Clinical Features of a Retinopathy Associated With a Dominant Allele of the RGR Gene

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PURPOSE. We describe the clinical features in two pedigrees with dominantly inherited retinopathy segregating the previously reported frameshifting mutation, c.836dupG (p.Ile280Asn*78) in the terminal exon of the RGR gene, and compare their haplotypes to that of the previously reported pedigree.

METHODS. The probands were ascertained at West Virginia University Eye Institute (WVU) and Moorfields Eye Hospital (MEH) through next generation sequencing (NGS) and whole genome sequencing (WGS) respectively. Clinical data included visual acuity (VA), visual fields, fundus autofluorescence (FAF), optical coherence tomography (OCT), and electroretinography (ERG). Haplotype analysis was performed using Sanger sequencing of the DNA from the molecularly ascertained individuals from the three pedigrees.

RESULTS. Nine heterozygous mutation carriers were identified in two families. Four carriers were asymptomatic; five carriers had variable VA reduction, visual field constriction, and experienced difficulty under dim illumination. Fundus examination of the asymptomatic carriers showed diffuse or reticular pigmentation of the retina; the symptomatic carriers had chorioretinal atrophy. FAF imaging showed widespread signal loss in advanced retinopathy, and reticular hyperautofluorescence in mild cases. OCT showed loss of outer retinal lamina in advanced disease. ERG showed moderate-to-severe rod-cone dysfunction in two symptomatic carriers; and was normal in three asymptomatic carriers. A shared haplotype flanking the mutation of up to 6.67 Mb was identified in both families. Within this region, 1.27 Mb were shared with the first family reported with this retinopathy.

CONCLUSIONS. The clinical data suggest a variable and slow degeneration of the RPE. A shared chromosomal segment surrounding the RGR gene suggests a single ancestral mutational event underlying all three families.

Keywords: autosomal dominant retinal dystrophy, deep retinal reticular pigmentation, next generation sequencing, whole genome sequencing, RGR

Inherited retinal dystrophies are a group of disorders in which retinal dysfunction and/or degeneration is due to disease-causing variants in one or both alleles of a single gene. They present with variable clinical features, and have high genetic and allelic heterogeneity. To date, disease-causing variants in more than 300 genes have been identified (available in the public domain at https://sph.uth.edu/retnet/; The University of Texas-Houston Health Science Center, Houston, TX, USA). Proteins encoded by these genes can be expressed ubiquitously, or by specific retinal cell types.1–4

The retinal G-protein coupled receptor gene (RGR, MIM_600342) is located on chromosome 10q23.1, and encodes an intracellular opsin localized to the retinal pigment epithelium (RPE) and Müller cells.5,7 To date, only one disease-causing
mutation in *RGR* has been described: a heterozygous frameshifting mutation (chr10:86018343dupG; NM_002921.3:c.836dupG; p.Ile280Asn*fs*78 — the same mutation as reported previously), which was reported in a pedigree with autosomal dominant retinopathy, but the clinical data were limited (RP44; MIM-613769). An allele reported to be associated with recessive retinal disease has been re-evaluated, with the disorder in those patients explained, instead, by biallelic mutation of *CDHR1*. Therefore, the consequences of biallelic loss of function of *RGR* on the human retina remain unknown. We characterized the clinical features secondary to the frameshifting mutation p.Ile280Asn*fs*78 in *RGR* in two not knowingly related Caucasian pedigrees from the United Kingdom and United States.

**METHODS**

Two probands (shown by arrows in Fig. 1) were initially ascertained through large sequencing studies. Family WVU was recruited from the medical retina clinic at the West Virginia University Eye Institute (WVU); family GC4177 was recruited from the inherited retinal disorders clinic at Moorfields Eye Hospital (MEH; Fig. 1). After obtaining informed consent, the probands and their family members donated blood for genetic testing. DNA analysis from the WVU family was performed as part of the National Ophthalmic Disease Genotyping and Phenotyping Network (eyeGENE) study, as a Health Insurance Portability and Accountability Act (HIPAA)-compliant study that was approved by the institutional review boards at WVU, and the National Eye Institute (Bethesda, MD, USA). DNA analysis from the GC4177 family was performed as part of the National Institute for Health Research BioResource - Rare Diseases (NIHRBR-RD) study, which was approved by MEH Research Management Ethics, and Cambridge South Research Committee, and adhered to the tenets of the Declaration of Helsinki. Details of the molecular investigations at WVU and MEH, including the haplotype analysis from the two pedigrees and the family reported by Morimura et al., are provided in the Supplementary Material.

