Autosomal Dominant Cone-Rod Dystrophy With Mutations in the Guanylate Cyclase 2D Gene Encoding Retinal Guanylate Cyclase-1

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Objective: To describe the phenotype in 4 families with dominantly inherited cone-rod dystrophy, 1 with an R838C mutation and 1 with an R838H mutation in the guanylate cyclase 2D (GUCY2D) gene encoding retinal guanylate cyclase-1.

Methods: Psychophysical and electrophysiological evaluation and confocal laser scanning ophthalmoscopic imaging was performed on 10 affected members of 4 British families.

Results: Although subjects had lifelong poor vision in bright light, a major reduction in visual acuity did not occur in most of them until after their late teens. Fundus abnormalities were confined to the central macula, and increasing central atrophy was noted with age. Increased background autofluorescence was observed surrounding the central atrophic area. Electrophysiological testing revealed a marked loss of cone function with only minimal rod involvement, even in older subjects. Photopic and scotopic static perimetry demonstrated central and peripheral cone-mediated threshold elevations with midperipheral sparing.

Conclusion: The phenotype associated with autosomal dominant cone-rod dystrophy with either an R838C or R838H mutation in GUCY2D is distinctive, with predominantly cone system involvement. There is some variation in severity within the 3 families with the R838C mutation.

Clinical Relevance: Families with the R838C or R838H mutation have a much milder phenotype than the family previously described that had 2 sequence changes, E837D and R838S, in GUCY2D.


ONE AND cone-rod dystrophies are a subgroup of the inherited retinal dystrophies. Cone dystrophies are characterized by poor central vision and an abnormal cone-isolated electroretinogram (ERG). In cone-rod dystrophy, the abnormality of rod function is less severe than that of cone function and may be detected later in the course of the disease than cone dysfunction. The diagnosis is established by electrophysiological evaluation; functional results depend on the stage of the disease and the age of the individual. The diagnosis of cone-rod dystrophy may be reinforced by the demonstration of peripheral as well as central visual field loss.

The most common symptoms of cone involvement include varying degrees of photophobia and loss of visual acuity, color vision, and central visual fields. Retinal appearance may be normal in the early stages of the disease, but with progression the retinal pigment epithelium (RPE) may take on a bull’s-eye pattern of change; later, central atrophy occurs. In some cases, temporal pallor of the optic nerve head is seen. Nystagmus may be present in early-onset disease. In cone-rod dystrophy, function that is dependent on rods, including visual fields and night vision, will be variably affected according to the degree of involvement. Varying degrees of intraretinal pigment and vessel attenuation occur.

To date, studies have shown that cone dystrophy and cone-rod dystrophy are genetically heterogeneous. Dominant, recessive, and X-linked inheritance patterns have been reported. Seven loci have been identified in autosomal dominant cone and cone-rod dystrophies. Loci associated with known genes include chromosome 6p12 with mutations in peripherin/RDS, chromosome 6p21 with mutations in GUCA1A, chromosome 17p with mutations in GUCY2D, and chromosome 17q with mutations in CRX. The other loci include 2 in chromosome 6q and a presumed autosomal dominant locus in chromosome 17q in association with neurofibromatosis. A sporadic case due
SUBJECTS AND METHODS

MOLECULAR ANALYSIS

The methods performed for linkage studies, mutation screening, heteroduplex electrophoresis, and direct genomic sequencing have been described previously.9

SUBJECTS

Three families with an R838C mutation and 1 family with an R838H mutation were invited to participate in the study. Five blood samples were available from family A, 8 from family B, 4 from family C, and 3 from family D. Three affected members from family A, 4 from family B, 2 from family C, and 1 from family D underwent phenotypic characterization. This research was performed in accordance with the Declaration of Helsinki, developed by the World Medical Association, and was approved by the Moorfields Eye Hospital Ethics Committee (London, England). Informed consent was obtained from all participants.

CLINICAL AND FUNCTIONAL INVESTIGATIONS

Phenotypic characterization included a full ophthalmic history and detailed fundus examination. Fundus photography, confocal scanning laser ophthalmoscopy, and psychophysical and electrophysiological evaluations were also performed.

Electrophysiology

Subjects underwent electrophysiological investigation using techniques in accord with the recommendations of the International Society for Clinical Electrophysiology of Vision.16-18 Electro-oculographic responses, full-field ERGs, and pattern ERGs were recorded for 10 subjects.

