Genotype-Phenotype Correlations in *ABCA4*-Associated Retinopathy: Insights From a Spanish Cohort of 245 Patients

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Purpose. *ABCA4*-associated retinopathy includes a broad range of inherited retinal dystrophies (IRDs), marked by notable genetic and phenotypic heterogeneity that complicates diagnosis and counseling. This study aims to evaluate genotype-phenotype correlations to improve clinical management and risk stratification.

Methods. In this multicenter, retrospective, cross-sectional study, we analyzed 245 patients with biallelic pathogenic *ABCA4* variants from 7 Spanish reference centers. Variants were classified by predicted severity, and patients were stratified into five phenotypic categories based on fundus findings. One-way ANOVA and Fisher's exact test were used to assess group differences. Spearman's correlation evaluated genotype-phenotype associations. Ordinal logistic regression, adjusted for age at symptom onset and disease duration, was used to assess the relationship between genotype severity and fundus phenotype.

Results. The mean age of the patients was 43.6 years. Earlier symptom onset and longer disease duration were significantly associated with more severe phenotypic features ($P \leq 0.0008$). Genotype severity correlated with phenotype severity (allele 1: $P = 0.0036$ and allele 2: $P = 0.02$). Severe variants were linked to more pronounced phenotypes, whereas milder alleles showed weaker associations. The presence of a predicted null (PVS1) variant in allele 1 significantly correlated with more advanced fundus changes ($P = 0.0029$).

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Conclusions. The severity of *ABCA4* variants correlate with the extent of retinal phenotype, emphasizing the importance of early molecular diagnosis. These findings support the refinement of variant classification and highlight the need for further studies to better understand the implications for clinical management and potential therapeutic strategies.

Keywords: *ABCA4*-associated retinopathy, inherited retinal dystrophies, genotype-phenotype correlation, variant severity, fundus phenotype

The *ABCA4* gene is a major contributor to inherited retinal dystrophies (IRDs), a clinically and genetically diverse group of conditions affecting photoreceptor function.[1,2] Initially linked to Stargardt's disease (STGD; MIM #248200),[3] *ABCA4* has since been recognized as causative of a broad phenotypic spectrum. This includes cone dystrophy (COD), cone-rod dystrophy (CORD; MIM #604116), retinitis pigmentosa (RP; MIM #601718), and late-onset STGD (L-STGD, defined as onset at or after 45 years), all inherited in an autosomal recessive manner.[4,5]

Advances in multimodal imaging and high-throughput sequencing have substantially deepened our understanding of *ABCA4*-associated IRDs. To date, over 2800 unique *ABCA4* variants have been documented (https://databases.lovd.nl/shared/variants/ABCA4/unique, accessed on April 30, 2025). Despite the implementation of American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines[6] and ClinGen expert recommendations,[7] variant classification remains challenging. Contributing factors include under-recognized pathogenic mechanisms, such as splicing defects caused by synonymous and deep-intronic variants,[8–10] as well as hypomorphic alleles that are relatively frequent in the general population but pathogenic when in trans with severe variants.[10,11] Moreover, complex alleles and potential modifier gene effects further complicate diagnostic interpretation.[2,12]

Historically, genotype-phenotype correlations have relied on the assumption that loss-of-function (LoF) variants are associated with more severe disease compared with missense variants.[10,13–15] However, emerging data suggest that synonymous, deep-intronic, and non-canonical splice variants may also result in early-onset, rapidly progressive phenotypes,[8,9,16] highlighting the need for refined frameworks to predict phenotype severity more accurately. A recent review of 2246 *ABCA4* variants provided important insights into variant interpretation and associated clinical outcomes.[12] Building on this work, Lee et al.[17] proposed a genotype–phenotype correlation matrix based on allele severity to predict disease outcomes.

Sex may also act as a potential modifier of disease expression in *ABCA4*-associated retinopathies. Runhart et al.[18] reported sex-specific penetrance for the c.5603A>T and c.5882G>A variants when present in trans with a severe allele, with penetrance estimates of approximately 6% in women and 4% in men for c.5603A>T, and 68% in women versus 32% in men for c.5882G>A. More recently, Cornelis et al.[19] supported these findings, observing a higher proportion of female patients harboring mild alleles, thus reinforcing the hypothesis of sex-influenced expressivity in *ABCA4*-related disease.

The present study aimed to contribute to a deeper understanding of *ABCA4* genotype-phenotype correlations by analyzing a large, clinically diverse cohort of patients with biallelic pathogenic variants. Leveraging updated variant classifications, we examined the impact of both well-established and under-recognized pathogenic mechanisms, including synonymous and deep-intronic variants, on clinical presentation. Our goal was to facilitate more precise patient stratification and to inform personalized genetic counseling and clinical management.

