathogenesis of strabismus. This will lead to improvement in the management of strabismus. It will also have a major impact medically and psychosocially, and also have an effect on the health care costs.

References

1. Robaei D, Rose KA, Kifley A, Cosstick M, Ip JM, Mitchell P. Factors associated with childhood strabismus: findings from a population-based study. Ophthalmology. 2006;113(7):1146–53.
2. Engle EC. The genetic basis of complex strabismus. Pediatr Res. 2006;59(3):343–8.
3. Multi-ethnic Pediatric Eye Disease Study. Prevalence of amblyopia and strabismus in African American and Hispanic children ages 6 to 72 months the multi-ethnic pediatric eye disease study. Ophthalmology. 2008;115(7):1229–36.

4. Chia A, Dirani M, Chan YH, Gazzard G, Au Eong KG, Selvaraj P, Ling Y, Quah BL, Young TL, Mitchell P, Varma R, Wong TY, Saw SM. Prevalence of amblyopia and strabismus in young Singaporean Chinese children. Investig Ophthalmol Vis Sci. 2010;51(7):3411–7.
5. Al-Tamimi ER, Shakeel A, Yassin SA, Ali SI, Khan UA. A clinic-based study of refractive errors, strabismus, and amblyopia in pediatric age group. J Family Community Med. 2015;22(3):158–62.
6. Kushner BJ. The efficacy of strabismus surgery in adults: a review for primary care physicians. Postgrad Med J. 2011;87:269–73.
7. Olitsky SE, Sudesh S, Graziano A, et al. The negative psychosocial impact of strabismus in adults. J AAPOS. 1999;3:209–11.
8. Tychsen LRM, Richards M, Wong AM, et al. Decorrelation of cerebral visual inputs as the sufficient cause of infantile esotropia. Am Orthopt J. 2008;58(1):60–9.
9. Ingram RMGL, Gill LE, Goldacre MJ. Emmetropisation and accommodation in hypermetropic children before they show signs of squint: a preliminary analysis. Bull Soc Belge Ophtalmol. 1994;253:41–56.
10. Hasany AWA, Wong A, Foeller P, Bradley D, Tychsen L. Duration of binocular decorrelation in infancy predicts the severity of nasotemporal pursuit asymmetries in strabismic macaque monkeys. Neuroscience. 2008;156(2):403–11.
11. Gwiazda JTF, Thorn F. Development of refraction and strabismus. Curr Opin Ophthalmol. 1999;10(5):293–9.
12. Birch EE, Fawcett S, Stager D. Co-development of VEP motion response and binocular vision in normal infants and infantile esotropes. Investig Ophthalmol Vis Sci. 2000;41(7):1719–23.
13. Ye XC, Pegado V, Patel MS, Wasserman WW. Strabismus genetics across a spectrum of eye misalignment disorders. Clin Genet. 2014;86:103–11.
14. Holmes JM, Lazar EL, Melia BM, Pediatric Eye Disease Investigator Group, et al. Effect of age on response to amblyopia treatment in children. Arch Ophthalmol. 2011;129(11):1451–7.
15. Hippocrates. Airs, water and places in the genuineworks of Hippocrates (trans: Adam F). New York, NY: WimWood & Co; 1886. p. 171.
16. Maconachie GDE, Gottlob I, McLean RJ. Risk factors and genetics in common comitant strabismus: a systematic review of the literature. JAMA Ophthalmol. 2013;131(9):1179–86.
17. Kruger JM, Mansouri B, Cestari DM. An update on the genetics of comitant strabismus. Seminars Ophthalmol. 2013;28(5–6):438–41.
18. Lorenz B. Genetics of isolated and syndromic strabismus: facts and perspectives. Strabismus. 2002;10(2):147–56.
19. Carter CO. Multifactorial genetic disease. In: McKusick V, Claiborne R, editors. Medical genetics. New York: H.P. Publishing; 1973. p. 199.
20. Gover M, Yankey J. Physical impairments of members of low-income farm families—1,490 persons in 2,477 farm security administration borrower families, 1940. Public Health Rep. 1944;59:1163–84.
21. Nordloew W. Squint: the frequency of onset at different ages and the incidence of associated defects in a Swedish population. Acta Ophthalmol (Copenh). 1964;42:1015–37.
22. Abrahamsson M, Magnusson G, Sjostrand J. Inheritance of strabismus and the gain of using heredity to determine populations at risk of developing strabismus. Acta Ophthalmol Scand. 1999;77:653–7.
23. Holm S. Le strabisme concomitant chez les palenegrides au Gabon, Afrique equatoriale francaise. Acta Ophthalmologica (Copenh). 1939;17:367–87.
24. Ing M, Pang S. The racial distribution of strabismus. In: Strabismus: proceedings of the third meeting of the International Strabismological Association. New York: Grune& Stratton; 1978.
25. Hu DN. Prevalence and mode of inheritance of major genetic eye diseases in China. J Med Genet. 1987;24:584–8.
26. Maumenee IH, Alston A, Mets MB, Flynn JT, Mitchell TN, Beaty TH. Inheritance of congenital esotropia. Trans Am Ophthalmol Soc. 1986;84:85–93.
27. Schlossman A, Priestley BS. Role of heredity in etiology and treatment of strabismus. AMA Arch Ophthalmol. 1952;47:1–20.

28. Ziakas NG, Woodruff G, Smith LK, Thompson JR. A study of heredity as a risk factor in strabismus. Eye Lond Engl. 2002;16:519–21.
29. Aurell E, Norrsell K. A longitudinal study of children with a family history of strabismus: factors determining the incidence of strabismus. Br J Ophthalmol. 1990;74:589–94.
30. Matsuo T, Yamane T, Ohtsuki H. Heredity versus abnormalities in pregnancy and delivery as risk factors for different types of comitant strabismus. J Pediatr Ophthalmol Strabismus. 2001;38(2):78–82.
31. Chew E, Remaley NA, Tamboli A, Zhao J, Podgor MJ, Klebanoff M. Risk factors for esotropia and exotropia. Arch Ophthalmol. 1994;112(10):1349–55.
32. Ferreira RDC, Oelrich F, Bateman B. Genetic aspects of strabismus. Arq Bras Oftalmol. 2002;65:171–5.
33. Scott MH, Noble AG, Raymond WR, Parks MM. Prevalence of primary monofixation syndrome in parents of children with congenital esotropia. J Pediatr Ophthalmol Strabismus. 1994;31:298–302.
34. Matsuo T, Hayashi M, Fujiwara H, Yamane T, Ohtsuki H. Concordance of strabismic phenotypes in monozygotic versus multizygotic twins and other multiple births. Jpn J Ophthalmol. 2002;46:59–64.
35. de Decker W, Feuerhake C. Squinting in monozygotic twins: an analysis of 30 pairs [in German]. Ber Zusammenkunft Dtsch Ophthalmol Ges. 1978;(75):490–3.
36. Podgor MJ, Remaley NA, Chew E. Associations between siblings for esotropia and exotropia. Arch Ophthalmol. 1996;114:739–44.
37. Sanfilippo PG, Hammond CJ, Staffieri SE, et al. Heritability of strabismus:genetic influence is specific to eso-deviation and independent of refractive error. Twin Res Hum Genet Off J Int Soc Twin Stud. 2012;15:624–30.
38. Wei NF. Genetic factors of concomitant strabismus. Zhonghua Yan Ke Za Zhi Chin J Ophthalmol. 1987;23:282–3.
39. Parikh V, Shugart YY, Doheny KF, et al. A strabismus susceptibility locus on chromosome 7p. Proc Natl Acad Sci USA. 2003;100:12283–8.
40. Rice A, Nsengimana J, Simmons IG, et al. Replication of the recessive STBMS1 locus but with dominant inheritance. Investig Ophthalmol Vis Sci. 2009;50:3210–7.
41. Shaaban S, Matsuo T, Fujiwara H, et al. Chromosomes 4q28.3 and 7q31.2 as new susceptibility loci for comitant strabismus. Investig Ophthalmol Vis Sci. 2009;50:654–61.
42. Zhang J, Matsuo T. MGST2 and WNT2 are candidate genes for comitant strabismus susceptibility in Japanese patients. Peer J. 2017;5:e3935.
43. Khan AO, Shinwari J, Abu Dhaim N, et al. Potential linkage of different phenotypic forms of childhood strabismus to a recessive susceptibility locus (16p13.12–p12.3). Mol Vis. 2011;17:971–6.
44. Altick AL, Feng CY, Schlauch K, et al. Differences in gene expression between strabismic and normal human extraocular muscles. Investig Ophthalmol Vis Sci. 2012;53:5168–77.
45. Liu G, Bai H, Yan Z, et al. Differential expression of proteins in monozygotic twins with discordance of infantile esotropic phenotypes. Mol Vis. 2011;17:1618–23.
46. Agarwal AB, Feng C-Y, Altick AL, et al. Altered protein composition and gene expression in strabismic human extraocular muscles and tendons. Investig Ophthalmol Vis Sci. 2016;57:5576–85.
47. Ma WX, Huang XG, Yang TK, Yao JY. Involvement of dysregulated coding and long noncoding RNAs in the pathogenesis of strabismus. Mol Med Rep. 2018;17(6):7737–45.
48. Shaaban S, Matsuo T, Strauch K, Ohtsuki H. Investigation of parent-of-origin effect in comitant strabismus using MOD score analysis. Mol Vis. 2009;15:1351–8.
49. Shaaban S, MacKinnon S, Andrew SC, for the Strabismus Genetics Research Consortium, et al. Genome- wide association study identifies a susceptibility locus for comitant esotropia and suggests a parent-of origin effect. Investig Ophthalmol Vis Sci. 2018;59:4054–64.
50. Plotnikov D, Shah RL, Rodrigues JN, et al. A commonly occurring genetic variant within the *NPLOC4–TSPAN10–PDE6G* gene cluster is associated with the risk of strabismus. Hum Genet. 2019;138:723–37.

