

Reevaluation of the Retinal Dystrophy Due to Recessive Alleles of *RGR* With the Discovery of a Cis-Acting Mutation in *CDHR1*

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PURPOSE. Mutation of *RGR*, encoding retinal G-protein coupled receptor was originally reported in association with retinal dystrophy in 1999. A single convincing recessive variant segregated perfectly in one family of five affected and two unaffected siblings. At least one further individual, homozygous for the same variant has since been reported. The aim of this report was to reevaluate the findings in consideration of data from a whole genome sequencing (WGS) study of a large cohort of retinal dystrophy families.

METHODS. Whole genome sequencing was performed on 599 unrelated probands with inherited retinal disease. Detailed phenotyping was performed, including clinical evaluation, electroretinography, fundus photography, fundus autofluorescence imaging (FAF) and spectral-domain optical coherence tomography (OCT).

RESULTS. Overall we confirmed that affected individuals from six unrelated families were homozygous for both the reported *RGR* p.Ser66Arg variant and a nearby frameshifting deletion in *CDHR1* (p.Ile841Serfs119*). All had generalized rod and cone dysfunction with severe macular involvement. An additional proband was heterozygous for the same *CDHR1/RGR* haplotype but also carried a second null *CDHR1* mutation on a different haplotype. A comparison of the clinical presentation of the probands reported here with other *CDHR1*-related retinopathy patients shows the phenotypes to be similar in presentation, severity, and rod/cone involvement.

CONCLUSIONS. These data suggest that the recessive retinal disorder previously reported to be due to homozygous mutation in *RGR* is, at least in part, due to variants in *CDHR1* and that the true consequences of *RGR* knock-out on human retinal structure and function are yet to be determined.

Keywords: RGR, CDHR1, whole genome sequencing, whole exome sequencing

To date, 140 genes have been implicated in nonsyndromic inherited retinal dystrophy (RetNet, available in the public domain, <http://www.sph.uth.tmc.edu/RetNet/>), a highly heterogeneous group of disorders characterized by progressive retinal dysfunction, degeneration, and visual failure. In most of the original discovery reports, multiple convincing mutations were identified in unrelated individuals. This reduces the likelihood of a false positive and also excludes the possibility of a cis-acting mutation in a distinct closely linked gene contained in the same ancestral chromosomal segment. A handful of

genes reported in the literature to cause retinal dystrophy have so far been associated with only one presumed disease-causing variant in all reported families. These include dominant disease due to mutation of *EFEMP1*,¹ *PRPF6*,² *RP9*,³ *GUCA1B*,⁴ *RIMS1*,⁵ and recessive disease due to mutation of *FSCN2*,⁶ *SEMA4A*,⁷ and *ZNF513*.⁸

One gene, *RGR*, encoding retinal G-protein coupled receptor (MIM *600342), was first reported in association with retinal dystrophy in 1999 when two distinct mutations were identified, each in one of two families. However, the