Materials and Methods

Study Design and Participants

A retrospective, cross-sectional analysis was conducted across seven Spanish reference centres: Bellvitge University Hospital, Clínic Hospital of Barcelona, Donostia University Hospital, SJD Barcelona Children's Hospital, Teknon Medical Centre, University and Polytechnic La Fe Hospital of Valencia, and Vall d'Hebron University Hospital. Data were collected between January 2024 and October 2024. The study adhered to the principles of the Declaration of Helsinki,[20] and written informed consent was obtained from all participants for the use of their medical and genetic data for research purposes. Genetic data were sourced from prior clinical genetic testing carried out at each participating center or external diagnostic laboratories. The study cohort consisted of 245 patients originating from 228 unique families, of whom 17 were affected siblings of index cases (representing sibling pairs or trios); no extended relatives were included. This research was approved by the Research Ethics Committee of Bellvitge University Hospital (reference number PR014/22).

Inclusion and Exclusion Criteria. Participants were included if they had biallelic pathogenic or likely pathogenic variants in the *ABCA4* gene, classified according to the most recent *ABCA4* variant's classification updates[12] and the ACMG/AMP standards.[6] Variants with sufficient supporting evidence to elevate them to likely pathogenicity (designated as true variants of unknown clinical significance [VUS]/LP) were included. Patients with exclusively true VUS without supporting pathogenic evidence were excluded. All patients were tested using comprehensive IRD multigene panels, and no additional pathogenic variants in other IRD genes were detected, although some were carriers

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Table 1. Severity Classification of *ABCA4* Variants

Severity Category	Description
Frequent hypomorphic	p.(Gly1961Glu)
Rare hypomorphic	p.(Asn1868Ile), p.(Ala1038Val), c.4253+43G>A, p.(Pro1486Leu), p.(Thr1526Met), p.(Ile1562Thr) and p.(Arg2030Gln)
Mild/moderate	Variants with reduced penetrance or mild/moderate effects
Moderate	Functional assays indicate a moderate effect or lack clear severity indicators
Severe	Variants typically found with hypomorphic alleles or demonstrated through functional assays to be severe
PVS1	Variants expected to result in null alleles, such as nonsense, frameshift, canonical splice site ±1,2 variants, or multi-exonic deletions

of recessive IRD gene variants not expected to affect the phenotype.

Given the symmetrical nature of the disease, one eye per patient was randomly selected for analysis. Exclusion criteria included cases with incomplete genetic data—such as the presence of only a single pathogenic *ABCA4* variant or only VUS without supporting evidence—as well as cases with insufficient clinical information or poor-quality imaging.

Data Collection. Demographic data, clinical diagnoses, phenotypic characteristics, and genetic results were retrieved from medical records. Variables collected included age, sex, clinical diagnosis (STGD, COD, CORD, RP, and L-STGD), family history, age at symptom onset (defined as the age when visual symptoms were first reported by the patient), and disease duration (years from onset to data collection).

Each center provided phenotypic data, including the presence of flecks, retinal atrophy, and retinal pigment epithelium (RPE) changes, with localization specified as macular, peripheral, or both, and peripapillary sparing when available. Genetic data included nucleotide and protein changes for both *ABCA4* alleles. The designation of allele 1 and allele 2 was assigned arbitrarily and does not reflect parental origin, phase, or severity. Data were compiled into a coded Excel sheet with systematically organized variables to ensure consistency across centers.

Genetic Analysis

All patients included in the study underwent targeted gene panel testing covering known IRD genes, excluding potentially disease-causing variants in other IRD-associated genes. *ABCA4* variants were reviewed and classified into distinct severity groups based on established criteria.[12] These categories range from hypomorphic variants with mild functional impact to severe and predicted null (PVS1) variants associated with significant functional loss. Complex alleles were treated as a single functional unit, and their combined effect was considered when assigning severity. Variants not included in this predefined classification were assigned a severity category based on existing literature or validated scientific evidence. Full details of the classification, including specific variants and their assigned severity, are provided in Table 1.

Phenotypic Assessment

Phenotypic data were obtained from multimodal imaging, including spectral-domain optical coherence tomography (SD-OCT; Topcon Triton, Topcon Corporation), fundus photography, and fundus autofluorescence (FAF) using Optos ultra-widefield retinal imaging (UWF; Optos California, Optos plc).

Phenotypic grading was performed according to established criteria,[4,17] assessing the extent of photoreceptor and RPE damage. The recorded features included the presence or absence of flecks, retinal atrophy, and RPE alterations, with localization specified as macular, peripheral, or both. Peripapillary sparing was noted where available.

Patients were classified into five phenotypic groups based on disease severity and fundus characteristics, taking into account the clinical staging progression described by Cremers et al. and Lee et al.[4,17] Patients assigned to phenotype 1 exhibited RPE changes visible on fundus examination, with or without flecks, corresponding to the mildest presentation, commonly referred to as fundus flavimaculatus.[21] Phenotype 2 included individuals with RPE changes with macular atrophy in the absence of flecks. Phenotype 3 was characterized by the presence of flecks alongside RPE changes and macular atrophy. Patients classified as phenotype 4 demonstrated macular atrophy limited to the posterior pole, alongside RPE alterations affecting both the macular and peripheral retina. Phenotype 5 represented the most severe stage, characterized by multifocal and extensive coalescing regions of chorioretinal atrophy extending beyond the macular area, with most flecks having undergone resorption.

