Genetic analysis of the guanylate cyclase activator 1B (GUCA1B) gene in patients with autosomal dominant retinal dystrophies

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Abstract

The guanylate cyclase activator proteins (GCAP1 and GCAP2) are calcium binding proteins which play a key role in the recovery phase of phototransduction. Recently a mutation in the GUCA1A gene (coding for GCAP1) mapping to the 6p21.1 region was described as causing cone dystrophy in a British family. In addition mutations in Ret-GC1 have been shown to cause Leber congenital amaurosis and cone-rod dystrophy. To determine whether GCAP2 is involved in dominant retinal degenerative diseases, the GCAP2 gene was screened in 400 unrelated subjects with autosomal dominant central and peripheral retinal dystrophies.

A number of changes involving the intronic as well as the coding sequence were observed. In exon 1 a T to C nucleotide change was observed leaving the tyrosine residue 57 unchanged. In exon 3 a 1 bp intronic insertion, a single nucleotide substitution G to A in the intron 3' of this exon, and a GAG to GAT change at codon 155 were observed. This latter change results in a conservative change of glutamic acid to aspartic acid. In exon 4 a 7 bp intronic insertion, a single nucleotide substitution G to A in the intron 5' of this exon, and a single base pair change C to G in the intron 3' of exon 4 were seen. None of these changes would be expected to affect correct splicing of this gene. All these changes were observed in controls. The results of this study do not show any evidence so far that GCAP2 is involved in the pathogenesis of autosomal dominant retinal degeneration in this group of patients. All the changes detected were found to be sequence variations or polymorphisms and not disease causing.

Keywords: guanylate cyclase activator protein 2 (GCAP2); retinal dystrophy; mutation screening

The guanylate cyclase activator proteins (GCAP1 and GCAP2) are calcium binding proteins which play a key role in the recovery phase of phototransduction. Light activation of rhodopsin stimulates a biochemical cascade, as part of which phosphodiesterase is activated and induces hydrolysis of cyclic guanosine monophosphate (cGMP), leading to low levels of cytoplasmic cGMP. This results in a reduction of the proportion of open cGMP gated channels and cellular hyperpolarisation. The cGMP channels therefore close and prevent entry of calcium and sodium. In addition, calcium is actively extruded by a light insensitive Na+/Ca2+ exchanger. Once the cytoplasmic calcium concentration falls to a critical level, the GCAP proteins are activated and stimulate the membrane bound photoreceptor guanylate cyclase(s) RetGC-1 and RetGC-2. These, in turn, accelerate synthesis of cGMP, resulting in opening of the cGMP channels, thus restoring the “dark current”.

The two GCAP proteins appear to have different activation specificities for the two RetGC proteins. GCAP1 modulates the activity of RetGC-1, but is almost inactive in stimulation of RetGC-2, while GCAP2, although it is comparatively much less effective, activates both RetGC proteins with similar affinities. The GCAP proteins are members of the EF hand containing superfamily of Ca2+ binding proteins, and display structural similarity to calmodulin, recoverin, and calcineurin B at the amino acid level.

GCAP1 has been localised in human retina using immunostaining techniques, and in particular to cone photoreceptor inner and outer segments. Immunoreactivity was weaker in rod inner segments and minimal in rod outer segments. This pattern parallels the immunolocalisation of RetGC-1. GCAP2 has been shown to be present in cones and in rods (rod outer segment immunostaining was more intense than in GCAP1). In addition, GCAP2 immunostaining was also seen in two types of ganglion cells, a type of amacrine cell, and more diffusely across the inner plexiform layer.

The human GCAP1 and GCAP2 genes are arranged in a tail to tail array on the short arm of chromosome 6p21.1. Both genes consist of four exons with greatest similarity between them limited to portions of exons 1 and 2. The 1.8 kb mRNA of GCAP1 and the 2.2 kb mRNA of GCAP2 are detectable solely in retina tissue by northern blotting. The two genes are thought to have arisen through a gene duplication event. As the GCAP proteins are important regulatory components of the phototransduction cascade, a mutation in either of the GCAP proteins would be expected to influence photoreceptor function.

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causing cone dystrophy in a British family was described recently. Additionally, mutations in RetGC-1 have been shown to cause Leber congenital amaurosis and cone-rod dystrophy.

To determine whether GCAP2 is involved in dominant retinal degenerative diseases, the GCAP2 gene was screened in 400 unrelated subjects with autosomal dominant central and peripheral retinal dystrophies.

**Materials and methods**

Peripheral blood was taken from 400 patients with autosomal dominant disorders seen at genetic clinics at Moorfields Eye Hospital. The diagnoses in these patients included retinitis pigmentosa (200 patients), cone (20) and cone-rod (80) dystrophy, and macular dystrophies (100). These patients were diagnosed as having an autosomal dominant mode of inheritance since one parent was known to have been affected. Peripheral blood was also collected from 50 unrelated normal subjects to act as normal controls. These controls were spouses of the patients and have been clinically examined; they are from the same ethnic background as the patients and are of a similar age.

