A New 2–Base Pair Deletion in the RPGR Gene in a Black Family With X-Linked Retinitis Pigmentosa

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Objective: To report the genetic and ophthalmic findings in a black family with X-linked retinitis pigmentosa resulting from a newly identified mutation in the RPGR (retinitis pigmentosa GTPase regulator) gene.

Patients: Four affected hemizygotes with retinitis pigmentosa and 2 obligate carriers were examined. Two unaffected family members, 1 woman and her unaffected son, were also examined.

Methods: Patients underwent a routine ocular examination including slitlamp examination and a dilated fundus examination. Certain patients also underwent testing with Goldmann visual field kinetic perimetry and electroretinography. DNA screening from affected male patients, 2 obligate carriers, and 2 unaffected family members was performed to determine the presence of any mutation in the RPGR gene.

Results: A 2–base pair deletion in exon 13 of the RPGR gene that creates a frameshift was found to segregate with the retinal disease in affected males and the carrier state in female heterozygotes in this family. The ophthalmic findings in hemizygotes and carriers were within the spectrum of findings characteristically noted in families with X-linked retinitis pigmentosa. In 2 obligate carriers, a tapetal-like reflex was not clinically apparent.

Conclusions: The described mutation is the first RPGR gene mutation reported in a black family. A 2–base pair deletion in exon 13 segregates with a clinical phenotype of X-linked retinitis pigmentosa.


**REPORT OF CASES**

**CASE 1**

The proband (Figure 1, patient II-1), a black man, was first seen by one of us (G.A.F.) at the age of 33 years. He had experienced poor night vision and impaired peripheral fields since early childhood. Several male members of the family were known to be affected with retinitis pigmentosa. His best-corrected visual acuity at that time was 20/50–2 OD and 20/50–1 OS. He was last examined at 41 years of age. The best-corrected visual acuity on his most recent visit was 20/80–2 with a refraction of −2.00+0.50×90 OD and 20/70+1 with a refraction of −1.25 OS. The intraocular pressure was normal in both eyes.

RETINITIS PIGMENTOSA is the term applied to a clinically and genetically heterogeneous group of progressive retinal dystrophies. Patients are afflicted with impairment of their night vision, peripheral vision, and, eventually, central vision.

Of the 3 genetically transmitted types, the X-linked form of retinitis pigmentosa (XIRP) is recognized as the most severe. In our population of patients with retinitis pigmentosa, the X-linked form accounts for 8% to 9% of families with retinitis pigmentosa.

By linkage analysis, 2 major genetic loci for XIRP on the short arm of the X chromosome have been identified. Retinitis pigmentosa type 2 (RP2) was linked to L1.28 at Xp11.3, while retinitis pigmentosa type 3 (RP3) was confirmed at the location Xp21.5,7 Multipoint linkage data have determined that the RP3 locus accounts for 70% or more of families with XIRP in different populations.6,11

A gene called RPGR (retinitis pigmentosa GTPase regulator) has recently been cloned, and mutations in this gene have been identified in approximately 10% of 74 XIRP-affected families from the United Kingdom and Germany.12 We report the ophthalmic findings in the first black family with a previously undescribed 2-base pair (bp) deletion in exon 13 of the RPGR gene.
PATIENTS AND METHODS

This black family was one of 17 families with XIRP who were screened for a possible mutation in the RPGR gene and examined by one of us (G.A.F.). The clinical and genetic criteria used to determine X-linked inheritance were previously described. The ocular examination in female family members focused on the presence or absence of peripheral pigmentary degenerative changes, as well as whether a tapetal-like reflex was present. An ophthalmic examination was performed by one of us (G.A.F.) on all family members who were available. This examination included best-corrected visual acuity with a Snellen projection chart with calibrated luminance. Slitlamp examination of the cornea, anterior chamber, lens, and vitreous was also performed. In selected patients, ocular pressure was determined byplanation tonometry. A dilated fundus examination with direct and indirect ophthalmoscopy was performed. Whenever possible, an electroretinogram (ERG) was obtained by means of 1 of 2 procedures previously described. These recording techniques adhere to the international standard for electrophysiologic measurements. All results were compared with the 90% tolerance limits or an appropriate range for a normal population.

A single-flash waveform was considered nondetectable if the amplitude was less than 10 µV, while a 30-Hz flickering stimulus was considered nondetectable if the response was less than 1 µV.

Visual field examination was performed monocularly with the use of a Goldmann perimeter using the II-4-e, III-4-e, and V-4-e test targets. The targets were moved from nonseeing to seeing regions. A review of the history of each patient disclosed when the patient first experienced nyctalopia or when the patient was first observed by a parent to have noticeable impairment in night vision. Each patient was also asked about whether and when impairment of peripheral vision was noted.

