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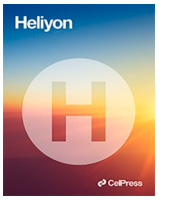

Research article

ABCA4-related retinopathies in Lebanon

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ABSTRACT

Variants in *ATP-binding cassette transporter type A4* (*ABCA4*) have been linked to several forms of inherited retinal diseases (IRDs) besides the classically defined Stargardt disease (STGD), known collectively as *ABCA4* retinopathies. *ABCA4* is a sizable locus harboring 50 exons; thus, its analysis has revealed over 2,400 variants described, of which more than 2,000 are causal. Due to the clinical and genetic heterogeneity, diagnosing *ABCA4* retinopathies is challenging. To date, no *ABCA4*-related retinopathy has been detected in Lebanon. Using next-generation sequencing, we analyzed our IRDs' cohort retrospectively (61 families) and identified five with *ABCA4*-related retinopathies, making it a relatively abundant cause of IRDs (about 8 %). Three families were diagnosed with rod-cone dystrophy (RCD), two with STGD, and one with cone-rod dystrophy (CRD). In conclusion, our study showed the presence of *ABCA4* variants with a high degree of heterogeneity in Lebanon.

1. Introduction

Inherited retinal dystrophies (IRDs) are a set of monogenic disorders marked by photoreceptor degeneration or impairment [1,2]. These diseases are well-defined by a high degree of clinical and genetic variation, with over 270 genes implicated [1]. Interestingly, the age of onset, the progression rate, manifestation with extra-ocular symptoms, and the etiological gene may assist in classifying IRDs into more than 20 distinct phenotypes [3,4]. The most prevalent form of IRDs is rod-cone dystrophy (RCD; MIM 613862), which affects over one million individuals worldwide and is defined by the primary death of rods subsequently accompanied by secondary deterioration of cone photoreceptors [3,5]. When cone photoreceptor degeneration occurs initially, followed by rod dysfunction in later stages, this is called cone-rod dystrophy (CRD; MIM 601777), and is represented by progressive degeneration and loss of the central retina [6,7]. Other aspects of IRDs that appear with central vision loss include macular dystrophies (MD) that affect mainly the macula [3,7]. With an incidence rate of 1 in 8,000–10,000 individuals, Stargardt disease (STGD; MIM 248200) emerges as the prevailing aspect of MD with an autosomal recessive mode of inheritance affiliated with etiological variants in the *ATP-binding cassette transporter type A4* (*ABCA4*) [8,9].

The *ABCA4* gene, initially identified by Allikmets and colleagues in 1997 as the causative gene for STGD [10], was later found to be

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associated with some forms of RCD and CRD, depending on the *ABCA4* variant type and the residual protein activity [11]. *ABCA4* is a 50 exons' locus on the short arm of chromosome 1 (1p21–p22.1) that encodes a single-chain ATP-binding cassette transporter protein situated at the outer segments of rod and cone photoreceptors [8,9]. ABCA4 protein moves all *trans*-retinal and toxic substances from the disc lumen to the photoreceptors' cytoplasm. It shows significant disparities in disease-causing alleles across racial and ethnic groups and exhibits founder mutations in different populations [12]. Considering the massive clinical and genetic heterogeneity, an accurate and thorough molecular diagnosis of *ABCA4-related* retinopathies is critical [13]. However, the allelic heterogeneity of *ABCA4* has made genetic analyses of these gene-associated IRDs very challenging [14]. Direct Sanger sequencing of all *ABCA4* exons has uncovered 60–80 % of the pathogenic alleles [13]. Notably, next-generation sequencing (NGS) platforms have found novel *ABCA4* variants, demonstrating their ability as more comprehensive approaches for systematic genetic screening of large cohorts [15,16]. Presently, NGS is critical for obtaining a prompt and precise genetic diagnosis, which is required to provide patients and their families with the appropriate genetic counseling [1]. The relevance of genetic diagnosis through the implementation of comprehensive and affordable sequencing technologies lies in identifying the disease-causing variants that may result in finding the genotype-phenotype correlations, establishing a clear interpretation of the pathophysiological mechanisms, and tailoring the personalized therapy approach [5]. Many *ABCA4* variants associated with different forms of IRDs have been reported worldwide, but none in Lebanon. Herein, we analyzed our IRDs' cohort retrospectively using NGS and identified five cases with *ABCA4*-related retinopathies (out of 61 families).

2. Materials and methods

2.1. Ethical considerations and clinical examinations

This study was reviewed and approved by the institutional review board committee of the Beirut Arab University, with the approval number 2017 H-0030-HS-R-0208. All the participants provided informed consent to participate in the study.

