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Genetic and Clinical Analysis of *ABCA4*-Associated Disease in African American Patients

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ABSTRACT: Autosomal recessive Stargardt disease (STGD1) is caused by hundreds of mutations in the *ABCA4* gene, which are often specific to racial and ethnic groups. Here, we investigated the *ABCA4* variation and their phenotypic expression in a cohort of 44 patients of African American descent, a previously under-characterized racial group. Patients were screened for mutations in *ABCA4* by next-generation sequencing and array-comparative genomic hybridization (aCGH), followed by analyses for pathogenicity by in silico programs. Thorough ophthalmic examination was performed on all patients. At least two (expected) disease-causing alleles in the *ABCA4* gene were identified in 27 (61.4%) patients, one allele in 11 (25%) patients, and no *ABCA4* mutations were found in six (13.6%) patients. Altogether, 39 different disease-causing *ABCA4* variants, including seven new, were identified on 65 (74%) chromosomes, most of which were unique for this racial group. The most frequent *ABCA4* mutation in this cohort was c.6320G>A (p.(R2107H)), representing 19.3% of all disease-associated alleles. No large copy number variants were identified in any patient. Most patients reported later onset of symptoms. In summary, the *ABCA4* mutation spectrum in patients of West African descent differs significantly from that in patients of European descent, resulting in a later onset and "milder" disease.

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KEY WORDS: ABCA4; Stargardt disease; next-generation sequencing; allelic heterogeneity; African American

Introduction

Mutations in the *ABCA4* gene are responsible for autosomal recessive Stargardt disease (STGD1; MIM #248200) [Allikmets et al., 1997], cone-rod dystrophy (CRD) [Cremers et al., 1998; Maugeri et al., 2000] and retinitis pigmentosa phenotypes [Cremers et al., 1998; Martinez-Mir et al., 1998; Shroyer et al., 2001]. *ABCA4*-associated diseases present with an extensive clinical and genetic heterogeneity, the current count of disease-associated *ABCA4* variants exceeds 800 [Allikmets, 2007] (RA and JZ, unpublished data). The most frequent disease-associated *ABCA4* variants have each been described in only ~10% of STGD1 patients of European descent [Burke et al., 2012]. However, many variants are more common in patients with specific geographic and ethnic backgrounds, such as the c.2588G>C (p.[G863A,G863del]) founder mutation in Northern European patients, [Maugeri et al., 1999] the c.[1622T>C; 3113C>T] (p.[L541P; A1038V]) complex allele in patients of mostly German origin [Maugeri et al., 2000; Rivera et al., 2000], the c.3386G>T (p.R1129L)founder mutation in Spain [Valverde et al., 2006], the c.2894A>G (p.N965S) variant in the Danish population [Rosenberg et al., 2007], and the c.5318C>T (p.A1773V) variant in Mexico [Chacon-Camacho et al., 2013].

Complete sequencing of the *ABCA4* coding and adjacent intronic sequences in patients with STGD1 routinely discovers ~75%–80% of mutations with the fraction of patients harboring the expected two disease-associated alleles comprising 65%–70%, with one mutation 15%–20%, and with no mutations in the remaining ~15% [Zernant et al., 2011]. These fractions depend on many variables, most importantly the quality of the clinical diagnosis and the ethnic composition of the cohort.

It is therefore clear that proper genetic diagnosis and interpretation of *ABCA4* alleles in ethnic and racial groups relies on many factors starting with a comprehensive database of disease-associated variants. Interestingly, there has been limited number of studies on STGD1 patients of African American descent. Only one case report [Huynh et al., 2014] and a small case series [Utz et al., 2013] have been described. The current study was designed to comprehensively address this issue by analyzing the clinical findings and, especially, the genetic composition of *ABCA4*-associated disease in African Americans.

