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Founder Effect of a c.828+3A>T Splice Site Mutation in Peripherin 2 (*PRPH2*) Causing Autosomal Dominant Retinal Dystrophies

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Abstract

Importance—Screening for splice site mutation c.828+3A>T in the peripherin 2 (*PRPH2*) gene should be a high priority in families with highly variable retinal dystrophies. The correction of missplicing is a potential therapeutic target.

Objective—To determine the prevalence, genetic origin, and molecular mechanism of a donor c. 828+3A>T mutation in the *PRPH2* (peripherin 2, retinal degeneration slow) gene in individuals with retinal dystrophies.

Design, Setting, and Participants—Case-control study that took place at the University of Texas Health Science Center, the University of Iowa, and the Retina Foundation of the Southwest, from January 1, 1987, to August 1, 2014, including affected individuals from 200 families with a diagnosis of autosomal dominant retinitis pigmentosa, 35 families with unspecified macular

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Study concept and design: Shankar, Birch, Bowne, Daiger.

Acquisition, analysis, or interpretation of data: Shankar, Birch, Ruiz, Hughbanks-Wheaton, Sullivan, Bowne, Stone.

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Study supervision: Shankar, Birch, Sullivan, Bowne, Stone, Daiger.

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dystrophies, and 116 families with pattern dystrophy. Participants were screened for the c.828+3A>T mutation by restriction-enzyme digest, single-strand conformational polymorphism screening, or bidirectional sequencing. Haplotypes of polymorphic markers flanking the *PRPH2* locus and sequence variants within the gene were determined by denaturing gel electrophoresis or automated capillary-based cycle sequencing. The effect of the splice site mutation on the *PRPH2* transcript was analyzed using NetGene2, a splice prediction program and by the reverse transcription polymerase chain reaction of illegitimate transcripts from peripheral white blood cells.

Main Outcomes and Measures—Results of testing for splice site mutation, haplotypes, and alternate transcripts.

Results—The *PRPH2* mutation was found in 97 individuals of 19 independently ascertained families with a clinical diagnosis of retinitis pigmentosa, macular dystrophy, and/or pattern dystrophy. All affected individuals also shared a rare haplotype of approximately 644 kilobase pairs containing the c.828+3A>T mutation, which extends from the short tandem repeat polymorphism D6S282 to c.1013G>A (rs434102, a single-nucleotide polymorphism) in exon 3 of *PRPH2*, suggesting this mutation is from a common ancestor and is a founder mutation. It has a prevalence of 2% in families diagnosed as having autosomal dominant retinitis pigmentosa and 10% in families with variable clinical diagnosis of pattern, macular, and retinal dystrophies. Individuals with the c.828+3A>T mutation expressed a *PRPH2* transcript not found in control participants and that was consistent with abnormal splicing.

Conclusions and Relevance—The *PRPH2* c.828+3A>T splice site mutation is a frequent cause of inherited retinal dystrophies and is owing to the founder effect. The likely cause of disease is the missplicing of the *PRPH2* message that results in a truncated protein product. Identifying the genetic etiology assists in more accurate management and possible future therapeutic options.

Peripherin 2 (*PRPH2*; also known as *retinal degeneration slow*) is a photoreceptor-specific transmembrane protein that plays a critical role in the formation and stabilization of outer segment discs in rods and cones.[1-3] Mutations in the *PRPH2* gene cause a wide range of autosomal dominant retinal dystrophies such as pattern dystrophy (PD), central areolar choroidal dystrophy, unspecified macular dystrophy (MD), and retinitis pigmentosa (RP).[4-6] A single mutation, the deletion of *PRPH2* codon 153, has been reported to cause RP, PD, and fundus flavimaculatus all within the same family.[7]

A donor splice site mutation in the *PRPH2* gene, c.828+3A>T, was initially identified in the proband of a large family diagnosed as having autosomal dominant RP.[8] The mutation has since been identified to cause PD, autosomal dominant RP, and MD/central areolar choroidal dystrophy in a number of other families, 10 of whom were reported previously.[9-11] In this study, we screened additional probands with retinal dystrophies to determine the prevalence of this splice site mutation. We hypothesized that the preponderance of this mutation was likely owing to a founder effect and tested this by analyzing an intragenic haplotype in exon 3 of the *PRPH2* coding region and genotyping short tandem repeat polymorphism markers near the *PRPH2* locus on chromosome 6.