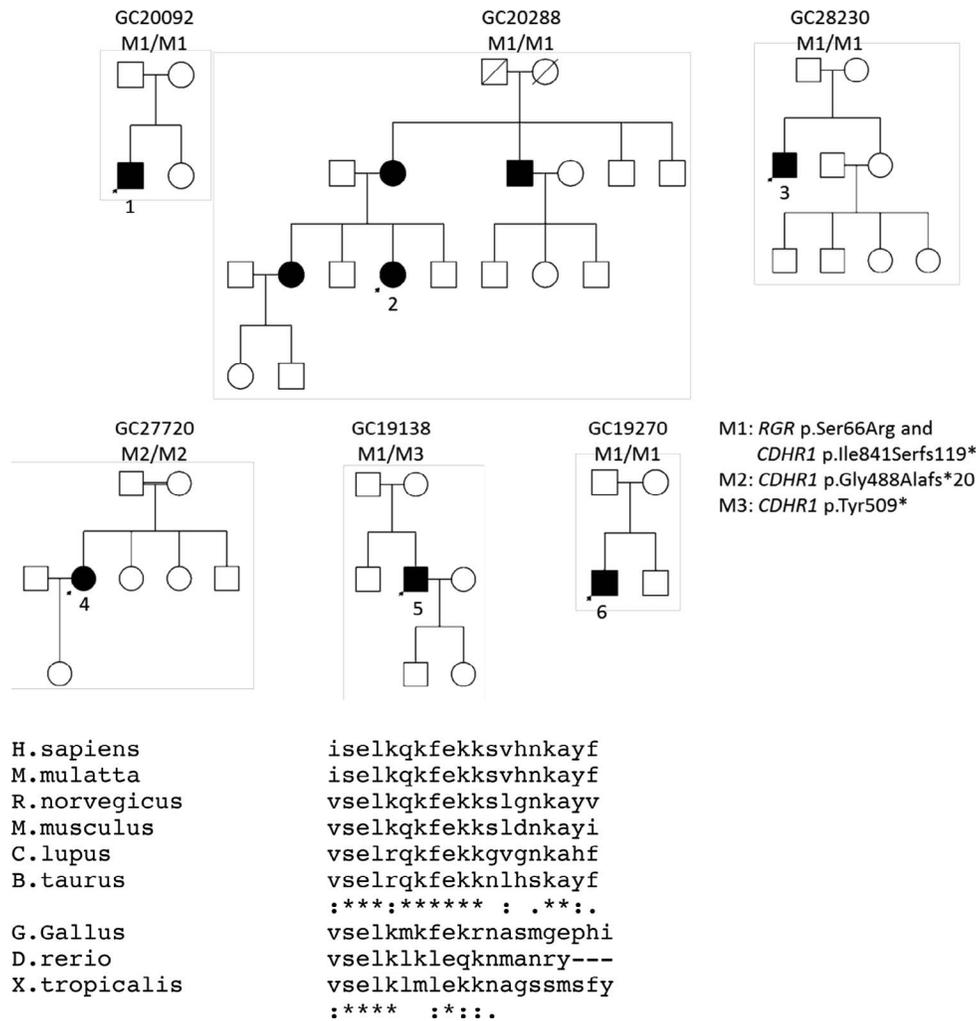


FIGURE 1. (a) Pedigrees and mutation found in families 1 to 6. (b) Clustal Omega alignment (EMBL-EBI, in the public domain, <http://www.ebi.ac.uk/Tools/msa/clustalo/>) of c-terminal 19 amino acid residues of six mammalian and three other vertebrate *CDHR1* orthologues showing high conservation. * = complete conservation, : = conservation between groups of strongly similar properties, . = conservation between groups of weakly similar properties.

mechanism appeared to be different for each, with one homozygous variant acting in a recessive fashion (c.196A>C; p.Ser66Arg), and another (c.824dupG; p.Ile276Asn*77) seemingly dominant.⁹ The same recessive *RGR* mutation was later identified by a different group in a proband using homozygosity mapping and direct sequencing of retinal dystrophy genes within homozygous regions.¹⁰

However, further data have resulted from whole-genome sequencing (WGS) of a cohort of retinal dystrophy patients, in whom all potential causative genes were interrogated in an unbiased fashion. Those data have enabled a reevaluation of the association between the recessive *RGR* p.Ser66Arg variant and human retinal disease. The data suggest that the ancestral chromosome harboring the recessive *RGR* variant also contains a convincing pathogenic variant in a nearby gene, *CDHR1*, and that is more likely to be the cause of the retinal degeneration in those individuals.

METHODS

The study protocol adhered to the tenets of the Declaration of Helsinki and received approval from the local ethics committee. Written, informed consent was obtained from all

participants or in the case of minors, their parents, before their inclusion in this study.

