Original Research

Clinical and genetic investigations in Chinese families with retinitis pigmentosa

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Impact Statement

Retinitis pigmentosa (RP) is a group of hereditary retinal diseases with progressive degeneration of the rod and cone photoreceptors. Although classified according to clinical presentation, RP evinces heterogeneous etiology that differs according to ethnicity. Moreover, systematic analysis in specific populations has been difficult owing to numerous identified genes. We utilized targeted exome sequencing to investigate variations in a total of 20 unrelated Chinese pedigrees with non-syndromic RP. We detected three novel (likely) pathogenic mutations and eight previously reported (likely) pathogenic mutations of genes known to be related to non-syndromic RP. We report novel mutations responsible for non-syndromic RP in Chinese pedigrees, expanding the number of gene mutations associated with this disorder and clarifying its genetic basis in the Chinese population.

Abstract

To describe clinical and genetic characteristics in a series of Chinese patients with non-syndromic retinitis pigmentosa, a total of 20 unrelated Chinese pedigrees with non-syndromic retinitis pigmentosa were evaluated. Complete ophthalmic examinations data including the Humphrey visual field, spectral domain-optical coherence tomography, full-field electroretinography, and fundus fluorescence were collected and analyzed. Targeted exome sequencing was utilized to investigate variations in 260 known genes of inherited retinal disease, including the 90 known causative retinitis pigmentosa genes. We initially identified the potential candidate variants in the pedigrees, then validated the variants using the Sanger sequencing and performed segregation analysis to verify that the variants constituted disease-causing mutations in these pedigrees. We detected three novel (likely) pathogenic and eight previously reported (likely) pathogenic variations in nine genes reported to be related to non-syndromic retinitis pigmentosa in nine of the pedigrees. We report clinical characteristics of Chinese patients with retinitis pigmentosa and novel mutations responsible for non-syndromic retinitis pigmentosa in Chinese pedigrees, expanding the number of gene mutations associated with this disorder and clarifying its genetic basis in the Chinese population. These data will help with rapid and efficient molecular diagnosis and the study of targeted treatment for retinitis pigmentosa in this population.

Keywords: Retinitis pigmentosa, gene, novel mutations, Chinese patients, targeted high-throughput DNA sequencing, clinical features

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Introduction

Retinitis pigmentosa (RP, OMIM 268000) comprises a group of hereditary diseases with progressive degeneration of the retinal cone and rod photoreceptors, characterized by night blindness, gradual decline of visual acuity, and progressive constriction of vision field. The common findings on retina include "bone spicule" pigmentary deposits initially from the mid-peripheral retina, retinal vessel attenuation, and waxy pale optic disks. In addition, characteristic changes in electroretinogram patterns include severely reduced or even extinguished signals, with the first affected rods and the lately affected cones. As RP advances, patients eventually develop complete blindness.[1–5] The disease most commonly affects the visual function in individuals of working age is inherited retinal disease (IRD). The worldwide incidence of RP is about one in 3500–5000 individuals, accounting for one-half of IRD cases with a significant disruptive impact on patients' health and life.[1–5]

RP is highly heterogeneous. Various inheritance modes include autosomal dominant (15–25%), autosomal recessive (5–20%), X-linked (5–15%), and unknown (sporadic) (10–40%).[1–8] RP may be transmitted as a digenic or mitochondrial trait rarely.[1–8] Moreover, more than 90 non-syndromic

RP-related genes have been identified and numerous different disease causative mutations have been identified in each gene.[1–15] Notably, different variations of the same gene may cause different clinical phenotypes. And different individuals may also present with different clinical features caused by the same variation in the same gene, even in the same pedigree. In addition, individual gene variations differ according to ethnicity, resulting in a mutation spectrum and frequency variation among different populations. However, systematic analysis in specific populations has been difficult owing to the large number of identified genes. Toward this end, two large cohort studies have provided an initial overview of the genetic landscape of Chinese patients with IRDs.[9,10] Few other studies that provide the genetic data of RP in the Chinese population are available,[11–13] highlighting the need for further investigation of this issue. Delineation of the RP genes mutation spectrum in individual populations would help with efficient and rapid molecular diagnosis and study of targeted therapeutics such as gene therapy for RP in the respective populations.[1–4,8–15]

In this study, the clinical and hereditary features in a series of patients with non-syndromic RP in Chinese population were described. We investigated variations in 260 known genes related to IRD, which included the 90 known RP-related genes, in probands of the 20 unrelated Chinese pedigrees with non-syndromic RP through targeted exome sequencing. The potential candidate pathogenic mutations were verified by the Sanger sequencing, and then segregation analysis in the available family members. We detected three novel (likely) pathogenic mutations and eight previously reported (likely) pathogenic mutations of genes known to be related to non-syndromic RP.