Materials and Methods

Patients

Patients (44) affected with STGD1 (41), including seven with bull's eye phenotype, and CRD (three) were, after consenting in writing, recruited and clinically examined over a 10-year period at the Department of Ophthalmology, Columbia University, at the

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University of Illinois at Chicago, and at the Pangere Center for Hereditary Retinal Diseases, The Chicago Lighthouse for People Who Are Blind or Visually Impaired. Onset decade was defined by the age at which symptoms were first reported. Visual acuity was measured using the Early Treatment Diabetic Retinopathy Study (EDTRS) Chart 1 or a Snellen acuity chart. Diagnosis was based on patient history and ophthalmic examination. Fundus photography, fundus autofluorescence (AF), spectral domain-optical coherence tomography (SD-OCT) (Heidelberg Spectralis, Heidelberg, Germany, or OPTOS Instrumentation, Marlborough, MA), and full-field electroretinography (ERG) were performed as needed using standard acquisition protocols following pupil dilation with tropicamide 1% and phenylephrine 2.5%.

Patients diagnosed with STGD1 were classified into one of the four stages as described previously [Fishman et al., 1999]. Stage I disease was characterized by parafoveal or perifoveal flecks with frequent atrophy of the central macula. Stage II disease was characterized by flecks that were more numerous and extended anterior to the vascular arcades and/or nasal to the optic disc, Stage III was defined by resorption of flecks. Choriocapillaris atrophy within the macula was often observed in stage III patients. Stage IV disease, characterized by widespread RPE and chorioretinal atrophy throughout the fundus, was not observed in our cohort of Stargardt disease patients [Fishman et al., 1999].

All genetic research was carried out with the approval of the Institutional Review Boards of Columbia University and the University of Illinois at Chicago, and in accordance with the Declaration of Helsinki.

Array Screening

Screening with the *ABCA4* array was performed on most study subjects followed by direct Sanger sequencing to confirm identified changes, as previously described [Jaakson et al., 2003]. Since the array screening had been performed over many years, different versions of the *ABCA4* chip had been used, from the least representative (~300 mutations) to the recent version of the array (>600 variants).

Sequencing and Data Analysis

All 50 exons and exon–intron boundaries of the *ABCA4* gene were amplified using Illumina TruSeq Custom Amplicon protocol (Illumina, San Diego, CA), followed by sequencing on Illumina MiSeq platform. The next-generation sequencing reads were analyzed and compared to the *ABCA4* reference sequence NG_009073.1, using the variant discovery software NextGENe (SoftGenetics LLC, State College, PA). All detected possibly disease-associated variants were confirmed by Sanger sequencing. Nucleotide positions and protein translation correspond to CCDS747.1 and NP_000341.2, respectively. Nucleotide numbering uses the A of the ATG translation initiation start site as nucleotide 1.

The allele frequencies of all variants were compared to the Exome Variant Server (EVS) dataset, NHLBI Exome Sequencing Project, Seattle, WA (http://snp.gs.washington.edu/EVS/; accessed March 2014). All *ABCA4* variants reported in this manuscript were submitted to the ABCA4 LSDB (http://www.lovd.nl/ABCA4) at the Leiden Open Variation Database 3.0 (http://www.lovd.nl/3.0/home).

Segregation of the new variants with the disease was analyzed in families if family members were available (Table 1).

The possible effect of all *ABCA4* variants was assessed using the combination of following prediction programs: Polyphen-2 [Adzhubei et al., 2010], Align-GVGD [Tavtigian et al., 2006], SIFT [Ng and Henikoff, 2001], MutationTaster [Schwarz et al., 2010], SpliceSiteFinder [Zhang, 1998], MaxEntScan [Yeo and Burge, 2004], NNSPLICE [Reese et al., 1997], GeneSplicer [Pertea et al., 2001], and Human Splicing Finder [Desmet et al., 2009]. All of these algorithms except for Polyphen-2 were accessed via Alamut software version 2.2 (Interactive Biosoftware, Rouen, France; http://www.interactive-biosoftware.com), using automated computation of this software version. Polyphen-2 results were retrieved from the single entry Web form (http://genetics.bwh.harvard.edu/pph2/) with the HumDiv-model and version 2.2.2 of the software. Predictions are included as supporting data to the conclusions that are based on different frequencies of the variants between the patients and general population.