51. Ye X, Lee R, Wasserman W. Curation and bioinformatic analysis of strabismus genes supports functional heterogeneity and proposes candidate genes with connections to RASopathies. Gene. 2019;697:213–26. https://doi.org/10.1016/j.gene.2019.02.020.
52. Appukuttan B, Gillanders E, Juo SH, et al. Localization of a gene for Duane retraction syndrome to chromosome 2q31. Am J Hum Genet. 1999;65(6):1639–46.
53. Graeber CP, Hunter DG, Engle EC. The genetic basis of incomitant strabismus: consolidation of the current knowledge of the genetic foundations of disease. Semin Ophthalmol. 2013;28(5-6):427–37.
54. Miyake N, Demer JL, Shaaban S, et al. Expansion of the CHN1 strabismus phenotype. Investig Ophthalmol Vis Sci. 2011;52(9):6321–8.
55. Park JG, Tischfield MA, Nugent AA, et al. Loss of MAFB function in humans and mice causes duane syndrome, aberrant extraocular muscle innervation, and inner-ear defects. Am J Hum Genet. 2016;98(6):1220–7.
56. Kohlhase J, Schubert L, Liebers M, et al. Mutations at the SALL4 locus on chromosome 20 result in a range of clinically overlapping phenotypes, including Okihiro syndrome, Holt-Oram syndrome, acro-renal-ocular syndrome, and patients previously reported to represent thalidomide embryopathy. J Med Genet. 2003;40(7):473–8.
57. Kohlhase J, Wischermann A, Reichenbach H, et al. Mutations in the SALL1 putative transcription factor gene cause Townes-Brocks syndrome. Nat Genet. 1998;18(1):81–3.
58. Pizzuti A, Calabrese G, Bozzali M, et al. A peptidase gene in chromosome 8q is disrupted by a balanced translocation in a duane syndrome patient. Investig Ophthalmol Vis Sci. 2002;43(12):3609–12.
59. Lehman AM, Friedman JM, Chai D, et al. A characteristic syndrome associated with microduplication of 8q12, inclusive of CHD7. Eur J Med Genet. 2009;52(6):436–9.
60. Abu-Amero KK, Kondkar AA, Alorainy IA, et al. Xq26.3 Microdeletion in a male with Wildervanck Syndrome. Ophthalmic Genetics. 2013;1:1–7.
61. Niyaz L, Tural S, Eski Yucel O, et al. Chromosomal microarray analysis of patients with Duane retraction syndrome. Int Ophthalmol. 2019;39:2057–67.
62. Bosley TM, Salih MA, Alkhalidi H, Oystreck DT, El Khashab HY, Kondkar AA, Abu-Amero KK. Duane retraction syndrome in a patient with Duchenne muscular dystrophy. Ophthalmic Genet. 2016;37(3):276–80.
63. Tischfield MA, Bosley TM, Salih MA, et al. Homozygous HOXA1 mutations disrupt human brainstem, inner ear, cardiovascular and cognitive development. Nat Genetics. 2005;37(10):1035–7.
64. Webb BD, Shaaban S, Gaspar H, et al. HOXB1 founder mutation in humans recapitulates the phenotype of Hoxb1-/- mice. Am J Hum Genet. 2012;91(1):171–9.
65. Yamada K, Andrews C, Chan WM, et al. Heterozygous mutations of the kinesin KIF21A in congenital fibrosis of the extraocular muscles type 1 (CFEOM1). Nat Genet. 2003;35(4):318–21.
66. Tischfield MA, Baris HN, Wu C, et al. Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. Cell. 2010;140(1):74–87.
67. Nakano M, Yamada K, Fain J, et al. Homozygous mutations in ARIX(PHOX2A) result in congenital fibrosis of the extraocular muscles type 2. Nat Genet. 2001;29(3):315–20.
68. Chew S, Balasubramanian R, Chan WM, et al. A novel syndrome caused by the E410K amino acid substitution in the neuronal beta-tubulin isotype 3. Brain J Neur. 2013;136(Pt 2):522–35.
69. Heidary G, Mackinnon S, Elliott A, Engle EC, Hunter DG. Outcomes of strabismus surgery in genetically confirmed congenital fibrosis of the extraocular muscles. J AAPOS. 2019;23:253.e1-6.
70. Chan WM, Traboulsi EI, Arthur B, et al. Horizontal gaze palsy with progressive scoliosis can result from compound heterozygous mutations in ROBO3. J Med Genet. 2006;43(3):e11.

Gene Therapy in Diabetic Retinopathy

15

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15.1 Introduction

The process of gene therapy involves introducing genetic material into the cells of the patients to recompense faulty genes or delivering therapeutic genes. The common complication of diabetes mellitus (DM) is diabetic retinopathy (DR) which is the leading cause of blindness among working age in both developed and developing countries [1]. International 2017 report stated that 159 million inthe western pacific and about 82 million in Southeast Asia are affected with DM [2]. The complex mechanism of DR is influenced by both genetics and environmental factors. Optimisation of glycaemic control, dyslipidaemia and blood pressure has reduced but has not stopped the progression of DR [3]. These findings explain other factors influencing the development and progression of DR, also several studies have implicated genetic associations. Heritability is estimated to account for about 18–27% in DR and 25–52% in proliferative diabetic retinopathy (PDR) [4]. World-wise incidence of diabetes mellitus (DM) is increasing at an alarming rate; about 422 million people are affected with DM, and among this, 35% of the population are affected with DR [5].

DR is a polygenetic and heterogenous disease; there is a wide array of gene involved in the DR pathogenesis established using candidate gene analysis [6]. Extensive analysis has been carried out with these genes;they include vascular endothelial growth factor (VEGF), aldose reductase, erythropoietin (EPO), and

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receptor of AGEs [6]. VEGF is well established for its part in DR neovascularization and breakdown of the blood–retinal barrier (BRB). VEGF is an apparent therapeutic mark in many DR gene therapies, and other characteristics of the DR pathogenesis of DR are polyol activation and oxidative stress as well as hexosamine pathway. The gene therapy could enable long-term treatment results with less adverse effects than other treatment strategies [1, 4]. Adeno associated virus (AAV) based gene therapy for Leber's congenital amaurosis (LCA) (associated gene RPE-65) in 2017 USA food and drug administration have made a landmark in gene therapy. This finding lead to the interest in gene therapy for complex diseases like DR, glaucoma, and age-related macular degeneration.

15.2 Pathogenesis and Genetics Involved in DR

DR is a gradually progressive disease characterised by microvascular damage of the retina. The progression is influenced by many factors like poor glycaemic control, chronic diabetes, elevated blood pressure and dyslipidaemia [2, 3]. Optimisation of glycaemic control, dyslipidaemia and blood pressure may reduce but not eliminate the progression [3]. Hereditability estimations account for about 18–27% in DR and 25–52% in PDR [4]. Many recent treatments for DR use anti-VEGF which demonstrates its vital role in the pathophysiology of DR, >100 genes may have been altered in DR which influences the suboptimal response for anti-VEGF treatment [7].