Materials and methods

Study participants

We evaluated 20 unrelated Chinese pedigrees with non-syndromic RP recruited from the Jinan University affiliated Shenzhen Eye Hospital. The study obtained the approval of the Ethics Committees of the Jinan University affiliated Shenzhen Eye Hospital. We performed the study in accordance with the Declaration of Helsinki and obtained written informed consent from all individuals. We collected and analyzed complete ophthalmic examinations data including best-corrected visual acuity, intraocular pressure, slit lamp examination, 30–2 visual field examination (VF, Humphrey Visual Field Analyzer, Carl Zeiss, Inc., San Diego, CA, USA), fundoscopy, fundus fluorescence imaging (FAF), spectral domain-optical coherence tomography (SD-OCT, SPECTRALIS® HRA + OCT, Heidelberg Engineering Inc., Heidelberg, Germany), and full-field electroretinogram (ERG, RETI-Port/Scan 21, Roland Consult Stasche & Finger GmbH, Brandenburg, Germany). Peripheral venous blood samples were also obtained from all study individuals.

Genetic analysis

DNA library preparation. The quality of DNA sample was ascertained by Nanodrop (Thermo Scientific, Waltham, MA, USA). We prepared DNA libraries according to the standard protocol of Illumina (San Diego, CA, USA). In brief, first nebulized 3 μg of genomic DNA to make it fragmented, ligated an "A" to the 3′ end, and ligated the Illumina adapters to the fragments, then size-selected the sample aiming for a 350–400 bp product. Then amplified the product by polymerase chain reaction (PCR), and then validated the final product using an Agilent Bioanalyzer (Santa Clara, CA, USA).

Targeted gene enrichment and sequencing. The amplified DNA product was captured with four IRD-related gene panels utilizing biotinylated oligo-probes (MyGenostics, Beijing, China). Although specific types of IRD have distinct terminology, there is considerable overlap between the clinical features of RP and other IRDs, as well as between diseases caused by RP gene mutations. We designed the probes to tile along 260 retinal disease-related genes, including those for syndromic and non-syndromic RP, Leber's congenital amaurosis, and other syndromic or non-syndromic diseases associated with IRD.

The capture next-generation sequencing procedure was conducted as the standard protocol. In brief, mixed 1 μg DNA library with the GenCap gene panel probe (MyGenostics), then held on a PCR thermocycler (TC-96/G/H(b)C, Bioer Technology, Hangzhou, China) first at 95°C for 7 min and then at 65°C for 2 min. And added 23 μL of 65°C Buffer HY (MyGenostics) to this mixture, and putted the mixture into the PCR thermocycler at 65°C for 22 h for hybridization. Next, added 64 μL of 2× binding buffer to the hybrid mixture, and transferred the mixture to a tube with 80 μL of preprepared MyOne beads (Life Technology, Carlsbad, CA, USA). Rotated the mixture for 1 h, washed the beads once for 15 min at room temperature (25°C) with WB1 buffer and then three times at 65°C with WB3 buffer for 15 min. And then amplified the eluted DNA by the following program: at first 98°C for 30 s, and 15 cycles of 98°C for 25 s, 65°C for 30 s, 72°C for 30 s, then 72°C for 5 min. After purifying the PCR product by SPRI beads (Beckman Coulter, Brea, CA, USA), sequenced the product on a sequencer (Illumina HiSeq X Ten sequencer) to obtain 100 bp paired reads.[16,17]

Bioinformatics analysis. Illumina HiSeq X Ten sequencing was performed, after which we retrieved high-quality reads using the Cutadapt program (http://cutadapt.readthedocs.io/en/stable/) to filter out low-quality reads and adaptor sequences from the raw reads. Next, to map clean reads to the human reference genome (hg19, we use hg19 as a reference genome because there are many software and databases that use hg19, such as HGMD, ProBean, PolyPhen-2, and MutationTaster2), we used the Burrows–Wheeler Alignment (BWA, http://bio-bwa.sourceforge.net/), and removed PCR duplicates utilizing Picard tools (http://broadinstitute.github.io/picard/), leaving only the uniquely mapped reads for variation detection. Subsequently, Genome Analysis Toolkit (GATK) HaplotypeCaller (https://software.broadinstitute.org/gatk/) was employed to detect variants containing single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels). We annotated the identified SNPs and InDels utilizing ANNOVAR (http://annovar.openbioinformatics.org/en/latest/).

