Whole Exome Sequencing in Thai Patients With Retinitis Pigmentosa Reveals Novel Mutations in Six Genes

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Purpose. To identify disease-causing mutations and describe genotype-phenotype correlations in Thai patients with nonsyndromic retinitis pigmentosa (RP).

Methods. Whole exome sequencing was performed in 20 unrelated patients. Eighty-six genes associated with RP, Leber congenital amaurosis, and cone-rod dystrophy were analyzed for variant detection.

Results. Seventeen variants (13 novel and 4 known) in 13 genes were identified in 11 patients. These variants include 10 missense substitutions, 2 nonsense mutations, 3 deletions, 1 insertion, and 1 splice site change. Nine patients with identified inheritance patterns carried a total of 10 potentially pathogenic mutations located in genes CRB1, C8orf37, EYS, PROM1, RP2, andUSH2A. Three of the nine patients also demonstrated additional heterozygous variants in genes ABCA4, GUCY2D, RD3, ROM1, and TULP1. In addition, two patients carried variants of uncertain significance in genes FSCN2 and NR2E3. The RP phenotypes of our patients were consistent with previous reports.

Conclusions. This is the first report of mutations in Thai RP patients. These findings are useful for genotype-phenotype comparisons among different ethnic groups.

Keywords: retinitis pigmentosa, whole exome sequencing, genotype-phenotype correlations, mutation screening

Retinitis pigmentosa (RP) is the most commonly inherited retinal disease and is both clinically and genetically heterogeneous. The worldwide prevalence of RP is approximately 1 in 5000 to 5000.1 The severity and age of onset of RP can vary among patients. Night blindness is usually the first symptom during adolescence with subsequent peripheral visual field loss occurring in young adulthood. In the later stages of disease, patients start to lose their central vision, usually between the ages of 40 to 60.2 Retinitis pigmentosa presents different modes of inheritance including autosomal recessive (arRP), autosomal dominant (adRP), X-linked recessive (xlRP), and at least two rare forms, digenic and mitochondrial trait.1

Mutations in at least 57 associated genes distributed among all RP modes of inheritance have been identified (The Retinal Information Network [RetNet]). The proteins encoded by these genes are involved in a variety of functions, including the phototransduction cascade, vitamin A metabolism, photoreceptor structural and cytoskeletal formation, cell-to-cell signaling or synaptic interaction, RNA intron-splicing factors, intracellular protein trafficking, pH regulation, and phagocytosis.2

There are many factors that contribute to the complexity of RP. Different mutations in the same RP-associated gene can cause different diseases. Furthermore, clinical variability, which may be due to modifier genes, has been observed in different affected individuals both in inter- and intrafamilial cases with the same mutation.3,4 Although many RP-associated genes have been reported, mutation screening of these genes fails to detect the disease-causing mutations in approximately 40% of RP patients.1 Due to genetic heterogeneity and the fact that no clear genotype-phenotype correlations have been described, sequence analysis of the entire coding region for each candidate gene is the most commonly used molecular genetic test for RP. However, this method is laborious, time-consuming, and expensive. Recent developments in next-generation sequencing (NGS) represent an efficient, time-saving, and cost-effective tool for variant detection in affected individuals.5 To date, NGS has been successfully applied to identify mutations in genes known to be associated with RP6–16 and
has also led to the discovery of several novel candidate RP genes.8,11–13

In this study, we present the first mutation analysis of 20 unrelated Thai patients with nonsyndromic RP using whole exome sequencing (WES). Seventeen different variants in 15 genes were identified in 11 patients. We also describe the genotype–phenotype correlation of each patient.

METHODS

Clinical Data and Sample Collection

Twenty unrelated Thai patients with nonsyndromic RP were evaluated at the Division of Ophthalmology, Siriraj Hospital, Bangkok. The ophthalmological examination included best-corrected visual acuity (BCVA) using Snellen chart, slit-lamp biomicroscopy, dilated fundus examination, optical coherence tomography (OCT, when available), full-field electroretinogram (ERG), and visual evoked potential (VEP). All patients were of Thai ancestry ranging in age from 5 to 69 years. This study was approved by the Institutional Review Board of Siriraj Hospital Mahidol University, being adherent to the Declaration of Helsinki. Peripheral blood samples were collected from all participants after informed consent was obtained. Deoxyribonucleic acid was extracted from leukocytes according to the salting-out method.17 Ethnically matched, unrelated healthy individuals were used for normal control screening.

