Mutations of the retinal specific ATP binding transporter gene (ABCR) in a single family segregating both autosomal recessive retinitis pigmentosa RP19 and Stargardt disease: evidence of clinical heterogeneity at this locus

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Abstract

Stargardt disease (STGD) is an autosomal recessive macular dystrophy of childhood characterised by bilateral loss of central vision over a period of several months. STGD has been mapped to chromosome 1p22.1 and recently ascribed to mutations in the retinal specific ATP binding transporter gene (ABCR). The fundus flavimaculatus with macular dystrophy (FFM), an autosomal recessive condition responsible for gradual loss of visual acuity in adulthood (second to third decade) has also been mapped to the same locus. However, a gene for autosomal recessive retinitis pigmentosa with distinctive features of choriocapillaris atrophy at an advanced stage (RP19) has been mapped to the genetic interval encompassing the STGD gene on chromosome 1p (D1S435-D1S236), raising the question of whether, despite striking differences in clinical course and presentation, RP19 and STGD might be allelic disorders at the ABCR locus.

In a family segregating RP and STGD in two first cousins, we found that heterozygosity for a splicing mutation in the ABCR gene (1938-1 G→A) resulted in STGD while hemizygosity for this splice mutation resulted in RP, and when studying the RP patient's parents, we found a maternal non-contribution with apparent segregation of a null allele ascribed to a partial deletion of the ABCR gene.

The present study shows that, despite striking clinical differences, RP19 and STGD are allelic disorders at the ABCR locus.

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Keywords: Stargardt disease; RP19; ABCR gene

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Stargardt disease (STGD) is an autosomal recessive macular dystrophy of childhood characterised by bilateral loss of central vision over a period of several months. It has an early onset (age 7 to 12 years), a rapidly progressive course, and a poor final outcome. This condition is associated with perimacular yellowish spots of depigmentation and atrophy of the retinal pigmentary epithelium. STGD has been mapped to chromosome 1p22.1[1 2] and recently ascribed to mutations in the retinal specific ATP binding transporter gene (ABCR).[3] The fundus flavimaculatus with macular dystrophy (FFM), an autosomal recessive condition responsible for gradual loss of visual acuity in adulthood (second to third decade), has also been mapped to the same locus.[4] However, a gene for autosomal recessive retinitis pigmentosa with distinctive features of choriocapillaris atrophy at an advanced stage (RP19) has been mapped to the genetic interval encompassing the STGD gene on chromosome 1p (D1S435-D1S236), raising the question of whether, despite striking differences in clinical course and presentation, RP19 and STGD might be allelic disorders at the ABCR locus.[5]

Patients and methods

PATIENTS

Two 34 year old paternal first cousins with early onset severe retinitis pigmentosa (II.2) and Stargardt disease respectively (II.1) were ascertained (fig 1) in our genetic clinic. Information regarding onset of visual symptoms, functional disability, visual acuity, fundus appearance, fluorescein angiography, Goldman perimetry, and electroretinogram (ERG) were obtained from both patients.

Blood samples from the patients and family relatives were obtained and DNA was prepared from lymphocyte pellets by SDS lysis, proteinase K digestion, phenol/chloroform extraction, ethanol precipitation, and Tris-EDTA resuspension.

METHODS

The 50 exons of the ABCR gene (200 ng genomic DNA) were amplified using intronic primers in a buffer (20 µl) containing 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.3), 1.5 mmol/l $MgCl_2$, 0.01% (w/v) gelatine, 160 mmol/l of each dNTP, 0.6 mmol/l of each primer, and 0.5 U *Taq* DNA polymerase (Gibco). After an initial denaturation for five minutes at 96°C, denaturation was at 96°C for 20 seconds, annealing at the exon specific temperature for 15 seconds, and extension at 72°C for 30 seconds, for 30 cycles followed by a final extension for seven minutes at 72°C. Oligonucleotide sequences have been previously reported.[3 6] Amplification products were loaded onto a 1% agarose gel, purified by

