Two heterozygous mutations identified in one Chinese patient with bilateral macular coloboma

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Abstract. Congenital macular coloboma is characterized by defined punched out atrophic lesions of the macula. The present study aimed to investigate the genetic alterations of one Chinese sporadic patient with bilateral large macular coloboma. Complete ophthalmic examinations, including best-corrected visual acuity, slit-lamp examination, fundus examination, fundus photograph and fundus fluorescein angiography imaging, Pentacam, and optical coherence tomography were performed on the patient. Genomic DNA was extracted from leukocytes in a peripheral blood sample collected from the patient, the patient's unaffected family members and from 200 unrelated control subjects from the same population. Next-generation sequencing of the known genes involved in ocular disease was performed. The functional effects of the mutation were analyzed using Polymorphism Phenotyping (PolyPhen) and Sorting Intolerant From Tolerant (SIFT). One heterozygous bestrophin 1 (*BEST1*) mutation c.1037C>A (p.Pro346His, p.P346H) in exon 9 and one heterozygous regulating synaptic membrane exocytosis 1 (*RIMS1*) mutation c.3481A>G (p.Arg1161Gly, p.R1161G) in exon 23 were identified in the patient being investigated, but not in the unaffected family members or unrelated control subjects. Polyphen and SIFT predicted that the amino acid substitution p.P346H in the BEST1 protein is damaging. In addition, Polyphen predicted that the amino acid substitution p.R1161G in the RIM1 protein is damaging. The results of the current study have increased the mutation spectrums of *BEST1* and *RIMS1*, and are valuable for improving the current genetic counseling process and developing novel therapeutic interventions for patients with macular coloboma.

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Introduction

Congenital coloboma is a very rare birth defect with a prevalence of 0.5-0.7/10,000 live births (1). Congenital macular coloboma is characterized by well-circumscribed, punched out atrophic lesions in the macula (2-4). Macular coloboma should be differentiate from other diseases, such as Best vitelliform macular dystrophy (BVMD), advanced cone-rod dystrophy (CORD), congenital toxoplasmosis macular scar, Leber's congenital amaurosis, and central areolar choroidal dystrophy (5,6). It is usually sporadic, although autosomal dominant or other inheritance patterns may be followed. It is thought to be caused by the failure of normal closure of the optic fissure between 5 and 7 weeks of development (1). Macular coloboma can be classified into three types, namely pigmented macular coloboma, non-pigmented macular coloboma, and macular coloboma associated with abnormal vessels (7).

The genetic changes responsible for the pathogenesis of congenital macular coloboma are not well studied. Identification of genetic mutations in congenital macular coloboma is the first step to unravel the pathogenesis of this disease and will be helpful for genetic counseling. In this

study, we aimed to characterize the clinical presentation of a 28-year-old female presented with bilateral large macular coloboma, and to identify the underlying genetic changes in this patient.

Patients and methods

Study participants. One patient presented with bilateral large atrophy at the macula in both eyes underwent complete ophthalmic examinations in Zhongshan Ophthalmic Center. Visual acuity was examined using the ETDRS chart (Precision Vision, La Salle, IL, USA). Anterior segment photograph was obtained using a BX 900 Slit Lamp (Haag-Streit, Bern, Switzerland). Anterior segment measurements were taken by Pentacam HR version 70700 (Oculus, Wetzlar, Germany). Fundus photograph were carried out using a Heidelberg Retina Angiograph (Heidelberg Engineering, Inc., Heidelberg, Germany). OCT was carried out by Cirrus HD-OCT (Carl Zeiss Meditec, Inc., Dublin, CA, USA). Physical examinations were performed to exclude systemic diseases. Venous blood samples from this patient, her unaffected family members, and 200 unrelated control subjects from the same population were collected.

Target capture and next-generation sequencing. A capture panel of inherited retinal-disease genes was previously designed and assessed by our group. The capture panel comprised 708,919 bp that covered all exons together with the flanking exon and intron boundaries (±15 bp) of 175 genes, including 138 genes causing common inherited nonsyndromic eye diseases and 54 genes causing syndromic eye diseases that have been previously reported and have been accepted by researcheres in this field.

