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The genotype-phenotype association of retinitis pigmentosa in a Chinese population: Analysis of three new cases and literature review

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Purpose: Retinitis pigmentosa (RP) is an inherited heterogeneous neurodegenerative retinal disease leading to blindness eventually. Currently, a large number of studies have explored its heterogeneity, but the genotype-phenotype correlation remains unclear. The present study aimed to explore genetic mutations and the correlation between genotype-phenotype in three RP families from the Chinese Han population.
Methods: Genomic DNA was obtained from peripheral blood samples of patients and their relatives and subjected to whole-exome and Sanger sequencing. The corresponding visual acuity and fundus examinations were also performed, including fundus photography and ophthalmologic examinations.
Results: In this study, three novel variants, including *CERKL* c.1482delT (p.Val495fs*), *RPRH2* c.-5_3dup (p.Ala2Glufs*6), and *RPGR* c.1539del (p.Lys513Asnfs*), and a heterozygous mutation c.239-2A>G from three families were identified from three inheritance formats. All above variants were cosegregated, with the *PRPH2* variant inherited in an autosomal dominant pattern, the *CERKL* variants in an autosomal recessive pattern, and the *RPGR* variant in an X-chromosome–linked recessive pattern, respectively.
Conclusions: This study laid the foundation for prenatal diagnosis of RP in three family pedigrees, offering a comprehensive understanding of the genetic and clinical characteristics of patients with RP, which provided theoretical support for addressing complex genetic heterogeneity to enable accurate prenatal screening and diagnosis, early detection, and treatment of RP.

Inherited retinal degeneration is a complex group of clinically heterogeneous retinal disorders characterized by photoreceptor degeneration or dysfunction, with clinical manifestations of predominantly nocturnal blindness and peripheral visual field defects with intraretinal bone pin pigmentation, optic disc pallor, and a weakened choroidal retinal system [1]. Inherited retinal degeneration is estimated to have a prevalence of 1/1,000 to 2,000 [2] worldwide, with retinitis pigmentosa (RP) being the most common and affecting approximately 1/4,000 people [3]. As an inherited heterogeneous neurodegenerative retinal disease, RP presents with degeneration and death of photoreceptors in optic rod cells and optic cones, including reduced and loss of night vision and central vision, as well as eventual blindness [3,4]. Hyperpigmentation is one of the most obvious fundus manifestations, except for macular cystoid edema, posterior subcapsular cataract, and myopia [5-7]. RP can be classified as nonsyndromic (not affecting other organs or tissues), syndromic (affecting other neurosensory systems such as hearing), or systemic (affecting multiple tissues) [4].

Generally, RP is predominantly inherited in an autosomal dominant manner (adRP, 15%-25% [caused by mutations in *PRPH2*, etc.]), followed by autosomal recessive RP (5%-20% [caused by mutations in *CERKL*, etc.]) and, to a lesser extent, X-linked RP (5%-15% [predominantly *RPGR*]). The other 40% to 50% of cases (sporadic RP) remain unclear for the genetic pattern [8]. Inherited most often as adRP with a high degree of genetic and phenotypic heterogeneity [9-11], peripherin 2 (*PRPH2*) localized at 6p21.2 encodes a transmembrane protein on the disc membranes of photoreceptors of optic rods and cones [12,13]. The mutations in *PRPH2* can lead to a variety of clinical manifestations, including pattern dystrophy (OMIM 169150) [14], vitelliform macular dystrophy (OMIM 608161) [15], RP (OMIM 608133) [5], cone-rod dystrophy, and central areolar choroidal dystrophy (OMIM 613105) [16]. Although a large number of studies have been conducted to explore heterogeneity, the genotype-phenotype correlation of *PRPH2* remains challenging [17].

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The ceramide kinase-like gene (*CERKL*) associated with autosomal recessive RP contains more than 20 transcripts and produces multiple protein isoforms, involved in proliferation, apoptosis, phagocytosis, and inflammation, which play an important role in protecting retinal photoreceptors from oxidative stress [18]. The *CERKL* mutations are likely to increase the susceptibility of retinal tissue to oxidative damage, leading to cell death and retinal neurodegeneration [19]. Recently, several RP-related *CERKL* mutations have been identified, but the exact function of *CERKL* is not fully understood. As the most common X-linked recessive cause of the RP gene [20], retinitis pigmentosa GTPase regulator (*RPGR*) is located at position Xp21.1 and consists of 19 exons [21]. Previous studies have demonstrated that mutations in only the first 15 exons can result in RP, and pathogenic variants are most frequently attributable to truncated proteins produced by frameshift mutations with early-onset and severe RP, but there are no effective treatments for *RPGR*-associated retinopathy [22]. In a large cohort study of Chinese patients with suspected RP, a total of 76 genes in 517 variants were identified by validated whole-exon sequencing of 1,243 patients with suspected RP, including *RPGR* in the top six of these genes, with a highly heterogeneous clinical phenotype [23]. RP has highly genetically heterogeneous and current treatments, such as macular edema treatment, cataract extraction, cryotherapy, and laser therapy, which focus on slowing disease progression and improving the quality of patients' lives rather than curing RP. Therefore, this study was designed to enrich the RP-causing variants through comparing clinical phenotypes and different inheritance modes, as well as enriching the genotype-phenotype mapping and various mutated genes, which can be used for prenatal screening, laying the foundation for early screening and diagnosis of patients with RP.

METHODS

Summary of patient clinical information: The three probands involved in this study from three RP family pedigrees were recruited through genetic counseling at the Prenatal Screening Centre of the Affiliated Hospital of Qingdao University. These individuals were diagnosed with RP based on fundamental optical examinations using next-generation sequencing, which included optical coherence tomography (OCT), color Doppler imaging, ultra-widefield fundus imaging, and fundus fluorescein angiography, which were conducted to exclude the remaining ophthalmological diseases (Figure 1).

