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Genotypic profile and phenotype correlations of *ABCA4*-associated retinopathy in Koreans

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Purpose: This study was conducted to analyze the clinical features associated with the pathogenic variants of *ABCA4* in Korean patients with inherited retinal dystrophies (IRDs).
Methods: We enrolled patients with IRDs who visited a tertiary referral hospital and identified the pathogenic variants of *ABCA4* through targeted gene panel sequencing and whole exome sequencing. We analyzed the clinical characteristics and phenotypic spectrum according to genotype.
Results: Eleven patients (from nine families) with IRDs and pathogenic variants in *ABCA4* were included. Eight patients (from seven families) with Stargardt disease (STGD), two (from one family) with cone-rod dystrophy (CRD), and one with early-onset retinitis pigmentosa (RP) were included. Two heterozygous mutations were identified in eight families, and one variant was found in a patient with fundus flavimaculatus. Two variants, p.Gln294Ter and p.Gln636Lys, were associated with severe phenotypes, such as early-onset RP and CRD. Four novel pathogenic variants, p.Gln636Lys, p.Ile1114del, p.Thr1117Ala, and p.Asn1588Tyr, were identified. p.Gln294Ter, p.Leu1157Ter, and p.Lys2049ArgfsTer12 were repeatedly detected in Koreans with *ABCA4*-associated retinal diseases (*ABCA4*-RD).
Conclusions: Various pathogenic variants of *ABCA4*, including four novel variants, were identified, and *ABCA4*-RD exhibited various phenotypes and disease severities in a Korean IRD cohort. These findings will be useful for understanding the clinical features of *ABCA4*-RD and ethnicity-specific variants in East Asians.

ABCA4, which is located at 1p22.1, encodes the ATP-binding cassette sub-family A member 4, a retina-specific protein that is exclusively expressed in the outer segments of photoreceptors [1]. This transmembrane protein facilitates the removal of toxic retinoid compounds from photoreceptor cells [2,3]. *ABCA4* is composed of 50 exons, and many variants of this gene have been reported [4]. Heterogeneous retinal dystrophies, including Stargardt disease (STGD1; OMIM 248200), cone-rod dystrophy (CRD3; OMIM 604116), retinitis pigmentosa (RP19; OMIM 601718), and early-onset severe retinal dystrophy (OMIM 248200), are associated with recessive mutations in *ABCA4* [5]. Numerous studies have reported phenotypic and allelic heterogeneity and variable severity in *ABCA4*-associated retinopathies (*ABCA4*-associated retinal diseases, *ABCA4*-RD). More than 800 disease-associated ABCA4 variants have been identified [1], and the deleterious effects of these variants, including missense, nonsense, splice-site, insertion-deletion, and frameshift mutations [6], have been associated with disease onset and phenotypic severity [7]. However, studying the genotype–phenotype correlation remains challenging because most patients harbor compound heterozygous mutations in *ABCA4*, and phenotypic variations have been observed among family members with the same mutations.

Moreover, more than half of such mutations have been detected only once, and the most frequent disease-associated *ABCA4* variants have been described only in approximately 10% of STGD patients [8]. This low detection rate of identical variations makes it difficult to validate their pathogenicity and to evaluate their clinical impacts and genotype–phenotype correlations. Thus, the extent to which each variant contributes to the etiology of *ABCA4*-RD in relation to other factors, such as age, lifestyle, environmental factors, and genetic modifiers, remains unknown. Furthermore, several studies have identified frequent ethnic group-specific *ABCA4* variants [5,8-11]. Ethnic differences and variant diversity cause highly variable *ABCA4*-RD phenotypes. However, clinical and genotypic data for *ABCA4*-RD in Asian populations are insufficient [1,5,12,13].

In this study, we analyzed the phenotypic spectrum and genotypes in 11 patients from 9 families with *ABCA4*-RD.

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We also identified four novel pathogenic variants of *ABCA4* and report the variants that have been repeatedly detected in Koreans.

METHODS

This study was conducted as part of a study to identify pathogenic variants in patients with inherited retinal dystrophies (IRDs) registered at Seoul National University Bundang Hospital between January 2009 and January 2018. Of these patients, those with pathogenic variants of *ABCA4* (11 patients from 9 families) were selected for the analysis. This study was conducted at Seoul National University Bundang Hospital. Institutional Review Board approval was obtained (IRB no. B-1105–127–014 and no. B-1901–519–103), and written informed consent was obtained from all the participants. The study protocols adhered to the tenets of the Declaration of Helsinki and its later amendments.

