Primary congenital and developmental glaucomas

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Abstract

Glaucoma is the leading cause of irreversible blindness worldwide. Although most glaucoma patients are elderly, congenital glaucoma and glaucomas of childhood are also important causes of visual disability. Primary congenital glaucoma (PCG) is isolated, non-syndromic glaucoma that occurs in the first three years of life and is a major cause of childhood blindness. Other early-onset glaucomas may arise secondary to developmental abnormalities, such as glaucomas that occur with aniridia or as part of Axenfeld-Rieger syndrome. Congenital and childhood glaucomas have strong genetic bases and disease-causing mutations have been discovered in several genes. Mutations in three genes (CYP1B1, LTBP2, TEK) have been reported in PCG patients. Axenfeld-Rieger syndrome is caused by mutations in PITX2 or FOXC1 and aniridia is caused by PAX6 mutations. This review discusses the roles of these genes in primary congenital glaucoma and glaucomas of childhood.

Introduction

Glaucoma is a heterogeneous group of optic nerve diseases that share two clinical features, a characteristic injury to the optic nerve (cupping) and a corresponding pattern of visual field loss. The anterior segment of the eye is filled with an aqueous fluid that is created in the ciliary body and exits the eye through the trabecular meshwork. The trabecular meshwork is a porous tissue located in the angle created where the cornea intersects the iris - the iridocorneal angle. Abnormalities in the structure or conformation of the iridocorneal angle may limit outflow of aqueous humor and cause a rise in intraocular pressure, which is a strong risk factor for developing glaucoma (1).

While the majority of glaucoma patients are adults, early-onset forms of glaucoma are also important causes of visual disability. Glaucomas that occur before three years of age without overt structural defects of the eye are termed primary congenital glaucoma (PCG). Conversely, developmental glaucomas occur secondarily to recognizable malformations of the anterior segment of the eye (iris, iridocorneal angle, etc.). PCG and developmental glaucomas have strong genetic bases and are the subject of this review.

Primary Congenital Glaucoma (OMIM: 231300)

PCG is the most common form of pediatric glaucoma (2) and accounts for up to 18% of childhood blindness (3,4). The incidence of PCG varies geographically, being most common in Saudi Arabia (1/2,500) (5) and among consanguineous populations (1/1,250 in Slovakian Roma) (6), and least common in Western countries (1/10,000) (7). Symptoms are nonspecific and include tearing, light sensitivity, eye rubbing, and irritability. High intraocular pressure leads to enlarged eyes, cloudy corneas, cracks in the cornea (Haab striae), and optic nerve cupping (8,9).

While the majority of PCG cases are sporadic, up to 40% are familial and follow autosomal recessive inheritance patterns

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with variable penetrance (7). Genetic analyses of affected families have yielded four loci associated with PCG: GLC3A on 2p21 (10), GLC3B on 1p36 (11), GLC3C (12) and GLC3D (13) adjacent to but not overlapping one another on 1q42. PCG-causing mutations have been identified in genes within two of the four loci. Cytochrome P450 1B1 (CYP1B1) mutations were discovered within the GLC3A locus and are the most common known cause of PCG (14). CYP1B1 encodes a metabolizing enzyme of the cytochrome P450 family. Mutations in latent transforming growth factor beta binding protein 2 (LTBP2), located in the GLC3D locus (15), have also been associated with PCG. LTBP2 encodes an extracellular matrix protein involved in cell adhesion and structural maintenance of connective tissues. PCG-causing mutations have not yet been found within the GLC3B or GLC3C loci. Most recently, mutations in a third gene, tunica interna endothelial cell kinase (TEK), have been reported in patients with PCG (16).

