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Outcome of *ABCA4* disease-associated alleles in autosomal recessive Retinal Dystrophies: Retrospective analysis in 420 Spanish families

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Abstract

Objective—To provide a comprehensive overview of all detected mutations in the *ABCA4* gene in Spanish families with autosomal recessive retinal disorders, including Stargardt disease (arSTGD), cone-rod dystrophy (arCRD), and retinitis pigmentosa (arRP). Also, to assess genotype-phenotype correlation and disease progression in 10 years by considering type of variants and age of onset.

Design—Case series.

Participants—A total of 420 unrelated Spanish families: 259 arSTGD, 86 arCRD and 75 arRP.

Methods—Spanish families were analysed through a combination of ABCR400 genotyping microarray, denaturing High-Performance Liquid Chromatography (dHPLC) and High Resolution Melting (HRM) scanning. Direct sequencing was used as confirmation technique for the identified variants. Screening by Multiple Ligation Probe Analysis (MLPA) was used in order to detect possible large deletions or insertions in the *ABCA4* gene. Selected families were further analysed by Next Generation Sequencing (NGS).

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Main Outcome Measures—DNA sequence variants, mutation detection rates, haplotypes, age of onset, central or peripheral vision loss, night blindness.

Results—Overall, we detected 70.5% and 36.6% of all expected *ABCA4* mutations in arSTGD and arCRD patient cohorts, respectively. In the fraction of the cohort where the *ABCA4* gene was completely sequenced the detection rates reached 73.6% for STGD and 66.7% for CRD. However, the frequency of possibly pathogenic *ABCA4* alleles in arRP families was only slightly higher to that in the general population. Moreover, in some families mutations in other known arRP genes segregated with the disease phenotype.

Conclusions—An increasing understanding of causal *ABCA4* alleles in arSTGD and arCRD facilitates disease diagnosis and prognosis and is also paramount in selecting patients for emerging clinical trials of therapeutic interventions. As *ABCA4*-associated diseases are evolving retinal dystrophies, assessment of age of onset, accurate clinical diagnosis and genetic testing are crucial. We suggest that *ABCA4* mutations may be associated with an RP-like phenotype often as a consequence of severe (null) mutations and/or in cases of long-term, advanced disease. Patients with “classical” arRP phenotypes, especially from the onset of the disease, should be first screened for mutations in known arRP genes and not *ABCA4*.

Keywords

ABCA4; Stargardt disease; cone-rod dystrophy; genotype-phenotype correlation

INTRODUCTION

The *ABCA4* (photoreceptor-specific ATP-binding cassette transporter 4; MIM: 601691; NM_000350) gene encodes a transmembrane protein exclusively localized at the rims of the outer segments of cone and rod photoreceptors.¹

Mutations in *ABCA4* are causal in autosomal recessive Stargardt disease (arSTGD) and fundus flavimaculatus (FFM),² and have also been implicated in more severe retinal dystrophies such as autosomal recessive cone-rod Dystrophy (arCRD) and autosomal recessive retinitis pigmentosa (arRP).^{3,4} The resultant phenotype with respective clinical manifestations has been explained through a disease model, which correlates severity of mutations with residual protein activity.⁵

Several genetic studies have been performed in cohorts of Spanish patients with arSTGD,⁶ arCRD and arRP,^{7,8} describing genotype-phenotype correlations. Whilst the implication of *ABCA4* mutations in arSTGD and arCRD is well known, the causative role of these mutations in arRP is not unequivocal. Previously, we have characterized one Spanish family segregating both arSTGD and arRP phenotypes due to mutations in two different genes, *ABCA4* and *CRB1*.⁹ These phenotypes had been previously associated only with *ABCA4* variants.¹⁰ We have also described several arRP families with one disease-associated *ABCA4* allele; however, haplotypes carrying these alleles did not co-segregate with the disease and were therefore considered a chance finding.⁷

Recently, the carrier frequency of *ABCA4* mutations in the Spanish population was estimated at ~6%,¹¹ resulting in a calculated arSTGD prevalence at about 1:1000. This agrees with the carrier frequency estimates in other studies,^{5,12,13} but suggests about 10X higher disease prevalence than previously suggested.¹⁴ While the frequency of *ABCA4*-associated disease is likely (much) higher than previously appreciated, it is also likely that some of these alleles do not cause the disease in homozygosity or in certain combinations with other *ABCA4* alleles since, even considering the wide range of phenotypes caused by *ABCA4* variants, altogether the *ABCA4*-associated diseases are certainly rarer than 1:1000.

The present study aims: 1) to correlate the spectrum of *ABCA4* disease-associated alleles with the two main phenotypes, arSTGD and arCRD and, 2) to further investigate the role of *ABCA4* mutations in arRP.

PATIENTS AND METHODS

Recruitment of subjects

This study was reviewed and approved by the Ethics Committee of the Fundacion Jimenez Diaz Hospital, and it was performed according to the tenets of the Declaration of Helsinki and further reviews (Seoul, 2008). The participating subjects signed a written informed consent form after the nature of procedures had been fully explained. The collection of samples belongs to the Biobank of the Fundacion Jimenez Diaz Hospital.

Clinical evaluation

Diagnoses of arSTGD, arCRD and arRP were determined according to a recessive mode of inheritance and were based on the following criteria:

- Diagnosis of STGD was determined according to a bilateral central vision loss; fundus presenting with a beaten-bronze appearance and/or the presence of orange-yellow flecks in the retina from the posterior pole to the mid-periphery; fluorescein angiography showing typical dark choroid; and normal to subnormal electroretinograms (ERGs).
- Diagnosis of CRD was based on initial complaints of blurred central vision without a history of night blindness, poor visual acuity, impairment of colour vision, funduscopy evidence of atrophic macular degeneration, peripheral disturbances including pigment clumping and/or pigment epithelial thinning, and greater or earlier loss of cone than rod ERG amplitude.
- RP was diagnosed in patients who developed night blindness, peripheral vision loss, pigmentary retinal degeneration and markedly reduced scotopic ERG.¹⁵

Molecular methods

A Spanish subset of 259 arSTGD, 86 arCRD and 75 arRP patients were tested for variants on the *ABCA4* gene using a combined strategy of genotyping tools: first, all patients were analysed by the ABCR400 microarray¹³; next, those patients harbouring only one mutated allele were subsequently screened by denaturing high-performance liquid chromatography (dHPLC),⁸ or High Resolution Melting (HRM) scanning.¹⁶ All identified variants were confirmed by direct sequencing and haplotype analysis was also performed.⁶ The pathogenicity for the *ABCA4* variants was assessed by several methods, namely: 1) their absence among control population; 2) segregation within the families; 3) analysis by the predictive program PolyPhen-2 (Polymorphism Phenotyping v2; <http://genetics.bwh.harvard.edu/pph2/>; accessed January 17, 2013). Following this multi-stage screening, patients who remained with one mutated allele were also analysed by Multiple Ligation Probe Analysis (MLPA) in order to detect possible large deletions or insertions in the *ABCA4* gene (SALSA MLPA P151 ABCA4 mix-1 probemix and SALSA MLPA P152 ABCA4 mix-2 probemix; MRC-Holland, Amsterdam, The Netherlands). No large deletions or insertions in the *ABCA4* gene were detected by MLPA in all 57 analysed cases.

Finally, 44 STGD families (42 with one disease-associated allele previously identified by the above-mentioned methods, and 2 without mutations) and 4 CRD families (2 with one disease-associated allele previously identified, and 2 without mutations) were further analysed by Next Generation Sequencing (NGS), as described elsewhere.¹⁷

In order to assess the potential implication of *ABCA4* mutant alleles in autosomal recessive retinitis pigmentosa, 75 Spanish arRP patients with or without identified variants in *ABCA4* after screening with the ABCR400 array, or those who were excluded by haplotype analysis, were further tested for mutations in other RP-related genes by genotyping with the Asper Biotech arRP microarray,¹⁹ homozygosity mapping, or whole exome sequencing (Avila-Fernandez 2012; submitted).

RESULTS

Genotype-phenotype correlation and mutation detection rates

a) arSTGD phenotype—Through the combined methodological approach, two *ABCA4* mutant alleles were identified in 159/259 families and one disease-associated allele was identified in 47 more families, resulting in a mutation detection rate of 70.5%. Haplotype analyses were performed for 133 of the 259 families (133/259; 51.3%), showing cosegregation with the disease with the *ABCA4* locus in all of them, except for seven families (1 with 1 mutation and 6 with no mutations). The NGS analysis detected the second mutant allele in 23/44 (52.3%) families, including 18 novel mutations. Also, a false negative for both dHPLC and HRM analyses was detected after NGS for MD-0167. (Table 1; available at <http://aaojournal.org>). Novel variants were considered as pathological when absent among population. Missense changes were considered as mutant alleles when PolyPhen-2 analysis showed predictions of “possibly damaging” or “probably damaging”. Finally, variant segregation was performed for every family.

Due to the existence of a prevalent disease-associated allele in the Spanish STGD patients, p.Arg1129Leu, 159 arSTGD patients were classified into 4 major categories: 1) those homozygous for p.Arg1129Leu (N=8), 2) those compound heterozygous for this mutation (N=58), 3) patients with complex alleles on at least one chromosome (N=10) and, 4) those harbouring compound heterozygous mutations excluding the p.Arg1129Leu allele (N=83). In addition, patients were grouped by the age of the disease onset (the age at which patients first experienced any symptom of visual impairment) and the mean age of onset was assessed for each group (Table 1; available at <http://aaojournal.org>).

Patients who are homozygous for the p.Arg1129Leu allele (i.e., in the first group) always present with an arSTGD phenotype, and the average age of onset of the disease was at the beginning of the 3rd decade of life (20.6), ranging from 12 to 40 years.

When this variant is combined with a different *ABCA4* mutation in a compound heterozygous state (the second group), the resulting phenotype is almost always STGD, with symptoms appearing from early in life (from 7 years of age) or much later; i.e., during the adulthood (39 years of age). As expected, patients with likely deleterious, that is nonsense, frameshift or splicing, variants as the second mutant allele, presented disease symptoms earlier, mainly during the 1st and 2nd decades of life and the mean age of onset was 17 years.

Patients with complex alleles (third group) start presenting symptoms between the 1st (5 yrs.) and the 4th (38 yrs.) decade of life, with an earlier mean age of onset (13.7 years old).

Finally, the largest group of patients were compound heterozygous for *ABCA4* mutations and did not harbour the p.Arg1129Leu variant. The age of onset of the disease in this large group, as expected, presents a wide spectrum ranging from 4 to 55 years, although the mean age of onset, 17.4 years, is practically identical to the second group. Again, patients harbouring nonsense, frameshift or splicing mutations showed earlier onset of the disease where symptoms appeared during the first decade of life, except for the family MD-0249, where the disease onset was at 13 years of age (Table 1; available at <http://aaojournal.org>).

Of all mutant alleles in this group, 66.7% were missense variants, 22.8% resulted in a frameshift, 6.3% were affecting splice sites and 4.2% were nonsense mutations.

b) arCRD phenotype—Of the 86 arCRD families, both *ABCA4* mutant alleles were identified in 26 families and one disease-associated variant was detected in 11 more families, yielding a mutation detection rate of 36.6% with methods not including complete sequencing of the *ABCA4* gene. Only four CRD families were analysed by NGS, and the second mutant allele was identified in one of them by NGS (MD-0565) and by complete Sanger Sequencing in RP-1715 (this was a NGS false negative). Haplotype analyses were performed for 36 of the 86 families (36/86; 41.8%), showing cosegregation of the *ABCA4* locus with the disease in all of them, except for families MD-0140 and MD-0526 (Table 2; available at <http://aaojournal.org>). Pathogenicity for the *ABCA4* variants was assessed similarly as described for the arSTGD phenotype.

arCRD patients start presenting symptoms mainly during the 1st decade of life (4 to 10 years), although some of them showed a later onset of the disease ranging from 12 to 24 years (Table 2; available at <http://aaojournal.org>). Overall, the mean age of onset was at the beginning of the 2nd decade (10.3 years).

