Clinical Investigation

Mutations in the gene coding for guanylate cyclase-activating protein 2 (GUCA1B) gene in patients with autosomal dominant retinal dystrophies

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Abstract

Background We investigated mutations in the gene coding for guanylate-cyclase activating protein 2 (GCAP2), also known as GUCA1B gene, in Japanese patients with retinitis pigmentosa (RP) and tried to identify phenotypic characteristics associated with mutations in the gene.

Subjects and methods Genomic DNA samples from 63 unrelated patients with autosomal dominant retinitis pigmentosa (ADRP) and 33 patients with autosomal recessive retinitis pigmentosa (ARRP) were screened by single-strand conformational polymorphism analysis followed by direct sequencing. Clinical features associated with a mutation were demonstrated by visual acuity, visual field testing, fundus photography, and electroretinography.

Results A novel transitional mutation converting GGA to AGA at codon 157 (G157R) was identified. This mutation has been found in three index patients from three independent families. Phenotypic examination of seven members of the three families revealed that this mutation was associated with RP with or without macular involvement in five members, macular degeneration in one member, and asymptomatic normal phenotype in one member. In addition, previously unknown polymorphic changes including V29V, Y57Y, T87I, and L180L were identified.

Conclusions A racial difference exists in the spectrum of mutations and/or polymorphisms in the GCAP2 gene between British and Japanese populations. Our findings suggest that the mutation in the GCAP2 gene can cause one form of autosomal dominant retinal dystrophy, with variable phenotypic expression and incomplete penetrance.

Introduction

Retinitis pigmentosa (RP) is a group of inherited neurodegenerative disorders of the retina. Patients with RP experience night blindness at an early stage and tunnel vision (constricted visual fields) in advanced stages of the disease. RP is both clinically and genetically heterogeneous. Autosomal dominant (ADRP), autosomal recessive (ARRP), X chromosome-linked, and digenic patterns of inheritance [1] occur in families with RP, and a non-allelic heterogeneity is present even among families with the same inheritance pattern.

Guanylate cyclase-activating proteins (GCAPs) are calcium-binding proteins that are closely related to recoverin, neurocalcin, and many other neuronal Ca2+-sensor proteins of EF-hand superfamily [2, 3]. To date, three homologous GCAPs (GCAPs 1–3) are known to express in the retina [4]. In the human retina, GCAP 1 is expressed at high levels in cones and at lower levels in rods, whereas GCAP 2 is intensely expressed in both rods and cones [4, 5]. Although the relative contribution of GCAPs to the kinetics of dim flash responses in rods and cones remains unclear, GCAPs 2 and 3 in vitro stimulate the activity of both known isozymes of membrane guanylate cyclase (retGC)-1 and (retGC)-2 that are present in photoreceptor membranes [4]. GCAP 1 can activate only retGC-1.

Because mutations in the retGC gene reportedly cause RP [13], mutations in the gene coding for GCAPs 1 and 2 that produce loss of function in GCAPs may also lead to retinal dystrophy. Although three mutations, Y99C, P50L, and E155G substitutions, have been identified in the GCAP1 (GUCA1A) gene associated with variable phenotypes, ranging from mild cone dystrophy to pronounced cone–rod dystrophy [4, 13, 20], no mutation has been previously detected in the GCAP 2 (GUCA1B) gene among 400 unrelated British patients with autosomal dominant retinal dystrophies [14]. However, other genes, such as the arrestin gene, have exhibited different patterns of mutations among different ethnic populations [11, 16]. The GCAP2 gene, therefore, may still be a candidate gene for autosomal dominant or recessive retinal dystrophies among non-British populations. In the present study, we screened Japanese patients with either ADRP or ARRP to determine whether the GCAP2 gene is involved in inherited retinal dystrophy.

Materials and methods

Subjects

This study conformed to the tenet of the 1964 Declaration of Helsinki and received approval from the Ethical Committee of the Hirosaki University School of Medicine. All patients enrolled in this study gave their informed consent before their inclusion in the study. We screened genomic DNA samples isolated from 96 unrelated patients with inherited retinal dystrophy, including ADRP (63 patients) and ARRP (33 patients), for mutations of the GCAP2 gene. All patients with dominantly inherited dystrophies had been evaluated already for mutations in the peripherin/RDS and GCAP1 genes and were found to be negative (data not shown). In addition, we screened 100 healthy control subjects for mutations in the gene. All individuals in the present study were Japanese.

