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Novel Deletion in the Pre-mRNA Splicing Gene *PRPF31* Causes Autosomal Dominant Retinitis Pigmentosa in a Large Chinese Family

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Abstract

We report the identification of a novel 12 bp deletion of the pre-mRNA splicing gene *PRPF31* in a large Chinese family with autosomal dominant retinitis pigmentosa (adRP). This mutation results in the deletion of four amino acids ($\Delta H_{111}K_{112}F_{113}I_{114}$) including H_{111}, an amino acid residue that is highly conserved throughout evolution. The 12 bp deletion co-segregates with the disease phenotype in 19 RP patients in the family, but is not present in unaffected relatives or 100 normal individuals. Our data indicate that the novel 12 bp deletion in *PRPF31* causes retinitis pigementosa in this Chinese adRP family. In contrast to the incomplete penetrance observed in most adRP families linked to chromosome band 19q13.4 (RP11), the 12 bp *PRPF31* deletion identified in this study appears to show high penetrance. These data expand the spectrum of *PRPF31* mutations causing adRP, and confirm the role of *PRPF31* in the pathogenesis of RP.

Keywords

retinitis pigmentosa; splicing; spliceosome; snRNP; *PRPF31* (*PRP31*); RP11; mutation; retinal degeneration and dystrophy; adRP

INTRODUCTION

Retinitis pigmentosa (RP) is a heterogeneous group of retinal dystrophies, characterized by photoreceptor cell degeneration, night blindness, a gradual loss of peripheral visual fields, and the eventual loss of central vision [Wang et al., 2001]. RP affects 1 in 4,000 persons and is

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responsible for visual handicap in 1.5 million individuals worldwide [Haim et al., 1992; Berson, 1993; Kumar-Singh et al., 1993]. RP exhibits high genetic heterogeneity with various inheritance modes including autosomal dominant, autosomal recessive, X-linked, and digenic forms [Dryja and Li, 1995; Inglehearn, 1998; van Soest et al., 1999; Phelan and Bok, 2000; Wang et al., 2001]. Autosomal dominant RP (adRP) and autosomal recessive RP (arRP) are each estimated to account for approximately 20% of RP cases, and X-linked RP accounts for 10% of cases [Jay, 1992; Ham, 1993]. Approximately 50% of RP cases are simplex RP, which represents either sporadic RP or arRP [Jay, 1992; Ham, 1993]. Digenic RP is rare. In this study, we genetically characterized a large Chinese family with adRP.

The genes that have been identified or cloned in adRP include *HPRP3* on chromosome band 1p13-q21, *RHO* at 3q21-24, *RDS*at 6p21.1-cen, *PAP1*at 7p14.2, *IMPDH1* at 7q31.1, *RP1* at 8p11-21, *RGR* at 10q23, *ROM1* at 11q13, *NRL* at 14q11.1-11.2, *PRPC8* at 17p13.3, *FSCN2* at 17q25, *CRX* at 19q13.3, and *PRPF31* at 19q13.4 [Vithana et al., 2001; Wang et al., 2001; Bowne et al., 2002; Keen et al., 2002; Kennan et al., 2002]. The chromosomal location of another adRP gene was mapped by linkage analysis, locus RP17 (17q22), but the responsible gene remains to be cloned or identified [Wang et al., 2001].

The adRP gene on chromosome 19q13.4 (*RP11*) was identified as the *PRPF31* gene that encodes a 499 amino acid protein with homology to the yeast pre-mRNA splicing factor PRP31 [Vithana et al., 2001]. PRPF31 is a component of the U4/U6 snRNP particle involved in pre-mRNA splicing [Makarova et al., 2002]. The *PRPF31* gene has 14 exons and spans about 18 kb [Vithana et al., 2001]. It is expressed in many tissues including retina [Vithana et al., 2001]. In this article, we describe a novel intragenic 12-bp deletion in *PRPF31* in a large Chinese family with 19 individuals affected with RP. This mutation causes the deletion of four amino acids ($\Delta H_{111}K_{112}F_{113}I_{114}$), including amino acid H_{111} that is evolutionally conserved from yeast to human.