Similar to the arSTGD phenotype, in arCRD patients two more prevalent disease associated alleles were found, p.Gly963fs (8 times in 6 patients) and p.Val1681_Cys1685del (4 times in 3 patients). In addition, the Spanish founder mutation p.Arg1129Leu was identified in only three families with arCRD (MD-0247, MD-0390 and RP-0532 (Table 2; available at <http://aaojournal.org>).

As expected, the arCRD patients harboured higher percentages of likely severe *ABCA4* mutations, i.e., those resulting in a frameshift effect (insertions/deletions; 30.2%) or in a premature stop codon (7.9%). Missense and splicing variants accounted for 53.9% and 7.9%, respectively.

c) arRP phenotype—Of the 75 patients presenting with retinitis pigmentosa, only 8 carried one *ABCA4* disease-associated variant, whilst no mutant alleles were detected in the remaining 67 families (mutation detection rate: 5.3%). Interestingly, all disease-associated variants identified in arRP families were missense mutations (predicted as “damaging” by PolyPhen-2), except for one mutation affecting splicing. In family RP-0280 the mutant allele did not co-segregate with the disease, therefore *ABCA4* was excluded as the causal gene. The actual causal gene, *CRB1*, was subsequently discovered in this family (Table 3; available at <http://aaojournal.org>).⁹

Subsequently, RP-0599 and RP-0759 families were additionally characterised, also presenting mutations in other RP-related genes (*LCA5* and *RPGR*; Table 3; available at <http://aaojournal.org>). Therefore, *ABCA4* only could potentially be the disease-causing gene in 5 of the 72 arRP patients (7%), although the second mutant allele was not detected.

Next, we compared the mutation detection rate in arRP patients (5.3%) to *ABCA4* carrier frequency among control population, as this frequency is well known in Spanish population and has been recently estimated, yielding a ~6–6.4%.¹¹ Carrier frequency differences between arRP patients (10.7%) and controls (6%) were compared using the chi-square test, but this analysis did not reveal a significant difference ($p = 0.05$).

DISCUSSION

Since the *ABCA4* gene was identified,² mutations in this gene have been associated with three autosomal recessive retinal phenotypes, Stargardt disease, cone-rod dystrophy and retinitis pigmentosa. The role of *ABCA4* mutations in arSTGD and arCRD has been extensively analysed in Spanish population,^{6–8} and is well known. The role of mutations in this gene in autosomal recessive retinitis pigmentosa still remains unclear. Therefore, this study was conducted to summarize and review the data and correlate the outcome of the different *ABCA4* disease-associated variants with disease phenotypes.

Genotype-phenotype correlation in arSTGD and arCRD

As summarized in Table 1 (available at <http://aaojournal.org>), all identified variants cosegregated with the Stargardt disease phenotype in all families except one, MD-0394, where the *ABCA4* allele is likely not disease-causing. The most prevalent p.Arg1129Leu allele, which accounts for 22.4% of the mutant alleles in Spanish STGD population, is mainly associated with arSTGD phenotype. In homozygotes, the age of onset is almost invariably in the second decade. Differences in the age of onset in compound heterozygotes with other pathogenic *ABCA4* alleles range from the first to the fourth decade of life, which is common in arSTGD.⁶ This variant is much less frequently found in patients presenting with more severe, arCRD, phenotype (6.4%; 4/62 mutant arCRD alleles). Thus, p.Arg1129Leu could be considered a relatively “moderate” allele and the severity of the phenotype is determined by the second mutation in compound heterozygotes (see below).

Complex alleles, i.e., where two or more mutations are in *cis* on the same chromosome, is a well known feature in *ABCA4*-associated diseases, where they account for up to 10% of all disease-associated variation.²¹ In the Spanish cohort, the complex alleles were only associated with the arSTGD phenotype (in 7.2% of cases) where they were associated with earlier mean age of onset of the disease (Table 1; available at <http://aaojournal.org>). This fact is readily explained by the cumulative deleterious effect of every new mutational event that occurs in “*cis*” over a previously mutated allele.

As expected, more severe effect on vision was observed in patients harbouring likely deleterious, or “null” mutations, such as deletions and insertions resulting in a frameshift, stop codons, or splicing variants (Tables 1 and 2; available at <http://aaojournal.org>). Comparison of mutation classes between arSTGD and arCRD phenotypes revealed that the percentage of missense mutations was lower in CRD (53.9% vs. 66.7%), while the percentage of frameshift (30.2% vs. 22.8%) and nonsense (7.9% vs. 4.2%) mutations was increased in arCRD as compared to STGD. This observation correlates well with the hypothesis that the severity *ABCA4* mutations (and the resulting loss of *ABCA4* activity) is directly associated with the severity of retinal disease.⁵

Obvious deleterious mutations, including those generating frame shift or stop in protein translation, are usually considered the most severe. However, it is known that missense mutations can sometimes be as, or even more, severe than deleterious mutations in recessive disease due to variable impact on the protein such as misfolding or mislocalization, which render the protein completely ineffective, or sometimes even eliciting a dominant negative effect. This phenomenon has been described²² or suggested²³ for many *ABCA4* missense alleles. This study has the advantage of the presence of a common missense mutation, p.Arg1129Leu, in the Spanish STGD population which accounts for almost 1/4 of all mutant alleles. As seen in Table 1 (available at <http://aaojournal.org>), 8 patients were homozygous for the p.Arg1129Leu variant and 58 we compound heterozygous, therefore reducing the variability in the patient pool and allowing comparing other mutations which are found in the same patients with the p.Arg1129Leu variant (which is constant). Based on our data, the

likely severe *ABCA4* missense mutations, resulting in an early disease onset and severe disease include, among others: p.Leu541Pro, p.Arg602Trp, p.Thr1019Met, p.Leu1940Pro and p.His1838Asp. In addition, the intronic IVS38-10T>C variant of unknown functional consequence is also associated with the severe disease. These results are supported by previous findings that the p.Leu541Pro and p.Arg602Trp variants result in mislocalized protein,²² the p.Leu1940Pro and IVS38-10T>C variants confer much earlier onset of the disease,²³ and the p.His1838Asp variant, in a complex allele with the p.Gly1961Glu mutation, results in an early onset, severe disease.²⁴ While the above estimates are simplified since they do not take into account environmental factors and genetic variation at other loci in these patients, they serve as a good basis for association with disease onset and disease severity.

Almost all disease-associated variants were found in both arSTGD and arCRD phenotypes in our cohort, with the exception of p.Leu11Pro, p.Arg18Trp, p.Ala538Val and p.Val1973*, which were associated only with CRD. Considering that we have previously reported 2 patients homozygous for the p.Gly963fs allele that initially presented with STGD phenotype and later evolved into CRD phenotype,⁷ we strongly recommend a close follow-up of these, and similar patients with 2 obviously deleterious alleles, and frequent ophthalmic reevaluation, as rapid worsening of the disease could be expected. In addition to obvious mutation severity, a STGD to CRD evolution as a consequence of a worsening of the disease, is more likely for patients presenting at early age (in our series, before 10 years of age) (Table 2; available at <http://aaojournal.org>). As an example, family MD-0213 was initially characterised as juvenile macular dystrophy (STGD) and later evolved to CRD. Other examples of genotypes associated with frequent worsening of the disease (i.e., found in both STGD and CRD) are summarized in Table 4 (available at <http://aaojournal.org>).

The increasing molecular understanding of *ABCA4* alleles related to arSTGD and arCRD facilitates an accurate disease diagnosis and prognosis. Conventional genetic screening of *ABCA4* has been particularly challenging until recently. Now, with the availability of NGS, we suggest that complete sequencing of the *ABCA4* coding regions, including flanking intronic sequences (splice sites), would be paramount for proper molecular diagnosis in every STGD or CRD patient, both in the context of genetic counselling and in the perspective of retinal gene therapy.

***ABCA4* mutant alleles in arRP Spanish families**

Mutations in *ABCA4* have been associated with the arRP phenotype on several occasions,⁴ since some patients at late stages of a severe form of the disease present typical RP-associated features (i.e., panretinal degeneration, bone spicule-shaped pigment deposits, etc.). However, in cases when patients present typical arRP symptoms from the disease onset, our results do not support the causal role of *ABCA4* variants in arRP, for the following reasons:

1) The carrier frequency of *ABCA4* disease-associated variants in the general population has been estimated at 5–6%¹¹; 2) Almost all *ABCA4* variants in arRP patients were missense (Table 3; available at <http://aaojournal.org>), which are considered as moderate alleles. Thus, it could be hypothesised that these variants are not causing this more severe retinal phenotype, although a phenotypic worsening effect cannot be ruled out; 3) The percentage of arRP families where *ABCA4* did not cosegregate with the disease is 21.7% (Table 3; available at <http://aaojournal.org>); 4) In 18/74 (24.3%) arRP patients we identified other causative mutations in other RP-associated genes (Table 3; available at <http://aaojournal.org>). The possibility of the existence of two different mutated genes segregating in the same family should be considered, as previously reported.⁹ Taking into account the

above, it is likely that most arRP patients are carriers of *ABCA4* alleles by chance due to the high prevalence of *ABCA4* variants in the general population.

Recent advances in high-throughput genotyping technologies, such as NGS, allow efficient screening for disease-associated variants in genes (including *ABCA4*¹⁷; Table 5; available at <http://aojournal.org>) and simultaneous screening of gene panels (e.g., all known genes for RP).²⁵ NGS is also the method of choice for finding new genes associated with retinal diseases, such as RP, by whole exome or whole genome sequencing approaches (Corton 2012; manuscript in preparation). Altogether, these approaches will identify causal genetic variation with great efficiency and will resolve many, or most, cases where the causal gene has not yet been identified.

In conclusion, the cases where *ABCA4* variation has been determined as causal in families segregating RP-like phenotype have been dealing mainly with advanced disease,^{3,4} where the disease phenotype had (likely) evolved from the CRD phenotype at early stages of the disease to that resembling arRP (bone spicules, extinguished ERG, etc.) on later, advanced stages. Therefore, it is likely that some or most of the reported cases represent CRD that had progressed to RP-like disease at the time of the clinical and genetic characterization. Therefore, the age of the disease onset, together with the age at precise clinical diagnosis and genetic testing are important variables to consider when suggesting the involvement of the *ABCA4* gene in various retinal phenotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Mutations in *ABCA4* in arSTGD families.

