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Prognosis for Splicing Factor PRPF8 Retinitis Pigmentosa, Novel Mutations and Correlation between Human and Yeast Phenotypes

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ABSTRACT: *PRPF8*-retinitis pigmentosa is said to be severe but there has been no overview of phenotype across different mutations. We screened RP patients for *PRPF8* mutations and identified three new missense mutations, including the first documented mutation outside exon 42 and the first *de novo* mutation. This brings the known RP-causing mutations in *PRPF8* to nineteen. We then collated clinical data from new and published cases to determine an accurate prognosis for *PRPF8*-RP. Clinical data for 75 *PRPF8*-RP patients were compared, revealing that while the effect on peripheral retinal function is severe, patients generally retain good visual acuity in at least one eye until the fifth or sixth decade. We also noted that prognosis for *PRPF8*-RP differs with different mutations, with p.H2309P or p.H2309R having a worse prognosis than p.R2310K. This correlates with the observed difference in growth defect severity in yeast lines carrying the equivalent mutations, though such correlation remains tentative given the limited number of mutations for which information is available. The yeast phenotype is caused by lack of mature spliceosomes in the nucleus, leading to reduced RNA splicing function. Correlation between yeast and human phenotypes suggests that splicing factor RP may also result from an underlying splicing deficit. ©2010 Wiley-Liss, Inc.

KEY WORDS: Retinitis pigmentosa, PRPF8, mRNA splicing, adRP

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INTRODUCTION

Retinitis Pigmentosa (RP) is a progressive rod-cone dystrophy leading to night-blindness, loss of peripheral visual fields and a reduced or absent electroretinogram (ERG), with later variable loss of central retinal function. It is an inherited disease primarily affecting the rod photoreceptors and the retinal pigment epithelium, and is the commonest inherited retinal dystrophy, affecting approximately 1 in 3500 people worldwide (Bunker, et al., 1984). Clinical manifestations include bone-spicule pigmentation in the retinal mid-periphery, arteriolar attenuation and waxy optic disc pallor.

RP is clinically and genetically heterogeneous, with all modes of Mendelian inheritance found in families with the condition. To date some forty-six loci have been identified for non-syndromic RP, and for these thirty six of the causative genes have been identified (see www.sph.uth.tmc.edu/RetNet/disease.htm for full list). These include genes encoding components of the phototransduction cascade and the visual cycle by which the chromophore component of rhodopsin is recycled, retinal transcription factors, photoreceptor structural proteins and cilia proteins, the latter being components of the highly evolved ciliary structure of the photoreceptor outer segments (Hims, et al., 2003; Roepman and Wolfrum, 2007). In addition, in a somewhat perplexing discovery, mutations in four ubiquitously expressed splicing factors, pre-mRNA processing factor 8 (*PRPF8* (MIM# 607300, McKie, et al., 2001), *PRPF31* (MIM# 606419, Vithana, et al., 2001), *PRPF3* (MIM# 607301, Chakarova, et al., 2002) and *PAP1* (MIM# 607331, Keen, et al., 2002; Maita, et al., 2004) were also found in dominant RP patients (reviewed by Mordes, et al., 2006). In total mutations in splicing factors are thought to be the second most common cause of autosomal dominant RP (Sullivan, et al., 2006).

PRPF8 is one of the largest and most highly conserved proteins found in the nucleus and is essential for pre-mRNA splicing (reviewed by Grainger and Beggs, 2005). It has remarkably low sequence similarity to other proteins, making it difficult to predict function from structure, though some domain structure has been determined (Boon, et al., 2006). It is a component of the U5 snRNP and U5·U4/U6 tri-snRNP (Boon, et al., 2007; Stevens and Abelson, 1999) and may function as a large protein scaffold in the spliceosome (Turner, et al., 2006, for review see Grainger and Beggs, 2005). In addition, recent crystal structures of C-terminal domains suggest a possible role in the catalysis of splicing (Abelson, 2008). Unlike other known splicing factors, as a component of the U5 snRNPs, PRPF8 participates in both U2 and U12 splicing (Luo, et al., 1999).

The *PRPF8* gene is located on the short arm of chromosome 17 and encodes 42 exons spanning ~36kb of genomic sequence. It is highly conserved in both sequence and size, which varies between 220 and 280kDa in different organisms (Hodges, et al., 1995; Luo, et al., 1999). Sixteen different *PRPF8* mutations, including missense, premature stop and deletions have been identified to date (De Erkenez AC, 2002; Kondo, et al., 2003; Martinez-Gimeno, et al., 2003; McKie, et al., 2001; Ziviello, et al., 2005), all but one of which cluster in a highly conserved region within the last exon. These mutations account for approximately 3% of dominant RP or around 1% of all RP cases (Sullivan, et al., 2006). Real-time PCR analysis has shown similar expression of both wild type and mutant alleles in cell lines from RP patients carrying a nonsense mutation (Gamundi, et al., 2008). This suggests that this transcript (and by inference other mutant transcripts) is not removed by nonsense mediated decay. This contrasts with the likely mechanism for mutations in PRPF31, for which evidence strongly suggests haploinsufficiency (Abu-Safieh, et al., 2006).

Mutations in *PRPF8* have been described as causing a severe form of dominant RP (Gamundi, et al., 2008; Tarttelin, et al., 1996; Walia, et al., 2008). However, in contrast to the phenotype for the commoner *PRPF31* form of splicing-factor RP, which has been well documented in patients with a variety of mutations (Al-Maghtheh, et al., 1996; Evans, et al., 1995) there has been no published overview of *PRPF8*-RP phenotype across a range of mutations. Patients and clinicians would value such an analysis since it could help provide a more accurate prognosis. Here we report three new *PRPF8* mutations, including one outside exon 42, and a de-novo case of a known mutation; review all published *PRPF8* mutations; present clinical data on the new cases and additional clinical data on two published *PRPF8*-RP families; and review the published clinical observations in eight further *PRPF8* families, in order to gain a clearer picture of this form of RP. The severity of retinal degeneration observed in patients with the three most common PRPF8 mutations correlates with the severity of the temperature-sensitive growth phenotype found in haploid yeast (*Sacchromyces cerivisia*) strains carrying the equivalent mutations. As

the mechanism underlying the yeast phenotype is known, this correlation between yeast and human phenotypes implies that a similar defect may underlie the human condition.

MATERIALS AND METHODS

Patients and Control subjects

Data presented in Table 1 include new clinical information on two families for which phenotypes were published previously only as brief summaries in the genetics literature (identified in Table 1 as British1 (McKie, et al., 2001; Tarttelin, et al., 1996) and South African (Greenberg, et al., 1994; McKie, et al., 2001)). In addition, published clinical information from eight families are summarised (identified in Table 1 as Dutch (van Lith-Verhoeven, et al., 2002) Japanese (Kondo, et al., 2003), Spanish1-4 (Martinez-Gimeno, et al., 2003), US1 (Walia, et al., 2008) and US2 (Kojis, et al., 1996). Lastly, clinical information is given for the *de novo* case (British2) and three new families (British 3, 4 and 5) described herein. No clinical information is available for patients with the remaining published mutations.