Array-Comparative Genome Hybridization

Custom array-comparative genome hybridization (aCGH) (Agilent Technologies, CA, United States) with high-density probes tiling the critical genetic loci of ABCA4-associated disease was designed to assess for copy number variations (CNVs) involving these genes. Each array slide was in 8 × 60K format, which tested eight separate samples with approximate 60,000 probes for each sample. Nine "primary" loci, including the *ABCA4* locus, other known genes often mimicking ABCA4-associated phenotypes (*ELOVL4*, *PRPH2*, *BEST1*, *RS1*, and *CNGB3*), and major age-related macular degeneration (AMD)-associated loci (*CFH*, *ARMS2*, and *C2/CFB*), were probed with ultra-high density (~20 probes per kilobase [kb] of DNA) throughout the entire genomic length of the genes plus the 5′ promoter regions and 3′ downstream regions, as well as at slightly lower density (~10 probes per kb) for flanking 5′ and 3′ conserved regions. Eighteen "secondary" AMD loci (*C3*, *APOE*, *CFI*, *LIPC*, *SYN3/TIMP3*, *CETP*, *COL8A1*, *BBX*, *PLD1*, *SPEF2*, *ADAM19*, *VEGFA*, *FRK*, *MEPCE*, *CHMP7/LOXL2*, *TGFBR1*, *NPS*, and *PICK1*) were probed with ultra-high density in exons (~8 probes per exon) and with lower resolution (~3 probes per kb) throughout each gene plus the flanking 5′ promoter regions and 3′ downstream regions.

DNA from 22 of the 44 African American patients diagnosed with *ABCA4*-associated disease, five with one mutation and 17 with two mutations in ABCA4, were used for aCGH analysis. For a positive control the DNA carrying a known, previously reported 1,030 bp heterozygous deletion of exon 18 in ABCA4, was used [Yatsenko et al., 2003]. Experimental procedures of aCGH were performed as described previously [Gonzaga-Jauregui et al., 2010] with minor modifications, and results were analyzed using Agilent Genomic Workbench version 7.0 software (Agilent Technologies, CA, United States). The called CNVs were filtered against several criteria and the plausible true-positive CNVs were tested by PCR for molecular validation.

Comparison with Patients of European Ancestry

The genetic and clinical data from patients of West African descent were compared with a Columbia in-house database of *ABCA4*-associated patients of European descent, which includes 244 patients. Comparisons were made for allele frequencies across patient and general populations, predicted or tested functional effect of selected mutations, disease onset, Fishman stage, and so on [Fishman et al., 1999].

Results

ABCA4 Mutations in African American Patients

After genetic analysis with a combination of *ABCA4* array screening and/or direct sequencing both *ABCA4* disease-causing alleles

