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A novel *PRPF31* splice-site mutation in a Chinese family with autosomal dominant retinitis pigmentosa

Kun Xia,[1] Duo Zheng,[1] Qian Pan,[1] Zheng Liu,[1] Xinghua Xi,[2] Zhengmao Hu,[1] Hao Deng,[1] Xiaoping Liu,[1] Deyong Jiang,[2] Hanxiang Deng,[1] Jiahui Xia[1]

(The first two authors contributed equally to this publication)

[1]National Laboratory of Medical Genetics of China and the [2]Department of Ophthalmology, The Second Xiangya Hospital, Central South University, Changsha, People's Republic of China

Purpose: The autosomal dominant form of retinitis pigmentosa (ADRP) can be caused by mutations in 13 genes and a further locus for which the gene remains to be identified. This study was intended to identify mutations in a large Chinese pedigree with ADRP.
Methods: A genome scan was conducted in the family. The whole coding sequences and the intron-exon boundaries of candidate genes were amplified and sequenced. The reverse transcriptase polymerase chain reaction (RT-PCR) was performed to amplify the mutated mRNA.
Results: The strongest evidence of linkage was detected with three adjacent microsatellite markers genotyped between D19S902 and D19S210 on chromosome 19q13.33-13.43. Within the region, a single nucleotide change (G>A) at position -1 of Intron 5 of *PRPF31* was found. The consensus AG doublet of the Intron 5 splice acceptor was changed to AA. The mutation co-segregated with the disease phenotype, suggesting that it was the disease-causing mutation in this family. This splicing site mutation is predicted to cause an erroneous splicing of Exon 6. By RT-PCR, we found the mutated nucleotide of Intron 5 (A) and the first nucleotide of Exon 6 (G) was regarded as a new splice acceptor, resulting in 1 bp deletion of the first codon of Exon 6 (GAG-to-AG) at the mRNA level. This change led to a frameshift and truncated protein of 196 amino acids with 56 novel amino acids prior to a premature stop.
Conclusions: A novel splicing mutation (IVS5-1G>A) in the pre-mRNA splicing-factor gene *PRPF31* causes retinitis pigmentosa in a large Chinese family. The mutation results in a truncated protein of *PRPF31*.

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of retinal dystrophies characterized by a progressive degeneration of photoreceptors, eventually resulting in severe visual impairment. RP is the most frequent form of inherited retinopathy, with an approximate incidence of 1 in 3,500 individuals worldwide. Clinical characteristics include night blindness and progressive degeneration of the midperipheral retina, accompanied by bone spicule-like pigmentary deposits and a reduced or absent electroretinogram. RP can be inherited either in an autosomal dominant, autosomal recessive or X-linked mode. So far, mutations in 13 genes have been identified to be associated with ADRP, including *HPRP3* at 1p13-q21, *RHO* at 3q21-24, *RDS* at 6q21.1-cen, *PAP1* at 7p14.2, *IMPDH1* at 7q31.1, *RP1* at 8p11-21, *RGR* at 10q23 [1], *ROM1* at 11q13, *NRL* at 14q11.1-11.2, *PRPF8* at 17p13.3, *FSCN2* at 17q25, *CRX* at 19q13.3, and *PRPF31* at 19q13.4. These are summarized at the RetNet web site. There is a further locus (RP17 at 17q22) for which the genes remain to be identified [2].

Correspondence to: Dr. Kun Xia, National Laboratory of Medical Genetics of China, Central South University, 410078 Hunan, People's Republic of China; Phone: +86-731-4472093; FAX: +86-731-4478152; email: nlmglcy@xysm.net

In this study, we found a large Chinese family with ADRP and intended to determinate the disease-causing gene in the family.

METHODS

Family data and DNA samples: A large Chinese family with autosomal dominant retinitis pigmentosa was collected from Hunan province (Figure 1). A complete family history was obtained and 34 individuals from the family were sampled to be used for linkage analysis with informed written consents. Family members were clinically diagnosed at the Second Xiangya Hospital of Central South University. 19 individuals were considered to be affected and 15 unaffected. 50 normal individuals with no personal or family history of RP were sampled as controls. Genomic DNA was extracted from peripheral blood by standard phenol extraction protocols.

