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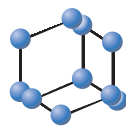
Potential Mutations in Chinese Pathologic Myopic Patients and Contributions to Phenotype

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Abstract: *Purpose:* Pathologic myopia is a leading cause of visual impairment in East Asia. The aim of this study was to investigate the potential mutations in Chinese pathologic myopic patients and to analyze the correlations between genotype and clinical phenotype.

Methods: One hundred and three patients with pathologic myopia and one hundred and nine unrelated healthy controls were recruited from Zhongshan Ophthalmic Center. Detailed clinical data, including ultra-widefield retinal images, measurements of best-corrected visual acuity, axial length, refractive error and ophthalmic examination results, were obtained. Blood samples were collected for high-throughput DNA targeted sequencing. Based on the screening results, phenotype-genotype correlations were analyzed.

Results: The study included 196 eyes of 103 patients (36 men and 67 women) with an average age of 52.19 (38.92 – 65.46) years, an average refractive error of -11.80 D (-16.38 – -7.22) and a mean axial length of 28.26 mm (25.79 – 30.73). The patients were subdivided into three groups: myopic chorioretinal atrophy (190 eyes of 101 patients), myopic choroidal neovascularization (17 eyes of 15 patients), and myopic traction retinopathy (71 eyes of 61 patients). Systematic analysis of variants in the 255 genes revealed six potential pathogenic mutations: PEX7, OCA2, LRP5 (rs545382, c.1647T>C), TSPAN12 (rs41623, c.765G>T), RDH5 (rs3138142, c.423C>T) and TTC21B (rs80225158, c.2385G>C). OCA2 mutations were primarily observed in patients with myopic traction maculopathy.

Conclusion: Genetic alterations contribute to various clinical characteristics in Chinese pathologic myopic patients. The study may provide new insights into the etiology of pathologic myopia and potential targets for therapeutic interventions.

Keywords : Pathologic myopia, genetics, targeted sequencing, genotype, phenotype, correlation analysis.

1. INTRODUCTION

The prevalence of myopia has dramatically increased in East Asian populations and exploded into a serious public health issue [1]. In parallel with the epidemic of myopia, an epidemic of high myopia has appeared, accompanied by a correspondingly disproportionate increase in the prevalence of pathologic myopia. Pathologic myopia, characterized by deteriorative complications, such as myopic foveoschisis (MF), retinal detachment, and myopic choroidal neovascularization (mCNV), may cause irreversible impairment of best-corrected visual acuity (BCVA) and blindness [2, 3]. The development of preventive strategies and effective treatment requires an understanding of the etiology that leads to the development of pathologic myopia.

To date, considerable debate regarding the exact mechanism of myopia remains. Emerging evidence has demonstrated significant associations between environmental factors and myopia [4-7]. However, previous data provided strong evidence that genetic factors also play a key role. Twin studies and genetic linkage analysis studies revealed a handful of potential loci for myopia-related genes [8, 9]. Recently, genome-wide association studies (GWAS) with large sample sizes identified more than 100 single nucleotide polymorphisms (SNPs) that influence myopia. These

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genes include: BMP3, GJD2, KCNQ5, LAMA2, PRSS56, RASGRF1, RBFOX1, RDH5, SHISA6, TJP2, TOX and ZIC2 [10, 11]. Analyses of syndromic forms of myopia implicated 21 novel candidate myopia genes: ERBB3, COL4A1, GJA1, IFIH1, OCA2, ASXL1, COL9A2, ADAMTS18, TFAP2A, FBN1, KIF11, LTBP2, ADAMTS2, POLR3B, PTPN11, ZNF469, ADAMTSL4, AGK, ALDH18A1, GNPTG and POMT1[12].

Nevertheless, previous studies primarily focused on the causal genetic influences on myopia or high myopia; few data regarding the genetic contributions to pathologic myopia in China are available. Furthermore, because the pathogenesis of high myopia is complex, these potential loci explain only a small percentage of the observed variation in myopia, and a large number of myopia-related genes have not yet been identified. Many retinal-related diseases might accompany this pathogenesis, such as cone and rod dystrophy, retinitis pigmentosa etc.[13]. Previous studies showed that patients with mutations responsible for inherited retinal diseases are much more than patients with mutations in genes responsible for nonsyndromic high myopia[14].

