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ORIGINAL PAPER

Spectrum of ABCR gene mutations in autosomal recessive macular dystrophies

Jean-Michel Rozet[1], Sylvie Gerber[1], Eric Souied[1], Isabelle Perrault[1], Sophie Châtelin[1], Imad Ghazi[2], Corinne Leowski[3], Jean-Louis Dufier[2], Arnold Munnich[1] and Josseline Kaplan[1]

[1]*Unité de Recherches sur les Handicaps Génétiques de l'Enfant INSERM U-393, Hôpital Necker-Enfants Malades, Paris, France*
[2]*Service d'Ophtalmologie, Hôpital Necker-Enfants Halades, Paris, France*
[3]*Institut National des Jeunes Avengles (INJA), Paris, France*

Stargardt disease (STGD) and late-onset *fundus flavimaculatus* (FFM) are autosomal recessive conditions leading to macular degenerations in childhood and adulthood, respectively. Recently, mutations of the photoreceptor cell-specific ATP binding transporter gene (*ABCR*) have been reported in Stargardt disease. Here, we report on the screening of the whole coding sequence of the *ABCR* gene in 40 unrelated STGD and 15 FFM families and we show that mutations truncating the ABCR protein consistently led to STGD. Conversely, all mutations identified in FFM were missense mutations affecting uncharged amino acids. These results provide the first genotype-phenotype correlations in ABCR gene mutations.

Keywords: ABCR mutations; macular dystrophies; Stargardt disease; genotype-phenotype correlations

Introduction

Stargardt disease (STGD), originally described in 1909, is an autosomal recessive condition of childhood, characterised by a bilateral loss of central vision over a period of several months.[1] This frequent cause of macular degeneration in children accounts for about 7% of all retinal dystrophies.[2] It has an early onset (between 7 and 12 years of age), a rapidly progressive course and a poor final visual outcome. The final visual acuity ranges from 1/60 to 3/60 and the peripheral visual field remains normal throughout life.

The *STGD* gene has been mapped to the short arm of chromosome 1[3] in a narrow genetic interval, subsequently assigned to band p22.1.[4] In addition, we have provided evidence of genetic homogeneity of the disease.[3] On the other hand, in 1965, Franceschetti described another form of fleck fundus disease termed late-onset *fundus flavimaculatus* (FFM)[5] with a Stargardt-like phenotype, but differing by a late-onset, more slowly progressive course and better prognosis. The decrease of vision is gradual and slowly progressive over many years, with a final visual acuity of 1/60–6/60. When visual acuity loss begins in the first two decades the disease is termed STGD, while the term 'FFM' is favoured when the disease begins later in life and has a slowly progressive course.[6] Yet the two conditions have

Correspondence: Dr J-M Rozet, Unité de Recherches sur les Handicaps Génétiques de l'Enfant INSERM U-393, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75743 Paris Cedex 15, France. Fax: 33 1 47 3485 14; Email: MUNNICH3@CIT12.FR
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long been regarded as varying manifestations of the same disease. Indeed, the *FFM* gene has been mapped to the STGD locus, supporting the view that the two conditions are allelic disorders.[7] Moreover, owing to the broad spectrum of age of onset in FFM (17 to 63 years), this genetic mapping addressed the intriguing question of the relevance of this locus in age-related macular degenerations (AMD) and, more generally, of a possible continuum of macular disorders at this locus from early childhood to old age.[7]

Mutations in the photoreceptor cell-specific ATP-binding transporter (*ABCR*) gene have been recently reported in STGD.[8] The ATP-Binding Cassette (ABC) family, also called 'traffic ATPases', belongs to a superfamily of membrane proteins involved in the energy-dependent transport of a wide variety of substrates across membranes[8,9] and the ABCR mouse orthologue has been identified (photoreceptor rim protein : RmP).[10] In addition, Allikmets *et al* reported on heterozygous *ABCR* gene mutations in age-related macular degenerations (AMD),[11] the most frequent cause of blindness after 70 years of age. Here we report on the screening of the 50 ABCR exons[12] in 55 unrelated STGD and FFM families. Preliminary results suggest that truncating ABCR mutations resulted in a Stargardt phenotype whereas they were consistently absent in our series of FFM patients.