Linkage analysis: Genome-wide screening was conducted using 382 fluorescent microsatellite markers from autosomes, at a resolution of approximately 10 cM (ABI PRISM Linkage Mapping Set, Version 2.0, Foster City, CA). Multiplex PCR was carried out in a 5 μl reaction mixture containing 30 ng of genomic DNA, primary PCR buffer, 200 μM of each dNTP, 3.0 mM $MgCl_2$, 80 pmol each of forward and reverse primers, and 0.2 U of Ampli*Taq* Gold DNA polymerase (Applied

Biosystems, Branchburg, NJ). Samples were incubated in a thermocycler for 10 min at 95 °C, 30 s at 94 °C; the annealing temperature was programmed to initiate from 63 °C at 1 min and decrease 0.5 °C every cycle; 72 °C for 110 s, for 15 cycles; followed by 94 °C for 30 s, 56 °C for 1 min, 72 °C for 1 min 110 s, for 24 cycles; a final extension at 72 °C for 10 min was performed. The PCR products were appropriately pooled, and an aliquot was loaded onto a 5% standard denaturing polyacrylamide gel and run in an Applied Biosystems 377XL DNA sequencer. The size of allele was determined on the basis of an internal size standard (Genescan-400HD ROX, Perkin Elmer, Foster City, CA) in each lane, and results were analyzed by Genescan 3.0 and Genotyper 2.1 software (Perkin Elmer). Two-point LOD scores between the disease locus and markers were calculated using the MLINK routine of the LINKAGE software package, version 5.1. The disease was specified to be an autosomal dominant trait with a disease-allele frequency of 0.0001. The allele frequencies for each marker were assumed to be equal, as well as the recombination frequencies in males and females. Genetic penetrance was assigned at 90%.

Mutation detection: Primers were designed to amplify all exons and flanking intronic splicing sites of *CRX*, *PRPF31*, and *PRKCG* from genomic DNA. PCRs were carried out using standard reaction mixtures and PCR products were sequenced directly by ABI Prism 377XL DNA sequencer to perform mutation analysis.

RT-PCR reactions: Lymphocytes from peripheral blood were isolated from the proband and two unaffected individuals from the family. Total RNA was extracted with TRIZOL Reagent (Invitrogen, Carlsbad, CA). Reverse transcription of isolated RNA was performed using Reverse Transcription System (Promega, Madison, WI) with random primers. The transcribed *PRPF31* fragment from exon 3 to exon 8 was amplified using paired primers: forward, 5'-aag tga tgg gac cag tgg ag-3'; and reverse, 5'-gta gac gag aag ccc gac ag-3'. The thermocycling profile was as follows: RT reaction (50 °C for 60 min, 80 °C for 2 min), followed by a three-step touchdown PCR (94 °C for 50 s; 65 °C for 1 min, drop 1 °C per cycle; 72 °C for 1 min) for 10 cycles, followed by a three-step PCR (94 °C for 50 s, 58 °C for 1 min, 72 °C for 1 min) for 24 cycles. PCR products were analyzed on a 6% polyacrylamide gel and sequenced.

RESULTS

By genome-wide scan and linkage analysis, the maximum two-point LOD score of 2.73 at θ=0.1 (Table 1) was found at D19S418 in this family, whereas two-point LOD scores were very low at other previously reported loci (Table 2). The strongest evidence of linkage was detected with three adjacent microsatellite markers genotyped between D19S902 and D19S210 on chromosome 19q13.33-13.43.

Three genes *CRX*, *PRPF31*, and *PRKCG* associated with ADRP have been mapped within the region of interest. To identify the disease-causing gene, we sequenced all exons and

Table 1. Two-point LOD scores between the causative gene and 8 markers of chromosome 19

	LOD score at θ					
Locus	0.0	0.1	0.2	0.3	0.4	0.5
D19S884	-18.08	-2.93	-1.23	-0.44	-0.07	0.00
D19S226	-25.95	-5.40	-2.60	-1.16	-0.36	0.00
D19S414	-12.57	-1.09	-0.43	-0.20	-0.1	0.00
D19S220	-16.19	-2.10	-0.59	-0.04	0.10	0.00
D19S420	-7.81	-0.41	0.12	0.23	0.17	0.00
D19S902	-11.67	-0.66	0.41	0.59	0.38	0.00
D19S418	-2.23	2.73	2.29	1.56	0.70	0.00
D19S210	-10.35	0.20	0.91	0.86	0.46	0.00

LOD scores were calculated under an autosomal dominant mode of inheritance and a penetrance of 90%.

