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Microarray-based mutation analysis of the *ABCA4* gene in Spanish patients with Stargardt disease: evidence of a prevalent mutated allele

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Purpose: To evaluate, in a pool of affected families, the mutation spectrum in Stargardt patients from Spain, using the ABCR400 microarray that contains described sequence variants in the gene encoding for the photoreceptor specific ATP-binding cassette transporter (*ABCA4*).
Methods: We analyzed 76 Spanish patients with STGD1 for a population-specific survey on the sequence variations in the *ABCA4* gene, using the ABCR400 microarray.
Results: Potential disease-associated alleles were identified in 91 of the 152 STGD1 chromosomes studied, resulting in a detection rate of 60%. The two mutant alleles were found in 33/76 patients (43%), whereas in 25/76 cases (33%) only one allele could be identified. In the remaining 18 patients no mutations were found. In total, we identified 40 sequence variations that could be related to the disease. The vast majority of these substitutions (35/40) were missense mutations. Three frameshift mutations and two splicing variants were also found.
Conclusions: We identified a major disease-associated allele, R1129L, which accounted for 24% of the mutated alleles detected, and a high frequency (12%) of complex alleles.

Stargardt disease (STGD1; OMIM 248200) is one of the most common forms of autosomal recessive macular degeneration. The disease has a juvenile to young adult onset and is characterized by bilateral loss of central vision, progressive bilateral atrophy of the macular retinal pigment epithelium (RPE), and accumulation of a lipofucsin-like substance in the RPE cells [1].

The gene responsible for STGD1 disease, *ABCA4* (OMIM 601691; GenBank U88667), has been mapped to chromosome 1p21-p22 [2] and contains 50 exons. The *ABCA4* gene encodes the ABCR protein, a member of the ATP-binding cassette transporters superfamily. It is involved in the transport of vitamin A derivatives across the disc membrane [3,4]. Photons of light convert the 11-*cis* retinal from rhodopsin into all-*trans* retinal. The all-*trans* retinal leaves the photoactivated rhodopsin molecule and enters the intradiscal space. Some of it combines with phosphatidylethanolamine (PE), forming a complex known as *N*-retinylidene-PE (*N*-RPE) to facilitate the all-trans retinal transport from the disk lumen to the photoreceptor cytoplasm. In Abca4 (-/-) mice, *N*-RPE is converted into A2E, a major component of lipofuscin. The A2E produced would accumulate in the RPE, dissolving cell membranes and destroying the RPE cells storing it [5].

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Different, albeit related, macular disorders have been associated with mutations in the *ABCA4* gene: Stargardt Disease (STGD1) [6], autosomal recessive cone rod dystrophy, CRD [7], autosomal recessive retinitis pigmentosa (ARRP) [8], and age-related macular degeneration (AMD) [9]. A model, based on the amount of residual protein activity produced by mutant *ABCA4* alleles, has been proposed to explain the severity of the *ABCA4*-related diseases [6,7,10-12]. Patients carrying two null mutations would manifest as ARRP. Cases combining a null allele and a moderately severe mutation would manifest as CRD. The inheritance of a null mutation together with a mild change or the inheritance of two moderately severe mutations would give rise to a STGD1 phenotype and individuals with one wild-type allele and one mutant allele would be at risk for AMD.

Numerous mutation analyses in patients with these inherited macular disorders have yielded more than 400 different *ABCA4* mutations [6,9,11,13-21]. Recently, a genotyping microarray (ABCR400 chip) was developed containing 438 variations that include all currently known disease-associated genetic variants and many common polymorphisms of the *ABCR* gene [22].

In this study we utilized the ABCR400 array to systematically screen for mutations in Spanish patients with STGD1 disease in order to assess the nature and frequency of *ABCR* mutant alleles in our population.

METHODS

Subjects and diagnostic criteria: Samples were collected all over Spain from groups belonging to the Retinal Dystrophy Investigation Spanish Network (EsRetNet). Informed consent was obtained from all participants. The ophthalmologic examination included documenting the personal and family history of the patient, visual acuity tests, central and peripheral visual fields tests, electroretinography, and a color vision test. The diagnosis of STGD1 was based on the following criteria: (1) a recessive mode of inheritance; (2) bilateral central loss of vision with a beaten-bronze appearance and/or the presence of orange-yellowish flecks in the retina from the posterior pole to the midperiphery; (3) normal caliber of the retinal vessels and no pigmented bone-spicules in the retinal periphery and (4) normal to subnormal electroretinograms (ERGs).

