Mutations in the Gene Coding for the Pre-mRNA Splicing Factor, *PRPF31*, in Patients with Autosomal Dominant Retinitis Pigmentosa

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Purpose. Retinitis pigmentosa is a clinically and genetically heterogeneous disorder. It is characterized by progressive degeneration of the peripheral retina, leading to night blindness and loss of the peripheral visual field. *PRPF31* is one of four pre-mRNA splicing factors identified as causing autosomal dominant retinitis pigmentosa, with incomplete penetrance being the unique feature associated with mutations in this gene. The purpose of this study was to identify *PRPF31* mutations in a cohort of 118 cases of autosomal dominant retinitis pigmentosa and determine the genotype–phenotype correlation emerging from the spectrum of mutations in this gene.

Methods. Probands with autosomal dominant retinitis pigmentosa underwent ophthalmic evaluation. Blood samples were obtained, genomic DNA was isolated, and *PRPF31* exons along with adjacent splice junctions were amplified by PCR and screened by direct sequencing.

Results. In the 118 individuals with autosomal dominant retinitis pigmentosa, six mutations were identified, of which four were novel. One previously known splice site mutation was identified in two other apparently unrelated families.

Conclusions. Mutations in *PRPF31* causing adRP were present in nearly 5% of a mixed U.K. population. The age of onset and the severity of the disease varied with different mutations. In addition, individuals carrying the same mutation showed a range of phenotypic variation, suggesting the involvement of other modifying genes. (*Invest Ophthalmol Vis Sci.* 2007;48: 1330–1334) DOI:10.1167/iovs.06-0963

Retinitis pigmentosa (RP) is the most common inherited retinal dystrophy, affecting approximately 1 in 3500 individuals worldwide. The mode of inheritance in RP may be autosomal recessive (ar), autosomal dominant (ad), X-linked recessive, or digenic. To date, 39 loci have been implicated in RP, of which 30 genes are known (http://www.sph.uth.tmc.edu/Retnet/home.htm/ provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX). These include genes encoding components of the phototransduction cascade, proteins involved in retinoid metabolism, cell–cell interaction proteins, photoreceptor structural proteins, transcription factors, intracellular transport proteins, and splicing factors (reviewed by Keenan et al.[1]). Four recently identified pre-mRNA splicing factors, *PRPF31*,[2] *PRPF8*,[3] *HPRP3*,[4] and *PAP-1*[5] correspond respectively to RP11, -13, -18, and -9.

PRPF31, located on chromosome 19, region q13.4, comprises 14 exons spanning approximately 16 kb of genomic DNA. It encodes a ubiquitously expressed 61-kDa splicing factor protein PRPF31, also referred to as splicing factor 61K. Incomplete penetrance is one of the unique features associated with mutations in *PRPF31*.[6] Asymptomatic carriers can have affected parents and children,[7,8] which can significantly complicate determining the mode of inheritance, thereby hindering the genetic counseling of the family. Previously, it has been reported that symptomatic individuals experience night blindness and loss of visual field in their teens and are typically registered as blind when they reach their 30s.[7,9] Detailed haplotype analysis in RP11-linked families indicated that asymptomatic patients inherit a different wild-type allele from the one inherited by their symptomatic siblings, suggesting the existence of differentially expressed wild-type alleles that can potentially determine the penetrance of the disease symptoms.[2] The high expression level of the wild-type allele may compensate for the nonfunctional mutant allele, whereas the low-expressing wild-type allele is inadequate to reach the required photoreceptor-specific PRPF31 activity threshold. The object of this study was to assess the prevalence of *PRPF31* mutations in a cohort of patients with adRP in the United Kingdom and to present the genotype–phenotype correlation in patients harboring *PRPF31* mutations.

Methods and Materials

Patients

Informed consent was obtained from the participants, in accordance with guidelines established by the Declaration of Helsinki and was approved by the Moorfield's Eye Hospital Ethics Committee. Genomic DNA was isolated (Nucleon DNA Isolation Kits for Mammalian Blood) according to the manufacturer's instructions (Tepnel Life Sciences, Manchester, UK).

Clinical Assessment of Patients

A full medical history was taken at the time of the first hospital visit and the first ophthalmic examination performed. Subjects underwent color fundus photography with a confocal scanning laser ophthalmoscope (cSLO; HRA; Heidelberg Engineering, Heidelberg, Germany). The criteria for the selection of subjects were the presence of at least two affected generations and male-to-male transmission. Of the 118 patients recruited for the study, 112 were of white origin, 5 were Asian, and 1 was of African origin.