DNA from 599 unrelated patients with inherited retinal disease, ascertained from the Inherited Eye Disease clinics at Moorfields Eye Hospital, London, underwent WGS as part of the NIHR BioResource-Rare Diseases project. Briefly, peripheral blood mononuclear cell-derived genomic DNA was processed using the Illumina TruSeq DNA PCR-Free Sample Preparation kit (Illumina, Inc., San Diego, CA, USA) and sequenced using an Illumina HiSeq 2500, generating minimum coverage of 15X for approximately 95% of the genome. Reads were aligned to the genome (GRCh37) using an Isaac aligner (Illumina, Inc., Great Chesterford, UK). Single-nucleotide variants (SNVs) and indels were identified using an Isaac variant caller. Variant examination was performed only on the SNVs and indels that met the following criteria: passed standard quality filters, predicted to alter the sequence of a protein, and had an allele frequency less than 0.01 in the 1000-genome database, the NHLBI GO Exome Sequencing Project (release 20130513, in the public domain, <http://evs.gs.washington.edu/EVS>), the UK10K database (in the public domain, <http://www.uk10k.org>), and the Exome Aggregation Consortium (ExAC) database (in the public domain, <http://>

Investigative Ophthalmology & Visual Science

TABLE 1. Clinical Summary

Patient	Country Origin	Age at Last rv, y/Sex/	Age of Onset/Symptoms	BCVA, logMAR (Snellen)	Refraction	Fundus	Age and Summary of Electrophysiology	Other Findings
1	35/M/Albania	25/poor dark adaptation, 30/central blur and photophobia	R 1.6 (20/800) L 1.58 (20/800)	R -2.00/-2.00 × 100 L -2.00/-2.25 × 95	Macular atrophy, attenuated vessels, midperipheral RPE atrophy, white dots, and pigment migration	ND		
2	43/F/Albania	20/nyctalopia, 24/ central vision loss, photophobia	R PL L PL	UK	Macular atrophy, attenuated vessels, peripheral scalloped atrophy	ND		
3	42/M/Albania	35/nyctalopia few weeks later reduced central vision, photophobia, rapid decline	R HM L HM	R -2.25/-3.00 × 70 L -3.00/-2.00 × 120	Macular atrophy, attenuated vessels, midperipheral RPE atrophy, and white dots	36/undetectable PERG, undetectable rod-specific ERG, severely reduced and delayed cone specific L > R	Ishihara: 1/17 R&L	
4	33/F/Mauritius	Infant/nyctalopia, 11/ central vision loss, 25/ photophobia	R 1.8 (3/200) L 1.8 (3/200)	R -3.50/-0.50 × 55 L -5.00/-1.50 × 180	Macular atrophy, attenuated vessels, midperipheral RPE atrophy, and white dots	33/undetectable PERG, undetectable rod-specific ERG, subnormal and delayed cone-specific ERG		
5	30/M/Romania	Infant/poor dark adaptation, 16/central vision loss, 20/ photophobia	R 0.60 (6/24) L 0.48 (6/18)	R -0.50/-1.25 × 50 L -0.75/-1.00 × 135	Macular atrophy, attenuated vessels, midperipheral RPE atrophy, white dots, small inferior circumscribed atrophic lesions	26/undetectable PERG and rod ERG, residual cone-specific responses	Ishihara: 0/17 R&L Goldmann visual fields: large central scotomas, relatively preserved nasal and temporal fields, small central islands of preserved vision R&L	
6	16/M/Albania	Infant/reduced vision, nyctalopia, photophobia	R 0.48 (20/60) L 0.30 (20/40)	R +3.25/-2.25 × 180 L +2.50/-1.75 × 10	Macular atrophy, attenuated vessels, midperipheral RPE atrophy, and white dots	16/residual PERG, undetectable ERG	Goldmann visual field: circumferential scotoma central 20 degrees, small target (I4e) visible centrally on R, not L, relative preservation of temporal fields	

BCVA, best-corrected visual acuity; F, female; L, left; M, male; ND, not determined; PL, perception of light; R, right; RPE, retinal pigment epithelium; rv, review; UK, unknown; y, years.

exac.broadinstitute.org), and less than 0.02 in approximately 6000 internal control genomes. The reads of the whole genome sequences were inspected manually across both *RGR* and *CDHR1* using the Integrated Genome Browser (in the public domain, <http://www.broadinstitute.org/igv/home>)^{11,12} in the appropriate probands.