Whole Exome Sequencing (WES)

Exome capture was performed using solution hybrid selection with a commercial enrichment kit (SureSelectXT Human All Exon Automated Target Enrichment Kit; Agilent Technologies, Santa Clara, CA, USA) for Illumina paired-end multiplexed sequencing. Exome capture libraries of 2 × 100 bp paired-end reads were sequenced on an array scanner (Illumina HiSeq2000; Illumina, Inc., San Diego, CA, USA) at the Department of Medical Genome Sciences, The University of Tokyo. Three DNA samples were pooled on each lane of the flow cell for sequencing.

Sequence Data Alignment, Variant Calling and Identification

The Illumina paired-end DNA sequence data were mapped and aligned to the reference human genome NCBI Build 37 (hg19), using the Burrows-Wheeler Aligner (BWA) program. A set of utilities (Sequence Alignment/Map [SAMtools], a tab-delimited text file that contains sequence alignment data; Wellcome Trust Sanger Institute, Hinxton, UK) was used to convert the alignments from SAM to BAM files (the binary version of a SAM file), sort and merge the alignments, remove PCR duplicates, and index the alignment data. A software package (The Genome Analysis Toolkit [GATK]; Broad Institute, Cambridge, MA, USA) was used for single nucleotide polymorphism (SNP) and indel calling. Visualization and filtration of the sequence variations were performed using a high-performance viewer (Integrative Genomics Viewer [IGV]; Broad Institute) in comparison with SNP information (common, flagged, and multiple-location mapped SNPs) from the dbSNP137 database.

Identification of Variants

Due to the overlap of clinical features and RP-associated genes with other inherited retinal diseases, 86 genes associated with RP, Leber congenital amaurosis (LCA), and cone-rod dystrophy (CRD) were analyzed for variant detection (Supplementary Fig. S1).

Filtration and Prioritization of Variants

To distinguish between pathogenic and nonpathogenic variants, the annotated sequencing data were filtered and prioritized based on the following criteria: (1) variant located in coding sequence; (2) nonsynonymous variant; (3) nonsense variant; (4) short insertion or deletion variant (indel) in coding sequence; (5) variant located at splice donor or acceptor site, and (6) variant reported in flagged SNPs but not in common or multiple-location mapped SNPs of the dbSNP137 database.

In Silico Prediction of Variants

Six computational tools were used to predict the effects of the identified amino acid substitutions on protein function: PolyPhen-2, (SIFT), VarisoWatch, MutationTaster, Prediction of Pathological Mutations (PMut), and Screening for Non-Acceptable Polymorphisms (SNAP). Only the following outputs were considered as deleterious variants: PolyPhen-2 (probably or possibly damaging); SIFT (damaging); VarisoWatch (very high or high); MutationTaster (disease causing); PMut (pathological); and SNAP (non-neutral). For estimating evolutionary conservation, multiple sequence alignments of the affected amino acids in different vertebrate species were performed using sequence alignment program (ClustalW; The Biology Workbench, San Diego, CA, USA).

Variant Validation

The workflow for variant validation is shown in Supplementary Figure S2. Variants were selected for confirmation by Sanger sequencing when three or more of the prediction outputs from the six computational prediction tools suggested that a variant was deleterious. The primer sequencing program (Primer3; University of Massachusetts, Boston, MA, USA) was used for designing amplicon-specific primers to amplify the regions containing each variant. The PCR products were treated with a reagent (Exonuclease I; Affymetrix, Santa Clara, CA, USA) before processing for direct sequencing. Sequencing was performed using a cycle-sequencing kits (ABI BigDye Terminator v3.1; Applied Biosystems, Grand Island, NY, USA) on an analyzer (ABI 3130xl Genetic Analyzer; Applied Biosystems). To verify the pathogenicity of novel variants identified in this study, denaturing HPLC (Transgenomic, Inc., San Jose, CA, USA) analysis was performed for detecting each variant on at least 110 DNA samples (220 chromosomes) from unrelated healthy individuals.

