Molecular Vision 2019; 25:35-46 <http://www.molvis.org/molvis/v25/35>
Received 18 July 2018 | Accepted 18 January 2019 | Published 20 January 2019

Whole exome sequencing reveals novel *EYS* mutations in Chinese patients with autosomal recessive retinitis pigmentosa

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Purpose: Retinitis pigmentosa (RP) belongs to a group of inherited retinal diseases with high genetic heterogeneity. This study aimed at identifying the disease-causing variants in patients with autosomal recessive RP.
Methods: Three RP families with autosomal recessive inheritance and 139 sporadic RP patients were included. Complete ophthalmic examinations were conducted in all the study subjects. DNA samples were extracted from patients' peripheral blood for whole exome sequencing (WES) analysis. Direct Sanger sequencing was conducted for validating the identified mutations and cosegregation pattern in the RP families.
Results: One novel (c.7492G>C:p.Ala2498Pro and c.8422C>T:p.Ala2808Thr) and one reported (c.8012T>A:p.Leu2671X and 6416G>A:p.Cys2139Tyr) pair of compound heterozygous mutations, as well as one reported compound homozygous mutation (c.6416G>A:p.Cys2139Tyr/c.8012T>A:p.Leu2671X), were identified in the *EYS* gene from three families with autosomal recessive RP. All the mutations were cosegregated with the RP phenotype in the RP families. For the sporadic RP patients, seven novel and seven reported *EYS* variants were identified in 19 patients, including two novel frameshift (c.8301dupT:p.Asp2767fs and c.9437_9440del:p.Glu3146fs), three novel missense (c.8297G>C:p.Gly2766Ala, c.9052T>C:p.Trp3018Arg, and c.8907T>G:p.Cys2969Trp), and one nonsense (c.490C>T:p.Arg164X) variants. All the novel mutations were confirmed by Sanger sequencing. Most of the variants were located at the C-terminus of the EYS protein. Bioinformatics analyses indicated that all detected variants were damaging or possibly damaging.
Conclusions: This study identified eight novel *EYS* variants and expanded the spectrum of *EYS* mutations in Chinese RP patients.

Retinitis pigmentosa (RP) belongs to a group of inherited retinal diseases with the initial symptom of night blindness and progressive visual field loss and even irreversible blindness, characterized by the sequential degeneration of photoreceptors and RPE [1,2]. The prevalence of RP in China is about 1 in 1,000 [1,2]. RP is a complex disease with clinical variability, genetic heterogeneity, and the existence of modifier genes [3]. The inheritance patterns of RP can be classified as autosomal dominant, autosomal recessive, X-linked, digenic, and mitochondrial [2]. To date, 58 genes and loci have been identified for autosomal recessive RP (RetNet) including *ABCA4*, *CDHR1*, *CERKL*, *CNGA1*, *CNGB1*, *CRB1*, and *EYS* [2,4]. *EYS* variants account for approximately 5–10% of all autosomal recessive RP patients, while other genes are responsible for 1–2% [1,5-7]. The autosomal recessive RP genes are involved in various biological functions, including cell metabolism, the phototransduction cascade, cell signaling, RNA and protein modification, and phagocytosis [3]. Identification of the disease-causing mutations in RP patients helps elucidate the genetic architecture and pathogenesis of RP, facilitating the development of novel treatments for RP patients [4,8-10].

With the rapid development of next-generation sequencing technology, whole exome sequencing (WES) analysis has been applied for identifying variants in exons and splicing sites at the genome-wide scale [4]. The mutations identified in exons or splicing sites are responsible for more than 85% of the disease-associated variants [8]. In this study, we aimed to delineate the disease-causing mutations in three RP families and sporadic RP patients by WES analysis. The identified mutations were also investigated by bioinformatics analyses.

METHODS

Study subjects and clinical examinations: This study was approved by the Ethics Committee of the Joint Shantou International Eye Center (JSIEC) of Shantou University and the Chinese University of Hong Kong, and it was performed in accordance with the Declaration of Helsinki. Informed