Molecular methods: Blood was collected by venipuncture, and genomic DNA was isolated by the salting-out method. All the exons of the *ABCA4* gene were PCR-amplified as described previously [23] and used in the primer extension reaction (APEX) on the ABCR400 microarray as described elsewhere in the literature [22].

Haplotypes were constructed after the analysis of six $[CA]_n$ microsatellite markers flanking the *ABCA4* gene (D1S435, D1S2804, D1S2868, D1S236, D1S2664, D1S2793) and were analyzed in an automatic genetic analyzer (ABIprism 3100, Applied Biosystems, Foster City, CA).

The presence of the R1129L mutation was analyzed in 100 control chromosomes by conventional PCR amplification, enzyme restriction digestion (37 °C overnight) with Acl I, and analyses of the restriction fragments in a 2% agarose gel.

TABLE 1.

Nucleotide change	Amino acid change	Cases	Exon	Reference
c.455G>A	R152Q	3	5	[30]
c.466A>G	I156V	3	5	[17]
c.634G>T	R212C	6	6	[31]
c.671 del C	p.V195fs	2	6	[22]
c.768G>T	V256 Splice	1	6	[16]
c.1140T>A	N380K	1	9	[21]
c.1804C>T	R602W	4	13	[15]
c.2300T>A	V767D	1	15	[23]
c.2546T>C	V849A	1	16	[21]
c.2588G>C	G863A/G863del	1	17	[9]
c.2690C>T	T897I	1	18	[16]
c.2701A>G	T901A	2	18	[18]
c.2791G>A	V931M	1	19	[15]
c.2888 del G	p.L934fs	2	19	[9]
c.3056C>T	T1019M	2	21	[11]
c.3163C>T	R1055W	1	21	[20]
c.3211_3212 ins GT	p.D1048fs	3	22	[9]
c.3322C>T	R1108C	5	22	[15]
c.3323C>A	R1108H	1	22	[21]
c.3364G>A	E1122K	1	23	[15]
c.3386G>T	R1129L	22	23	[6]
c.3758C>T	T1253L	1	25	[18]
c.4139C>T	P1380L	1	28	[15]
c.4222T>C	W1408R	2	28	[15]
c.4457C>T	P1486L	2	30	[15]
c.4469G>A	C1490Y	1	30	[21]
c.4926C>G	S1642R	1	34	[32]
c.4918C>T	R1640W	2	35	[11]
c.5395A>G	N1799D	1	38	[21]
c.5547+5G>A	Splice	3	40	[7]
c.5653G>A	E1885K	1	40	[18]
c.5819T>C	L1940P	3	41	[20]
c.5882G>A	G1961E	10	42	[9]
c.5929G>A	G1977S	3	43	[11]
c.6179T>G	L2060R	1	45	[20]
c.6320G>C	R2107P	1	46	[9]
c.6320G>A	R2107H	4	46	[11]
c.6449G>A	C2150Y	1	47	[33]
c.6721C>G	L2241V	1	48	[18]
c.6764G>T	S2255I	10	49	[25]

ABCA4 mutations found in patients with Stargardt disease in this study.

RESULTS

Results for families 14 and 16 were improved with the detection of another mutation by using of the ABCR400 chip. For family 14 which has been described as homozygous for the complex allele R152Q+R2107H, the ABCR400 chip detected another mutation in homozygosis, the R1108C mutation. Thus the affected in this family was homozygous for the complex allele R152Q+R1108C+R2107H. For family 16 only the N1799D mutation had been described in this family, but the use of the ABCR400 chip allowed the detection of the complex allele N1799D+N380K in heterozygosis.

Two potential mutant alleles were found in 33/76 patients (43%), whereas in 25/76 cases (33%) only one allele could be identified. In the remaining 18 patients, no mutations were found.

Several sequence changes were detected. A total of 40 different sequence variants related to the disease were identified. The vast majority of these substitutions (35/40) were missense mutations. Three frameshift mutations and two splicing variants were also found (Table 1).