Therefore, the purpose of the current study was to identify mutations associated with pathologic myopia and to investigate genotype-phenotype interactions. Our study expands the list of potential loci associated with pathologic myopia.

2. MATERIALS AND METHODS

2.1. Patients

This study was approved by the Institutional Review Board of Zhongshan Ophthalmic Center affiliated with Sun Yat-sen University (Guangzhou, China) and conducted in accordance with the World Medical Association Declaration of Helsinki. Written informed consent was obtained from each participant.

A total of 196 eyes of 103 patients (36 men and 67 women) with a final diagnosis of pathologic myopia were recruited from nine provinces of China at the Zhongshan Ophthalmic Center. Detailed clinical information for these patients is listed in Table 1. The criteria for patients with pathologic myopia were as follows: 1) spherical refraction ≤–6.00 D and/or axial length >26 mm; 2) the presence of myopic chorioretinal atrophy, and/or mCNV, and/or myopic traction retinopathy. The exclusion criteria were as follows: 1) history of other ocular pathologies, such as other retinal vascular diseases, diabetic retinopathy, age-related macular degeneration, glaucoma and uveitis; 2) previous history of ocular surgery or trauma; 3) other related systemic diseases. In total, one hundred and nine ethnically matched participating individuals without a history of ocular diseases were enrolled as controls.

Comprehensive ophthalmological examinations, including BCVA, slit-lamp examination of the anterior segment, dilated fundus examination by a +120 D lens, intraocular pressure, optical coherence tomography (OCT), refractive error and measurement of axial length, were performed in every patient. The axial length was determined by IOLMaster 5 times for each eye, and the mean value was used for the statistical analysis.

2.2. Sample Selection and DNA Extraction

Peripheral venous blood samples were collected from ethylenediaminetetraacetic acid (EDTA) vials. In each sample, genomic DNA was extracted from whole blood according to the standard protocol of PureLink Genomic DNA Kits (Life Technologies, Carlsbad, CA, USA).

Next-generation targeted sequencing was performed on the genomic DNA fragments using the Ion Torrent PGM™ Platform (Life Technologies, Carlsbad, CA, USA). To accurately evaluate the purity of DNA, NanoDrop spectrophotometers were used. Pure DNA required an A260/280 ratio greater than 1.6. Additionally, the ratio between the absorbance at 260 and 230 nm was required to be greater than 1.2 for "pure" nucleic acid samples.

2.3. Library Preparation

Multiplex amplification was performed with the Ion AmpliSeq™ Library Kit 2.0. The target amplification reaction consisted of 2 µL of 5 × Ion AmpliSeq™ HiFi Mix, 5 µL of 2 × primer, 10 ng of genomic DNA and 2 µL of nuclease-free water. To amplify target regions, the following parameters were recommended: activation of the enzyme, 99°C for 2 min; denaturing, 99°C for 15 s; annealing and extension, 15 cycles of 60°C for 4 min; and holding at 10°C. To partially digest amplicons, we added 1 µL of FuPa Reagent to each amplified sample and ran the following program: 50°C for 10 min, 55°C for 10 min, 60°C for 20 min, and holding at 10°C.

For each multiplex-PCR product, barcode adapters were ligated to the amplicons. Accurate evaluation and quantification of libraries were achieved using the Agilent 2100 Bioanalyzer.

Library concentration was determined by a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and normalized to an equimolar concentration as recommended by the manufacturer.

2.4. Template Preparation, Enrichment of ISPs and Sequencing

We prepared and enriched template-positive Ion PGM ™ Hi-Q ™ Ion Sphere ™ Particles (ISPs) containing clonally amplified DNA using the Ion PGM ™ Hi-Q ™ OT2 Kit on the Ion OneTouch ™ 2 Instrument and the Ion OneTouch ™ enrichment system. The enriched samples of ISPs were loaded onto Ion 314/316 sequencing chips (Life Technologies, Carlsbad, CA, USA). Finally, sequencing was performed on the Ion PGM (Life Technologies, Carlsbad, CA, USA) system using the Ion PGM ™ Hi-Q ™ View Sequencing Kit (Life Technologies, Carlsbad, CA, USA).