Sanger sequencing of all coding exons and exon-intron boundaries of *RGR* in 18 unrelated probands from MEH, whose retinal features were similar to those of the MEH proband and WVU: III-8, and were negative for *CHM* mutations, did not identify further carriers of this change or other likely disease-causing variants in *RGR* (primers available upon request).

The probands and available family members underwent ophthalmic examination including best-corrected Snellen visual acuity (VA), dilated fundus examination, optical coherence tomography (OCT), fundus autofluorescence (FAF) imaging (Spectralis HRA+OCT; Heidelberg Engineering GmbH, Heidelberg, Germany), color fundus photography, Goldmann perimetry, and electrophysiologic testing using protocols that incorporated the recommendations of the International Society of Electrophysiology of Vision (ISCEV) for electroretinography (ERG), pattern electroretinography (PERG), and electrooculography (EOG) at the time of examination.

**RESULTS**

**Molecular Genetic Analysis**

A previously reported heterozygous variant in *RGR* (NM_002921.3:c.836dupG; p.Ile280Asn*fs*78, MIM_600342.0002) was detected in the genomic DNA from both probands WVU-IV-5, and GC4177-IV-1 by targeted next generation sequencing (NGS) and whole genome sequencing (WGS), respectively.
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## Table 2. Clinical Features of RGR Retinopathy in Two Unrelated Pedigrees

| Subject | Age, y | Presentation | BCVA | Visual Field | Fundus Features | FAF | OCT | ERG |
|---------|--------|--------------|------|--------------|----------------|-----|-----|-----|
| WVU: III-6; 81 | Visual field defects | RE: 20/30; LE: 20/25 | Enlarged blind spots, BE | Bilateral peripapillary atrophy | Reduced peripapillary AF, extending nasally & temporally, relative foveal sparing, reticular pattern of increased AF in the posterior pole, speckled AF inferiorly | Relative sparing of the fovea, interlaminar bridge nasal to the fovea, peripapillary loss of outer retinal layers, thin choroid | Moderate-to-severe reduction of scotopic and photopic responses |
| WVU: III-8; 59 | Reduced VA | RE: 20/25; LE: 20/60 | Enlarged blind spot & large nasal scotomata, BE | Extensive peripapillary atrophy, relative foveal sparing. At age 77: bilateral chorioretinal atrophy in the posterior pole | Reduced AF with islands of preserved AF in the posterior pole | At age 77: interlaminar bridge temporal to the fovea; extensive loss of the outer retina, thin choroid | Moderate reduction of scotopic bright flash response; mildly subnormal photopic b-wave amplitude |
| WVU: IV-2; 60 | Asymptomatic | RE: 20/20; LE: 20/20 | NA | Bilateral peripapillary atrophy & peripheral reticular pigmentary | Reduced peripapillary AF, reticular pattern of increased AF sparing of the posterior pole. | Preservation of the foveal structure, peripapillary loss of outer retinal layers | NA |
| WVU: IV-5; 50 | Asymptomatic; peripapillary atrophy | RE: 20/20; LE: 20/20 | Enlarged blind spots, BE | Bilateral peripapillary atrophy & peripheral reticular pigmentary | Reduced peripapillary AF, reticular pattern of increased AF and patches of reduced AF nasally | Preservation of the foveal structure, deep peripapillary excavation & loss of outer retinal layers | Normal scotopic & photopic responses |
| WVU: IV-6; 48 | Difficulty driving at night | RE: 20/25; LE: 20/25 | NA | Bilateral peripapillary atrophy & peripheral reticular pigmentary | Widespread reduced AF with islands of preserved AF in the posterior pole | Extensive loss of the outer retina at the posterior pole; ORT | NA |
| MEH-GC4177: IV-1; 73 | Reduced VA, difficulty seeing under dim light, & visual field defects | RE: 20/1000; LE: 20/80 | NA | Bilateral chorioretinal atrophy, no retinal vascular attenuation, patches of intraretinal pigmentation in the temporal periphery | Widespread reduced AF with islands of preserved AF in the posterior pole | Extensive loss of the outer retina at the posterior pole; ORT | NA |
| MEH-GC4177: V-1; 52 | Progressive visual field constriction | RE: 20/20; LE 20/30 | NA | Bilateral peripapillary atrophy & peripheral reticular pigmentary | Reduced peripapillary AF, reticular AF pattern, patches of reduced AF nasally, relative sparing of the parafoveal region | Mild disruption of the EZ & the IZ; focal thickening of the RPE band nasal to the fovea | NA |
| MEH-GC4177: V-2; 51 | Asymptomatic | RE: 20/20; LE: 20/20 | NA | Bilateral peripapillary atrophy & peripheral reticular pigmentary | Reduced peripapillary AF, reticular pattern of increased AF nasally | Marked attenuation of the ONL & EZ nasal to the fovea; ORT | Normal scotopic & photopic responses |
| MEH-GC4177: VI-1; 25 | Asymptomatic | RE: 20/15; LE: 20/20 | Normal | Bilateral peripapillary atrophy & diffuse retinal pigment alteration | Reduced peripapillary AF, widespread “salt-and-pepper” hypo-AF anterior to the vascular arcades | Preservation of the foveal structure, peripapillary loss ONL & EZ; ORT | Normal scotopic & photopic responses |

