ABCA4 and *ROM1:* Implications for Modification of the *PRPH2*-Associated Macular Dystrophy Phenotype

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Purpose. To identify the causative mutation leading to autosomal dominant macular dystrophy, cone dystrophy, and cone–rod dystrophy in a five-generation family and to explain the high intrafamilial phenotypic variation by identifying possible modifier genes.

Methods. Fifteen family members were investigated by detailed ophthalmic and electrophysiologic phenotyping. Mutation screening was initially performed with microarrays that detect known mutations in genes associated with retinal degeneration. Furthermore, the patients' genomic DNA was analyzed by sequencing analysis of *PRPH2*, *ABCA4*, and *ROM1*.

Results. Heterozygous mutations were identified in three genes and showed five different combinations within the studied family. All clearly affected family members carried the heterozygous *PRPH2* mutation p.R172W. Patients with heterozygous sequence alterations only in *ROM1* (p.R229H) or *ABCA4* (p.V2050L) showed a mild ocular phenotype and were otherwise asymptomatic. The phenotypic severity of patients carrying the *PRPH2* mutation increased with an additional mutation in *ROM1*. Patients carrying all three mutations were the most severely affected.

Conclusions. Features of a *PRPH2*-associated phenotype might be modulated by additional mutations in other genes (in this family *ABCA4* and/or *ROM1*) accounting for intrafamilial variability and resulting in a cumulative effect worsening the phenotype. Families showing a variable macular dystrophy phenotype caused by mutations in *PRPH2* should be tested for additional mutations in *ABCA4* and *ROM1*, as they may alter the progression of the *PRPH2* phenotype. This testing will influence genetic counseling, as patients with additional mutations may be confronted with a faster progression of visual loss. (*Invest Ophthalmol Vis Sci.* 2010;51:4253–4265) DOI:10.1167/iovs.09-4655

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Mutations in the gene *PRPH2* cause a wide phenotypic spectrum of autosomal dominant retinal dystrophies, including retinitis pigmentosa,[1–7] retinitis punctata albescens,[8] cone–rod dystrophy,[9–11] cone dystrophy,[12,13] adult vitelliform macular dystrophy,[14–16] fundus flavimaculatus,[17] pattern dystrophy,[18,19] and macular dystrophy.[2,20]

There are several explanations for a variable phenotype of a presumed monogenic disorder. The variation may be caused by allelic heterogeneity, environmental factors, or genetic modifiers.[21] Modifiers are genes whose influence on the properties of the primary disease gene leads to phenotypic variability.[22]

In mice, modifier loci in hereditary retinal disease phenotypes have been identified in the retinal degeneration mouse model rd3 and rd7,[23,24] in a murine retinoschisis 1 model,[25] and in ocular retardation mice.[26] Mouse models with modifying genetic backgrounds have also been developed for retinitis pigmentosa (RP).[27,28] *Rpe65* has been suggested to modulate rhodopsin regeneration in a transgenic mouse model of autosomal dominant RP.[29] In patient-derived cell lines, modifier loci have been mapped in RP (Mendelian Inheritance in Man [MIM] 600138; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) caused by mutations in *PRPF31*.[30]

The *PRPH2* gene on chromosome 6 encodes 346 amino acids.[31,32] The gene product is a membrane-spanning glycoprotein located in the disc membranes of cone and rod photoreceptors. Peripherin is thought to act as a membrane stabilizer of the outer segment discs arrangement. This notion is further supported by the finding that knockout mice carrying a homozygous null mutation in perhipherin*/rds* fail to develop photoreceptor outer segments.[33,34] Peripherin assembles into heterotetrameric complexes with the rod outer segment protein 1 (ROM1).[35,36] ROM1 is a disc rim integral membrane protein[37,38] that is localized to the rod and cone outer segments.[35–37] It seems to be especially essential for rod photoreceptor survival.[39]

It has been reported that the *PRPH2* p.Leu185Pro allele and two protein-truncating *ROM1* mutations show a digenic inheritance, where only double heterozygotes are affected by RP.[6,7] A third *ROM1* missense mutation has also been found to be associated with digenic RP.[7] So far, no cases of RP have been reported that are caused by sequence changes in *ROM1* alone.

In contrast to *ROM1*, morphologically normal asymptomatic heterozygous *ABCA4* mutation carriers showed functional impairment as revealed by reduced contrast sensitivity and reduced amplitudes in the multifocal (mf)ERG.[40] The *ABCA4* gene[41] codes for a retina specific membrane transporter protein. Similar to peripherin and ROM1, ABCA4 is situated in rod and cone outer segments.[42,43] It is thought to play a major role in the recycling of all-*trans* retinal during the visual cycle.[44,45]

ABCA4 mutations have been found to cause autosomal recessive Stargardt disease (STGD1, MIM 248200)[41,46]; fundus flavimaculatus, which is considered to be an allelic disor-

der[47,48]; autosomal recessive cone rod dystrophy[49–54]; and autosomal recessive RP.[50,55–58] An increased susceptibility for age-related macular degeneration due to *ABCA4* sequence variations had also been postulated.[59] However, this association was not confirmed in other studies.[60–62]

Although *PRPH2* mutations frequently are associated with variable phenotypes, the mutation p.R172W so far has shown an exceptional high intra- and interfamilial consistency for central retinal manifestations.[13,63–67] Only a single study described considerable phenotypic variability associated with this mutation.[9]

Herein, we describe a five-generation family with the p.R172W mutation in *PRPH2* whose members show a remarkable intrafamilial variation in ocular phenotype. Our data suggest that *ABCA4* and *ROM1* act as modifier genes of the *PRPH2* p.R172W-associated phenotype.

METHODS

Patients and Clinical Investigation

Informed consent was obtained before examination from all participants. All agreed to diagnostic services to identify disease-associated mutations. The study adhered to the tenets of the Declaration of Helsinki[68] and was approved by the ethics committees of the University of Freiburg, Germany.

Peripheral blood samples were obtained from 18 subjects. Fifteen members of this five-generation Caucasian German family with autosomal dominant retinal dystrophy were included in the study (Fig. 1): patient identification numbers 28586 (III-1), 28589 (III-2), 28630 (III-4), 28628 (III-6), 28633 (III-7), 28590 (IV-1), 28588 (IV-2), 28585 (IV-3), 26593 (IV-4), 28632 (IV-7), 28629 (IV-9), 28582 (V-1), 28587 (V-2), 28584 (V-3), and 28583 (V-4). Patients IV-5, V-5, and V-6 did not agree to scientific analysis of their DNA samples and were thus excluded from the scientific molecular genetic analysis.

