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Poloschek, C M ; Bach, M ; Lagrèze, W A ; Glaus, E ; Lemke, J R ; Berger, W ; Neidhardt, J

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ABCA4 and *ROM1:* Implications for modification of the *PRPH2*-associated macular dystrophy phenotype

Poloschek CM[1], Bach M[1], Lagrèze WA[1], Glaus E[2], Lemke JR[3], Berger W[2], Neidhardt J[2**]*

1 Department of Ophthalmology, University of Freiburg, Freiburg, Germany

2 Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland

3 Institute of Medical Genetics, University of Tübingen, Germany

Addresses for correspondence

*Charlotte M. Poloschek, Department of Ophthalmology, University of Freiburg, Killianstr. 5, 79106 Freiburg, Germany

Phone: + 49 (761) 270-4001

Fax: + 49 (761) 270-4114

Email: charlotte.poloschek@uniklinik-freiburg.de

**John Neidhardt, Institute of Medical Genetics, Division of Medical Molecular Genetics and Gene Diagnostics, University of Zurich, Schorenstr. 16, 8606 Schwerzenbach, Switzerland

Phone: + 41 (44) 655 7389

Fax: + 41 (44) 655 7213

Email: neidhardt@medgen.unizh.ch

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Abstract

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: We investigated 15 family members by detailed ophthalmic and electrophysiologic phenotyping. Mutation screening was initially performed with microarrays that detect known mutations in genes associated with retinal degeneration. Furthermore, 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. We suggest that families showing a variable macular dystrophy phenotype caused by mutations in *PRPH2* are tested for additional mutations in *ABCA4* and *ROM1* as they might alter the progression of the *PRPH2* phenotype. This will influence genetic counseling as patients with additional mutations might be confronted with a faster progression of visual loss.

Keywords

Macular dystrophy, cone-rod dystrophy, Stargardt disease, fundus autofluorescence, modifier gene, *ABCA4*, *ROM1*, *RDS*, *PRPH2*, peripherin

Introduction

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 might be caused either 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 for autosomal dominant RP.[29] In patient-derived cell lines, modifier loci have been mapped in RP (Mendelian Inheritance in Man [MIM] #600138) 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 is further supported by the finding that knock out 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 disk rim integral membrane protein[37, 38] and localizes to the rod and cone outer segments.[35-37] ROM1 seems to be especially required for rod photoreceptor survival.[39]

It has been described 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 ERG.[40] The *ABCA4* gene [41] codes for a retina specific membrane transporter protein. Similar as peripherin and ROM1, ABCA4 is situated in rod and cone outer segments.[42, 43] It is suggested 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 an allelic disorder,[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]

Here we describe a five-generation family with the p.R172W mutation in *PRPH2* showing a remarkable intrafamilial variation of the 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 prior to 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 drawn from 18 subjects. 15 members of this five-generation Caucasian German family with an autosomal dominant retinal dystrophy were included in the study according to Figure 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), 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.

12 members (see Fig. 1) underwent a complete ophthalmic examination. They were queried about subjective complaints of loss of visual acuity, loss of visual fields, difficulties to see in dim/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 preceding retinal detachment surgery. Member III-1 died shortly after genotyping and thus was not available for phenotyping.

Phenotyping included Goldmann and Octopus perimetry. 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 (Zeiss FF450 fundus camera, Carl Zeiss Jena GmbH, Jena, Germany).

The severity of fundus changes was judged using a grading system of "minimal" – “mild" – "moderate" – "severe". Fundus autofluorescence (FAF) was recorded with a confocal scanning laser ophthalmoscope.[69] Full-field electroretinograms (ERG, maximum flash intensity 1.8 cd·s/m^2; Nicolet, Madison, USA), multifocal electroretinograms (mfERG; VERIS 4.8, Electro-Diagnostic Imaging, Redwood City, California, USA) and electrooculograms (EOG, Nicolet, Madison, USA) were analyzed using binocular stimulation according to the ISCEV (International Society for Clinical Electrophysiology of Vision) guidelines and standards.[70-72] ERG results concentrate on the scotopic 1.8 b-wave, i.e. the maximal rod-cone response, and cone flicker amplitude as they showed the pathologies of the rod and cone system most clearly for the different family members.

DNA preparation and mutation analysis

Genomic DNA was isolated from blood samples of the 18 family members using the Chemagic 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 (Fig. 1, Fig. 2). PCR conditions using 50-100 ng of genomic DNA as a template and HotFire polymerase (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 55-63°C, 1 minute incubation at 72°C for 35 cycles, and a final extension at 72°C for 10 minutes. PCR primers are available upon 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/).

Results

Molecular genetic findings

We analyzed 15 members of a Caucasian family as shown in Figure 1 using molecular genetic techniques to identify the causative mutation and/or additional sequence alterations. Index patient IV-4 was initially screened using 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 additionally screen 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 cosegregates 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, III-6), cone dystrophy (III-4), and cone rod dystrophy (IV-4, III-2).