The R1129L allele accounts for 24% of the disease-associated alleles. We found the following (1) five homozygous patients (ARDM-47, ARDM-111, ARDM119, ARDM-128, and B-223) for this mutation belonging to unrelated families. Only one patient (B-223) belongs to a consanguineous family; (2) we found nine patients in whom the R1129L mutation was inherited along with another mutation and (3) three heterozygous cases in which only the R1129L allele was identified (Table 2). The clinical data of the patients harboring the R1129L mutation are also shown in Table 2. None of the 100 control chromosomes analyzed harbored the R1129L mutation.

In the patient belonging to the ARDM-61 family, the microarray results indicated that this patient was homozygous for the R1129L mutation. In our analysis the parents, we found the father showed a heterozygous pattern for the R1129L mutation but the mother did not. Co-segregation analysis with microsatellite markers showed that the R1129L mutation was provided by the father and that there was a deletion compassing the *ABCA4* gene in the maternal allele.

The next most common potential mutations from the total detected, sorted by decreasing frequency, were G1961E (9%), R212C (5%), R1108C (4%), R2107H (4%), and R602W (4%).

For patients with more than one mutation, molecular analysis of the parents was performed to establish the haplotype. The presence of a complex allele was detected in eleven patients. The different groups of variants acting in *cis* (complex alleles) are shown in Table 3.

TABLE 2.

Family number	Allele 1	Allele 2	Age	Sex	Age at onset	Central scotoma	Actual visual acuity	ERG	Mac	Additional findings
ARDM-76	Ex.23 (3386G>T) R1129L	Ex.6 (768G>T)V256 Splice	32	F	9	Yes	0.1/0.1	-	No	RPE atrophy
B262	Ex.23 (3386G>T) R1129L	Not detected	31	F	10	Yes	0.06/0.08	-	Yes	Optic pit left eye
ARDM-78	Ex.23 (3386G>T) R1129L	Not detected	37	F	10	No	0.1/0.1	-	No	
B270	Ex.23 (3386G>T) R1129L	Not detected	37	F	10	Yes	0.05/0.2	2	Yes	
ARDM-155	Ex.23 (3386G>T) R1129L	Ex 13 (1804C>T) R602W	21	M	11	Yes	0.3/0.3	1	Yes	RPE atrophy
ARDM-57	Ex.23 (3386G>T) R1129L	Ex.42 (5882G<A) G1961E+Ex.48 IVS+21C>T Splice	23	F	15	Yes	0.3/0.4	1	Yes	Photosensitive
ARDM-82	Ex.23 (3386G>T) R1129L	Ex.23 (3364G>A) E1122K	17	M	15	Yes	0.2/0.3	2	Yes	Myopia, photosensitive
ARDM-162	Ex.23 (3386G>T) R1129L	No detected	48	F	16	Yes	0.1/0.2	-	Yes	
ARDM-66	Ex.23 (3386G>T) R1129L	Ex.41 (5819T>C) L1940P	34	F	17	Yes		x	No	Photosensitive
ARDM-139	Ex.23 (3386G>T) R1129L	Ex 5 (455G>A) R152Q+Ex 22 (3322C>T) R1108C+Ex 46 (6320G>A) R2107H	42	M	20	Yes	0.1/0.1	-	Yes	RPE atrophy
ARDM 96	Ex.23 (3386G>T) R1129L	Ex.35 (4918C>T) R1640W+Ex.28 (4222T>C) W1408R	40	M	21	Yes	0.15/0.1	-	No	RPE atrophy
B258	Ex.23 (3386G>T) R1129L	Ex 6 671 del C	45	M	25	Yes	HM/0.1	-	Yes	Accidental retina detachment
ARDM-62	Ex.23 (3386G>T) R1129L	Ex.43 (5929G>A) G1977S	52	M	29	Yes	CF/HM	2	Yes	Cataract, RPE atrophy, chronic glaucoma
ARDM-31	Ex.23 (3386G>T) R1129L	Ex.35 (4926C>G) S1642R	51	M	40	Yes	0.2/0.5	1	Yes	RPE atrophy, parafoveolar teleangiectasia, photosensitive
ARDM-61	Ex.23 (3386G>T) R1129L	Deletion	38	F	9	Yes	0.5/0.5	x	Yes	Strabismus (2-3 y), RPE atrophy, FFM
ARDM-111	Ex.23 (3386G>T) R1129L	Ex.23 (3386G>T) R1129L	35	F	31	Yes	0.1/0.8	1	Yes	
B223	Ex.23 (3386G>T) R1129L	Ex.23 (3386G>T) R1129L	33	F	21	No	0.16/0.125	1	No	Consanguinity
ARDM-119	Ex.23 (3386G>T) R1129L	Ex.23 (3386G>T) R1129L	32	F	19	Yes	0.4/0.1	2	Yes	RPE atrophy, dischromatopsy
ARDM-47	Ex.23 (3386G>T) R1129L	Ex.23 (3386G>T) R1129L	27	F	19	Yes	0.1/0.1	2	Yes	Strabismus, RPE atrophy, FFM
ARDM-128	Ex.23 (3386G>T) R1129L	Ex.23 (3386G>T) R1129L	41	F	12	Yes	0.1/0.1	-	Yes	RPE atrophy

