Genotype–Phenotype Correlations in a Spanish Cohort of 506 Families With Biallelic *ABCA4* Pathogenic Variants

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• PURPOSE: To define genotype–phenotype correlations in the largest cohort study worldwide of patients with biallelic *ABCA4* variants, including 434 patients with Stargardt disease (STGD1) and 72 with cone-rod dystrophy (CRD).

• DESIGN: Cohort study.

• METHODS: We characterized 506 patients with *ABCA4* variants using conventional genetic tools and next-generation sequencing technologies. Medical history and ophthalmologic data were obtained from 372 patients. Genotype–phenotype correlation studies were carried out for the following variables: variant type, age at symptom onset (AO), and clinical phenotype.

• RESULTS: A total of 228 different pathogenic variants were identified in 506 *ABCA4* patients, 50 of which were novel. Genotype–phenotype correlations showed that most of the patients with biallelic truncating variants presented with CRD and that these cases had a significantly earlier AO than patients with STGD1. Three missense variants are associated with CRD for the first time (c.1804C > T; p.[Arg602Trp], c.3056C > T; p.[Thr1019Met], and c.6320G > C; p.[Arg2107Pro]). Analysis of the most prevalent *ABCA4* variant in Spain, c.3386G > T; p.(Arg1129Leu), revealed that is correlated to STGD1, later AO, and foveal sparing.

• CONCLUSIONS: Our study, conducted in the largest *ABCA4*-associated disease cohort reported to date, updates the genotype–phenotype model established for *ABCA4* variants and broadens the mutational spectrum of the gene. According to our observations, patients with *ABCA4* presenting with 2 truncating variants may first present features of STGD1 but eventually develop rod dysfunction, and specific missense variants may be associated with a different phenotype, underscoring the importance of an accurate genetic diagnosis. Also, it is a prerequisite for enrollment in clinical trials, and to date, no other treatment has been approved for STGD1. (Am J Ophthalmol 2020;219:195–204.

CAUSATIVE VARIANTS IN THE *ABCA4* GENE (MIM 601691) are associated with several inherited retinal dystrophies. Biallelic *ABCA4* variants are mostly found in patients with Stargardt disease (STGD1)[1] but have also been described in patients with cone-rod dystrophy (CRD) and retinitis pigmentosa (RP).[2,3]

ABCA4 comprises 50 exons and encodes the multidomain transmembrane protein ABCA4, located at the rim of disc membranes in the outer segments of both cone and rod photoreceptors of the human retina.[4] The role of ABCA4 in the visual cycle is to transport or flip N-retinylidene-phosphatidylethanolamine from the lumen to the cytoplasmic side of the disc membrane.[4] Mutant ABCA4 proteins usually induce the accumulation in disc membranes of all-trans retinal and N-retinylidene-PE, which react to produce fluorophore A2E precursors, leading to photoreceptor degeneration.[5]

STGD1 (248200) is the most common juvenile macular dystrophy, with an estimated prevalence of 1:10,000 and a carrier frequency of approximately 2%.[6] However, previous studies suggested a higher prevalence (6%) of carriers in

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Spain.[7] STGD1 is characterized by a disease onset affecting central vision usually within the first 2 decades of life; however, early onset and late onset cases exist. Ophthalmoscopic examinations reveal atrophy of the retinal pigment epithelium and the presence of yellow flecks around the macula and midperiphery.[8] In contrast, CRD is defined as a progressive loss of cone function followed by rod function loss, resulting in further impairment of peripheral vision and night blindness. Ophthalmoscopic examinations in patients with CRD showed perifoveal atrophy of the outer retina and bull's eye maculopathy.[9,10] To explain the differences in the clinical inherited retinal dystrophy subtypes induced by *ABCA4* variants, a genotype–phenotype model was proposed based on the functional consequences of the combination of *ABCA4* variants.[11,12] Persons carrying 2 severe variants are expected to present with severe forms of CRD, which could be misdiagnosed as retinitis pigmentosa because of progression of the disease, which closely resembles CRD.[7]

To date, >1200 *ABCA4* variants have been reported in the Human Gene Mutation Database. Most STGD1 patients seem to carry biallelic coding *ABCA4* variants, whereas unsolved cases carrying no *ABCA4* mutated alleles or one such allele can be explained by the presence of deep intronic variants[13–17] or by the low penetrant c.5603A>T; p.(Asn1868Ile) variant.[18,19]

In this study, we report findings from the largest cohort of patients with *ABCA4* described to date, consisting of 506 families with biallelic variants. In addition, to precisely assess the prevalence of *ABCA4* variants in this Spanish cohort, we describe new genotype–phenotype correlations for *ABCA4* causal variants and STGD1 or CRD phenotypes.