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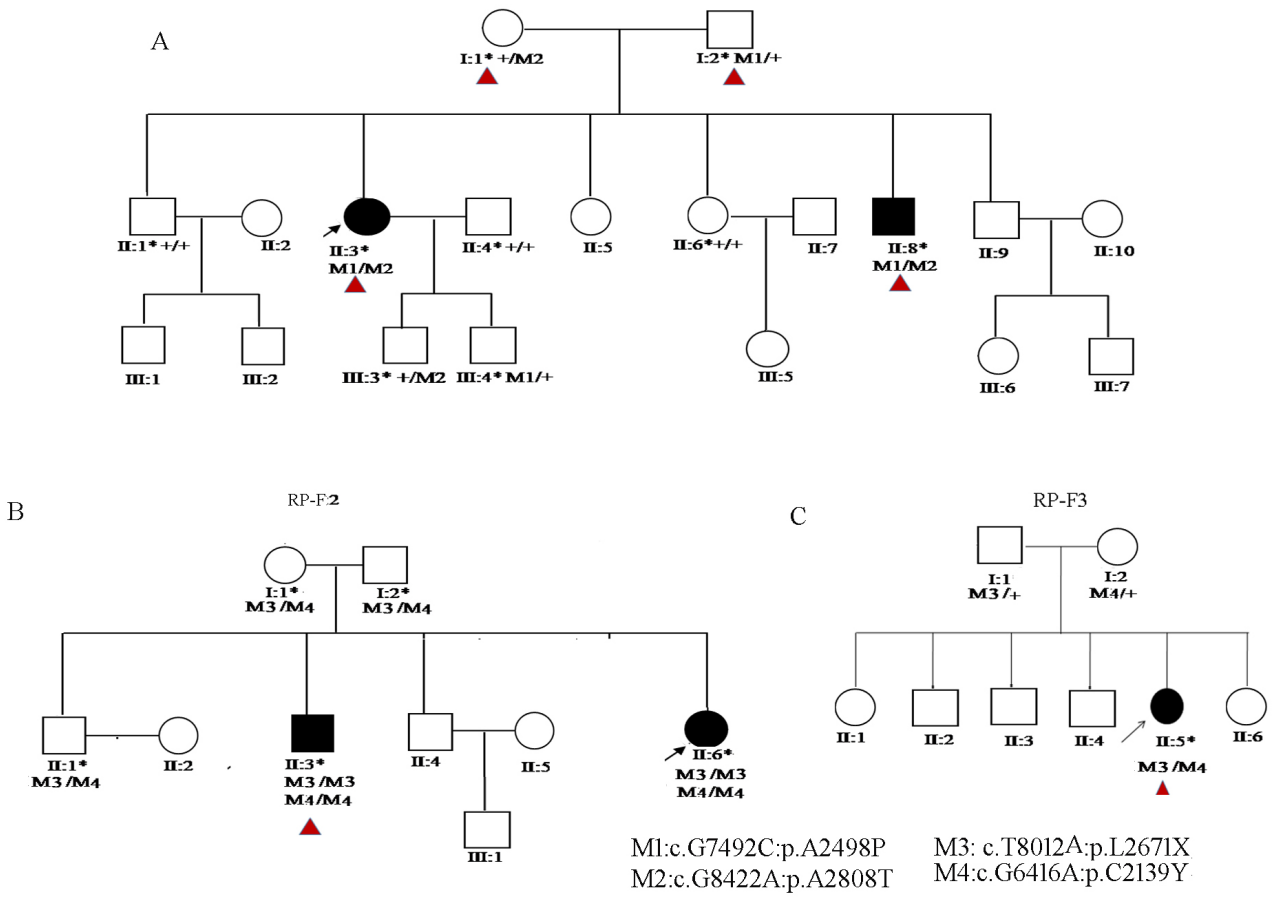

Figure 1. Pedigree of retinitis pigmentosa (RP) in families F1, F2, and F3. The asterisks signify that the patients' blood was collected, red-filled triangles show that the patients' DNA was sent to whole exome sequencing (WES), black arrows represents the probands, question marks represents a lack of clinical data, filled square (male) or circle (female) represents RP patients for male or female, unfilled square (male) or circle (female) represents healthy individuals, square (male) or circle (female) with slash represents the individuals are dead.

consent was obtained from all the study subjects before recruitment. Three Chinese RP families (RP-F1–3), 139 sporadic RP patients, and 200 senile cataract controls were recruited in this study. Complete ophthalmic examinations were conducted, including visual acuity, fundus photography, visual field test, slit-lamp examination, optical coherence tomography (OCT) and full-field electroretinogram (ERG). Peripheral blood samples were collected in all study subjects.

WES analysis: Genomic DNA from the whole blood was extracted by the TIANGEN Blood DNA kit DP318 (TIANGEN, Beijing, China) according to the manufacturer's protocol. The genomic DNA of four affected patients and two unaffected family members from the three RP families (Figure 1) and 139 sporadic RP patients were subjected to WES analysis (ANOROAD, Beijing, China). Briefly, sequencing libraries were prepared using the SureSelect XT Target Enrichment Kit (Agilent Technologies, Santa Clara, CA) and captured using the Agilent Sure Select Human All Exon Kit V5 (Agilent Technologies). Paired-end sequencing was conducted using the HiSeq 2500PE100 platform (Illumina, San Diego, CA) with a read length of 100 bp and average coverage depth of at least 100X for each sample.

Mutation analyses: All reads from the WES analysis were aligned to the human genome (GRCh37/hg19) with an alignment tool, BWAMEM. The reads with low-mapping quality, PCR duplication, low alignment score and mismatch rate, or high suboptimal alignment score and mismatch rate ≥5% were removed with an in-house program. The candidate single-nucleotide polymorphism (SNPs) and insertion/deletion variants were identified with multiple filtering steps: First, high-frequency variants minor allele frequency (MAF ≥ 0.05) in the 1000 Genome project and EXAC, intergenic variants, intronic variants, and synonymous variants were excluded. Second, the potential variants in the reported RP

genes were compared with the RetNET database. Finally, the candidate variants were further analyzed by Polymorphism Phenotyping v2 (Polyphen-2) Sorting Intolerant from Tolerant (SIFT), and Mutation Taster to predict the potential effect on the protein function.

Sanger sequencing confirmation: The identified novel variants were validated by PCR and Sanger sequencing analysis in the remaining affected and unaffected family members. PCR was performed in BioRad PCR machines with specific primers (Table 1). The PCR products were purified (Omega, GA) and sequenced by Guangzhou IGE Biotechnology Ltd. (Guangzhou, China). The cosegregation pattern was analyzed in the RP families. The identified variants were confirmed with 200 control subjects by Sanger sequencing.

RESULTS

Clinical characteristics of the RP patients: The fundus photographs of the proband from RP-F1 showed typical RP phenotypes, with the bone-spicule pigmentation of the retina and waxy pallor optic disc (Figure 2A,B). The OCT analysis presented the disappearance of the photoreceptor layer (Figure 2C,D), and the visual field test indicated a visual field defect (Figure 2E,F). The ERG analysis showed no response to the stimulus for both cone and rod photoreceptors (Figure 2G,H). The affected members in RP-F2 and RP-F3, as well as the sporadic RP patients, showed similar RP phenotypes (Table 2), whereas the unaffected family members and control subjects did not present any RP phenotype. The parents and children of the affected study subjects were not diagnosed with RP, indicating the autosomal recessive inheritance in the three recruited families (Figure 1).