We also determined the consequence of the c.828+3A>T mutation on *PRPH2* transcript splicing in peripheral white blood cells (WBCs). The third base of the donor-splice junction is either an A (58%) or a G (40%) in 98% of all eukaryotic donor splice sites; a T occurs in just 2% of the splice sites.[12] The nucleotide change at the third base from an A to a T could result in either exon skipping or activation of a cryptic splice site and intron retention that leads to aberrant transcripts or it may result in a null allele.[13] Alternatively, the weakening or strengthening of the splicing motif could be leaky and result in variable levels of normal and aberrant transcripts.

Unfortunately, *PRPH2* is only expressed in retina, a tissue not readily accessible for transcript studies; however, illegitimate transcripts in readily accessible cells, such as WBCs and cultured lymphoblasts or fibroblasts, provide a way of examining the effect of a mutation on transcripts when a gene is expressed in tissues not available for biopsy.[13-18] We analyzed the pathogenic consequence of this mutation by NetGene2, a splice prediction program, and by the reverse transcription polymerase chain reaction of illegitimate *PRPH2* transcripts in WBCs.

Methods

Study Design

This study conformed to the Declaration of Helsinki and received institutional review board approval from the University of Texas Health Science Center, the University of Iowa, and the Retina Foundation of the Southwest. Patient recruitment and the study took place between January 1, 1987, and August 1, 2014. Written informed consent was obtained from all participants enrolled in studies at the Laboratory for Molecular Diagnosis of Inherited Eye Diseases at the University of Texas Health Sciences at Houston and at the Molecular Ophthalmology Laboratory at the University of Iowa. Nineteen families with the *PRPH2* splice site mutation were studied (Table). Ten families (1-10) were ascertained by D.G.B. and underwent clinical examination and visual function tests at the Retina Foundation of the Southwest in Dallas, Texas. Family 11 was examined by R.S.R. at the Department of Ophthalmology and Visual Sciences at the University of Texas Health Sciences, and family 12 was examined at Cole Eye Institute, by Jonathon Sears, MD, Cole Eye Institute. Family 14 was examined by Richard Weleber, MD, Casey Eye Institute, at the University of Oregon, Portland. Family 16 was examined by Gerald Fishman, MD, at the Department of Opthalmology, University of Illinois. Families from Iowa (13, 15, and 1-17) were recruited by E.M.S. from the University of Iowa hospitals and clinics or from family studies conducted in the communities where the patients resided.

Individuals were considered affected if they had peripheral or macular lipofuscin deposits, retinal pigment epithelium, choroidal atrophy or bone spicules on evaluation of fundus photographs, and/or electroretinographic evidence of reduced or absent function. If medical records were available, diagnoses of retinal diseases per patients' ophthalmologists were entered.

The study included 235 probands (200 with a diagnosis of autosomal dominant RP[11] and 35 with unspecified MD) who were screened by sequencing for mutations in the *PRPH2* gene

in the University of Texas Health Sciences molecular diagnostic lab. In a molecular lab at the University of Iowa, 116 individuals from families with predominantly PD and some with unspecified retinal dystrophy were screened for the *PRPH2* splice variant c.828+3A>T by restriction enzyme digest and then confirmed by bidirectional sequencing. A total of 245 unrelated healthy control participants, 100 unrelated parents from the Center for the Study of Human Polymorphisms, and 145 control participants at the University of Iowa were tested for the *PRPH2* splice site mutation by either restriction digest or by bidirectional sequencing. Probands and control participants who were tested were predominantly US individuals of European origin.