Twelve members (see Fig. 1) underwent a complete ophthalmic examination. They were queried about subjective complaints of loss of visual acuity, loss of visual fields, difficulty seeing in dim or dark light conditions, photophobia, and color vision problems. In two subjects, detailed clinical records of 13 years of preceding ophthalmic evaluation were available. Thorough medical histories were recorded. Member III-7 was excluded from the study, as both eyes had undergone retinal detachment surgery. Member III-1 died shortly after genotyping, before phenotyping was performed.

Phenotyping included Goldmann and Octopus perimetry (Haag Streit, Köniz, Switzerland). Color vision was assessed with the Panel D 15 color vision test. For better comparison after ophthalmoscopy, fundus photographs were taken from the central and peripheral retina (model FF450 fundus camera; Carl Zeiss Meditec GmbH, Jena, Germany).

The severity of fundus changes was classified as minimal, mild, moderate, or severe. Fundus autofluorescence (FAF) was recorded with a confocal scanning laser ophthalmoscope.[69] Electrooculograms (EOG; Nicolet, Madison, WI) and full-field electroretinograms (ERGs; maximum flash intensity 1.8 cd · s/m^2; Nicolet) were recorded binocularly according to ISCEV (International Society for Clinical Electrophysiology of Vision) standards.[70,71] The ERG analysis concentrates on the scotopic 1.8 b-wave (i.e., the combined rod-cone response, and cone flicker amplitude as they showed the diseases of the rod and cone system most clearly for the different family members). Multifocal electroretinograms (mfERG; VERIS 4.8; Electro-Diagnostic Imaging, Redwood City, CA) were recorded binocularly according to the ISCEV guideline.[72] Since mfERG-detected disease in the present cases was not patchy but was always cumulative with rising eccentricity (i.e., with increasing radius from the center of the stimulated retinal area), the analysis was based on the laboratory norm for ring averages. Highest sensitivity was achieved by analyzing not amplitudes but ring ratios (the ratio of any given ring amplitude relative to the average of all other rings), which we embodied in a Z-classification as follows: Z1, only relative macular (ring 1) impairment; Z2, relative impairment of rings 1 and 2, normal ratios for rings 3 to 5; Z3, relative impairment of rings 1 to 3, normal ratios for rings 4 and 5; etc.

DNA Preparation and Mutation Analysis

Genomic DNA was isolated from blood samples of the 18 family members (Magnetic Separation Module I; Chemagen, Baesweiler, Germany) and underwent routine diagnostic testing of molecular genetic defects in genes associated with retinal degenerative diseases. The genomic DNA of index patient IV-4 (26593) was analyzed by commercially available genotyping microarrays detecting known sequence alterations that have been described in genes causing autosomal dominant retinitis pigmentosa or in the *ABCA4* gene (AD RP and *ABCA4* panel; Asper Biotech, Tartu, Estonia). These analyses included the following genes: *ABCA4*, *CA4*, *CRX*, *FSCN2*, *IMPDH1*, *NRL*, *PNR*, *PRPF3*, *PRPF31*, *PRPF8*, *RDS*, *RHO*, *ROM1*, *RP1*, *RP9*, and *TOPORS*. Detected sequence alterations were verified by sequencing (ABI3100; Applied Biosystems, Rotkreuz, Switzerland). In addition, we sequenced the *PRPH2* and *ROM1* coding exons including flanking intronic sequences of IV-4 and IV-6. *ABCA4* was screened in patient V-4 by sequencing. We further verified pathogenic sequence alterations of *PRPH2*, *ROM1*, and *ABCA4* in all family members (Figs. 1, 2). PCR conditions using 50 to 100 ng of genomic DNA as a template and polymerase (HotFire; Solis BioDyne, Tartu, Estonia) were as follows: After an initial 15 minutes at 95°C, we performed a denaturing step at 95°C, followed by annealing at 55°C to 63°C, a 1-minute incubation at 72°C for 35 cycles, and a final extension at 72°C for 10 minutes. PCR primers are available on request. Ahead of sequencing, the quality of amplified DNA fragments was controlled by agarose gel electrophoresis. Sequence variations on DNA and protein level are described as recommended by the Human Genome Variation Society (HGVS, http://www.hgvs.org/ provided in the public domain by the Genomic Disorders Research Center, Carlton South, VIC, Australia).

RESULTS

Molecular Genetic Findings

We analyzed 15 members of a Caucasian family, as shown in Figure 1, by using molecular genetic techniques to identify the causative mutation and/or additional sequence alterations. Index patient IV-4 was initially screened with genotyping microarrays for known mutations described in genes associated with autosomal dominant retinitis pigmentosa and macular dystrophy. The initial referral diagnosis was Stargardt disease (STGD1), which prompted us to screen as well for *ABCA4* mutations by genotyping microarrays. In both analyses, heterozygous mutations were identified either in *PRPH2* or *ABCA4*. The *PRPH2* mutation locates to exon 1 and constitutes a C-to-T exchange at nucleotide position 514 (c.514C>T). This missense mutation is predicted to cause an amino acid substitution (p.Arg172Trp, p.R172W). The *PRPH2* mutation cosegregated with the disease in all affected family members and thus is considered to be the causative mutation leading to macular dystrophy (V-1, V-2, V-3, V-4, IV-1, IV-7, IV-9, and III-6), cone dystrophy (III-4), and cone–rod dystrophy (IV-4, III-2).

To verify whether *ROM1* mutations occurred in the family, we sequenced all coding and flanking intronic regions of *ROM1* in patients IV-4 and IV-7. We detected several polymorphic variants (IV-4: rs35904570, rs1801144, rs1799959, and rs4387351; and IV-7: rs1799959 and rs4387351) in addition to a sequence alteration in exon 2 (c.686G>A; p.Arg229His, p.R229H),[73] which so far has been described as a rare, nonpathogenic variant. The occurrence of this rare sequence alteration was tested in all other individuals and was identified in seven members (III-2, IV-1, V-1, IV-4, V-4, III-6, and