Clinical investigations: Clinical diagnoses and classifications were based on patient age at the time of symptom onset, visual acuity, and findings from fundus examinations, autofluorescence (AF), optical coherence tomography (OCT), and electroretinograms (ERGs). All patients underwent comprehensive ophthalmic examinations, including slit-lamp and dilated fundus examinations. Vision was assessed by measuring best-corrected visual acuity (BCVA). BCVAs were measured using a decimal chart and converted to Snellen equivalents. Spectral domain-OCT (SD-OCT) scans and fundus autofluorescence (FAF) imaging were conducted using a confocal scanning laser ophthalmoscope (SPECTRALIS HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). The areas of definitely decreased autofluorescence (DDAF) and questionably decreased autofluorescence (QDAF) were measured and analyzed in FAF images according to a previous report [14]. When AF of the optic disc was defined as 100% black, DDAF was defined as more than 90% black, and QDAF was defined as 50 to 90% black. The size of the area was semi-automatically evaluated using the area-calculating module built in Spectralis HRA + OCT. Full-field ERGs were performed for all patients, and all procedures complied with the guidelines and recommendations of the International Society for Clinical Electrophysiology of Vision standard available at the time of recording.

Blood sampling and DNA collection: A professional nurse for clinical research collected blood from the patients' antecubital fossa vein after alcohol disinfection and stored blood in EDTA bottles. DNA was extracted using a DNA extraction kit (QIAamp DNA Blood Maxi Kit, Qiagen, Hilden, Germany) for targeted exome sequencing. DNA sampling and basic ophthalmic examinations were also performed for the family members of the patients who showed no visual symptoms.

Sequencing and variant identification: ABCA4 variants were identified using two methods: whole-exome sequencing for the samples from patients H75 and H830, and targeted gene panel sequencing for the samples from the other patients. Both methods covered all 50 exons and exon-intron boundaries of *ABCA4*. For targeted gene panel sequencing, pre-capture libraries (Illumina, Inc., San Diego, CA) were prepared, and the capture process (Roche NimbleGen, Madison, WI) was performed according to the manufacturer's protocols. SureSelect Human All Exon V6 (Agilent Technologies, Santa Clara, CA) was used for exome capture in whole-exome sequencing. All captured libraries were sequenced using the Illumina HiSeq 2000 platform (paired-end, average depth ≥100x). Burrows-Wheeler Aligner was used to align the sequence reads in the human reference sequence (hg19, GRCh37). Genome analysis toolkit (GATK) packages, SAMtools, and Dindel were used for variant calling, local realignment, and variant recalibration. ANNOVAR was used for variant annotation. NextGENe software (SoftGenetics, State College, PA) was also used for the analysis. We compared the allele frequencies of all variants to the 1000 Genome database, Exome Variant Server of the NHLBI Exome Sequencing Project, and a genome aggregation database (gnomAD; Cambridge, MA) to filter out the common variants. Exonic or splicing variants with allele frequencies <0.1 in the 1000 Genome database and the gnomAD browser (accessed: February 12, 2019) were used for variant prioritization. Prediction software programs CADD, Polyphen, SIFT, and MutationTaster were used to evaluate the pathogenicity of the novel *ABCA4* variants. Sanger sequencing was performed to validate the candidate variants in additional familial samples.

RESULTS

In this study, 11 patients (7 females and 4 males from 9 families) with pathogenic variants of *ABCA4* were evaluated (Table 1). The median BCVA was 20/320 and ranged from the ability to distinguish hand motions to 20/20 vision. The median age at the onset of symptoms was 11 years (range: 4–48 years). Eight STGD patients from seven families, two CRD patients from one family, and one patient with early-onset RP were involved. Two heterozygous mutations were identified in eight unrelated individuals, and one variant was found in a patient (H830) with fundus flavimaculatus. All patients exhibited autosomal recessive or isolated inheritance patterns in their pedigrees (Figure 1). For three families (F4, F6, and F9), the parents' DNA was collected and sequenced.

Table 1. Clinical features of *ABCA4*-associated retinopathies in Korean patients.

