

# Phenotype of autosomal dominant cone–rod dystrophy due to the R838C mutation of the *GUCY2D* gene encoding retinal guanylate cyclase-1

M Smith<sup>1</sup>, N Whittock<sup>2</sup>, A Searle<sup>3</sup>, M Croft<sup>3</sup>,  
 C Brewer<sup>3</sup> and M Cole<sup>1</sup>

## Abstract

**Aims:** To describe the phenotype of members of a large Caucasian British family affected by autosomal dominant cone–rod dystrophy due to an R838C mutation in the guanylate cyclase 2D (*GUCY2D*) gene encoding retinal guanylate cyclase-1 (*RETGC-1*).

**Methods:** Retrospective review of 29 patients from four generations of the same family.

**Results:** Visual symptoms usually commenced in childhood. Only two patients, aged 14 and 25 years, had visual acuity compatible with driving. Of the 12 patients aged over 40 years, eight (66%) had vision of counting fingers or worse and were eligible for blind registration in the UK. Of the 29 patients, 18 (62%) had myopia greater than 5 D in at least one eye. Most had discernible macular changes on biomicroscopy, which varied from subtle RPE change to gross macular atrophy. All patients who underwent computerised perimetry exhibited a central or paracentral scotoma with normal peripheral field of vision. Of the 21 patients who underwent electrodiagnostic testing, all exhibited decreased cone function, but rod function was normal in 12 (57%) patients.

**Conclusion:** We believe this report highlights the importance of phenotype–genotype correlation in cone and cone–rod dystrophies. Increased understanding of the varying phenotypes associated with different genetic mutations allows appropriate counselling of patients. In addition, the phenotypic characterisation of the natural history of these conditions may prove valuable in the future

**should therapeutic interventions become available.**

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**Keywords:** cone dystrophy; cone–rod dystrophy; *GUCY2D*; *RETGC-1*

## Introduction

Cone dystrophies are a genetically heterogeneous group of disorders characterised by early deterioration of visual acuity and colour vision.<sup>1</sup> Although cone dystrophies with normal rod function may occur, in most cases there is a degree of rod impairment, albeit less severe than that of cone function and often not evident until late in the disease. These patients are described as having cone–rod dystrophies.

Cone and cone–rod dystrophy are genetically heterogeneous with dominant, recessive, and X-linked inheritance patterns described. To date, nine loci for autosomal dominant cone and cone–rod dystrophies have been identified: *CORD4*, *RCD1*, *AIPL1*, *CRX*, *GUCA1A*, *GUCY2D*, *RIMS*, *UNC119*, and *SEMA4A*.

In 1997, Kelsell *et al*<sup>2</sup> localised a gene responsible for autosomal dominant cone–rod dystrophy (ADCRD) in a British family (CORD6) to chromosome 17p. Further work revealed mutations in the guanylate cyclase 2D (*GUCY2D*) gene encoding retinal guanylate cyclase-1 (*RETGC-1*) to be responsible.<sup>3,4</sup> Analysis of the original CORD6 family and other families with ADCRD identified single or multiple mutations in codons 837, 838, or 839

<sup>1</sup>Department of Ophthalmology, Torbay Hospital, Torquay, Devon, UK

<sup>2</sup>Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, UK

<sup>3</sup>Department of Clinical Genetics, Royal Devon and Exeter Hospital, Exeter, UK

Correspondence: M Smith, Department of Ophthalmology, Torbay Hospital, Lawes Bridge, Torquay, Devon TQ2 7AA, UK  
 Tel: +01803 614567;  
 Fax: +01803 616334.  
 E-mail: SMITH@mic73.freereserve.co.uk

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of the *GUCY2D* gene.<sup>4-7</sup> The phenotypes of these families appears to vary depending on the specific mutation and the presence or absence of multiple mutations. There is also some phenotype variation both between and within families with the same mutation.

