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Unveiling the Genetic and Phenotypic Landscape of a Chinese Cohort With Retinitis Pigmentosa

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ABSTRACT

Introduction: Retinitis pigmentosa (RP) is a type of inherited retinal degeneration (IRD) that typically leads to vision loss in individuals of working age. Currently, over 100 genes and loci, as well as over 1000 individual variants, have been identified in relation to RP. The aim of this study was to investigate the genetic distribution and characteristics of Chinese patients with RP, as well as to describe and analyze the genetic features of the high-frequency variant from the *RPGR* gene.

Methods: A total of 69 Chinese patients diagnosed with RP from 36 families were included in this study. Blood samples were collected, and DNA was extracted for genetic analysis. A custom panel targeting 822 genes associated with RP was designed for next-generation sequencing (NGS) analysis. The sequenced data were processed and analyzed using bioinformatics tools to identify genetic variants. Variant classification followed the guidelines provided by the American College of Medical Genetics and Genomics (ACMG), taking into consideration functional effects, population frequencies, and previous literature reports. Variant validation was performed using Sanger sequencing to confirm the presence of identified variants. The inheritance pattern of RP-associated variants was determined by analyzing the segregation pattern within families. Pedigrees were constructed based on the clinical and genetic information of the participants. Statistical analysis was conducted to summarize the clinical characteristics of the RP patients using descriptive statistics. Ethical considerations were strictly followed throughout the study, with approval obtained from the ethics committee and informed consent obtained from all participants.

Results: Following this, the identified variants were classified and subjected to statistical analysis. A total of 15 candidate genes associated with RP were identified, along with 39 variants, consisting of 36 reported variants and 3 novel variants. The majority of these variants were classified as pathogenic. The most common changes observed in this study were substitutions, followed by missense variants. Genetic analysis indicated that all variants occurred in the exon region. In the *RPGR* gene, half the variants are located in the ORF15. Gene, with half of variants located in ORF15. The most frequent variant within this group was *RPGR* NM_001034853.1: c.2236_2237del, which was identified in a large five-generation pedigree. The three novel variants reported in this study include NM_015629.3: c.1168_1169insGATTCAGCCTGGCC of *PRPF31,* NM_001034853.1: c.3026_3027insAGAGGGAGAGGAAGAAGG and NM_000328.2: c.611T>G of *RPGR*.

He-nan Sun and Kai-li Du are co-first authors for this work.

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Conclusions: The findings of this study offer valuable insights into the genetic variants responsible for RP in affected individuals, which can be utilized for genetic counseling and diagnosis. This underscores the significance of genetic testing in the management and treatment of RP.

1 | Introduction

Retinitis pigmentosa (RP) is a type of retinal degenerative disease caused by the loss of retinal photoreceptors and retinal pigment epithelial cells, which leads to gradual degeneration of vision and eventually blindness (Hartong, Berson, and Dryja 2006; Zhang 2016). The clinical manifestation of this disease is night blindness in the initial stage, which gradually progresses to a reduced visual field and decreased visual acuity. In the later stages, symptoms of photophobia and photopsia may occur, eventually leading to severe blindness. Fundus examination reveals a triad of retinal bone spicule pigmentation, optic disc pallor, and retinal vascular degeneration (Gasparini et al. 2019; Shintani, Shechtman, and Gurwood 2009). RP is the most common heterogeneous, hereditary retinal dystrophy eye disease with prevalence of approximately 1/5000–1/3000 (Hu et al. 2019; Verbakel et al. 2018; O'Neal and Luther 2024; Chizzolini et al. 2011). And it is a disorder with high genetic variability that can be inherited in an autosomal recessive (ar), autosomal dominant (ad), or X-linked recessive (xl) pattern (Ayuso and Millan 2010). A rough estimate for the distribution of each inheritance pattern may be around 15%–25% for autosomal-dominant RP (adRP), 35%–50% for autosomal-recessive RP (arRP), 7%–15% for X-linked RP (xlRP), and 25%–60% for syndromic RP (Ayuso and Millan 2010). Compared to other genetic types of RP, such as recessive and dominant RP, X-chromosome-linked RP has an earlier onset time and a more severe degree of onset. Additionally, male patients exhibit more serious clinical characteristics than female patients (Tsang and Sharma 2018; Kurata et al. 2019; Beltran et al. 2014).

Currently, the disease-causing genes associated with X-chromosome-linked RP have been identified as *RPGR* (OMIM 312610), *RP2* (OMIM 312600), and *OFD1* (OMIM 300170) (Meindl et al. 1996; Schwahn et al. 1998; Webb et al. 2012; Campochiaro and Mir 2018). According to statistics, 70%–80% of X chromosome-linked RP is caused by *RPGR* gene variants (Tsang and Sharma 2018). The *RPGR* gene, also known as retinitis pigmentosa GTPase regulator, is located on chromosome Xp21.1 and was first discovered as the pathogenic gene of XLRP in 1996 (Meindl et al. 1996). *RPGR* proteins are involved in cilia formation and actin stability, and they play an important role in effective intra-flagellar transport (IFT) (Patnaik et al. 2015; Gakovic et al. 2011; Murga-Zamalloa, Swaroop, and Khanna 2010). Various forms of transcripts are expressed through alternatively spliced *RPGR* genes, among which the two most important transcripts are *RPGR* ex1-14 and *RPGR* ORF15 (Roepman et al. 1996; Vervoort and Wright 2002). The most common protein isomer in the retina is *RPGR* ORF15 (Roepman et al. 1996; Jin et al. 2006). This transcript spans 15 exons and part of intron 15, encoding 1152 amino acids (Roepman et al. 1996). ORF15 contains a highly repetitive and purine-rich sequence that encodes 567 amino acids rich in glycine and glutamate residues. In the human retina, *RPGR* ORF15 is preferentially expressed in retinal photoreceptor junction cilia, which is required for *RPGR* function (Vervoort and Wright 2002; Khanna et al. 2005). ORF15 is a hotspot for variants of the *RPGR* gene, and most pathogenic variants are predicted to promote polymerase arrest and slipped strand mispairing, resulting in protein truncation and loss of C-terminal domain (Vervoort et al. 2000; Rao, Anand, and Khanna 2016). Therefore, the detection of variants in the ORF15 region is necessary for the diagnosis of XLRP.

