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, CDH1, 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 consent was...
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 (Ilumina, 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

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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.
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).
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 \textit{EYS} variant, and the remaining unaffected members (RP-F1-II:1 and RP-F1-II:6) did not carry any \textit{EYS} variants (Figure 1). These findings indicated that the two \textit{EYS} variants followed the cosegregation pattern of recessive inheritance. The \texttt{c.8422G>A:p.Ala2808Thr} variant has been previously reported (rs111991705), whereas the \texttt{c.7492G>C:p.Ala2498Pro} \textit{EYS} variant was neither found in the \texttt{1000 genome} and \texttt{dbSNP} nor previously reported, suggesting that it is a novel mutation. Furthermore, these two variants were not found in

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.
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 | −32.41 db | −31.55 db | NA |
| OCT OD | ISe loss | NA | ISe loss | ISe loss | ISe loss |
| OCT OS | ISe loss | NA | ISe loss | 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.
| Family | Gene | Nucleotide /Amino acid change | Previously reported | Variant type | ExA frequency | C | SIFT | Polyphen2 | 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 | D | D | D | 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 | rs18234561 | Missense | 0.0001 | T | D | N | Homozygous |
| RP-F3  | EYS  | NM_001292009:exon41: c.8012T>A:p.Leu2671X | PMID:24652164 | Nonsense | Absent | D | D | D | 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 reported.
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 located 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].
| Patients ID | Gene | Chromosome position | Novelty | Nucleotide change | Amino acid Change | Polyphen2 HDIV | SIFT Mutation Taster | Frequency 1000G | ExAC | Genotype |
|-------------|------|---------------------|---------|-------------------|------------------|----------------|----------------------|----------------|------|----------|
| J-RP007     | EYS  | Chr6:64431689       | Novel   | c.8301dupT(Insert A) | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |
| J-RP013     | ZNF513 | Chr2:27601385      | novel   | rs200255167       | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |
| J-RP021     | EYS  | Chr6:64431689       | novel   | 8297G>C           | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |
| J-RP021     | CRB1 | Chr1:197313422      | c.457G>A | p.Asp2767fs       | .                |                      | .              | .    | Heterozygous |
| J-RP011     | USH2A | Chr1:216138711      | rs20003892 | p.Asp2767fs       | .                |                      | .              | .    | Heterozygous |
| J-RP021     | GUCA1B | Chr6:64431689      | Reported in database | c.130C>T         | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |
| J-RP111     | MAK  | Chr6:10773343       | Novel   | .                 | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |
| J-RP111     | EYS  | Chr6:64431689       | Novel   | rs8907T>G         | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |
| J-RP28      | EYS  | Chr6:64431689       | Novel   | rs144892841       | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |
| J-RP031     | EYS  | Chr6:64431689       | Novel   | .                 | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |
| J-RP031     | FSCN2 | Chr17:79495999     | Reported in database | c.442C>T         | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |
| J-RP039     | EYS  | Chr6:64431689       | Novel   | rs20003892        | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |
| J-RP057     | EYS  | Chr6:64431689       | Novel   | rs20003892        | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |
| J-RP059     | EYS  | Chr6:64431689       | Novel   | rs20003892        | p.Asp2767fs     | .                |                      | .              | .    | Heterozygous |

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 | EYS | Chr6:64431689 | Novel | 8297G>C | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP21 | EYS | Chr6:64431689 | Novel | rs8907T>G | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP11 | USH2A | Chr1:216138711 | rs20003892 | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP021 | GUCA1B | Chr6:64431689 | Reported in database | c.130C>T | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP11 | MAK | Chr6:10773343 | Novel | . | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP11 | EYS | Chr6:64431689 | Novel | rs8907T>G | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP28 | EYS | Chr6:64431689 | Novel | rs144892841 | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP31 | EYS | Chr6:64431689 | Novel | . | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP31 | FSCN2 | Chr17:79495999 | Reported in database | c.442C>T | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP39 | EYS | Chr6:64431689 | Novel | rs8907T>G | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP57 | EYS | Chr6:64431689 | Novel | rs8907T>G | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP59 | EYS | Chr6:64431689 | Novel | rs8907T>G | p.Asp2767fs | . | . | . | . | . | Heterozygous

Patients carried homozygous or compound heterozygous EYS variant

J-RP007 | EYS | Chr6:64431689 | Novel | c.8301dupT(Insert A) | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP028 | EYS | Chr6:64431689 | Novel | rs144892841 | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP031 | EYS | Chr6:64431689 | Novel | rs20003892 | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP031 | FSCN2 | Chr17:79495999 | Reported in database | c.442C>T | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP039 | EYS | Chr6:64431689 | Novel | rs8907T>G | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP057 | EYS | Chr6:64431689 | Novel | rs8907T>G | p.Asp2767fs | . | . | . | . | . | Heterozygous

J-RP059 | EYS | Chr6:64431689 | Novel | rs8907T>G | p.Asp2767fs | . | . | . | . | . | Heterozygous

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| Patients ID | Gene | Chromosome position | Novelty | Nucleotide change | Amino acid Change | Polyclen2 HDIV | SIFT Mutations | Taster | Frequency 1000G | ExAC | Genotype |
|------------|------|---------------------|---------|------------------|-------------------|---------------|----------------|--------|----------------|------|----------|
| J-RP066   | EMC1 | Chr1:19577999       | Reported in database | c.5C>T | p.Ala2Val | D | D | D | . | 0.0001 | Heterozygous |
|           | FAM16IA | Chr2:62067223 | rs185615774 | 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      | PMC49191908 | 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 |
|           | EYS  | Chr6:64431689      | Novel | c.9437_9440del(AGTT) | p.Glu3146fs | . | . | . | . | . | . | Heterozygous |
| J-RP122   | EYS  | Chr6:64431689      | DOI:10.3760/cma.j.issn.1674-845X.2016.01.006 | c.8012T>A | p.Leu2671X | . | . | D | . | . | . | Heterozygous |
|           | 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      | PMID: 25,753,737 | c.6416G>A | p.Cys2139Tyr | D | D | D | . | 0.00005274 | Heterozygous |
| J-RP131   | EYS  | Chr6:64431689      | PMID: 25,753,737 | c.6416G>A | p.Cys2139Tyr | D | D | D | . | 0.00005274 | Heterozygous |
|           | PRPF6 | Chr20:62663398 | Reported in database | . | . | . | . | . | . | . | Heterozygous |
|           | 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 |
|           | EYS  | Chr6:64431689      | Novel | c.4908delA | p.Ala1636fs | . | . | . | . | . | . | Heterozygous |

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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.

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