Genomic DNA from peripheral blood leucocytes was extracted using the QIAamp DNABlood Midi Kit (Qiagen, Hilden, Germany). Then the genomic DNA was fragmented by Covaris LE220 (Covaris, Inc., Woburn, MA, USA) to generate paired-end library (200-250 bp). The library was enriched by array hybridization as previously described (8), followed by elution and post-capture amplification. The products were then subjected to Agilent 2100 Bioanalyzer and ABI StepOne for estimating the magnitude of enrichment. After quality control, captured library sequencing was carried out on Illumina HiSeq2500 Analyzers (Illumina, San Diego, CA, USA) for 90 cycles per read to generate paired-end reads. Image analysis, error estimation, and base calling were performed using Illumina Pipeline software (version 1.3.4) to generate raw data.

Data analysis and interpretation of genetic variants. To detect the potential variants in the family, we performed bioinformatics processing and data analysis after receiving the primary sequencing data. We used previously published filtering criteria to generate 'clean reads' for further analysis (8). The 'clean reads' (with a length of 90 bp) derived from targeted sequencing and filtering were then aligned to the human genome reference (hg19) using the BWA (Burrows Wheeler Aligner) Multi-Vision software package (9). After alignment, the output files were used to perform sequencing coverage and depth analysis of the target region, single-nucleotidevariants (SNVs) and INDEL calling. We used SOAPsnp software (9) and Samtools (10) to detect SNVs and indels. All SNVs and indels were filtered and estimated via multiple databases, including NCBI dbSNP, HapMap, 1,000 human genome dataset and a database of 200 Chinese healthy adults.

To predict the effect of missense variants, SIFT and PolyPhen were used to predict the possible impact of an amino acid substitution on the protein structure and function using straightforward physical and comparative considerations. Variants were predicted to be pathogenic only when at least one of the two programs predicted deleterious effect of the amino acid substitution on the protein structure and function. The Human Gene Mutation Database (HGMD) was used to screen mutations reported in published studies.

We also used PolyPhen to check whether the mutations affected highly conserved amino acid residues.

Mutation validation. The two novel pathogenic mutations were validated using conventional polymerase chain reaction (PCR) -based sequencing methods (11-13). Exon 9 of the *BEST1* gene and the Exon 23 of *RIMS1* were amplified by PCR with respective primers (Table I). Briefly, PCR was conducted in 50 μl reactions. The cycling profile included one cycle at 94˚C for 5 min, followed by 40 cycles at 94˚C for 45 sec, 59-60˚C for 45 sec, 72˚C for 45 sec, and one cycle at 72˚C for 10 min. The PCR products were sequenced from both directions with an ABI3730 Automated Sequencer (PE Biosystems, Foster City, CA, USA). The sequencing results were analyzed using Seqman (version 2.3; Technelysium Pty, Ltd., Brisbane, QLD, Australia), and compared with the reference sequences in the database at the National Center for Biotechnology Information.

All experimental protocols were carried out according to the guidelines approved by the Ethics Committee of Zhongshan Ophthalmic Center, and in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects. The data generated or analyzed in the current study are included herein.

Results

Clinical data. The patient studied in this report was from the southern area of China. Patient is a 28-year-old female without known familial history of ocular disease. Her best-corrected visual acuity was 1.3 LogMAR in the right eye, and figure count/40 cm (FC/40 cm) in the left eye. Anterior segment photograph showed some opacities in the lens of both eyes (Fig. 1). Fundus examination revealed bilateral large atrophy in the macula of each eye with well-circumscribed borders (Fig. 2). OCT showed that the foveal region of both eyes were abnormally thin. A large cave in the macular area and retinal schisis were observed in the left eye (Fig. 3).

Mutation screening. A heterozygous *BEST1* mutation c.1037C>A (p.Pro346His, p.P346H) in exon 9 and a heterozygous *RIMS1* mutation c.3481A>G (p.Arg1161Gly, p.R1161G) in exon 23 were identified in the affected case, but not in any of the normal controls (Fig. 4). The first mutation we identified has previously been reported in Japanese

Table I. Primers used for the amplification of the *BEST1* and *RIMS1* in this study.

Gene	Exon	Forward (5'-3')	Reverse (5'-3')	Product size (bp)	Annealing temperature (˚C)
BEST1	9	CAGGGAAACTGAGGTCCAGA	AGGCTGTCCTTCGAGTAGCA	539	60
RIMS1	23	GGCGGATTCCAAACATCTTCC	AGGTGCTTTACCAGAGTTGGC	487	60