The agreement between two senior observers (authors E.C. and J.C.) when classifying the patient's phenotype was determined for a subgroup of the dataset using the weighted kappa index.[22]

Statistical Analysis

Continuous variables (e.g., age and disease duration) were summarized as means ± and standard deviation (SD), whereas categorical variables (e.g., sex and familial history) were presented as frequencies and percentages. Between-group differences were analyzed using 1-way ANOVA for quantitative variables and Fisher's exact test for categorical variables. Spearman's correlation coefficient assessed associations between genotype and phenotype severity.

Ordinal logistic regression models,[23,24] adjusted for age at onset and disease duration, evaluated relationships between genotype severity and fundus findings. Univariate and multivariable models were constructed for each allelic severity combination. Given the ordinal nature of fundus findings, regression models were used, with Wald tests assessing the impact of allele severity. Missing data were handled using multiple imputation methods where applicable.

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Statistical significance was defined as a two-tailed P value < 0.05. All analyses were performed using Stata IC version 15.1 (StataCorp, College Station, TX, USA). No adjustments for multiple comparisons were performed.[25]

Results

Demographics and Clinical Diagnosis

A total of 273 patients were initially enrolled in the study. Of these, 28 were excluded due to incomplete genotypic data (resulting from unresolved segregation analyses or pending variant reclassification) or missing clinical imaging information. Thus, the final cohort included 245 patients with available clinical and genetic data (Supplementary Table S1).

The mean age of participants was 43.6 ± 19.0 years, with a near-equal sex distribution (135 female patients [55.10%] and 110 male patients [44.90%]). The average age at symptom onset was 23.6 ± 15.2 years, and the mean disease duration was 20.1 ± 16.1 years. A positive family history (first-degree or second-degree relative reported to have a clinical diagnosis of IRD) was reported in 29% of cases. Most individuals (87.3%) were diagnosed with STGD, followed by COD (5.3%), CORD (2.9%), and RP (4.5%).

ABCA4 Variant Distribution and Severity Classification

A total of 490 pathogenic or likely pathogenic *ABCA4* variants were identified across 245 individuals, comprising 159 unique variants (Supplementary Table S2). The 3 most prevalent variants in this cohort were c.3386G>T (16.33%), c.5882G>A (7.26%), and c.634C>T (3.02%), consistent with previously reported allele frequencies in European populations.

Most of the unique variants were missense (82/159, 51.57%), followed by frameshift (21/159, 13.21%), nonsense (20/159, 12.58%) and non-coding variants (20/159, 12.58%), complex alleles (14/159, 8.81%), synonymous variant (1/159, 0.63%), and insertion/deletion variant (1/159, 0.63%).

Of the 159 variants, 133 were classified according to established severity criteria[12] and 26 were newly assigned a severity levels.

Phenotypic Stratification and Disease Severity

Significant differences in current age, age at onset, and disease duration were observed across the five fundus-based on fundus appearance ($P \leq 0.0008$), with earlier onset and longer disease duration correlating with more severe phenotypes. No differences were observed in sex distribution across phenotypic groups (Table 2). Phenotype classifications followed a predictable severity pattern, with phenotype 5 representing the most severe stage. All phenotypic groups were comparably represented, except for phenotype 1, which was less frequent (5.3%). This classification of phenotypes, based on disease severity, was a key endpoint of the study.

The agreement in phenotype classification, assessed using the weighted κ coefficient, was 0.66 (standard error = 0.08, $n = 72$; see details in Supplementary Table S3). The expected agreement due to chance was 63.9%, whereas the observed agreement was 87.9% ($P < 0.0001$), indicating substantial inter-rater reliability.

Genotype Frequencies and Severity

Allelic combinations were classified according to variant severity (Table 3). Most individuals carried combinations involving moderate to severe variants, with the highest frequencies observed in moderate-moderate (8.6%) and moderate-severe (8.6%) groups. Severe variants were present in 16 individuals (6.5%) and PVS1 variants were present in 10 individuals (4.1%).

Table 2. Patient Demographics by Phenotype

	Phenotype 1 ($n = 13$)	Phenotype 2 ($n = 63$)	Phenotype 3 ($n = 66$)	Phenotype 4 ($n = 43$)	Phenotype 5 ($n = 60$)	*P* Value
Demographics						
Female	5 (38.5%)	35 (55.6%)	36 (54.6%)	27 (62.8%)	32 (53.3%)	0.64
Current age, y	42.7 (15.9)	35.6 (19.5)	44.0 (16.8)	46.8 (19.4)	49.6 (18.8)	0.0008
Age at onset, y	33.7 (18.1)	21.8 (13.9)	27.5 (16.1)	25.7 (16.4)	17.3 (10.8)	0.0001
Duration	9 (6.0)	13.7 (12.1)	16.5 (12.4)	21.1 (13.9)	32.3 (19.1)	<0.0001

Quantitative values are presented as means with standard deviations, whereas categorical variables are shown as counts and percentages.