Patients and Methods

Patients

A total of 58 patients belonging to 55 unrelated families were split into two groups based on their age at onset of the disease (STGD before 12 years, FFM after 17 years). Among them, 40 families had STGD (15 multiplex families) and 15 families had late-onset FFM (four multiplex families). Ophthalmological data were available for each affected individual. The time course of the disease over approximately 10 years (1–40 years) was obtained by interviewing the patients or their parents and a pedigree was established. The minimal criteria for diagnosis of STGD and FFM have been previously described.[3,7] Only 34/110 living parents were older than 60 and 49/220 living grand-parents were older than 70. Among them, 1/34 parent and 2/49 grand-parents were known to be affected with AMD.

Methods

For single strand conformation polymorphism analysis and direct sequencing of the 50 exons of the *ABCR* gene, genomic DNA (200 ng) was submitted to PCR amplification using 1 μM of intronic primers[10] and alpha^{33}P dCTP (0.1 μl, 10 mCi/ml NEN) in an amplification mixture (20 μl) containing 200 mM dNTPs and 0.5 U Taq DNA polymerase (Life Technologies). Amplified DNA (2 μl) was mixed with an equal volume of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), denatured for 10 min at 95°C, loaded on to a polyacrylamide gel (20 × 45 × 0.04 cm, MDE) and electrophoresed at room temperature for 14 h at 3 W. Gels were transferred on to 3 mm Whatman paper, dried and autoradiographed with Kodak X-OMAT films for 16–48 h.

For direct sequencing, amplification products were loaded on to a 0.7% agarose gel, purified by phenol-chloroform extraction and recovered by ethanol precipitation. Purified fragments were directly sequenced using the same specific intronic primers and the PRISM™ Ready Reaction Sequencing Kit (Perkin Elmer Cetus), on an automatic fluorometric DNA sequencer (Applied Biosystems).

Results

A total of 25 different mutations was identified in 21/40 STGD and 5/15 FFM patients, respectively. Interestingly, the truncating mutations (10/25) were identified in STGD only (3 nonsense, 2 frameshift and 5 splicing mutations, *n* = 11). In addition, 9 different missense mutations were detected in 10 additional STGD cases. Most of them (5/9) converted an arginine into an uncharged amino acid (Table 1 and Figure 1). By contrast, only missense mutations (*n* = 7) were identified in FFM patients (*n* = 5). All of them involved uncharged amino acids (4 leucines, 1 alanine and 2 glycines, Table 1 and Figure 1). On the other hand, all ABCR missense mutations identified in STGD and FFM concerned amino acids conserved in RmP[10] contrasting with the other members of the ABC family[8,9] (see Table 1).

So far, only two unrelated STGD patients were found to be homozygotes for a mutant genotype in our series (the 571–2A→G splice site mutation in intron 5 in a consanguineous family of Moroccan origin, an arginine to tryptophane substitution at codon 1640 in exon 35). The vast majority of our patients was either compound heterozygotes (5 STGD and 2 FFM) or heterozygotes for one single mutation (14 STGD and 3 FFM). None of these sequence changes have been previously reported[8] nor were they identified in any of 50 healthy controls (100 chromosomes).

It is worth noting that no mutation has been hitherto identified in the first family segregating both STGD and AMD in the patient and his mother, respectively. By contrast, in the second and third families, the grandmothers affected with AMD were found to carry one of the two different mutations identified in their grandsons affected with Stargardt disease (R1107C, family JUL; R212C, family JEG; see Table 1).

Discussion

The ABCR protein is a member of the adenosine triphosphate (ATP) binding cassette transporter superfamily[9] involved in transport of a wide variety of substrates across cellular membranes. Recently, Allikmets *et al* reported *ABCR* gene mutations in STGD.[8] The purpose of the present study was to investigate the allelic heterogeneity at the *ABCR* locus and to look for possible phenotype–genotype correlations in a large series of STGD/FFM patients.

