Novel compound heterozygous EYS variants may be associated with arRP in a large Chinese pedigree

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As a genetically heterogeneous ocular dystrophy, gene mutations with autosomal recessive retinitis pigmentosa (arRP) in patients have not been well described. We aimed to detect the disease-causing genes and variants in a Chinese arRP family. In the present study, a large Chinese pedigree consisting of 31 members including a proband and another two patients was recruited; clinical examinations were conducted; next-generation sequencing using a gene panel was used for identifying pathogenic genes, and Sanger sequencing was performed for verification of mutations. Novel compound heterozygous variants c.G2504A (p.C835Y) and c.G6557A (p.G2186E) for the EYS gene were identified, which co-segregated with the clinical RP phenotypes. Sequencing of 100 ethnically matched normal controls didn't found these mutations in EYS. Therefore, our study identified pathogenic variants in EYS that may cause arRP in this Chinese family. This is the first study to reveal the novel mutation in the EYS gene (c.G2504A, p.C835Y), extending its mutation spectrum. Thus, the EYS c.G2504A (p.C835Y) and c.G6557A (p.G2186E) variants may be the disease-causing missense mutations for RP in this large arRP family. These findings should be helpful for molecular diagnosis, genetic counseling and clinical management of arRP disease.

Introduction

It is well known that retinitis pigmentosa (RP) is a large group of genetically heterogeneous ocular dystrophies [1–4], including autosomal recessive (arRP), autosomal dominant (adRP), and X-linked inheritance (xlRP). The EYS (Eyes shut homolog) gene (GenBank access number: NM_001142800.1), also known as SPAM, bA307F22.3, bA166P24.2, bA74E24.1, C6orf179, C6orf178, C6orf180, dJ1018A4.2, dJ22I17.2, EGFL10, dJ303F19.1, EGFL11, or RP25, is mapped on human chromosome 6q12; the EYS protein (GenBank access number: NP_001136272.1) encodes 3449-amino acids with a predicted molecular mass of 350 kDa. The EYS protein contains multiple epidermal growth factor (EGF)-like and LamG domains [5]. EGF-like domains have calcium-binding capability for protein–protein interactions, whereas LamG domains have binding sites for steroids, integrins, sulfatides, etc., which is important for maintaining the integrity of photoreceptor cells [6]. Indeed, the Eys knockout in zebrafish caused mislocalization of outer segment proteins, such as rhodopsin, opn1lw, opn1sw1, GNB3 and PRPH2, and disrupted actin filaments in photoreceptors [7].

Mutations in EYS (OMIM 612424) leading to retinitis pigmentosa 25 (RP25) for arRP (OMIM 602772) were first reported by two groups in 2008 [5,6]. Abd El-Aziz et al. analyzed six candidate genes within RP25 of 6q12 and identified six different EYS gene mutations in five unrelated families from ten Spanish pedigrees with arRP, leading to premature stop codons [6]. In another group, Collin et al. revealed homozygosity for a nonsense variant (p.Y3156X) and a deletion of 1 bp by analyzing the EYS gene in 10 arRP probands [5]. Mutations occurring in the EYS C-terminus in RP patients presented with hyperautofluorescent rings.
Figure 1. M195 pedigree with arRP

Normal individuals are shown as empty circles (females) or squares (males), and affected individuals are shown as filled symbols. The patient designated by the arrow is the proband (II:1), who has the compound heterozygous variants of the EYS gene: NM_001142800.1: c.G2504A:p.C835Y; c.G6557A:p.G2186E.

on fundus fluorescent photographs, elucidating genotype–phenotype correlations in EYS-associated RP (EYS-RP) [8]. More variants of the EYS gene were revealed [9–14] that caused changes in retinal structure and function [12,15,16]. Although the EYS gene mutations accounted for ~5% of arRP in a cohort of RP patients who were mainly of western Europe ancestry [17], the EYS mutations of patients with arRP and genotype–phenotype correlations in the Chinese population have not been well described. Here, next-generation sequencing (NGS) was conducted to identify novel, compound heterozygous variants in EYS from a large Chinese arRP family, thereby extending its mutation spectrum.

