Discordant Phenotypes in Fraternal Twins Having an Identical Mutation in Exon ORF15 of the RPGR Gene

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Objective: To report discordant phenotypes, resulting from the same mutation in exon ORF15 (GenBank AF286472) of the retinitis pigmentosa GTPase regulator gene (RPGR) (GenBank U57629), in 2 presumed dizygotic twin brothers with X-linked retinal disease.

Methods: The 2 brothers underwent complete ophthalmic examination that included best-corrected visual acuity, slitlamp biomicroscopy, and detailed fundus examination. Visual field recording using Goldmann kinetic perimetry and a full-field electroretinogram were also obtained in both patients. Mutational screening was performed for RPGR because of an X-linked pattern of inheritance indicated by pedigree analysis.

Results: One brother had a phenotypic expression of cone-rod dystrophy, while the other exhibited X-linked retinitis pigmentosa. A 1-nucleotide deletion was identified in the 3′ region of exon ORF15 of RPGR (ORF15 + 1339delA).

Conclusions: An identical mutation in RPGR-ORF15 manifested distinct clinical phenotypes in individuals of the same family. Our data provide strong evidence in support of additional modifier genes that can produce diverse disease phenotypes in patients with RPGR mutations.

Clinical Relevance: The clinical observation of different retinal phenotypes in a family with the same mutation in exon ORF15 of RPGR implicates the potential importance of modifier genes for the phenotypic expression of this form of X-linked retinal disease.

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Mutations in the retinitis pigmentosa (RP) GTPase regulator gene (RPGR) account for 80% to 90% of X-linked RP and almost 25% of male subjects with simplex RP.1,4 Most RPGR mutations are detected in exon ORF15, which encodes a repetitive glycine and glutamic acid–rich domain of unknown function.5 The RPGR-ORF15 isoforms are preferentially expressed in cells with primary cilia.6,8 The high mutability of exon ORF15 seems to be related to unusual nucleotide composition or to the repetitive nature of its sequence. This type of sequence may adopt unusual conformations, including triplex structures, which are associated with reduced fidelity of replication.9 Exon ORF15 contains numerous potential polymerase arrest sites, suggesting that arrest may occur during replication, leading to slipped-strand mispairing events, as many mutations involve direct repeats.10

Patients with mutations in the 3′ region of exon ORF15 have primarily demonstrated cone-rod dystrophies and milder forms of RP,2,11,12 while mutations closer to the 5′ region are detected in more severe forms of X-linked RP.1,5,13-15 Mutations in this exon have also been shown to cause cone dystrophy5,16 and atrophic macular degeneration.17 However, discordant phenotypes within the same family have not been reported, to our knowledge. Herein, we describe a family with 2 male presumed dizygotic twins in whom an identical mutation in exon ORF15 resulted in 2 different phenotypes.

Methods

Patients and Clinical Analysis

Informed consent was obtained from the patients. The research protocol was approved by institutional review boards at the University of Illinois, Chicago, and the University of Michigan, Ann Arbor.

A 52-year-old man (patient III-1) reported a history of decreased vision in his left eye at the age of 49 years (Figure 1). Previous records showed that at 14 years of age his visual...
acuity was 20/20 OD and 20/30 OS. The fundus appearance at that time suggested early central choroidal atrophy. Progressive worsening of vision in both eyes continued thereafter. The patient reported being extremely sensitive to bright light but had no subjective loss of night or peripheral vision. Difficulty with color perception had also been noted by the patient. He had undergone a laser in situ keratomileusis (LASIK) procedure in both eyes 4 years previously. His medical history was significant for hypertension, which was well controlled with medication. His family history revealed a pedigree consistent with an X-linked pattern of inheritance.

On ophthalmic examination, best-corrected visual acuity on a Snellen visual acuity chart was 20/400 OD and 20/200 OS. He could read 20/50 OU for near vision with a ×7 magnifier. On testing for color vision using Ishihara pseudoisochromatic plates, he was able to identify only a single plate with either eye. Anterior segment examination using slitlamp bio-

microscopy showed the presence of corneal scars temporally in both eyes from the LASIK procedure flaps. The intraocular pressure was 15 mm Hg OU. A fundus examination showed grossly normal optic discs and retinal vessel attenuation in both eyes. A bilateral bull’s eye-appearing macular lesion was present in each eye with a tapetal type sheen temporal to the macula (Figure 2).

Visual field examination using Goldmann kinetic perimetry showed only mild peripheral restriction with the V-4-e, III-4-e, and II-4-e test targets and the presence of a central scotoma to the III-4-e and II-4-e targets in the right eye and to the II-4-e target in the left eye (Figure 3 and Figure 4). An electroretinogram (ERG) was obtained using a unipolar Burian-Allen contact lens electrode, as described previously.18 Stimuli were presented in a commercial recording unit (Nicolet Gan
gfeld; Nicolet Biomedical Inc, Madison, Wisconsin), and signals were acquired (Nicolet Viking IV system, Nicolet Biomedical...

Figure 1. Pedigree analysis shows the presence of an X-linked recessive disorder in male members (indicated by shading). Squares indicate men; circles, women; diamond, person’s sex is unknown; dot, an obligate carrier; arrow, proband; the twins, X; P, pregnancy (sex unknown).

Figure 2. Fundus photographs of patient III-1 with cone-rod dystrophy showing an area of atrophy in the macular region and the absence of peripheral pigmentation. The right eye (A) and the posterior pole (B) and peripheral retina (C) of the left eye are shown.
Figure 3. Goldmann kinetic perimetry of the right eye of patient III-1 showing mild peripheral restriction of the visual field and the presence of a central scotoma.

