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Original Research Article

Distinct Phenotypic Patterns of *ABCA4* Associated Retinal Disease in a Unique Saudi Consanguineous Cohort

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Abstract

Purpose: This study aimed to establish phenotype- genotype correlations in a unique cohort of thirteen patients from eight unrelated consanguineous Saudi families with variable inherited retinal phenotypes caused by *ABCA4* mutations.

Methods: A detailed ophthalmological assessment followed by next-generation sequencing (NGS) screening using vision panel was performed in the affected patients. Reference sequence (*ABCA4*): NM_000350.

Results: Clinically, all patients had reduced visual acuity and nystagmus documented in early childhood. A considerable phenotypic variability of *ABCA4* associated disease (AAD) was observed including: Stargardt disease in 2/13 (15%), Retinitis Pigmentosa in 5/13 (39%) and Cone- Rod dystrophy in 6/13 (46%) and). Genetically; potentially eight distinct homozygous ABCA4 mutations were identified in all the studied patients (100%), five of which are novel.

Conclusion: Our study represents a unique cohort of patients originating from consanguineous families with variable retinal phenotypes caused by known and novel recessive *ABCA4* mutations. This study expands the phenotypic – genotypic spectrum of AAD and provides an accurate genetic diagnosis that paves the gates for the opportunity of gene-based therapies in AAD within the scope of precision medicine

Keywords: *ABCA4*; Stargardt Disease; Mutation; Cone-Rod Dystrophy; Rapid-Onset Chorioretinopathy

Introduction

Inherited retinal diseases (IRDs) is a heterogeneous group of visually debilitating diseases that are caused by mutations in critical genes to retinal function [1]. Out of this, *ABCA4* associated disease (AAD) is a unique spectrum of IRDs that has been linked to mutations in the *ABCA4* (encoding the transmembrane protein ABCA4 (ATP-binding cassette (ABC), subfamily A, member 4, MIM 601691) [2].

ABCA4 was found to be highly expressed in the outer-segment of rod and cone photoreceptors and plays a pivotal role in the visual cycle by preventing premature retinal pigmented epithelium (RPE) cell death and photoreceptors degradation [3]. Although, week expressions of *ABCA4* was detected in fibroblasts, lymphocytes, keratinocytes, kidney and brain but no clear functional correlation was generated so far.

Up to date, biallelic mutations in *ABCA4* can lead to a wide spectrum of AAD phenotypes including: Stargardt disease 1 (STGD1), cone-rod dystrophy (CRD), a typical retinitis pigmentosa (RP), fundus flavimaculatus (FFM), rapid onset Chorioretinopathy (ROC) and generalized choriocapillaris atrophy (GCCD). On the other hand, these distinct phenotypes are based on age of onset, disease progression and the spectrum of retinal degeneration [1].

Here, we aimed to clinically and genetically and characterize a unique cohort of 13 individuals from 8 unrelated consanguineous

families with autosomal recessive AAD, to unequivocally confirm the diagnosis and to establish phenotype- genotype correlations.

Methods

Patients and clinical assessment

This study was conducted following the IRB-approved written informed consent by King Saud University Institutional Review Board, (KSU- IRB Project NO. E-23-7817). Thirteen patients out of eight unrelated consanguineous Saudi families with variable inherited retinal phenotypes (simplex = 5, complex = 8) were recruited from the ophthalmic genetics clinic at Department of Ophthalmology, College of Medicine, King Saud University (KSU), Riyadh, Saudi Arabia. These patients underwent a detailed ophthalmological examination including best corrected visual acuity (BCVA), dilated fundus examination, retinal fundus imaging, fundus autofluorescence (FAF) imaging and optical coherence tomography (OCT) scans. Full-field electroretinography (ERG) was performed according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards [4,5]. Genomic DNA was extracted from EDTA blood using standard procedures.

Mutation screening by next-generation multiplexing -Vision panel assay

Genomic DNA was extracted from EDTA blood using standard procedures. All index patients were initially run on Vision panel as a first-tier test. A panel of 322 genes known to be mutated in various ophthalmic genetic diseases including those involving in inherited retinal conditions was designed as previously described [6]. Genes were amplified using the AmpliSeq HiFi mix and proprietary primer design (Thermo Fisher, Carlsbad, CA), followed by sequencing on the Ion Proton platform following the manufacturer's protocol.

Variant interpretation

Variant classification as pathogenic, likely pathogenic or variants of unknown significance (VOUS) was performed following ACMG guidelines [7]. The variant filtering pipeline was applied using the unique Saudi Human Genome in addition to other general genomic databases including dbSNP build 145 (http://www.ncbi.nlm.nih.gov/SNP/), ExAC (http://exac.broadinstitute.org) and gnomAD (http://gnomad.broadinstitute.org). Moreover, *in silico* prediction tools were used: Align GVGD, Sorting Intolerant From Tolerant [SIFT], MutationTaster, and PolyPhen-2, Grantham score calculation, conservation. Mutation nomenclature uses numbering with the A of the initiation codon ATG as +1 (http://varnomen.hgvs.org/) based on the following RefSeqs: NM_000350 (*ABCA4*).

