Autosomal Dominant Retinal Dystrophies Caused by a Founder Splice Site Mutation, c.828+3A>T, in PRPH2 and Protein Haplotypes in trans as Modifiers

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PURPOSE. We determined the phenotypic variation, disease progression, and potential modifiers of autosomal dominant retinal dystrophies caused by a splice site founder mutation, c.828+3A>T, in the PRPH2 gene.

METHODS. A total of 62 individuals (19 families) harboring the PRPH2 c.828+3A>T mutation had phenotype analysis by fundus appearance, electrophysiology, and visual fields. The PRPH2 haplotypes in trans were sequenced for potential modifying variants and generalized estimating equations (GEE) used for statistical analysis.

RESULTS. Several distinct phenotypes caused by the PRPH2 c.828+3A>T mutation were observed and fell into two clinical categories: Group I (N = 44) with mild pattern dystrophies (PD) and Group II (N = 18) with more severe cone-rod dystrophy (CRD), retinitis pigmentosa (RP), and central aracral chorioretal dystrophy (CACD). The PRPH2 Gln304-Lys310-Asp358 protein haplotype in trans was found in Group I only (29.6% vs. 0%), whereas the Glu304-Lys310-Gly358 haplotype was predominant in Group II (94.4% vs. 70.4%). Generalized estimating equations analysis for PD versus the CRD/CACD/RP phenotypes in individuals over 43 years alone with the PRPH2 haplotypes in trans and age as predictors, adjusted for correlation within families, confirmed a significant effect of haplotype on severity (P = 0.03) with an estimated odds ratio of 7.16 (95% confidence interval [CI] = [2.8, 18.4]).

CONCLUSIONS. The PRPH2 c.828+3A>T mutation results in multiple distinct phenotypes likely modified by protein haplotypes in trans; the odds of having the CACD/RP-like phenotype (versus the PD phenotype) are 7.16 times greater with a Glu304-Lys310-Gly358 haplotype in trans. Further functional studies of the modifying haplotypes in trans and PRPH2 splice variants may offer therapeutic targets.

Keywords: phenotype, genetic modifiers, retinal dystrophy

Mutations in the PRPH2 gene (OMIM:179605) are known to cause a wide range of autosomal dominant retinal dystrophies (adRD; phenotype MIM: 613105, 608133, 169150, 608161, 608133, 136880), but the source of this variation is poorly understood. We previously identified a PRPH2 (NM_000322.4) splice site mutation, c.828+3A>T, in numerous patients with retinal dystrophies. Haplotype analysis revealed that the mutation derives from a common ancestor and its prevalence is due to a founder effect. Individuals with the c.828+3A>T mutation express a PRPH2 splice variant that was not found in controls and is consistent with abnormal splicing. Clinical diagnoses in individuals with this mutation range from pattern dystrophies (PD) to retinitis pigmentosa (RP), cone-rod dystrophy (CRD), and/or unspecified macular dystrophy. This phenotypic variation is comparable to other mutations in PRPH2, for example, a 3-bp deletion of codon 153, and a P210R missense change that were reported to cause multiple phenotypes within the same nuclear families. Another PRPH2 mutation, R172W, also exhibits a founder effect but with similar phenotypes in most families, although intrafamilial variation also has been described in two families.

In this study, we evaluated clinical records, fundus photographs, and visual function data to characterize the phenotype of the PRPH2 c.828+3A>T splice site mutation. Due to the founder effect, this cohort is particularly well suited for identifying possible causes of variation that are not in cis to the splice site mutation.