Methods

Exons 1 through 4 of the GCAP2 gene were amplified by polymerase chain reaction (PCR) using the DNA Thermal Cycler 9700 (Perkin Elmer Applied Biosystems, USA).
Foster City, CA, USA) and four sets of oligonucleotide primer pairs (Table 1). The PCR products were visualized in 1.5% agarose gel electrophoresis by ethidium bromide staining as single bands. Subsequently, they were analyzed for the presence of mutations by single-strand conformational polymorphism (SSCP) using the GenePhor system (Phamacia, Upsala, Sweden). PCR products that showed abnormal mobility on SSCP were directly sequenced to identify mutations by an automated sequencer (310 Genetic Analyzer, Perkin–Elmer Applied Biosystems, Foster City, CA, USA), according to the manufacturer’s protocol.

**Table 1** Nucleotide sequences of oligomers to generate exons of the GCAP2 gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sequence</th>
<th>Annealing temperature (°C)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Forward</td>
<td>5'-TCA GGC CTC CTG GAA AGG G-3'</td>
<td>54</td>
<td>353</td>
</tr>
<tr>
<td>1 Reverse</td>
<td>5'-GGT GGA CTG GCC ACT GTG C-3'</td>
<td>54</td>
<td>305</td>
</tr>
<tr>
<td>2 Forward</td>
<td>5'-AGA AGC CCT GTG TTT GCA GG-3'</td>
<td>58</td>
<td>272</td>
</tr>
<tr>
<td>2 Reverse</td>
<td>5'-TTG TGG CCA ACC TTC AGA GC-3'</td>
<td>58</td>
<td>281</td>
</tr>
<tr>
<td>3 Forward</td>
<td>5'-GGA AGT GGT GCT GGG GGA TG-3'</td>
<td>58</td>
<td>272</td>
</tr>
<tr>
<td>3 Reverse</td>
<td>5'-GAC GTG CGG CCA GAA AGT GG-3'</td>
<td>58</td>
<td>281</td>
</tr>
<tr>
<td>4 Forward</td>
<td>5'-CAT CCT GGG AGC GAG GTC TC-3'</td>
<td>58</td>
<td>272</td>
</tr>
<tr>
<td>4 Reverse</td>
<td>5'-AGC CAG GAC CCT CTC ACT AC-3'</td>
<td>58</td>
<td>281</td>
</tr>
</tbody>
</table>

Clinical examination

Clinical findings were characterized by visual acuity, kinetic visual field testing using Goldmann perimetry, slit-lamp biomicroscopy, fundus findings, and electroretinography (ERG) by a method previously reported [22]. Briefly, ERGs were obtained using a full-field stimulus with 200 cd s/m² white flash in the dark-adapted condition (30 min) for maximal responses of both rods and cones. The isolated cone responses were recorded by 10 cd s/m² 30-Hz white flicker stimulation under light-adapted conditions (10 min). The instrument we used was LE-1000 (Tomey, Nagoya, Japan).

**Results**

Mutation screening

We identified a novel heterozygous transition mutation from guanine (G) to adenine (A) nucleotide in codon 157 of the GCAP 2 gene that resulted in an amino acid substitution of arginine (AGA) for glycine (GGA) in three unrelated patients (Nos. 128, 120, and 230) with ADRP (Fig. 1, Table 1). This nucleotide change, G157R, was not found in any of the 33 patients with ARRP or in the 100 normal subjects. We also identified another transition mutation from cytosine (C) to thymine (T) in codon 87, resulting in an amino acid substitution of isoleucine (ATC) for threonine (ACC) in a patient with ADRP. This missense mutation (T87I), however, did not segregate with the disease but was likely considered a polymorphism, as described in [14] Segregation study and clinical features [17] (Table 2). In addition, we detected three other previously unreported silent mutations, Y57Y, V29V, and L180L, that appeared unrelated to the disease (Table 2).

**Fig. 1** A novel G157R missense mutation was found in our patients. The bi-directional sequencing data showed heterozygous G157R (GGA to AGA) mutation in both forward (left) and reverse (right) strands

**Table 2** Nucleotide substitutions in the GCAP2 gene found in patients with retinitis pigmentosa (RP)
<table>
<thead>
<tr>
<th>Type of RP</th>
<th>Exon</th>
<th>Codon #</th>
<th>Base change</th>
<th>Amino acid change</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADRP (n=63)</td>
<td>1</td>
<td>57</td>
<td>TAT → TAC</td>
<td>Y57Y</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>87</td>
<td>ACC → ATC</td>
<td>T87I</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>157</td>
<td>GGA → AGA</td>
<td>G157R</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>180</td>
<td>CTG → CTA</td>
<td>L180L</td>
<td>1</td>
</tr>
<tr>
<td>ARR (n=33)</td>
<td>1</td>
<td>57</td>
<td>TAT → TAC</td>
<td>Y57Y</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>1</td>
</tr>
</tbody>
</table>