METHODS

• **SUBJECTS AND SAMPLES:** Five hundred six Spanish families with a clinical diagnosis of STGD1 or CRD were recruited at the Fundación Jiménez Díaz University Hospital (Madrid, Spain). A solved genotype with biallelic *ABCA4* variants was used for the inclusion criteria. This study was performed in accordance with the tenets of the Declaration of Helsinki and subsequent reviews, and the procedure for patient enrollment was approved by the Research Ethics Committee of the Fundación Jimenez Diaz University Hospital. DNA samples were collected from the Fundación Jiménez Díaz University Hospital biobank. Informed consent was obtained from all subjects.

• **MOLECULAR SCREENING:** Index cases were from 506 unrelated families that had undergone molecular characterization over the past 29 years. A total of 299 index cases were characterized using previously described conventional genetic tools[7,20] and 207 index cases were studied by different next-generation sequencing (NGS) strategies, including targeted gene panels, clinical exome, and/or whole-exome sequencing, as previously described.[21,22] Depending on the screening technique used at the time of diagnosis, subjects with 1 identified *ABCA4* allele underwent either Sanger sequencing of known deep intronic variants or multiplex ligation probe amplification using *ABCA4* probes (Probemis P-151 and P-152; MRC-Holland, Amsterdam, the Netherlands), copy number variation (CNV) analysis of NGS data, or a combination of these. In addition, to complete the genotype data for 34 cases, the entire *ABCA4* gene was sequenced using single-molecule molecular inversion probe–based technology.[23]

The pathogenicity of *ABCA4* variants was established according to their allele frequency appearing in gnomAD (http://gnomad.broadinstitute.org/); in silico prediction tools were used to classify new splice and missense variants, including SIFT,[24] PolyPhen,[25] CADD,[26] and M-CAP.[27] In addition, we conducted cosegregation studies in family members when other relatives were available for study. For variant classification, we followed the guidelines of the American College of Medical Genetics and Genomics[28] and the recent study by Cornelis and associates.[29] Stop, frameshift, and splice variants were considered as truncating variants because of their presumable effect on the protein, including unreported noncanonical splice site variants.[23] Complex alleles are defined when 2 *ABCA4* variants were present on the same allele. Complex alleles carrying a truncating variant were considered truncating alleles.

Five microsatellite markers (D1S2804, D1S2868, D1S236, D1S2664, and D1S2793) and 3 single nucleotide polymorphisms (rs769211, rs1801555, and rs4148058) flanking 3.74 Mb around *ABCA4* were studied in 6 families with the variant c.699_768+341del.

• **CLINICAL ASSESSMENT:** A comprehensive review of the ophthalmologic data available in the clinical examination notes of the 506 *ABCA4* patients was carried out to record the following data: age at onset of visual acuity (VA) loss, visual field constriction, and night blindness; best-corrected VA (BCVA) measurements, in decimal scale; full-field electroretinography (ffERG) responses; and fundus appearance. In some cases, spectral-domain optical coherence tomography (OCT) and fundus autofluorescence (FAF) images were examined. The age of onset (AO) of the disease was defined as the patient age at VA loss or at initial diagnosis.

Diagnoses of STGD1 or CRD were based on the following criteria: STGD1 was determined according to initial symptoms of VA loss; fundus images showing orange-yellow flecks in the retina, a beaten-bronze appearance; and normal or cone-altered ffERG results; CRD was based on initial symptoms of loss of central vision and/or night blindness; fundus images showing atrophic macular degeneration and peripheral alterations including pigment epithelial thinning, pigment deposits, or both; and a

