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# Mutation Analysis Identifies *GUCY2D* as the Major Gene Responsible for Autosomal Dominant Progressive Cone Degeneration

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## Abstract

**Purpose—**Heterozygous mutations in the *GUCY2D* gene, which encodes the membrane-bound retinal guanylyl cyclase-1 protein (RetGC-1), have been shown to cause autosomal dominant inherited cone degeneration and cone–rod degeneration (adCD, adCRD). The present study was a comprehensive screening of the *GUCY2D* gene in 27 adCD and adCRD unrelated families of these rare disorders.

**Methods—**Mutation analysis was performed by direct sequencing as well as PCR and subsequent restriction length polymorphism analysis (PCR/RFLP). Haplotype analysis was performed in selected patients by using microsatellite markers.

**Results—***GUCY2D* gene mutations were identified in 11 (40%) of 27 patients, and all mutations clustered to codon 838, including two known and one novel missense mutation: p.R838C, p.R838H, and p.R838G. Haplotype analysis showed that among the studied patients only two of the six analyzed p.R838C mutation carriers shared a common haplotype and that none of the p.R838H mutation carriers did.

**Conclusions—***GUCY2D* is a major gene responsible for progressive autosomal dominant cone degeneration. All identified mutations localize to codon 838. Haplotype analysis indicates that in most cases these mutations arise independently. Thus, codon 838 is likely to be a mutation hotspot in the *GUCY2D* gene.

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Inherited progressive cone–rod dystrophies (CRDs) are characterized by progressive loss of cone photoreceptor function followed by progressive loss of rod photoreceptor function, often accompanied by retinal degeneration.[1–5] In contrast, in inherited progressive cone dystrophies (CDs), only cone function is impaired, and retinal degeneration is often minimal and confined to the macula. All modes of Mendelian inheritance have been observed, and genetic heterogeneity is a hallmark of both CD and CRD.[1]

Heterozygous mutations in the *GUCY2D* gene have been shown to cause autosomal dominantly inherited CD and CRD (adCD, adCRD; OMIM 601777 and 600977; http://www.ncbi.nlm.nih.gov/omim/ Online Mendelian Inheritance in Man; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD),[6,7] whereas homozygous or compound heterozygous mutations cause autosomal recessively inherited Leber congenital amaurosis (OMIM 204000).[8] *GUCY2D* encodes the membrane bound retinal guanylyl cyclase-1 protein (RetGC-1) which is expressed in both cone and rod photoreceptors, but predominantly in the cone outer segments.[9,10] To date, several studies have been conducted to investigate the spectrum of *GUCY2D* mutations associated with retinal degenerations.[6–8,11–16] However, the prevalence of *GUCY2D* gene mutations in adCD and adCRD have been evaluated in only two studies with relatively small sample sizes.[13,17] Thus, there has been a lack of robust data regarding the frequency of *GUCY2D* mutations in adCD and adCRD.

The purpose of this study was therefore to determine the prevalence of *GUCY2D* gene mutations in a group of 27 unrelated patients affected by adCD and adCRD and to evaluate the associated phenotype.

## Methods

### Subjects and Clinical Examination

Patients diagnosed with CD or CRD according to standard diagnostic criteria[2] and a family history consistent with an autosomal dominant mode of inheritance were included in the study and recruited at the Center for Ophthalmology, Tübingen, Germany, and ophthalmic specialist centers throughout Europe and the United States of America. The diagnosis of adCD or adCRD was mainly based on the results of full field electroretinography (ERG), performed according to ISCEV (International Society for Clinical Electrophysiology of Vision) standard.[18] Patients with reduced cone ERGs and normal rod ERGs received a diagnosis of adCD, whereas those with reduced cone and rod ERGs were deemed to have adCRD. Characteristic symptoms and signs, fundus appearance, and visual field results were used to corroborate the diagnosis. The study was performed according to the tenets of the Declaration of Helsinki and approved by the ethics committees of the participating institutions. Informed consent was obtained from all patients and examined family members.

Phenotype analysis consisted of clinical ophthalmic examination, static and kinetic perimetry, Panel D15 color testing, dark-adapted final thresholds, Ganzfeld electroretinography, and multifocal electroretinography (mfERG). Ganzfeld electroretinography was recorded according to the ISCEV standard in all participating centers by one of three recording systems (Espion e$^2$ system and ColorDome Ganzfeld

stimulator, Diagnosys UK Ltd., Cambridge, UK, with DTL electrodes; Nicolet Spirit and Ganzfeld, Nicolet Biomedical, Madison, WI; and RetiScan and Ganzfeld; Roland Consult, Brandenberg, Germany). White flashes were used at a standard flash intensity of 2.25 or 3 cd-s/$m^2$. mfERG was performed according to the method described by Sutter and Tran[19] (VERIS system; EDI, San Francisco, CA).

### Mutation Analysis

Mutation analyses of all coding exons of the *GUCY2D* gene plus flanking intron sequences were performed in 19 subjects by polymerase chain reaction (PCR) amplification of genomic DNA, with 13 sets of gene-specific primer pairs (Table 1) and subsequent DNA sequencing. DNA sequencing was performed (BigDye Sequencing Chemistry; Applied Biosystems, Inc. [ABI], Darmstadt, Germany), and products were separated on a capillary sequencer (model 3100; ABI). In another eight patients, genotyping for the prevalent mutations at codon 838 in exon 13 of the *GUCY2D* gene was performed by means of PCR and subsequent restriction length polymorphism analysis (PCR-RFLP) with the restriction enzyme *Hha*I, according to the manufacturer's procedure (New England Biolabs, Beverly, MA). *Hha*I has the recognition sequence -GCGC- and thus covers the nucleotides c.2511 to c.2514 of exon 13 (the last nucleotide of codon 837 and all three nucleotides of codon 838). PCR products were digested overnight and the RFLP pattern was evaluated by agarose gel electrophoresis. Mutations detected by PCR/RFLP were confirmed by DNA sequencing.

