Further support of the role of CYP1B1 in patients with Peters anomaly

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Purpose: Peters anomaly is a developmental anomaly of the eye frequently associated with glaucoma. The aim of this study was to further define the molecular basis of this condition.

Methods: The role of four candidate genes implicated in ocular development or glaucoma, PAX6, PITX2, MYOC, and CYP1B1, was studied in 15 patients with Peters anomaly. Mutational analysis used a combination of single strand conformation polymorphism (SSCP) and direct cycle sequencing.

Results: Four mutations in CYP1B1 were found in 3/15 (20%) affected individuals compared with 1/140 (0.7%) control individuals.

Conclusions: This study supports the role of CYP1B1 as a causative gene in Peters anomaly. Furthermore, this emphasizes the broad range of phenotypic expression for CYP1B1 mutations, and its role in eye development.

Peters anomaly (OMIM 604229) is a developmental disorder of the anterior segment of the eye, associated with glaucoma in over 50% of affected individuals. This disorder may be unilateral or bilateral. The phenotype is characterized by a variable degree of corneal opacity with defects in the posterior corneal structures, in addition to cornea-lens and/or iris-lens adhesions [1].

Mutations in eye development genes such as PAX6 (OMIM 607108), PITX2 (OMIM 601542), FOXC1 (OMIM 601090), and CYP1B1 (OMIM 601771) have been documented in association with some cases affected with Peters anomaly [2-4]. PAX6 is a homeobox gene that is critical to ocular development. Mutations are most often associated with aniridia but may also be causative of other phenotypes such as isolated macular hypoplasia, nuclear cataract, superficial vascularized keratitis, or Peters anomaly [1,2]. PITX2 mutations are usually associated with Axenfeld-Rieger syndrome, iridogoniodysgenesis, and iris hypoplasia but a mutation was observed in one case of Peters anomaly [3]. FOXC1 mutations are most often with iridogoniodysgenesis and Axenfeld-Rieger phenotypes, but two associations with Peters anomaly are reported [4]. We recently reported mutations in CYP1B1 in one patient with Peters anomaly associated with congenital glaucoma [5]. CYP1B1 mutations are usually observed in autosomal recessive primary congenital glaucoma [6], accounting for 20-30% of cases in an ethnically mixed population [7]. CYP1B1 is a member of the cytochrome P450 gene superfamily, with mRNA expression in the trabecular meshwork, iris, and ciliary body. Because of its association with juvenile and primary open angle glaucoma, MYOC was also considered as a candidate gene.

Our study was designed to examine the relative role of mutations in PAX6, PITX2, CYP1B1, and MYOC in an ethnically mixed population of patients affected with Peters anomaly.

METHODS

This project was approved by the Research Ethics Board of The Hospital for Sick Children (HSC), Toronto, Canada respecting the Tenets of the Declaration of Helsinki. Patients meeting the diagnostic criteria for Peters anomaly [1] were recruited through the Ocular Genetics Program of the Hospital for Sick Children and the New York Eye and Ear Infirmary, New York, NY. DNA extraction followed standard protocols [8]. Mutational analysis was undertaken of the coding sequences of four genes associated with ocular development and/or glaucoma; CYP1B1, PITX2, PAX6, and MYOC. All protocol used are available on request. Mutational analysis used a combination of single strand conformational polymorphism (SSCP) and bi-directional direct-cycle sequencing according to previously described protocols [9], and using previously published primers [10-14]. Control individuals (140) of mixed ethnicity (including 40 of East Indian descent) were recruited with a full ocular examination excluding the presence of glaucoma.
RESULTS

Fifteen patients affected with Peters anomaly were recruited (including the previously reported individual, Case 3 in Table 1 [5]). No disease causing mutation was identified in the coding sequences of MYOC, PITX2, or PAX6. Mutations in CYP1B1 were identified in three patients affected with Peters anomaly (20%). In two of these two patients, only one disease-causing mutation was identified despite bidirectional sequencing of all coding exons. The details of these findings are summarized in the Table 1.