MATERIALS AND METHODS

Genomic DNA, Genotyping, and Linkage Analyses

Informed consent was obtained from the participants in accordance with guidelines established by local institutional review boards. Human genomic DNA was isolated using the DNA Isolation Kits for Mammalian Blood according to the manufacturer's instructions (Roche Diagnostics Corporation, Indianapolis, IN). Genotyping and linkage analysis were carried out as described previously [Wang et al., 1995; Wang et al., 1996]. Lod scores were calculated for each marker by two-point linkage analysis using the linkage package 5.2 [Lathrop et al., 1985].

Mutation Analysis

PCR-amplification of *PRPF31* exons was carried out using primers listed in Table I. Mutation analysis was carried out by direct DNA sequence analysis by BigDye™ terminator cycle sequencing with an ABI-3100 Genetic Analyzer.

Mutation analysis was also carried out using single-strand conformational polymorphism (SSCP) analysis as described previously [Zhao et al., 2001a,b]. The aberrant SSCP conformer was cut directly from dried gels, rehydrated in water, re-amplified by PCR, and sequenced.

RESULTS

We have identified a large Chinese family (kindred RPYT) with clear diagnosis of RP (Fig. 1). The inheritance pattern in kindred RPYT appears to be autosomal dominant (adRP) (Fig. 2a). Because many genetic loci have been identified in adRP, our initial genetic study of kindred

RPYT was focused on linkage analysis with markers linked to known genetic loci for adRP: *RHO* (*D3S3023, D3S1764*), *RDS* (*GATA11E02, D6S1053*), *NRL* (*D14S1280, D14S608*), *RP1* (*D8S1110*), *CRX* (*D19S420, D19S902, D19S571*), *FSCN2* (*D17S785, D17S928*), *RP9* (*D7S516, D7S484*), *RP10* (*D7S486, D7S530, D7S640*), *RP11* (*D19S418, D19S210*), *RP13* (*D17S849, D17S831*), *RP17* (*D17S1868, D17S787*), and *RP18* (*D1S252, D1S498, D1S484*). Two-point lod scores varied from 0.000022 to 0.000018 with all markers tested except *D19S418*. Because *D19S418* yielded a positive lod score of 2.8 and it is located 5 cm from the RP11 gene *PRPF31*, our genetic analysis of kindred RPYT was then shifted to mutation analysis of *PRPF31*.

Single strand conformation polymorphism (SSCP) and DNA sequence analysis demonstrated a novel 12 bp deletion in exon 5 of *PRPF31* (Fig. 2b). This deletion results in an in-frame deletion of four amino acids, $H_{111}K_{112}F_{113}I_{114}$ (Fig. 2c). The 12-bp deletion was shown to co-segregate with the RP phenotype in 18 affected members of kindred RPYT by SSCP analysis (Fig. 2a). The deletion was not observed in DNA samples from 11 unaffected members in the family (Fig. 2a) and was not present in more than 100 controls (data not shown).

DISCUSSION

We report the identification of a novel 12-bp deletion of the pre-mRNA splicing gene *PRPF31* in a large Chinese family. We provide two lines of evidence that strongly suggests that the 12-bp deletion is causal. First, the 12bp deletion co-segregates with the RP phenotype in 19 patients in kindred RPYT, but not with 11 unaffected family members and 100 normal controls. Secondly, this mutation deletes four amino acids ($\Delta H_{111}K_{112}F_{113}I_{114}$), one of which is evolutionally conserved amino acid residue H_{111} [Vithana et al., 2001]. The 12 bp deletion is located in exon 5 of *PRPF31* in which no mutations have been identified to date. The previously identified mutations were clustered in more 3′-end exons of the gene, including exons 6, 7, 8, and 11 [Vithana et al., 2001]. Our study expands the spectrum of *PRPF31* mutations causing adRP, and confirms the role of pre-mRNA splicing factor PRPF31 in the pathogenesis of RP.