Cytochrome P450 1B1 (CYP1B1)

Autosomal recessive mutations in CYP1B1 are the most common known cause of PCG. Over 150 variants have been identified worldwide and the prevalence of CYP1B1 mutations in PCG varies greatly with ethnicity (17). A few specific CYP1B1 mutations account for a majority of PCG cases in select populations (i.e. E387K in Slovakian Roma and G61E in Saudi Arabia) (18,19). One mutation, R390H, is especially common among Chinese, Iranian, Indian, and Pakistani cases of PCG (17,20–22). Pathogenic CYP1B1 alleles have been detected in a smaller fraction of PCG cases in Japan (20%) (23) and the United States (14.9%) (24). Although CYP1B1 mutations are most commonly detected in PCG patients, mutations have also been rarely reported in patients with a range of other phenotypes including aniridia (25), Peters anomaly (26) Axenfeld-Rieger syndrome (27), juvenile open angle glaucoma (28), and primary open angle glaucoma (29).

CYP1B1 is a member of the cytochrome P450 family of membrane-bound oxidase enzymes that have broad roles in metabolism and produce hormones and other metabolic intermediates during development (30). While most other cytochromes are highly expressed in the liver, CYP1B1 is more abundant in extra-hepatic tissues including lung, colon, kidney and eye (31). The precise mechanism by which CYP1B1 mutations cause PCG is unknown. However, CYP1B1 is expressed in the ciliary body and trabecular meshwork, tissue of the eye that regulate intraocular pressure (14,32). Recent studies propose that CYP1B1 may be essential in the development and function of the trabecular meshwork (33,34). Moreover, glaucoma-causing mutations reportedly decrease CYP1B1 enzyme stability, abundance, or catalytic activity (35–41). Together these data suggest that CYP1B1 mutations may alter trabecular meshwork function, cause dysregulation of intraocular pressure, optic nerve damage, and ultimately PCG.

Latent transforming growth factor beta binding protein 2 (LTBP2)

Autosomal recessive LTBP2 variants are a rare cause of PCG, with cases primarily reported in consanguineous families from Pakistan and Iran (15,42). LTBP2 mutations are also responsible for many cases of PCG in Slovakian Roma, with the R399X mutation in LTBP2 accounting for over half of CYP1B1-negative cases (43). However, LTBP2 mutations have not been detected in PCG cohorts from northern India, the United Kingdom, the United States, or China (24,44–46). Recently, mutations in LTBP2 were found to cause congenital glaucoma in Siamese cats, providing additional support for the role of this gene in PCG (47).

LTBP2 encodes an extracellular matrix protein with putative roles in cell adhesion (48,49) and elastin microfibril assembly (50–52). LTBP2 is highly expressed in tissues that are rich in elastic fibers, such as lungs and arteries (53). LTBP2 is also expressed in ocular tissues that are vital to regulation of intraocular pressure and glaucoma biology, including the trabecular meshwork and ciliary body. Moreover, LTBP2 is essential for development of the anterior chamber and ciliary zonules (15,53). Mutations in LTBP2 are, consequently, a plausible cause of congenital abnormalities of ocular structures that may lead to increased intraocular pressure and PCG.

Homozygous LTBP2 mutations have also been reported in rare cases with a range of other ocular abnormalities (megalocornea, microspherophakia, ectopia lentis, primary open angle glaucoma, pseudoxfoliation syndrome, primary angle-closure glaucoma and Weill-Marchesani syndrome) (54–59).

Tunica interna endothelial cell kinase (TEK)

Recently, transgenic “knock-out” mice that are deficient for the angiopoietin 1 and 2 (Angpt1 and Angpt2) genes or the angiopoietin receptor also known as tunica interna endothelial cell kinase (Tek) gene were shown to have signs of PCG including enlarged lenses, high intraocular pressure, retinal ganglion cell loss, and optic nerve damage. These phenotypes of the transgenic mice were attributed to an absence of ocular structures required for fluid drainage (i.e. Schlemm canal) and have implicated the angiopoietin signaling pathways in PCG pathogenesis (60). Furthermore, a dose response to Tek deficiency was demonstrated. Mice heterozygous for the Tek “knock-out” mutation develop abnormalities in outflow structures and elevation in intraocular pressure with intermediate severity. Tek haploinsufficiency can compromise aqueous humor outflow in mice (16).

