A novel splicing mutation in the PRPH2 gene causes autosomal dominant retinitis pigmentosa in a Chinese pedigree

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1 | INTRODUCTION

Retinitis pigmentosa (RP) (OMIM: 268000) is a rare, heterogeneous group of inherited ocular disorders that results in a progressive retinal degeneration.1,2 The PRPH2 gene (NM_000322.4) (OMIM: 179605), also known as RDS, AOFMD, AVMD, CACD2, DS, MDBS1, PRPH, rd2, RP7 and TSPAN22, is located on chromosome 6p21.1 with three exons spanning 26,395 bp length in human genome (GRCh38/hg38) that encodes a putative protein with 346 amino acids.3 The PRPH2 protein (NP_000313.2) is a member of the transmembrane 4 superfamily, also known as the tetraspanin family. The majority of the members are cell-surface proteins which were identified by the presence of four hydrophobic domains. The PRPH2 protein is a membrane-associated glycoprotein, which is restricted to the area of photoreceptor outer segment discs.4 PRPH2 functions as an adhesion molecule by glycosylation and compaction of outer segment discs. PRPH2 and ROM1 (OMIM: 180721) can be assembled into noncovalent tetramers (heterodimer) in vivo using disulphide bonds and higher order disulphide-linked oligomers, thereby involving in photoreceptor disc morphogenesis.5

Mutations in the PRPH2 gene are involved with assorted blinding diseases of the retina, inducing degenerations in both central retinal and peripheral retinal.6-8 The relationships between the mutations in the PRPH2 gene and the resultant diseases in the patients are variable; making genotype/phenotype correlations different. PRPH2 mutation in RP patients and genotype/phenotype relationship have not been well described in the Chinese population.

2 | MATERIALS AND METHODS

2.1 Ethics statement, proband, pedigree and clinical assessment

The study was approved by the Ethics Committee of Southwest Medical University. Written informed consent was obtained from the participants, in accordance with the guidelines of the Declaration of Helsinki (2013 Revision). The pedigree (M074) consisted of a...
proband (Figure 1A, pedigree II: 1, arrow). For detailed clinical assessments, a clinical history and ophthalmic examination were previously described. Genomic DNA (gDNA) was extracted using a reported phenol/chloroform method.

2.2 | Target sequencing and data analysis

Targeted next-generation sequencing (TGS) analyses were performed on the gDNA sample of the proband from family M074. The capture Agilent probes were used as in previously published studies with a retinal disease capture panel with 195 retinal disease-causing genes. Library construction and sequencing were used according to the manufacturer's protocols. Data analysis and sequenced variants identification were described in detail previously.

2.3 | Primer design and PCR amplification

A primer pair M074-PRPH2 was designed containing the NM_000322.4: c.582-2A>T in the PRPH2 gene (M074-PRPH2-868L: 5-ttcagcgcctagaacagtga -3; M074-PRPH2-868R: 5- tcgaagagccaaatgaggag-3, 411 bp). For variant validation and pedigree segregation analysis, PCR amplification was used to by using gDNA of all available individuals. PCR amplification for sequencing was performed in a total volume of 20 µL.

2.4 | Sanger sequencing and co-segregation analysis

The amplified PCR products were then Sanger sequenced using a primer M074-PRPH2-868L. Co-segregation analysis in the pedigree was finished by using Sanger sequencing results.

2.5 | Protein structure prediction and bioinformatics analysis

A search for conserved domains in protein or nucleotide sequence was done through the online program (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The PRPH2 gene homologs were analysed using previously described HomoloGene system: (https://www.ncbi.nlm.nih.gov/homologene?Db=homologene&Cmd=Retrieve&list_uids=273).