To verify whether *ROM1* mutations occurred in the family described herein, 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, rs4387351; IV-7: rs1799959, 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, non-pathogenic variant. The occurrence of this rare sequence alteration was tested in all other individuals and was identified in 7 members (III-2, IV-1, V-1, IV-4, V-4, III-6, IV-9) as well as excluded in 5 family members (III-4, IV-7, V-2, III-1, V-3).

A second mutation was detected by 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 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 of *PRPH2* (p.R172W)

The youngest member of this group V-2 (22 years old) shows perifoveloar drusen and corresponding spots of increased FAF (Fig. 3). Her mfERG amplitudes are within the lower normal limit without eccentricity dependence, i.e. responses have the same amplitude across the retina from the center outward to the periphery of the stimulated area. This is in contrast to the young members from group 2 (her brother V-1, 26 years) and 3 (V-4, 22 years). The same is found, to a lesser extent, by comparing the older generation members IV-7 (this group) and IV-1 (group 2) (Fig. 4A).

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

Group 2: Mutation of *PRPH2* and *ROM1* (p.R172W, p.R229H)

Member V-1 complained about a flickering light sensation at age 22 at which his sister V-2 (group 1) was still asymptomatic. V-1's FAF is more severely affected than the FAF of V-2 as it shows a speckled pattern with a surrounding ring of increased FAF measuring 2 optic disc diameters (ODD) (Fig. 3). In the mfERG V-1 shows a more pronounced central amplitude reduction relative to the peripheral rings compared to his sister V-2 (group 1) (Fig. 4A). ERG rod responses are 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) shows a severe loss of rod function. Cone responses are similar to group 1 with a reduction only at older ages (Fig. 5).

Group 3: Mutation of *PRPH2*, *ROM1* and *ABCA4* (p.R172W, p.R229H, p.V2050L)

V-4 shows more advanced macular degeneration on fundoscopy and FAF as the same aged member V-2 (group 1). Even the four years older V-1 (group 2) has less macular degeneration on funduscopy than V-4. However, FAF is already markedly altered. V-4's mother IV-4 shows moderate to severe macular atrophy compared to her six years older brother IV-1 (group 2) who shows only mild RPE atrophy. Her FAF is also more severely altered as the affected speckled area extends beyond the arcades whereas her brother displays speckles that stay within the vascular arcades (Fig. 3).

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

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

Group 4: Mutation of *ROM1* (p.R229H)

Members of this group show only minor fundus changes, e.g. minor RPE granularity in the older member III-6 and her son IV-9. FAF is normal (Fig. 3). However, the central mfERG amplitudes of both members are 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 Fig. 4A, this factors out the interindividual amplitude variability and thus is more sensitive for radially localized loss. It reveals mild but clear central macular dysfunction for both members.

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

Group 5: Mutation of *ABCA4* (p.V2050L)

V-3 shows minimal perifoveolar RPE defects and a normal FAF (Fig. 3). However, the central amplitude of the mfERG is reduced below the normal limit in both eyes. The pathologic amplitude reduction is more pronounced than in group 4 (Fig. 4A). ERG rod and cone responses are normal (Fig. 5).

In conclusion, we observed that 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*. Although the ERG alone does not discriminate differences in disease severity at young ages (e.g. generation V), those patients carrying all three mutations seem to be more strongly effected than carriers of only one or two mutations.

Discussion

We found high phenotypic variability within a family where the p.R172W mutation in *PRPH2* cosegregates with the disease. We provide support for a model where 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 worsening the patient's phenotype.

In members who carry only the *PRPH2* mutation p.R172W (group 1), cone function seems to be predominantly affected with stable rod function. This indicates that an isolated p.R172W mutation leads to a macular dystrophy phenotype.

Known *ROM1* sequence alterations comprise 8 polymorphisms and 9 rare variants including the p.R229H rare 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 the same age when his sister of group 1 (V-2) carrying only the *PRPH2* p.R172W mutation still did not display any symptoms. He also showed more pronounced FAF alterations than his sister.

Additionally 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 non-pathogenic rare variant should be reassessed.