Segregation Analysis

To assess variant segregation within each patient’s family, Sanger sequencing was also performed on the family members from whom DNA was available.

Protein Structure and Functional Prediction

The protein structures and functions resulting from missense variants identified in this study were predicted using the freely available web service HOPE (Have Your Protein Explained).18

RESULTS

Twenty unrelated Thai patients were recruited for this pilot study. Most patients experienced poor night vision. The BCVA ranged from hand motion (HM) to 6/6. The OCT was performed in two patients (RP023 and RP069), which demonstrated no evidence of cystoid macular edema. A summary of clinical information and family pedigrees are
### Table 1. Clinical Information for Patients in this Study

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sex</th>
<th>Onset</th>
<th>At Exam</th>
<th>Presenting Symptoms</th>
<th>BCVA</th>
<th>ERG</th>
<th>VEP</th>
<th>Fundus Examination</th>
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<td>M</td>
<td>27</td>
<td>30</td>
<td>Poor night vision; flashing</td>
<td>6/6</td>
<td>6/9</td>
<td>NR</td>
<td>NR Normal</td>
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<td>F</td>
<td>60</td>
<td>69</td>
<td>Progressive visual loss</td>
<td>6/24</td>
<td>NLP</td>
<td>NR</td>
<td>NR Normal</td>
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<tr>
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<td>29</td>
<td>Poor night vision</td>
<td>6/9</td>
<td>6/9</td>
<td>NR</td>
<td>NR Normal</td>
</tr>
<tr>
<td>RP016</td>
<td>M</td>
<td>26</td>
<td>36</td>
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<td>CF</td>
<td>CF</td>
<td>NR</td>
<td>Moderately decreased amplitude</td>
</tr>
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<td>F</td>
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<td>49</td>
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<td>6/24</td>
<td>6/18</td>
<td>NR</td>
<td>Normal</td>
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<tr>
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<td>11</td>
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<td>6/60</td>
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<td>51</td>
<td>Poor night vision</td>
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<td>6/24</td>
<td>NR</td>
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</tr>
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<td>Progressive visual loss; flashing</td>
<td>HM</td>
<td>CF</td>
<td>NR</td>
<td>Markedly decreased amplitude</td>
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<td>HM</td>
<td>NR</td>
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<td>5</td>
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<td>6/36</td>
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<td>Normal</td>
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<tr>
<td>RP087/2*</td>
<td>M</td>
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<td>10</td>
<td>Blurred distant vision; poor night vision; mild myopia with high astigmatism</td>
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<td>6/60</td>
<td>NR</td>
<td>Normal</td>
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</tbody>
</table>

**Notes:**
- RE, right eye; LE, left eye; CF, counting fingers; VF, visual field; NR, nonrecordable; NLP, no light perception due to anterior ischemic optic neuropathy.
- * Consanguineous family.
shown in Table 1 and the Figure, respectively. The average read depth of WES observed in the 20 samples was approximately 150. The putative deleterious variants were detected in 11 out of 20 patients (55%). Nine of them carried a total of 10 potentially pathogenic mutations and five additional heterozygous variants, while the other two having two variants of uncertain significance (VUS).

### Potentially Pathogenic Mutations

Ten different putative, disease-causing mutations, eight novel and two known, were identified in nine patients (Table 2, Supplementary Table S1). These include five autosomal recessive genes (CRB1, C8orf37, EYS, PROM1, and USH2A) and one X-linked recessive gene (RP2). These mutations include five missense substitutions, two nonsense mutations, two deletions, and one splice site change. None of the novel missense variants were detected in at least 220 alleles from the unrelated normal controls.