15.3 Metabolic Memory Phenomenon

The term metabolic memory or legacy effect is a phenomenon, where the harmful effects of uncontrolled glycaemia remain for years in the body even after attaining good glycaemic control [4]. Studies like the diabetes control complications trial (DCCT) and the epidemiology of diabetes interventions and complications (EDIC) projected the idea of metabolic memory [4]. Intensive insulin therapy resulted in fewer complications like nephropathy, neuropathy or retinopathy after 6.5 years; EDIC study reported that patients who received intensive insulin therapy formerly continued to have lesser complications [4]. These studies explain the benefits of early treatment and the harmful effects of chronic uncontrolled glycaemia resulting in metabolic phenomenon. Many accumulating studies report that these mechanisms could be related to the epigenetic modifications in DR even after attaining normal glycaemic levels [5–8]. It is also noted in patients with type 1 diabetes where most of the complications are mediated by the "cumulative glycaemic effects" without requiring metabolic memory. Mitochondrial oxidative damage is mediated through ROS overproduction which leads to DR; hence, mitochondria and its role in oxidative stress suggested a therapeutic target for retinopathy [9].

15.4 Epigenetic Modifications in DR

Epigenetic modifications are the heritable alterations in the DNA but the DNA sequence doesnot get altered. These alterations play a vital role in the gene modifications of DR. The three main epigenetic modifications in DR which are associated with metabolic memory are DNA methylation, histone modifications and miRNA [3, 7]. These modifications result in raised oxidative stress, angiogenesis, apoptosis and inflammations. DNAme at CpGs (cytosine-phosphodiester bond-guanine) of the candidate gene could be the mechanism leading to sustained diabetic complications [10]. DNAme and genetic variants at some of the HbA1c-associated CpGs are also reported to be associated. Persistent hyperglycaemia could probably induce DNAme changes at target genes like thioredoxin-interacting protein (TXNIP), in haematopoietic stem cells and others that are epigenetically retained in myeloid cells to enable metabolic memory, which modifies enhancer activity at the adjacent genes [10].

15.5 Candidate Gene Analysis

Pathogenesis of DR initiates the identification of candidate genes, the frequency of a specific genetic variant in subjects with or without DR is compared with candidate gene analysis. Multiple gene association with DR was revealed through this analysis [11] (Table 15.1).

15.6 Gene Wide Association Studies (GWAS)

These studies investigate the association between single nucleotide polymorphisms (SNPs) throughout the whole human genome and multiple phenotypes of the disease. This method has aided in the identification of hundreds of genetic variants related tocomplex diseases [11]. Recently, sight-threatening DR was found to be associated with growth factor receptor-bound protein genes (GBR2) on chromosome 17q25.1 [24]. Another important gene found from GWAS was the centrosome protein 162 kDA (CEP162) gene, which is reported to play a role in the formation of the ciliary transition zone. Dysregulation of ciliary-associated genes is susceptible to DR [25]. One more GWAS reported an SNP (rs9362054), which was found to have a borderline genome-wide significance among Japanese [26].

15.7 Limitations in Currents Treatments for DR

Many of the current treatment modalities for DR can be highly effective but also includes drawbacks, which directs the way for the need for novel treatment methods like gene therapy. Glycaemic control is the mainstay for DR management, but the

Table 15.1 Summary of susceptible genes associated with DR identified using candidate gene technique

Gene names (Gene symbols)	Function	Ch	Type of DM	Population	Disease association	Ref
Candidate gene analysis						
Angiotensin -1 converting enzyme (ACE-1)	Renin angiotensin system activation of angiotensin-2	17	1 and 2	Caucasian, Japanese	NPDR	[12]
Aldose reductase (ALR2)	Polyol pathway-Converts glucose into sorbitol	7	1 and 2	Euro-Brazilian, Mainland Chinese, Japanese	PDR	[13]
Erythropoietin (EPO)	Regulator of erythropoiesis, stimulate proliferation, migration, and hypoxic cell angiogenesis	7	1 and 2	American and Australian	PDR	[14]
Receptor for advanced glycation end products (RAGE)	Initiation of pro-inflammatory genes	6	2	Chinese and Malaysians	NPDR	[15]
Endothelial nitric oxide synthase (eNOS3)	Synthesis of nitric oxide (Vasodilation)	7	2	Caucasian French	Severe NPDR and PDR	[16]
Complement factor H (CFH)	Regulation of complement activation	1	2	Chinese	NPDR	[17]
Chimerin 2 (CHN2)	Cell growth, proliferation, and migration regulation	7	2	Taiwanese	NPDR	[18]
Methylenetetrahydrofolate reductase (MTHFR)	Remethylation of homocysteine to methionine	1	2	Turkish, Chinese, and Japanese	NPDR and PDR and diabetic neuropathy	[19]
Osteoprotegerin (OPG)	Cytokine receptor	8	2	Slovenian	NPDR	[20]
Monocyte chemoattractant protein (MCP-1)	Cytokine- activation of monocytes,macrophages, and lymphocytes	17	1, 2	Japanese	PDR	[21]
Insulin like Growth factor 1 (IGF-1)	Increased vitreous IGF-1 levels trigger molecular events initiating retinal angiogenesis	12	2	South Indian	DR and NPDR	[22]
Polymorphisms in protein kinase C β-1 (PRKCβ-1)	Triggers amplified vascular permeability and angiogenesis by increasing VEGF expression	16	2	South Indian	PDR	[23]
Pigment epithelium derived factor (PEDF)	Activates ocular angiogenesis, downregulation of PEDF is associated with increase VEGF expression in the diabetic eye	17	2	South Indian	PDR	[23]

Ch chromosome, *DM* diabetes mellitus, *Ref* references

metabolic memory phenomenon limits the efficacy in poor glycaemic control cases with DM [24, 25]. Diabetic macular oedema is usually intervened with laser photocoagulation which is a destructive treatment; this can induce scarring of the retina and apoptosis of the retinal pigment epithelium (RPE) which causes diminution of the visual acuity [1]. Intraocular corticosteroid treatment has an incidence of cataracts, elevated IOP, haemorrhage and endophthalmitis. Intravitreal triamcinolone injections also show an increase in IOP; other complications are secondary ocular hypertension and nuclear cataract [25]. Intravitreal anti-VEGF includes a high prevalence of non-responders; a serious sight-threatening adverse effect of anti-VEGF in proliferative diabetic retinopathy is tractional retinal detachment. They often result in a reduction of the visual field, decreased contrast sensitivity and impaired colour perception, resistance to therapy is seen in repeated injections [24].

15.8 Gene Therapies for DR

The aim of gene therapy is to attain an adequate expression of a transgene at a level to decrease or cure disease conditions with negligible complications. Gene therapy allows the intervention of the condition during the early stages before progression to vascular and neuronal damage. The established gene therapy strategies are gene augmentation, gene-specific targeting and genome editing [1]. Gene augmentation is the introduction of a functional gene into the host cell to repair a defective gene; this method is often used for monogenic diseases [4]. Gene-specific targeted therapy is designed to modify the function of an existing defective gene. Genome editing repairs a mutant gene into a functional gene [4].Gene therapy possesses many advantages over other treatments for DR; it enables longer therapeutic effect, easier administration, early intervention and fewer adverse effects [4, 7].

15.9 Vectors of Gene Therapy

A gene vector is a molecular device thatcarries it into the cell nucleus for transcription. Retinal diseases are commonly cured with adeno-associated viral (AAV) vectors and lentivirus [27, 28]. The two main routes for vector delivery are intravitreal and sub-retinal injections and subretinal delivery to photoreceptors and RPE [29, 30]. The right choice of vector is the integral part ofgene therapy for favourable outcomes. The most often used vector for ocular gene therapies isviral vectors-based delivery [31]. Other vector models are non-viral vectorsthatare engineered to transmit large gene loads;large-scale production is easier and cheaper [4].

15.10 Targeting Retinal Vasculopathy

DME and PDR are managed with intravitreal anti-VEGF agents;they bind through-VEGF and stops the pathway to decrease neovascularisation. Various experiments have been conducted to inhibit the intraocular VEGF pathway both extra- and intracellularly [32]. sFlt-1is a soluble splice variant of VEGF receptor-1 (VEGFR) that

acts as a decoy VEGF of the extracellular space, various studies have been reported that this gene reduces neovascularisation [26, 27, 33]. Flt23k is reported to be a novel intraceptor consisting of binding domains 2 and 3 of VEGFR1; they couple with the endoplasmic reticulum retention signal sequence lysine-aspartic acid glutamic acid leucine [1]. One more method to suppress retinal angiogenesis is through the incorporation of inhibitors, namely pigment epithelium-derived factor(PEDF) which could downregulate VEGF. Other transgenes that have the potential to reduce angiogenesis include angiotensin, endostatin, tissue inhibitor metalloprotein-3 and calreticulin anti-angiogenic domain (CAD) [1–4].