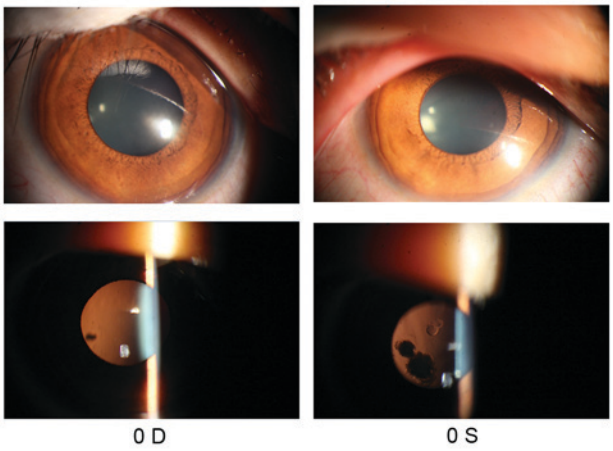

Figure 1. Anterior segment photograph showed some opacities in the lens of both eyes.

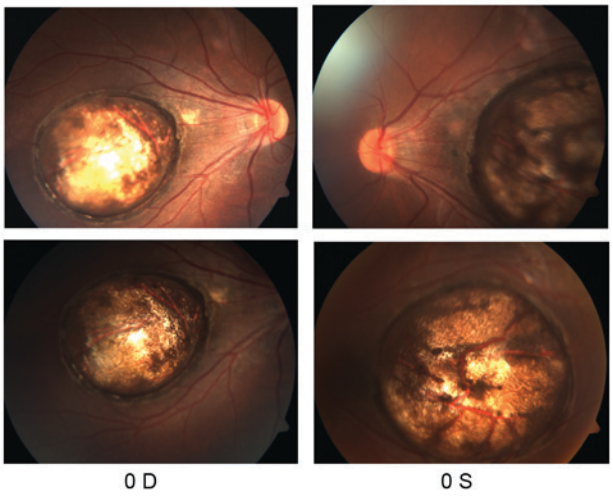

Figure 2. Fundus examination revealed bilateral large atrophy in the macula of each eye with well-circumscribed borders.

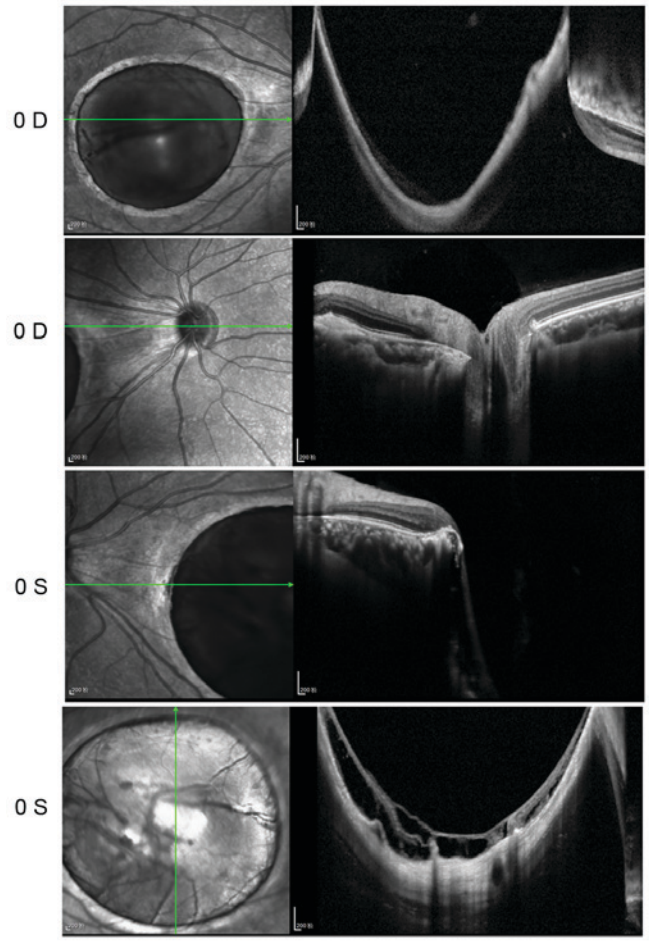

Figure 3. OCT showed that the foveal region of both eyes were abnormally thin. A large cave in the macular area and retinal schisis were observed in the left eye.

patients (14). Since we were unable to obtain information of the patient's parents, we could not determine the inheritance pattern of the mutations. Polyphen and SIFT predicted that the amino acid substitution p.P346H in protein bestrophin 1 is damaging (Fig. 5), and Polyphen predicted that the amino acid substitution p.R1161G in protein RIM1 is damaging (Fig. 6). Multiple sequenced alignment indicated that the residue at position 346 of bestrophin-1 and the residue at position 1161 of RIM1 are highly conserved across species.