Family ID	Patients ID	Sex	Age (years)	Age at onset (years)	Clinical diagnosis	CDS variants	Protein variation	BCVA (Snellen)		Fundus, FAG findings	Full field ERG findings
								Right	Left		
F1	H27	F	28	NA	STGD	c.6146delA	p.Lys2049ArgfsTer12	20/250	HM	Flecks, macular atrophy	Normal
						c.1933G>A	p.Asp645Asn			Dark choroid	
F2	H62	M	23	11	STGD	c.6146delA	p.Lys2049ArgfsTer12	20/500	20/320	Flecks, macular atrophy	Normal
						c.3349A>G	p.Thr1117Ala			Dark choroid	
F3	H75	M	21	14	STGD	c.4762A>T	p.Asn1588Tyr	20/500	20/200	Flecks, macular atrophy	Normal
						c.1760+2T>G	Splice site			Dark choroid	
F4	H147	F	28	11	STGD	c.3420C>G c.3342_3344delCAT	p.Cys1140Trp	20/500	20/500	Flecks, macular atrophy	Cone↓, Rod ↓
							p.Ile1114del			Dark choroid, BM hole	
F4	H148	F	20	NA	STGD	c.3420C>G	p.Cys1140Trp	20/320	20/500	Flecks, macular atrophy	Cone↓
						c.3342_3344delCAT	p.Ile1114del			Dark choroid	
F5	H234	F	22	16	STGD	c.3470T>G	p.Leu1157Ter	20/125	20/125	Macular atrophy	Rod↓↓
						c.869G>A	p.Arg290Gln			Dark choroid	
F6	H278	M	11	11	STGD	c.4762A>T	p.Asn1588Tyr	20/63	20/100	Flecks	Normal
						c.3470T>G	p.Leu1157Terr			Macular atrophy	
F7	H830	F	51	48	FF	c.575C>T	p.Ala192Val	20/20	20/20	Flecks	Rod↓
F8	H91	F	18	4	RP	c.1906C>A	p.Gln636Lys	CF	HM	Extensive CR atrophy	No cone & rod response
					(early-onset)	c.880C>T	p.Gln294Ter			Diffuse pigmentation	

Family ID	Patients ID	Sex	Age (years)	Age at onset (years)	Clinical diagnosis	CDS variants	Protein variation	BCVA (Snellen)		Fundus, FAG findings	Full field ERG findings
								Right	Left		
F9	H144	F	15	5	CRD	c.4748T>C	p.Leu1583Pro	20/500	20/200	Macular atrophy	Cone↓↓, Rod↓↓
						c.1906C>A	p.Gln636Lys				
F9	H145	M	19	4	CRD	c.4748T>C	p.Leu1583Pro	20/200	20/500	Macular atrophy	Cone↓↓, Rod↓↓
						c.1906C>A	p.Gln636Lys				

BCVA, best-corrected visual acuity; STGD, Stargardt disease; FF, fundus flavimaculatus; RP, retinitis pigmentosa; CRD, cone-rod dystrophy; BM, Bruch's membrane; CR, chorioretinal; HM, hand motion; CF, counting finger. p.R290Q in H234, a patient with STGD features, is a rare variant (allele frequency: 3.995e-6 (%) in gnomAD) and has not been reported. However, the pathogenicity of this variant was predicted as benign by most prediction programs, including polyphen, SIFT, and MutationTaster.

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Retinal phenotypes and visual function: Clinically, all patients exhibited mild to severe phenotypes consistent with the described spectrum for *ABCA4*-RD. Patients with STGD showed typical features: a "beaten bronze metal" appearance in their macula and bilateral atrophic macular changes with or without diffuse pisciform flecks (Figure 2A, Figure 3, and Table 1). Patient H234 showed only bull's-eye lesions without flecks. Fundus fluorescein angiography revealed a dark choroid sign in some patients. Multiple hyper-autofluorescent dots observed at earlier ages changed into larger hypo-autofluorescent lesions during follow-up periods. Outer retinal foveal atrophy and loss of the inner ellipsoid zone were observed after OCT scanning (Figure 2B). Patients with STGD showed a normal or subnormal response in their scotopic and photopic ERGs (Figure 2C). A patient with fundus flavimaculatus (H830) showed yellowish flecks in their RPE with no other anatomic or functional changes. Early clinical features in H144 and H145 of the F9 family appeared in the form of CRD. The cone response significantly decreased, while the rod response remained relatively unchanged on the ERG, and the fundus examination revealed macular dystrophy without flecks (Figure 2D,F). After five years, the retinal degenerative areas progressed beyond the macula, and bony spicule pigments appeared similar to those observed in RP. The most severe phenotype characterized by early-onset and extensive atrophy in the entire fundus was observed in patient H91 (Figure 2G–I). The BCVAs for H91 were 20/63 in the right eye and 20/200 in the left eye, and 20 prism diopters of the exotropia and nystagmus were observed at the age of nine years, when the patient first visited the hospital. During the next five years, retinal degeneration rapidly progressed, and severe macular atrophy and pigmentary changes were observed. At 18 years, BCVAs were lower (ability to count fingers through the right eye and respond to hand motions through the left eye) than those in CRD and STGD, and both rod and cone responses on ERGs were extinct. In patients with STGD, the DDAF and QDAF areas were not directly correlated with visual acuities, visual fields, or ERG response (Table 2). As shown in Figure 3 and Table 2, the DDAF and QDAF areas in patient H27 were relatively small, but the BCVA in her left eye was hand motions; on the contrary, the DDAF and QDAF areas in patients H75 and H278 were large, but their BCVAs were relatively high. All STGD patients showed central scotomas of less than five degrees to as much as 20 degrees in their visual fields.

