Novel Complex \textit{GUCY2D} Mutation in Japanese Family with Cone-Rod Dystrophy

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\textbf{Purpose.} All mutations in the retinal guanylate cyclase gene (\textit{GUCY2D}) that causes autosomal dominant cone-rod dystrophy (CORD) are associated with an amino acid substitution in codon 838. A novel heterozygous complex missense mutation of I915T and G917R in the \textit{GUCY2D} gene was found in a Japanese family with autosomal dominant CORD. The clinical features associated with this mutation were described.

\textbf{Methods.} Blood samples were collected from 27 patients with cone-rod or cone dystrophies and from 11 patients with macular dystrophy. Genomic DNA was extracted from peripheral leukocytes. All 18 coding exons of the \textit{GUCY2D} gene were directly sequenced. The PCR product carrying a novel mutation was subcloned, and each allele was sequenced. A complete ophthalmologic examination was performed in members of the family with the novel mutation.

\textbf{Results.} A novel heterozygous complex missense mutation of T2817C and G2822C that would predict I915T and G917R amino acid substitutions, respectively, was found in an autosomal dominant CORD family. The two nucleotide changes were located on the same allele, and segregated with the disease. Two other known missense mutations of R838H and R838C were found in two other CORD families. The clinical phenotype associated with the novel mutation was similar to that with the Arg838 mutations.

\textbf{Conclusions.} A heterozygous complex mutation of I915T and G917R in the \textit{GUCY2D} gene caused autosomal dominant CORD, indicating that a heterozygous mutation that does not include a codon 838 substitution can lead to this ocular phenotype. (\textit{Invest Ophthal Mol Vis Sci.} 2004;45:1480–1485) DOI:10.1167/iovs.03-0315

\textbf{Cone-rod dystrophy (CORD)} is a subgroup of inherited chorioretinal dystrophies that is associated with progressive impairment of central vision and a central scotoma with atrophic retinal changes in the macula. The typical symptoms at onset include loss of color discrimination and central visual disturbances. The electrophysiological results are a greater reduction of the cone electroretinograms (ERGs) than the rod ERGs. In patients with cone dystrophy (COD), cone function is depressed progressively but rod function is well preserved until the late stages.

CORD is genetically heterogeneous, and according to RETNET (http://www.sph.uth.tmc.edu/retnet/ home.htm), nine genes have been identified to cause CORD or COD: \textit{CRX}, \textit{GUCY2D}, \textit{AIPL1}, \textit{GUCA1A}, \textit{RIMS1}, \textit{UNC119}, \textit{ABCA4}, and \textit{RDH15} are causative genes for autosomal dominant CORD; \textit{ABCA4} and \textit{RDH15} are causative genes of autosomal recessive CORD; and \textit{RPGR} is a causative gene of X-linked recessive CORD. Also, six loci of the CORD-causing genes have been mapped; RCD1 on 6q25-q26, COD2 on Xq27, CORD4 on 17q, CORD5 on 17p13-p12, CORD8 on 1q12-q24, and CORD9 on 8p11.

The \textit{GUCY2D} gene encodes human photoreceptor-specific guanylate cyclase (RetGC-1) which catalyzes the conversion of guanosine triphosphate (GTP) to cyclic 5',5'-guanosine monophosphate (cGMP) in mammalian photoreceptor cells. The gene was first proved to be responsible for autosomal recessive Leber congenital amaurosis (LCA) as a homozygous or a compound heterozygous mutation. In 1998, a heterozygous mutation of the \textit{GUCY2D} gene was found to cause another ocular phenotype. Thus, a heterozygous complex mutation of E837D and R838S was identified in a large British family with autosomal dominant CORD (CORD6). Subsequently, three additional heterozygous mutations in the \textit{GUCY2D} gene in ten unrelated European families with CORD have been reported; R838C in six families, R838H in two families, and a triple mutation of E837D and R838C + T839M in one family. All of these mutations causing CORD are present in the same or in adjacent codons including codon 838, and are located in the putative dimerization domain of the RetGC-1 protein.

A novel heterozygous complex missense mutation, T to C at nucleotide 2817 predicting an I915T amino acid substitution, and a G to C at nucleotide 2822 predicting a G917R amino acid substitution was analyzed. This mutation was found in a family with autosomal dominant CORD. Their clinical phenotypes indicated that a heterozygous mutation not involving codon 838 can cause an ocular phenotype of autosomal dominant CORD.

\textbf{Patients and Methods}

\textbf{Subjects} Based on ERG examinations, 27 families that had CORD or COD and 11 families with macular dystrophy (MD) were chosen for this study. Nine families with CORD or COD and one family with MD showed an autosomal dominant inheritance pattern. To the best of our knowledge, the families were not related.

