An Insight into Ocular Genetics

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Abstract

Over the past decade, there has been an exponential increase in the database of heritable eye disorders. More than 100,000 germline mutations reported in more than 3,700 different human nuclear genes are associated with inherited diseases. Continuously increasing mutation database has revolutionized the study of human genome and facilitated “personalized genomics.” With more than 300 new “inherited disease genes” being identified annually, it is essential to ask how many “inherited disease genes” are in the human genome, containing how many mutations, and where they are located? Knowledge of the clinical and molecular features of ocular genetics and inherited eye diseases is important for appropriate diagnosis and patient management. Genetic causes for a wide range of eye diseases have been identified, leading to discovery of genes associated with the eye disorder. Discovery of these new genes has led to a rethinking and a reclassification of eye disorders that were earlier based only on classical clinical signs, but now also on underlying genetic aetiology. Some of these disorders include the corneal dystrophies, rare forms of strabismus, ocular disorders resulting from mutations in transcription factors, cataract that result from mutations in crystallins and other structural lens components, retinal dystrophies that result from defects in phototransduction or visual cycle defects and many more. This article discusses molecular basis of some of these eye disorders and also advances in the field of ophthalmic genetics.

Keywords: genetics, epigenetics, microRNAs, inheritance, mitochondrial inheritance, tumor suppressor, genetic testing, neurodegeneration, pathogenesis

Introduction

Genetic disorders have been classified as single gene (monogenic disorders), polygenic and complex disorders. The single gene disorders were further classified as autosomal dominant autosomal recessive X-linked dominant X-linked recessive and Y-linked trait depending on the pattern of inheritance. Complex or multifactorial disorders are associated with effects of multiple genes in combination with lifestyle and environmental factors. There has been dramatic advances in the elucidation of the genetic aetiology of inherited eye diseases. The genes for a wide range of eye diseases have been identified and have led to a rethinking and a reclassification of disorders that is based not only on classical clinical signs, but also on underlying genetic aetiology. What allowed gene discovery in most of these conditions were the concerted efforts to collect families and refine phenotypic delineation over decades by clinicians and clinician-scientists interested in genetics. This permitted genetic mapping and, through classic candidate gene or other approaches, leading to the identification of the responsible gene(s). Laboratories around the world are offering genetic testing for increasing numbers of ocular malformations and retinal dystrophies. This is done either through direct sequencing of the whole gene or of exons most likely to carry mutations, or through the use of microarrays that detect mutations that have been reported in the literature. The contribution of ocular genetics to human discovery has been exceptional, beginning with a strong interest in ocular genetics by many clinical ophthalmologists who carefully described the patterns of inheritance of familial eye disorders. Of the approximately 4000 genetic diseases and syndromes known to affect humans, at least one-third involve the eye. These single-gene defects are the types of genetic abnormality called the Mendelian disorders. They are subdivided into autosomal dominant, autosomal recessive and X-linked or sex-linked. Epigenetic mechanisms such as aberrant DNA methylation have been found to be associated with various ocular pathologies such as age-related macular degeneration, susceptibility to oxidative stress, cataract, pterygium, and retinoblastoma. Changes in histone modifications have also been observed in experimental models of diabetic retinopathy and glaucoma. The expression levels of specific microRNAs have also been found to be altered in ocular inflammation, retinal degeneration, pathological angiogenesis, diabetic retinopathy, and ocular neoplasms. Although the complete spectrum of epigenetic modifications and how the underlying mechanism is operative in various ocular diseases remains to be fully elucidated, it is clear that epigenetic deregulation is an important contributor to common ocular diseases and may be a relevant therapeutic target for future therapies.

Some Common Ocular Genetic Disorders

A genetic or inherited disease is a condition that may be passed on from parent to their children through the coded information contained in the genes. Inherited disorders differ from the medical disorders in that they tend to recur within families and often, the risk of occurrence for other family members can be predicted.