WES analysis in RP families: Each WES analysis resulted in a total of 12 GB of sequence data, and 95.5% of the sequence reads originated from the exons, with a mean coverage of 100-fold. The total numbers of variants (SNPs and indels) of exons and splice sites identified were as follows: 25,403 for RP-F1-II:3, 25,554 for RP-F1-II:8, 25,523 for RP-F1-I:2, 25,583 for RP-F1-I:1, 23,148 for RP-F2-II:2, and 23,846 for RP-F3-II:5 (**Figure 3**). After filtering the synonymous, intergenic, intronic, and common variants, the candidate variants of known RP genes in recessive inheritance were reduced to two for RP-F1, three for RP-F2, and two for RP-F3.

For the family RP-F1, two compound heterozygous variants in *EYS* gene were identified in the two affected members (RP-F1-II:3 and RP-F1-II:8), namely a missense variant c.7492G>C:p.Ala2498Pro in exon 38 and a missense variant c.8422G>A:p.Ala2808Thr in exon 43 (Table 3).

Table 1. Primers and Usage for EYS confirmation.

Primer name	Sequence(5′-3′)	Usage
EYS-F1F1	GTTTGTGGAAGTGACGAAGGA	PCR-Confirmation
EYS-F1R1	AAGCTGACGGAACTCCTGAA	
EYS_F1F2	CAACTTGGCCAGAAACAGCA	PCR-Confirmation
EYS_F1R2	TCACCTACATTTGAGCCACCT	
EYS-F2F	ACTGAAAACATCTTAGGAGGCT	PCR-Confirmation
EYS-F2R	ACTTCTGTCAGCCCCTCT	
EYS-41F	AGGCTCCCAGAGATGAAGTC	PCR-Confirmation
EYS-41R	TGACAAGTTAGCATCAGGGC	
EYS-1F	AGGGCTTCTAAATTCATACGCA	c.8301dupT:p.D2767fs
EYS-1R	TCTTTCTCTCCTTCCCTCAGC	
EYS-2F	ACAATCAGAACCTTCAGTGACA	c.9437_9440del:p.E3146fs
EYS-2R	GTGGCTCTAAACTATGATGGCA	
EYS-3F	GCACCAACTCTTCCTGCTTT	c.G8297C:p.G2766A
EYS-3R	TCAATGAGAACTGTCCACAACT	
EYS-4F	ACATGCATCAAGTTCCTGGC	c.C490T:p.R164X
EYS-4R	TGTTCCCCAGATTTGCCCT	
EYS-5F	GCCATCATAGTTTAGAGCCACA	c.T9052C:p.W3018R
EYS-5R	GTGTACTTTGGGTTGGGTGG	
EYS-6F	GGAGACCAATTGCCAGAAAATC	c.T8907G:P.C2969W
EYS-6R	GCAGAAATGGAGGTGAATGTACA	