This study included a total of 255 genes associated with retinal diseases from the RetNet database (https://sph.uth.edu/retnet/, a website that provides data on genes and loci associated with inherited retinal diseases).

2.5. Bioinformatics

Primary data obtained from the PGM system were processed to generate sequence reads by the Ion Torrent platform-specific pipeline software Torrent Suite (Life Technologies, Carlsbad, CA, USA). FastQC plug-in and Coverage Analysis plug-in software were used for quality control. Files in variant caller format (VCF) were generated by the Torrent Suite Variant Caller (TSVC) plug-in and further annotated using ANNOVAR software. The filtering strategy for the variants was as follows: variant positions with the coverage depth less than 10 were excluded. SNPs were filtered against data from dbSNP and Exome Aggregation Consortium (ExAC, Cambridge, MA, USA) . Variants predicted to be benign by the online tool of SIFT were also excluded.

2.6. Statistical Analysis

All data analyses were performed with SPSS software ver. 24.0 (SPSS Inc., Chicago, IL, USA). The frequencies of variants were compared between pathologic myopic patients and healthy participants using the chi-square test. Differences with *P*-values less than 0.05 were considered statistically significant.

3. RESULTS

3.1. Baseline Characteristics and Clinical Findings in Patients with Pathologic Myopia

One hundred and three unrelated Chinese individuals with the clinical diagnosis of pathologic myopia were recruited. In summary, 36 men and 67 women with an average age of 52.19±13.27 years at blood draw were included. The average refractive error was -11.80±4.58D, and the average axial length was 28.26±2.47 mm. The clinical features of these pathologic myopic patients are listed in Table **1**.

According to the long-term clinical observations and serious complications, pathologic myopia lesions were categorized into 3 types: myopic chorioretinal atrophy, mCNV and myopic traction maculopathy [15, 16]. The clinical features of the 196 pathologic myopic eyes of the 103 patients were summarized in Table 2. At blood draw, only 10 patients had lesions in one eye, while the other 93 patients had bilateral lesions. Eighty eyes of 70 patients had at least 2 types of lesions in the same eye and 116 eyes of 93 patients had only 1 type of lesion in the same eye. Myopic chorioretinal atrophy was the most commonly observed pattern. The differences in axial length among the three types of lesions were statistically significant. Eyes with myopic traction maculopathy had the longest axial length

Table 1. Clinical information on pathologic myopic patients.

Characteristics	Pathologic Myopic Patients
Mean age at blood draw (yrs)	
No.	103
Mean (95% CI)	52.19 (38.92 – 65.46)
Gender (frequency), no. (%)	
No.	103
Female	67 (65.05)
Male	36 (34.95)
Visual acuity in worse eye (frequency), no. (%)	
No.	103
20/25 or better	1 (0.97)
20/30 – 20/50	9 (8.74)
20/60 -20/160	22 (21.36)
20/200 or worse	71 (68.93)
Fundus lesion at blood draw (frequency), no. (%)	
No.	103
Myopic traction maculopathy	61 (59.22)
Myopic choroidal neovascularization	15 (14.56)
Myopic chorioretinal atrophy	101 (98.06)
Spherical equivalent (D), mean (95% CI)	-11.80 (-16.38 ~ -7.22)
Axial length (mm), mean (95% CI)	28.26 (25.79 ~ 30.73)

CI = confidence internal;

(average, 30.01±2.08 mm). Representative images of the three types of lesions were shown in Fig. **1**.

3.2. Sequencing Quality Control

Pseudo-color imaging showed that the ISP density was 77% (Fig. **2A**), showing potentially addressable wells.

To ensure that only high-quality reads were written to the final results, a collection of filters was applied. A total of 66% of library ISPs passed all filters, resulting in 5,714,297 final reads (Fig. **2B**). The median read length generated during the sequencing run was 277 bp (Fig. **2C**). As shown in Fig. 2D, the total percentage of aligned bases out of all the reads was 98%. At every position in an aligned sequence, the height of the blue area showed the number of aligned reads at that position. The purple area revealed the number of unaligned bases at that position. Unaligned reads were shown by the difference between the purple height and the blue heights. The average raw accuracy plotted by

Table 2.   Clinical characteristics of eyes at blood draw.