In recent years, next-generation sequencing (NGS) has emerged as a precise and high-throughput detection technique, playing a crucial role in the diagnosis and treatment of ocular genetic diseases. However, accurate detection of variants in this particular region has been challenging due to the highly repetitive sequence of ORF15, which adversely affects sequencing coverage and depth (Huang et al. 2015). Therefore, Sanger sequencing technology, known as the gold standard in traditional sequencing, plays a vital role in the clinical detection of ORF15 variants (Ayyagari et al. 2002; Daiger, Sullivan, and Bowne 2013).

In this study, we conducted panel testing on 822 genes and analyzed 69 RP patients. Additionally, we utilized Sanger sequencing technology to confirm variants in the suspected ORF15 region and to identify the frequency and types of ORF15 variants. The findings of this study offer valuable insights for the genetic diagnosis of RP patients.

2 | Materials and Methods

2.1 | Ethical Statement

This study was approved by the Ethics Committee of the Shenyang, He Eye Specialist Hospital, He University, China. Blood samples were collected from all participants with their written informed consent obtained either from the individuals themselves or their guardians. The entire procedure was conducted in accordance with the principles of the Declaration of Helsinki.

2.2 | Clinical Evaluation

All individuals obtained the medical and ophthalmic history and underwent a comprehensive ophthalmologic examination, which included Best-Corrected Visual Acuity (BCVA), visual field examination, fundus photography, full-field electroretinography (following the standards of the International Society for Clinical Electrophysiology of Vision; available at www.iscev.org), and Optical Coherence Tomography (OCT).

2.3 | Sample Collection and Next-Generation Sequencing

This study selected the patients based on the principles referred to previous articles: (a) night blindness; (b) progressive loss of

vision; (c) visual field narrowing to the center; (d) fundus lesion, optic disc atrophy with wax yellow, osteoblast-like pigmentation of the retina, blue-gray retinal, thinner blood vessels; (e) full-field electroretinography results showed declined rod function, and decrease in cone function at the advanced stage (Hartong, Berson, and Dryja 2006; Sun et al. 2020). And all of them were from northern China. Blood samples were collected from a total of 69 patients (36 probands) and their available family members who visited the Shenyang He Eyes Specialist Hospital between 2019 and 2024.

The collection of venous blood involved the use of EDTA anticoagulant tube with 5 mL, followed by storage at −20°C. Genomic DNA extraction from blood was performed using FlexiGene DNA Kit (Qiagen, Venlo, Netherlands) according to the standard protocol provided by Qiagen. NGS was conducted to analyze all genomic DNA samples from the probands. DNA fragments were amplified by polymerase chain reaction using custom primers for all open reading frames and flanking intronic sequences, and then hybridized to a DNA-capture probe designed for the target gene. The captured DNA fragments were eluted, amplified again, and finally analyzed on Illumina NextSeq550 (Illumina; Inc) platform for sequencing analysis. Target Eye 822 NGS gene capture panels were designed and customized by Eye Genebank (Eye Genebank-Shenyang, China). These panels code for exons, introns, and untranslated regions of 822 genes that are associated with common hereditary eye diseases.

2.4 | Genetic and Bioinformatics Analysis of Sequencing Results

The coverage of the target region reached 99.96%, and the average sequencing depth was over 300×. The Burrows-Wheeler algorithm Version 0.7.11 (BWA-MEM) was used to align the sequencing data to the human reference Genome (UCSC hg19) sequence, followed by the removal of repetitive sequences. Variant identification was performed using the Genome Analysis Tool Kit (GATK, version 4.1), and the detected variants were annotated using Annovar and SnpEff. The identified variants were evaluated using the 1000 Genomes, dbSNP, ExAC, and ESP6500 databases to filter out benign variants. Finally, the variants were classified based on common databases such as the Human Gene Mutation Database, the Online Mendelian Inheritance in Man, ClinVar, and the Retinal Information Network, following the guidelines of the American College of Medical Genetics (ACMG).

2.5 | Sanger Verification

To confirm the presence of candidate variants that co-segregated between the gene and phenotype, the variants were further validated using Sanger sequencing in probands and their family members. PCR primers were designed in the ORF15 region of the PRGR gene using Primer 5 software (Forward Primer-TCCTTTCCCCTCCTCTACTT; Reverse Primer-GGGGAGAAAGACAAGGGTAG). The PCR amplification products were purified and sequenced using the ABI 3730XL automated DNA sequencer.

3 | Results

3.1 | Cohort Characteristic and Clinical Assessments

A total of 69 patients (62 males, 7 females) with clinically diagnostic RP from 36 unrelated families (21 families and 15 sporadic) were recruited for this study. Male accounted for 91.67% among the probands. And their ages at their first visits ranged from 3 to 55 years old, with a mean age of 47.84 ± 15.99 years old. The average best-corrected visual acuity (BCVA) of both eyes and overview clinical information of probands could be found in Table 1. The majority of probands experienced night blindness, with symptoms appearing in early childhood. And the specific symptoms of these patients are shown in Table 1. Some probands also had complications such as cataracts ($n = 11$, 30.56%), refractive errors ($n = 4$, 11.11%), and pathologic myopia ($n = 4$, 11.11%). Among the total probands, 80.56% of them had a known family history of RP.

