Phenotypic Expression of a PRPF8 Gene Mutation in a Large African American Family

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Objectives: To describe the phenotype and determine the genetic cause of autosomal dominant retinitis pigmentosa (adRP) in a large African American family.

Methods: Fourteen members from 4 generations were evaluated clinically. Visual field measurements were made for most, and electroretinography, Tubinger perimetry, and optical coherence tomographic testing were done for individual family members. Genetic screening was performed on a recently introduced adRP microarray that contains approximately 400 mutations from 13 genes.

Results: All of the affected members had a type 1 form of adRP, characterized by early onset of symptoms for visual impairment, marked central and peripheral vision loss, nondetectable electroretinographic responses, and decreased macular thickness on optical coherence tomographic testing. Two variants in the PRPF8 gene were identified in the proband, H2309R and IVS41-4G→A. The H2309R mutation segregated with the disease in the family, whereas the IVS41-4G→A variant did not.

Conclusions: The severe form of adRP was caused by the PRPF8 H2309R variant, whereas the IVS41-4G→A variant was benign.

Clinical Relevance: PRPF8 mutations should be suspected in patients with a type 1 form of adRP. A combination of advanced clinical workup and comprehensive genetic testing is essential for the precise diagnosis of diseases with high genetic heterogeneity such as RP.
The pedigree (Figure 1) portrays an autosomal dominant form of inheritance in an African American family. Fourteen members from 4 successive generations were examined by one of us (G.A.F.) at the University of Illinois at Chicago. The project was approved by an institutional review board at the University of Illinois, and informed consent was obtained from all of the participating family members. The research was conducted according to the tenets of the Declaration of Helsinki.

Proband II:5 was the first to be examined by one of us (G.A.F.). She had symptoms of nyctalopia and photosensitivity. Other members of the family were subsequently examined.

Best-corrected visual acuity was recorded in all of the patients. Complete clinical examination including slitlamp and dilated fundus examinations were performed. Visual fields were tested using a Goldmann perimeter with 2 or more target sizes (II-4-e, III-4-e, and V-4-e). Electroretinography results were obtained in 5 members of the family using a previously described procedure.17 Electroretinography was not performed when either there was profound visual field restriction or the patient was unwilling to undergo the examination. Threshold perimetry was performed in 1 family member (IV:16) using a Tu¨ binger perimeter and a previously described procedure.17 Optical coherence tomography was performed on patient III:10 (RTVue software version 2.0.3.2; Optovue Inc, Fremont, California). Detection thresholds were measured at different retinal locations along the horizontal meridian using a Tu¨ binger perimeter.

We extracted DNA from blood collected from the proband (II:5) and subjected it to screening with the adRP geno-
typing microarray. For these purposes, 42 amplicons from 13 genes (RP1, RHO, RDS, IMPDH1, PRPF3, PRPF8, PRPF31, NRL, CA4, ROM1, FSCN2, and CRX) were amplified by polymerase chain reaction as described previously (details of manufacturing microarrays, primer extension, screening procedures, and analysis are given in the articles by Zernant et al and Allikments and Zernant). Array-identified variants were confirmed by direct sequencing with the Taq Dyedeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California) according to the manufacturer’s instructions. Sequencing reactions were resolved on an ABI 377 automated sequencer (Applied Biosystems).

**RESULTS**

The patients examined within this family were aged 5 to 54 years. All of the patients reported a history of nyctalopia starting from the first decade of life. With few exceptions, visual field loss was also reported at an early age. The youngest family member (aged 5 years) to be examined, patient IV:21, had a visual acuity of 20/25−2 in each eye as measured on a Snellen visual acuity chart. The best-corrected visual acuity in the better eye was 20/200 or better in all of the patients except patient IV:20, who had a best-corrected visual acuity of 8/350 in her better seeing eye as measured by a Feinbloom visual acuity chart (Table 1).

Anterior segment examination results were within normal limits in 10 patients, whereas 4 patients showed minimal to moderate posterior subcapsular cataractous changes (Table 1).