Peripherin/PRPH2 is a photoreceptor-specific membrane protein that homodimerizes with itself. Polymorphic protein haplotypes in trans were considered as modifiers of dominant diseases because the two alleles are expressed at the same time in the same tissues, translation is concurrent, and protein
products may follow identical pathways and interact directly. Therefore, sequence variants in the PRPH2 gene in trans that code for polymorphic protein isoforms (haplotypes), Glu304Lys/Gly358 (G910A>G1013), Glu404Arg/Asp358 (G910>G929 A1013), and Glu304Lys/Asp358 (G910>A929 A1013), coded by three polymorphic cSNPs, rs590659 c.910G>C (GAG>CAG) p.Glu304Gln, rs429876, c.929A>G (AAG>AGG) p.Lys310Arg, and rs454102 c.1013G>A (GGC>GAC) p.Gly338Asp were considered as potential modifiers of the disease phenotype.2,9,10

We also considered 3 other candidate genes as modifiers, ROM1, GUCA1A, and NXNL1. ROM1 is a photoreceptor-specific gene that encodes a rod outer-membrane protein localized to the photoreceptor outer segment disk membranes. It has 35% homology to PRPH2 and interacts with PRPH2-forming heterotetramers.11 Mutations in this gene alone have not been shown conclusively to cause any retinal disease, although a digenic form of RP with a PRPH2 mutation has been reported.12 Therefore, ROM1 was considered as a strong candidate modifier gene, particularly in individuals with the retinitis pigmentosa phenotype. The GUCA1A gene encodes a photoreceptor-specific guanylate cyclase-activator protein, a novel Ca(2+) binding protein that stimulates synthesis of cGMP in photoreceptors.13 Mutations in this gene are known to cause autosomal dominant cone dystrophy, making it a likely modifier gene.14,15 Another gene, NXNL1, encodes a thioredoxin-like protein 6, which is specifically expressed in photoreceptors and required for cone viability.16 We considered GUCA1A and NXNL1 as potential modifier genes, especially in those patients exhibiting predominant macular disease and cone loss.

METHODS

Subjects and Clinical Studies

All subjects were ascertained in the United States after obtaining written informed consent and all procedures conformed to the Declaration of Helsinki and were approved by the Institutional Review Board (IRB) board of each respective institution as described previously.2 We studied 19 families (FAM) harboring the splice site mutation. Families 1 to 10 were ascertained by David Birch, PhD, at the Retina Foundation of the Southwest (RFSW; Dallas, TX, USA); Fam11 by Richard Ruiz, MD, at University of Texas Health Science Center (Houston, TX, USA); Fam12 by Jonathon Sears, MD, at Cole Eye Institute (Cleveland, OH, USA); Fam13, Fam15, and Fam17 to Fam19 by Edwin Stone, MD, PhD, at the University of Iowa (Iowa City, IA, USA); Fam14 at the University of Oregon (Eugene, OR, USA) by Richard Weleber, MD, and Fam16 by Gerald Fishman, MD, at the University of Illinois (Chicago, IL, USA). Screening for sequence variants was performed at the Molecular Ophthalmology Laboratory at the University of Iowa and the Laboratory for Molecular Diagnosis of Inherited Eye Diseases at the University of Texas Health Science Center.

Clinical findings were analyzed to determine the phenotype of the individuals and classify them into defined diagnostic groups. Fundus photographs were available for 62 individuals from 19 families with the splice site mutation. To maintain consistent standards, we used electrophysiology data from RFSW alone for analysis. Electroretinographic (ERG) data were available for 25 individuals, dark adaptometry for 24 individuals, and Humphrey visual fields for 10 individuals.

Visual Acuity

Best-corrected visual acuity was recorded using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart or standard Snellen visual acuity charts.

Visual Fields

Visual fields were tested at RFSW using standard spot size 3, program 30-2 and/or 60-4 SITA Fast on a Humphrey field analyzer (Humphrey Instruments, Inc., San Leandro, CA, USA).