Our study is a retrospective analysis of six Lebanese families having *ABCA4*-related retinopathies; those families are part of a larger IRDs' cohort collected since 2015. All our included participants showed at least one *ABCA4* causal variant and were recruited at Beirut Eye and ENT Specialist Hospital (Lebanon), where they carried out a clinical ophthalmologic assessment, as previously described [17]. The color fundus and the fundus autofluorescence (FAF) images were captured using a Maestro2 (Topcon Corporation, Tokyo, Japan), the spectral-domain optical coherence tomography (SD-OCT) images were captured on 3D OCT 2000 (Topcon Corporation, Tokyo, Japan). The electrophysiology tests; the electroretinogram (ERG) and the electro-oculograpm (EOG) were done using the MonPack3 (Métrovision, Pérenchies, France). All patients were classified according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards (https://iscev.wildapricot.org/standards).

2.2. Variant screening

All our participants gave whole blood samples. Genomic DNA extraction was done by Qiagen's QIAamp DNA Mini Kit (Hilden, Germany). DNA samples from probands F31:II.1, F37:II.1, F41:II.1, F56:II.1, and F58:II.1 were sequenced with whole exome sequencing (WES), whereas that of F3:II.1 underwent targeted NGS. Both WES and targeted NGS were performed as described previously [16]. The targeted NGS panel was selected from the SureSelect Human All Exon Kits Version 4 (Agilent, Massy, France). Enriched DNA samples were then sequenced on an Illumina GAIIx as paired-end 75 bp reads, and base calling was carried out using the Illumina Real Time Analysis (RTA) Pipeline. For WES, all the exon regions of all human genes (~22,000) were captured by xGen Exome Research Panel v2 (Integrated DNA Technologies, Coralville, Iowa, USA). The captured regions of the genome were sequenced with Novaseq 6000 (Illumina, San Diego, CA, USA).

The DNA sequencing data analysis, including alignment to the GRCh37/hg19 human reference genome, variant calling, and annotation, was conducted with open-source bioinformatics tools and in-house software. Common polymorphisms (outside the *ABCA4* locus) that had a minor allele frequency (MAF) greater than 0.01 were all omitted using various public databases, including the Genome Aggregation Database (GnomAD, https://gnomad.broadinstitute.org/) [18] and the Iranome (if available): http://www.iranome.ir/gene/ENSG00000198691 [19]. If no variant was found with the MAF = 0.01 threshold, a second filtering approach was done with a higher MAF threshold (0.05). Since *ABCA4* harbors several common disease-causing mutations [20], we manually searched for these variants (p.(Asn1868Ile), p.(Gly1961Glu) and many others) in the unsolved cases. Annotation type-based filtering, where we removed in-frame insertions-deletions (indels), intronic variants, synonymous variants, and variants in untranslated regions followed this step. Contrariwise, priority was given to nonsense variants, frameshift variants, missense variants and splice site variants. Next, we checked whether the candidate variants were reported as homozygous in the supposed healthy individuals of GnomAD. Ideally (except for some *ABCA4* variants), bi-allelic disease-causing variants are absent in GnomAD database. Variants that are never observed in the homozygous state in GnomAD may be more likely to cause severe phenotypes. In contrast, GnomAD homozygous variants may be more likely to be benign or have mild effects.

2.3. Pathogenicity assessment of the candidate variants

The conservation of substituted amino acids in various species, such as primates and main placental mammals, was checked using the University of California at Santa Cruz (UCSC) genome browser across 46 different species [21]. Information regarding the details was previously described [22], very briefly, if no amino acid change was found among all species or was different in just one species,

Table 1
Clinical results of six Lebanese patients with *ABCA4* variants.

Family	F3	F31	F37	F41	F56	F58
Individual	F3:II.1	F31:II.1	F37:II.1	F41:II.1	F56:II.1	F58II.1
Gender	M	M	M	M	F	F
Age	27	32	40	42	27	33
Disease	STGD	RCD	RCD	CRD	STGD	RCD
Age of diagnosis	21	24	33	30	26	adolescence
ERG	reduced photopic and scotopic	reduced photopic, Very reduced scotopic	reduced photopic and scotopic	NA	Slightly reduced photopic and scotopic	Reduced photopic, very reduced scotopic
Color Photography	showing mild pigmentary changes in the posterior pole and outside the vascular arcades	mild pigmentary changes in the posterior pole and outside the vascular arcades	optic disc pallor and atrophy in the posterior pole	Pigmentary macula, marked pigmentary changes in the posterior pole with marked vascular attenuation and optic disc pallor	Relative preservation of the macular function, abnormal reflex at the macula	marked pigmentary changes in the posterior pole and outside the vascular arcades with marked vascular attenuation and optic disc pallor
Autofluorescence / Fluorescein angiography	NA/granular hyper-fluoresce in the posterior pole with focal hyper-fluorescence at the macula	increased hyper-fluorescence at the macula/NA	NA/increased hyperfluorescence at the macula	NA	NA/increased hyper-fluorescence at the macula	NA/diffuse granular hyper-fluorescence in the posterior pole with decreased fluorescence at the macula
OCT	diffuse thinning of the retinal layers	NA	Thinning of the retinal layers and hyper-reflectivity at the level of the choroid	diffuse thinning of the retinal layers with cystic changes and focal scarring	NA	diffuse thinning of the retinal layers
EOG	NA	Reduced	Reduced	Reduced	NA	NA