In mice with a *Rom1*-null allele the maximal scotopic response was lowered by 50% in comparison to that of age-matched controls.[39] It might thus be possible that the loss of rod function observed in the oldest subject of 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) carrying 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 additionally minor fundus abnormalities. This suggests that the p.V2050L mutation in the heterozygous state is capable to mildly reduce 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. described and clinically characterized a heterozygous *ABCA4* p.V2050L carrier within a family with Stargardt disease.[40] MfERG amplitudes were found to be smaller than normal yet higher than those of

Stargardt disease patients despite otherwise normal findings. This can be attributed to the commonly accepted hypothesis that the severity of the phenotype is inversely correlated with residual *ABCA4* function[76] although there is evidence that *ABCA4* disease severity might 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 a period of two years. In contrast, normal flicker amplitudes and stable rod function as shown in a follow-up exam 13 years after the initial presentation was found in her brother (group 2, IV-1) not carrying *ABCA4* p.V2050L. This might 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* 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 suffering from RP carrying a heterozygous p.V2050L mutation. Thus, it was postulated that *ABCA4* mutations might 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 previously been reported in 9 British families with the *PRPH2* p.R172W mutation.[63] Nakawaza et al. found only moderately reduced rod function in a Japanese family with the p.R172W mutation. [64] Rod function was reduced in a British family in which 2 of 6 affected members showed rod involvement in the ERG.[13, 63] Downes et al. attributed the effect to an unidentified second mutation in a gene other than *PRPH2*.

Michaelides et al. were the first to describe a high intrafamilial variability in a family with the *PRPH2* p.R172W mutation and a cone-rod dystrophy pattern.[9] However, other genes, esp.

ROM1, were not screened. Leroy et al. excluded *ROM1* as a modifier gene in two families with *PRPH2* mutations different from p.R172W for those with retinitis pigmentosa.[80]

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 additionally 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 might 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. Additionally, 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 indicated that three mutations in two different BBS genes where required to cause the disease. The same group described BBS families where two mutated alleles were sufficient to cause the disease, but the phenotype of 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 where 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* might have an effect on generalized rod and cone function in the presence of the *ROM1* sequence variation. Of course these findings are based on one family only with correspondingly low numbers for each genotype and additional sequence alterations cannot be excluded completely. However, our data support a model where in addition to a disease causing mutation in *PRPH2* modifying sequence alterations influence the disease expression. As to *ROM1*, we suggest that the p.R229H sequence change, that has been described as a non-pathogenic 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 might alter the progression of the *PRPH2* phenotype. This will aid genetic counseling as patients with additional mutations might have to 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 might have cumulative effects on retinal cell function.

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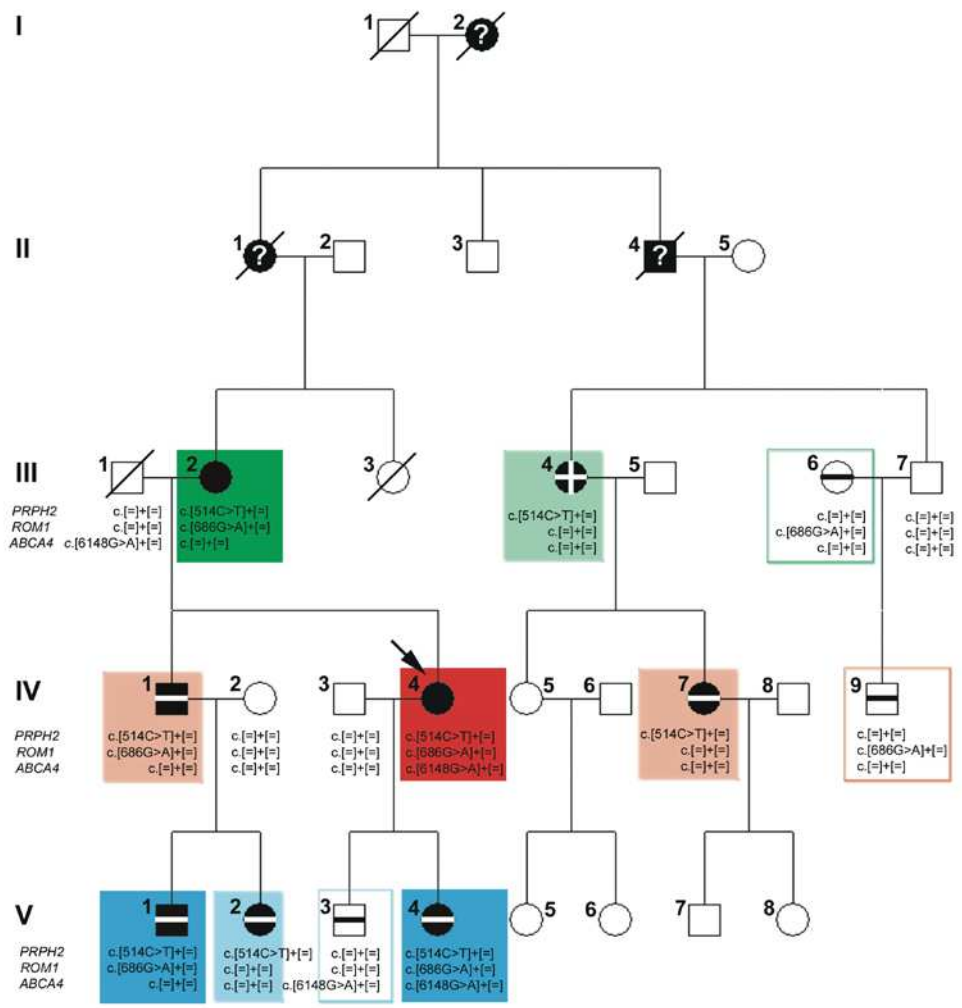

