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Mutation Spectra in Autosomal Dominant and Recessive Retinitis Pigmentosa in Northern Sweden

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Abstract

Retinal degenerations represent a heterogeneous group of disorders affecting the function of the retina. The frequency of retinitis pigmentosa (RP) is 1/3500 worldwide, however, in northern Sweden it is 1/2000 due to limited migration and a 'founder' effect. In this study we identified genetic mechanisms underlying autosomal dominant and recessive RP present in northern Sweden. Several novel mutations unique for this region were found. In an autosomal recessive form of RP, Bothnia dystrophy caused by mutations in the *RLBP1* gene, bi-allelic mutations R234W, M226K and compound heterozygosity, M226K+R234W was detected.

In dominant form of RP mapped to 19q13.42 a 59 kb genomic deletion including the *PRPF31* and three other genes was found.

These data provide additional information on the molecular mechanisms of RP evolvement and in the future might be useful in development of therapeutic strategies. Identification of the disease-causing mutations allowed introducing molecular genetic testing of the patients and their families into the clinical practice.

29.1 Introduction

Retinitis pigmentosa (RP) is a group of inherited retinal disorders with a considerable genetic variation. Typical signs of the disease are night blindness and progressive loss of the peripheral visual field, characteristic pigment deposition in the retina, attenuation of the retinal blood vessels, and optic disc pallor.

A form of autosomal dominant RP (adRP) in patients with night blindness in the first and second decade, progressive visual field loss in later life, along with asymptomatic individuals though having an affected parent and an affected child was mapped to 19q13.4 (Al-Maghtheh et al., 1994) (RP11, MIM 600138). A putative human ortholog of yeast pre m-RNA splicing factor, *PRPF31* was reported as a disease causing gene (Vithana et al., 2001). To date 42 *PRPF31* mutations are listed in the Human Genome Mutation Database and among these only 9 are missense, while the rest are deletions, insertions, indels and

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splicing mutations (http://www.hgmd.cf.ac.uk/ac/all.php). The majority of the mutations would result in truncated proteins due to exon skipping and premature stop codons (Vithana et al., 2001; Martinez-Gimeno et al., 2003; Sato et al. 2005; Sullivan et al., 2006), therefore, haploinsuffiency was suggested as a mechanism of RP11 evolvement (Vithana et al., 2001).

A rather large group of patients with a variant of autosomal recessive RP (arRP), Bothnia dystrophy (BD) (MIM 180090) has been identified in northern Sweden (Burstedt et al. 1999; 2001). The phenotype is characterized by night blindness in early childhood, retinitis punctata albescens (RPA) in young adulthood and a progressive macular and peripheral retinal degeneration (Burstedt et al. 2001).

The disease was reported to be associated with a bi-allelic c.700C>T mutation in the *RLBP1* gene (p.R234W). The majority of the reported cases carried *RLBP1* mutations in homozygous state although compound heterozygotes have also been described (http://www.hgmd.cf.ac.uk/ac/all.php). Sequence changes involving single nucleotides are not the only type of mutation affecting the *RLBP1* gene. In a patient with RPA a large homozygous deletion was recently described (Humbert et al. 2006).

In our inventory work on retinal dystrophies in northern Sweden, we found among patients with a BD phenotype homozygotes and heterozygotes for the c.700C>T mutation. The patients heterozygous for the c.700C>T mutation appeared to be carriers of the second mutation in the *RLBP1* gene, c.677T>A.

Thus, in this study we report a novel genomic deletion including almost the entire *PRPF31* gene in two families with adRP linked to 19q13.42 and compound heterozygosity in the *RLBP1* gene in arRP of Bothnia type.

29.2 Materials and Methods

29.2.1 Patients and Ophthalmologic Examinations

Patients residing in the four counties of northern Sweden with a population of 880,000 were included in this study. All of them had a history of night blindness and a clinical diagnosis of either arRP or adRP. The study followed the tenets of the Declaration of Helsinki, and consent was obtained from all individuals. Standard ophthalmologic examination included fundus photography and visual field testing. Dark adaptation tests and full-field ERGs were performed in selected cases.