The molecular mechanism underlying pathogenesis of the *PRPF31* 12-bp deletion in RP is not clear. *PRPF31* encodes a 61 kDa pre-mRNA splicing protein that is required for U4/U6.U5 tri-snRNP (small nuclear ribo-nucleoprotein) formation [Makarova et al., 2002]. The tri-snRNP formation starts with the association between the U4 and U6 snRNPs through extensive RNA-RNA base-pairing, which is followed by the association of the U4/U6 snRNP complex with U5 snRNP [Staley and Guthrie, 1998]. The PRPF31 protein appears to tether U4/U6 to U5 snRNP to form the tri-snRNP complex [Makarova et al., 2002]. The tri-snRNP formation is a critical step in the assembly of the catalytically active spliceosome. Lack of the PRPF31 protein blocks tri-snRNP formation and pre-mRNA splicing [Makarova et al., 2002]. It is interesting to note that two other newly-identified adRP genes also encode proteins involved in pre-mRNA splicing: *PRPC8* at 17p13.3 (*RP13*) coding for PRP8 (a core component of the U5 snRNP) [McKie et al., 2001], and *HPRP3* at 1p13-q21 (*RP18*) for PRP3 (a component of the U4/U6) [Chakarova et al., 2002]. These data together suggest that disruptions in tri-SNP formation and function contribute to the pathogenesis of adRP. Thus, we speculate that the 12-bp deletion of the *PRPF31* gene identified in this study may disrupt the association of the U4/U6 snRNP complex to U5-snRNP, leading to defective pre-mRNA splicing. It is puzzling as why these splicing defects cause a defect in the vision system only. Deery et al. [2002] recently found that two missense mutations (A194E, A216P) in *PRPF31* may affect splicing by impeding the translocation of PRPF31 into the nucleus. The splicing defect of A216P was further demonstrated by the finding that PRPF31 with A216P failed to fully complement functional deficiency in a temperature-sensitive, *PRP31p*-deficient yeast strain at the high restrictive temperature (higher growth rate, high demand for splicing) [Deery et al., 2002]. It

was argued that rod photoreceptors may have a high demand for splicing of important molecules such as opsin mRNA, and subtle defects in splicing due to loss-of-function of PRPF31 may lead to a disease in this system (RP).

The chromosome 19q13.4 linked RP (RP11) is thought to be a frequent cause of adRP [Al Maghtheh et al., 1996]. It is noteworthy that mutations at the RP11 locus show “all or none” form of incomplete penetrance, where gene carriers display either fully symptomatic phenotype or completely asymptomatic phenotype [Al Maghtheh et al., 1994, 1996; Evans et al., 1995; McGee et al., 1997]. Interestingly, the 12 bp deletion of *PRPF31* identified in this study shows high penetrance (Fig. 2a). The cause of the high penetrance phenotype associate with the 12 bp deletion is unknown. One possible explanation is that the 12 bp deletion may be a highly severe mutation, and reduces the splicing activity in the photoreceptors below the threshold level that is sufficient for the development of RP.