In Case 1, the missense mutation 7940G>A, (Arg 368 His) involved one allele and was previously reported to segregate with congenital glaucoma [15]. In addition, this patient was also heterozygous for the leucine432 allele of the previously studied Val432Leu polymorphism [16]. Case 1 (Arg368His) had bilateral Peters anomaly with full thickness corneal opacity involving the inferotemporal quadrant in one eye, and the inferonasal quadrant in the other eye, both extending into the visual axis, with iridolenticular and iridocorneal adhesions (Figure 1). She developed glaucoma at age eight years, for which bilateral trabeculectomies were performed. Her glaucoma is now controlled with the addition of topical latanoprost and timolol. Case 1 is of east Indian ancestry and the only affected family member. This sequence change was observed in 1 of 140 controls (0.7%), 40 of which were of east Indian descent (Table 1). The positive control was of Saudi Arabian ancestry.

In Case 2 (a Caucasian child), a novel missense mutation, 4157C>A (Pro118Thr), was predicted to change the highly conserved proline residue in position 118 to threonine. This Pro118Thr mutation was not observed in 100 controls of mixed ethnicity. Immediately adjacent to this mutation, case 2 was also homozygous for the less common serine form of the Ala119Ser polymorphism. Previous studies consistently show the frequency of the serine/serine polymorphism in populations of different ethnicities ranges from 4-9% [11,15,17]. In addition case 2 was also heterozygous for the leucine432 allele of the previously studied Val432Leu polymorphism [16]. Case 2 has not developed glaucoma at the age of nine years. No other family members were available for this study. Slit lamp photographs were not available.

Case 3 (previously published) was a compound heterozygote for the missense mutation 3807T>C which is predicted to result in the amino acid change Met1Thr, and the nonsense mutation 3976G>A, which is predicted to truncate the P450I1B1 polypeptide (Trp57Stop,) and possibly cause nonsense-mediated decay of the CYP1B1 mRNA [5]. This child had early onset glaucoma. No mutation was identified in MYOC, which had not previously been sequenced in this individual.

DISCUSSION

The identification of mutations in two additional cases of Peters anomaly reported herein suggests that mutations in CYP1B1 could be contributory in up to 20% (3/15) of cases of Peters anomaly. For the two patients where only one change was identified, a second mutation in CYP1B1 on the other allele may have been missed by the techniques used. It is also possible that a second change may involve regulatory regions surrounding the gene, or mRNA processing. It could also be that the single abnormal CYP1B1 allele may be sufficient to alter the expression of CYP1B1 or that it may modify the expression of another gene involved in anterior segment development. This type of interaction was recently proposed to play a role in primary open angle glaucoma (POAG) [18]. In addition, recent research by Melki et al. [19] demonstrated that 4.6% of French POAG patients carried one or two mutated CYP1B1 gene(s) and no MYOC mutation. It was proposed that

<table>
<thead>
<tr>
<th>Case</th>
<th>Mutation(s) identified</th>
<th>Predicted amino acid change</th>
<th>Control individuals with a mutation</th>
<th>Phenotype</th>
<th>Leu432Val CTG&gt;GTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7940G&gt;A</td>
<td>Arg368His</td>
<td>1/140</td>
<td>Bilateral Peters anomaly and juvenile glaucoma</td>
<td>C/G</td>
</tr>
<tr>
<td>2</td>
<td>4157C&gt;A</td>
<td>Pro118Thr</td>
<td>0/100</td>
<td>Bilateral Peters anomaly and no glaucoma (age 9 years)</td>
<td>C/G</td>
</tr>
<tr>
<td>3*</td>
<td>3807T&gt;C</td>
<td>Met1Thr</td>
<td>0/100</td>
<td>Bilateral Peters anomaly and congenital glaucoma</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>3976G&gt;A</td>
<td>Trp57Stop</td>
<td>0/100</td>
<td>Bilateral Peters anomaly and congenital glaucoma</td>
<td>C/C</td>
</tr>
</tbody>
</table>

Details of mutational analysis of CYP1B1 in patients affected with Peters anomaly (n=15). The asterisk indicates that the mutation has been previously reported [5]. In the “Leu432Val” column, the changed base is marked in red.
a heterozygous change in CYP1B1 could act as a modifier of another gene and could contribute to a milder phenotype than seen in a homozygote or compound heterozygote. The phenotypes of cases 1 and 2 are less severe than that seen in our previously published case, who had bilateral corneal opacities and congenital glaucoma manifest within days of birth and two significant mutations [5].