Segregation study and clinical features

**G157R mutation**

We subsequently performed family analyses of three pedigrees harboring the G157R mutation (Families A, B, and C in Fig. 2). In Family A, we examined six members; four members have the heterozygous G157R mutation in the GCAP 2 gene and two members do not (Fig. 2a). Among the four who harbor the mutation, we found three members (III-7, III-8, III-9, No. 128) who have RP and one member (III-10) who has macular degeneration (Fig. 2a–c). The regional distribution of retinal degeneration in patients with RP was variable, including diffuse pigmentary degeneration in the mid-peripheral area (III-7 and III-9, Fig. 2a,b), and focal pigmentary retinal degeneration located mainly in the nasal area associated with macular degeneration (III-8, Fig. 2b,c). Results of kinetic visual field tests revealed that the visual field defects corresponded to the area affected by the retinal degeneration (Fig. 4). The ERG findings demonstrated that patients III-7 and III-9 showed severe deterioration of both rods and cones, while patient III-8 still had recordable responses of both rods and cones (Fig. 5). Because we could not perform ERGs and visual field examination on patient III-10, we do not know his exact clinical features. We speculated, however, that his phenotypic expression is probably macular degeneration, because he has reported no night blindness or constricted visual field. Also, he had no abnormal fundus findings except in the macula (Fig. 2c).

![Pedigrees showing affected members with autosomal dominant retinal dystrophies associated with G157R (Family A in a and Families B and C in b) mutation in the GCAP2 gene. Open symbols indicate unaffected subjects; solid symbols, affected persons; X, individuals examined in this study; symbols with slashes, deceased members; (+), subjects with the mutation; (−), subjects without the mutation; arrows, proband; a, pedigree of Family A; and b, pedigrees of Families B and C.](http://www.springerlink.com/media/64T4F2WUXQXHNBCCL/Contributions/7/G/6/2/7G626UGK1TAPM8CR_html/fulltext.html)
Fundus photographs of affected family members. a, Diffuse RP with asteroid hyalosis in III-7 of Family A; b, Focal RP in the nasal mid-peripheral area in III-8 of Family A; c, Macular degeneration in the same patient as Family A; d, Diffuse RP in III-9 of Family A; e, Macular degeneration in III-10 of Family A; f, Diffuse RP in II-1 of Family B; g, Diffuse RP in II-4 of Family C

Fig. 3 Fundus photographs of affected family members. a, Diffuse RP with asteroid hyalosis in III-7 of Family A; b, Focal RP in the nasal mid-peripheral area in III-8 of Family A; c, Macular degeneration in the same patient as Family A; d, Diffuse RP in III-9 of Family A; e, Macular degeneration in III-10 of Family A; f, Diffuse RP in II-1 of Family B; g, Diffuse RP in II-4 of Family C
Results of Goldmann kinetic visual field testing of affected members. Visual fields of III-7 of Family A (top), III-8 of Family A (middle upper), III-9 of Family A (middle lower), and II-4 of Family C (bottom) show various degrees of visual field impairment corresponding to the area affected by the retinal degeneration.

Fig. 4
Electroretinograms (ERGs) of affected members. Both the maximal responses of combined rods and cones and those of 30-Hz flicker stimuli of III-7 and III-9 of Family A and II-1 of Family B show severe deteriorations in amplitudes, whereas responses of III-8 of Family A demonstrate reduced amplitudes in both rods (maximal response) and cones (30-Hz flicker). Conditions of ERG recordings are described in Methods.

Although we examined only two members of Family B (Fig. 2b), this pedigree was anamnetically a branch of a family with ADRP. The two members showed quite different phenotypes, although both carried the same G157R mutation (Table 3). Patient II-1 (No. 120) exhibited typical features of diffuse pigmentary degeneration, with severe deterioration of both rods and cones (Table 3 and Figs. 3f, 3d), whereas her mother, patient I-2 showed normal findings (data not shown). Because members of Family C live in remote areas, we were able to examine only one member (II-4, No. 230), who demonstrated a diffuse type of RP similar to that of patient No. 120 in Family B (Table 3 and Figs. 3g, 3d).

Table 3 Summary of clinical findings associated with G157R mutation of the GCAP2 gene
Heterozygous G157R mutation.
bd, not done.
rcr, non-recordable.
ddeg, degeneration.

T87I mutation

Because members of Family D showed a previously unknown heterozygous missense mutation, T87I of the GCAP2 gene, we also examined affected members of this family. Two members (a daughter and her mother) showed the same mutation, but another daughter had a normal sequence of the gene in spite of having RP. We considered, therefore, that this missense mutation most likely did not cause RP in this family, but that this nucleotide change was a newly identified polymorphism. There is a possibility that this mutation may play the role of a genetic modifier.