STGD: Stargardt disease; RCD: rod-cone dystrophy; CRD: cone-rod dystrophy; ERG: electroretinogram; OCT: optical coherence tomography; EOG: Electrooculogram; NA: not available.

then the residue was considered "highly conserved." If a different change was seen in less than four species and not in the primates, then it was considered "moderately conserved," and if a change was present in 5–7 species, it was considered "marginally conserved"; otherwise, the amino acid residue was considered "not conserved." The possible effect of the amino acid substitution was assessed by Sorting Intolerant From Tolerant (SIFT) [23], Polymorphism Phenotyping v2 (PolyPhen-2) [24], and MutationTaster2 [25]. Several public databases were utilized to determine if the candidate variant causing IRD was previously known, including the Human Gene Mutation Database (HGMD, http://www.hgmd.org) [26] the Leiden Open Variant Database (LOVD, https://www.lovd.nl) [27], PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), and the Online Mendelian Inheritance in Man (OMIM, https://omim.org/). All the candidate variations were classified according to the American College of Medical Genetics (ACMG) standards [28].

2.4. Segregation analysis

DNA was amplified by conventional polymerase chain reaction (T100, Biorad, Kaki Bukit, Singapore) and then the candidate variants were validated (Applied Biosystems 3730xl DNA Sequencer, Courtaboeuf, Les Ulis, France) to exclude the possibility of false positive results. Unidirectional Sanger sequencing was applied to all available family members' DNA for segregation analysis.

3. Results

3.1. Ophthalmic data

Out of 61 IRD families, five had *ABCA4*-related retinopathies (about 8 %). In family 3, proband F3:II.1 is a 27-year-old male diagnosed with STGD at age 21 (Table 1). Parents of F3:II.1 are third cousins (Fig. 1). Color fundus photographs of F3:II.1 revealed mild pigmentary changes in the posterior pole and outside the vascular arcades (Fig. 2a). Fluorescein angiography showed granular hyperfluorescence in the posterior pole with focal hyperfluorescence at the macula (Fig. 2b). Additionally, OCT exhibited diffuse thinning of the retinal layers (Fig. 2c). EOG demonstrated no light rise and a subnormal Arden ratio of 1.68 on the right eye and a reduced Arden ratio of 1.51 on the left eye, which are below the normal value (>1.8); ERG revealed reduced scotopic and photopic responses (Table 1).

Family 31 has a 32-year-old affected male; F31:II.1, diagnosed with RCD at 24 with no known family history (Table 1). Color fundus photography of this patient showed mild pigmentary changes in the posterior pole and outside the vascular arcades (Fig. 2a). His FAF examination showed increased hyperfluorescence at the macula (Fig. 2d). Besides, this individual's ERG demonstrated reduced photopic and significantly reduced scotopic responses. Additionally, F31:II.1 exhibited reduced EOG with an Arden ratio of 1.29 on the right eye and 1.25 on the left eye (Table 1).