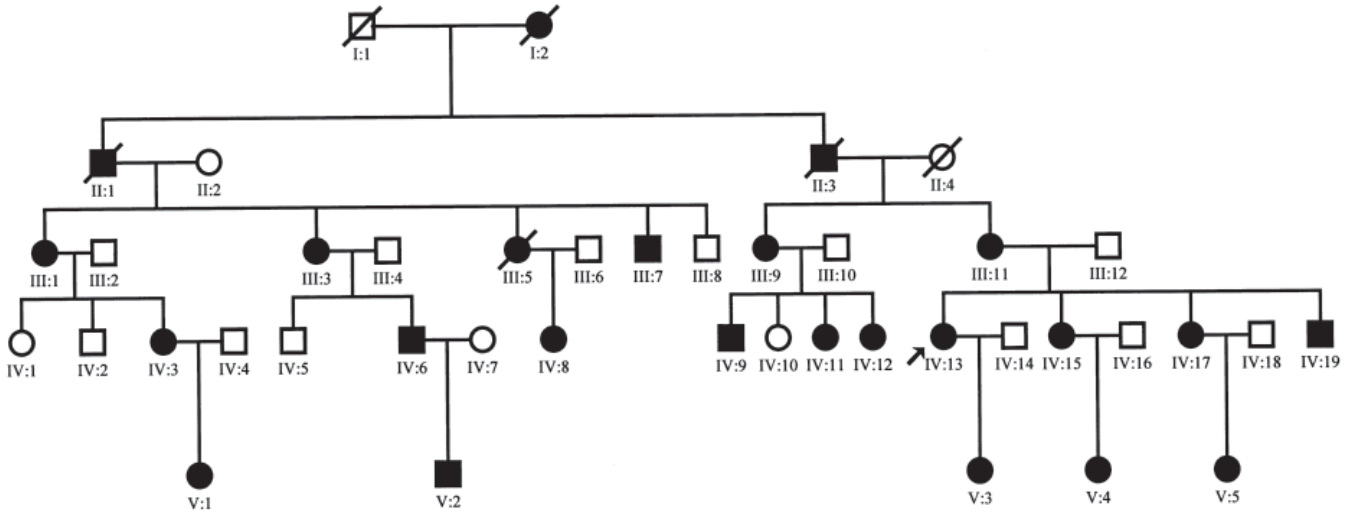

Figure 1. A Chinese family with autosomal dominant retinitis pigmentosa. Normal individuals are shown as clear circles (female) or squares (male), and affected individuals are shown as solid symbols. Deceased individuals are shown with a slash. The arrow indicates the proband.

intron-exon boundaries of *CRX*, *PRKCG*, and *PRPF31* in the family. No mutation was detected in *CRX* and *PRKCG*. But a single nucleotide change (G>A) at the position -1 of the Intron 5 of *PRPF31* was found in all affected members and an unaffected individual (V-5) who was regarded as an asymptomatic carrier (Figure 2). The same splice-site mutation was not detected in 14 unaffected members in the family and 50 controls (data not shown). The mutation was shown to cosegregate with the RP phenotype, suggesting it was the disease-causing mutation in the family.

To test whether the splice-site mutation lead to a defective mRNA, we performed an RT-PCR assay to amplify *PRPF31* mRNA from total RNA isolated from peripheral blood of the proband and two unaffected members. A fragment of about 559 bp was obtained. The results of sequencing revealed that the RT-PCR product from the proband actually contained a normal and a 1 bp deletion fragment. The deletion occurred in the first nucleotide of Exon 6 in codon glu141 (GAG-to-AG) leading to a frameshift and truncated protein of 196 amino acids with 56 novel amino acids prior to a premature stop (Figure 3). The mutated mRNA was not detected in samples from two unaffected members in the family.

Table 2. Two-point LOD scores between the causative gene and 14 markers of chromosome 3

	LOD score at θ					
Locus	0.0	0.1	0.2	0.3	0.4	0.5
D3S1297	-9.88	-1.54	-0.46	-0.07	-0.00	0.00
D3S1263	-22.81	-4.60	-2.15	-0.93	-0.28	0.00
D3S1266	-8.35	-1.12	-0.14	0.14	0.10	0.00
D3S1289	-17.74	-2.74	-1.21	-0.51	-0.16	0.00
D3S1300	-24.97	-1.71	-0.48	-0.11	-0.03	0.00
D3S3681	-13.17	-2.89	-1.18	-0.42	-0.10	0.00
D3S1271	-20.13	-3.18	-1.34	-0.50	-0.13	0.00
D3S1292	-11.57	-0.83	0.09	0.32	0.21	0.00
D3S1569	-22.29	-5.17	-2.64	-1.31	-0.49	0.00
D3S1279	-18.45	-5.59	-2.77	-1.31	-0.46	0.00
D3S1614	-18.09	-2.79	-1.14	-0.45	-0.12	0.00
D3S1262	-12.83	-2.07	-0.95	-0.40	-0.12	0.00
D3S1580	-18.23	-4.19	-2.05	-0.94	-0.31	0.00
D3S1311	-13.14	-2.36	-1.28	-0.72	-0.31	0.00

LOD scores were calculated under an autosomal dominant mode of inheritance and a penetrance of 90%.