Aniridia

Aniridia is a rare disorder that causes corneal pannus, cataract, glaucoma, partial or complete absence of the iris,
and foveal hypoplasia. The degree of visual impairment is quite variable and the phenotype of aniridia can include cases of autosomal dominant keratitis. This is an autosomal dominant disorder inherited directly from a parent who has aniridia. It is the result of a mutation in a gene called PAX6 (Figure 1). Aniridia is associated with many serious ocular (eye) conditions, including cataract, glaucoma and corneal pannus. Each child of a person with aniridia will have a 50% chance of inheriting the gene mutation.4 Riccardi and co-workers described several cases of aniridia and Wilms tumor associated with an interstitial 11p deletion and proposed a contiguous gene syndrome (WAGR = Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation) due to genes in the region.9 The syndromic form of aniridia (WAGR) results from deletion of the contiguous genes: PAX6 and the Wilms tumor suppressor gene 1 (WT1).10

**Glaucoma**

Glaucoma includes a set of ocular neurodegenerative disorders that have in common a distinct type of optic nerve damage due to increased intraocular pressure (IOP) causing irreversible defects in the visual field and vision loss if left undetected or untreated.11 When the accepted range for IOP (10-22 mm Hg) increases, it results progressive degeneration of retinal ganglion cells, thinning of the retinal nerve fibre layer, cupping of optic nerve head.22 It is considered as one of the most frequent causes of irreversible blindness worldwide, affecting around 60 million people. It can be manifested early at birth (Figure 2) or can have a late onset. Broadly, it is classified into 3 types i.e. primary open angle glaucoma (POAG; no OMIM entry), primary angle closure glaucoma (POAG; OMIM 137760), and primary congenital glaucoma (PCG; OMIM 231300).13 Altered immunity, impaired microcirculation, oxidative stress and excitotoxicity may cause secondary neurodegeneration of retinal neurons and cells involved in central visual pathway by altering their environment and increasing susceptibility to damage.14 Although the molecular mechanism behind the pathogenesis of glaucoma is largely unknown, but numerous studies suggested familial clustering in all forms of glaucoma which indicate presence of substantial genetic component.15

GLC1A is the first reported locus for POAG on chromosome number 1 (1q24.3-q25.2) containing gene MYOC, which encodes the protein myocilin. In juvenile or early adult form of POAG, disease-associated less soluble forms of myocilin (result from disease associated mutation of MYOC) interfere with protein trafficking and result in aggregation of misfolded protein in endoplasmic reticulum which in turn causes loss of trabecular meshwork cells, resulting in high IOP. Optineurin (OPTN) at GLC1E (10p15-p14), is primarily responsible for familial cases of normal tension glaucoma (NTG), a type of POAG in which there is progressive retinal ganglion cell loss despite normal IOP. WD repeat domain 36 (WD36) at GLC1G (5q21.3-q22.1) appears to contribute to POAG severity rather than its development.22 An expanded genome-wide association study (GWAS) and replication study of PACG has identified total eight susceptibility loci at EPDR1 rs3816415, CHAT rs1258267, GLIS3 rs736893, FERM2 rs7494379, DPM2–FAM102A rs3739821, PLEKH7 rs11024102, PCMTD1–ST18 rs1015213, COL11A1 rs3753841.15 This indicates that POAG and PACG are distinct genetic entities with different genes associated with each disease. Mitochondrial sequence variations have been associated with POAG, PCG, PACG, Leber’s hereditary optic neuropathy (LHON), pseudoexfoliation glaucoma (PEG), and other spontaneous optic neuropathies.17 Mitochondrial DNA variations adversely affect the respiratory chain; impair the oxidative phosphorylation pathway, resulting in low ATP production; and impair the growth, development, and differentiation of trabecular meshwork.18 Missense mutations, nonsense mutations, frameshifts and large-gene deletions in CYP1B1, encoding cytochrome P450B1 (GLC3A), MYOC, FOXC1, LTBP2 (latent transforming growth factor binding protein 2) have been associated with PCG, an autosomal recessive disease. CYP1B1, a dioxin-inducible enzyme belonging to the cytochrome P450 superfamily, plays important role in a variety of developmental processes including in utero development of ocular structures, hence its malfunction can lead to ocular developmental defects leading to PCG.19