### Haplotype Analysis

Haplotype analysis was performed in patients with the mutations c.2512C>T and c. 2513G>A. Three polymorphic single-copy microsatellite markers (*D17S720*, *D17S1796*, and *D17S1812*) with a heterozygosity greater 0.6 flanking the *GUCY2D* gene and located in the same recombination block according to Rutgers Combined Linkage-Physical Map[20] were selected. Microsatellite markers were PCR amplified and subsequently resolved and analyzed on a DNA sequencer (model 377; Applied Biosystems).

## Results

### Mutation Analysis of *GUCY2D* in Patients with adCD or adCRD

A group of 27 unrelated patients with adCD or adCRD were recruited for mutation screening. Nineteen were screened for mutations in all coding exons of the *GUCY2D* gene, and another eight were genotyped for mutations at codon 838 of the *GUCY2D* gene by means of PCR-RFLP. Thereby, mutations in 11 patients were discovered: c.2512C>T (p.R838C) in 7; c.2513G>A (p.R838H) in 3, both previously identified; and a novel nucleotide substitution c.2512C>G (p.R838G) in 1 (Table 2). The novel mutation c. 2512C>G was excluded in 100 chromosomes of normal control subjects by DNA sequencing. Segregation of the mutant allele with the disease phenotype was demonstrated in all families for which samples from additional family members were available (Fig. 1).

The prevalence of *GUCY2D* gene mutations in our adCD and adCRD patient group was thus 11 of 27 patients (40%), and all mutations affected codon 838. Combining our and

previous data showed the prevalence of *GUCY2D* gene mutations in adCD and adCRD to be 35% (Table 2). Again virtually all mutations in these studies are located at codon 838.

### Haplotype Analysis of *GUCY2D* in Patients with c.2512C>T or c.2513G>A Mutations

To address the question of whether this accumulation of mutations at codon 838 is due to a founder effect or results from independent mutational events (a mutation hotspot), haplotype analysis was performed in three suitable families (ZD131, ZD181, ZD260) for the c.2512C>T mutation, applying markers *D17S720*, *D17S1796*, and *D17S1812*, which flank the *GUCY2D* gene and which are located in the same recombination block. In addition, sequence variants identified within the *GUCY2D* gene were used to construct the haplotype. Haplotype reconstruction revealed a common haplotype in families ZD131 and ZD260, both of German origin, whereas the third family, of Italian descent, had a different haplotype. In addition, carriers of the *GUCY2D* mutations c.2512C>T (patients ZD111/7301, ZD204/13670, ZD138/9058) and c.2513G>A (patients ZD73/2132, ZD174/11824/, ZD197/13045), all without large enough families to reconstruct a haplotype, were genotyped for the three markers. Even without phase information, the data clearly showed no common disease-associated haplotype either for the c.2512C>T or the c.2513G>A mutation carriers. Thus, it appears that the high prevalence of these specific mutations cannot be explained by a founder effect.

### Phenotype of Patients with adCD or adCRD with *GUCY2D* Gene Mutations

The clinical data of all 11 independent index patients with identified *GUCY2D* gene mutations and 12 available affected relatives are given in Table 3. Most patients had adCD (8/11) with reduced cone system–driven responses and essentially normal rod system–driven responses in the ERG. Three patients showed a phenotype of adCRD, presenting with both rod and cone ERG responses but more severely reduced cone responses. Disease onset ranged from infancy to young adulthood. Visual acuity was reduced in all patients, with a wide range from only mildly reduced (0.8) to severely reduced visual acuity (light perception). Glare sensitivity (6/11) and color vision abnormalities (9/11) were common findings. Most patients experienced normal night vision and had normal dark-adaptation thresholds. Scattered relative or absolute scotomas within the 30° visual field were observed in all but one patient. Visual field outer borders were mostly normal (6/11, no information for three patients), but two patients (RCD62/5127 and ZD174/11824) presented with concentric narrowing.

Of note, fundus alterations were typically confined to the macula and presented even in advanced stages only with mottling or circumscribed atrophy of the RPE (Figs. 2A, 2B, 2E). Within families the older subjects typically had a more severe phenotype compared with the younger generations. Figure 2E shows the fundus of a 6-year-old subject (ZD249/15965/M) with normal appearance despite markedly changed ERG recordings. The fundus of his great uncle is shown in Figure 2G whose fundus presented a clear macular atrophy but an otherwise unremarkable optic disc, retinal vessels, and retinal periphery.

Pronounced atrophic lesions and a bone spicule–like pigmentation in the periphery were found in only one family (RCD62), whose affected family members also suffered from rod dysfunction (Figs. 2C, 2D).

In conclusion, the phenotype caused by *GUCY2D* gene mutations at codon 838 presented in most cases as CD with increased glare sensitivity, color vision abnormalities, and central scattered absolute and relative scotomas with preserved outer visual field border, and fundus changes typically confined to the macula. In general, apart from age-dependent disease progression interindividual variability was modest. However, all members of family RCD62 diverted from this commonly found phenotype, as they presented with extinguished cone and reduced rod responses, central and peripheral RPE atrophy, bone spicule–like peripheral pigmentation, narrowed visual field, and cecocentral scotoma, perhaps because they carried the mutation c.2512C>G (p.R838G), whereas all other patients carried c.2512C>T (p.R838C) or c.2513G>A (p.R838H), suggesting a more severe phenotype associated with this novel mutation. In addition, patient (ZD174/11824/F) with the c.2513G>A (p.R838H) mutation had a more severe phenotype with markedly reduced visual acuity, extinguished cone and rod system–driven ERG, and constricted peripheral visual field.