Initially, likely disease-causing variants in a panel of 192 genes previously associated with inherited retinal disease were interrogated (gene list available on request). Variants were ranked based on previous identification in retinal disease in the literature and/or a predicted impact on protein function, including high pathogenicity scores for missense variants using the predictive algorithms of "Sorting Intolerant From Tolerant" (SIFT; in the public domain, <http://sift.jcvi.org>), and Polymorphism Phenotyping v2 (PolyPhen-2; in the public domain, <http://genetics.bwh.harvard.edu/pph2>).^{13,14} Five patients were ascertained from these data.

Arrayed primer extension (APEX) microarray (Asper Biotech Ltd., Tartu, Estonia) for previously identified Leber congenital amaurosis (LCA) disease-associated mutations (including the *RGR* p.Ser66Arg mutation), and subsequent direct Sanger sequencing of *CDHR1* exon 17 using standard PCR and sequencing techniques (primers available on request), were used in one individual. Direct Sanger sequencing of *CDHR1* exon 17 was also performed in the two individuals that had previously been reported in the literature to harbor the p.Ser66Arg mutation in *RGR* in the homozygous state.^{9,10}

The minimal shared haplotype was determined for all patients analyzed by WGS and homozygous for the *RGR* p.Ser66Arg. From the variant call data for these patients, all variants that passed the standard quality filter, had a read depth ≥ 10 , and were located ± 1.5 Mb from the *RGR* p.Ser66Arg variant (Chr10:86007463) were extracted and analyzed for identical homozygous genotype.

The clinical phenotype of these patients was reviewed and compared with nine families with distinct *CDHR1* mutations. Retinal fundus imaging was performed by conventional 35-degree color fundus photography (Topcon Great Britain Ltd, Berkshire, UK) or by ultrawide field confocal scanning laser imaging (Optos plc, Dunfermline, UK); 30-degree or 55-degree fundus autofluorescence (FAF) imaging (Spectralis; Heidelberg Engineering Ltd, Heidelberg, Germany); and Spectralis optical coherence tomography (OCT). Full-field and pattern electroretinography (ERG, PERG) were performed in four patients using gold foil electrodes to incorporate the International Society for Clinical Electrophysiology of Vision (ISCEV) standards.^{15,16} Dynamic perimetry with Goldmann visual fields was additionally performed in two patients.

RESULTS

In total, eight individuals were identified to harbor *CDHR1* mutations through this study. Of 599 WGS probands with inherited retinal disease, three unrelated patients (patients 1-3) with progressive retinal dystrophy who were homozygous for the published *RGR* missense mutation (Chr10:g.86007463A>C, NM_002921.3 - c.196A>C; p.Ser66Arg) (Fig. 1) were identified additionally to harbor a novel homozygous frameshift mutation in *CDHR1* (Chr10:g.85974319_85974325del, NM_033100.3 - c.2522_2528delTCTCTGA; p.Ile841Serfs119*). The variants in all three individuals were confirmed by direct Sanger sequencing using standard techniques.

The *CDHR1* variant, within 33,145 bp of the *RGR* change, occurs in the final exon (exon 17) of the canonical transcript of *CDHR1*, 19 codons upstream of the termination codon. It is unknown if this mutation would lead to mature protein. However, assuming a protein product to be expressed, the C-

terminal 19 amino acid residues of the normal protein would be replaced by a 119-residue out-of-frame extension. The c-terminal 19 residues are well conserved in mammalian *CDHR1* orthologues with only two divergent residues (Fig. 1) and are divergent from paralogues. This may suggest a functional role or a role in targeting the protein in the mammalian photoreceptor. There are two major protein-coding transcripts of *CDHR1*: the mutation is located in exon 17 of transcript variant 1 (NM_033100.3) but not transcript variant 2 (NM_001171971.2), which uses an alternative final exon; transcript variant 1 is the major retinal transcript identified in RNaseq analysis of retinal tissue, suggesting that mutations located here would alter *CDHR1* in the photoreceptor.¹⁷