15.11 Vascular and Neuronal Protection

Vascular and neuronal degeneration could be intervened with gene therapy before apparent clinical pathologies. Retinal neurons and BRB can be protected from membrane attack complex damage by using a soluble cluster of differentiation-59 (sCd59) [29, 30]. Increasing neurotrophic factors like brain-derived neurotrophic factor (BDNF) have been aimed to decrease oxidative stress through manganese-dependent superoxide dismutase (MsSOD) delivery. Similarly, other goals are to regulate renin–angiotensin system with ACE 2 and Mas receptors [31].

15.12 Future Directions

Despite the intense accumulation of studies, the genetics of DR remain unclear. The majority of the studies report single gene targeting pathological changes. Gene therapy may be effective in treating monogenic defects, but in complex diseases like DR, delivery of multiple transgenes should be focused [34]. Another approach is transgene expression regulation according to the disease mechanism, this allowsan increase of gene expression during disease progression or vice versa [4]. Targeting specific ethnic populations may be beneficial to narrow down the associations. Another gene therapy strategy is targeting the renin–angiotensin system (RAS) pathway and antioxidant which hold effective management for microvascular complications [2]. Future studies and funding agencies should concentrate on human trials as diabetes mellitus is one of the rapidly progressing diseases in epidemic proportions.

15.13 Conclusion

Diabetic retinopathy is a polygenic complex disease that requires novel treatments like gene therapy. However, further research must be conducted to explore the molecular genetics which influence the progression, to halt the disease at an early stage.

References

1. Wang JH, Roberts GE, Liu GS. Updates on gene therapy for diabetic retinopathy. Curr Diab Rep. 2020;20(7):22.
2. Nare S, Mohan S, Satagopan U, Natarajan S, Kumaramanickavel G. Diabetic retinopathy: clinical, genetic, and health economics (An Asian perspective). Adv Vision Res. 2019;2:345–56.
3. Kumari N, Karmakar A, Ganesan SK. Targeting epigenetic modifications as a potential therapeutic option for diabetic retinopathy. J Cell Physiol. 2020;235(3):1933–47.
4. Wang JH, Ling D, Tu L, van Wijngaarden P, Dusting GJ, Liu GS. Gene therapy for diabetic retinopathy: are we ready to make the leap from bench to bedside? Pharmacol Ther. 2017;173:1–18.
5. Arar NH, Freedman BI, Adler SG, et al. Heritability of the severity of diabetic retinopathy: the FIND-Eye study. Investig Ophthalmol Vis Sci. 2008;49(9):3839–45.
6. Han J, Lando L, Skowronska-Krawczyk D, Chao DL. Genetics of diabetic retinopathy. Curr Diab Rep. 2019;19(9):67.
7. Agarwal A, Ingham SA, Harkins KA, Do DV, Nguyen QD. The role of pharmacogenetics and advances in gene therapy in the treatment of diabetic retinopathy. Pharmacogenomics. 2016;17(3):309–20.
8. Barr CC. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive insulin therapy, by the diabetes control and complications trial/Epidemiology of Diabetes Interventions and Complications Research Group. N. Engl. J. Med 342:381-9, 2000. Surv Ophthalmol. 2001;45(5):459–60.
9. Cabrera AP, Monickaraj F, Rangasamy S, Hobbs S, McGuire P, Das A. Do genomic factors play a role in diabetic retinopathy? J Clin Med. 2020;9(1):216.
10. Burdon KP, Fogarty RD, Shen W, et al. Genome-wide association study for sight-threatening diabetic retinopathy reveals association with genetic variation near the GRB2 gene. Diabetologia. 2015;58(10):2288–97.
11. Awata T, Yamashita H, Kurihara S, et al. A genome-wide association study for diabetic retinopathy in a Japanese population: potential association with a long intergenic non-coding RNA. PLoS One. 2014;9(11):e111715.
12. Calcedo R, Wilson JM. AAV natural infection induces broad cross-neutralizing antibody responses to multiple AAV serotypes in chimpanzees. Hum Gene Ther Clin Dev. 2016;27(2):79–82.
13. Liang S, Pan M, Hu N, et al. Association of angiotensin-converting enzyme gene 2350 G/A polymorphism with diabetic retinopathy in Chinese Han population. Mol Biol Rep. 2013;40(1):463–8.
14. Dos Santos KG, Canani LH, Gross JL, Tschiedel B, Souto KE, Roisenberg I. The -106CC genotype of the aldose reductase gene is associated with an increased risk of proliferative diabetic retinopathy in Caucasian-Brazilians with type 2 diabetes. Mol Genet Metab. 2006;88(3):280–4.
15. Abhary S, Burdon KP, Casson RJ, Goggin M, Petrovsky NP, Craig JE. Association between erythropoietin gene polymorphisms and diabetic retinopathy. Arch Ophthalmol. 2010;128(1):102–6.
16. Zhang HM, Chen LL, Wang L, et al. Association of 1704G/T and G82S polymorphisms in the receptor for advanced glycation end products gene with diabetic retinopathy in Chinese population. J Endocrinol Investig. 2009;32(3):258–62.
17. Taverna MJ, Sola A, Guyot-Argenton C, et al. eNOS4 polymorphism of the endothelial nitric oxide synthase predicts risk for severe diabetic retinopathy. Diabet Med. 2002;19(3):240–5.
18. Wang J, Yang MM, Li YB, Liu GD, Teng Y, Liu XM. Association of CFH and CFB gene polymorphisms with retinopathy in type 2 diabetic patients. Mediators Inflamm. 2013;2013:748435.
19. Chen M, Lin WR, Lu CH, et al. Chimerin 2 genetic polymorphisms are associated with non-proliferative diabetic retinopathy in Taiwanese type 2 diabetic patients. J Diabetes Complications. 2014;28(4):460–3.

20. Maeda M, Yamamoto I, Fukuda M, et al. MTHFR gene polymorphism is susceptible to diabetic retinopathy but not to diabetic nephropathy in Japanese type 2 diabetic patients. J Diabetes Complications. 2008;22(2):119–25.
21. Katakami N, Matsuhisa M, Kaneto H, et al. Monocyte chemoattractant protein-1 (MCP-1) gene polymorphism as a potential risk factor for diabetic retinopathy in Japanese patients with type 2 diabetes. Diabetes Res Clin Pract. 2010;89(1):e9–e12.
22. Uthra S, Raman R, Mukesh BN, et al. Diabetic retinopathy and IGF-1 gene polymorphic cytosine-adenine repeats in a Southern Indian cohort. Ophthalmic Res. 2007;39(5):294–9.
23. Uthra S, Raman R, Mukesh BN, et al. Protein kinase C beta (PRKCB1) and pigment epithelium derived factor (PEDF) gene polymorphisms and diabetic retinopathy in a south Indian cohort. Ophthalmic Genet. 2010;31(1):18–23.
24. Whitehead M, Wickremasinghe S, Osborne A, Van Wijngaarden P, Martin KR. Diabetic retinopathy: a complex pathophysiology requiring novel therapeutic strategies. Expert Opin Biol Ther. 2018;18(12):1257–70.
25. Zhao Y, Singh RP. The role of anti-vascular endothelial growth factor (anti-VEGF) in the management of proliferative diabetic retinopathy. Drugs Context. 2018;7:212532.
26. MankočRamuš S, Kumše T, GlobočnikPetrovič M, Petrovič D, Cilenšek I. SNP rs2073618 of the osteoprotegerin gene is associated with diabetic retinopathy in Slovenian patients with type 2 diabetes. Biomed Res Int. 2013;2013:364073.
27. Pechan P, Rubin H, Lukason M, et al. Novel anti-VEGF chimeric molecules delivered by AAV vectors for inhibition of retinal neovascularization. Gene Ther. 2009;16(1):10–6.
28. Cho H, Sobrin L. Genetics of diabetic retinopathy. Curr Diab Rep. 2014;14(8):515.
29. Lalit PS, Thangal Y, Fayi Y, Takhellambam SD. Potentials of gene therapy for diabetic retinopathy: the use of nucleic acid constructs containing a TXNIP promoter. Open Access J Ophthalmol. 2018;3(2):147.
30. Planul A, Dalkara D. Vectors and gene delivery to the retina. Annu Rev Vis Sci. 2017;3:121–40.
31. Adhi M, Cashman SM, Kumar-Singh R. Adeno-associated virus mediated delivery of a non-membrane targeted human soluble CD59 attenuates some aspects of diabetic retinopathy in mice. PLoS One. 2013;8(10):e79661.
32. Lamartina S, Cimino M, Roscilli G, et al. Helper-dependent adenovirus for the gene therapy of proliferative retinopathies: stable gene transfer, regulated gene expression and therapeutic efficacy. J Gene Med. 2007;9(10):862–74.
33. Jiang J, Xia XB, Xu HZ, et al. Inhibition of retinal neovascularization by gene transfer of small interfering RNA targeting HIF-1alpha and VEGF. J Cell Physiol. 2009;218(1):66–74.
34. Ludwig PE, Freeman SC, Janot AC. Novel stem cell and gene therapy in diabetic retinopathy, age related macular degeneration, and retinitis pigmentosa. Int J Retina Vitreous. 2019;5:7.