RESULTS

DNA ANALYSIS

An R838C mutation in GUCY2D was identified in 3 families (families A, B, and C), and an R838H mutation was discovered in 1 family (family D) (Figure 1). The mutation was found to segregate with disease in all 4 families. Within these 3 families and including the family with the 2 sequence changes in GUCY2D (R838S and E837D),9 there is some evidence of linkage disequilibrium between the disease allele and one of the flanking markers, indicating that this mutation may have had a common origin in a relatively distant ancestor. However, it was not possible to prove this conclusively.

CLINICAL FINDINGS

Clinical characteristics are documented in the Table. Most subjects became symptomatic during the first 2 decades of life, and the affected members of family B reported more disabling symptoms in the early stages of their disease than other families. All subjects were aware of lifelong poor vision in bright sunlight followed by reduced central vision and color vision. Eccentric fixation was common. No subject complained of nyctopia.

Visual acuities in families A, C, and D were found to be better than those of family B at a comparable age. However, no subject older than 50 years had a visual acuity better than 20/200.

FUNDUS APPEARANCE

Soon after the onset of visual symptoms, mild RPE granular abnormalities were apparent at the fovea (Figure 2A and 2B). At this stage, autofluorescence imaging showed...
Figure 1. Pedigrees of families A, B, C, and D. Roman numerals indicate generations; solid symbols, affected family members; open symbols, unaffected family members; squares, males; circles, females; and slash, deceased.

### Clinical Characteristics and Electrophysiological Results in 4 Families With Dominantly Inherited Cone-Rod Dystrophy

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Age, y</th>
<th>Age of Symptom Onset, y</th>
<th>P</th>
<th>Visual Acuity</th>
<th>EOG Rod-Specific ERG, µV</th>
<th>Bright White Flash ERG, µV</th>
<th>Photopic ERG b Wave, µV</th>
<th>Cone Flicker Response, µV</th>
<th>Color Contrast Sensitivity</th>
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</thead>
<tbody>
<tr>
<td>A:II/3</td>
<td>60</td>
<td>&lt;3</td>
<td>+++</td>
<td>Counting fingers OU 20/60 OU</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>A:III/3</td>
<td>32</td>
<td>17</td>
<td>+</td>
<td>Counting fingers OU 20/120 OU</td>
<td>170</td>
<td>170</td>
<td>a, 305; b, 360</td>
<td>30</td>
<td>Absent</td>
</tr>
<tr>
<td>A:III/4</td>
<td>29</td>
<td>3</td>
<td>+</td>
<td>Counting fingers OU 20/30 OU</td>
<td>225</td>
<td>160</td>
<td>a, 250; b, 350</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td>A:III/5</td>
<td>25</td>
<td>20</td>
<td>+</td>
<td>Counting fingers OU 20/400 OD 20/400 OU</td>
<td>205</td>
<td>140</td>
<td>a, 220; b, 305</td>
<td>45</td>
<td>↑↑↑ IMP</td>
</tr>
<tr>
<td>B:II/2</td>
<td>49</td>
<td>2</td>
<td>++</td>
<td>Counting fingers DS 20/400 OD 20/400 OU</td>
<td>180</td>
<td>90</td>
<td>a, 130; b, 210</td>
<td>Absent</td>
<td>Absent</td>
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<tr>
<td>B:II/4</td>
<td>46</td>
<td>6</td>
<td>++</td>
<td>Counting fingers DS 20/220 OU</td>
<td>205</td>
<td>165</td>
<td>a, 290; b, 350</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>B:III/1</td>
<td>28</td>
<td>6</td>
<td>+++</td>
<td>Counting fingers OU 20/220 OU</td>
<td>185</td>
<td>140</td>
<td>a, 195; b, 280</td>
<td>b, 10</td>
<td>15 ↑↑↑ IMP</td>
</tr>
<tr>
<td>B:III/3</td>
<td>16</td>
<td>14</td>
<td>++</td>
<td>Counting fingers OU 20/40 OU</td>
<td>260</td>
<td>120</td>
<td>a, 200; b, 325</td>
<td>b, 45</td>
<td>14 ↑↑ IMP</td>
</tr>
<tr>
<td>C:IV/2†</td>
<td>68</td>
<td>40s</td>
<td>+</td>
<td>Counting fingers DU 20/220 OS perception of light OD 20/120 OU</td>
<td>185</td>
<td>N</td>
<td>N</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>C:IV/6</td>
<td>78</td>
<td>20s</td>
<td>+</td>
<td>Counting fingers DU 20/220 OS perception of light OD 20/120 OU</td>
<td>Flats</td>
<td>65</td>
<td>a, 115; b, 135</td>
<td>Absent</td>
<td>Absent</td>
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<tr>
<td>D:IV/3</td>
<td>35</td>
<td>20s</td>
<td></td>
<td>Counting fingers DU 20/220 OS perception of light OD 20/120 OU</td>
<td>N</td>
<td>120</td>
<td>a, 200; b, 380</td>
<td>70 ↑TIMP</td>
<td>55 ↑TIMP</td>
</tr>
</tbody>
</table>

*No patient had nyctalopia. P indicates protan; EOG, electro-oculogram; ERG, electoretinogram; NP, not performed; a, a wave; b, b wave; IMP, implicit times; N, normal; D, deutan; and T, tritan. Plus signs indicate the presence of photophobia. One arrow indicates mild increase; 2 arrows, moderate increase; and 3 arrows, severe increase.