In family #6 with a novel complex mutation, 6 affected members and 4 unaffected members were used for a segregation study and clinical examinations (Fig. 1). Other members of the family would not consent to participate in this study.
**RESULTS**

**Genetic Analysis**

The screening of GUCY2D in 38 patients with CORD, COD, or MD revealed three disease-causing mutations in three separate Japanese families with autosomal dominant CORD. In one family (#65; Fig. 1), novel double heterozygous sequence changes were detected in the proband (IV-7). The changes were T to C transition at nucleotide 2817 and G to C transition at nucleotide 2822 in exon 14, resulting in missense substitutions of threonine for isoleucine in codon 915 and arginine for glycine in codon 917, respectively (Fig. 2A). Direct genomic DNA sequencing of the GUCY2D gene in the other 9 available members of the family showed that all 5 affected members had both heterozygous sequence changes, and none of the unaffected members had either of the two sequence changes. These results led to the suspicion that the novel double sequence changes would be located on the same allele. To confirm this, the PCR product of exon 14 of the gene from the proband (IV-7) was cloned into a pCR2.1-TOPO vector and the sequences of each allele were determined. Six out of ten clones had both sequence changes (Fig. 2C), whereas the remaining four showed the wild-type sequence (Fig. 2B). Thus, the two missense sequence changes were considered to be located on the same allele.

We further confirmed the segregation of the mutation by SSCP analysis using the PCR products of exon 14 of the GUCY2D from all available family members. Affected individuals with the I915T and G917R mutations showed an excess band due to possible unusual homoduplex or heteroduplex molecules on the gel, which were presented neither in unaffected individuals nor in a normal control (data not shown).

Two other heterozygous mutations in two other autosomal dominant CORD families were identified from this screening; a missense mutation of G to A at nucleotide 2586, predicting an R838H amino acid substitution, and a missense mutation of C to T at nucleotide 2585, predicting an R838C amino acid substitution (Table 1). These two mutations were the same as those reported in white people,\(^5,^{14-16}\) indicating that codon 838 is a focus for autosomal dominant CORD internationally. The detailed clinical features of the families with the codon 838 mutations will be described elsewhere.

Eleven other sequence variants were noted, and five were predicted to cause amino acid changes. However, whether they were disease-causing mutations could not be determined. All mutations and polymorphisms found are listed in Table 1. A heterozygous T134C sequence change (W21R) was found in a female patient and was not present in 100 normal control alleles. This mutation was believed not to be disease-causing but a rare polymorphism because the patient’s father had the sequence change homozygously without any ocular abnormality.

A heterozygous G1697A sequence change (G542S) was found in a male proband but not in 100 normal control alleles. However, whether it was a disease-causing mutation or a rare polymorphism could not be determined, because no other family members were available for a segregation study. A G227T (A52S) sequence change was found in 31 probands, homozygously in 13, and heterozygously in 18. However, it was also detected in normal control individuals homozygously in 25 and heterozygously in 23, and was considered to be a polymorphism.

A C2174T (P701S) variant, that was found in 17 probands, homozygously in 2 and heterozygously in 15, was also considered to be unrelated to the disease because it was detected in 19 normal control individuals heterozygously. A C257T (T55M) sequence change was found in 3 healthy individuals heterozygously, but was not found in patients. Three heterozygous silent mutations, C154T (P27P), G2167A (P658P), and C2257T (D728D), were detected in only one proband and were not found in control alleles. Another silent mutation, G2575A (L834L), was found heterozygously in two normal control individuals but not in patients. Two other silent mutations, C814T (H247H) and C1444T (C457C), were identified in both patients and controls (Table 1).

**Phenotype of Family #65 (I915T+G917R)**

Family #65 with CORD showed a typical autosomal dominant hereditary pattern through four generations (Fig. 1). The male proband (IV-7) who first noticed a reduction of visual acuity at approximately 20 years of age was referred to our hospital at age of 25 years. His best-corrected visual acuity was 0.1 in the right eye and 0.09 in the left eye with severe myopic refractive errors of approximately −10 diopters in each eye. He failed all of the Ishihara color plates, and the panel D-15 test revealed tritanomaly.

Fundus examination showed only minimal disruption with tiny yellow deposits in the fovea (Fig. 3E), and fluorescein angiography showed no apparent abnormality (Fig. 3F). No visual field defect was detected by Goldmann kinetic perimetry in both eyes (Fig. 4C).
FIGURE 2. (A) Nucleotide sequences of GUCY2D using sense primers in the patients of the family (#65). Two closely-located heterozygotic missense mutations of T to C at nucleotide 2817 (I915T) and G to C at nucleotide 2822 (G917R) were found in exon 14. (B) An allele of exon 14 of the gene from the proband (IV-7) was the wild type without either mutation. (C) Another allele of exon 14 of the gene from the proband (IV-7) carried both mutations of I915T and G917R showing that the two missense sequence changes were located on the same allele. (D) Amino acid sequence alignment of human RetGC-1, human RetGC-2,25 rat GC-E, GC-F26 and bovine ROS-GC.27 Asterisks identify residues of identity, and residues Ile915, which is replaced by Thr, and Gly917 which is replaced by Arg are indicated by arrows.