The genomic DNAs were obtained from the peripheral blood of patients and their families by the TIANGEN Blood DNA Kit (TIANGENBIOTECH, Beijing, China), homogenizing all DNA samples to 100 ng/μl with their consent. The study adhered to the principles of the Declaration of Helsinki and received approval from the Ethics Committee of the Affiliated Hospital of Qingdao University, Shandong, China. Written informed consent was procured from all participants or their legal guardians.

Whole-exon sequencing and bioinformatics analysis: After purifying and electrophoresing the extracted DNA, 100 ng DNA was added to the Illumina DNA Prep with the Exome 2.5 Enrichment kit for DNA enrichment and library preparation. The prepared library mixture was up-sampled into the S4 flow-through tank of the NovaSeq 600 sequencer system (Illumina, San Diego, CA), with more than 99% of the sample coverage at ≥40×.

The generated sequencing information was saved in fastq files, compared with the human reference genome (GRCh38), and then aligned by BWA software (version 0.7.15). After removing duplicates through Picard (version 2.18.7), the variants were annotated using ANNOVAR, whose pathogenicity ratings were obtained from the guidelines of the American College of Medical Genetics and Genomics (ACMG) and the ClinGen sequence variant interpretation to exclude a minor allele frequency (MAF) >0.1 and nonfunctional mutations in the 1000 Genomes (1000G), exome aggregation consortium, genome aggregation database, and Shenzhou Genome Database. By integrating the analysis of genotype-phenotype association, relevant databases, and literature searches, the likely pathogenic variants were identified using Sanger sequencing and pedigree analysis.

Sanger sequencing: The patients' and their families' genomic DNA were extracted to be amplified by PCR with primers designed by Premier 5 software to gain the upstream and downstream sequences involved in the mutations. PCR amplification products were analyzed by 2% agarose gel electrophoresis, followed by product purification and mutation analysis on an ABI 3730 analyzer (Applied Biosystems, ThermoFisher, Los Angeles, CA). Mutations were identified by comparison with reference sequences on the NCBI website.

Genetic analyses: We performed amino acid sequence comparisons of *CERKL*, *PRPH2*, and *RPGR* using MEGA 11, selecting sequences from the corresponding transcripts of other species at the site of the mutation. Subsequently, the protein structural domains of the three genes were predicted by InterPro.

Literature review: We reviewed a total of seven studies published in PUBMED in the past decade on Chinese patients with RP caused by *PRPH2*, removing two studies related to

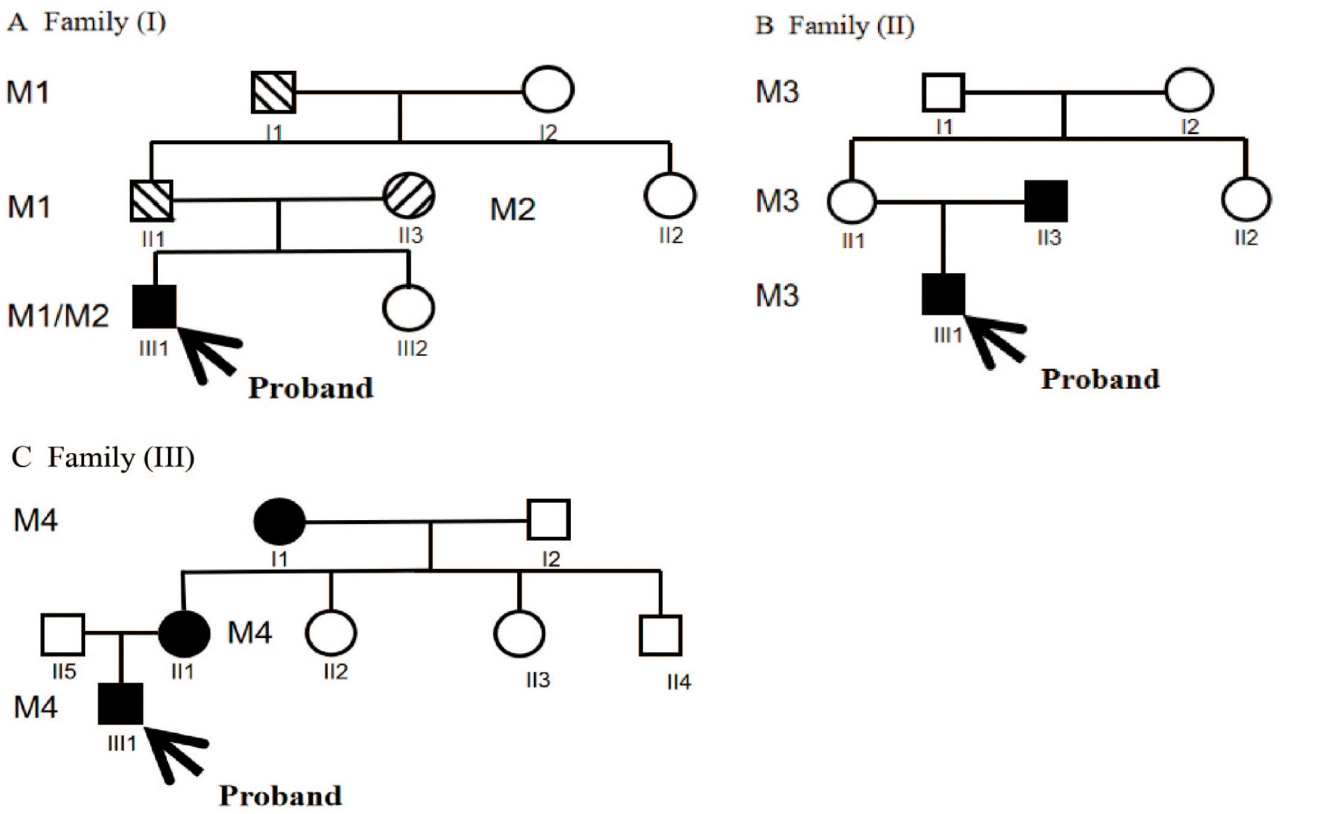

Figure 1. The mapping of three probands' pedigrees. **A**: M1: CERKL c.1482delT (p.Val495fs*), M2: CERKL c.239–2A>G, slashes are drawn to carry a single mutant, and hatched areas are diseased proband; **B**: M3: RPRH2 c.-5_3dup (p.Ala2Glufs*6), hatched areas are diseased proband; **C**: M4. RPGR c.1539del (p.Lys513Asnfs*), hatched for diseased proband.