Results

Clinical assessment

A total of 13 patients were ophthalmologically evaluated. The age of onset varied after five years of age to early twenties, and the BCVA ranged from 20/60 to no light perception. All the studied patients were noticed to have nystagmus of either a pendular or jerk waveform, photophobia, The clinical characteristics of the 13 index patients are summarized in Table 1.

FA#	PT#	Gender	Age	Pt status	Pheno.	Age of onset (yrs.)	Nystagmus	Anterior segment	Fundus	ERG
F1	P1	M	23	Index	STGD	10	*Yes*	NL	Pale optic disc, retinal vessels attenuation with well demarcated macular coloboma.	Extinguished
F2	P2	F	34	Index	STGD	20	*Yes*	Cataract	Pale optic disc, retinal vessels attenuation with well demarcated macular coloboma.	Extinguished
F3	P3	F	26	Index	CRD	5	*Yes*	NL	Pale optic disc, retinal vessels attenuation and intraretinal bone spicules	(Photobic > Scotopic) dysfunction
	P4	F	15	Sister	CRD	7	*Yes*	NL	Pale optic disc, retinal vessels attenuation with no sign of RPE pigmentation	(Photobic > Scotopic) dysfunction
	P5	F	9	Sister	CRD	5	*Yes*	NL	Pale optic disc, retinal vessels attenuation with RPE well-defined, oval-shaped retinal pigment epithelial defects at the foveal are	(Photobic > Scotopic) dysfunction
	P6	F	8	Sister	CRD	6	*Yes*	NL	Pale optic disc, retinal vessels attenuation with general RPE mottling	(Photobic > Scotopic) dysfunction

F4	P7	M	15	Index	CRD	7	*Yes*	NL	Pale optic disc, retinal vessels attenuation with no sign of RPE pigmentation	(Photobic > Scotopic) dysfunction
	P8	F	10	Sister	CRD	5	*Yes*	NL	Pale optic disc, retinal vessels attenuation with no sign of RPE pigmentation	(Photobic > Scotopic) dysfunction
F5	P9	F	13	Index	RP	8	*Yes*	NL	Pale optic disc, retinal vessels attenuation and intraretinal bone spicules	Non-recordable responses
	P10	M	8	Brother	RP	5	*Yes*	NL	Pale optic disc, retinal vessels attenuation and intraretinal bone spicules	Non-recordable responses
F6	P11	M	40	Index	RP	18	*Yes*	Cataract	Pale optic disc, retinal vessels attenuation and intraretinal bone spicules	Non-recordable responses
F7	P12	M	22	Index	RP	20	*Yes*	NL	Pale optic disc, retinal vessels attenuation and intraretinal bone spicules	Non-recordable responses
F8	P13	M	16	Index	RP	5	*Yes*	NL	Pale optic disc, retinal vessels attenuation and intraretinal bone spicules	Non-recordable responses

Table 1: Clinical features of thirteen patients from eight unrelated consanguineous families with *ABCA4*-associated diseases. Abbreviations used: FA#: family number; PT#: patient number; M: male; F: female; BCVA: best-corrected visual acuity; ERG: electroretinogram; OU: both eyes; M: male;; NA: not available; NL: normal limit; NR: non-recordable; SN: subnormal; STGD: Stargardt disease; RP: retinitis pigmentosa; CRD: cone-rod dystrophy.

Overall, the phenotypes on retinal imaging represent the typical known phenotypic heterogeneity of AAD, where the retina may appear normal initially, while later, a variety of abnormalities may develop either isolated or combined with vascular attenuation, intraretinal pigment migration, macular coloboma and optic disc abnormalities. Several phenotype such as in 2/13 (15%), Retinitis Pigmentosa in 5/13 (39%) and Cone- Rod dystrophy in 6/13 (46%) and) were observed in the studied patients. Noteworthy, there were a clear phenotypic variability in the fundus photos within affected patients in the same family (Figure 1).

Concerning the ERG data both rod (scotopic) and cone (photopic) responses were abnormal in all patients, ranging between extinguished to non-recordable responses.

Novel and known variants in *ABCA4*

Overall, eight distinct biallelic *ABCA4* mutations were found in all the studied 13 patients (100%). A summary of all variants identified Table 2. All of them the ACMG criteria for (likely) pathogenicity.

Discussion

This genetic and phenotypic study characterizes thirteen patients from eight unrelated consanguineous Saudi families; simplex (n = 5) and complex (n = 8).