Table 3. Description and Frequency of Each Allele Combinations in the Study Group ($n = 245$)

	Allele 2					
Allele 1	**Freq Hypo**	**Rare Hypo**	**Mild/Moderate**	**Moderate**	**Severe**	**PVS1**
Freq hypo	0 (0.0%)	1 (0.4%)	0 (0.0%)	7 (2.9%)	6 (2.5%)	6 (2.5%)
Rare hypo	2 (0.8%)	2 (0.8%)	2 (0.8%)	3 (1.2%)	4 (1.6%)	4 (1.6%)
Mild/moderate	2 (0.8%)	0 (0.0%)	5 (2.0%)	8 (3.3%)	1 (0.4%)	6 (2.5%)
Moderate	4 (1.6%)	3 (1.2%)	10 (4.1%)	21 (8.6%)	21 (8.6%)	17 (6.9%)
Severe	20 (8.2%)	8 (3.3%)	0 (0.0%)	5 (2.0%)	16 (6.5%)	6 (2.5%)
PVS1	16 (6.5%)	19 (7.8%)	5 (2.0%)	20 (8.2%)	15 (6.1%)	10 (4.1%)

Freq hypo, frequent hypomorphic; Rare hypo, rare hypomorphic; PVS1, predicted null variant.

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Table 4. Phenotype-Genotype Correlation and Distribution

Allele 1	Allele 2	Ph1	Ph2	Ph3	Ph4	Ph5	Disease duration (range)
Freq hypo	*Freq hypo*	0%	0%	0%	0%	0%	N/A
	Rare hypo	0%	0%	0%	100% (1)	0%	43
	Mild/Moderate	0%	0%	0%	0%	0%	N/A
	Moderate	0%	71.4% (5)	28.6% (2)	0%	0%	10 (5 - 18)
	Severe	16.7% (1)	66.7% (4)	0%	0%	16.7% (1)	10.5 (3 - 44)
	PVS1	50% (3)	16.7% (1)	33.3% (2)	0%	0%	6 (2 - 17)
Rare hypo	*Freq hypo*	0%	0%	50% (1)	50% (1)	0%	25.5 (19 - 32)
	Rare hypo	0%	0%	50% (1)	0%	50% (1)	24.5 (14 - 35)
	Mild/Moderate	0%	0%	100% (2)	0%	0%	16 (7 - 25)
	Moderate	0%	0%	0%	66.7% (2)	33.3% (1)	23 (18 - 37)
	Severe	25% (1)	50% (2)	0%	25% (1)	0%	10.5 (4 - 34)
	PVS1	0%	0%	50% (2)	25% (1)	25% (1)	14 (8 - 23)
Mild/Moderate	*Freq hypo*	0%	100% (2)	0%	0%	0%	5.5 (2 - 9)
	Rare hypo	0%	0%	0%	0%	0%	N/A
	Mild/Moderate	0%	20% (1)	0%	20% (1)	60% (3)	42 (3 - 66)
	Moderate	0%	0%	25% (2)	37.5% (3)	37.5% (3)	22 (4 - 50)
	Severe	0%	0%	0%	100% (1)	0%	33
	PVS1	16.7% (1)	0%	66.7% (4)	0%	16.7% (1)	9.5 (7 - 56)
Moderate	*Freq hypo*	25% (1)	0%	50.0% (2)	0%	25% (1)	4.5 (3 - 9)
	Rare hypo	33.3% (1)	0%	33.3% (1)	33.3% (1)	0%	15 (2 - 19)
	Mild/Moderate	0%	20.0% (2)	40.0% (4)	10.0% (1)	30.0% (3)	35.5 (10 - 53)
	Moderate	9.5% (2)	47.6% (10)	14.3% (3)	14.3% (3)	14.3% (3)	15 (1 - 63)
	Severe	4.8% (1)	42.9% (9)	28.6% (6)	14.3% (3)	9.5% (2)	12 (3 - 56)
	PVS1	0%	23.5% (4)	29.4% (5)	29.4% (5)	17.7% (3)	15 (1 - 43)
Severe	*Freq hypo*	25% (1)	0%	50% (2)	25% (1)	0%	15 (11 - 31)
	Rare hypo	12.5% (1)	50% (4)	12.5% (1)	12.5% (1)	12.5% (1)	13.5 (1 - 30)
	Mild/Moderate	0%	0%	0%	0%	0%	N/A
	Moderate	0%	40% (2)	20% (1)	0%	40% (2)	13 (6 - 28)
	Severe	0%	0%	12.5% (2)	25% (4)	62.5% (10)	36 (2 - 72)
	PVS1	0%	0%	16.7% (1)	16.7% (1)	66.7% (4)	12 (9 - 47)
PVS1	*Freq hypo*	0%	31.3% (5)	56.3% (9)	12.5% (2)	0%	18.5 (4 - 39)
	Rare hypo	0%	20% (1)	60% (3)	0%	20% (1)	11 (8 - 28)
	Mild/Moderate	0%	40% (2)	40% (2)	0%	20% (1)	8 (7 - 36)
	Moderate	0%	25% (5)	20% (4)	25% (5)	30% (6)	13 (3 - 50)
	Severe	0%	13.3% (2)	26.7% (4)	20% (3)	40% (6)	9 (2 - 64)
	PVS1	0%	20% (2)	0%	20% (2)	60% (6)	16.5 (4 - 63)

Ph, phenotypes (fundus features).

