Autosomal Dominant Retinitis Pigmentosa with Intrafamilial Variability and Incomplete Penetrance in Two Families Carrying Mutations in PRPF8

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PURPOSE. The aim of this study was to report detailed genotype/phenotype correlation in two British autosomal dominant retinitis pigmentosa (adRP) families with recently described mutations in PRPF8.

METHODS. Ten affected members from the two families (excluded for PRPF31 mutations) were assessed clinically. Seven subjects had fundus photography; some had electrophysiology, autofluorescence imaging, and visual field testing. Linkage analysis was performed from genomic DNA in one family. RNA was extracted from lymphocytes of the proband from both families, reverse transcribed into cDNA and subsequently screened for mutations in PRPF8. Segregation of mutations in each family was tested by direct genomic sequencing of the specific exons carrying the mutation.

RESULTS. All affected members complained of nyctalopia with variable age of onset. In the first family, there was marked variation in the clinical phenotype among affected individuals ranging from severe rod-cone dystrophy to a 67-year-old patient with a normal retinal appearance and mild rod dysfunction on scotopic electrophotoreitngiography (ERG). The second family demonstrated similar variability and a history of a nonpenetrant individual. Linkage analysis in the first family showed strong evidence for linkage to markers on chromosome 17p implicating PRPF8 as a candidate gene. A c.6353 C>T change causing a nonconservative missense mutation p.S2118F was found in exon 38 of PRPF8 by direct sequencing of the cDNA. The mutation c.6930G>C (p.R2310S) was found in the second family.

CONCLUSIONS. This is the first report of marked intrafamilial variability associated with mutations in the PRPF8 gene, including incomplete penetrance. PRPF8 mutations should be suspected in patients with adRP and variable expressivity. (Invest Ophthalmol Vis Sci. 2011;52:9304–9309) DOI:10.1167/iovs.11-8372

Retinitis pigmentosa (RP) is term used to describe a group of inherited retinal disorders in which there is degeneration of rod and cone photoreceptors. There is considerable genetic and phenotypic heterogeneity. Typically the first symptom is night blindness reflecting early rod photoreceptor involvement and later there is peripheral field loss and ultimately visual loss due to involvement of cones in the central retina. RP is the most frequent inherited form of blindness with a prevalence of approximately 1 in 4000. The disorder may be inherited as an autosomal dominant, autosomal recessive, or X-linked recessive trait; maternal, mitochondrial inheritance has also been reported. RP may result from mutations in more than 45 genes. Dominant transmission occurs in approximately one third of cases and at least 17 genes have been implicated in autosomal dominant RP (adRP). Three of these genes, PRPF3, PRPF8, and PRPF31 encode splicing factors which together account for approximately 15% of families with adRP in the United Kingdom (RM, ARW, personal communication, 2011). There are few detailed descriptions of the clinical phenotype associated with PRPF8 mutation. The present report describes the detailed clinical phenotype in two British families with adRP associated with mutations in PRPF8.

PATIENTS AND METHODS

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Local Research Ethics Committee.

Clinical Assessment

Two families, a six-generation (family 1) and another three-generation (family 2), were ascertained from the inherited eye disease clinic of the Moorfields Eye Hospital (Fig. 1). Eight members of family 1, seven affected and one mildly symptomatic, underwent ophthalmic examination including best-corrected visual acuity, slit lamp examination and fundoscopy. Full medical histories were obtained. Color fundus photography was performed in five out of eight patients; three had autofluorescence imaging (AF) and spectral-domain optical coherence tomography (SD-OCT) and four had Goldmann visual field examination (VF). Four affected members of family 2 were examined and underwent color fundus photography and three had AF and SD-OCT.

Full field electroretinography (ERG) was performed using the International Society for Clinical Electrophysiology of Vision (ISCEV) standards in two of the milder affected members of family 1 (IV.8 and V.12; Fig. 1) and a young (6-year-old) affected (III.2; Fig. 1) from family 2 who underwent a modified pediatric ERG protocol.

Molecular Genetic Analysis

After obtaining written consent, blood samples from affected and unaffected family members were collected for DNA and RNA extractions. RNA was isolated from lymphocytes of one affected member from each family (Trizol; Invitrogen, Paisley, UK), genomic DNA was extracted using commercially-available kits (Nucleon DNA Isolation Kit for Mammalian Blood; Tepnel Life Sciences, Manchester, UK) according to the manufacturer’s instructions. Microsatellite markers flanking known genes for adRP were selected from a linkage mapping set (ABI Prism Linkage Mapping Set v 2.5; Applied Biosystems, Foster City, CA) and additional FAM-labeled microsatellite markers were selected from the Ensembl database. PCRs were done (Absolute QPCR; Thermo-Fisher, Epsom, UK) according to manufacturer’s instructions. The resultant PCR products were loaded in a DNA sequencer (ABI model 3730; Applied Biosystems) and the genotyping calls and Mendelian error checks were performed with commercial software (GeneMarker, version 1.70; Biogene, Cambridgeshire, UK) for linkage analysis.