Nucleotide numbering in the text and tables reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (accession number NM_006445.3), according to journal guidelines (www.hgvs.org/mutnomen). Mutations are described at the protein level by numbered codons based on the same reference sequence, with the initiation codon as codon 1.

For the new cases, after obtaining informed consent, a full ophthalmic examination was conducted, including Goldman perimetry, Optical Coherence Tomography (OCT) and, where possible, photopic and scotopic ERG testing. A medical history was also obtained and a sample of peripheral blood taken. Genomic DNA was extracted from the blood using the QIAamp DNA Blood Midi Kit (Qiagen, Crawley, United Kingdom) according to the manufacturer's instructions.

Although the clinical data in Table 1 are incomplete, mean acuity in the better seeing eye was calculated for the three most common and the remaining mutations where this information was available. This calculation was made by converting Snellen acuity into an ETDRS letter score. Acuities of hand movements and counting fingers were assumed to be equivalent to 0 ETDRS letters. The Mann-Whitney *U* test was used to evaluate the significance of apparent differences in age of onset between patients with the H2309P mutation and those with R2310K or H2309R, since the H2309P data are not normally distributed. The significance of the difference in age of onset between patients with R2310K and patients with H2309R was tested by the *t* test since the values of these samples were normally distributed.

Microsatellite analysis to confirm paternity for the de novo mutation case

Aliquots of DNA from affected and unaffected family members were genotyped for seven different microsatellite markers on three different chromosomes. The products were mixed with the size standard GeneScan 500-ROX (Applied Biosystems, Warrington, United Kingdom) and resolved by electrophoresis on a 3130xl Genetic Analyser (Applied Biosystems). The results were analysed using the GeneMapper version 4.0 software (Applied Biosystems).

DNA sequencing

Specific primer pairs were selected encompassing all exons of *PRPF8* (Table 2). These were utilised in the PCR to amplify products which were initially digested with ExoSAP-IT (GE Healthcare, Chalfont St. Giles, United Kingdom) according to the supplier's instructions. The digested DNA was sequenced directly using the BigDye Terminator version 3.1 Cycle Sequencing Kit and the 3130xl Genetic Analyser according to the manufacturer's instructions (Applied Biosystems).

RESULTS

Clinical and genetic analysis of new cases

Table 3 summarises all *PRPF8* RP causing mutations known to date. Most have been reported elsewhere but we report three novel mutations, S2118F, R2310S and Y2334N.

Y2334N was identified in a Caucasian father and son diagnosed with dominant RP at Moorfields Eye Hospital. The father was referred by his GP while the son, who accompanied his father, was unaware of symptoms on the first visit. Subsequently that grandfather was seen aged 67 years. He reported symptoms from late teens but maintained central macular function. Fundus photography and autofluorescence in all three generations revealed a typical RP fundal appearance with bone-spicule pigmentation in the mid periphery and extensive retinal pigment epithelial atrophy (Figs 1a-c and Fig 2). OCT analysis revealed cystoid macular oedema (CMO) in the son.

R2310S was identified in a single patient with RP seen at Moorfields eye hospital. The patient gave a family history consistent with dominant inheritance but no further family members were examined. He was first aware of symptoms in his late teens and had severely constricted visual fields on examination age 35. Ophthalmic examination revealed bone-spicule pigmentation and RPE atrophy.

The S2118F mutation was identified in a large family segregating RP in a dominant fashion. Linkage analysis implicated a mutation in the *PRPF8* gene but screening of *PRPF8* exon 42 failed to reveal a mutation. It was then decided to sequence additional 3' exons, leading to the identification of the S2118F missense change in exon 38. Only clinical details for the proband were available for this study. Examination of this 57 year old male revealed extensive bone-spicule pigmentation, cataracts and constricted visual fields.

Each of these mutations change amino-acids conserved in all eukaryotes. The substitutions involved are non-conservative, giving negative scores on the Blosum62 matrix. These mutations were excluded from over 120 ethnically matched Caucasian control DNAs and Y2334N was also shown to segregate with RP in the family. These mutations are therefore highly likely to be the cause of RP in these families and cases, as defined by the criteria used by Stone 2003 (Stone, 2003).

We also identified a case with a *de novo* missense mutation. This 9-year-old Caucasian boy was a tertiary referral to the eye department, St James's hospital, Leeds, to investigate symptoms of night blindness. On ophthalmic examination RP was diagnosed, but both parents showed normal visual acuity, fundi and visual fields to confrontation. DNA was obtained and screened for known exonic hotspots for retinal degeneration causing mutations. This identified the known H2309P mutation in *PRPF8*. However, on testing, this change was found to be absent from both parents. Paternal status was confirmed by genotyping seven microsatellite markers on three different chromosomes in the boy and both parents (data not shown). This confirmed that RP in this patient was the result of a *de novo* mutation event. Fundus photography in the patient revealed a typical RP fundal appearance with bone-spicule pigmentation in the mid periphery (Fig 1d). OCT analysis showed CMO in both eyes, with a central retinal thickness in the right eye of 591 microns and an indistinct photoreceptor layer (Fig 3).

Genotype-Phenotype analysis

The data presented in Table 1 are derived from fourteen families with various *PRPF8* mutations. In all, clinical and genetic information is presented for 75 *PRPF8* RP patients, together with a limited overview of phenotype for one further family. The clinical information available for each case is often incomplete, since the clinical notes and/or published descriptions varied widely in the detail available. Nevertheless, data were available in most cases on two key clinical indicators, age at onset of night blindness or visual field loss and visual acuity at age of examination, and these have allowed a number of observations to be made.

Table 1. Summary clinical data for individuals from published PRPF8-RP families and for the three new families and the de novo case.

Legend: NR= non-recordable; VA= visual acuity; CMO= central macular oedema; CF= counting fingers; HM= hand movements; PSCLO= posterior subcapsular lens opacity; PSCC= posterior subcapsular cortical cataract. References; Dutch family (van Lith-Verhoeven, et al., 2002), British 1 (McKie, et al., 2001; Tarttelin, et al., 1996), British 2 (described herein), South African (Greenberg, et al., 1994; McKie, et al., 2001), US1 (Walia, et al., 2008), Japanese (Kondo, et al., 2003), Spanish 1-4 (Martinez-Gimeno, et al., 2003), British 3, 4 and 5 (described herein) and US2 (Kojis, et al., 1996).