PRPF31 and *RPGR*, as well as two cases of RP accompanied by retinitis punctata albescens and concomitant angle-closure glaucoma, and finally selected three studies [16,24,25]. This was followed by downloading all *RPGR* data from the LOVD database, selecting pathogenicity and likely pathogenicity with only RP in Chinese. In addition, a large cohort study of suspected RP in China was conducted, including genotype-phenotype association analyses of *PRPH2* and *RPGR*. The study presented and plotted two tables of *PRPH2* and *RPGR*, respectively, including mutation type, mutation site, ACMG and human gene mutation database (HGMD) scores, mutation classification, and references [26].

RESULTS

Clinical phenotypes: Patient 1 (family 1 III-1), a 28-year-old man with congenital RP, presented with the following clinical findings: visual acuity was 0.2 in both eyes, intraocular pressure was normal, and anterior segment examination revealed transparent corneas with intermediate anterior chamber depth and clear aqueous humor in both eyes. Pupils were 2.5 mm with normal light reflexes. Fundus examination findings included a grayish-white aspect in the retinal periphery, slightly pale papilla in the temporal area, and decreased retinal vessel size. Ocular ultrasound demonstrated increased ophthalmic artery flow velocity, decreased perfusion in the central retinal artery and short posterior ciliary arteries, and abnormal intraocular echoes with mild vitreous opacities. Fluorescein angiography showed compromised central retinal artery perfusion bilaterally. Macular OCT identified neuroepithelial/retinal pigment epithelial complex atrophy in both eyes. In addition, an ophthalmological examination was performed on his grandfather with the following results: visual acuity in both eyes of 0.01, macular degeneration, and advanced cataracts in both eyes.

Patient 2 (family 2, III-1) is a 26-year-old man with congenital RP who had the following clinical findings: visual acuity of 0.4 in the right eye and 0.2 in the left eye, normal intraocular pressure in both eyes, the conjunctiva in both eyes having no obvious congestion or edema, and anterior segment examination revealing a clear cornea with intermediate anterior chamber depth and clear aqueous humor in both eyes. The pupils measured approximately 3 mm in diameter

and were responsive to light. Fundus examination revealed increased lens density with bilateral pigmentary deposits in the posterior pole. The right eye demonstrated diffuse yellow-white punctate lesions with partial confluence at the posterior pole, accompanied by macular edema and subretinal hemorrhage. Additionally, his mother has had a history of high myopia accompanied by poor night vision and bilateral blurred vision for over 20 years.

Patient 3 (family 3, III-1) is a 29-year-old man with the following clinical findings: visual acuity was near-blindness in the right eye and 0.01 cm in the left eye. Macular OCT showed the retinal pigment epithelial cell layer thickness in the central subregion and approximately 200 μm bilaterally, with a mean thickness ranging from 160 to 180 μm (Figure 2). High lens density was observed. Fundus examination showed peripheral retinal pigmentation and bone spicule–like deposits, and the retina appeared pale with numerous pigmented and osteocyte-like changes. No foveal reflex was detected. In addition, the patient's mother had experienced night blindness for many years, and ophthalmologic examination showed that her mother's conjunctiva was not congested or edematous. The cornea was clear, the anterior chamber was deep, and the pupil was round and reactive to light. However, the lens cortex was unevenly cloudy, there was posterior capsula opacity, the fundus of the eye was blurred and visible, and the optic disc border was clear and pale. There was also a great deal of hyperpigmentation and osteoclast-like changes in the retina.

Genetic analysis: Patient 1 in family 1 (III 1) carried a compound heterozygous mutation c.239-2A>G and c.1482delT (p. Val495fs*) in *CERKL*. The c.239-2A>G mutation is a splice acceptor variant in intron 1 that does not produce protein, while the c.1482delT (p. Val495fs*) mutation is a nonsense mutation that changes the codon for valine at