Table 1. Clinical features and family history of 20 pedigrees with non-syndromic retinitis pigmentosa.

Family	Clinical features of the proband	Family history
1	Poor vision, night blindness from age 3 years	Grandfather, mother, two aunts, and two cousins affected with RP
2	Poor vision, night blindness complicated by nystagmus and exotropia from age 4 years	Not present
3	Poor vision and night blindness from age 10 years	Great-grandfather, grandfather, father, aunt, and uncle affected with RP
4	Bietti crystalline corneoretinal dystrophy	Elder sister affected with RP
5	Diagnosed with RP since childhood, with poor vision, night blindness complicated by nystagmus	Parents consanguineous
6	RP sine pigmento, poor vision for counting fingers, intolerance of light complicated by nystagmus	Not present
7	RP complicated by glaucoma	Mother and maternal uncle affected with RP
8	Poor vision and night blindness since childhood	Not present
9	Diagnosed with RP with poor vision and narrowing of field from the age of 20 years	Two great aunts affected with RP
10	Diagnosed with RP with poor vision and narrowing of field	Not present
11	Poor vision, night blindness complicated by nystagmus since childhood	Parents consanguineous
12	Reverse(central) RP complicated by exotropia, with poor vision and night blindness since childhood	Not present
13	Night blindness since childhood, decreased vision from the age of 35 years	Uncle affected with RP, consanguinity within five generations
14	Fundus albipunctatus (retinitis punctata albescens), night blindness, and color weakness since childhood	Grandfather affected
15	Poor vision and tubular field of vision since childhood	Grandfather affected with RP
16	Poor vision, RP sine pigmento diagnosed age of 30 years, associated with macular edema in the left eye	Not present
17	Night blindness since childhood	Parents consanguineous, uncle and aunt consanguineous, and four cousins affected with RP
18	Night blindness since childhood	Grandfather, father, and two aunts affected with RP
19	Night blindness since childhood	Not present
20	RP complicated with choroidal neovascularization and vitreous hemorrhage in both eyes	Not present

RP: retinitis pigmentosa.

Finally, we utilized Integrative Genomics Viewer (http://www.igv.org/) to identify short read alignments and verify the SNPs and InDels. We focused on identifying rare variants (minor allele frequency $\leqslant$0.05 based on the 1000 Genomes, ESP6500, and ExAC databases), as well as pathogenic variants that had been predicted via algorithms (SIFT, PolyPhen-2, MutationTaster, and GERP++).[16,17] We utilized the online ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php), the Online Mendelian Inheritance in Man (OMIM) (https://www.omim.org/), the American College of Medical Genetics and Genomics (ACMG) guidelines, and the Combined Annotation-Dependent Depletion (CADD) (https://cadd.gs.washington.edu) to classify the pathogenicity of each genetic variation. CNVkit (https://cnvkit.readthedocs.io/en/stable/) software was used to obtain copy number variation information.

Expanded validation DNA samples were obtained from the probands of the pedigrees for the targeted exome sequencing and filtering analysis. To validate all likely pathogenic mutations and allow familial segregation analyses whenever possible, conventional Sanger bidirectional sequencing was conducted on all affected and unaffected subjects of the families. We sequenced the PCR products (ABI 3500 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA) and analyzed the results using the Mutation Surveyor (Softgenetics, State College, PA, USA) (The primers for the Sanger sequencing were shown in Supplementary Material STable1.).

Results

Clinical findings

We evaluated a total of 20 unrelated Chinese pedigrees with RP, without syndromic or systemic abnormality. The clinical features and family history of the 20 families are shown in Table 1 and Supplementary Material SFigure1. Among these, 10 families presented with family history of RP, whereas the other families did not present with family history. Consanguinity was noted in four families (families 5, 11, 13, and 17). The patients exhibited typical characteristics including early night blindness, gradual decline of visual acuity, constriction of the visual field, pigment abnormality initially occurred in the mid-peripheral area of the retina, attenuated retinal artery, and pale optic disks (Figure 1). Electroretinogram recordings were severely reduced or even extinguished, with the rods affected before the cones. In addition to the typical characteristics, some families presented with specific characteristics. Specifically, one family exhibited the Bietti crystalline corneoretinal dystrophy (families 4), one family exhibited fundus albipunctatus (retinitis punctata albescens, families 14), two showed sine pigmento (families