The Arg368His mutation appears to be the predominant glaucoma mutation in an Indian primary congenital glaucoma (PCG) population, accounting for 17% of mutations in PCG patients [20]. This sequence change was found at disproportionately very low frequency (one individual, 0.7%) in our ethnically matched controls, as in a previous study which supports its pathogenic role [18]. The Arg368 residue is highly conserved and predicted to be involved in protein folding or heme binding [21]. The functional effect of Arg368His is currently not known. Functional studies of other CYP1B1 mutant proteins (Gly61Glu and Arg469Trp) demonstrate altered activity on estradiol substrates [22].

In vitro studies exploring the potential functional implication of the polymorphic leucine variant of the Val432Leu polymorphism (8131C>G) suggest that the heterozygous “polymorphism” results in altered 4-hydroxylation of 17-β-estradiol [23,24]. Whether this is enough to destabilize CYP1B1 activity to impact anterior segment formation remains to be investigated.

The combination of heterozygote CYP1B1 mutations (Arg368 His and Pro118Thr) with this leucine432 variant may result in a functional disadvantage and lead to the observed phenotype. Further study is required to test this hypothesis, as our techniques did not determine whether the mutation and polymorphisms reside within the same allele.

The role of CYP1B1 mutations in early onset glaucoma is well documented in association with either homozygous or compound heterozygous mutations. However, there are numerous patients with PCG who are heterozygous for a CYP1B1 mutation in whom the second mutation has not been identified [15,25,26]. The identification of case 2 who does not have glaucoma supports the role of CYP1B1 mutations in anterior segment development, beyond congenital glaucoma pathology.

Peters anomaly appears to be genetically heterogeneous. The association of Peters anomaly with PAX6 mutations remains controversial because of the phenotype and inheritance pattern described [2,27,28]. The PAX6 mutation database lists 309 unique mutations, of which two are associated with a Peters phenotype. Only one PITX2 mutation has been documented with Peters anomaly [3]. The association of FOXC1 mutations and Peters anomaly has been reported in two cases. Nishimura et al. [4,29] described a duplication of 6p25 with anterior chamber defects only [4] and a case with an unbalanced translocation [29]. Honkanen et al. [30] reported a case of Peters anomaly among a family affected with Rieger malformation. All affected individuals carried a Phe112Ser mutation in FOXC1. Although Peters anomaly is an atypical feature of FOXC1 mutations, this observation could suggest that in some cases Peters anomaly and Rieger malformation are allelic variants and are part of spectrum of anterior segment dysgenesis.

Although our sample size is small, PAX6, PITX2, and MYOC appear not to play prominent roles in the genesis of Peters anomaly. However the study of our patient cohort does support the pathogenic role of CYP1B1 in Peters anomaly. This collective information suggests that the Peters phenotype represents a variant of a genetically heterogeneous group of conditions referred to as anterior segment dysgenesis. The severity of the resulting phenotypes may depend on various parameters including either the timing or the cumulative severity of the insult(s).

Our findings support a significant role of CYP1B1 mutations in Peters anomaly and its likely contribution to anterior segment development beyond the primary congenital glaucoma phenotype.

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Figure 1. Ocular phenotype of Case 1 (Arg368His). Slit lamp photograph of the right eye following trabeculectomy and cataract extraction showing a corneal opacity involving the inferotemporal quadrant of the cornea. The brown ring in the center reflects the site of the preoperative iridolenticular adhesion. Iridocorneal adhesions are seen to the inferotemporal aspect of the opacity.
REFERENCES