Electrophysiology

Patients were dilated using tropicamide 1% and phenylephrine 2.5% and then dark-adapted for 45 minutes before testing. Usually only the eye with the poorer visual acuity was tested. The ERG was recorded from the dilated, dark-adapted eye following the International Society for Clinical Electrophysiology of Vision (ISCEV) standards as described previously.17

Dark Adaptometry

Patients’ eyes were dilated using tropicamide 1% and phenylephrine 2.5% and dark-adapted completely for 45 minutes before testing. Final dark-adapted thresholds were measured on a Goldmann-Weekers adaptameter according to standard protocols.18–20

Fundus Photographs

Fundus photographs were obtained from each site whenever available. Phenotypic classification was based on fundus findings available in 62 individuals. All individuals with macular and/or peripheral yellow, orange, or gray (dot and halo) deposits were grouped into various types of PD as described previously.21–23 Patients with macular pigmentation and atrophy preceding peripheral retinal changes were classified as having CRD.24 If the fundus appeared to have peripheral intraretinal bone spicules, mottling, and granularity of the RPE, and/or had optic nerve pallor and retinal vessel attenuation, patients were classified as having RP.25 Individuals with symmetric, sharply outlined areas of geographic atrophy of the RPE and choroid, approximately 3 to 4 disc diameters in size, and having a yellow-white appearance of the choroidal vessels, were classified as central areolar chorioretinal dystrophy (Cacd).26

These diagnoses were combined into two groups to analyze the effect of modifier genes: Group I – milder phenotype with PD: The presence of yellow or pigmented lesions in the retina and/or retinal atrophy confined to the macula alone; and Group II – more severe phenotype with CRD, RP and CACD: These were characterized by photoreceptor loss, RPE, and choroidal atrophy extending outside the macula and/or presence of intraretinal bone spicules.

Molecular Studies

DNA extraction and screening by SSCP or bidirectional fluorescent di-deoxy sequencing of the genes, PRPH2 (NM_000322.4), ROM1 (NM_000327.3), GUCA1A (NM_000409.3), and NXNL1 (NM_138454.1) were performed using standard conditions as described previously.1,27 PRPH2 haplotypes in trans were determined by subtraction of the known c.828+5A>T haplotype from the genotype determined via sequencing. Primers used in the study are available in Supplementary Table S1.
Statistical Analysis

Linear regression was used to analyze the relation between age and visual function parameters in patients with the splice site PRPH2 mutation. The best-fit values of the slope and intercept, along with their standard errors and 95% confidence intervals (CIs), were calculated. Associations between the two phenotype groups and the three protein haplotypes in trans were tested by Fisher’s exact probability test. To confirm that significant associations were not due to age differences or familial clustering of genotypes, generalized estimating equation (GEE) models were fit using the “geepack” R package. These regressions modeled phenotype group as a function of haplotype and age via a logistic link, and robustly accounted for genetic correlation within families. An exchangeable correlation structure was specified for each familial cluster, but it is important to note that GEE models are robust even if the correlation structure is misspecified. For all haplotype analyses, we excluded all individuals younger than 43 years, taking into consideration the fact that the youngest age of an individual with the more severe phenotype in our cohort was 43 years.

RESULTS

The presenting symptoms of the individuals with the c.828+3A>T splice site mutation include reduced, altered, or distorted central vision, sensitivity to bright lights, and reduced night vision.

Four distinct phenotypes were observed consistent with PDs, CRD, RP, or CACD with extensive intra- and interfamilial variation. Three types of PD, adult-onset vitelliform macular dystrophy (AVMD), butterfly-shaped macular dystrophy (BMD), and adult-onset flavimaculatus-like macular dystrophy (AOFMD) of three individuals in their 40s are shown in Figures 1A through 1C. Individuals having AVMD and BMD exhibited a milder phenotype at age 40 than the individual with AOFMD,
who showed more severe ERG and field loss at age 28 years. A fluorescent angiogram shows hyperfluorescent lesions that are not visible by ophthalmoscope (Fig. 1C, inset). The absence of “dark choroid” distinguishes these from Stargardt’s and fundus flavimaculatus caused by mutations in the \textit{ABCA4} gene.\textsuperscript{30} Individuals with more extensive vision loss, as in CRD, CACD, and RP phenotypes, are shown in Figures 2A through 2C.