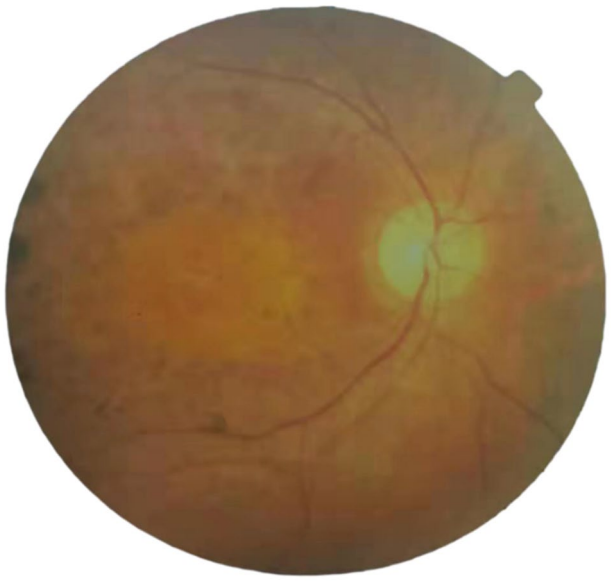

Figure 1. Fundus of the proband of family 17 with retinitis pigmentosa. Pigment abnormality initially occurred in the mid-peripheral retina, pale optic disks, and attenuated retinal artery.

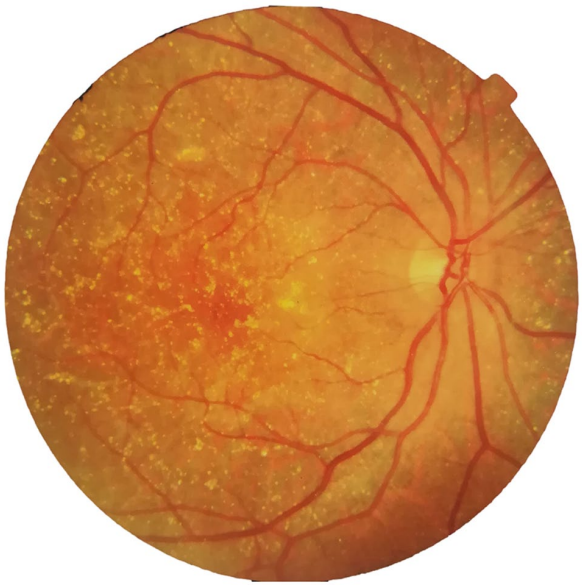

Figure 2. Fundus of the proband of family 4 with the Bietti crystalline corneoretinal dystrophy.
Chorioretinal atrophy and atrophy in the retinal pigment epithelium (RPE) in association with multiple shimmering yellow-white deposits occurred in the posterior area of the retina are observed.

6 and 16), one patient exhibited RP sine pigmento associated with macular edema (family 6), one patient complicated with choroidal neovascularization and vitreous hemorrhage in both eyes (family 20), and one exhibited reverse (central) RP (family 12). Notably, family 4 presented with atrophy of the retinal pigment epithelium (RPE), chorioretinal atrophy, and with multiple shimmering yellow-white spots occurred in the posterior area of the retina (Figure 2).

Mutation analysis

Evaluation of the quality of the capture next-generation sequencing results revealed that the average coverage of the target regions was over 98.0% and the average sequencing depth was 200 reads. We processed the data using the BWA program, then called and annotated the variants by GATK to identify non-synonymous variants. These were searched against multiple databases including the ESP6500 (http://evs.gs.washington.edu/EVS/), 1000 Genome (http://www.1000genomes.org/), and a 702-sample in-house exome database as normal controls. Variants with allele frequencies >1% for autosomal recessive and allele frequencies >0.1% for autosomal dominant in these databases were excluded. The possible influences of the variants upon the function or structure of the encoded proteins and the pathogenicity of the variants were analyzed by bioinformatics tools. A list of selected candidate variants was obtained for the probands by this stepwise analysis method. Finally, we identified several variants as the potential causative mutations in the pedigrees. We then performed the Sanger bidirectional sequencing and segregation analysis, which verified that the variants were disease-causing mutations in these pedigrees.