29.2.2 Molecular Genetic Analysis

DNA was extracted from peripheral blood and used for linkage analysis with the ABI PRISM Linkage Mapping set version 2.5 (Applied Biosystems) as described elsewhere (Köhn et al. 2007). Sequence analysis of coding exons and adjacent intronic sequences of candidate genes was performed as described by Köhn et al. (2007). The products of the sequencing reactions obtained with Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) were run on a 3730 xl DNA analyzer (Applied Biosystems).

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DNA from two BD patients carrying the *RLBP1* c.700C>T mutation on one allele were subjected to microarray genotyping designed and manufactured according to the APEX technology (Pastinen et al. 1997). (http://www.asperbio.com). Genetic testing was performed using the arRP array for both patients and in addition patient 223:3 was analysed with the autosomal dominant retinitis pigmentosa (adRP) array taking into account that only one mutation was detected in the BD patients. The arRP array included testing for 501 known mutations in 16 genes. adRP panel comprised 347 mutations in 13 genes (information about testing is available on http://www.asperophthalmics.com).

MLPA was done according to Sullivan et al. (2006) with a set of probes designed by this group in combination with the Retinitis Pigmentosa Kit (MRC Holland, http://www.mrc-holland.com/pages/indexpag.html). Additionally, we used *VSTM1* probe with following sequences forward 5′ – GGGTTCCCTAAGGGTTGGAccttcacggacctgaagcctaaggatgctgggag; reverse 5′ – gtacttttgtgcctacaagacaacagcctcccatgagtggTCTAGATTGGATCTTGCTGGCAC (DNA Technology, Denmark). The collected raw data were analysed with ABI Prism GeneMapper Software v3.0 (Applied Biosystems). The ratio of 1.0 indicates the presence of two alleles (normal diploid) and 0.5 or 1.5 suggests either deletion or duplication of the target sequence, respectively. The breakpoint region was defined using long range PCR across the deletion with forward primer *VSTM* F1 5′– GATAGAGGAGGTTTTGCTCTGAC and reverse primer *PRPF31* 13R 5′ – CGGACCCTGCAGAAGCAGAGCGTCGTAT. PCR product was cloned into pGEM-T Easy (Promega) vector and positive clones were sequenced. Allele specific PCR on genomic DNA was done as described elsewhere using specific primers for both mutant and wild type alleles (BP-F 5′ –TGAAAGAGAGAAGGGGCTCA, BP-R 5′ – GTGGCCTCGTTTACCTGTGT, cDNA *PRPF31* 12F 5′ – ATCGAGGAGGACGCCT).

29.3 Results and Discussion

29.3.1 adRP

In one of two families with adRP at least 2 individuals were obligate carriers of the mutation since they had both an affected parent and offspring (VII:8, VII:15) (Fig. 29.1). Significant LOD scores with a maximum of 7.58 at the marker D19S926 at 19q13.42 were revealed in the region spanning nearly 1.77 Mb. The reconstructed haplotypes in both families confirmed segregation of adRP with markers D19S924, D19S927, D19S926, D19S418 and D19S605. Since no disease causing mutations were detected by PCR-based methods in *RDH13, SYT5, PPP1R12C, PRKCG, CACNG6, 7, 8* and *PRPF31*genes we considered testing for large genomic deletions using MLPA examining *PRPF31*, *RHO*, *RP1,* and *IMPDH1* genes. A large genomic deletion including *TFPT, NDUFA3, OSCAR* genes and 11 exons of the *PRPF31* gene was detected (Fig. 29.2). Based on the MLPA results indicating a normal ratio for the *VSTM1* probe and probes for exons 12–14 of the *PRPF31,* we applied a long range PCR with *VSTM1* and exon 13 *PRPF31* primers (Fig. 29.2b) and obtained a ~7 kb PCR fragment. Sequencing of this product revealed a deletion of 58,733 nucleotides with breakpoints in intron 11 of the *PRPF31* gene and in LOC441864 (ref|NT_011109.15|Hs19_11266), similar to osteoclast-associated receptor isoform 5 (Fig. 29.2b–d). Allele specific PCR with primers set shown at Fig. 29.2c revealed presence of the mutation in

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affected individuals, obligatory carriers and also several asymptomatic members (Fig. 29.1b). None of 20 simplex adRP cases or 94 healthy controls (188 control chromosomes) from the matched population demonstrated the mutant allele (data not shown).