Materials and methods

Pedigree and clinical assessment

This research study was carried out in accordance with the World Medical Association Declaration of Helsinki. This was approved by the ethical committee at the Southwest Medical University and written informed consent was obtained from all subjects. A Chinese proband (Figure 1, pedigree II: 1, arrow) was recruited. For clinical assessment, an ophthalmic examination was conducted, including best-corrected Snellen visual acuity, Humphrey visual fields, optical coherence tomography (OCT), slit-lamp biomicroscopy, fundoscopy, fundus photograph (FP) and fundus fluorescein photograph (FFP), as in previous studies [18,19].

Sample collection and gDNA extraction

Fresh peripheral blood samples (2 ml each) from seven people in this family were collected, and gDNA was extracted by using our phenol/chloroform method that has been previously described [20–22]. One hundred healthy and ethnically matched individuals were sampled for DNA extraction as controls. A NanoDrop spectrophotometer (NanoDrop 2000, Wilmington, DC, U.S.A.) was used to measure the extracted DNA quality.

Capture panel design, sequencing and data analysis

To characterize the disease-causing genes in the M195 family, TES (targeted next-generation sequencing) analyses were performed on the proband (M195), according to the Illumina paired-end libraries as reported previously [23,24]. The capture Agilent probes were applied in the previous reports [23–27], using a retinal disease capture panel. Then, paired-end sequencing Illumine reads were applied to align to the human hg19 reference genome by BWA (version 0.6.1) and the UCSC database [28]. Variations of SNPs and INDELs were refined to look for the causative mutations in suspected genes. The pathogenicity in each variant was estimated using the following programs: Mutation Taster, PolyPhen-2, SIFT, and I-Mutant2.0. The ExAC and HGMD databases were used to search for novel mutations.

Primer design and PCR amplification

Locus-specific primer pairs (EYS-M195-16, EYS-M195-32), through the Primer 3 program with online website http://primer3.ut.ee/ containing mutations in the EYS gene, were designed [29] (Table 1). PCR products for EYS that were
Table 1 The sequences of PCR primers and PCR product sizes

| Primer name   | Left primer | Sequence (5′-3′)       | Right primer | Sequence (5′-3′)       | Size  | °C |
|---------------|-------------|------------------------|--------------|------------------------|-------|----|
| EYS-M195-16  | EYS-M195L16 | tggatggactggacagaact   | EYS-M195R16  | gtccttctccacatgtaca    | 374   | 60 |
| EYS-M195-32  | EYS-M195L32 | cagtcttttcctgtactggt   | EYS-M195R32  | cttcatgcactggtctggaa   | 478   | 60 |

374 bp and 478 bp in length were amplified using gDNA as a template. PCR amplification and DNA sequencing in the EYS variants were used to all the available gDNA for variant verification and segregation analysis [19,30].

Sanger sequencing and co-segregation analysis
All the PCR products were then directly used for Sanger method sequencing using a machine of ABI-3500DX sequencer from Applied Biosystems Inc. in our laboratory through the specific primers EYS-M195L16 or EYS-M195L32 (Table 1). All controls with unrelated ethnical-matched were also used to sequence by aforementioned primers in Table 1. For co-segregation, we conducted an analysis based on our sequenced results and the patient's clinical phenotype.

The structure of the EYS protein and bioinformatics
A search of conserved domains from coding nucleotide sequences or proteins by inputting 'EYS' was performed using an online NCBI system (Conserved Domain Search website: https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?INPUT_TYPE=precalc&SEQUENCE=224451128) [31,32]. Homologs were determined by the online NCBI system for the EYS protein: https://www.ncbi.nlm.nih.gov/homologene?Db=homologene&Cmd=Retrieve&list_uids=129971.

RNA expression profiles
The EYS mRNA expression profiles in 27 normal human tissues were obtained by RNA-sequencing to determine tissue-specificity through an online NCBI database (https://www.ncbi.nlm.nih.gov/gene/346007#gene-expression) [33]. Since no retinal tissue was obtained from RNA sequencing, the EYS mRNA expression profiles in different tissues, including the human retina were also obtained from the three transcriptomic datasets (https://www.proteinatlas.org/ENSG00000188107-EYS/summary/rna).