Fundus Lesion	Age (yrs)	Gender (Male/Female)	Refractive Error (D)	Axial Length (mm)*
Myopic chorioretinal atrophy (190 eyes/ 101 patients)	52.45±13.31	67: 123	-12.09±4.5	28.40±2.41
Myopic choroidal neovascularization (17 eyes/ 15 patients)	51.71±6.47	6:11	-15.16±4.25	29.44±1.41
Myopic traction maculopathy (71 eyes/ 61 patients)	54.64±13.59	25:46	-12.89±4.91	30.01±2.08

 * $P < 0.05$
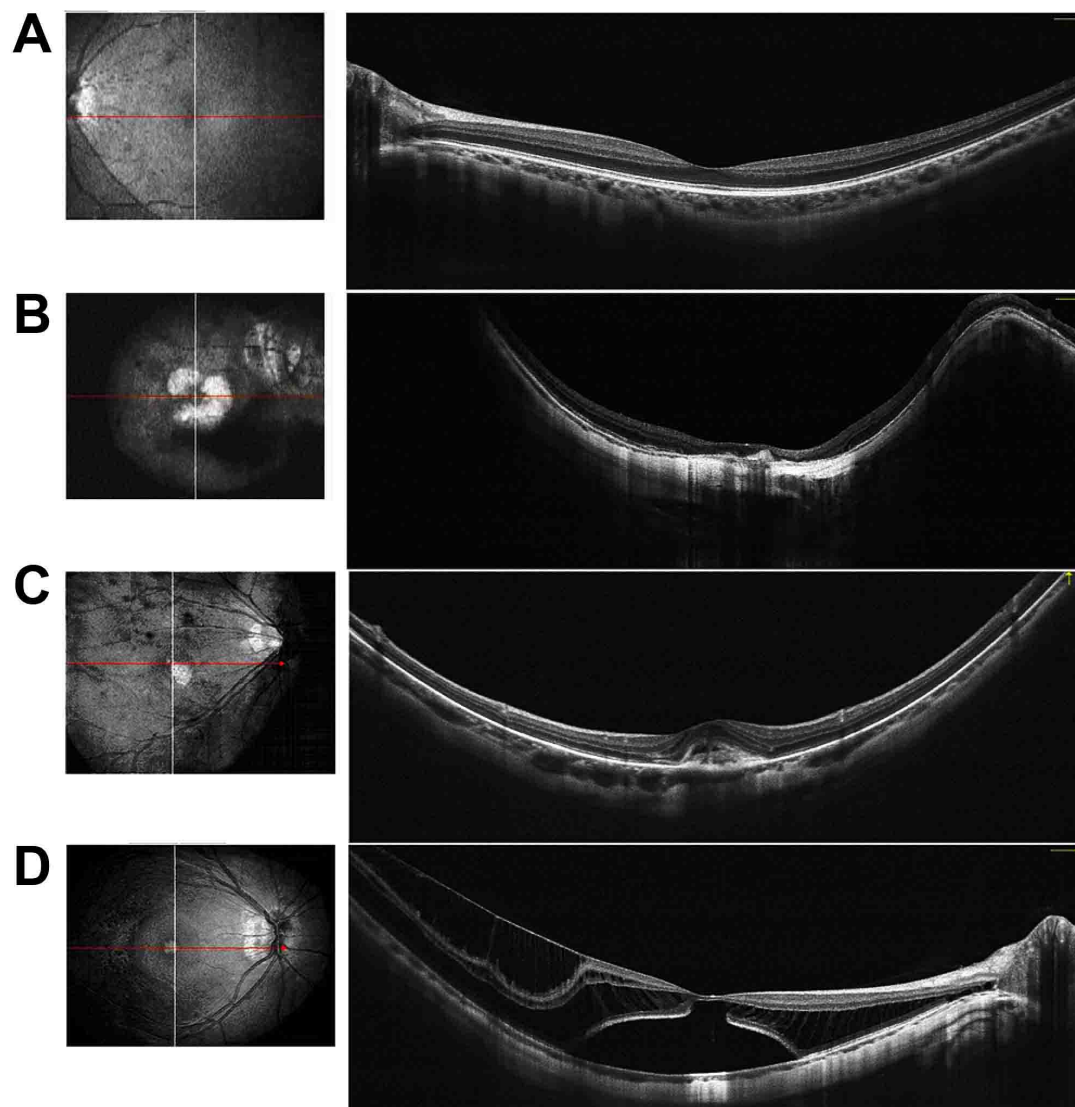
Fig. (1). Optical coherence tomography images showing three types of macular changes in highly myopic eyes. **(A)** Healthy control. **(B)** Myopic chorioretinal atrophy. **(C)** Myopic choroidal neovascularization. **(D)** Myopic traction maculopathy.