Analysis of ABCA4 variants in Korean patients: In our analysis, 13 rare variants of *ABCA4* were identified, and four novel variants, p.Gln636Lys, p.I1114del, p.Thr1117Ala, and p.Asn1158Tyr, were observed (Table 3). All the novel variants were predicted to be deleterious mutations by the pathogenicity prediction program. The novel variant p.Gln636Lys was present in each patient with RP and CRD. In-frame deletion p.Ile1114del was found in two siblings of the F4 family with a known pathogenic variant, p.Cys1140Trp. Novel variant p.Asn1588Tyr was repeatedly present in two different STGD families. Two patients commonly harbored variant c.6146delA (p.Lys2049ArgfsTer12), which has not been uploaded in ClinVar or other databases but

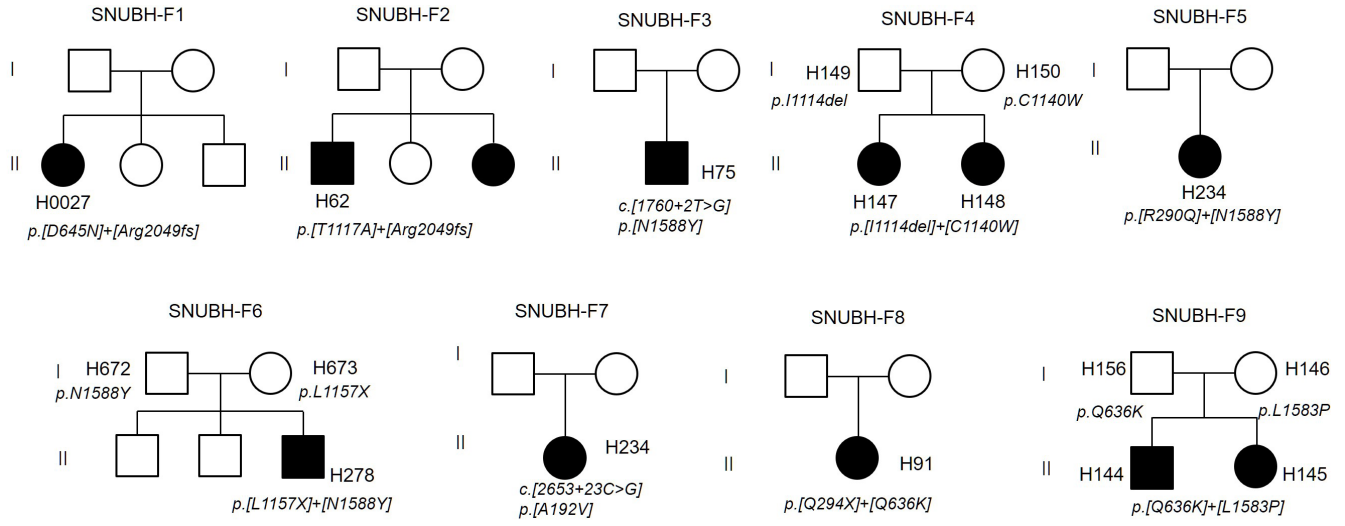

Figure 1. Pedigrees of *ABCA4*-associated retinopathies. SNUBH-F1–F7 were diagnosed as Stargardt disease (STDG). SNUBH-F8 (H91) showed retinitis pigmentosa (RP), and SNUBH-F9 members (H144 and H145) showed cone-rod dystrophies (CRDs). The pathogenic variants of SNUBH-F4, F6, and F9 were also confirmed with their parents' genotypes.