The fundus features of 62 individuals, along with their diagnoses, age, and sex, and the \textit{PRPH2} mini-haplotypes in \textit{trans}, are shown in Table 1 and pedigrees for 14 families are available in the Supplementary Material. Of the individuals, 44 were graded as Group I and had the clinical diagnosis of PD, and 18 were graded as Group II and had CRD, RP, or CACD.

\textbf{Age}

The age range of our patients was 16 to 90 years, with majority in the 40- to 50-year range. The youngest individual examined, a 16-year-old male, had minimal yellow lesions in the peripheral retina and the oldest individual, a 90-year-old female, had geographic atrophy in the macula; both belonged to the same family. The majority of the individuals with the splice site mutation fell into the PD Group I (44), with ages ranging from the 20s to the 70s (Table 1). Individuals having a fundus appearance suggestive of CRD (8), RP (6), and CACD (4) were in their fifth to sixth decades of life (Table 1).

\textbf{Visual Acuity}

Visual acuity data were available for 46 individuals with the splice site mutation, and the best-corrected visual acuity of the better eye was used for plotting (Fig. 3A). Most of the PD patients retain fairly good visual function, with their acuity remaining above 20/40 (0.5) until 65 years of age (green symbols). There was a modest decrease of visual acuity in PD with age due to photoreceptor and RPE atrophy in the macular region (Fig. 3A, solid line). In contrast, patients with CACD and CRD exhibited significantly lower visual acuity (red symbols), with more than half of the patients over age 45 having VA below 20/100 (0.2). Retinitis pigmentosa patients had VA ranging from 20/40 to 20/400 (0.2 to <0.08).
| No. | Individual ID | Haplotype in trans | Age, y | First Visit | Sex | Phenotype – Clinical Features |
|-----|---------------|--------------------|--------|-------------|-----|------------------------------|
| 1   | Fam13-3       | C-A-A              | 16     | M           | PD  | few lipofuscin deposits      |
| 2   | Fam13-35      | G-A-G              | 24     | F           | PD  | lipofuscin deposits          |
| 3   | Fam14-7       | C-A-A              | 25     | M           | PD  | few lipofuscin deposits      |
| 4   | Fam13-68      | C-A-A              | 26     | F           | PD  | few lipofuscin deposits      |
| 5   | Fam5-5738     | G-A-G              | 27     | F           | PD  | lipofuscin deposits in macula and ST arcade |
| 6   | Fam2-4957     | G-A-G              | 28     | F           | PD  | granular appearance in macula, flecks in peripapillary region and arcades, confluent, pigment + |
| 7   | Fam14-16      | G-A-G              | 30     | F           | PD  | lipofuscin deposits in macula and around arcades |
| 8   | Fam2-5878     | C-A-A              | 30     | M           | PD  | minimal lipofuscin deposits  |
| 9   | Fam8-6226     | G-A-G              | 35     | F           | PD  | grayish yellow lesion in macula with min flecks |
| 10  | Fam13-91      | G-A-G              | 37     | M           | PD  | minimal macular lesion       |
| 11  | Fam13-41      | G-A-G              | 38     | F           | PD  | subtle adult vitelliform lesions in fovea, lipofuscin accumulation near the arcades |
| 12  | Fam13-92      | G-A-G              | 38     | M           | PD  | macular pigmentary changes with peripheral drusen-like subretinal deposits |
| 13  | Fam14-21      | G-A-G              | 39     | M           | PD  | yellow deposit in macula with min flecks |
| 14  | Fam6-5755     | G-A-G              | 39     | M           | PD  | yellow deposit in macula, flecks in peripapillary region and arcades |
| 15  | Fam12-3       | C-A-A              | 40     | F           | PD  | yellow deposit in macula, RPE atrophy with flecks |
| 16  | Fam7-7082     | G-A-G              | 41     | M           | PD  | yellow deposit in macula with min flecks |
| 17  | Fam11-3       | G-A-G              | 41     | F           | PD  | yellow deposits in macula     |
| 18  | Fam2-9205     | G-A-G              | 43     | M           | CRD | extensive RPE atrophy in macula, flecks around atrophic zone |
