A Novel ABCR Nonsense Mutation Responsible for Late-Onset Fundus Flavimaculatus

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PURPOSE. To report the ophthalmologic features of a novel truncating mutation in the ABCR gene in a patient affected with late-onset fundus flavimaculatus (FFM).

METHODS. A complete ophthalmologic examination was performed in a 70-year-old patient, including best-corrected visual acuity measurement, slit lamp and fundus examination, fundus photographs, frequent fluorescein and indocyanine green angiographies, visual field testing, color vision analysis, electroretinogram, and electro-oculogram. The 50 exons of the ABCR gene were analyzed using direct sequencing.

RESULTS. Fluorescein and indocyanine green angiographies confirmed the diagnosis of FFM. A heterozygous base change was found, resulting in the substitution of an arginine to a stop at codon 152 of the ABCR gene.

CONCLUSIONS. A heterozygous nonsense ABCR gene mutation was found in a patient affected with FFM. No other mutation has been identified in the entire coding sequence and the promoter region, suggesting that a heterozygous severe ABCR mutant may be responsible for a mild and delayed FFM phenotype, different from that of age-related macular degeneration. (Invest Ophthalmol Vis Sci. 1999;40:2740–2744)

S targardt disease (STGD) is an autosomal recessive macular dystrophy of childhood, characterized by juvenile onset, a rapidly progressive course, and poor visual outcome.1 The STGD gene has been mapped to 1p22.1, with evidence of genetic homogeneity of the disease, and has been physically mapped to a specific YAC contig.2,3 In contrast, fundus flavimaculatus (FFM), an STGD-like phenotype, described by Franceschetti and François, is characterized by late-onset and a more slowly progressive course.4 The disease is usually termed STGD when visual acuity loss begins in the first 2 decades, whereas the term FFM is favored when the disease begins at the end of the second decade or within the third decade and has a slowly progressive course.5,6 The FFM gene has been mapped to the STGD locus, supporting the idea that the two conditions are allelic disorders.3,7

The ABCR protein belongs to a superfamily of membrane proteins involved in energy-dependent transport of a wide variety of substrates across membranes.8 Mutations in the ABCR gene encoding a rod cell–specific ATP-binding cassette (ABC) transporter have been associated recently with at least four inherited retinal dystrophies: STGD, FFM, cone–rod dystrophy, and retinitis pigmentosa (RP19).8–13 Mutation screening of the ABCR gene in large series of patients affected with STGD, FFM, or retinitis pigmentosa allowed genotype-phenotype correlations.8–13 These studies suggest that homozygosity for truncating ABCR gene mutations results in retinitis pigmentosa phenotypes, whereas compound heterozygosity for frameshift and missense mutations or two missense mutations at this locus results in STGD disease. Conversely, FFM phenotype has always been reported as the result of two missense mutations. We report the ophthalmologic consequence of a novel mutation truncating ABCR in a patient affected with late-onset FFM.

METHODS

Patient

A 70-year-old man attended our department for moderate loss of central vision in the left eye (LE; 20/30). He did not mention any prior visual symptom. A complete ophthalmologic examination was performed including best-corrected visual acuity measurement, slit lamp and fundus examination, fundus photography, fluorescein angiography (FA), indocyanine green (ICG) angiography, visual field testing, color vision analysis, electroretinogram (ERG) and electro-oculogram. This patient was subsequently examined at ages 71, 72, 73, 74, 75, and 76 years. Fundus photography and FA were performed using a Topcon camera (model 50IA; Tokyo, Japan). ICG angiography was performed using a scanning laser ophthalmoscope (Heidelberg Engineering, Heidelberg, Germany). Visual field analysis (static perimetry), color vision analysis (28-hue Farnsworth–Munsell), ERG, and electro-oculogram were performed according to standardized protocols.

The patient had no siblings and was born to nonconsanguineous parents. His two healthy sons underwent best-corrected visual acuity measurement, slit lamp and fundus examination, and FA.