Pedigrees	Gene	Allele 1				Allele 2				Age of onset (years)	Haplotype analysis	Methods	References
		Nucleotide change		Amino acid change		Nucleotide change		Amino acid change					
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change				
MD-0061	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	23	c.3386G>T	p.Arg1129Leu	12	Yes	ABCR400	Valverde et al. 2006 (6)		
MD-0317	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	23	c.3386G>T	p.Arg1129Leu	15	NP	ABCR400			
MD-0111	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	23	c.3386G>T	p.Arg1129Leu	18	Yes	ABCR400	Valverde et al. 2006 (6)		
MD-0119	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	23	c.3386G>T	p.Arg1129Leu	19	Yes	ABCR400	Valverde et al. 2006 (6)		
MD-0128	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	23	c.3386G>T	p.Arg1129Leu	19	Yes	ABCR400	Valverde et al. 2006 (6)		
MD-0047	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	23	c.3386G>T	p.Arg1129Leu	21	Yes	ABCR400	Valverde et al. 2006 (6)		
MD-0410	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	23	c.3386G>T	p.Arg1129Leu	21	Yes	ABCR400			
MD-0252	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	23	c.3386G>T	p.Arg1129Leu	40	NP	ABCR400			
Mean age of onset (years)										20.6			

Pedigrees	Gene	Allele 1				Allele 2				Age of onset (years)	Haplotype analysis	Methods	Reference
		Nucleotide change		Amino acid change		Nucleotide change		Amino acid change					
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change				
MD-0277	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	19	c.2888delG	p.Gly963fs	7	Yes	ABCR400			
MD-0547	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	39; 42	c.[5512C>G; 5882G>A]	p.[His1838Asp; Gly1961Glu]	7	Yes	ABCR400			
MD-0466	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	22	c.3211_3212insGT	p.Ser1071fs	8	Yes	ABCR400			
MD-0076	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	6	c.768G>T	p.Val256Val or splicing defect	9	NP	ABCR400	Valverde et al. 2006 (6)		
MD-0427	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	13	c.1832T>C	p.Leu611Pro [^]	9	Yes	ABCR400 + NGS	This study		
MD-0078	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	48	c.6559C>T	p.Gln2187*	10	Yes	ABCR400 + SS	Valverde et al. 2006 (6)		
MD-0287	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	21	c.3056C>T	p.Thr1019Met	10	Yes	ABCR400			
MD-0291	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	45	c.6179T>G	p.Leu2060Arg	10	Yes	ABCR400			
MD-0370	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	21	c.3056C>T	p.Thr1019Met	10	Yes	ABCR400			
MD-0155	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	13	c.1804C>T	p.Arg602Trp	11	Yes	ABCR400	Valverde et al. 2006 (6)		
MD-0164	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	6	c.700C>T	p.Gln234*	11	Yes	ABCR400 + dHPLC + HRM + MLPA	Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010		
MD-0359	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	38	c.5395A>G	p.Asn1799Asp	12	NP	ABCR400			

Pedigrees	Gene	Allele 1				Allele 2				Age of onset (years)	Haplotype analysis	Methods	Reference
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change						
MD-0445	ABCA4	23	c.3386G>T	p.Arg1129Leu	19	c.2888delG	p.Gly963fs	12	Yes	ABCR400	This study		
MD-0354	ABCA4	23	c.3386G>T	p.Arg1129Leu	8	c.1022_1035delAAACAATAAATAT	p.Asp342_Lys346del	12	Yes	ABCR400 + NGS			
MD-0227	ABCA4	23	c.3386G>T	p.Arg1129Leu	41	c.5819T>C	p.Leu1940Pro	13	NP	ABCR400			
MD-0254	ABCA4	23	c.3386G>T	p.Arg1129Leu	5	c.454C>T	p.Arg152*	13	Yes	ABCR400			
MD-0281	ABCA4	23	c.3386G>T	p.Arg1129Leu	36	c.5041_5055del	p.Val1681_Cys1685del	14	Yes	ABCR400			
MD-0264	ABCA4	23	c.3386G>T	p.Arg1129Leu	47	c.6449G>A	p.Cys2150Tyr	14	Yes	ABCR400			
MD-0334	ABCA4	23	c.3386G>T	p.Arg1129Leu		c.5461-10T>C	Unknown effect	14	NP	ABCR400			
MD-0498	ABCA4	23	c.3386G>T	p.Arg1129Leu	14	c.2041C>T	p.Arg681*	14	NP	ABCR400			
MD-0342	ABCA4	23	c.3386G>T	p.Arg1129Leu	9	c.1222C>T	p.Arg408*	15	NP	ABCR400			
MD-0057	ABCA4	23	c.3386G>T	p.Arg1129Leu	42	c.5582G>A	p.Gly1961Glu	15	Yes	ABCR400	Valverde et al. 2006 (6)		
MD-0283	ABCA4	23	c.3386G>T	p.Arg1129Leu	19	c.2888delG	p.Gly963fs	15	NP	ABCR400	This study		
MD-0451	ABCA4	23	c.3386G>T	p.Arg1129Leu		c.2382+5G>C ^	Splicing defect	15	NP	ABCR400 + NGS			
MD-0324	ABCA4	23	c.3386G>T	p.Arg1129Leu	1	c.3G>A	p.Met11le ^	15	Yes	ABCR400 + NGS	This study		
MD-0244	ABCA4	23	c.3386G>T	p.Arg1129Leu	14	c.1957C>T	p.Arg653Cys	16	Yes	ABCR400	ABCR400 + HRM + MLPA		
MD-0300	ABCA4	23	c.3386G>T	p.Arg1129Leu	39	c.5549T>C	p.Leu1850Pro	16	NP	ABCR400			
MD-0560	ABCA4	23	c.3386G>T	p.Arg1129Leu	47	c.6410G>A	p.Cys2137Tyr	16	NP	ABCR400			
MD-0200	ABCA4	23	c.3386G>T	p.Arg1129Leu	14	c.2041C>T	p.Arg681*	17	Yes	ABCR400			
MD-0096	ABCA4	23	c.3386G>T	p.Arg1129Leu	28; 35	c.[4222T>C; 4918C>T]	p.[Trp1408Arg; Arg1640Trp]	17	Yes	ABCR400	Valverde et al. 2006 (6)		
MD-0238	ABCA4	23	c.3386G>T	p.Arg1129Leu	41	c.5819T>C	p.Leu1940Pro	17	NP	ABCR400	Valverde et al. 2006 (6)		
MD-0066	ABCA4	23	c.3386G>T	p.Arg1129Leu	41	c.5819T>C	p.Leu1940Pro	17	Yes	ABCR400			
MD-0519	ABCA4	23	c.3386G>T	p.Arg1129Leu	43	c.5929G>A	p.Gly1977Ser	17	NP	ABCR400			
MD-0191	ABCA4	23	c.3386G>T	p.Arg1129Leu	13	c.1804C>T	p.Arg602Trp	18	Yes	ABCR400			
MD-0412	ABCA4	23	c.3386G>T	p.Arg1129Leu	28	c.4139C>T	p.Pro1380Leu	18	Yes	ABCR400	Aguirre-Lamban et al. 2009 (8)		
MD-0452	ABCA4	23	c.3386G>T	p.Arg1129Leu	45	c.6229C>T	p.Arg2077Trp	18	Yes	ABCR400			
MD-0198	ABCA4	23	c.3386G>T	p.Arg1129Leu	19	c.2888delG	p.Gly963fs	19	Yes	ABCR400 + dHPLC + MLPA			
MD-0082	ABCA4	23	c.3386G>T	p.Arg1129Leu	23	c.3364G>A	p.Glu1122Lys	19	Yes	ABCR400	Valverde et al. 2006 (6)		
MD-0262	ABCA4	23	c.3386G>T	p.Arg1129Leu	13	c.1819G>A	p.Gly607Arg	19	NP	ABCR400			

Pedigrees	Gene	Allele 1			Allele 2			Age of onset (years)	Haplotype analysis	Methods	Reference
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change				
MD-0518	ABCA4	23	c.3386G>T	p.Arg1129Leu	6	c.742_768+29del56bp [^]	Frameshift defect	19	NP	ABCR400 + NGS	This study
MD-0196	ABCA4	23	c.3386G>T	p.Arg1129Leu	40	c.5644A>G	p.Met1882Val	20	Yes	ABCR400	
MD-0139	ABCA4	23	c.3386G>T	p.Arg1129Leu	22; 46	c.[3322C>T; 6320G>A]	p.[Arg1108Cys; Arg2107His]	20	Yes	ABCR400	
MD-0607	ABCA4	23	c.3386G>T	p.Arg1129Leu	43	c.5929G>A	p.Gly1977Ser	21	NP	ABCR400	
MD-0345	ABCA4	23	c.3386G>T	p.Arg1129Leu	13	c.1804C>T	p.Arg602Trp	22	NP	ABCR400	
MD-0428	ABCA4	23	c.3386G>T	p.Arg1129Leu	43	c.5929G>A	p.Gly1977Ser	22	Yes	ABCR400	
MD-0497	ABCA4	23	c.3386G>T	p.Arg1129Leu	1	c.52C>T	p.Arg18Trp	23	NP	ABCR400	
MD-0465	ABCA4	23	c.3386G>T	p.Arg1129Leu		c.5714+5G>A	Splicing defect	24	NP	ABCR400	
MD-0435	ABCA4	23	c.3386G>T	p.Arg1129Leu	12	c.1609C>T	p.Arg537Cys	25	NP	ABCR400	
MD-0481	ABCA4	23	c.3386G>T	p.Arg1129Leu	39; 42	c.[5512C>G; 5882G>A]	p.[His1838Asp; Gly1961Glu]	25	NP	ABCR400	
MD-0363	ABCA4	23	c.3386G>T	p.Arg1129Leu		c.5461-10T>C	Unknown effect	28	Yes	ABCR400	
MD-0433	ABCA4	23	c.3386G>T	p.Arg1129Leu	30	c.4457C>T	p.Pro1486Leu	36	NP	ABCR400	
MD-0331	ABCA4	23	c.3386G>T	p.Arg1129Leu	21	c.3056C>T	p.Thr1019Met	37	NP	ABCR400	
MD-0062	ABCA4	23	c.3386G>T	p.Arg1129Leu	43	c.5929G>A	p.Gly1977Ser	39	Yes	ABCR400	
MD-0270	ABCA4	23	c.3386G>T	p.Arg1129Leu	6	c.634C>T	p.Arg212Cys	N/A	Yes	ABCR400	
MD-0437	ABCA4	23	c.3386G>T	p.Arg1129Leu	6	c.634C>T	p.Arg212Cys	N/A	Yes	ABCR400	
MD-0545	ABCA4	23	c.3386G>T	p.Arg1129Leu	46	c.6329G>A	p.Trp2110*	N/A	NP	ABCR400	
MD-0577	ABCA4	23	c.3386G>T	p.Arg1129Leu	43	c.5929G>A	p.Gly1977Ser	N/A	Yes	ABCR400	
MD-0585	ABCA4	23	c.3386G>T	p.Arg1129Leu	13	c.1766G>A	p.Trp589* [^]	N/A	NP	ABCR400 + NGS	This study

Pedigrees	Gene	Allele 1				Allele 2				Age of onset (years)	Haplotype analysis	Methods	References
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change	Exon	Amino acid change				
MD-0014	ABCA4	22; 46	c.[3322C>T; 6320G>A]	p.[Arg1108Cys; Arg2107His]	22; 46	c.[3322C>T; 6320G>A]	p.[Arg1108Cys; Arg2107His]			5	Yes	ABCR400	Valverde et al. 2006 (6)
MD-0017	ABCA4	22; 46	c.[3322C>T; 6320G>A]	p.[Arg1108Cys; Arg2107His]	46	c.6320G>C	p.Arg2107Pro			8	Yes	ABCR400	Valverde et al. 2006 (6)
MD-0110	ABCA4	22; 46	c.[3322C>T; 6320G>A]	p.[Arg1108Cys; Arg2107His]	42	c.5582G>A	p.Gly1961Glu			8	Yes	ABCR400	Valverde et al. 2006 (6)
MD-0015	ABCA4	17; 21	c.[2588G>C; c.3163C>T]	p.[Gly863Ala; Arg1055Trp]	19	c.2888delG	p.Gly963fs			11	Yes	ABCR400	Valverde et al. 2006 (6)
MD-0116	ABCA4	22; 46	c.[3322C>T; 6320G>A]	p.[Arg1108Cys; Arg2107His]	30	c.4469G>A	p.Cys1490Tyr			11	Yes	ABCR400	Valverde et al. 2006 (6)
MD-0479	ABCA4	12; 12	c.[1751T>A; 1753delA]	p.[Ile584Asn; Asp586fs] ^b	45	c.6179T>G	p.Leu2060Arg			12	Yes	ABCR400 + NGS	This study