Identifier	Sex	Age @ onset	Age @ exam	Visual field	Origin	VA Rt	VA Lt	ERG	Comments	Mutation	Reference
III-1	f	10	69	10°	dutch	CF	6/9	NR		R2310K	van Lith-Verhoeven, et al., 2002
III-5	m	40	64	temp scotoma	dutch	6/7.5	6/19	NR	milder phenotype	R2310K	"
III-6	f	6	76	10°	dutch	6/120	6/7.5	NR		R2310K	"
III-7	m	40	68	10°	dutch	6/19	6/9	NR		R2310K	"
III-10	f	16	65	10°	dutch	6/30	6/15	NR		R2310K	"
IV-1	m	16	36	10°	dutch	6/7.5	6/9	NR		R2310K	"
IV-4	m	35	36	concentric depression	dutch	6/7.5	6/9	abnormal		R2310K	"
IV-6	f	15	36	ring scotoma	dutch	6/15	6/15	NR		R2310K	"
IV-7	m	20	13	ring scotoma	dutch	6/4.8	6/4.8	abnormal		R2310K	"
FH	m	5	38	10°	british1	6/18	6/60		blind @ 30, cataract surgery @ 46	H2309P	McKie, et al., 2001; Tarttelin, et al., 1996
TH	m	5			british1				registred blind @ 25	H2309P	"
DH	f	5	10	5°	british1	6/18	6/18		no change in VA in past 10 years	H2309P	"
LH	m	>10	14		british1	HM	HM		blind at 14	H2309P	"
JH	f		37	5°	british1	6/36	6/24		PSCLO	H2309P	"
DL	f	9	24	10°	british1	6/12	6/18		CMO	H2309P	"
LL	f	4	4		british1	6/9	6/9			H2309P	"

Identifier	Sex	Age @ onset	Age @ exam	Visual field	Origin	VA Rt	VA Lt	ERG	Comments	Mutation	Reference
NT	m	7	11	15°	british1	6/24	6/36		CMO	H2309P	"
DT	f	10	33	5°	british1	6/36	6/36		CMO	H2309P	"
GFH	m	5	72		british1	6/18	6/36		cataract surgery @ 66	H2309P	"
1014144	f		34	50°	british1	6/12	6/9.5			H2309P	"
176571	f		29	40°	british1	6/6	6/9	NR		H2309P	"
547129	f	5	64	<10°	british1	6/60	6/60		cataract	H2309P	"
1111882	f	24	34	15°	british1	6/15	6/15		mild PSCC	H2309P	"
MCEC7507	m	7	7	unknown	british1	3/3.8	3/3.8			H2309P	"
De novo case	m	8	11	10°	british2	6/12	6/12	NR	CMO	H2309P	Unpublished
RPD 5.21	m	9	45	constriction	south afr	CF	6/30		ON drusen, deterioration @ 30	H2309R	Greenberg, et al., 1994; McKie, et al., 2001
RPD 5.22	f	13	17		south afr	6/9	6/20	abnormal	early cataracts	H2309R	"
RPD 5.38	m	<10	18		south afr				cataracts, osteogenesis imperfecta	H2309R	"
RPD 5.36	f	15	40		south afr				vision deteriorated in mid-40s	H2309R	"
RPD 5.3	f	15			south afr					H2309R	"
RPD 5.4	m	<10	72	constriction	south afr	CF	CF	NR	cataract surgery @ 65	H2309R	"
RPD 5.8	m	7	7		south afr	6/18	6/18			H2309R	"
RPD 5.9	m	<10			south afr				early PSCLO	H2309R	"
RPD 5.12	f	<10			south afr	6/7.5	6/7.5			H2309R	"
RPD 5.16	f				south afr	6/12	6/9			H2309R	"
RPD5.21	m	9			south afr				deteriorated @ 40, optic nerve drusen	H2309R	"
RPD5.23	m	10			south afr				cataracts	H2309R	"

Identifier	Sex	Age @ onset	Age @ exam	Visual field	Origin	VA Rt	VA Lt	ERG	Comments	Mutation	Reference
RPD 5.26	f	17			south afr				cataracts	H2309R	"
RPD 5.31	m				south afr	6/7.5	6/7.5		cataracts	H2309R	"
RPD5.58	f	4	10		south afr	6/12	6/12		ostogenesis imperfecta	H2309R	"
RPD5.59	m	4		constriction	south afr	6/12	6/18			H2309R	"
II:5	f		54		US1	6/60	6/60		CMO	H2309R	Walia, et al., 2008
III:6	f		43		US1	6/9	6/9	NR		H2309R	"
III:7	f		44		US1	6/7.5	6/7.5			H2309R	"
III:10	f		28		US1	6/60	6/24		CMO	H2309R	"
III:11	f		35		US1	6/9	HM		CMO	H2309R	"
III:12	f		26		US1	6/12	6/12	NR	CMO	H2309R	"
IV:8	m		33		US1	6/12	6/9	NR		H2309R	"
IV:12	m		8		US1	6/7.5	6/7.5			H2309R	"
IV:16	m		14		US1	6/6	6/7.5	NR		H2309R	"
IV:19	f		29		US1	6/60	6/120	NR	CMO	H2309R	"
IV:20	f		25		US1	CF	CF		CMO	H2309R	"
IV:21	f		5		US1	6/7.5	6/7.5			H2309R	"
IV:25	m		12		US1	6/7.5	6/7.5			H2309R	"
V:6	m		7		US1	6/12	6/12			H2309R	"
II-4	m	10	56	10°	japanese	48/60	48/60			E2324fsX2359	Kondo, et al., 2003
	m		3		japanese					E2324fsX2359	"
	f		2		japanese					E2324fsX2359	"
	m	10	59		japanese	HM	HM			E2324fsX2359	"
m323 II2	m	20	77	<10°	spanish1	6/60	6/60	NR		R2310G	Martinez-Gimeno, et al., 2003
m323 III1	m	21	50	10°	spanish1	6/48	6/38	NR		R2310G	"

Identifier	Sex	Age @ onset	Age @ exam	Visual field	Origin	VA Rt	VA Lt	ERG	Comments	Mutation	Reference
m323 IV2	m	7	18	annular scotoma	spanish1	6/30	6/30	NR		R2310G	"
m618 II1	f	5	45	10°	spanish2	6/36	6/36		cataract age 15	L2298fsX2337	"
m618 III1	f	5	19	30°	spanish2	6/12	6/12		cataract age 18	L2298fsX2337	"
v17 II1	f	20	64	10°	spanish3	6/60	6/60	NR		L2315fsX2358	"
v17 III1	m	10	40	10°	spanish3	6/38	6/48	NR		L2315fsX2358	"
v17 III2	m	17	34	10°	spanish3	6/7.5	6/6	NR		L2315fsX2358	"
sjd III2	f	10	70		spanish4					V2325fsX2329	"
sjd III4	m	10	65	10°	spanish4	12/60	6/60	NR		V2325fsX2329	"
sjd IV2	f	10	29	10°	spanish4	18/60	24/60	NR		V2325fsX2329	"
MEH-GC16993	m	late teens	67	5°	british3	6/18	6/24			Y2334N	Unpublished
MEH-GC16993	m	childhood	48		british3	6/9	6/9		cataract age 50	Y2334N	Unpublished
MEH-GC16993	m	16	15		british3	6/9	6/9	NR	CMO	Y2334N	Unpublished
MEH-GC16352	m	18	35	5°	british4	6/24	2/60		Cataract age 24	R2310S	Unpublished
MEH-GC171	m	Early twenties	57	15°	british5	HM	6/18		Cataract age 55	S2118F	Unpublished
ucla RP09	family			severe constriction 4 patients	US2			NR in 4 patients		E2331fsX2358	Kojis, et al., 1996