Clinical data of Stargardt disease patients with the R1129L mutation. “ERG” column lists electroretinographic features. A “1” indicates normal and a “2” indicates minimal rod or cone abnormalities. A hyphen indicates that an ERG was unrecordable. An “x” indicates that no information about an ERG, whether recorded or unrecordable, was obtained. There were no ERGs recorded that showed severe dysfunction. In the “Mac” column, an asterisk indicates the presence of the characteristic macular changes of Stargardt disease. CF represents counting fingers; HM represents hand movements; RPE represents retinal pigmented epithelium; FFM represents fundus flavimaculatus.

DISCUSSION

The *ABCA4* gene, the largest of the ATP-binding cassette transporter genes, comprises 50 exons and has an open reading frame of 6,819 base pairs. To detect mutation in this gene constitutes a real challenge that can be overcome by using high-throughput technologies such as the ABCR400 microarray developed by Asper Biotech, Tartu, Estonia [22].

More than 200 families with patients clinically diagnosed with STGD1 disease are registered in the EsRetNet databases. To determine the genetic variation underlying STGD1 in Spanish population, we selected 76 patients that met the clinical criteria as detailed in the Materials and Methods section.

Jaackson et al. [22] 2003 reported that the ABCR400 array is theoretically able to detect about 56% of the disease-associated alleles in populations of European origin. The experimental data obtained by those authors correlated with the theoretical calculations. The ABCR400 array detected 54-56% of all possible disease-associated *ABCA4* alleles in European cohorts. In our set of samples including 76 unrelated STGD1 patients, a total of 91 potential disease-associated alleles were identified, yielding a detection rate of 60% by the ABCR400 chip. These authors also calculated that both disease-causing alleles would be detected in approximately 31% of patients; one mutated allele would be found in about 49%, whereas no mutation would be identified in the remaining 20% of the cases. Their experimental data indicate that the detection of both disease-associated alleles was higher than predicted (36,6%) and that the fraction of patients detected with no apparent STGD1 alleles was also higher than expected (24,4%). In our series of Spanish patients, the two mutant alleles were found in 43% of the patients; only one allele could be identified in 33%, whereas no mutations were found in the remaining 24% of the cases.

The patterns of the disease-associated alleles identified in this cohort of Spanish patients differ from other European populations previously analyzed. Table 4 shows the differences in the frequencies of the three most prevalent alleles reported in Europe.

In our Spanish STGD1 patients, the most frequent disease-associated variant detected is the missense R1129L mutation, which accounts for 24% of all identified STGD1 alleles in this study. A prevalence for this allele of 1% has been found in STGD1 patients from North America [21]. The biochemical characterization of a recombinant ABCR protein with the R1129L mutation revealed a substantial reduction in both expression and ATP-binding activity, when compared with the wild-type ABCR, which supports its role in the pathogenesis of the STGD1 disease [24].