Discussion

Macular coloboma may result from intrauterine inflammation (15), and can be associated with systemic developmental abnormalities. Notably, it may be difficult to distinguish macular coloboma with macular atrophy if medical history is not provided. The underlying biological mechanism for development of macular coloboma is unclear. Interestingly, we found that this patient had two different mutations simultaneously, reminding us that some serious congenital defects can be caused by multiple mutations on multiple genes, making gene therapy more challenging.

BVMD is one of the most frequent form of autosomal dominant macular dystrophy (16). It is associated

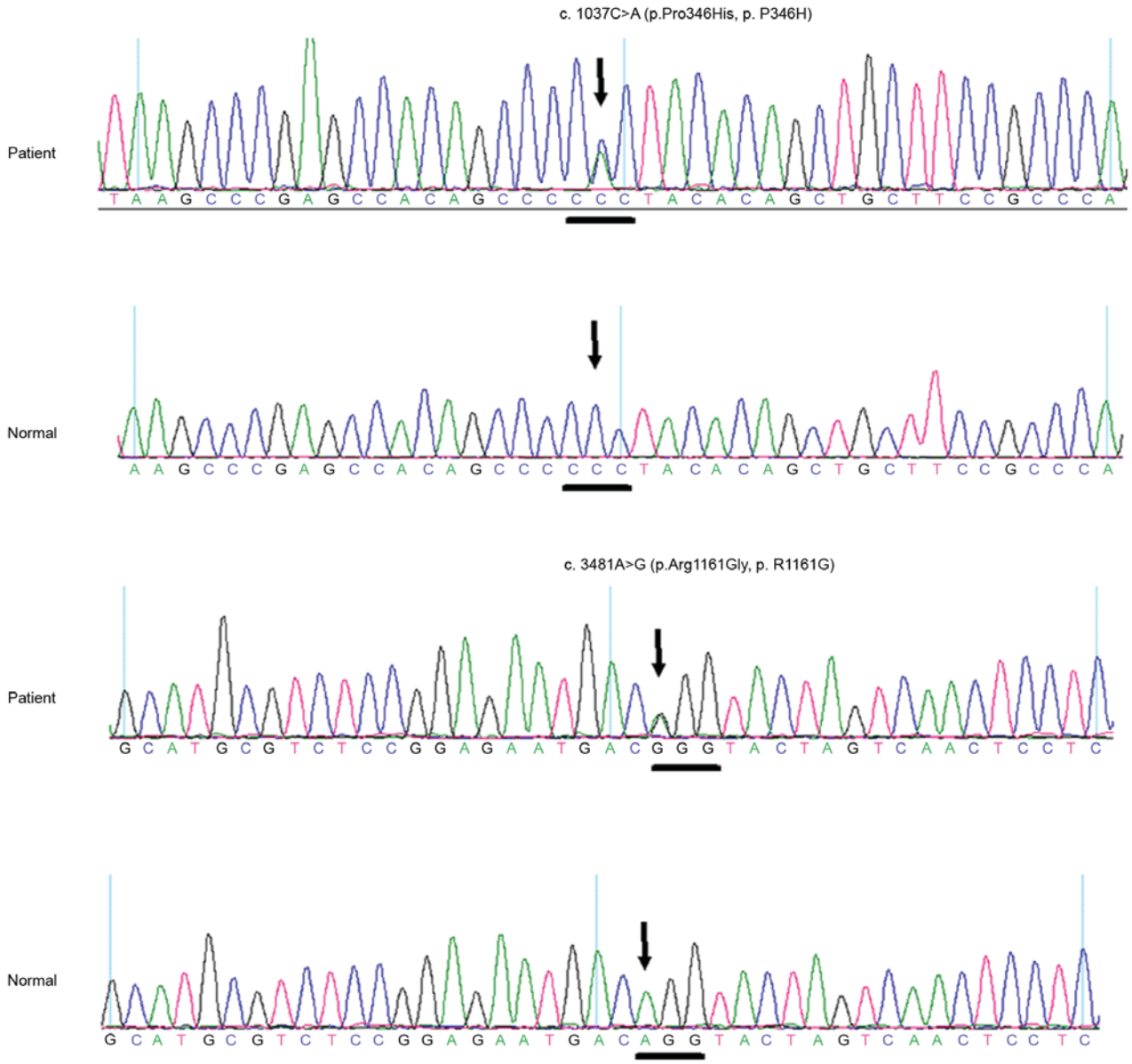

Figure 4. A heterozygous *BEST1* mutation c.1037C>A (p.Pro346His) in exon 9 and a heterozygous *RIMS1* mutation c.3481A>G (p.Arg1161Gly) in exon 23 were identified in the affected case, but not in the unaffected family members or unrelated control subjects.