Disease duration represent median years. Percentages may not add to 100% due to rounding, and numbers in parentheses represent individual counts.

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Table 5. Univariate and Multivariate Ordinal Regression Models to Evaluate the Relationship of These Variables With Severity of Fundus Findings for Each Allele Combination

	Univariate			Multivariate		
Allele 2	**OR (SE)**	**95% CI**	***P* Value**	**OR (SE)**	**95% CI**	***P* Value**
Allele 1: Frequent hypomorphic ($n = 20$)						
FH	Reference	–	–	Reference	–	–
RH	NA	–	–	NA	–	–
M/M	NA	–	–	NA	–	–
Mod	0.05 (0.10)	0.00 to 2.20	0.12	10.12 (32.51)	0.02 to 5479.74	0.47
Sev	0.03 (0.06)	0.00 to 1.58	0.08	7.44 (26.11)	0.01 to 7220.70	0.57
PVS1	0.01 (0.03)	0.00 to 0.79	0.038*	4.44 (15.07)	0.01 to 3437.42	0.66
Onset	0.97 (0.03)	0.91 to 1.04	0.36	1.01 (0.05)	0.92 to 1.10	0.91
Duration	1.23 (0.09)	1.06 to 1.41	0.005*	1.25 (0.12)	1.04 to 1.51	0.016*
Allele 1: Rare hypomorphic ($n = 17$)						
FH	Reference	–	–	Reference	–	–
RH	3.02 (5.97)	0.06 to 145.67	0.58	4.83 (9.35)	0.11 to 214.69	0.42
M/M	0.28 (0.51)	0.01 to 9.79	0.48	0.12 (0.24)	0.00 to 6.40	0.29
Mod	5.76 (9.45)	0.23 to 143.66	0.29	2.31 (4.20)	0.06 to 82.10	0.65
Sev	0.03 (0.07)	0.00 to 1.61	0.09	0.00 (0.01)	0.00 to 0.36	0.017*
PVS1	1.54 (2.39)	0.07 to 32.41	0.78	0.70 (1.22)	0.02 to 21.14	0.84
Onset	1.01 (0.02)	0.96 to 1.06	0.79	1.10 (0.05)	1.00 to 1.20)	0.05*
Duration	1.03 (0.05)	0.94 to 1.12	0.53	1.03 (0.06)	0.92 to 1.15	0.62
Allele 1: Mild/moderate ($n = 22$)						
FH	Reference	–	–	Reference	–	–
RH	–	–	–	–	–	–
M/M	108.37 (197.07)	3.07 to 3826.8	0.010*	27.89 (51.75)	0.73 to 1059.09	0.073
Mod	61.96 (102.47)	2.42 to 1584.2	0.013*	49.93 (88.59)	1.54 to 1616.19	0.027*
Sev	45.89 (98.26)	0.69 to 3050.6	0.074	7.94 (18.18)	0.09 to 706.48	0.37
PVS1	8.01 (12.36)	0.39 to 164.95	0.18	2.54 (4.14)	0.10 to 61.99	0.57
Onset	0.98 (0.03)	0.94 to 1.03	0.53	1.05 (0.04)	0.98 to 1.13	0.16
Duration	1.08 (0.03)	1.02 to 1.15	0.010*	1.10 (0.04)	1.02 to 1.18	0.008*
Allele 1: Moderate ($n = 76$)						
FH	Reference	–	–	Reference	–	–
RH	0.63 (0.94)	0.03 to 11.50	0.76	0.26 (0.40)	0.01 to 5.06	0.38
M/M	1.94 (2.16)	0.22 to 17.10	0.55	0.44 (0.55)	0.04 to 5.04	0.51
Mod	0.57 (0.59)	0.07 to 4.40	0.59	0.24 (0.27)	0.03 to 2.19	0.21
Sev	0.69 (0.71)	0.09 to 5.21	0.72	0.45 (0.50)	0.05 to 4.01	0.47
PVS1	1.72 (1.79)	0.22 to 13.28	0.60	1.00 (1.12)	0.11 to 8.96	1.00
Onset	1.00 (0.01)	0.97 to 1.02	0.78	1.02 (0.02)	0.98 to 1.05	0.29
Duration	1.05 (0.02)	1.02 to 1.08	<0.001*	1.06 (0.02)	1.03 to 1.10	<0.001*
Allele 1: Severe ($n = 39$)						
FH	Reference	–	–	Reference	–	–
RH	0.59 (0.66)	0.06 to 5.37	0.64	0.80 (0.97)	0.07 to 8.56)	0.85
M/M	–	–	–	–	–	–
Mod	2.07 (0.61)	0.17 to 24.64	0.56	2.21 (2.96)	0.16 to 30.50	0.55
Sev	13.85 (14.72)	1.72 to 111.26	0.013*	5.95 (7.15)	0.56 to 62.67	0.14
PVS1	15.21 (1922)	1.28 to 181.20	0.031*	14.43 (20.65)	0.87 to 238.31	0.062
Onset	0.94 (0.02)	0.90 to 0.98	0.006*	0.98 (0.03)	0.92 to 1.04	0.48
Duration	1.06 (0.02)	1.02 to 1.11	0.005*	1.05 (0.03)	1.00 to 1.11	0.059
Allele 1: PVS1 ($n = 71$)						
FH	Reference	–	–	Reference	–	–
RH	1.68 (1.49)	0.29 to 9.55	0.56	0.85 (0.86)	0.12 to 6.13	0.87
M/M	1.05 (0.99)	0.17 to 6.63	0.96	2.19 (2.23)	0.30 to 16.10	0.44
Mod	3.24 (1.97)	0.98 to 10.67	0.054	8.12 (5.68)	2.06 to 32.01	0.003*
Sev	5.09 (3.35)	1.40 to 18.49	0.013*	23.24 (20.08)	4.27 to 126.37	<0.001*
PVS1	10.85 (8.74)	2.24 to 52.65	0.003*	38.23 (38.22)	5.39 to 271.30	<0.001*
Onset	0.99 (0.01)	0.97 to 1.02)	0.69	1.06 (0.02)	1.01 to 1.10	0.011
Duration	1.05 (0.02)	1.02 to 1.09	0.001*	1.07 (0.02)	1.03 to 1.11	0.001