| 19  | Fam14-9       | G-A-G              | 45     | F           | PD  | pattern-like maculopathy     |
| 20  | Fam1-177      | C-A-A              | 45     | F           | PD  | min Flecks, hypopigmentation in macula |
| 21  | Fam14-14      | G-A-G              | 46     | F           | PD  | pattern-like maculopathy     |
| 22  | Fam5-9226     | G-A-G              | 46     | F           | PD  | extensive confluent flecks   |
| 23  | Fam7-5790     | C-A-A              | 46     | F           | PD  | vitelliform-like lesion at macula, min flecks |
| 24  | Fam13-39      | G-A-G              | 47     | F           | PD  | very early pattern lesion in fovea, abnormal lipofuscin deposits beyond the arcades |
| 25  | Fam1-262      | G-A-G              | 48     | M           | RP  | extensive atrophy – bone spicules all quadrants |
| 26  | Fam12-2       | G-A-G              | 49     | M           | PD  | yellow lesion in macula and flecks |
| 27  | Fam14-15      | G-A-G              | 49     | F           | PD  | pattern-like maculopathy     |
| 28  | Fam1-214      | G-A-G              | 49     | F           | PD  | lipofuscin deposits – macula and periphery |
| 29  | Fam1-194      | C-A-A              | 49     | F           | PD  | lipofuscin deposits in macula, min flecks |
| 30  | Fam7-6221     | C-A-A              | 49     | F           | PD  | grayish yellow deposits in macula with min flecks |
| 31  | Fam8-9048     | G-A-G              | 50     | M           | PD  | multiple flecks, flavimaculatus-like, mottling of RPE |
| 32  | Fam2-9207     | G-A-G              | 51     | F           | PD  | yellow lesion at macula, min flecks |
| 33  | Fam4-5249     | G-A-G              | 51     | F           | PD  | gray, yellow lesions in macula, min flecks |
| 34  | Fam13-6       | G-A-G              | 53     | F           | CRD | extensive RP and choroidal atrophy in macula and periphery with bone spicules |
| 35  | Fam2-4920     | G-A-G              | 54     | F           | CRD | extensive RP and choroidal atrophy in macula and periphery with bone spicules |
| 36  | Fam12-1       | G-A-G              | 55     | F           | PD  | yellow lesions in macula, RPE degeneration with flecks |
| 37  | Fam13-18      | G-A-G              | 56     | M           | PD  | macular dot and halo lesion, lipofuscin deposits around arcades |
| 38  | Fam5-9234     | G-A-G              | 57     | F           | CRD | choriotreital atrophy in macular region min flecks |
| 39  | Fam15-1       | G-A-G              | 57     | F           | RP  | initially ad retinal dystrophy with flecks; 2 y later RPE atrophy w/ clumped RPE – pavingstone and photoreceptor degeneration |
| 40  | Fam13-1       | G-A-G              | 58     | M           | PD  | fleck-like collections of lipofuscin in macula extending beyond arcade |
| 41  | Fam1-302      | G-A-G              | 58     | F           | PD  | yellow flecks in macula      |
| 42  | Fam1-212      | G-A-G              | 58     | F           | CRD | extensive atrophy macular region and around peripapillary region |
| 43  | Fam13-17      | G-A-G              | 59     | M           | PD  | large reticular lipofuscin deposits extending from the arcades to the perifoveal area |
| 44  | Fam14-3       | G-A-G              | 59     | M           | CACD | central RPE and choroidal atrophy |
| 45  | Fam15-6       | G-A-G              | 59     | F           | PD  | lipofuscin deposits, mild arterial attenuation |
| 46  | Fam8-6138     | G-A-G              | 60     | F           | RP  | macula – hypopigmentation, peripheral BSs, and drusen-like deposits |
| 47  | Fam15-16      | G-A-G              | 61     | M           | CRD | RPE atrophy w/pigment clumps, arterial attenuation, cobblestone |
| 48  | Fam1-34       | C-A-A              | 65     | F           | PD  | 5-pointed butterfly lesion in macula |
| 49  | Fam1-365      | G-A-G              | 65     | M           | PD  | yellow-gray lesion with confluent flecks |
| 50  | Fam13-87      | G-A-G              | 65     | F           | CACD | multifocal choroidal atrophy, optic atrophy/arterial attenuation |
TABLE 1. Continued