Overall, we detected eleven (likely) pathogenic gene mutations among nine families with RP, including three novel and eight previously identified mutations in RP-related genes (Table 2, Supplementary Material SFigure2 and STable1) and did not detect disease causative mutation in the other families by targeted exome sequencing and clinical exome sequencing analysis. We detected three mutations of the ATP-binding cassette subfamily A member 4 (*ABCA4*) gene. A homozygous variation, NM_000350:c.2424C>G, p.Y808X of *ABCA4* gene was detected in the proband of pedigree 11. In addition, two novel compound heterozygous variations, NM_000350:c.4604dup T, p.T1537Nfs*18 and NM_000350:c.4195G>T, p.E1399X of *ABCA4* gene were detected in the proband of pedigree 12. In addition, a heterozygous deletion of the total exon region of the carbonic anhydrase 4 (*CA4*) gene was detected in the proband of pedigree 8 (Table 2 and Supplementary Material SFigure2). Moreover, in family 20, a variation in *RPGR* gene, NM_000328:c.2289G>C, p.K763N located at chrX:38129038 (GRCh37/hg19) (Table 2, Supplementary Material SFigure2 and STable1) was detected in the male proband. Consistent with this, the inheritance mode of this family was X-linked dominant.

Discussion

In this study, genetic analysis of 20 families with RP confirmed the highly heterogeneous nature of RP despite the small sample size, identifying different mutations in different genes among these families with no gene or mutation detected at high frequency. Moreover, we observed extensive heterogeneity in clinical phenotypes, including RP sine pigmento, reverse (central) RP, retinitis punctata albescens, and the Bietti crystalline corneoretinal dystrophy presenting with poor vision, night blindness, and tubular field of vision complicated by nystagmus, exotropia, or choroidal neovascularization and vitreous hemorrhage. Although other RP or IRDs studies in Chinese population indicated that some genes

Table 2. Mutations detected in 20 pedigrees with non-syndromic retinitis pigmentosa.

Family	Gene	Reference sequence	DNA	Amino acid	Gene type	ACMG classification	ACMG evidence levels	CADD (PHRED score)	Known/ novel	References
1	*RHO*	*NM_000539*	*c.392T>C*	*p.L131P*	*Heterozygous*	*Likely pathogenic*	*PM3 Strong, M2, PP3*	*28.8*	*Known*	*Fuchs, et al. Hum Mol Genet, 3, 1203, 1994*
3	*BEST1*	*NM_004183*	*c.715G>A*	*p.V239M*	*Heterozygous*	*Likely pathogenic*	*PS4, PM1, PM2, PP3*	*26.4*	*Known*	*Yardley, et al. Invest Ophthalmol VisSci, 45, 3683, 2004*
4	*CYP4V2*	*NM_207352*	*c.802-8_810del TCATACAGGTCATCGCTinsGC*	*p.268_270del*	*Homozygous*	*Pathogenic*	*PVS1, PM3 Strong, PM2*	*–*	*Known*	*Wada Y.et.al. Am J Ophthalmol. 2005 May; 139(5):894–9*
5	*CRB1*	*NM_201253*	*c.663T>A*	*p.C221X*	*Homozygous*	*Pathogenic*	*PVS1, PM3_ Strong, PM2*	*36*	*Known*	*Wang, et al. Invest Ophthalmol Vis Sci, 56, 3642, 2015*
6	*IMPG2*	*NM_016247*	*c.1173C>A*	*p.H391Q*	*Heterozygous*	*Uncertain*	*PM2, BP4*	*22.7*	*Known*	*ClinVar database: Illumina Clinical Services Laboratory, Illumina*
8	*CA4*	*NM_000717*	*Deletion of the total CA4gene exon-containing region*	*–*	*Heterozygous*	*–*		*–*	*–*	*This study*
11	*ABCA4*	*NM_000350*	*c.2424C>G*	*p.Y808X*	*Homozygous*	*Pathogenic*	*PVS1, PM3_ Strong, PM2*	*39*	*Known*	*Zhou, et al. PLoS ONE, 9, e91962, 2014*
12	*ABCA4*	*NM_000350*	*c.4604dupT*	*p.T1537Nfs*18*	*Heterozygous*	*Pathogenic*	*PVS1, PM3_ Strong, PM2*	*–*	*Novel*	*This study*
			c.4195G>T	*p.E1399X*	*Heterozygous*	*Pathogenic*	*PVS1, PM3_ Strong, PM2*	*49*		
13	*SLC7A14*	*NM_020949*	*c.988G>A*	*p.G330R*	*Heterozygous*	*Uncertain*	*PP3, BS1*	*32*	*Known*	*Jin, et al. Nat Commun, 5, 3517, 2014*
14	*RDH5*	*NM_002905*	*c.272A>C*	*p.Q91P*	*Heterozygous*	*Uncertain*	*PM2*	*0.004*	*–*	*This study*
			c.928delCinsGAAG	*p.L310delinsEV*	*Heterozygous*	*Likely pathogenic*	*PM3_Strong, PM2, PM4*	*–*	*Known*	*Nakamura, et al. Invest Ophthalmol VisSci, 41, 3925, 2000*
15	*KCNJ13*	*NM_001172416*	*c.275C>A*	*p.S92X*	*Heterozygous*	*Uncertain*	*PM2*	*24.8*	*–*	*This study*
17	*PDE6A*	*NM_000440*	*c.1957C>T*	*p.R653X*	*Homozygous*	*Pathogenic*	*PVS1, PM3_ Strong, PM2*	*41*	*Known*	*Perez-Carro, et al. Sci Rep, 6, 2016*
18	*PROM1*	*NM_006017*	*c.1117C>T*	*p.R373C*	*Heterozygous*	*Uncertain*	*PM3_Strong, PM2, BP4*	*16.88*	*Known*	*Yang, et al. J Clin Invest, 118, 2908, 2008*
19	*EYS*	*NM_001142800*	*c.6416G>A*	*p.C2139Y*	*Heterozygous*	*Likely pathogenic*	*PM3_Strong, PM1, PM5, PP3*	*29.5*	*Known*	*Audo, et al. Hum Mutat, 31, E1406, 2010*
			c.4628C>G	*p.S1543X*	*Heterozygous*	*Likely pathogenic*	*PVS1, PM2*	*40*	*Novel*	*This study*
20	*RPGR*	*NM_000328*	*c.2289G>C*	*p.K763N*	*Homozygous*	*Uncertain*	*PM2, BP4*	*6.27*	*–*	*This study*