Genomic DNA was extracted using the Nucleon II extraction kit (Scotlab Bioscience). This DNA was used to perform mutation analysis by polymerase chain reactions (PCR) as described below.

**Heteroduplex analysis**

Genomic DNA was amplified using primers that allowed amplification of the complete coding region of GCAP2. The primer sequences are as follows: (forward/reverse, 5'-3') GUC41B exon 1, tcaagctctggaagggccaggtg (305 bp), exon 2, gagaagctctgtgctagggcttcgacctgctgc (305 bp), exon 3, gagaagctctctctgttcagagc (272 bp), exon 4, ctcgcagcagcgtcctctgacgagc (281 bp). Annealing temperature for all primers was 58°C. The PCR reaction buffer contained 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.3), 1.5 mmol/l MgCl₂, 0.01% (w/v) gelatin, 160 µmol/l of each primer, and 0.5 U Taq DNA polymerase (Promega). The amplified exons were analysed by electrophoresis using MDE gel run at 180 V overnight using Hoeffer 600S apparatus.

**Direct Sequencing**

Products of PCR amplification were sequenced using the PRISM™ Ready Reaction Sequencing Kit (Perkin Elmer Cetus) and the products were analysed on an ABI 373 automated sequencer. All PCR products were sequenced in the forward and reverse directions.

**Results**

Heteroduplexes were seen in DNA from patients in exons 1, 3, and 4. These PCR products were sequenced to determine the nucleotide changes in these patients.

**EXON 1**

The change was identified as a T to C nucleotide change that resulted in a third base pair substitution giving a silent polymorphism leaving the tyrosine residue 57 unchanged. This polymorphism has been identified in approximately 35% of our normal and diseased populations.

**EXON 3**

Three changes were observed in this exon. The first was a 1 bp intronic insertion −22 bp from the 3' splice site of exon 3. This is predicted not to affect the correct splicing of this exon, and indeed the insertion did not segregate with disease in the pedigree. The insertion was only seen in one patient of the 400 affected subjects and in none of our controls, which suggests a rare sequence variation. The second change seen in six of our patients was a single nucleotide substitution G to A in the intron 9 bp from the 3' splice site of this exon; again this is not predicted to alter splicing. The third change seen in two patients was a GAG to GAT change at codon 155. This results in a conservative change of glutamic acid to aspartic acid. The Glu is within the EF4 hand predicted calcium binding site of the molecule and is conserved in the human and bovine amino acid sequences. However, the change still conforms to the consensus sequence for EF hands. In addition, this change and the others seen in exon 3 were also detected in our normal controls.

**EXON 4**

Three changes were observed in the affected group. The first was a 7 bp intronic insertion −59 bp from the 5' splice site of exon 4. The insertion was only seen in two patients in this group and in none of our controls, suggesting that it is a rare sequence variation. The second change seen in 10 of the affected group was a single nucleotide A to G substitution in the intron 30 bp from the 5' splice site of this exon. The third change was again a single base pair change C to G in the intron 34 bp from the 3' splice site of exon 4. These changes would not be expected to affect correct splicing of this exon and were observed in controls.

These results are shown in table 1.

**Discussion**

The results of this study did not show any evidence that GCAP2 was involved in the pathogenesis of autosomal dominant retinal degener-

**Table 1** Sequence variations and polymorphisms found in the GCAP2 gene and their frequency in the disease and control populations studied

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Frequency in patients</th>
<th>Frequency in controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T to C</td>
<td>141</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>1 bp intronic ins</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>−22 bp from 3’ splice site</td>
<td>Intron G to A</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>−9 bp from 3’ splice site</td>
<td>G to T</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>7 bp intronic ins</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>−59 bp from 5’ splice site</td>
<td>Intron A to G</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>−30 bp from 5’ splice site</td>
<td>Intron C to G</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>−34 bp from 3’ splice site</td>
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</table>
eration in this group of patients. In addition, the changes detected were considered to be sequence variations or polymorphisms and not disease causing.

The biochemical studies on GCAP2 show that it is less effective than GCAP1 at stimulating RetGC-1 suggesting that it plays a lesser role in photoreceptor recovery than GCAP1. GCAP2 has, however, been shown to have a unique role in stimulating RetGC-2 (structurally similar to RetGC-1 and also found in the retina) in vitro. Both GCAP2 and RetGC-2 have an as yet undefined function in the retina, neither being part of the phototransduction pathway. RetGC-2 (located on chromosome Xq22) has been screened for mutations in patients with X linked retinal degenerations (Xq22) has been screened for mutations in the 6p21.1 region. In autosomal dominant retinal degenerative diseases, it is not a common cause of retinal degeneration in our population, they should be considered as a possible cause of degeneration in families linked to the 6p21.1 region.

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