Table 2. Primers used to amplify the coding exons of PRPF8 and also including the 5' non-coding exon

Name	Sequence	Name	Sequence
r1F1	CGCTGGGAACGCGGAGCGG	r6R	GAGTGCCTTGGACGTAAAGAAG
r6F	ACACTACCTATGATGTCGACTCT	r10R	ATGCTTCAGGGCATTCAGCACA
r9F	CAATACAGCCAATGGCATTGCC	r16R	GTTGTAGTGGGCAGTGTTGGTC
r16F	CGCTGCTGGAAAGCCAACATTC	rR20R	CACGTCCTGCAGGTTATTGATG
r19F	CATGGATCTGTATAGCCACCTC	r23R	GTAACCTCATTCTGCAGGTTCC
r23F	GCGGTATTCTGGGACATCAAGA	r27R	TGTTCTGAGCAATGGCCTCTTG
r26F	CGTTCAGGAATGAGCCATGAAG	r32R	GTGGTGTAGTCCAGGAACTTG
r31F	TGCCTCCTATAAGTGGAATGTCT	r43R	AGAGGCCAAACTGCTGAATGTC
e43F	TAGCAGTAGGGATAAGGTGAGG	e43R	CAGCATCTTGCTGTGAACCGC

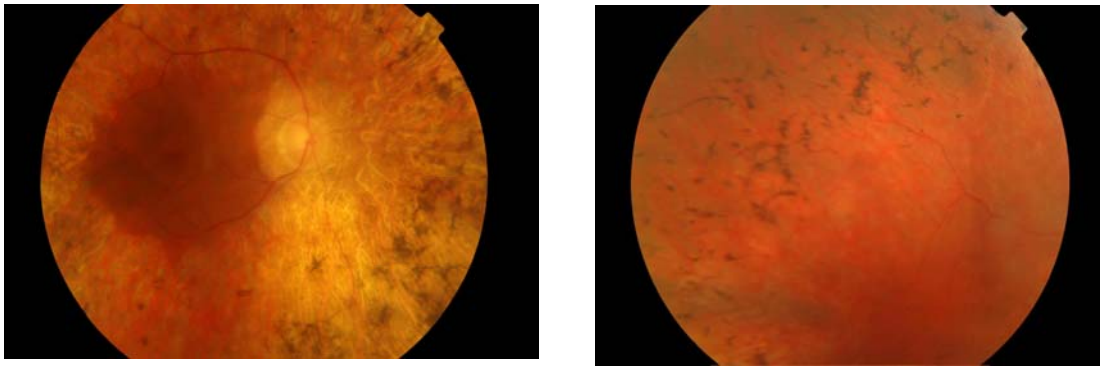
Primer numbers include this non-coding exon, so that primers e43F and e43R in fact amplify what has been referred to in the previous literature on this gene as exon 42.

Table 3. Table summarising all known PRPF8 (RP13) RP causing mutations.

PRPF8 nucleotide change	**Mutation Type**	**Amino Acid change**	**Reference**
c.6353C>T	missense	S2118F	new
c.6901C>A	missense	P2301T	(McKie, et al., 2001)
c.6901C>T	missense	P2301S	(Testa, et al., 2006; Ziviello, et al., 2005)
c.6912C>G	missense	F2304L	(McKie, et al., 2001)
c.6926A>C	missense	H2309P	(McKie, et al., 2001)
c.6926A>G	missense	H2309R	(McKie, et al., 2001)
c.6928A>G	missense	R2310G	(McKie, et al., 2001)
c.6929G>A	missense	R2310K	(McKie, et al., 2001)
c.6930G>C	misense	R2310S	new
c.6942C>A	missense	F2314L	(McKie, et al., 2001)
c.6943-6944delC	frame-shift	L2315fsX2358	(Martinez-Gimeno, et al., 2003)
c.6974-6994del21bp	frame-shift	V2325fsX2329	(Martinez-Gimeno, et al., 2003)
c.6893-6896delins7bp	frame-shift	L2298fsX2336	(Martinez-Gimeno, et al., 2003)
c.6972-6977del6bpins11bp	frame-shift	2325fsX2359	(Kondo, et al., 2003)
c.6991delG	frame-shift	E2331fsX2358	(Sullivan, et al., 2006)
IVS exon 41/ 42 junction IVS41-4G>A	Splice site change		(Sullivan, et al., 2006)
c.6961CAG>TAG	Stop	Q2321X	(De Erkenez AC, 2002)
c.7000T>A	missense	Y2334N	new
c.7006T>C	frame-shift	Stop2336fsX2377	(Martinez-Gimeno, et al., 2003)

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (accession number NG_009118), according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

A. B.



C. D.

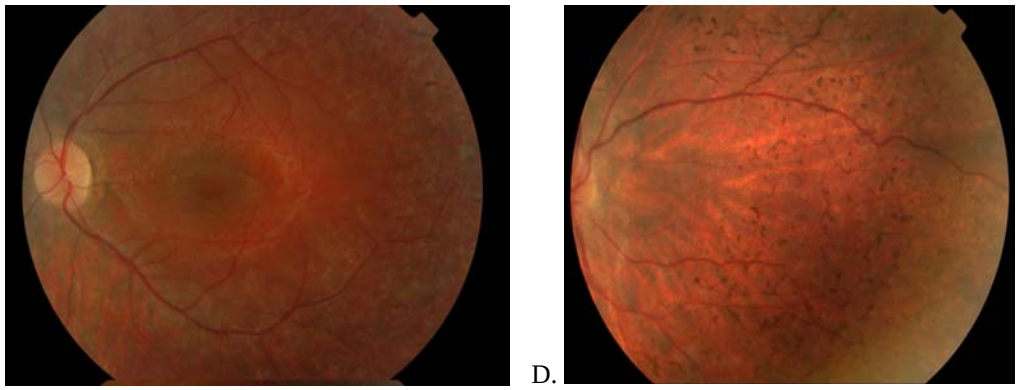


Figure 1. Fundus photography in the left eyes of A) Grandfather, B) father and C) son with the Y2334N mutation and D) the *de novo* case with H2309P, reveal characteristic bone-spicule pigmentation in the mid periphery.