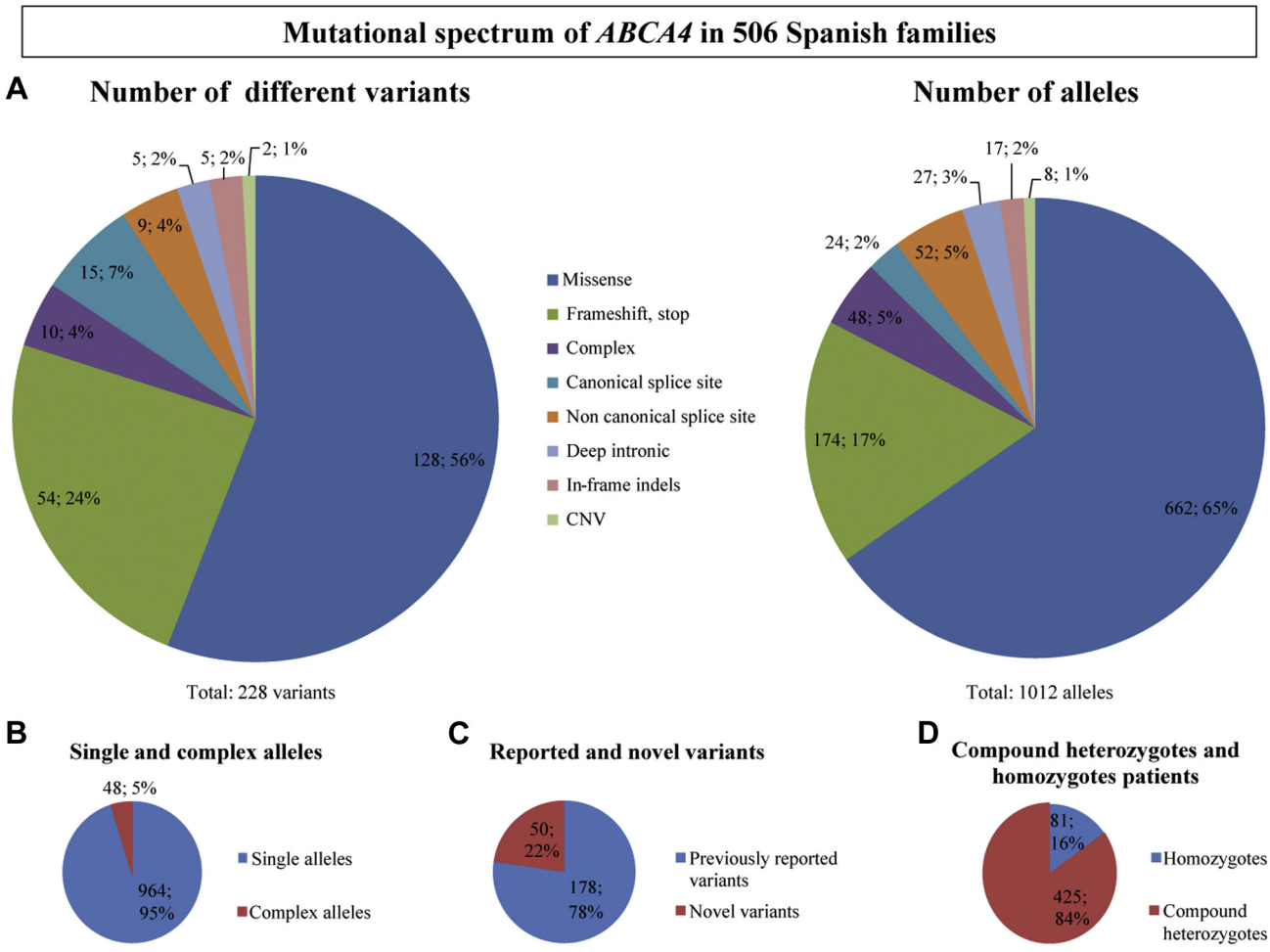

FIGURE 1. Mutational spectrum of *ABCA4* in 506 Spanish patients. (A) Percentage distribution of 228 different variants identified in 1012 patient alleles by variant type. (B) Percentage distribution of single and complex alleles. (C) Percentage distribution of novel and reported variants. (D) Representation of persons carrying homozygous or compound heterozygous *ABCA4* variants. Complex variants include variants present in complex alleles and not in single alleles. CNV = copy number variation.

decrease in cone-rod ffERG responses. When clinical information was not available, the diagnosis referred by each patient's ophthalmologist was used.

• **GENOTYPE–PHENOTYPE CORRELATIONS:** To perform genotype–phenotype correlations, all 1012 alleles from the 506 families were classified into the following 12 categories: A, B, and C included patients carrying missense-missense, missense-truncating, and truncating-truncating variants, respectively; A2, B2, and C2 included patients carrying missense-missense, missense-truncating, and truncating-truncating variants excluding the c.3386G>T variant, respectively; D, E, and F included patients carrying the c.3386G>T variant in homozygosis, in combination with a different missense variant, and in combination with a truncating variant, respectively; and G, H, and I included patients carrying the c.5882G>A variant in homozygosis, in combination with a different missense variant, and in combination with a truncating variant, respectively.

Categories were compared based on AO and clinical diagnosis of STGD1 or CRD. Because of the nonnormal distribution of data, the Wilcoxon rank sum test was used to perform comparisons between groups. Medians and interquartile ranges (IQRs) were represented. For missense variants, 95% confidence intervals (CIs) for percentages were calculated using the binomial exact method. Odds ratios (ORs) and their respective 95% CIs were calculated by median unbiased estimation. Statistical analyses and graphical representation were done using *R* software version 3.6.0.

RESULTS

• **MUTATIONAL SPECTRUM OF *ABCA4* VARIANTS:** A total of 228 different variants in the *ABCA4* gene were found in 1012 alleles from our Spanish cohort of 506 index patients

TABLE 1. Most Prevalent *ABCA4* Variants Found in 506 Spanish Families

Variant	Exon	Nucleotide	Protein	Families, n	Alleles, n
Single					
	23	c.3386G>T	p.(Arg1129Leu)	170	190
	42	c.5882G>A	p.(Gly1961Glu)	64	64
	22	c.3210_3211dup	p.(Ser1071Cysfs*14)	30	34
	13	c.1804C>T	p.(Arg602Trp)	26	30
	41	c.5819T>C	p.(Leu1940Pro)	26	29
	30	c.4457C>T	p.(Pro1486Leu)	23	26
	19	c.2888del	p.(Gly963Alafs*14)	23	25
	45	c.6179T>G	p.(Leu2060Arg)	21	24
Complex					
	22; 46	c.[3322C>T; 6320G>A]	p.[Arg1108Cys; Arg2107His]	12	13
	23; 48	c.[3386G>T; 6718A>G]	p.[Arg1129Leu; Thr2240Ala]	5	6
	35; 36	c.[4926C>G; 5044_5058del]	p.[Ser1642Arg; Val1681_Cys1685del]	4	6

(Supplemental Tables S1 and S2). Their classification by type of variant is shown in Figure 1, A.