Figure 1: Pedigree and genotype of a five-generation family. Circles: females; squares: males; slashed symbols: deceased family members; symbols with horizontal bar: macular dystrophy; black symbols with horizontal and vertical bar: cone dystrophy; black symbols: affected by cone-rod dystrophy; black symbols with question mark: affected according to interview of family members without possibility of phenotyping; arrow: index patient. Genotype data are presented underneath the patient from whom DNA was available: upper genotype: *PRPH2*; middle genotype: *ROM1*; lower genotype: *ABCA4*. Colored boxes around affected individuals illustrate phenotype severity that increases with increasing saturation. Importantly, we only compare phenotype severity within the same generation (green: 3rd generation, red: 4th generation, blue: 5th generation) to exclude a possible age confounding effect. 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.

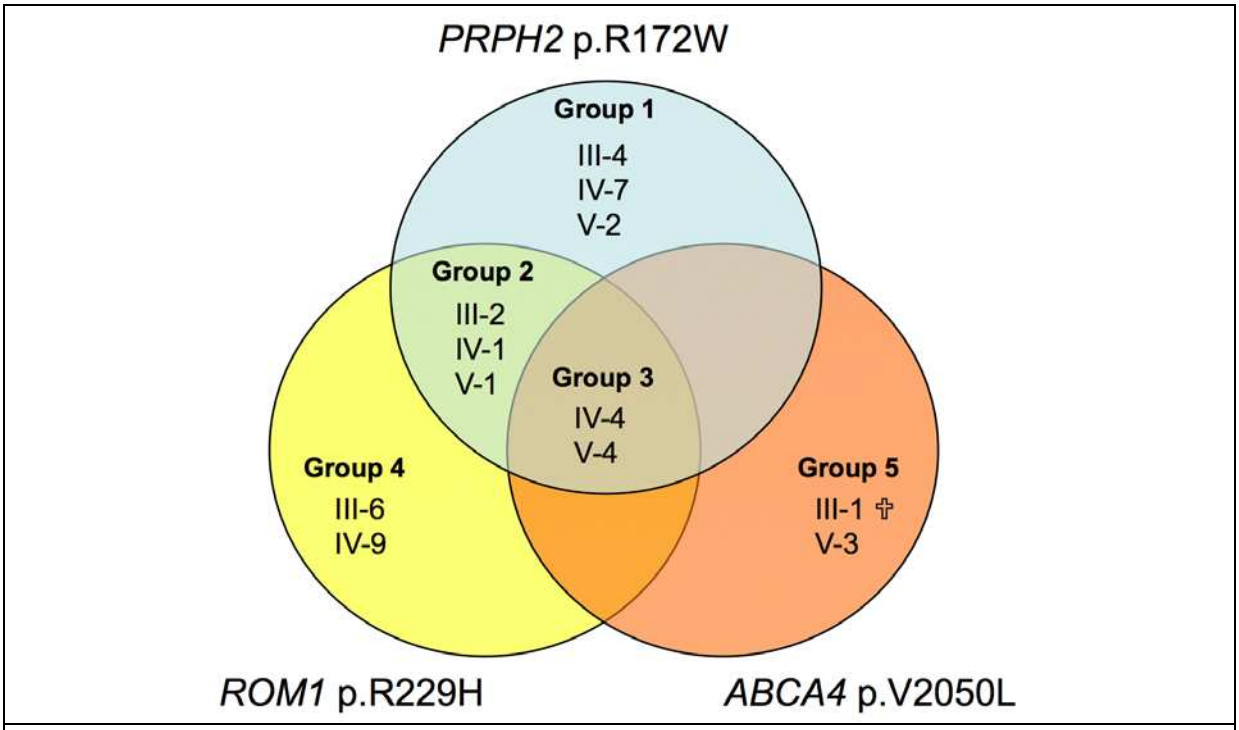

Figure 2. Five different heterozygous genotypes were found in the family. Family members with a similar genotype were grouped accordingly. Mutations in *PRPH2* (group 1); mutations in *PRPH2*, *ROM1* (group 2); mutations in *PRPH2*, *ROM1*, *ABCA4* (group 3); mutations in *ROM1* (group 4); mutations 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.

