Clinical Case Report

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Novel splice receptor-site mutation of RPGR in a Chinese family with X-linked retinitis pigmentosa

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Abstract

Rationale: Retinitis pigmentosa (RP) is a group of clinically and genetically heterogeneous diseases; X-linked retinitis pigmentosa (XLRP) is the most serious type. Mutations in RP GTPase regulator (RPGR) account for over 70% of patients with XLRP.

Patient concerns: We report a Chinese family with RP, 5 males presented with night blindness and decreased vision, and 8 females showed different severities of myopia.

Diagnoses: Targeted exome capture sequencing was performed in 2 affected males, which revealed a novel variant (NM_000328.2, c.470-1G>A) in the *RPGR* gene. The mis-splicing causes a substitution of the 157th amino acid from glutamic acid to glycine and finally the 165th codon is changed to stop codon, possibly resulting in a truncated protein and/or a nonsense-mediated mRNA decay. The mutation cosegregated with the disease phenotype in the family.

Interventions: Medication and cataract surgery.

Outcomes: The phenotype of affected males is more serious than that of the carrier females, and the effect of clinical treatment is not very well.

Lessons: Next-generation sequencing is a suitable method for early detection of pathogenic mutations in RP, which would be helpful for prenatal diagnosis of the disease.

Abbreviations: NGS = next-generation sequencing, RP = retinitis pigmentosa, RPGR = retinitis pigmentosa GTPase regulator, XLRP = X-linked retinitis pigmentosa.

Keywords: novel splice receptor-site mutation, RPGR, X-linked retinitis pigmentosa

1. Introduction

Retinitis pigmentosa (RP), which is a group of inherited ocular diseases with extensive clinical and genetic heterogeneity, affects approximately 1 in 1,798–5,260 people.[1,2] RP results in progressive retinal degeneration, with the main symptoms being night blindness, decreased visual acuity, and constricted visual fields, and can occur at any stage of a person's life. The characteristics of the fundus changes include "bone corpuscle" lumps of pigment, cystoid macular edema, attenuated retinal vessels, fine pigmented vitreous cells, and waxy optic disc pallor. RP is mainly inherited in an autosomal-recessive mode (~50%–60%), followed by the autosomal-dominant mode (30%–40%), and 5% to 15% of cases are inherited through X linkage.[3]

X-linked RP (XLRP), the most serious type of RP, primarily affects the rod photoreceptors.[4] It typically causes early-onset night blindness and loss of peripheral vision and often develop into total blindness by the age of 30 to 40 years. Mutations in RP GTPase regulator (RPGR) occur in over 70% of patients with XLRP, and in 11% of patients with RP, and can also cause a syndromic form of RP as well as other eye phenotypes.[5] Herein, we report on the use of an ophthalmic disease assay and targeted exome capture sequencing to identify the causative variant in a Chinese family with RP.

2. Patients and methods

2.1. Subjects

The family enrolled in the present study comprised 23 individuals and 1 fetus. Five males in the family presented with night blindness, mainly with decreased vision, and 8 females showed different severities of myopia. Clinical data were collected from all family members. Blood samples were collected from all the affected males (cases II-3, III-2, III-5, III-9, and III-13), the 8 females with myopia (cases II-2, II-4, II-6, II-10, III-3, III-6, III-14, and IV-3), and the other asymptomatic members. The study was approved by the ethics committee of West China Second University Hospital of Sichuan University, and informed consent was provided by the family before collection of the clinical data, blood, and amniotic fluid samples. Informed written consent was obtained from the patients for publication of this case report and accompanying images. The pedigree of the family is shown in Figure 1.