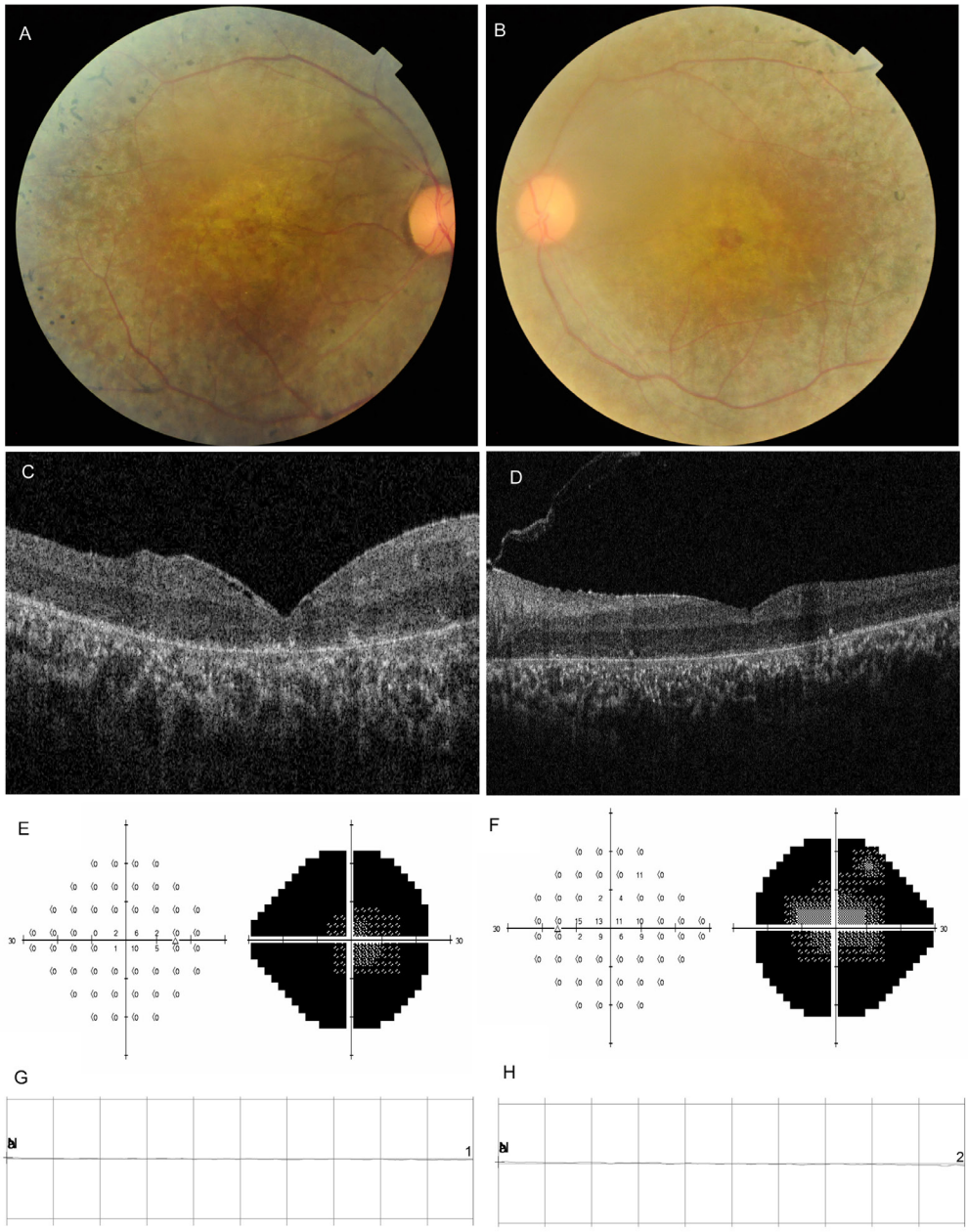

Figure 2. Clinical information for the proband of retinitis pigmentosa family 1 (RP-F1). **A,B**: Fundus picture of the right (A) and left (B) eyes. **C,D**: Optical coherence tomography (OCT) scans of the right (C) and left (D) eyes. **E,F**: Visual fields of the right (E) and left (F) eyes. **G,H**: Electroretinogram (ERG) results of the right (E) and left (F) eyes.

Their unaffected parents (RP-F1-I:1 and RP-F1-I:2) and the unaffected third-generation male subjects (RP-F1-III:3 and RP-F1-III:4) only carried either one of the heterozygous *EYS* variant, and the remaining unaffected members (RP-F1-II:1 and RP-F1-II:6) did not carry any *EYS* variants (Figure 1). These findings indicated that the two *EYS* variants followed the cosegregation pattern of recessive inheritance. The c.8422G>A:p.Ala2808Thr variant has been previously reported (rs111991705), whereas the c.7492G>C:p.Ala2498Pro *EYS* variant was neither found in the 1000 genome and dbSNP nor previously reported, suggesting that it is a novel mutation. Furthermore, these two variants were not found in

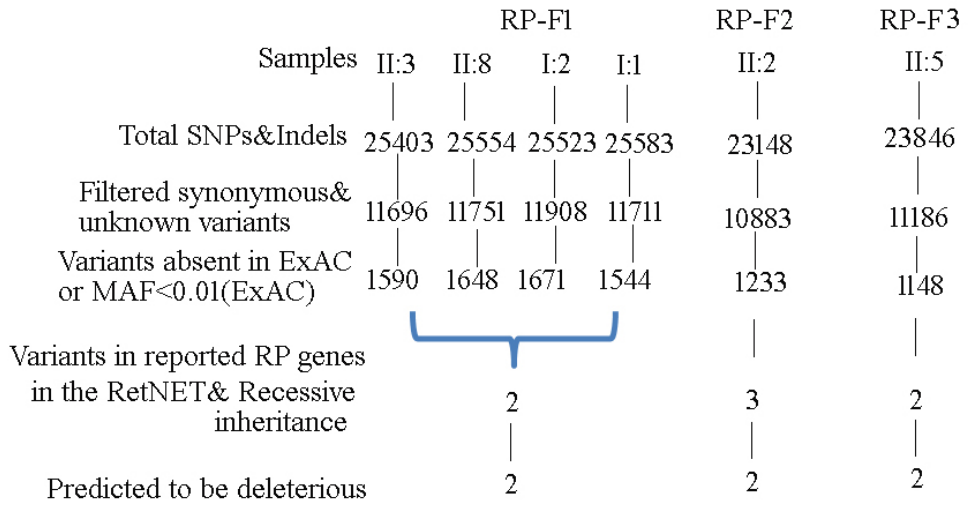

Figure 3. Pipeline of mutation screening for whole exome sequencing (WES) data.

200 control subjects from our cohort. Therefore, compound heterozygous c.7492G>C:p.Ala2498Pro and c.8422G>A:p.Ala2808Thr variants should be the causative mutations for the family RP-F1.

For the family RP-F2, two homozygous variants in the *EYS* gene and one homozygous variant in the *RPGR* gene were identified in the affected member (RP-F2-II:3), as follows: a nonsense *EYS* variant c.8012T>A:p.Leu2671X in exon 41, a missense *EYS* variant c.6416G>A:p.Cys2139Tyr in exon 31 and a missense RPGR variant c.C1282G:p.Leu428Val in exon

Table 2. Clinical information of retinitis pigmentosa patients in the included pedigrees.

Patient	F1-II:3	F1-II:8	F2-II:3	F2-II:6	F3-II:5
Gender	Female	Male	Male	Female	Female
Age of diagnosis	44	35	32	28	61
Visual acuity OD	HM	FC	0.6	0.6	0.3
Visual acuity OS	HM	FC	0.8	0.6	0.3
Macular dystrophy OD	Severe	Severe	Mild	Mild	Mild
Macular dystrophy OS	Severe	Severe	Mild	Mild	Mild
Optic disc OD	Waxy	Waxy	Mild	Mild	Waxy
Optic disc OS	Waxy	Waxy	Mild	Mild	Waxy
Artery attenuation OD	Yes	Yes	Mild	Mild	Yes
Artery attenuation OS	Yes	Yes	Mild	Mild	Yes
Pigment deposits OD	Yes	Yes	Mild	Mild	Yes
Pigment deposits OS	Yes	Yes	Mild	Mild	Yes
Electroretinogram OD	Diminished	NA	Diminished	Diminished	NA
Electroretinogram OS	Diminished	NA	Diminished	Diminished	NA
Visual Field MD OD	−33.30 db	NA	−32.46 db	−31.54 db	NA
Visual Field MD OS	−33.31 db	NA	−33.19 db	−33.39 db	NA
OCT OD	ISe loss	NA	NA	ISe loss	ISe loss
OCT OS	ISe loss	NA	NA	ISe loss	ISe loss

MD: mean defect; OCT: optical coherence tomography; HM: hand movement; FC: finger counting; NA: not available; ISe: inner segment ellipsoid zone.