Pedigrees	Gene	Allele 1				Allele 2				Age of onset (years)	Haplotype analysis	Methods	References
		Nucleotide change		Amino acid change		Nucleotide change		Amino acid change					
		Exon	Nucleotide change	Exon	Amino acid change	Exon	Nucleotide change	Exon	Amino acid change				
MD-0137	ABCA4	22; 46	c.[3322C>T; 6320G>A]		p.[Arg1108Cys; Arg2107His]		c.5714+5G>A		Splicing defect	13	Yes	ABCR400	Valverde et al. 2006 (6)
MD-0494	ABCA4	22; 46	c.[3322C>T; 6320G>A]		p.[Arg1108Cys; Arg2107His]	42	c.5582G>A		p.Gly1961Glu	18	Yes	ABCR400	
MD-0012	ABCA4	25; 42	c.[3758C>T; 5582G>A]		p.[Thr1253Met; p.Gly1961Glu]	27	c.3943C>T		p.Gln1315*	38	Yes	ABCR400 + SS	Valverde et al. 2006 (6)
MD-0524	ABCA4	28; 35	c.[4222T>C; 4918C>T]		p.[Trp1408Arg; Arg1640Trp]	42	c.5582G>A		p.Gly1961Glu	N/A	Yes	ABCR400	
Mean age of onset (years)										13.7			
Pedigrees	Gene	Allele 1				Allele 2				Age of onset (years)	Haplotype analysis	Methods	References
		Nucleotide change		Amino acid change		Nucleotide change		Amino acid change					
		Exon	Nucleotide change	Exon	Amino acid change	Exon	Nucleotide change	Exon	Amino acid change				
MD-0298	ABCA4	43	c.5929G>A		p.Gly1977Ser	8	c.982G>T		p.Glu328*	4	Yes	ABCR400	
MD-0605	ABCA4		c.5461-10T>C		Unknown effect		c.5461-10T>C		UnkNown effect	4	Yes	ABCR400	
MD-0288	ABCA4	22	c.3322C>T		p.Arg1108Cys	8	c.982G>T		p.Glu328*	6	Yes	ABCR400	
MD-0307	ABCA4	28	c.4200C>A		p.Tyr1400*		c.5018+2T>C		Splicing defect	6	Yes	ABCR400	
MD-0329	ABCA4	41	c.5819T>C		p.Leu1940Pro	41	c.5819T>C		p.Leu1940Pro	6	NP	ABCR400	
MD-0432	ABCA4	22	c.3211_3212insGT		p.Ser1071fs	14	c.2041C>T		p.Arg681*	6	NP	ABCR400	
MD-0084	ABCA4	47	c.6410G>A		p.Cys2137Tyr	47	c.6410G>A		p.Cys2137Tyr	7	Yes	ABCR400 + dHPLC + HRM + MLPA	Valverde et al. 2006 (6); Aguirre-Lamban et al. 2010 (16)
MD-0581	ABCA4	42	c.5582G>A		p.Gly1961Glu		c.4668-1G>A [^]		Splicing defect	7	Yes	ABCR400 + NGS	This study
MD-0038	ABCA4	13	c.1804C>T		p.Arg602Trp	33	c.4739delT		p.Leu1580fs	8	Yes	ABCR400 + dHPLC + HRM + SS	Valverde et al. 2006 (6); Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)
MD-0040	ABCA4	21	c.3056C>T		p.Thr1019Met	27	c.3943C>T		p.Gln1315*	8	Yes	ABCR400 + dHPLC + HRM + SS	Valverde et al. 2006 (6); Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)
MD-0090	ABCA4	43	c.5929G>A		p.Gly1977Ser		c.3329-2A>T		Splicing defect	8	Yes	ABCR400 + dHPLC + HRM + SS	Valverde et al. 2006 (6); Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)
MD-0158	ABCA4	22	c.3211_3212insGT		p.Ser1071fs	30	c.4537delC		p.Gln1513fs	9	NP	ABCR400 + dHPLC + HRM + MLPA	Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)
MD-0125	ABCA4	22	c.3211_3212insGT		p.Ser1071fs	40	c.5629_5643dup		p.Lys1877_Ala1881dup	9	Yes	ABCR400 + dHPLC + HRM	Valverde et al. 2006 (6); Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)

Pedigrees	Gene	Allele 1				Allele 2				Age of onset (years)	Haplotype analysis	Methods	References
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change						
MD-0194	ABCA4	19	c.2791G>A	p.Val931Met	22	c.3211_3212insGT	p.Ser1071fs	9	Yes	ABCR400	Valverde et al. 2006 (6); This study		
MD-0178	ABCA4	46	c.6320G>C	p.Arg2107Pro	22	c.3211_3212insGT	p.Ser1071fs	9	NP	ABCR400			
MD-0360	ABCA4		c.4253+4C>T	Splicing defect		c.4253+4C>T	Splicing defect	9	Yes	ABCR400			
MD-0474	ABCA4	12	c.1622T>C	p.Leu541Pro	28	c.4234C>T	p.Gln1412*	9	NP	ABCR400			
MD-0516	ABCA4	36	c.5041_5055del	p.Val1681_Cys1685del	36	c.5041_5055del	p.Val1681_Cys1685del	10	Yes	ABCR400			
MD-0408	ABCA4	41	c.5819T>C	p.Leu1940Pro	47	c.6449G>A	p.Cys2150Tyr	10	NP	ABCR400			
MD-0416	ABCA4	41	c.5819T>C	p.Leu1940Pro	42	c.5582G>A	p.Gly1961Glu	10	NP	ABCR400			
MD-0420	ABCA4	48	c.6563T>C	p.Phe2188Ser	22	c.3211_3212insGT	p.Ser1071fs	11	Yes	ABCR400			
MD-0600	ABCA4	23	c.3364G>A	p.Glu1122Lys	42	c.5582G>A	p.Gly1961Glu	11	NP	ABCR400			
MD-0364	ABCA4	28	c.4139C>T	p.Pro1380Leu	22	c.3211_3212insGT	p.Ser1071fs	11	Yes	ABCR400 + NGS		This study	
MD-0349	ABCA4	20	c.2966T>C	p.Val989Ala	27	c.3988G>T	p.Glu1330*	11	NP	ABCR400 + NGS		This study	
MD-0022	ABCA4	45	c.6179T>G	p.Leu2060Arg	19	c.2888delG	p.Gly963fs	12	Yes	ABCR400		Valverde et al. 2006 (6)	
MD-0207	ABCA4	42	c.5582G>A	p.Gly1961Glu	30	c.4537_4538insC	p.Gln1513fs	12	Yes	ABCR400			
MD-0215	ABCA4	47	c.6449G>A	p.Cys2150Tyr	36	c.5041_5055del	p.Val1681_Cys1685del	12	NP	ABCR400			
MD-0506	ABCA4	22	c.3292C>T	p.Arg1098Cys	35	c.4919G>A	p.Arg1640Gln	12	NP	ABCR400			
MD-0072	ABCA4	13	c.1804C>T	p.Arg602Trp	22	c.3287C>T	p.Ser1096Leu [^]	12	Yes	ABCR400 + NGS		Valverde et al. 2006 (6); This study	
MD-0146	ABCA4	21	c.3056C>T	p.Thr1019Met	44	c.6140T>A	p.Ile2047Asn	13	Yes	ABCR400 + dHPLC + HRM + MLPA	Valverde et al. 2006 (6); Aguirre-Lamban et al. 2010 (16)		
MD-0203	ABCA4	35	c.4918C>T	p.Arg1640Trp	42	c.5582G>A	p.Gly1961Glu	13	Yes	ABCR400	Valverde et al. 2006 (6); This study		
MD-0249	ABCA4	36	c.5041_5055del	p.Val1681_Cys1685del		c.5714+5G>A	Splicing defect	13	Yes	ABCR400			
MD-0170	ABCA4	30	c.4457C>T	p.Pro1486Leu	9	c.1222C>T	p.Arg408*	14	Yes	ABCR400			
RP-0280_1	ABCA4	38	c.5413A>G	p.Asn1805Asp	38	c.5413A>G	p.Asn1805Asp	14	Yes	ABCR400			
MD-0240	ABCA4	42	c.5582G>A	p.Gly1961Glu	15	c.2285C>A	p.Ala1762Glu	14	Yes	ABCR400 + dHPLC + HRM + MLPA		Valverde et al. 2006 (6); Aguirre-Lamban et al. 2010 (16)	
MD-0482	ABCA4	14	c.2057T>C	p.Leu686Ser	27	c.4069G>A	p.Ala1357Thr [^]	14	NP	ABCR400 + NGS		This study	
MD-0590	ABCA4	42	c.5582G>A	p.Gly1961Glu	27	c.4000_4001delCC	p.Pro1335Argfs [^]	14	NP	ABCR400 + NGS		This study	
MD-0318	ABCA4	27	c.4069G>A	p.Ala1357Thr		c.4253+5G>A	Splicing defect	15	Yes	ABCR400		Valverde et al. 2006 (6)	
MD-0064	ABCA4	6	c.634C>T	p.Arg212Cys	43	c.5929G>A	p.Gly1977Ser	15	Yes	ABCR400			
MD-0168	ABCA4	42	c.5582G>A	p.Gly1961Glu	45	c.6179T>G	p.Leu2060Arg	15	Yes	ABCR400			
MD-0582	ABCA4	34	c.4793C>A	p.Ala1598Asp	41	c.5819T>C	p.Leu1940Pro	15	NP	ABCR400			

Pedigrees	Gene	Allele 1			Allele 2			Age of onset (years)	Haplotype analysis	Methods	References
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change				
MD-0088	ABCA4	28	c.4139C>T	p.Pro1380Leu		c.5714+5G>A	Splicing defect	16	NP	ABCR400	Valverde et al. 2006 (6)
MD-0181	ABCA4	22	c.3323G>A	p.Arg1108His		c.5460+5G>A	Splicing defect	16	Yes	ABCR400 + dHPLC + HRM	Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)
MD-0486	ABCA4	17	c.2588G>C	p.Gly863Ala	45	c.6179T>G	p.Leu2060Arg	16	NP	ABCR400	This study
MD-0529	ABCA4	42	c.5582G>A	p.Gly1961Glu	39	c.5531G>A	p.Gly1844Asp [^]	16	Yes	ABCR400 + NGS	
MD-0060	ABCA4	13	c.1804C>T	p.Arg602Trp	42	c.5582G>A	p.Gly1961Glu	17	Yes	ABCR400	
MD-0260	ABCA4	13	c.1804C>T	p.Arg602Trp		c.5714+5G>A	Splicing defect	17	Yes	ABCR400	Valverde et al. 2006 (6)
MD-0305	ABCA4	21	c.3056C>T	p.Thr1019Met	22	c.3323G>A	p.Arg1108His	17	Yes	ABCR400	
MD-0588	ABCA4	22	c.3323G>A	p.Arg1108His		c.3862+1G>A	Splicing defect	17	NP	ABCR400	
MD-0081	ABCA4	29	c.4297G>A	p.Val1433Ile	42	c.5582G>A	p.Gly1961Glu	18	Yes	ABCR400 + SS	Valverde et al. 2006 (6)
MD-0267	ABCA4	19	c.2791G>A	p.Val931Met		c.5714+5G>A	Splicing defect	18	Yes	ABCR400	
MD-0218	ABCA4	19	c.2894A>G	p.Asn965Ser	19	c.2894A>G	p.Asn965Ser	20	Yes	ABCR400	
MD-0431	ABCA4	30	c.4457C>T	p.Pro1486Leu	22	c.3211_3212insGT	p.Ser1071fs	20	NP	ABCR400	
MD-0308	ABCA4	12	c.1592A>G	p.Glu531Gly		c.4253+4C>T	Splicing defect	21	Yes	ABCR400 + HRM	
MD-0284	ABCA4	42	c.5582G>A	p.Gly1961Glu	45	c.6179T>G	p.Leu2060Arg	22	NP	ABCR400	
MD-0225	ABCA4	42	c.5582G>A	p.Gly1961Glu	48	c.6559C>T	p.Gln2187*	23	Yes	ABCR400 + dHPLC + HRM	Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)
MD-0167	ABCA4	22	c.3211_3212insGT	p.Ser1071fs	22	c.3281C>G	p.Pro1094Arg [^]	23	Yes	ABCR400 + dHPLC + HRM + MLPA + NGS	Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16); This study
MD-0138	ABCA4	17	c.2588G>C	p.Gly863Ala	30	c.4537_4538insC	p.Gln1513fs	24	Yes	ABCR400	Valverde et al. 2006 (6)
MD-0153	ABCA4	22	c.3211_3212insGT	p.Ser1071fs	42	c.5881G>A	p.Gly1961Arg	24	Yes	ABCR400	
MD-0135	ABCA4	42	c.5582G>A	p.Gly1961Glu	8	c.1029_1030insT	p.Asn344*	24	NP	ABCR400 + dHPLC + HRM + MLPA	Valverde et al. 2006 (6); Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)
MD-0326	ABCA4	30	c.4457C>T	p.Pro1486Leu	43	c.5929G>A	p.Gly1977Ser	27	Yes	ABCR400	
MD-0266	ABCA4	42	c.5582G>A	p.Gly1961Glu	45	c.6179T>G	p.Leu2060Arg	28	Yes	ABCR400	
MD-0544	ABCA4	22	c.3292C>T	p.Arg1098Cys		c.4773+1G>T	Splicing defect	28	NP	ABCR400	
MD-0535	ABCA4	35	c.4918C>T	p.Arg1640Trp	5	c.457A>T	p.Ile153Leu [^]	28	NP	ABCR400 + NGS	This study
MD-0163	ABCA4	30	c.4457C>T	p.Pro1486Leu	36	c.5172G>T	p.Trp1724Cys	32	Yes	ABCR400 + dHPLC + HRM + MLPA	Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)