CI, confidence interval; FH, frequent hypomorphic; M/M, mild/moderate; Mod, moderate; NA, not applicable; OR, odds ratio; RH, rare hypomorphic; SE, standard error; Sev, severe.

Age was omitted in all models due to collinearity with disease duration.

* These *P* values are statistically significant.

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Genotype-Phenotype Correlation

The distribution of allele combinations according to phenotype severity is presented in Table 4. A clear correlation was observed, with increasing variant severity associated with more severe phenotypes. Statistically significant associations were found between allele severity and phenotypic progression for both allele 1 ($\rho = +0.19$, $P = 0.0036$) and allele 2 ($\rho = +0.14$, $P = 0.02$).

For example, patients with PVS1 or severe variants tended to present more frequently with phenotype 5, and disease duration was generally longer in these groups.

Statistical Models and Clinical Implications

Univariate and multivariable ordinal regression models were used to assess the influence of genotype and clinical variables on phenotype severity (Table 5). In univariate analyses where allele 1 was classified as frequent hypomorphic variant, the severity of fundus findings was inversely related to severe and PVS1 variants in allele 2 and increased with longer disease duration. In the multivariable model (see Table 5, right), only disease duration was positively associated with worsening fundus findings (odds ratio [OR] = 1.25, 95% confidence interval [CI] = 1.04 to 1.51, $P = 0.016$). Overall, in patients with a frequent hypomorphic variant in allele 1, variants in allele 2 were not associated with a more advanced phenotype ($P \geq 0.21$).

In cases where allele 1 was a rare hypomorphic variant (see Table 5, left), no statistically significant results were found in univariate models. However, multivariable models showed that a rare hypomorphic-severe variant combination decreased the likelihood of more severe phenotypes, with disease duration increasing the odds of a more severe phenotype. Similarly, in patients with a rare hypomorphic variant in allele 1, variants in allele 2 were not associated with cross-sectional severity ($P \geq 0.09$).

When allele 1 was classified as a mild/moderate variant (see Table 5, middle left), univariate analyses showed greater odds of a more severe phenotype for longer disease duration and a trend toward decreasing odds of progression with increasing severity of allele 2. However, in multivariable models, only disease duration remained statistically significant, with longer disease duration remained statistically significant, with longer disease duration increasing the risk of a more severe phenotype.

When allele 1 was classified as a moderate variant (see Table 5, left), the only statistically significant variable associated with severity was time since diagnosis, which increased the likelihood of severity by 5% (univariate) and 6% (multivariable; $P < 0.001$). In these cases, variants in allele 2 were not significantly associated with phenotype severity ($P \geq 0.31$).

In patients with a severe variant in allele 1 (see Table 5, left), univariate analyses showed increased odds of a more severe phenotype when severe and PVS1 variants were present in allele 2 and with increased time since diagnosis. Unexpectedly, older patients showed a decreased odd of cross-sectional severity. No variable was significantly associated with phenotype severity in multivariable analyses.