Results
Pedigree and clinical characteristics
A large Chinese pedigree, consisting of 31 members including a proband and two additional patients was recruited (Figure 1, pedigree II: 1 with arrow indicated). The proband, who was from a non-consanguineous family, is a 56-year-old male, who first showed night blindness symptom at 15 years of age. The FPs and FFPs of the proband (II:1) in both eyes and control images are shown in Figure 2. The proband clearly showed clear severe RPE atrophic changes, pigmentation with bone spicules in the retina of the peripheral-mid and transparent macula. The vessels were extremely small, and the optic disc was pale or waxen in both eyes (Figure 2A–D). Electroretinography (ERG) showed reduced cone and core responses or low amplitude ERG in the patient (data not shown). Two younger brothers of the proband showed similar RP feature. The proband’s parents and other family members didn’t exhibit any RP features, suggesting an autosomal recessive inheritance pattern. Based on pedigree analysis, the cases in this family are likely considered arRP.

NGS analysis and screening of pathogenic mutations
Causative mutations were revealed by targeted capture high-throughput sequencing (Figure 1, pedigree II: 1) [23,24]. Compound heterozygous variants, including a missense variant c.G2504A in exon 16 and a missense variant c.G6557A in exon 32 of the EYS gene (NM_001142800.1, isoform 1) in the M195 proband, were identified, leading to amino acid substitutions, from Cysteine (Cys, C) to Tyrosine (Tyr, Y) at position 835 (p.C835Y), and from Glycine (Gly, G) to Glutamic acid (Glu, E) at position 2186 (p.G2186E) of the EYS protein (NP_001136272.1), respectively (Figure 1 II: 1). The possible deleterious and pathogenic variants: c.G2504A (p.C835Y) and c.G6557A (p.G2186E) in the EYS gene are presented in Table 2.
Figure 2. Representative fundus photographs (FP) and fundus fluorescein photographs (FFP) of the proband II:1 from both eyes.

(A and B) FP and FFP of the proband in the right eye respectively. (C and D) FP and FFP of the proband in the left eye respectively. (E and F) Normal control images for FP and FFP in the right eye, respectively.

Table 2 Characteristics of EYS variants and analysis of disease-causing effects

| Gene | Exon | Variation | Protein* | Type | Status |
|------|------|-----------|----------|------|--------|
| EYS  | 16   | c.G2504A  | p.C835Y  | Missense | Heter | B(0.012) | DC (0.999) | DS | T(0.3) | Novel |
|      | 32   | c.G6557A  | p.G2186E | Missense | Heter | PD (0.98) | DC (0.999) | DS | T(0.31) | known |

Abbreviations: B, benign; c, variation at cDNA level; DC, disease causing; DS, decreased stability; ExAC, Exome Aggregation Consortium; EYS, eyes shut homolog; Heter, heterozygote; p, variation at protein level; PD, probably damaging; T, tolerated.* All nucleotides and amino acids are abbreviated according to the International Union of Pure and Applied Chemistry (IUPAC).

Variant confirmation and analysis of co-segregation results

Although deficient, variant confirmation and analysis of co-segregation for EYS were conducted by Sanger sequencing (Figure 3). The c.G2504A and c.G6557A variants of EYS were confirmed in the mutant compound heterozygous types in the proband (pedigree II:1; Figure 3A,F); and we revealed wild-type without RP in the proband’s wife (pedigree II:2; Figure 3B,G), mutant heterozygous types without RP symptoms in the proband’s mother (pedigree II:2; Figure 3C,H) and his younger sister (pedigree II:8; Figure 3D,I), wild-type without RP symptoms in the proband’s daughter (pedigree III:2; Figure 3E,J), and mutant compound heterozygous types in the proband’s two younger brothers (II:3 and II:5 with RP symptoms, data not shown). Thus, these findings show co-segregation with disease in this family we tested, and suggest its role in pathogenesis. Furthermore, 100 ethnically matched normal controls were sequenced for both variants of EYS; no variants were revealed (data not shown).

Effects of function in EYS variants c.G2504A (p.C835Y) and c.G6557A (p.G2186E)

Searching of the Conserved Domain Database (CDD) from NCBI was performed. Comparing human EYS protein to other species indicated that EYS is conserved among chicken, dog, rhesus monkey, and zebrafish (Figure 4A).
Figure 3. Photogram profiles for EYS verification by Sanger sequencing
Panels (A–E) indicate the sequencing results for the EYS mutant allele of the c.G2504A or its wild-type, whereas panels (F–J) indicate the sequencing results for the EYS mutant allele of the c.G6557A or its wild-type. The arrows indicate mutations at the nucleotide positions: c.G2504A or c.G6557A in the EYS gene. W, wild-type allele.