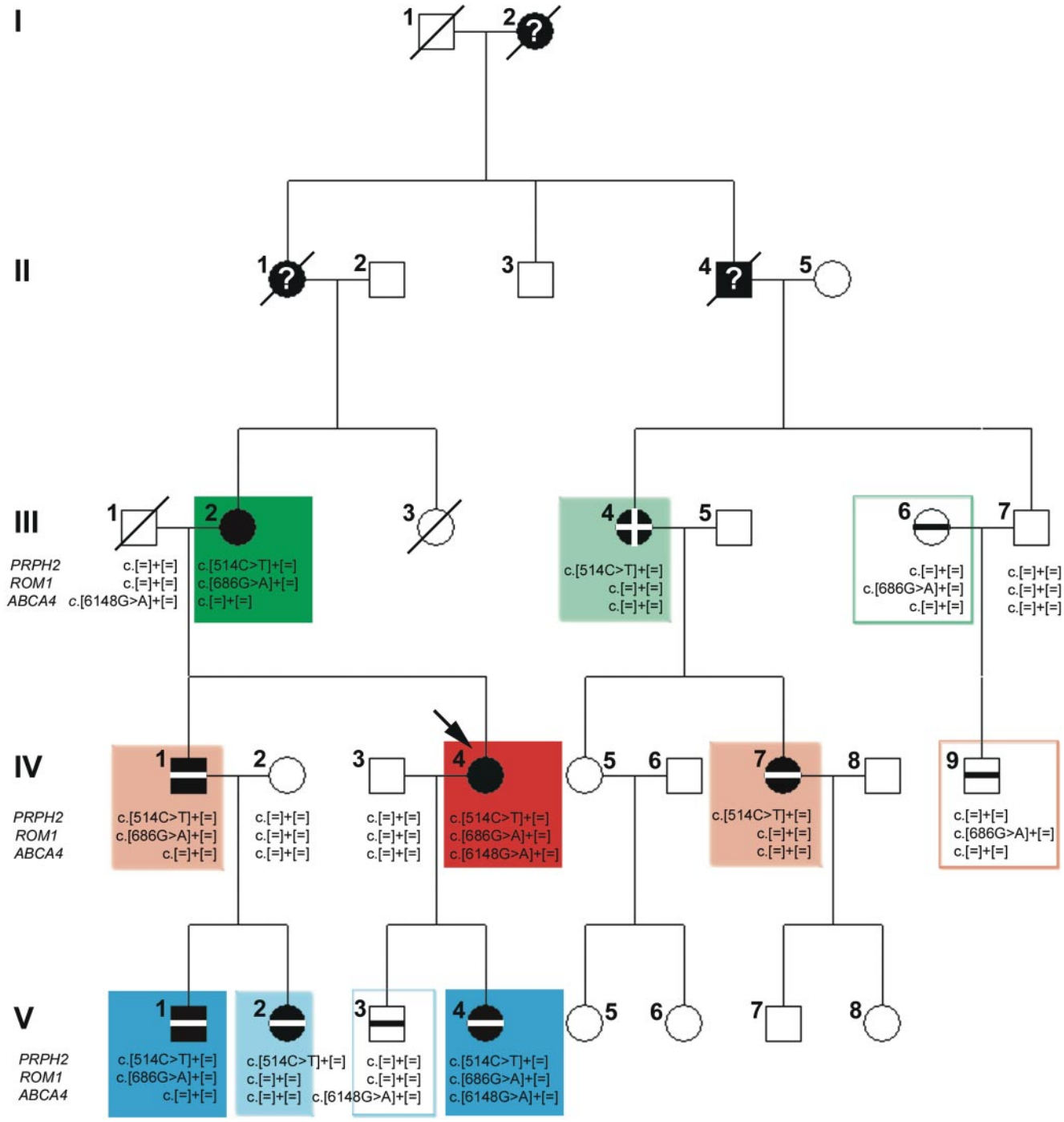

Figure 1. Pedigree and genotype of a five-generation family. *Circles*: females; *squares*: males; *slashed symbols*: deceased family members; *symbols with horizontal bar*: macular dystrophy; *filled symbols with horizontal and vertical bar*: cone dystrophy; *filled symbols*: affected by cone-rod dystrophy; *filled symbols with question mark*: affected according to interview of family members without possibility of phenotyping; and *arrow*: index patient. Genotype data are presented beneath the patient from whom DNA was available: *top*: *PRPH2*; *middle*: *ROM1*; *bottom*: *ABCA4*. *Colored boxes* around affected individuals illustrate phenotype severity, which increased with increasing saturation. Phenotype severity was compared only within the same generation (*green*: third generation; *red*: fourth generation; *blue*: fifth generation), to exclude the possible confounding effect of age. The increase in severity of the retinal degeneration phenotype (caused by a *PRPH2* mutation) correlates with the occurrence of additional mutations in *ABCA4* and *ROM1*. Patient III-1 deceased shortly after genotyping so that a clinical evaluation was not possible.

IV-9) as well as excluded in five family members (III-4, IV-7, V-2, III-1, and V-3).

A second mutation was detected by sequencing and genotyping microarrays affecting exon 45 of *ABCA4* (c.6148G>C). It is predicted to lead to an amino acid substitution (valine to leucine at position 2050, p.V2050L). The mutation was analyzed in all family members. The presence of additional pathogenic sequence alterations in *ABCA4* was excluded by sequencing the complete coding region including exon-flanking intronic parts in V-3.

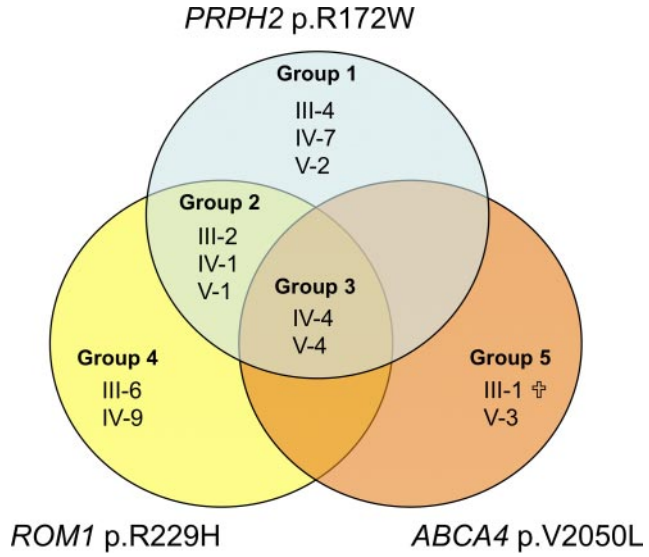

FIGURE 2. Five different heterozygous genotypes were found in the family. Family members with a similar genotype were grouped accordingly. Mutations in *PRPH2* (group 1); mutations in *PRPH2*, *ROM1* (group 2); mutations in *PRPH2*, *ROM1*, and *ABCA4* (group 3); mutations in *ROM1* (group 4); and mutations in *ABCA4* (group 5). ✞: Member III-1 died shortly after genotyping. Members IV-5, V-5, and V-6 decided to not participate in scientific DNA analysis and consequently are not listed. Patient III-7 was excluded from the study due to bilateral retinal detachment surgery.

Figure 2 summarizes the five different genotypes found in the family. For phenotypic description we divided the family members into five corresponding groups based on genotype (Fig. 1).

Clinical Findings

Family members are grouped according to Figure 2. A detailed description of the clinical and electrophysiologic examination results is shown in Table 1. After a thorough analysis of all clinical data we identified the following differences between the five genotypes.