| No. | Individual ID | Haplotype in trans | Age, y First Visit | Sex | Phenotype – Clinical Features |
|-----|---------------|---------------------|-------------------|-----|--------------------------------|
| 51  | Fam11-1       | G-A-G               | 65                | F   | PD – atrophic patches in foveal region with yellow flecks extending to arcades |
| 52  | Fam11-2       | G-A-G               | 67                | M   | PD – RPE atrophy confined to macular area, surrounded by flecks |
| 53  | Fam14-1       | G-A-G               | 69                | M   | CACD – central RPE and choroidal atrophy |
| 54  | Fam14-17      | G-A-G               | 69                | M   | RP – central RPE and choroidal atrophy and peripheral bone spicules |
| 55  | Fam13-60      | C-A-A               | 70                | M   | PD – butterfly lesion in macula and scattered lesions along the arcades |
| 56  | Fam6-5809     | G-A-G               | 70                | M   | CACD – central RPE and choroidal atrophy |
| 57  | Fam15-95      | C-A-A               | 71                | F   | PD – over 17 y changes from macular lesion to central atrophy |
| 58  | Fam13-15      | G-A-G               | 75                | F   | CRD – RPE atrophy macula and peripapillary region along with bone spicules |
| 59  | Fam3-5097     | G-A-G               | 76                | F   | RP – peripheral bone spicules |
| 60  | Fam1-178      | G-A-G               | 84                | M   | RP – extensive photoreceptor loss, RPE and choroidal atrophy along with bone spicules |
| 61  | Fam15-14      | C-G-A               | 85                | M   | CRD – atrophy and drusen, lots of hyperplasia, cobblestone, geographic atrophy |
| 62  | Fam13-33      | C-A-A               | 90                | F   | PD – peripheral lipofuscin deposits, peripapillary atrophy and scarring, as well as geographic atrophy in macula |

The age, sex, and fundus appearance of the affected individuals harboring the splice site mutation, and haplotypes in trans, Glu304-Lys310-Gly338 (G910-A929-G1014), Gln304-Arg310-Asp338 (C910-G929-A1014), and Gln304-Lys310-Asp338 (C910-A929-A1014) in exon 3 of PRPH2 are shown. Classification of the individuals into three phenotypes, PD, CACD, and RP, was based on fundus appearance and other visual function data including ERG and visual fields when available. Min, minimum.
Visual Fields

Humphrey visual fields were available in 10 individuals. Individuals with PD had better visual fields than those with CRD, CACD, or RP (Figs. 1, 2). Individuals with vitelliform-like PD had the most well-preserved visual fields (Fig. 1A). Butterfly-type PD (Fig. 1B) and flavimaculatus-like PD (Fig. 1C) showed preserved central and mild to moderate peripheral field loss. The CRD and CACD patients had central and paracentral scotoma (Figs. 2A, 2B) and RP patients had primarily peripheral visual field loss progressing to loss of central visual fields as expected (Fig. 2C).

Dark Adaptometry

Final dark-adapted thresholds were available from 24 patients (Fig. 3B). The majority of patients with PD (green symbols) had thresholds near the upper limit of normal. Thresholds were elevated approximately 2 to 4 log units above normal in individuals with the RP, CRD, and CACD phenotypes (red symbols).