Mutation Screening

cDNA was synthesized from leukocyte RNA (patient IV.2 in family 1 and II.2 in family 2) using reverse transcriptase (Superscript III Reverse Transcriptase; Invitrogen) according to manufacturer’s instructions. Mutation analysis was performed by Sanger sequencing of the cDNA using a terminator sequencing kit (BigDye ver 3.1; Life Technologies, Paisley, UK) on the ABI 3730 machine (Applied Biosystems). cDNA sequences obtained for patient IV.2 and II.2 were compared with the reference sequence of PRPF8 from the Ensembl database. Primers used for PCR and sequencing are described in Table 2 of Towns et al. Exons and nucleotides were numbered according to OMIM (Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) ID 607300. For the other members of the families, genomic DNA was sequenced using primers e38F/e39R in family 1 and e42F/e42R in family 2. DNA from 130 healthy Caucasian controls (Sigma-Aldrich, Dorset, UK) was sequenced for the two identified mutations from family 1 and family 2.

RESULTS

Clinical Assessment

Family 1. Seven clinically affected members aged between 37 and 82 years and one mildly symptomatic, obligate carrier (68 years) were assessed. Six affected subjects complained of night blindness as the first symptom. The age of onset of the night blindness varied within family members from early childhood (patients V.10, V.11, V.12) to mid-thirties (IV.12). Visual field constriction was reported in late teens in patients III.2 and V.11 and after the age of forty in patient IV.12. Visual field constriction was reported in late teens in patients III.2 and V.11 and after the age of forty in patient IV.12. Patient IV.8, an obligate carrier, reported no problems driving at night or looking at stars in the night sky although had confessed to mild difficulty in different lighting conditions previously. He did not complain of any visual field constriction.

Best corrected visual acuity (BCVA) in the better eye was 6/12 or better in five out of seven symptomatic patients. In the 82-year-old female (III.2), BCVA was 6/18 in the right and 6/36 in the left eye while in a 59-year-old male (IV.2), it was hand movements right and 6/18 left eye.

Bilateral subcapsular cataract was observed in patients III.2 at a young age, IV.2 (age 34 years), and V.11 (age 33 years). In all patients fundoscopy revealed attenuated retinal vessels, pale optic nerve head and variable degrees of diffuse bone spicule-like pigment clumping within the neurosensory retina (Fig. 2A). Asteroid hyalosis was observed in the left eye of patient IV.12. Patient V.11 had bilateral optic nerve drusen. Patient IV.2 developed Coats-like retinal telangiectasia in his right retinal periphery resulting in a vitreous hemorrhage when aged 34 years. Patient IV.12 had an episode of angle closure glaucoma at 41 years of age. Cystoid macular edema of various degrees was observed in four symptomatic patients (IV.2, V.10, V.11, V.12).
FIGURE 2. Variability of phenotype of PRPF8 retinitis pigmentosa. (A) Family 1; (B) family 2. (A1) Right fundus of 60-year-old male shows extensive intraretinal pigmentation with mid-peripheral and macular atrophy. Spectral domain OCT shows areas of outer retinal atrophy in right (A2) and left eye (A3). (A4, A5) Right fundus and AF of a 51-year-old female shows little intraretinal pigmentary deposition but widespread retinal pigment epithelial atrophy. (A6, A7) The right fundus of a 38-year-old male shows patchy intraretinal pigmentation with AF showing a small peri-foveal hyperfluorescent ring. (B) Family 2; (B1, B2) show extensive RPE atrophy involving the macula in the the fundus and AF of a 49-year-old male. His daughters showed little pigmentary deposition in their fundi (B3, B5) but hyperfluorescent rings in AF at age 15 and 11 years of age respectively (B4, B6). (C) Asymptomatic: fundus photographs, autofluorescence, spectral-domain OCT, and Goldmann visual fields of asymptomatic individual IV.8 of family 1 showing relative preservation of normal structure and function at 63 years of age. OCT of the right eye shows the presence of an epiretinal membrane causing flattening of the foveal depression.
V.11, and V.12). Individual IV.8 had a normal retinal appearance (Fig. 2C).