## Discussion

We report the result of a mutation screening of the *GUCY2D* gene in 27 unrelated patients with adCD or adCRD. Families with dominant CD and CRD are exceptionally rare.[1] This study includes by far the largest patient sample screened so far for these conditions, enabling now a more solid estimate of the prevalence of mutations in this gene. We identified *GUCY2D* gene mutations in 11 of 27 patients (40%), indicating that *GUCY2D* is a major disease gene for adCD and adCRD. All identified mutations clustered to codon 838. The most frequent mutation was c.2512C>T (p.R838C) in seven patients, followed by c. 2513G>A (p.R838H) in three, and the novel mutation c.2512C>G (p.R838G) in one.

The combined data of our study and two smaller previous studies suggests that approximately one third of adCD and adCRD is caused by mutations in *GUCY2D*. Almost all *GUCY2D* gene mutations identified so far in patients with adCD or adCRD are located at codon 838 or the two adjacent codons 837 and 839. As a consequence for the diagnostic routine, we therefore suggest that all adCD and adCRD patients be prioritized for codon 838 genotyping which can be easily performed by PCR-RFLP in a cost- and time-efficient manner.

In accordance with previous reports[6–8,11–16] the typical phenotype of c.2512C>T (p.R838C) and c.2513G>A (p.R838H) mutation carriers was CD, with disease onset in childhood or early adolescence characterized by increased glare sensitivity, color vision abnormalities, and central scotomas, but preserved outer visual field border. Retinal morphology was relatively well preserved in young affected individuals and a certain degree of progression was seen with age. But also, in older subjects, retinal changes were subtle and confined to the macula. However, mutations in *GUCY2D* may also cause a more severe adCRD phenotype as observed in patient ZD174/11824/F and family RCD62. The latter carry a new

mutation, c.2512C>G (p.R838G). Whether this novel mutation itself or other modifying factors cause this more severe phenotype is yet unknown.

We also investigated whether the observed clustering of mutations to codon 838 of the *GUCY2D* gene is caused by a common founder of the individuals carrying the same mutation. Haplotype analysis showed that among the studied patients, only two families of German origin share a common haplotype, whereas the other eight analyzed patients do not. This indicates that the mutations at codon 838 most likely arose independently in most of the analyzed families and that codon 838 is likely to be a mutation hotspot for the adCD and adCRD phenotype. Codon 838 (nucleotide sequence -CGC-) comprises a typical mutable motif in human genes, the -CpG-dinucleotides. [21,22] It has been shown that spontaneous -CG- to -TA- mutations occur at these sites and are thought to be caused by deamination of methylated cytosine. Thus, the mutations observed at codon 838 of the *GUCY2D* gene, c. 2512C>T and 2513G>A, could be due to this common mutable motif.

Biochemical analyses demonstrated dominant negative effects for the RetGC-1 mutants p.R838C and p.R838H. They are less sensitive to high intracellular calcium concentrations in comparison to the wild-type protein and retain residual catalytic activity, even at high calcium levels.[23,24] Similar to p.R838C expressed alone, coexpressed p.R838C and wild-type RetGC-1 are less sensitive to calcium negative feedback,[23] which indicates that the reduced calcium sensitivity of p.R838C is dominant in the presence of wild-type RetGC-1. In contrast to the dominant negative effects observed for mutations at codon 838 in cone and cone rod dystrophy, for most *GUCY2D* gene mutations observed in Leber congenital amaurosis, a loss of function was demonstrated.

On a cellular level, the reduced calcium sensitivity of the RetGC-1 mutants p.R838C and p.R838H may lead to increased cGMP synthesis in the dark and increased calcium influx through cGMP-gated cation channels. Consequently, calcium concentration in the photoreceptor may be elevated, which eventually leads to apoptosis of the photoreceptor. However, currently there is no animal model available for the mutations p.R838C and p.R838H in *GUCY2D*, and therefore pathophysiologic effects that take place in the photoreceptor are unknown. The phenotype observed in our patients suggests events that leave the retina morphologically relatively intact, but impair its function. Moreover, in conclusions also drawn from the phenotype in our patients, these events may predominantly affect the cone system and only secondarily the rod system. This may result from the fact that RetGC-1 is predominantly expressed in cones,[9,10] which could support this observation.

In conclusion, we evaluated the prevalence of *GUCY2D* gene mutations in 27 unrelated patients with adCD and adCRD. We found that more than one third of the patients had mutations at codon 838 of the *GUCY2D* gene. We therefore propose that *GUCY2D* is to date the major disease gene involved in the pathogenesis of adCD and adCRD.