Among the 25 hitherto unreported *ABCR* gene mutations described here, 10/25 were truncating mutations. Interestingly, mutations truncating the *ABCR* gene product were found in STGD patients only. In addition, all except one (HAD family) were either heterozygote for a frameshift mutation or compound heterozygote for frameshift and missense mutations. Based on this observation and on previously reported *ABCR* gene mutations in retinitis pigmentosa (RP)[14,15] we suggest that homozygosity for truncating *ABCR* gene mutations results in RP phenotype, while compound heterozygosity for frameshift and missense mutations or two missense mutations at this locus results in STGD. Conversely, FFM phenotype is always the result of two missense mutations. Concerning the consanguineous STGD family with an homozygous splice site mutation (HAD, see Table 1), it is most likely that this mutation will not affect the reading frame.

Most of the missense mutations detected in STGD (5/9) converted charged into uncharged amino acids. By contrast, all mutations identified in FFM patients were missense mutations involving uncharged amino acids and none of them (except the A1038V mutation) were detected in STGD. Interestingly, the A1038V mutation was found in compound heterozygotes (one STGD, family CHE and one FFM, family VIL), the second mutation being another missense mutation in the FFM family (L541P) but a frameshift mutation in STGD (Table 1). Furthermore, all ABCR missense mutations

Table 1 Mutations in the ABCR gene in STGD and FFM families

			Conserved aa in:				
Nucleotide change	*Amino acid change*	*Domain*	*ABCs*	*RmP*	*Phenotype*	*Families*	*Comment*
(571–2)A□ G	**splicing mutation**				**STGD**	**1**	**HAD[1]**
(1938–2)A□ G	**splicing mutation**				**STGD**	**1**	
(4668+2)T□ C	**splicing mutation**				**STGD**	**1**	
(4735+2)T□ A	**splicing mutation**				**STGD**	**1**	
del(5196+1–5196+6	**splicing mutation**				**STGD**	**1**	**LOZ[2]**
2570 delT	**frameshift mutation**				**STGD**	**1**	
3209insGT	**frameshift mutation**				**STGD**	**2**	**CHE[2]**
G3754T	**E1252X**				**STGD**	**1**	
C3994T	**Q1332X**				**STGD**	**1**	
C6337G	**I2113X**				**STGD**	**1**	**JEG[2]**
C52T	R18W	IC	–	+	STGD	1	
C634T	R212C	EC	–	+	STGD	5	GEN[2], JEG[2]
G1908T	Q636H	IC	–	+	STGD	1	LOZ[2]
C3056T	T1019M	IC	–	+	STGD	1	
C3322T	R1107C	IC	–	+	STGD	1	JUL[2]
C4916T	R1640W	IC	+	+	STGD	2	MAR[1]
G5929A	G1977S	ATP2	+	+	STGD	1	GEN[2]
G6320A	R2107H	IC	+	+	STGD	1	JUL[2]
C3114T	A1038V	IC	–	+	STGD	2	CHE[2]
					+FFM	+1	VII[2]
T1622C	L541P	EC	–	+	FFM	1	VII[2]
T31C	L11P	IC	+	+	FFM	1	
G3272A	G1090E	IC	+	+	FFM	1	
G4522T	G1508C	IC	+	+	FFM	1	
C5908T	L1970F	IC	+	+	FFM	1	GON[2]
T5912G	L1971R	IC	+	+	FFM	1	GON[2]

Mutations refer to the standard nomenclature. Truncating mutations are indicated by bold letters. EC: extracellular domain, IC: intracytoplasmic domain; ATP2: second ATP-binding site; RmP: mouse Rim protein, ABCs: other members of the ABC subfamily (mouse ABC1, mouse ABC2, human ABCC), +: conserved amino acid, –: non-conserved amino acid. The comment field indicates [1]homozygous families and [2]compound heterozygous families in which two independent altered alleles were identified.