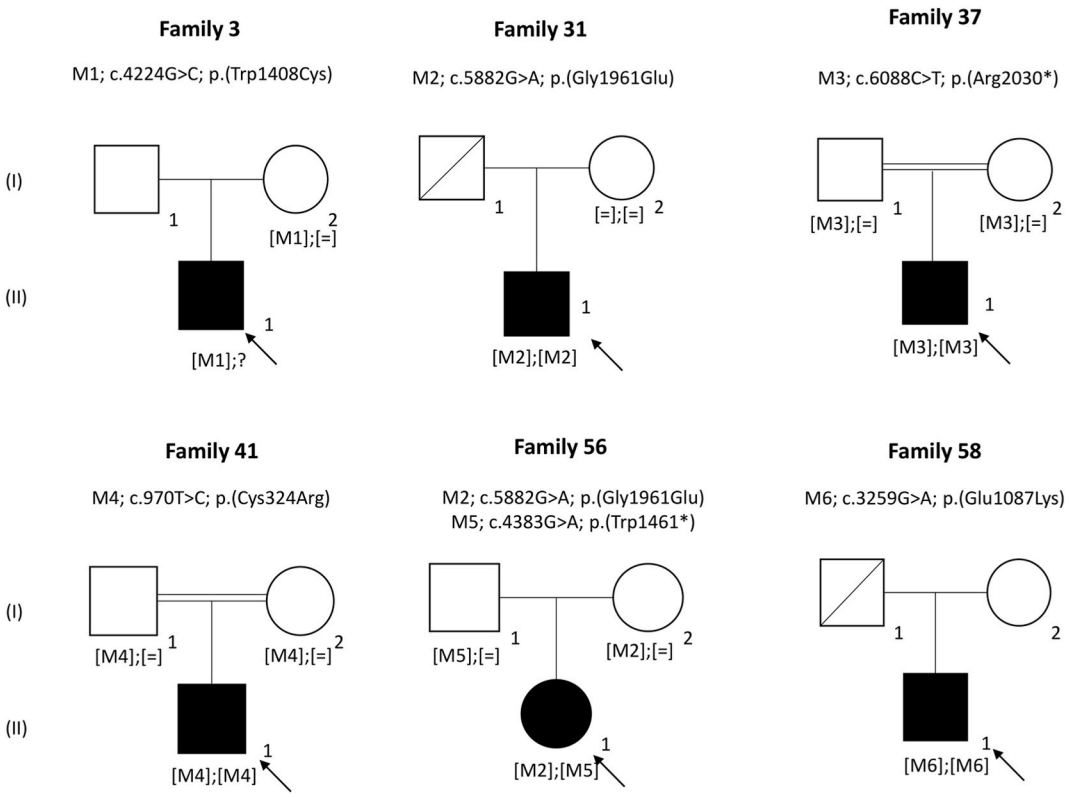

Fig. 1. Pedigrees of six families with *ABCA4* variants. White symbols represent unaffected members. Symbols in black denote affected individuals. Arrows indicate probands. Males and females are represented by square and round symbols, respectively. A slash denotes individuals who have died. Double horizontal lines represent consanguineous marriages. M: mutation.

The proband of family 37, F37:II.1, is a male aged 40, issued from a consanguineous marriage, and was diagnosed with RCD at 33 (Table 1). Clinical findings of this patient indicated reduced photopic and scotopic ERG responses (Table 1). Moreover, the EOG of this patient exhibited a reduced Arden ratio of 1.25 and 1.24 on the right and left eyes, respectively (Table 1). Color photographs revealed optic disc pallor and atrophy in the posterior pole (Fig. 2a). OCT scans showed thinning of the retinal layers and hyper-reflectivity at the choroid level (Fig. 2c).

Family 41 exhibited consanguinity among parents (Fig. 1). This family has an affected descendant, F41:II.1. Color fundus examination showed marked pigmentary changes in the posterior pole with marked vascular attenuation and optic disc pallor (Fig. 2a). Additionally, OCT demonstrated diffuse thinning of the retinal layers with cystic changes and focal scarring (Fig. 2c). The ERG displayed diminished photopic and scotopic responses. The clinical findings indicated a CRD (Table 1).

Parents in family 56 are phenotypically normal but have an affected descendant, F56:II.1, a 27-year-old female diagnosed with STGD at age 26 (Table 1). Her color photograph showed an abnormal reflex at the macula (Fig. 2a). Besides, fluorescein angiography revealed increased hyperfluorescence at the macula (Fig. 2b). Clinical diagnosis of F56:II.1 revealed macular dystrophy with relative preservation of macular function. ERG of this patient demonstrated slightly reduced photopic and scotopic responses (Table 1).

The proband F58:II.1 has phenotypically non-affected parents, but she was diagnosed clinically with RCD. ERG revealed reduced photopic and very reduced scotopic (Table 1). Color photographs showed marked pigmentary changes in the posterior pole and outside the vascular arcades with marked vascular attenuation and optic disc pallor (Fig. 2a). Fluorescein angiography demonstrated diffuse granular hyperfluorescence in the posterior pole with decreased fluorescence at the macula (Fig. 2b). Additionally, OCT scan of the patient exhibited diffuse thinning of the retinal layers (Fig. 2c).

3.2. Genetic findings

We detected five probands with *ABCA4*-related retinopathies (Table 2). For the proband F3:II.1 of family 3, we found a heterozygous variant in *ABCA4*. However, the second mutated allele remains missing. The detected mono-allelic variant is a missense variant in exon 28 of the *ABCA4* gene, [M1]: c.4224G > C, p.(Trp1408Cys). The variant M1 did not appear in the ExAC, GnomAD, or TopMed populations. Based on the UCSC genome browser, the amino acid Trp1408 was 'marginally conserved' as the Tryptophan amino-acid at this position was found in 44 (out of 46) different species. The substitution was also predicted to be probably damaging on PolyPhen-2 and diseases-causing on MutationTaster, while tolerated on the SIFT software. Sanger sequencing validated the occurrence of M1 in a