Pedigrees	Gene	Allele 1			Allele 2			Age of onset (years)	Haplotype analysis	Methods	References
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change				
MD-0530	ABCA4	39	c.5512C>G	p.His1838Asp	42	c.5582G>A	p.Gly1961Glu	33	NP	ABCR400	This study
MD-0464	ABCA4	17	c.2588G>C	p.Gly863Ala		c.6147+2T>A	Splicing defect	33	NP	ABCR400 + NGS	
MD-0539	ABCA4	13	c.1804C>T	p.Arg602Trp	30	c.4457C>T	p.Pro1486Leu	35	NP	ABCR400	
MD-0450	ABCA4	42	c.5582G>A	p.Gly1961Glu	8	c.1022_1035delAAGACAATAACTAT	p.Asp342_Lys346deifs [^]	37	Yes	ABCR400 + NGS	This study
MD-0216	ABCA4	31	c.4577C>T	p.Thr1526Met		c.5714+5G>A	Splicing defect	38	Yes	ABCR400	This study
MD-0187	ABCA4	28	c.4139C>T	p.Pro1380Leu	42	c.5582G>A	p.Gly1961Glu	39	NP	ABCR400	
MD-0388	ABCA4	47	c.6449G>A	p.Cys2150Tyr	45	c.6230G>A	p.Arg2077Gln [^]	54	NP	ABCR400 + NGS	
MD-0183	ABCA4	44	c.6079C>T	p.Leu2027Phe	43	c.5929G>A	p.Gly1977Ser	55	Yes	ABCR400 + dHPLC + HRM	Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)
MD-0514	ABCA4	22	c.3323G>A	p.Arg1108His	36	c.5041_5055del	p.Val1681_Cys1685del	N/A	Yes	ABCR400	This study
MD-0528	ABCA4	41	c.5819T>C	p.Leu1940Pro	44	c.6089G>A	p.Arg2030Gln	N/A	Yes	ABCR400	
MD-0559	ABCA4	35	c.4919G>A	p.Arg1640Gln	41	c.5819T>C	p.Leu1940Pro	N/A	Yes	ABCR400	
MD-0509	ABCA4	17	c.2588G>C	p.Gly863Ala	44	c.6118C>T	p.Arg2040*	N/A	NP	ABCR400	This study
MD-0523	ABCA4	41	c.5819T>C	p.Leu1940Pro	42	c.5582G>A	p.Gly1961Glu	N/A	NP	ABCR400	
MD-0597	ABCA4	42	c.5582G>A	p.Gly1961Glu	12	c.1714C>T	p.Arg572*	N/A	NP	ABCR400	
MD-0439	ABCA4	23	c.3364G>A	p.Glu1122Lys	8	c.871C>G	p.Pro291Ala [^]	N/A	NP	ABCR400 + NGS	This study
MD-0534	ABCA4	48	c.6718A>G	p.Thr2240Ala [^]		c.4253+5G>A	Splicing defect	N/A	NP	ABCR400 + NGS	This study
MD-0553	ABCA4	44	c.6089G>A	p.Arg2030Gln	6	c.735T>G	p.Tyr245*	N/A	NP	ABCR400 + NGS	This study
MD-0565	ABCA4	41	c.5819T>C	p.Leu1940Pro	23	c.3380G>A	p.Gly1127Glu	N/A	NP	ABCR400 + NGS	This study
											17.4

Pedigrees	Gene	Allele 1				Allele 2				Age of onset (years)	Haplotype analysis	Methods	References
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change						
MD-0423	ABCA4		c.4253+4C>T	Splicing defect			Not detected		2	NP	ABCR400 + NGS	This study	
MD-0415	ABCA4	13	c.1819G>A	p.Gly607Arg			Not detected		6	NP	ABCR400 + NGS	This study	
MD-0248	ABCA4	19	c.2861A>C	p.Tyr954Ser			Not detected		7	Yes	ABCR400 + dHPLC + HRM + MLPA	Aguirre-Lamban et al. 2010 (16)	
MD-0569	ABCA4	45	c.6148G>C	p.Val2050Leu			Not detected		8	Yes	ABCR400		
MD-0599	ABCA4	23	c.3386G>T	p.Arg1129Leu			Not detected		8	NP	ABCR400 + SS		
MD-0016	ABCA4	9; 38	c.[1140T>A; 5395A>G]	p.[Asn380Lys; p.Asn1799Asp]			Not detected		8	Yes	ABCR400	Valverde et al. 2006 (6)	

Pedigrees	Gene	Allele 1				Allele 2				Age of onset (years)	Haplotype analysis	Methods	References
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change				
MD-0286	ABCA4	21	c.3056C>T	p.Thr1019Met		Not detected		9	Yes	ABCR400 + dHPLC + MLPA + NGS	This study	Valverde et al. 2006 (6); This study	
MD-0039	ABCA4		c.5714+5G>A	Splicing defect		Not detected		10	Yes	ABCR400 + SS + NGS			
MD-0395	ABCA4	23	c.3386G>T	p.Arg1129Leu		Not detected		11	NP	ABCR400 + NGS	This study	Valverde et al. 2006 (6); This study	
MD-0065	ABCA4	42	c.5582G>A	p.Gly1961Glu		Not detected		12	NP	ABCR400 + dHPLC + MLPA + SS + NGS			
MD-0091	ABCA4	19	c.2791G>A	p.Val931Met		Not detected		12	Yes	ABCR400 + NGS	This study	Valverde et al. 2006 (6); This study	
MD-0304	ABCA4	29	c.4297G>A	p.Val1433Ile		Not detected		12	Yes	ABCR400 + HRM +MLPA			
MD-0401	ABCA4	19	c.2791G>A	p.Val931Met		Not detected		12	NP	ABCR400 + NGS	This study	Valverde et al. 2006 (6)	
MD-0279	ABCA4	43	c.5929G>A	p.Gly1977Ser		Not detected		14	Yes	ABCR400			
MD-0456	ABCA4	41	c.5761G>A	p.Val1921Met		Not detected		14	NP	ABCR400			
MD-0075	ABCA4	48	c.6721C>G	p.Leu2241Val		Not detected		15	NP	ABCR400 + dHPLC + MLPA			
MD-0197	ABCA4	36	c.5172G>T	p.Trp1724Cys		Not detected		15	Yes	ABCR400 + dHPLC + HRM + MLPA	Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)	Valverde et al. 2006 (6); Aguirre-Lamban et al. 2009 (8); This study	
MD-0162	ABCA4	23	c.3386G>T	p.Arg1129Leu		Not detected		16	Yes	ABCR400 + dHPLC + MLPA + NGS			
MD-0373	ABCA4		c.6147+2T>A	Splicing defect		Not detected		18	Yes	ABCR400 + NGS	This study	This study	
MD-0580	ABCA4	42	c.5582G>A	p.Gly1961Glu		Not detected		18	NP	ABCR400 + NGS			
MD-0583	ABCA4	23	c.3386G>T	p.Arg1129Leu		Not detected		18	NP	ABCR400		Valverde et al. 2006 (6)	
MD-0242	ABCA4	12	c.1715G>C	p.Arg572Pro		Not detected		18	Yes	ABCR400			
MD-0051	ABCA4	22	c.3292C>T	p.Arg1098Cys		Not detected		18	Yes	ABCR400 + MLPA	Valverde et al. 2006 (6)	Aguirre-Lamban et al. 2009 (8)	
MD-0595	ABCA4		c.6147+2T>A	Splicing defect		Not detected		19	NP	ABCR400			
MD-0222	ABCA4	19	c.2791G>A	p.Val931Met		Not detected		23	Yes	ABCR400 + dHPLC + MLPA		Aguirre-Lamban et al. 2010 (16); This study	
MD-0301	ABCA4	43	c.5914G>A	p.Gly1972Arg		Not detected		25	NP	ABCR400 + HRM +MLPA			
MD-0463	ABCA4	22	c.3211_3212insGT	p.Ser1071fs		Not detected		28	Yes	ABCR400		Aguirre-Lamban et al. 2009 (8)	
MD-0257	ABCA4	22	c.3211_3212insGT	p.Ser1071fs		Not detected		28	Yes	ABCR400 + dHPLC + HRM + MLPA + NGS			
MD-0166	ABCA4	46	c.6320G>A	p.Arg2107His		Not detected		30	Yes	ABCR400 + dHPLC + MLPA	Aguirre-Lamban et al. 2009 (8)	Aguirre-Lamban et al. 2009 (8)	
MD-0165	ABCA4	22	c.3211_3212insGT	p.Ser1071fs		Not detected		30	NP	ABCR400 + dHPLC + MLPA + NGS			
MD-0478	ABCA4	45	c.6148G>C	p.Val2050Leu		Not detected		34	NP	ABCR400		Aguirre-Lamban et al. 2009 (8); This study	
MD-0472	ABCA4	29	c.4297G>A	p.Val1433Ile		Not detected		39	Yes	ABCR400 + NGS			

Pedigrees	Gene	Allele 1		Allele 2		Age of onset (years)	Haplotype analysis	Methods	References
		Exon	Nucleotide change	Amino acid change	Exon				
MD-0243	ABCA4	45	c.6148G>C	p.Val2050Leu	Not detected	40	NP	ABCR400 + NGS	Valverde et al. 2006 (6)
MD-0031	ABCA4	23	c.3386G>T	p.Arg1129Leu	Not detected	41	Yes	ABCR400	
MD-0493	ABCA4	30	c.4457C>T	p.Pro1486Leu	Not detected	44	NP	ABCR400 + NGS	
MD-0285	ABCA4	23	c.3386G>T	p.Arg1129Leu	Not detected	51	NP	ABCR400 + dHPLC + MLPA + NGS	
MD-0536	ABCA4		c.4253+5G>A	Splicing defect	Not detected	55	NP	ABCR400	
MD-0205	ABCA4	35	c.4919G>A	p.Arg1640Gln	Not detected	N/A	Yes	ABCR400 + dHPLC + MLPA + NGS	Aguirre-Lamban et al. 2009 (8); This study
MD-0245	ABCA4		c.4253+5G>A	Splicing defect	Not detected	N/A	Yes	ABCR400 + dHPLC + MLPA	
MD-0402	ABCA4	12	c.1622T>C	p.Leu541Pro	Not detected	N/A	Yes	ABCR400	
MD-0548	ABCA4	6	c.634C>T	p.Arg212Cys	Not detected	N/A	Yes	ABCR400	
MD-0571	ABCA4		c.5461-10T>C	Unknown effect	Not detected	N/A	Yes	ABCR400 + NGS	
MD-0394	ABCA4	5	c.454C>T	p.Arg152*	Not detected	N/A	No	ABCR400	This study
MD-0323	ABCA4	41	c.5819T>C	p.Leu1940Pro	Not detected	N/A	NP	ABCR400	
MD-0414	ABCA4	35	c.4918C>T	p.Arg1640Trp	Not detected	N/A	NP	ABCR400	
MD-0572	ABCA4	23	c.3386G>T	p.Arg1129Leu	Not detected	N/A	NP	ABCR400	
MD-0589	ABCA4	23	c.3386G>T	p.Arg1129Leu	Not detected	N/A	NP	ABCR400	