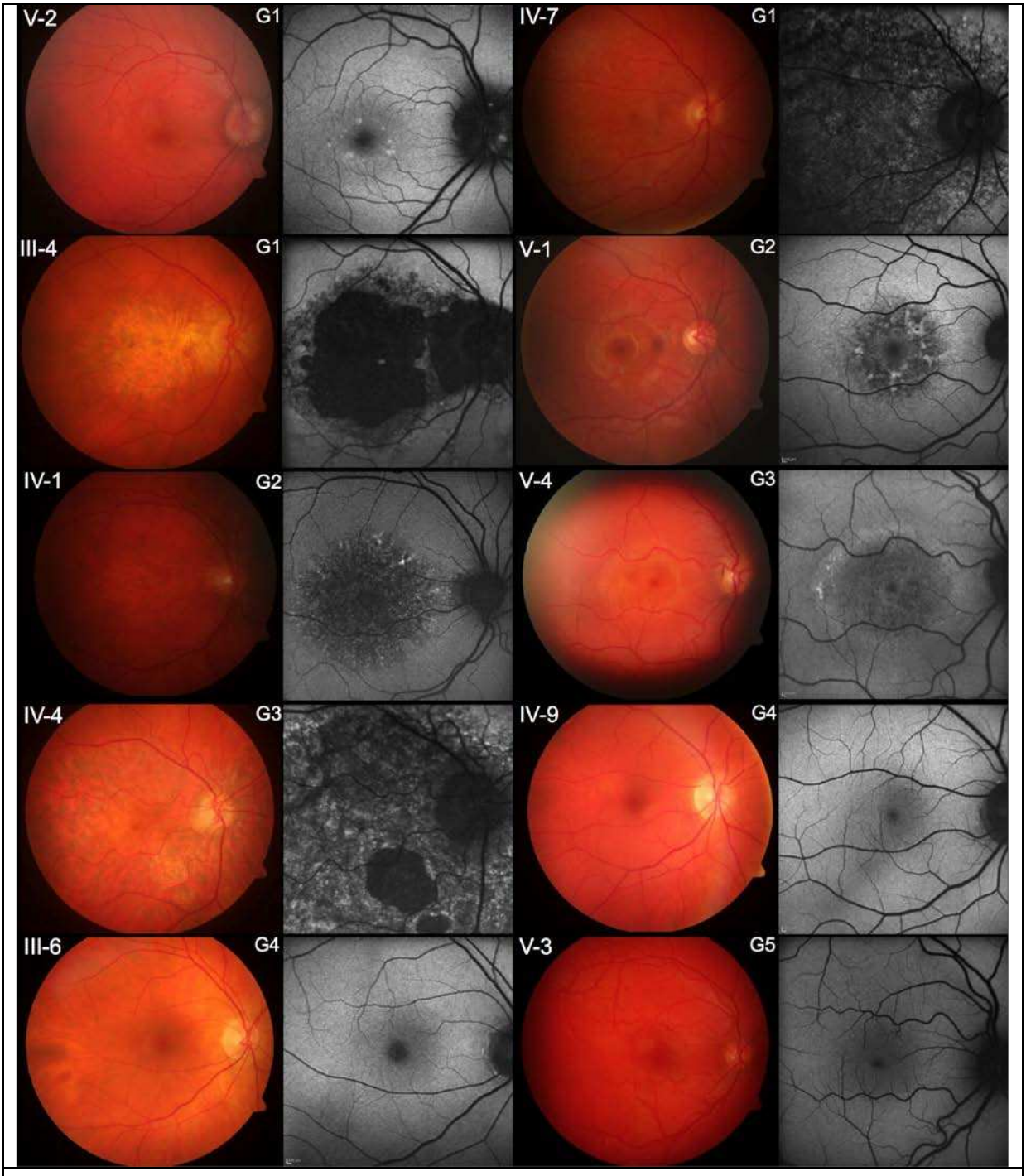

Figure 3. For each patient fundus photographs and corresponding FAF recordings of the right eye are shown. Due to high intraocular symmetry left eyes are not shown. Patient III-2 is not included in this figure due to dense cataracts in both eyes and corresponding poor quality of fundus photographs (FAF was not possible). Patient III-7 was not included in the study due to bilateral retinal detachment. For a detailed description please refer to the Results section. G1–5: groups 1–5.