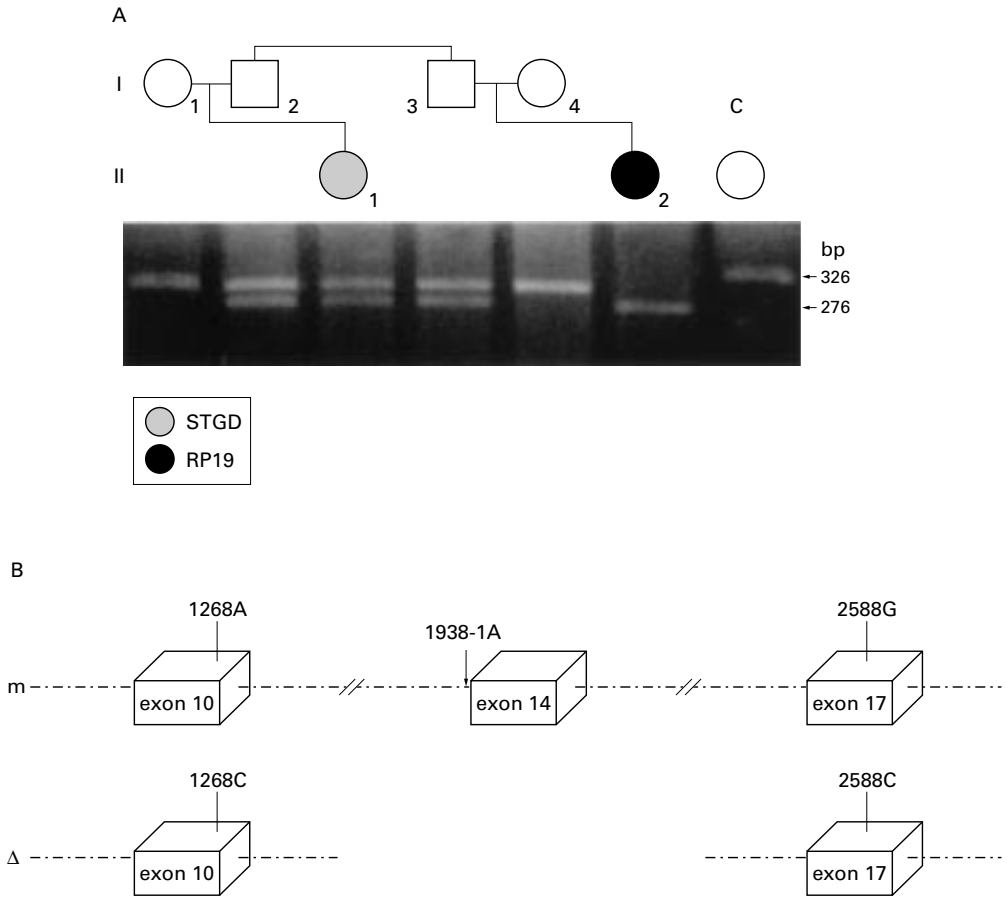

Figure 1 (A) Pedigree of the family and restriction analysis of the 1938-1 G→A mutation. The 1938-1 G→A mutation created a MseI site. PCR amplified genomic DNA was digested using MseI. Heterozygous carriers of the 1938-1 G→A (I.2, I.3, II.1) display three fragments of 326 bp (undigested), 276 bp, and 50 bp (not visible). There is no maternal contribution at the ABCR locus, as the RP patient (II.1) is hemizygous for the ABCR gene mutation while her mother (I.4) displays the 276 bp fragment only. (B) Schematic representation of the two ABCR alleles of I.4. m: allele harbouring the 1938-1 G→A mutation, Δ: allele carrying a large deletion of unknown size, encompassing exon 14 and flanked by exons 10 and 17 as shown by two heterozygous polymorphisms, 1268A/C and 2588 G/C, respectively.

phenol-chloroform extraction, and recovered by ethanol precipitation. Purified fragments were directly sequenced using intronic primers (3.2 pmol) and the PRISM™ Ready Reaction Sequencing Kit (Perkin Elmer Cetus) on an automatic fluorometric DNA sequencer (Applied Biosystems).

