Molecular Vision 2025; 31:555-567 <http://www.molvis.org/molvis/v31/555>
Received 13 November 2024 | Accepted 26 December 2025 | Published 28 December 2025

Molecular epidemiology of *ABCA4*-related retinopathies in Pakistan: report of disease-causing variants in 10 families and review of the literature

Zainab Akhtar,[1] Sumaira Altaf,[2] Yumei Li,[3] Asma Arshad,[1] Sawaira Subhani,[1] Nomana Malik,[1] Meng Wang,[3] Kiran Afshan,[1] Amtul Aziz,[4] Rui Chen,[3] Sabika Firasat[1,5]

[1]Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan; [2]Department of Pediatric Ophthalmology and Strabismus, Al-Shifa Trust Eye Hospital, Jhelum Road, Rawalpindi, Pakistan; [3]Department of Ophthalmology, Center for Translational Vision Research, University of California, Irvine School of Medicine, CA; [4]Diagnostic Center, Al-Shifa Trust Eye Hospital, Jhelum Road, Rawalpindi, Pakistan; [5]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX

Purpose: ABCA4 retinopathies are a group of ABCA4 gene-associated disorders with phenotypic heterogeneity and variable disease severity. The genotype-phenotype correlation is a great challenge for exact disease diagnosis because of symptom variations even within the same family members and the same gene variants giving altered disease phenotypes. This study describes the molecular epidemiology of 10 Pakistani families segregating pathogenic variants of the ABCA4 gene and summarizes *ABCA4*-associated genetic findings from Pakistani families reported until October 2024.
Methods: We enrolled consanguineous Pakistani families having at least one child affected with retinal dystrophy. DNA was extracted from blood samples. Probands were analyzed using capture panel sequencing of 344 known genes for retinal dystrophies. Sanger sequencing was used to perform family segregation testing. To review previously published *ABCA4* disease-causing variants from Pakistan, data were extracted by retrieving articles through online sources—specifically, PubMed and Google Scholar.
Results: Out of 72 families, 10 revealed a total of five reported (c.6658C>T, c.214G>A, c.6088C>T, c.6729+5_6729+19del, and c.6218G>C) and two novel (c.2790C>A and c.1099+5G>A) variants in the ABCA4 gene were segregating in each respective family. Furthermore, one of the novel variants, c.1099+5G>A, was segregating in a compound heterozygous manner along with a c.6658C>T stop-gain variant of the ABCA4 gene in one family. All identified *ABCA4* variants were segregated in an autosomal recessive manner.
Conclusions: The variant c.6658C>T was detected in 50% of families analyzed in this study, but previous reports from Pakistan highlight the c.214G>A variant as a frequent *ABCA4* mutation in cases of Pakistani decent. Identification of two novel pathogenic variants in the present study reaffirms the allelic and genetic heterogeneity of ABCA4 retinopathies in Pakistani patients. The clinical variability or discordance among individuals carrying the same pathogenic variant may be due to other factors influencing the phenotype, including variables such as sex of the individual or role of modifiers that have yet to be identified.

The ATP-binding cassette transporter type A4 (ABCA4; OMIM: 601691) consists of 50 exons that encode a transmembrane protein located at the outer segments of the photoreceptor and internal membrane of retinal pigment epithelial (RPE) cells [1,2]. The molecular mass of the ABCA4 protein is 250 kDa, and it plays an essential role in retinoid recycling in the visual cycle [3]. The human ABCA4 protein, also called ABCR, a transporter of 2,273 amino acids, consists of two dissimilar tandem halves, each half containing a transmembrane domain (TMD), a nucleotide-binding domain (NBD), and a large glycosylated exocytoplasmic domain (ECD), which is present after the transmembrane segment of each half [4,5]. Six membrane-spanning α-helical segments are present as a bundle in each TMD and serve as a pathway for translocation of substrates across the membrane [6,7]. Both TMDs contain an extracellular helix pair, likely to play a role in its proper folding [8] and short transverse intracellular helical segments that function as coupling helices by coordinating the conformational changes in the NBDs upon nucleotide binding [9]. The NBDs in the cytoplasmic region also have two regulatory domains, RD1 and RD2 downstream to NBD1 and NBD2, respectively, which are present close to each other in a head-to-tail arrangement and help to keep the domains close to each other by opposite interactions [10,11]. Among the two ECDs, ECD1 is larger than ECD2, with each domain having four N-linked glycosylation sites [11].

Correspondence to: Sabika Firasat; Quaid-i-Azam University, Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, 44000, Pakistan; Phone: +9290643199; FAX: +9290643170; email: sabika.firasat@qau.edu.pk

In the visual transduction cycle, the ABCA4 protein is the importer of N-retinylidene phosphatidylethanolamine (NrPE) and phosphatidylethanolamine (intermediate compounds in recycling 11-*cis*-retinal) [12]. NrPE and all-*trans*-retinal accumulate within the disc membrane of photoreceptors and condense to form phosphatidyl-pyridinium bisretinoid (A2PE) due to defective ABCA4 protein [13]. The A2PE is hydrolyzed by lysosomal enzymes to nonmetabolizing bisretinoid N-retinyl-N-retinylidene ethanolamine (A2E) [14]. The resulting A2E accumulates within the RPE and forms a major component of lipofuscin, which is toxic to the RPE, accounting for deterioration of the RPE and subsequent loss of the photoreceptor cells [3]. This accumulation of lipofuscin causes diverse phenotypes, also called ABCA4 retinopathies, resulting from dysfunctional ABCA4 protein [12].

ABCA4 retinopathies include a highly variable phenotype of autosomal recessive Stargardt disease (STGD), fundus flavimaculatus, bull's-eye maculopathy, macular atrophy, cone-rod dystrophy (CRD), retinitis pigmentosa (RP), and age-related macular degeneration (AMD) arising from mutations in the ABCA4 gene [15-18]. The estimated incidence of ABCA4 retinopathies is 1 in 8,000 to 10,000 people [15]. *ABCA4* variants cause more than 95% of STGD, 30% of CRD, and 8% of autosomal recessive RP [1,19], and heterozygous mutations are associated with an increased risk of an AMD-like phenotype [20]. In the present study, we identify disease-causing variants in the ABCA4 gene in 10 families using a customized panel sequencing approach and discuss phenotypic and genetic heterogeneity of ABCA4 retinopathies in a Pakistani population based on our findings alongside published literature until October 2024.

METHODS

Ethical approval and enrollment of families: The Bio-Ethical Review Committee of the Faculty of Biologic Sciences, Quaid-i-Azam University Islamabad, Pakistan (protocol # BEC-FBS-QAU2023–491), and Ethical Review Committee, Al-Shifa Trust, Rawalpindi, Pakistan (Reference No: ERC-09/AST-23), approved this study. Families were clinically assessed by ophthalmologists at Al-Shifa Trust Eye Hospital, Rawalpindi, Pakistan, and recruited following the principles of the Declaration of Helsinki for molecular genetic analysis. Blood samples were collected after informed written consent from each participating adult and from parents of participating children of each enrolled family. Genomic DNA extraction and quantification were performed at the Department of Zoology, Quaid-i-Azam University, Islamabad, Pakistan, as per our previously reported method [21].