Pedigrees	Gene	Allele 1		Allele 2		Haplotype analysis	Methods	References
		Exon	Nucleotide change	Amino acid change	Exon			
MD-0068	ABCA4		Not detected		Not detected	NP	ABCR400	Valverde et al. 2006 (6)
MD-0151	ABCA4		Not detected		Not detected	NP	ABCR400	
MD-0156	ABCA4		Not detected		Not detected	No	ABCR400	
MD-0173	ABCA4		Not detected		Not detected	Yes	ABCR400 + dHPLC + MLPA	Aguirre-Lamban et al. 2009 (8)
MD-0177	ABCA4		Not detected		Not detected	NP	ABCR400 + MLPA	
MD-0208	ABCA4		Not detected		Not detected	NP	ABCR400 + MLPA	
MD-0211	ABCA4		Not detected		Not detected	NP	ABCR400 + MLPA	
MD-0223	ABCA4		Not detected		Not detected	No	ABCR400	
MD-0236	ABCA4		Not detected		Not detected	No	ABCR400 + dHPLC + MLPA	This study
MD-0255	ABCA4		Not detected		Not detected	No	ABCR400	
MD-0258	ABCA4		Not detected		Not detected	NP	ABCR400 + HRM + MLPA + NGS	
MD-0261	ABCA4		Not detected		Not detected	NP	ABCR400	

Pedigrees	Gene	Allele 1				Allele 2				Haplotype analysis	Methods	References
		Exon		Amino acid change		Exon		Amino acid change				
		Nucleotide change	Nucleotide change	Amino acid change	Amino acid change	Nucleotide change	Nucleotide change	Amino acid change	Amino acid change			
MD-0269	ABCA4			Not detected			Not detected			NP	ABCR400 + MLPA	ABCR400 + MLPA
MD-0297	ABCA4			Not detected			Not detected			No	ABCR400 + MLPA	
MD-0312	ABCA4			Not detected			Not detected			No	ABCR400	ABCR400
MD-0313	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0314	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0315	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0316	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0335	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0338	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0340	ABCA4			Not detected			Not detected			Yes	ABCR400	
MD-0350	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0352	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0353	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0365	ABCA4			Not detected			Not detected			Yes	ABCR400	
MD-0381	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0383	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0384	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0391	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0396	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0424	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0455	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0460	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0470	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0476	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0477	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0483	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0484	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0491	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0511	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400
MD-0517	ABCA4			Not detected			Not detected			NP	ABCR400	
MD-0522	ABCA4			Not detected			Not detected			NP	ABCR400	ABCR400

Pedigrees	Gene	Allele 1					Allele 2			Haplotype analysis	Methods	References
		Exon		Amino acid change		Exon	Nucleotide change		Amino acid change			
		Nucleotide change	Amino acid change	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change	Amino acid change			
MD-0537	<i>ABCA4</i>		Not detected		Not detected				Not detected	NP	ABCR400	This study
MD-0541	<i>ABCA4</i>		Not detected		Not detected				Not detected	NP	ABCR400	
MD-0543	<i>ABCA4</i>		Not detected		Not detected				Not detected	NP	ABCR400	
MD-0564	<i>ABCA4</i>		Not detected		Not detected				Not detected	NP	ABCR400	
MD-0567	<i>ABCA4</i>		Not detected		Not detected				Not detected	NP	ABCR400	
MD-0568	<i>ABCA4</i>		Not detected		Not detected				Not detected	NP	ABCR400	
MD-0570	<i>ABCA4</i>		Not detected		Not detected				Not detected	NP	ABCR400	
MD-0575	<i>ABCA4</i>		Not detected		Not detected				Not detected	NP	ABCR400	
MD-0579	<i>ABCA4</i>		Not detected		Not detected				Not detected	NP	ABCR400 + NGS	
MD-0601	<i>ABCA4</i>		Not detected		Not detected				Not detected	NP	ABCR400	

Families were classified over the presence of the p.Arg1129Leu mutation either in a homozygous or heterozygous state, compound heterozygous mutations not presenting this allele, or patients presenting complex alleles. Mean age of onset was also assessed for each group. Patients with one mutant allele are also shown. Families and mutations identified by Next Generation Sequencing are shown in bold. Additionally, patients were sorted by age of onset of the disease. Haplotype analysis and cosegregation of mutations with the disease is also indicated.

[^] Novel mutation; (NP) Not performed; (N/A) Not available; (ABCR400) ABCR400 genotyping microarray; (dHPLC) denaturing High-Performance Liquid Chromatography; (HRM) High Resolution Melting; (MLPA) Multiple Ligation Probe Analysis; (SS) Sanger Sequencing; (NGS) Next Generation Sequencing.

Table 2

Mutations in *ABCA4* in arCRD families.

Pedigrees	Gene	Allele 1			Allele 2			Age of onset (years)	Haplotype analysis	Methods	References
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change				
MD-0174	<i>ABCA4</i>	35	c.4918C>T	p.Arg1640Trp		c.6147+2T>A	Splicing defect	4	Yes	ABCR400 + dHPLC + HRM	Aguirre-Lamban et al. 2010 (16)
RP-0998	<i>ABCA4</i>	14	c.2041C>T	p.Arg681*	22	c.3211_3212insGT	p.Ser1071fs	5	Yes	ABCR400	
RP-0267	<i>ABCA4</i>	36	c.5041_5055del	p.Val1681_Cys1685del	36	c.5041_5055del	p.Val1681_Cys1685del	6	Yes	ABCR400	Valverde et al. 2006 (6)
MD-0295	<i>ABCA4</i>	43	c.5917delG	p.Val1973*	43	c.5917delG	p.Val1973*	7	Yes	ABCR400	
MD-0467	<i>ABCA4</i>	12	c.1622T>C	p.Leu541Pro	43	c.5917delG	p.Val1973*	7	NP	ABCR400	Valverde et al. 2006 (6)
MD-0079	<i>ABCA4</i>	19	c.2888delG	p.Gly963fs	19	c.2888delG	p.Gly963fs	8	Yes	ABCR400	
MD-0086	<i>ABCA4</i>	19	c.2888delG	p.Gly963fs	19	c.2888delG	p.Gly963fs	8	Yes	ABCR400	Valverde et al. 2006 (6)
MD-0133	<i>ABCA4</i>	1	c.32T>C	p.Leu11Pro	19	c.2888delG	p.Gly963fs	8	Yes	ABCR400	
MD-0206	<i>ABCA4</i>		c.5461-10T>C	Unknown effect	36	c.5041_5055del	p.Val1681_Cys1685del	8	Yes	ABCR400	Valverde et al. 2006 (6)
RP-0741	<i>ABCA4</i>	43	c.5917delG	p.Val1973*	43	c.5917delG	p.Val1973*	8	NP	ABCR400	
RP-1126	<i>ABCA4</i>		c.3862+1G>A	Splicing defect	46	c.6329G>A	p.Trp2110*	8	NP	ABCR400	This study
MD-0392	<i>ABCA4</i>	46	c.6320G>C	p.Arg2107Pro	46	c.6329G>A	p.Trp2110*	8	Yes	ABCR400	
RP-0266	<i>ABCA4</i>	45	c.6179T>G	p.Leu2060Arg		c.4253+5G>A	Splicing defect	8	NP	ABCR400	This study
MD-0565	<i>ABCA4</i>	41	c.5819T>C	p.Leu1940Pro	23	c.3380G>A	p.Gly1127Glu [^]	8	NP	ABCR400 + NGS	
MD-0290	<i>ABCA4</i>	13	c.1804C>T	p.Arg602Trp	35	c.4919G>A	p.Arg1640Gln	9	NP	ABCR400	Aguirre-Lamban et al. 2009 (8); Aguirre-Lamban et al. 2010 (16)
MD-0126	<i>ABCA4</i>	43	c.5929G>A	p.Gly1977Ser	43	c.5929G>A	p.Gly1977Ser	10	Yes	ABCR400	
MD-0176	<i>ABCA4</i>	45	c.6179T>G	p.Leu2060Arg	19	c.2888delG	p.Gly963fs	10	Yes	ABCR400	This study
RP-0714	<i>ABCA4</i>		c.4253+4C>T	Splicing defect		c.4253+4C>T	Splicing defect	10	Yes	ABCR400	
RP-1680	<i>ABCA4</i>	13	c.1804C>T	p.Arg602Trp	36	c.5041_5055del	p.Val1681_Cys1685del	11	NP	ABCR400	This study
MD-0247	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	47	c.6410G>A	p.Cys2137Tyr	12	Yes	ABCR400 + dHPLC + HRM	
MD-0299	<i>ABCA4</i>	1	c.52C>T	p.Arg18Trp	45	c.6179T>G	p.Leu2060Arg	13	Yes	ABCR400	This study
MD-0213	<i>ABCA4</i>	13	c.1804C>T	p.Arg602Trp	13	c.1804C>T	p.Arg602Trp	15	Yes	ABCR400	
MD-0336	<i>ABCA4</i>	44	c.6088C>T	p.Arg2030*	44	c.6088C>T	p.Arg2030*	16	Yes	ABCR400	This study
MD-0302	<i>ABCA4</i>	12	c.1622T>C	p.Leu541Pro	42	c.5882G>A	p.Gly1961Glu	17	Yes	ABCR400	
MD-0390	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu	19	c.2888delG	p.Gly963fs	20	Yes	ABCR400	This study
RP-1715	<i>ABCA4</i>	13	c.1804C>T	p.Arg602Trp	27	c.3874C>T	p.Gln1292*	N/A	Yes	ABCR400 + SS + NGS	

Pedigrees	Gene	Allele 1			Allele 2			Age of onset (years)	Haplotype analysis	Methods	References						
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change										
		Mean age on onset years															
10.3																	
Pedigrees	Gene	Allele 1			Allele 2			Age of onset (years)	Haplotype analysis	Methods	References						
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change										
		MD-0526	ABCA4	12	c.1613C>T		p.Ala538Val					Not detected		Congenital	No	ABCR400	
		MD-0557	ABCA4	45	c.6148G>C		p.Val2050Leu					Not detected		4	NP	ABCR400 + SS	
		RP-1058	ABCA4	29	c.4297G>A		p.Val1433Ile					Not detected		6	NP	ABCR400 + dHPLC + MLPA	Aguirre-Lamban et al. 2009 (8)
		MD-0190	ABCA4	22	c.3211_3212insGT		p.Ser1071fs					Not detected		8	Yes	ABCR400	
		RP-1769	ABCA4	22	c.3211_3212insGT		p.Ser1071fs					Not detected		9	NP	ABCR400	
		MD-0604	ABCA4	19	c.2888delG		p.Gly963fs					Not detected		10	NP	ABCR400 + SS	
		RP-1354	ABCA4	23	c.3386G>T		p.Arg1129Leu					Not detected		12	NP	ABCR400	
		RP-1177	ABCA4	46	c.6320G>C		p.Arg2107Pro					Not detected		22	Yes	ABCR400 + HRM + MLPA	
		MD-0099	ABCA4	29	c.4297G>A		p.Val1433Ile					Not detected		46	NP	ABCR400 + dHPLC + MLPA	Aguirre-Lamban et al. 2009 (16)
		RP-1539	ABCA4	22	c.3211_3212insGT		p.Ser1071fs					Not detected		59	NP	ABCR400	
		RP-0532	ABCA4	23	c.3386G>T		p.Arg1129Leu					Not detected		24	Yes	ABCR400 + HRM	
		Pedigrees	Gene	Allele 1			Allele 2					Haplotype analysis	Methods	References			
Exon	Nucleotide change			Amino acid change	Exon	Nucleotide change	Amino acid change										
MD-0023	ABCA4				Not detected		Not detected		NP	ABCR400	Valverde et al. 2006 (6)						
MD-0049	ABCA4				Not detected		Not detected		Yes	ABCR400	Valverde et al. 2006 (6)						
MD-0069	ABCA4				Not detected		Not detected		NP	ABCR400	Valverde et al. 2006 (6)						
MD-0085	ABCA4				Not detected		Not detected		NP	ABCR400	Valverde et al. 2006 (6)						
MD-0100	ABCA4				Not detected		Not detected		Yes	ABCR400 + dHPLC + MLPA	Aguirre-Lamban et al. 2009 (8)						
MD-0123	ABCA4				Not detected		Not detected		NP	ABCR400	Valverde et al. 2006 (6)						
MD-0131	ABCA4				Not detected		Not detected		Yes	ABCR400 + dHPLC + MLPA	Aguirre-Lamban et al. 2009 (8)						
MD-0140	ABCA4				Not detected		Not detected		No	ABCR400							
MD-0142	ABCA4				Not detected		Not detected		Yes	ABCR400 + dHPLC + MLPA	Aguirre-Lamban et al. 2009 (8)						
MD-0159	ABCA4				Not detected		Not detected		NP	ABCR400							
MD-0169	ABCA4				Not detected		Not detected		Yes	ABCR400							
MD-0171	ABCA4				Not detected		Not detected		NP	ABCR400 + MLPA							