One further unrelated proband from this same experiment (patient 4) was identified as a homozygote for a distinct null variant in *CDHR1* (Chr10:g.85970899delG, NM_033100.3 - c.1463delG; p.Gly488Alafs*20, previously reported as p.Gly487-Glyfs*20).¹⁸ In addition, one heterozygous carrier of the *RGR* missense/*CDHR1* frameshift variants was identified (patient 5). This patient also carried a second heterozygous null variant of *CDHR1* (Chr10:g.85971445T>G, NM_033100.3 - c.1527T>G; p.Tyr509*), but no further rare variants in *RGR* were detected. Family DNA samples were unavailable for co-segregation of the two *CDHR1* variants.

It is hypothesized that the *RGR* and *CDHR1* variants were present on an ancestral haplotype. One patient (patient 6), who had been identified by APEX microarray to harbor the *RGR* p.Ser66Arg variant in the homozygous state, was ascertained for the present study. Subsequent direct Sanger sequencing of *CDHR1* exon 17 in this proband identified the homozygous c.2522_2528delTCTCTGA; p.Ile841Serfs119* mutation. An identical haplotype of 1.71Mb (Chr10:85631818-87350435) in patients 1 to 3 identified by WGS to be homozygous for the *RGR/CDHR1* variants was demonstrated using the variant call data.

DNA from the family members of the two previously reported families^{9,10} was sequenced for the *CDHR1* mutation and each affected individual was found to be homozygous for the *CDHR1* allele (patients 7 and 8), supporting complete linkage disequilibrium of the two variants.

The clinical features of patients 1 to 6 are summarized in Table 1. All but one patient described nyctalopia or poor dark adaptation preceding central vision loss, dyschromatopsia, and intolerance of bright lights. Patients 1 to 3 presented in their third to fourth decades, patients 4 to 6 from infancy. Vision was universally reduced ranging from 0.30 logMAR to perception of light only. The best visual acuity was found in patient 6 at age 16 years. Patients 1 to 3 with adult-onset disease were noted to have a rapid decline in their vision to legal blindness (visual acuity <1.0 logMAR) over a period of 5, 4, and 1 years respectively. The decline in the infant-onset patients was more gradual with only patient 4 legally blind at the age of 28 years. All patients had macular atrophy, midperipheral hypopigmentary RPE change, and relatively preserved retinal thickness on OCT particularly notable in patients 1 to 4 with the poorest vision (Fig. 2). Full-field ERGs were undetectable in one 16-year-old individual and showed severe reduction and delay in three others (aged 25-36 years), consistent with severe generalized rod and cone photoreceptor dysfunction. Pattern ERG reduction indicated severe macular dysfunction in all cases.

There have been eight previously reported families with *CDHR1*-related retinal dystrophy with onset of symptoms mainly in the second to fourth decades, but onset in infancy also has been reported (Table 2). In four families, nyctalopia was the presenting symptom, with central vision disturbance, dyschromatopsia, and photophobia reported later. In four families, the reverse was true. Legal blindness occurred in the fourth to fifth decades. The ERG was severely reduced when