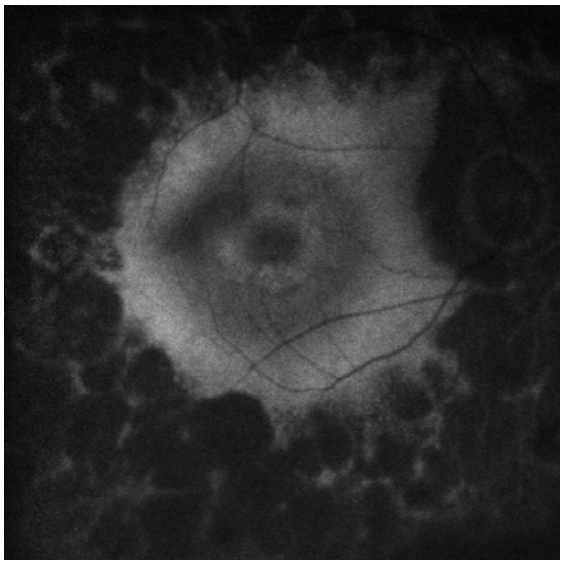


Figure 2. Fundus autoflourescence of the grandfather in the family segregating the Y2334N mutation, showing marked loss of RPE to within 10 degrees of fixation.

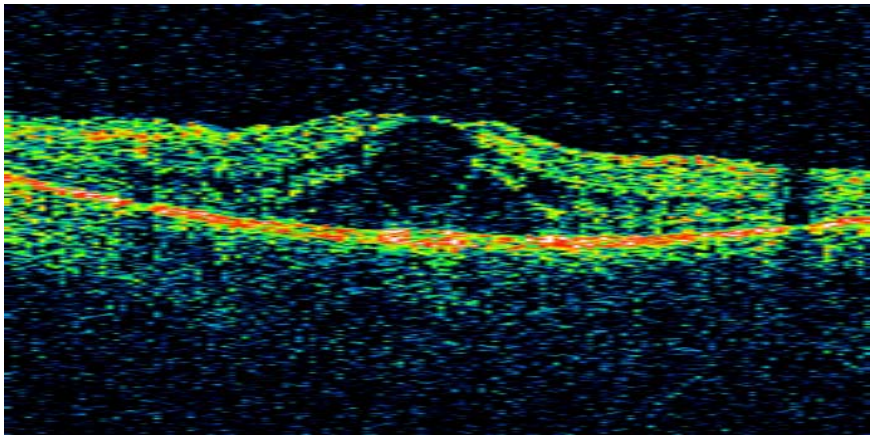


Figure 3. OCT image of the left eye in the *de novo* H2309P mutation-carrying case, showing cystoid macula oedema

Visual field loss to within 10 degrees of fixation was recorded in 23 of the 29 subjects for whom numeric data were available. In most cases, loss of peripheral retina function or night blindness was first reported between the first and second decades. Statistical analysis was performed to determine whether there are significant differences between age of onset of disease in patients with different disease-causing mutations. Due to the limited information available and the small sample sizes of some of the mutation categories, this was only possible for three of the RP-causing mutations; H2309P, H2309R and R2310K. The average age of onset of nyctalopia for each of these mutations is 6.6, 10.7 and 22 years respectively. Statistical analysis showed significant differences between H2309P and R2310K (p=0.001), between H2309R and R2310K (p=0.02) and between H2309P and H2309R (p=0.026). This implies that patients carrying H2309P or H2309R mutations have a more severe phenotype, reflected by earlier onset of disease, than patients carrying the R2310K mutation.

Acuity in the better eye was compared between the three most common mutations, all other *PRPF8* mutations and with cumulated average data on generic RP obtained from the literature (Marmor 1980, Grover et al 1999) (see Table 4). This suggests that the R2310K mutation is associated with relatively good acuity in all age ranges, by comparison with approximate figures for RP in general, but patients with the H2309P or H2309R mutations appear to have similar or worse visual loss than would be expected for combined RP. The scatterplot (Figure 4) suggests a trend towards worse acuity with increasing age for all published *PRPF8* cases combined and for each of the three common mutations. However, the inclination of the trend for the combined data is not particularly steep, indicating that visual loss with *PRPF8* mutations tends to be similar in late adulthood as it was at presentation. Comparison with combined RP figures suggest that the overall progression of PRPF8-RP is the same or slightly worse than expected for RP in general. However separate plots of the same measurements in cases with the three different mutations for which information is available again reveal that RP in patients with the H2309P or H2309R mutations progress more rapidly and have a poorer outcome than those with the R2310K mutation.

CMO was reported in 11 of 75 subjects and posterior subcapsular cataract or other significant cataract was noted in 17 of 75 subjects. Both these figures are the minimum prevalence for these phenotypic variables as reporting is almost certainly incomplete.

Table 4. ETDRS letter score in the better seeing eye for the three most common and other PRPF8 mutations and comparison with cumulated average acuities from previous RP progression studies.

	<20 years	**20-39 years**	**30-59 years**	**>60 years**
R2310K	92 (n=1)	75 (n=3)		75 (n=5)
H2309P	56 (n=6)	65 (n=7)		47 (n=2)
H2309R	75 (n=8)	51 (n=6)	60 (n=4)	0 (n=1)
Others	60 (n=2)	70 (n=2)	43 (n=4)	40 (n=4)
Combined RP Marmor 1980	75		60	
Combined RP Grover et al 1999			68	54