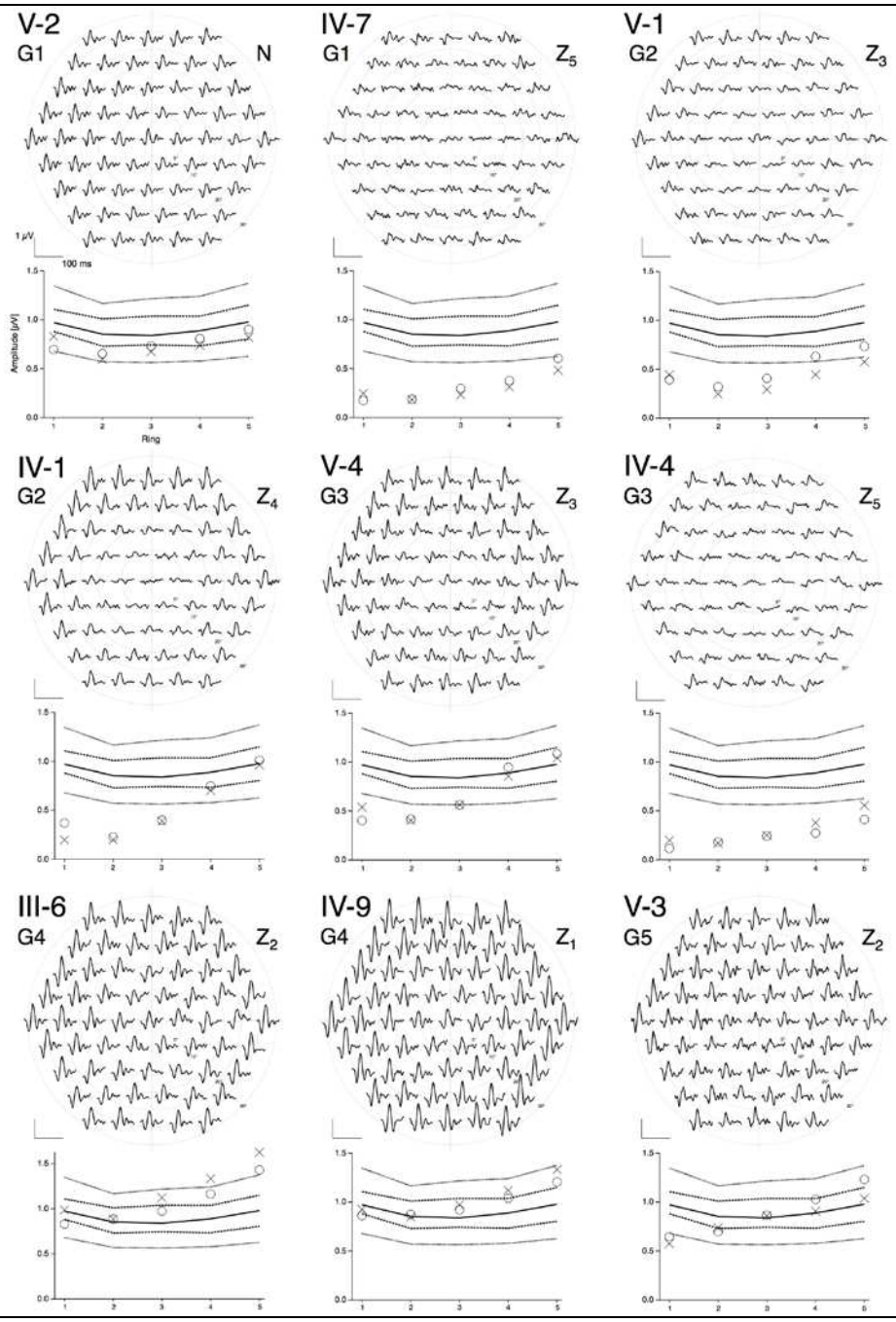

Figure 4A. For each of the nine patients the top parts show the mfERG trace array of the right eye. Below the trace arrays are amplitudes averaged for each ring. Crosses: right eye; open circles: left eye; Lower and upper dotted lines: 5% resp. 95% normal confidence interval; solid line: normal median; the electrophysiologic categories are indicated top right of each trace array and subdivided as follows: N: constant amplitude throughout the stimulated area; $Z_{1–4}$: amplitude reduction (low ratio center/periphery–high ratio center/periphery); Z_5: additional to central reduction peripheral amplitudes pathologically reduced; G1–5: groups 1–5. Patients III-2 and III-4 showed a global amplitude reduction and are not displayed in this figure due to economy of space.