Table 3. Identified variants in recessive inheritance, population frequencies, and in silico predictions of pathogenic function.

Family	Gene	Nucleotide /Amino acid change	Previously reported	Variant type	ExAC frequency	SIFT	Polyphen2 HDIV	MT	Genotypes
RP-F1	EYS	NM_001292009:exon44: c.8422G>A:p.Ala2808Thr	rs111991705	Missense	0.0098	T	P	N	Heterozygous
	EYS	NM_001292009:exon38: c.7492G>C:p.Ala2498Pro	Novel	Missense	Absent	D	D	D	Heterozygous
RP-F2	EYS	NM_001292009:exon41: c.8012T>A:p.Leu2671X	PMID:24652164	Nonsense	Absent	.	.	.	Homozygous
	EYS	NM_001292009:exon31: c.6416G>A:p.Cys2139Tyr	PMID:25753737	Missense	0.00005274	D	D	D	Homozygous
	RPGR	NM_000328:exon11 c.C1282G:p.Leu428Val	rs182345461	Missense	0.0001	T	D	N	Homozygous
RP-F3	EYS	NM_001292009:exon41: c.8012T>A:p.Leu2671X	PMID:24652164	Nonsense	Absent	.	.	.	Heterozygous
	EYS	NM_001292009:exon31: c.6416G>A:p.Cys2139Tyr	PMID: 25,753,737	Missense	0.00005274	D	D	D	Heterozygous

11 (Table 3). The affected sister (RP-F2-II:6) also carried the homozygous *EYS* variants of c.8012T>A:p.Leu2671X and c.6416G>A:p.Cys2139Tyr, while the unaffected parents (RP-F2-I:1 and RP-F2-I:2) carried the heterozygous *EYS* variants (Figure 1). These results suggested that the two *EYS* variants were on the same allele, and the cosegregation pattern of recessive inheritance was confirmed. Both c.8012T>A:p.Leu2671X and c.6416G>A:p.Cys2139Tyr variants have been previously reported, and they were not found in 200 control subjects from our cohort. Therefore, compound homozygous c.8012T>A:p.Leu2671X and c.6416G>A:p.Cys2139Tyr variants should be the causative mutations for the family RP-F2.

For the family RP-F3, two compound heterozygous variants in the *EYS* gene were identified in the affected member (RP-F3-II:5): the nonsense *EYS* variant c.8012T>A:p.Leu2671X in exon 41 and missense *EYS* variant c.6416G>A:p.Cys2139Tyr in exon 31 (Table 3). Her unaffected parents (RP-F3-I:1 and RP-F3-I:2) only carried either one of the heterozygous *EYS* variants (Figure 1). This indicated that the two *EYS* variants were on different alleles and followed the cosegregation pattern of recessive inheritance. Therefore, compound heterozygous c.8012T>A:p.Leu2671X and c.6416G>A:p.Cys2139Tyr variants should be the causative mutations for the family RP-F3. Sanger sequencing analysis has confirmed all identified variants in the affected patients (Figure 4), and they were not found in 200 control subjects from our cohort.

WES analysis in sporadic RP patients: To extend the discovery of variants in the *EYS* gene, 139 sporadic RP patients were screened by WES analysis. Fourteen *EYS* variants were identified in 18 Chinese sporadic RP patients (Table 4), including seven novel variants and seven previously