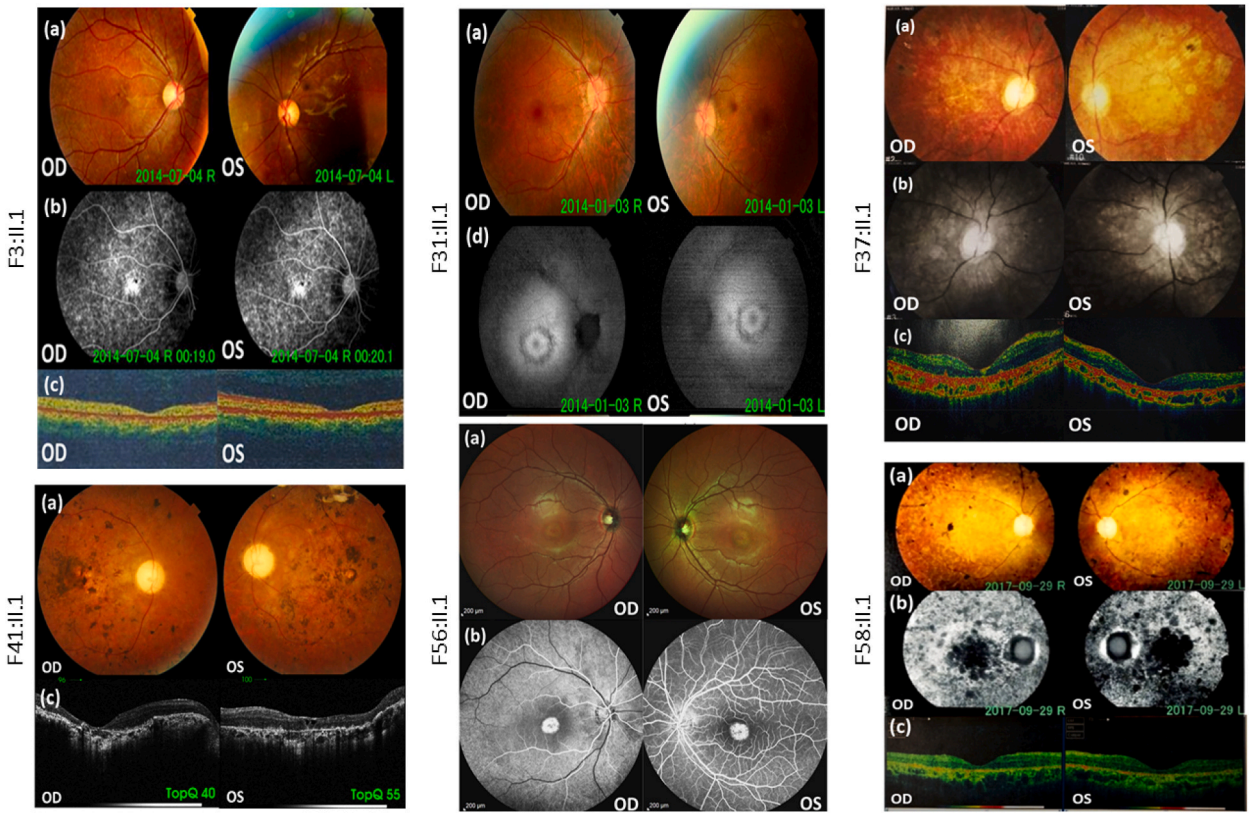

Fig. 2. Color fundus photographs (a), fluorescein angiography (b), optical coherence tomography (OCT) scans (c), and fundus autofluorescence (FAF) pictures (d) of patients F3:II.1, F31:II.1, F37:II. 1,F41:II.1, F56:II.1 and F58:II.1. OD: oculus Dexter; OS: ocular sinister. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

heterozygous state in the patient F3:II.1 of this family (Sigure S1 a). The mother was a heterozygous carrier of M1, while the father carried the wild-type allele. M1 is a known variant that has been reported [29]. According to the ACMG standards; M1 is likely pathogenic.

The proband of family 31, F31:II.1, carries a homozygous missense variant within exon 42 of *the ABCA4* gene. This variant is well-known in exon 42, [M2]: c.5882G > A; p.(Gly1961Glu), rs1800553. M2 exhibited a rare occurrence and was observed to be homozygous in some individuals from ExAC, GnomAD, and TOPmed population databases (respective frequencies = 0.005054, 0.003406, and 0.00284). This variant affects a highly conserved residue (Gly1961). Furthermore, according to the predictions made by PolyPhen-2, SIFT, and MutationTaster, the M2 variant was determined to be probably damaging, damaging, and disease-causing, respectively. Sanger sequencing analysis validated the presence of this homozygous variant in F31:II.1 (Figure S1 b). The father of the affected patient was deceased; however, the mother was found to be homozygous for the wild type (WT) allele. According to the ACMG standards, M2 is likely pathogenic.