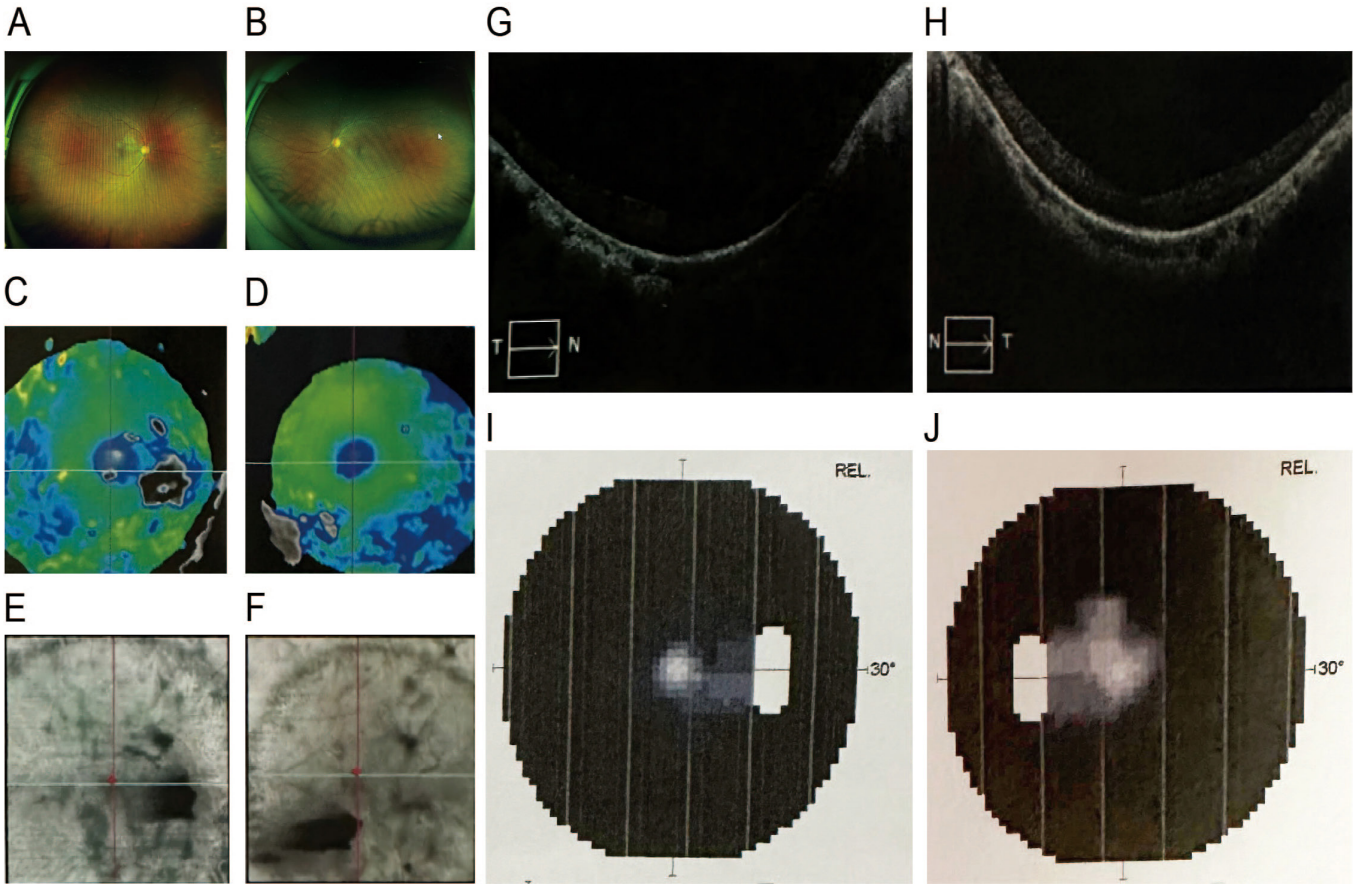

Figure 2. The clinical phenotype of proband (III). **A** and **B** images show macular OCT: thinning of the nerve fibre layer thickness in the macula; **C**: OD ILM-PRE thickness, fovea centralis (depression in the macular retina, most sensitive optic region): 248, 76; **D**: OS ILM-PRE thickness, fovea centralis (depression in the macular retina, most sensitive optic region): 249, 72; **E**: OCT fundus for OD (including choroid, retina, optic nerve, etc.); **F**: OCT fundus for OS (including choroid, retina, optic nerve, etc.); **G**: Brightness-mode ultrasound for OD; **H**: Brightness-mode Ultrasound for OS; **I** and **J** images demonstrate tubular visual field.