PRPF31 codes for a protein needed for splicing in all cell types although its pathologic effect is seen only in rod photoreceptors, causing adRP with incomplete penetrance. PRPF31 is a 61 kDa protein, part of the U4/U6-U5 tri-snRNP complex (Makarova et al. 2002). The proposed mechanism of adRP (RP11) evolvement is haploinsufficiency rather than a dominant negative effect (Vithana et al. 2001). Identification of the large genomic deletion with almost entire loss of *PRPF31* gene in this study provides additional evidence for haploinsuffciency as the mechanism in adRP pathogenesis.

Molecular methods used for mutation detection are mainly based on PCR and, therefore, large genomic rearrangements are easily missed. The deletion encompassing almost 59 kb of genomic sequence includes three genes additional to *PRPF31* and breakpoints occur in intron 11 of *PRPF31* and within the predicted gene, LOC441864, annotated as 'similar to osteoclast-associated receptor isoform 5'. A number of *Alu*-repeats in *PRPF31* introns can prone to internal unequal recombination resulting in a deletion; however the exact mechanism is not known.

Due to the size of the deletion we could expect a severe phenotype in our families as reported previously (Abu-Safieh et al. 2006). However, among our patients there were individuals with quite preserved visual fields and recordable ERGs at their 50 s. No additional symptoms associated with the genetic defects in *NDUFA3, TFPT* or *OSCAR* was observed. In conclusion, identification of such large deletion involving the *PRPF31* gene reveals additional evidence that haploinsufficency is a molecular mechanism of evolvement of adRP with incomplete penetrance. Identification of deletion breakpoint provides an important tool for molecular testing and genetic counselling of these patients.

29.3.2 Bothnia Dystrophy

67 out of 121 individuals affected with arRP were homozygous and 10 were heterozygous for the c.700C>T mutation in the *RLBP1*. Simultaneous evaluation of 501 mutations known as a cause of arRP performed by arrayed APEX technology in two BD patients (027:4 and 223:3, Fig. 29.3a) revealed besides the one known to us *RLBP1* c.700C>T (p.R234W) a second mutation, c.677T>A, resulting in p.M226K. Segregation analysis in five tested families showed that c.700C>T and c.677T>A were allelic and the patients were compound heterozygotes, [677T>A]+[700C>T] (Fig. 29.3). None of BD patients homozygous for c. 700C>T carried c.677T>A mutation. Allele frequency of the c.677T>A mutation was 1 in 233. Two homozygotes for the *RLBP1* c.677T>A mutation were found in RP population from northern Sweden.

Testing of a BD patient (223:3) with the adRP panel which included 347 known mutations in 13 genes resulted in detection of only one sequence change, c.40C>T in exon 1 of carbonic anhydrase, *CAIV* (p.R14W). The p.R14W mutation was reported as a cause of adRP, RP17 (Rebello et al. 2004; Yang et al. 2005). Testing of all compound *RLBP1* heterozygotes for the presence of c.40C>T revealed absence of the *CAIV* c.40C>T in nine

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carriers of *RLBP1* c.[677T>A]+[700C>T] while its presence was detected in 223:3 and his unaffected mother (223:2). 6 carriers of *CAIV* c.40C>T were detected among 143 healthy blood donors (data not shown).

29.4 Conclusions

The prevalence of nonsyndromic RP is approximately 1/2000 in Västerbotten County in the northern part of Sweden. This can be explained by 'founder' effect, isolation of population in small villages and low migration rate. To date, at least 40 causative genes and loci have been identified in nonsyndromic RP but one can expect that only a limited number of mutated genes causes retinal degenerations in such populations like the northern Swedish.

We identified a large group of patients with similar clinical appearance and an identical underlying genetic defect. 67 patients have a biallelic mutation in the *RLBP1* gene, c. 700C>T (p.R234W). Molecular testing in 10 patients with a phenotype similar to BD revealed two mutations, c.700C>T and c.677T>A. Based on allele frequency of c.700C>T (3/200) and c.677T>A (1/233) in a control population we expect that c.700C>T was the first mutation to appear in northern Sweden. Analysis of all ten c.[677T>A]+[700C>T] BD patients and 143 healthy control individuals for the p.R14W mutation in CAIV revealed the presence of this sequence variant in 4% of the population from northern Sweden. The phenotype of the *RLBP1* compound heterozygote carrying also CAIV p.R14W could not be distinguished from the other BD patients and no signs of retinal degenerative changes were detected in his 61 year-old mother carrying the same sequence variant.