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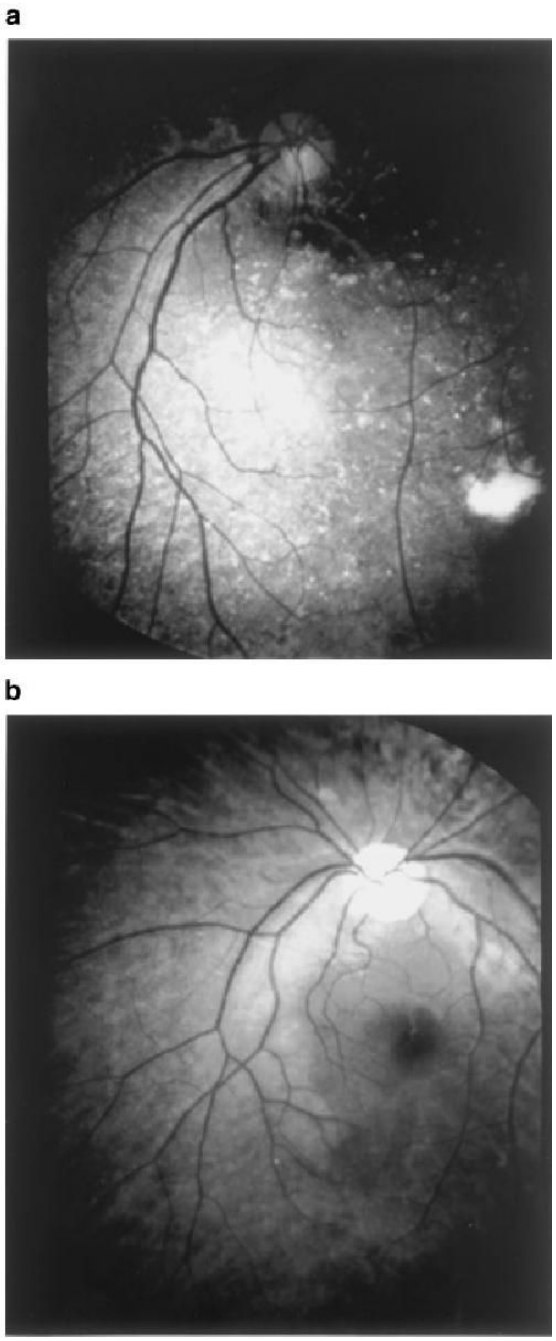
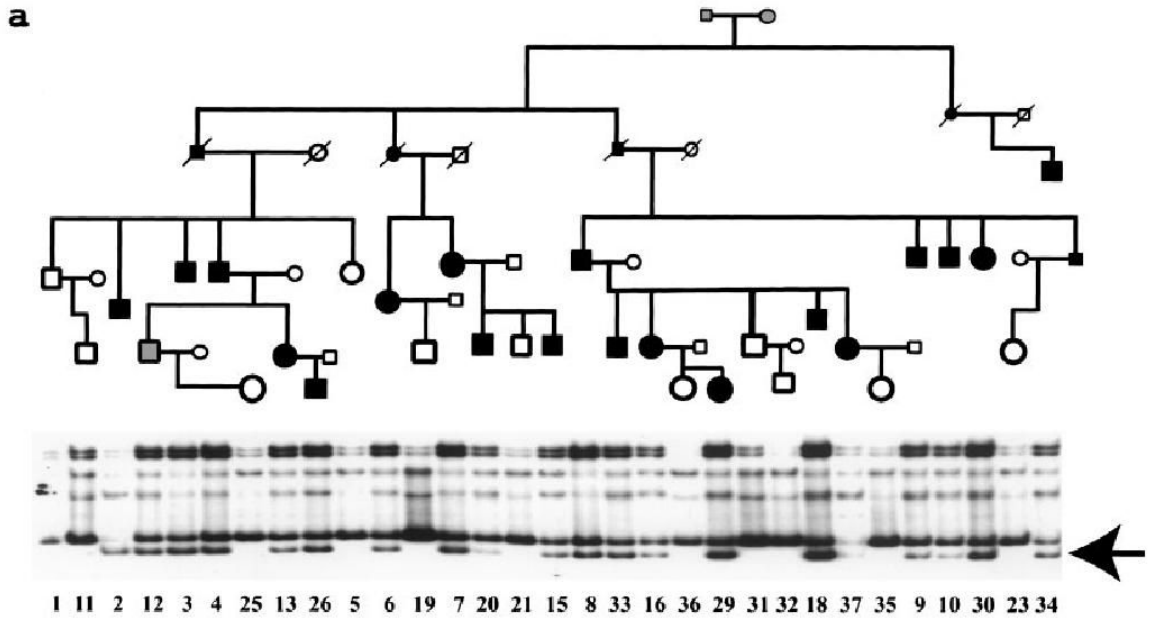
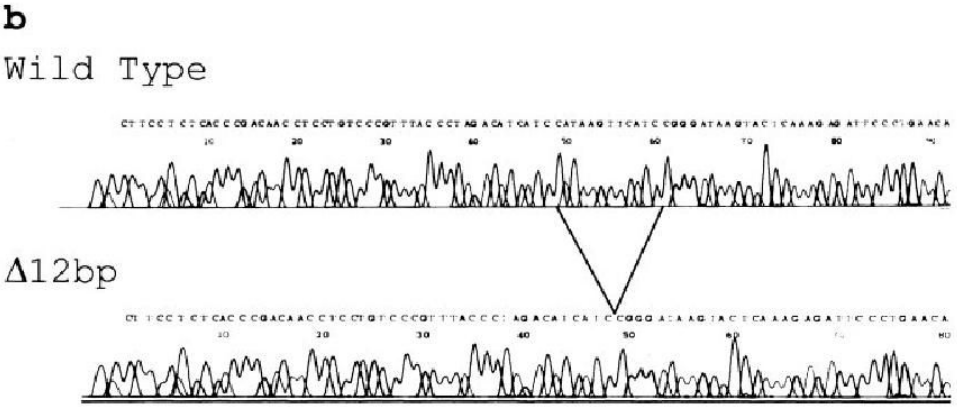