Principles of Genetic Counseling in Eye Diseases

16

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Abbreviations

AD	Autosomal dominant
AR	Autosomal recessive
CMA	Chromosomal microarray
DNA	Deoxyribonucleic acid
NF1	Neurofibromatosis type 1
NGS	Next-generation sequencing
RP	Retinitis pigmentosa
VOUS	Variant of unknown significance

16.1 Introduction

Molecular genetic testing has rapidly advanced in recent years, allowing early and accurate diagnosis of inherited eye diseases. Simultaneously, it has led to complexities in counseling the families, as the approach varies from case to case, depending upon the diagnosis, inheritance pattern, genetic heterogeneity or variable penetrance. These advances have led to genetic counseling being an integral part of the management of inherited eye disorders, to help the patient and the family, understand and accept the disease in their lives. Moreover, exciting therapeutic

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development in genetic therapies for ocular disorders makes it important that the families get the latest information and make informed decisions. Majority of ocular disorders are genetic and have varying ages at onset from birth till mid-adulthood posing different challenges in counseling. In this chapter, we would deal with the common case scenarios that pose different challenges during counseling after a brief introduction about genetic counseling and the patterns of inheritance.

Genetic counseling is defined as a systematic way of providing information to the patient and/or the family about the genetic disorder, including details about the diagnosis, causes, recurrence risk and options available for prevention and treatment [1]. An ocular genetic counseling session would include providing the latest information about the:

- Eye problem and its underlying basis
- Available genetic tests, their cost and the diagnostic yield
- Implications of diagnosis on management, prognosis and preventive strategies
- Information on recurrences and the possibility of prenatal diagnosis
- Information about any relevant research options for better understanding the disorder or treatment
- Information about the support groups if available.

While communicating the information to the proband (the individual with the disease, who brings the family to attention)/consultand (person seeking the genetic counseling), basic ***ABC*** of ethical principles must be considered.

Autonomy Autonomy is a long-recognised ethical principle in medical practice [2]. While counseling, the patient/consultand should be given full power to make his/her decisions regarding genetic testing, treatment options, pre-symptomatic or prenatal testing, etc. They should be given the best possible medical information to empower them to make further decisions or to opt out at any time.

Beneficence The counselor needs to act in the best interest of the consultand and help the individual make the best out of the present available opportunities.

Confidentiality It is an important aspect as information about a particular genetic disorder running in any family, a person's carrier status and associated reproductive risks are quite sensitive. It is not only potentially stigmatising in employments and insurance, but also can raise complex family, interpersonal and social issues.

Privacy The patient should have full right to privacy of his/her genetic condition or test results, even when sharing with his/her family. A detailed discussion should be held before disclosing the results to the family members/relatives.

Informed Consent The patient/consultand should be given full information before undergoing any genetic testing or treatment about the procedure to be carried out, detection rate, limitations, the possibility of incidental findings in the test results and possible outcomes of all treatment options. The patient should have the right to choose the tests or the treatment options if available.

Non-directive Counseling Genetic counseling must be non-directive, implying that the patient/consultand should be given full information and full right to make his/her own choices regarding the testing or treatments, and the decisions of the patient/consultant should not be steered in a particular direction. Also, the genetic counselor should be non-judgmental about whatever decision the patients, their families or the consultand makes, even if it is contrary to the belief of the counselor.

16.2 Components of Genetic Counseling

16.2.1 Gathering Information and Pedigree Drawing

A genetic counseling session usually begins with obtaining medical history, family history and pedigree drawing. A pedigree is an indispensable tool that helps in inferring the possible mode of inheritance of the disease in a particular family. In simplex cases, it might be difficult to interpret the mode of inheritance. It is drawn using standardised symbols. The various symbols used for pedigree drawing and their interpretation are shown in Table 16.1.

16.2.2 Establishing a Genetic Diagnosis

Accurate diagnosis forms the cornerstone of effective genetic counseling as it helps in providing disease prognosis, access to treatment if available and recurrence risk assessment. As per the clinical suspicion and clinical geneticist evaluation, a certain set of investigations are performed to make a specific diagnosis. These include chromosomal analysis (Karyotype) for any dysmorphic syndrome with ocular involvement, amino acid analysis as in gyrate atrophy, galactosemia enzyme assay in congenital cataract or specific gene testing for Peter plus syndrome. Next-generation sequencing (NGS) is used for genetically heterogeneous disorders such as retinitis pigmentosa, cataract and ocular disorders with large genes. These tests are special tests that are expensive and require great expertise and in-depth knowledge. These tests have varying diagnostic yield, and should be accompanied by extensive pre- and post-test counseling [3].

Pre-test counseling should include an explanation about what will be tested (chromosomes/genes), what sample would be required (blood or tissue) and what method would be used [(karyotype/Chromosomal microarray (CMA)/NGS]. The detection rates, false-positive rates of the test/s must be told, including the

Table 16.1 Various symbols used for pedigree drawing and their interpretation

Symbol			Interpretation
Male	Female	Gender unspecified	Individual
			
			Affected (with one condition)
			Affected with two or more than two conditions
			Unaffected Carrier
			Consultand
			Proband
Parents	Consanguinity	Divorce	Relationship
			
Pregnancy (singleton)	Monozygotic twins	Dizygotic twins	Pregnancy
			
Missed abortion	Medical termination of pregnancy	Stillbirth	
			
No children (By choice)	Infertility		No children
			
Donor sperm	Donor Ovum	Surrogate	Assisted Reproduction
			
Adoption in	Adoption out		Adoption
			

limitations of the tests and turn-around time. It should address as to how the test results may alter the diagnosis/management or options for prenatal testing. A further explanation of what test results we might get, positive/negative or "secondary

findings" (An incidental finding is an additional finding for which the patient was not actually tested for and may be encountered while doing NGS), and what further testing options would be available, if the test results are negative should be provided. Also, a possibility of getting a variant of uncertain significance (VOUS) after CMA/NGS testing should be explained [4].

Similarly, the post-test counseling should include the discussion on the diagnosis (if confirmed on genetic testing), how the mutation affects the body and causes the disease, the inheritance pattern, treatment options available and any further testing (biochemical validation, sanger validation, variant testing in parents) required. If a VOUS is found, methods to validate the variant (in silico tools, biochemical analysis) should be explained and advised. The risk of transmission (based on inheritance pattern and penetrance) should be calculated and explained, along with the options for prenatal testing. Further, implications for the extended family, e.g. first-degree relatives (siblings/parents) and requirement of clinical examination or genetic testing based on the test results should be discussed.

16.2.3 Risk Assessment

The process of genetic counseling involves risk assessment based on the pattern of inheritance concluded from pedigree analysis and/or diagnosis confirmed from cytogenetic or molecular testing. The various patterns of inheritance and their important characteristics are described below.

16.2.3.1 Autosomal Dominant

Autosomal dominant (AD) traits are those that manifest even in the heterozygous state, i.e. the individual possesses both the abnormal (mutant) allele and the normal allele. The AD disorders show "vertical transmission" and are usually seen in multiple generations. Some of the AD disorders may have new mutations, where family history of such disorders would not be obtained. Both males and females are equally affected and at each subsequent pregnancy, there is a risk of 50% for the offsprings to be affected with the same disorder, e.g. Marfan's syndrome (manifesting ectopia lentis, myopia, tall stature, arachnodactyly and aortic root dilation).

Some AD traits disorders show a difference in the phenotypic expression of the disease, also known as *variable expressivity*. For example, in families with neurofibromatosis type 1, some individuals manifest cafe-au-lait-macules with Lisch nodules and have no neurofibromas, while some family members have neurofibromas, although, all of them harbour the same genetic variant in the NF1 gene.

