# Retinitis pigmentosa caused by a homozygous inactivating mutation in the Stargardt disease gene *ABCR*

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Retinitis pigmentosa (RP) is a group of inherited eye disorders characterised by loss of rod photoreceptor function leading to a progressive degeneration of the retina. It constitutes a major cause of genetic blindness in adults with a prevalence of 1/3000-7000. One of the main features of RP is its extreme heterogeneity, both clinical and genetic. It can be inherited as an X-linked, an autosomal dominant or an autosomal recessive trait. Additionally, non-allelic heterogeneity is documented by over twenty loci and genes described so far, which account for less than 50% of RP cases[1]. Mutations in ten of these loci can cause autosomal recessive RP (arRP)[1-6]. Recently, we have mapped a new arRP locus on 1p13-p21 (*RP19*)[6] which colocalises with the Stargardt (*STGD1*) and fundus flavimaculatus (*FFM*) loci. The causal gene for these allelic disorders has been recently identified as the ATP-binding cassette transporter, retina-specific (*ABCR*) gene[7]. Interestingly, mutations in *ABCR* have also been identified in age-related macular degeneration (AMD)[8]. Although its function as a transporter is not yet well defined, ABCR is located exclusively in the outer segments of rods, and this makes it an excellent candidate for the *RP19* locus. To test this hypothesis, we have performed a mutational analysis of the *ABCR* gene in the *RP19* consanguineous family (M-33).

Exons 1-50 of the proband were analysed by PCR-SSCP. An aberrant pattern was detected for exon 13 (Fig.1 *a*). Sequencing of the PCR product revealed a homozygous 1bp deletion at cDNA position 1847 (Fig.2 *a*). The segregation of this variant (1847delA) in the family was tested by PCR-SSCP (Fig.1 *a*) and restriction analysis (Fig.1 *b*). Direct sequencing was also performed in the mother and the non-affected sister (Fig.2 *b*). All six affected members are homozygous for the deletion and the mother and the healthy sister are both carriers of one mutated allele, as shown previously by linkage studies[6]. The mutation generates a frameshift early in the coding region (codon 616, in exon 13) that adds 32 new residues and a premature STOP codon.

Screening the proband's entire *ABCR* coding sequence and intron/exon boundaries led to the identification of two other variants: 3523IVS-28T→C (intron 23) and 5584IVS+34C→T (intron 40; -*Hae*III), both in homozygosity. As neither of them alters any consensus splicing sequence, they probably do not contribute to the M-33 phenotype. None of the three variants identified have been found in over 100 control chromosomes. To evaluate the contribution of the *ABCR* gene to arRP, a cosegregation analysis with the *RP19* markers in 50 non-related Spanish pedigrees is currently being undertaken.

The *ABCR* gene has been identified as a member of the ABC transporter superfamily. The predicted protein consists of two halves, each presenting an ATP-binding region and a transmembrane domain consisting of six membrane-spanning segments[7]. As the mutated position described here lies between the first and second transmembrane segments, the resulting protein would lack most of the first and the whole second transmembrane domains as well as both ATP-binding domains.

Allikmets *et al.*[7] have shown that the *ABCR* gene is expressed only in the retina, and particularly in the rod cells. Immunohistochemical analysis on macaque, bovine and mouse retinas[9-11] showed that the ABCR/RmP protein is restricted to the rim and incisures of the rod outer segment (ROS) discs and absent from cone and retinal pigment epithelium (RPE) cells.

The restricted pattern of ABCR expression supports a specific role in the ROS, either in phototransduction or in disc morphology. Accordingly, an ABCR contribution to the transport/cycling of substrates has been proposed[9,10]. Alternatively, the highly curved structure at the disc rim and incisures would require specialised proteins, such as ROM1 (ref 12) and peripherin/RDS[13], among others.