In summary, the high frequency of arRP observed in northern Sweden is due to the presence of two mutations in the *RLBP1* gene, c.677T>A and c.700C>T. The patients are either homozygotes or compound heterozygotes. All 79 patients originating from Västerbotten County, with a population of 257,000 inhabitants presented the BD-like phenotype. Bothnia dystrophy is caused by loss of CRALBP function due to changed physical features and impaired activity of retinoid binding. The CAIV p.R14W known as a cause of RP17 found in one of BD patients is not pathogenic in population of northern Sweden.

Furthermore, a novel mutation unique in patients of Swedish origin, a large genomic deletion resulting in almost entire loss of *PRPF31*and three additional genes was identified as the cause of adRP with reduced penetrance. Identification of the deletion breakpoints allowed development of a simple tool for molecular testing of this genetic subtype of adRP.

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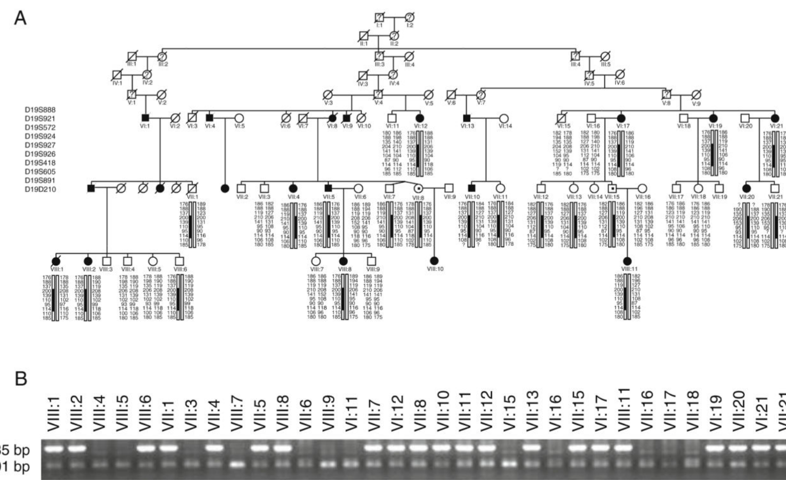

Fig. 29.1.

Haplotype analysis (**a**) and segregation of the mutation (**b**) in the family 78. **a** – filled symbols indicate affected individuals, while empty symbols indicate unaffected. Symbols with represent an asymptomatic gene carrier. Only disease haplotypes shared by affected individuals in both families are boxed. **b** – allele-specific PCR where a band of 385 bp indicates the mutant allele and a band of 101 bp indicates presence of the internal PCR control, as a result of wild type and mutant allele's amplification

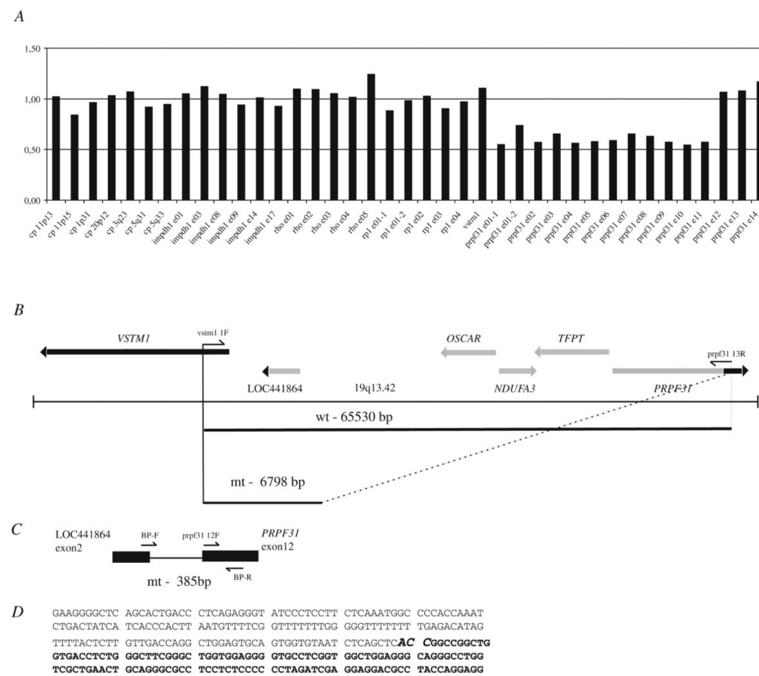

Fig. 29.2.