Thirty-three different variants were part of 21 complex assortments and accounted for 5% (48/1012) of all alleles. Ten variants were only found in complex alleles and not as single alleles (Supplemental Table S2; Figure 1, A and B). The following were the 3 most frequent complex variant combinations: the previously reported c.[3322C>T; 6320G>A] and c.[4926C>G; 5044_5058del],[29] as well as 1 novel variant, c.[3386G>T; 6718A>G], representing 12.5% of the total complex alleles in our Spanish cohort. In 7 families with 3 *ABCA4* variants identified, the correct phase could not be established because no samples from relatives were available; therefore, these 7 complex alleles could be in other combination in these patients (Supplemental Table S3).

The most frequent variants are shown in Table 1, with the missense c.3386G>T; p.(Arg1129Leu) being present in 33.6% of the patients with an allelic frequency of 18.8% (190/1012). This variant was found in 183 single and 7 complex alleles.

In this genetic screening, 50 variants were as yet unpublished, representing 22% of the total number of different variants and 7.2% of all patient alleles (73/1012) (Figure 1, C and Supplemental Table S4). Three (c.6071A>G, c.2481del, and c.2483C>T) were present as 2 complex allele assortments, since the last 2 variants were observed in *cis*. All novel variants were segregating with the disease or predicted as pathogenic by at least 3 of the 4 programs used, and their population frequency was absent or <0.002.

CNVs were found in 7 families, representing 0.7% of the total number of alleles. Family MD-0401 carried a deletion of intron 11. A novel 411-bp deletion [c.699_768+341del; p.(Gln234Phefs*5)] covering 70 bp of exon 6 and 341 bp of intron 6 was identified in 6 unrelated Spanish families (MD-0162, MD-0039, RP-2668, MD-0166, MD-0460, and RP-2531). This deletion was found in a heterozygous state in 5 families and in homozygosis in 1 family. Segregation studies confirmed the presence of the novel deletion in combination with a second unshared variant in *trans* in 4 families. Haplotype analysis of 8 markers in *ABCA4* revealed a common minimal and maximal shared region of 1.58 Mb (chr1:94360107-95946135) and 3.29Mb (chr1:93335742-96628133) in all the families, respectively (Supplemental Figure S1). In addition, 3 families shared the same haplotype for all the markers used (MD-0039, MD-0162, and RP-2531).

Deep intronic variants were found in 28 patients, with 2.8% allele frequency (Supplemental Table S5). The most prevalent was c.4539+2064C>T, which was present in 14 patients (1 homozygote), representing 1.5% of all alleles.

The screening of the complete *ABCA4* gene in 7 patients with c.6148G>C; p.(Val2050Leu), a variant that was previously classified as pathogenic but now recognized as benign, allowed us to identify additional pathogenic variants in all of them. In addition, 9 patients with the low-penetrant variant c.5603A>T; p.(Asn1868Ile) also underwent this screening. In this case, further pathogenic intronic variants in *cis* were found in only 2 patients (MD-1075 and MD-1279; Supplemental Tables S1 and S3).

Homozygous variants were carried by 81 patients (Figure 1, D). Cosegregation and existence of consanguinity or endogamy allowed us to confirm their homozygous state in 56 (69%) of cases. Sixteen of the remaining patients in whom cosegregation analysis was not performed carried variants found to be prevalent among the Spanish population shown in Table 1, thus explaining homozygosity. In homozygotes for c.3386G>T, CNV studies including multiplex ligation probe amplification or NGS were performed to discard gross deletions.

• **CLINICAL CHARACTERISTICS OF *ABCA4* PATIENTS:** A diagnosis of STGD1 was established for 434 patients; the remaining 72 patients presented with CRD. Clinical information of 372 patients including AO, age at diagnosis,