A large Caucasian family of British origin with ADCRD due to an arginine to cysteine missense mutation at amino-acid residue 838 (R838C) has been attending our department for many years. This paper describes the phenotype of 29 affected members of this family.

## Materials and methods

### Patients

A pedigree of this family identified 35 members with likely cone-rod dystrophy. Of the 35 family members, 29 were current patients in our department or had attended within the last 5 years. The case notes of all 29 were reviewed. Of the 29 patients, nine had undergone molecular genetic investigation.

### Clinical and functional investigations

All 29 patients had undergone clinical examination including Snellen visual acuity and dilated retinal examination. A total of 18 patients had undergone colour vision testing using Ishihara pseudo-isochromatic plates and 13 patients static threshold perimetry using the standard 24-2 threshold protocol on the Humphrey Field Analyser. A total of 21 patients had undergone electrodiagnostic investigation.

### Genotyping

Nine of the 29 patients and one unaffected family member had undergone molecular genetic investigation.

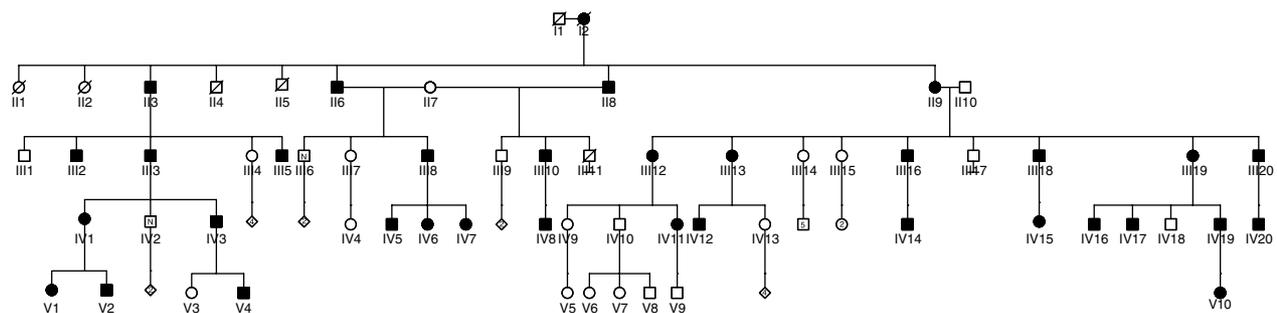
### Haplotype analysis

Microsatellite markers proximal and distal to the *RDS* gene on 6p (D6S1582, D6S1552, D6S1017, D6S1672,

D6S1549), *GUCY2D* on 17p (D17S1353, D17S960, D17S786, D17S1796), and the *CRX* gene on 19q13.3 (D19S902, D19S604) were amplified in a mix containing 40 ng genomic DNA, 5 pmol of forward and reverse primers, 2.5 nmol each dNTP, 100 mM Tris-HCl, 500 mM KCl, 15 mM MgCl<sub>2</sub>, and 0.25 U Amplitaq Gold *Taq* polymerase (Applied Biosystems). Forward or reverse primers were labelled with 6-FAM or HEX fluorescent dyes. Reaction conditions were initial denaturation of 95°C for 12 min, followed by 10 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and then 30 cycles of 89°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final step of 72°C for 7 min. PCR products were separated by polyacrylamide gel electrophoresis on an ABI 377 DNA sequencer and analysed using Genescan 3.0 and Genotyper 2.5 software (Applied Biosystems). Primers sequences were obtained from the Genome Database (<http://www.gdb.org/>). Tails to reduce stutter bands (GTTTCTT) were introduced to the 5' end of either the forward or reverse primer.