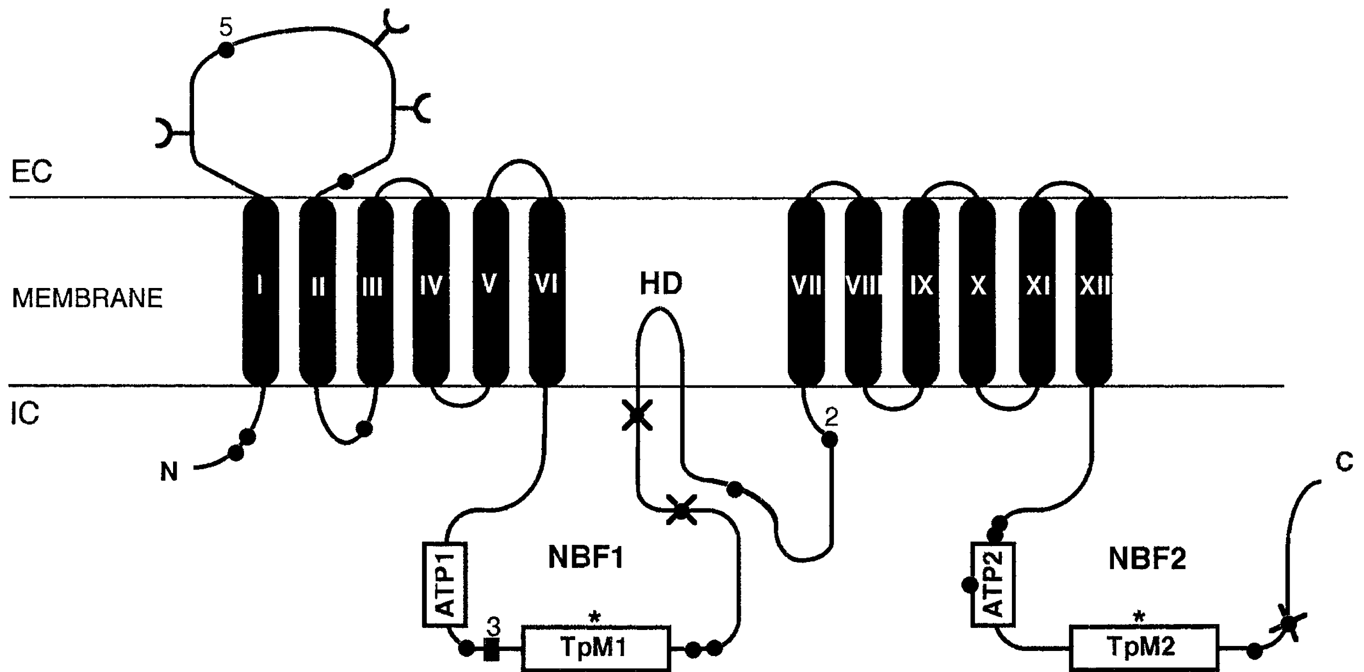

Figure 1 *Predicted topology of ABCR and position of point mutations associated with STGD and FFM. EC : extracellular domain and IC: cytoplasmic domain. The 12 predicted membrane-spanning α-helical segments are indicated with roman numbers. ⋎ predicted N-glycosylation site. ATP1 : first ATP-binding site; ATP2 : second ATP-binding site;* TpM_1 *: first transporter signature-motif;* TpM_2*: second transporter signature-motif; NBF1 : first nucleotide binding fold; NBF2 : second nucleotide binding fold; HD: proline rich hydrophobic domain. *PKA-phosphorylation sites. The approximate positions of missense mutations identified in STGD, FFM and in both phenotypes are indicated by dots, triangles and a dark square, respectively. × shows the approximate positions of nonsense mutations and* $\overset{n}{\bullet}$ *indicates that the mutation was found more than once (in n families).*

detected in STGD and FFM patients involved amino acids conserved in the mouse orthologue[10], (see Table 1). Nevertheless, no correlation between the severity of the phenotype and the conservation of the mutated amino acid in other members of the ABC subfamily could be established.[8,9] (see Table 1).

On the other hand, it is worth noting that no mutation has been hitherto identified in 29/55 families with either STGD or FFM and that 17/26 patients were found to be heterozygotes for one single mutation (although 7/17 cases were multiplex, strongly suggesting an autosomal recessive inheritance). This point could be partly explained by the defective sensitivity of the PCR–SSCP method of detecting mutations (about 80%).[13] Nevertheless, it is also likely that some of the allelic mutations lie in an unscreened region of the gene, such as the introns or the promotor region. Finally, only two patients' relatives affected with AMD were found to carry an ABCR mutation identified in a STGD proband. It is particularly important to look for macular degeneration in parents and grandparents of STGD and FFM patients so as to decide whether the incidence of AMD in this subgroup is indeed higher than in a control population.

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