†This value was obtained without using the standard of the International Society for Clinical Electrophysiology of Vision.
Figure 2. A, The left macula of subject A:III/3, aged 32 years, shows minimal granular retinal pigment epithelial abnormalities. B, Reflectance imaging of the right macula of subject B:III/3, aged 16 years, shows a depressed foveal reflex. C, Autofluorescence imaging of the right macula of subject D:IV/3, aged 35 years, shows an area of decreased autofluorescence surrounding a small central area of retained autofluorescence at the fovea. D, Autofluorescence imaging of the left macula of the same subject with similar findings. E, The right macula of subject B:II/4, aged 46 years with a visual acuity of 20/400 OU, shows central atrophy. F, Autofluorescence imaging of the same eye shows a ring of autofluorescence surrounding this atrophy. G, The left macula of subject B:II/2, aged 49 years with a visual acuity of counting fingers OU, shows central atrophy with pigmentation. H, Autofluorescence imaging of the same fundus shows an annulus of increased autofluorescence surrounding the atrophic center shown on the left of Figure 2G. I, Macula of subject C:IV/2, aged 68 years with a visual acuity of counting fingers OU, shows central atrophic changes. J, Autofluorescence imaging in the same subject with increased autofluorescence surrounding the atrophic area.
little or no abnormality (Figure 2C and 2D). All other subjects had well-defined atrophy centered on the fovea without central sparing. No gross abnormalities of the peripheral retina were detected; in particular, there was no intraretinal pigmentation. Striking abnormalities of autofluorescence were visible including an annulus of increased autofluorescence around the perimeter of the atrophy (Figure 2E-J).

**ELECTROPHYSIOLOGY**

The electrophysiological results are presented in the Table and Figure 3. Pattern ERGs were extinguished in all patients; only 1 subject (A:II/3) did not have a pattern ERG. The electro-oculographic light rise was normal in each subject except for subject C:IV/6, who was the oldest one examined. The responses in each family were broadly similar, although family B had the more severe disease. Cone function was consistently more affected than rod function. All subjects had abnormal full-field cone responses characterized by markedly reduced amplitudes of photopic ERG a and b waves, and some had flicker responses with prolonged implicit time. Rod function was outside the normal range in only 3 subjects, including a 16-year-old boy. In the remaining subjects, the rod-specific ERG was at the lower end of the normal range.

**PSYCHOPHYSICS**

Sixty-degree photopic visual fields showed primarily central losses within 20° of fixation of greater than 30 dB. At 50° to 60°, a band of decreased sensitivity was also observed during photopic testing in all patients, the loss of sensitivity being less severe in family A (Figure 4A and B). Dark-adapted perimetry demonstrated losses of both rod and cone function primarily localized to the central macular region and extending to about 20° from fixation. The extent and magnitude of central rod and cone-mediated losses were similar (Figure 4C). The youngest individual (B:III/3) showed early losses of photopic sensitivity in the central macula of between 8 and 17 dB at the age of 16 years. His dark-adapted perimetry to both the red and blue stimuli showed sensitivities within 5 dB of normal values throughout the visual field.

Color vision was assessed in all subjects, and protan, deutan, and tritan thresholds were found to be grossly elevated with no axis predominantly affected.

The phenotype of cone-rod dystrophy reported in this article would be classified as type 1a according to Szlyk.

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**Figure 3.** The electrophysiology is shown of 2 subjects from family B: B:II/2, aged 49 years, and B:III/3, aged 16 years; 1 subject is from family A, and 1 is from family D. The youngest subject from family B has a rod electroretinogram (ERG) with a subnormal amplitude, as does subject D:IV/3, aged 35 years. The bright white flash ERG is subnormal in amplitude in all but subject B:II/2. Photopic and flicker ERGs are grossly delayed and reduced in amplitude in all subjects.