Genomic DNA was isolated from lymphocytes by standard methods. Screening for mutations in the RPGR gene was performed by sequence analysis of the genomic DNA.

Examination of the cornea and anterior chamber in each eye revealed no clinically significant findings. Slitlamp examination showed mild posterior subcapsular changes in the right eye and moderate changes in the left eye. Both eyes had moderate anterior cortical changes. The vitreous showed a small number of cells in each eye. Fundus examination showed pale optic discs with a refraction of −0.75+1.50

Goldmann visual field testing showed markedly restricted fields with targets V-4-e, III-4-e, and II-4-e (Figure 3). There was a remaining central 40 degrees of visual field with target V-4-e. An ERG demonstrated nondetectable cone or rod responses to single-flash or flicker stimuli.

A set of primers was used, allowing the polymerase chain reaction (PCR) amplification of single exons (2-19) from genomic DNA. A total of 200 ng of genomic DNA was amplified in 50 µL reactions containing the following concentrations: potassium chloride, 30 mmol/L; TRIS hydrochloride (pH 8.3), 10 mmol/L; deoxyribonucleoside triphosphates, 200 µmol/L; magnesium chloride, 1.5 mmol/L; each primer, 1 µmol/L; and Taq polymerase, 0.5 U (Gibco-BRL-Life Technologies, Gaithersburg, Md).

Cycling conditions included 5 minutes of initial denaturation at 94°C, 1 minute of annealing at appropriate temperature, and 2 minutes of extension at 72°C, with a final 7-minute extension at 72°C.

After amplification products were analyzed in 2% agarose gel, they were subjected to direct sequencing by a dideoxynucleotide chain termination method with the use of a reaction buffer (Sequenase, US Biochemicals, Amersham, Cleveland, Ohio). Amplified DNA samples (5-50 ng) were treated with 10 U of exonuclease 1 and 2 U of shrimp alkaline phosphatase for 30 minutes at 37°C.

The same primers that were used for PCR amplification were used in the sequencing reactions. Templates were denatured for 30 minutes at 100°C and annealed with primers at 4°C for 20 minutes.

The sequencing reactions (10 µL total volume) contained 2 µL of Sequenase reaction buffer, 1 µL of a 0.1-mol/L concentration of dithiothreitol, 0.4 µL of labeling buffer, 0.5 µL of sulfur 35-deoxyadenosine triphosphate, 1.9 µL of glycerol diluent, 0.125 µL of inorganic pyrophosphatase (5 U/µL), and 0.125 µL of Sequenase (13 U/µL). The reactions were kept at 4°C for 5 minutes.

The completed reactions were electrophoresed in a 6% sequencing gel (Sequagel, National Diagnostics, Atlanta, Ga). Dried gels were exposed to x-ray film for 40 hours at room temperature. Sequence variations were confirmed by repeating PCR amplification followed by sequencing.

Twenty-microliter aliquots of the exon 19 PCR product were digested with 10 U of the BsrI restriction endonuclease at 37°C for 2 hours. DNA fragments were separated in a 2% agarose gel and visualized by staining with ethidium bromide.

CASE 2

A 43-year-old obligate carrier (Figure 1, patient II-5), the mother of patient III-2, was examined in 1993. There were no ocular symptoms of poor night vision, side vision, or central vision.

The best-corrected visual acuity was 20/25+1 with a refraction of −0.75+1.50×110 OD and 20/25+1 with a refraction of −2.50+1.75×40 OS. The intraocular pressure was normal in both eyes, and the cornea and vitreous were clear. The lenses showed trace nuclear sclerosis and minimal anterior subcapsular changes in both eyes.

The fundus examination showed normal optic discs and retinal vessels in both eyes. However, the retina showed pigment mottling at the posterior pole and isolated pigment clumping in the midperiphery (Figure 4).
Visual fields and an ERG were not obtained because the patient refused to participate in any further visual field or electrophysiologic testing.

**CASE 3**

A 28-year-old obligate carrier (Figure 1, patient II-11), the mother of patients III-15 and III-16, was examined in 1989. She had no subjective ocular symptoms of night blindness or impairment of peripheral vision.

Her best-corrected visual acuity was 20/25 with a refraction of +0.50+0.50×90 OD and 20/70 with a refraction of +0.50+3.00×90 OS. The cornea, lens, and vitreous were clear in each eye.

The fundus examination showed a normal optic disc and retinal vessels in both eyes. There was no evidence of any peripheral pigmentary changes in the retina. Although a typical tapetal-like reflex was not present, a nonspecific pigmentary mottling in the posterior pole was noted.