**Leber’s Hereditary Optic Neuropathy**

Leber’s Hereditary Optic Neuropathy (LHON) was first reported in a patient more than 150 years ago but it was (OMIM 535000) first described as a distinctive clinical entity in 1871 by the German ophthalmologist Theodore Leber (1840–1917).19 The prevalence of LHON is estimated to be 1:50,000 which can occur at any age20 with acute, painless loss of central vision. In about 25% of LHON cases, visual loss is bilateral at onset. Unilateral optic nerve involvement in LHON is exceptionally rare; if it is present, another underlying pathologic process should be actively excluded.21 If unilateral, the fellow eye is usually affected within six to eight weeks. LHON is a maternally inherited disease and

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**Figure 1:** Bilateral Congenital Aniridia condition caused by mutation in PAX6 gene

**Figure 2:** Bilateral Primary Congenital Glaucoma condition caused by mutation in glaucoma associated gene.
shows variable penetrance with a male preponderance of 86%. LHON often progresses rapidly which leads to severe visual loss with only little probability of visual recovery. LHON is usually caused by mtDNA mutations residing in genes encoding subunits of complex I (component of mitochondrial respiratory chain). Ophthalmologic findings in LHON patients are variable but classical LHON cases exhibit abnormalities like vascular tortuosity of the central retinal vessels, swelling of the retinal nerve fibre layer, a circumpapillary telangiectatic microangiopathy and a cecocentral scotoma develops with variable preservation of peripheral vision.

Nearly all patients worldwide carry one of three mtDNA pathogenic point mutations at positions ND4:p.R340H, ND1:p.A52T, and ND6:p.M64V. Other pathogenic mtDNA LHON variants have also been described in various studies, with some still awaiting full confirmation for pathogenicity but MTND1 and MTND6 genes of mitochondria are thought to be "mutational hotspots" for LHON-causing mutations, in addition to primary LHON mutations.

Retinoblastoma
Retinoblastoma (Rb) (OMIM 180200) is the most common neoplasm of the eye occurring in early childhood and accounts for about 2.5-4% of all the childhood malignancies. Rb affects very young children where two-third children are diagnosed before the age of 2 years and more than 90% are diagnosed before the age of 5 years (Figure 3). The worldwide incidence is of Rb is 1 case in every 15,000-20,000 live births which corresponds to nearly 9,000 new cases every year. The basis for Rb development was first explained by Alfred G. Knudson’s Two-hit hypothesis which explains that “Two mutational events are rate limiting for the development of Rb and all cases of Rb arise as a consequence of loss-of-function of both the alleles of RB1 tumor suppressor gene located on 13q14.2”. Rb is clinically distinguished as bilateral or multifocal and unilateral or unifocal. The bilateral or multifocal form of Rb (40% of all cases) is heritable in nature and associated with biallelic inactivation of RB1 gene where the first hit is constitutional, and the second hit occurs somatically in one or more retinal cells. The unilateral or unifocal form of Rb (60% of all cases) is non-familial in nature where both RB1 alleles are damaged only in the developing retina, but 15% of unilateral cases also carry a heritable constitutional mutation in the RB1 gene.