Group 1: Mutation in *PRPH2* (p.R172W). The youngest member of this group V-2 (22 years old) showed perifoveolar drusen and corresponding spots of increased FAF (Fig. 3). Her mfERG amplitudes were within the lower limit of normal with no eccentricity dependence (i.e., responses had the same amplitude across the retina from the center outward to the periphery of the stimulated area). This result is in contrast to the young members from groups 2 (her brother V-1, 26 years) and 3 (V-4, 22 years). The same was found, to a lesser extent, by comparing the older generation members IV-7 (group 1) and IV-1 (group 2; Fig. 4A).

These family members (also including the oldest III-4; 75 years old) had normal or only slightly reduced rod responses. Cone responses were reduced in III-4 but not in the younger members (Fig. 5).

Group 2: Mutations in *PRPH2* and *ROM1* (p.R172W, p.R229H). Member V-1 reported a flickering light sensation at age 22 at which time his sister V-2 (group 1) was still asymptomatic. V-1's FAF was more severely affected than the FAF of V-2, as it showed a speckled pattern with a surrounding ring of increased FAF measuring two optic disc diameters (ODDs; Fig. 3). In the mfERG, V-1 showed a more pronounced central amplitude reduction relative to the peripheral rings compared with his sister V-2 (group 1; Fig. 4A). ERG rod responses were normal or only slightly reduced for young subject V-1 and 56-year-old subject IV-1. However, in contrast to group 1, the oldest subject (III-2; 84 years old) showed a severe loss of rod function. Cone responses were similar to those in group 1 with a reduction only at older ages (Fig. 5).

Group 3: Mutations in *PRPH2*, *ROM1*, and *ABCA4* (p.R172W, p.R229H, and p.V2050L). V-4 showed more advanced macular degeneration on fundoscopy and FAF as the same-aged member V-2 (group 1). Even the 4 years older member V-1 (group 2) had less macular degeneration on funduscopy than did V-4. However, FAF was already markedly altered. V-4's mother IV-4 showed moderate to severe macular atrophy compared with her 6 years older brother IV-1 (group 2) who showed only mild RPE atrophy. Her FAF was also more severely altered, as the affected speckled area extended beyond the arcades, whereas her brother displayed speckles that stayed within the vascular arcades (Fig. 3).

mfERG amplitudes of both group members IV-4 and V-4 were more severely reduced than her approximately age-matched relatives IV-1 (group 2) and V-2 (group 1). IV-4 showed the most severe mfERG amplitude reduction of all family members (Fig. 4A).

Only subjects of group 3 complained of nyctalopia. This could be explained by the rod findings in the ERG: IV-4 showed the most severe reduction of rod b-waves. Moreover, there was a rapid decline of rod function over a period of 2 years. In contrast, her brother IV-1 (group 2) had stable rod function over a 13-year period (Fig. 5).

Group 4: Mutation of *ROM1* (p.R229H). Members of this group showed only minor fundus changes (e.g., minor RPE granularity in the older member III-6 and her son IV-9). FAF was normal (Fig. 3). However, the central mfERG amplitudes of both members were lower than their peripheral amplitudes (Fig. 4A). Figure 4B shows the macular dysfunction in a more obvious way: The lower part shows normalized ring ratios. Each ring is displayed in relation to the sum of all normal ring values and is normalized to the largest value. In contrast to the raw ring amplitudes of Figure 4A, this method factors out the interindividual amplitude variability and thus is more sensitive to radially localized loss. It revealed mild but clear central macular dysfunction in both family members.

Scotopic and photopic ERGs were normal (Fig. 5).

Group 5: Mutation of *ABCA4* (p.V2050L). V-3 showed minimal perifoveolar RPE defects and a normal FAF (Fig. 3). However, the central amplitude of the mfERG was reduced below the normal limit in both eyes. The pathologic amplitude reduction was more pronounced than in group 4 (Fig. 4A). ERG rod and cone responses were normal (Fig. 5).

In conclusion, the increase in severity of the retinal degeneration phenotype caused by a *PRPH2* mutation correlated in this family with the occurrence of additional mutations in *ABCA4* and *ROM1*. Although the ERG alone did not discriminate differences in disease severity at young ages (e.g., generation V), those patients carrying all three mutations seemed to be more strongly affected than did the carriers of only one or two mutations.

DISCUSSION

We found high phenotypic variability within a family in which the p.R172W mutation in *PRPH2* cosegregated with the disease. We provide support for a model in which features of a mutation-induced phenotype can be modulated by additional mutations in other genes, accounting for the intrafamilial variability observed in this family. It seems that additional mutations in *ABCA4* and *ROM1* result in a cumulative age-dependent effect that worsens the patient's phenotype.

In members who carried only the *PRPH2* mutation p.R172W (group 1), cone function seemed to be predomi-

TABLE 1. Detailed Phenotypic Data of All Examined Family Members Sorted by Groups

Patient	Group Genotype	Age At Ex.	VA OD/OS	Age (y) at Onset of Symptoms	Visual Field	Color Vision (Panel D 15)	Fundus	FAF	ERG Scotopic 1.8 B-Wave Amplitude	ERG 30-Hz Flicker Amplitude	mfERG	EOG Arden Ratio
V-2	1 p.R172W	22	1.25/1.25	Asymptomatic	OP: OD nasal sup small scotoma, OS normal; GP: OD normal	OD desaturated: 2 unspecific defects	OU: ON drusen, perifoveolar drusen OD > OS	OU: ON drusen, peri-foveolar punctual increase OD > OS	P_1	N	N	OD N
						OS desaturated: 1 unspecific defect						OS NA
IV-7	1 p.R172W	45	0.2/0.1	40; VA loss 35; photophobia	GP: OU central scotoma 40° (I/2)	OD: mild protan, deutan and tritan defects, OS: unspecific defects	OU: macular RPE granularity, OS: 0.5 ODD temporal MA	OU: speckled beyond arcades; OS: reduced at lower arcade indicating atrophy, 1 ODD	N	N	Z_5	ND*
III-4	1 p.R172W	75	0.05/0.07	42; VA loss photophobia (onset unknown)	GP: OU central scotoma 30° (I/3)	OU: marked protan and deutan defects	OU: severe MA, 4 ODD	OU: reduced CTA, 4 ODD, surrounding speckles	N	P_2	A	P_1
V-1	2 p.R172W, p.R229H	26	0.9/0.9	22; flickering light sensation	OP: OD normal OS paracentral defects 10° from center	OU: normal	OU: mild macular RPE clumping	OU: speckled, 2 ODD, surrounding increase, OD: reticular increase	P_1	P_1	Z_3	N
		21		Asymptomatic	NRe	NRe	NRe	ND	ND	ND	Z_1	ND
IV-1	2 p.R172W, p.R229H	56	0.8/0.7	501 adaptation difficulties	GP: OU paracentral scotoma: 1–10° radius (I/2, I/3 inf, I/4 sup)	OU: mild unspecific defects OS: 1 tritan defect	OU: mild RPE atrophy	OU: speckled, 3 ODD	N	P_1	Z_4	P_1
				32; photophobia	OP: as in GP		OD: moderate temporal optic nerve atrophy					
				30; flickering light sensation, relative paracentral scotoma								
		47	1.0/1.0	32; photophobia	GP: OU stable	ND	OU: mild RPE irregularity	ND	ND	ND	Z_3	ND
		43	0.9/0.9	30; flickering light sensation, relative paracentral scotoma	GP: OU paracentral scotoma: 3–10° radius (I/2 inf, I/4 sup)	OU: moderate tetartan defects	OU: minor RPE irregularity	ND	P_2	N	ND	P_1

