Identification of Novel Mutations in Pakistani Families With Autosomal Recessive Retinitis Pigmentosa

Retinitis pigmentosa (RP) is a major cause of inherited blindness and accounts for 20% of children attending blind schools in Pakistan. Based on the European and American populations, the prevalence is estimated at 1 in 4000 individuals but has been reported to be as high as 1 in 372 in rural areas of South India. Eight loci and 44 genes have been associated with RP (RetNet, http://www.sph.uth.tmc.edu/RetNet). Pakistan is among the countries with the highest prevalence of consanguineous marriages. Consanguineous families are ideally studied by homozygosity mapping to identify the locus harboring the mutated gene causing the disease phenotype. To identify novel mutations and genes causing RP, we studied a panel of 23 consanguineous families with autosomal recessive RP (arRP) from different areas of Pakistan.

Methods. This study adhered to the tenets of the Declaration of Helsinki and has been approved by the Shifa College of Medicine and Shifa International Hospital institutional review boards. In most of the 23 families, there was consanguinity involving first-cousin marriages. Furthermore, in the nonconsanguineous families, most marriages were between individuals from the same ethnic group. Most of the families have more than 5 affected individuals with RP. In all 23 families, the onset of the disease was in the first or second decade of life. The initial clinical diagnosis was based on the onset of night blindness followed by clinical diagnostic tests including funduscopy and electroretinography. Homozygosity for the known arRP genes and loci was evaluated in 15 families through microsatellite marker analysis, and genome-wide analyses using single-nucleotide polymorphism arrays were performed in 13 selected families. The subsequent mutation analysis of the known RP genes residing in the homozygous regions was then carried out.

Results. The homozygosity mapping approach resulted in linkage of the retinal phenotypes to known retinal disease genes in 11 families and in identification of the genetic defect in 9 of these families. The length of the homozygous regions ranged from 3.29 to 34.2 megabases (Table). Most of the mutations identified in Pakistani patients were found to affect components of the phototransduction cascade. Four novel mutations were identified in this study: 1 missense mutation (c.3296C>T, p.T1099K) in CRB1, 1 missense mutation (c.2284C>T, p.R762C) and 1 splice-site mutation (c.412-1G>A) in CNGB1, and 1 splice-site mutation (c.1722+1G>A) in PDE6B.

Comment. Through homozygosity mapping in large families with and without reported consanguinity, we searched for the genetic causes of arRP in Pakistan. Causative mutations were identified in 9 of 23 families (39%). In 3 families, the diagnosis was corrected after the genetic defects were identified. Eleven families (48%) could not be linked to any known arRP gene or locus and likely harbor mutations in novel arRP genes. Molecular genetic analyses performed in this study were helpful to establish a proper diagnosis and provide genetic counseling in those areas of Pakistan where there is less awareness about this disease. Establishing a molecular diagnosis is also a prerequisite to enter patients into future gene augmentation trials for retinal degeneration.

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Table. Linkage and Mutation Analysis Results of Known Retinal Dystrophy Genes in Pakistani Families

<table>
<thead>
<tr>
<th>Family</th>
<th>Method</th>
<th>Chromosome</th>
<th>Flanking Markers</th>
<th>LOD Score</th>
<th>arRP Gene in the Region</th>
<th>Gene Function</th>
<th>Size of Homozygous Region, Mb</th>
<th>Identified Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP04</td>
<td>Microsatellite</td>
<td>1q31.3</td>
<td>D1S1189-D1S1660</td>
<td>ND</td>
<td>CRB1</td>
<td>Structural integrity</td>
<td>3.29</td>
<td>c.3296C&gt;T, p.T1099K</td>
</tr>
<tr>
<td>RP12</td>
<td>Microsatellite</td>
<td>16p13</td>
<td>D16S3039-D16S3050</td>
<td>ND</td>
<td>CNGB1</td>
<td>Signal transduction (rods)</td>
<td>9.1</td>
<td>c.412-1G&gt;A</td>
</tr>
<tr>
<td>RP19</td>
<td>10K SNP array</td>
<td>13q34</td>
<td>rs1927724-rs723995</td>
<td>3.29</td>
<td>GRK1</td>
<td>Inactivation of phototransduction</td>
<td>15.2</td>
<td>c.614C&gt;A, p.S205X</td>
</tr>
<tr>
<td>RP21</td>
<td>Microsatellite</td>
<td>3p21.3</td>
<td>D3S1269-D3S3546</td>
<td>2.6</td>
<td>RH0</td>
<td>Light absorption</td>
<td>18.5</td>
<td>c.448G&gt;A, p.E150K</td>
</tr>
<tr>
<td>RP23</td>
<td>10K SNP array</td>
<td>4p16.3</td>
<td>rs718429-rs742858</td>
<td>2.57</td>
<td>PDE6B</td>
<td>Hydrolysis of cGMP</td>
<td>6.8</td>
<td>c.1722+1G&gt;A</td>
</tr>
<tr>
<td>RP24</td>
<td>10K SNP array</td>
<td>16p13</td>
<td>rs1582594-rs3852784</td>
<td>3.46</td>
<td>CNGB1</td>
<td>Signal transduction (rods)</td>
<td>14.3</td>
<td>None</td>
</tr>
<tr>
<td>RP26</td>
<td>10K SNP array</td>
<td>2q11.2</td>
<td>rs178159-rs1373002</td>
<td>3.81</td>
<td>CNGB3</td>
<td>Signal transduction (cones)</td>
<td>9.0</td>
<td>c.822G&gt;T, p.R274S</td>
</tr>
<tr>
<td>RP42</td>
<td>10K SNP array</td>
<td>2q11.2</td>
<td>rs282571-rs275517</td>
<td>3.0</td>
<td>CNGB1</td>
<td>Signal transduction (rods)</td>
<td>4.2</td>
<td>c.2284C&gt;T, p.R762C</td>
</tr>
<tr>
<td>RP43</td>
<td>10K SNP array</td>
<td>4p15.32</td>
<td>rs909015-rs965978</td>
<td>2.1</td>
<td>PROM1</td>
<td>Structural integrity</td>
<td>20.6</td>
<td>None</td>
</tr>
<tr>
<td>RP44</td>
<td>10K SNP array</td>
<td>8q21.3</td>
<td>rs1545881-rs1116032</td>
<td>1.22</td>
<td>CNGB3</td>
<td>Signal transduction (cones)</td>
<td>34.2</td>
<td>c.1825delG, p.V609WfsX9</td>
</tr>
<tr>
<td>RP53</td>
<td>Microsatellite</td>
<td>2q35.3</td>
<td>D3S3606-D3S3694</td>
<td>ND</td>
<td>RH0</td>
<td>Light absorption</td>
<td>11.3</td>
<td>c.448G&gt;A, p.E150K</td>
</tr>
</tbody>
</table>