Patients were examined at WVU or MEH-GC4177. BCVA, best-corrected visual acuity; RE, right eye; LE, left eye; BE, both eyes; DS, spherical dioptr; FAF, fundus autofluorescence; AF, autofluorescence; OCT, optical coherence tomography; EZ, ellipsoid zone; IZ, inter-digitation zone; RPE, retinal pigment epithelium; ONL, outer nuclear layer; ORT, outer retinal tubulation; ERG, full-field electroretinogram; NA, not available.
asymptomatic carriers WVU: IV-5 (note the normative data in Supplementary Table S2) and GC4177: V-2, VI-1 had normal scotopic and photopic full field ERGs. The PERG was normal in the youngest carrier GC4177:VI-1, but showed mildly reduced P50 amplitude in GC4177: V-2. Subjects WVU: III-6 and WVU: III-8 were symptomatic, and the ERG from WVU: III-6 showed moderately severe reduction of the scotopic amplitudes at the age of 81 years, with delayed and subnormal photopic responses. The ERG recordings from WVU: III-8 (at the age of 59 years with limited outer retinal atrophy) showed moderate reduction of the scotopic bright flash response; and subnormal photopic b-wave amplitude with normal peak time, in addition to mild reduction of the EOG light rise (light peak-dark trough ratio of 1.6) in both eyes. Because of the putative role of RGR in the visual cycle,15 ERG was performed in subject GC4177: V-2 after extended monocular dark adaptation overnight. The amplitude of the scotopic mixed bright flash response of the eye that underwent extended dark adaptation was higher than that of the eye that underwent standard dark adaptation for 20 minutes, but this difference was not clinically significant.

**DISCUSSION**

We described the clinical features of retinal dystrophy associated with a rare, heterozygous frameshifting mutation in RGR in two families from the United States and the United Kingdom with a haplotype that is shared with the first reported family,8 and demonstrated the spectrum of retinal changes. These ranged from seemingly innocuous reticular or diffuse deep retinal pigment alteration, normal visual acuity, and normal ERG, to diffuse atrophy of the retina and choroid with severe visual loss. Longitudinal data for the asymptomatic carriers were limited and, therefore, it is unknown if the retinal changes in those subjects are nonprogressive signs of the carrier state or represent an early stage of a slowly progressive retinal dystrophy. The features of RGR-retinopathy emphasize the importance of FAF imaging in recognizing mild retinal manifestations and guiding the molecular investigation in individuals with poor vision under dim lighting conditions and peripapillary RPE atrophy. Variable expressivity (variable phenotypic severity) and low penetrance (mutation carriers with no associated phenotype) also have been observed in pedigrees heterozygous for the p.Asp477Gly allele in RPE65, another dominant disease affecting the RPE.15