Figure 4. Goldmann kinetic perimetry of the left eye of patient III-1 showing a central scotoma and mildly reduced peripheral boundaries.
The patient’s dark-adapted b-wave responses to short wavelength and to maximal flash stimuli were 296 µV (within the lower normal range of 273-684 µV) and 359 µV (22% below the lower range of normal, 461-908 µV), respectively. The light-adapted brief flash b-wave amplitude was 34 µV (82% below the lower range of normal, 133-320 µV), and the amplitude for the light-adapted 32-Hz flicker was 25 µV (81% below the lower range of normal, 131-354 µV) (Figure 5). The clinical phenotype and ERG recordings were consistent with cone-rod dystrophy in this patient.

The presumed dizygotic twin brother of patient III-1 (patient III-2 in Figure 1) was seen at the University of Illinois at the age of 52 years. He gave a history of impaired peripheral vision and nyctalopia since the age of 10 years. Retinitis pigmentosa was diagnosed when the patient was 12 years old. His visual acuity at age 14 years was 20/30 OD and 20/50 OS; the fundus had a tesselated myopic appearance. At that time, a non-Ganzfeld ERG was nondetectable under light-adapted and dark-adapted conditions. Progressive worsening of impaired peripheral vision and nyctalopia, more in the left eye, was noted by the patient, and he reported having very poor vision in the left eye for the past 20 years. There was less severe subjective impairment of central acuity in the right eye. His other symptoms included photosensitivity and difficulty with color vision. His medical history was significant for asthma, hypertension, and gastric reflux disease.

The patient’s most recent best-corrected visual acuity was 20/50−1 OD measured by Snellen visual acuity chart and light perception in the left eye with temporal projection. He could read J1 on a Jaeger near vision chart using an ×7 magnifier with his right eye. He had 30° to 40° of left exotropia. Anterior segment examination showed the presence of pseudophakia in the right eye and moderate posterior capsular, nuclear, and anterior cortical cataract in the left eye. Fundus examination showed the presence of bilateral optic disc pallor and retinal vessel attenuation. There was an atrophic appearance of the retinal pigment epithelium within the posterior pole in both eyes, with relative sparing of the foveal region in the right eye. Moderately extensive midperipheral bone spicule pigment clumping was also seen in both eyes (Figure 6). Visual field testing
using a Goldmann perimeter showed severe peripheral field loss in each eye to even a V-4-e test target (Figure 7). An ERG recording showed nondetectable cone or rod responses. This patient’s phenotype was consistent with an X-linked form of RP.

**GENETIC ANALYSIS**

Blood samples were obtained from the twins for the purpose of genetic analysis. Lymphocyte DNA was used for amplification of exon ORF15 of RPGR using 1 forward and 4 reverse primers as described by Demirci et al.11

High-fidelity DNA Taq polymerase (AccuPrime; Invitrogen, San Diego, California) was used to amplify a fragment of approximately 1.9 kilobase (kb). Polymerase chain reaction (PCR) reagents were 5 µL of the enzyme PCR buffer, 200nM of each forward and reverse primer, and 1 µL of DNA at 50 to 100 ng/µL. The reaction volume was made to 50 µL with PCR water. Reaction tubes were placed in a PCR machine (model 9700; Applied Biosystems, Norwalk, Connecticut) and were run on the following program: 94°C for 2 minutes, followed by 10 cycles at 92°C for 30 seconds, and then 56°C annealing and 68°C for 2 minutes. This was followed by 25 cycles at 92°C for
30 seconds, annealing at 60°C for 30 seconds, and extension at 68°C for 2.5 minutes. Further extension was done at 68°C for 20 minutes and then held at 4°C. The PCR-amplified products were run on 1% agarose gels with a 1-kb ladder to check the product size and quality before sending the PCR products for sequencing. Sequencing was performed by the Biomedical Research Core Facility at the University of Michigan. RPGR-ORF15 PCR products were sequenced using the relative primers as in the referenced article.11 Chromatograms were read using a demonstration version of the sequencer software to help in identifying the sequence variants compared with normal sequences.

The mutation identified in each subject was ORF15 + 1339delA. This mutation is predicted to result in a frameshift and premature truncation of the protein. It was previously identified by Bader et al10 and by Sandberg et al19 in patients with X-linked RP.

Previous studies1,4,11-16,19 identified that ORF15 mutations can cause cone-rod dystrophy or an RP phenotype. Our findings show that even among individuals of the same family the same genetic mutation may present distinct phenotypes. In a study by Demirci et al,11 a 2-nucleotide deletion involving the same location, ORF15 + 1339_1340delAG, was shown to be associated with X-linked cone-rod dystrophy. A more severe form of X-linked RP was believed to be associated with mutations at the 5’ region of RPGR-ORF15.2 However, 1 of our patients demonstrates that a mutation at the 3’ end of exon ORF15 may also express a severe form of X-linked RP, as measured by visual field and ERG testing. A similar observation was reported by Sandberg et al.10

It would be valuable to identify the factors responsible for the different phenotypes that can occur with the same genotype. Our findings in the 2 presumed dizygotic twins carrying the same RPGR-ORF15 mutation suggest that modifier genes are likely to significantly contribute to an individual’s phenotype. It is possible that environmental factors may also modulate a disease phenotype to some degree. Nevertheless, additional genetic analysis would seem prudent in families that show intrafamilial variation in the phenotype of their retinal disease yet carry similar causative mutations.

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