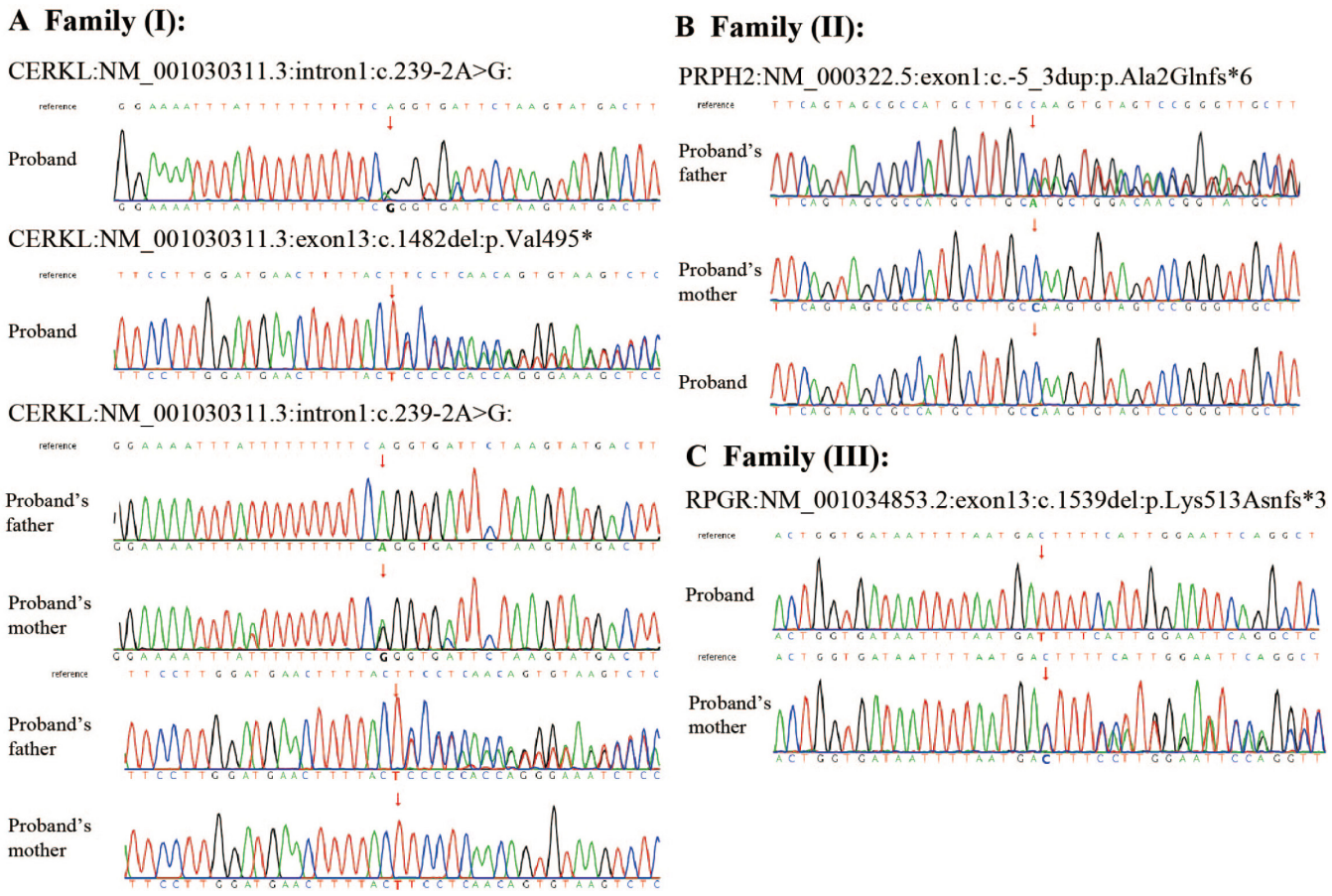

Figure 3. Probands (I, II, III) and their pedigrees sequencing information. Family (I): Proband has c.239–2A>G and c.1482del heterozygous mutations of CERKL; Proband's father: c.1482delT; Proband's mother: c.239–2A>G. Family (II): Proband has a c.-5_3dup mutation of PRPH2; Proband's father: c.-5_3dup mutation. Family (III): Proband has a c.1539delC shift mutation of RPGR; Proband's mother: c.1539delC.

position 495 into a termination codon, producing a truncated protein located in exon 13. Derived from cosegregation, the c.239-2A>G mutation was inherited from his mother, whereas the c.1482delT mutation was passed on by his father and ultimately came from his grandfather. Combined with clinical genotype-phenotype association, it demonstrated that the c.239-2A>G/ c.1482delT mutations in *CERKL* are transmitted in the format of autosomal recessive mutations.

Patient 2 in family 2 (III 1) had a mutation c.-5_3dup (p. Ala2Glufs*6) in *PRPH2* that is a frameshift variant that occurred in exon 1, encoding a truncated protein, inherited from his father by family pedigree cosegregation. Then, it was confirmed that the c.-5_3dup mutation in *PRPH2* is an autosomal dominant variant.

Patient 3 in family 3 (III 1) harbored the c.1539delC (p. Lys513Asnfs*) mutation in *RPGR*. Due to a frameshift mutation leading to a truncated protein, this mutation occurs in exon 1 and cosegregates with his pedigree, which was inherited from his mother. Subsequently, an extended validation of the mutation in the family indicated that his aunts and uncles of the proband did not carry the causative mutations, and the relevant phenotypes were not detected in their offspring, revealing that this *RPGR* mutation was an X-chromosome–linked recessive genetic variant (Figure 3).

According to the Sequence Variant Interpretation guidelines specified by the ACMG and based on screenings from the Human Phenotype Ontology, Online Medline Inheritance in Man, Genetics Home Reference, and other databases, the c.239-2A>G and c.1483delT mutations of *CERKL* were pathogenic. In addition, the c.-5_3dup mutation in *PRPH2* and the c.1539delC mutation within *RPGR* were identified as likely pathogenic in this study (Appendix 1). By analyzing the conservation of protein structural domains, the mutated amino acids are located in the protein's conserved structural domains, so that the production of truncated proteins can lead to the loss of functional domains and ultimately result in pathogenic mutation (Figure 4).