3.2 | Genetic Findings and Novel Variants

Target genes were captured and NGS was performed on the 36 probands and their family members. Out of all cohort, 15 candidate genes were detected, including *ADGRV1*, *ALMS1*, *BBS2*, *CERKL*, *CNGA1*, *EYS*, *NR2E3*, *PRCD*, *PROM1*, *PRPF8*, *PRPF31*, *RHO*, *RPGR*, *SPG11*, and *USH2A* (Figure 1A). Compound heterozygous variants of seven genes which included *ALMS1, BBS2, CERKL*, *CNGA1*, *EYS*, *SPG11*, and *USH2A* were detected in eight families (No. 04, 06, 10, 11, 12, 32, 34, and 36, see Table S1). Interestingly, the nucleotide changes NM_206933.2: c.2802T>G of *USH2A* were identified in both unrelated families (No. 10 and 32).

A total of 39 variants from the genes were located in the exon regions. From the identified variants, four nucleotide change types were classified, including substitution, insertion, deletion, and duplication (Figure 1B). Remarkably, substitution and deletion had the highest ratio of total types, with substitution occurring in 12 out of 15 genes accounted for 64.1% ($n = 25$) (Figure 1B). Additionally, these variants consisted of three distinct variant types, including frameshift, missense, and nonsense. The occurrences of each variant type in patient cohorts were displayed (Figure 1C). The highest frequency of occurrences among all variant types was missense obviously, and the next was frameshift.

According to the ACMG genetic variant classification criteria, all variants were identified as pathogenic, likely pathogenic, and variant of uncertain significance (VUS). The main type of variation was pathogenic ($n = 18$), accounting for 46.15% of all variations. Likely pathogenic and VUS variants accounted for 20.51% and 33.33%, respectively. Among these variants, 33.33% ($n = 6$) variants were classified as pathogenic in frameshift, 44.44% ($n = 8$) variants were classified as pathogenic in missense, and 22.22% ($n = 4$) variants were classified as pathogenic in nonsense (Table 1).

In total identified variants, 35 variants were previously reported, and 3 variants were first reported herein (Figure 1A).

TABLE 1 | Basic clinical information of the probands with Retinitis pigmentosa.

Family ID and probands ID	Age	Time of first symptom detection	Family history	Sex	Mode of onset	BCVA		Gene (OMIM ID)	Transcript	Nucleotide changes	Amino acid changes	Classification
						OD	OS					
F01P01	54	From childhood	Yes	F	No	HM/10 cm	0.4	*PROM1* (604365)	NM_001145848	c.139del	p.His47Ilefs*12	Likely pathogenic
F02P01	40	19	Yes	M	No	0.5	0.3	*PRPF31* (606419)	NM_015629.3	c.693_695del	p.I231del	Vus
F03P01	46	From childhood	Yes	M	No	0.7	0.7	*PRPF31* (606419)	NM_015629.3	c.1168_1169insGATTCAGCCTGGCC*	p.Glu390Glyfs*28	Vus
F04P01	23	From childhood	No	F	No	0.25	0.5	*ALMS1* (606844)	NM_015120.4	c.3902C>A	p.Ser1301Ter	Pathogenic
								ALMS1 (606844)	NM_015120.4	c.8008C>T	p.Arg2670Ter	Pathogenic
F05P01	64	10	Yes	M	Cataracts, Glaucoma	HM/10 cm	LP(+)	*PRPF8* (607300)	NM_006445.3	c.6994G>T	p.Asp2332Tyr	Vus
F06P01	60	From childhood	Yes	M	No	LP(−)	LP(+)	*SPG11* (610844)	NM_025137.3	c.566A>G	p.Asn189Ser	Vus
								SPG11 (610844)	NM_025137.3	c.6284T>C	p.Leu2095Ser	Vus
F07P01	19	4	Yes	M	Refractive errors	0.8	0.8	*PRPF31* (606419)	NM_015629.3	c.615del	p.Tyr205Terfs*1	Pathogenic
F08P01	61	33	Yes	M	Cataracts	LP(+)	LP(+)	*PRCD* (610598)	NM_001077620.2	c.2T>C	p.Met1Thr	Pathogenic
F09P01	60	3	Yes	M	Cataracts, Pathologic Myopia	HM/100 cm	HM/80 cm	*RPGR* (312610)	NM_001034853.1	c.905G>A	p.Cys302Tyr	Pathogenic
F10P01	68	38	Yes	M	Pathologic Myopia	0.05	0.05	*USH2A* (608400)	NM_206933.2	c.7075_7076del	p.Leu2359Asnfs*17	Likely Pathogenic
								USH2A (608400)	NM_206933.2	c.2802T>G	p.Cys934Trp	Pathogenic
F11P01	17	From childhood	No	M	No	0.15	0.2	*BBS2* (606151)	NM_031885.3	c.2107C>T	p.Arg703Ter	Pathogenic
								BBS2 (606151)	NM_031885.3	c.700C>T	p.Arg234Ter	Likely pathogenic

(Continues)

TABLE 1 | (Continued)