with mutations in the *BEST1* gene (17) and results from dysfunction of the retinal pigment epithelium (RPE) (18). Bestrophin-1 is the product of the gene *BEST1*. This protein is mainly expressed in the basolateral plasma membrane of the RPE (19). This protein contains several domains with a high degree of evolutionary conservation. The function of Bestrophin-1 remains unclear, and some studies proposed that it acts as a Cl^- channel activated by intracellular Ca^{2+} and/or as a channel regulator (20,21). A previous study conducted by Katagiri *et al* (14). identified a novel mutation p.P346H on *BEST1* in a 38-year-old patient who was in the vitelliruptive stage, which was less serious than the patient in our study. It is likely that in BVMD, the clinical manifestations of transheterozygous mutations may be more serious than a single mutation.

CORD is one of the common forms of inherited retinal degeneration with a prevalence of 1/40 000 (22,23). CORD is characterized by the impairment of cone photoreceptors with or without dysfunction of rod photoreceptors (24,25). Clinical manifestations in CORD include photophobia, reduced visual acuity, color vision defects, and central scotomata (26). At present, a total of 30 genes have been found associated with CORD, including 10 genes related to autosomal dominant CORD (*AIPL1, CRX, GUCA1A, GUCY2D, PITPNM3, PROM1, PRPH2, RIMS1, SEMA4A, UNC119*) (27,28). RIM1, the protein product of *RIMS1*, localizes to the presynaptic active zones in brain and retinal tissue, and plays an important role in regulating synaptic vesicle release and presynaptic plasticity (29). RIM1 is a large multi-domain protein that interacts with multiple molecules at different regions (30).

In this case, we consider the patient had BVMD and CORD simultaneously. Mutations on *BEST1* and *RIM1* affected the RPE and photoreceptors, respectively. Therefore, the atrophy of the retina was very serious with only a little retina tissue remained. For these patients, transplantation of the retina