2.6 | RNA extraction and reverse transcription-PCR

RNA was extracted from mice according to our previously reported standard protocol. Semi-quantitative RT-PCR was
performed with the primer pair RT-m-Prph2 (RT-m-Prph2-L: 5'-tgctcaacctctccgtcgg-3'; RT-m-Prph2-R: 5'-catcgctgcatacgtaagc-3', 457 bp) targeting the mouse Prph2 gene; the mouse β-actin gene was used as an internal control, which was described previously.\(^2\)

3 | RESULTS

3.1 | Proband and clinical characteristics

The proband (Figure 1A, II: 1) was a 60-year-old Chinese male, and claimed a reduction in his visual acuity and peripheral field loss in the fourth decade of life. Fundus examination revealed refractive medium opacity, fundus blurring, macular degeneration and posterior pole retinal atrophy in both eyes (Figure 1B and C). Yellow-white deposits varying in shape and size within the macula were also noticed. The retinal pigment epithelium atrophy and small retinal vessels were observed. Electroretinogram (ERG) assessments showed that amplitudes of rod-isolated responses and amplitudes of cone-isolated responses were almost extinguished. The proband’s parents and his daughter was normal without retinal disease. As a result, the proband was characterized as adult-onset RP.

3.2 | TGS results and putative pathogenic mutation screening

By TGS with the proband gDNA (Figure 1A, pedigree II: 1). A heterozygous, splice site mutation (c.582-2A>T) at the exon 3 boundary in the PRPH2 gene (NM_000322.4) was revealed, leading to unknown amino acid changes of the PRPH2 protein C-terminus (NP_000313.2) (Figure 1A, II: 1). These deleterious and pathogenic aspects of the PRPH2 gene (c.582-2A>T) variant are listed in Table 1. Thus, this splicing mutation c.582-2A>T in the PRPH2 gene most likely damaged the protein function in this Chinese RP pedigree. This variant was demonstrated to be novel by searching in database ExAC (http://exac.broadinstitute.org/gene/ENSG00000112619) and HGMD (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=PRPH2).

3.3 | Variant verification and segregation analysis

Confirmation of the variant and co-segregation analysis was done by Sanger sequencing (Figure 1). The c.582-2A>T mutation of the PRPH2 gene was verified to be heterozygous in the proband (pedigree II: 1; Figure 1D), while we revealed the wild-type gene in the proband’s daughter without RP symptoms till test at the ages of 30 (pedigree III: 1; Figure 1E), and the proband’s wife had two copies of the wild-type allele and a normal phenotype (pedigree II: 2; Figure 1F). Therefore, we validated that the c.582-2A>T mutation in the PRPH2 gene is co-segregated with the RP disease phenotype in these pedigree members. Furthermore, the c.582-2A>T variant was absent in 100 normal, ethnically matched controls by Sanger sequencing. Comprehensively, this finding shows co-segregation of the variant in this RP pedigree and pinpoints c.582-2A>T variant role in pathogenesis of this RP disease. Unfortunately, no DNA samples were available due to the death of proband’s parents. The c.582-2A>T variant of the PRPH2 gene might be de novo as no any RP phenotypes were claimed in the proband’s parents.

3.4 | Functional effects of the pathogenic mutation c.582-2A>T for PRPH2

Conserved Domain rpsblast searching found that PRPH2 has two conserved domains (Figure 2A), namely tetraspanin, extracellular domain or large extracellular loop (LEL) (tetraspanin_LEL, cl02781) or the tellurite-resistance/Dicarboxylate Transporter (TDT) family (TDT, cl04176) (Figure 2B). The c.582-2A>T variant is located in the tetraspanin_LEL domain in H sapiens (aa.120-aa.262), leading to amino acid change after 194 (Figure 2C, arrow of wild protein). By orthologous comparison of H sapiens PRPH2 to nine other species, including Pan troglodytes, Macaca mulatta, Bos taurus, Canis lupus, Mus musculus, Rattus norvegicus, Gallus gallus, Xenopus tropicalis and Danio rerio (two isoforms), we found that this protein is highly conserved (Figure 2A). Altogether, our investigation revealed that the PRPH2 heterozygous variant, c.582-2A>T, might cause adRP disease.