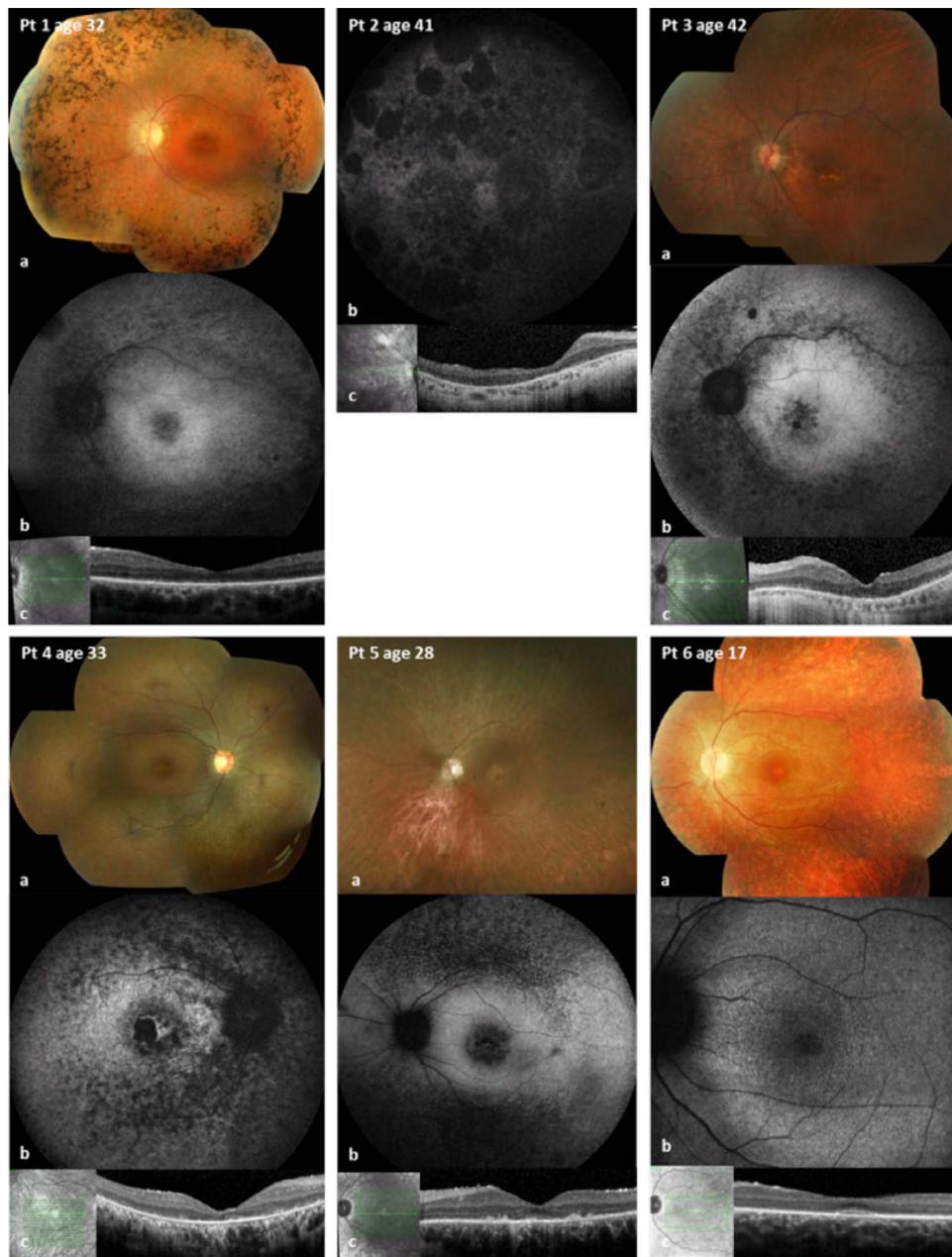


FIGURE 2. Retinal imaging of patients 1 to 6. Patients 1 to 3 and 6 are *CDHR1/RGR* homozygous, patient 4 is *CDHR1* p.Gly488Alafs*20 homozygous, patient 5 is *CDHR1/RGR* and *CDHR1* p.Tyr509* heterozygous. (a) Color fundus photography, (b) fundus autofluorescence, (c) OCT. Patients 1, 3 to 6: attenuated vessels, macular atrophy, midperipheral RPE atrophy and *white dots*; reduced autofluorescence centrally and in midperiphery (not shown for patient 6); loss of outer retina on OCT. Patient 1 in addition midperipheral pigmentary migration. Patient 2: color images not available; generalized loss of autofluorescence; loss of outer retina on OCT. Patient 6: preserved central inner segment ellipsoid. Patient 5 in addition midperipheral pigmentary migration with multiple small atrophic round lesions inferiorly.

tested. There were common fundus features of macular atrophy and peripheral RPE hypo/hyperpigmentary change and in addition peripheral circumscribed atrophic patches were described in two families reminiscent of the changes in patients 2 and 5 in the present series.