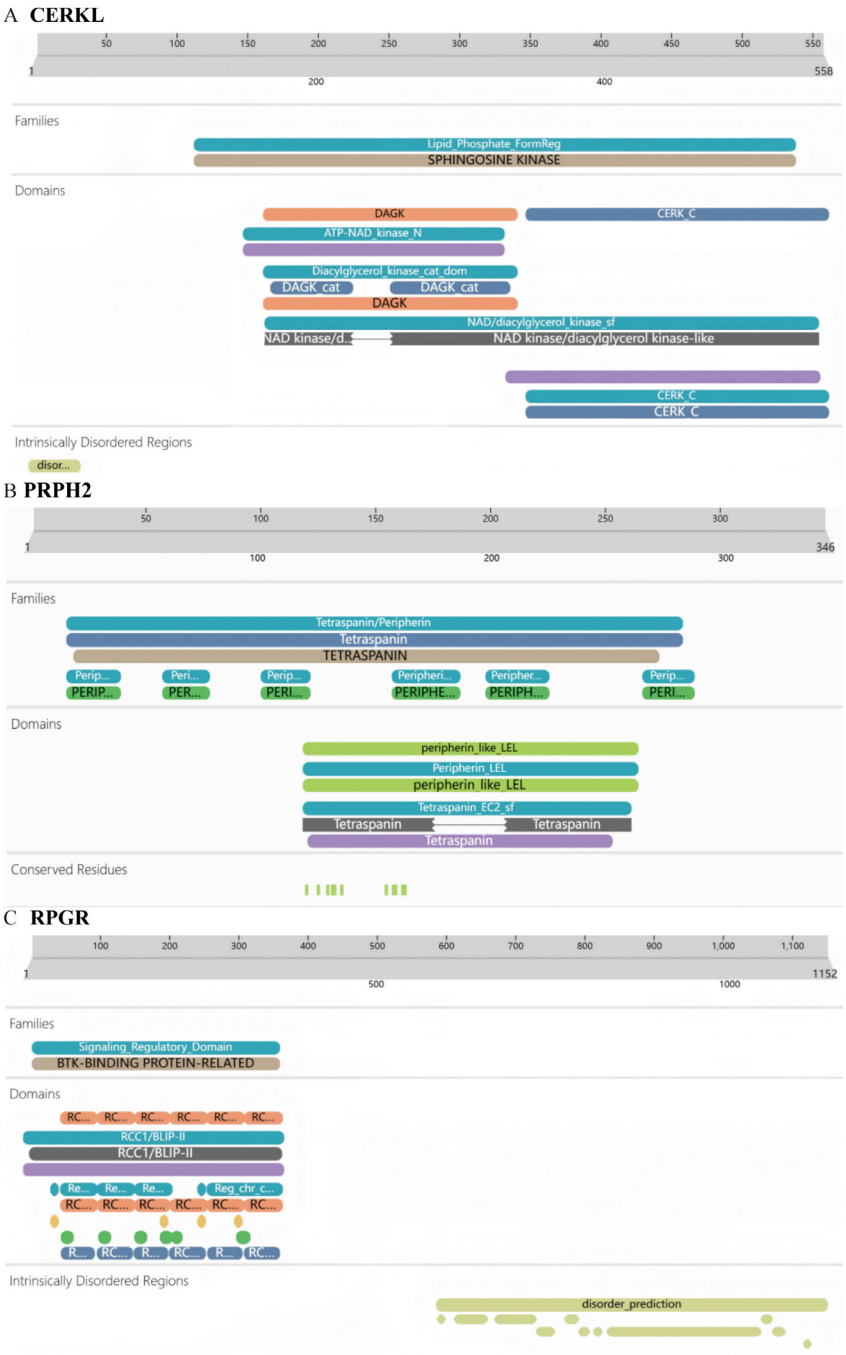

Figure 4. Bioinformatics prediction for three proteins. **A**: Structural domain prediction of CERKL protein by Interpro; **B**: Structural domain prediction of PRPH2 protein by Interpro; **C**: Structural domain prediction of RPGR protein by Interpro.

Literature review of RP cases in China: When comparing the clinical phenotypes caused by different mutations listed in Table 1 of *PRPH2*, we found that there was genetic heterogeneity in the families in these studies. Furthermore, the different *RPGR* mutations listed in Table 2 can result in various pathogenicity and phenotypes that differ in severity and onset time, indicating phenotypic heterogeneity, as detailed in Table 1 and Table 2.

Table 1. Four studies associated with PRPH2 mutations causing RP in Chinese indexed from PubMed and their detailed.

Variation type	Position	Mutation site	Protein	ACMG	HGMD	Classification	Reference
Truncation	42,666,162	c.914del	p.G305Afs*19	PVS1+PS4+PM2+PP1+PP4	DM	P	Wang Y et al.
	42,689,650	c.423C>G	p.Y141*	PVS1+PS4+PM2+PP4	DM	P	
	42,672,134	c.797G>A	p.G266D	PS1+PS4+PM2+PP3+PP4	DM	P	
	42,672,273	c.658C>T	p.R220W	PS4+PM2+PP3+PP4	DM	LP	
	42,689,538	c.535T>C	p.W179R	PS1+PS4+PM2+PP1+PP3+PP4	DM	LP	
	42,689,621	c.452T>G	p.F151C	PS4+PP2+PP3+PP4	DM	LP	
Missense	42,672,298	c.633C>G	p.F211L	PS1+PS4+PM2+PP3+PP4	DM	P	
	42,672,347	c.584G>A	p.R195Q	PS1+PS4+PM2+PM5+PP3+PP4	DM	P	
	42,672,347	c.584G>T	p.R195L	PS1+PS4+PM5+PP3+PP4	DM	P	
	42,689,559	c.514C>T	p.R172W	PS1+PS3+PS4+PM1+ PM2+PM5+PP3+PP4	DM	P	
		c.460A>C	p.Lys154Gln			P	Gao FJ et al.
		c.946T>G	p.Trp316Gly			P	
		c.946T>G	p.Trp316Gly			P	
		c.232G>C;c. 232_233insT	p.Ala78Leufs*99		DM	P	Lim KP et al.
		c.518A>T	p.Asp173Val				
Splicing		c.582-2A>T			DM	LP	Cheng J et al.

Note: Described from the guidlines of ACMG. Very strong evidence of pathogenicity: PVS1: Loss of function (LOF) caused null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene led to disease. Strong evidence of pathogenicity: PS1: A previously pathogenic resulted by same amino acid change regardless of nucleotide changing; PS3: Functional studies in vitro or in vivo supported a damaging effect on the gene or gene product; PS4: Comparing with the prevalence in controls, the prevalence of the variants in affected individuals is significantly increasing; Moderate evidence of pathogenicity: PM1: The functional domain located in a mutational hot spot hadn't benign variation; PM2: Absent from controls (or at extremely low frequency if recessive) in 1000 Genomes or ExAC; PM5: Novel missense changing in an amino acid residue differentiated from pathogenic missense reported before. Supporting evidence of pathogenicity: PP1: Disease resulted by known genes cosegregated with family members; PP2: Missense variants in a gene had a low rate of benign missense variation that are a common mechanism of disease; PP3: Several computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc); PP4: Patient's phenotype and their trios were highly specific for a disease with a single genetic etiology. p=pathogenic; LP=likely pathogenic; DM=damaging mutations.