The clinical findings on fundus examination are listed in Table 2. The optic disc was observed to be clinically normal in 7 patients, waxy disc pallor was observed in 6 patients, and optic atrophy was seen in 1 patient. Four members of the family showed cystoid macular edema on at least 1 of their clinical examinations, which had further progressed to either a macular hole or an atrophic-appearing lesion in the macular region in 3 of them (Figure 2). Eight family members had a clinically normal appearance of the macula and 2 others had already developed atrophic-appearing lesions in the macula on their initial visit. All

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**Table 2. Characteristics of Fundus Examination, Electroretinography Results, and Pattern of Visual Field Loss**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Optic Disc</th>
<th>Macula</th>
<th>ERG Response</th>
<th>Pattern of Visual Field Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>II:5</td>
<td>Atrophy</td>
<td>Atrophy</td>
<td>Not done</td>
<td>I</td>
</tr>
<tr>
<td>III:6</td>
<td>Normal</td>
<td>Normal</td>
<td>Nondetectable</td>
<td>IIA</td>
</tr>
<tr>
<td>III:7</td>
<td>Waxy pallor</td>
<td>Waxy pallor</td>
<td>Not done</td>
<td>I</td>
</tr>
<tr>
<td>III:10</td>
<td>Normal</td>
<td>Atrophy</td>
<td>Not done</td>
<td>I</td>
</tr>
<tr>
<td>III:11</td>
<td>Waxy pallor</td>
<td>Waxy pallor</td>
<td>Macular hole</td>
<td>I</td>
</tr>
<tr>
<td>III:12</td>
<td>Normal</td>
<td>Normal</td>
<td>Nondetectable</td>
<td>IIA</td>
</tr>
<tr>
<td>IV:8</td>
<td>Waxy pallor</td>
<td>Waxy pallor</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>IV:12</td>
<td>Normal</td>
<td>Normal</td>
<td>Nondetectable</td>
<td>I</td>
</tr>
<tr>
<td>IV:16</td>
<td>Waxy pallor and drusen</td>
<td>Waxy pallor and drusen</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>IV:19</td>
<td>Waxy pallor</td>
<td>Atrophy</td>
<td>Nondetectable</td>
<td>IIA</td>
</tr>
<tr>
<td>IV:20</td>
<td>Waxy pallor</td>
<td>Macular hole</td>
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<td>I</td>
</tr>
<tr>
<td>IV:21</td>
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<td>Not done</td>
</tr>
<tr>
<td>IV:25</td>
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<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>V:6</td>
<td>Normal</td>
<td>Normal</td>
<td>Not done</td>
<td>Not done</td>
</tr>
</tbody>
</table>

Abbreviations: CME, cystoid macular edema; ERG, electroretinography; ERM, epiretinal membrane.

**Figure 2. Fundus photographs.** A, Macular hole along with retinal vessel attenuation and pigment clumping in the left eye of patient III:11. B, Waxy pallor of the optic disc, attenuated retinal vessels, extensive bone spicule–like pigmentation, and retinal pigment atrophy with mild pigment clumping and atrophic-appearing changes in the macula in patient II:5.
Electroretinography measurements showed non-detectable cone and rod responses in 5 patients. This procedure was not performed on additional family members. The test stimulus for both the 500-nm and 656-nm wavelengths was detected by the cone system at all of the eccentricities in the dark-adapted state by Tubinger perimetry testing (Figure 5).

An optical coherence tomographic image of patient III:10 showed marked thinning of the retina at the center of the fovea, visualized as atrophic changes on clinical examination. There was absence of the inner-outer segment juncture. The outer nuclear layer in this patient was thin as compared with a visually normal control subject (Figure 6).

In the genetic analysis, the proband possessed 2 variants, H2309R and IVS41-4G→A, in the PRPF8 gene. Eleven other members of the family were screened for the 2 variants by direct sequencing. Of those screened, 11 family members (I:10, II:5, III:6, III:7, III:11, III:12, IV:12, IV:16, IV:19, IV:20, and IV:21) who were clinically diagnosed with RP had the H2309R mutation, whereas the only person without RP (II:3) had no mutation. The IVS41-4G→A variant was detected in only 3 affected family members (II:5, III:6, and III:7) and was therefore defined as a benign intronic variant, although it has been previously suggested but not confirmed as a possibly pathogenic splice mutation.22

Autosomal dominant RP is a group of genetically heterogeneous retinal degenerations estimated to contribute from 20% to between 30% and 40% of all RP cases.23,24 Mutations in the PRPF8 gene are estimated to be involved in 2% to 3% of adRP and cause a more severe (sub)phenotype.23,24