Capture panel sequencing and bioinformatic analysis: Capture panel sequencing was performed using genomic DNA of two affected individuals of each enrolled family at Baylor College of Medicine, Houston, Texas. Exome enriched genomic libraries were prepared using the KAPA HyperPrep Kit (Roche, Basel, Switzerland) following the manufacturer's protocol, then pooled together for targeted enrichment of a panel of 344 known and candidate inherited retinal disease–related genes (as described in our previous article [21]) with the SureSelect Target Enrichment System for the Illumina Platform (Agilent, Santa Clara, CA [22];). Captured DNA was quantified and sequenced using a Novaseq 6000 (Illumina, San Diego, CA). Variant calling, data alignment, and filtration were performed at the Functional Genomics Core at Baylor College of Medicine, as described in our previous articles [21,22]. Variants passing the filtering steps were evaluated as suggested by the American College of Medical Genetics and Genomics (ACMG) guidelines for their interpretation. Previously reported pathogenic variants were detected and searched through HGMD, ClinVar, and LOVD databases. Novel variants were evaluated for their potential impact on protein function using multiple in silico tools. Nonsense and splice site variants were classified as likely loss-of-function alleles. Missense variants were evaluated based on sequence conservation and in silico predictions.

Sanger sequencing and segregation test: We used the Primer3 web resource to design primers for validating each candidate pathogenic variant using Sanger sequencing. Sanger sequencing was performed on the amplified fragments using genomic DNA of the proband, as well as other affected and unaffected members of each family based on availability of DNA samples.

RESULTS

In the pursuit of determining the disease-causing variants of inherited retinal dystrophies (IRDs), we have solved a large cohort of 72 IRD segregating highly inbred Pakistani families using capture panel sequencing. A total of 10 of 72 families (13.9%) reported in this study were segregating disease-causing variants in the ABCA4 gene (Appendix 1).

Clinical and demographic profiles: Among ABCA4 segregating families, seven families (RD013, RD018, RD030, RD033, RP053, RP171, and RP179) were Punjabi, two (RD008 and RP186) were Pashtun, and one was Muhajir family (RP074). Clinical data of the affected individuals of these families at the time of enrollment are detailed in Table 1. Detailed interviews of elders of each family revealed ethnicity, onset ages, symptoms, and complete family history for drawing pedigrees (Figure 1, Figure 2, Appendix 2 and

Appendix 3). Probands of six families (i.e., RD008, RD013, RD018, RD030, RD033, and RP053) were diagnosed with STGD or juvenile macular dystrophy, whereas probands of four families (i.e., RP074, RP171, RP179, and RP186) were segregating the RP phenotype.

All probands with STGD phenotypes were enrolled at the second decade of their life and had an age of onset by birth (RD008, RD018, RD033, and RP053) or the first decade of life (RD013 and RD030) while those of RP families had congenital onset of disease (Table 1). Disease-associated symptoms were highly variable among families. Visual acuities, a common symptom of ABCA4 retinopathies, were much reduced in clinically affected members, and complete blindness was seen in three affected sisters of family RP179 (IV.V, IV.VI, and IV.IX).

Progressive loss of color vision was observed in cases of RD018 (IV.IV and IV.V), RD030 (IV.I, IV.II, and IV.III), RD033 (IV.IV), and all affected members of RP074 and complete loss in RP179 (IV.V, IV.VI, and IV.IX). Photophobia was observed in RD033 (IV.I), RP171 (IV.I), and all affected members of families RD018, RP074, RP179, and RP186. All RP-diagnosed patients had nyctalopia, while it was only in two patients with the STGD phenotype (i.e., RD008 [III.V] and RD033 [IV.I]). Hemeralopia was present only in affected members of RP179 and RP186. Nystagmus eyes were observed in RD013 (IV.III), RD018 (IV.IV), RD030 (IV.III), RD033 (IV.IV), and RP179 (IV.IX). Representative fundus photographs of affected individuals III.IV, IV.I, and III.III of families RP074, RP171, and RP186, respectively, are shown in Figure 3.

Genetic analysis: For the genetic screening of 72 consanguineous IRD-affected families, capture panel sequencing followed by Sanger sequencing validation was performed. Ten families (13.9%) were identified with seven pathogenic variants in the ABCA4 gene, including five loss-of-function alleles (three stop-gain and two splice site) and two missense variants. One stop-gain variant (i.e., c.6658C>T) in the ABCA4 gene was detected in 5 of 10 families (50%) with different disease phenotypes (Table 2). From identified variants, five have been previously reported, and two are newly identified in the current study. Eight disease-causing variants segregated with the disease phenotype in an autosomal recessive homozygous form, whereas in one family (RP171), a missense variant c.6218G>C causing p.(Gly2073Ala) was found as a homozygous allele in one affected case (IV.III in Appendix 3) and as a heterozygous allele in three affected and two phenotypically normal individuals. Furthermore, two heterozygous variants (i.e., c.6658C>T and c.1099+5G>A) were segregating in a compound heterozygous manner with disease phenotype in one family (i.e., RP186). Detailed pedigrees with identified novel and reported variants showing protein change are given in Figure 1, Figure 2, Appendix 2 and Appendix 3, respectively. All the identified variants were classified as per ACMG guidelines, and their details are provided in Table 2.

Among ABCA4 gene variants identified in this study, a previously reported stop-gain variant, c.6658C>T in exon 48, causing premature protein termination (i.e., p.(Gln2220*)), was segregating in a homozygous recessive manner with disease phenotype in one Muhajir (RP074) and three Punjabi consanguineous families (i.e., RD013, RD018, and RP179). Segregation of this variant was confirmed in two affected and one unaffected member of STGD family RD013, two affected and two unaffected members of STGD family RD018, and three affected and three unaffected members of RP segregating families RP074 and RP179, supporting pathogenicity (Appendix 2and Appendix 4). This variant has a gnomAD (v2.1.1) frequency of 0.00005171. The same variant was observed in the RP186 family with Pashtun ethnicity in a compound heterozygous form with a novel splice site variant c.1099+5G>A (Table 2). Both heterozygous variants were segregating in two affected and two unaffected members with the RP phenotype (Figure 2A-C). The novel splice site variant is absent from the gnomAD (v2.1.1) database and is predicted to alter the correct splicing of the transcript with the Splice AI score for acceptor loss (delta score of 0.36, 245 bp), donor loss (delta score of 0.51, 5 bp), acceptor gain (delta score of 0.01, −66 bp), and donor gain (delta score of 0.47, 88 bp). Among splice site variants identified in the current study, a previously reported splice site variant, c.6729+5_6729+19del, was found recessively segregating with the STGD phenotype in family RP053. The segregation of the variant was confirmed by genotyping five affected and three unaffected family members (Appendix 3 and Appendix 4). The reported gnomAD (v2.1.1) allele frequency of this large deletion is 0.00006783.

A novel stop-gain variant, c.2790C>A, was observed as a homozygous allele in a Pashtun family (RD008) with one STGD disease-affected male member who had congenital onset of phenotype and one asymptomatic female (III.I in Figure 3A) who was 6 years old at the time of enrollment for this study (Figure 3A, B, Table 1). Both homozygous children belonged to unaffected nonconsanguineous parents of the same ethnicity. This observed variant in exon 19 of the ABCA4 gene is absent from the gnomAD (v2.1.1) database and results in premature termination of protein p.(Cys930*) and is predicted to remove 59% of the encoded protein that may cause nonsense-mediated decay (NMD). Another

Table 1. Demographic and clinical features of probands of 10 *ABCA4* retinopathies families described in this study.