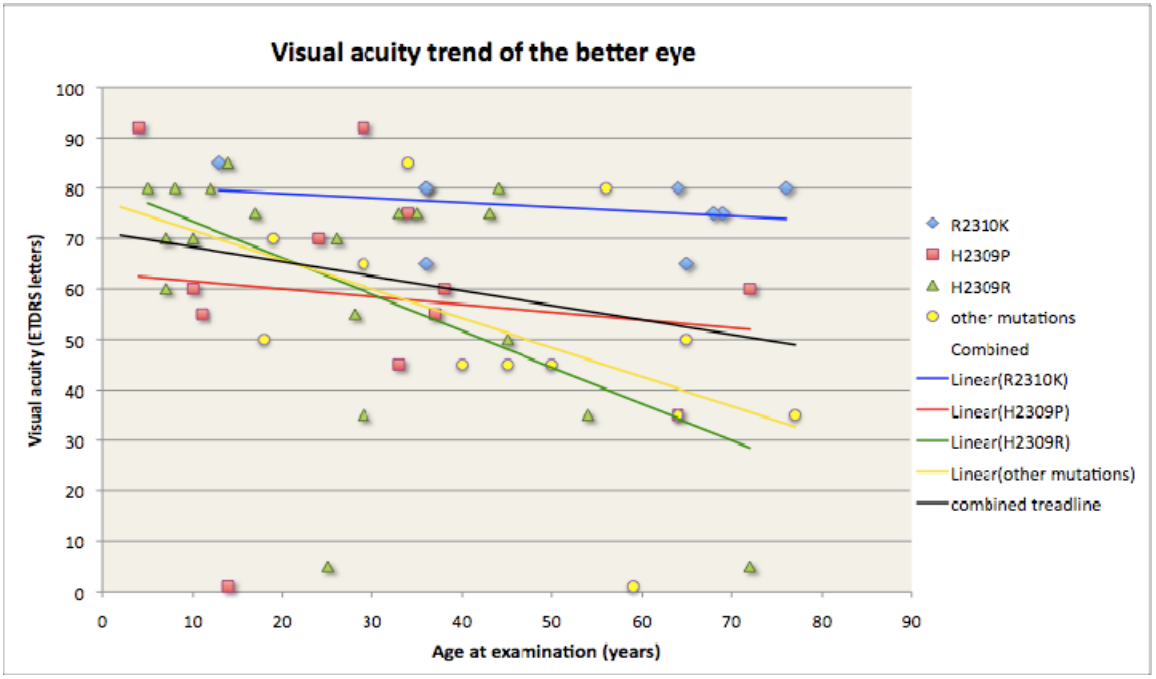


Figure 4. Graph comparing visual acuity in the better eye between the different *PRPF8*-causing mutations.

DISCUSSION

The report of S2118F, R2310S and Y2334N mutations brings the total number of *PRPF8*-RP mutations to nineteen, accounting for approximately 1% of all RP cases. S2118F is the first *PRPF8* mutation found outside exon 42, which has significant implications for future genetic screening in RP. Nevertheless, these mutations are highly clustered at the C-terminus and are either missense changes or are nonsense mutations that change or delete at most only the last 37 amino-acids of this remarkably conserved 2335 amino acid protein. This pattern of mutations makes it likely that mutated PRPF8 protein will be present in patient cells, a theory supported by the observation that a *PRPF8* mRNA containing one of the C-terminal frameshift mutations was not subject to nonsense mediated decay (Gamundi, et al., 2008). Furthermore, a mouse knock-in model with the H2309P *PRPF8* mutation exhibits sub-retinal pigment epithelium (RPE) deposits at two years of age (Graziotto et al 2010) but a PRPF8 knockout has no observable abnormalities (J.Graziotto, Q.Zhang and E.A.Pierce, unpublished). These data are all consistent with the hypothesis that PRPF8-RP is the result of presence of abnormal protein. However, in contrast, haploid yeast with H2387P, equivalent to the human H2309P mutation, have a growth sensitive phenotype, but the diploid heterozygous yeast grow completely normally (Boon, et al., 2007). This finding suggests that gene replacement therapy could still prove effective in this form of RP.

The H2309P mutation has been reported previously in a large UK family (british1) and therefore when it was identified in another UK patient the initial expectation was that this individual was related to the original family. However, our analysis showed that it was in fact a *de novo* mutation. Such a finding is not unprecedented but is a relatively rare occurrence (Schwartz, et al., 2003). This observation could imply that this codon, and potentially this region of the gene, may in fact represent a mutation hotspot, rather than a domain essential to retinal function as previously proposed (McKie, et al., 2001). The existence of such mutations also confirms that it is important for clinicians to consider the possibility of dominant disease even in isolated patients with RP, who are generally considered more likely to have recessive disease.

Much of the published literature on *PRPF8*-RP (also known as RP13) concentrates on genetic analysis, with clinical findings often described only briefly and in limited detail. Four papers (Kondo, et al., 2003; Martinez-Gimeno, et al., 2003; van Lith-Verhoeven, et al., 2002; Walia, et al., 2008) provide more detailed descriptions of the phenotype associated with individual mutations but give at best only a limited comparison with clinical findings in other families. Here we present clinical data on a family and two cases with new mutations, a *de novo* case of a known mutation, additional clinical data on two published families, and an overview of previously published clinical findings in eight further families. On the basis of these data, while dominantly inherited RP due to mutations in the *PRPF8* gene can indeed be severe, overall prognosis for visual acuity is not as bleak as some previous reports have suggested. Nyctalopia, visual field constriction and an abnormal or non-recordable ERG are consistent early findings. However, visual acuity in the better eye often remains normal or near-normal into middle-age. Furthermore Figure 4 shows only a modest overall trend towards a decline in vision with age, suggesting that this phenotype is not necessarily associated with profound or rapidly deteriorating visual loss. Comparison with acuity figures for progression of combined RP suggests that *PRPF8*-RP in general is similar or slightly worse than RP in general, but that at least one PRPF8 mutation, R2310K, gives rise to milder form of RP, while others are more severe. Some individuals with *PRPF8*-RP retain good visual acuity into the sixth or even seventh decade, and only 9 of the 64 cases for which acuity data are available have acuity in the better eye of 6/60 or worse. A similar range in severity has been documented for other forms of autosomal dominant RP (Jacobson, et al., 2000).

The data most consistently available for patients, and which therefore allow comparison between patients with the different mutations, are age at onset of nyctalopia or visual field loss and visual acuity at a given age. We therefore compared these measurements in patients grouped according to the mutation they carried. This was only possible for the H2309P, H2309R and R2310K mutations, for which data were available on larger numbers of patients than the remaining mutations. Statistical analysis showed that H2309P conferred the earliest onset of night blindness and had the most severe prognosis for visual acuity, that H2309R mutation carriers have less severe symptoms and that R2310K carriers have the best outcome for these indicators.

It is interesting to compare these findings with two recent studies performed in yeast (Boon, et al., 2007; Maeder, et al., 2009). Both studies showed that the mutations which cause RP in human patients, when present in yeast Prp8 protein, result in its reduced association with the DEAD-box helicase Brr2p in the yeast nucleus (Boon, et al., 2007; Maeder, et al., 2009). The association of Prp8p with Brr2p is thought to be essential for the

production of a mature spliceosome and yeast carrying the RP-causing mutations have reduced splicing activity. These studies also noted differences in the specific effects of the different RP-causing mutations. Yeast Prp8p protein with mutations equivalent to H2309P and H2309R showed no association with Brr2p, while that carrying the R2310K-equivalent was still capable of interaction but at a reduced level. Furthermore, haploid yeast with the RP-causing mutations show a temperature sensitive growth phenotype, with the yeast equivalents of H2309R and H2309P having more severe phenotypes than R2310K. It is therefore evident that the phenotype caused by these mutations in yeast, and their effect on interaction with Brr2, correlates with the severity of RP caused by their human equivalent mutations. This correlation is tentative since information is only available for three of the nineteen mutations identified in human patients, and additional studies are required to determine whether other pathogenic mechanisms also contribute to clinical expression in patients with *PRPF8* mutations. Nevertheless these observations provide suggestive evidence that human RP may be a consequence of defective splicing due to a mechanism similar to that observed by Boon and colleagues in yeast (Boon, et al., 2007). However, splicing deficiency studies in human cell lines have been equivocal. Several laboratories reported that splicing factor RP-causing mutations caused measurable abnormalities in splicing *in vitro* (Ivings, et al., 2008; Mordes, et al., 2007; Wilkie, et al., 2008), but microarray analysis *in vivo* revealed no consistent splicing defects (Gamundi, et al., 2008; Rivolta, et al., 2006). Furthermore, the question as to why only the retina is affected in human patients remains unanswered.