3.5 | Expression profiles of Prph2 mRNA level

The Prph2 expression in the indicated tissues and developmental retinal stages was studied in mice (Figure 2D and E). We found that Prph2 transcript is highly expressed in the retina, lens, sclera and cornea of the eye; is weakly expressed in the testis; have no detectable expression in the uterus, ovary, breast, spleen, kidney, liver, brain, intestine, skeletal muscle and blood (Figure 2D); and is highly expressed at the latter four different developmental stages of retinal tissue (Figure 2E). The very high expression of Prph2 in the retinal tissue and ubiquitous expression in other tissues of eyes demonstrated that Prph2 should play an important role in the retinal/eye function.

| Gene     | Exon | Nucleotide# | Protein# | Type    | Status   | Disease-causing | ExAC |
|----------|------|-------------|----------|---------|----------|----------------|------|
| PRPH2    | 3    | c.582-2A>T  | NA       | Splicing| Heter    | Damaged        | Novel|

All nucleotide and amino acid are abbreviated according to the International Union of Pure and Applied Chemistry (IUPAC). c: variation at cDNA level; ExAC: Exome Aggregation Consortium; Heter: heterozygote; NA: not available; p: variation at protein level; PRPH2: Homo sapiens peripherin 2.
DISCUSSION

Earlier diagnosis and management result in a better prognosis. The PRPH2 mutation in RP patients and genotype/phenotype relationship with RP have not been well described in the Chinese population. In this study, we have successfully revealed a heterozygous, splicing mutation of the PRPH2 gene, **c.582-2A>T**, in a Chinese pedigree, which led to the adRP disease. The patient’s parents did not show any RP phenotypes till death, suggesting that this variant might be de novo. By searching HGMD (access date, September 24, 2018), 125 pathogenic mutations in the PRPH2 gene have been found, including missense/nonsense (83), small deletions (28), small insertions (5), small indels (4), a gross deletion (1) and a gross insertions/duplication (1). To the best of our knowledge, the PRPH2 mutation **c.582-2A>T** is novel, thereby extending mutation spectrums.

The PRPH2 protein belongs to the transmembrane 4 superfamily-tetraspanin family, which mediates signal transduction events by playing roles in the regulation of cell development, activation, growth and motility. As a membrane-associated glycoprotein, it was found in the outer segment of rod/cone photoreceptor cells. PRPH2 functions as an adhesion molecule involving in stabilization and compaction of outer segment discs. PRPH2 and ROM1 (OMIM: 180721) are assembled into noncovalent tetramers (heterodimer) using disulfide bonds and disulfide-linked oligomers, thus involved in photoreceptor disc morphogenesis. Prph2 and Rom1 oligomerization are essential for forming photoreceptor outer segment by an intermolecular disulfide bond at Prph2-C150/Rom1-C153; disrupting this bond in a C150S-Prph2 knockin mouse loses complex formation, normal OS structure and function. PRPH2 has the tetraspanin-LEL domain where it may act as molecular facilitator relating the ability, specific cell-surface...
proteins grouping and of signalling complex formation and stability. Variant c.582-2A>T of PRPH2 is located on the tetraspanin-LEL domain and near to dimer interface region (Figure 2C), which might affect the formation of outer segment morphogenesis and stability of signalling complexes. Thus, this PRPH2 mutation might affect the formation and heterodimerization, inhibit signalling, thereby we explain the genetic dominance of the PRPH2 mutant allele in our pedigree.

Orthologous comparison of H. sapiens PRPH2 to nine other species revealed that this protein is highly conserved. Our quantitative RT-PCR results in mouse showed that Prph2 mRNA is only highly expressed in the retina, lens and sclera and cornea of the eye, indicating that PRPH2 plays an important role in the retina/eye functions. Comprehensively, our study found that the PRPH2 heterozygous mutation, c.582-2A>T, might causes adRP disease.