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## References


1. Hamel C. Cone rod dystrophies. Orphanet J Rare Dis. 2007; 2:7–7. [PubMed: 17270046]
2. Merin, S. Inherited Eye Diseases: Diagnosis and Management. New York: Taylor & Francis; 2005.
3. Michaelides M, Hardcastle A, Hunt D, Moore A. Progressive cone and cone-rod dystrophies: phenotypes and underlying molecular genetic basis. Surv Ophthalmol. 2006; 51:232–258. [PubMed: 16644365]
4. Szlyk JP, Fishman GA, Alexander KR, Peachey NS, Derlacki DJ. Clinical subtypes of cone-rod dystrophy. Arch Ophthalmol. 1993; 111:781–788. [PubMed: 8512479]
5. Yagasaki K, Jacobson SG. Cone-rod dystrophy: phenotypic diversity by retinal function testing. Arch Ophthalmol. 1989; 107:701–708. [PubMed: 2719580]
6. Kelsell R, Gregory-Evans K, Payne A, et al. Mutations in the retinal guanylate cyclase (RETGC-1) gene in dominant cone-rod dystrophy. Hum Mol Genet. 1998; 7:1179–1184. [PubMed: 9618177]
7. Udar N, Yelchits S, Chalukya M, et al. Identification of GUCY2D gene mutations in CORD5 families and evidence of incomplete penetrance. Hum Mutat. 2003; 21:170–171.
8. Perrault I, Rozet J, Calvas P, et al. Retinal-specific guanylate cyclase gene mutations in Leber's congenital amaurosis. Nat Genet. 1996; 14:461–464. [PubMed: 8944027]
9. Dizhoor A, Lowe D, Olshevskaya E, Laura R, Hurley J. The human photoreceptor membrane guanylyl cyclase, RetGC, is present in outer segments and is regulated by calcium and a soluble activator. Neuron. 1994; 12:1345–1352. [PubMed: 7912093]
10. Liu X, Seno K, Nishizawa Y, et al. Ultrastructural localization of retinal guanylate cyclase in human and monkey retinas. Exp Eye Res. 1994; 59:761–768. [PubMed: 7698269]
11. Downes S, Payne A, Kelsell R, et al. Autosomal dominant cone-rod dystrophy with mutations in the guanylate cyclase 2D gene encoding retinal guanylate cyclase-1. Arch Ophthalmol. 2001; 119:1667–1673. [PubMed: 11709018]
12. Gregory-Evans K, Kelsell R, Gregory-Evans C, et al. Autosomal dominant cone rod retinal dystrophy (CORD6) from heterozygous mutation of GUCY2D, which encodes retinal guanylate cyclase. Ophthalmology. 2000; 107:55–61. [PubMed: 10647719]
13. Payne A, Morris A, Downes S, et al. Clustering and frequency of mutations in the retinal guanylate cyclase (GUCY2D) gene in patients with dominant cone-rod dystrophies. J Med Genet. 2001; 38:611–614. [PubMed: 11565546]
14. van Ghelue M, Eriksen H, Ponjavic V, et al. Autosomal dominant cone-rod dystrophy due to a missense mutation (R838C) in the guanylate cyclase 2D gene (GUCY2D) with preserved rod function in one branch of the family. Ophthalmic Genet. 2000; 21:197–209. [PubMed: 11135490]
15. Weigell-Weber M, Fokstuen S, Torok B, Niemeyer G, Schinzel A, Hergersberg M. Codons 837 and 838 in the retinal guanylate cyclase gene on chromosome 17p: hot spots for mutations in autosomal dominant cone-rod dystrophy? Arch Ophthalmol. 2000; 118:300. [PubMed: 10676808]
16. Yoshida S, Yamaji Y, Yoshida A, et al. Novel triple missense mutations of GUCY2D gene in Japanese family with cone-rod dystrophy: possible use of genotyping microarray. Mol Vis. 2006; 12:1558–1564. [PubMed: 17200655]
17. Ito S, Nakamura M, Ohnishi Y, Miyake Y. Autosomal dominant cone-rod dystrophy with R838H and R838C mutations in the GUCY2D gene in Japanese patients. Jpn J Ophthalmol. 2004; 48:228–235. [PubMed: 15175914]
18. Marmor M, Holder G, Seeliger M, Yamamoto S, et al. Standard for clinical electroretinography (2004 update). Doc Ophthalmol. 2004; 108:107–114. [PubMed: 15455793]
19. Sutter E, Trana D. The field topography of ERG components in man- I. The photopic luminance response. Vision Res. 1992; 32:433–446. [PubMed: 1604830]
20. Kong X, Murphy K, Raj T, He C, White P, Matise T. A combined linkage-physical map of the human genome. Am J Hum Genet. 2004; 75:1143–1148. [PubMed: 15486828]
21. Cooper D, Youssoufian H. The CpG dinucleotide and human genetic disease. Hum Genet. 1988; 78:151–155. [PubMed: 3338800]
22. Rogozin I, Kondrashov F, Glazko G. Use of mutation spectra analysis software. Hum Mutat. 2001; 17:83–102. [PubMed: 11180592]

23. Tucker C, Woodcock S, Kelsell R, Ramamurthy V, Hunt D, Hurley J. Biochemical analysis of a dimerization domain mutation in RetGC-1 associated with dominant cone-rod dystrophy. Proc Natl Acad Sci USA. 1999; 96:9039–9044. [PubMed: 10430891]

24. Wilkie S, Newbold R, Deery E, et al. Functional characterization of missense mutations at codon 838 in retinal guanylate cyclase correlates with disease severity in patients with autosomal dominant cone-rod dystrophy. Hum Mol Genet. 2000; 9:3065–3073. [PubMed: 11115851]