Genomic deletion including *PRPF31* gene in adRP. **a** – a deletion detected by MLPA in VII: 20 from family 078. **a** P235 Retinitis Pigmentosa kit (MRC Holland) along with the *VSTM1* probe was applied. The graph indicates presence of one gene copy with probes for exons 1–11 in the *PRPF31* gene (ratio is ~0.5). Two gene copies are present with the probes for *IMPDH1*, *RHO*, *RP1* and for exons 12–14 in the *PRPF31* (ratio is ~1.0). **b** – schematic representation of the genomic region 19q13.42 in proximity to *PRPF31*. PCR primers *VSTM1* 1F and *PRPF31* 13R were applied to amplify across the deleted region (the estimated size of a wild type allele is 65,530 bp and can not be amplified by PCR). PCR fragment of ~7 kb (mutant allele) was obtained by long range PCR, subcloned into pGEM-T Easy vector and sequenced. **c** – localisation of allele specific primers. Primers sequences and allele specific PCR are described in Materials and Methods and Results and Discussion. PCR with allele-specific primers BP-F and BP-R primers used for segregation analysis (Figs. 29.1B and 29.2B) resulted in 385 bp fragment representing a mutant allele. **d** – a partial sequence across the deletion. Nucleotide sequence belonging to the predicted gene 'similar to osteoclast-associated receptor isoform 5' and intron 11 in the *PRPF31* gene is shown. ACC at breakpoint shown in bold can be part of either LOC441864 or *PRPF31*

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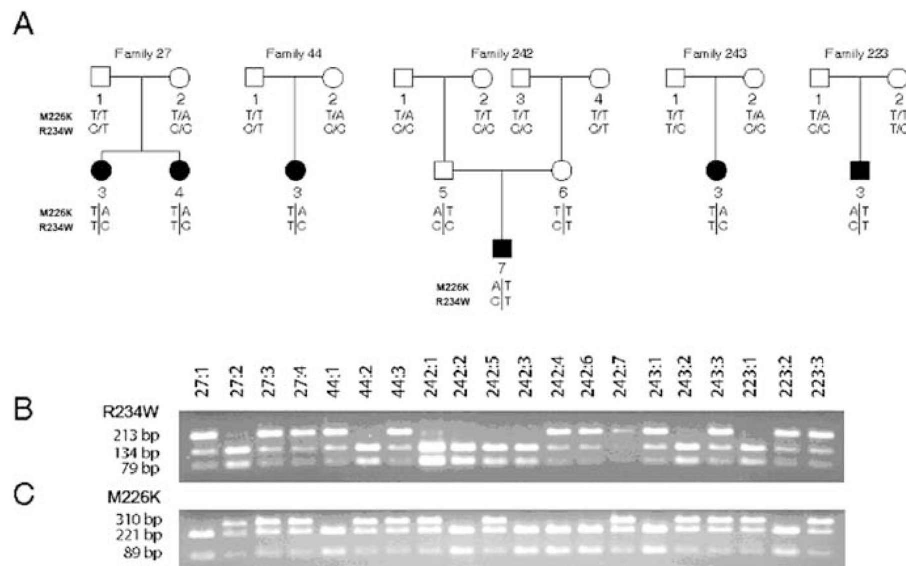

Fig. 29.3.

Allelic c.677T>A and c.700C>T mutations in families to probands diagnosed with arRP of Bothnia dystrophy. Detection of both mutations was done by PCR-RFLP. **a**. Pedigree charts of 5 families where filled symbols indicate affected individuals, while empty symbols indicate unaffected. **b**. c.700C>T mutation abolishes a *Msp*I restriction site and results in one fragment of 213 bp. **c**. c.677T>A mutation abolishes a *Nsp*I restriction site and results in one fragment of 310 bp