Electrophysiology

Electroretinographic data were available for 25 individuals, with many patients tested on multiple occasions. Summary ERG parameters are shown in Table 2 and Figure 3. In general, cone responses were affected less than rod responses. As shown in Figure 3C, patients with PD showed a gradual loss with age of cone amplitude to 30 Hz flicker. The solid green symbols show repetitive measures on the same individual, with mild decline evident between visits, especially after 40 years of age. In contrast, red symbols show that patients with CRD, CACD, and RP typically had smaller cone amplitudes than patients with PD. Cone b-wave implicit times showed greater delays in patients with more widespread disease than in patients with PD, where cone implicit times were only slightly delayed.

Long-term follow-up of some individuals over several years show differences in severity of progression. Patients with PD showed milder progressive vision loss (Fig. 4) than patients with CACD/RP-like phenotype (Fig. 5).

Modifier Gene Screening

Peripherin/PRPH2. The distribution of PRPH2 (NM_000322.4) haplotypes in trans for PD, CRD, CACD, and RP phenotypes are shown in Tables 3 and 4. For individuals over 43 years, in Group I, representing milder disease with PD, where cone implicit times were only slightly delayed.

Table 2. Mean and Standard Deviations of ERG Values for Affected (Aff) Individuals Harboring the c.828+3A>T Splice Site Mutation and Normal (Nor) Controls Are Shown

| Parameters          | Aff Mean | Nor Mean | Aff SD | Nor SD | Aff Min | Nor Min | Aff Max | Nor Max |
|---------------------|----------|----------|--------|--------|---------|----------|---------|---------|
| Age at exam         | 48       | 114      | 24     | 76     | 20      | 84       |         |         |
| Rod B amp           | 50       | 85       | 42     | 24     | 31      | 79       | 108     | 93      |
| Max cone amp        | 195      | 35       | 13     | 2     | 13      | 28       | 35      | 28      |
| Cone 30 Hz amp      | 34       | 55       | 13     | 12     | 0       | 29       | 159     | 107     |
| Max cone IT         | 42       | 35       | 34     | 2     | 0       | 54       | 108     | 93      |
| SF cone amp         | 28       | 79       | 7       | 0      | 0       | 29       | 54      | 29      |
| Dark adaptometry    | 3        | 1        | 1      | 1      | 1       | 7        |         |         |

Amplitudes (amp) are in microvolts, and implicit times (IT) are in milliseconds.

Table 3. Frequency of Haplotypes in trans in the Two Groups

| Haplotypes | Group I, PD (%) | Group II, CRD, CACD, & RP (%) | Total (%) |
|------------|-----------------|-------------------------------|-----------|
| C-A-A      | 13 (29.6)       | 0 (0)                         | 13 (21)   |
| G-A-G      | 31 (70.4)       | 17 (94.4)                     | 48 (77.4) |
| C-G-A      | 0 (0)           | 1 (5.6)                       | 1 (1.6)   |
| Total      | 44 (71)         | 18 (29)                       | 62 (100)  |

Group I with PD and milder phenotype (44 individuals) and Group II with CRD, RP and CACD with more severe phenotype (18 individuals). Skew in haplotype with Group I having more C-A-A (29.6% vs. 0%) and Group II having more G-A-G (94.4% vs. 70.4%).
Gly338 haplotype among individuals with CRD, RP, and CACD was significant with a relevant $P$ value of $<2e-16$. However, because the asymptotic $P$ value may not be robust to the “zero” cell counts in two of the haplotype-phenotype categories (see Tables 3, 4), we reran the GEE model taking the conservative approach of collapsing the Gln304-Lys310-Asp338 (C-A-A) and Gln304-Arg310-Asp338 (C-G-A) haplotypes into a single category ($P = 0.03$, $N = 45$ due to removal of individuals with age $<43$). The estimated odds ratio for this comparison is $7.16$ ($95\% CI = [2.8–18.4]$, suggesting that the odds of having the CACD/RP-like phenotype (versus the PD phenotype) are $7.16$ times greater for affected individuals with a Gln304-Lys310-Gly338 haplotype (G-A-G) haplotype in $\text{trans}$ than for affected individuals with one of the other two haplotypes in $\text{trans}$ (Gln304-Lys310-Asp338 [C-A-A] and Gln304-Arg310-Asp338 [C-G-A]).