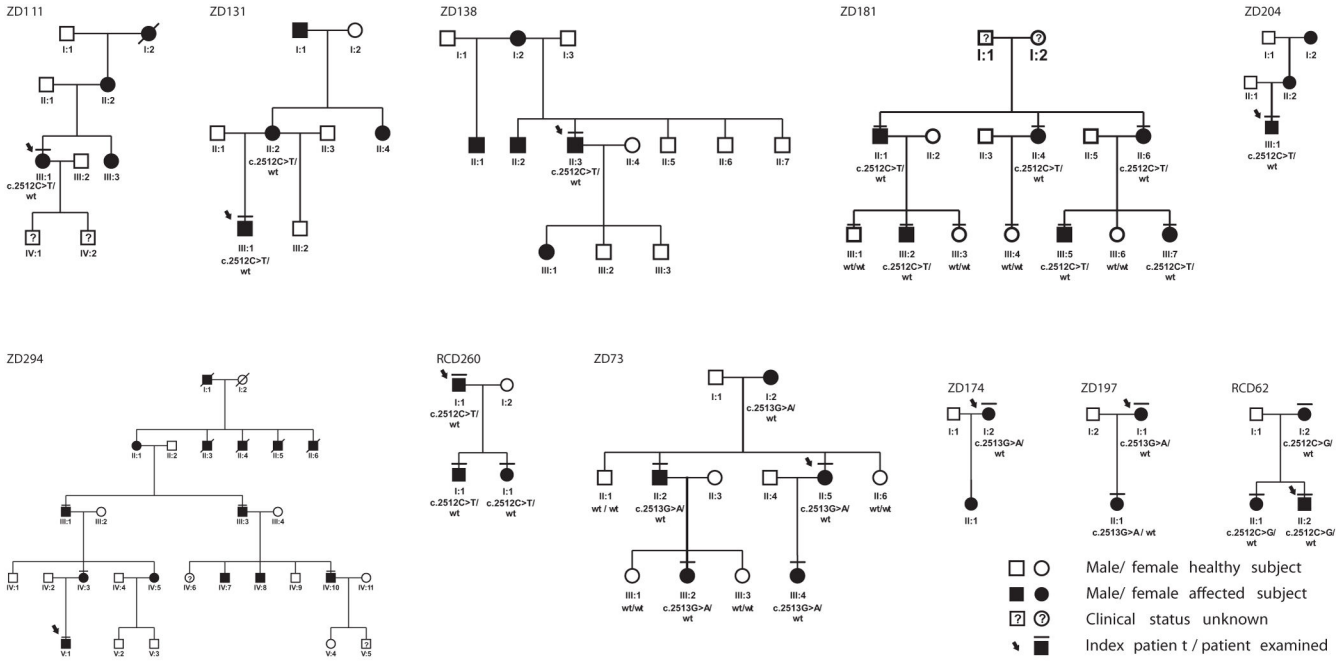


**Figure 1.**

Pedigrees of adCD and adCRD families segregating GUCY2D gene mutations. *Arrows*: index patients initially screened for GUCY2D mutations. Genotypes of family members whose DNA samples were available are listed below the respective subject. *Horizontal bars above symbols*: patients who underwent clinical examination. Pedigrees are arranged from *left* to *right* and *top* to *bottom* in the same order as patients are listed in Table 3. The pedigree number is given *above* and to the *left* of each pedigree.

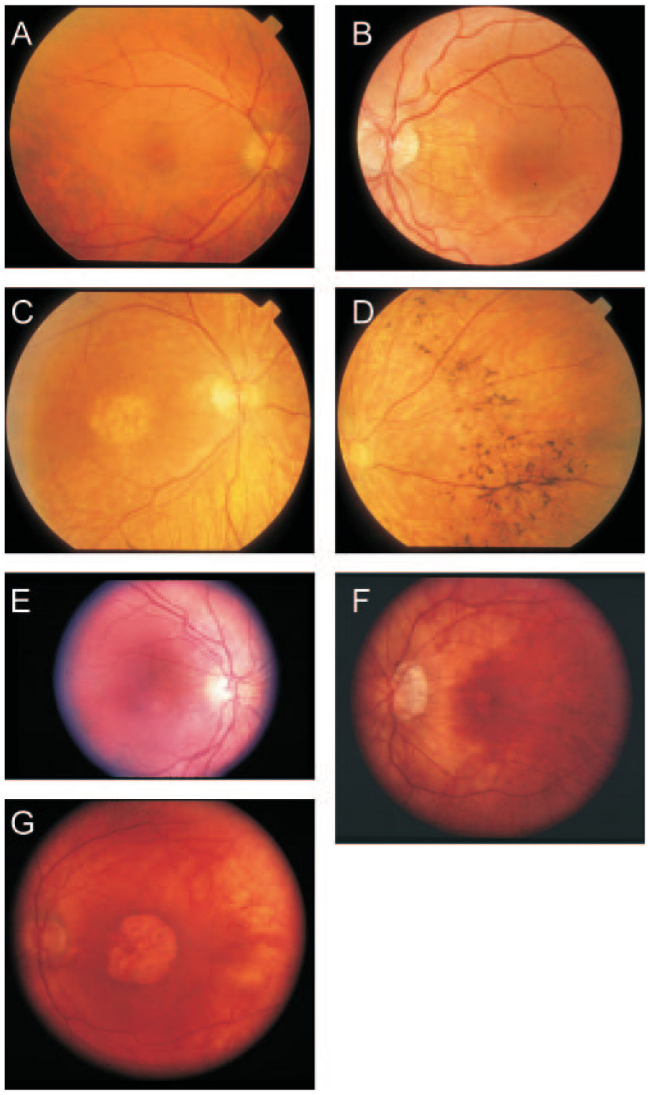


**Figure 2.**

Patients with GUCY2D gene mutations typically had only mild fundus alterations. Apart from slightly narrowed retinal vessels, the fundus of patient ZD138/9058 showed no alterations of the central and peripheral retina (**A**). Patient ZD181/5003 had a slightly pale optic disc, a somewhat mottled macular RPE, and thinning of the RPE nasally to the macula, but no alterations of the peripheral retina (**B**, peripheral retina not shown). Within families, fundus alterations were more pronounced in the older generations. For example, 6-year-old patient ZD249/15965 had a subtle central RPE atrophy (**E**), but his grand uncle at 61 years of age had marked macular RPE atrophy (**G**). His son at the age of 31 showed less severe atrophy of the macular RPE (**F**). All three, however, had a normal peripheral retina. Whereas most patients had only changes in the macula, one patient (RCD62/5127) had alterations both in the macula and periphery (**C**, **D**) with marked narrowing of the retinal vessels, widespread RPE atrophy and bone spicules in the periphery, which were in part more pronounced around the vessels.