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et al,\textsuperscript{12} having relatively mild involvement of the peripheral field and rod-driven ERG responses. The cone-rod dystrophy phenotype we reported was less severe than that reported in a family with sequence changes at adjacent codons (R838S and E837D) of \textit{GUCY2D},\textsuperscript{10} which was categorized as type 2b using the classification of Szlyk and colleagues. Although difficulty seeing in bright light was present at an early age, the photophobia was much less disabling; in addition, our patients denied nyctopia. Peripheral visual field loss was mild, and intraretinal pigmentation was not seen in the midperipheral or peripheral retinas in our families. Finally, the electrophysiological abnormalities were less marked than those associated with sequence changes at codons R838S and E837D.\textsuperscript{10}

Of the genes described to date in association with cone-rod dystrophies, mutations in \textit{RDS} also exhibit early predominant cone involvement and have been classified as a type 2a cone-rod dystrophy; however, the phenotype associated with mutations in \textit{CRX} exhibits earlier and more severe rod involvement and is classified as type 2b.\textsuperscript{6,7,23}

Autofluorescence imaging demonstrates the distribution of disease at the level of the RPE better than any other technique and sheds light on the cellular changes. In vivo imaging and histopathologic investigations have provided evidence that the autofluorescent material is lipofuscin located in the RPE.\textsuperscript{24-26} Abnormally high levels of autofluorescence may occur if the metabolic demand is increased or the RPE function is compromised. The earliest abnormality of increased autofluorescence at the fovea implies that this is the site of initial dysfunction in our families, in contrast to bull's-eye dystrophies in which there is central sparing in the early stages of the disease. The lack of autofluorescence at the site of atrophy has been well recorded and is indicative of the loss of photoreceptor cells, or at least their outer segments.\textsuperscript{27} Increased autofluorescence at the edge of atrophy is likely to indicate an area destined to become atrophic. The dysfunction causing the autofluorescence may directly account for cell death. Alternatively, the high levels of lipofuscin may contribute to cell loss due to the release of free radicals, as has been claimed by Rozansowska et al.\textsuperscript{28} Finally, high levels of autofluorescence may indicate an inability to recycle products of phagosomal degradation that are essential for outer-segment renewal.\textsuperscript{29}

Retinal guanylate cyclase-1 (retGC-1) is a key component in the phototransduction cascade and synthesis of cyclic guanosine monophosphate (cGMP) from 5'-GMP. Cyclic GMP levels are responsible for increasing the proportion of open cGMP-gated channels in the dark-adapted state. Therefore, mutations in \textit{GUCY2D}-encoding retGC-1 could give rise to retinal disease. Leber congenital amaurosis is also associated with mutations in this gene, but in recessive disease.\textsuperscript{30}

The phenotype of a dystrophy predominantly of the cone variety with some involvement of rods is consistent with the tissue localization studies of retGC-1.\textsuperscript{31} Immunoeimaging with confocal and electron microscopy in human and monkey retinas has demonstrated that retGC-1 is primarily located in the photoreceptor layer, and outer segments of cones are more densely labeled with antibodies than those of rods. This finding has been confirmed by others.\textsuperscript{32,35}

Possible factors causing the variability observed in subjects with mutations in the same gene include the character or position of the mutation, the effect of the second allele exerting either a protective or deleterious influence, and the susceptibility of the genotype to environmental or stochastic phenomena as yet unrecognized. In the original family with autosomal dominant cone-rod dystrophy, adjacent R838S and E837D mutations were present,\textsuperscript{10} and Perrault et al\textsuperscript{34} reported a family with early-onset and severe retinal degeneration in which the disease gene encoded a protein with 3 amino acid substitutions at positions 837, 838, and 839. The common feature in these 2 latter cases and in all of the single mutants is the presence of a substitution at position 838; this substitution would appear to be the disease-determining change, with severity depending on the precise amino acid replacement and the presence of additional substitutions in adjacent codons. Codon 838 seems particularly prone to mutational change; the R838H mutation described in this article as well as mutations in this codon in a French and Swiss family all support this observation.\textsuperscript{34,35}

Sites 837 to 839 are located within the putative dimerization domain of the retGC-1 protein. An inves-
tigation of the Ca\(^{2+}\) sensitivity and the catalytic activity of the R838C, R838H, and R838S mutants and wild-type retGC-1 has shown that significantly higher concentrations of Ca\(^{2+}\) are required to deactivate the enzyme. The overall effect is the constitutive activation of mutant retGC-1 by GCAP-1 at physiological Ca\(^{2+}\) concentrations.\(^{36,37}\) This gain in function may result in elevated levels of cyclic GMP and Ca\(^{2+}\) in the photoreceptors. Precisely which effect is more detrimental to photoreceptor survival remains to be established.

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