their position in an aligned sequence was 99.5% (Fig. **2E**). In the plot of alignment quality control (Fig. **2F**), AQ20 denoted an error rate of 1% or less. At the AQ20 level, the total number of bases was 1.24Gb, the average segment length was 238 bp, the longest alignment was 392 bp, and the mean coverage depth was $0.4\times$.

3.3. Causative Mutations Identified in Patients with Pathologic Myopia

Based on an evaluation of the targeted sequencing data of the 255 genes (Supplementary Table S1) of the patients with pathologic myopia and healthy controls, six potential pathogenic mutations were identified in the

Fig. (2). Quality control. Representative summary report generated after a typical run through the Ion Torrent platform. **(A)** Ion sphere particles (ISPs) density. **(B)** Library ISP summary. **(C)** Read length histogram. **(D)** The numbers of aligned (blue) and unaligned (purple) reads by position are shown in an aligned sequence. **(E)** Mean raw accuracy. **(F)** Alignment quality.

PEX7, OCA2, LRP5, TSPAN12, RDH5 and TTC21B genes (Table **3**), including intronic mutations in PEX7 and OCA2, and synonymous mutations in LRP5, TSPAN12, RDH5 and TTC21B. The variant of OCA2 is novel, *i.e.,* not previously reported in dbSNP138 or 1000 Genomes. Mutations in PEX7 and LRP5 were novel variants that were not reported to be associated with myopia or high myopia. Detailed data on the potential pathological mutations identified were shown in Table **4**.

3.4. The Correlation Between Genotype and Phenotype

To evaluate the potential genotype and phenotype correlation in patients with potential mutations, the 103

patients were categorized into three groups: myopic chorioretinal atrophy (190 eyes of 101 patients), mCNV (17 eyes of 15 patients) and myopic traction retinopathy (71 eyes of 61 patients). Table **5** shows the distribution of the six mutations in 103 patients. Mutation in OCA2 was observed in no mCNV patients, three patient with myopic chorioretinal atrophy and 46 patients with myopic traction retinopathy.

4. DISCUSSION

Various GWASs [10, 17] and candidate gene studies [18-21] have been conducted to investigate genetic factors associated with high myopia. A large number of loci and genes have been identified. Nevertheless, little is known about the genes that may

Table 3.   Potential mutations identified in patients with pathologic myopia.

Gene	CytoBand	SNP138	Nucleotide Change	Protein change	Reported or novel	HM percent	Healthy percent	*P* value
PEX7	6q23.3	rs927181	-	-	novel	93.20%	3.67%	4.47E-46
LRP5	11q13.2	rs545382	c.1647T>C	p.F549F	novel	84.47%	6.42%	7.67E-34
TSPAN12	7q31.31	rs41623	c.765G>T	p.P255P	reported	78.64%	5.50%	2.66E-30
RDH5	12q13.2	rs3138142	c.423C>T	p.I141I	reported	79.61%	11.01%	1.65E-25
OCA2	15q13.1	.	-	-	reported	47.57%	6.42%	2.95E-12
TTC21B	2q24.3	rs80225158	c.2385G>C	p.L795L	reported	66.99%	7.34%	1.03E-20

Table 4.   Detailed data on the potential pathologic mutations identified in this study.

Gene	Chr.	Start	End	Ref	Alt	Status	Func. refGene	Exonic Func. refGene
PEX7	chr6	137166682	137166682	G	A	homo	intronic	.
LRP5	chr11	68171013	68171013	T	C	homo	exonic	synonymous
TSPAN12	chr7	120428799	120428799	C	A	homo	exonic	synonymous
RDH5	chr12	56115585	56115585	C	T	het	exonic	synonymous
OCA2	chr15	28228435	28228438	CTAA	-	het	intronic	.
TTC21B	chr2	166767913	166767913	C	G	het	exonic	Synonymous

Table 5.   Phenotype-genotype analysis.