CADD: Combined Annotation-Dependent Depletion; ACMG: American College of Medical Genetics and Genomics.

were collectively responsible for disease in the majority of the families, and the most commonly implicated genes were *CYP4V2*, *USH2A*, *ABCA4*, *RPGR*, *EYS*, *RHO*, and *RP1*.[9–11]

Among the 20 families, 10 exhibited a family history of RP whereas no family history of RP was apparent in the remainder. It has been reported that sporadic cases (i.e. a single patient in a pedigree) had the proportion 10–40% of all patients with RP.[1–8] But in the two recent large cohort RP or IRDs studies in Chinese population, of all the patients in the studies, 53.34% ($n = 663/1243$) or 75.6% ($n = 605/800$) had no known family history.[9,10] Possible explanations for the relatively high proportion of simplex cases (50%) in this study include the following: incomplete or inaccurate reporting of family history, autosomal recessive inheritance, incomplete penetrance in the prior generations in autosomal dominant inheritance or X-linked inheritance, and *de novo* pathogenic variants as X-linked or autosomal dominant inheritance. The inheritance modes of these sporadic patients would be refined if likely pathogenic variants or pathogenic mutations were identified in these patients. One large cohort IRDs study in the Chinese population indicated that the inheritance modes in 330 cases among 605 sporadic patients were refined because likely pathogenic or pathogenic variations were identified in these patients. In the study, the inheritance models of sporadic cases were mostly inherited as autosomal recessive (275 among 330), with a small proportion inherited as autosomal dominant (26 among 330) or X-linked (29 among 330).[10] We identified (likely) pathogenic variations in three patients with no known family history (family 12, family 19, and family 20) in this study. The (likely) pathogenic compound heterozygous variations NM_000350:c.4604dupT, p.T1537Nfs*18 and NM_000350:c.4195G > T, p.E1399X of the *ABCA4* gene were detected in the proband of family 12. The (likely) pathogenic compound heterozygous variations NM_001142800:c.6416G > A, p.C2139Y and NM_001142800:c.4628C > G, p.S1543X in the gene *EYS* were detected in the proband of family 19. And the variant NM_000328:c.2289G > C, p.K763N in the gene *RPGR*, located at chrX:38129038 (GRCh37/hg19) which is X-linked mode of inheritance, was detected in the proband of family 20. So, family history and personal clinical characteristics are not reliable to determine the mode of inheritance or the recurrence risk in future pregnancies and the occurrence in other family members, but the genetic analysis will be helpful.