**Table 1**

PCR Primers for Amplification of Coding Sequences of the *GUCY2D* Gene

| Amplicon | Forward Primer (5′-3′) | Reverse Primer (5′-3′) | Size (bp) |
|---|---|---|---|
| Exon 2 | CTCGGGCTTGGAGAAACTCGGG | CACTGCTGCGGACAGAGGCTTG | 906 |
| Exon 3 | ACAGGTAGGCTCCCTTGCAG | GCTGCCAGTGGTTCTTTCTC | 494 |
| Exon 4 | TGGGCTTGACAGGCAGTG | CTAGAAGGGCATCGAAGACG | 542 |
| Exon 5–6 | CCTAGAGCCTCTCTGGGC | GGGGTAGAAATCAGGCTTCC | 703 |
| Exon 7 | CCAAAACTCAGCCTGACCTC | AGAGTGCGCCTCCCCTC | 259 |
| Exon 8 | AGCCAATGGAAATGAGGGG | GAGACCTACCTCTGTACCCAGC | 261 |
| Exon 9–10 | AAATCTCATCTTCTGGGTCTGG | AGAGGTAGGGAGGAAGCGG | 649 |
| Exon 11 | TGGTGGTGTCTGGGTGC | GTTTCATCACTGGGCTTTGC | 338 |
| Exon 12 | CTTGGTCTTCAACAGTCAGGC | TCTGCAGCTGTCTCAGGTTG | 314 |
| Exon 13–14 | GTAGATGAATGGTGGCAGCG | GATTGGGCAGGTAGGCTAGG | 680 |
| Exon 15 | TTCTGCACTAACCCCAGGTG | TCCATGAGTTGCCTCCTCTAC | 363 |
| Exon 16–17 | GATAATGGGTGCGAAGATCC | GTCAGAAGGGTGAGCTGAGG | 466 |
| Exon 18–19 | CAAACCTCAGCTCACCCTTC | CTGCAGGCAGCAGAGGG | 514 |

**Table 2**

Prevalence of *GUCY2D* Gene Mutations in Different Cohorts of adCD and adCRD Patients

| Study | Analyzed Patients | Patients Identified with *GUCY2D* Mutations | Identified Mutations | | Frequency of Mutation |
|---|---|---|---|---|---|
| | | | cDNA* | Protein† | |
| Payne et al.[13] | 13 | 3 | c.2512C>T | p. R838C | 2 |
| | | | c.2513G>A | p. R838H | 1 |
| Ito et al.[17] | 9 | 3 | c.2512C>T | p. R838C | 1 |
| | | | c.2513G>A | p. R838H | 1 |
| | | | [c.T2817C; c.G2749C] | [p.I915T; p.G917R] | 1 |
| Current study | 27 | 11 | c.2512C>T | p. R838C | 7 |
| | | | c.2513G>A | p. R838H | 3 |
| | | | c.2512C>G | p. R838G | 1 |
| | 49 | 17 (35%) | | | |

*cDNA sequence: NM_000180.2.

†Protein sequence: NP_000171.1.

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**Table 3**

Phenotype and Electrophysiological Data of Index Patients with *GUCY2D* Gene Mutations

| Family ID/ Patient ID/ Gender, Pedigree ID | Mutation | Diagnosis | Onset Age | Age | BCVA (OD/OS) | Spherical Refraction (OD/OS) | Color Vision * | Glare Sensitivity | Dark Adaptation * | Night Vision | Fundus Findings * | | Visual Field * | Scotopic GF-ERG *† | | | | Photopic GF-ERG *† | | | | mfERG * |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | Rod Response | | Mixed Rod Cone Response | | Single Flash | | 30 Hz Flicker | | |
| | | | | | | | | | | | | | | A | I | A | I | A | I | A | I | |
| ZD111/7301/F | c.2512C>T, p.R838C | CD | 19 | 32 | 0.8/0.8 | NI | PD15 sat; protan/deutan defect | NI | Normal | Abnormal | Slight macular RPE mottling, vessels slightly narrowed | C | Absolute and relative paramacular defects; some scattered relative and absolute defects within the central 30° VF | N | N | ↑/↓ | ↑/↓ | ↑↑/↓↓ | ↑↑/↓↓ | ↑/↓ | N | In the four central rings heavily reduced amplitudes, near normal amplitudes only in the outer ring |
| III:1, index patient | | | | | | | | | | | | P | Normal outer borders | | | | | | | | | |
| ZD 131/8570/M | c.2512C>T, p.R838C | CRD | NI | 30 | 0.8/0.6 | −2.5/−3.0 | NI | NI | NI | NI | Subtle temporal atrophy of optic disc, subtle macular and peripheral RPE mottling | C | Absolute and relative central defects | OD ↑/↓, OS bN | N | OD ↑↑/↓↓, OS↑/↓ | N | ↑↑/↓↓ | ↑↑/↓↓ | OD N, OS ↑/↓ | ↑/↓ | Reduced at all eccentricities |
| III:1, index patient | | | | | | | | | | | | P | NI | | | | | | | | | |
| ZD138/9058/M | c.2512C>T, p.R838C | CD | Infancy | 36 | 0.2/0.1 | −5.75/−2.5 | PD15 sat: OD protan/deutan defect, OS chaotic; Nagel anomaloscope: OD/OS no color discrimination | Increased | Normal | Normal | Slightly narrowed vessels, otherwise unremarkable fundus on ophthalmoscopy | C | Absolute (OD) and relative (OS) paramacular defects; few scattered relative and absolute defects within the central 30° VF | bN | bN | ↑/↓ | ↑/↓ | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | Reduced at all eccentricities; reproducible amplitudes only in outer ring |
| II:3, index patient | | | | | | | | | | | | P | NI | | | | | | | | | |
| ZD181/5003/M | c.2512C>T, p.R838C | CD | Infancy | 23 | 0.4/0.5 | −2.5/−3.5 | PD15 sat: only minor errors | Increased | Normal | Normal | Slight macular RPE mottling; RPE thinning nasally to the macula; pale optic disc; vessels normal | C | Absolute and relative paramacular defects; few scattered relative and absolute defects within the central 30° VF | N | N | N | | ↑↑/↓↓ | ↑/↓ | bN | ↑↑/↓↓ | Reduced at all eccentricities, centrally more than peripherally |
| III:5, index patient | | | | | | | | | | | | P | Normal outer borders | | | | | | | | | |
| ZD204/13670/M | c.2512C>T, p.R838C | CD | Childhood | 14 | 0.4/0.3 | 0.5/0.5 | Many errors | Normal | Normal | Normal | Subtle macular RPE mottling | C | Absolute central scotoma and few absolute relative paracentral scotomas | N | N | N | N | ↑↑/↓↓ | ↑/↓ | ↑↑/↓↓ | ↑/↓ | NI |
| III:1, index patient | | | | | | | | | | | | P | Normal outer borders | | | | | | | | | |
| ZD249/15965/M | c.2512C>T, p.R838C | CD | Infancy | 6 | 0.5/0.5 | NI | Normal | NI | Threshold slightly elevated (0.3 log unit) | Normal | Subtle central RPE atrophy | C | NI | N | N | N | | ↑↑/↓↓ | N | ↑↑/↓↓ | N | NI |
| V:1, index patient | | | | | | | | | | | | P | Normal outer borders | | | | | | | | | |
| ZD249/F | DNA unavailable for testing | CD | Infancy | 34 | 0.5/0.5 | NI | PD15 sat: protan/deutan defect | Increased | Normal | Normal | Subtle macular RPE mottling | C | NI | N | N | N | | ↑↑/↓↓ | ↑/↓ | ↑↑/↓↓ | ↑/↓ | NI |
| IV:3, relative | | | | | | | | | | | | P | Normal outer borders | | | | | | | | | |
| ZD249/M | DNA unavailable for testing | CD | Infancy | 56 | 0.17/0.125 | NI | PD15 sat; chaotic | Increased | Threshold slightly elevated (0.3 log unit) | Normal | NI | C | NI | ↑/↓ | ↑/↓ | ↑/↓ | ↑/↓ | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | NI |
| III:1, relative | | | | | | | | | | | | P | Constricted | | | | | | | | | |
| ZD249/M | DNA unavailable for testing | CD | Infancy | 62 | 0.08/0.1 | NI | PD15 sat: chaotic | Increased | Normal | Normal | Macular RPE atrophy, vessels narrowed | C | NI | ↑/↓ | ↑/↓ | ↑/↓ | ↑/↓ | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | NI |
| III:3 | | | | | | | | | | | | P | Constricted | | | | | | | | | |
| ZD249/M | DNA unavailable for testing | CD | Infancy | 31 | 0.25/0.2 | −14.25/−13.25 | Subjectively poor | Increased | Normal | Normal | Macular RPE atrophy, vessels narrowed | C | NI | N | N | N | ↑/↓ | ↑↑/↓↓ | ↑/↓ | ↑↑/↓↓ | ↑/↓ | NI |