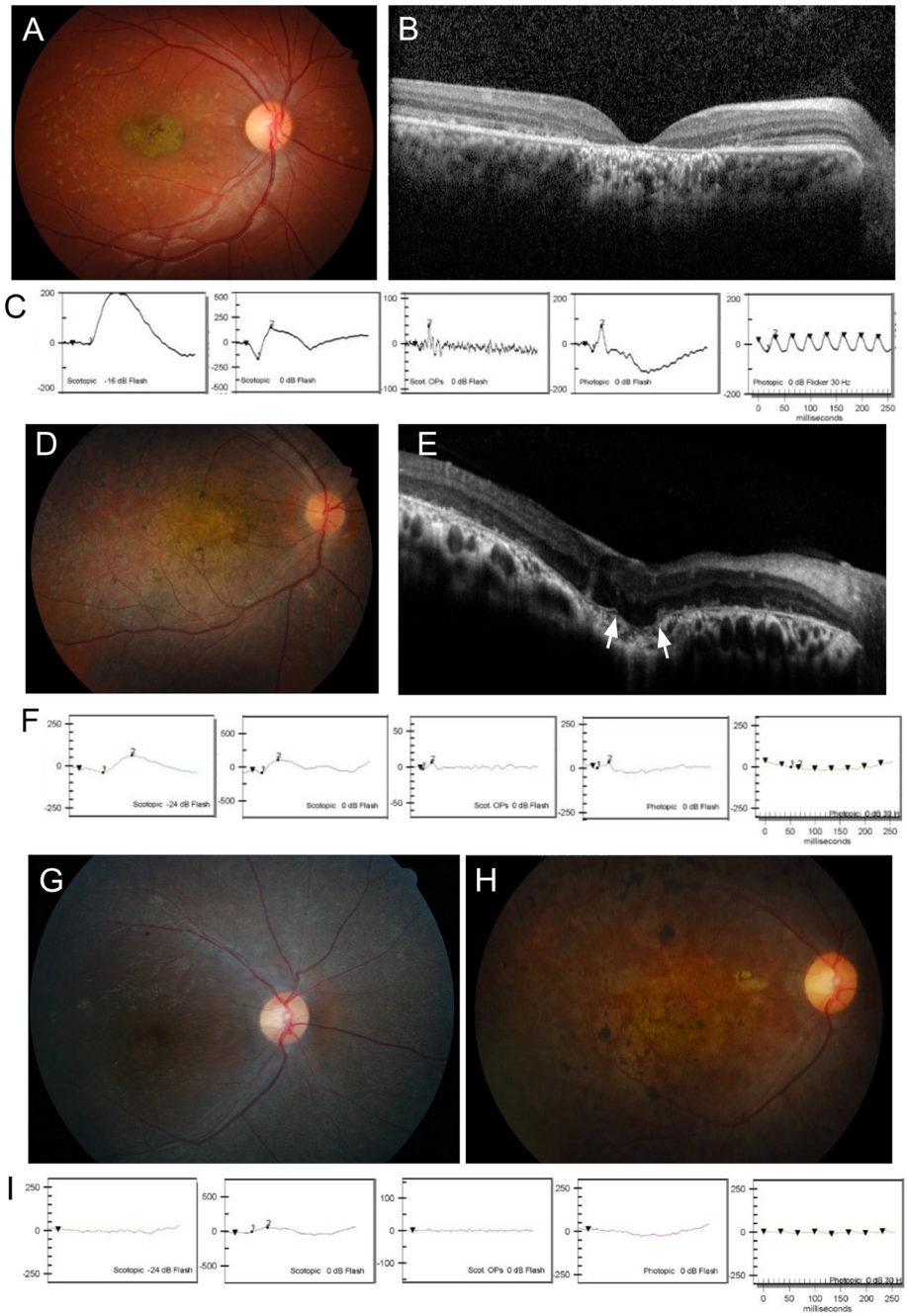

Figure 2. Clinical features of *ABCA4*-associated retinopathies. **A**, **D**, **G**, **and H**: Fundus photography. **B and E**: Optical coherence tomography (OCT). **C, F**, **and I**: Electroretinograms (ERGs). **A**, **B**, **and C**: The ophthalmologic examination of patient H147 showed typical features of STDG. Atrophy of photoreceptors and RPE in the macula is shown on fundus photography and OCT. **D**, **E**, **and F**: Patient H145 showed CRD with macular degeneration and reduced cone response. The white arrows indicate choroidal excavation and defect of the Bruch's membrane. **G and H**: Fundus examination of patient H91 at 10 and 19 years of age, respectively. I: Both the cone and the rod responses on the ERG at the age of 10 years were strikingly decreased, suggesting typical features of RP.

was recently reported in two Korean patients, suggesting that it is frequently present in Korean STGD with ethnic specificity [12]. Two known variants, c.3342_3344delCAT (p.Ile1114del) and c.3349A>G, (p.Thr1117Ala), were also identified repeatedly in different families. All variants were typically predicted to be deleterious except two missense variants, p.Ala192Val and p.Arg290Gln. The previously reported p.Ala192Val variant showed low allele frequency (2.131e-4) and was predicted to be a disease-causing variant in MutationTaster but not in Polyphen or SIFT. Despite its rarity, variant p.R290Q was predicted as benign by Polyphen, SIFT, and MutationTaster and showed a low Phred score (7.2) in CADD.

