Phenotypic heterogeneity of *CYP1B1*: mutations in a patient with Peters' anomaly

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Congenital glaucoma refers to a genetically heterogeneous group of distinctive clinical diseases characterised by increased intraocular pressure most often associated with increased corneal diameter, corneal oedema, and consequent visual impairment. Primary congenital glaucoma (PCG) is associated with a primary angle defect, whereas secondary congenital glaucoma is associated with a more generalised developmental anomaly of the anterior segment such as seen in Peters' anomaly. The inheritance of PCG is usually autosomal recessive.

Peters' anomaly consists of corneal opacity, defects in the posterior structures of the cornea, and iridocorneal and/or keratolenticular adhesions, and it most frequently occurs sporadically.¹ Over 50% of subjects develop glaucoma in childhood. Numerous aetiologies have been proposed including chromosomal abnormalities, teratogens,¹ and mutations in the eye developmental genes *PAX6*^{2–4} and *PITX2*.⁵ However, large subsets of Peters' anomaly cases are without molecular characterisation.

Primary congenital glaucoma has been linked to chromosomes 1p36 (*GLC3B*) and 2p21 (*GLC3A*), but only the *GLC3A* gene has been identified. This gene, *CYP1B1*, encodes a broadly expressed cytochrome P450 enzyme (*P4501B1*) whose natural substrate is unknown.^{6–7} It is proposed to be the major gene for disease causing mutations in primary congenital glaucoma.^{6–11} A variety of chain terminating and missense *CYP1B1* mutations have been described.^{6–9} We report two novel mutations in *CYP1B1* in a patient who had Peters' anomaly with secondary congenital glaucoma.

Case report

The subject, a male of Native Indian (Mohawk)/French Canadian background, presented with a history of bilateral cloudy corneas and tearing since birth. Examination at 3 weeks of age showed bilateral corneal oedema with central corneal opacities, superficial pannus (corneal vascularisation), and iridocorneal adhesions with a well formed anterior chamber (fig 1, above). Iris was present for 360° in both eyes. Corneal diameters were 10.75 mm in the right eye (OD) and 11.25 mm in the left eye (OS), which was slightly increased for age.¹² No view was available of the angle or optic nerve. Applanation tonometry under general anaesthesia documented intraocular pressures (IOPs) up to 49 mmHg in the right eye and 50 mmHg in the left eye, which were well beyond the normal range. The child was otherwise

entirely well with no other malformations and no significant family history.

A diagnosis of congenital glaucoma and Peters' anomaly with no significant family history was made. Bilateral trabeculotomies were performed to decrease the IOP followed by a left corneal transplant for visual rehabilitation. At surgery, the undersurface of the cornea showed a central, Y shaped, posteriorly projecting opacity that corresponded to a raised opacity on the anterior capsule of the lens. This overlapped the position of the fetal sutures and suggested the existence of previous keratolenticular adhesions during development. The lens also showed a pulverulent (powdery) cataract through which there was a good red reflex. Subsequently, the IOPs were difficult to control in both eyes, requiring frequent surgery and medication. At the most recent examination at 6 years of age, his right eye showed signs of advanced but controlled glaucoma with a cup:disc ratio of 0.8 to 0.9 OD. His left eye became phthisical (shrunken).

Microscopic examination of the left corneal button removed at transplantation showed that the corneal epithelium was of normal thickness, although there was some oedema of the basal cells (fig 1, below). Bowman's membrane was not recognisable and the anterior stroma was hypercellular with a disordered lamellar pattern. The posterior stroma was absent centrally, and there was a basophilic granular deposit containing a small amount of extracellular melanin. Descemet's membrane was not identified in this half of the button. However, transmission electron microscopy showed a recognisable Bowman's membrane, though abnormal and containing scattered keratocytes. Also a small segment of Descemet's was observed, although thin (approximately 1.5 µm) with a rather poorly defined banding pattern. No endothelial cells were seen.