Heterozygous mutations in the human TEK gene were discovered in 10 of 189 unrelated human PCG patients. Seven of these ten PCG cases had no family history of glaucoma, while additional affected family members were identified for three PCG probands. Pedigree analyses reported autosomal dominant inheritance with variable expressivity and incomplete penetrance (16). Several of the TEK mutations detected in human PCG patients were shown to have functional effects in cell culture assays including altered transcription, reduced protein production, altered post-translational modification, and aberrant trafficking. TEK regulates vasculogenesis and is highly expressed in blood vessels and lymphatic endothelia, as well as in the endothelium of Schlemm canal (61,62). Although their specific role in glaucoma pathogenesis is unknown, it is likely that TEK mutations cause alterations in the development of ocular structures necessary for normal aqueous outflow and regulation of intraocular pressure and subsequently predispose development of congenital glaucoma.

Developmental Glaucomas

Axenfeld-Rieger syndrome (OMIM: 180500)

Axenfeld-Rieger syndrome encompasses a heterogeneous collection of disorders with ocular and systemic features. Axenfeld-Rieger syndrome is rare and has a prevalence of
Various congenital heart defects may also be present, including atrial septal defects (69,70).

Linkage analysis of large pedigrees with dominantly inherited Axenfeld-Rieger syndrome led to the discovery of disease-causing mutations in two genes, paired-like homeodomain 2 (PITX2) (68) and forkhead box C1 (FOXC1) (71).

Paired-like homeodomain transcription factor 2 (PITX2)

A range of PITX2 (originally termed RIEG1) mutations have been associated with Axenfeld-Rieger syndrome including missense mutations, nonsense mutations, splice site mutations (68), and copy number variations (72). PITX2 encodes a member of the bicomod class of homeodomain transcription factors, which play critical roles in embryonic development and tissue morphogenesis. PITX2 encodes several alternatively spliced isoforms, which all contain a partial or complete DNA-binding homeodomain termed solufull. The majority of Axenfeld-Rieger-causing defects are missense mutations within the homeodomain (73).

The precise mechanisms by which PITX2 mutations cause Axenfeld-Rieger syndrome are not fully known, but relate to PITX2 haploinsufficiency and dominant negative effects (74–76). Some PITX2 mutations detected in Axenfeld-Rieger patients have been shown to impact functional changes in cell-based in vitro assays, including impaired ability to bind DNA and altered transactivation activity in reporter assays (76). In rare cases, hypermorphic alleles of PITX2 were identified in Axenfeld-Rieger patients. These cases suggest that strict regulation of PITX2 dosage is necessary for normal development and function in the eye (76).

PITX2 is expressed in the tissues most affected by Axenfeld-Rieger syndrome (68,77). Moreover, studies using experimental mouse models have demonstrated that PITX2 is required for normal ocular development and have helped to reveal a range of abnormalities arising from PITX2 deficiencies (78,79). Knockout of Pitx2 reduces the abundance of astrocytes and retinal vasculature in neural crest-derived tissues (78). These deficits suggest that developmental abnormalities of the optic nerve and retina may contribute to glaucoma susceptibility in Axenfeld-Rieger patients. Mice heterozygous for a Pitx2 null mutation also recapitulate the ocular malformations of Axenfeld-Rieger syndrome and associated glaucoma (80). Together, these data confirm a role for PITX2 mutations in the development of Axenfeld-Rieger syndrome.

PITX2 mutations have also been associated with another developmental abnormality characterized by adhesions between the central cornea and lens known as Peters anomaly (81). Mutations in PITX2 have also been discovered in patients diagnosed with iris hypoplasia, iridogoniodysgenesis, mesodermal dysgenesis, and anterior segment cleavage syndrome, which likely represent conditions located on a spectrum of phenotypes best described as Axenfeld-Rieger syndrome (63).

Forkhead box transcription factor C1 (FOXC1)

Missense mutations, deletions, and duplications of FOXC1 (previously termed FKHL5) are another cause of Axenfeld-Rieger syndrome (71,82–84). FOXC1 encodes a member of the forkhead box family of transcription factors, which function as important regulators of embryogenesis, cell migration, differentiation, and fate determination (85). The defining and functional feature of this family of transcription factors is the conserved 110-amino

Figure 1. Clinical features of Axenfeld-Rieger Syndrome in a patient with a PITX2 mutation. DNA sequencing of the PITX2 gene in a female with Axenfeld-Rieger syndrome identified a novel heterozygous mutation of a canonical splicing sequence within intron 3, IVS3-1delG. This patient had classic features of Axenfeld-Rieger syndrome including bilateral iris hypoplasia (panel A and B); posterior embryotoxon (panel A and B indicated with black arrows); and corectopia (panel B indicated with an asterisk). This patient also had hypodontia and microdontia (panel C).
acid forkhead domain, which mediates DNA binding and protein-protein interactions.