Mutation Analysis

The ABCR gene was analyzed using both single-strand conformation polymorphism (SSCP) and sequencing of each exon. Informed consent was obtained, as required by French bioethics legislation, in agreement with the Declaration of Helsinki for research involving human subjects. The entire coding region and intronic sequences flanking the 50 exons were analyzed using the previously described primers.14 The DNA (100 ng) from peripheral blood leukocytes was amplified using the primers and 0.1 µl [α-32P]dCTP (10 mCi/ml) in a 25-µl amplification mixture containing 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM dNTPs, and 0.5 units Taq polymerase. The PCR conditions were set for each exon. Amplified DNA (6 µl) was mixed with an
equal volume of formamide loading dye (95% formamide, 20 mM EDTA, 0.005% bromophenol blue, and 0.05% xylene cyanol). The samples (5 μl) were denatured for 10 minutes at 95°C, quickly cooled, loaded onto a polyacrylamide gel, and electrophoresed at 4 W for 18 hours at room temperature in 0.6× TBE running buffer. Gels were transferred onto Whatman paper (Maidstone, UK), dried, and autoradiographed (X-OMAT film; Eastman Kodak, Rochester, NY) for 24 hours. Exons that displayed abnormal profiles were compared with control samples from 90 unrelated and healthy French subjects.

Purified fragments were directly sequenced using the specific primers (3.2 picomoles) and a sequencing kit (PRISM Ready Reaction; Perkin Elmer–Cetus, Überlingen, Germany) on an automatic fluorometric DNA sequencer (Applied Biosystems, Foster City, CA). Direct sequencing was repeated twice, to rule out errors of the Taq polymerase.

RESULTS
Ophthalmologic Examination
Best-corrected visual acuity slowly decreased from 20/20 right eye (RE) and 20/30 LE at age 70, to 20/30 RE and 20/40 LE at age 76. No cataract was detected at slit lamp examination. Fundus exam-
inination revealed the presence of bilateral FFM yellowish flecks in the posterior pole. FA confirmed the diagnosis of FFM (Fig. 1). The "young" flecks, yellowish, sharply outlined and hypofluorescent on FA, turned to "old" flecks, gray, less defined and hyperfluorescent on FA. Two patches of perimacular retinal pigment epithelial atrophy were noted in the left eye at age 76. Dark choroid slowly increased at FA examinations. Retinal flecks, whatever their age, were constantly hypofluorescent on ICG angiography, as previously described15 (Fig. 2). Findings in visual field analysis, color vision analysis, ERG, and electro-oculogram, recorded at age 70, were all perfectly normal. FA performed in the two healthy sons (aged 44 and 38 years) showed normal eyes.

**ABCR Gene Mutation**

One abnormal pattern of migration was found in exon 5 of the *ABCR* gene by SSCP analysis. DNA sequence analysis showed that this bandshift resulted from a heterozygous C-to-T transition, at nucleotide 450, resulting in the substitution of an arginine to a stop codon at position 152 of *ABCR* (Fig. 3). Subsequently, the patient was carefully examined for a mutation on the second allele by direct sequencing of the entire promoter sequence and the 49 remaining exons. The *ABCR* gene promoter sequence16 was amplified as four partially overlapping segments (Table 1). No polymorphism, variant, or deleterious base change was found in this careful analysis. Of the two children, only the youngest (aged 38) harbored this truncating mutation. This sequence change was not observed in the 90 healthy control subjects (180 chromosomes).

**DISCUSSION**

We report a first case of an *ABCR* nonsense mutation in a patient affected with late-onset and mild FFM, subtly different from AMD. Previous screening of the *ABCR* gene in large series of STGD and FFM patients allowed the identification of numerous mutations.8–13,17,18 Phenotype-genotype correlations in a

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933584/ on 11/06/2018)

**Figure 2.** ICG angiogram of the patient at age 76. The infrared frame of the right eye (A) showed typical FFM flecks (*arrows*). The hypofluorescence, 5 minutes after ICG injection, showed precisely the extent of the flecks (B). The left eye showed on the infrared frame (C) the two well-delimited areas of retinal pigment epithelial atrophy (*open arrows*). Some peripheral flecks (*arrows*) were still obvious. The two macular atrophic areas (*open arrows*), suggesting AMD in the 76-year-old patient, were surrounded with hypofluorescent spots typical of FFM on ICG angiograms at 5 minutes (D).