The proband F37:II.1 of family 37 had a homozygous nonsense variant in the exon 44 of *ABCA4*. This substitution resulted in the appearance of a stop codon; [M3]: c.6088C > T; p.(Arg2030*), rs61751383. According to population databases, M3 is a rare and heterozygous variant with frequencies equal to 0.00002471, 0.00001425, and 0.00000756 in ExAC, GnomAD and TOPMed, respectively, affecting a highly conserved amino acid based in the UCSC genome browser. This variant was validated by Sanger sequencing in F37:II.1. Moreover, parents were found to be heterozygous carriers of M3 (Figure S1 c). M3 is not a novel variant [30]; according to the ACMG standards, it is pathogenic.

The proband F41:II.1 presented a homozygous variant [M4]: c.970T > C; p.(Cys324Arg) in exon 8. The parents were heterozygous for M4 (Figure S1 d), which was extremely rare and absent in online databases. Prediction tools revealed it as probably damaging, damaging, and disease-causing on PolyPhen-2, SIFT and MutationTaster, respectively. Based on the UCSC genome browser, the impacted amino acid (Cys324) is moderately conserved. This variant was previously reported in a Chinese population [31]. According to the ACMG standards, M4 is a variant of unknown significance (VUS).

In family 56, F56:II.1 harbors compound heterozygous variants. The first is the missense substitution in exon 42 [M2], while the second is a nonsense variant in exon 30, [M5]: c.4383G > A; p.(Trp1461*). M5 is extremely rare in GnomAD with allele frequency of 6.842e-7, and never homozygous. Sanger sequencing validated the presence of M2 and M5 in F56:II.1. Besides, it revealed that the disease co-segregated within the family, where the father was found heterozygous for M5 and the mother was heterozygous for M2 (Figure S1 e). This finding confirms the autosomal recessive inheritance pattern observed in F56:II.1. According to the ACMG

Table 2
ABCA4 mutations in six Lebanese families with *ABCA4* variants.

Family	Disease	Gene Reference Sequence	Exon	rs ID	Nucleotide Exchange	Amino Acid Change	Frequencies	PolyPhen-2 (Scour)	SIFT (Score)	MutationTaster (Score)
F3	STGD	*ABCA4* NM_000350.2	28		c.4224G > C	p.(Trp1408Cys)	–	Probably damaging (1)	Tolerated (0.13)	Disease causing (0.999)
			–	–	–	–	–	–	–	–
F31	RCD	*ABCA4* NM_000350.2	42	rs1800553	c.5882G > A	p.(Gly1961Glu)	0.005054 (ExAC)/4hom 0.003406 (GnomAD)/ 44hom 0.00284 (TopMed)/1hom 0.02562 (Iranome)/1hom	probably damaging (1)	damaging (0)	disease causing (0.999)
F37	RCD	*ABCA4* NM_000350.2	44	rs61751383	c.6088C > T	p.(Arg2030*)	0.00002471 (ExAC)/ 0hom 0.00001425 (GnomAD)/ 0hom 0.00000756 (Topmed)/ 0hom			disease causing (1)
F41	CRD	*ABCA4* NM_000350.2	8		c.970T > C	p.(Cys324Arg)	Not reported	possibly damaging (0.754)	damaging (0)	disease causing (0.999)
F56	STGD1	*ABCA4* NM_000350.2	42	rs1800553	c.5882G > A	p.(Gly1961Glu)	0.005054 (ExAC)/4hom 0.003406 (GnomAD)/ 44hom 0.00284 (TopMed)/1hom 0.02562 (Iranome)/1hom	probably damaging (1)	damaging (0)	disease causing (0.999)
			30	rs1347261858	c.4383G > A	p.(Trp1461*)	6.842e-7 (GnomAD)/ 0hom			disease causing (1)
F58	RCD	*ABCA4* NM_000350.2	22	rs61751398	c.3259G > A	p.(Glu1087Lys)	0.00003296 (ExAC)/ 0 hom 0.00002416 (GnomAD)/ 0hom 0.0000227 (Topmed)/ 0hom 0.000625 (Iranome)/ 0hom	probably damaging (1)	damaging (0)	disease causing (0.999)

F: Family, STGD: stargardt diseases; STGD1: *ABCA4*-STGD CRD: cone rod dystrophy; RP: Retinitis pigmentosa; hom: homozygous; rs: reference SNP.

standards, M2 is likely pathogenic and M5 is pathogenic. Notably, this variant was found before along with another allele in a patient diagnosed with CRD [32].

Variant analysis in the proband F58:II.1 revealed a homozygous missense variant [M6] in exon 22. M6: c.3259G > A; p.(Glu1087Lys), rs61751398 is rare and never homozygous with an allele frequency of 0.00003296 in ExAC, 0.00002416 in GnomAD and 0.0000227 in TOPMed. M6 is likely damaging on PolyPhen-2, damaging on SIFT, and disease-causing on MutationTaster. The affected amino acid (Gly1087) is also highly conserved, as found in the UCSC genome browser. The zygosity of the M6 variant was verified through Sanger sequencing in the proband. In contrast, M6 was heterozygous in the mother (Figure S1 f). As the father was deceased, we were unable to confirm the segregation in this family. The literature search showed M6 as a known variant [30]. According to the ACMG standards, M6 is a VUS.