In conclusion, our research is the first to identify the novel heterozygous mutation c.582-2A>T of PRPH2, which might causes RP disease in our Chinese family, thereby extending mutation spectrums. Our findings can also help in further understanding of adRP molecular pathogenesis, and assist the diagnosis and genetic counseling of the RP disease.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHORS’ CONTRIBUTIONS
JF was in charge of the idea, project design and concept of the study; JC, JIF, LY, SF and CW performed DNA extraction, PCR amplification, sequencing and data analysis; HL, QZ and XX recruited the clinical patients and were in charge of the clinical assessments; JF, JC, SF and MK wrote the manuscript; JF revised the manuscript.

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REFERENCES
1. Ali MU, Rahman M, Cao J, Yuan PX. Genetic characterization and disease mechanism of retinitis pigmentosa: current scenario. J Cell Mol Med. 2017;7:251.
2. Fu J, Ma L, Cheng J, et al. A novel, homozygous nonsense variant of the CDHR1 gene in a Chinese family causes autosomal recessive retinal dystrophy by NGS-based genetic diagnosis. J Cell Mol Med. 2018;22:5662-5669.
3. Travis GH, Christerson L, Danielson PE, et al. The human retinal degeneration slow (RDS) gene: chromosome assignment and structure of the mRNA. Genomics. 1991;10:733-739.
4. Travis GH, Sutcliffe JG, Bok D. The retinal degeneration slow (rds) gene product is a photoreceptor disc membrane-associated glycoprotein. Neuron. 1991;6:61-70.
5. Loewen CJ, Moritz OL, Molday RS. Molecular characterization of peripherin-2 and rom-1 mutants responsible for digenic retinitis pigmentosa. J Biol Chem. 2001;276:22388-22396.
6. Kajiwara K, Hahn LB, Mukai S, Travis GH, Berson EL, Dryja TP. Mutations in the human retinal degeneration slow gene in autosomal dominant retinitis pigmentosa. Nature. 1991;354:480-483.
7. Wells J, Wroblewski J, Keen J, et al. Mutations in the human retinal degeneration slow (RDS) gene can cause either retinitis pigmentosa or macular dystrophy. Nat Genet. 1993;3:213-218.
8. Farrar GJ, Kenna P, Jordan SA, et al. A three-base-pair deletion in the peripherin-RDS gene in one form of retinitis pigmentosa. Nature. 1991;354:478-480.
9. Huang J, Fu J, Fu S, et al. Diagnostic value of a combination of next-generation sequencing, choriorietal imaging and metabolic analysis: lessons from a consanguineous Chinese family with gyrate atrophy of the choroid and retina stemming from a novel OAT variant. Br J Ophthalmol. 2019;103:428-435.
10. Yang L, Fu J, Cheng J, et al. A novel variant of the FZD4 gene in a Chinese family causes autosomal dominant familial exudative vitreoretinopathy. Cell Physiol Biochem. 2018;51:2445-2455.
11. Fu J, Li L, Lu G. Relationship between microdeletion on Y chromosome and patients with idiopathic azoospermia and severe oligozoospermia in the Chinese. Chinese Med J. 2002;115:72-75.
12. Wang F, Wang H, Tuan HF, et al. Next generation sequencing-based molecular diagnosis of retinitis pigmentosa: identification of a novel genotype-phenotype correlation and clinical refinements. Hum Genet. 2014;133:331-345.
13. Zhang Q, Xu M, Verriotto JD, et al. Next-generation sequencing-based molecular diagnosis of 35 Hispanic retinitis pigmentosa probands. Sci Rep. 2016;6:32792.
14. Zhu L, Cheng J, Zhou B, et al. Diagnosis for choroideremia in a large Chinese pedigree by next-generation sequencing (NGS) and non-invasive prenatal testing (NIPT). Mol Med Rep. 2017;15:1157-1164.
15. Salvo J, Lyubavskiy V, Xu M, et al. Next-generation sequencing and novel variant determination in a cohort of 92 familial exudative vitreoretinopathy patients. Invest Ophthalmol Vis Sci. 2015;56:1937-1946.

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