Sr. No.	Family ID	Proband ID	Ethnicity	No. of cases in family	Disease	Age in years at		Visual acuity		Symptoms			
						Enrollment	onset	(OD)	(OS)	DCV	Photophobia	Nyctalopia	Others
1	RD008	III.V	Pashtun	2	STGD	12	By birth	6/60*	6/60*	-	+	+	Hyperopia, Astigmatism
2	RD013	IV.IV	Punjabi	2	STGD	18	5	6/96*	7/76*	-	-	-	-
3	RD018	IV.IV	Punjabi	2	STGD	11	By birth	6/120*	6/76*	+	+	-	Hyperopia, Nystagmus
4	RD030	IV.III	Punjabi	5	STGD	19	8	6/120*	6/120*	+	-	-	Nystagmus
5	RD033	IV.I	Punjabi	3	STGD	18	By birth	6/120*	6/60*	+	+	-	Astigmatism, Nystagmus
6	RP053	V.IX	Punjabi	7	STGD	12	By birth	N/A	N/A	-	-	+	-
7	RP074	III.IV	Muhajir	3	RP	34	By birth	N/A	N/A	+	+	+	-
8	RP171	IV.I	Punjabi	5	RP	28	By birth	6/24**	6/9**	-	+	+	-
9	RP179	IV.V	Punjabi	4	RP	70	By birth	No vision	No vision	+	+	+	Hemeralopia, cataract, Nystagmus
10	RP186	III.III	Pashtun	2	RP	26	By birth	1/60**	2/60**	-	+	+	Hemeralopia

Abbreviations: STGD: Stargardt disease, RP: Retinitis Pigmentosa OD: right eye OS: left eye DCV: Defective Color Vision, N/A: Not Available. The * symbol indicates the visual acuity was calculated using Early Treatment Diabetic Retinopathy Study (ETDRS) chart, The ** symbol indicates the visual acuity was measured using Cardiff card.

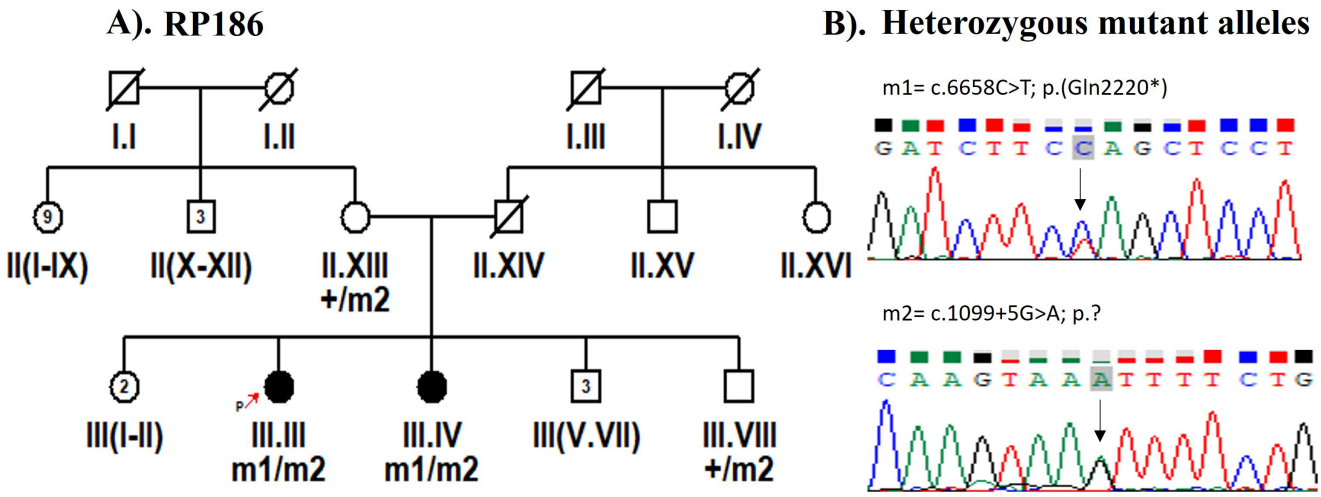

Figure 1. Pedigree of family RP186. **A**. Pedigree of family RP186 shows the segregation of the two heterozygous *ABCA4* single nucleotide substitutions, c.6658C>T causing p.(Gln2220*) and a novel splice site change c.1099+5G>A in a compound heterozygous manner, in two affected and two unaffected individuals. **B**. The sequence chromatograms highlight the heterozygous mutant allele, c.6658C>T and c.1099+5G>A of *ABCA4*, on the left side. Empty squares and circles in the pedigree show the unaffected males and females, respectively. The filled shapes show the affected individuals. The symbol labeled with a red arrow in the pedigree highlights the proband.

stop-gain variant, c.6088C>T in exon 44 of the ABCA4 gene, was found segregating with the STGD phenotype in a Punjabi family (RD033). Validation of segregation of this variant in an autosomal recessive manner with the STGD phenotype was confirmed by genotyping three affected and five unaffected members (Appendix 3 and Appendix 4). This premature termination of the p.(Arg2030*) variant has a reported gnomAD (v2.1.1) frequency of 0.00002784 and is predicted to remove more than 10% of the encoded protein that may lead to NMD.

Two Punjabi families, RD030 and RP171, were segregating previously reported missense variants. The variant p.(Gly72Arg), caused by a single base transition (i.e., c.214G>A in exon 3), was segregating in an autosomal recessive manner in family RD030 (Appendix 3 and Appendix 4). This family had five affected members diagnosed with the STGD phenotype in two consecutive generations. Three siblings were homozygous for the variants from a heterozygous carrier mother. This variant is classified as pathogenic or likely pathogenic in ClinVar for ABCA4 retinopathies with an allele frequency of 0.00002784 in gnomAD (v2.1.1). Interestingly, all affected individuals of the RP171 family except one (IV.III) were heterozygous for disease-causing variant c.6218G>C, which substitutes glycine to alanine, p.(Gly2073Ala; Appendix 3 and Appendix 4). Family RP171 is a consanguineous Punjabi family with an affected father and four affected children in two generations, among whom one affected (IV.III) was homozygous for a mutant allele (Appendix 3). This variant is reported as a variant of uncertain significance in ClinVar for ABCA4 retinopathy and had an allele frequency of 0.00009546 in gnomAD (v2.1.1).

Next, we performed an extensive literature search for previously reported *ABCA4* variants from Pakistani families until October 2024 and listed them in Table 3 along with findings of the current study (Table 3). In conclusion, in addition to two novel disease-causing variants p.(Cys930*) and c.1099+5G>A, the two previously reported mutations p.(Arg2030*) and p.(Gly2073Ala) were identified for the first time in patients of Pakistani descent in this study.

DISCUSSION

In this study, we report 10 families ascertained from different regions of Pakistan, segregating different retinopathies due to disease-causing variants in *ABCA4* genes. Our literature search to catalog all the disease-causing variants described until October 2024 in the *ABCA4* gene from Pakistan and identification of the two novel disease-causing variants described in the present study summarizes a total of 16 disease-causing variants in *ABCA4* from sporadic and familial cases of the study population (Table 3). These include 10 missense, 4 nonsense, and 2 splice site mutations (Table 3). These mutations are scattered across the gene (Table 3), suggesting that its entire coding region needs to be sequenced in an affected individual of Pakistani decent for molecular genetic testing.

The results of our genetic analysis affirmed the allele c.6658C>T causing p.(Gln2220*), with a disease allele frequency of 45% (9/20 mutant alleles) as the most common *ABCA4* variant in our analyzed families (Table 2), but our literature review revealed p.(Gly72Arg) as the most common *ABCA4* mutant allele in cases of Pakistani descent, followed by p.(Gln2220*; Table 3) [23-29], with an allele frequency of 37.5% (21/56) and 19.6% (11/56), respectively (Table 3). It has been reported that severity of disease due to ABCA4 gene variants varies according to genotype, which can be specific to ethnic and racial groups [27]. Therefore, the report of the c.214G>A variant as a founder mutation from northwestern Pakistan with the presence in 50% of analyzed families [23] and increased frequency of the p.(Gln2220*) variant in our studied families, including three Punjabi, one Muhajir, and one of Pashtun ethnicity, explains ethnicity-specific variability of *ABCA4* retinopathies in Pakistan. Consistent with these findings, a splice site variant (i.e., c.6729+5_6729+19del) has been previously reported to cause IRD in four families from Sindh, Pakistan [24], but we identified this variant in only one family of Punjabi origin (RP053; Table 1, Table 2, Table 3). The association of region-specific homozygous founder mutations with a disease explains an increased risk of autosomal recessive diseases in our population due to high rates of consanguineous unions.