Genotype–phenotype correlation: Patients who harbored the c.1760+2TG, c.1933G>A, c.3342_3344delCAT, c.3349A>G, c.3420C>G, c.3470T>G, c.4762A>T, and c.6146delA variants presented with typical STGD features—bilateral macular atrophy (bull's-eye maculopathy) and blocked fluorescence—in fluorescein angiography. All variants in exon 23 of *ABCA4* were associated with STGD in our cohort. One patient (H234) harboring the c.3470T>G (p.Leu1157Ter) and c.869G>A (p.Arg290Gln) variants showed bull's-eye maculopathy without flecks. Two patients (H144 and H145) with CRD with c.1906C>A (p.Gln636Lys) and c.4748T>C (p.Leu1583Pro) showed a central defect in the RPE-Bruch's membrane with choroidal excavation (Figure 2E). These patients also showed hypo-autofluorescence over the entire macula, consistent with fundus and OCT findings (Figure 3C). The patient with the p.Gln294Ter variant showed the early-onset RP phenotype, and the p.Gln636Lys variant was associated with early-onset RP and CRD in our cohort. Compared to patients with STGD, patients with RP and CRD showed a tendency to harbor at least one causative variant (p.Gln294Ter or p.Gln636Lys) in earlier exon regions (exons 8 and 13) of *ABCA4* (Table 3). No other significant associations between genotypes, FAF, and visual function were found in our cohort.

DISCUSSION

One of the most frequent genetic causes of retinal dystrophies is mutation in *ABCA4*, which encodes a retina-specific ABC transporter expressed in rod and cone photoreceptors [15]. *ABCA4* mutations lead to the accumulation of toxic bisretinoid adducts of all-trans retinal within the photoreceptors and RPE [3,16]. In this study, all the STGD cases showed typical clinical features, with patients exhibiting symptoms in their teen years and visual acuity below 20/125 owing to the disease progression in their 20s. Although *ABCA4*-RD is considered to initiate in the vicinity of the macula and cause a decrease in central vision, the disease progresses to various extents and is not limited to the macular area; progression often occurs from the macula to the extramacular regions. Thus, RP-like disease patterns can be observed in patients with *ABCA4* mutations, as observed in patient H91. This patient showed the most severe phenotype, characterized by aggressive, early-onset RP, and harbored an early nonsense mutation, p.Gln294Ter, in exon 8 of *ABCA4* and a deleterious missense mutation from glutamine to lysine (p.Gln636Lys) in exon 13 of *ABCA4*. Glutamine is an amino acid with a polar uncharged side chain; the substitution of glutamine with lysine causes this side chain to be electrically charged. In this study, this mutation was found in both early-onset RP and CRD. On the basis of initial features and electrophysiologic findings, CRD was diagnosed in patients H144 and H145 who harbored the p.Gln636Lys mutation; however, the disease rapidly progressed beyond the macula, and profound destruction of both the rod and cone areas occurred, as

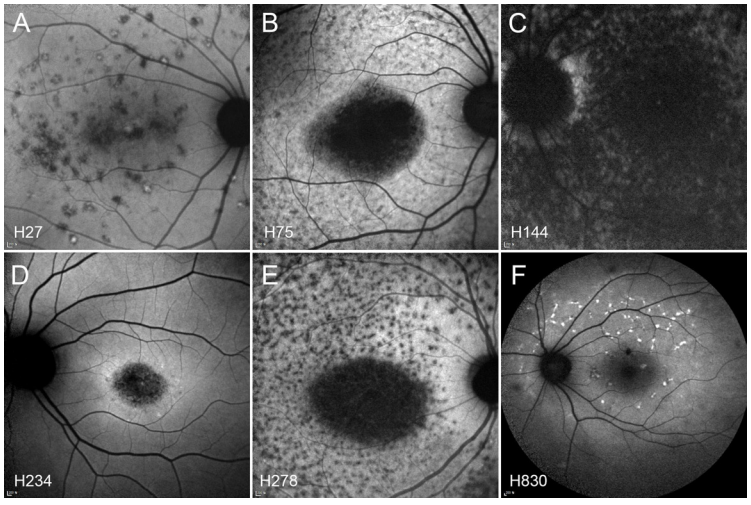

Figure 3. Fundus autofluorescence (FAF) images. **A**: Multiple hypo-autofluorescence dots without a bull's-eye pattern in patient H27. **B and E**: Typical FAF images of STGD, with round macular hypo-autofluorescence and multiple dots (H75 and H278). **C**: Hypo-autofluorescence on the entire macula in a patient with CRD (H144). **D**: Bull's-eye maculopathy without flecks (H234). **F**: FAF images in a patient with fundus flavimaculatus (H830).

Table 2. Area of decreased autofluorescence and functional measurement.