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TABLE 1. Mutations Identified in *PRPF31* in a Cohort of 118 Patients with adRP

Patient	Visual Acuity	Exon/Intron	Nucleotide Change	Codon Change
2076	RE 6/60, LE hm	Exon 2	c. 79 G → T	p.Glu27X
2181	RE6/12, LE6/18	Exon 5	c.412 C → A	p.Thr138Lys
85	RE 6/6, LE 6/6	Intron 6	c.527+3A → G	Exon6/IVS6junction
15590	RE 6/18, LE 6/12	Intron 6	c.527+3A → G	Exon6/IVS6juction
16220	RE 6/9, LE 6/9	Intron 11	c.1146+2T → C	Exon11/IVS11junction
16722	RE 6/60, LE 6/18	Intron 6	c.528−1G → A	IVS6/exon7

Mutation Analysis

All 14 exons of *PRPF31* were screened by direct sequencing using primers reported previously.[2] Mutation analysis was performed by direct DNA sequencing (BigDye terminator cycle sequencing kit ver. 1.1; Applied Biosystems [ABI], Cheshire, UK) on a genetic analyzer (model 3100; ABI). One hundred ninety-two normal white DNAs were purchased from the European Collection of Cell Cultures (Wiltshire, UK).

RESULTS

All patients recruited for this study attended the Medical Retina Clinic at Moorfield's Eye Hospital, London. A total of 118 apparently unrelated patients were selected for the study.

DNAs from probands were screened for all 14 exons of the *PRPF31*. Six individuals carried *PRPF31* mutations (Table 1). Of these, three were splice site (c.527+3A→G identified in two individuals, c.528−1G→A, and c.1146+2T→C), one missense mutation (p.Thr138Lys) and one null mutation (p.Glu27X). All the mutations were identified in white patients, and none was found in 192 white normal control subjects.

Description of Mutations

c.527+3A→G (formerly IVS6+3A→G). We identified two apparently unrelated adRP families who carried this change. c.527+3A→G is a known mutation first identified by Vithana et al.[2] (patient RP1907) in a white woman attending the Medical Retina Clinic. The two other families carrying this change reported in this study show incomplete penetrance in their pedigrees (Fig. 1).

The splice site change will lead to retention of intron 6, in turn leading to premature termination of PRPF31. Vithana et al.[2] also predicted a reduction in splicing efficiency from 0.99 to 0.68. We further investigated the family of RP1907 to see whether this change is indeed pathogenic. Six unaffected (III-3, III-4, III-8, IV-5, IV-6, V-7, and V-9) and three affected (IV-4, IV-8, and V-8) members were screened for segregation of this splice site change. None of the tested unaffected individuals along