The first evidence of involvement of *ABCA4* variants in retinopathies was provided by Allikrnets et al. in 1997 [30]. Since then, homozygous and compound heterozygous mutations of *ABCA4* have been identified as a cause of various autosomal recessive retinopathies, whereas heterozygous mutations were found responsible for dominantly inherited age-related macular degeneration in both humans and mice [30-32]. Our results are consistent with these data as we identified homozygous disease-causing variants in nine consanguineous families and compound heterozygous pathogenic variants in one nonconsanguineous family (RP186; Table 2, Figure 1A-C). One interesting observation is the segregation of the c.6218G>C variant causing p.(Gly2073Ala) in

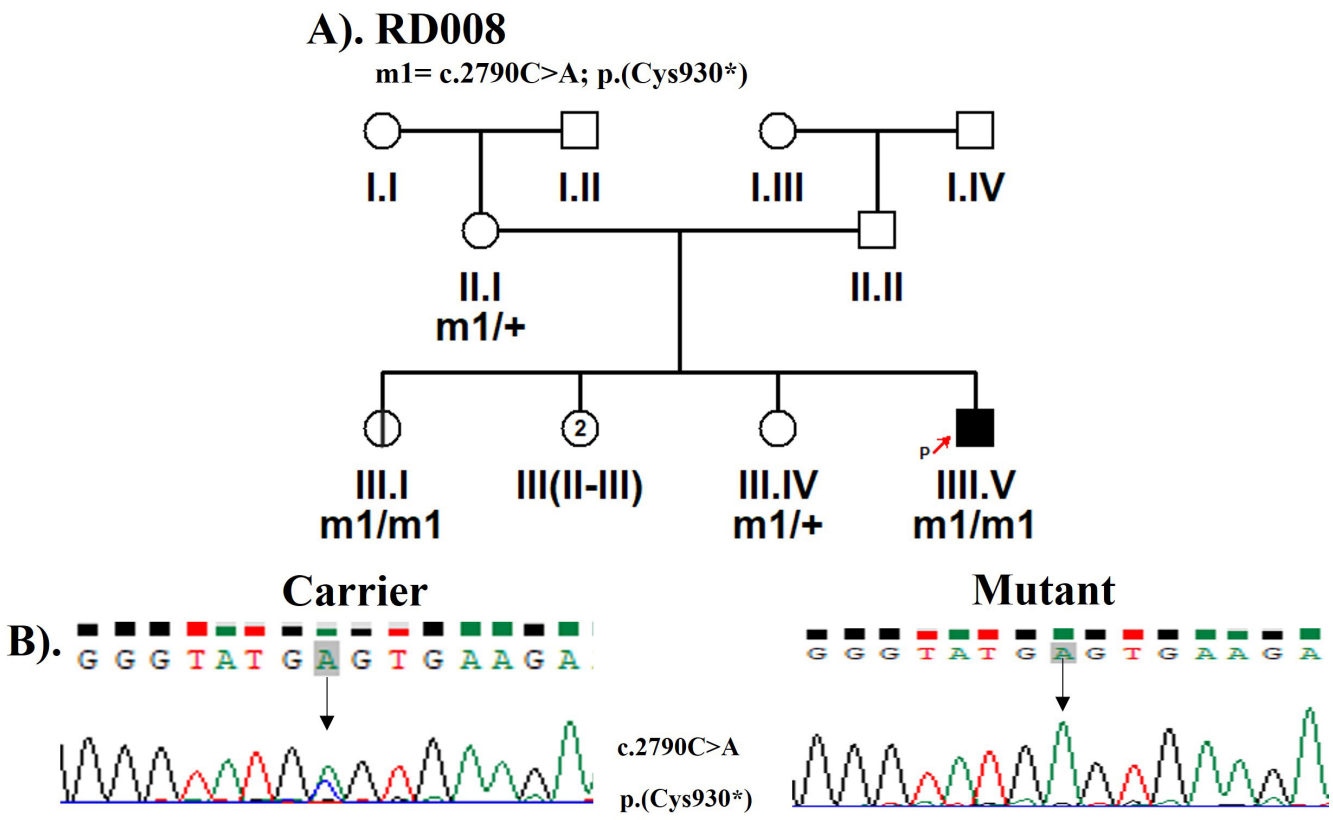

Figure 2. Pedigree of family RD008. **A**. Pedigree of family RD008 shows the segregation of the novel *ABCA4* variant, c.2790C>A leading to p.(Cys930*), in an autosomal recessive manner. **B**. The sequence chromatograms highlight carrier status and a homozygous mutant allele on the right and left sides, respectively. Empty squares and circles in the pedigree show the unaffected males and females, respectively. The filled shapes show the affected individuals. The symbol labeled with a red arrow in the pedigree highlights the proband. The circle with a vertical line in the center shows the presymptomatic female with a homozygous mutant allele who may manifest disease later.

the RP171 family as this variant is found in a homozygous condition in one affected individual, but, like the unaffected individual, three other affected cases are heterozygous (Appendix 3). This variant has been previously reported as a likely pathogenic allele in a patient with unsolved Stargardt disease by Corradi et al. [33]. They labeled this patient as unsolved as they performed single-molecule molecular inversion probes (smMIP)s-based sequencing of the ABCA4 gene only, and a monoallelic variant was identified in this sample that is not expected to lead to *ABCA4*-associated retinopathy on its own, which is consistent with our findings in RP171. Here, we performed capture panel sequencing; thus, the missing second mutant allele could be identified through whole-genome sequencing of the RP171 family in the future to identify the other variant in a novel IRD gene yet to be identified or a potential regulatory variant in an upstream and downstream region of *ABCA4*. However, based on an estimated 14% to 15% carriership of *ABCA4* pathogenic variants in the general population [34] and most *ABCA4* variant alleles being missense (Table 3), the identification of a heterozygous missense variant could be a chance finding. There is a possibility that the father and the other two affected siblings of IV.III of RP171 (Appendix 3 and Appendix 4) may have a homozygous disease-causing variant in an IRD-associated gene yet to be identified since in highly consanguineous populations, segregation of two disease-causing genes within the same family is also reported [26,35].