To analyse the segregation of the mutation, genomic DNA was submitted to PCR amplification using specific primers (1938F, 1938R).[6] Amplified products (20 µl) were ethanol precipitated. Purified fragments were digested for one hour at 37°C using restriction enzyme *Mse*I (10 units) in the buffer supplied by the manufacturer (Life Technologies).

Results

CLINICAL FINDINGS

The two paternal first cousins (II.1 and II.2, fig 1) suffered distinct retinal diseases. II.1 had typical early onset Stargardt disease from the age of 14 years and developed a slowly progressive loss of visual acuity over a period of several years. Ophthalmoscopic investigations showed typical macular dystrophy surrounded by yellowish flecks (fig 2A, B) confirmed by fluorescein angiography (not shown, available on request). The visual field showed a central scotoma (fig 3A and B). The ERG was originally normal (fig 4A) but has become photopically abnormal in the last few years (not shown).

Her cousin (II.2) developed typical RP by the end of the first decade with progressive night blindness and a concentric reduction of the visual field. At 34 years, she is almost totally blind, with a visual acuity reduced to light perception. Her fundus displays aspects of RP with pigmentary deposits all over the retina with choriocapillaris atrophy (fig 2C, D). Goldman perimetry showed a severe concentric reduction of the visual field (fig 3C, D) and an unrecordable ERG was obtained for at least 15 years (fig 4B). Her retinal disease and clinical course strictly fulfil the criteria of RP19.[5] It is worth noting that the flat trace of this patient (fig 4B) shows the abrogation of rod function while the normal trace of her cousin (fig 4A) allows exclusion of the diagnosis of RP.

MUTATION SCREENING

In the STGD patient (II.1), sequence analysis of the 50 exons and intron-exon boundaries of the ABCR gene showed an apparently heterozygous mutation in the acceptor splice site of intron 13 (1938-1 G→A). Potential splice