Patients	DDAF (mm^2)		QDAF (mm^2)		Visual field	Dark-adapted 0.01 ERG		Light-adapted 3 ERG	
ID	Right	Left	Right	Left	(method)	Rod amp	Rod IT	Cone amp	Cone IT
H27	0.13	0	0.13	1.68	≤10° CS (GVF)	1.93	0.79	2.03	0.97
H62	NA	NA	NA	NA	NA	1.12	0.97	1.5	1.05
H75	6.84	6.61	12.3	13.34	≤20° CS (GVF)	1.75	0.99	0.72	0.98
H147	NA	NA	NA	NA	≤10° CS (GVF)	0.57	1.08	0.75	1
H148	NA	NA	NA	NA	≤5° CS (GVF)	1.02	1.14	0.7	1.04
H234	0	0	2.67	2.46	≤5° CS (HVF)	0.48	1.02	1.29	0.92
H278	0.58	0.44	8.19	8.08	≤10° CS (HVF)	3.04	1.03	4.8	0.95
H830	0	0	0	0	Normal	0.84	1.12	2.49	0.92
H91	NA	NA	NA	NA	Extrafoveal VF island	0	0	0	0
H144	EA	EA	EA	EA	No detected	0.43	1.25	0.64	0.83
H145	EA	EA	EA	EA	No detected	0.41	1.29	0.53	1.09

DDAF, definitely decreased autofluorescence; QDAF, questionably decreased autofluorescence; GVF, Goldmann visual field test (III4e); HVF, Humphery visual field 24–2; amp, the ratio of b-wave amplitude compared to the normative value; IT, the ratio of b-wave implicit time compared to the normative value; NA, not applicable; CS, central scotoma; EA, the entire macula

Table 3. Profiles of pathogenic variants in Korean families with *ABCA4*-associated retinopathies.

No. exon /intron	Nucleotide change	Protein variant / annotation	No. family†	Disease	Polyphen (score)	SIFT (score)	Mutation taster (ENST00000370225)	CADD Phred score (GRCh37-v1.4)‡	Allele frequency (%) in gnomAD¶	Clinical significance (ClinVar)
6	c.575C>T	p.Ala192Val	1	FF	Benign -0.353	Tolerated (0.58)	Disease causing	19.32	0.000213 (58/272,116)	Not provided§
8	c.880C>T	p.Gln294Ter	1	RP	NA	NA	Disease causing	35	None	Pathogenic
12	c.1760+2T>G	Splice site	1	STGD	NA	NA	NA	23.1	None	Pathogenic
13	c.1906C>A	p.Gln636Lys	2	RP, CRD	Possibly damaging -0.634	Deleterious 0	Disease causing	26	None	Novel
13	c.1933G>A	p.Asp645Asn	1	STGD	Probably damaging, (0.954)	Deleterious (0)	Disease causing	28.4	1.99E-05 (5/251,094)	Not provided
23	c.3342_3344delCAT	p.Ile1114del	1	STGD	NA	NA	Disease causing	NA	None	Novel
23	c.3349A>G	p.Thr1117Aal	1	STGD	Damaging	Deleterious (0)	Disease causing	26.5	None	Novel
23	c.3420C>G,	p.Cys1140Trp,	1	STGD	Probably damaging, (0.997)	Deleterious (0)	Disease causing	25.8	None	Reported#[18]
23	c.3470T>G,	p.Leu1157Ter,	2	STGD	NA	NA	Disease causing	43	None	Reported[12]
33	c.4748T>C	p.Leu1583Pro	1	CRD	Possibly damaging, (0.878)	Deleterious (0)	Disease causing	25.9	7.95E-06 (2/251,468)	Not provided
33	c.4762A>T,	p.Asn1588Tyr	2	STGD	Probably damaging, (0.962)	Deleterious (0.04)	Disease causing	26.7	None	Novel
44	c.6146delA,	p.Lys2049ArgfsTer12	2	STGD	NA	NA	Disease causing	NA	None	Reported[12]

†No. family, the number of families with the same variant; ‡CADD, https://cadd.gs.washington.edu/; ¶gnomAD, https://gnomad.broadinstitute.org/ §Not provided, reported but clinical significance was not determined; #Reported, recently reported but no information in ClinVar or other database.

previously observed in rapid onset *ABCA4*-RD [17]. Therefore, p.Gln294Ter and p.Gln636Lys are associated with rapidly progressive, early-onset *ABCA4*-RD with the severe phenotype.