Group	N	PEX7	LRP5 (rs545382)	TSPAN12 (rs41623)	RDH5 (rs3138142)	OCA2	TTC21B (rs80225158)
Myopic chorioretinal atrophy	101	32	25	23	35	3	28
Myopic choroidal neovascularization	15	14	11	12	3	0	2
Myopic traction Retinopathy	61	50	51	46	44	46	39
Total	103	96	87	81	82	49	69

lead to the onset of pathologic myopia, one of the leading causes of irreversible visual loss worldwide.

The current study was designed to identify genes associated with pathologic myopia in patients of Chinese descent. By analyzing the targeted sequencing data of 255 genes in the pathologic myopic cohort, six variants were identified in the PEX7, OCA2, LRP5, TSPAN12, RDH5 and TTC21B genes. Verifying genes related to pathologic myopia in this high-risk population may provide important data for developing predictive models for early diagnosis and progression as well as for the discovery of new targets for drug therapies.

The TSPAN12 gene, located on human chromosome 7q31.31, encodes a protein member of the transmembrane 4 superfamily [22]. The expression of Tspan12 protein is restricted to the retinal vasculature. Tspan12 is involved in the Wnt/Norrin-β-catenin signaling pathways, which play key roles in retinal vascular development [23, 24]. Familial exudative vitreoretinopathy (FEVR) is a genetically related ocular disease characterized by developmental disorders of the retinal vasculature. Studies in Tspan12 mutant mice and TSPAN12 screening in 70 FEVR patients showed that TSPAN12 mutations were the cause of autosomal-dominant FEVR [24]. A study based on exome sequencing in 298 patients with early-onset high myopia identified a TSPAN12 mutation in

one proband [21]. TSPAN12 has also been reported to be responsible for ocular phenotypes accompanied by high myopia [25]. In the current study, a synonymous mutation in TSPAN12 was identified in patients with pathologic myopia. Although synonymous SNPs do not alter amino acid sequences, they have been demonstrated to influence mRNA splicing, protein synthesis and folding [26, 27]. Therefore, it is possible that mutations in TSPAN12 may affect mRNA splicing stability and contribute to the development of pathologic myopia.

The RDH5 gene is mapped onto the chromosome 12q13q14. 11-cis retinol dehydrogenase, encoded by the RDH5 gene, is involved in the retinoid cycle, the metabolic pathway that regenerates the visual chromophore following light exposure. The RDH5 protein is abundant in the smooth endoplasmic reticulum of the retinal pigment epithelium (RPE) cells of the eye [28]. Mutations in the RDH5 gene are the most common cause of fundus albipunctatus (FA), one form of stationary night blindness [29, 30]. Genome-wide meta-analyses including 8,376 individuals of Asian ancestry and 37,382 individuals of European ancestry identified RDH5 (rs3138144) as a novel susceptibility gene for refractive error [10]. Retinoic acid is produced in the retina and is predominantly expressed in the choroid. It may lead to scleral ECM remodeling and contribute to eye growth in experimental myopia models [31]. Therefore, it was inferred that mutations in RDH5 may alter scleral ECM components, resulting in increased axial length. By annotating information from GWAS data, researchers demonstrated that RDH5 (synonymous rs3138142) was a GWAS-significant SNP, falling within the binding site of POLR2A. POLR2A is an enzyme involved in RNA synthesis, transcription regulation and mRNA processing. RDH5/rs3138142 has a close relationship with DNase sites and histone markers in many cell types, affecting the proximal transcription of RDH5 [32]. In the current study, we identified a potentially functional variant located in RDH5 (synonymous rs3138142), in accordance with previous studies.

OCA2 encodes a protein involved in tyrosine transport, a precursor of melanin that contributes to mammalian pigmentation. Mutations in this gene lead to oculocutaneous albinism (type 2) [33, 34]. Many patients with oculocutaneous albinism (type 2) have clinical features of myopia. Gene-based analyses identified 21 novel candidate myopia genes, including OCA2 [12]. In the current study, we implicated a possible functional polymorphism located in the intron of OCA2, suggesting that the non-coding variants might alter gene expression and be crucial for disease phenotypes [35].