DNA extraction followed standard protocols.¹³ Amplification of the coding sequence of *CYP1B1* used previously published primers.^{7–8} Additional primers for exon 3 were designed from the genomic DNA sequence (Acc No U56438). Forward primer: 5'-ctataaagcttcctctaagc-3', reverse primer: 5'-ttgtccaagaatcgagctgg-3'. Mutational analysis of the amplicons by single strand conformational polymorphism (SSCP) and direct cycle sequencing used protocols previously described.^{11–14} We identified two novel *CYP1B1* mutations in exon 2 making our subject a compound heterozygote for the missense mutation 3807T→C, which is predicted to result in the amino acid change Met1Thr (ATG→ACG), and the nonsense mutation 3976G→A, which

Figure 1 (Above) Photo of the right cornea in the first year of life with central corneal leucoma (opacity). (Below) Histopathology of the central cornea with scarring of anterior stroma, posterior stromal defect (D), and basophilic granular deposit. Where the full thickness of the stroma is seen (arrow), Descemet's membrane is not present. (Haematoxylin and eosin.)

Figure 2 Chromatograms of direct cycle sequencing of *CYP1B1*, exon 2 showing (left) substitution of 380T→C compared with normal, above and (right) 3976G→A resulting in a premature stop codon, also compared with the normal sequence shown above.

is predicted to truncate the P4501B1 polypeptide (Trp57Stop, TGG→TAG) and possibly cause nonsense mediated decay of the *CYP1B1* mRNA (fig 2). This latter mutation was seen in the mother's DNA who was clinically normal.

The father was not available for testing. These mutations were not seen in 100 normal controls of mixed ethnicity. Furthermore, mutational analysis of *PITX2* and *PAX6* using previously described protocols¹⁵⁻¹⁷ failed to document any pathogenic sequence change in this patient. Ten additional unrelated subjects with Peters' anomaly were screened for mutations in *CYP1B1*, *PITX2*, and *PAX6*. Only one patient showed a sequence change in *PAX6* and none in *CYP1B1*.

Discussion

Peters' anomaly is a congenital abnormality that shows a wide range of histopathological changes.¹⁸⁻²¹ It has been proposed that a normal Descemet's/endothelial unit is necessary for maintenance of the integrity of the corneal stroma.²² The posterior stromal abnormalities seen in our patient may be a consequence of the abnormal segment of Descemet's membrane. The nature of the basophilic deposit in the posterior stromal defect remains uncertain but may represent denatured lens protein associated with the presumed developmental keratolenticular adhesion noted at the time of the corneal transplant. The absence of Bowman's membrane on light microscopy, albeit not complete, is also a characteristic feature of Peters' anomaly.

It has been suggested that both Peters' anomaly and primary congenital glaucoma arise from defective neural crest cell migration in the 4th to 7th week of fetal development, during which period the anterior segment of the eye forms.²³ The relationship between these two diseases is otherwise not well understood. This is the first documentation of *CYP1B1* mutations causing a phenotype other than congenital glaucoma. We report two new *CYP1B1* mutations, the combination of which is associated with a different phenotype, Peters' anomaly. These findings strengthen the potential role of this gene in anterior segment eye development. One of the *CYP1B1* mutations identified (3976G→A, Met1Thr) would probably disrupt translation initiation, as the methionine codon surrounded by a Kozak sequence is crucial for ribosomal recognition.²⁴ The next conserved Kozak sequence where translation could be initiated occurs in frame, but would result in truncation of 131 amino acids from the amino terminus of the protein. Translation can be initiated at non-AUG codons, including an ACG, but this is extremely rare and requires favourable mRNA secondary structure.²⁵ The second mutation in this patient (3976G→A) results in a premature stop codon (Trp57Stop). Expression of this allele would therefore result in a protein truncated by 486 amino acids and is thus also likely to represent a functional null allele.

Our observations suggest that a normal *CYP1B1* product is required for differentiation of the anterior segment, and that some cases of Peters' anomaly and PCG may share a common aetiology. Homozygous *CYP1B1* null mutations have been described in cases of congenital glaucoma^{6-9, 11}; however, insufficient clinical information is available to establish

phenotype-genotype correlations. We hypothesise that the severity of the Peters' phenotype described correlates with the extent of predicted protein truncation.

The spectrum of the phenotypes associated with *CYP1B1* mutations is broader than anticipated as is the genetic heterogeneity of Peters' anomaly. The findings reported here suggest that the role of *CYP1B1* is not solely confined to the pathogenesis of primary congenital glaucoma but may play a more general role in eye development. This work emphasises the genetic heterogeneity and the complexity behind anterior segment development disorders and early glaucoma.

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