The mean age of presentation of bilateral and unilateral Rb tumors is 15 months and 27 months respectively. Genetic testing in unilateral cases is essential as preliminary investigation for Rb because in the absence of a positive family history, unilateral cases may involve the germ line RB1 mutation and thus, are capable of being transmitted to the next generation. When Rb remains confined to the eye, it has one of the best survival rates of all the childhood cancers, but once the spread occurs outside the globe, many children do not survive. Rb ranks 4th in the list of mortalities in Indian children. Treatment of Rb is risk adapted. Factors to be considered in the treatment decisions include intraocular and extracocular stage, laterality, and potential for vision. Ocular salvage treatments include systemic or intra-arterial chemotherapy, aggressive focal treatments (photocoagulation, thermotherapy, cryotherapy, and brachytherapy), and external beam radiation therapy.

Most germline mutations in non-familial sporadic heritable Rb cases are paternal in origin. Studies have also documented an association between paternal age, particularly advanced paternal age (>40 years) and poor lifestyle habits such as smoking, excessive alcohol consumption, pesticide exposure, exposure to harmful radiations, etc. are associated with an increased risk of developing Rb in their children.

Retinitis pigmentosa
Retinitis pigmentosa is a group of clinically and genetically heterogeneous retinal degenerative hereditary diseases. The disease displays X-linked, autosomal dominant and autosomal recessive inheritance patterns. Clinically, the disease is characterized by night blindness, narrowing of the visual field and pigmentary changes or alterations of the retina, eventually leading to complete loss of vision. Mutations in RPID gene is the cause of autosomal dominant retinitis pigmentosa. Mutations in the RPGR gene are the most frequent cause of X-linked retinitis pigmentosa. The genes more frequently involved in autosomal recessive retinitis pigmentosa are the genes encoding the subunits α and β of the cGMP Phosphodiesterase, RHO and the cGMP gated ion channel protein CNCG.

Another form of retinal dystrophy is Leber’s congenital amaurosis which is an inherited condition where clinical findings commonly first appear after 2-3 months of life. Visual function is usually poor and often accompanied by nystagmus, sluggish or near-absence pupillary responses, photophobia, high hyperopia, keratoconus and enopthalmos. In contrast to original thinking, Leber’s congenital amaurosis is now known to be caused by at least seven genes. Mutations in RPE65, RetGC1, AIPL1, RPGR1P1,
TULPI, CRB1 and CRX are known to be the cause of the condition.\textsuperscript{36}

**Keratoconus**
Keratoconus is a bilateral and asymmetric corneal degeneration characterized by localized corneal thinning, which leads to protrusion of the thinned cornea.\textsuperscript{40} It is the most common primary ectasia.\textsuperscript{41} It usually occurs in the second decade of life and affects both genders and all ethnicities.\textsuperscript{42}

The cause and possible mechanisms for the development of keratoconus remains poorly understood. However, several hypotheses have been proposed on the genetic mechanism of development.\textsuperscript{43} Heon et al (2002)\textsuperscript{44} identified four mutations of the VSX1 gene (R166W, L159M, D144E and H244R) in different keratoconic patients (Figure 4). Recently, Liskova et al (2007)\textsuperscript{45} and Tang et al (2008)\textsuperscript{46} have shown that mutations of D144E, L159M, R166W and H244R are not related to keratoconus. The etiology of keratoconus is complicated but the genetic basis of the disease has started to be uncovered. More recently, Guan et al (2012)\textsuperscript{47} have shown the potential involvement of TGFB1 gene in the keratoconus Chinese population.

**Corneal dystrophies**
The word “dystrophy” is derived from Greek (dys=wrong or difficult, trophe=nourishment). Corneal dystrophies are a group of inherited corneal diseases that are typically bilateral, symmetric, and slowly progressive and without relationship to environment or systemic factors.\textsuperscript{47} These autosomal dominant diseases are characterized by progressive accumulation of corneal deposits beginning in the first or second decade of life. Mutations in KRT3 and KRT12 are the causes of dystrophies.\textsuperscript{47} Meesmann corneal dystrophy is an autosomal dominant disorder that affects only the corneal epithelium.