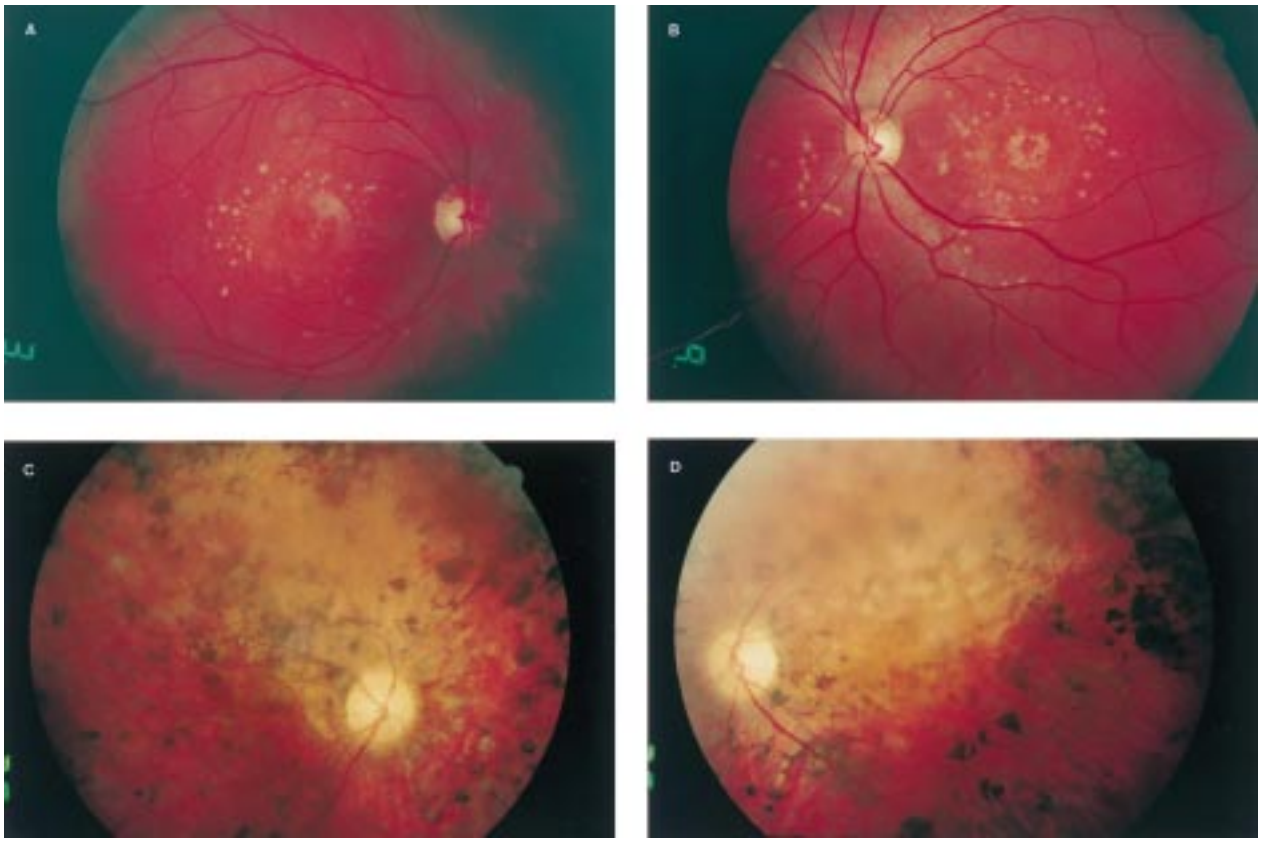

Figure 2 Ophthalmologic findings. (A, B) Fundus of II.1 showing the typical findings of Stargardt disease with macular dystrophy and whitish perimacular flecks (fundus flavimaculatus): (A) right eye, (B) left eye. (C, D) Fundus of II.2 showing typical findings of RP with pigmentary deposits, reduction of the retinal vessel calibre, and choriocapillaris atrophy: (C) right eye, (D) left eye.

scores were calculated using the "Splice Site Prediction Network" (http://www-hgc.lbl.gov/projects/splice.html) for the normal and the mutated sequence. The potential score for the normal splice site is 0.94. Another putative splice site was identified in the normal sequence at position nt 1938-43 with a potential score of 0.85. When the sequence carries the 1938-1G→A mutation, the only splice site that might be used is located at nt 1938-43 probably leading to the production of an mRNA longer (+43 nt) than the normal one, and resulting in a frameshift.

This mutation created a *Mse*I restriction site. Restriction analysis of the entire family showed that the patient and her healthy father (I.2) were both heterozygous for the splice mutation (fig 1A).

In the patient with RP19 (II.2), apparent homozygosity for the same mutation was observed. Subsequent restriction analysis showed that her father (I.3) was also heterozygous for the 1938-1 G→A splice mutation (fig 1A). Most interestingly, her mother (I.4) was apparently homozygous for the normal allele providing strong evidence for lack of maternal contribution at the ABCR locus (fig 1A, B). Unambiguous maternal contribution was observed at the other polymorphic loci tested (including flanking intragenic polymorphisms 1268 A→C and 2588 G→C, fig 1B). Although no direct evidence for deletion of the ABCR gene could be found, the apparent segregation of a null allele through the pedigree is consistent with deletion of the genomic region encompassing the acceptor splice site in intron 13.

Discussion

Here we report on a splicing mutation of the ABCR gene in first cousin patients with RP19 and STGD, respectively. It is important to note the two first cousins were of the same age and displayed unambiguously different retinal disorders. Indeed, STGD in the first patient (II.1) started at the age of 14 years and only affected the macular area, while RP in the second patient (II.2) had an early onset (before the age of 10) and involved the whole retina.