A heterozygous variant, NM_016247:c.1173C > A, p.H391Q in the *IMPG2* gene located at chr3:100972606 (GRCh37/hg19) was detected in the proband of family 6. The mode of inheritance of *IMPG2*-associated RP was autosomal recessive RP. This variant in *IMPG2* has been reported as uncertain significance in ClinVar database, and the frequency of this variant in population was 0.001. *IMPG2* mutations cause a relatively more severe pattern of RP (RP56). The RP symptoms caused by *IMPG2* gene mutations manifest in the first two decades of life, moreover, are commonly accompanied by macular involvement with chorioretinal atrophy. These damages result in decline in central vision together with the severe tunnel visual field leading to severe visual loss.[1,3–5,7,8,18–22] The clinical characteristics of the patient from family 6 were consistent with those previously reported for *IMPG2*-associated RP. The patient presented with poor vision for counting fingers and intolerance to light complicated by nystagmus. The patient also exhibited RP sine pigmento, which constitutes a normal retinal appearance despite documented abnormalities of photoreceptor function, with extremely poor vision, intolerance to light, nystagmus, and severe abnormality of dark adaptation and photopic response by the flash electroretinogram test. Although optical coherence tomography for detecting the macular structure could not be performed in the patient due to the severe nystagmus, damaged macular function was inferred based on the poor central vision in this patient. She had no family history of RP. The mode of inheritance of *IMPG2*-related RP is reported to be autosomal recessive, but only one heterozygous variation in the *IMPG2* gene was identified in this patient. Further studies are necessary to elucidate the relationship between this variant and RP.

In addition, we detected heterozygous deletion of the total exon region of *CA4* (carbonic anhydrase 4) in the proband of family 8. The *CA4* gene cannot be detected in the retina but is expressed with very high degree in the choriocapillaris of the eye. *CA4* gene encodes carbonic anhydrase 4 (CAIV), which is the enzyme to catalyze the carbon dioxide hydration reaction ($H_2O + CO_2 <-> HCO_3^- + H^-$). The photoreceptors in the retina produce abundant acidic metabolites because of the high metabolic requirements. To maintain pH homeostasis, it is key to remove acid load efficiently from the RPE and the retina by the choriocapillaris in the choroid. The coordinated action of plasma membrane bicarbonate transporters and carbonic anhydrases to move bicarbonate to the circulatory system is necessary for HCO_3^- shuttling. Because the retinal phototransduction is regulated by the changes of pH value surrounding it, the pH value in the retina is significant for the photoreceptors to exert normal function. CAIV plays a crucial role in maintaining the pH value in the retina.[23–25] Mutations of the *CA4* gene, including a missense mutation (R14W) in the *CA4* gene which is at signal sequence, a missense variation (C > A transversion) that replaces Arg219 with Ser (R219S) in exon7, a G to A transition at 59 of the 3′UTR, a missense mutation within exon3 (R69H), and a missense mutation within exon1 (A12T) have been reported as associated with RP with rod-cone dystrophy. These mutations were proposed to abolish the function of *CA4* on electrogenic sodium bicarbonate co-transporter 1 activity, either owing to decreased physical interaction or altered catalytic activity. No notable difference was observed in the distribution pattern between the wild-type and mutant proteins. However, decreased mRNA expression of the mutant CAIV was observed compared to the wild-type.[1–8,11,23–25] A macromutation of c.198_199delACinsG, p.L67Wfs*24 in *CA4* gene has also been reported as associated with RP.[26]

The most recent article reported that the identification of complex structural variants (SVs) on chromosome Chr17q22, involving *YPEL2* (OMIM, 609723) topologically associating domains and resulting in increased expression of the *GDPD1* gene (OMIM, 616317) in the retina, which was detected by whole-genome sequencing (WGS), was the genomic cause of adRP at the RP17 locus.[27] And the variants in *CA4* gene, previously titled RP17 (OMIM, 600852), have been re-classified to be variants of unknown significance based on this report.[27] In comparison, the heterozygous deletion of the total exon

region of *CA4* detected in this study has not been reported before. The patient presented with poor vision and night blindness since childhood. Further studies are necessary to elucidate the relationship between this variant and RP. This variation which was detected by targeted exome sequencing should be validated by multiplex ligation-dependent probe amplification (MLPA) or Quantitative Real-time polymerase chain reaction (qPCR). And further studies such as WGS is necessary to evaluate whether the deletion is not a more complex SV and to understand the pathogenic mechanism of this deletion.