Abbreviations: arRP, autosomal recessive retinitis pigmentosa; cGMP, cyclic guanosine monophosphate; LOD, logarithm of the odds; Mb, megabases; ND, not determined; SNP, single-nucleotide polymorphism.
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Report of a Case. A 49-year-old Asian Indian man had decreased vision in both eyes for 3 months, with pain and redness in the right eye for 1 month. Both eyes had a best-corrected visual acuity of counting fingers at 1 m with nongranulomatous anterior uveitis, posterior synechiae, cataracts, and dense vitritis. In addition, the right eye had an iris bombe configuration with an intraocular pressure of 44 mm Hg. Systemic history and examination results were unremarkable. Antiglaucoma medication, topical steroids, and cycloplegics were started. A motile worm (approximately 200 µm long) was subsequently observed on the anterior capsule of the left eye. Peripheral blood smear showed microfilariae of Wuchereria bancrofti. Stool examination was negative for cysts or ova of parasites.

Oral prednisone (1 mg/kg/d) was started, and antifilarial treatment (diethylcarbamazine, 6 mg/kg/d for 3 weeks, and albendazole, 400 mg once daily for 1 week) was initiated 3 days later. Treatment with oral doxycycline hydrochloride, 100 mg twice daily, was given for 4 weeks to eradicate Wolbachia. Glaucoma was unresponsive to medical management. Therefore, a trabeculectomy with phacoemulsification and intraocular lens implantation was performed on the right eye within 1 week of commencing treatment with steroids. The postoperative period was uneventful. Six months later, phacoemulsification with intraocular lens implantation was performed on the left eye. Steroids were gradually tapered and stopped. Both eyes have been quiet for 6 months, with a visual acuity of 6/12 J2 due to an epiretinal membrane over the macula. Peripheral blood smear 1 year after doxycycline treatment did not show any microfilaria.

Comment. Uveitis secondary to intraocular filariasis in the Indian subcontinent is mainly due to W bancrofti and Brugia malayi.1 Intraocular filariasis is caused more commonly by microfilariae than by adult worms.2 This is an unusual case of microfilariae causing bilateral uveitis, which to our knowledge has been reported in only 1 other article.3

The role of antifilarial drugs is controversial because of the possibility of increased uveitis due to the killing of microfilaria as seen in the Mazzotti reaction. However, several reports have used these successfully under steroid cover without untoward effects.2,4 Diethylcarbamazine and ivermectin clear the microfilaria from the blood but do not act on the adult worm, which lives in the lymphatic system for 10 to 15 years.2,3 Therefore, repeated treatments may be necessary to prevent recurrent episodes of uveitis.2,4 Albendazole can reduce the microfilaria possibly due to its embryotoxic effect on the adult worms.2 Recently, an endobacterium of the Wolbachia species that belongs to the family Rickettsiaceae cline hydrochloride, which sterilizes adult worms by eliminating their symbiotic bacteria and may prevent recurrences of uveitis.

Management of Bilateral Uveitis Secondary to Intraocular Filariasis

Management of uveitis secondary to filariasis has been inadequately described as these cases are rare. We report a case of bilateral uveitis due to intraocular filariasis and discuss its medical and surgical treatment. We also document the use of doxycycline hydrochloride, which sterilizes adult worms by eliminating their symbiotic bacteria and may prevent recurrences of uveitis.

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