Table 1. Mutations and Polymorphisms in the GUCY2D Gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide Variant*</th>
<th>Amino Acid Change</th>
<th>CORD Proband (%)</th>
<th>Normal Controls (%)</th>
<th>Type of Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>134T→C</td>
<td>W21R</td>
<td>1/76 (1.3)</td>
<td>0/100</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>2</td>
<td>154C→T</td>
<td>P27P</td>
<td>1/76 (1.3)</td>
<td>0/100</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>2</td>
<td>227G→T</td>
<td>A52S</td>
<td>44/76 (58)</td>
<td>75/100 (73)</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>2</td>
<td>237C→T</td>
<td>T55M</td>
<td>0/76</td>
<td>3/100 (3.0)</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>3</td>
<td>814C→T</td>
<td>H247R</td>
<td>20/76 (26)</td>
<td>15/100 (15)</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>3</td>
<td>1444C→T</td>
<td>C457C</td>
<td>6/76 (7.9)</td>
<td>11/100 (11)</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>7</td>
<td>1697G→A</td>
<td>G542S</td>
<td>1/76 (1.3)</td>
<td>0/100</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>10</td>
<td>2167G→A</td>
<td>P698P</td>
<td>1/76 (1.3)</td>
<td>0/100</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>10</td>
<td>2174C→T</td>
<td>P701S</td>
<td>19/76 (25)</td>
<td>19/100 (19)</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>11</td>
<td>2257C→T</td>
<td>D728D</td>
<td>1/76 (1.3)</td>
<td>0/100</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>13</td>
<td>2575G→A</td>
<td>L834H</td>
<td>0/76</td>
<td>2/100 (2.0)</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>13</td>
<td>2585C→T</td>
<td>R838C</td>
<td>1/76 (1.3)</td>
<td>0/100</td>
<td>Missense</td>
</tr>
<tr>
<td>13</td>
<td>2586G→A</td>
<td>R838H</td>
<td>1/76 (1.3)</td>
<td>0/100</td>
<td>Missense</td>
</tr>
<tr>
<td>14</td>
<td>2817T→C + 2822G→C</td>
<td>I915T + G917R</td>
<td>1/76 (1.3)</td>
<td>0/100</td>
<td>Missense + missense</td>
</tr>
</tbody>
</table>

* Nucleotides numbered from the ATG initiator codon based on a cDNA reference (GenBank M92432).
† Not determined because a segregation analysis could not be performed.
The best-corrected visual acuity of the proband’s affected father (III-10) was 0.09 in each eye at the age of 57 years. He apparently had macular degeneration in both eyes (Figs. 3C, 3D), with bilateral paracentral scotoma (Fig. 4B). He failed all of the Ishihara color plates, and also failed the panel D-15 test without any axis.

The proband’s 83-year-old grandmother (II-4) had a more severe phenotype. Her best-corrected visual acuity was 0.05 in the right eye and 0.01 in the left. The central atrophic retinal lesions and the central scotoma were larger than that of her son (Figs. 3A, 3B, 4A). She was barely able to perform the Ishihara color vision and panel D-15 tests because of her poor vision.

Another young affected member in another branch of this family (IV-2; 29-years-old, with corrected visual acuity of 0.2 in each eye) had only mild fundus changes (Fig. 3H) with almost normal visual field, that were similar to those of the proband (IV-7). She failed the panel D-15 test without any axis. Her 62-year-old affected father (III-2) showed macular dystrophy (Fig. 3G) and paracentral scotoma above the center in each eye (Fig. 4D), that were similar to those in the proband’s affected father (III-10).

All four unaffected family members had good best-corrected visual acuities of > 1.2 in both eyes with normal visual fields, normal color visions, and normal fundus appearances. The refractive errors were either mild myopia or hyperopia in all of the affected and unaffected family members except for the high myopic proband (IV-7) and an unaffected man (IV-6) who had moderate myopia of approximately −5.0 diopters. Astigmatism was not high in all subjects.

Full-field ERGs recorded from the proband (IV-7) according to the ISCEV protocol showed that the photopic and 30 Hz flicker ERGs were almost nonrecordable, and that the amplitude of scotopic b-wave was subnormal. In the bright-flash mixed rod-cone ERG, the a- and b-waves as well as the oscillatory potentials (OPs) were within the normal range (Fig. 5A). Focal macular cone ERGs, which represent responses of the 15°, 10°, or 5° of the macula, were recorded from proband (IV-7), showing that the a- and b-waves as well as OPs were very reduced (Fig. 5B). Full-field ERGs and focal macular ERGs of the other family members were not performed.