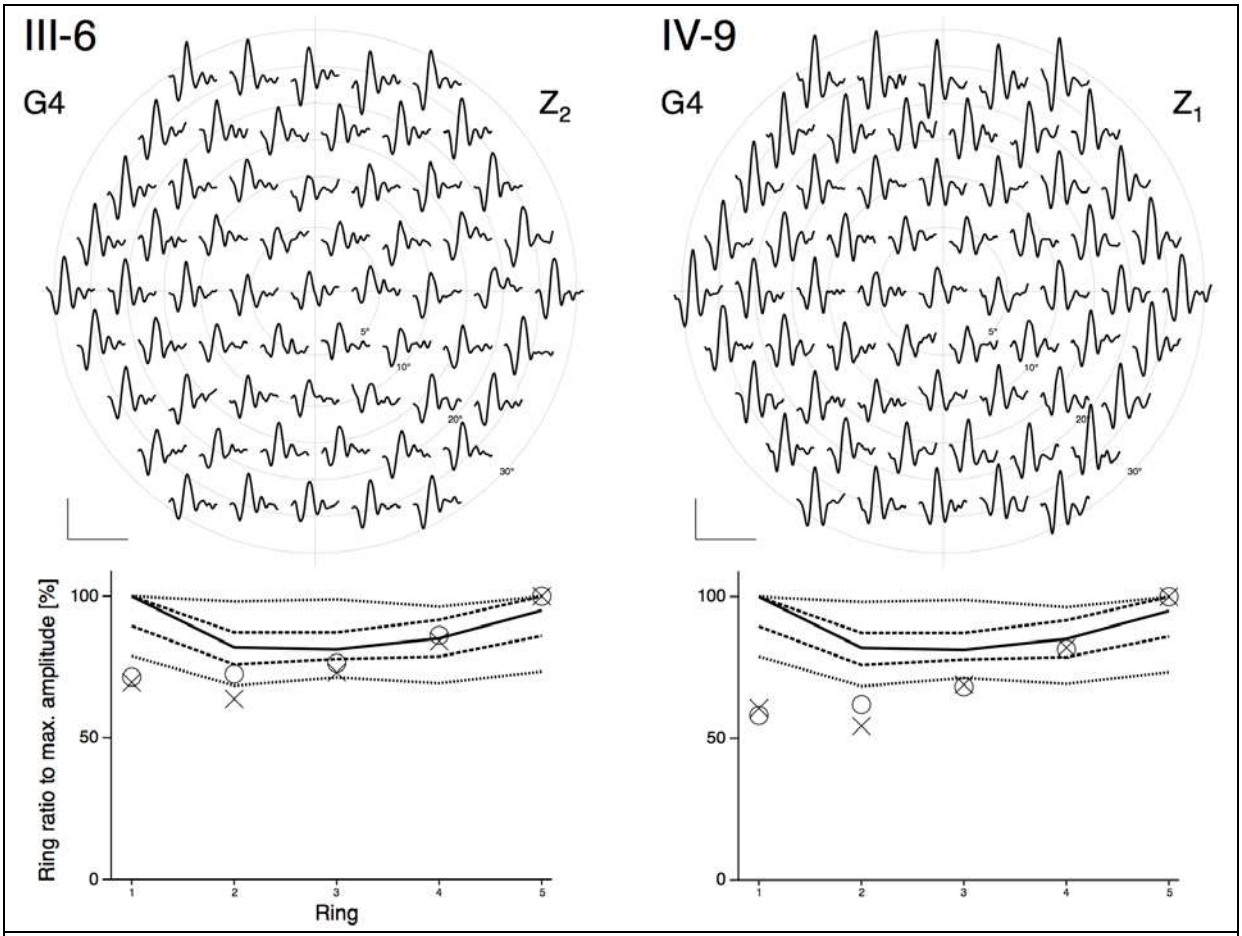

Figure 4B. Mild central macular dysfunction of group 4 (G4). Top parts show the mfERG trace array of the right eye of members III-6 and IV-9. Below the trace arrays there are normalized ring ratios: Each ring is displayed in relation to the sum of all normal ring values and normalized to the largest value. This analysis brings out localized loss across eccentricity. Both members show a mild but clear central macular dysfunction as central amplitudes are reduced below the 5th percentile (ring1 in member III-6, rings 1 and 2 in member IV-9). Crosses: right eye; open circles: left eye; lower and upper dotted lines: 5% resp. 95% normal confidence interval; solid line: normal median.