Figure 4. EYS structure and conservation domains
(A) EYS ortholog, structure and domains. (B) Comparison of the conservation domain of EYS and the position of variant p.C835Y. Conserved amino acids are highlighted in yellow.
(C) Comparison of the conservation domain of EYS and the position of variant p.G2186E. Conserved amino acids are highlighted in yellow.
EYS mRNA expression profiles

(A) Expression profiles for EYS mRNA in 27 indicated human samples. RPKM: Reads Per Kilobase Million. (B) Expression profiles for EYS mRNA in 55 indicated tissue types and 6 blood cell types; NX, consensus normalized expression.

Figure 5. EYS mRNA expression profiles

(A) Expression profiles for EYS mRNA in 27 indicated human samples. RPKM: Reads Per Kilobase Million. (B) Expression profiles for EYS mRNA in 55 indicated tissue types and 6 blood cell types; NX, consensus normalized expression.

has two repeated conserved domains: calcium-binding EGF-like domain (EGF_CA, cl09941) and laminin G domain (LamG, cl17353). EYS variant p.C835Y, located in the EGF_CA domain (amino acid 810~846), and p.G2186E, located in the LamG domain (amino acid 2149~2315), were also highly conserved (Figure 4B,C), which may affect protein function. However, no EYS ortholog in mice was found.

MutationTaster revealed disease-causing, SIFT revealed tolerated, and I-Mutant2.0 showed decrease stability for both p.C835Y and p.G2186E variants; Polyphen 2 for p.G2186E showed probably damaging, whereas Polyphen 2 for p.C835Y showed benign (Table 2). Thus, these compound and heterozygous variants c.G2504A (p.C835Y) and c.G6557A (p.G2186E) in EYS might damage protein function in this arRP family. The variant c.G2504A (p.C835Y) revealed a novel mutation even though c.G6557A (p.G2186E) was not found [16] in a search of the HGMD and ExAC databases (Table 2).

Comprehensively, the present study shows that compound heterozygous, pathogenic missense variants of EYS c.G2504A (p.C835Y) and c.G6557A (p.G2186E) may cause arRP in this large Chinese pedigree.

Expression profiles of EYS mRNA

RNA-seq data showed that EYS has low expression in 27 tested different human tissues but fat has highest expression (RPKM value: 0.397 ± 0.042), followed by the testis (RPKM value: 0.258 ± 0.112); the pancreas has the lowest expression (RPKM value: 0.008 ± 0.002) (Figure 5A and Table 3). The protein is expressed in the photoreceptor layer of the retina [34,35]. However, no eye tissue data were shown by RNA-seq; no mouse EYS ortholog existed. Then the EYS mRNA expression profiles in more different tissues, including the retina and cells (55 tissue types and 6 blood cell types) were also obtained, and the results showed that EYS is most highly expressed in the retina of humans, with an NX value of 40.7 in the retina, but an NX value of only 2.9 in the testis (Figure 5B). Thus, this demonstrated that EYS is only highly expressed in retinal tissue and probably only plays a vital role in the retina of the eye.
Table 3 Expression of EYS mRNA in human different tissues

| Sample          | Numbers | RPKM values | Counts |
|-----------------|---------|-------------|--------|
| Adrenal         | 3       | 0.036 ± 0.007 | 5527   |
| Appendix        | 3       | 0.045 ± 0.01  | 6330   |
| Bone marrow     | 4       | 0.016 ± 0.001 | 6091   |
| Brain           | 3       | 0.055 ± 0.012 | 9646   |
| Colon           | 5       | 0.122 ± 0.086 | 61,808 |
| Duodenum        | 2       | 0.047 ± 0.014 | 4398   |
| Endometrium     | 3       | 0.031 ± 0.015 | 5475   |
| Esophagus       | 3       | 0.019 ± 0.006 | 4820   |
| Fat             | 3       | 0.397 ± 0.042 | 61,275 |
| Gall bladder    | 3       | 0.036 ± 0.007 | 9284   |
| Heart           | 4       | 0.019 ± 0.012 | 7054   |
| Kidney          | 4       | 0.058 ± 0.009 | 11,252 |
| Liver           | 3       | 0.056 ± 0.017 | 9539   |
| Lung            | 5       | 0.019 ± 0.013 | 5828   |
| Lymph node      | 5       | 0.038 ± 0.013 | 17,239 |
| Ovary           | 2       | 0.052 ± 0.004 | 10,458 |
| Pancreas        | 2       | 0.008 ± 0.002 | 1487   |
| Placenta        | 4       | 0.042 ± 0.011 | 14,889 |
| Prostate        | 4       | 0.049 ± 0.014 | 9969   |
| Salivary gland  | 3       | 0.018 ± 0.003 | 5785   |
| Skin            | 3       | 0.041 ± 0.026 | 11,209 |
| Small intestine | 4       | 0.045 ± 0.023 | 9755   |
| Spleen          | 4       | 0.035 ± 0.008 | 10,262 |
| Stomach         | 3       | 0.03 ± 0.013  | 6032   |
| Testis          | 7       | 0.258 ± 0.112 | 1,79,148 |
| Thyroid         | 4       | 0.071 ± 0.016 | 26,332 |
| Urinary bladder | 2       | 0.023 ± 0.007 | 3920   |