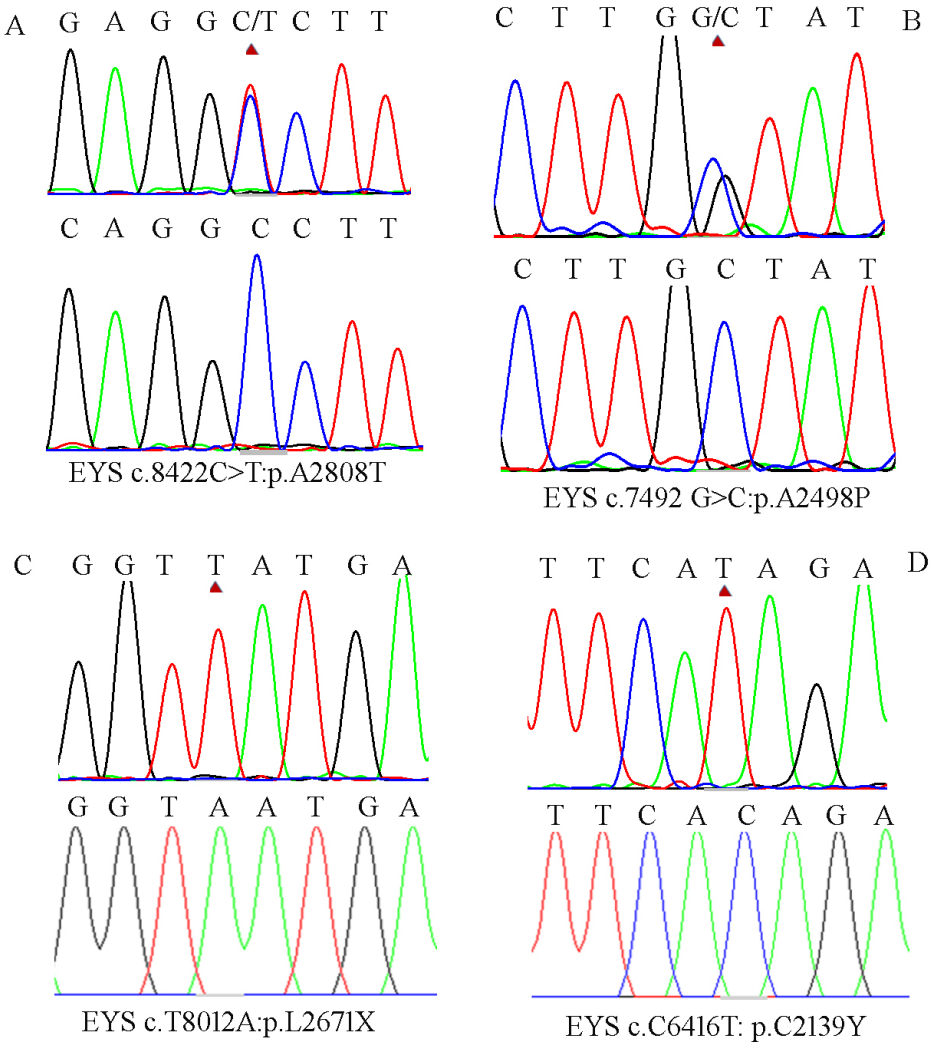

Figure 4. PCR–Sanger sequencing validating the candidate *EYS* variants in the retinitis pigmentosa family 1 (RP-F1) and RP-F2 DNA sequencing profiles of the identified mutations (upper) and their wild-type form (lower). Red arrows indicate the position of the mutated nucleotide. **A,B**: From RP-F1; **C,D**: from RP-F2.

reported variants. Novel frameshift variants (c.8301dupT:p.Asp2767fs and c.9437_9440del:p.Glu3146fs) were discovered in three independent sporadic RP patients, whereas other novel variants were found in only one RP patient. All novel variants were validated by Sanger sequencing analysis. The previously reported variant c.6416G>A:p.Cys2139Tyr in exon 31 was found in eight sporadic RP patients. Two sporadic patients carried homozygous *EYS* variants, seven had compound heterozygous *EYS* variants, and nine had only one *EYS* variant.

In addition to the *EYS* variants, 12 heterozygous variants of other inherited retinal dystrophy genes were identified in seven sporadic patients (Table 4). Six of the genes were inherited in an autosomal dominant manner, including *CRB1*, *FSCN2*, *GUCA1B*, *IMPDH1*, *PDE6B*, and *RPRF6*. Other autosomal recessive RP genes would be unlikely to cause RP in these patients because of heterozygous carriers.

Bioinformatics analyses: In silico analyses by SIFT, Polyphen2, and MutationTaster bioinformatics programs showed that all identified *EYS* variants were predicted to be deleterious or possibly damaging (Table 3 and Table 4). Most of the variants are located at the C-terminal of the EYS protein. The novel variants are localized in the region between the third and the fifth LamG domain at the C-terminal of the EYS protein (Figure 5).

DISCUSSION

In the present study, eight novel and seven reported variants were identified in the *EYS* gene by WES analysis in three Chinese autosomal recessive RP families and 139 sporadic patients. The human *EYS* gene is a homolog of the Drosophila eye spacemaker (SPAM) gene. EYS protein specifically expressed in the photoreceptor cell layer of the retina, and it is essential for the development and morphology of photoreceptors [11]. Currently, more than 270 variants have been reported in *EYS* for autosomal recessive RP patients [5], and the mutations include missense, nonsense, insertion, deletion, and splice site mutations [12-14]. The EYS protein contains 3,165 amino acids encoded by 43 exons, which is composed of 21 epidermal growth factor (EGF)-like domains, EGF-like and laminin A G-like domains; CA-calcium-binding domains and 5 LamG domains [15]. In addition, patients carrying *EYS* mutations progress more rapidly than those with RP caused by other autosomal recessive genes, such as *USH2A* and *MAK* [16].

One novel mutation, c.7492G>C:p.Ala2498Pro, was identified in family RP-F1, which caused an amino acid change from alanine into proline. This could influence the proper folding of protein, and thus, the protein function could be affected. This variant was also confirmed by in silico prediction (Table 3). The heterozygous carrier of this mutation did not show any observable RP symptom. Because of the autosomal recessive inheritance, the second *EYS* mutation is c.8422G>A:p.Ala2808Thr in the affected family members. This variant has been reported in an Indian autosomal recessive RP family with another *EYS* variant, c.7868G>A:p.Gly2623Glu [12]. This further support the causal role of our novel *EYS* mutation, c.7492G>C:p.Ala2498Pro, in autosomal recessive RP.

In the family RP-F2, compound homozygous variants (nonsense c.8012T>A:p.Leu2671X and missense c.6416G>A:p.Cys2139Tyr) are the causative mutations for the autosomal recessive RP (Figure 1B). Comparatively, in the family RP-F3, the same mutations but expressed in a compound heterozygous manner caused the RP phenotype, which has also been reported in other Chinese RP families [1,17]. This could be explained by the mutations locating in the same allele in RP-F2 but different alleles in RP-F3. Moreover, the onset age of patients from RP-F2 with compound homozygous mutations was younger than that from RP-F3 with compound heterozygous mutations. The missense mutation in RP-F3 could still contain some EYS protein function compared with the truncating protein in RP-F2. This was also observed in another Chinese autosomal recessive RP family and other sporadic RP patients, in which the patients with a nonsense mutation p.[Arg164*] showed an earlier age of onset than those without this mutation [1,3,17].

Most of the variants are localized at the C-terminus of the EYS protein (**Figure 5**). Some nonsense mutations (p.Leu2671X and p.Tyr2956X) cause truncation in the EYS protein and partially delete the LamG domains. Other truncating mutations, such as p.Ala1636fs and p.Asp2767fs, could be insertions or deletions. Previous studies showed that the LamG domain is required for EYS function in interrhabdomeral space formation [5]. Therefore, these mutations could affect the EYS functions through disruption of the LamG domain. The C-terminus localization of *EYS* mutations has also been reported in other studies [5,6,12,13]. However, variants from a cohort of Spanish origin did not exhibit this trend [18].