Table 1. Pathogenic *ABCA4* Variants in Patients of African American Descent

Patient	Clinical Assessment			BCVA		CNV	
ID	*Diagnosis*	*Stage*	*Onset decade*	*OD*	*OS*	analysis	ABCA4 mutations
1	STGD1	2	8th	20/25	20/25		p.[(P1380L)];[?]
2	STGD1	2	5th	10/100	10/160	X	p.[(R220C(;)R2107H)]
3	STGD1	1	4th	20/30	20/200		p.[(G1961E(;)D403V)]
4	STGD1	2–3	3rd	20/400	20/100	X	p.[(W1408L(;)R2107H)]
5	STGD1	0	5th	20/200	20/50	X	p.[(P62S)];[?]
6	STGD1	1	7th	20/25-2	20/25-3	X	p.[(R2107H)];[?]
7	STGD1	N/A	4th	20/70+2	20/25-2	X	p.[(P309R(;)R681∗)]
8	STGD1	1	4th	20/15-3	20/20+1	X	p.[(G991R(;)L1126P)]
9	STGD1	N/A	2nd	20/400	20/400	X	c.5289del(;)p.(V989A)
10	STGD1	N/A	N/A	N/A	N/A		p.[(R2077G(;)R2107H)]
11	STGD1	2	3rd	20/200	20/200	X	c.3523-1G>A(;)p.(R2107H)
12	STGD1	2	4th	20/20	20/20	X	p.[(G991R)];[?]
13	STGD1	1	4th	20/70+2	20/25-2		no mutations found
14	STGD1	3	1st	20/400	20/400	X	c.768G>T(;)p.(R2107H)
15	STGD1	1	5th	20/50-2	20/40	X	p.[(V989A(;)R2107H(;)P870S)]
16	STGD1	2–3	1st	20/400	20/300	X	p.[(G607R(;)R2040Q)]
17[a]	STGD1	1	2nd	10/100-1	10/60-1	X	p.[(P309R(;)R537C(;)R2107H)]
18	STGD1	2	1st	20/400	20/400	X	c.5461-10T>C(;)p.(R2040Q)
19	STGD1	2	2nd	10/120+1	10/100-1		c.5461-10T>C(;)p.(E531G)
20	STGD1	1	3rd	20/80-2	20/125+1		No mutations found
21[a]	STGD1 (BEM)	1	2nd	20/100	20/150	X	p.[(G991R)];[(L1138P)]
22[a]	STGD1	2	3rd	20/400	20/50-2		c.4537dup;p.(V1686M)
23	STGD1	2	2nd	20/125	20/125		p.[(R1640W)];[?]
24	CRD	3	1st	CF	CF		p.[(T983A(;)L1729P)]
25	STGD1	1	4th	20/30+2	20/40-2	X	p.[(V989A)];[(V989A)]
26	STGD1	3	2nd	20/150	20/25		p.[(N965S(;)R2040Q)]
27[a]	CRD	3	1st	CF	CF	X	c.4540-2A>G;p.(R2107H)
28	STGD1	2	6th	20/30	20/40-1	X	c.5461-10T>C;[?]
29	STGD1	1	6th	20/20	20/20	X	p.[(F1015L)];[?]
30	STGD1 (BEM)	1	5th	20/50	20/30		No mutations found
31	STGD1 (BEM)	1	6th	20/20	20/20		p.[(R2107H)];[?]
32	STGD1 (BEM)	1	3rd	20/20	20/20	X	p.[(R1300∗(;)R2106C)]
33	STGD1 (BEM)	1	5th	20/100	20/125		p.[(R2107H)];[?]
34	STGD1	3	3rd	LP	LP		p.[(R2107H)];[?]
35	STGD1	2	5th	20/40+1	20/40	X	p.[(W339G(;)R2107H)]
36	STGD1	3	1st	20/30	20/40		No mutations found
37	STGD1	1	5th	20/25	20/25-1		p.[(I975M(;)K1978E)]
38	STGD1	2	2nd	20/200	20/200		p.[(V989A)];[?]
39	STGD1	1	3rd	20/100-1	20/200+1		c.302+1G>A(;)p.(R2107H)
40	STGD1 (BEM)	1	7th	20/20-3	20/20-3		p.[(R2107H)];[(R2107H)]
41	STGD1	1	3rd	30/30-2	20/100		No mutations found
42	STGD1 (BEM)	1	5th	20/40-2	20/50-2		No mutations found
43	CRD	N/A	3rd	5/180+1	5/160+1	X	p.[(V989A)];[?]
44[a]	STGD1	2	N/A	N/A	N/A		c.4253+4C>T(;)p.[(G1961E(;) R2107H)]

Nucleotide positions and protein translation correspond to CCDS747.1 and NP_000341.2, respectively. Nucleotide numbering uses the A of the ATG translation initiation start site as nucleotide 1.
BCVA: best corrected visual acuity; CNV: copy number variation; N/A: not available; CF: counting fingers; LP: light perception.
[a]The variants are confirmed on different chromosomes.

were identified in 27/44 (61.4%) of African American patients, one disease-causing allele in 11 (25%) patients, and no *ABCA4* mutations were found in six (13.6%) patients. Altogether, 65 (73.9%) disease-causing *ABCA4* alleles were identified in the cohort of 44 patients of African American origin presenting with clinically diagnosed *ABCA4*-associated disease. These data correlate well with the comparative numbers/fractions of patients of mostly, or exclusively, European descent. In a cohort of 496 patients presenting with likely *ABCA4*-associated diseases of mostly European origin, we identified 698 *ABCA4* mutations (70.4%), whereas in the cohort of 244 patients of definitely European origin, this fraction was 352 (72.1%).