(*continues*)

TABLE 1 (*continued*). Detailed Phenotypic Data of All Examined Family Members Sorted by Groups

Patient	Group Genotype	Age At Ex.	VA OD/OS	Age (y) at Onset of Symptoms	Visual Field	Color Vision (Panel D 15)	Fundus	FAF	ERG Scotopic 1.8 B-Wave Amplitude	ERG 30-Hz Flicker Amplitude	mfERG	EOG Arden Ratio
III-2	2 p.R172W, p.R229H	84	0.05/0.02	56; photophobia 30; VA loss	GP stable	OU: not possible	OU: severe MA	OU: NR due to dense cataracts	P_2	P_2	Z_4	P_2
		71	0.02/0.03		GP: OU sup VF loss, OS inf, VF constriction to 70°	OU: not possible	OU: severe MA	ND	ND	ND	ND	ND
V-4	3 p.R172W, p.V2050L, p.R229H	22	1.0/1.2	22; photophobia in bright light 22; mild nyctalopia	GP: OU sup mild constriction (I/3, I/2, I/1), OS paracentral scotoma 5° (I/1), OP: reduction central 20°	OU: normal	OU: mild-moderate MA	OU: speckled, 3 ODD, encircling increase	P_1	N	Z_3	N
IV-4	3 p.R172W, p.V2050L, p.R229H	50	0.2/0.3	49; nyctalopia 40; photophobia 35; VF defects 13; mild VA loss	GP: OD paracentral scotoma (III/4) 10°, sensitivity loss for I/1, I/2, OS central scotoma 40° (I/3) extending temporally	OU: marked protan and deutan defects	OU: moderate MA extending beyond arcades; temporally >1 ODD severe MA; midperipheral RPE clumping; moderate temporal ON atrophy	OU: speckled beyond arcades, reduced CTA (mild progression)	P_3	P_2	Z_5	P_1
		48	0.2/0.5	40; photophobia 35; VF defects 13; mild VA loss	GP: OD central scotoma 35° (I/3) extending temporally, OS central scotoma 40° (I/3)	OD: marked protan and deutan defects OS: marked protan, deutan, and tritan defects	OU: mild pericentral MA to arcades; mid-peripheral RPE clumping; nasally 1 ODD RPE atrophy; moderate temporal ON atrophy	OU: speckled beyond arcades, reduced CTA	P_2	P_2	Z_5	ND
IV-9	4 p.R229H	36	1.25/1.25	Asymptomatic	OP and GP: normal	OS: unsaturated: 2 unspecific defects	OU: normal	OU: normal	N	N	Z_1	N

(*continues*)

TABLE 1 (*continued*). Detailed Phenotypic Data of All Examined Family Members Sorted by Groups

Patient	Group Genotype	Age At Ex.	VA OD/OS	Age (y) at Onset of Symptoms	Visual Field	Color Vision (Panel D 15)	Fundus	FAF	ERG Scotopic 1.8 B-Wave Amplitude	ERG 30-Hz Flicker Amplitude	mfERG	EOG Arden Ratio
III-6	4 p.R229H	68	1.0/1.0	Asymptomatic	OP and GP: normal	OS: unsaturated: 1 unspecific defect	OU: mild arteriolar narrowing; arteriovenous nipping	OU: normal	N	N	Z_2	N
V-3	5 p.V2050L	24	1.25/1.25	Asymptomatic	OP and GP: Normal	OD desaturated: 1 unspecific defect	OU: minimal perifoveolar RPE irregularity	OU: subtle perifoveolar increase	N	N	Z_2	N

A, global amplitude reduction; arcades, temporal vascular arcades; CTA, corresponding to atrophy; EOG, electrooculogram; ERG, full-field electroretinogram; ex., examination; FAF, fundus autofluorescence; GP, Goldmann perimetry; inf, inferior; MA, macular atrophy; mfERG, multifocal electroretinogram; NA, not analyzable due to artifact; N, normal; ND, not done; ND*, not done because patient refused the examination; NL, normal limit; NR, no records; ON, optic nerve; OP, Octopus perimetry; P_{1-3}, amplitude (ERG) or Arden ratio (EOG, normal range 1.7–3.3) below the lower normal limit (increasing reduction); ODD, optic disc diameter; sup, superior; VA, visual acuity; VF, visual field; Z_{1-5}, please see Methods for an explanation. All degrees indicate diameter unless otherwise indicated.

nantly affected with stable rod function. This finding indicates that an isolated p.R172W mutation leads to a macular dystrophy phenotype.

Known *ROM1* sequence alterations comprise eight polymorphisms and nine rare variants, including the rare p.R229H variant.[7,73–75] This variant was identified in two autosomal dominant retinitis pigmentosa patients from two different families. In one family the alteration did not segregate with the disease.

However, in the family described herein, members III-6 and IV-9 (group 4) who carried only the *ROM1* p.R229H alteration displayed minor RPE granularity and a mild yet distinct loss of macular function in the mfERG. Thus, we suggest that the heterozygous change p.R229H causes a mild macular dysfunction. This effect could explain that member V-1 of group 2 (*PRPH2* p.R172W and *ROM1* p.R229H mutations) was already symptomatic at an age when his sister in group 1 (V-2), who carried only the *PRPH2* p.R172W mutation, still did not display any symptoms. He also showed more pronounced FAF alterations than his sister. In addition, mfERGs of V-1 and IV-1 showed a distinct eccentricity dependence that was not observed in members of group 1. Since three affected family members (group 1) did not carry the p.R229H mutation in *ROM1*, the sequence alteration is unlikely to be associated with digenic inheritance of the different phenotypes in the family described herein.

Our data suggest that the presence of an additional *ROM1* p.R229H mutation worsens the *PRPH2* macular dystrophy phenotype and that the former description as a nonpathogenic rare variant should be reassessed.