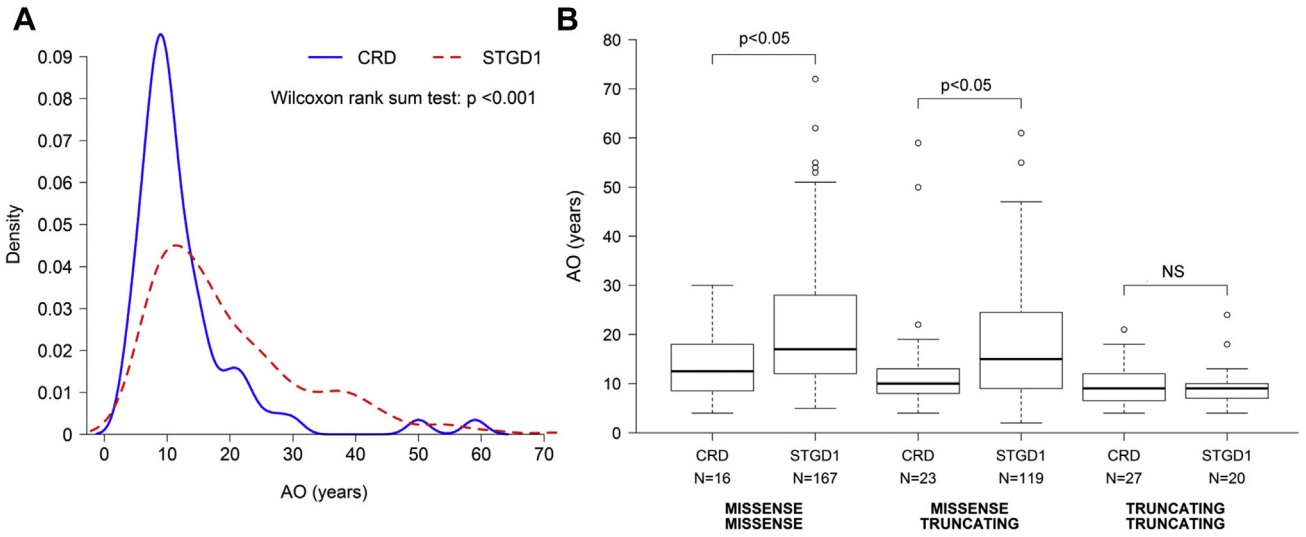

FIGURE 2. Distribution of age of onset and genotype–phenotype correlation in patients with CRD vs patients with STGD1. (A) Patients with CRD presented with a statistically significant earlier AO than patients with STGD1. (B) The AO of patients with CRD and STGD1 carrying ≥1 missense variant showed statistically significant differences. Patients with biallelic truncating variants presented with a similar AO. Missense variants c.1804C > T; p.(Arg602Trp), c.3056C > T; p.(Thr1019Met), and c.6320G > C; p.(Arg2107Pro) were overrepresented in patients with CRD, with c.3386G > T; p.(Arg1129Leu) overrepresented in patients with STGD1. AO = age of onset; CRD = cone-rod dystrophy; STGD1 = Stargardt disease; NS = not significant.

BCVA, and ffERG results is summarized in Supplemental Table S1.

The median AO (IQR) of 66 CRD and 306 STGD1 patients was 10 (6) and 16 (15) years, respectively (Supplemental Table S6). Patients with CRD presented an onset of disease during the first and early second decade of life, while the disease onset in patients with STGD1 was in the second and third decades, revealing a statistically significant difference in distribution according to this variable (Figure 2, A).

Some patients presented with a good BCVA at age at diagnosis, not showing symptoms of loss of VA. A well-preserved foveal structure together with a good BCVA was described in 8 STGD1 patients from families MD-0853, MD-0991, MD-0959, MD-1106, MD-1110, MD-1146, MD-1356, and MD-1381, ranging in age from 19–72 years. Spectral-domain OCT and FAF images of 4 of them are shown in Figure 3. FAF images revealed macular atrophy sparing the fovea in patients MD-0959, MD-1146, and MD-1381, while MD-1356 revealed a hyperautofluorescent halo surrounding areas of nondefinitive dark hypoautofluorescence in the macula.

• **GENOTYPE–PHENOTYPE CORRELATIONS:** To determine whether the combination of *ABCA4* variants in our cohort, regardless of diagnosis, reflected the established genotype–phenotype model, the AO of 372 index patients was compared between genotype categories A, B, and C. The median AO (IQR) was 17 (15), 14 (14), and 9 (3.5) years, respectively. There were statistically significant differences between patients with biallelic truncating variants (category C) and those with both biallelic missense (category A) and missense-truncating (category B) variants (Supplemental Figure S2 and Supplemental Table S7). Patients carrying 2 missense (category A) and missense-truncating variants (category B) also showed statistically significant differences.

Analysis of patients with the c.3386G>T variant revealed that the median AO (IQR) in categories D, E, and F was 21.5 (18.5), 17 (8.5), and 14 (10) years, respectively (Table 2). There were statistically significant differences between c.3386G>T homozygotes (category D) and compound heterozygotes carrying a truncating variant (category F), and between patients carrying the c.3386G>T in combination with a missense variant (category E) and patients carrying the c.3386G>T in combination with a truncating variant (category F). Comparisons between the patients carrying the c.3386G>T variant and non-3386G>T patients showed statistically significant differences when all patients were taken into account, the median AO (IQR) among these patients was 16.5 (10.8) and 13 (15) years, respectively (Table 2). There were no statistically significant differences when comparing categories A2–C2 with D–F (Supplemental Tables S8 and S9). The same analysis was carried out for the c.5882G>A variant, excluding category G because there were no homozygous patients in our cohort. In this case, median AO (IQR) for categories H and I were 17 (13.8) and 20 (15) years, respectively, and no statistically significant differences were found (Table 2). The comparison