The present study shows that hemizygosity for a splice mutation at the ABCR locus (with maternal non-contribution at this locus) resulted in severe retinitis pigmentosa, RP19. This observation is consistent with the report of RP19 in a Spanish family harbouring a homozygous frameshift deletion of the ABCR gene. On the other hand, compound heterozygosity for the same splice mutation resulted in STGD in the first cousin of the RP19 patient. Although we have sequenced the entire ABCR coding sequence, we have not at present been able to identify the second ABCR gene mutation in our STGD patient, as previously reported in several other patients.[3 8] Based on this observation, we suggest giving consideration to the view that homozygosity for truncating ABCR gene mutations results in a severe RP phenotype, while compound heterozygosity for missense (or frameshift and missense) mutations at this locus

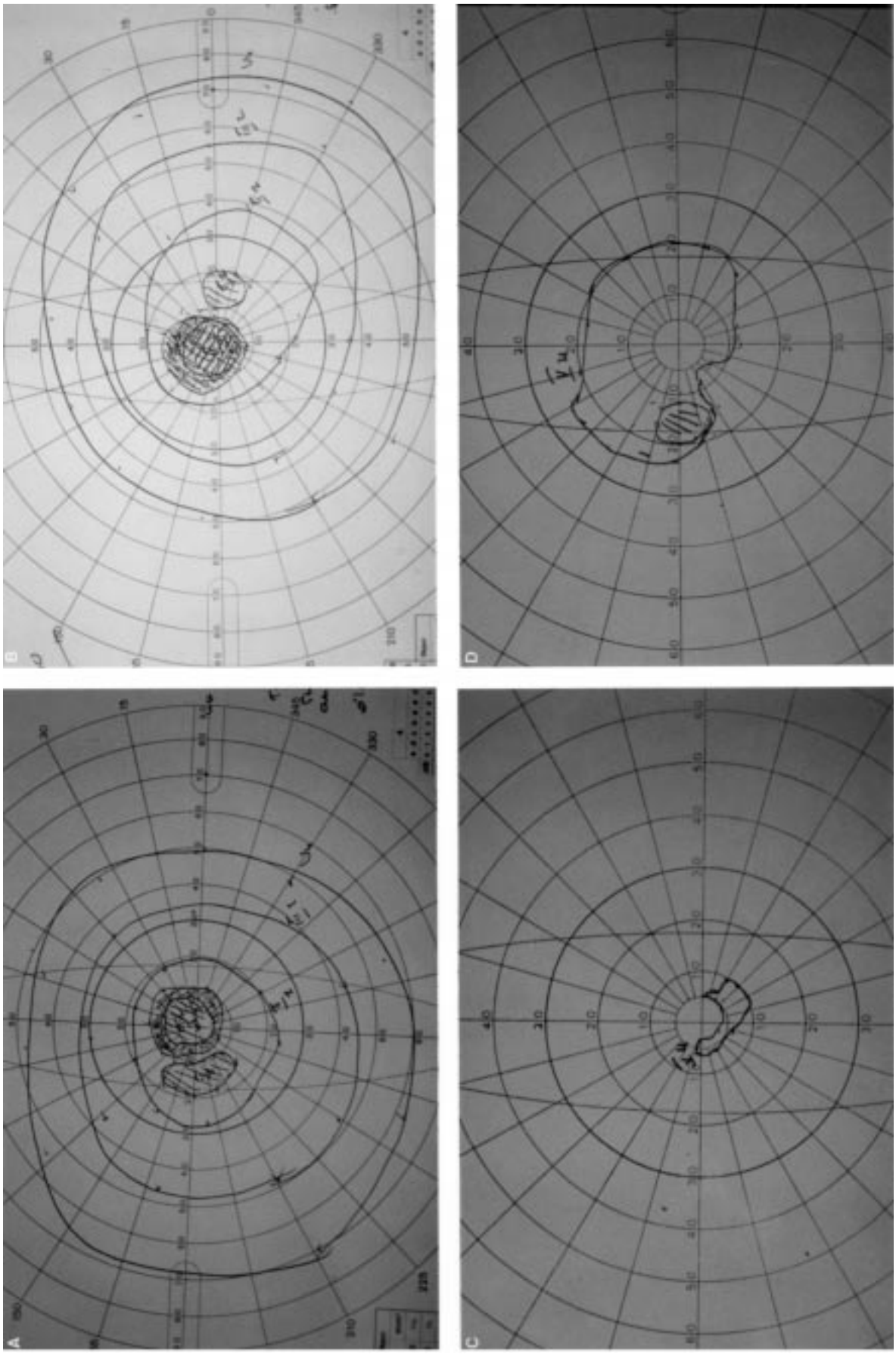

Figure 3 Goldman perimetry. (A, B) Visual field (VF) of II.1 showing a central scotoma while the peripheral VF is normal: (A) right eye, (B) left eye. (C, D) Visual field of II.2 showing a severe concentric reduction: (C) right eye, (D) left eye.