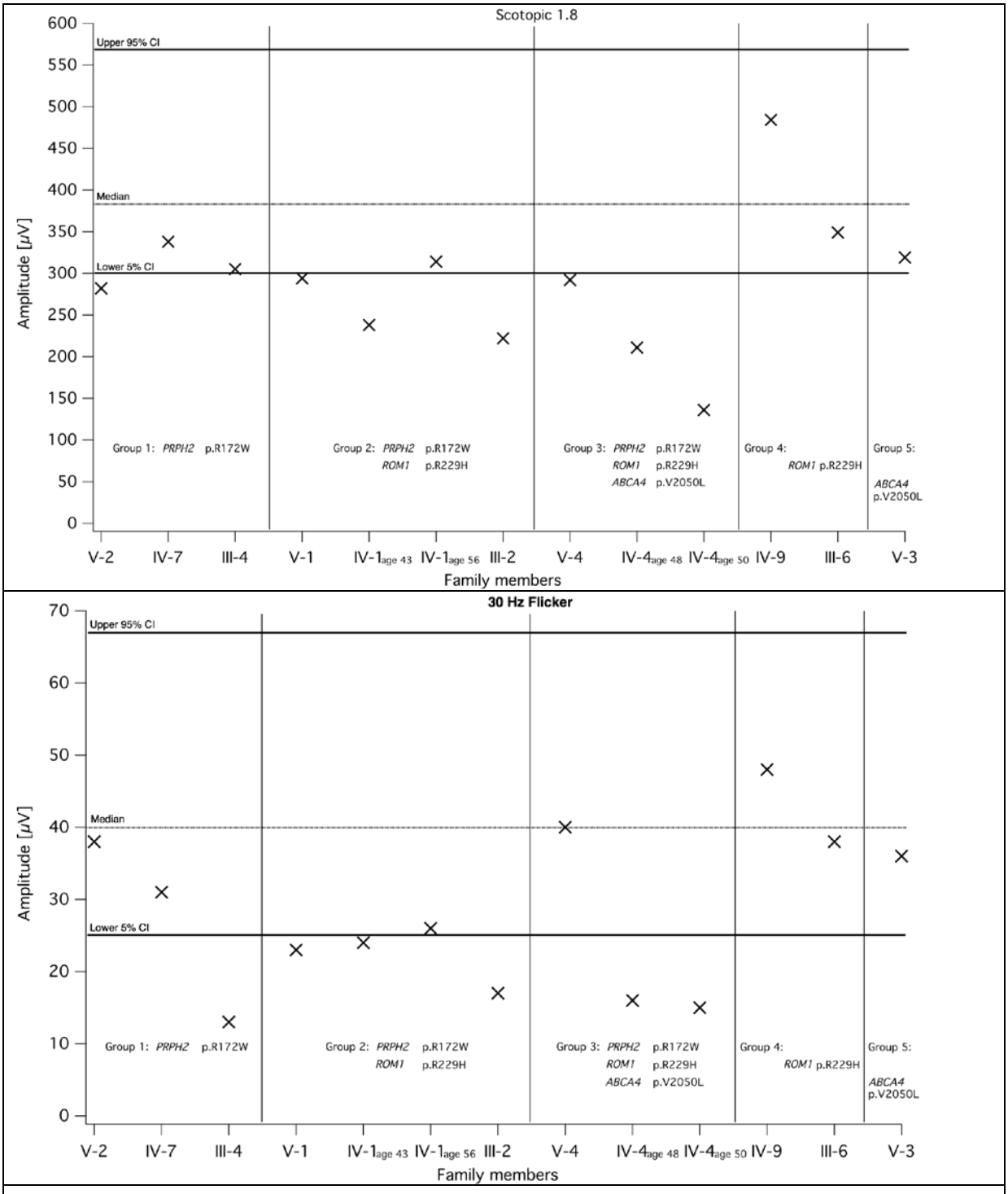

Figure 5. ERG results. For each family member the scotopic b-wave amplitude (upper graph, recorded with a flash of 1.8 cd·s/m^2) and the 30 Hz flicker amplitude (lower graph) is displayed. Rod responses are normal in group 1, lowered with age in group 2, reduced early in group 3, and normal in groups 4 and 5. Cones 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.

Table 1. Detailed phenotypic data of all examined family members sorted by groups. 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; n.a.: not analyzable due to artifact; N: normal; ND: not done; ND*: not done because patient refused the examination; NL: normal limit; NR: not recordable; NR: no records; ON: optic nerve; OP: Octopus perimetry; P_{1-2}: 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_{1-4}: amplitude reduction across eccentricity (the smaller the Z-number the more central the amplitude reduction); Z_5: additional to central reduction peripheral amplitudes pathologically reduced. All degrees indicate diameter unless otherwise indicated.

Patient	Group Genotype	Age at ex.	VA OD / OS	Age 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 OS desaturated: 1 unspecific defect	OU: ON drusen, perifoveolar drusen OD > OS	OU: ON drusen, perifoveolar punctual increase OD > OS	P_1	N	N	OD N OS n.a.
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,	56	0.8 / 0.7	50 – adaptation difficulties	GP: OU paracentral	OU: mild unspecific defects, OS: 1 tritan	OU: mild RPE atrophy	OU: speckled, 3 ODD	N	P_1	Z_4	P_1

	p.R229H			32 – photophobia 30 – flickering light sensation, relative paracentral scotoma	scotoma from 1 - 10° radius (I/2, I/3 inf, I/4 sup) OP: as in GP	defect	OD: moderate temporal optic nerve atrophy					
		47	1.0 / 1.0	32 – photophobia 30 – flickering light sensation, relative paracentral scotoma	GP: OU stable	ND	OU: mild RPE irregularity	ND	ND	ND	Z_3	ND
		43	0.9 / 0.9		GP: OU paracentral scotoma from 3 - 10° radius (I/2 inf, I/4 sup)	OU: moderate tetartan defects	OU: minor RPE irregularity	ND	P_2	N	ND	P_1
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 con-striction 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 constric-tion (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
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

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