In mice with a *Rom1*-null allele, the maximum scotopic response was lowered by 50% in comparison with that in age-matched control animals.[39] It thus is possible that the loss of rod function observed in the oldest subject in group 2 (III-2) is attributable to the heterozygous *ROM1* p.R229H mutation, which is supported by the fact that the oldest subject of group 1 (III-4), who carried only the *PRPH2* mutation, showed normal rod responses. Nevertheless, we cannot exclude that this effect is partially attributable to the age difference between the two subjects.

Usually, *ABCA4* mutations are inherited in an autosomal recessive manner. The *ABCA4* p.V2050L heterozygous carrier reported herein (group 5, V-3) showed centrally reduced mfERG amplitudes and additional minor fundus abnormalities. This finding suggests that the p.V2050L mutation in the heterozygous state is capable of mildly reducing macular function without an additional mutation on the second allele. Indeed, heterozygous mutations in *ABCA4* have been reported to cause electrophysiologically detectable dysfunction in individuals who had no obvious clinical signs: Maia-Lopes et al.[40] described and clinically characterized a heterozygous *ABCA4* p.V2050L carrier within a family with Stargardt disease. mfERG amplitudes were found to be smaller than normal yet higher than those in Stargardt disease patients despite otherwise normal findings. These amplitude variations can be attributed to the commonly accepted hypothesis that the severity of the phenotype correlates inversely with residual *ABCA4* function,[76] although there is evidence that *ABCA4* disease severity may be modified by other factors, too.[77]

Flicker amplitudes of the older group 3 member IV-4 were reduced below the normal limit in the ERG, and rod function declined rapidly over 2 years. In contrast, normal flicker amplitudes and stable rod function as shown in a follow-up examination 13 years after the initial presentation was found in her brother (group 2, IV-1) not carrying *ABCA4* p.V2050L. This finding may hint at an additional effect of p.V2050L on generalized cone function and an acceleration of loss of rod function if the genotype also contains *ROM1* p.R229H and *PRPH2*

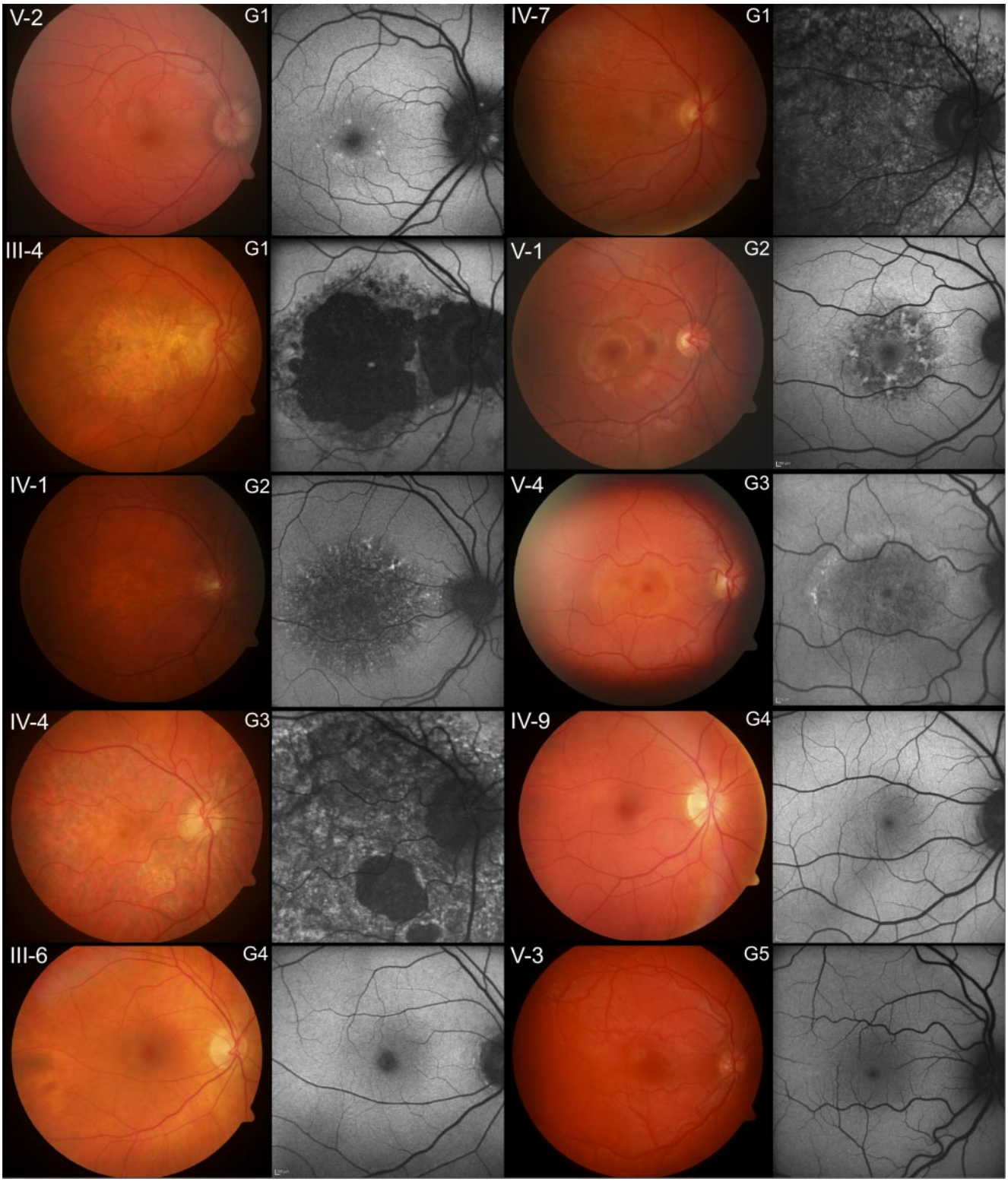

FIGURE 3. Fundus photographs and corresponding FAF recordings of the right eye are shown for each patient. Because of high intraocular symmetry, left eyes are not shown. Patient III-2 is not included in this figure because of dense cataracts in both eyes and corresponding poor quality of fundus photographs (FAF was not possible). Patient III-7 was not included in the study because of bilateral retinal detachment. G1–5: groups 1–5.

p.R172W. In support of this idea, it has been reported previously that *ABCA4* p.V2050L can be associated with a rod–cone pattern of functional loss as described in one patient with RP who bears a heterozygous p.V2050L mutation. Thus, it was postulated that *ABCA4* mutations contribute to the phenotypic variability of retinitis pigmentosa.[78] Furthermore, delayed dark adaptation was found in heterozygous *Abca4* transgenic mice.[79]

