RGR variants in different forms of retinal diseases: The undetermined role of truncation mutations

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Abstract. It has been previously reported that mutations in retinal G protein coupled receptor (RGR) are associated with retinitis pigmentosa. The present study aims to systematically analyze the potential role of variants of RGR in retinal diseases. Variants in coding regions and splice sites of RGR were selected from a whole exome sequencing dataset of 820 probands with various forms of genetic ocular diseases. Potential variants of RGR were further confirmed by Sanger sequencing and analyzed in available family members. Clinical data was reviewed for patients with RGR variants. As a result, a total of five variants in RGR were detected in six probands with different types of ocular diseases. Of the five variants, two were novel heterozygous truncation variations, c.266C>A (p.S89*) and c.722_723delCC (p.S241Yfs*29), identified in two probands with high myopia and confirmed by Sanger sequencing. Segregation analysis on available family members demonstrated p.S89* and p.S241Yfs*29 were also present in unaffected relatives. The other three variants of RGR were heterozygous missense variants randomly occurring in patients with different genetic ocular diseases. No homozygous or compound heterozygous variants were detected. The results of the present study suggested that the heterozygous truncation variants in RGR were less likely to be pathogenic. Further studies are expected to evaluate the pathogenicity of variants in RGR.

Introduction

Retinal G protein coupled receptor (RGR) [Online Mendelian Inheritance in Man (MIM) 600342] encodes a putative retinal G-protein coupled receptor, a rhodopsin homologue, expressed exclusively in the retina (1-3). RGR is essential for the visual cycle as it is involved in the production of 11-cis-retinal (4). An abnormal visual cycle affects visual perception and ultimately leads to ocular disorders (5). However, the association of RGR with specific ocular diseases has been rarely reported. Only a homozygous missense mutation and a heterozygous frameshift mutation have been reported to be associated with retinitis pigmentosa and choroidal sclerosis, respectively (5). However, the involvement of RGR in the pathogenesis of retinitis pigmentosa has not been implicated in subsequent studies (6,7). The potential role of RGR in retinal diseases remains to be elucidated. Thus, the present study aims to systematically evaluate and analyze the potential role and pathogenicity of variants in RGR. This will be done with reference to a whole exome sequencing dataset from 820 probands with different forms of genetic ocular diseases.

Materials and methods

Patients. The present study is part of a project to investigate genetic defects associated with genetic ocular diseases using whole exome sequencing. Whole exome sequencing was performed on samples from 820 probands with different forms of genetic ocular diseases. All patients were recruited from the clinic of the Zhongshan Ophthalmic Center (Guangzhou, China). Written informed consent was obtained from the participants or their guardians, following the tenets of the Declaration of Helsinki. The present study was approved by the Institutional Review Board of Zhongshan Ophthalmic Center.

Sequencing. Whole exome sequencing was performed using a SureSelect Human All Exon Enrichment kit V4 (Agilent Technologies, Inc., Santa Clara, CA, USA) or TruSeq Exome Enrichment Kit (Illumina, Inc., San Diego, CA, USA) as previously described (8,9). Variants in coding regions and splice sites in RGR were selected from the whole exome sequencing data of 820 probands with various genetic ocular diseases. Those variants with minor allele frequency (MAF) ≤0.01 were further analyzed by functional prediction using online methods, including SIFT (sift.jcvi.org/www/SIFT_enst_submit.html) (10), PolyPhen-2 (genetics.bwh.harvard.edu/phy2/) (11), and Berkeley Drosophila Genome Project (www.fruitfly.org/) (12). The MAF of each variant was obtained from the public databases,
Table I. Primers used for amplification and sequencing of RGR.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward primer (5'-3')</th>
<th>Reverse primer (5'-3')</th>
<th>Amplicon (bp)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGR-86008695</td>
<td>GCAGCATTCCAGGAACACACA</td>
<td>CCCCTGCTCTTTATCTCTCC</td>
<td>283</td>
<td>65-58³</td>
</tr>
<tr>
<td>RGR-86017741</td>
<td>TGCTGACCTGGTTTTCTTGGG</td>
<td>AGGAAGAGACTGACACAGAGG</td>
<td>300</td>
<td>65-58³</td>
</tr>
</tbody>
</table>

³Gradient annealing temperatures from 65 to 58°C. RGR, retinal G protein coupled receptor.