PRPF8 encodes Prp8, a key factor in messenger RNA splicing. PRPF8 is highly conserved across species and is expressed in all cell types.10,23,26 However, mutations in this gene are only expressed in the retinal tissue. It has been observed that PRPF8 mutations weaken but do not abolish interactions of Prp8 with its spliceosome partners, which may not be sufficient for retina-specific splicing events as opposed to the same events in other tissues.23 It has also been hypothesized that PRPF8 mutations may disrupt the interactions between Prp8 and a partner yet to be identified, which may reflect an unknown function of Prp8 specific to the retina.26

Several mutations in PRPF8 are associated with adRP. Although other studies have documented a severe form of disease associated with mutations in this gene, limited clinical description is available. One study has documented a mild phenotype with partial preservation of the cone function in a family with a point mutation in PRPF8.25 To our knowledge, this is the first report of a PRPF8 mutation in a family of African descent; prior observations are reported in British, Dutch, Italian, and Spanish families.5,7,13,16

All of the patients in our family had an early onset of nystagmus in the first decade of life. van Lith-Verhoeven et al13 reported a late onset of symptoms in 3 male mem-

Figure 3. Pattern IA of visual field loss (A-C) in proband IV:20 over a period of 14 years.

of the patients showed attenuated retinal vessels and diffuse bone spicule-like pigment clumping for 360°.

Using Goldmann perimetry, 2 patterns of visual field loss were observed.21 The type IA pattern, consisting of concentric visual field loss, was observed in 6 patients (Figure 3). Five patients demonstrated a type IIA pattern of visual field loss, with initial nasal or superior nasal restriction; the scotoma then wound around inferiorly from the nasal side, leaving a central field and a temporal island (Figure 4). No records were available on the visual fields of 3 family members.
Figure 4. Sequence of pattern II A visual field loss (A-D). Visual fields from patients IV:8, IV:19, and IV:25 are included.

bers of a Dutch family, which was not observed in our African American family. However, 6 other members of the Dutch family described previously were reported to have had the onset of their symptoms between ages 6 and 20 years. The range of visual acuity in our family was from 20/20 to 8/350, which is similar to previous descriptions.13,15,16 Posterior subcapsular lens changes were observed in 5 of the 14 members of our family. Tarttelin et al15 also noted cataracts as a late complication of this mutation.

Optic disc pallor, bone spicule–like pigmentation, attenuated vessels, and cystoid macular edema have been mentioned previously.13,16 In our family, waxy pallor of the optic disc was noted in 6 members and optic atrophy in 1; attenuated vessels and diffuse bone spicule–like pigmentation were observed in all of the patients. Cystoid macular edema was observed in 4 members photographically and by clinical examination of the family, and it may occur with notable frequency in patients with a PRPF8 mutation.

Restriction of the visual field “up to 10°” in most members of a Dutch family was described previously.13 In our family, 2 distinct patterns of visual field loss were observed. Six members showed progressive concentric visual field loss, whereas 5 others showed midperipheral visual field loss that progressed from the nasal to the temporal side. Electoretinography showed nondetectable rod and cone function in 5 members who were tested; similar responses have been cited previously.13 Testa et al16 reported recordable photopic electoretinography responses with a mean amplitude of 47 µV in 5 of the 6 members in a family with a point mutation in the PRPF8 gene and a recordable scotopic response in the youngest member of the same family.

Genetic testing revealed a missense mutation, H2309R, that segregated with the disease, ie, it was present in 11 affected members and absent in 1 unaffected member. Three affected family members were also found to have a splice-site variant IVS41-4G→A in addition to the missense mutation. No difference in phenotypic expression was seen in these 3 family members as compared with the others. Based on our observations, we conclude that this splice-site variant is most likely a benign polymorphism and probably not pathogenic as previously suggested.22

Based on our findings, PRPF8 should be one of the first genes to be analyzed in any patient with a severe form of adRP showing early onset of symptoms, diffuse involvement of the retina, cystoid macular edema, characteristic marked visual field loss, and electoretinographic changes. The adRP genotyping microarray is suggested as the preferred first-pass screening tool before more expensive and laborious screening methods such as direct sequencing are used.

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Figure 5. Dark-adapted (DA) threshold profile measurement in patient IV:16 showing cones mediating threshold for 656- and 500-nm stimulus wavelengths. The broken line represents the reference range for thresholds.

Figure 6. Horizontal optical coherence tomographic scans. A, Left eye from patient III:10 showing marked thinning of the retina in the foveal region. B, Eye from a visually normal control subject.

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