An ERG recording from each eye showed a moderate and symmetric reduction in the scotopic and photopic a- and b-wave amplitudes (Figure 5). The single-flash dark-adapted b-wave responses for rod or rod-dominant function and flicker-response implicit times were normal, while the light-adapted single-flash cone b-wave and 30-Hz flicker-response implicit times were prolonged.

**CASE 4**

A 6-year-old boy (Figure 1, patient III-2), a nephew of the proband, was first examined at 3 years of age because of problems seeing at night. He had been born 2 months prematurely and had received oxygen after birth. He had an extra digit on each hand.

At his most recent visit, the best-corrected visual acuity was 20/200 OD and 10/200 OS with a correction of −15.0 in each eye. The cornea and lenses were clear on slitlamp examination.

The fundus examination showed a prominent choroidal pattern with isolated areas of pigment clumping and mottling in both eyes. Accurate visual fields could not be obtained because of his young age. An ERG showed nondetectable cone and rod responses.
CASE 5

A 30-year-old woman (Figure 1, patient III-14), the mother of patient IV-1 and sister of patient III-2, was examined in 1996 with the complaint of slightly decreased central visual acuity since 9 years of age. She also had a history of slight impairment in night vision; she bumped into objects in a dark environment and took relatively more time to adapt to darkness. There were no subjective difficulties with peripheral vision or color vision.

The best-corrected visual acuity was 20/25-2 with a refraction of −0.50+0.50\(^\circ\) OD and 20/25-2 with a refraction of −0.75 OS. The intraocular pressure was 14 mm Hg in each eye.

The ocular media were clear. The fundus examination showed a normal optic disc, fovea, and retinal arterioles in both eyes with no evidence of bone-spicule pigmentation in the retinal periphery or a tapetal-like reflex in the central area. Goldmann visual fields were within normal limits with II-4e and III-4e test targets.

An ERG showed normal cone function to single-flash and 30-Hz flicker stimuli, and the isolated rod function was normal. The rod-dominant b-wave response of 380 µV was minimally reduced below the lower limit of 400 µV.

Despite her subjective complaints of impaired night vision, the normal findings of the ocular examination and ERG recordings were not diagnostic of a carrier state of XIRP.

CASE 6

A 12-year-old boy (Figure 1, patient III-15) also complained of poor night vision from the first decade of life. The best-corrected visual acuity was 20/200-1 with a refraction of −2.00+0.50\(^\circ\) OD and 20/60-2 with a refraction of −2.00+1.00\(^\circ\) OS. The anterior segment showed a clear cornea and lens by slitlamp examination. There were a few isolated cells in the vitreous of each eye. The fundus examination showed attenuated and sheathed retinal arterioles with a mottled appearance to the retinal pigment epithelium in each macula. There was moderate pigment mottling with no evidence of bone-spicule–like pigment clumping in the midperipheral retina.

Visual fields and ERG recordings were not obtained because of poor compliance.

CASE 7

A 13-year-old boy (Figure 1, patient III-16), the brother of patient III-15, had complained of poor night vision since 5 years of age, and peripheral visual field impairment since 10 years of age. He had also noticed poor central vision for as long as he could remember. There was a medical history of chronic bronchitis.

The best-corrected visual acuity was 20/30−2 with a refraction of −1.00 OD and 20/80 with a refraction of −2.00+0.50\(^\circ\) OS. The cornea and lenses were clear. The fundus examination showed attenuated retinal arterioles with moderate midperipheral pigment clumping, predominantly in the inferior and inferior nasal areas (Figure 6). There was pigment mottling of the retinal pigment epithelium in the maculae of both eyes.

Visual fields and ERG recordings were not obtained because of poor compliance.
CASE 8

A 9-year-old boy (Figure 1, patient IV-1) complained of a recent onset of difficulty with night vision and having failed a visual examination at school. There was an indication of a possible attention deficit disorder. The best-corrected visual acuity was 20/20-3 with a refraction of −0.75+1.75×105 OD and 20/20 with a refraction of −0.75+1.00×75 OS. He could correctly identify all the Ishihara test color plates with each eye. The external and slitlamp examinations revealed no clinically significant findings. There were no cells in the vitreous.

The fundus examination showed normal optic discs, foveas, retinal arterioles, and retinal periphery. There was no sign of pigmentary degeneration. Goldmann visual fields with targets V-4-e and II-4-e were normal in both eyes.

The ERG findings showed that cone function to single-flash and 30-Hz flicker was within the lower limits of normal, as was the isolated rod function to a low-intensity blue flash and dark-adapted rod-dominant response to a high-intensity stimulus.