### Additional Heterozygous Variants

Among the nine patients with potentially pathogenic mutations, three (RP009, RP022, and RP038 probands) also carried a heterozygous variant in one of five genes (ABCA4, GUCY2D, RD3, ROM1, and TULP1; Fig., Table 2). The RP009 proband carried four variants in three genes (USH2A, ABCA4, and RD3) (Fig., Tables 2, 3, Supplementary Fig. S3). A compound heterozygous mutation of a known variant, c.4732C>T (p.Arg1578Cys), and a novel variant, c.773A>C (p.Gln258Pro), were identified in USH2A. The variant p.Arg1578Cys was previously reported as a disease-causing mutation in a patient with Stargardt disease type 1,20 c.71G>A (p.Arg24His), was detected. Finally, a novel heterozygous frameshift variant, c.94_95delG (p.Glu32Serfs*2), was found in the RD3 gene.
In addition, the RP022 proband carried two variants, but three alleles, in genes \textit{CRB1} and \textit{GUCY2D} (Fig.). In the \textit{CRB1} gene, a novel homozygous mutation, c.3442T>C (p.Cys1148Arg), was detected. The occurrence of this mutation at a highly conserved cysteine (Supplementary Fig. S3), its absence in the 242 normal chromosomes screened, and the results of the six prediction tools support its true pathogenicity (Table 3). In addition, HOPE prediction showed that the substitution from cysteine 1148 to arginine changed a highly conserved cysteine in the EGF-like 15 domain and caused a disruption of the disulfide bridge with Cys1163, which in turn could lead to misfolding and thereby affect the binding properties of \textit{CRB1} product (crumbs homolog 1). Both asymptomatic parents and a brother of the patient were found to be heterozygous carriers of p.Cys1148Arg.

Another novel heterozygous variant in the \textit{GUCY2D} gene, c.1138C>T (p.Arg380Cys), was detected in the patient and his unaffected mother.

Variants of Uncertain Significance (VUS)

Single novel heterozygous variants predicted to be deleterious were detected in genes \textit{FSCN2} and \textit{NR2E3} in two isolated RP patients (Fig., Table 2). These include a c.1264C>T (p.Arg422Trp) variant in \textit{FSCN2} and a c.424C>T (p.Arg142Trp) variant in the \textit{NR2E3} identified in the RP016 and RP010 probands, respectively.


discussion

We present here the first mutation report in Thai patients with nonsyndromic RP using WES. Eighty-six genes associated with RP, LCA, and CRD were analyzed to identify potentially pathogenic variants. Our study in 20 unrelated patients uncovered 10 potentially pathogenic mutations and two VUSs in nine and two patients, respectively. We also describe genotype-phenotype correlations of the identified genetic variants (Tables 1, 2).

Potentially Pathogenic Mutations With and Without the Additional Variants

The RP038 proband carries three variants, but four alleles in three genes, \textit{C8orf37}, \textit{ROM1}, and \textit{TULP1}. A novel homozygous splice-donor-site mutation, c.243+2T>C, identified in \textit{C8orf37} is most likely to be the cause of arRP in this family. This change is predicted to introduce a premature termination codon (PTC) that could lead to activation of the nonsense-mediated mRNA decay (NMD) pathway.\textsuperscript{21} The proband’s phenotype was accordant with a previous report in which all affected members demonstrated early impairment of the macula with age of onset ranging from infancy to late teenage years.\textsuperscript{13} Furthermore, two additional variants were identified in the \textit{ROM1} and \textit{TULP1} genes. A frameshift insertion in \textit{ROM1}, c.531_532insG (dbSNP rs137955062), was previously described in monogenic adRP\textsuperscript{22,23} with disease severity ranging from asymptomatic to severe, and in digenic RP when co-inherited with a p.Leu185Pro mutation in the \textit{PRPH2} gene.\textsuperscript{24} This variant was also detected in two out of 240 normal chromosomes, indicating that the c.531_532insG variant is not the causative mutation for the RP038 proband.

A novel heterozygous variant, c.43G>A, in \textit{TULP1} was identified in the RP038 proband and his unaffected elder brother. Previous reports have described \textit{TULP1} variants as the causes of both arRP and arLCA (RetNet). These data imply that the change in \textit{TULP1} in the RP038 proband does not correlate with the disease.