Loss of cone function with normal rod function has also been reported in nine British families with the *PRPH2* p.R172W muta-

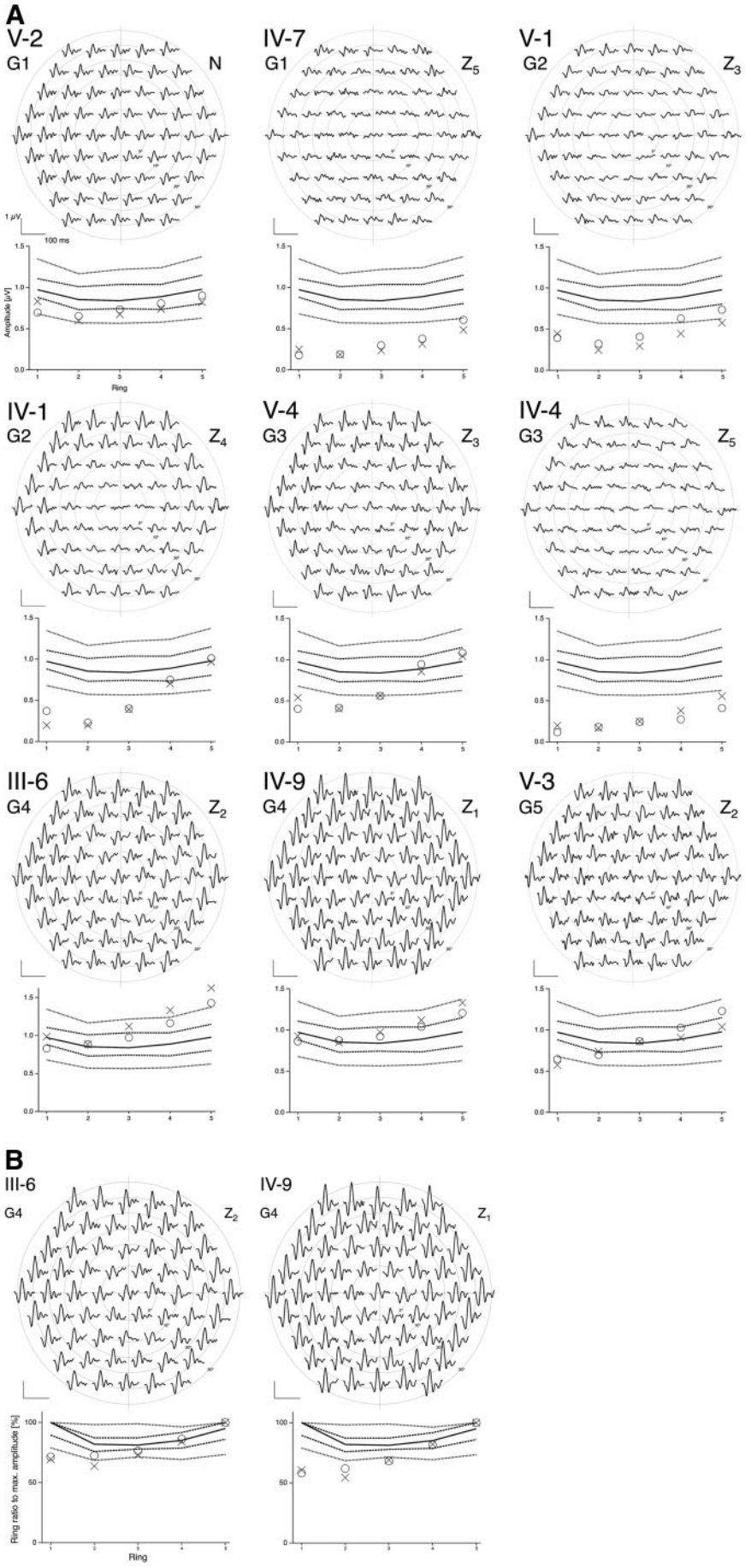

FIGURE 4. (**A**) For each of the nine patients the top parts show the mfERG trace array of the right eye. Below the trace arrays are amplitudes averaged for each ring. *Crosses*: right eye; *open circles*: left eye; *dotted lines*: 5% resp. 95% normal confidence interval [CI]; *solid line*: normal median. The electrophysiologic categories are indicated at the *top right* of each trace array and subdivided as follows: N: constant amplitude throughout the stimulated area; Z_{1-5}: see Methods for an explanation; G1–5: groups 1–5. Patients III-2 and III-4 showed a global amplitude reduction and are not displayed in this figure because of space considerations. (**B**) Mild central macular dysfunction of group 4 (G4). Top parts show the mfERG trace array of the right eye of members III-6 and IV-9. Below the trace arrays, there are normalized ring ratios: Each ring is displayed in relation to the sum of all normal ring values and normalized to the largest value. This analysis brings out localized loss across eccentricity. Both members showed a mild but clear central macular dysfunction, as central amplitudes were reduced below the fifth percentile (ring 1 in member III-6, rings 1 and 2 in member IV-9). *Crosses*: right eye; *open circles*: left eye; *dotted lines*: 5% resp. 95% normal confidence interval; *solid line*: normal median.