Family ID and probands ID	Age	Time of first symptom detection	Family history	Sex	Mode of onset	BCVA		Gene (OMIM ID)	Transcript	Nucleotide changes	Amino acid changes	Classification
						OD	OS					
F12P01	32	From childhood	Yes	F	No	LP(+)	LP(+)	*CERKL* (608381)	NM_001030311.2	c.836del	p.Met279Argfs*7	Pathogenic
								CERKL (608381)	NM_001030311.2	c.626A>T	p.Glu209Val	Vus
F13P01	57	From childhood	Yes	M	No	0.1	HM/10 cm	*RHO* (180380)	NM_000539.3	c.26T>C	p.Phe9Ser	Vus
F14P01	48	14	Yes	M	Cataracts	0.6	0.5	*ADGRV1* (602851)	NM_032119.3.3	c.10648G>T	p.Asp3550Tyr	Vus
								ADGRV1 (602851)	NM_032119.3.3	c.13340G>A	p.Gly4447Asp	Vus
F15P01	71	From childhood	Yes	M	No	UK	UK	*NR2E3* (604485)	NM_014249	c.960_963del	p.Phe320Phefs*3	Pathogenic
F16P01	52	From childhood	Yes	M	Cataracts	HM/10 cm	HM/10 cm	*RHO* (180380)	NM_000539.3	c.632A>G	p.His211Arg	Pathogenic
F17P01	62	From childhood	Yes	M	No	0.1	0.05	*RHO* (180380)	NM_000539.3	c.541G>A	p.Glu181Lys	Pathogenic
F18P01	31	6	Yes	M	Refractive Errors	0.5	0.5	*RPGR* (312610)	NM_001034853.1	c.2236_2237del	p.Glu746Argfs*23	Pathogenic
F19P01	51	5	Yes	M	Cataracts	FC/50 cm	0.3	*RPGR* (312610)	NM_001034853.1	c.747_742dup	p.Gly251Leufs*33	Pathogenic
F20P01	49	From childhood	Yes	M	Cataracts	HM/30 cm	HM/80 cm	*RPGR* (312610)	NM_001034853.1	c.2218G>T	p.Glu740Ter	Pathogenic
F21P01	45	From childhood	Yes	M	Cataracts, Pathologic Myopia	FC/100 cm	FC/100 cm	*RPGR* (312610)	NM_001034853.1	c.2426_2427del	p.Glu809fs	Pathogenic
F22SP01	64	From childhood	Yes	M	Cataracts	0.05	HM/30 cm	*RPGR* (312610)	NM_001034853.1	c.904del	p.Cys302Valfs*8	Likely Pathogenic
F23SP01	43	From childhood	Yes	M	No	0.2	0.3	*RPGR* (312610)	NM_001034853.1	c.2383G>T	p.Glu795Ter	Likely Pathogenic
F24SP01	26	14	Yes	M	Refractive Errors	0.4	0.4	*RPGR* (312610)	NM_001034853.1	c.2236_2237del	p.Glu746Argfs*23	Pathogenic
F25SP01	60	12	Yes	M	No	0.2	0.2	*RPGR* (312610)	NM_001034853.1	c.904del	p.Cys302Valfs*8	Likely Pathogenic
F26SP01	28	4	Yes	M	No	0.3	0.4	*RPGR* (312610)	NM_001034853.1	c.137A>G	p.His46Arg	Vus

(Continues)

TABLE 1 | (Continued)

Family ID and probands ID	Age	Time of first symptom detection	Family history	Sex	Mode of onset	BCVA		Gene (OMIM ID)	Transcript	Nucleotide changes	Amino acid changes	Classification
						OD	OS					
F27SP01	55	From childhood	Yes	M	No	0.03	0.02	*RPGR* (312610)	NM_001034853.1	c.904del	p.Cys302Valfs*8	Likely Pathogenic
F28SP01	71	55	Yes	M	No	UK	UK	*RPGR* (312610)	NM_001034853.1	c.3026_3027insAGAGGGAGAGGAAGAAGG*	p.Gly1009_Glu1010insLeuLeuProLeuProLeu	Vus
F29SP01	54	From childhood	Yes	M	No	LP(+)	LP(+)	*RPGR* (312610)	NM_001034853.1	c.2426_2427del	p.Glu809fs	Pathogenic
F30SP01	36	20	Yes	M	No	1	1	*RPGR* (312610)	NM_001034853.1	c.3170_3172del	p.Arg1057_Asn1058delinsAsn	Vus
F31SP01	37	From childhood	No	M	Cataracts	UK	UK	*RPGR* (312610)	NM_000328	c.1345C>T	p.Arg449Ter	Likely Pathogenic
F32SP01	73	24	Yes	M	No	HM/30 cm	0.3	*USH2A* (608400)	NM_206933.2	c.8232G>C	p.Trp2744Cys	Pathogenic
								USH2A (608400)	NM_206933.2	c.2802T>G	p.Cys934Trp	Likely Pathogenic
F33SP01	21	3	No	M	Refractive Errors	FC/80 cm	0.04	*RPGR* (312610)	NM_000328.2	c.611T>G*	p.Phe450Ser	Vus
F34SP01	35	From childhood	No	M	No	0.1	0.15	*EYS* (612424)	NM_001292009.1	c.6557G>A	p.Gly2186Glu	Pathogenic
								EYS (612424)	NM_001292009.1	c.6416G>A	p.Cys2139Tyr	Pathogenic
F35SP01	44	From childhood	No	M	Cataracts, Pathologic Myopia	UK	UK	*RPGR* (312610)	NM_001034853.1	c.905G>A	p.Cys302Tyr	Pathogenic
F36SP01	31	From childhood	No	M	No	UK	UK	*CNGA1* (123825)	NM_001142564.1	c.1744G>A	p.Gly582Arg	Likely pathogenic
								CNGA1 (123825)	NM_001142564.1	c.472del	p.Leu158Phefs*4	Likely pathogenic

* represents the novel variant.