**Congenital Cataract**
The transparency of the lens depends on a highly structured arrangement of lens proteins and lens fibers. About 90% of the lens proteins are crystallins. The crystallins are long-lived proteins located inside lens fibers, and are essential in the maintenance of transparency and refractive power. These proteins that function in maintaining the clarity of the lens could be the potential genes causing heritable cataract (Figure 5). Congenital cataract can occur either due to genetic or non-genetic factors. Worldwide significant inroads are being made into understanding the genetics of human congenital cataract. Cataract resulting from chromosomal translocations has been documented and mostly associated with other abnormalities as more than one gene may be disrupted by the translocation. Autosomal dominant congenital cataract has been described in a three-generation family with reciprocal translocation between chromosomes 2p22 and 16p13.\textsuperscript{48} Over 26 of the 39 mapped loci for congenital cataract have been associated with mutations in specific genes. About half have mutations in crystallins, about a quarter have mutations in connexins, with the remainder divided among the genes for heat shock transcription factor 4 (HSF4), aquaporin-0 (AQP0, MIP), and beaded filament structural protein-2 (BFSP2). There is often some correlation between the pattern of expression of the mutant protein and the morphology of the resulting cataract.\textsuperscript{49} Inheritance of same mutation in different families or even the same mutation within the same family can result in radically different cataract morphologies and severities.

**Techniques used for Genetic Testing:** In general, there are three categories of genetic testing: cytogenetic, biochemical, and molecular are available to detect abnormalities in chromosome structure, protein function, and DNA sequence, respectively.

A) Cytogenetic Testing: Conventional cytogenetic testing referred to as karyotyping, involves the examination of chromosomes to identify structural abnormalities (Figure 6). Karyotyping involves cell culture, followed by chromosome harvesting and banding; microscopic analysis; and karyotype production.\textsuperscript{49} Fluorescent in situ hybridization (FISH) testing is a method by which an assessment is made for the presence, absence, relative positioning and/or the copy number of specific DNA segments by fluorescence microscopy. Depending upon the application, FISH can be applied to metaphase chromosome preparations and/or interphase cell nuclei.\textsuperscript{50}

B) Biochemical testing: Clinical testing for a biochemical disease utilizes techniques that examine the protein instead of the gene. Depending on the disease, tests can be developed to directly measure protein activity or to measure the level of metabolites, and the size or quantity of protein. Because gene products may be more unstable...
than DNA or RNA and can degrade quickly, the sample must be collected, stored properly, and shipped promptly according to the laboratory’s specifications. The methodology employed for biochemical testing includes high performance liquid chromatography (HPLC) (Figure 7), gas chromatography/mass spectrometry (GC/MS), and tandem mass spectrometry (MS/MS).51

DNA microarray also referred to as gene, genome, or DNA chip, is a tool for determining gene expression. Molecules of mRNA bind, or hybridize, specifically to a DNA template, typically a gene or portion of a gene, from which it originated (Figure 9).52

C) Molecular Testing: Direct DNA analysis is applicable when the gene sequence of interest is known but for small DNA mutations, direct DNA testing is typically

Figure 6: Karyotype of Rb patient with 13q14 deletion

Figure 7: HPLC Instrument

Figure 8: PCR Thermal Cycler

Figure 9: Gene Chip
Conclusion
With the current detailed delineation of the clinical manifestations of genetic eye diseases, and with the availability of precise gene testing, sophisticated retinal imaging and electrophysiological testing modalities, accurate diagnosis of individual retinal dystrophies is possible in many but not in all patients. There is good reason to be hopeful that therapy will be available soon, at least for some of these devastating ocular diseases. In the last two decades, molecular genetics has been able to discover the complexity of genetics of various ocular disorders. New genes associated with different eye diseases have been mapped and cloned at an incredible rate. This article provides a clear summary of the genetics of heritable ocular disorders and can serve as an initiating point for the investigation of many patients and families.

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