4. Discussion

To date, the analysis of the *ABCA4* gene has disclosed a bulk of genetic data with more than 2,000 variants underlying IRDs of different severity and manifestations [12]. We found six variants in the *ABCA4* gene in a small Lebanese group. Biallelic variants were detected in five probands, while in one proband, a heterozygous variant was detected. Sanger sequencing verified the putatively pathogenic variants in the probands.

Determining the biallelic variants may be challenging due to the *ABCA4*'s large size, the wide range of pathogenic variants it exhibits, such as hypomorphic variants [33–35], non-canonical splice site variants [36], and lately, deep-intronic variants [36,37]. In line with previous literature [38], targeted sequencing of the patient in family 3 revealed only one mutant allele, while the second variant is still missing, thus rendering the case of this family genetically unsolved. According to Nassisi et al. there are two basic explanations for this performance's relative poorness: (1) because the whole gene was not scanned, the second allele may be located in the gene's promoter, untranslated regions (UTRs), or another deep intronic region. Additionally, the phenotype may be caused by unrecognized copy number variations (CNVs) in exonic or intronic areas [39]. Hence, sequencing of *ABCA4* locus could genetically resolve this case [37]. (2) As there are multiple phenocopies associated with STGD, it may be necessary to examine the exome or genome of the patient to identify the existence of pathogenic variants in other genes, possibly additional genes not previously linked with IRDs [37].

Herein, we report the first detection of the variant c.5882G > A p.(Gly1961Glu) in the Lebanese population. The presence of the p.(Gly1961Glu) allele (homozygous or compound heterozygous) is associated with markedly different phenotypes [12]. In this study, p.(Gly1961Glu) was detected in two probands in both homozygosity and compound heterozygosity states. Variant analysis revealed p.(Gly1961Glu) in a homozygous state in the proband of family 31 diagnosed with RCD, while it was in compound heterozygous state along with c.4383G > A; p.(Trp1461*) in the proband of family 56. Initially, it was thought that p.(Gly1961Glu) was not likely pathogenic, mainly when found in the homozygous state. A homozygous c.5882G > A; p.(Gly1961Glu) variant was described in an asymptomatic 25-year-old Somali male with normal vision assessment [40]. However, this was later justified by a study in which patients who were homozygous for c.5882G > A; p.(Gly1961Glu) were reported to have later disease onset (>25 years old) than would be seen in STGD in typical cases [40,41]. Our analysis of WES data in this patient did not reveal conclusive modifier variants. It is still plausible that in rare cases, modifiers are environmental, which can still result in changes in disease pattern expression. The fact that M2; c.5882G > A; p.(Gly1961Glu) was homozygous in the proband F31:II.1 but absent in his mother reveals that either; (1) c.5882G > A is a de novo variant that arose on the maternal allele, (2) or the mother carries an undetected structural variant. (3) Alternatively, there is uniparental isodisomy from the paternal side. Because of the absence of paternal DNA and the absence of the sequencing raw data of the proband, we could not determine the p.(Gly1961Glu) inheritance mechanism (uniparental isodisomy or a structural deletion); which is a limitation of our study.

The missense variant allele c.5882G > A; p.(Gly1961Glu) has been observed to be linked with retinal impairment that is localized to the macula, without being widespread [42]. The c.5882G > A; p.(Gly1961Glu) is expected to affect protein function by reducing ATP binding and ATPase activity, as shown by indirect functional testing [43,44]. Generally, c.5882G > A; p.(Gly1961Glu) was reported to cause milder phenotypes. However, it may be associated with phenotypes of varying severity [42]. Its actual clinical manifestation may rely on the severity of the other paired mutant allele, as revealed by previous genotype-phenotype investigations [42,45]. Hence, the type of the combined *ABCA4* mutant alleles in compound heterozygosity determines the phenotype severity, including the age of onset and functional effects [42,46]. Our proband F31:II.1 demonstrated a RCD disease pattern, similar to what Burke et al. reported in a Somali patient with a homozygous p.(Gly1961Glu) variant, showing the diversity of phenotypes caused by *ABCA4* variants [40]. Interestingly, Burke et al. have examined 12 individuals with homozygous p.(Gly1961Glu) and found that all of them have *ABCA4*-related retinopathies, with severe phenotypes consistent with the existence of additional (modifier) *ABCA4* variants [40].