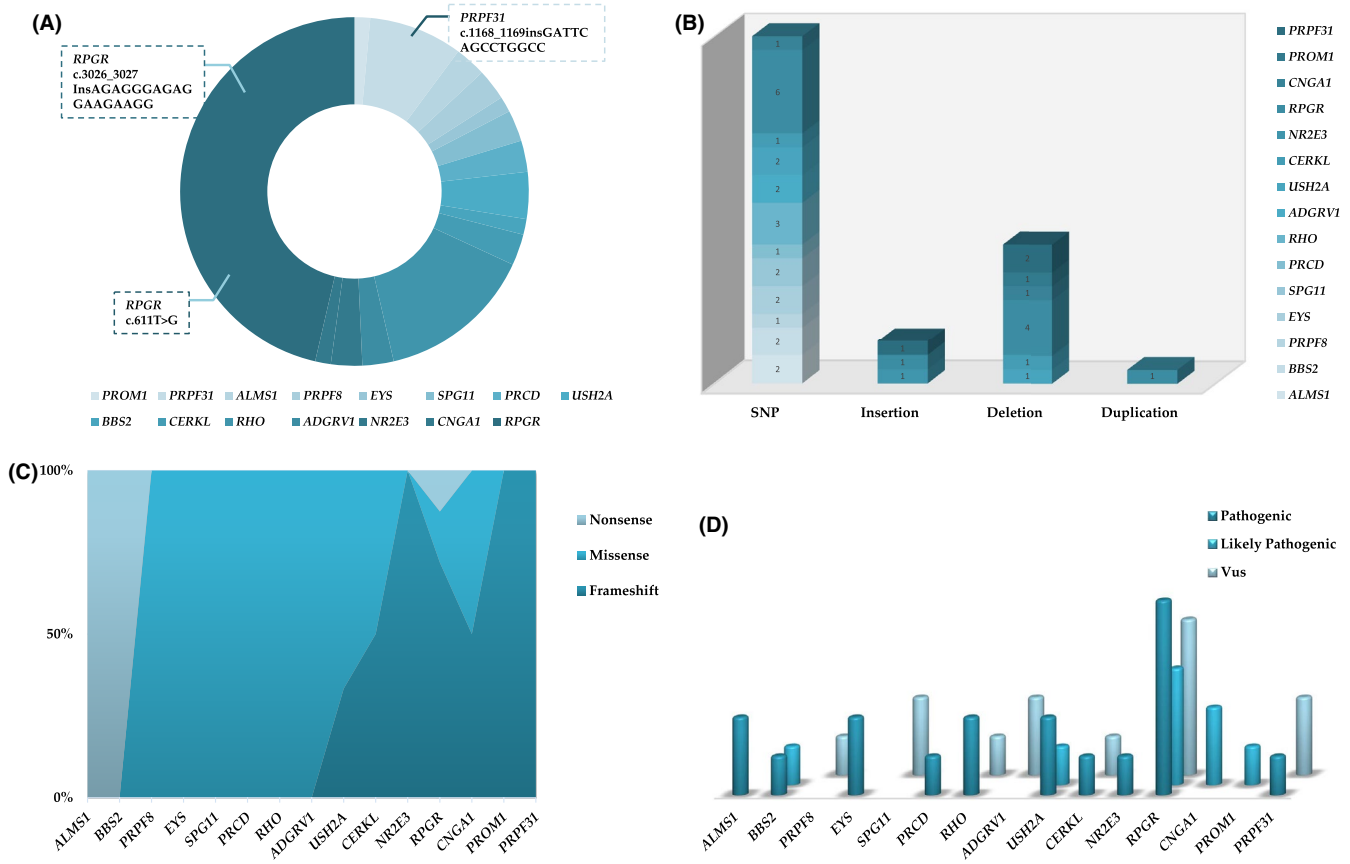

FIGURE 1 | Overview of the genetic analysis conducted in the cohort. (A) Distribution of gene types in retinitis pigmentosa cohorts as well as novel variants found in the detected genes (in the dotted boxes). (B) Different types of nucleotide changes observed in the genes. (C) Occurrences of variant types within the genes. (D) Classified statistics of gene variants according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

The novel variants in the genes including *PRPF31* and *RPGR* were identified in a family and two sporadic patients (No. 03, 28, and 33, see Figure 1A and Table S1). In family No. 03, a novel frameshift variant was identified in exon12 of *PRPF31*. And other two novel variants were identified in *RPGR* of sporadic patients (c.611T>G, c.3026_3027insAGAGGGAGAGGAAGAAGG). The above four variants were classified as VUS because of low incidence exceptionally in the healthy individuals (PM1).

3.3 | RPGR Variation Types and High Frequency Variant

Certainly, among the candidate genes, the *RPGR* gene variants accounted for the largest proportion (47.22%) in 17 families (Figure 1A). There were 28 males and 4 females (Table S1). Genetic analysis confirmed that the variants were hemizygous in all males and heterozygous in all females (Table S1). A total of 12 *RPGR* variants were identified among all cases, located in six sub-regions. Specifically, the genetic analysis revealed that six variants (50%) were located on ORF15, while the remaining variants were distributed across exons 2, 7, 8, 10, and 11 (Table 2). As mentioned above, two novel variants of *RPGR*, c.611T>G and c.3026_3027insAGAGGGAGAGGAAGAAGG, were identified in exon 10 and 15, respectively.

In the *RPGR* gene variants, the most prevalent type of nucleotide changes identified were substitution at 50% ($n=6$), followed by deletions at 33.33% ($n=4$), duplications at 8.33% ($n=1$), and insertions at 8.33% ($n=1$). Frameshift variants were the most common type at 50% ($n=6$), with four (66.67%) occurring in ORF15, while nonsense variants accounted for 25% ($n=3$) and missense variants for 25% ($n=3$) (Figure 1C and Table 2). According to the ACMG guidelines for variant classification, the majority of *RPGR* variants were classified as pathogenic at 41.67% ($n=5$), likely pathogenic at 25% ($n=3$), and variants of uncertain significance (VUS) at 33.33% ($n=4$) (Figure 1C). Notably, only frameshift and nonsense variants were observed in ORF15, with more severe impact on protein function compared to other types of variants. Additionally, half of the variants in ORF15 were classified as pathogenic.

The nucleotide change (NM_001034853.1: c.2236_2237del) contributed to frameshift was found in the ORF15 region with the highest frequency, and it was identified in two unrelated families among the subjects. One was No. 18 family, which is a five-generation pedigree (Figure 2). Gene detection of 12 family members (11 males and 1 female) in this pedigree who were able to be contacted were all verified by Sanger sequencing (Figure 3). The 11 males harbored hemizygous variants, and 1 female was affected by heterozygosis variants. The 7 carriers were identified as heterozygotes. The proband was a 31-year-old male.

TABLE 2 | 39 variants presented on 15 genes in a cohort of 36 probands.