![Figure 3](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933584/ on 11/06/2018)

**Figure 3.** Identification of the heterozygous mutant genotype of the *ABCR* gene. Top: the normal sequence of exon 5 of the *ABCR* gene. Bottom: the automatic sequence analysis of the patient. *Arrowhead* indicates the heterozygous base substitution shown by the superposition of a normal C in blue and an abnormal T in red. The abnormal amino acid sequence resulting from the base substitution Arg→Stop is represented.
large series of patients showed that compound heterozygosity for frameshift and missense mutations or two missense mutations results in STGD, whereas the FFM phenotype is always associated with two missense mutations.8–15,17,18

Surprisingly, in this sporadic late-onset case of FFM, a heterozygous truncating mutation was identified in the ABR gene. STGD and FFM are usually considered to be autosomal recessive conditions, and it cannot be ruled out that we failed to detect a mutation in the other allele. Indeed, previous studies using SSCP screening identified only 60% of the disease-causing mutations.8,12 In the present study, we subsequently carefully screened the 50 exons and the promoter region of the ABR gene using direct sequencing, but no other mutation was found on the second allele.

However, according to the previous phenotype–genotype correlations, a compound heterozygosity for frameshift and missense mutations would be expected to induce a severe form of the disease. Conversely, our patient was affected with very late onset of FFM and a slowly progressive course. Age at first symptoms was 70 years, which was very late, even for FFM. It is notable that the retinal dystrophy turned into retinal pigment epithelium atrophy, retinal flecks, or dark choroid. However, analysis of the ABR gene revealed that the youngest, aged 38, had inherited the deleterious mutation. Considering the age of onset of the proband, we cannot exclude that macular dystrophy would appear later in life.

In conclusion, we have found a heterozygous truncating ABR gene mutation associated with a very mild and late-onset form of FFM. Although the question of whether the ABR gene is involved in AMD has become controversial, we demonstrated in this study that the ABR gene was involved in this “age-related FFM.” No other mutation on the second allele has been detected in the entire coding sequence, splice junctions, or the promoter region, suggesting that a truncating mutation could lead to a FFM phenotype late in life. This hypothesis remains open to debate. Further ABR gene analyses of late-onset FFM cases are awaited.

References

Enhanced Secretory Group II PLA₂ Activity in the Tears of Chronic Blepharitis Patients

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PURPOSE. Phospholipase A₂ (PLA₂) hydrolyzes phospholipids, one of the important constituents of human meibomian gland secretions. This study was performed to investigate PLA₂ type and activity in the tears of chronic blepharitis patients compared to those of normal persons.

METHODS. Tear samples of 36 patients and 10 normal persons were collected in non-heparinized microcapillary tubes. PLA₂ activity in the tears was measured by Dole’s method, and the results of the blepharitis patients were compared to those of the normal persons. The characterization of PLA₂ was performed by the head group preference test and the dithiothreitol (DTT) sensitivity test. The classification of PLA₂ type was done using Western blot analysis with anti-human secretory PLA₂ antibody.

RESULTS. No statistically significant differences were found among the six categories of chronic blepharitis. However, the mean PLA₂ activity in the tears of the chronic blepharitis patients was about two times higher than that of the normal controls with statistical significance (P < 0.05). The PLA₂ substrate specificity test revealed group II PLA₂ activity. Furthermore, the group II PLA₂ was identified as a 14 kDa band in Western blot analysis using an antibody raised against human secretory group II PLA₂.

CONCLUSIONS. Secretory group II PLA₂ activity was significantly enhanced in the tears of the chronic blepharitis patients compared with that of the normal controls. It is suggested that this increased enzymatic activity may decrease the tear film stability through increased hydrolysis of phospholipids. (Invest Ophthalmol Vis Sci. 1999;40: 2744-2748)

Chronic blepharitis, a commonly encountered condition, is characterized by variable pictures of eyelid inflammation and is difficult to manage.

There are several classifications of chronic blepharitis. Dougherty and McCulley have expanded the two fundamental categories of marginal lid inflammation into six categories, while at the same time placing a strong emphasis on the role of the meibomian glands in the inflammatory picture.

The tear film lipid layer, derived primarily from secretions of meibomian glands, is important in tear film stability. The composition of human meibomian gland secretions have been reported as hydrocarbons (7%), sterol esters (27%), wax esters (32%), triglycerides (4%), polar lipids (15%), free sterols (2%), and free fatty acids (2%). Significant differences have been discovered in meibomian lipid composition between chronic blepharitis patients and normal individuals. The constituents of polar lipids as well as sterol esters and wax esters are important.