Molecular Methods

In this study, DNA was extracted from whole blood using standard procedures as described previously.[10,19] Mutation nomenclature was assigned in accord with GenBank accession number NT_007592. Detection of the *PRPH2* splice site change was performed by single-strand conformational polymorphism assay,[10,20] DNA sequencing,[9-11] or restriction digestion. Single-strand conformational polymorphism variants were confirmed by sequencing. The oligonucleotide primer sequences used to screen across intron-exon junctions and across all 3 exons were reported previously.[10,11] Bidirectional sequencing was performed on polymerase chain reaction (PCR) products analyzed on an ABI 3100-Avant Genetic Analyzer (Applied Biosystems). Restriction digestion was conducted using the Eco0109I enzyme (New England BioLabs Inc) on a PCR product amplified using the primers 5′-GTTACGACCACCAGACGGAG-3′ and 5′-CTTACCCTCTACCCCCAGC-3′. The restriction digest was performed at 37°C for 1 hour with approximately 1 μg of PCR product and 10 units of enzyme using the buffer recommended by the manufacturer. This was followed by electrophoresis in 2% agarose gels. Digested bands were visualized by ethidium bromide staining and ultraviolet transillumination. All of the exons and intron-exon junctions of *PRPH2* as well as 2000 base pairs (bp) 5′ of the start of transcription were sequenced in 4 affected individuals (GenBank NT_007592).

Intragenic polymorphic protein isoforms (haplotypes), Glu_{304}-Lys_{310}-Gly_{338} (G_{910}-A_{929}-G_{1013}), Gln_{304}-Arg_{310}-Asp_{338} (C_{910}-G_{929}-A_{1013}), and Gln_{304}-Lys_{310}-Asp_{338} (C_{910}-A_{929}-A_{1013}) coded by the following 3 polymorphic coding single-nucleotide polymorphisms: rs390659 c.910G>C (GAG->GCT) p.Glu304Gln, rs425876 c.929A>G (AAG->AGG) p.Lys310Arg, and rs434102 c.1013G>A (GGC->GAC) p.Gly338Asp in exon 3 of *PRPH2* were determined as previously described using allele-specific amplification with a biotinylated forward PCR primer, magnetic bead mutation of the labeled single-strand product, and detection by mutation detection enhancement gel electrophoresis.[10] This test was performed in 14 affected members of families 1, 2, 3, 4, and 11; 5 unaffected members of family 1; 72 Center for the Study of Human Polymorphisms samples; and 133 control participants from the diagnostic laboratory. For all remaining individuals with the *PRPH2* splice site mutation, the haplotype was determined by bidirectional sequencing of exon 3 and the phase was derived by pedigree inspection and inference.

Probands and affected family members with the *PRPH2* splice site mutation were genotyped with the short tandem repeat polymorphism markers D6S1549, D6S1017, D6S1552,

D6S1582, D6S282, and D6S1650 flanking the *PRPH2* gene. Genotyping was done by performing PCR amplification using fluorescent-labeled primers followed by capillary electrophoresis on an ABI 3100-Avant Genetic Analyzer at the University of Texas or by denaturing gel electrophoresis and silver staining as described previously at the University of Iowa.[21] Allele sizes were determined using GeneMapper software (Applied Biosystems) or by visual inspection.