Gene	Number of patients	Count of occurrences	Nucleotide changes	Amino acid changes	Location
PROM1	1	1	c.139del	p.His47Ilefs*12	EX2
PRPF31	6	2	c.693_695del	p.I231del	EX7
		2	c.1168_1169insGA TTCAGCCTGGCC	p.Glu390Glyfs*28	EX12
		2	c.615del	p.Tyr205Terfs*1	EX7
ALMS1	2	2	c.3902C>A	p.Ser1301Ter	EX8
		1	c.8008C>T	p.Arg2670Ter	EX10
PRPF8	2	2	c.6994G>T	p.Asp2332Tyr	EX43
EYS	1	1	c.6557G>A	p.Gly2186Glu	EX32
		1	c.6416G>A	p.Cys2139Tyr	EX31
SPG11	2	2	c.566A>G	p.Asn189Ser	EX3
		2	c.6284T>C	p.Leu2095Ser	EX33
PRCD	2	2	c.2T>C	p.Met1Thr	EX1
USH2A	3	2	c.7075_7076del	p.Leu2359Asnfs*17	EX37
		3	c.2802T>G	p.Cys934Trp	EX13
		1	c.8232G>C	p.Trp2744Cys	EX42
BBS2	1	1	c.2107C>T	p.Arg703Ter	EX17
		1	c.700C>T	p.Arg234Ter	EX6
CERKL	2	2	c.836del	p.Met279Argfs*7	EX6
		2	c.626A>T	p.Glu209Val	EX4
RHO	10	1	c.26T>C	p.Phe9Ser	EX1
		4	c.632A>G	p.His211Arg	EX3
		5	c.541G>A	p.Glu181Lys	EX3
ADGRV1	2	1	c.10648G>T	p.Asp3550Tyr	EX51
		2	c.13340G>A	p.Gly4447Asp	EX66
NR2E3	2	2	c.960_963del	p.Phe320Phefs*3	EX7
CNGA1	1	1	c.1744G>A	p.Gly582Arg	EX10
		1	c.472del	p.Leu158Phefs*4	EX5
RPGR	32	3	c.905G>A	p.Cys302Tyr	EX8
		13	c.2236_2237del	p.Glu746Argfs*23	ORF15
		2	c.747_742dup	p.Gly251Leufs*33	EX7
		2	c.2218G>T	p.Glu740Ter	ORF15
		3	c.2426_2427del	p.Glu809fs	ORF15
		1	c.2383G>T	p.Glu795Ter	ORF15
		3	c.904del	p.Cys302Valfs*8	EX8
		1	c.137A>G	p.His46Arg	EX2
		1	c.3026_3027insAGAG GGAGAGGAAGAAGG	p.Gly1009_Glu1010insLeu LeuProLeuProLeu	ORF15

(Continues)

TABLE 2 | (Continued)

Gene	Number of patients	Count of occurrences	Nucleotide changes	Amino acid changes	Location
		1	c.3170_3172del	p.Arg1057_Asn1058delinsAsn	ORF15
		1	c.1345C>T	p.Arg449Ter	EX11
		1	c.611T>G	p.Phe450Ser	EX10

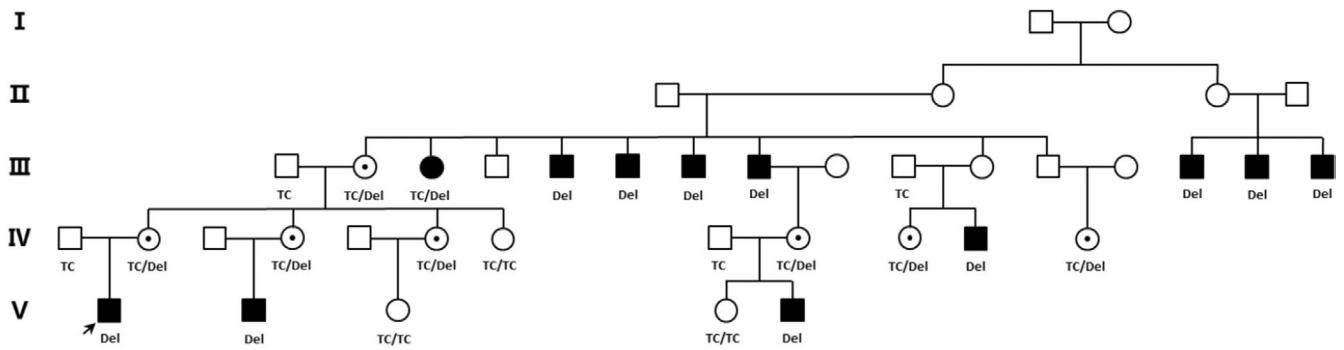

FIGURE 2 | Pedigree of the Chinese No. 18 family with the X-Linked retinitis pigmentosa. The square represents male, while the circle represents females. The filled shape represents individuals affected by RP, while the dot in the circle represents asymptomatic carrier. The arrow indicates the proband.

At the age of 6 years old, the patient was diagnosed with night blindness, while day vision was normal. The Electroretinogram (ERG) with dark-adapted 0.01 flash stimulation shows the absence of an a-wave and b-wave, exhibiting an "extinguished" pattern. The ERG with light-adapted 3.0 flash stimulation displays an unstable b-wave waveform, significantly prolonged latency, and a notable decline in amplitude, with the possibility of it being completely absent (Figure S1). The perimetry showed the visual fields of the patient were narrowed significantly in both eyes (24-2) (Figure S2). The SD-OCT scan of the macula reveals degenerative changes in the retinas of both eyes, particularly in the outer layer structures. These changes include thinning of the retina, severe disorganization, and atrophy of the outer nuclear layer, near disappearance, and a blurred ellipsoid zone, making it difficult to discern (Figure 4A). And the fundus photography examination indicated the pigment of the optic disc was pale, white, and precipitated (Figure 4B). Seven female carriers in the family had pathologic myopia and refractive errors. As they showed no signs or symptoms of retinitis pigmentosa, they were assumed to be unaffected.

4 | Discussion

In our study, we conducted a sequencing analysis of 822 genes in blood samples collected from 69 patients diagnosed with RP and provided detailed genetic profiles. By utilizing bioinformatics tools for NGS analysis of the 822 genes, we identified a total of 39 variants, with the majority of them being located in the *RPGR* variants caused XLRP. It was possible that the participants we recruited were most males with rapid progression of vision loss and early onset. XLRP is extremely severe in males with early onset and rapidly progressive vision loss (Comander et al. 2015; Lam et al. 2024; Ferrari et al. 2011). In previous research, Liu et al. (2022) identified that 97.4% *RPGR* variants caused XLRP in a Chinese cohort. Xinhua Shu et al. found that 95% of *RPGR* mutations are related to XLRP in Caucasians (Shu et al. 2007).