### Identification of mutations in the *GUCY2D* gene

Exon 13 of the human *GUCY2D* gene was directly amplified from genomic DNA using primers GUCY2D 13F (5'-GTA GAT GAA TGG TGG CAG CG-3') and GUCY2D 13R (5'-GAT GGC AGG CAG TGA GGT C-3'). Intronic primers were designed based on the genomic sequence of *GUCY2D* (<http://genome.ucsc.edu/>). Approximately 200 ng of patient or control genomic DNA was used in a 50 µl reaction mixture containing 20 pmol forward and reverse primers, 10 nmol each of dTTP, dCTP, and dATP, 7.6 nmol dGTP, 2.5 nmol deaza GTP, 2.5 µl DMSO, 50 µmol betaine, 100 mM Tris-HCl, 500 mM KCl, 15 mM MgCl<sub>2</sub>, and 1.0 U Amplitaq Gold *Taq* polymerase (Applied Biosystems). After an initial denaturation of 95°C for 12 min, 40 cycles were performed of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min, with a final extension step of 72°C for 10 min. The PCR products were examined by 2% agarose gel electrophoresis, purified using spin columns (Qiagen, Crawley, England) and directly sequenced using Big



**Figure 1** Pedigree of family with autosomal dominant cone dystrophy. Roman numerals indicate generations; solid symbols, affected family members; open symbols, unaffected family members; squares, males; circles, females; slash, deceased.

**Table 1** Clinical characteristics and electrophysiological results

Pedigree no.	Age (years)	Sex	Decade of symptom onset	VA OD	VA OS	Refraction OD	Refraction OS	Fundus	Rod-specific ERG (b-wave)		Single flash cone ERG (b-wave)		30 Hz flicker cone ERG (b-wave)		
									LAT	AMP	LAT	AMP	LAT	AMP	
II-8	78	M	1	CF	CF	-6.00/-2.50	-5.50/-1.50	3	Not performed	—	—	—	—	—	—
II-9	80	F	1	CF	CF	-2.00	-2.00	2	Not performed	—	—	—	—	—	—
III-3	57	M	1	CF	CF	+1.00/+1.25	+1.00/+0.75	3	Not performed	—	—	—	—	—	—
III-8	56	M	3	CF	6/24	-6.75	-6.50/-2.00	1	N	N	↑	↓	↑	↓	↓
III-10	48	M	1	6/60	6/60	-3.00/-2.00	-2.50/-1.50	2	N	N	N	N	↑	↓	↓
III-12	61	F	1	CF	CF	-11.00/-2.00	-15.00/-2.50	3	↑	↓	↑	↓	↑	↓	↓
III-13	60	F	1	HM	HM	-4.50/-3.00	-5.00/-3.50	3	Not performed	—	—	—	—	—	—
III-16	51	M	2	6/60	6/60	-0.25	+0.25/+0.25	3	N	N	N	↓	N	↓	↓
III-18	53	M	1	CF	CF	-10.00/-2.00	-10.00	3	Not performed	—	—	—	—	—	—
III-19	66	F	1	CF	CF	-8	-8.5	3	N	N	ABS	ABS	ABS	ABS	ABS
III-20	55	M	1	CF	CF	-6.00/-2.50	-6.00/-2.00	3	Not performed	—	—	—	—	—	—
IV-1	38	F	1	6/36	6/60	-7.00/-1.00	-9.00/-2.25	1	N	N	↑	↓	↑	↓	↓
IV-3	38	M	3	6/60	6/60	+1.5	+2.25/-1.50	2	↑	↓	ABS	ABS	ABS	ABS	ABS
IV-5	22	M	1	6/36	6/18	-5.50/-3.5	-3.75/-4.5	0	N	↓	N	↓	↑	↓	↓
IV-6	25	F	2	6/6	6/6	+1.00/-2.50	-0.50/-1.50	1	N	N	N	↓	N	↓	↓
IV-7	21	F	1	6/18	6/18	+0.75/-1.50	+0.25/-1.50	1	Not performed	—	—	—	—	—	—
IV-8	28	M	1	CF	CF	-6.00/-1.00	-5.50/-1.00	0	↑	↓	ABS	ABS	ABS	ABS	ABS
IV-11	32	F	1	6/36	6/24	-3.75/-0.75	-3.25/-0.75	1	N	N	↑	↓	↑	↓	↓
IV-12	30	M	3	6/12	6/12	-1.00/-1.25	-0.75/-1.25	1	↑	↓	↑	↓	↑	↓	↓
IV-14	12	M	1	6/36	6/36	+3.75/-5.00	+3.75/-3.50	1	N	N	N	↓	↑	↓	↓
IV-15	34	F	1	6/36	6/36	-10.75/+0.50	-13.25/+4.25	2	N	N	N	N	↑	↓	↓
IV-16	41	M	1	6/18	6/18	-6.50/+1.50	-7.00/+1.25	1	Not performed	—	—	—	—	—	—
IV-17	32	M	1	6/24	6/18	-9.50/-0.50	-8.50/-1.00	0	↑	↓	↑	↓	↑	↓	↓
IV-19	40	M	3	6/60	6/60	-8.25	-9.00/+0.50	2	↑	↓	↑	↓	↑	↓	↓
IV-20	32	M	3	CF	CF	-1.75/-3.00	-2.00/-4.00	2	N	↓	N	↓	ABS	ABS	ABS
V-1	17	F	2	6/18	6/24	-7.00/-2.00	-1.75/-2.00	2	N	N	↑	↓	↑	↓	↓
V-2	14	M	1	6/18	6/60	-4.50/-2.00	-4.50/-2.00	1	N	N	↑	↓	↑	↓	↓
V-4	14	M	1	6/36	6/9	-6.00/-1.75	-4.25/-1.00	1	N	N	N	↓	N	↓	↓
V-10	19	F	1	6/18	6/18	-7.25/-3.50	-8.75/-5.25	1	↑	↓	↑	↓	↑	↓	↓