Ribonucleic Acid Isolation and Reverse Transcription PCR

Total ribonucleic acid (RNA) was isolated from 6 healthy individuals and 5 affected individuals with the *PRPH2* splice site mutation, either from freshly drawn anticoagulated whole blood or from anticoagulated whole-blood samples to which RNA later (RiboPure kit; Ambion) was added, followed by storage at −20°C for 1 to 2 days. The eluted RNA was then subjected to enzymatic removal of genomic DNA by DNase I digestion followed by treatment with DNA-free reagents (Ambion Inc) to remove any contaminating DNA. Total RNA (500-1000 ng) was reverse transcribed with SuperScript III (Invitrogen) using a *PRPH2* gene–specific primer within exon 3 (5′-GAGGGGGAGATCCACGTTTC-3′), 5 first-strand buffers (reaction buffer, magnesium chloride, deoxyribonucleotide triphosphates, a 1-to-1 mixture of random hexamers, and oligo dT primers), 1M dithiothreitol, and SuperScript III reverse transcriptase (Invitrogen). The first PCR reaction was performed in a total reaction volume of 50 μL using 3 μL of first-strand complementary DNA and the following primers to amplify the region of *PRPH2* transcript containing 110 bp of the 3′ end of exon 2 and 250 bp of the 5′ end of exon 3: 5′-ACAGTTACGACCACCAGACG-3′ (within *PRPH2* exon 2) and 5′-GAGGGGGAGATCCACGTTTC-3′(within *PRPH2* exon 3). Ten microliters of this PCR product was used as the template for the second nested PCR reaction in a total volume of 100 μL. The internal primers for the second PCR reaction were 5′-AGGAGCTCAACCTGTGGG-3′ (within *PRPH2* exon 2) and 5′-TGCACTATTTCTCAGTGTTCGGG-3′ (within *PRPH2* exon 3). Polymerase chain reaction conditions for both reactions were initial denaturation for 5 minutes followed by incubation for 35 cycles of 95°C for 60 seconds, 60°C for 60 seconds, and 72°C for 60 seconds in a DNA thermocycler. The final PCR products were electrophoresed on 2% agarose gels and individual bands were purified using the QIA quick gel extraction kit (Qiagen) and the manufacturer's protocol. To control for genomic DNA contamination, parallel reactions without reverse transcriptase were included and carried out through the nested PCR steps.

The likely consequences of the splice site mutation on splicing efficiency were evaluated using the Netgene2 program (http://www.cbs.dtu.dk/services/NetGene2).[22] The protein products from aberrant transcripts were predicted using the program MacVector (MacVector Inc; http://www.macvector.com), a comprehensive Macintosh application used for translation and protein prediction. Linkage testing was done using the LINKAGE Package version 5.2.[23]

Results

A *PRPH2* splice site mutation, c.828+3A>T, was identified in a total of 97 patients belonging to 19 putatively unrelated families. Ten of these families were reported

previously.[9,10] The initial clinical diagnoses in the proband of these 19 families are detailed in the Table and range from RP, unspecified MDs and RDs, PDs, and cone-rod dystrophy. In families 9, 17, 18, and 19, each had a diagnosis of PD but without a family history of eye disease. No other potentially pathogenic variants were found in linkage disequilibrium to the splice site variant on screening the coding region of the entire gene and 2000-bp promoter region. The c.828+3A>T splice sitemutation was found in 19 probands among 351 unrelated individuals, including 4 of 200 (2%) with autosomal dominant RP[11] and 15 of 151 (10%) with PDs and unspecified MDs, and was absent from 245 control participants (Fisher exact test; $P = .006$). This variant was also not found in the 1000 Genomes Project database. All families harboring the splice site mutation were US individuals of European origin.

Analysis of the short tandem repeat polymorphism markers D6S1549, D6S1017, D6S1552, D6S1582, D6S282, and D6S1650 flanking the *PRPH2* gene showed that all affected individuals shared common alleles for D6S1582 and D6S282 located towards the 5′ end of the *PRPH2* gene. Linkage analysis in family 13 showed significant linkage to the *PRPH2* locus (D6S271; $z = 5.4$ at $\theta = 0$) and recombinations at markers D6S1650 and D6S1552 defined a linkage interval of 5 cM (approximately 1.38 Mb), making it the shortest interval with a known phase[24] (Figure 1). Towards the 3′ end of *PRPH2* gene, all affected individuals belonging to 19 families shared the Gln_{304}-Lys_{310}-Asp_{338} (C_{910}-A_{929}-A_{1013}) haplotype in *cis* to the splice site mutation. This defines a 644-kbp rare haplotype extending from the short tandem repeat polymorphism D6S282 (43 342 553 - 43 342 923) to the SNP rs434102 c.1013G>A (42 698 322) including the c.828+3A>T mutation. This haplotype is rare and was absent in unaffected family members. In addition, it has not been observed in any HapMap populations tested, suggesting that the splice site mutation likely arose from a common ancestor and the prevalence was owing to a founder effect.

The Netgene2 splice site prediction program[22] comparing the wild-type *PRPH2* sequence, c. 828+3A, with the c.828+3T allele scored the likelihood of the canonical splice site as being active more than 95% whereas the mutant site with the substituted T was less than 70%. Further, a cryptic splice site 29 bp downstream in intron 2 was predicted to have a 93% likelihood, suggesting that this was a better alternate than the mutated site.