Discussion

In the present study, we initially found a novel missense mutation, G157R, in the GCAP2 gene in three patients of three unrelated Japanese families with ADRP. A subsequent investigation of family members revealed that the phenotypic characteristics associated with the G157R mutation included not only RP but also macular degeneration, a combination of RP and macular degeneration, and funduscopy normal retina. Based on our examinations, all family members who had any form of retinal degeneration also had the mutation. Considering the possibility of incomplete penetrance, therefore, it is reasonable that a mutation in the GCAP2 gene may cause one form of autosomal dominant retinal dystrophy with phenotypic variation among Japanese populations. In addition, the factors that support the pathogeneity of the G157R mutation are first, that this mutation was independently found in the three unrelated families; second, that this mutation was not found in 100 control subjects or 33 unrelated patients with ADRP; and third, that the amino acid substitution is relatively severe. Although the exact genetic frequency of the mutation should be determined after haplotype analysis of these three families, it is interesting that the G157R mutation is found only in members of families with autosomal dominant inheritance.

Although the precise molecular mechanism is uncertain, we can speculate that the defective GCAP2 eventually leads to photoreceptor degeneration. Both GCAPs 1 and 2 are known to play a key role in the recovery of phototransduction [2]. Photoactivation of rhodopsin eventually causes deprivation of cytoplasmic cGMP, leading to closing of cGMP-gated cation channels and hyperpolarization of photoreceptors. These biochemical events induce deprivation of intracellular Ca²⁺, and GCAPs 1 and 2 are subsequently activated to stimulate membrane-bound ret-GC 1 and 2 to maintain the intracellular concentration of cGMP. Because mutations in genes coding for ret-GC and GCAP 1 have been known, respectively to cause retinal dystrophies [4, 13, 15, 20], mutations in the GCAP 2 gene, and defective GCAP 2 protein may have a potential to cause insufficient regulation of activities of ret-GC 1 and/or 2, eventually leading to photoreceptor dysfunction or even cell death. In animal experiments, GCAP 2 knockout mice showed normal responses to the paired-flash, if the intact GCAP 1 gene was introduced by transgenic techniques [7], suggesting that intact GCAP 1 can compensate for a role in GCAP 2. However, because the patterns of intraretinal regional expression of GCAPs 1 and 2 proteins are different between mice and humans [3], the role of GCAP 2 may be different between different species. It is also possible that the presence of GCAP 2 is mandatory for human photoreceptors. In particular, the EF-hand motif is a key domain for GCAPs to act as activator proteins. Because the glycine residue at amino acid position 157 is involved in the fourth EF-hand motif, EF-4 (Fig. 6), a conversion from glycine to arginine, a highly charged amino acid, at this position can deteriorate the function of GCAP 2. As has been experimentally proven, for the Y99C mutant GCAP 1 to constitutively stimulate ret-GC even at high calcium concentration [3, 17], the G157R mutant GCAP 2 would also be expected to persistently stimulate ret-GC 1 and/or 2 under dark conditions (high Ca²⁺), where these enzymes should be inactive. The consequence is elevated levels of cGMP, which cause photoreceptor degeneration.

![Fig. 6 Amino acid sequence around EF-4 region of GCAP2. G157R mutation causes conversion of glycine to highly charged amino acid arginine in the stretch of the EF-4 region](http://www.springerlink.com/media/64T4F2UWXQJWENXHLBCL/Contributions/7/G/6/2/7G626UGK1TAPM8CR_html/fulltext.html)
In the present study, we identified variable phenotypes. Among seven subjects harboring the mutation, four members had diffuse RP, one had focal RP with macular degeneration, one had isolated macular degeneration, and one had normal fundus. Such variable expressivity is somewhat similar to the phenotypic variety caused by mutations in the peripherin/RDS gene [1, 2]. Diffuse peripheral retinal degeneration, pattern dystrophy of the macula, and even normal phenotype have been reported to be associated with the P210R mutation of the peripherin/RDS gene [3]. Although the mechanism is uncertain for the phenotypic variability in mutations of the peripherin/RDS gene, a possible interaction between peripherin/RDS protein and the highly homologous protein rom1 has been hypothesized [4]. Similarly, it can be speculated that the presence of intact GCAP1 can modify the effect of mutant GCAP2 on the formation of retinal degeneration.

The presence of incomplete penetrance is characteristic for phenotypic expression of RP11, which has been known to be caused by mutation in the PRPF31 gene in ADRP [10, 13]. It is suggested that one of the putative phenotypic characteristics associated with mutations in the GCAP2 gene is also present in subjects with apparently normal fundi. In addition, there is certainly a racial difference in terms of kinds of nucleotide substitutions between British and Japanese patient populations. Our G157R mutation was not found in the previous study of British patients, and all of our polymorphisms were different from those found previously [14]. Further investigations including more extended segregation study and haplotype analysis are needed to clarify the role of mutations in the GCAP2 gene in the process of retinal degeneration.

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References