| Family ID/ Patient ID/ Gender, Pedigree ID | Mutation | Diagnosis | Onset Age | Age | BCVA (OD/OS) | Spherical Refraction (OD/OS) | Color Vision * | Glare Sensitivity | Dark Adaptation * | Night Vision | Fundus Findings * | | Visual Field * | Scotopic GF-ERG *† | | | | Photopic GF-ERG *† | | | | mfERG * |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | Rod Response | | Mixed Rod Cone Response | | Single Flash | | 30 Hz Flicker | | |
| | | | | | | | | | | | | | | A | I | A | I | A | I | A | I | |
| IV:10, relative | | | | | | | | | | | | P | NI | | | | | | | | | |
| RCD260/14324/M | c.2512C>T, p.R838C | CRD | Childhood, amblyopia OD | 53 | 0.02/0.04 | 1-Apr | Subjectively poor | Increased | NI | NI | Macular RPE atrophy and clumping | C | Central scotoma | EX | EX | ↑↑/↓↓ | ↑↑/↓↓ | EX | EX | EX | EX | Few residual peripheral responses with prolonged implicit time |
| I:1, index patient | | | | | | | | | | | | P | NI | | | Reduced b/a ratio, negative ERG | | | | | | |
| RCD260/14324/M | c.2512C>T, p.R838C | CRD | Childhood | 23 | 0.1/0.1 | 3.5/3 | Subjectively poor | Increased | NI | NI | Normal on ophthalmoscopy | C | Central scotoma | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | EX | EX | EX | EX | Reduced responses with prolonged implicit time |
| II:1, relative | | | | | | | | | | | | P | NI | | | Reduced b/a ratio, negative ERG | | | | | | |
| RCD260/14326/F | c.2512C>T, p.R838C | CRD | Childhood | 20 | 0.05/0.05 | 2/2.5 | Subjectively poor | Increased | NI | NI | Normal on ophthalmoscopy | C | Central scotoma | EX | EX | ↑↑/↓↓ | ↑↑/↓↓ | EX | EX | EX | EX | |
| II:2, relative | | | | | | | | | | | | P | NI | | | Reduced b/a ratio, negative ERG | | | | | | |
| ZD73/2132/F | c.2513G>A, p.R838H | CD | Childhood | 36 | 0.3/0.05 | 0.5/0.5 | PD15 rat; unable to discriminate colors; Nagel anomaloscope; protan defect | Increased | NI | Normal | Subtle central RPE atrophy, vessels slightly narrowed | C | OD scotoma, OS centrocecal | N | N | bN | bN | N | ↑↑/↓↓ | bN | ↑↑/↓↓ | NI |
| II:5, index patient | | | | | | | | | | | | P | Slight concentric narrowing | | | | | | | | | |
| ZD73/2135/F | c.2513G>A, p.R838H | CD | 15 | 16 | 0.8/0.8 | 4.0/4.24 | PD15 desat: normal | Increased | NI | | OS: two RPE atrophy lesions below the lower arcade; otherwise unremarkable fundus OD/OS on ophthalmoscopy | C | Normal central DLS | N | N | N | N | N | ↑↑/↓↓ | N | N | NI |
| III:4, relative | | | | | | | Nagel anomaloscope: pseudoprotanomaly | | | | | P | Normal outer borders | | | | | | | | | |
| ZD73/3792/M | c.2513G>A, p.R838H | CD | 10 | 40 | 0.5/0.4 | −3.0/−4.75 | PD15 sat: unable to discriminate colors | Increased | NI | Normal | Subtle macular RPE atrophy, vessels slightly narrowed | C | Absolute and relative paramacular defects; few scattered relative and absolute defects within the central 30° VF | OD N, OS bN | N | OD bN, OS ↑↑/↓↓ | N | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | Markedly reduced at all eccentricities; reproducible amplitudes only in outer ring |
| II:2, relative | | | | | | | | | | | | P | Normal outer borders | | | | | | | | | |
| ZD73/3790/F | c.2513G>A, p.R838H | CD | NI | 23 | 0.05/0.02 | −8.0/−7.75 | PD15 sat: tritan defect | Increased | NI | Normal | Central and peripheral RPE atrophy, vessels narrowed | C | OS: many relative defects within the central 30° VP | N | N | ↑/↓ | N | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | ↑↑/↓↓ | NI |
| III:2, relative | | | | | | | | | | | | P | Normal outer borders | | | | | | | | | |
| ZD174/11824/F | c.2513G>A, p.R838H | CD | Childhood | 48 | HM/LP | NI | No color discrimination | NI | NI | NI | Macular RPE atrophy and clumping, vessels narrowed | C/P | Concentric narrowing to 8–17° | | | NI | | EX | EX | EX | EX | NI |
| I:2, index patient | | | | | | | | | | | | | | | | | | | | | | |
| ZD197/13045/F | c.2513G>T, p.R838H | CD | 25–30 | 50 | 0.1/0.05 | 0.75/0.75 | No color discrimination | Increased | Normal | Normal | Subtle macular RPE mottling and white spots centrally | C | Small absolute central scotoma | N | N | N | | ↑↑/↓↓ | NI | ↑↑/↓↓ | NI | NI |
| I:1, relative | | | | | | | | | | | | P | Normal outer borders | | | | | | | | | |
| ZD197/13047/F | c.2513G>A, p.R838H | CD | 17 | 23 | 0.4/0.4 | −7.75/−7.75 | No color discrimination | Increased | Normal | Normal | Very subtle macular RPE mottling | C | Small relative central scotoma | N | N | N | | ↑↑/↓↓ | NI | ↑↑/↓↓ | NI | NI |
| II:1, index patient | | | | | | | | | | | | P | Normal outer borders | | | | | | | | | |
| RCD62/5127/F | c.2512C>G, p.R838G | CRD | Infancy | 32 | 0.03/HM | 2.0/2.0 | PD15 sat: unable to discriminate colors | Increased | Threshold greatly elevated (2.5 log units) | Normal | Macular RPE atrophy and clumping and choroidal atrophy; peripheral RPE atrophy, bone spicules in the periphery, partly accentuated in the vessels; | C/P | Large centrocecal scotoma with concentric narrowing of the outer border, leaving a ringshaped visual field of 5–50° in the midperiphery | EX | EX | ↑↑/↓↓ | bN | EX | EX | EX | EX | NI |