ERG = electroretinogram; LAT = latency; AMP = amplitude; N = normal; ↑ = increased; ↓ = decreased; ABS = absent.

Pedigree numbers as in Figure 1.

Fundus abnormality: 0 = normal, 1 = subtle abnormality, 2 = obvious RPE change, 3 = gross macular atrophy.

Dye terminators on an ABI 377 genetic analyser (ABI, Foster City, USA) and analysed using Sequence Navigator 1.0.1 software (Applied Biosystems).

## Results

### Clinical and functional investigations

Figure 1 shows the pedigree of this family. There is clearly autosomal dominant inheritance, with no evidence of nonpenetrance. The phenotype was similar in male and female subjects. In all, 34 family members were reported to be affected, and of these 29 had been examined in our department, the remaining five (I.2, II.3, II.6, III.2, and III.5) were either deceased or had moved away from the area. A diagnosis of cone-rod dystrophy had been made on the basis of clinical examination with or without electrodiagnostic testing. The main clinical and electrophysiological features of these patients are summarised in Table 1.

Visual symptoms usually commenced in childhood with 72% of patients experiencing symptoms in the first decade of life and a further 10% in the second decade. Five patients (17%), however, did not notice any problems until the third decade. Only two patients (7%), aged 14 and 25 years, had visual acuity compatible with driving. Of the 12 patients aged over 40 years, eight

(66%) had vision of counting fingers or worse and are eligible for blind registration in the UK. Although photophobia was a common symptom, no patients reported problems with nyctalopia. Of the 29 patients, 18 (62%) have myopia greater than 5 D in at least one eye, many of these with myopic astigmatism. A total of 26 patients (90%) had discernible macular changes on biomicroscopy, which varied from subtle RPE change (11 patients) to gross macular atrophy (eight patients). Figure 2 shows a typical fundus appearance in advanced disease with macular atrophy. All patients who underwent computerised perimetry exhibited a central or paracentral scotoma with normal peripheral field of vision. All patients with visual acuity good enough to allow testing with Ishihara pseudo-isochromatic plates were only able to see the test plate. Of the 21 patients who underwent electrodiagnostic testing, all exhibited decreased cone function but rod b-wave electroretinogram (ERG) was of normal latency and amplitude in 12 (57%). Examples of a reduced photopic 30 Hz flicker ERG response are shown in Figure 3.