Homozygous or compound heterozygous *EYS* mutations were also identified in sporadic RP patients from our study (Table 4). The frequency of RP patients with *EYS* mutations was 6.47% (9/139) in our cohort. *EYS* mutations are common in RP [12-17]. The variant c.6416G>A:p.Cys2139Tyr was not only frequently found in RP patients in our cohort, but it is also frequently identified in other populations [13,19].

Table 4. Table 4. Variant identification by whole exome sequencing analysis in sporadic RP patients.

Patients ID	Gene	Chromosome position	Novelty	Nucleotide change	Amino acid Change	Poly-phen2 HDIV	SIFT	Muta-tion Taster	Frequency		Genotype
									1000G	ExAC	
Patients carried one EYS variant or together with variants from other known RP genes											
J-RP007	*EYS*	Chr6:64431689	Novel	c.8301dupT(Insert A)	p.Asp2767fs	.	.	.		.	Heterozygous
J-RP013	*ZNF513*	Chr2:27601385	rs200255167	c. 748C>T	p.Arg250Trp	D	D	N	0.000998403	0.0003	Heterozygous
	EYS	Chr6:64431689	novel	c. 8297G>C	p.Gly2766Ala	D	D	D	.	.	Heterozygous
J-RP021	*EYS*	Chr6:64431689	PMID:24652164	c. 8868C>A	p.Tyr2956X	.	T	D	.	.	Heterozygous
J-RP011	*CRB1*	Chr1:197313422	rs114846212	c. 457G>A	p.Glu153Lys	D	D	D	0.00139776	0.0007	Heterozygous
	USH2A	Chr1:216138711	rs200038092	c. 7068T>G	p.Asn2356Lys	P	T	D	0.00159744	0.0008	Heterozygous
	GUCA1B	Chr6:42162429	Reported in database	c. 130C>T	p.Arg44Cys	D	D	D	.	0.0000992	Heterozygous
	EYS	Chr6:64431689	PMC4911908	c. 6416G>A	p.Cys2139Tyr	D	D	D	.	0.00005274	Heterozygous
J-RP111	*MAK*	Chr6:10773343	Novel	.	.	.	.	.	.	.	Heterozygous
	EYS	Chr6:64431689	Novel	c. 8907T>G	p.Cys2969Trp	D	D	D	.	.	Heterozygous
RP016	*USH2A*	Chr12:16019180	rs144892841	c. 9041C>A	p.Thr3014Asn	D	T	D	0.000399361	0.0001	Heterozygous
	EYS	Chr6:64431689	PMID:22302105	./ splicing	.	.	.	D	.	.	Heterozygous
Patients carried homozygous or compound heterozygous *EYS* variant											
	EYS	Chr6:64431689	Novel	c.8301dupT(Insert A)	p.Asp2767fs	.	.	.	.	.	Heterozygous
J-RP028	*EYS*	Chr6:64431689	PMID:24652164	c. 8012T>A	p.Leu2671X	.	.	D	.	.	Heterozygous
	EYS	Chr6:64431689	PMID: 25,753,737	c. 6416G>A	p.Cys2139Tyr	D	D	D	.	0.00005274	Heterozygous
	EYS	Chr6:64431689	Novel	c.9437_9440del (AGTT)	p.Glu3146fs	.	.	.	.	.	Heterozygous
J-RP031	*EYS*	Chr6:64431689	PMC4911908	./splicing	.	.	.	D	.	.	Heterozygous
	FSCN2	Chr17:79495999	Reported in database	c. 442C>T	p.Arg148Trp	D	D	D	.	0.0008	Heterozygous
J-RP039	*EYS*	Chr6:64431689	PMID: 25,753,737	c. 6416G>A	p.Cys2139Tyr	D	D	D	0.00005274	0	Heterozygous
J-RP057	*EYS*	Chr6:64431689	Novel	c. 490C>T	p.Arg164X	.	.	A	.	.	Heterozygous
	SPATA7		Novel	c.20_23del(TCAG)	p.Val7fs	.	.	.	.	.	Heterozygous
J-RP059	*EYS*	Chr6:64431689	DOI:10.3760/cma.j.issn.1674-845X.2016.01.006	c. 8012T>A	p.Leu2671X	.	.	D	.	.	Heterozygous
	EYS	Chr6:64431689	PMID: 25,753,737	c. 6416G>A	p.Cys2139Tyr	D	D	D	.	0.00005274	Homozygous