This study also documented three novel variants associated with RP in male patients. *PRPF31* is the second most common causative gene of autosomal dominant RP (Chen et al. 2022). In our study, we discovered a novel insertion variation of *PRPF31* (NM_015629.3: c.1168_1169insGATTCAGCCTGGCC) caused by the insertion of "GATTCAGCCTGGCC" between nucleic acid 1168 and1169, leading to frameshift on 390th amino acid.

Furthermore, the two novel variants of *RPGR*, NM_001034853.1: c.3026_3027insAGAGGGAGAGGAAGAAGG and NM_000328.2: c.611T>G, were identified in this research. *RPGR* is one of the gene loci associated with X-linked retinitis pigmentosa (XLRP), one of the most severe clinical phenotypes of RP (Meindl et al. 1996; Roepman et al. 1996). The variant, NM_001034853.1: c.3026_3027insAGAGGGAGAGGAAGAAGG, is located on ORF15 of *RPGR*. Frameshift variants in ORF15 were found to be the most common among all types of variants in our analysis. This finding aligns with previous studies that have emphasized the high frequency of variants in the ORF15 region of *RPGR* (Sharon et al. 2003; Ruddle et al. 2009). *RPGR* ORF15 is a crucial alternative spliced transcript in the retina that is associated with XLRP (Vervoort and Wright 2002; Vervoort et al. 2000). The N-terminal regulator of chromatin condensation 1 (RCC1)-like domain (RLD) of *RPGR* ORF15, a repetitive domain rich in glycine and glutamic acid, may contribute to the prevalence of variants in ORF15 (Wright and Shu 2007). The glutamate- and glycine-rich sequence in ORF15 is difficult to fold into a spatial stereo structure, making it likely to act as a flexible linker connecting the N-terminal and C-terminal of the protein. This can lead to slip chain mismatches

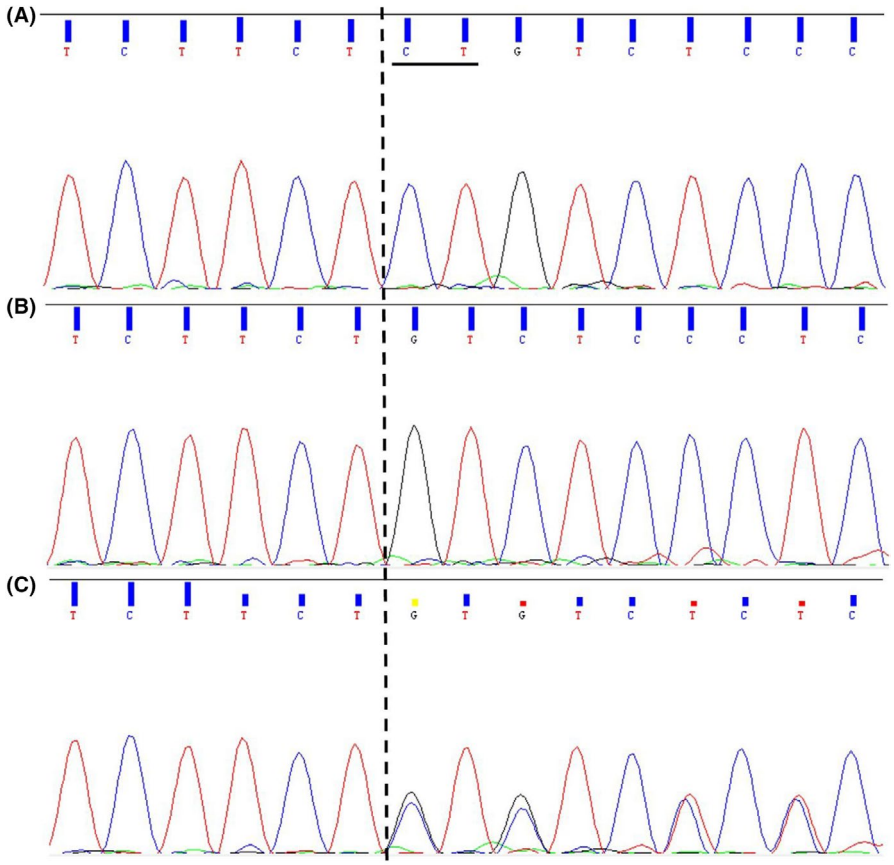

FIGURE 3 | Sanger sequencing results of the *RPGR* gene ORF15 in the No. 18 family. (A) The result for the normal individual. (B) The proband and other male members carried the hemizygous variants (c.2236_2237del p. Glu746Argfs23). (C) The female carrier in the third generation was confirmed to have the heterozygous variant (c.2236_2237del p. Glu746Argfs23). The black horizontal line represents the site of the variant.

of the polymerase, resulting in frameshift variants (Vervoort et al. 2000; Hong and Li 2002). Since ORF15 is a C-terminal exon and transcripts with frameshift variants are stable, translated *RPGR* ORF15 may accumulate, potentially causing functional gain or a toxic phenotype (Sharon et al. 2003). Research indicates that diseases tend to be more severe when the abnormal amino acid sequence is longer (Sharon et al. 2003; Wright and Shu 2007). This observation is consistent with our findings that most *RPGR* variants are pathogenic, with their pathogenicity attributed to frameshift mutations.