### Genotyping

Nine of the 29 patients and one unaffected family member had undergone molecular genetic investigation. Haplotype analysis demonstrated linkage to the



Figure 2 Fundus photograph of patient III-16 with bilateral macular atrophy.

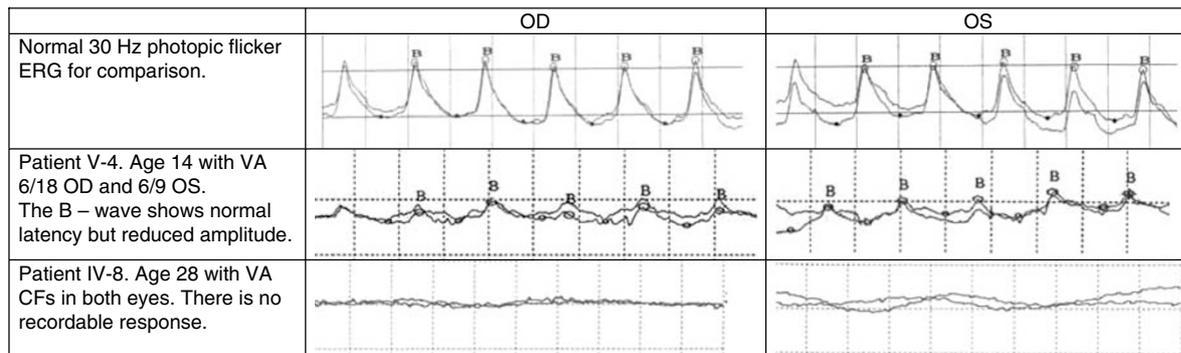
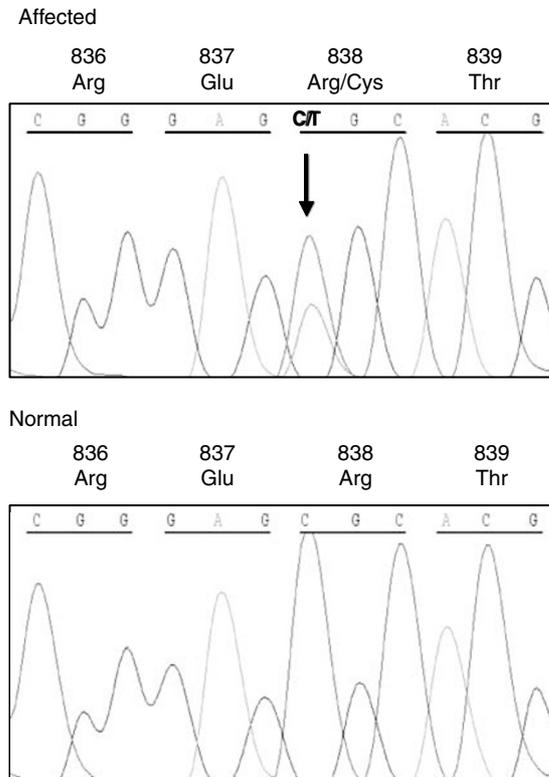


Figure 3 Examples of photopic 30 Hz flicker electroretinograms. Patient numbers as in Figure 1.



**Figure 4** A heterozygous C>T mutation results in an arginine to cysteine mutation at codon 838 of the *GUCY2D* gene.

*GUCY2D* gene on chromosome 17 in all nine affected patients, but not the unaffected control. Direct sequencing of the *GUCY2D* exon 13 in the nine affected family members revealed heterozygosity for a C>T substitution that leads to an arginine to cysteine missense mutation at the amino-acid residue 838 (R838C) (Figure 4). The unaffected family member showed no mutation in exon 13.