We have confirmed that the two previously reported families with homozygous p.Ser66Arg *RGR*-related retinal dystrophy also carry the homozygous p.Ile841Serfs119* mutation in *CDHR1* and have a similar phenotype. Five affected siblings from one family had markedly reduced vision in their fourth to fifth decades with macular atrophy, diffuse peripheral RPE atrophy, and hyperpigmentary change and ERG findings consistent with

generalized loss of retinal function.⁹ In the other family, a proband was diagnosed with retinitis pigmentosa (RP) at 6 years of age and had perception of light vision only by 36 years of age. His fundus was characterized by macular atrophy, peripheral RPE atrophy, bone spicule pigmentation, and peripheral paving-stone-like degeneration.¹⁰

DISCUSSION

Next generation sequencing (NGS), and in particular WGS, is producing unexpected findings in the field of rare disease,

TABLE 2. Summary of Key Features of Previously Reported Families With *CDHRI*-related Retinal Dystrophy

Paper/Family (No. Examined)/ Country Origin	<i>CDHRI</i> Mutation	Onset/Symptoms	Age of Legal Blindness	Fundus Feature	Age, If Known/PERG, ERG	Other Features
Henderson/family 1 (4)/Middle East	p.Gly113Alafs*1 Homozygous	Teenage years/ nyctalopia Mid-20s/photophobia	Early 40s	Diffuse RPE change, sparse pigment migration, macular atrophy later (OCT not reported)	42,40,3,4/rod and cone responses present 32/cone-rod pattern	Low myope, severe color vision defect
Henderson/family 2 (1)/South Asia	p.Gly487Glyfs*20 Homozygous	18/nyctalopia, later reduced central vision	44	Macular atrophy, later peripheral circular atrophic patches	No detectable responses	Myope Goldmann visual field age 46, peripheral islands of vision
Ostergaard/family (6, but 2 excluded from analysis as coincident oculocutaneous albinism)/Faroe Islands	p.Gln175Glnfs*47 Homozygous	Infancy/nyctalopia for 1 patient, unknown for others 17/decreased vision for 3 patients	Not present	Macular atrophy, diffuse peripheral RPE atrophy and hyperpigmentary change	2 patients undetectable, 2 reduced and delayed both rod and cone responses	2 low myopes, 2 emmetropic Severe color vision defect Goldmann visual fields variable loss of peripheral fields
Cohen/family (2)/Israeli Christian/ Arab	c.1485+2T>G Homozygous	20s/reduced central vision	30s	Macular atrophy, peripheral pigmentary change	20s/cone response absent, rod response markedly reduced	High myope (-11, -13) Severely impaired color vision
Duncan/family (3)/Palestinian Muslim	p.Gln461* Homozygous	Childhood/glare and photosensitivity Teenage/nyctalopia 20s/color vision loss	30s	Macular atrophy, diffuse midperipheral RPE changes with peripheral patches circumscribed RPE atrophy	32-45 y/undetectable for both rod and cone responses	Myope range -1 to -11
Ba-Abbad/family (3)/Indian subcontinent	p.Gly488Alafs*20 Homozygous	16-24 y/blurred central vision, dyschromatopsia, photoaversion	Not present	Macular atrophy, varying from subtle to more marked, including elevated lesion, periphery not reported	Severe generalized dysfunction with undetectable PERG	Severe color vision defects Central scotoma for 2 patients, normal 1 patient
Nikopoulos/family 1 (1)/Spanish	p.Pro574Ala Homozygous	30s/photophobia, dyschromatopsia, photopsia, later nyctalopia, reduced central vision, field loss	40s	Macular atrophy, peripheral pigment	Undetectable ERG	Small central island of vision on field testing age 42
Nikopoulos/family 2 (2)/Spanish	c.1485+2T>C Homozygous	20s/nyctalopia and field constriction	40s	Macular atrophy, central photoreceptor sparing, midperipheral pigment	Undetectable ERG	Low myope (1 patient)

including nonsyndromic RP consequent on mutations in syndromic disease genes^{19–21} and potentially allows the reclassification of previously assigned disease mutations as benign variants. This study reports the identification of a *CDHR1* mutation that is likely to cause recessive retinal degeneration previously reported to be caused by a homozygous *RGR* missense mutation.