In some AD disorders, few individuals harbouring the disease causing variants do not manifest the disease, which is referred to as *incomplete penetrance.* The

Table 16.2 Characteristics of autosomal dominant inheritance

Autosomal dominant inheritance
• Manifest even in heterozygous state
• Multiple generations affected
• Equal sex distribution in males and females
• Vertical transmission
• Variable expression- a person with mutant gene manifests the trait but severity of expression varies among different individuals
• Reduced penetrance- a person with a mutant gene may or may not manifest the disease
• Risk of transmission to next generation by an affected parent—50%
• Examples:Neurofibromatosis, Axenfeld–Reiger syndrome

Table 16.3 Characteristics of autosomal recessive inheritance

Autosomal recessive inheritance
• Manifests in homozygous state
• Multiple members in one generation affected (horizontal transmission)
• Equal sex distribution in males and females
• Parents asymptomatic, can be carriers for the mutant allele
• Consanguinity may be present
Examples: Leber's congenital amaurosis, Bardet–Biedl syndrome, Costeff syndrome

factors like incomplete penetrance and variable expressivity need to be considered while assessing the risk for a consultand. The characteristics of autosomal dominant inheritance are shown in Table 16.2.

16.2.3.2 Autosomal Recessive

When a disease manifests only in the homozygous or compound heterozygous state, it is said to have an autosomal recessive inheritance. The carriers in these disorders (harbouring only one mutant allele) do not manifest the disease. Consanguinity in families increases the risk of autosomal recessive disorders, and at each conception, there is a risk of 25% for the offsprings to be affected, if both parents are heterozygous (carriers) of the disease causing variant. For example, Usher syndrome (retinitis pigmentosa with sensorineural hearing loss) shows an autosomal recessive inheritance pattern. The characteristics of autosomal recessive inheritance are shown in Table 16.3.

16.2.4 X-Linked Inheritance

16.2.4.1 X-Linked Recessive

X-linked recessive conditions are those caused by the genes located on the X-chromosome and usually manifest only in males, owing to the presence of single X-chromosome, that harbours the mutant allele. The females with two

Table 16.4 Characteristics of X-linked recessive inheritance

X-linked recessive inheritance
• Males are predominantly affected as they have single X chromosome
• Affected males have carrier daughters
• Carrier females transmit disorder to 50% of their sons
• Skip generation can be seen
• No male to male transmission
• "Diagonal" or "knight's move" pattern of transmission
Examples: Norrie disease, X-linked macular dystrophy, X-linked retinitis pigmentosa, Colour blindness

Table 16.5 Characteristics of X-linked dominant inheritance

X-linked dominant inheritance
• Males and females are affected but often an excess of females
• Females less severely affected
• No male to male transmission
• Affected males will transmit the disease to all daughters
• Affected females will transmit the disease to 50% sons and 50% of daughters
• Some disorders are lethal in males
Examples: Incontinentia pigmenti, Aicardi syndrome

X-chromosomes are asymptomatic carriers, who can transmit the disease to 50% of their sons. Males, with the disease, will transmit the disease to all of their daughters and none of their sons. Hence, in these pedigrees, male-to-male transmission is not seen, e.g. X-linked red-green colour blindness. The characteristics of X-linked recessive inheritance are listed in Table 16.4.

16.2.4.2 X-Linked Dominant

X-linked dominant conditions are also caused by the mutations in genes carried on X-chromosome, but they manifest even in the heterozygous females. Seen across the population, more number of females are affected, but males are more severely affected. Some disorders may show male lethality. The affected females can transmit the disease to 50% of their sons and 50% of their daughters, while affected males will transmit the disease to all of their daughters and none of their sons. For example, incontinentia pigmenti (characteristic skin lesions with retinal abnormalities). The characteristics of X-linked dominant inheritance are presented in Table 16.5.

16.2.4.3 Mitochondrial Inheritance

This type of inheritance is exclusively maternal, as all the mitochondria are transmitted through oocyte only. Apart from nuclear DNA, there is mitochondrial DNA, that has 37 genes. Hence, a female carrying the mutation can transmit the disease to both her sons and daughters, but a male carrying a mutation, cannot transmit the disease to any of his children,e.g. Leber's hereditary optic neuropathy.

16.2.4.4 Digenic Inheritance

It is a non-mendelian pattern of inheritance, where mutations in two genes at two different loci are required to produce a disease phenotype. Mutation in either one of those genes does not lead to the disease. A classic example of digenic inheritance is retinitis pigmentosa.

16.2.4.5 Psychosocial Counseling

An ideal counseling session should be undertaken in strict privacy, in a quiet and comfortable place. While counseling, one should be a sympathetic listener, to promote coping and help with the adjustment. One should be cautious while dealing with the issues related to blaming self or other partner, social stigma and family conflicts.

16.3 Common Counseling Scenarios That Illustrate the Various Principles of Genetic Counseling

16.3.1 Case Scenario 1: Previous Child with Bilateral Retinoblastoma

A 32-year-old female has come for pre-conceptional counseling as her 4-year-old son had bilateral retinoblastoma. The child underwent enucleation of the right eye, followed by chemotherapy. He was developmentally normal, and there was no history of any other cancers in the family (Fig. 16.1).

16.3.1.1 Genetic Counseling

Retinoblastoma provides a special model for genetic counseling, as many factors are involved. About 15% of the unilateral tumours and almost 99% of the bilateral tumours are heritable (can be passed on to the nextgeneration) [5]. Further, the penetrance is only 90%, implying that only 90% of those harbouring the mutant allele will manifest the disease. In a few patients, spontaneously regressed tumour (retinoma) is seen; hence, parents of an affected child must be examined for a retinoma, as it changes the recurrence risks in future pregnancies. A few cases of retinoblastoma are associated with developmental delay, dysmorphism or malformations, which are usually caused by 13q microdeletions.

When counseling the female with the previous child affected with bilateral retinoblastoma, a detailed discussion about the disease, treatment options, survival outcomes is followed by the recurrence risks. Since most of the bilateral retinoblastomas are heritable, the first step in determining heritability is the examination of the parents for any spontaneously regressed tumour. The parents should be explained about the autosomal dominant inheritance and that each child of the affected parent has a 45% chance of developing retinoblastoma (considering 90% penetrance).

The option of molecular testing by sequencing and detection of deletions/duplications of RB1 gene should be discussed. Possibilities of not finding a causative variant should be explained. Once a variant is found in the molecular test results, the parents

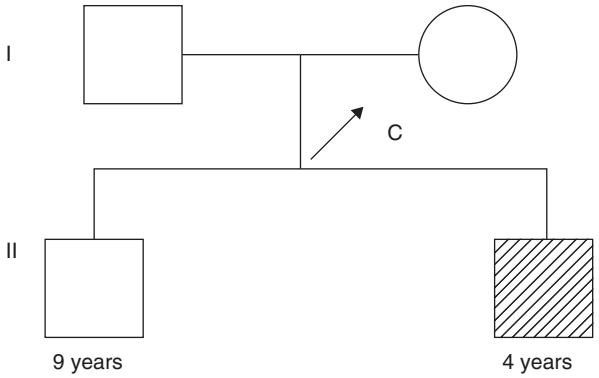

Fig. 16.1 Pedigree of the family with a child with bilateral retinoblastoma

should be tested for the variant. If the parent harbours the same variant, the recurrence risk is 45%, but if not found in the parent, the recurrence risk remains around 2% due to the risk of germline mosaicism. Also, if the variant is found in the parents, implications to the parent in view of developing non-ocular tumours (osteosarcoma, melanoma etc.) should be discussed, along with the options for prenatal testing in future pregnancies. If the parents are not able to get the molecular testing, then the empirical risk of recurrence will be around 2% for bilateral RB and 1% for unilateral RB in clinically unaffected parents and 45% if one of the parent has unilateral retinoblastoma with a positive family history, or a bilateral retinoblastoma, irrespective of the family history.

In the above-mentioned case, genetic testing showed c.2489+1G>A variant in the RB1 gene, and the parents were clinically and molecularly normal; therefore, the family can be given a risk estimate of 2%.

16.3.2 Case Scenario 2: Previous Child with Cataract and a Positive Family History of Cataract

A 4-year-old child presented with a bilateral congenital cataract, which was operated on. There was a history of similar cataract in the elder sister, father and paternal grandmother. No other organ system was involved and all the family members were intellectually normal. Also, his mother was 12 weeks pregnant, and concerned about the risk of the next child developing similar condition (Fig. 16.2).