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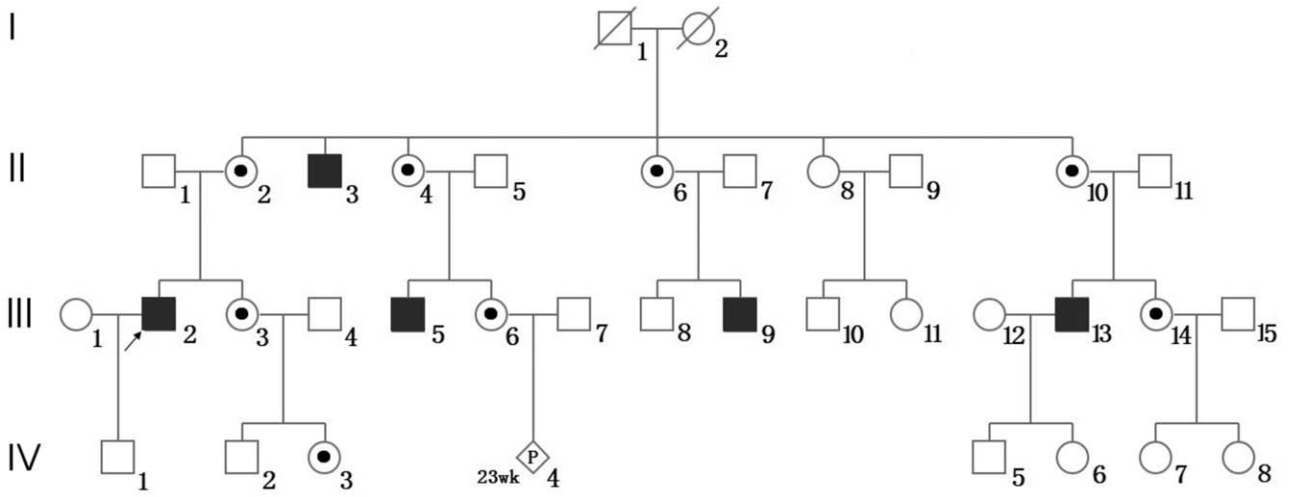

Figure 1. Pedigree of the present family. Black symbols represent affected individuals, and open symbols represent healthy individuals. Squares indicate males, circles indicate females, rhombi indicate babies of unknown gender. The proband is indicated by an arrow. The carrier female is designated by a circle-enclosed dot.

2.2. Targeted exome capture sequencing and Sanger sequencing

Genomic DNA (gDNA) was isolated from 2 mL of EDTA whole blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The concentration and purity of the gDNA were detected using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Fisher Scientific, Massachusetts). For cases III-2 and III-5, targeted exome capture sequencing was performed using the MG16219 Targeted Exome Capture Kit (MyGenostics, Beijing, China) according to the manufacturer's protocol; this kit targets 660 genes known to cause ophthalmic diseases, including 148 genes associated with nonsyndromic or syndromic forms of RP. The enrichment libraries were sequenced using a NextSeq 500 sequencer (Illumina, California). The novel pathogenic mutation identified by next-generation sequencing (NGS) was confirmed and checked for cosegregation by Sanger sequencing in all family members from which gDNA was available.

2.3. RT-PCR and cDNA sequencing in peripheral blood leukocyte

Peripheral blood leukocyte from the proband (case III-2) and 1 normal male (case III-7, wild-type) were used to obtain total RNA using the TRIzol Plus RNA Purification Kit (Invitrogen, California) according to the manufacturer's protocol. ReverTra Ace qPCR RT Master Mix Kit (Toyobo, Osaka, Japan) was used to generate cDNA from the RNA. Primers (forward 5'-gtgtcaacagaaggaggcaa-3' and reverse 5'-gacatccaccacaag-caacc-3') were designed to amplify wild-type and mis-spliced cDNA.

3. Results

3.1. Clinical data

Clinical data were collected before mutation identification. The proband (case III-2) is a 33-year-old man who began to show night blindness at the age of 2 years and had developed a progressive decline in vision. Cataract surgery was performed at the age of 29 years. Fundus examination indicated a waxy optic disc pallor, retinal vascular stenosis, and "bone corpuscle" lumps of pigment. The clinical diagnosis was RP, and he currently has bilateral near-total blindness. Case III-5 is an 18-year-old man with a 15-year history of night blindness and myopia, with mild decline in vision. Cases II-3, III-9, and III-13 all have night blindness and varying degrees of vision decline. Cases II-2, II-4, II-6, II-10, III-3, III-6, III-14, and IV-3 are all females showing different severities of myopia. Case IV-4 was a fetus at 23 weeks of gestation at the time of the sequencing study. With the exception of Case III-2, all the other patients in the family refused further specialized investigations.

3.2. Results of NGS and Sanger sequencing of gDNA from the family members

Targeted exome capture sequencing revealed a novel variant (NM_000328.2, c.470-1G>A) in the *RPGR* gene of cases III-2 and III-5, which was confirmed by Sanger sequencing. c.470-1 is the splice receptor site at the intron 5-exon 6 junction. This variant was checked by Sanger sequencing in the other family members for whom gDNA was available. The 8 affected females (cases II-2, II-4, II-6, II-10, III-3, III-6, III-14, and IV-3) showed heterozygous peaks (G/A, heterozygous carrier) at this site, and all 5 affected males (cases II-3, III-2, III-5, III-9, and III-13) showed 1 peak (A, hemizygous mutation), whereas the healthy members (cases II-8 and III-8) showed 1 peak (G, reference nucleotide). The results of Sanger sequencing are shown in Figure 2. The mutation cosegregated with the disease phenotype in the family.