Discussion

The EYS protein (NP_001136272.1) has different isoforms. Consistent with RNA expression data (Figure 5), expression analysis by PCR of cDNA from tissues and cell lines showed that the EYS gene is only expressed in the retina and in a retinoblastoma cell line in humans [6]. Immunohistochemistry (IHC) in mature pig retinas showed Eys expression at the photoreceptor layer. In a zebrafish model, Eys absence led to degeneration in the photoreceptor outer segments, photoreceptor death, disorganized retinal architecture, decreased ERG responses and caused visual dysfunction [7,36,37], whereas in humans, by novel EYS mutations, IHC revealed advanced retinal degenerative changes of in all eyes with rod photoreceptor absence [38]. Collin et al. in 2008 independently identified a large transcript, encoding 3165 amino acids (isoforms 4, NP_001278938) with a signal peptide, and domains with EGF-like and laminin A G-like. All four EYS transcripts were expressed in the retina and the Y79 cell line of humans, whereas isoforms 2 and 3 of EYS are also expressed in the testis [35]. BLAST analyses found that this EYS gene was the true ortholog of the Drosophila 'eyes shut' (eys) gene. Surprisingly this EYS gene is abundantly expressed in the retina in humans, but Eys is completely absent in mouse. Expression profiles of EYS mRNA showed that it is only highly expressed in the retina tissue of humans but not in other tissues (Figure 5), demonstrating that EYS probably only plays a vital role in the retina of the eye.

The targeted NGS approach has been proven to be efficient in the genetic diagnosis of RP [9,10,30]. In this paper, we identified that compound heterozygous, missense variants (c.G2504A, p.C835Y; c.G6557A, p.G2186E) of EYS may cause arRP in the large Chinese family by targeted NGS. Analyses by MutationTaster revealed disease causing, SIFT revealed tolerated, and I-Mutant2.0 showed decrease stability for both variants; Polyphen 2 for p.G2186E was probably damaging, whereas Polyphen 2 for p.C835Y was benign. Thus, combined all information, these compound and heterozygous variants in EYS might be pathogenic in this large Chinese pedigree by damaging and reducing the stability of the EYS protein. Variant p.C835Y for EYS is located in a conserved EGF,CA domain that may be crucial for protein–protein interactions, whereas variant p.G2186E for EYS is located at a conserved LamG domain that has the role in signal transduction, adhesion, migration and differentiation by mediating cell adhesion molecule through binding. In this regard, the EYS variants p.C835Y and p.G2186E may interrupt protein–protein interactions and
signal transduction, leading to the pathogenesis of arRP. To the best of our knowledge, the EYS mutation of c.G2504A (p.C835Y) is novel and enriches the EYS mutation spectrum, and is linked with arRP phenotypes. Therefore, these findings will help in understanding the molecular pathogenesis of RP for diagnosis, prevention as well as genetic counseling.

Conclusion
In summary, our study identified the compound heterozygous variants c.G2504A (p.C835Y) and c.G6557A (p.G2186E) of EYS, which may cause arRP in this large Chinese family. It is the first study to reveal the novel EYS mutation (c.G2504A, p.C835Y) for RP disorder in our Chinese patients, expanding the EYS mutation spectrum. These findings should help in understanding the molecular pathogenesis of arRP disease for diagnosis, clinical management and genetic counseling.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Funding
The authors gratefully thank the patients and their family for providing samples. We thank R.C. for help with NGS analysis. This work was funded by the National Natural Science Foundation of China [grant numbers 81672887, 31701087, and 30371493]; and the Joint Research Foundation of Luzhou City and Southwest Medical University [grant number 2018LZXNYD-YL01].