In summary, we report three new mutations and a de novo mutation in *PRPF8*, bringing the total number of RP-causing mutations in this gene to nineteen. We have reviewed the RP phenotype associated with these mutations and find that, while the effect on peripheral retinal function is severe, patients generally retain good visual acuity in at least one eye until the fifth or sixth decade. We also find that patients with the H2309P or H2309R mutations have a worse prognosis than those with R2310K, and note that yeast lines carrying the equivalent mutations show the same severity trend for a temperature-sensitive growth phenotype and for interaction with Brr2, which may imply a common mechanism underlying the human and yeast phenotypes.

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REFERENCES

Abelson J. 2008. Is the spliceosome a ribonucleoprotein enzyme? Nat Struct Mol Biol 15(12):1235-7.

Abu-Safieh L, Vithana EN, Mantel I, Holder GE, Pelosini L, Bird AC, Bhattacharya SS. 2006. A large deletion in the adRP gene PRPF31: evidence that haploinsufficiency is the cause of disease. Mol Vis 12:384-8.

Al-Maghtheh M, Vithana E, Tarttelin E, Jay M, Evans K, Moore T, Bhattacharya S, Inglehearn CF. 1996. Evidence for a major retinitis pigmentosa locus on 19q13.4 (RP11) and association with a unique bimodal expressivity phenotype. Am J Hum Genet 59(4):864-71.

Boon KL, Grainger RJ, Ehsani P, Barrass JD, Auchynnikava T, Inglehearn CF, Beggs JD. 2007. prp8 mutations that cause human retinitis pigmentosa lead to a U5 snRNP maturation defect in yeast. Nat Struct Mol Biol 14(11):1077-83.

Boon KL, Norman CM, Grainger RJ, Newman AJ, Beggs JD. 2006. Prp8p dissection reveals domain structure and protein interaction sites. RNA 12(2):198-205.

Bunker CH, Berson EL, Bromley WC, Hayes RP, Roderick TH. 1984. Prevalence of retinitis pigmentosa in Maine. Am J Ophthalmol 97(3):357-65.

Chakarova CF, Hims MM, Bolz H, Abu-Safieh L, Patel RJ, Papaioannou MG, Inglehearn CF, Keen TJ, Willis C, Moore AT, Rosenberg T, Webster AR, Bird AC, Gal A, Hunt D, Vithana EN, Bhattacharya SS. 2002. Mutations in HPRP3, a third member of pre-mRNA splicing factor genes, implicated in autosomal dominant retinitis pigmentosa. Hum Mol Genet 11(1):87-92.

De Erkenez AC BE, Dryja TP. 2002. Novel mutations in the PRPC8 gene, encoding a pre-mRNA splicing factor in patients with autosomal dominant Retinitis Pigmentosa. Invest. Ophthalmol. Vis. Sci.(43; ARVO abstract 791).

Evans K, al-Maghtheh M, Fitzke FW, Moore AT, Jay M, Inglehearn CF, Arden GB, Bird AC. 1995. Bimodal expressivity in dominant retinitis pigmentosa genetically linked to chromosome 19q. Br J Ophthalmol 79(9):841-6.

Gamundi MJ, Hernan I, Muntanyola M, Maseras M, Lopez-Romero P, Alvarez R, Dopazo A, Borrego S, Carballo M. 2008. Transcriptional expression of cis-acting and trans-acting splicing mutations cause autosomal dominant retinitis pigmentosa. Hum Mutat 29(6):869-78.

Grainger RJ, Beggs JD. 2005. Prp8 protein: at the heart of the spliceosome. RNA 11(5):533-57.

Graziotto JJ, Farkas MH, Bujakowska K, Deramaudt, BM, Zhang Q, Nandrot EF, Inglehearn CF, Bhattacharya SS, Pierce EA. 2009. Three gene-targeted mouse models of RNA splicing factor retintis pigmentosa show late onset RPE and retinal degeneration. Invest. Ophthalmol. Vis. Sci. Submitted

Greenberg J, Goliath R, Beighton P, Ramesar R. 1994. A new locus for autosomal dominant retinitis pigmentosa on the short arm of chromosome 17. Hum Mol Genet 3(6):915-8.

Grover S, Fishman GA, Anderson RJ, Tozatti MSV, Heckenlively JR, Weleber RG, Edwards AO, Brown J. 1999. Visual acuity impairment in patients with retinitis pigmentosa at age 45 years and older. Ophthalmology 106:1780-85.

Hims MM, Daiger SP, Inglehearn CF. 2003. Retinitis pigmentosa: genes, proteins and prospects. Dev Ophthalmol 37:109-25.

Hodges PE, Jackson SP, Brown JD, Beggs JD. 1995. Extraordinary sequence conservation of the PRP8 splicing factor. Yeast 11(4):337-42.

Ivings L, Towns KV, Matin MA, Taylor C, Ponchel F, Grainger RJ, Ramesar RS, Mackey DA, Inglehearn CF. 2008. Evaluation of splicing efficiency in lymphoblastoid cell lines from patients with splicing-factor retinitis pigmentosa. Mol Vis 14:2357-66.

Jacobson SG, Cideciyan AV, Iannaccone A, Weleber RG, Fishman GA, Maguire AM, Affatigato LM, Bennett J, Pierce EA, Danciger M, Farber DB, Stone EM. 2000. Disease expression of RP1 mutations causing autosomal dominant retinitis pigmentosa. Invest Ophthalmol Vis Sci 41(7):1898-908.

Keen TJ, Hims MM, McKie AB, Moore AT, Doran RM, Mackey DA, Mansfield DC, Mueller RF, Bhattacharya SS, Bird AC, Markham AF, Inglehearn CF. 2002. Mutations in a protein target of the Pim-1 kinase associated with the RP9 form of autosomal dominant retinitis pigmentosa. Eur J Hum Genet 10(4):245-9.