Mechanisms by which FOXC1 mutations cause Axenfeld-Rieger syndrome are not precisely known, but may often relate to FOXC1 haploinsufficiency. Mutations that alter the FOXC1 gene dosage in mice confirm a crucial role in the development of the eye and Axenfeld-Rieger syndrome. Haploinsufficiency of FOXC1 is associated with iris hypoplasia, corectopia, and embryotoxon in mice (86).

Many (71,87), but not all (71) FOXC1 mutations in human Axenfeld-Rieger syndrome patients influence the forkhead domain and can impair localization (88,89), DNA-binding, and transactivation (90,91). In rare cases, mutations in the forkhead domain have been identified in Axenfeld-Rieger patients that result in expression of mutant FOXC1 at levels similar to that of wild-type, but with differential phosphorylation. Abnormal phosphorylation of FOXC1 hinders nuclear localization, DNA-binding, and transactivation (92). Each mutation that interferes with FOXC1’s function as a transcription factor has the potential to cause Axenfeld-Rieger syndrome. FOXC1 has recently been identified as a risk factor for primary open angle glaucoma, suggesting a role in adult-onset glaucoma as well (93).

FOXC1 is expressed in the tissues with key roles in the pathophysiology of Axenfeld-Rieger syndrome. FOXC1 is expressed in the neural crest-derived tissues that form the drainage structures of the eye (trabecular meshwork) during development and in the adult iris (94). FOXC1 is also detectable in non-ocular tissues affected by Axenfeld Rieger syndrome including the heart and heart valves (95,96) with alternative transcripts expressed in a tissue-specific manner (94). These expression patterns along with several cases of heart valve and atrial septal defects in patients with Axenfeld-Rieger syndrome suggest that congenital heart disease may be an additional component of this syndrome associated with FOXC1 mutations (95).

PITX2 and FOXC1 both encode transcription factors that interact directly and influence their respective transcriptional activity. PITX2 is a negative regulator of FOXC1. Consequently, mutations in PITX2 may cause Axenfeld-Rieger syndrome, at least in part, by altering the function of FOXC1 (87).

Axenfeld-Rieger syndrome is heterogeneous. Additional Axenfeld-Rieger syndrome loci have been mapped to chromosomes 13q24 and 16q24, however, the specific disease-causing genes in these loci have not yet been discovered (97).

Aniridia (OMIM: 106210)

Aniridia is a rare developmental eye disease characterized by an underdeveloped iris that has a prevalence of 1/164,000 to 1/96,000 and either autosomal dominant inheritance or sporadic occurrence (98). Although the name “aniridia” suggests a complete absence of the iris, all patients have some iris tissue ranging from a tiny vestige that may be difficult to recognize to a normal appearing iris (Fig. 2B and C). These abnormal irides may collapse against the peripheral cornea and obstruct outflow of aqueous humor, leading to increased intraocular pressure, optic nerve damage (Fig. 2D and E) and a secondary glaucoma. Corneal pannus, foveal hypoplasia, and cataract (Fig. 2B and C) are other important features of aniridia (99). Pannus alters the transparency of the cornea and may significantly compromise vision, while foveal hypoplasia is failure of the retina to develop structures required for high-resolution central vision. Aniridia has variable expressivity, with patients showing a range of severity for corneal pannus, foveal hypoplasia, and iris abnormalities (98).