X-linked recessive RP is recognized for its uncommon mode of inheritance (Li et al. 2005; Chivers et al. 2021). In our study, the inheritance mode of No. 18 family was determined to be X-linked through NGS analysis. This determination was based on the fact that all affected individuals in the family were male (with the exception of one female who carried heterozygous variants confirmed by Sanger sequencing), and both parents were unaffected. However, this variant was inherited from carrier mother side. Furthermore, male patients only transmit the disease-causing gene to their daughters. It is crucial to conduct precise genetic examinations and establish inheritance models for RP patients, as these will serve as crucial evidence for future treatments. In China, there remains a significant population of RP patients who do not have access to genetic testing. Therefore, it is strongly recommended to implement measures that encompass a broader range of RP variants. There is a clear gender bias in our study center, with a higher proportion of males than females. Thus, our study has certain limitations. The RP cohort, which is clearly focused on a specific population, may not be applicable to other groups.

5 | Conclusions

In conclusion, this study provided a comprehensive genetic and phenotypic analysis of Chinese patients with RP. By sequencing 822 RP-related genes, we identified a total of 39 variants in RP patients and their families. The majority of these variants were found in the *RPGR* gene, with a novel variant identified in *PRPF31* gene. Of particular interest were the six variants located in the ORF15 region of the *RPGR* gene, which are known to be associated with X-linked RP. Our findings further support the significance of ORF15 variants in the diagnosis and understanding of RP in Chinese populations. The genetic classification of the identified variants according to the ACMG guidelines revealed numerous pathogenic or likely pathogenic variants, emphasizing their clinical relevance.

Additionally, the investigation of inheritance patterns allowed us to determine the mode of inheritance for these RP-associated variants within the families. This information is crucial for genetic counseling and prognosis prediction in affected individuals and their relatives. The clinical and genetic data presented in our

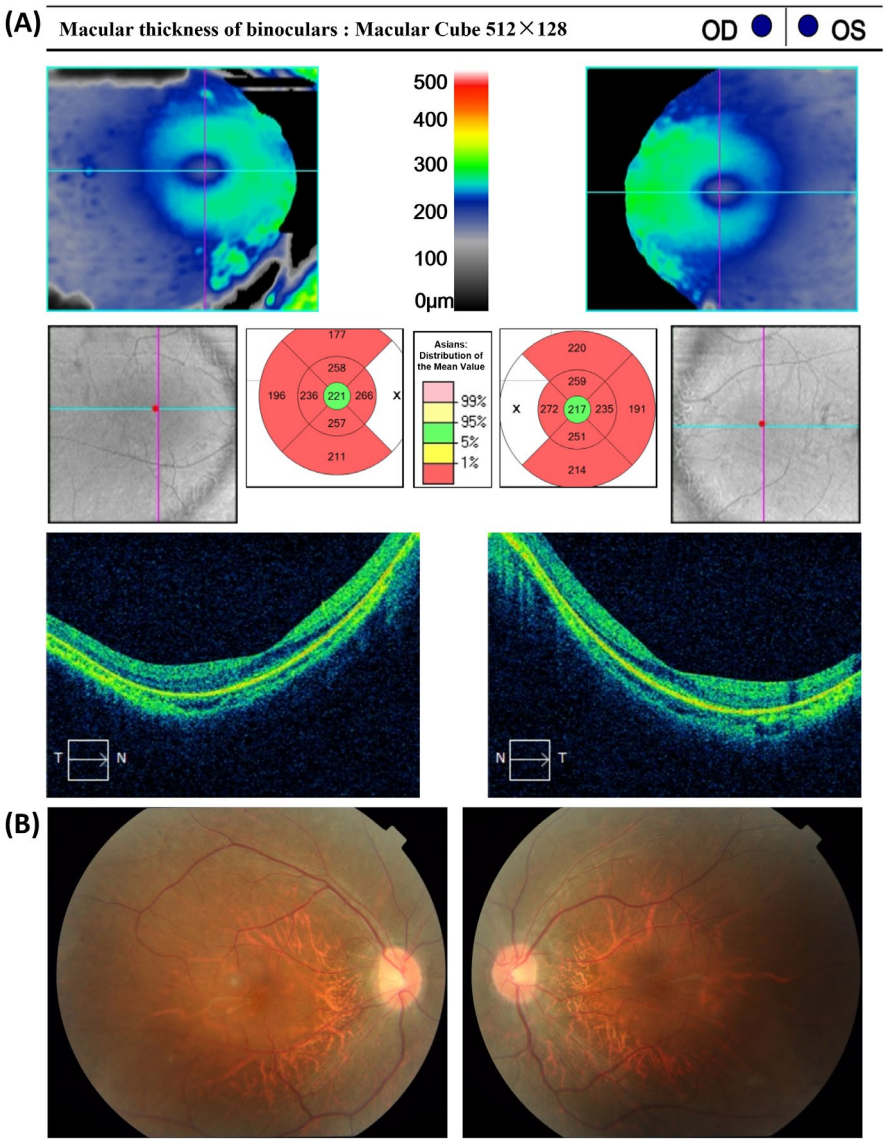

FIGURE 4 | OCT images and fundus photography of the proband from Family 18. (A) OCT images indicated retinal atrophy in both eyes of the proband. (B) Fundus photography indicated the optic disc was lightly pigmented with precipitation of both eyes.

study contribute to expanding the knowledge and understanding of RP genetics, specifically in the Chinese population.

Overall, this study enhances our understanding of the genetic landscape of RP in Chinese patients and highlights the importance of comprehensive genetic analysis in the diagnosis and management of this complex disease. The identified variants may serve as valuable resources for future genetic studies, as well as for developing targeted therapies and personalized treatment approaches for individuals with RP.

Author Contributions

Zhuo-Shi Wang, Jian-Kang Li and Ji-jing Pang designed the research study. He-nan Sun, Xin-xin Wang and Hui-hui Yu performed the research. Yan Sun, Ye Liu, Jia-yuan Ge, Jia Rong, Di Wang and Yue Ren provided help and advice on clinical samples collection and information collation. Cong Liu, Jin-hui Xue analyzed the data. He-nan Sun and Kai-li Du wrote and revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Ethics Statement

This study was approved by the Ethics Committee of the Shenyang He Eye Specialist Hospital, He University, China.

Consent

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.