Pedigrees	Gene	Allele 1				Allele 2				Haplotype analysis	Methods	References
		Exon		Amino acid change		Exon		Amino acid change				
		Nucleotide change	Nucleotide change	Amino acid change	Amino acid change	Nucleotide change	Nucleotide change	Amino acid change	Amino acid change			
MD-0186	<i>ABCA4</i>			Not detected				Not detected		Yes	ABCR400 + dHPLC + MLPA	This study This study
MD-0192	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0195	<i>ABCA4</i>			Not detected				Not detected		Yes	ABCR400 + MLPA	
MD-0212	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0265	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400 + MLPA	
MD-0278	<i>ABCA4</i>			Not detected				Not detected		Yes	ABCR400	
MD-0292	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400 + MLPA	
MD-0309	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0327	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0366	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0367	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0380	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0385	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0397	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0398	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0411	<i>ABCA4</i>			Not detected				Not detected		Yes	ABCR400	
MD-0422	<i>ABCA4</i>			Not detected				Not detected		Yes	ABCR400	
MD-0426	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0440	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0442	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0443	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0446	<i>ABCA4</i>			Not detected				Not detected		Yes	ABCR400	
MD-0448	<i>ABCA4</i>			Not detected				Not detected		Yes	ABCR400 + NGS	
MD-0475	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400 + NGS	
MD-0488	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0490	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0495	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0503	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0504	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0507	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	
MD-0527	<i>ABCA4</i>			Not detected				Not detected		NP	ABCR400	

Pedigrees	Gene	Allele 1			Allele 2			Haplotype analysis	Methods	References
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change			
MD-0554	<i>ABCA4</i>		Not detected			Not detected		NP	ABCR400	Valverde et al. 2006 (6) Valverde et al. 2007 (7)
MD-0562	<i>ABCA4</i>		Not detected			Not detected		NP	ABCR400	
MD-0596	<i>ABCA4</i>		Not detected			Not detected		NP	ABCR400	
RP-0577	<i>ABCA4</i>		Not detected			Not detected		NP	ABCR400 + dHPLC + MLPA	
RP-0827	<i>ABCA4</i>		Not detected			Not detected		NP	ABCR400	
RP-0964	<i>ABCA4</i>		Not detected			Not detected		NP	ABCR400 + dHPLC	

Patients were sorted by age of onset. Mean age of onset and cosegregation of mutations with the disease were also stated. Families and mutations identified by Next Generation Sequencing are shown in bold.

[^] Novel mutation; (NP) Not performed; (N/A) Not available; (ABCR400) ABCR400 genotyping microarray; (dHPLC) denaturing High-Performance Liquid Chromatography; (HRM) High Resolution Melting; (MLPA) Multiple Ligation Probe Analysis; (SS) Sanger Sequencing; (NGS) Next Generation Sequencing.

Table 3

Mutations in *ABCA4* in arRP families.

Pedigrees	Gene	Allele 1			Allele 2			Haplotype analysis	Methods	Mutations in other arRP genes	References
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change				
RP-0280	<i>ABCA4</i>	38	c.5413A>G	p.Asn1805Asp			Not detected	No	ABCR400	<i>CRBB1</i> p.[Tp822*];[Cys948Tyr]	Riveiro-Alvarez et al. 2008 (9)
RP-0599	<i>ABCA4</i>	43	c.5929G>A	p.Gly1977Ser			Not detected	NP	ABCR400	<i>RPGR</i> [g. ORF15 481_482delAG]	Garcia-Hoyos et al. 2006 (19)
RP-0759	<i>ABCA4</i>	17	c.2588G>C	p.Gly863Ala			Not detected	Yes	ABCR400 + HRM +MLPA	<i>LCA5</i>	Under publication
RP-0773	<i>ABCA4</i>	23	c.3386G>T	p.Arg1129Leu			Not detected	NP	ABCR400 + dHPLC + MLPA		Valverde et al. 2006 (6); Aguirre-Lamban et al. 2009 (8)
RP-0834	<i>ABCA4</i>		c.5714+5G>A	Splicing defect			Not detected	NP	ABCR400 + dHPLC		Valverde et al. 2006 (6); Valverde et al. 2007 (7)
RP-0988	<i>ABCA4</i>	45	c.6148G>C	p.Val2050Leu			Not detected	NP	ABCR400		
RP-1484	<i>ABCA4</i>	42	c.5882G>A	p.Gly1961Glu			Not detected	Yes	ABCR400		
RP-1578	<i>ABCA4</i>	34	c.4793C>A	p.Alal598Asp			Not detected	NP	ABCR400		
RP-0003	<i>ABCA4</i>		Not detected				Not detected	Yes	ABCR400		
RP-0012	<i>ABCA4</i>		Not detected				Not detected	No	ABCR400	<i>RP1</i>	Under publication
RP-0017	<i>ABCA4</i>		Not detected				Not detected	NP	ABCR400		
RP-0055	<i>ABCA4</i>		Not detected				Not detected	NP	ABCR400	<i>EYS</i>	Under publication
RP-0082	<i>ABCA4</i>		Not detected				Not detected	NP	ABCR400		Valverde et al. 2006 (6)
RP-0137	<i>ABCA4</i>		Not detected				Not detected	NP	ABCR400	<i>RP1</i>	Under publication
RP-0160	<i>ABCA4</i>		Not detected				Not detected	NP	ABCR400	<i>RP1</i>	Under publication
RP-0180	<i>ABCA4</i>		Not detected				Not detected	NP	ABCR400		
RP-0181	<i>ABCA4</i>		Not detected				Not detected	NP	ABCR400		
RP-0201	<i>ABCA4</i>		Not detected				Not detected	No	ABCR400		
RP-0231	<i>ABCA4</i>		Not detected				Not detected	NP	ABCR400 + MLPA		
RP-0260	<i>ABCA4</i>		Not detected				Not detected	No	ABCR400	<i>USH2A</i> p.[Cys3267Arg];[Cys3358Tyr]	Avila-Fernandez et al. 2010 (18)
RP-0261	<i>ABCA4</i>		Not detected				Not detected	NP	ABCR400		
RP-0285	<i>ABCA4</i>		Not detected				Not detected	No	ABCR400	<i>LCA5</i>	Under publication
RP-0289	<i>ABCA4</i>		Not detected				Not detected	No	ABCR400		
RP-0341	<i>ABCA4</i>		Not detected				Not detected	No	ABCR400	<i>PDE6A</i> c.[998+1G>A];[1705C>A]	Avila-Fernandez et al. 2010 (18)
RP-0407	<i>ABCA4</i>		Not detected				Not detected	Yes	ABCR400		
RP-0417	<i>ABCA4</i>		Not detected				Not detected	NP	ABCR400		

Pedigrees	Gene	Allele 1		Allele 2		Haplotype analysis	Methods	Mutations in other arRP genes	References
		Exon	Nucleotide change	Amino acid change	Nucleotide change				
RP-0503	<i>ABCA4</i>		Not detected		Not detected	No	ABCR400	<i>MERTK</i>	Under publication
RP-0509	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400	<i>CNGBI</i>	Under publication
RP-0578	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400	<i>CRBI</i>	Under publication
RP-0586	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400		
RP-0643	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400		
RP-0657	<i>ABCA4</i>		Not detected		Not detected	No	ABCR400	<i>CERKL</i> p.[Arg257*];[Arg257*]	Avila-Fernandez et al. 2010 (18)
RP-0682	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400		
RP-0687	<i>ABCA4</i>		Not detected		Not detected	No	ABCR400		
RP-0700	<i>ABCA4</i>		Not detected		Not detected	No	ABCR400		Valverde et al. 2006 (6)
RP-0716	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400 + dHPLC + MLPA		Valverde et al. 2006 (6); Valverde et al. 2007 (7)
RP-0757	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400		
RP-0766	<i>ABCA4</i>		Not detected		Not detected	No	ABCR400 + dHPLC + MLPA		Valverde et al. 2006 (6); Valverde et al. 2007 (7)
RP-0768	<i>ABCA4</i>		Not detected		Not detected	Yes	ABCR400		
RP-0775	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400 + dHPLC + MLPA		Valverde et al. 2006 (6); Valverde et al. 2007 (7)
RP-0813	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400		Valverde et al. 2006 (6)
RP-0818	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400 + dHPLC + MLPA		Valverde et al. 2006 (6); Valverde et al. 2007 (7)
RP-0828	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400	<i>CERKL</i> p.[Arg257*];[Arg257*]	Valverde et al. 2006 (6); Avila-Fernandez et al. 2008 (20)
RP-0854	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400 + dHPLC + MLPA		Valverde et al. 2007 (7)
RP-0857	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400		Valverde et al. 2006 (6)
RP-0870	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400		
RP-0888	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400		
RP-0901	<i>ABCA4</i>		Not detected		Not detected	No	ABCR400	<i>CEP290</i>	Under publication
RP-0927	<i>ABCA4</i>		Not detected		Not detected	No	ABCR400 + HRM +MLPA	<i>EYS</i>	Under publication
RP-0937	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400		
RP-0942	<i>ABCA4</i>		Not detected		Not detected	No	ABCR400		
RP-0959	<i>ABCA4</i>		Not detected		Not detected	Yes	ABCR400 + dHPLC + MLPA		Aguirre-Lamban et al. 2009 (8)
RP-1000	<i>ABCA4</i>		Not detected		Not detected	NP	ABCR400		

Pedigrees	Gene	Allele 1		Allele 2		Haplotype analysis	Methods	Mutations in other arRP genes	References
		Exon	Nucleotide change	Amino acid change	Exon	Nucleotide change	Amino acid change		
RP-1174	<i>ABCA4</i>		Not detected			Not detected		ABCR400	Under publication
RP-1175	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1205	<i>ABCA4</i>		Not detected			Not detected		ABCR400 + HRM + MLPA	
RP-1222	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1231	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1280	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1288	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1511	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1529	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1535	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1557	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1562	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1569	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1600	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1654	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1664	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1668	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1673	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1698	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1714	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1743	<i>ABCA4</i>		Not detected			Not detected		ABCR400	
RP-1747	<i>ABCA4</i>		Not detected			Not detected		ABCR400	

Cosegregation of the mutant allele with the disease is also indicated. Additionally, mutations identified by further analyses in other arRP related genes are shown.

(NP) Not performed; (ABCR400) ABCR400 genotyping microarray; (dHPLC) denaturing High-Performance Liquid Chromatography; (HRM) High Resolution Melting; (MLPA) Multiple Ligation Probe Analysis.

Table 4

Spectrum of *ABCA4* mutations and associated phenotypes in Spain.