ROM1 Gene. No significant sequence variants were identified by bidirectional sequencing of ROM1 (NM_000327.3), in 10 affected individuals representing different phenotypes.

GUCA1A Gene. A GUCA1A (NM_000409.3), c.149C>T (p.Pro50Leu), sequence variant was identified in 4 individuals (3 affected—1 with macular atrophy and 2 with PD; 1 individual had normal retina) by bidirectional sequencing of GUCA1A in 62 individuals.

**FIGURE 4.** Pattern dystrophy in a female aged 36 years (Fam86226). Milder vision loss and slow progression over a 9-year period, with relatively preserved rod and cone function at age 45. Lower limits of normal ($P < 0.05$) are 85 $\mu$V for rod ERG, 200 $\mu$V for standard combined ERG, 50 $\mu$V for 30-Hz flicker ERG, and 75 $\mu$V for single flash cone ERG.
**NXNL1 Gene.** Several variants including c.46G>A (p.Asp16Asn), c.83G>C (p.Arg28Pro), c.172_174delTTC, (p.del-Phe58), c.190G>A (p.Glu64Lys), and c.461A>T (p.Glu154Val), were identified by bidirectional screening of *NXNL1* (NM_138454.1) in 62 affected individuals and 103 CEPH normal controls. However, none showed any significant association.

**DISCUSSION**

This study demonstrated that the c.828+3A>T splice site mutation in the *PRPH2* gene is the primary cause of retinal disease in all 62 affected individuals in these 19 families with complete penetrance but highly variable expressivity. It results in clinically diverse, autosomal dominant retinal dystrophies, including PDs, CRD, CACD, and RP (Figs. 1, 2), in 19 families derived from a common ancestor. The phenotypes fall into two broad clinical categories: Individuals in Group I (N=44; AVMD, BMD, and AOFMD types of PD) have milder disease than individuals in Group II (CRD, CACD, and RP). There is limited progression from Groups I to II. In our cohort, almost all individuals had reduced photoreceptor responses relative to age-matched controls, regardless of the phenotype (Fig. 5).

We previously had established a founder effect for the *PRPH2* c.828+3A>T mutation by haplotype analysis which
excludes sequence variants in cis within 5 megabases of the PRPH2 locus as modifiers. In this study, we analyzed the haplotypes in trans and showed that Gln304-Lys310-Asp338 (G910A-G929A-G1013A) is significantly associated with Group 1 pattern dystrophies and Gln304-Lys310-Gly338 (G910A-G929G-G1013A) with Group II (P value = 0.03 taking family structure, progression with age and restricting our analysis to only individuals (N = 45) older than 43 years of age. This suggested the possible modifier role of the polymorphic protein haplotypes in trans, with an estimated 7.16-fold increase in the odds of the more severe phenotype for affected individuals harboring a Gln304-Lys310-Gly338 (G-A-G) haplotype in trans compared to other affected individuals, and a possible protective role for having a second Gln304-Lys310-Asp338 (C-A-A) haplotype in trans. Given that the two alleles are similar in their temporal and spatial expression and interact directly, the protein haplotype in trans is likely to contribute to the phenotypic variation seen in the PRPH2 splice site mutation families.

Several sequence variants were found in the other potential modifier genes that were screened; however, none of them had a significant association with any one phenotype. Progressive photoreceptor loss with age explains continued vision loss in a significant association with any one phenotype. Progressive phenotypic variation seen in the protein haplotype in their temporal and spatial expression and interact directly, the A) haplotype in haplotypes in trans

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