16.3.2.1 Genetic Counseling

An isolated congenital cataract is caused by mutations in a large number of genes (genetic heterogeneity), and all forms of inheritance (AD, AR, X-linked recessive) are seen, but most of the cases show AD inheritance. In the given pedigree, vertical transmission and affection in all the generations can be appreciated. Both males and females are affected and male-to-male transmission is seen. Hence, an autosomal dominant inheritance is likely. As per the inheritance pattern, a recurrence risk of 50% in each subsequent pregnancy may be given to the family.

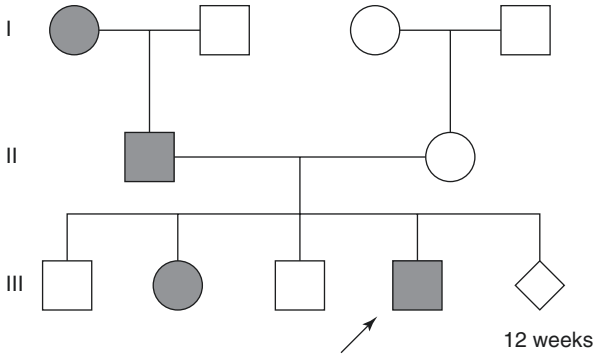

Fig. 16.2 Pedigree of the family with congenital bilateral cataract

Options for molecular testing using NGS should be discussed, explaining the possibility of also getting incidental findings or even not finding a disease causing variant at all. If the family opts for molecular testing, and a disease causing variant is found, prenatal testing in subsequent pregnancies can be done, by chorionic villus sampling at 11–13 weeks of pregnancy.

16.3.3 Case Scenario 3: Leber's Hereditary Optic Neuropathy (LHON)

Three siblings from a family presented with progressive loss of central vision and were diagnosed as Leber's hereditary optic neuropathy. There was a history of similar manifestations in mother and maternal uncle and aunt along with maternal grandmother (Fig. 16.3).

16.3.3.1 Genetic Counseling

LHON is caused by point mutations in the mitochondrial DNA, but the inheritance is complicated by the incomplete penetrance, which implies that a few individuals with the mutant allele, do not develop optic neuropathy. Approximately 50% of the males and 10% of the females with pathogenic LHON mutation develop the disease [6]. Another factor affecting the disease expression is heteroplasmy, which is seen in 10–15% of individuals with LHON mutations [7]. Heteroplasmy refers to a mixture of mitochondria with mutation and without the mutation (wild type). A higher level of mutated mitochondria would lead to an expression of the disease, while a low level would lead to incomplete penetrance.

In this pedigree, the disease is being transmitted to all the children of an affected mother, but none of the children of an affected father. This is a pattern of inheritance typical to mitochondrial disorders (caused by mutations in the mitochondrial genome). Since the mitochondria in an embryo are contributed exclusively by the oocyte, the mutant mitochondrial genes are transmitted to all the offsprings of an affected mother, while the affected father does not transmit the mutant allele to any of his children.

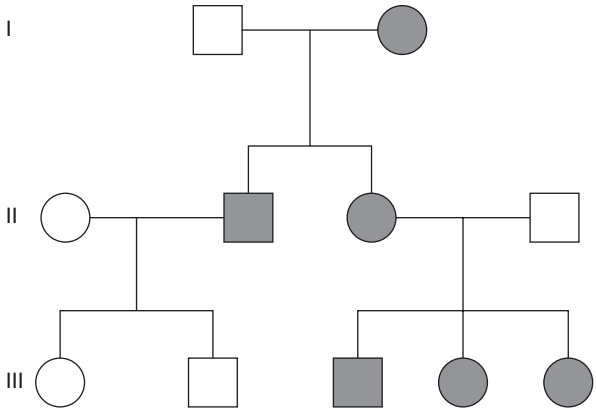

Fig. 16.3 Pedigree ofthe family with Leber's hereditary optic neuropathy (LHON)

A detailed explanation of the diagnosis, visual outcomes and treatment options, followed by a risk of recurrence and options for molecular testing of the mitochondrial genome should be discussed. Since the mother is affected in this family, there is a 100% risk of foetus being affected in subsequent pregnancies, but the phenotype, age of onset and visual outcomes cannot be predicted.

16.3.4 Case Scenario 4: Retinitis Pigmentosa

A 12-year-old male child presented with slowly progressive loss of vision in the dark and on examination was diagnosed as retinitis pigmentosa. Father was similarly affected, and the other three siblings were normal. The molecular testing using next-generation sequencingfor the proband revealed heterozygous mutations in ROM1 gene and peripherin/RDS gene. The same variants were found in father (Fig. 16.4).

16.3.4.1 Genetic Counseling

Retinitis pigmentosa is an inherited retinal degeneration and shows genetic heterogeneity (caused by many genes) and AD, AR, X-linked patterns of inheritance. A particular type of retinitis pigmentosa (RP7) is caused by heterozygous mutations in both the ROM1 gene and peripherin/ RDS gene [8]. Mutations in either of the gene alone do not result in the RP phenotype. This type of inheritance pattern is termed as a *digenic inheritance.*

The family should be counseled regarding the disease course, visual outcomes and pattern of inheritance, explaining the family that the next child can be affected if both the mutant alleles are inherited, and will be unaffected if either of them is present. Prenatal testing can be carried out in the next pregnancy by testing the chorionic villus samples for both variants.

Another issue to be addressed here is the late-onset nature of the disease, which may manifest during adolescence or adulthood. In this case, the youngest sibling is currently 3 years old and is asymptomatic. The molecular testing of the child would

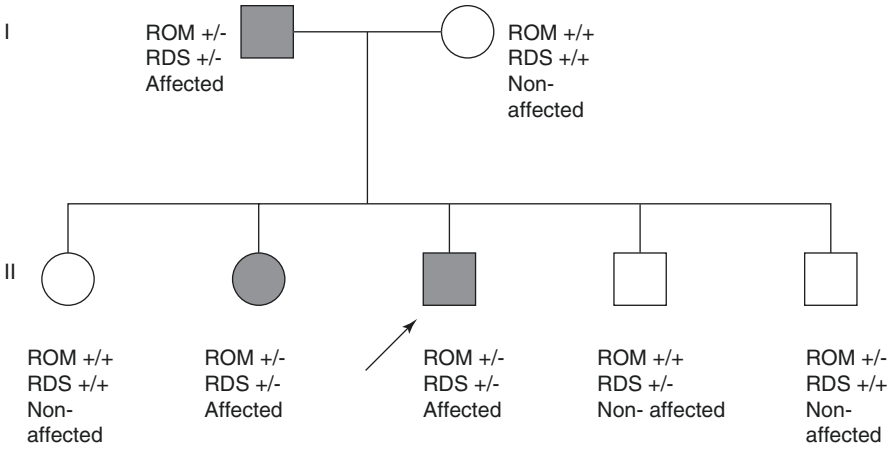

Fig. 16.4 Pedigree of a family with retinitis pigmentosa

determine if he has inherited the pathogenic variants or not, but would not determine the age of onset if the variants are present. This type of testing for late-onset disorders, before the symptoms appear is known as *pre-symptomatic testing* or *predictive testing*. There has been a debate regarding the offering of predictive testing for non-treatable disorders. Some parents affirm in knowing the status of their child, as it would help them guide their child towards the most appropriate support and be compliant to a better follow up, it could also be argued that this testing compromises the child's future autonomy and may harm the child's psyche, as he/she would grow up with the knowledge of developing a disorder later in life. Hence, most geneticists recommend delaying the testing for the disorders for which immediate treatment is not available, until 18 years of age when an informed decision is possible.

16.3.5 Case Scenario 5: Leber's Congenital Amaurosis (LCA)

The family has been sent for genetic counseling as three children are affected by Leber's congenital amaurosis to a consanguinous couple. Parents are unaffected. The couple wants to know the risks of having a future affected baby (Fig. 16.5).