Author Contribution
C.W., J.F., L.Y. and J.C. were in charge of PCR, sequencing and data analysis. J.C. and T.X. performed DNA extraction. H.L. and Q.Z. enrolled the clinical patients and performed clinical assessments. J.F. designed the project, wrote and revised the manuscript.

Ethics Approval
The study has the Ethical Committees approval granted by the Southwest Medical University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent for Publication
Written informed consent was obtained from all participants or their guardians.

Abbreviations
arRP, autosomal recessive retinitis pigmentosa; adRP, autosomal dominant retinitis pigmentosa; EGF, epidermal growth factor; FP, fundus photograph; FFP, fundus fluorescein photograph; RP25, retinitis pigmentosa 25; xIRP, X-linked inheritance retinitis pigmentosa.

References
1 Ali, M.U., Rahman, M. S.U., Cao, J. and Yuan, P.X. (2017) Genetic characterization and disease mechanism of retinitis pigmentosa; current scenario. J Biotech 7, 251, https://doi.org/10.1007/s13205-017-0878-3
2 Maubaret, C. and Hamel, C. (2005) Genetics of retinitis pigmentosa: metabolic classification and phenotype/genotype correlations. J. Francais d’ophtalmologie 28, 71–92, https://doi.org/10.1016/S0181-5512(05)81029-0
3 Ferrari, S., Di Iorio, E., Barbaro, V., Ponzin, D., Sorrentino, F.S. and Parmeggiani, F. (2011) Retinitis pigmentosa: genes and disease mechanisms. Curr. Genomics 12, 238–249
4 Allard, R.E. (1983) Retinitis pigmentosa—an overview. J. Am. Optom. Assoc. 54, 793–800
5 Collin, R.W., Littink, K.W., Klevering, B.J., van den Born, L.I., Koenekoop, R.K., Zonneveld, M.N. et al. (2008) Identification of a 2 Mb human ortholog of Drosophila eyes shut/spacemaker that is mutated in patients with retinitis pigmentosa. Am. J. Hum. Genet. 83, 594–603, https://doi.org/10.1016/j.ajhg.2008.10.014
6 El-Aziz, Abd, M., Barragan, I., O’Driscoll, C.A., Goodstadt, L., Prigmore, E. et al. (2008) EYS, encoding an ortholog of Drosophila spacemaker, is mutated in autosomal recessive retinitis pigmentosa. Nat. Genet. 40, 1285–1287, https://doi.org/10.1038/ng.241
7 Lu, Z., Hu, X., Liu, F., Soares, D.C., Liu, X., Yu, S. et al. (2017) Ablation of EYS in zebrafish causes mislocalisation of outer segment proteins, F-actin disruption and cone-rod dystrophy. Sci. Rep. 7, 46098, https://doi.org/10.1038/srep46098
8 Sengillo, J.D., Lee, W., Nagasaki, T., Scheruch, K., Yannuzzi, L.A., Freund, K.B. et al. (2018) A Distinct Phenotype of Eyes Shut Homolog (EYS)-Retinitis Pigmentosa Is Associated With Variants Near the C-Terminus. Am. J. Ophthalmol. 190, 99–112, https://doi.org/10.1016/j.ajo.2018.03.008
9 Gu, S., Tian, Y., Chen, X. and Zhao, C. (2016) Targeted next-generation sequencing extends the phenotypic and mutational spectrums for EYS mutations. Mol. Vis. 22, 646–657

10 Chen, X., Liu, X., Sheng, X., Gao, X., Zhang, X., Li, Z. et al. (2015) Targeted next-generation sequencing reveals novel EYS mutations in Chinese families with autosomal recessive retinitis pigmentosa. Sci. Rep. 5, 8927, https://doi.org/10.1038/srep08927

11 Hirashima, T., Miyata, M., Ishihara, K., Hasegawa, T., Sugahara, M., Ogino, K. et al. (2017) Choroidal Vasculature in Bietti Crystalline Dystrophy With CYP4V2 Mutations and in Retinitis Pigmentosa With EYS Mutations. Invest. Ophthalmol. Vis. Sci. 58, 3871–3878, https://doi.org/10.1167/iovs.17-21515