We also detected mutations of the gene *ABCA4* located at 1p22.1 in this study. The gene encodes a retina-specific transmembrane protein ABCA4 which is exclusively expressed in the outer segments of photoreceptors. This protein is involved in facilitating the clearance of toxic retinoid compounds from photoreceptor cells. Damage to this protein caused by *ABCA4* gene mutations causes lipofuscin accumulation within the photoreceptors and RPE, which can be damaging to the RPE and photoreceptors. Pathogenic variants in the gene *ABCA4* are reported to be related to various retinal dystrophies with macular involvement. For example, mutations in *ABCA4* are reported to be responsible for over 95% of the Stargardt disease cases, along with some cases of RP19 and cone-rod dystrophy cases.[1–8,28–34] However, a missense variant c.1268A>G was reported to play protective role in one family, to delay the onset or reduce the risk of the Stargardt disease.[35] Herein, we detected a homozygous (NM_000350:c.2424C>G, p.Y808X) mutation in the proband of family 11. The patient exhibited severe RP, presenting with very poor vision and serious night blindness complicated by nystagmus since childhood. His parents were consanguineous. The compound heterozygous variations of the gene of *ABCA4* p.G607R and p.Y808X have previously been reported to be linked with the Stargardt disease.[30] It has been reported that the non-sense mutation c.C2424G, p.Y808X, located within exon16, caused a stop codon removing 1465 amino acids from the protein ABCA4 (2273 amino acids). As the non-sense-mediated mRNA decay response degenerates the mRNA, this mutation is predicted to lead to a decreased expression of ABCA4. Moreover, novel compound mutations, heterozygous NM_000350:c.4604dupT, p.T1537Nfs*18 and heterozygous NM_000350:c.4195G>T, p.E1399X were identified in the proband of family 12. The patient exhibited severe RP, presenting with reverse (central) RP complicated by exotropia, with poor vision and night blindness since childhood. Notably, the heterozygous mutation c.4604dupT, p.T1537Nfs*18 has been reported as linked to cone-rod dystrophy,[31] whereas the heterozygous c.4195G>T, p.E1399X mutation is reported to be related to the Stargardt disease.[32] The results in this study thus expanded the mutation spectrum of gene *ABCA4* associated with RP.

The results of (likely) benign variants detected in this study were not shown. In family 2, the proband exhibited severe RP, presenting with poor vision and night blindness since the age of 4 years, complicated by nystagmus and alternating exotropia with the angle of squint 40°, and no systemic syndromic or systemic abnormality was found. The patient had no family history of RP. In family 16, the proband exhibited RP sine pigmento diagnosed at the age of 30 years, presented with poor vision on both eyes associated with macular edema on the left eye. The patient has no syndromic or systemic abnormality including hearing, intelligence, heart, kidney, endocrine, and so on. She has no family history of RP. And she has given birth to four children, and there was no abnormality found in her children. In this study, only one heterozygous variant was identified in a gene which is reported to be related to autosomal recessive RP in the patient in two families (variant of *IMPG2* in family 6 and variant of *PROM1* in family 18). We detected one heterozygous variant NM_006017:c.1117C>T, p.R373C in *PROM1*, which has been reported to be pathogenic in ClinVar database in the patient of family 18, and the inheritance mode of RP caused by *PROM1* mutations is autosomal recessive. But we only detected the heterozygous variant in this study. It is warranted to perform further studies to elucidate the relationship between this variant and RP in this family.

Overall, we report clinical features of Chinese patients with RP including typical characteristics and specific characteristics, the Bietti crystalline corneoretinal dystrophy, retinitis punctata albescens, reverse (central) RP, sine pigmento, associated with macular edema or choroidal neovascularization and vitreous hemorrhage. We detected three novel (likely) pathogenic mutations and eight previously reported (likely) pathogenic mutations in genes known to be related to non-syndromic RP. These results expand the mutation spectrum of these genes related to RP. It is fundamental to screen new found mutations in the general population, in order to evaluate their frequencies and impacts. As few studies have provided the genetic data of RP in the Chinese population, the genetic basis for RP in this population remains largely unknown. Our identification of mutations responsible for RP in the Chinese population may, therefore, help with rapid and efficient molecular diagnosis and facilitate the development of targeted therapeutics, including gene therapy for RP. As this study was limited by the small sample size and the inability to study the probands' family members in some pedigrees, the relationships between some mutations and RP were uncertain. Further studies are necessary to clarify the pathogenic mechanism of these mutations. Additional studies of family members and additional molecular and *in vitro* studies might yield new information. Several mutated genes identified to date have been studied in association with angiogenesis, mitochondria activity, and ion-channels.[36–38] To improve our understanding of the possible pathophysiological effects, future studies should evaluate the pathways in which mutated genes are involved.

AUTHORS' CONTRIBUTIONS

All authors play role in the design of the study, analysis of the data and review of the manuscript. L.C., N.W., and M.L. designed the study. L.C., M.L., F.H., J.H., X.Y., R.W., and X.F. collected the samples and clinical data. L.C. performed the study, analyzed the data, interpretated the research, and contributed to the manuscript preparation and editing. All authors approved the final manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

The study obtained the approval of the Ethics Committees of the Jinan University affiliated Shenzhen Eye Hospital. We conducted the study in accordance with the Declaration of Helsinki and obtained written informed consent from all subjects.

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SUPPLEMENTAL MATERIAL

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