DISCUSSION

An ADRP locus on 19q13.4 (RP11) was first identified by linkage analysis in a large British family [3]. The genomic region of the autosomal dominant RP11 locus was narrowed to a 600 kb region between markers D19S927 and D19S781.2 on 19q13.4 [4]. Within this region, mutations in the *PRPF31* gene, a homolog of the yeast pre-mRNA splicing gene *Prp31*

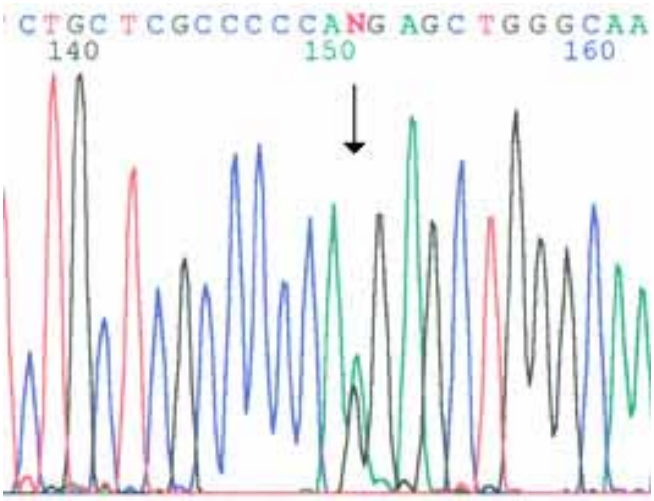

Figure 2. Mutation in *PRPF31* associated with ADRP. A single nucleotide change (G>A) at the position -1 of intron 5 in the Chinese family.

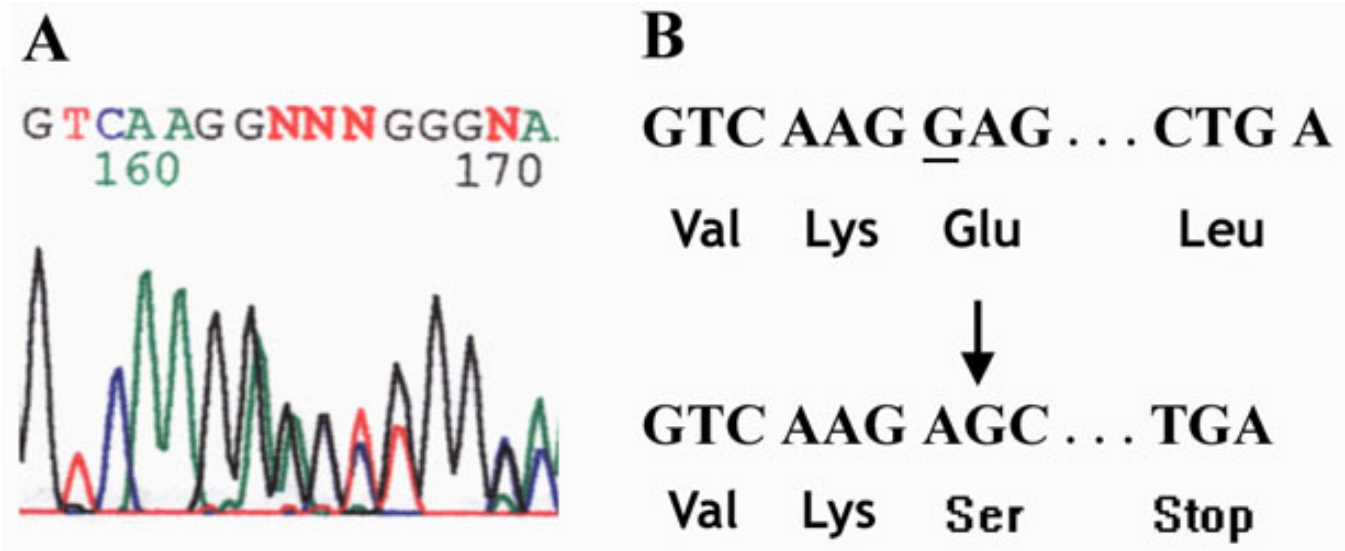

Figure 3. Result of mRNA analysis *PRPF31* (proband). **A**: Sequencing of mRNA. **B**: One base deletion of G in codon glu141 (GAG-to-AG).