Exon	Allele		Associated phenotypes (N° cases)	Zygosity
	Nucleotide change	Amino acid change		
1	c.3G>A	p.Met1Ile	STGD (N=1)	Heterozygous
1	c.32T>C	p.Leu11Pro	CRD (N=1)	Heterozygous
1	c.52C>T	p.Arg18Trp	STGD (N=1), CRD (N=1)	Heterozygous
5	c.454C>T	p.Arg152*	STGD (N=2)	Heterozygous
5	c.457A>T	p.Ile153Leu	STGD (N=1)	Heterozygous
6	c.634C>T	p.Arg212Cys	STGD (N=4)	Heterozygous
6	c.768G>T	p.Val256Val or splicing defect	STGD (N=1)	Heterozygous
6	c.700C>T	p.Gln234*	STGD (N=1)	Heterozygous
6	c.735T>G	p.Tyr245*	STGD (N=1)	Heterozygous
6	c.742_768+29del56bp	Frameshift defect	STGD (N=1)	Heterozygous
8	c.871C>G	p.Pro291Ala	STGD (N=1)	Heterozygous
8	c.982G>T	p.Glu328*	STGD (N=2)	Heterozygous
8	c.1022_1035delAAAGACAAATAACTAT	p.Asp342_Lys346delifs	STGD (N=1)	Heterozygous
8	c.1029_1030insT	p.Asn344*	STGD (N=1)	Heterozygous
9	c.1222C>T	p.Arg408*	STGD (N=2)	Heterozygous
12	c.1592A>G	p.Glu531Gly	STGD (N=1)	Heterozygous
12	c.1609C>T	p.Arg537Cys	STGD (N=1)	Heterozygous
12	c.1613C>T	p.Ala538Val	CRD (N=1)	Heterozygous
12	c.1622T>C	p.Leu541Pro	STGD (N=2), CRD (N=2)	Heterozygous
12	c.1714C>T	p.Arg572*	STGD (N=1)	Heterozygous
12	c.1715G>C	p.Arg572Pro	STGD (N=1)	Heterozygous
13	c.1766G>A	p.Trp589*	STGD (N=1)	Heterozygous
13	c.1804C>T	p.Arg602Trp	STGD (N=8), CRD (N=2)	Heterozygous
13	c.1819G>A	p.Gly607Arg	CRD (N=1)	Homozygous
13	c.1832T>C	p.Leu611Pro	STGD (N=2)	Heterozygous
14	c.1957C>T	p.Arg653Cys	STGD (N=1)	Heterozygous
14	c.2041C>T	p.Arg681*	STGD (N=3), CRD (N=1)	Heterozygous

Exon	Allele		Associated phenotypes (N° cases)	Zygosity
	Nucleotide change	Amino acid change		
14	c.2057T>C	p.Leu686Ser	STGD (N=1)	Heterozygous
15	c.2285C>A	p.Ala762Glu	STGD (N=1)	Heterozygous
	c.2382+5G>C	Splicing defect	STGD (N=1)	Heterozygous
17	c.2588G>C	p.Gly863Ala	STGD (N=4)	Heterozygous
19	c.2888delG	p.Gly963fs	STGD (N=6), CRD (N=4)	Heterozygous
19	c.2791G>A	p.Val931Met	STGD evolving to CRD (N=2)	Homozygous
19	c.2861A>C	p.Tyr954Ser	STGD (N=5)	Heterozygous
19	c.2894A>G	p.Asn965Ser	STGD (N=1)	Heterozygous
20	c.2966T>C	p.Val989Ala	STGD (N=1)	Homozygous
21	c.3056C>T	p.Thr1019Met	STGD (N=7)	Heterozygous
22	c.3211_3212insGT	p.Ser1071fs	STGD (N=14), CRD (N=4)	Heterozygous
22	c.3281C>G	p.Pro1094Arg	STGD (N=1)	Heterozygous
22	c.3287C>T	p.Ser1096Leu	STGD (N=1)	Heterozygous
22	c.3292C>T	p.Arg1098Cys	STGD (N=3)	Heterozygous
22	c.3322C>T	p.Arg1108Cys	STGD (N=1)	Heterozygous
22	c.3323G>A	p.Arg1108His	STGD (N=4)	Heterozygous
	c.5018+2T>C	Splicing defect	STGD (N=1)	Heterozygous
23	c.3364G>A	p.Glu1122Lys	STGD (N=3)	Heterozygous
23	c.3380G>A	p.Gly1127Glu	STGD (N=1), CRD (n=1)	Heterozygous
23	c.3386G>T	p.Arg1129Leu	STGD (N=8)	Homozygous
	c.3862+1G>A	Splicing defect	STGD (N=66), CRD (N=4)	Heterozygous
27	c.3874C>T	p.Gln1292*	STGD (N=1), CRD (N=1)	Heterozygous
27	c.3943C>T	p.Gln1315*	CRD (N=1)	Heterozygous
27	c.3988G>T	p.Glu1330*	STGD (N=2)	Heterozygous
27	c.4000_4001delCC	p.Pro1335Argfs	STGD (N=1)	Heterozygous
27	c.4069G>A	p.Ala1357Thr	STGD (N=2)	Heterozygous
28	c.4139C>T	p.Pro1380Leu	STGD (N=4)	Heterozygous
28	c.4200C>A	p.Tyr1400*	STGD (N=1)	Heterozygous

Exon	Allele		Associated phenotypes (N° cases)	Zygosity
	Nucleotide change	Amino acid change		
28	c.4234C>T	p.Gln1412*	STGD (N=1)	Heterozygous
	c.4253+4C>T	Splicing defect	STGD (N=1), CRD (N=1)	Homozygous
29	c.4297G>A	p.Val1433Ile	STGD (N=6), CRD (N=1)	Heterozygous
30	c.4457C>T	p.Pro1486Leu	STGD (N=3), CRD (N=2)	Heterozygous
30	c.4469G>A	p.Cys1490Tyr	STGD (N=7)	Heterozygous
30	c.4537delC	p.Gln1513fs	STGD (N=3)	Heterozygous
31	c.4577C>T	p.Thr1526Met	STGD (N=1)	Heterozygous
	c.4668-1G>A	Splicing defect	STGD (N=1)	Heterozygous
33	c.4739delT	p.Leu1580fs	STGD (N=1)	Heterozygous
	c.4773+1G>T	Splicing defect	STGD (N=1)	Heterozygous
34	c.4793C>A	p.Ala1598Asp	STGD (N=1)	Heterozygous
35	c.4918C>T	p.Arg1640Trp	STGD (N=3)	Heterozygous
35	c.4919G>A	p.Arg1640Gln	STGD (N=3), CRD (N=1)	Heterozygous
	c.5018+2T>C	Splicing defect	STGD (N=1)	Heterozygous
36	c.5041_5055del	p.Val1681_Cys1685del	STGD (N=1), CRD (N=1)	Homozygous
	c.5172G>T	p.Trp1724Cys	STGD (N=4), CRD (N=2)	Heterozygous
38	c.5395A>G	p.Asn1799Asp	STGD (N=2)	Heterozygous
38	c.5413A>G	p.Asn1805Asp	STGD (N=1)	Heterozygous
	c.5460+5G>A	Splicing defect	STGD (N=1)	Heterozygous
	c.5461-10T>C	Unknown effect	STGD (N=3), CRD (N=1)	Heterozygous
39	c.5512C>G	p.His1838Asp	STGD (N=1)	Homozygous
39	c.5531G>A	p.Gly1844Asp	STGD (N=1)	Heterozygous
39	c.5549T>C	p.Leu1850Pro	STGD (N=1)	Heterozygous
40	c.5629_5643dup	p.Lys1877_Ala1881dup	STGD (N=1)	Heterozygous
40	c.5644A>G	p.Met1882Val	STGD (N=1)	Heterozygous
	c.5714+5G>A	Splicing defect	STGD (N=1)	Heterozygous
41	c.5761G>A	p.Val1921Met	STGD (N=8)	Heterozygous
			STGD (N=1)	Heterozygous

Exon	Allele		Associated phenotypes (N° cases)	Zygosity
	Nucleotide change	Amino acid change		
41	c.5819T>C	p.Leu1940Pro	STGD (N=11), CRD (N=1)	Heterozygous
42	c.5881G>A	p.Gly1961Arg	STGD (N=1)	Homozygous
42	c.5582G>A	p.Gly1961Glu	STGD (N=1)	Heterozygous
43	c.5914G>A	p.Gly1972Arg	STGD (N=26), CRD (N=1)	Heterozygous
43	c.5917delG	p.Val1973*	STGD (N=1)	Heterozygous
43	c.5929G>A	p.Gly1977Ser	CRD (N=2)	Homozygous
44	c.6079C>T	p.Leu2027Phe	CRD (N=1)	Heterozygous
44	c.6088C>T	p.Arg2030*	CRD (N=1)	Homozygous
44	c.6089G>A	p.Arg2030Gln	STGD (N=2)	Heterozygous
44	c.6118C>T	p.Arg2040*	STGD (N=1)	Heterozygous
44	c.6140T>A	p.Ile2047Asn	STGD (N=1)	Heterozygous
45	c.6147+2T>A	Splicing defect	STGD (N=3), CRD (N=1)	Heterozygous
45	c.6179T>G	p.Leu2060Arg	STGD (N=7), CRD (N=3)	Heterozygous
45	c.6148G>C	p.Val2050Leu	STGD (N=3), CRD (N=1)	Heterozygous
45	c.6229C>T	p.Arg2077Trp	STGD (N=1)	Heterozygous
45	c.6230G>A	p.Arg2077Gln	STGD (N=1)	Heterozygous
46	c.6320G>A	p.Arg2107His	STGD (N=1)	Heterozygous
46	c.6320G>C	p.Arg2107Pro	STGD (N=2), CRD (N=2)	Heterozygous
46	c.6329G>A	p.Trp2110*	STGD (N=1), CRD (N=2)	Heterozygous
47	c.6410G>A	p.Cys2137Tyr	STGD (N=1), CRD (n=1)	Heterozygous
47	c.6449G>A	p.Cys2150Tyr	STGD (N=1)	Homozygous
48	c.6559C>T	p.Gln2187*	STGD (N=4)	Heterozygous
48	c.6563T>C	p.Phe2188Ser	STGD (N=2)	Heterozygous
48	c.6718A>G	p.Thr2240Ala	STGD (N=1)	Heterozygous
48	c.6721C>G	p.Leu2241Val	STGD (N=1)	Heterozygous
9; 38	c.[1140T>A; 5395A>G]	p.[Asn380Lys; p.Asn1799Asp]	STGD (N=1)	Heterozygous

Exon	Allele		Associated phenotypes (N° cases)	Zygosity
	Nucleotide change	Amino acid change		
12; 12	c.[1751T>A; 1753delA]	p.[Ile584Asn; Asp586fs]	STGD (N=1)	Heterozygous
17; 21	c.[2588G>C; c.3163C>T]	p.[Gly863Ala; Arg1055Trp]	STGD (N=1)	Heterozygous
22; 46	c.[3322C>T; 6320G>A]	p.[Arg1108Cys; Arg2107His]	STGD (N=7)	Homozygous or heterozygous
25; 42	c.[3758C>T; 5582G>A]	p.[Thr1253Met; p.Gly1961Glu]	STGD (N=1)	Heterozygous
28; 35	c.[4222T>C; 4918C>T]	p.[Trp1408Arg; Arg1640Trp]	STGD (N=2)	Heterozygous
39; 42	c.[5512C>G; 5882G>A]	p.[His1838Asp; Gly1961Glu]	STGD (N=2)	Heterozygous

Numbers of cases harbouring each mutation are shown for the respective phenotypes and a homozygous or heterozygous state is listed.

Table 5

Mutation detection rates and screening methods.

Phenotypes	Increase of mutation detection rate with additional methods			Mutation detection rate after complete <i>ABCA4</i> sequencing
	<i>ABCR400</i> array (n)	Added screening with dHPLC and HRM (n)	Added full gene sequencing with SS or NGS (n)	
arSTGD	48.3% (259)	55.4% (45/259)	70.5% (53/259)	73.6% (53)
arCRD	28.5% (86)	33.1% (13/86)	36.6% (6/86)	66.7% (6)
arRP	5.3% (75)	5.3% (11/75)	ND	ND

The mutation detection rate increases, as expected, with the addition of other screening methods to *ABCR400* microarray. The last column shows the mutation detection rate in the subset of the cohort where the entire *ABCA4* gene was directly sequenced. The number (or the fraction) of samples analysed by each method is given in parentheses.
(n) number; (dHPLC) denaturing High-Performance Liquid Chromatography; (HRM) High Resolution Melting; (SS) Sanger Sequencing; (NGS) Next Generation Sequencing.