Interestingly, we identified *ABCA4* as the commonly mutated gene with disease-causing alleles in 10 of 72 (13.9%) analyzed families in contrast to the recent report by Munir et al., who reported PDE6A as the topmost mutated gene in patients with IRDs from Pakistan [36]. Furthermore, 8 of

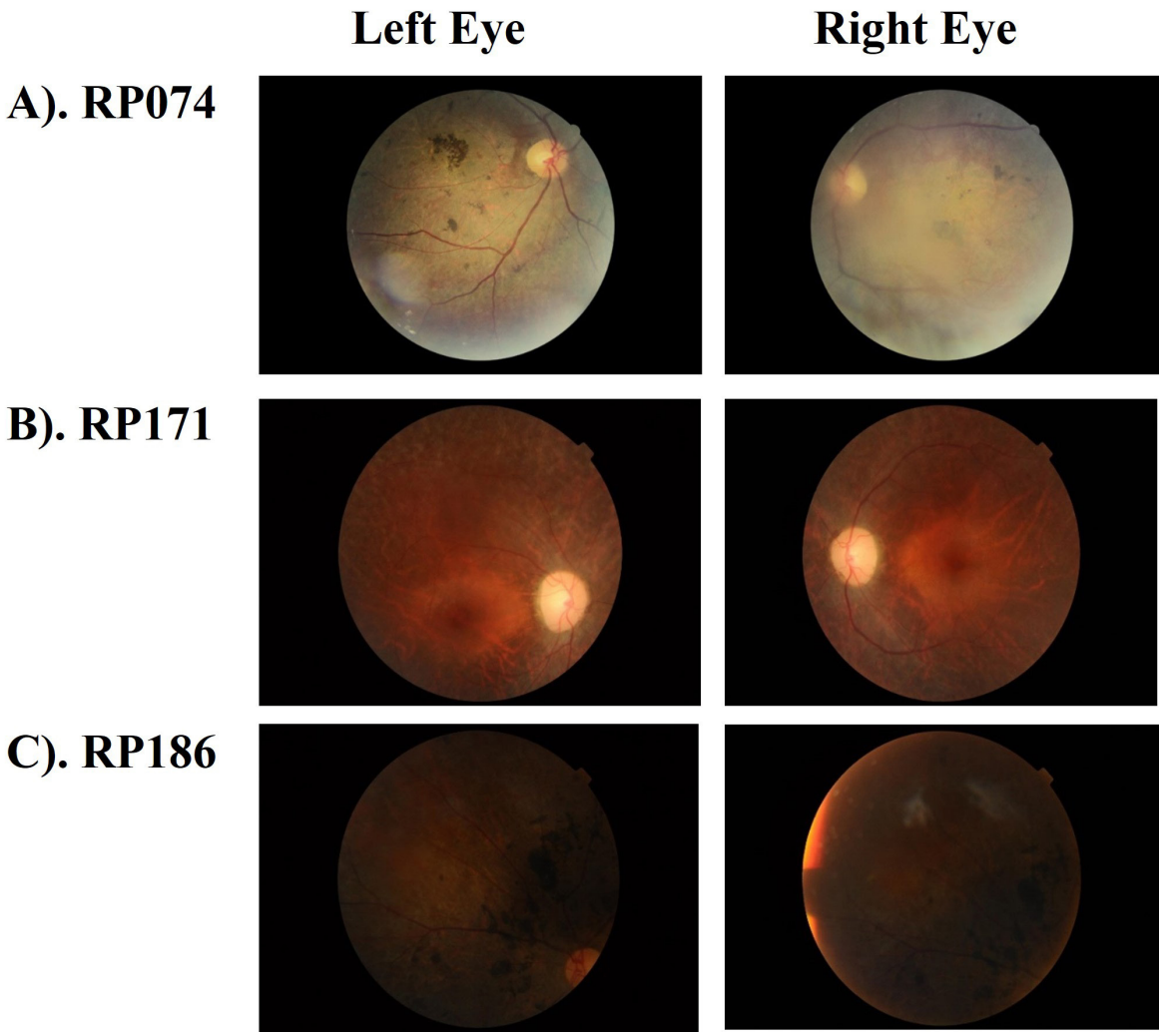

Figure 3. Fundus photographs. Fundus photographs of the left and right eyes of probands of family RP074 (**A**), RP171 (**B**), and RP186 (**C**).

Table 2. List of identified variants of *ABCA4* gene in 10 consanguineous Pakistani families analyzed in this study.

Sr. No.	Family ID	Accession number	Zygosity	Variant	Nucleotide change	Protein change	db SNP ID	ClinVar ID/Classification/Curated classification (codes met)
1	RD008	(NM_000350.3)	Homo	CHR1:94512603G>T	c.2790C>A	p. (Cys930*)	NA	NA/ Likely Pathogenic (PM2, PVS1) *
2	RD013	(NM_000350.3)	Homo	CHR1:94463488G>A	c.6658C>T	p. (Gln2220*)	rs61753046	99,476/ Pathogenic
3	RD018	(NM_000350.3)	Homo	CHR1:94463488G>A	c.6658C>T	p. (Gln2220*)	rs61753046	99,476/ Pathogenic
4	RD030	(NM_000350.3)	Homo	CHR1:94577082C>T	c.214G>A	p. (Gly72Arg)	rs61751412	99,120/ Pathogenic
5	RD033	(NM_000350.3)	Homo	CHR1:94471056G>A	c.6088C>T	p. (Arg2030*)	rs61751383	7907/ Pathogenic
6	RP053	(NM_000350.3)	Homo	CHR1:94463397GTGCCCCAGGGCCAAC>G	c.6729+5_6729+19del	p.?	rs749526785	283,573/ Pathogenic
7	RP074	(NM_000350.3)	Homo	CHR1:94463488G>A	c.6658C>T	p. (Gln2220*)	rs61753046	99,476/ Pathogenic
8	RP171	(NM_000350.3)	Homo/ Hetero	CHR1:94467478C>G	c.6218G>C	p. (Gly2073Ala)	rs752850266	429,847/ Uncertain significance (PM2_Supporting, PP3, PP1) *
9	RP179	(NM_000350.3)	Homo	CHR1:94463488G>A	c.6658C>T	p. (Gln2220*)	rs61753046	99,476/ Pathogenic
10	RP186	(NM_000350.3)	Hetero	CHR1:94463488G>A	c.6658C>T	p. (Gln2220*)	rs61753046	99,476/ Pathogenic
		(NM_000350.3)	Hetero	CHR1:94546029C>T	c.1099+5G>A	p.?	NA	NA/ Likely Pathogenic (PM2, PVS1) *

*Curated variant classification of novel and previously reported variants of uncertain significance is given based on data of present study as per ACMG guidelines. PM2: Pathogenic moderate 2 [Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium] PVS1: Pathogenic very strong [null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene here LOF is a known mechanism of disease)] BS1: Benign Strong 1 [Allele frequency is greater than expected for disorder] PP1:[Cosegregation with disease in multiple affected family members in a gene definitively known to cause disease] PP3: Pathogenic supporting 3 [Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)] Sequence variant nomenclature was obtained according to the guidelines of the Human Genome Variation Society (HGVS) by using Mutalyzer. The position of variants is according to GRCh37/hg19 reference genome assembly.

Table 3. A list of *ABCA4* variants previously reported and identified in this study (those identified for the first time in this study are highlighted as red) in cases of Pakistani decent along with disease sub type.

Sr. No.	Exon	Nucleotide cChange	Protein change	Variant type	Familial/ sporadic	No of alleles	Onset age	Disease	Reference
						2	8 years	STGD	This study
1	3	c.214G>A	p. (Gly72Arg)	Missense	Familial	19	NA	MD	[23]
						NA	NA	IRD	[24]
2	19	c.2790C>A	p. (Cys930*)	Stop gain	Familial	2	By birth	STGD	This study
3	21	c.3081T>G	p. (Tyr1027*)	Stop gain	Familial	1	NA	MD	[23]
4	23	c.3364G>A	p. (Glu1122Lys)	Missense	Familial	2	NA	STGD	[25]
					Familial	2	2 years	IRD	[26]
5	42	c.5882G>A	p. (Gly1961Glu)	Missense	Sporadic	1	2nd decade	IRD	[27]
6	44	c.6088C>T	p. (Arg2030*)	Stop gain	Familial	2	By birth	STGD	This study
7	45	c.6218G>C	p. (Gly2073Ala)	Missense	Familial	1	By birth	RP	This study
						5	By birth	STGD	This study
8	48	c.6658C>T	p. (Gln2220*)	Stop gain	Familial	4	By birth	RP	This study
						2	10–15 years	RP	[28]
						2	By birth	RP	This study
9	Intron 48	c.6729+5_6729+19del	p.?	Splice Site	Familial	NA	NA	IRD	[24]
					Sporadic	2	30 years	CRD	[29]
10	Intron 8	c.1099+5G>A	p.?	Splice Site	Familial	1	By birth	RP	This study
11	2	c.91T>C	p. (Trp31Arg)	Missense	Sporadic	2	1st decade	IRD	[27]
12	14	c.2023G>A	p. (Val675Ile)	Missense	Sporadic	1	5th decade	IRD	[27]
13	37	c.5243G>A	p. (Gly1748Glu)	Missense	Sporadic	1	2nd decade	IRD	[27]
14	40	c.5646G>A	p. (Met1882Ile)	Missense	Sporadic	1	5th decade	IRD	[27]
15	44	c.6098T>A	p. (Leu2033His	Missense	Sporadic	1	2nd decade	IRD	[27]
16	46	c.6317G>A	p. (Arg2106His)	Missense	Sporadic	1	2nd decade	IRD	[27]