The reverse-transcription PCR of illegitimate *PRPH2* transcripts from peripheral WBCs detected an aberrant band on the gel electrophoresis of the nested PCR product across exon 2 and exon 3 of *PRPH2* in all 5 affected individuals with the *PRPH2* splice site mutation, in addition to the normally spliced *PRPH2* transcript (Figure 2). The aberrant band was absent from 8 healthy individuals: 5 were unaffected relatives and 3 were unrelated healthy control individuals. This test result was reproducible among affected individuals, although the strength of the band varied. Gel extraction and sequencing of the higher–molecular weight aberrant product (Figure 2) showed a transcript with the c.828+3A>T change along with the inclusion of 29 bp of intron 2, demonstrating aberrant splicing in affected individuals only (Figure 3). This aberrantly spliced transcript was consistent with cryptic splicing in intron 2 as predicted by Netgene2. Gel extraction and sequencing of the lower–molecular weight product (Figure 2) showed normally spliced *PRPH2* exon 2 and exon 3 transcripts. Gel extraction and sequencing of the lower molecular weight product (Figure 2) showed normally spliced *PRPH2* exon 2 and exon 3 transcripts, shown in the upper chromatogram

in Figure 3. To determine whether the aberrant transcript was specific to individuals with the splice site mutation alone or whether it had been reported earlier, we performed a basic local-alignment search tool search of human expressed sequence tags (National Center for Biotechnology Information) with the aberrant sequence (including the final 29 bp of exon 2). The results failed to match any reported expressed sequence tags, demonstrating it was a novel transcript. An additional higher–molecular weight band seen in both unaffected and affected individuals was sequenced and found to have a 153-bp intron 2 inclusion. This sequence had a stop codon in the reading frame and thus would be subject to nonsense-mediated decay.[25] It was likely a normal splice variant found in illegitimate transcripts. The predicted protein product from the aberrant transcript included 10 additional amino acids at the end of the canonical exon 2 using 29 bp of intron 2 and the first base pair of exon 3. Splicing into exon 3, the last exon, resulted in a premature stop, thus making a truncated protein product that likely escaped nonsense-mediated decay.[25]

Discussion

Our study demonstrated a founder effect of the *PRPH2* splice site mutation c.828+3A>T, given the rare 644-kbp haplotype that is shared among all affected individuals and is absent in unaffected family members and the International HapMap data. This mutation accounts for more than10% of all RDs and is the most prevalent *PRPH2* variant known to cause such diverse clinical phenotypes. Mutations in *PRPH2* result in more than 13 different types of retinal disease including the phenotypes described in our families.[26,27] This mutation has also been associated with unspecified age-related maculopathy by independent investigators.[28]

Because the splice site mutation is at the third base pair of the splice junction, it is theoretically possible that the A>T change may not have been the actual disease-causing variant but was in linkage disequilibrium with another pathologic variant. However, we presented several lines of evidence to support the conclusion that the c.828+3A>T sequence variant was, in fact, pathogenic. First, the splice site mutation segregated among all affected members of 19 independently ascertained families and was absent from 245 control participants and in the 1000 Genomes Project database (http://www.1000genomes.org/). Second, the screening of the putative *PRPH2* promoter region 2000 bp upstream of the start site, the entire coding region, and intron-exon junctions failed to show any other significant variants. Third, analysis of homologues in other species (chick, mouse, chimp, fugu, and rat) showed conservation of A in the c.828+3 position. In addition, analysis of illegitimate transcripts in peripheral WBCs revealed an aberrant transcript with the addition of 29 bp of intron 2 among individuals harboring the splice site change. Finally, basic local-alignment search tool searches for the aberrant transcript failed to identify any known human expressed sequence tags. The limitation of the study was that *PRPH2* expression studies could not be performed in the retina, the ideal tissue of expression of the gene, because it is not a readily accessible tissue. Although animal models would be an attractive option to test this effect, it would necessitate the creation of a transgenic mouse model and would have time constraints.