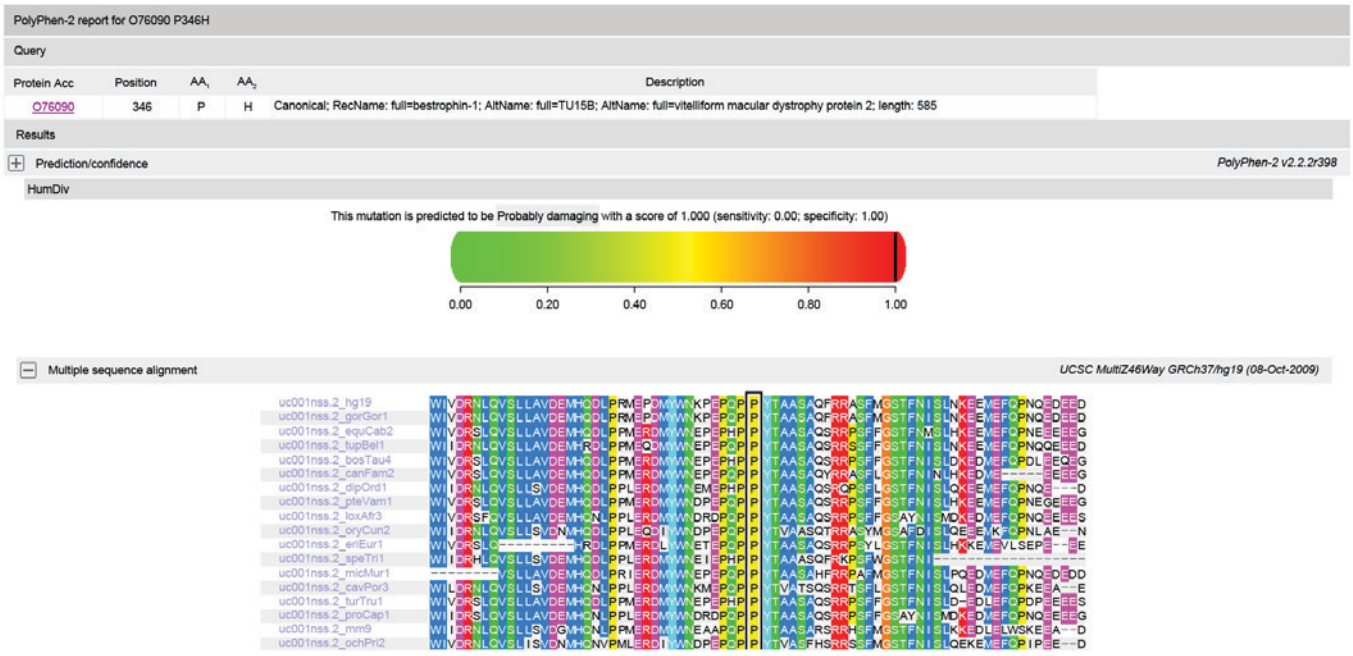

Figure 5. Polyphen and SIFT predicted that the amino acid substitution p.P346H in protein bestrophin 1 is damaging. Multiple sequenced alignment (Basic Local Alignment Search Tool) indicated that the residue at position 346 of bestrophin-1 is highly conserved across species.

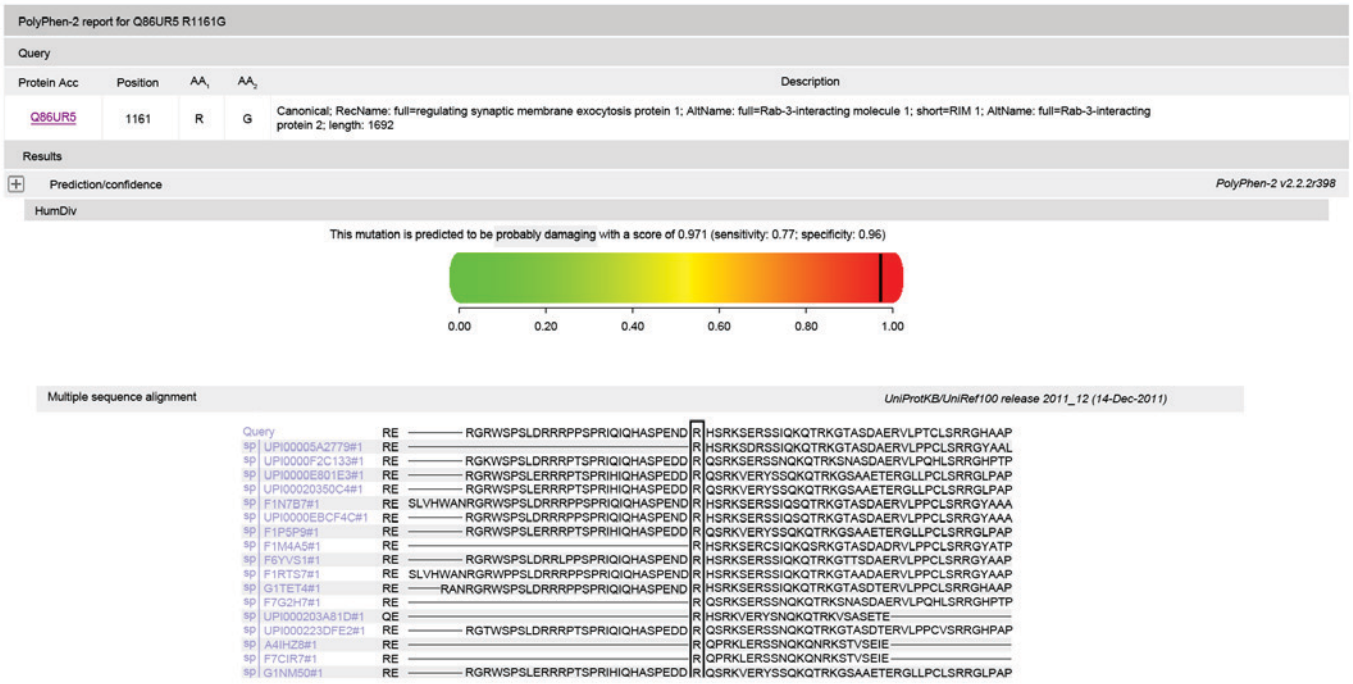

Figure 6. Polyphen predicted that the amino acid substitution p.R1161G in protein RIM1 is damaging. The residue at position 1161 of RIM1 is highly conserved across species.

stem cells or retina cell membrane may be more feasible for treatment.

In summary, our study identified two mutations of *BEST1* and *RIMS1* in one Chinese patient with bilateral macular coloboma. These findings expand the mutation spectrums of *BEST1* and *RIMS1*, and will be valuable for genetic counseling and development of therapeutic interventions for patients with macular coloboma.

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