Table II. Summary of variants in RGR detected in probands with different forms of genetic ocular diseases.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Position</th>
<th>Sample</th>
<th>Variation</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>Status</th>
<th>SIFT</th>
<th>Phen-2</th>
<th>Poly</th>
<th>MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGR</td>
<td>chr10</td>
<td>86007503</td>
<td>HM345, QT371</td>
<td>c.236G&gt;A</td>
<td>p.R79H</td>
<td>Hetero</td>
<td>D</td>
<td>B</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>RGR</td>
<td>chr10</td>
<td>86007377</td>
<td>QT1072</td>
<td>c.110C&gt;T</td>
<td>p.T37I</td>
<td>Hetero</td>
<td>D</td>
<td>B</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>RGR</td>
<td>chr10</td>
<td>86008695</td>
<td>HM723</td>
<td>c.266C&gt;A</td>
<td>p.S89*</td>
<td>Hetero</td>
<td>-</td>
<td>-</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>RGR</td>
<td>chr10</td>
<td>86012764</td>
<td>QT90</td>
<td>c.522C&gt;G</td>
<td>p.D174E</td>
<td>Hetero</td>
<td>T</td>
<td>D</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>RGR</td>
<td>chr10</td>
<td>86017741</td>
<td>HM824</td>
<td>c.722_723delCC</td>
<td>p.S241Yfs29</td>
<td>Hetero</td>
<td>-</td>
<td>-</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

D, damaging; B, benign; T, tolerate; NA, not available/not applicable; EVS, Exome Variant Server; MAF, minor allele frequency; RGR, retinal G protein coupled receptor; 1000G, 1000 Genomes database.

dbSNP (www.ncbi.nlm.nih.gov/projects/SNP/), 1000 Genomes (www.1000genomes.org/), and the Exome Variation Server (evs.gs.washington.edu/EVS/). Potential variants of RGR were further confirmed by Sanger sequencing and validated in available family members. Primers used for amplification of fragments were designed using the Primer3 online tool (bioinfo.
The methods used for amplification, sequencing, and analysis of the target fragments were as previously described (13). The descriptions of the variants are consistent with the nomenclature for sequence variations (www.hgvs.org/mutnomen) (14).

Results

Following a review of the whole exome dataset of 820 probands with different forms of genetic ocular diseases, a total of 5 variants of RGR were detected in 6 of the 820 probands. Of the five variants, two were heterozygous truncation variants, c.266C>A (p.S89*) and c.722_723delCC (p.S242Yfs*29), identified in two probands with early-onset high myopia (Fig. 1A and Table II). These two variants were further confirmed by Sanger sequencing (Fig. 1A). Segregation analysis on available family relatives identified that p.S89* and p.S242Yfs*2 did not co-segregate with high myopia, they were present in the unaffected relatives but absent in the affected relatives (Fig. 1A). The other three variants were heterozygous missense variants and identified in four probands, one with high myopia, one with cone-rod dystrophy, and two with Leber congenital amaurosis (Table II). No homozygous or compound heterozygous variants in RGR were detected.

The two probands with RGR truncation variants complained of poor vision at younger than primary school age, but denied photophobia and night blindness (Table III). Fundus examination demonstrated tigroid fundus and temporal crescent of optic nerve head (Fig. 1B and C), which was consistent with the diagnosis of high myopia. Neither marked retinal vessel attenuation nor bone corpuscle type of pigmentation were observed (Fig. 1B and C). However, additional family members with RGR truncation variants (HM723II4 and HM824I1) were unaffected individuals without high myopia (Table III) and did not have any notable signs of abnormal fundus changes (Fig. 1D).

Discussion

Based on the whole exome sequencing dataset from 820 probands with different forms of genetic ocular diseases, two heterozygous truncation variants in RGR were identified in two probands with high myopia, but these did not co-segregate with high myopia. The other three variants in RGR were heterozygous missense variants, and occurred randomly in four patients with different forms of genetic ocular diseases. No homozygous or compound heterozygous variants in RGR were detected.

Only a limited number of RGR variants have been previously reported (5-7). Among them, only two have been identified in two families with either retinitis pigmentosa or choroidal sclerosis (5), a homozygous c.196A>C (p.Ser66Arg) variant identified in a family with autosomal recessive retinitis pigmentosa and a heterozygous c.824dupG (p.M275Ifs*83) insertion identified in a small family with autosomal dominant choroidal sclerosis (5). Subsequently, screening of RGR in two independent studies only identified a number of less likely pathogenic variants and polymorphisms, as reviewed in Table IV. Of the five variants detected in the current study, two were heterozygous novel truncations, p.S89* and p.S242Yfs*29, which were not present in two probands with high myopia. These two variants and the previously reported heterozygous variant, c.824dupG, were
that the potential role of heterozygous truncation of RGR in ocular diseases remains to be determined. Additional studies are required to provide further understanding.

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**References**