10 of these families were segregating null variants of the ABCA4 gene, which is considered an important determinant of the disease severity and age of onset [4,31]. Here, the age of onset of disease was by birth or within 5 to 7 years of age in phenotypically affected individuals of all our analyzed families carrying null variants (Table 1 and Table 2), and patients reported more rapid disease progression as it is documented that patients with *ABCA4* retinopathy with early onset typically have a more severe phenotype and more rapid disease progression [4]. Furthermore, we observed clinical discordance in siblings of the RD008 family carrying the same homozygous *ABCA4* mutation (Figure 3A). One of the siblings (III.V in Figure 3A), homozygous for the c.2790C>A disease-causing variant causing p. Cys930*, was by birth a phenotypically affected male, whereas the other sibling (III.I in Figure 3A) was an asymptomatic female until 6 years of age with the same homozygous genotype for the mutant allele. Previous studies have highlighted incomplete penetrance and clinical discordance of disease in siblings carrying the same *ABCA4* gene mutations [37-39]. Close monitoring of an asymptomatic female (III.I in Figure 3A) of RD008 is crucial to identify difference in age of onset and discordant phenotype between siblings, but further research is required to unveil the unknown disease modifier.

The ABCA4 gene is reported in association with diverse IRD phenotypes, including STGD, RP, and CRD [19]. We identified a previously reported stop-gain variant, c.6658C>T: p.(Gln2220*), segregating in a homozygous manner in four families, each of which was initially diagnosed having a different phenotype: STDG (RD013 and RD018) and RP (RP074 and RP179). Two affected individuals of a Pashtun family (RP186) were segregating the p.(Gln2220*) variant along with a novel splice site variant c.1099+5G>A in a compound heterozygous manner with the RP phenotype. Previously, this variant, p.(Gln2220*), was first identified as a heterozygous allele in a single affected case of CRD in a study conducted on populations from Germany and the Netherlands [19]. The same variant was reported later in two male cousins affected with CRD whose parents were consanguineous and heterozygous carriers for this variant [40]. Interestingly, this variant is also reported in a Pakistani consanguineous family having seven affected members with the RP phenotype [28]. Similarly, the reported single nucleotide substitution c.6088C>T, leading to a stop-gain variant p.(Arg2030*) identified in STGD-segregating Punjabi family RD033, has also been previously reported to cause the STGD phenotype [41-43], rapid-onset chorioretinopathy phenotype [44], and RP [45] in cases from different ethnicities. Furthermore, a large splice site variant, c.6729+5_6729+19del, observed in family RP053 diagnosed with STGD, was reported for the first time in a nonconsanguineous patient with CRD of Pakistani descent [29]. Later on, this variant was reported in an Indian male patient [46] as well as in four Pakistani families from Sindh province, Pakistan [24], with a general diagnosis of IRD. In this study, the main limitations explaining genetic variants to phenotypic diversity are the unavailability of advanced clinical tests, including electroretinography and optical coherence tomography, due to a lack of these diagnostic facilities at the collaborating hospital(s). The observations of differences in the clinical manifestation, variable penetrance, and clinical variability of the disease in patients carrying the same mutant allele highlight the challenge of diagnosing IRDs, more specifically *ABCA4*-related retinopathies, and explain the hindrance to define genotype-phenotype correlations in this and similar studies. This shows the infrastructure challenges of Pakistan's health care system and a need to invest in better clinical diagnostic capabilities to capture more comprehensive clinical data for future research. Furthermore, the availability of molecular genetic evaluation should be an unavoidable benchmark for the diagnosis and management of patients with these conditions.

APPENDIX 1. FREQUENCY DISTRIBUTION OF IDENTIFIED VARIANTS IN 72 IRDS ANALYZED FAMILIES.

To access the data, click or select the words "Appendix 1." The Pie chart show frequency distribution of identified variants in IRDs reported genes in seventy two Pakistani families. Each color is representing frequency of respective gene while the category "others" is used for the group of retinal dystrophy genes identified only in each single family.

APPENDIX 2. PEDIGREES OF FOUR FAMILIES RD013, RD018, RP074 AND RP179.

To access the data, click or select the words "Appendix 2." Pedigrees of four families (RD013, RD018, RP074 and RP179) in which same reported variant c.6658C>T; p. (Gln2220*) of *ABCA4* was identified. Empty squares and circles show the unaffected males and females, respectively. The filled shapes show the affected individuals. The symbol labeled with a red arrow in each pedigree highlights the proband. Consanguineous unions are indicated by double lines.

APPENDIX 3. PEDIGREES OF FOUR INBRED FAMILIES RD030, RD033, RP053 AND RP171.

To access the data, click or select the words "Appendix 3." Pedigrees of four inbred families RD030, RD033, RP053 and RP171 in which reported variants c.214G>A; p. (Gly72Arg), c.6088C>T; p. (Arg2030*), c.6729+5_6729+19del; p.? and

c.6218G>C; p. (Gly2073Ala) respectively in *ABCA4* were identified respectively. Empty squares and circles show the unaffected males and females, respectively. The filled shapes show the affected individuals. The symbol labeled with a red arrow in each pedigree highlights the proband. Consanguineous unions are indicated by double lines.

APPENDIX 4. SEQUENCE CHROMATOGRAMS OF FAMILIES WITH REPORTED VARIANTS IN *ABCA4.*

To access the data, click or select the words "Appendix 4." (A-E). The wild-type/carrier sequences are given on the left side and the variant sequences are given on the right side.

ACKNOWLEDGMENTS

We thank patients and their families for their participation in this study. Rui Chen is a co-corresponding author and email addresses of both corresponding authors are sabika.firasat@qau.edu.pk and ruic20@hs.uci.edu. Author Contribution: Sabika Firasat, Kiran Afshan and Rui Chen contributed to the conceptualization and design. Sumaira Altaf and Amtul Aziz performed clinical evaluation of patients. Zainab Akhtar, Asma Arshad, Sawaira Subhani and Nomana Malik enrolled families and collected data. Zainab Akhtar, Yumei Li and Sabika Firasat performed experiments and data analysis. Zainab Akhtar, Yumei Li, Kiran Afshan, Sabika Firasat and Rui Chen prepared the first draft of the manuscript. All authors read and approved the content and submission of the final manuscript. Conflict of interest: We have no conflict of interest. Funding: This study was financially supported (in part) by the University Research Fund (URF) from Quaid-i-Azam University, Islamabad, Pakistan and access to scientific instrumentation grant (ASIP/R&D/HEC/2023/10005/73043/88) from Higher Education Commission, Islamabad, Pakistan. This work is also supported by grants from the National Eye Institute [EY022356, EY018571, EY002520, P30EY010572, EY09076, EY030499]; Retinal Research Foundation; NIH shared instrument grant [S10OD023469]. The authors acknowledge support to the Gavin Herbert Eye Institute at the University of California, Irvine from an unrestricted grant from Research to Prevent Blindness and from NIH grant P30 EY034070. Rui Chen