Maugeri et al. have suggested a genotype-phenotype correlation model which demonstrated an opposite relation between the *ABCA4* residual activity and the level of severity of the IRD [47]. According to this model, compound heterozygosity for two severe (null) *ABCA4* variants causes RCD, the most severe phenotype [47]. Whereas, in case of partial retention of *ABCA4* activity, CRD will result due to compound heterozygosity of a severe and moderately severe variant, while STGD1 macular degeneration will appear if a severe variant is inherited along with a mild *ABCA4* variant [47]. Applying the Maugeri et al. model to our genotype-phenotype correlations reveals that in family 3, the missing variant probably has a mild effect (Table 3). In contrast, the genotype-phenotype association in family 31 does not follow the Maugeri model unless an additional modifier variant with severe effect is found (Table 3). The genotype-phenotype correlations in families 37, 56, and 58 fit the Maugeri model (Table 3). We could not find a severity classification for M4 in F41:II.1; thus, we did not draw additional conclusions in this family (Table 3).

Family 37 presented a consanguinity case with a child diagnosed with RCD. The homozygous nonsense variant revealed in the

Table 3
ABCA4 genotype-phenotype correlations using the Maugeri model and the Cornelis severity classification.

ABCA4 Genotypes				Severity	IRD	Correlation
Family	Variant	Nucleotide Exchange	Amino Acid Change	Cornelis, 2022.		
3	M1	c.4224G > C	p.(Trp1408Cys)	N.A	**STGD**	+
31	M2	c.5882G > A	p.(Gly1961Glu)	Mild	**RCD**	–
31	M2	c.5882G > A	p.(Gly1961Glu)	Mild		–
37	M3	c.6088C > T	p.(Arg2030*)	Severe	**RCD**	–
37	M3	c.6088C > T	p.(Arg2030*)	Severe		–
41	M4	c.970T > C	p.(Cys324Arg)	N.A	**CRD**	N.A
41	M4	c.970T > C	p.(Cys324Arg)	N.A		N.A
56	M2	c.5882G > A	p.(Gly1961Glu)	Mild	**STGD1**	+
56	M5	c.4383G > A	p.(Trp1461*)	Severe		+
58	M6	c.3259G > A	p.(Glu1087Lys)	Severe	**RCD**	+
58	M6	c.3259G > A	p.(Glu1087Lys)	Severe		+

STGD: Stargardt disease; CRD: cone-rod dystrophy, RCD: rod-cone dystrophy, STGD1: *ABCA4*-STGD.
(+): expected correlation, (−): not expected.
N.A: not applicable.

proband of this family, c.6088C > T, causing a stop codon at Arg2030, is likely to destabilize the messenger RNA by the nonsense-mediated decay mechanism in case the protein is expressed because the affected arginine residue at position 2030 is situated in the second nucleotide-binding domain of ABCA4 protein [48,49]. This variant has been identified before as being associated with STGD1 or CRD [50–52]. The patient of family 41, F41:II.1, whose parents were first cousins, was diagnosed with CRD and was shown to harbor the homozygous missense variant, c.970T > C; p.(Cys324Arg). Interestingly, this variant was only seen once in a compound heterozygous state with c.4316G > T; p.(Gly1439Val) in a Chinese patient with STGD1 [31]. Similarly, the homozygous missense variant in exon 22 of *ABCA4*: c.3259G > A; p.(Glu1087Lys) was associated with RCD in the proband of family 58. This variant was previously found in the compound heterozygous state associated with STGD1 and CRD [53,54].

Although we cannot build on our limited sample size to provide an exact prevalence and frequency for *ABCA4*-related retinopathies, it clearly shows that *ABCA4*-related retinopathies constitute a sizable fraction among the Lebanese IRDs' patients. This observation is supported by worldwide studies which showed *ABCA4* as a major IRD gene [55,56] and a mojor contributor to worldwide carrier frequency [57].

In conclusion, *ABCA4*-related retinopathies were detected in five probands, while one case remained genetically unsolved. When combined with the phenotypic data, our findings show the significant allelic heterogeneity of the *ABCA4* gene. The expanded capabilities of genetic screening, assisted by utilizing high-resolution diagnostic imaging technologies, have enlarged the phenotypic expression spectrum of *ABCA4*-related retinopathies. A thorough understanding of the *ABCA4* variants and their correlations with the phenotype is indispensable to comprehending the association of this gene with different forms of IRDs.

Data availability

The data associated with our study has been deposited into the lovd database (https://databases.lovd.nl/whole_genome/genes/ABCA4). The data is included in the article and the supplementary material.

Confidentiality

The patient and the family IDs are unknown to anyone except the PI: SES.

CRediT authorship contribution statement

Mariam Ibrahim: Writing – original draft, Visualization, Investigation. **Lama Jaffal:** Writing – original draft, Software, Investigation, Data curation. **Alexandre Assi:** Software, Resources. **Charles Helou:** Software, Resources, Investigation, Formal analysis. **Said El Shamieh:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30304.

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