R1129L can be regarded as a moderately severe mutation, given that the five unrelated homozygous patients were diagnosed with STGD1 in their early adulthood (ages 12, 19, 19, 21, and 31; Table 2). In cases in which R1129L was inherited along with a second identified mutation, the range of the age of onset of the disease is wider (9 to 40 years; Table 2). In these cases, the severity of the symptoms must be related to the nature of the second mutated allele. In two of these patients, the onset was before the age of 10 years: In patient ARDM-76, the first symptoms appeared at an early age (9 years). She is a compound heterozygous R1129L/c.768G->T. In this patient the nucleotide substitution of the third position in codon 256, which does not change the amino acid residue, creates an alternative splice site that generates an aberrant transcript [16], leading to an absence of normal gene product. Patient ARDM-61 has a large deletion encompassing the genomic region flanked by the extragenic markers D1S435 and D1S2793 as the second mutation, hence the early age of onset could be associated to this deletion. In contrast, patient ARDM-31, with an age of onset of 40 years, is a compound heterozygous R1129L/S1642R. This last substitution is located in the transmembrane domain of the protein and can be regarded as a mild mutation. Thus, the position and type of mutation, together with the selected combination of mutant alleles, are important determinants for the onset of visual impairment.

One of the most frequently mutated alleles in several populations analyzed is the G1961E mutation. A frequency of 6,6% (10 alleles in 76 patients) was found in Spanish STGD1 pa-

Table 3.

Family number	Allele 1	Allele 2
12	T1253L+G1961E	Not detected
110	R1108C+R2107H	G1961E
116	R1108C+R2107H	C1490Y
14	R152Q+R1108C+R2107H	R152Q+R1108C+R2107H
17	R152Q+R1108C+R2107H	R2107P
139	R152Q+R1108C+R2107H	R1129L
15	G863A+R1055W	2888 del G FS
16	N380K+N1799D	Not detected
96	W1408R+R1640W	R1129L
RP193	W1408R+R1640W	G1961 E
RP224	R1108H+R212C	V849A

Shown are complex alleles of the *ABCA4* gene that were identified in this study.

Table 4.

Mutation	Stargardt disease population	Allele frequency	Reference
G1961E	Slovenian	21%	[22]
	Italian	13%	[22]
		10%	[25]
	German	12%	[18]
	Dutch	11%	[22]
	Spanish	6.57%	This report
G863A	Dutch/German	18.75%	[16]
	English	3.6%	[17]
	French	2.8%	[34]
	Spanish	0.6%	This report
		3%	[20]
	Italian	0%	[23]
R1129L	Spanish	14.47%	This report

Allele frequencies of the most prevalent mutations in the *ABCA4* gene in distinct European populations of Stargardt disease patients.

tients, which represents the lowest range observed in the European studies. This mutation has a frequency of 21% in the Slovenian population, in contrast with an average frequency for the central European populations of 10%, being the frequency rate even lower in the case of the Spanish population. Fumagalli et al. [25] performed a polymorphism analysis in exon 42 and defined a common haplotype linking the G1961E mutation to three single nucleotide polymorphysm (SNPs; c.5836-43A->C; c.5836-11A->G and c.5844A->G), suggesting a common origin from the same ancestral chromosome for this mutation. In the Spanish STGD1 patients analyzed, the G1961E mutation was always in *cis* with two SNPs detected in the chip c.5682G->C, c.5603A->T. These SNP are not the same as the evaluated by Fumagalli but they are suggestive of a common origin for the mutation in these patients.

The missense R212C mutation has an allele frequency of 5% in our Spanish population. This variant is located in the intradiscal domain of the protein, and has been considered as a mild allele. It has been reported in a homozygous state in patients with an early onset of the STGD1 disease and in patients with CRD [23,26]. In our set of patients, the R212C mutation was detected in five families. Only one STGD1 patient was homozygous for this R212C mutation; the second disease-associated allele was characterized in two additional families (G1977S classified as a mild mutation and c.3211_3212 ins GT classified as a severe mutation, as it leads to protein termination 11 amino acids further downstream); for another family, the R212C substitution was found in a complex allele (R212C+R1108H) in combination with the V849A mutation (family RP224 in Table 3) and the second mutated allele was not identified in the remaining family. For the first three patients (R212C/R212C; R212C/c.3211_3212insGT; R212C/G1977S) the ages of onset were around 14-15 years with severe reduced visual acuity and ERG normal or with minor alterations. For the patient carrying the R212C/G1977S genotype, a diminished audition was also referred. The age of onset in the patient carrying the complex allele (R212C+R1108H) with the V849A mutation was 26 years, later than the others. He has low visual acuity (counting fingers) and a typical STGD1 fundus with minor abnormalities in the ERG pattern.