12 Hashimi, J.A., Albarry, M.A., Almatrafi, A.M., Albalawi, A.M., Mahmood, A. and Basit, S. (2018) Whole exome sequencing identified a novel single base pair insertion mutation in the EYS gene in a six generation family with retinitis pigmentosa. Congenit. Anom. (Kyoto) 58, 10–15, https://doi.org/10.1093/cga/122255

13 Di, Y., Huang, L., Sundaresan, P., Li, S., Kim, R., Ballav Saikia, B. et al. (2016) Whole-exome Sequencing Analysis Identifies Mutations in the EYS Gene in Retinitis Pigmentosa in the Indian Population. Sci. Rep. 6, 19432, https://doi.org/10.1038/srep19432

14 Messchaert, M., Haer-Wigman, L., Khan, M.I., Cremers, F.P.M. and Collin, R.W.J. (2018) EYS mutation update: In silico assessment of 271 reported and 26 novel variants in patients with retinitis pigmentosa. Hum. Mutat. 39, 177–186, https://doi.org/10.1002/humu.23731

15 Mcguigan, D.B., Heon, E., Cideciyan, A.V., Ratnapriya, R., Lu, M., Sumaroka, A. et al. (2017) EYS Mutations Causing Autosomal Recessive Retinitis Pigmentosa: Changes of Retinal Structure and Function with Disease Progression. Genes 8, 178, https://doi.org/10.3390/genes8070178

16 Xiao, X., Cao, Y., Chen, S., Chen, M., Mai, X., Zheng, Y. et al. (2019) Whole exome sequencing reveals novel EYS mutations in Chinese patients with autosomal recessive retinitis pigmentosa. Mol. Vis. 25, 35–46

17 Littink, K.W., van den Born, L.I., Koenekoop, R.K., Collin, R.W., Zonneveld, M.N., Blokland, E.A. et al. (2010) Mutations in the EYS gene account for approximately 5% of autosomal recessive retinitis pigmentosa and cause a fairly homogeneous phenotype. Ophthalmology 117, 2026e1–7-2033e1-7, https://doi.org/10.1016/j.ophtha.2010.01.040

18 Imani, S., Mobasher-Jannat, A., Wei, C., Fu, S., Yang, L. et al. (2018) Identification of a novel RPGRIP1 mutation in an Iranian family with leber congenital amaurosis by exome sequencing. J. Cell. Mol. Med. 22, 1733–1742, https://doi.org/10.1111/jcmm.13454

19 Fu, J., Ma, L., Cheng, J., Yang, L., Wei, C., Fu, S. et al. (2018) A novel, homozygous nonsense variant of the CDH1 gene in a Chinese family causes autosomal recessive retinal dystrophy by NGS-based genetic diagnosis. J. Cell. Mol. Med. 22, 5662–5669, https://doi.org/10.1111/jcmm.13841

20 Fu, J., Li, L. and Lu, G. (2002) Relationship between microdeletion on Y chromosome and patients with idiopathic azoospermia and severe oligozoospermia in the Chinese. Chin. Med. J. 115, 72–75

21 Cheng, J., Fu, J., Zhou, Q., Xiang, X., Wei, C., Yang, L. et al. (2019) A novel splicing mutation in the PRPH2 gene causes autosomal dominant retinitis pigmentosa in a Chinese pedigree. J. Cell. Mol. Med. 23, 3776–3780, https://doi.org/10.1111/jcmm.14278

22 Cheng, J., Peng, J., Fu, J., Khan, M.A., Tan, P., Wei, C. et al. (2020) Identification of a novel germline BRCA2 variant in a Chinese breast cancer family. J. Cell. Mol. Med. 24, 1676–1683, https://doi.org/10.1111/jcmm.14861

23 Wang, F., Wang, H., Tuan, H.H., Nguyen, D.H., Sun, V., Keser, V. et al. (2014) Next generation sequencing-based molecular diagnosis of retinitis pigmentosa: identification of a novel genotype-phenotype correlation and clinical refinements. Hum. Genet. 133, 331–345, https://doi.org/10.1007/s00439-013-1381-5

24 Zhang, Q., Xu, M., Verrott, J.D., Li, Y., Wang, H., Gan, L. et al. (2016) Next-generation sequencing-based molecular diagnosis of 35 Hispanic retinitis pigmentosa probands. Sci. Rep. 6, 32792, https://doi.org/10.1038/srep32792