REFERENCES

1. Sangermano R, Khan M, Cornelis SS, Richelle V, Albert S, Garanto A, Elmelik D, Qamar R, Lugtenberg D, van den Born LI, Collin RWJ, Cremers FPM. *ABCA4* midigenes reveal the full splice spectrum of all reported noncanonical splice site variants in Stargardt disease. Genome Res 2018; 28:100-10. [PMID: 29162642].
2. Ng ESY, Kady N, Hu J, Dave A, Jiang Z, Pei J, Gorin MB, Matynia A, Radu RA. Membrane attack complex mediates retinal pigment epithelium cell death in Stargardt macular degeneration. Cells 2022; 11:3462-[PMID: 36359858].
3. Tsybovsky Y, Molday RS, Palczewski K. The ATP-binding cassette transporter ABCA4: structural and functional properties and role in retinal disease. Adv Exp Med Biol 2010; 703:105-25. .
4. Al-Khuzaei S, Broadgate S, Foster CR, Shah M, Yu J, Downes SM, Halford S. An overview of the genetics of ABCA4 retinopathies, an evolving story. Genes (Basel) 2021; 12:1241-[PMID: 34440414].
5. Bungert S, Molday LL, Molday RS. Membrane topology of the ATP binding cassette transporter ABCR and its relationship to ABC1 and related ABCA transporters: identification of N-linked glycosylation sites. J Biol Chem 2001; 276:23539-46. [PMID: 11320094].
6. Coleman JA, Quazi F, Molday RS. Mammalian P4-ATPases and ABC transporters and their role in phospholipid transport. Biochim Biophys Acta 2013; 1831:555-74. [PMID: 23103747].
7. Thomas C, Tampé R. Structural and mechanistic principles of ABC transporters. Annu Rev Biochem 2020; 89:605-36. [PMID: 32569521].
8. Garces FA, Scortecci JF, Molday RS. Functional Characterization of ABCA4 Missense Variants Linked to Stargardt Macular Degeneration. Int J Mol Sci 2020; 22:185-[PMID: 33375396].
9. Kim Y, Chen J. Molecular structure of human P-glycoprotein in the ATP-bound, outward-facing conformation. Science 2018; 359:915-9. [PMID: 29371429].
10. Qian H, Zhao X, Cao P, Lei J, Yan N, Gong X. Structure of the human lipid exporter ABCA1. Cell 2017; 169:1228-39. .
11. Liu F, Lee J, Chen J. Molecular structures of the eukaryotic retinal importer ABCA4. Elife 2021; 10:e63524[PMID: 33605212].
12. Cremers FPM, Lee W, Collin RWJ, Allikmets R. Clinical spectrum, genetic complexity and therapeutic approaches for retinal disease caused by ABCA4 mutations. Prog Retin Eye Res 2020; 79:100861[PMID: 32278709].
13. Ben-Shabat S, Parish CA, Vollmer HR, Itagaki Y, Fishkin N, Nakanishi K, Sparrow JR. Biosynthetic studies of A2E, a major fluorophore of retinal pigment epithelial lipofuscin. J Biol Chem 2002; 277:7183-90. [PMID: 11756445].
14. Sparrow JR, Kim SR, Cuervo AM, Bandhyopadhyayand U. A2E, a pigment of RPE lipofuscin, is generated from the precursor, A2PE by a lysosomal enzyme activity. Recent Advances in Retinal Degeneration: Springer; 2008. p. 393–8.
15. Mizobuchi K, Hayashi T, Tanaka K, Kuniyoshi K, Murakami Y, Nakamura N, Torii K, Mizota A, Sakai D, Maeda A, Kominami T, Ueno S, Kusaka S, Nishiguchi KM, Ikeda Y, Kondo M, Tsunoda K, Hotta Y, Nakano T. Genetic and

Clinical Features of ABCA4-Associated Retinopathy in a Japanese Nationwide Cohort. Am J Ophthalmol 2024; 264:36-43. [PMID: 38499139].

16. Klevering BJ, Deutman AF, Maugeri A, Cremers FPM, Hoyng CB. The spectrum of retinal phenotypes caused by mutations in the ABCA4 gene. Graefes Arch Clin Exp Ophthalmol 2005; 243:90-100. [PMID: 15614537].

17. Cremers FPM, van de Pol DJR, van Driel M, den Hollander AI, van Haren FJJ, Knoers NVAM, Tijmes N, Bergen AA, Rohrschneider K, Blankenagel A, Pinckers AJ, Deutman AF, Hoyng CB. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. Hum Mol Genet 1998; 7:355-62. [PMID: 9466990].

18. Maugeri A, van Driel MA, van de Pol DJ, Klevering BJ, van Haren FJ, Tijmes N, Bergen AA, Rohrschneider K, Blankenagel A, Pinckers AJ, Dahl N, Brunner HG, Deutman AF, Hoyng CB, Cremers FP. The 2588G–>C mutation in the ABCR gene is a mild frequent founder mutation in the Western European population and allows the classification of ABCR mutations in patients with Stargardt disease. Am J Hum Genet 1999; 64:1024-35. [PMID: 10090887].

19. Maugeri A, Klevering BJ, Rohrschneider K, Blankenagel A, Brunner HG, Deutman AF, Hoyng CB, Cremers FP. Mutations in the ABCA4 (ABCR) gene are the major cause of autosomal recessive cone-rod dystrophy. Am J Hum Genet 2000; 67:960-6. [PMID: 10958761].

20. Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, Zabriskie NA, Li Y, Hutchinson A, Dean M, Lupski JR, Leppert M. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science 1997; 277:1805-7. [PMID: 9295268].

21. Tehreem R, Chen I, Shah MR, Li Y, Khan MA, Afshan K, Chen R, Firasat S. Exome Sequencing Identified Molecular Determinants of Retinal Dystrophies in Nine Consanguineous Pakistani Families. Genes (Basel) 2022; 13:1630-[PMID: 36140798].

22. Wang F, Wang H, Tuan H-F, Nguyen DH, Sun V, Keser V, Bowne SJ, Sullivan LS, Luo H, Zhao L, Wang X, Zaneveld JE, Salvo JS, Siddiqui S, Mao L, Wheaton DK, Birch DG, Branham KE, Heckenlively JR, Wen C, Flagg K, Ferreyra H, Pei J, Khan A, Ren H, Wang K, Lopez I, Qamar R, Zenteno JC, Ayala-Ramirez R, Buentello-Volante B, Fu Q, Simpson DA, Li Y, Sui R, Silvestri G, Daiger SP, Koenekoop RK, Zhang K, Chen R. Next generation sequencing-based molecular diagnosis of retinitis pigmentosa: identification of a novel genotype-phenotype correlation and clinical refinements. Hum Genet 2014; 133:331-45. [PMID: 24154662].

23. Rehman AU, Peter VG, Quinodoz M, Rashid A, Khan SA, Superti-Furga A, Rivolta C. Exploring the genetic landscape of retinal diseases in North-Western Pakistan reveals a high degree of autozygosity and a prevalent founder mutation in ABCA4. Genes (Basel) 2019; 11:12-[PMID: 31877759].

24. Fatima M, Zakaria M, Akbar F, Jeeva I, Tayyab H, Siddiqui R, Kirmani S. P368: Disease-causing variants in inherited retinal diseases genes in an ethnically diverse Pakistani population: Experience from an academic medical center. Genetics in Medicine Open. 2023; 1:100396.