In the first report of RGR retinopathy, the proband showed bilaterally subnormal VA, macular atrophy, and severely reduced ERGs, but the milder and early manifestations of this retinopathy were not described.8 The clinical features of retinal dystrophies with presumed primary RPE pathology differ from those of retinitis pigmentosa by the paucity of intraretinal bone spicule pigment deposition, and relative preservation of the retinal vascular caliber. The phenotype described in patients WVU: III-8 and GC4177: IV-1 resembles
that observed in choroideremia (CHM), patients heterozygous for the p.Asp477Gly mutation in RPE65, some PRPH2 mutations, and the p.M216K mutation in RHO.13-16 CHM, and RPE65- and RHO-dominant retinopathies affect initially the RPE due to the expression of their respective genes, and, therefore, are likely to have similar clinical features. Early RPE involvement in some cases of PRPH2-retinopathy and the p.M216K mutation in RHO remain unexplained as both genes are expressed in the photoreceptors.15,16 One early symptom common to these disorders is reduced vision in the dark; it is plausible to speculate that the impaired night vision is caused by an abnormal visual cycle in the affected RPE while the cone function is spared due to the Müller cell-mediated visual cycle.17

Electrophysiologic testing in one individual with advanced retinopathy showed loss of retinal function without shifting the peak-time, suggesting that the main pathology in some cases is loss of photoreceptor function without dysfunction.

The subnormal EOG in this patient can be associated with reduction of the scotopic ERG and is milder than the reported abolished EOG light rise in a patient with a similar retinal phenotype in dominant RPE dystrophy due to the p.Asp477Gly allele in RPE65.13 RGR and RPE65 are expressed in the RPE and these alleles likely result in cellular toxicity.5,13,18 The subnormal macular function, as shown on PERG in one asymptomatic carrier, suggested that this retinopathy can involve the macula.

To date, there are no other known disease-causing mutations in RGR. It is informative to examine the population variants in the gnomAD database (available in the public domain at http://gnomad.broadinstitute.org/gene/ENSG00000148604; the Broad Institute). The presence of numerous predicted loss-of-function mutations suggests that haploinsufficiency is too common to be responsible for retinal disease and supports our hypothesis that the reported allele has a toxic or gain-of-function effect. Moreover, eight instances of one specific final exon frame-shifting mutation in 24028 African alleles also are present (NM_002921.3:c.796_797insCC; p.Ile267Profs*37; rs770085833). No other C-terminal frame-shifting alleles are reported. The c.796_797insCC variant, unlike the c.836dupG variant reported in our study, affects the noncanonical reading-frame and produces a different carboxyl terminal peptide (by an out-of-frame extension through the canonical stop codon, adding seven novel amino-acids at the carboxyl terminal, and replacing 29 amino-acids in the cytoplasmic domain of RGR). In addition to containing distinct amino-acids, it also is shorter than that predicted from the c.836dupG variant. This observation further supported the assertion that the retinopathy reported here is due to toxicity conferred by the specific abnormal carboxyl peptide.

The shared haplotype, in the three pedigrees, suggests that the p.Ile280Asnfs*78 allele is a founder mutation. Recognition of this phenotypic variability could lead to identification of further affected families, and analysis of their respective haplotypes.
Our study reinforced the notion that, although candidate gene testing—when a distinct phenotype is observed—often is helpful, unbiased genetic screening using large NGS panels or whole exome/genome sequencing can reveal unexpected genotype-phenotype associations and expand the list of candidate genes.\textsuperscript{19,20}

In conclusion, this report exemplified the success of the unbiased approaches of WGS and NGS in the molecular diagnosis of a highly genetically heterogeneous disorder, such as retinal dystrophy. The clinical features suggested a variable severity of retinopathy consequent upon heterozygosity of a specific allele of the \textit{RGR} gene. The slow progression of this retinopathy and possible primary involvement of the RPE make this disorder tractable to potential therapeutic interventions.

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