RESULTS OF DNA ANALYSIS

The RPGR gene spanning the genomic region of approximately 70 kilobases consists of 19 exons. Because the primers for exon 1 did not produce the consistent results in repeated experiments, we used 18 primer sets (exons 2-19) to amplify genomic DNA obtained from the patients and screen it for mutations by direct sequencing of PCR products. Exon 1 represents less than 2% of the coding region.

The RPGR sequence obtained from patient II-1 showed a 2-bp deletion of nucleotides 1571 and 1572 (CA dinucleotide; numbering according to the human RPGR sequence, U5762912) in exon 13, which would create a frameshift (Figure 7).

This mutation does not affect any restriction site, and its segregation with the disease was confirmed by direct sequencing. The mutation was shown to be present in the patient’s sister, who is a carrier (II-11), as well as in her 2 affected sons (III-15 and III-16).

Patient II-1 also had another sequence variant, a C to A transversion, at nucleotide 2361 of exon 19, changing proline to threonine. This sequence change destroys the restriction site for the BsrBI restriction endonuclease. DNA samples from all available family members were PCR amplified with exon 19 primers, and the products were digested with this endonuclease. The results indicated that this sequence variant does not segregate with the disease in the family members studied (data not shown). This C to A transversion sequence variant was not observed in 79 additional male patients with XIRP from other unrelated families.19
The recent cloning of the RPGR gene has provided an opportunity to elucidate the nature of the mutations responsible for the disease and to study whether different mutations result in different phenotypes. The functional consequences of observed mutations are difficult to predict, because they depend on the amino acid changes and their precise location within the protein.

The small deletion in exon 13 in patient II-1 leads to a frameshift alteration and premature stop codon, resulting in a protein truncation. Of note, this observed 2-bp deletion within exon 13 in the family we studied occurred outside of the region homologous to RCC1, where most of the reported RPGR mutations have been found. However, its causative role in retinitis pigmentosa was confirmed by segregation with the disease in affected family members.

The ocular findings in the affected male members of this family were similar to those we have encountered in other families with XIRP. Such patients tend to manifest impairment of night vision within the first 2 decades of life and show appreciable impairment of their photoreceptor cell function. A high frequency of spherical myopia and plus cilindrical refractive errors is also noted. Reduction in central vision at an early age is also a characteristic feature.

Carrier females can also manifest certain ocular findings, such as a tapetal-like fundus reflex, isolated or occasionally extensive pigmentary degenerative changes, and reductions in ERG amplitudes. Asymmetry of fundus degenerative changes is not an uncommon finding. As in affected hemizygotes, carriers show a high frequency of spherical myopia and plus cilindrical refractive errors.

Neither of 2 obligate carriers in our pedigree who were examined showed a tapetal-like reflex. Only 1 showed evidence of peripheral pigmentary degenerative changes with isolated bone-spicule–like pigment clumping.

The family in our report was included among a total of 80 apparently unrelated families with XIRP who were screened for mutations in the RPGR gene. In these 80 families, 15 different putative disease-causing mutations were identified; these include 4 nonsense mutations, 1 missense mutation, 6 microdeletions, and 4 intronic sequence substitutions resulting in splice defects.

The clinical phenotypes of 3 such families of Swedish ancestry were reported by Andréasson and coworkers. Two families with single base pair mutations in the introns 10 and 13 of the RPGR gene were found to have a less severe phenotype in both affected males and carrier females when compared with a third family with a microdeletion spanning portions of exons 8 through 10 in which a more severe visual handicap was noted in affected males and female carriers.

Another family was studied by Jacobson and coworkers. That family showed a single base pair change, a G to T transversion sequence change in the RPGR gene that converts codon 52 GGA (Gly) to TGA (stop codon). In that family, the heterozygote carriers varied in the degree of disease expression from mild to severe. Unlike the family we studied, a tapetal-like reflex was present in 1 female carrier. The affected male patients did not show fundus features that would have distinguished them from our 4 affected male patients. Because the mutation in the family we studied has not been previously reported, overall categorical conclusions about the expression of the clinical phenotype of affected persons and carriers cannot be made with confidence. Furthermore, only 2 carrier females were available for examination. Therefore, additional families with this RPGR mutation must be observed to determine whether the absence of a tapetal-like reflex in heterozygotes is a characteristic feature of this 2-bp deletion in exon 13 of the RPGR gene. Similarly, additional pedigrees of black families with XIRP must be studied for mutations in the RPGR gene to ascertain whether mutations in this gene, found in approximately 20% of families with XIRP who are not black, are seen with a similar frequency.

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