25. D'Atri I. Defining the genetic and molecular basis of inherited eye diseases present in Pakistan: University of Exeter (United Kingdom); 2020.

26. Biswas P, Duncan JL, Maranhao B, Kozak I, Branham K, Gabriel L, Lin JH, Barteselli G, Navani M, Suk J, Parke M, Schlechter C, Weleber RG, Heckenlively JR, Dagnelie G, Lee P, Riazuddin SA, Ayyagari R. Genetic analysis of 10 pedigrees with inherited retinal degeneration by exome sequencing and phenotype-genotype association. Physiol Genomics 2017; 49:216-29. [PMID: 28130426].

27. Lee W, Schuerch K, Zernant J, Collison FT, Bearelly S, Fishman GA, Tsang SH, Sparrow JR, Allikmets R. Genotypic spectrum and phenotype correlations of ABCA4-associated disease in patients of south Asian descent. Eur J Hum Genet 2017; 25:735-43. [PMID: 28327576].

28. Hussain A, Shahzad A, Venselaar H, Bokhari H, de Wijs IJ, Hoefsloot LH. Homozygosity mapping identifies genetic defects in four consanguineous families with retinal dystrophy from Pakistan. GENETIC BASIS OF INHERITED EYE DISEASES. 2013:107.

29. Littink KW, Koenekoop RK, van den Born LI, Collin RW, Moruz L, Veltman JA, Roosing S, Zonneveld MN, Omar A, Darvish M, Lopez I, Kroes HY, van Genderen MM, Hoyng CB, Rohrschneider K, van Schooneveld MJ, Cremers FP, den Hollander AI. Homozygosity mapping in patients with cone-rod dystrophy: novel mutations and clinical characterizations. Invest Ophthalmol Vis Sci 2010; 51:5943-51. [PMID: 20554613].

30. Allikmets R, Singh N, Sun H, Shroyer NF, Hutchinson A, Chidambaram A, Gerrard B, Baird L, Stauffer D, Peiffer A, Rattner A, Smallwood P, Li Y, Anderson KL, Lewis RA, Nathans J, Leppert M, Dean M, Lupski JR. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat Genet 1997; 15:236-46. [PMID: 9054934].

31. Wiszniewski W, Zaremba CM, Yatsenko AN, Jamrich M, Wensel TG, Lewis RA, Lupski JR. ABCA4 mutations causing mislocalization are found frequently in patients with severe retinal dystrophies. Hum Mol Genet 2005; 14:2769-78. [PMID: 16103129].

32. Mata NL, Tzekov RT, Liu X, Weng J, Birch DG, Travis GH. Delayed dark-adaptation and lipofuscin accumulation in abcr+/- mice: implications for involvement of ABCR in age-related macular degeneration. Invest Ophthalmol Vis Sci 2001; 42:1685-90. [PMID: 11431429].

33. Corradi Z, Khan M, Hitti-Malin R, Mishra K, Whelan L, Cornelis SS, Hoyng CB, Kämpjärvi K, Klaver CCW, Liskova P, Stöhr H, Weber BHF, Banfi S, Farrar GJ, Sharon D, Zernant J, Allikmets R, Dhaenens CM, Cremers FPM. ABCA4-Study Group. Targeted sequencing and in vitro splice assays shed light on ABCA4-associated retinopathies missing heritability. HGG Adv 2023; 4:100237[PMID: 37705246].

34. Cornelis SS, Runhart EH, Bauwens M, Corradi Z, De Baere E, Roosing S, Haer-Wigman L, Dhaenens CM, Vulto-van Silfhout AT, Cremers FPM. Personalized genetic counseling for Stargardt disease: Offspring risk estimates based on variant severity. Am J Hum Genet 2022; 109:498-507. [PMID: 35120629].

35. Marwan M, Dawood M, Ullah M, Shah IU, Khan N, Hassan MT, Karam M, Rawlins LE, Baple EL, Crosby AH, Saleha S. Unravelling the genetic basis of retinal dystrophies in Pakistani consanguineous families. BMC Ophthalmol 2023; 23:205-[PMID: 37165311].

36. Munir A, Afsar S, Rehman AU. A systematic review of inherited retinal dystrophies in Pakistan: updates from 1999 to April 2023. BMC Ophthalmol 2024; 24:55-[PMID: 38317096].

37. Runhart EH, Khan M, Cornelis SS, Roosing S, Del Pozo-Valero M, Lamey TM, Liskova P, Roberts L, Stöhr H, Klaver CCW, Hoyng CB, Cremers FPM, Dhaenens CM. Disease Consortium Study Group. Association of Sex With Frequent and Mild ABCA4 Alleles in Stargardt Disease. JAMA Ophthalmol 2020; 138:1035-42. [PMID: 32815999].

38. Valkenburg D, Runhart EH, Bax NM, Liefers B, Lambertus SL, Sánchez CI, Cremers FPM, Hoyng CB. Highly Variable Disease Courses in Siblings with Stargardt Disease. Ophthalmology 2019; 126:1712-21. [PMID: 31522899].

39. Burke TR, Tsang SH, Zernant J, Smith RT, Allikmets R. Familial discordance in Stargardt disease. Mol Vis 2012; 18:227-33. [PMID: 22312191].

40. Shanks ME, Downes SM, Copley RR, Lise S, Broxholme J, Hudspith KA, Kwasniewska A, Davies WI, Hankins MW, Packham ER, Clouston P, Seller A, Wilkie AO, Taylor JC, Ragoussis J, Németh AH. Next-generation sequencing (NGS) as a diagnostic tool for retinal degeneration reveals a much higher detection rate in early-onset disease. Eur J Hum Genet 2013; 21:274-80. [PMID: 22968130].

41. Lewis RA, Shroyer NF, Singh N, Allikmets R, Hutchinson A, Li Y, Lupski JR, Leppert M, Dean M. Genotype/Phenotype analysis of a photoreceptor-specific ATP-binding cassette transporter gene, ABCR, in Stargardt disease. Am J Hum Genet 1999; 64:422-34. [PMID: 9973280].

42. Maggi J, Koller S, Bähr L, Feil S, Kivrak Pfiffner F, Hanson JVM, Maspoli A, Gerth-Kahlert C, Berger W. Long-range PCR-based NGS applications to diagnose mendelian retinal diseases. Int J Mol Sci 2021; 22:1508-[PMID: 33546218].

43. Fujinami K, Zernant J, Chana RK, Wright GA, Tsunoda K, Ozawa Y, Tsubota K, Robson AG, Holder GE, Allikmets R, Michaelides M, Moore AT. Clinical and molecular characteristics of childhood-onset Stargardt disease. Ophthalmology 2015; 122:326-34. [PMID: 25312043].

44. Tanaka K, Lee W, Zernant J, Schuerch K, Ciccone L, Tsang SH, Sparrow JR, Allikmets R. The rapid-onset chorioretinopathy phenotype of ABCA4 disease. Ophthalmology 2018; 125:89-99. [PMID: 28947085].

45. . Pozo MG-d. Méndez-Vidal C, Bravo-Gil N, Vela-Boza A, Dopazo J, Borrego S, Antinolo G. Exome sequencing reveals novel and recurrent mutations with clinical significance in inherited retinal dystrophies. PLoS One 2014; 9:e116176.

46. Duncker T, Tsang SH, Lee W, Zernant J, Allikmets R, Delori FC, Sparrow JR. Quantitative fundus autofluorescence distinguishes ABCA4-associated and non-ABCA4-associated bull's-eye maculopathy. Ophthalmology 2015; 122:345-55. [PMID: 25283059].

Articles are provided courtesy of Emory University and The Abraham J. & Phyllis Katz Foundation. The print version of this article was created on 28 December 2025. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.