When allele 1 was a PVS1 variant (see Table 5, bottom), univariate models showed an increased likelihood of a more severe phenotype when allele 2 was either severe or PVS1, and with longer time since diagnosis. The association was borderline statistically significant for moderate variants in allele 2. In multivariable models, moderate, severe, and PVS1 variants in allele 2, higher age at diagnosis, and increased disease duration raised the likelihood of a more severe phenotype.

After applying false discovery rate (FDR) correction (Supplementary Table S4), most associations lost statistical significance, except for disease duration in patients with a moderate allele in allele 1 ($q = 0.0365$) and for combinations involving PVS1 variants in allele 1 with severe or PVS1 variants in allele 2 ($q = 0.0365$). These results indicate that the strongest predictors of advanced more severe phenotype are disease duration and the presence of two highly deleterious alleles.

In summary, when one of the alleles is a severe variant or a PVS1 variant, univariate analysis reveal a statistically significant association with fundus phenotype. Nevertheless, this association reaches significance in multivariable models adjusted for age at disease diagnosis and disease duration only when the allele 1 is a PVS1 variant.

Discussion

A precise molecular diagnosis in IRDs is crucial, not only for confirming clinical suspicion but also to guide genetic counseling, inform reproductive decisions, and enable access to gene-targeted therapies or clinical trials. However, in *ABCA4*-associated retinopathies, the considerable phenotypic variability limits the prognostic value of clinical findings alone. Many patients seek information on how their disease might evolve; thus, incorporating genotype-based prognostic indicators into routine care remains an urgent unmet need.

In this study, we conducted a comprehensive genotype–phenotype correlation analysis in a large cohort of 245 individuals with biallelic *ABCA4* variants. Our data confirm a significant association between severe and PVS1 variants and more severe retinal phenotypes. These findings are consistent with prior reports by Cremers et al. and Lee et al.,[4,17] reinforcing the major influence of variant type on the severity of clinical manifestations. Notably, the classification of variant severity in our study was based on the recent framework proposed by Cornelis et al.[12] Although this ensures consistency and standardization, it is important to recognize that some variants may be classified differently in other studies or as new functional data become available, as illustrated by Bauwens et al.[26]

We stratified patients into five distinct phenotypic groups based on multimodal retinal imaging, which allowed us to capture the full clinical spectrum of *ABCA4*-associated retinopathy. Individuals with the most severe group (phenotype 5) were typically older and had an earlier onset of symptoms and longer disease duration. Compared with earlier classification systems, such as that proposed by Ferreira et al.,[27] our phenotyping framework includes a very mild form (phenotype 1), characterized by RPE mottling without central atrophy. This phenotype likely represents underrecognized or late-onset disease, whereas subtle fundus changes may delay diagnosis. The intergrader agreement regarding the 5-category phenotype classification was $\kappa = 0.66$, which is considered substantial.[28]

The FDR-adjusted analysis confirmed that only the most biologically plausible associations remained significant: disease duration and combinations of highly deleterious alleles (PVS1 with severe/PVS1). This reinforces the idea that genotype severity and disease duration are major

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factors influencing phenotype severity, whereas other associations observed in unadjusted models may reflect residual confounding or limited sample size.

As expected, patients harboring two severe alleles, or one severe and one PVS1 variant, exhibited the most severe phenotypic manifestations. Our data also suggest that severe and PVS1 variants contribute equivalently to disease severity, reinforcing findings from Lee et al.[17] In contrast, the presence of a hypomorphic variant did not consistently correlate with milder phenotypes, possibly due to limited subgroup sizes or diagnostic delays in these patients. One illustrative case is patient P-201, who carries 2 rare hypomorphic variants and nonetheless presented with symptom onset at age 6 years. This early onset may be partially explained by earlier screening due to affected family members. It is also important to clarify that the term "rare hypomorph" was used in our study to denote alleles of likely mild effect, consistent with what other studies refer to as "mild" variants. Discrepancies in terminology across studies underscore the need for continued functional validation and harmonized classification criteria.

In rare cases, unexpected genotype–phenotype correlations were observed. For example, patient P-153 was found to be homozygous for the deep-intronic variant c.5196+1137G>A, which has been previously reported as insufficient to cause disease on its own. One possible explanation is the presence of an undetected third pathogenic variant in trans, such as a structural variant or complex rearrangement not captured by conventional sequencing methods. Alternatively, the variant may exert pathogenic effects in specific *cis*-regulatory contexts or be modulated by trans-acting genetic or epigenetic factors.[26]

We also highlight the frequent co-occurrence of the variant c.2588G>C with c.5603A>T in *cis*, a pattern that complicates genotype interpretation. Because c.5603A>T is a common hypomorphic allele whose pathogenicity is highly dependent on the variant in trans, its presence alongside c.2588G>C necessitates careful phasing to avoid misclassification. The rarity of c.2588G>C occurring without c.5603A>T further supports the importance of considering complex alleles in clinical interpretation.[2,4]

A noteworthy observation in our cohort is the overrepresentation of female patients carrying hypomorphic *ABCA4* variants, including but not limited to c.5603A>T. Although *ABCA4* is autosomal, this sex imbalance, consistent with findings by Runhart et al.[18] and Cornelis et al.,[19] suggests a possible sex-influenced component to disease expression. Potential explanations include sex-specific genetic modifiers, hormonal effects, or detection bias in milder phenotypes. Although not the main focus of this study, these findings raise the possibility that a subset of *ABCA4*-associated retinopathies may not follow classical Mendelian inheritance, with implications for counseling and risk assessment.