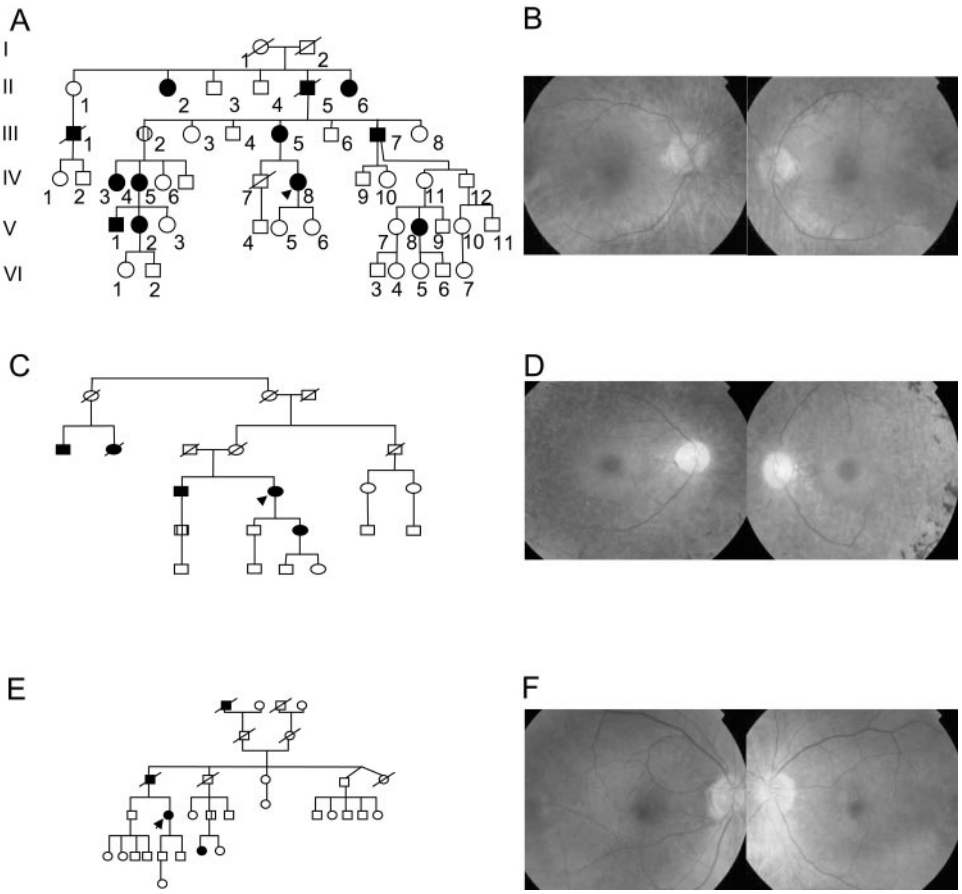

FIGURE 1. Pedigree of families carrying c527+3A→G mutation in *PRPF31* and fundus photograph of probands (*arrow*) harboring this change. (**A**) Family pedigree of RP1907. (**B**) Fundus photographs of RP1907 showing a normal appearance of the optic nerve, macula, and vessels. There are few pigmented bone spicules in the far periphery. (**C**, **D**) Pedigree and fundus photographs of patient 85, respectively. Fundus photographs show advanced RP with pigmented bone spicules in the midperiphery. The peripheral retina is mottled, and only the central part of the macula appears to be preserved. (**E**, **F**) Pedigree and fundus photographs of patient 15590, respectively. The fundus photographs show a few pigmented bone spicules in the far periphery. The optic nerve, macula, and vessels were within normal limits.

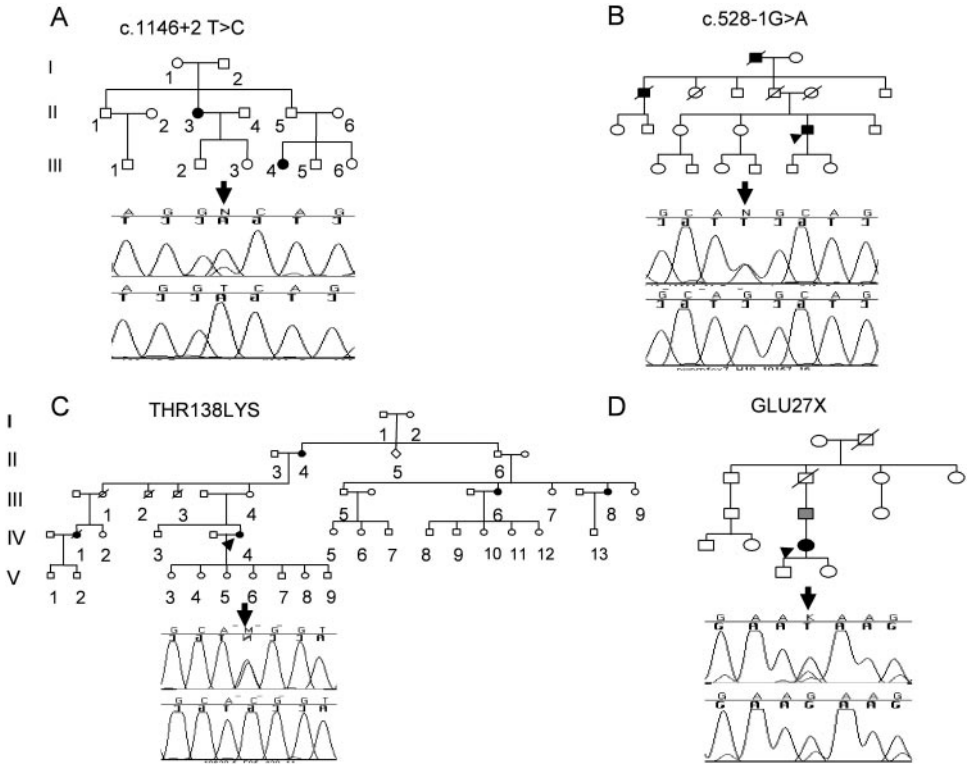

FIGURE 2. Family pedigrees of patients harboring *PRPF31* mutations. All the families showed incomplete penetrance in their pedigrees.

with 192 normal white control subjects carried this change, strongly suggesting that c.527+3A→G is indeed pathogenic.