The TTC21B gene of human maps to chromosome 2q24.3 and encodes a member of the TTC21 family, a component of the cilium axoneme. This protein is required for retrograde intraflagellar transport in the cilium. Mutations in the TTC21B gene have been pro-posed to be associated with various ciliary dysfunctions [36]. A study on massive parallel sequencing in

patients with glomerular and cystic kidney diseases revealed that myopia was present in 5 of 23 patients (22%), two of whom had high myopia [37]. These 5 patients carried the p.P209L or p.H426D TTC21B variant. Studies in shRNA-mediated knockdown rats showed that the TTC21B protein was a novel photoreceptor sensory cilium (PSC) protein that is important for normal ciliary formation in PSCs. Dysfunction of the TTC21B protein causes progressive disorder and photoreceptor death, leading to inherited retinal degenerations [38]. In the present study, we found a synonymous polymorphism, rs80225158 (c.2385G>C), in the TTC21B gene that may alter the overall expression of TTC21B mRNA in the retina of pathologic myopic patients. This result suggests that TTC21B (rs80225158, c.2385G>C) may influence the structure and function of photoreceptors and contribute to retinal degenerations in pathologic myopia.

The human PEX7 gene, encoding the type-2 peroxisome targeting signal (PTS2) receptor, is required for peroxisome assembly [39]. PEX7 variants clinically cause peroxisome biogenesis disorders, including Refsum disease [40] and rhizomelic chondrodysplasia punctata (RCDP) [41]. Some patients with PEX7 mutations have the clinical features of bilateral cataracts and/or retinitis pigmentosa [41, 42]. However, the current study reports intronic mutations in PEX7 (rs927181) related to pathological myopia for the first time. The mechanism of the relationship between rs927181 and the development of pathological myopia needs to be further investigated.

The LRP5 gene, mapped to the chromosome 11q13.2, encodes a protein in the low-density lipoprotein receptor family that plays a key role in eye development. Defects in this gene causes failed involution of primary vitreal vasculature and contribute to persistent hyperplasia of the primary vitreous (PHPV) [43]. In addition, linkage and sequencing analysis of three consanguineous families diagnosed with autosomal recessive FEVR demonstrated that mutations in LRP5 were associated with autosomal recessive FEVR [44]. In the present study, a synonymous polymorphism in the LRP5 gene (rs545382, c.1647T>C) was discovered. This synonymous polymorphism in the LRP5 gene may not change the encoded protein, but it may alter the secondary structures of mRNA, ultimately influencing protein folding and gene expression.

To evaluate the potential correlation between genotype and phenotype, patients were classified into three groups. Patients with mutations in PEX7 (rs927181), LRP5 (rs545382, c.1647T>C) and TSPAN12 (rs41623, c.765G>T) were primarily observed in the mCNV group, suggesting that these three variants have a close relationship with the development of mCNV. Mutations in OCA2 were mainly observed in the myopic traction retinopathy group. This finding suggests that the OCA2 mutation might lead to myopic traction retinopathy in Han Chinese individuals.

CONCLUSION

In summary, we performed a comprehensive evaluation of the potential causative mutations underlying the pathogenesis of pathologic myopia. This evaluation may provide important information for the diagnosis and prediction of prognosis. To validate the correlations between phenotype and clinical features, further studies should be performed to evaluate gene mutations identified in the present study in a larger cohort of patients and normal controls. Animal model studies should be conducted to investigate the functional consequences and the molecular mechanisms of these mutations in the development of pathologic myopia.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Institutional Review Board of Zhongshan Ophthalmic Center affiliated with Sun Yat-sen University (Guangzhou, China).

HUMAN AND ANIMAL RIGHTS

No animal were used in this study, Reported experiments on humans were in accordance with the ethical standards of the committee responsible for human experimentation (institutional national), and with the *Helsinki Declaration* of 1975, as revised in 2008 (http://www.wma.net/ en/20activities/10ethics/ 10helsinki/).

CONSENT FOR PUBLICATION

Written informed consent was obtained from each participant.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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