The predicted effect of the aberrant *PRPH2* transcript on translation indicated that translation would result in an abnormal truncated protein product. Past studies have suggested that the protein product localization signal and membrane fusogenic signal are in the C terminal end of the *PRPH2* protein by deletion analysis of exon 3. Thus, this loss may result in *PRPH2* not being transported to outer segments of photoreceptors[29] and cause haploinsufficiency or result in gain-of-function effects, leading to loss of photoreceptor function and eventual cell death. The most striking feature in families with the splice site mutation was the marked clinical diversity despite the founder effect in all families reported thus far. This clinical heterogeneity could be the result of either variations in the splicing of the mutant copy, expression, or functioning of the *PRPH2* allele in trans or it could be owing to variation in other proteins that might interact with *PRPH2*.[27]

Conclusions

This study suggests that the c.828+3A>T variant in the *PRPH2* gene is a pathogenic and relatively common founder splice site mutation that should be considered when screening for disease-causing genes in white families of European origin with marked diversity in their retinal phenotypes.

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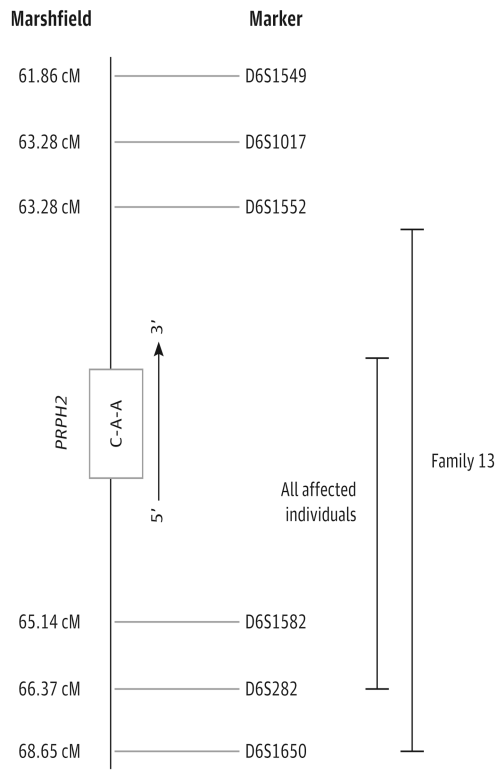

Figure 1. Shared Haplotype

Chromosome 6 showing a rare 644–kilobase pair haplotype from D6S282 to c.1013G>A in exon 3 of peripherin 2 (*PRPH2*) shared among all affected individuals harboring the c. 828+3A>T splice site mutation. Family 13 shares a 5-cM haplotype between D6S1552 and D6S1650.

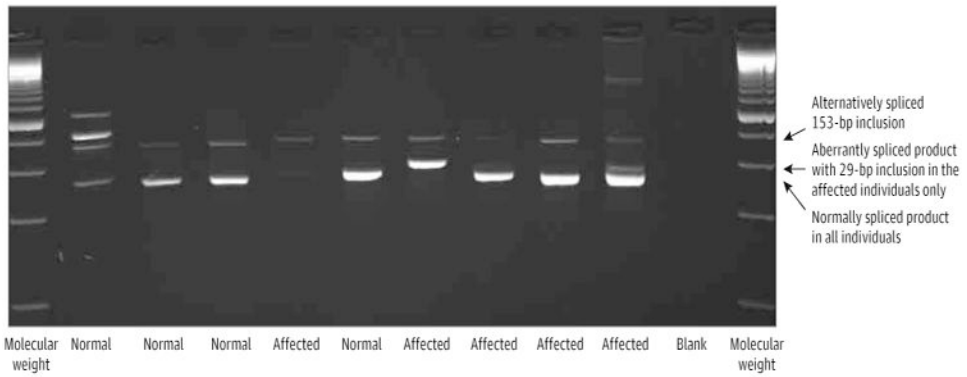

Figure 2. Reverse-Transcription Polymerase Chain Reaction Products

Illegitimate peripherin 2 gene transcripts from white blood cells showing normally spliced product in all individuals, aberrantly spliced product with 29–base pair (bp) inclusion in the affected individuals only, and alternatively spliced 153-bp inclusion in all individuals.