Two symptomatic (IV-8 and V-8) and one asymptomatic (IV-11) member of this family were clinically investigated in detail. RP1907 (IV-8) was mildly affected, with the onset of night blindness when she was aged 20. Her peripheral vision was within 15°, and the central vision was 6/9 in her right eye (RE) and 6/12 in her left eye (LE) at her last visit at 52 years of age (Figs. 1A, 1B). On examination, she had macular edema in both eyes, and the peripheral retina showed mild to moderate intraretinal pigment deposits. Another symptomatic individual (V-8) showed a much more severe phenotype with onset of night blindness at the age of 13 years. At the age of 23 years, her visual acuity was reduced to 6/9 RE and 6/12 LE. She also had macular edema and the peripheral retina examination showed bone spicules in the midperiphery. Her vision improved to 6/6 in the right and 6/9 in the left eye after successful treatment with acetazolamide. Her visual field was within 10° at the last examination at 29 years. Her asymptomatic mother (IV-11) had visual acuity of 6/6 in both eyes at 45 years which changed to 6/9 at 61 years. She did not report any night blindness; however, episodic disturbance in vision was reported in her right eye. The fundus examination showed few pigment deposits on the nasal peripheral retina. Her general medical history was remarkable for myasthenia gravis since the age of 24 years. These observations suggest that c.527+3A→G causes RP with intrafamilial variation.

This wide range of disease symptoms was also observed in two other individuals (patients 85 and 15590). The first individual in our adRP cohort (patient 85) was moderately affected at the age of 58 and had well-preserved central vision. She had had night blindness since the age of 30 years (Figs. 1C, 1D). The second individual (patient 15590) was 50 when she first noticed night blindness (Figs. 1E, 1F). At the age of 56, she had very mild generalized retinal dysfunction and normal central vision. Haplotype analysis using three markers (*D19S912*, *D19S572*, and *D19S210*) and three single nucleotide polymorphisms (SNPs) within *PRPF31*—rs12985735, rs11556769, and rs1042816—on these three individuals suggest that this could be a founder mutation.

c.1146+2T→C. This change destroys the splice donor site and will result in the inclusion of 70 new amino acids before termination. The 18-year-old patient (III-4, Fig. 2A) had severe generalized retinal dysfunction with bilateral cystoid macular edema, causing reduced central vision. She reported first symptoms of RP at the age of 11 and the disease was diagnosed when she was 16 years of age. Her asymptomatic father (II-5) had vision of 6/6 in both eyes with no night blindness and a fundus with subtle motling. Another affected in this family, II-3, had night blindness since 10 years of age, with diagnosis of RP at 20 years. Her fundus at 44 years showed advance signs of photoreceptor loss and her visual field was within 10°.

c.528−1G→A. This splice site change, shown in Figure 2B, leads to the skipping of exon 7 resulting in the addition of 44 new amino acids due to the change of reading frame in exon 8 before terminating prematurely. This individual had blindness since the age of 20 and had a severe form of RP. He also had a cataract early in life that was removed at the age of 30 years. No other affected members of this family were available for study.

Threonine138Lysine (p.Thr138Lys). This mutation was identified in a large family showing incomplete penetrance (Fig. 2C). The proband carrying Thr138Lys mutation (IV-4) had night blindness at the age of 20 and showed clinical signs of RP at the age of 30. Her asymptomatic 77-year-old mother, who is a carrier of this change, showed normal retinal appearance. The proband's seven children have not been tested for ethical reasons. However, four of them are undergoing electrophysiological testing to confirm the diagnosis of RP. Patient III-6 also carries this change. Phenotypically, III-6 is more severely affected compared with IV-4 with an earlier age (15 years) of onset of RP.

Glutamine27Termination (Glu27X). The Glu27X mutation, as shown in Figure 2D, leads to premature termination of PRPF31. This nonsense mutation was identified in a 43-year-old

TABLE 2. Isocoding Changes and Polymorphisms Identified in 118 Patients with adRP

Nucleotide Change	Codon Change	Patients Harboring the Change (*n*)
c.564T → A	p.Asp188Asp	3
c.735C → T	p.Pro245Pro	25
c.1096C → T	p.Leu346Leu	1
c.1467C → T	p.Val469Val	3
c.420+81T → C	—	27
c.420+82C → G	—	48
c.855+40G → C	—	22
c.1073+55C → T	—	7

white woman who had a diagnosis of a severe form of RP with poor residual central vision (RE, 6/60; LE, hand motions). She has been night blind since the age of 15 years, was red-green color blind, and had several other retinal signs of RP: cystoid macular edema, telangiectasis, and vitritis. Her asymptomatic father was red-green color blind and showed irregularity in the RPE.