AF imaging revealed extensive peripheral atrophy in two affected individuals with a small peri-foveal ring of increased autofluorescence (Fig. 2A). OCT showed preserved outer retinal structure in the area delineated by the ring of hyperautofluorescence (Fig. 2A). In another patient (IV.2), OCT showed disruption of the outer retinal structure in the fovea (Fig. 2A). AF and OCT of the only mildly symptomatic obligate carrier (IV.8) (Fig. 2C) were normal.

Goldman perimetry showed a variable degree of visual field loss with patient V.12 retaining approximately 50° of horizontal visual field in the better eye with the biggest and brightest target (V4e) at 34 years of age while V.10 had near normal fields at 37 years of age. Subject IV.2 had a small central field of approximately 15° at 38 years of age with retention of a temporal island of preserved field. Individual IV.8 had normal visual fields at 63 years of age (Fig. 2C).

Electrophysiological testing of the patient (IV.8) who complained of only mild contrast adjustment showed mildly abnormal rod photoreceptor function (rod specific ERGs were normal but bright flash DA 11.0 a-wave was mildly reduced). Cone ERGs were normal (Fig. 3A). Subjects V.10 and V.12 had only residual detectable rod and cone ERGs in keeping with a severe rod-cone dystrophy (data not shown).

Family 2. The proband, a 35-year-old man (Fig. 1, lower panel, II.2), had night vision problems from early childhood and was diagnosed with RP in his late teens. He later developed deterioration of central vision and when examined aged 35 years, right eye BCVA was 6/24 with hand movements in the left eye. At that time his Esterman supra-threshold bilateral visual fields were reduced to <5° (data not shown). He developed bilateral subcapsular cataracts in his mid-twenties. His half-brother (II.1), sharing the same mother, was also affected and had early onset of subcapsular cataracts for which he underwent bilateral cataract surgery. His BCVA at 48 years of age was 3/60 right and left. His older daughter (III.2) developed night blindness in early childhood. BCVA at age 15 years was 6/10 right eye; 6/5 left eye. The youngest daughter (III.3) was noted to be night blind in the first decade and at age 11 years had BCVA of 6/15 in both right and left eyes. According to reports from her sons, the deceased grandmother of the two affected females (I.2) had no visual symptoms when she died at the age of 69 years.

All symptomatic family members examined showed severe attenuation of arterioles, pale optic discs, and sparse intraretinal pigment migration (Fig. 2B). AF in patient II.1 showed peripheral and central macular atrophy, while there was preservation of central macula with perifoveal hyperautofluorescent ring in the two young females (III.2 and III.3) (Fig. 2B). AF and OCT revealed bilateral macular edema in both III.2 and III.3 with the younger more affected (Fig. 2B). OCT of II.1 showed central macular atrophy.

Electrophysiological assessment of III.2 at 6 years of age using pediatric protocols revealed severe photoreceptor dysfunction with undetectable waveforms (Fig. 3B).

Mutational Screening

Family 1. Linkage analysis with flanking microsatellite markers to all known adRP loci showed strong evidence for linkage at the RP13 locus (markers D17S849, D17S831, D17S1840, D17S1574, and D17S525). Because PRPF8 is the gene implicated in RP13, it was initially decided to directly sequence exon 42 as all published mutations have been found in this exon. No variant was found by direct sequencing of the proband IV.2. Therefore, RNA from IV.2 was extracted, reverse transcribed, and PRPF8 cDNA was directly sequenced allowing for a quick investigation of possible splicing defect and/or exonic mutation. The variant c.6353 C>T, was identified in exon 38 in a heterozygous state (Fig. 1, upper panel). Direct sequencing of the PRPF8 exon 38 from the genomic DNA of the whole family revealed that all the affected patients, and the one mildly symptomatic carrier harbored this change, which was not present in unaffected family members. The mutation c.6353 C>T (p.S2118F) was not found in 130 unrelated controls from the

![Figure 3](https://iovs.arvojournals.org/figures/)
same ethnic origin indicating that this change is likely to be causative of the disorder in this family.

**Family 2.** The entire cDNA of affected proband (II.2) was sequenced and the mutation c.6930G>C (Fig. 1, lower panel) leading to p.R2310S missense change in the protein was identified in exon 42. The same variant was present in all affected family members and was not found in 130 control individuals.

**DISCUSSION**

This report describes the detailed phenotype of affected members of a large British family carrying the missense PRPF8 mutation c.6353 C>T (p.S2118F) in exon 38 recently reported by Towns et al. All previous PRPF8 mutations had been reported to occur in exon 42 of the gene. Mutations in PRPF8 gene are rare, accounting for 2%–3% of patients with adRP in Spanish and American studies and 5.5% in a large United Kingdom cohort (RM, ARW, unpublished observations, 2011). These percentages are likely to be underestimates given the current restriction of screening to exon 42 of the gene.