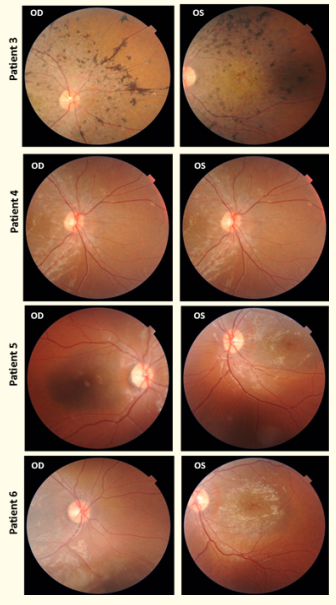

Figure 1: Retinal imaging of four affected siblings with ABCA4-associated diseases in Family 3.
Color Fundus of right eye (OD) and Left eye(OS) displaying; (patient 3): pale optic disc, retinal vessels attenuation and intraretinal bone spicules, (patient 4) pale optic disc, retinal vessels attenuation with no sign of RPE pigmentation; (Patient 5) pale optic disc, retinal vessels attenuation with RPE well-defined, oval-shaped retinal pigment epithelial defects at the foveal are; (Patient 6) pale optic disc, retinal vessels attenuation with general RPE mottling.

FA#	PT#	Gender	Pt status	Pheno.	Mutation	Geno.	Type	ACMG	Reference
F1	P1	M	Index	STGD	*ABCA4:*NM_000350: c.4739T>C:p.Leu1580Ser	HOM	Missense	4	Known [10]
F2	P2	F	Index	STGD	*ABCA4:*NM_000350:c.913C>T:p.Q305*	HOM	Nonsense	5	This study
F3	P3	F	Index	CRD	*ABCA4:*NM_000350:c.G4867C:p.G1623R	HOM	Missense	4	This study
	P4	F	Sister	CRD		HOM	Missense		
	P5	F	Sister	CRD		HOM	Missense		
	P6	F	Sister	CRD		HOM	Missense		
F4	P7	M	Index	CRD	*ABCA4:*NM_000350:c.1633_1634dupGAAA:p.Asn545Lysfs*24	HOM	Frame-shift	5	This study
	P8	F	Sister	CRD		HOM			
F5	P9	F	Index	RP	*ABCA4:*NM_000350: c.4316G>A; P.Gly1439Asp	HOM	Missense	4	Known [10]
	P10	M	Brother	RP		HOM			
F6	P11	M	Index	RP	*ABCA4:*NM_000350:c.4852T>A:p.Trp1618Arg	HOM	Missense	5	This study
F7	P12	M	Index	RP	*ABCA4:*NM_000350: c.5391_5392del:p.Ala1798*	HOM	Nonsense	5	This study
F8	P13	M	Index	RP	*ABCA4:*NM_000350: c.5460+1G>A	HOM	Splicing	4	Known [11]

Table 2: Variant assessment of the identified *ABCA4* mutations in patients with *ABCA4*-associated diseases.
Abbreviations used: FA#: family number; PT#: patient number; M: male; F: female; Geno: genotype; HOM.: STGD: Stargardt disease; RP: retinitis pigmentosa; CRD: cone-rod dystrophy; Geno.: genotype; HOM: homozygous;; Class 4: likely pathogenic and Class 5: pathogenic.

In our study, biallelic pathogenic variants in eight distinct ABCA4 biallelic mutations were identified in all the studied patients (100%), five of which are novel supporting autosomal recessive inheritance.

Other genomic studies of Saudi cohorts have reported a clinical detection rate of 63% but when solely considering autosomal recessive cases this percentage steeply increases to 93%, with almost exclusively homozygous pathogenic variants being identified which emphasizes the advantage of studying consanguineous families [8].

Herein, a clinical diagnosis of AAD-STGD was made mainly in 2/13 (15%). On the other hand, the majority of the studied families exhibited the diagnosis of either AAD-RP in 5/13 (39%) and AAD-CRD in 6/13 (46%) respectively.

Noteworthy, in family 3, with four affected siblings, despite the confirmed genotype with a novel missense *ABCA4:*NM_000350:c.G4867C:p.G1623R, distinct clinical phenotypes where observed in their color fundus photos ranging between no sign of RPE pigmentation to intraretinal bone spicules with variable range of macular defects. Thus, the dysfunction of *ABCA4* could give rise to a variety of retinal phenotypes classified mostly according to prognostic and genotypic presdpostion [9]. Thus, understanding the genotype-phenotype correlation will improves the clinical management and pave the gate to understand the natural history of the disease pathogenesis.

In conclusion, we took advantage of studying a highly consanguineous families and be able to solve them al. Our study represents a unique cohort of patients originating from consanguineous families with variable retinal phenotypes caused by known and novel recessive *ABCA4* mutations. This study expands the phenotypic – genotypic spectrum of AAD and provides an accurate genetic diagnosis that paves the gates for the opportunity of gene-based therapies in AAD within the scope of precision medicine.

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