25 Fu, H., Xu, M., Chen, X., Sheng, X., Yuan, Z., Liu, Y. et al. (2017) CEP78 is mutated in a distinct type of Usher syndrome. J. Med. Genet. 54, 190–195, https://doi.org/10.1136/jmedgenet-2016-104166

26 Hu, P., Wu, S., Yuan, L., Lin, Q., Zheng, W., Xia, H. et al. (2017) Compound heterozygous POMT1 mutations in a Chinese family with autosomal recessive muscular dystrophy-dystroglycanopathy C1. J. Cell. Mol. Med. 21, 1388–1393, https://doi.org/10.1111/jcmm.13086

27 Zhang, L., Zhou, Q., Cheng, J., Khan, M.A., Fu, J., Duan, C. et al. (2020) Targeted next-generation sequencing identified novel compound heterozygous variants in the CDH23 gene causing Usher syndrome type ID in a Chinese patient. Front. Genet. 11, 422, https://doi.org/10.3389/fgene.2020.00422

28 Li, H. and Durbin, R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754–1760, https://doi.org/10.1093/bioinformatics/btp324

29 Imani, S., Cheng, J., Fu, J., Mobasher-Jannat, A., Wei, C., Mohazzab-Torabi, S. et al. (2019) Novel splicing variant c.208+2T>C in BBS segregates with Bardet-Biedl syndrome in an Iranian family by targeted exome sequencing. Biosci. Rep. 39

30 Wei, C., Yang, L., Cheng, J., Imani, S., Fu, S., Lv, H. et al. (2018) A novel homozygous variant of GPR98 causes usher syndrome type IIIC in a consanguineous Chinese family by next generation sequencing. BMC Med. Genet. 19, 99, https://doi.org/10.1186/s12881-018-0602-0

31 Marchler-Bauer, A., Bo, Y., Han, L., He, J., Lanczycki, C.J., Lu, S. et al. (2017) CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. Nucleic Acids Res. 45, D200–D203, https://doi.org/10.1093/nar/gkw1129

32 Imani, S., Ijaz, I., Shasaltaneh, M.D., Fu, S., Cheng, J. and Fu, J. (2018) Molecular genetics characterization and homozygote modeling of the CHM gene mutation: A study on its association with choroideremia. Mutat. Res. 775, 39–50, https://doi.org/10.1016/j.mrrv.2018.02.001

33 Zhou, B., Wei, C., Khan, M.A., Chen, H. and Fu, J. (2019) Characterization and molecular cloning of novel isoforms of human spermatogenesis associated gene SPATA3A. Mol. Biol. Rep. 46, 3827–3834, https://doi.org/10.1007/s12051-019-04825-4

34 Guduric-Fuchs, J., Ringland, L.J., Gu, P., Dellett, M., Archer, D.B. and Cogliati, T. (2009) Immunohistochemical study of pig retinal development. Mol. Vis. 15, 1915–1928

35 Alfano, G., Krucek, P.M., Shah, A.Z., Kramarz, B., Jeffery, G., Zelhof, A.C. et al. (2016) EYS Is a Protein Associated with the Ciliary Axoneme in Rods and Cones. PLoS ONE 11, e0166397, https://doi.org/10.1371/journal.pone.0166397

36 Messcher, M., Dona, M., Broekman, Ş., Peters, T.A., Corral-Serrano, J.C., Slijkerman, R.W.N. et al. (2018) Eyes shut homolog is important for the maintenance of photoreceptor morphology and visual function in zebrafish. PLoS ONE 13, e0200789, https://doi.org/10.1371/journal.pone.0200789
37 Yu, M., Liu, Y., Li, J., Natale, B.N., Cao, S., Wang, D. et al. (2016) Eyes shut homolog is required for maintaining the ciliary pocket and survival of photoreceptors in zebrafish. Biol. Open 5, 1662–1673, https://doi.org/10.1242/bio.021584

38 Bonilha, V.L., Rayborn, M.E., Bell, B.A., Marino, M.J., Pauer, G.J., Beight, C.D. et al. (2015) Histopathological comparison of eyes from patients with autosomal recessive retinitis pigmentosa caused by novel EYS mutations. Graefe's Archive Clin. Exp. Ophthalmol. = Albrecht von Graefes Archiv fur Klinische und Experimentelle Ophthalmologie 253, 295–305, https://doi.org/10.1007/s00417-014-2668-z