In the gnomAD browser (accessed: February 13, 2019), 1,303 missense variants and 87 loss-of-function variants of *ABCA4* were observed. *ABCA4* encodes a large protein composed of 2,272 amino acids and more than 11 transmembrane domains, as well as an ATP-binding site, a Walker A/P loop, and D-, H-, and Q-loops (NP_000341.2). This complexity is associated with numerous variants, and the identification of disease-causing mutations can be challenging. Thus, categorizing new *ABCA4* variants as pathogenic is likely highly important for molecular diagnosis in some patients. In this study, all four novel mutations (p.Gln636Lys, p.Ile1114del, p.Thr1117Ala, and p.Leu1588YTyr) were highly deleterious and are not listed in gnomAD, indicating that they have extremely low allele frequencies. Amino acids 1114 and 1117 in *ABCA4* protein are included in the H-loop/switch region (1,114–1,120) and at the ATP-binding site (966–1,118), suggesting that deletion of isoleucine 1114 or substitution of threonine 1117 can result in a dysfunctional *ABCA4* protein. Variant c.4762A>T (p.Asn1588Tyr) was detected in two different families with STGD, suggesting that this variant is the disease-causing mutation for STGD. Variant c.575C>T (p.Ala192Val) was predicted as a pathogenic variant through a FATHMM prediction (score 0.94) and MutationTaster. Moreover, in the same locus as p.Ala192Val but involving a different protein change, the Ala192Thr variant (alanine to threonine) in *ABCA4* was reported previously in an individual with STGD who was compound heterozygous for the Ala192Thr variant and another variant [18,19]. However, p.Ala192Val was predicted to be benign by Polyphen and SIFT, and its allele frequency in gnomAD (58/272,116) was higher than that of other variants. Thus, p.Ala192Val may cause STGD; however, this could not be confirmed. Variants p.Cys1140Trp and p.Leu1157Ter are not present in the ClinVar database; however, they were identified in patients with STGD in recent reports as well as in two different families in our study, which suggests that they cause STGD [12,20].

The regional and ethnic specificity of several variants has been reported [5]. The founder variant c.2588G>C (p.[Gly863Ala, Gly863del]) was reported to be associated with the European population, and the complex alleles c.[1622T>C;3113C>T] (p.[Leu541Pro;Ala1038Val]) and the variant c.2894A>G (p.Asn965Ser) were predominantly found in individuals of German and Danish descent, respectively [21-24]. Moreover, the c.6320G>A (p.Arg2107His) variant accounted for 19.3% of all disease-associated alleles in a large cohort of African-American patients with STGD [11]. Although several studies of the genotype spectrum and phenotypic correlation in Asian *ABCA4*-RD have recently been conducted, the data remain insufficient. Recently, c.859–9T>C was reported as a highly frequent variant in an Indian *ABCA4*-RD cohort [5]. Three novel *ABCA4* mutations, IVS7–45_952delinsTCTGACC, IVS12+2T>G, and p.Arg2149Ter, were reported in Japanese patients with STGD [13]. Moreover, c.5646G>A (p.Met1882Ile) and c.3523–2A>G were recently identified as disease-causing novel variants in a Chinese STGD pedigree [25]. Such regional and ethnic specificities of the same variants were also observed in our cohort. In a recent report, three novel variants, c.880C>T (p.Gln294Ter), c.3470T>G (p.Leu1157Ter), and c.6146delA (p.Lys2049ArgfsTer12), were identified in Korean patients with STGD [12]. In the present study, the same variants were also found in patients with *ABCA4*-RD, despite the small number of people in the cohort. These variants have not previously been reported, and no information was available in gnomAD. Variant p.Gln294Ter induces early truncation of the *ABCA4* protein, leading to a profound loss of function, and p.Leu1157Ter induces premature termination at the approximate midpoint of the *ABCA4* protein. MutationTaster predicted that p.Lys2049ArgfsTer12 causes the resultant protein to undergo nonsense-mediated decay through selective degradation at the mRNA level. Two pathogenic variants, p.Arg2040Ter and p.Lys2056Ter, which induce early truncation of *ABCA4* near the region of p.Lys2049ArgfsTer, have been identified as pathogenic variants of *ABCA4*-RD. Considering the rarity of p.Gln294Ter, p.Leu1157Ter, and p.Lys2049ArgfsTer12, as well as their repeated detection in Koreans with *ABCA4*-RD, these variants may be more frequent in Korean patients with *ABCA4*-RD than in patients of other ethnicities.

In summary, we described the clinical and genetic spectra of *ABCA4*-RD in Korean patients. Four novel variants, p.Gln636Lys, p.Ile1114del, p.Thr1117Ala, and p.Asn1588Tyr, were identified, and p.Gln294Ter, p.Leu1157Ter, and p.Lys2049ArgfsTer12 were frequently detected in a Korean population with *ABCA4*-RD. This information will be useful for understanding the clinical features of *ABCA4*-RD and ethnicity-specific variants in East Asians.

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