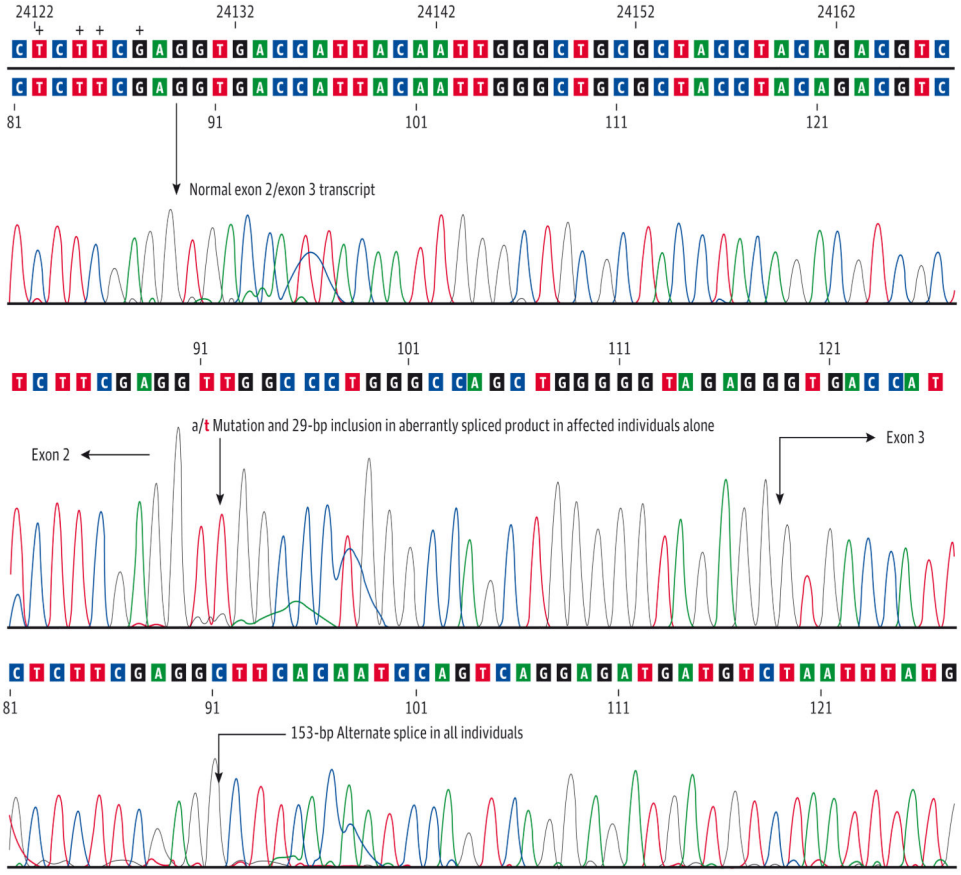

Figure 3. Peripherin 2 (*PRPH2*) Chromatographs of Gel-Extracted Bands

Bp indicates base pair.

Table

Families With Peripherin2 (*PRPH2*) Splice Site Mutation and Initial Diagnoses in the Probands

Family, No.	No. of Affected Individuals Examined	Diagnosis in Probands
1	10	Autosomal dominant retinitis pigmentosa
2	6	Cone-rod dystrophy
3	1	Autosomal dominant retinitis pigmentosa
4	3	Autosomal dominant macular dystrophy
5	7	Autosomal dominant macular dystrophy
6	6	Autosomal dominant retinitis pigmentosa
7	3	Autosomal dominant macular dystrophy
8	4	Juvenile macular dystrophy
9	1	Pattern dystrophy
10	1	Autosomal dominant macular dystrophy
11	3	Autosomal dominant macular dystrophy
12	3	Pattern dystrophy
13	29	Pattern dystrophy
14	11	Chorioretinal atrophy
15	4	Autosomal dominant atypical retinitis pigmentosa
16	2	Pattern dystrophy
17	1	Pattern dystrophy
18	1	Pattern dystrophy
19	1	Pattern dystrophy