[5], were first identified in 4 RP11-linked British families and 3 sporadic RP cases. The mutations included missense substitutions, deletions, insertions and splice-site mutations [4]. Afterwards insertion-deletion alterations in exon 8 of *PRPF31* were detected in Spanish families [6]. Recently, a novel 12 bp deletion in exon 5 of *PRPF31* was reported in a large Chinese family with ADRP [7].

In this study, we report the identification of a novel splice-site mutation (IVS5-1G>A) of pre-mRNA splicing gene *PRPF31* in a large Chinese family. The mutation co-segregates with RP phenotype in 19 patients in kindred ADRP, but not with 14 unaffected family members and 50 controls, which strongly supports that this mutation may be the cause of ADRP in this family. It is still unclear how this mutation changes the splicing of mRNA. The substitution of nucleotide (G>A) occurs at the splice acceptor of Intron 5, usually leading to erroneous skipping of Exon 6. Interestingly, the first nucleotide of Exon 6 is G, and therefore the substituted A in Intron 5 forms a new splice acceptor together with the G. This alteration results in deletion of the first nucleotide of Exon 6, which leads to a frameshift and truncated protein of 196 amino acids with 56 novel amino acids prior to the premature stop. 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.

PRPF31 was previously shown to be a human homolog of the yeast pre-mRNA splicing gene, *Prp31* [4]. *PRPF31* encodes a 61 kDa pre-mRNA splicing protein that is required for U4/U6-U5 tri-snRNP (small nuclear ribonucleoprotein) formation. Tri-snRNP formation is critical in the assembly of an active spliceosome. Lack of the PRPF31 protein blocks tri-snRNP formation and pre-mRNA splicing [8]. Besides *PRPF31*, *PRPF8* (RP13), and *HPRP3* (RP18) are also ADRP genes encoding the pre-mRNA splicing-factor components of U4/U6-U5 tri-snRNP particle [9,10]. These data suggest that disruptions in tri-snRNP formation and function contribute to the pathogenesis of ADRP. The mutation in the *PRPF31* detected in our Chinese family is a novel splice-site mutation that leads to a truncated protein. It is predicted to be a loss of function mutation, and unlikely to promote formation of functional tri-snRNP. Thus, the functional U4/U6-U5 tri-snRNP may be decreased to 50% that would lead to lower the rate of splicing in the cell.

An alternative mechanism for the autosomal dominant character of this mutation is a gain-of-function property of the mutant protein. This might occur if the truncated protein, which might be misfolded, somehow causes cellular toxicity. The mutant protein might have its toxic effect anywhere in the photoreceptor cell, and further studies are needed to elucidate the mechanism by which the mutant protein causes ADRP.

It is not clear why these splicing defects cause a defect in the vision system only. Two missense mutations (A194E, A216P) in *PRPF31* were found to affect splicing by impeding PRPF31 into the nucleus, and may cause an insufficiency in splicing function. 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) [11].

Incomplete penetrance was reported in many families affected with RP11. 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 [12-14]. In the ADRP family reported in this study, an asymptomatic carrier of the splice-site mutation (IVS5-1G>A) in the *PRPF31* gene was identified, suggesting incomplete penetrance in this pedigree. The incomplete penetrance has also been reported in previously studied ADRP pedigrees carrying deletion or nonsense mutations in *PRPF31* [4,6]. It is possible that variations in wild-type alleles at the RP11 locus influence the penetrance of pathogenic alleles. A significant difference in wild-type *PRPF31* mRNA levels was observed between symptomatic and asymptomatic individuals, indicating that symptomatic patients inherit a relatively poorly expressed *PRPF31* wild-type allele from their parent compared with asymptomatic patients [15]. The high expression of wild-type allele may be able to compensate for the presumptive nonfunctional mutant allele [4,15]. It may be proposed that haploinsufficiency rather than dominant negative effect is the pathogenic mechanism underlying this disease. These results may also imply that a proportion of sporadic RP, previously assumed to be recessive, might result from mutations at this locus with incomplete penetrance [12].

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