Functional splicing assays demonstrated that the c.859-442C>T deep-intronic variant leads to the insertion of a 238 bp pseudoexon, causing a frameshift and premature stop codon, likely abolishing *ABCA4* protein function.[29] This finding not only expands the mutational spectrum of *ABCA4* but also reinforces the diagnostic importance of investigating non-coding regions in genetically unsolved cases.[30]

Multimodal imaging, including SD-OCT, ultra-widefield fundus imaging, and full-field electroretinogram (ffERG), enabled detailed phenotypic assessment and precise disease staging. This comprehensive approach enhances diagnostic accuracy, supports longitudinal follow-up, and ensures appropriate selection for clinical trials.

Looking ahead, artificial intelligence may further enhance phenotype classification and disease modeling. For example, Glinton et al.[31] showed that machine learning applied to ffERG can distinguish *ABCA4*-related phenotypes, whereas deep-learning models based on fundus imaging have demonstrated promise in predicting causative genes in IRD cohorts. Integration of these tools with large-scale imaging repositories could refine genotype-phenotype predictions, particularly in intermediate disease stages (phenotypes 2 and 3), and facilitate early identification of biomarkers for progression or therapeutic response.

Finally, our findings have direct implications for clinical translation. Several therapeutic strategies targeting *ABCA4* are currently under development, including adeno-associated virus (AAV)-mediated gene therapy, antisense oligonucleotides (AONs), and small-molecule therapies.[4,32,33] The genotype-based severity scoring proposed in this study could aid in patient stratification, inform trial design, and support the implementation of precision medicine approaches in this genetically complex disorder.[34]

Limitations

Interpretation of the findings should consider certain methodological constraints.

Age at onset was patient-reported and subject to recall bias and variable symptom recognition, which introduced misclassification, particularly for earlier childhood onset. Nevertheless, patient-reported age of onset is generally more accurate than the one reported in the patient's medical record.

The lack of a centralized imaging review may have contributed to inter-center variability in phenotype classification. Similarly, differences in the detection and reporting of deep-intronic variants across centers may have led to their under-representation. Differences in genetic testing pipelines across participating centers represent an important limitation of this study. Although *ABCA4* is generally well-covered by contemporary next-generation sequencing (NGS) methods, the lack of uniformity in sequencing platforms, coverage thresholds, copy number variant (CNV) detection, and inclusion of deep-intronic regions may have led to variability in variant ascertainment. In particular, deep-intronic *ABCA4* variants—known contributors to disease—were assessed only in a subset of centers. As a result, some pathogenic or likely pathogenic variants may have remained undetected in individuals sequenced using more restricted pipelines.

The cross-sectional nature of the study limits the ability to evaluate disease progression. Longitudinal data will be necessary to validate the severity classification and its prognostic value. Longitudinal studies with serial imaging are required to validate these associations as predictors of temporal change. Larger multicenter studies are needed to validate genotype-phenotype correlations in rarer *ABCA4*-associated diagnoses (e.g., CORD, COD, and RP).

After applying FDR correction for multiple testing, most associations observed in unadjusted models did not remain statistically significant, likely reflecting the exploratory nature of subgroup analyses and limited sample sizes within certain allele combinations. However, the strongest and most biologically plausible associations—disease duration

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and combinations of highly deleterious alleles (PVS1 with severe/PVS1)—remained significant, supporting the robustness of our main conclusions.

Despite the large overall cohort size, subgroup analyses—particularly those stratified by the severity of alleles 1 and 2—resulted in relatively small sample sizes within certain categories, which may limit statistical power and generalizability. In addition, it was not possible to confirm systematic screening for the c.5603A>T p.(Asn1868Ile) variant. Phasing analysis to determine cis/trans configuration was performed when parental or familial data were available; in cases without such data, trans configuration was inferred, which introduces a residual uncertainty. Due to the multicentric design, access to raw sequencing data or DNA samples was not consistent across all cases. Although now recognized as pathogenic when in trans with a severe allele, this variant was previously considered benign or of uncertain significance and may have been overlooked or misclassified in earlier analyses, introducing a residual risk of underdetection or misinterpretation. Finally, the cohort included a small number of related individuals (17 affected siblings among 245 patients), which introduces a degree of non-independence; however, sensitivity analyses excluding second affected siblings yielded results consistent with the full cohort, suggesting that this had minimal impact on the overall findings.

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