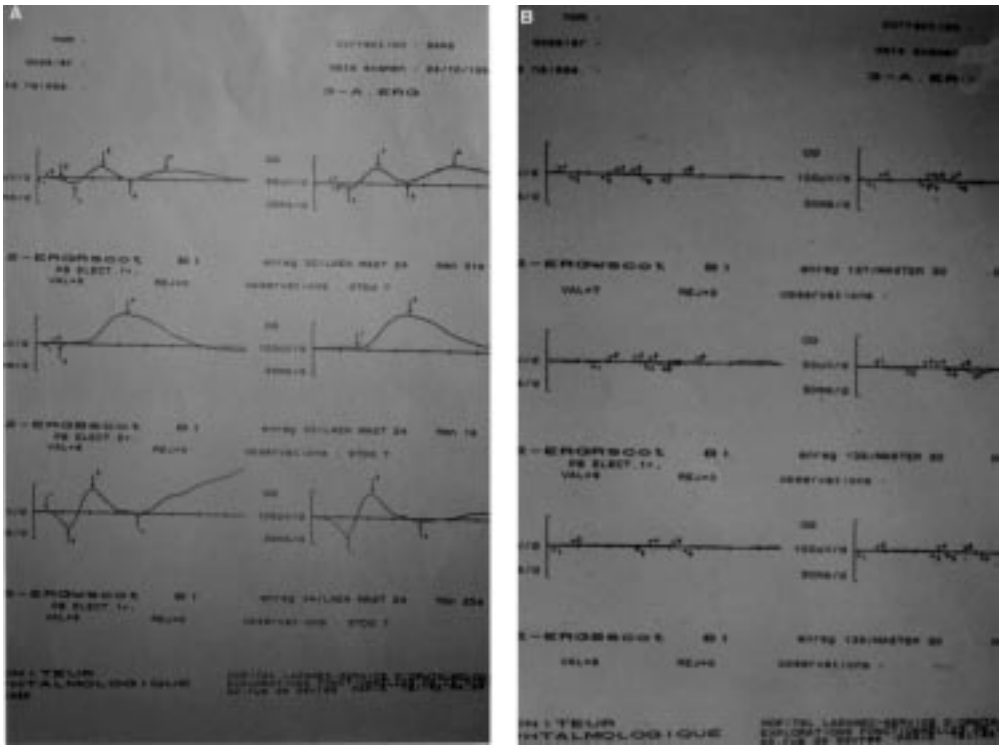

Figure 4 Scotopic electroretinogram (ERG) of right eye (left) and left eye (right). The three traces were recorded for each eye after three successive flashes (13 seconds, 38 seconds, and 95 seconds from top to bottom). (A) Normal ERG of II.1. (B) ERG of II.2: extinguished.

results in a macular disorder. Whatever the mechanism, this observation gives support to the view that despite striking clinical differences, RP19 and STGD are allelic disorders at the ABCR locus.

Clinical heterogeneity at a single gene locus has been previously reported in retinal dystrophies. For example, mutations of the peripherin gene have been associated with autosomal dominant RP, retinitis punctata albescens, and several forms of macular dystrophy.[9–11] Similarly, mutations in the β-PDE and rhodopsin genes have resulted in either RP or congenital stationary night blindness.[12–15] Considering the variable clinical expression of ABCR gene mutations and their wide range of age of onset (including perhaps age related macular dystrophies),[16–18] the present study addresses the intriguing question of the relevance of the gene in other retinal dystrophies, especially as ABCR is expressed in rods[3] and is a major gene in the ATP dependent transport of a wide variety of substrates and cellular debris across the cellular membranes of the retinal pigment epithelium. More generally, a variety of ABCR gene mutations could possibly account for a continuum of macular/retinal degenerations that has onset from early childhood to late adulthood.

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