16.3.5.1 Genetic Counseling

A detailed explanation about the genetic disorder, prognosis, treatment options and options for molecular testing should be discussed. There are at least 19 different types of LCA described, each one caused by a different gene, the molecular testing can be done using a targeted panel. Since the disease is inherited in an autosomal recessive manner, as is evident in the pedigree analysis too, the recurrence risk in future pregnancies would be 25%. The prenatal testing can be done, once the variant is known in the proband. One particular form, LCA2 (caused by mutations in the RPE65 gene), is amenable to treatment, by using FDA approved gene therapy. Clinical trials have

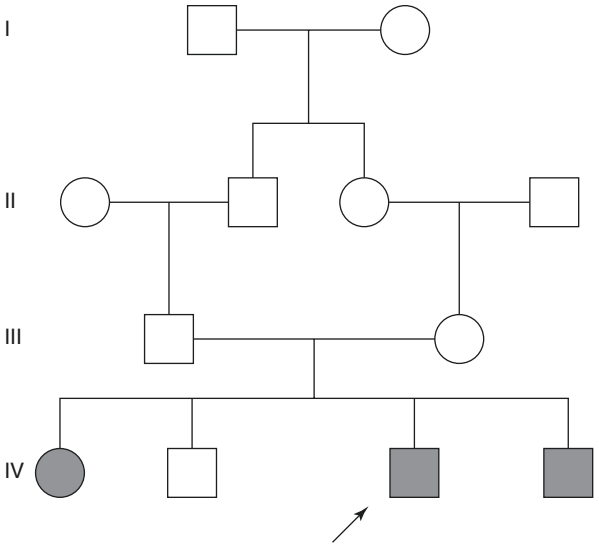

Fig. 16.5 Pedigree of a family with Leber's congenital amaurosis (LCA)

shown improvement in vision, after a sub-retinal injection of AAV (adeno-associated virus) containing the RPE65 gene. The information about this treatment can be given to the patient after the molecular testing has confirmed a variant in the RPE65 gene, but the cost and non-availability of this treatment in India should also be explained.

16.3.6 Case Scenario 6: Norrie Disease, X-Linked Disorder

A 3-year-old child presented with retinal detachment and visual loss along with developmental delay and autistic features. A clinical diagnosis of Norrie disease was suspected (Fig. 16.6).

16.3.6.1 Genetic Counseling

A provisional clinical diagnosis of Norrie disease was made based on the X-linked pattern of inheritance seen on pedigree analysis, which shows only males being affected and the disease being transmitted through carrier females.

Norrie disease is a rare disorder, that affects primarily the males, and leads to early-onset blindness (at birth or soon after birth). It is caused by mutations in the NDP gene. Besides, the patients may have progressive hearing loss and developmental delay. The visual prognosis is poor, and additional neurological features apart from developmental delay may include seizures or autistic features in a few individuals.

Since the maternal grandfather of the child was also affected, the mother is an obligate carrier here, which implies that the disease can be transmitted to 50% of her sons, and 50% of her daughters will be carriers. The prenatal testing at 11–13 weeks of gestation can be done, once the variant is identified in the NDP gene using direct gene sequencing.

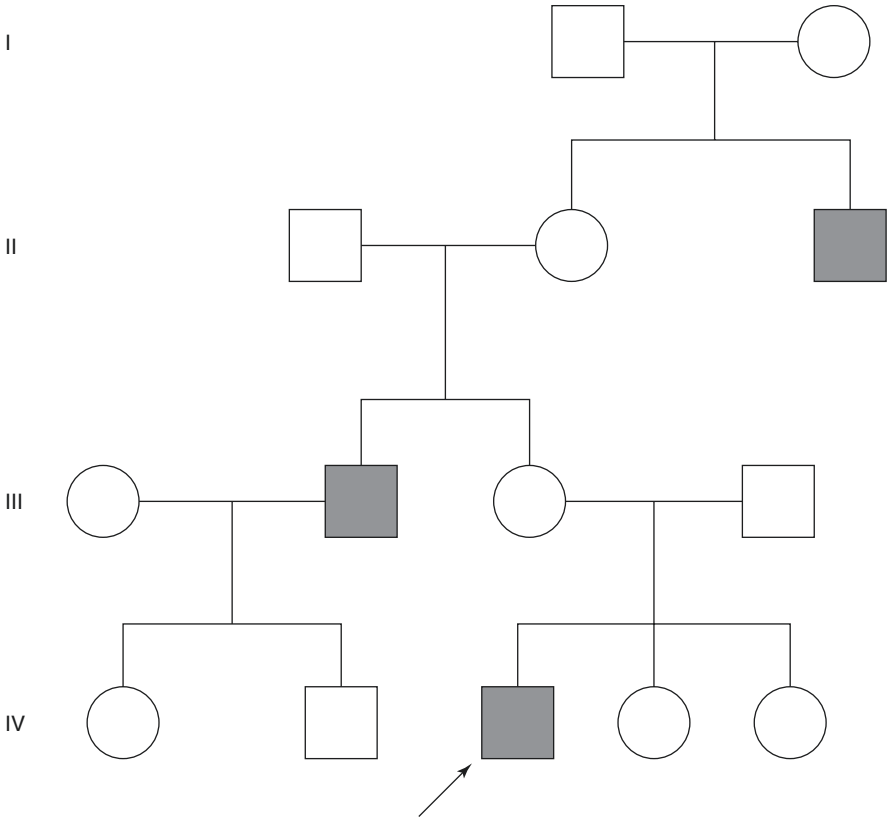

Fig. 16.6 Pedigree of a family with Norrie disease

16.3.7 Case Scenario 7: Stickler Syndrome

A 3-year-old child was brought for evaluation in view of non-progressive high myopia. On examination, the child also had a cleft palate and short stature, along with a flat facial profile. There was no family history (Fig. 16.7).

16.3.7.1 Genetic Counseling

A clinical diagnosis of Stickler syndrome was made and advised for molecular testing by NGS. Stickler syndrome is a collagenopathy, and results due to mutations in any one of the six genes—*COL2A1*, *COL11A1*, *COL11A2*, *COL9A1*, *COL9A2*, *COL9A3.*

The pattern of inheritance here, cannot be deduced from the pedigree, hence, molecular confirmation of the mutation would help to predict the risk of transmission. Careful parental clinical evaluation is needed to assess for the mild clinical features of Stickler syndrome. Stickler syndrome caused by mutations in *COL2A1*,

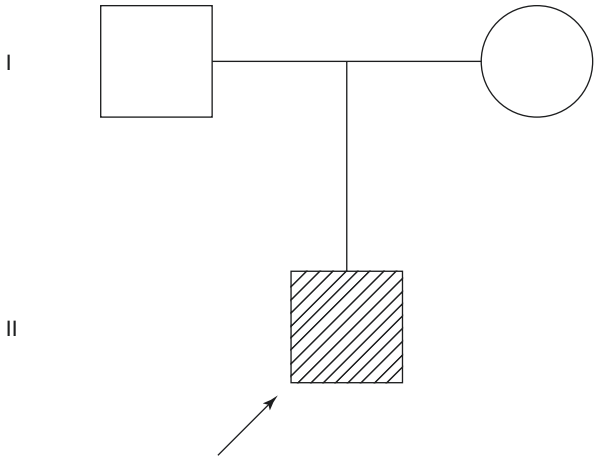

Fig. 16.7 Pedigree of a family with Stickler syndrome

COL11A1, or *COL11A2* genes is transmitted in an autosomal dominant manner and that caused by mutations in genes *COL9A1*, *COL9A2*, or *COL9A3* have an autosomal recessive inheritance. Counseling should include issues related to variable expression and penetrance. Pre-test and post-test counseling should be done.

16.4 Conclusion

To conclude, genetic counseling has become an integral component of ophthalmology due to a wider availability of genetic testing and recognition of underlying genetic etiology for an ocular manifestation. Effective counseling needs interaction with clinical geneticists and genetic counselors due to its implication on family members and preventing recurrences. Latest information should be provided in a simplified language along with appropriate psychosocial counseling.

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References

1. Turnpenny PD, Ellard S. Emerys elements of medical genetics. Philadelphia: Elsevier; 2017.
2. Page K. The four principles: can they be measured and do they predict ethical decision making? BMC Med Ethics. 2012;13:1–8.
3. Lenassi E, Clayton-Smith J, Douzgou S, et al. Clinical utility of genetic testing in 201 preschool children with inherited eye disorders. Genet Med. 2020;22(4):745–51.
4. Matthijs G, Souche E, Alders M, et al. Guidelines for diagnostic next-generation sequencing. Eur J Hum Genet. 2016;24:2–5.
5. Houdayer C, Gauthier-Villars M, Castéra L, Desjardins L, Doz F, Stoppa-Lyonnet D. Retinoblastoma – genetic counseling and molecular diagnosis. London: IntechOpen Limited; 2012. https://doi.org/10.5772/34069.

6. Man PYW, Turnbull DM, Chinnery PF. Lebers hereditary optic neuropathy. J Med Genet. 2002;39:162–9.
7. Phasukkijwatana N, Chuenkongkaew WL, Suphavilai R, Luangtrakool K, Kunhapan B, Lertrit P. Transmission of heteroplasmic G11778A in extensive pedigrees of Thai Leber hereditary optic neuropathy. J Hum Genet. 2006;51(12):1110–7.
8. Kajiwara K, Berson E, Dryja T. Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. Science. 1994;264(5165):1604–8.