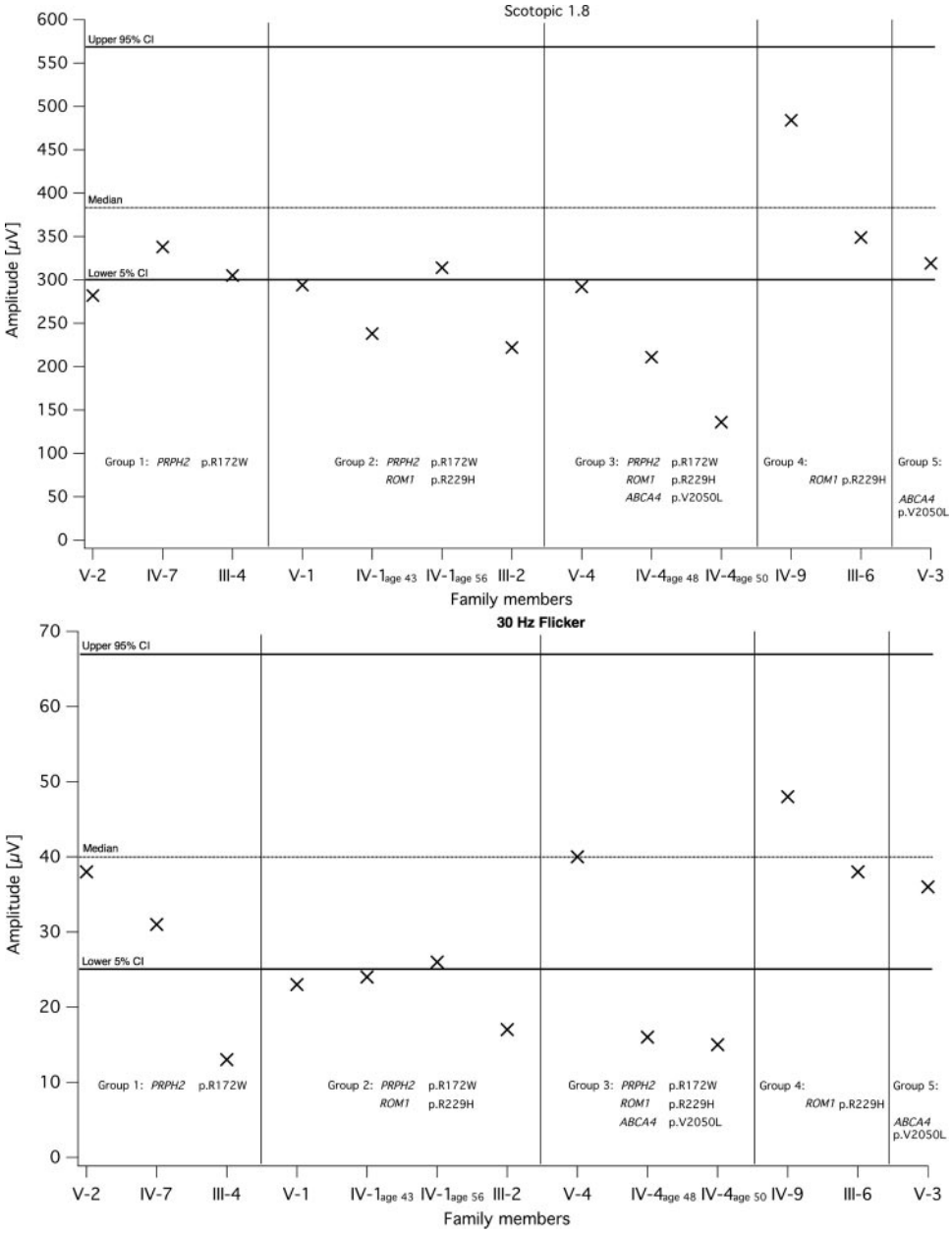

FIGURE 5. ERG results. The scotopic b-wave amplitude (*top*, recorded with a flash of 1.8 cd · s/m^2) and the 30-Hz flicker amplitude (*bottom*) are displayed for each family member. Rod responses were normal in group 1, decreased with age in group 2, decreased early in group 3, and normal in groups 4 and 5. Cone responses are reduced with age in groups 1 and 2, early reduced in group 3, and normal in groups 4 and 5. X, average amplitude of right and left eye; *solid lines*: 5% resp. 95% normal confidence interval; *dashed line*: normal median.

tion.[63] Nakawaza et al.[64] found only moderately reduced rod function in a Japanese family with the p.R172W mutation. Rod function was reduced in a British family in which two of six affected members showed rod involvement in the ERG.[13,63] Downes et al.[63] attributed the effect to an unidentified second mutation in a gene other than *PRPH2*.

Michaelides et al.[9] were the first to describe a high intrafamilial variability in a family with the *PRPH2* p.R172W mutation and a cone–rod dystrophy pattern. However, other genes, especially *ROM1*, were not screened. Leroy et al.[80] excluded *ROM1* as a modifier gene in two families with *PRPH2* mutations different from p.R172W for those with retinitis pigmentosa.

PRPH2 has been shown to cause digenic forms of retinitis pigmentosa by genetic interaction with *ROM1.* In some families, carriers of the *PRPH2* mutation p.Leu172Pro were not affected unless they also carried a mutation in the unlinked *ROM1* gene.[3] Peripherin and ROM1 build tetrameric protein complexes, a finding that provides the molecular link to the digenic inheritance pattern.[81,82] One may consider the finding of digenic inheritance as an extreme example of phenotype modification by an independent gene.

Another exceptional example has been published for the recessively inherited Bardet-Biedl syndrome (BBS). In BBS, the clinical variability ranges from mild to severe phenotypes. In addition, the same combination of mutations can be associated with different combinations of phenotypes. It has been shown that this phenotypic variability can be explained by different genotypes within the 12 BBS-associated genes. Moreover, in a two-generation pedigree, the presence of the disease itself was dependent on the presence of three mutated alleles in the same patient: one individual carried two nonsense *BBS2* mutations without having a phenotype, whereas his affected brother carried a third nonsense mutation in *BBS6.*[83] This difference in manifestation of the phenotype indicates that three mutations in two different BBS genes are necessary to cause the disease. The same group described BBS families in which two mutated alleles were sufficient to cause the disease,

but the phenotype in the individuals was stronger when a third mutation in a different BBS gene was present in the same patient.[84] Together, these findings show that genetic modifiers can potentiate the disease phenotype. Furthermore, it is likely that different mutations cause distinct variations in the patients' phenotype.

It is well documented that the *PRPH2* mutation p.R172W is associated with variable phenotypes such as cone dystrophy, cone–rod dystrophy, and various types of macular dystrophies. In the case described herein, we detected different phenotypic expressions within one family in which all patients carried the *PRPH2* mutation p.R172W. The presence of a known mutation in the unlinked gene *ABCA4* and a sequence variant in *ROM1* correlated with a modified phenotype which showed a more pronounced macular dysfunction. This observation was confirmed by funduscopy, mfERG, and FAF. There were also hints that *ROM1* alters rod function and *ABCA4* may have an effect on generalized rod and cone function in the presence of the *ROM1* sequence variation. Of course, these findings are based on only one family with correspondingly low numbers for each genotype, and additional sequence alterations cannot be excluded completely. However, our data support a model in which, in addition to a disease-causing mutation in *PRPH2*, modifying sequence alterations influence disease expression. As for *ROM1*, we suggest that the p.R229H sequence change, which has been described as a nonpathogenic rare variant so far, should be considered a mild mutation. Since heterozygous sequence alterations in *ABCA4* have been described to cause mild forms of macular degeneration, it is plausible that the combination of *PRPH2* and *ABCA4* mutations leads to an altered phenotype.

We suggest testing those families that show a variable macular dystrophy phenotype caused by mutations in *PRPH2* for additional mutations in *ABCA4* and *ROM1*, as they may alter the progression of the *PRPH2* phenotype. Such testing will aid genetic counseling, as patients with additional mutations may face a faster progression of visual loss.

Taken together, our data support the idea that the phenotypic expression of patients with retinal degenerations is influenced by a specific genetic environment as sequence alterations in several genes relevant for survival and maintenance of retinal cells may have cumulative effects on retinal cell function.

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