Patients ID	Gene	Chromosome position	Novelty	Nucleotide change	Amino acid Change	Poly-phen2 HDIV	SIFT	Muta-tion Taster	Frequency		Genotype
									1000G	ExAC	
J-RP066	*EMC1*	Chr1:19577999	Reported in database	c. 5C>T	p.Ala2Val	D	D	D	.	0.0001	Heterozygous
	FAM161A	Chr2:62067223	rs183615774	c. 916C>T	p.Arg306Trp	D	D	D	0.000798722	0.0002	Heterozygous
	PDE6B	Chr4:619675	Novel	c. 260T>C	p.Leu87Pro	D	D	D	.	0.0000845	Heterozygous
	TULP1	Chr6:35471544	Novel	c. 1194C>G	p.Ser398Arg	D	D	D	.	0.00003798	Heterozygous
	EYS	Chr6:64431689	rs184722374	c.9437_9440del(AGTT)	p.Glu3146fs	.	.	.	.	.	Heterozygous
	EYS	Chr6:64431689	Reported in database	c. 8170G>T	p.Glu2724X	.	.	D	0.000199681	0.00005069	Heterozygous
	EYS	Chr6:64431689	Novel	c. 9052T>C	p.Trp3018Arg	D	D	D	.	.	Heterozygous
J-RP069	*EYS*	Chr6:64431689	DOI:10.3760/cma.j.issn.1674-845X.2016.01.006	c. 8012T>A	p.Leu2671X	.	.	D	.	.	Heterozygous
	EYS	Chr6:64431689	PMID: 25,753,737	c. 6416G>A	p.Cys2139Tyr	D	D	D	.	0.00005274	Heterozygous
J-RP092	*EYS*	Chr6:64431689	PMC4911908	c. 6557G>A	p.Glu2186Glu	P	P	D	.	.	Heterozygous
	EYS	Chr6:64431689	PMID: 25,753,737	c. 6416G>A	p.Cys2139Tyr	D	D	D	.	0.00005274	Heterozygous
J-RP122	*EYS*	Chr6:64431689	Novel	c.9437_9440del (AGTT)	p.Glu3146fs	.	.	.	.	.	Heterozygous
	EYS	Chr6:64431689	rs184722374	c. 8170G>T	p.Glu2724X	.	.	D	0.000199681	0.00005069	Heterozygous
J-RP131	*IMPDH1*	Chr7:128040533	Novel	c. 310G>T	p.Asp104Tyr	D	D	D	.	.	Heterozygous
	C2orf71	Chr2:29297043	rs201706430	c. 85C>T	p.Arg29Trp	D	D	N	0.000199681	0.0003	Heterozygous
	EYS	Chr6:64431689	DOI:10.3760/cma.j.issn.1674-845X.2016.01.006	c. 8012T>A	p.Leu2671X	.	.	D	.	.	Heterozygous
	EYS	Chr6:64431689	PMID: 25,753,737	c. 6416G>A	p.Cys2139Tyr	D	D	D	.	0.00005274	Heterozygous
	PRPF6	Chr20:62663398	Reported in database	./ splicing	.	.	.	D	.	.	Heterozygous
RP020	*EYS*	Chr6:64431689	Novel	c.8301dupT (Insert A)	p.Asp2767fs	.	.	.	.	.	Heterozygous
	EYS	Chr6:64431689	rs150951106	c. 3489T>A	p.Asn1163Lys	D	T	D	0.00179712	0.0004	Heterozygous
RP022	*EYS*	Chr6:64431689	Novel	c.4908delA	p.Ala1636fs	.	.	.	.	.	Homozygous

Molecular Vision 2019; 25:35-46 <http://www.molvis.org/molvis/v25/35>

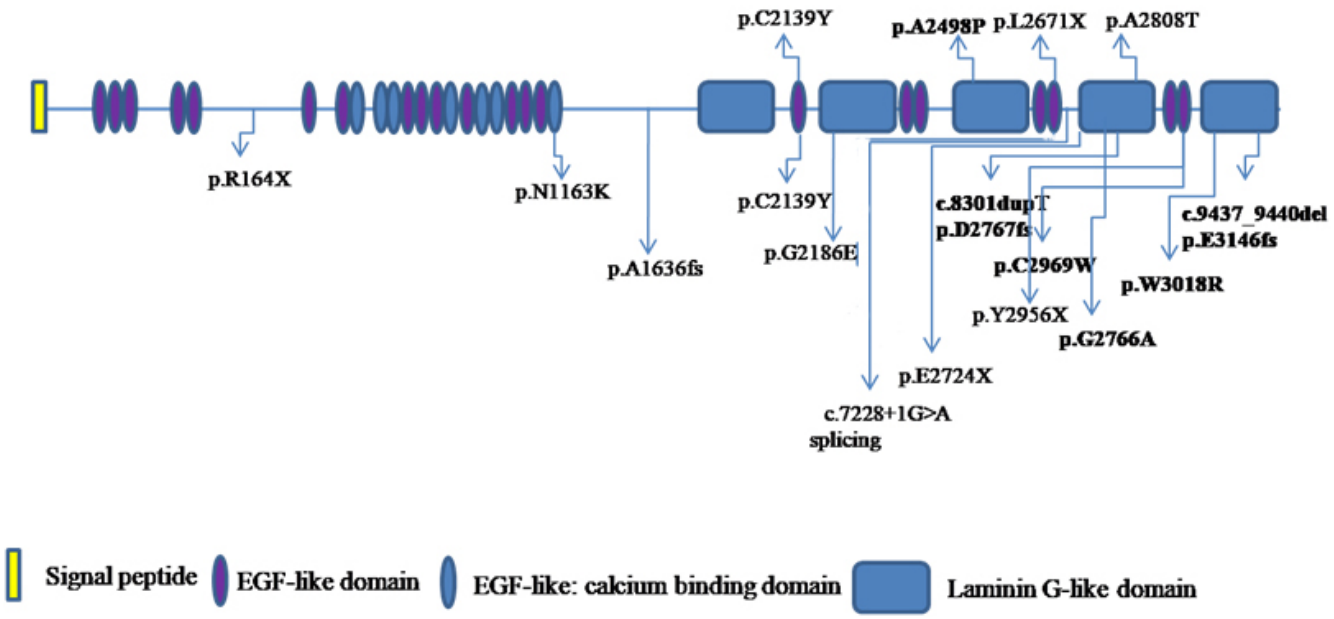

Figure 5. Localization of identified mutations in the schematic structure of EYS protein.

Nevertheless, further investigations are required to delineate the pathological functions of our novel *EYS* mutations.

In summary, this study revealed eight novel and seven reported mutations in the *EYS* gene in Chinese autosomal recessive RP families and sporadic RP patients through WES analysis. Our results further expand the spectrum of *EYS* variants in RP and further confirm the reported mutations. Genetic diagnosis is a critical strategy for aiding clinical diagnosis to bring about better clinical management and counseling. It should be recommended as a routine examination for RP patients.

ACKNOWLEDGMENTS

We are grateful to the participants in this study. This study is supported by the National Nature Science Foundation of China (30,901,646 and 81,170,853), YangFan Program, and TeZhi Program of Guangdong Province, China.

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Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 20 January 2019. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.