In this study we report the association of an *ABCR* frameshift mutation with arRP, adding further allelic and non-allelic heterogeneity to the complex genetic scenario of human vision disorders. It is now challenging to envision how different *ABCR* mutations are related to the clinically distinct phenotypes of STGD1, AMD and RP19. Based on the data gathered, we propose a preliminary model. The family described here is homozygous for a frameshift early in the coding region of the gene and would be predicted to have no functional ABCR protein on the rod disc membrane. Since *ABCR* is expressed exclusively in the rods, this could lead to their degeneration, a loss of peripheral vision and an RP phenotype. In all STGD1 patients characterised to date at least one of the two mutated *ABCR* alleles encodes a protein which could be directed to the membrane and retain some function. Thus rods in STGD1 patients may remain partially active, but lead to the accumulation of the lipofuscin-like material observed in the underlying RPE. The highest concentration of rods is 5 mm out from the fovea[14], within the zone that is affected in macular degeneration. If STGD1 and AMD rods produce an aberrant product, it could reach the highest concentration in this region. Patients with one wild-type and one mutant *ABCR* allele (either missense, splice-site or frameshift) would be predisposed to a late-onset accumulation of cellular debris (drusen) and the development of AMD[8]. The fact that the heterozygous parents of family M-33, aged 72 and 82, show no signs of AMD does not argue against haploinsufficiency as a cause of the disease. Given that AMD is a mutifactorial disorder, *ABCR* mutations would only play a predisposing role. Similarly, most of the parents of STGD1 patients are not affected of AMD. A clearer picture may emerge when more data are gathered on *ABCR* mutations associated with retinal degeneration phenotypes.

In summary, we describe a consanguineous arRP family carrying two putative *ABCR* null alleles. The ABCR expression, confined to rods, and the fact that these photoreceptors are the cell type primarily affected in RP, support *ABCR* as the gene responsible for arRP linked to *RP19*.

**Acknowledgements**

*The authors thank the members of family U.G. for their generous cooperation. This work was supported by CIC YT (SAF96-0329), FIS96-0065-01E, and FAARPE. A. M-M. is recipient of a fellowship from the Generalitat de Catalunya.*

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**Figure Legends**

Fig.1 *a,* Segregation of SSCP patterns of exon 13 in family M-33. All affected sibs present a band distinct from that of a control individual (C). The mother and unaffected sister display b oth mutant and wild-type bands. *b,* Restriction analysis of the 1847delA mutation. A mismatc hed primer that creates an *Mbo* II site in the wild-type sequence was used to show that the pro band (P) is homozygous for the deletion. The PCR fragment (194 bp) contains two *Mbo* II sit es besides the diagnostic site. Controls (C) give rise to the series: 72 + 62 + 51 + 9 (the last fr agment is undetectable), while patients show 72 + 62 + 60. (M) molecular weight marker.

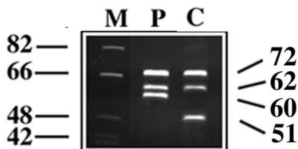
Fig.2 Chromatograms of a partial sequence of exon 13 from the proband and one control indi vidual *a,* and the proband's mother *b,*. An overlapping pattern (boxed) is observed starting at t he deletion point.

A

C

mutant allele
wT allele

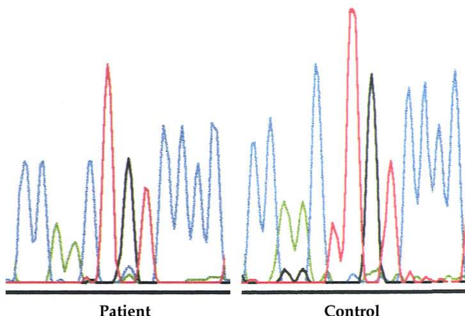
B



**A**

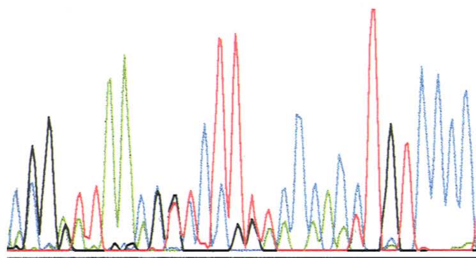
5'GCCTATCTGCAGGACATGGTTGAACAGGGGATCACAAGGAGCCAGGT3'
3'CGGATAGACGTCCTGTACCAACTTGTCCCCTAGTGTTCCTCGGTCCA5'

3'CCAACTGTCCCC5'    3'CCAACTTGTCCCC5'




**B**

Carrier



wT allele 3'CGGATAGACGTCCTGTACCAACTTGTCCCC5'
mutant allele 3'CGGATAGACGTCCTGTACCAACTGTCCCC5'