In our study, two \textit{CRB1} gene mutations in exons 9 (RP022 proband) and 7 (RP023 proband) were identified. This is in accordance with previous publications that showed mutations mainly in exons 9 (41%) and 7 (27%).\textsuperscript{25} These findings confirm the importance of the domains included in these two exons, exon 7: laminin G-like 2, exon 9: laminin G-like 3 and EGF-like 15 through 17. Previous reports of \textit{CRB1} gene mutations in retinal dystrophies showed variable age of onset and BCVA.\textsuperscript{25,26} In the RP022 proband, who showed onset of blurred vision since birth, a homozygous mutation (p.Cys1148Arg) in \textit{CRB1} and a heterozygous variant (p.Arg380Cys) in \textit{GUCY2D} were detected, whereas a homozygous mutation (p.Phe847Ile) in

\textbf{Figure.} Continued.

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<table>
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<th>Sample</th>
<th>MOI in Family</th>
<th>Gene</th>
<th>MOI of Gene</th>
<th>Chr</th>
<th>Exon</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
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<td>USH2A</td>
<td>arRP</td>
<td>1</td>
<td>4</td>
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<td>USH2A</td>
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<td>22</td>
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<td>C-CAP/cofactor C-like</td>
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**Potentially pathogenic mutations**

**Additional heterozygous variants**

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<th>Gene</th>
<th>MOI of Gene</th>
<th>Chr</th>
<th>Exon</th>
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<td>Het</td>
<td>Transmembrane</td>
<td>Reported</td>
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**Variants of uncertain significance**

<table>
<thead>
<tr>
<th>Sample</th>
<th>MOI in Family</th>
<th>Gene</th>
<th>MOI of Gene</th>
<th>Chr</th>
<th>Exon</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>State</th>
<th>Domain</th>
<th>Reference</th>
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<tbody>
<tr>
<td>RP010</td>
<td>Isolated RP</td>
<td>NR2E3</td>
<td>arRP and adRP</td>
<td>15</td>
<td>4</td>
<td>c.42C&gt;T</td>
<td>p.Arg42Trp</td>
<td>Het</td>
<td>-</td>
<td>Novel</td>
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</tbody>
</table>

*Nucleotide numbering reflects cDNA numbering with position 1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (provided in the public domain by The Human Genome Variation Society, http://www.hgvs.org/mutnomen). The initiation codon is codon 1. The nucleotide position of each gene was based on these GenBank cDNAs (accession number): ABCC4 (NM_000350.2), C8orf37 (NM_177965), CRB1 (NM_201253.2), EYS (NM_001142800.1), FSCN2 (NM_012418.3), GUCY2D (NM_000180.3), NR2E2 (NM_014249.2), PROM1 (NM_006017.2), RD1 (NM_001166688.1), ROM1 (NM_000327.3), RP2 (NM_006915.2), TULP1 (NM_003522.5), and USH2A (NM_206935.2). MOI, mode of inheritance; Het, heterozygous; Hom, homozygous; Hem, hemizygous.*
<table>
<thead>
<tr>
<th>Sample</th>
<th>Gene</th>
<th>Amino Acid Change</th>
<th>PolyPhen-2</th>
<th>SIFT</th>
<th>VarioWatch</th>
<th>MutationTaster</th>
<th>PMut</th>
<th>SNAP</th>
<th>Familial Segregation</th>
<th>Alleles in Control†</th>
<th>Vertebrate Conservation‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP009</td>
<td>USH2A</td>
<td>p.Gln258Pro</td>
<td>Probably damaging</td>
<td>Damaging</td>
<td>High</td>
<td>Disease causing</td>
<td>Neutral</td>
<td>Non-neutral</td>
<td>NA</td>
<td>0/224</td>
<td>Yes</td>
</tr>
<tr>
<td>RP011</td>
<td>EYS</td>
<td>p.Asn3141Metfs*8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>RP019</td>
<td>EYS</td>
<td>p.Cys2139Cys</td>
<td>Probably damaging</td>
<td>Damaging</td>
<td>High</td>
<td>Disease causing</td>
<td>Non-neutral</td>
<td>NA</td>
<td>Yes</td>
<td>NA</td>
<td>Yes</td>
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<tr>
<td>RP022</td>
<td>CRB1</td>
<td>p.Arg1578Cys</td>
<td>Possibly damaging</td>
<td>Damaging</td>
<td>High</td>
<td>Disease causing</td>
<td>Pathological</td>
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<tr>
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<td>CRB1</td>
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<td>Tolerated</td>
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<tr>
<td>RP027</td>
<td>EYS</td>
<td>p.Glu2703Ter</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>RP038</td>
<td>CRB1</td>
<td>p.Phe847Ile</td>
<td>Possibly damaging</td>
<td>Tolerated</td>
<td>High</td>
<td>Disease causing</td>
<td>Neutral</td>
<td>Non-neutral</td>
<td>Yes</td>
<td>NA</td>
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<tr>
<td>RP069</td>
<td>PROM1</td>
<td>p.Lys1148Ter</td>
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<tr>
<td>RP087</td>
<td>RP2</td>
<td>p.Trh1346Glnfs*15</td>
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### Potentially pathogenic mutations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gene</th>
<th>Amino Acid Change</th>
<th>PolyPhen-2</th>
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<th>Alleles in Control†</th>
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</thead>
<tbody>
<tr>
<td>RP009</td>
<td>ABCA4</td>
<td>p.Arg24His</td>
<td>Possibly damaging</td>
<td>Tolerated</td>
<td>High</td>
<td>Disease causing</td>
<td>Pathological</td>
<td>Non-neutral</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
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<tr>
<td>RP022</td>
<td>GUCY2D</td>
<td>p.Arg380Cys</td>
<td>Possibly damaging</td>
<td>Damaging</td>
<td>High</td>
<td>Disease causing</td>
<td>Neutral</td>
<td>Neutral</td>
<td>Op</td>
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<td>Yes</td>
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<tr>
<td>RP038</td>
<td>ROM1</td>
<td>p.Leu141Alafs*18</td>
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<td>Yes</td>
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<tr>
<td>TULP1</td>
<td>p.Asp15Asn</td>
<td>p.Arg380Cys</td>
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<td>Damaging</td>
<td>High</td>
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### Additional heterozygous variants