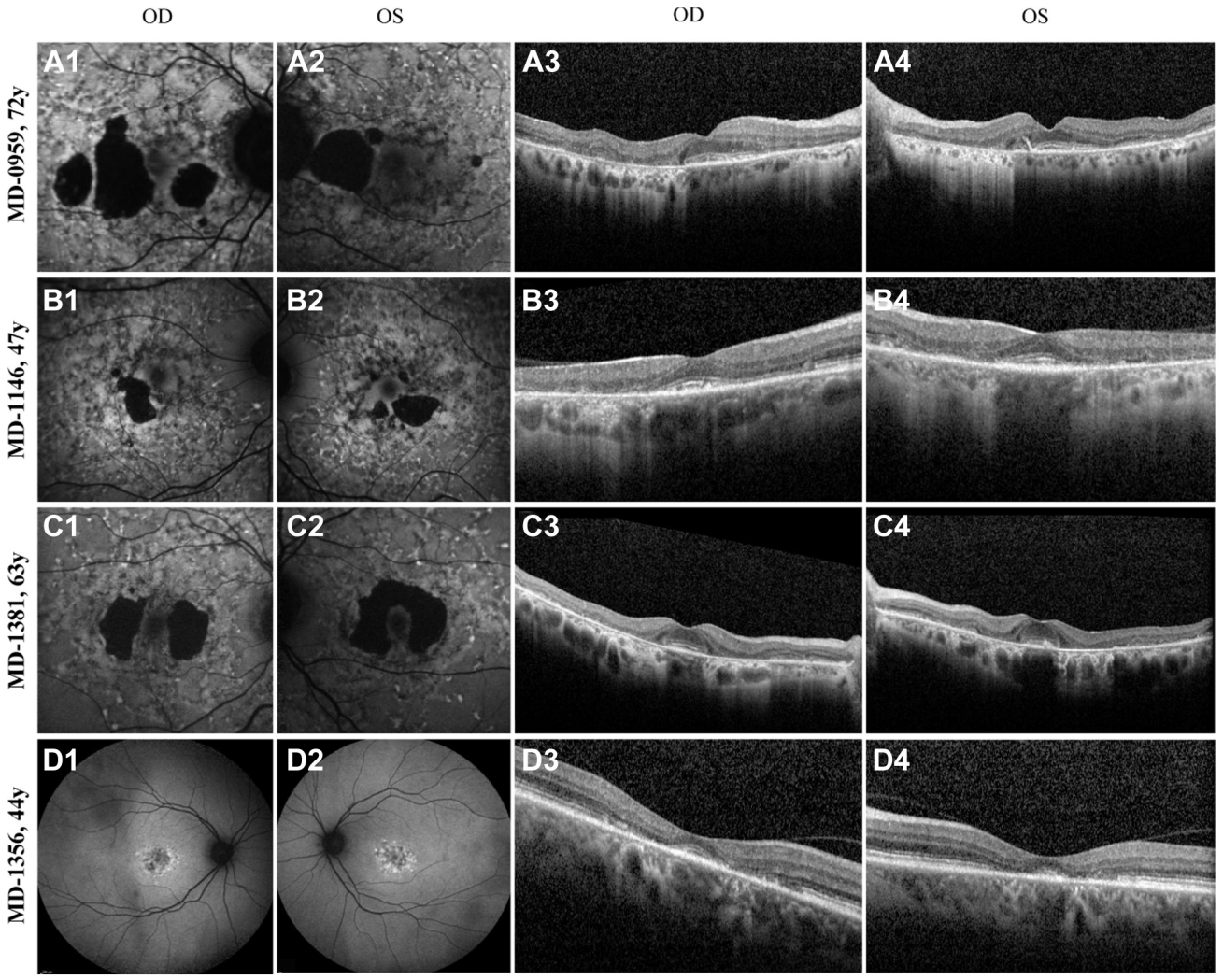

FIGURE 3. Fundus autofluorescence and spectral-domain optical coherence tomography images of patients presenting with foveal sparing. Fundus autofluorescence images A1/A2 to C1/C2 and D1/D2 at 35° and 55° center in the macula, respectively, showing areas of definitive dark autofluorescence sparing in the foveal area in patients MD-0959, MD-1146, and MD-1381. Images obtained of patient MD-1356 images show nondefinitive dark autofluorescence with scattered hyperautofluorescent lesions in the perifoveal area. Spectral-domain optical coherence tomography images A3/A4 to D3/D4 evidence disruption of the ellipsoid zone and external layers in the perifoveal area with subfoveal preservation in all patients. Right eye OD = right eye oculus dexter; left eye OS = left eye oculus sinister.

between patients carrying c.3386G>T and c.5882G>A did not reveal statistically significant differences (Supplemental Table S10).