For the missense S2255I mutation, the allelic frequency in our study is 9%. This variant has been described as enriched among patients with Stargardt disease but not to the extent that would be expected if they were fully penetrant Stargardt alleles [21]. These alleles could be defined as having limited pathogenicity. Thus, they would not be expected to cause the disease, if paired with themselves or each other. However, they could cause the disease, if paired with another allele of higher pathogenicity. Therefore, the modifying effect of this variant on the course of the disease is not clear, but defining the role of this particular change in the pathology of this disease could be interesting.

In this study, the R1108C and R2107H mutations were always identified in the context of complex alleles. In four out of six of these alleles, the two mutations were linked to a third substitution (R152Q; Table 3).

The existence of two or more alterations in the *ABCA4* gene occurring in *cis* configuration ("complex alleles") was first reported by Shroyer et al. [27], who detected complex alleles in a family manifesting both STGD1 disease and RP [28]. Recently, Klevering et al. [29], using the *ABCA4* chip, found a complex allele in 26% of the *ABCA4* mutated alleles in CRD patients. In the present study, the complex alleles account for 12% of the mutated alleles. These data show that the detection of complex segregating alleles in STGD1 families is much higher when the *ABCA4* chip is used. This could be due to the limitations of the technique used. Another explanation could be that when classic methods (SSCP, DGGE, DHPLC, etc.) are employed to screen for mutations in a given gene with a recessive pattern of inheritance, a definitive genotype is assumed when two likely pathogenic mutations are detected, leaving some exons unevaluated. This limitation is overcome by the use of the array that can simultaneously detect more than 400 variants in the *ABCA4* gene. For the samples retested (families 12, 14, 15, 16), the use of the ABCR chip improved the mutation detection rate obtained using SSCP analysis only. Functional analysis of the complex alleles should improve our understanding of the way in which mutations interact within the same mutant protein.

The considerable size of the *ABCA4* gene and its remarkable allelic heterogeneity complicates the mutation screening. In this regard, the *ABCA4* array proved to be an efficient screening tool for known mutation/variants in the gene. However, the ABCR400 chip could help to provide evidence of new deletions or rearrangements in this gene. We assessed the mutation spectrum of the *ABCA4* gene underlying STGD1 in Spain and a prevalent disease-associated allele was identified, which accounted for 14.3% of the STGD1 alleles. Further studies will help in the definition of the genotype-phenotype relationship in *ABCA4*-associated retinal dystrophies, clarifying the role of complex alleles and of those with limited pathogenecity.

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REFERENCES

1. Hadden OB, Gass JD. Fundus flavimaculatus and Stargardt's disease. Am J Ophthalmol 1976; 82:527-39.
2. Gerber S, Rozet JM, Bonneau D, Souied E, Camuzat A, Dufier JL, Amalric P, Weissenbach J, Munnich A, Kaplan J. A gene for late-onset fundus flavimaculatus with macular dystrophy maps to chromosome 1p13. Am J Hum Genet 1995; 56:396-9.
3. Sun H, Molday RS, Nathans J. Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease. J Biol Chem 1999; 274:8269-81.
4. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the function of Rim protein in photoreceptors and