### Discussion

The structural changes of the protein retinal guanylate cyclase (*RETGC-1*), which arise from mutations in codons 837, 838, and 839 of exon 13 of the *GUCY2D* gene, are known to cause ADCRD. This protein plays a key role in the phototransduction cascade by controlling intracellular calcium concentration via the influence on cation channels of its enzymatic product, guanosine 3', 5'-cyclic monophosphate (cGMP). The amino acids affected by these mutations play a role in the dimerisation of the enzyme into its active form. The mutations result in increased stability of the dimer, resulting in raised intracellular calcium concentration in the photoreceptor outer segment.<sup>8,9</sup> It is this high intracellular calcium concentration which is proposed to damage the photoreceptor, resulting in ADCRD.

Recessive mutations in this same gene are responsible for a significant proportion of cases of Lebers Congenital Amaurosis (LCA), a hereditary retinal dystrophy characterised by severe visual impairment from birth.<sup>10</sup> Although LCA mutations have been identified in several locations throughout the gene, no case has been localised to exon 13, and it seems likely that the mutations associated with LCA have a more severe effect on protein function than the mutations which result in ADCRD.

In addition, there are differences between the phenotype of families identified to have exon 13 mutations. The original *CORD6* family had a missense mutation resulting in the replacement of glutamate by aspartate at codon 837 (E837D) and a mutation at codon 838 which results in the substitution of arginine by serine (R838S).<sup>4</sup> Perrault *et al*<sup>5</sup> reported a family with amino-acid substitutions at codons 837, 838, and 839 and there have been descriptions of several single mutations at codon 838 resulting in ADCRD. Although the common feature of these cases is a mutation at codon 838, the families with additional mutations in the adjacent codons do seem to exhibit a severe phenotype with earlier onset and more pronounced rod involvement and these additional changes may have a cumulative effect on protein function.

The family in this report has the R838C mutation. This mutation was first reported by Kelsell *et al*<sup>3</sup> in 1998 as a single mutation resulting in ADCRD in three British families. The same mutation was one of the three mutations in a single family described by Perrault *et al*<sup>5</sup> as discussed above. Although the phenotype of several members of each of the three original British families was described by Downes *et al*,<sup>11</sup> the only previous phenotypic description of a multigenerational family with the R838C mutation was by Van Ghelue *et al*<sup>7</sup> who described the characteristics of 11 members of an affected Norwegian family. The British family in this report appears to exhibit similar characteristics to the Norwegian family. Symptoms tended to begin in the first decade and visual acuity was generally poor, with only two of our patients maintaining visual acuity that met the legal standard for driving. Like the Norwegian family, myopia and astigmatism is common in this family and fundus appearance varies from fine pigment change in the early stages to advanced macular atrophy later in the disease. Unlike the Norwegian report where preserved rod function was limited to one branch of the family, 57% of our family who underwent electrodiagnostic testing exhibited normal rod b-wave ERG. These patients were not restricted to one branch of the family and included patients with very poor visual acuity and absent cone responses on electrodiagnostic testing.<sup>7</sup> Rod involvement does not therefore seem to be a predominant feature of ADCRD due to R838C mutations, and this appears to

apply to all of the single *GUCY2D* gene mutations, in contrast to the original *CORD6* family and the other reported families with multiple *GUCY2D* mutations.

Our description of the phenotype of a large caucasian family of British origin with ADCRD due to an R838C mutation in the *GUCY2D* gene confirms this as a disease with variable phenotype in the younger patient. Although most children affected by this condition will be able to be educated in mainstream schools with appropriate support, few will maintain adequate visual acuity to allow them to drive, and by the age of 40 years most will have very poor visual acuity. Rod dysfunction is not a predominant feature in this family and many had a normal rod ERG.

We believe this report highlights the importance of phenotype-genotype correlation in cone-rod dystrophies. Increased understanding of the varying phenotypes associated with different genetic mutations allows appropriate counselling of patients. In addition, the phenotypic characterisation of the natural history of these conditions may prove valuable in the future, should therapeutic interventions become available.

#### Acknowledgements

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