3.3. cDNA sequencing

cDNA sequencing in the peripheral blood leukocyte showed the first 2 bases (AG) of exon 6 were spliced out in the proband, while it is expressed normally in the healthy male. The mis-splicing causes a substitution of the 157th amino acid from glutamic acid (E) to glycine (G) and finally the 165th codon is changed to stop codon, possibly resulting in a truncated protein and/or a nonsense-mediated mRNA decay (Fig. 3).

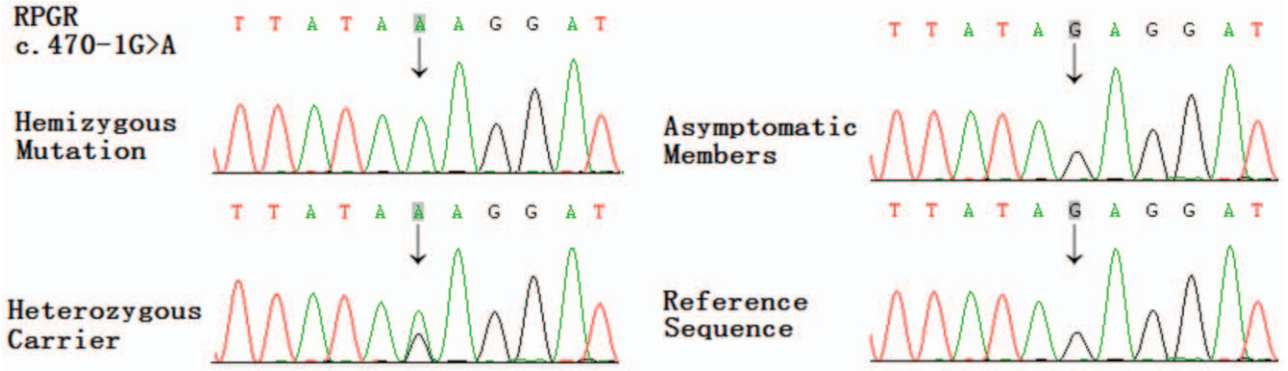

Figure 2. Sequence analysis of the *RPGR* gene in affected males (hemizygous mutation), affected females (heterozygous carrier), asymptomatic members, and reference sequence. Arrows indicate the positions of the novel mutations (c.470-1). *RPGR*=retinitis pigmentosa GTPase regulator.

3.4. Prenatal diagnosis

Because she was genetically diagnosed as a heterozygous carrier of XLRP, case III-6 requested for detection of this site for her fetus (case IV-4). The fetal DNA was extracted from amniotic fluid cells and analyzed using direct sequencing, where the results showed 1 peak (G) in the site. Copy number variation sequencing suggested that the number of copies of the X and Y chromosomes was 2 and 0, respectively, indicating that the fetus was female and unaffected.

4. Discussion

The *RPGR* locus on chromosome Xp11.4 was identified in isolated and sequenced cosmids from the region of microdeletions in patients with RP3 (OMIM: 300029) in 1996.[6] The gene covers 58.4 kb, is composed of 19 exons, and codes for a protein of 815 amino acids. Owing to a substantial contribution of *RPGR* mutations to simplex RP, some researchers have suggested that *RPGR* should be considered as a first-tier gene for screening in males with RP.[7] The identified mutation (c.470-1G>A) in the *RPGR* gene, which cosegregated with the disease phenotype in the family, has not been reported in the literature. All affected males have night blindness and varying degrees of vision decline, and the phenotype is more serious than that of the carrier females, who only showed different severities of myopia. In RP3, affected males generally have a severe phenotype, whereas carrier females show a wide spectrum of clinical features, ranging from being completely asymptomatic to having severe RP, and most carriers have mildly or moderately reduced visual function but rarely progress to legal blindness.[8,9] The results of our study are in accordance with these previous reports.

cDNA sequencing in the peripheral blood leukocyte showed the first 2 bases of exon 6 were spliced out in the proband, likely