Kojis TL, Heinzmann C, Flodman P, Ngo JT, Sparkes RS, Spence MA, Bateman JB, Heckenlively JR. 1996. Map refinement of locus RP13 to human chromosome 17p13.3 in a second family with autosomal dominant retinitis pigmentosa. Am J Hum Genet 58(2):347-55.

Kondo H, Tahira T, Mizota A, Adachi-Usami E, Oshima K, Hayashi K. 2003. Diagnosis of autosomal dominant retinitis pigmentosa by linkage-based exclusion screening with multiple locus-specific microsatellite markers. Invest Ophthalmol Vis Sci 44(3):1275-81.

Luo HR, Moreau GA, Levin N, Moore MJ. 1999. The human Prp8 protein is a component of both U2- and U12-dependent spliceosomes. RNA 5(7):893-908.

Maeder C, Kutach AK, Guthrie C. 2009. ATP-dependent unwinding of U4/U6 snRNAs by the Brr2 helicase requires the C terminus of Prp8. Nat Struct Mol Biol 16(1):42-8.

Maita H, Kitaura H, Keen TJ, Inglehearn CF, Ariga H, Iguchi-Ariga SM. 2004. PAP-1, the mutated gene underlying the RP9 form of dominant retinitis pigmentosa, is a splicing factor. Exp Cell Res 300(2):283-96.

Marmor MF. 1980. Visual loss in retinitis pigmentosa. Am J Ophthalmol 89:692-8

Martinez-Gimeno M, Gamundi MJ, Hernan I, Maseras M, Milla E, Ayuso C, Garcia-Sandoval B, Beneyto M, Vilela C, Baiget M, Antinolo G, Carballo M. 2003. Mutations in the pre-mRNA splicing-factor genes PRPF3, PRPF8, and PRPF31 in Spanish families with autosomal dominant retinitis pigmentosa. Invest Ophthalmol Vis Sci 44(5):2171-7.

McKie AB, McHale JC, Keen TJ, Tarttelin EE, Goliath R, van Lith-Verhoeven JJ, Greenberg J, Ramesar RS, Hoyng CB, Cremers FP, Mackey DA, Bhattacharya SS, Bird AC, Markham AF, Inglehearn CF. 2001. Mutations in the pre-mRNA splicing factor gene PRPC8 in autosomal dominant retinitis pigmentosa (RP13). Hum Mol Genet 10(15):1555-62.

Mordes D, Luo X, Kar A, Kuo D, Xu L, Fushimi K, Yu G, Sternberg P, Jr., Wu JY. 2006. Pre-mRNA splicing and retinitis pigmentosa. Mol Vis 12:1259-71.

Mordes D, Yuan L, Xu L, Kawada M, Molday RS, Wu JY. 2007. Identification of photoreceptor genes affected by PRPF31 mutations associated with autosomal dominant retinitis pigmentosa. Neurobiol Dis 26(2):291-300.

Rivolta C, McGee TL, Rio Frio T, Jensen RV, Berson EL, Dryja TP. 2006. Variation in retinitis pigmentosa-11 (PRPF31 or RP11) gene expression between symptomatic and asymptomatic patients with dominant RP11 mutations. Hum Mutat 27(7):644-53.

Roepman R, Wolfrum U. 2007. Protein networks and complexes in photoreceptor cilia. Subcell Biochem 43:209-35.

Schwartz SB, Aleman TS, Cideciyan AV, Swaroop A, Jacobson SG, Stone EM. 2003. De novo mutation in the RP1 gene (Arg677ter) associated with retinitis pigmentosa. Invest Ophthalmol Vis Sci 44(8):3593-7.

Stevens SW, Abelson J. 1999. Purification of the yeast U4/U6.U5 small nuclear ribonucleoprotein particle and identification of its proteins. Proc Natl Acad Sci U S A 96(13):7226-31.

Stone EM. 2003. Finding and interpreting genetic variations that are important to ophthalmologists. Trans Am Ophthalmol Soc 101:437-84.

Sullivan LS, Bowne SJ, Birch DG, Hughbanks-Wheaton D, Heckenlively JR, Lewis RA, Garcia CA, Ruiz RS, Blanton SH, Northrup H, Gire AI, Seaman R, Duzkale H, Spellicy CJ, Zhu J, Shankar SP, Daiger SP. 2006. Prevalence of disease-causing mutations in families with autosomal dominant retinitis pigmentosa: a screen of known genes in 200 families. Invest Ophthalmol Vis Sci 47(7):3052-64.

Tarttelin EE, Plant C, Weissenbach J, Bird AC, Bhattacharya SS, Inglehearn CF. 1996. A new family linked to the RP13 locus for autosomal dominant retinitis pigmentosa on distal 17p. J Med Genet 33(6):518-20.

Testa F, Ziviello C, Rinaldi M, Rossi S, Di Iorio V, Interlandi E, Ciccodicola A, Banfi S, Simonelli F. 2006. Clinical phenotype of an Italian family with a new mutation in the PRPF8 gene. Eur J Ophthalmol 16(5):779-81.

Turner IA, Norman CM, Churcher MJ, Newman AJ. 2006. Dissection of Prp8 protein defines multiple interactions with crucial RNA sequences in the catalytic core of the spliceosome. RNA 12(3):375-86.

van Lith-Verhoeven JJ, van der Velde-Visser SD, Sohocki MM, Deutman AF, Brink HM, Cremers FP, Hoyng CB. 2002. Clinical characterization, linkage analysis, and PRPC8 mutation analysis of a family with autosomal dominant retinitis pigmentosa type 13 (RP13). Ophthalmic Genet 23(1):1-12.

Vithana EN, Abu-Safieh L, Allen MJ, Carey A, Papaioannou M, Chakarova C, Al-Maghtheh M, Ebenezer ND, Willis C, Moore AT, Bird AC, Hunt DM, Bhattacharya SS. 2001. A human homolog of yeast pre-mRNA splicing gene, PRP31, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (RP11). Mol Cell 8(2):375-81.

Walia S, Fishman GA, Zernant-Rajang J, Raime K, Allikmets R. 2008. Phenotypic expression of a PRPF8 gene mutation in a Large African American family. Arch Ophthalmol 126(8):1127-32.

Wilkie SE, Vaclavik V, Wu H, Bujakowska K, Chakarova CF, Bhattacharya SS, Warren MJ, Hunt DM. 2008. Disease mechanism for retinitis pigmentosa (RP11) caused by missense mutations in the splicing factor gene PRPF31. Mol Vis 14:683-90.

Ziviello C, Simonelli F, Testa F, Anastasi M, Marzoli SB, Falsini B, Ghiglione D, Macaluso C, Manitto MP, Garre C, Ciccodicola A, Rinaldi E, Banfi S. 2005. Molecular genetics of autosomal dominant retinitis pigmentosa (ADRP): a comprehensive study of 43 Italian families. J Med Genet 42(7):e47.