etiology of Stargardt's disease from the phenotype in abcr knockout mice. Cell 1999; 98:13-23.
5. Glazer LC, Dryja TP. Understanding the etiology of Stargardt's disease. Ophthalmol Clin North Am 2002; 15:93-100,viii.
6. Allikmets R, Singh N, Sun H, Shroyer NF, Hutchinson A, Chidambaram A, Gerrard B, Baird L, Stauffer D, Peiffer A, Rattner A, Smallwood P, Li Y, Anderson KL, Lewis RA, Nathans J, Leppert M, Dean M, Lupski JR. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat Genet 1997; 15:236-46. Erratum in: Nat Genet 1997; 17:122.
7. Cremers FP, van de Pol DJ, van Driel M, den Hollander AI, van Haren FJ, Knoers NV, Tijmes N, Bergen AA, Rohrschneider K, Blankenagel A, Pinckers AJ, Deutman AF, Hoyng CB. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. Hum Mol Genet 1998; 7:355-62.
8. Martinez-Mir A, Paloma E, Allikmets R, Ayuso C, del Rio T, Dean M, Vilageliu L, Gonzalez-Duarte R, Balcells S. Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. Nat Genet 1998; 18:11-2.
9. Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, Zabriskie NA, Li Y, Hutchinson A, Dean M, Lupski JR, Leppert M. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science 1997; 277:1805-7.
10. Sun H, Nathans J. Stargardt's ABCR is localized to the disc membrane of retinal rod outer segments. Nat Genet 1997; 17:15-6.
11. Rozet JM, Gerber S, Souied E, Perrault I, Chatelin S, Ghazi I, Leowski C, Dufier JL, Munnich A, Kaplan J. Spectrum of ABCR gene mutations in autosomal recessive macular dystrophies. Eur J Hum Genet 1998; 6:291-5. Erratum in: Eur J Hum Genet 1999; 7:102.
12. Cideciyan AV, Aleman TS, Swider M, Schwartz SB, Steinberg JD, Brucker AJ, Maguire AM, Bennett J, Stone EM, Jacobson SG. Mutations in ABCA4 result in accumulation of lipofuscin before slowing of the retinoid cycle: a reappraisal of the human disease sequence. Hum Mol Genet 2004; 13:525-34.
13. Nasonkin I, Illing M, Koehler MR, Schmid M, Molday RS, Weber BH. Mapping of the rod photoreceptor ABC transporter (ABCR) to 1p21-p22.1 and identification of novel mutations in Stargardt's disease. Hum Genet 1998; 102:21-6.
14. Rozet JM, Gerber S, Ghazi I, Perrault I, Ducroq D, Souied E, Cabot A, Dufier JL, Munnich A, Kaplan J. 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. J Med Genet 1999; 36:447-51.
15. Lewis RA, Shroyer NF, Singh N, Allikmets R, Hutchinson A, Li Y, Lupski JR, Leppert M, Dean M. Genotype/Phenotype analysis of a photoreceptor-specific ATP-binding cassette transporter gene, ABCR, in Stargardt disease. Am J Hum Genet 1999; 64:422-34.
16. Maugeri A, van Driel MA, van de Pol DJ, Klevering BJ, van Haren FJ, Tijmes N, Bergen AA, Rohrschneider K, Blankenagel A, Pinckers AJ, Dahl N, Brunner HG, Deutman AF, Hoyng CB, Cremers FP. The 2588G—>C mutation in the ABCR gene is a mild frequent founder mutation in the Western European population and allows the classification of ABCR mutations in patients with Stargardt disease. Am J Hum Genet 1999; 64:1024-35.
17. Papaioannou M, Ocaka L, Bessant D, Lois N, Bird A, Payne A, Bhattacharya S. An analysis of ABCR mutations in British patients with recessive retinal dystrophies. Invest Ophthalmol Vis Sci 2000; 41:16-9.
18. Rivera A, White K, Stohr H, Steiner K, Hemmrich N, Grimm T, Jurklies B, Lorenz B, Scholl HP, Apfelstedt-Sylla E, Weber BH. A comprehensive survey of sequence variation in the ABCA4 (ABCR) gene in Stargardt disease and age-related macular degeneration. Am J Hum Genet 2000; 67:800-13.
19. Briggs CE, Rucinski D, Rosenfeld PJ, Hirose T, Berson EL, Dryja TP. Mutations in ABCR (ABCA4) in patients with Stargardt macular degeneration or cone-rod degeneration. Invest Ophthalmol Vis Sci 2001; 42:2229-36.
20. Paloma E, Martinez-Mir A, Vilageliu L, Gonzalez-Duarte R, Balcells S. Spectrum of ABCA4 (ABCR) gene mutations in Spanish patients with autosomal recessive macular dystrophies. Hum Mutat 2001; 17:504-10.
21. Webster AR, Heon E, Lotery AJ, Vandenburgh K, Casavant TL, Oh KT, Beck G, Fishman GA, Lam BL, Levin A, Heckenlively JR, Jacobson SG, Weleber RG, Sheffield VC, Stone EM. An analysis of allelic variation in the ABCA4 gene. Invest Ophthalmol Vis Sci 2001; 42:1179-89.
22. Jaakson K, Zernant J, Kulm M, Hutchinson A, Tonisson N, Glavac D, Ravnik-Glavac M, Hawlina M, Meltzer MR, Caruso RC, Testa F, Maugeri A, Hoyng CB, Gouras P, Simonelli F, Lewis RA, Lupski JR, Cremers FP, Allikmets R. Genotyping microarray (gene chip) for the ABCR (ABCA4) gene. Hum Mutat 2003; 22:395-403.
23. Simonelli F, Testa F, de Crecchio G, Rinaldi E, Hutchinson A, Atkinson A, Dean M, D'Urso M, Allikmets R. New ABCR mutations and clinical phenotype in Italian patients with Stargardt disease. Invest Ophthalmol Vis Sci 2000; 41:892-7.
24. Sun H, Smallwood PM, Nathans J. Biochemical defects in ABCR protein variants associated with human retinopathies. Nat Genet 2000; 26:242-6.
25. Fumagalli A, Ferrari M, Soriani N, Gessi A, Foglieni B, Martina E, Manitto MP, Brancato R, Dean M, Allikmets R, Cremonesi L. Mutational scanning of the ABCR gene with double-gradient denaturing-gradient gel electrophoresis (DG-DGGE) in Italian Stargardt disease patients. Hum Genet 2001; 109:326-38.
26. Maugeri A, Klevering BJ, Rohrschneider K, Blankenagel A, Brunner HG, Deutman AF, Hoyng CB, Cremers FP. Mutations in the ABCA4 (ABCR) gene are the major cause of autosomal recessive cone-rod dystrophy. Am J Hum Genet 2000; 67:960-6.
27. Shroyer NF, Lewis RA, Lupski JR. Complex inheritance of ABCR mutations in Stargardt disease: linkage disequilibrium, complex alleles, and pseudodominance. Hum Genet 2000; 106:244-8.
28. Shroyer NF, Lewis RA, Yatsenko AN, Lupski JR. Null missense ABCR (ABCA4) mutations in a family with stargardt disease and retinitis pigmentosa. Invest Ophthalmol Vis Sci 2001; 42:2757-61.
29. Klevering BJ, Yzer S, Rohrschneider K, Zonneveld M, Allikmets R, van den Born LI, Maugeri A, Hoyng CB, Cremers FP. Microarray-based mutation analysis of the ABCA4 (ABCR) gene in autosomal recessive cone-rod dystrophy and retinitis pigmentosa. Eur J Hum Genet 2004; 12:1024-32.
30. Souied EH, Ducroq D, Rozet JM, Gerber S, Perrault I, Sterkers M, Benhamou N, Munnich A, Coscas G, Soubrane G, Kaplan J. A novel ABCR nonsense mutation responsible for late-onset fundus flavimaculatus. Invest Ophthalmol Vis Sci 1999; 40:2740-4.
31. Gerber S, Rozet JM, van de Pol TJ, Hoyng CB, Munnich A, Blankenagel A, Kaplan J, Cremers FP. Complete exon-intron structure of the retina-specific ATP binding transporter gene