DISCUSSION

Currently, distributed throughout the whole gene, the variants in *CERKL* reflect genetic heterogeneity due to their different mutation sites, resulting in different phenotypes. Previous studies reported that a white male carried a compound heterozygous mutation for CERKL c.847C>T (p. Arg283*) as a nonsense mutation and c.566_569delinsGTG (p. Lys189Serfs*5) as a frameshift mutation, which both produced a truncated protein [27]. The white male onset of RP occurred at the age of 25 years, with an ophthalmologic examination demonstrating disc pallor, a markedly diminished choroid retinal system, and central macular atrophy, whose clinical phenotype is similar to patient 1 in this study [28]. Previous experimental research has identified that *CERKL* downregulation affects mitochondrial homeostasis and impairs the mitochondrial response to oxidative stress, whereas *CERKL* overexpression protects cells from acute oxidative stress [18]. For example, García demonstrated that *CERKL* knockout (ko) in a mouse model increases reactive oxygen species levels and alters glutathione metabolism and stress granule production, leading to increased basal stress and multiple cell deaths in the retina [29]. Therefore, the truncated protein production consistent with the *CERKL* ko both reduced its expression, which can lead to dysregulation of basal oxidative stress in the retina, ultimately leading to blindness.

As the most typical gene in adRP [10,11], *PRPH2* mutations can lead to a variety of clinical manifestations,

Table 2. The RPGR mutations are downloaded from LOVD and a large cohort study from Chinese suspected RP patients.

Variant type	Gene	Protein	Classification	Reference
Delins	c.ORF15+483_484delGA	p.ORF15+E161fs	P	Li Y et al.
	c.ORF15+694_708del15	p.ORF15+N231_P235del	P	Yang Z et al.
	c.ORF15+1343_1344delGG		P	
	c.1088_1089delinsA	p.Val363Aspfs*18	P	Wang X et al.
	c.197A>G	p.Gln66Arg	P	
Nonsynonymous	c.284G>A	p.Glu95Glu	LP	Huang L et al.
Frameshift	c.1477delC	p.Gly494Glufs*7	LP	
	c.2236_2237delCT	p.Glu746Argfs*23	LP	
	c.2333delA	p.E778Pfs*83	P	Zhang Q et al.
	c.G494A	p.G165D	LP	
Ins	c.3240del		P	Sun W et al.
	c.3361del		P	
	c.139_140insTCTGC		LP	
	c.1282C>G		LP	
	c.1967A>T		LP	
	c.2135A>G		LP	
	c.2200G>A		LP	
	c.2245G>T	p.Glu749*	P	Ge Z et al.
	c.3039_3040del	p.Glu1014Glyfs*64	P	
	c.1495_1496insA	p.Ile499Asnfs*14	P	
	c.2377C>T	p.Q793X	LP	Huang XF et al.
	c.1202_1206del5	p.V401Afs*50	LP	Wang J et al.
	c.92G>A	p.W31*		
	c.905G>C	p.C302S	LP	
	c.1709C>T	p.T570M		
	c.153C>T	p.T51T		
	ORF15+650_653delAGAG	p.Thr801ThrfsX813	LP	
	c.2218G>T	p.Glu740Ter	P	
	c.3092del	p.Glu1031GlyfsTer58	P	
Splice	c.310+3A>G		P	
	c.29–2A>T		P	Wang L et al.
	c.1754–3C>G		LP	
	c.2695G>T	p.Glu899Ter	P	

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Variant type	Gene	Protein	Classification	Reference
	c.1399C>T	p.Gln467Ter	P	
Ins	c.2405_2406del	p.Glu802GlyfsTer32	P	
	c.2964_2965del	p.Glu989GlyfsTer89	P	
	c.373G>T	p.Glu125Ter	P	
	c.2038_2041delGACA	p.Asp680Argfs*16	P	Chen X et al.
	c.248–1G>T		P	Wang X et al.
	c.1377_1378del		P	
	c.2158C>T		P	
	c.2359G>T		P	
	c.1375_1376del		P	
	c.223A>G		LP	Song J et al.
Subsititution	c.352G>A	p.Gln118*	P	Wang P et al.
	c.473del	p.Asp158Valfs*17	P	
	c.532dup	p.Ser178Lysfs*2	P	
	c.494G>T	p.Gly165Val	P	Xu K et al.
Splice	c.1573–2A>G		P	
	c.778+2T>C		P	
	c.2592_2593insA	p.Gly865Argfs*214	LP	Chen ZJ et al.
Subsititution	c.2032G>T	p.Glu678*	LP	
	c.2006G>A	p.Trp669*	LP	Dan H et al.
	c.905G > A	p.Cys302Tyr	LP	Sun Y et al.
	c.1694_1695delAA	p.Gln565Argfs*17	P	Zhang X et al.
	c.284G>A	p.Gly95Glu	P	Xiao T et al.
	c.904T>C	p.Cys302Arg	P	
	c.3160G>T	p.Glu1054Ter	P	Chen PJ et al.
	c.614dupT	p.Thr206AsnfsTer5	P	
	c.2425G>T	p.Glu809Ter	P	
	c.1991C>G	p.Ser664Ter	LP	
	c.646G>T	p.Glu216Ter	LP	Zhu J et al.
	c.2894del	p.Glu965Glyfs*124	P	
	c.2777_2778del	p.Glu926Glyfs*152	P	
Delins	c.2293delG	p.Glu765Argfs*50	LP	
Missense	c.818A>G	p.Gln273Arg	LP	
	c.469+2T>C			Gao FJ et al.

Variant type	Gene	Protein	Classification	Reference
	c.2321_2330del	p.Glu774Glyfs38		
	c.154G>A	p.Gly52Arg		
	c.2730_2731delGG	p.Glu911Glyfs*167		
	c.1041delG	p.Leu347LeufsX4		
	c.1681C>T	p.Gln561Ter		
	c.2899_2902delGAAG			
	c.3220G>A	p.Glu1074Lys		
	c.1207C>T	p.Gln403Ter		
	c.2323_2324delAG	p.Arg775Glufs59		
	c.1115delC	p.Ala372Glufs9		
	c.2007G>A	p.Trp669Ter		
	c.380_383delGAAA	p.Arg127Thrfs5		
	c.2293G>T	p.Glu765Ter		
	c.785C>G	p.Ala262Gly		

Note: Note: P means pathogenic, LP means likely pathogenic.