**DISCUSSION**

The *GUCY2D* gene is the third gene identified as causing autosomal dominant CORD. Mutations in this gene also cause...
Leber congenital amaurosis (LCA), a more severe, early onset autosomal recessive retinal dystrophy. However, the mutations for LCA are causative in the homozygous or compound heterozygous state, and heterogeneous carriers of the mutations show essentially normal clinical phenotype except for subclinical mild reduction of cone and rod ERGs. Only mutations associated with codon 838 have been reported to cause the apparent ocular phenotype, CORD, in the heterozygous state.

These results would indicate that codon 838 is an important amino acid in RetGC-1, and in fact, an in vitro study has shown that the R838C mutation increases the affinity of recombinant RetGC-1 for GCAP1 and alters the Ca$^{2+}$ sensitivity of GCAP1 response. The mutant can be stimulated by GCAP1 at higher Ca$^{2+}$ concentrations than the wild type, which then causes a higher than normal rate of cGMP synthesis in dark-adapted photoreceptors, resulting in cone and rod degeneration.

The report of novel I915T>G917R mutations in an autosomal dominant CORD family is the first to show that a heterozygous mutation of GUCY2D not involving codon 838 is causative for CORD. Although an in vitro study was not performed, the codons 915 and/or 917 were assumed to be important for the function of the RetGC-1, because amino acid substitutions of these codons caused apparent ocular phenotypes in a heterozygous state similar to those of codon 838. In addition, alignment of part of this domain from human RetGC-1 and four other members of the subgroup (human RetGC-2, rat GC-E, GC-F, and bovine ROS-GC) show that both Ile915 and Gly917 are fully conserved among sensory cyclase family members (Fig. 2D). With the I915T>G917R mutations, the characteristics of the amino acid change from hydrophobic to hydrophilic in codon 915, and from neutral to basic in codon 917. The codons 915 and 917 are located within the putative catalytic domain of the RetGC-1 protein, and the secondary structure of the RetGC-1 protein predicted using the 3D-ID compatibility algorithm from the SSThread Program (http://www.ddbj.nig.ac.jp/E-mail/ssthread/welcome.html) was significantly changed in both the dimerization and catalytic domains by both amino acid changes (data not shown).

Whether only one of the mutations caused the clinical phenotype or both are concerned with the disease cooperatively was not determined. Possibly, one of the mutations is critical and another is a secondary mutation.

In the clinical phenotype of the affected members of the family with the I915T>G917R mutations, the visual acuity of the young 20's patients (IV-7 and IV-2) was between 0.1 and 0.2 with almost normal fundus and normal visual fields. The ERGs in the young proband (IV-7) demonstrated severely reduced cone function and nearly normal rod function (Fig. 5A). The visual acuity in patients at approximately 60 years of age (III-2 and III-10) was < 0.1 when macular degeneration became apparent with the presence of central scotoma. Color vision was impaired without any axis. The peripheral visual fields were relatively well-preserved even in the elderly patients (Fig. 4), and the central scotomas were more likely to be the upper area of the macula. These findings were similar to the previous descriptions of the phenotypes associated with codon 838 mutations, indicating that patients with CORD caused by the GUCY2D mutations have an overall clinical phenotype similar to that with codon 838 even when the mutation is not associated with a codon 838 substitution.

Until now, only one large mutation screening of this gene has been conducted. A group of 40 patients, 27 with autosomal dominant MD and 13 with autosomal dominant CORD or COD, was screened, and three probands (7.5%) were identified as having mutations in the gene. In the present study, the frequency was almost the same; 3 out of 38 probands (7.8%) with CORD had GUCY2D mutations. However, the frequency became higher at 30% if we limited the subjects only to apparent autosomal dominant families, because only 10 out of the 38 families had apparent autosomal dominant inheritance pattern. These results suggested that GUCY2D mutations are relatively common as the cause of autosomal dominant CORD in the Japanese population.

Whether the codon 838 mutations arose separately or from a common ancestor was not determined, because all previously reported GUCY2D mutations were found in white populations. A haplotype analysis conducted among five families with R838C substitution could not answer this question. Our results that R838H and R838C mutations were present in Japanese patients far from Europe would suggest that codon 838 is a determinant for dominant CORD.

In conclusion, novel complex mutations of I915T>G917R in the GUCY2D gene were analyzed and the phenotypes described in an autosomal dominant CORD family. This is the first report showing that a GUCY2D mutation not involving codon 838 can cause autosomal dominant CORD.

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