Isocoding Changes in *PRPF31*

Besides the six mutations identified in *PRPF31*, four isocoding changes were also identified in 37 of the 118 patients with adRP. Twenty-five patients carried c.735C→T (p.Pro245Pro), c.564 T→A (p.Asp188Asp) was identified in three individuals, c.1096C→T (p.Leu346Leu) was identified in one individual, and c.1467C→T (p.Val469Val) was identified in three individuals. Various other polymorphisms detected in *PRPF31* are shown in Table 2.

DISCUSSION

This study reports six mutations in *PRPF31* in a cohort of 118 patients with adRP; of these, four are novel. The prevalence of 5% is likely to be an underestimation due to the methodology used in the study for mutational screening. Any large deletions, insertions, and inversions would be missed by direct sequencing of the exons along with adjacent intronic sequences.

In this report, all six individuals with *PRPF31* mutations showed nonpenetrance in their pedigrees. These results are similar to those obtained in a Japanese cohort in which all the three patients carrying mutations in *PRPF31* showed incomplete penetrance in their pedigrees. This finding was also noted in 2 of the 3 patients in Spanish cohort,[10] and in 5 of 11 patients reported by Sullivan et al.[11] Taken together, these results strongly suggest that mutations in *PRPF31* cause adRP with incomplete penetrance, and therefore genetic counseling to families should be offered with caution.

One previously reported splice site mutation, c.527+3A→G, formerly IVS6+3A→G, was identified in two other apparently unrelated families. The age of onset of night blindness and severity of disease is quite varied in the families harboring this splice site change. Patient RP1907 had symptoms of night blindness at the age of 20, patient 85 had a very early onset of night blindness, and patient 15590 presented with night blindness in her 50s. These observations suggest that there may be another modifier gene, besides the high- and low-expressing alleles, which affects the outcome of the genetic mutations in *PRPF31*. This change appears to show a similar kind of intrafamilial variation as reported for Arg172Trp (R172W) peripherin/*RDS* mutation by Michaelides et al.[12] in a five-generation family.

There appears to be a high percentage of mutations that lead to gross deletion in PRPF31. In this report, five of six *PRPF31* mutations identified lead to premature termination of PRPF31, due to exon skipping, insertion of additional amino acids, or a premature stop codon. This observation is consistent with all the previously reported *PRPF31* mutations, 7 of 9 reported by Vithana et al.,[2] 3 of 3 by Martinez-Gimeno et al.[10] and Sato et al.,[13] and 9 of 11 by Sullivan et al..[11] These results could be an underestimation as any large deletion, insertion, duplication, and inversion will be missed by the present screening procedures which only screen the exons along with adjacent intronic sequences. Furthermore, it has been estimated that at least 15% of point mutations exert their effect on the standard consensus intronic splice sites, resulting in exon skipping, or less commonly, in the creation of an ectopic splice site or activation of a cryptic splice site.[14] This has recently been shown for the missense mutation p.Leu107Val in *PRPF31*.[15] This change creates a new splice site that results in a 4-bp deletion in exon 4 resulting in a frameshift and premature termination of the resultant protein. A single missense mutation identified in this study, C412A, also appears to create a weak splice donor site. The splice site prediction program NetGene2 (http://www.cbs.dtu.dk/databases/ provided in the public domain by the Center for the Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark) shows that C412A appears to create a splice donor site, TCCGCAAGGTCAAGGT, (confidence increases from 0 to 0.55) that results in the deletion of 6 bp at the end of exon 5. Taken together, these results suggest that haploinsufficiency, rather than the dominant negative affect of the mutant protein, appear to be the cause of disease in patients with adRP, due to *PRPF31* mutations. This conclusion is also consistent with the fact that there is a reduction of *PRPF31* mRNA derived from mutant alleles,[15] most likely due to nonsense-mediated decay, which can be triggered by transcripts bearing premature translation termination codons.

In addition to the mutations necessary for the disease, the wild-type allele appears to influence the penetrance of the disease. Symptomatic patients primarily differ from asymptomatic gene carriers in the levels of *PRPF31* mRNA produced from the normal, wild-type allele.[16] The clinical phenotype only results from co-inheritance of a mutated allele and a wild-type low-expressed allele. High prevalence of low-expressing alleles in certain populations may account for the *PRPF31* mutations being identified in patients with adRP with apparent complete penetrance.[17] The molecular basis of high- and low-expressing alleles of *PRPF31* is not known. Identifying these alleles would help in early diagnosis and disease management by possible therapeutic intervention.

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