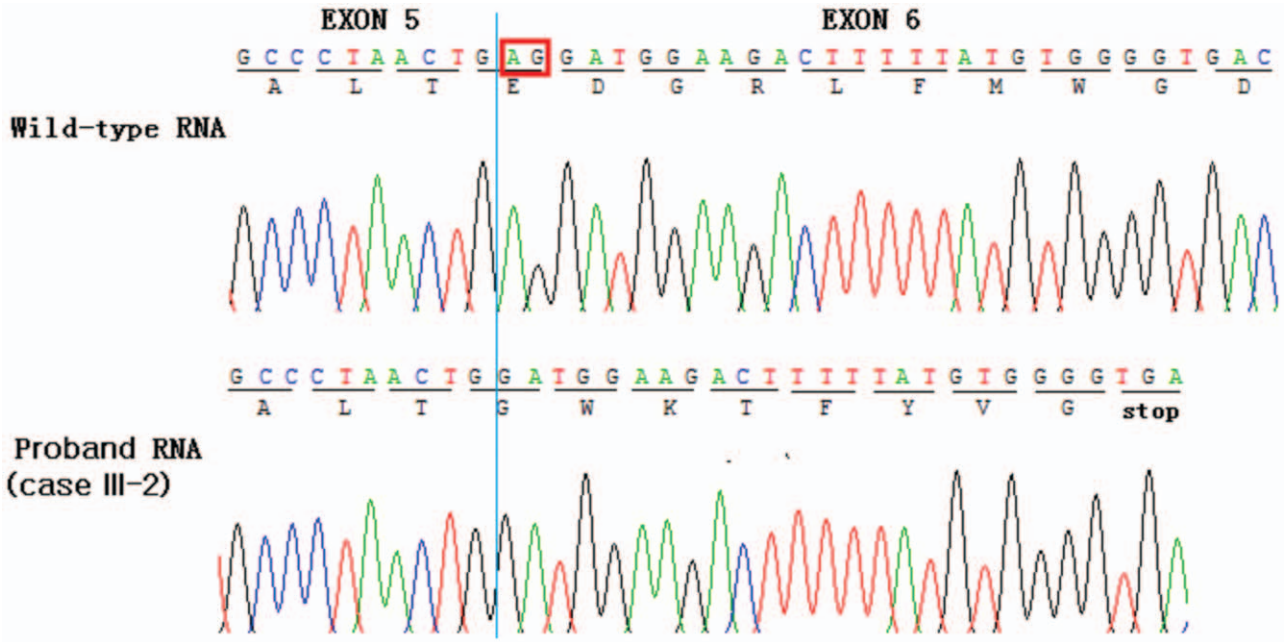

Figure 3. cDNA sequencing in the peripheral blood leukocyte shows the first 2 bases (AG) of exon 6 were spliced out in the proband (case III-2), likely resulting in a truncated protein.

causing a truncated protein. In addition, according to the latest American College of Medical Genetics and Genomics guidelines,[10] the variant has 1 very strong (canonical ± 1 or 2 splice sites can often be assumed to disrupt gene function), 1 moderate (the mutation is absent in the Exome Sequencing Project, 1000 Genomes Project, Exome Aggregation Consortium, and Genome Aggregation Database), and 2 supporting evidence for its pathogenicity (segregation with the disease and specific patients' phenotype). Therefore, this variant can be currently annotated as "pathogenic". To date, 77 nonsyndromic RP-related genes have been included in the Online Mendelian Inheritance in Man database (http://www.omim.org/phenotypicSeries/PS268000). Meanwhile, because patients with RP generally show a wide spectrum of clinical features, it is difficult for clinicians to judge the pathogenic genes through the clinical manifestations of the patients. Therefore, compared with direct sequencing, NGS is a more comprehensive, accurate, rapid, and economical method for the detection of pathogenic mutations in RP. NGS would help to provide both medical suggestions for affected family members and prenatal diagnosis for high-risk fetuses.

5. Conclusions

Compared with direct sequencing, NGS is a more suitable method for the detection of pathogenic mutations in RP. This is the first report of a c.470-1G>A mutation at the splice receptor site of the *RPGR* gene causing XLRP. Its detection would facilitate genetic counseling and aid prenatal diagnosis for the affected family members.

Author contributions

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Resources: Jing Wang.
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Validation: Cong Zhou.
Writing – original draft: Jing Wang.
Writing – review & editing: Hongqian Liu.

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