Paired box 6 (PAX6)

Missense mutations in PAX6 have been associated with autosomal dominant inheritance of aniridia (100), while deletions and rearrangements have been associated with sporadic cases (101). PAX6 encodes a transcription factor that has profound effects on ocular development. PAX6 activates expression of other genes via its DNA-binding domains (a paired and a paired-type homeodomain) and a proline, serine, threonine-rich transactivating domain. Mutations that cause aniridia may alter PAX6 functional domains (i.e. missense mutations), cause a truncation of the encoded protein, or disrupt enhancer sequences, such as a distant downstream regulatory region (DRR) element (102–105). A common feature of disease-causing mutations is that they alter the dose of functional PAX6 transcription factor, which leads to dysregulation of downstream transcription factors and gene expression patterns, resulting in congenital malformations that may promote glaucoma (106).

Phenotypes similar to aniridia have been detected in animal models with mutations in PAX6 orthologues. Small eye (Sey) mice do not develop eyes or noses and die soon after birth (107). This developmental abnormality in mice was shown to be due to a PAX6 mutation in parallel with discoveries of PAX6 mutations in human patients with aniridia (100,102). A similar mutation of the Drosophila Pax6 gene is associated with an eyeless (ey) phenotype (108). PAX6 has a highly conserved and central role in ocular development and aniridia (109).

Sporadic cases of aniridia may be caused by a large deletion of chromosome 11p13 spanning the PAX6 gene, which has been associated with a contiguous gene syndrome, WAGR (Wilms tumor, Aniridia, Genitourinary abnormalities, and mental Retardation). In addition to deletion of the PAX6 gene, sporadic aniridia patients may also have a deletion of the neighboring tumor suppressor gene, WT1, and increased risk for Wilms tumor. All children with sporadic cases of aniridia should be investigated for a chromosome 11p13 deletion and the associated risk for a potentially lethal Wilms tumor (110).

Conclusion

Glucomas of infancy and childhood have important genetic components to their pathogenesis. Over the last two decades, many disease-causing mutations have been identified. These include mutations in three genes (CYP1B1, LTB2, and TEK) that have been associated with primary congenital glaucoma (PCG). Mutations in a different set of genes have been detected in patients with secondary glaucomas of childhood. Specifically, PITX2 and FOXC1 mutations have been associated with Axenfeld-Rieger syndrome, while mutations in PAX6 have been associated with aniridia. Patients with Axenfeld-Rieger syndrome or with aniridia are at high risk for developing secondary glaucoma. Notably, the genes that have been associated with these secondary glaucomas (PITX2, FOXC1, and PAX6) are all transcription factors that control ocular development. In each of these forms of glaucoma, disease-causing mutations lead to microscopic and/or visible developmental malformations of ocular structures that regulate drainage of fluid from the eye and ultimately cause elevated intraocular pressure and optic nerve damage. Despite the significant discoveries of many
glaucoma-causing mutations in several genes, a large proportion of PCG, Axenfeld-Rieger syndrome, and aniridia cases do not have known molecular genetic causes. Continuing research is needed both to identify the additional glaucoma-causing mutations in novel genes that are responsible for disease in these patients and to translate this knowledge into new treatments.

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References

Figure 2. Clinical features of aniridia in a patient with a PAX6 mutation. Whole genome DNA sequencing of the PAX6 gene in a female with aniridia and secondary glaucoma detected a tandem inversion [hg19:chr11:31763505-40323405inv;chr11:40323414-4353635inv] on chromosome 11p13 (panel A) including the PAX6 gene. This inversion moved PAX6 away from a vital enhancer (DRR) (105) that is normally downstream of the PAX6 gene (top, wild-type configuration) to an inactive position millions of base pairs away (bottom, mutant configuration). This patient had an absence of visible iris tissue in both eyes (panel B, right eye; panel C, left eye). The edges of the natural lenses (indicated with black arrows) were visible due to the absence of visible iris tissue. There is bilateral cataract indicated by black arrowheads. Finally, the white arrowheads indicate Ahmed drainage valves that were surgically implanted to control intraocular pressure. This patient has severe secondary glaucoma with a near total cupping of the right optic nerve head (panel D) and a large optic cup with a thin rim of neural tissue remaining on the left optic nerve head (panel E). A normal optic nerve head is shown in panel F for comparison.


glaucoma by reduction of either activity or abundance of the enzyme. *Hum. Mutat.*, 29, 1147–1153.