(ABCR) allows the identification of novel mutations underlying Stargardt disease. Genomics 1998; 48:139-42.

32. Birch DG, Peters AY, Locke KL, Spencer R, Megarity CF, Travis GH. Visual function in patients with cone-rod dystrophy (CRD) associated with mutations in the ABCA4(ABCR) gene. Exp Eye Res 2001; 73:877-86.
33. Fishman GA, Stone EM, Grover S, Derlacki DJ, Haines HL, Hockey RR. Variation of clinical expression in patients with Stargardt dystrophy and sequence variations in the ABCR gene. Arch Ophthalmol 1999; 117:504-10.
34. Maugeri A, Flothmann K, Hemmrich N, Ingvast S, Jorge P, Paloma E, Patel R, Rozet JM, Tammur J, Testa F, Balcells S, Bird AC, Brunner HG, Hoyng CB, Metspalu A, Simonelli F, Allikmets R, Bhattacharya SS, D'Urso M, Gonzalez-Duarte R, Kaplan J, te Meerman GJ, Santos R, Schwartz M, Van Camp G, Wadelius C, Weber BH, Cremers FP. The ABCA4 2588G>C Stargardt mutation: single origin and increasing frequency from South-West to North-East Europe. Eur J Hum Genet 2002; 10:197-203.

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