Previous reports have documented a severe form of RP in British, Spanish, Dutch, and African American families, with mutations in exon 42 of PRPF8. The previous phenotype descriptions were consistent with an infantile onset of night blindness, followed by visual field loss a few years later. All the published adult patients have unrecordable ERG values and the rod function in affected pediatric population is severely abnormal. The phenotype is relatively uniform and there is no evidence of variability or incomplete penetrance. The present study reveals a high degree of intrafamilial phenotypic variability. The age of onset of night blindness varied from early childhood to late thirties. One 67-year-old individual heterozygous for the c.6353 C>T mutation who was only mildly symptomatic, had a completely normal visual acuity, when available, revealed a ring of increased autofluorescence surrounding the central macula (Fig. 2). Hyperfluorescent rings of various types are observed in RP or Leber congenital amaurosis (usually surrounding healthy macula) and cone or cone-rod dystrophy or X-linked retinoschisis (usually surrounding dystrophic macula). However, in primary RPE retinal dystrophies, such as those caused by mutations in MERTK or RPE65, such rings have not been described. Furthermore, SD-OCT images in affected patients demonstrate an intact hyperreflective layer at the junction of dystrophic retina and choroid which might represent intact RPE-Bruch’s membrane complex. However, in the mouse model there is evidence of defective RPE function. It is possible that human PRPF8 retinopathy may have thus different pathophysiology with relative late demise of RPE.

The ERG in the mildly symptomatic obligate carrier showed a DA 0.01 rod ERG within the normal range but a subnormal DA 11.0 bright flash ERG a-wave. Cone full-field ERGs and pattern ERGs were normal. The consequences of this mutation, which in other family members caused legal blindness, were those of minor rod photoreceptor function. Affected status was not evident from fundus examination or perimetry. This suggests caution in reassuring members of families with PRPF8-related disease of their unaffected status and confirms the importance of electroretinography in the detection of subclinical disease.

There is a history of incomplete penetrance in two individuals in the second family, but neither was available for examination. The mutations p.R2310K and p.R2310G have previously been reported and suggests that c.6930G>C (R2310S) in exon 42 is a hot spot for mutation.

**PRPF8, PRPF3, and PRPF31 genes** are involved in the assembly and function of the spliceosome, which clips introns out of pre-mRNA. The gene PRPF8 encodes a large and highly conserved nuclear protein which stimulates a helix and Br2, required for activation of the spliceosome. Mutations which cause adRP inhibit this function. More than PRPF8, PRPF3, and PRPF31 genes are associated with RP and presumably inhibit the interaction of the PRPF8 protein with Br2. The only detected mutation in PRPF8 outside exon 42 is p.S2118F, which was reported recently, occurring in family 2 of this report. This nonconservative amino acid change is predicted to cause significant alteration to the structure and function of the PRPF8 protein.

The function of the C-terminal of PRPF8 protein has been investigated using various methods by different laboratories. Grainger and Beggs suggested that the nine RP13 missense mutations reported in exon 42 of PRPF8 affect seven very highly conserved amino acid residues indicating that those residues have a conserved function which is altered in case of mutation. Those nine amino acids belong to the region of PRPF8p that interacts with Br2p, one of the spliceosomal RNA helicases, and is close to the prp8-1 (G2347D) mutation, which ablates the binding of C-terminal yPrp8 peptides by Br2p in vitro. By yeast two-hybrid, Fan et al. have demonstrated that C-terminal 94-amino acid region of hPRPF8 interacts with the multifunctional plasmidogen activator inhibitor type-2 protein (PAI-2). Therefore, it is reasonable to assume that the interaction PRPF8/PAI-2 could also be altered in RP13 patients.

Recently, two different groups have established independently the crystal structure of PRPF8. Both groups proposed that the C-terminal domain of PRPF8 including the MPN domain (Mpr-1, Pad-1, N-terminal) domain is an interaction domain. They suggest that the reported residues mutated in RP13 constitute a binding surface between PRPF8 and other partner(s) and the disruption of this interaction provides a plausible molecular mechanism for RP13. It is notable that exon 38 encodes a part of the MPN domain of PRPF8 and S2118 is part of an α helix which is may be altered by the mutation in the present report.

To conclude, this is the first report of marked intrafamilial variation and nonpenetrance associated with two PRPF8 mutations: c.6353 C>T (p.S2118F) in exon 38 and c.6930G>C (p.R2310G) in exon 42. The data suggest that all exons of PRPF8 should be screened in families with adRP and incomplete penetrance. The important role of electrophysiological assessment is noted. The findings extend the range of phenotypes seen in association with PRPF8 gene mutations and provide important information to assist the management of families in whom this form of adRP is suspected.

**Acknowledgments**

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