<table>
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<th>Sample</th>
<th>Gene</th>
<th>Amino Acid Change</th>
<th>PolyPhen-2</th>
<th>SIFT</th>
<th>VarioWatch</th>
<th>MutationTaster</th>
<th>PMut</th>
<th>SNAP</th>
<th>Familial Segregation</th>
<th>Alleles in Control†</th>
<th>Vertebrate Conservation‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP010</td>
<td>NR2E3</td>
<td>p.Arg95Ile</td>
<td>Probably damaging</td>
<td>NA</td>
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<td>RP016</td>
<td>FSCN2</td>
<td>p.Arg129Ile</td>
<td>Possibly damaging</td>
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<td>High</td>
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<td>Pathological</td>
<td>Non-neutral</td>
<td>NA</td>
<td>0/224</td>
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</tbody>
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### Variants of uncertain significance

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<thead>
<tr>
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<th>SIFT</th>
<th>VarioWatch</th>
<th>MutationTaster</th>
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<th>SNAP</th>
<th>Familial Segregation</th>
<th>Alleles in Control†</th>
<th>Vertebrate Conservation‡</th>
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</thead>
<tbody>
<tr>
<td>RP009</td>
<td>ABCA4</td>
<td>p.Arg24His</td>
<td>Possibly damaging</td>
<td>Tolerated</td>
<td>High</td>
<td>Disease causing</td>
<td>Pathological</td>
<td>Non-neutral</td>
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<tr>
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<td>GUCY2D</td>
<td>p.Arg380Cys</td>
<td>Possibly damaging</td>
<td>Damaging</td>
<td>High</td>
<td>Disease causing</td>
<td>Neutral</td>
<td>Neutral</td>
<td>Op</td>
<td>0/220</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### NA, not available; Op, observed in proband only; Opo, observed in proband and other(s).


† Control—Allele frequencies were measured in at least 110 controls (220 alleles) from unrelated normal controls.

‡ Vertebrate conservation—Yes, conserved in more than 80% of all vertebrates aligned; No, conserved in less than 80% of all vertebrates aligned.