The retinal dystrophy affecting the individuals from six families with homozygous mutations described in this report is likely to be consequent on the frameshift mutation in *CDHR1*. Four homozygous patients described are of Albanian origin. This is likely to represent an ancestral disease allele, the phenotype of which is indistinct from that caused by other frameshift mutations in *CDHR1*. The key finding to support this view is patient 5, who is heterozygous for the *CDHR1/RGR* haplotype and a second *CDHR1* null mutation in the absence of a second *RGR* mutation. This strongly supports the view that the *CDHR1* variant contained in the ancestral allele is pathogenic. A modifier role for the *RGR* variant cannot be ruled out in these patients, but this must anyway be subtle, given the comparable phenotype with other *CDHR1* patients.

Mutations in *CDHR1* encoding the cadherin-related family member 1 protein were implicated in recessively inherited retinopathy ranging from RP to cone-rod dystrophy.¹⁸ This photoreceptor-specific cadherin colocalizes with nascent disks at the base of photoreceptor outer segments and is predicted to have a role alongside prominin 1 in photoreceptor disk morphogenesis.^{22–25} To date, eight families have been described as having recessively inherited retinal dystrophy consequent on homozygous mutations in *CDHR1*.^{18,26–30} With one exception, identified mutations are predicted to eliminate the *CDHR1* protein by nonsense-mediated decay due to a splicing, frameshift, or nonsense mutation. The patients described herein have similar symptoms, fundus features, and ERG findings, with mixed rod and cone involvement being evident. Comparison of these patients with the previously reported *CDHR1* families demonstrates strong phenotypic similarities.

Retinal G-protein-coupled receptor is thought to modulate the visual cycle in the RPE cell by regulating the activity of lecithin retinol acyltransferase in the RPE cell.³¹ *RGR* knockout mice (*Rgr*^{−/−}) have a mild phenotype, developing morphologically normal retinae and exhibit no RPE or photoreceptor degeneration.^{32,33} If the p.Ser66Arg mutation found in the human were to have a similar effect on human retinal physiology, any associated dysfunction is likely to be masked by the degeneration caused by *CDHR1* disease in our patients. In contrast, studies in mice reveal that *CDHR1* is expressed by photoreceptors at the base of the outer segment and that lack of the protein results in disorganized outer segments and progressive photoreceptor cell death.²⁴

There are seven *RGR* variants reported in the Human Gene Mutation Database (available in the public domain; <http://www.hgmd.cf.ac.uk/ac/index.php> and Supplementary Table), although there is no evidence of pathogenicity for these except one (c.874dupG; p.Ile280Asnfs*78).⁹ It is likely that the dominant disease caused by the terminal exon frameshift mutation of *RGR* results in an out of frame extension and represents a toxic allele similar to the dominant disease associated with the *RPE65* c.1430G>A, p.Asp477Gly mutation.³⁴ The dominant *RGR* disease resembles that of choroideremia caused by mutation in the *CHM* gene encoding the RPE-specific RAB escort protein 1 characterized by RPE cell death followed by photoreceptor loss.^{9,35}

In conclusion, six patients with rod and cone photoreceptor dystrophy who are homozygous for the *RGR* p.Ser66Arg mutation have been identified to harbor a novel additional *CDHR1* frameshift mutation 34 kb away. One further patient,

heterozygous for this allele, carries a second *CDHR1* null mutation in the absence of a second variant in *RGR*. These observations and the fact that the retinopathy in these patients is similar to others harboring distinct *CDHR1* mutations, make it highly likely that *CDHR1* mutation is sufficient to explain the recessive disease previously ascribed to *RGR*.

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