Genotype–phenotype correlation regarding the clinical CRD and the STGD1 phenotypes evidenced statistically significant differences between patients from the 2 classes carrying bialellic missense variants (12.5 [8.3] and 17 [16] years, respectively) and a missense with a truncating variant (10 [5] and 15 [15.5] years, respectively; Supplemental Table S6). Patients with CRD and STGD1 who were carrying biallelic truncating variants had a similar AO (9 [5.5] and 9 [3] years; Figure 2, B and Supplemental Table S6). Remarkably, most of the patients with CRD (41%, 27/66) belonged to the latter group, thus contrasting with patients with STGD1 (6.5%, 20/306).

The number of alleles carrying different missense variants was compared between patients with CRD and those with STGD1. Four variants showed statistically significant differences (Supplemental Table S11). Variants c.1804C>T; p.(Arg602Trp) (OR 5.31 [95% CI 2.27–11.7]), c.3056C>T; p.(Thr1019Met) (OR 7.58 [95% CI 2.12–25.1]), and c.6320G>C; p.(Arg2107Pro) (OR 10.5 [95% CI 1.08–102]) were overrepresented in patients with CRD while the c.3386G>T variant was the only variant overrepresented in patients with STGD1 (OR 0.37 [95% CI 0.14–0.80]). In addition, the c.3386G>T variant was also overrepresented in the foveal sparing

TABLE 2. Genotype–Phenotype Correlation for Prevalent *ABCA4* Variants c.3386G>T; p.(Arg1129Leu) and c.5882G>A; p.(Gly1961Glu)

Genotype–Phenotype Correlation	Category	Median (IQR) AO, Years	*P* Value
c.3386G>T variant	D (c.3386G>T-c.3386G>T)	21.5 (18.5), n = 12*	NS
	E (c.3386G>T-missense)	17.0 (8.50), n = 68	
	D (c.3386G>T-c.3386G>T)	21.5 (18.5), n = 12*	<.05
	F (c.3386G>T-truncating)	14.0 (10.0), n = 43	
	E (c.3386G>T-missense)	17.0 (8.50), n = 68	<.05
	F (c.3386G>T-truncating)	14.0 (10.0), n = 43	
	c.3386G>T patients	16.5 (10.8), n = 126	<.05
	Non-c.3386G>T patients	13.0 (15.0), n = 246	
	c.3386G>T patients	16.5 (10.8), n = 126	NS
	All patients	15.0 (15.0), n = 372	
	Non-c.3386G>T patients	13.0 (15.0), n = 246	NS
	All patients	15.0 (15.0), n = 372	
c.5882G>A variant	H (c.5882G>A-missense)	17.0 (13.8), n = 28	NS
	I (c.5882G>A-truncating)	20.0 (15.0), n = 15	

AO = age of onset; IQR = interquartile range; NS = not significant.
*Three patients were excluded because they carried another variant in *cis* together c.3386G>T variant.

cohort: it was carried by MD-0959 and MD-1356 in homozygosis, and MD-1106 carried it in heterozygosis. Missense variants previously described as severe variants did not show statistically significant differences (Supplemental Table S11).

DISCUSSION

WE REPORT THE LARGEST COHORT OF PATIENTS WITH *ABCA4* variants ever analyzed to date, consisting of 434 patients with STGD1 and 72 patients with CRD, providing an accurate analysis of the genomic and phenotypic landscape of different combinations of variants in this gene. Two hundred twenty-eight different DNA changes were identified, most of which were missense changes (56%).

Novel variants accounted for 22% of all variants and 7.2% of *ABCA4* patient alleles, and other studies based on large cohorts of patients with STGD1 have found similar rates of novel variants.[17,30,31] Using comprehensive targeted NGS-based screening, we were able to observe the highly diverse allelic and mutational spectrum of the *ABCA4* gene.

By screening CNVs and/or deep intronic variants we were able to solve 8 and 25 families, respectively. CNVs in the *ABCA4* gene do not usually account for a representative proportion of variants[16,32]; the same holds for our cohort as well, for which they represent <1% of all alleles. Interestingly, a novel 411-bp deletion partially encompassing the sixth exon and intron of *ABCA4* was found in 6 families, in whom a common region of 1.58 Mb was found, suggesting a possible founder mutation in the Spanish population. Sequencing of *ABCA4* introns enabled us to explain some of the missing heritability, thanks to the identification of several deep intronic variants that affect the correct splicing of primary *ABCA4* transcripts, as previously reported.[13–17] In our cohort, 2.6% of all alleles were found to be deep intronic variants, a rate that closely matches the 2% to 2.4% reported by Schulz and associates[30] and Fujinami and associates[31] in large cohorts of >300 STGD1 cases. However, in Khan and associates,[17] these variants represented 15% of the missing alleles, most likely because these patients had been previously screened for coding variants and because all studied probands were analyzed for deep intronic variants.