| Family ID/ Patient ID/ Gender, Pedigree ID | Mutation | Diagnosis | Onset Age | Age | BCVA (OD/OS) | Spherical Refraction (OD/OS) | Color Vision * | Glare Sensitivity | Dark Adaptation * | Night Vision | Fundus Findings * | | Visual Field * | Scotopic GF-ERG *† | | | | Photopic GF-ERG *† | | | | mfERG * |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | Rod Response | | Mixed Rod Cone Response | | Single Flash | | 30 Hz Flicker | | |
| | | | | | | | | | | | | | | A | I | A | I | A | I | A | I | |
| | | | | | | | | | | | atrophy of the optic disc; vessels narrowed | | | | | | | | | | | |
| II:1, index patient | | | | | | | | | | | | | | | | | | | | | | |
| RCD62/5128/F | c.2512C>G, p.R838G | CRD | Infancy | 35 | 0.03/0.04 | −6.5/−8.25 | PD15 sat: unable to discriminate colors | Increased | Threshold slightly elevated (1 log unit) | Normal | Macular RPE atrophy and clumping; vessels narrowed; OD: two circumscribed atrophic lesions in the periphery | C/P | Large centrocecal scotoma with concentric narrowing of the outer border, leaving a ringshaped visual field of 10–20° in the midperiphery | EX | EX | ↑↑/↓↓ | bN | EX | EX | EX | EX | NI |
| II:1, relative | | | | | | | | | | | | | | | | | | | | | | |
| RCD62/9149/F | c.2512C>G, p.R838G | CRD | Childhood | 54 | 0.03/0.03 | −3.25/−4.75 | PD15 sat: unable to discriminate colors | Increased | Threshold greatly elevated by (2.5 log units) | Normal | Zone of RPE and choroidal atrophy spanning from the macula in the optic disc; RPE atrophy and bone spicules in the periphery; vessels greatly narrowed | C/P | Large centrocecal scotoma with concentric narrowing of the outer border, leaving a ringshaped visual field of 5–30° in the midperiphery | ↑↑/↓↓ | bN | ↑↑/↓↓ | bN | EX | EX | EX | EX | NI |
| I:2, relative | | | | | | | | | | | | | | | | | | | | | | |

ID, identification number; Dis. dur., disease duration (y); BCVA, best corrected Snellen visual acuity; OD, right eye; OS, left eye; RPE, retinal pigment epithelium; GF-ERG, full field electroretinography; A, amplitude; I, implicit time; HM, perceiving hand movements; LP, light perception; PD15, Panel D15 Color vision test; sat, saturated; desat, desaturated; NI, no information; PD, papilla diameter; N, normal; bN, borderline normal data within normal range but near the 5th quantile of normal values; EX, below detection limit; ↑/↓, value just outside normal range; ↑↑/↓↓, value outside normal range.

*Both eyes are identical for all features unless stated.

†Normal range 5–95% confidence interval of normal values.