Fig. 1.

Fundus photographs of two patients with the 12-bp deletion of *PRPF31*. **a**: individual no. 7 in Figure 2a; **(b)**: individual no. 20 in Figure 2a. Note the typical findings associated with RP: bone-spicule pigmentation, precipitates of golden particles, and dystrophy of optic nerves.





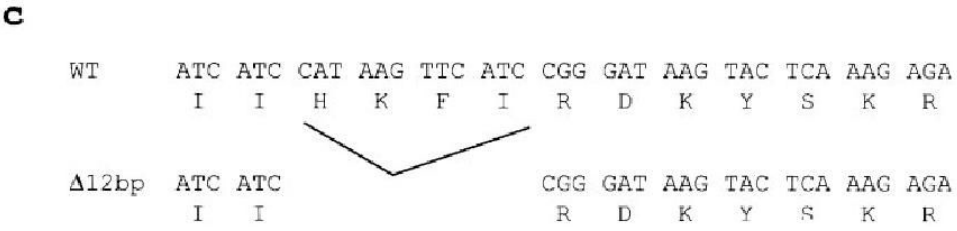


Fig. 2.

A 12-bp deletion in *PRPF31* causes autosomal dominant RP in a large Chinese kindred (RPYT). **a**: The pedigree of RPYT is shown at the top. Normal individuals are shown as clear circles (females) or squares (males), and affected individuals are shown as solid symbols. The individual with a possible diagnosis of RP (no. 12) is shown with a gray square. The deceased individuals are shown with "/". Results of SSCP analysis are shown below the pedigree structure. The arrow indicates the abnormal SSCP band. The numbers beneath the SSCP image are unique identification codes for individual family members. Note that only affected individuals carry the abnormal SSCP band, and all normal family members do not carry it. **b**: Sequence analysis of normal (wild type) and abnormal (Δ12bp) SSCP conformers. The deletion

spot is indicated. **c**: Δ12bp leads to deletion of four amino acids of PRPF31, $H_{111}K_{112}F_{113}I_{114.}$ Amino acid H_{111} is evolutionally conserved from yeast, *A. thaliana*, *Drosophila*, to humans.

TABLE I

PCR Primers for Amplification of *PRPF31* Exons*

Exon(s)	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Annealing temperature°C
2, 3	GTCGGGGCAAGTTTTTAGGG	GAGATGGGGAGGGGCACAGAGT	64
4	CCGAGAGGGGGTAGGGATTTAGAT	AGGCCAGTGGGGAAGGGAGAGG	64
5	TTAGGGCCAACCAGCAGAGTC	GAGGGGGTCCGAGAGTGAGC	64
6, 7	GTTCCCGAGCCTCCCCTATCTTCT	CGCTCCAGCTCCTCCTCCGACAG	64
8	CCGGCGGCCTGACCAACC	GGGAGGGGCCATGACGCAGTG′	64
9	GCGCGGTTGCTTTGCTGTTA	ACTGCCTCCGCCTTGGTAG	64
10, 11	GTGGCGGTGAGGCAGCATTAGGTG	CTGGCTGGCTGTGGGGTTGAGGA	55
12, 13	GGGCCTGGTCGCTGA	GGGGAGGTACCTGGAGTGG	64
14	GGTCACAGTTGGGGCCTTCTCCTC	TACTGGGCGGTGATCTCGGTCCTG	64

*Exon 1 was not studied because it is outside the coding region.

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