A recent in silico meta-analysis provided a pathogenic classification for all reported *ABCA4* variants based on their frequency in control subjects and in patients with inherited retinal dystrophies.[29] Based on these findings, the complete gene was also sequenced in a parallel study in 7 cases carrying c.6148G>C, a variant previously classified as pathogenic. The variant was found in combination with another pathogenic *ABCA4* variant in *cis* in all cases. One of these cases is family RP-0674, previously reported by Corton and associates.[33] The new variant identified was a coding variant filtered out on the whole-exome sequencing analysis because of extremely low coverage. According to these data and findings from recent studies,[34] c.6148G>C should therefore be considered a likely benign variant. On the other hand, the frequent variant c.5603A>T, recently considered a low-penetrant variant,[18,19] was identified in 9 cases, allowing us to consider them solved, and only 2 carried an additional intronic variant in *cis*. Further analysis of negative results together with review and reclassification of variants is needed to solve these cases.

Genotype–phenotype correlations for the AO of disease in patients were assessed following stratification by *ABCA4*-variant categories, regardless of phenotype, and by clinical STGD1 or CRD phenotypes. Our results showed that patients with biallelic truncating variants have a statistically significant earlier AO than other combinations of variants and most of them presented with a CRD phenotype. Combinations of missense variants with another missense or truncating variant were overrepresented in patients with STGD1. Our data suggest that patients with STGD1 who are carrying 2 truncating variants could evolve to be CRD; as a result, further ophthalmologic examinations, including ffERG, should be considered. The proposed genotype–phenotype correlation model suggests that the phenotype can be predicted by the *ABCA4* variant type, depending on the residual function of the ABCA4 protein.[35] We believe that our findings provide further insights into the accuracy of this model based on AO data of 372 patients, a sample size that confers greater statistical weight. It is also true that the classification of truncating variants included splice variants that produce partial truncations, and there are also missense variants that cause severe functional effects.[36–38] None of these missense variants (c.[1622T>C; 3113C>T]; p.[Leu541Pro; Ala1038Val], c.2894A>G; p.[Asn965Ser], and c.4918C>T; p.[Arg1640Trp]) were related with a CRD phenotype in our cohort. However, variants c.1804C>T; p.(Arg602Trp), c.3056C>T; p.(Thr1019Met), and c.6320G>C; p.(Arg2107Pro) were associated with a CRD phenotype, while c.3386G>T was correlated with patients with STGD1. To our knowledge, this is the first time that these specific *ABCA4* missense variants are clinically associated to a different phenotype, which could be used to evaluate the prognosis of patients who are diagnosed at early ages with mild clinical manifestations.

We also performed genotype–phenotype correlations for the most prevalent Spanish variant, c.3386G>T,[20] as well as the common c.5882G>A variant. Homozygous patients for c.3386G>T presented later AO and represent only 11% of all patients with this variant, as seen also in homozygous cases for the c.5882G>A variant, though these cases were absent from our cohort. It has been reported that c.5882G>A in a homozygous state typically causes a milder phenotype than when it is present in combination with other variants.[36] Our data could suggest that homozygous patients for c.5882G>A could have a mild phenotype, even without manifestation of visual symptoms. The Spanish variant c.3386G>T should be considered mild, although we previously proposed that it could have a moderately severe effect.[20]

Severe CRD phenotypes could be diagnosed as RP.[7] In this work, all patients with CRD presented with rod disfunction because of ERG findings or symptoms associated with rod degeneration. Six cases in which these data were not available were referred with a diagnosis of CRD. At the other end of the severity spectrum, 8 patients with clinical features of STGD1 but without clinical symptoms at the age of diagnosis had good VA and well-preserved foveal structure. Later onset or preserved VA has been described in patients with STGD1[39,40] associated with a milder phenotype and foveal sparing.[41–44] A previous study reported that the c.6089G>A; p.(Arg2030Gln) change, which we did not identify in our patients, was overrepresented in cases with foveal sparing compared with typical STGD1 cases.[41] In our cohort, 2 patients were homozygous for the Spanish c.3386G>T variant, a finding that supports the mild effect of this variant and the possibility of an underdiagnosis of additional homozygotes because of the lack of visual disabling symptoms. However, 1 patient with CRD carried this variant in homozygosis. Further studies sequencing the entire *ABCA4* gene or regulatory regions would be needed to determine if additional variants in *cis* could be modifying the penetrance of these variants in homozygotes.

In summary, this study supports the role played by genetic diagnosis in predicting the progression of the disease, and the difficulty of obtaining a correct clinical diagnosis when nontypical STGD1 features are present or electrophysiology data are not available. Certain combinations of variants in homozygosis state may not always be associated with a diagnosed clinical phenotype. Alternatively, onset of symptoms may occur later in life, as in the case of patients with foveal sparing. Given the wealth of gene-based therapy initiatives under way involving patients with *ABCA4* causative variants, a precise identification of the genetic makeup of STGD1 or CRD cases, including the presence of missing alleles, is a crucial step toward enrolling these patients in clinical trials.

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