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including RP, macular dystrophy, and others [30]. Additionally, *PRPH2*-associated diseases usually exhibit phenotype heterogeneity, and patients with the same mutation usually have significant differences in disease manifestations, progression, and recurrence. In a previous study, Ruiz found a PRPH2 c.584 G>T (p.Arg195Leu) mutation in a male with RP from a large Spanish family, which had been transmitted for at least 11 generations [31]. This mutation causes central areolar choroidal dystrophy, presenting a different clinical diagnostic phenotype compared to the RP caused by the PRPH2 c.-5_3dup (p.Ala2Glufs*6) mutation discussed in this study. The latter mutation was identified to affect both the optic cone cell (light vision) response and the mixed (dark vision) response by establishing a knock-in point mutation mice model, which had a slightly greater effect on the rod cell response than on the cone cell response [12]. The predominant phenotype of *PRPH2* in Asian populations was associated with RP, whereas in Caucasians, it was more likely to be macular dystrophy [32]. Currently, the analysis of *PRPH2* genotype-phenotype correlation has been performed mainly in Caucasians, but there are fewer studies on *PRPH2*-associated retinopathy in Asians, especially in Chinese. We compared RP phenotypes caused by *PRPH2* variants in other races, and our findings indicated that there were race-specific phenotype differences between Caucasians and Chinese, along with a relatively high mutation frequency in European ancestry, which reflects inherited heterogeneity. The novel *PRPH2* mutations in this study provide support for genotype-phenotype correlation and expand the clinical phenotype mapping in the Chinese population.

As the first X-linked gene, RPGR variants occurred most commonly in open reading frame 15 (ORF15), which is also the hotspot of mutation-rich in glutamine and glycine residues demonstrated in a mouse model, leading to 60% of X-linked RPs [33,34]. A previous study indicated that overexpression of the full-length *RPGR* isoform in the mouse model can lead to photoreceptor degeneration, demonstrating the effect of this proportion on photoreceptor integrity [35]. *RPGR* protein is mainly located in the connecting cilia of photoreceptor cells involved in the regulation of cilia structure and protein transport. Mutations in *RPGR* can lead to cilia dysfunction, affecting the molecular transport of light signaling, ultimately causing photoreceptor cell degeneration, which can also result in a variety of disease patterns, including rod-cone (70%), cone-rod (6%-23%), and cone dystrophy [20]. In this study, the c.1539delC mutation is a frameshift mutation in exon 1 of *RPGR* with the highly repetitive structural domains, which produces truncated proteins and is a dominant reason to cause RP eventually in patients. Compared with other types of autosomal inherited RP, X-linked RP has a more severe phenotype, whose onset usually occurs in childhood with a more rapid rate of visual deterioration, eventually progressing to legal blindness at about 4% to 5% per year [36].

More than 80 genes are known to be involved in the development of RP. Kinesin-2 motor *KIF3B* has an autosomal dominant inheritance. Unlike *PRPH2* mutations, which interfere with the structure of the photoreceptor outer segment disc membranes, KIF3B mutations affect kinesin complex function through a dominant-negative effect, leading to abnormal cilia length and impaired transport [37]. These multiple pathogenic mechanisms of different genes in adRP enriched genotype-phenotype correlations. Previous studies demonstrated that the *KIF3B* variants in the zebrafish model caused photoreceptor cilia lengthening and defective rhodopsin transport, whose phenotype is consistent with that of *CERKL*-ko mice [29,37]. Given RP is highly genetically heterogeneous, this study will enrich the genotype-phenotype correlates of RP and provide useful insights for genetic and clinical counseling of patients with RP, thereby contributing to ongoing and future gene therapy trials.

Contributors: J. Liu, S. Liu, and M. Han designed and performed the study and wrote the manuscript; X. Zhang, S. Liu, and N. Jiang collected the samples and information; J. Liu participated in data analysis; and J. Liu and M. Li coordinated the study over the entire time. All the authors were involved in the development of the primary manuscript and have read and approved the final version.

APPENDIX 1. THE DETAILED INFORMATION OF MUTATIONS FOUND IN THIS STUDY.

To access the data, click or select the words "Appendix 1." Note: I CERKL: c.239–2A>G, Very strong (PVS1): this mutation occurs in the splicing region resulting in protein functional altering in CERKL; Moderate (PM2): the variant is not found in the 1000G and Shenzhou Genome databases, with frequencies of 1.9e-05 and 1.16e-04 in ExAC and gnomAD, respectively; Moderate (PM3): there is no corresponding mutation detected in the complementary strand for recessive inheritance; I CERKL: c.1482delT, Very strong (PVS1): this mutation causes an alteration in the open reading frame (ORF) to influencing protein function; Moderate (PM2): the mutation is not found in the 1000G, ExAC and Shenzhou Genome databases, with a frequency of 5.76e-04 in gnomAD; Moderate (PM3): there is no corresponding mutation detected in the complementary strand for recessive inheritance. II PRPH2: c.-5_3dup, Very strong (PVS1): this mutation leads to an alteration in the ORF in PRPH2, changing protein function; Moderate (PM2): the mutation frequency is not found in 1000G, Shenandoah Genome Database, ExAC,

and gnomAD. III RPGR: c.1539delC, Very strong (PVS1): the ORF in RPGR was changed by this mutation, resulting protein function altered; the mutation is not found in 1000G, Shenzhou Genome Database, ExAC and gnomAD.

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