§ Known variant.
CRB1 was identified in the RP023 proband with onset of RP occurring at age 25 years. Given the fact that various GUCY2D gene mutations have been reported to cause both adCRD and arlCA (RetNet), and that the p.Arg380Cys variant was predicted to be deleterious, GUCY2D cannot be excluded from acting as a possible modifier gene. Combination of variants in both CRB1 and GUCY2D might explain the earlier onset of the disease in the RP022 proband compared with the RP023 proband.

In a previous report, a compound heterozygous and a homozygous mutation identified in the USH2A gene were associated with USH2 and RP without hearing loss, respectively.27 Here, four variants in three different genes (USH2A, ABCA4, and RD3) were detected in the RP009 proband. Among the identified variants, a compound heterozygous mutation (p.Arg1578Cys and p.Gln258Pro) detected in USH2A is likely the cause of the disease. While the p.Arg1578Cys mutation was previously reported in a patient with USH2,19 the RP009 proband has no hearing loss at age 30 years. Previous reports showed a wide range of BCVA among patients with USH2A mutations.28 The BCVA and the macula of the RP009 proband were both normal. Although single heterozygous ABCA4 gene variants have been reported to cause age-related macular degeneration,4,30 this finding was not observed in the RP009 proband at the time of examination. For RD3, all causative mutations were reported only in arlCA. Due to the lack of cosegregation analysis in family members, it is difficult to describe the ABCA4 and RD3 gene variants as possible modifier genes or as possible causes of phenotypic variation within the patient’s family.

Reported EYS mutations are distributed along the length of the gene.31,32 In this study, a reported homozygous missense mutation (p.Cys2139Tyr) in exon 31 and a novel homozygous nonsense mutation (p.Glu2703Ter) in exon 42 of EYS were detected in the RP019 and RP027 probands, respectively. Normally, the Cys residue at position 2139 forms a disulfide bridge with Cys2130 (provided in the public domain by The Universal Protein Resource [UniProt], http://www.uniprot.org/uniprot/Q5T1H1/). Substitution of p.Cys2139Tyr may affect the conformation of the EYS protein. In the RP011 proband, a compound heterozygous mutation of a novel frameshift mutation (p.Asn421Metfs*8) in exon 8 and the same missense mutation as in the RP019 proband were observed (Table 2). The p.Glu2703Ter mutation is located near the C-terminal, leading to the loss of 441 amino acids in the EYS protein, while the p.Asn421Metfs*8 mutation introduces eight new amino acids. Both null alleles are predicted to induce a PTC that can lead to the loss of 441 amino acids in the EYS protein, while the p.Asn421Metfs*8 mutation introduces eight new amino acids. Both null alleles are predicted to induce a PTC that can lead to activation of the NMD pathway.21 The RP011 proband developed poor night vision at age 19. This data supports the previous report in a French family with a compound heterozygous mutation of p.Cys2139Tyr and c.2847-1G►A reported in Chinese arRP patients.51 A homozygous nonsense mutation (p.Glu1836Ter) in exon 26 reported in a Chinese arRP patient showed 33 disease-causing mutations in both CRB1 and GUCY2D.46 The CRB1 gene was identified in the RP010 proband. A previous study showed 35 disease-causing mutations with only one mutation causing adRP and two mutations causing arRP.46 The RP010 proband demonstrated a relatively good prognosis with late disease onset and 6/24 vision in her right eye. Her left eye had no light perception due to anterior ischemic optic neuropathy. Although the identified variant (p.Arg422Trp) was predicted to be deleterious, it would require the family’s DNA samples to confirm the significance of this missense variant.

In summary, WES is a powerful approach for the identification of pathogenic genes in RP. Further analysis will reveal other known or novel genes contributing to the disease.

**Web Resources**

The URLs for data presented herein are provided in the public domain as follows:

5. Integrative Genomics Viewer (IGV), http://www.broadinstitute.org/igv
7. Sorting Intolerant From Tolerant (SIFT), http://sift.jcvi.org/www/SIFT_enst_submit.html
11. SNAP, http://snapstablab.org/services/snap/
12. HOPE, http://www.cmbi.ru.nl/hope/home

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References