The combination of array screening and sequencing identified 47 different *ABCA4* variants in the 44 patients (Tables 1–3). Eight variants (Tables 2 and 3) were deemed benign based on similar allele frequencies between patients and the general population of African American descent (data derived from screening of 2203 individuals on the EVS), and/or by predictive programs. These include frequent variants c.3602T>G (p.L1201R), c.3899G>A (p.R1300Q), c.1927G>A (p.V643M), and c.4925C>A

Table 2. Analysis of the *ABCA4* (GenBank Reference Sequence NG_009073.1) Variants by Predictive Programs

Exon/ intron	Nucleotide change	Protein variant	Polyphen-2 prediction	AGVGD class	SIFT prediction	TASTER prediction	Predicted effect on splicing
3	c.184C>T	p.P62S	Probably damaging	C65	Deleterious	Disease causing	
IVS3	c.302+1G>A						Eliminates the donor
6	c.658C>T	p.R220C	Possibly damaging	C25	Deleterious	Polymorphism	
6	c.768G>T						Donor decrease by 47%
8	c.926C>G	p.P309R	Benign	C65	Deleterious	Disease causing	
8	c.1015T>G	p.W339G	Possibly damaging	C65	Deleterious	Disease causing	
9	**c.1208A>T**	**p.D403V**	Benign	C65	Deleterious	Disease causing	
11	c.1538T>C	p.V513A[a]	Benign	C0	Tolerated	Polymorphism	
12	**c.1592A>G**	**p.E531G**	Probably damaging	C65	Deleterious	Disease causing	
12	c.1609C>T	p.R537C	Probably damaging	C45	Deleterious	Disease causing	
13	c.1819G>A	p.G607R	Probably damaging	C65	Deleterious	Disease causing	
13	c.1927G>A	p.V643M[a]	Probably damaging	C15	Deleterious	Disease causing	
14	c.2041C>T	p.R681*					
16	c.2546T>C	p.V849A	Benign	C25	Deleterious	Polymorphism	
17	**c.2608C>T**	**p.P870S**	Possibly damaging	C65	Deleterious	Disease causing	
19	c.2791G>A	p.V931M[a]	Possibly damaging	C0	Deleterious	Disease causing	
19	c.2894A>G	p.N965S	Probably damaging	C45	Deleterious	Disease causing	New acceptor site
20	**c.2925C>G**	**p.I975M**	Possibly damaging	C0	Deleterious	Disease causing	
20	c.2947A>G	p.T983A	Probably damaging	C55	Deleterious	Disease causing	New acceptor site
20	c.2966T>C	p.V989A	Benign	C25	Deleterious	Disease causing	
20	c.2971G>C	p.G991R	Probably damaging	C65	Deleterious	Disease causing	
20	**c.3043T>C**	**p.F1015L**	Possibly damaging	C15	Deleterious	Disease causing	
23	c.3377T>C	p.L1126P	Probably damaging	C65	Deleterious	Disease causing	
23	c.3413T>C	p.L1138P	Probably damaging	C65	Deleterious	Disease causing	
23	c.3416T>C	p.Y1139C[a]	Benign	C55	Deleterious	Disease causing	
IVS23	c.3523-1G>A						Eliminates the acceptor
24	c.3602T>G	p.L1201R[a]	Benign	C65	Deleterious	Disease causing	
27	c.3898C>T	p.R1300*					
27	c.3899G>A	p.R1300Q[a]	Benign	C0	Tolerated	Polymorphism	
28	c.4139C>T	p.P1380L	Probably damaging	C65	Deleterious	Disease causing	
28	c.4223G>T	p.W1408L	Probably damaging	C55	Deleterious	Disease causing	
IVS28	c.4253+4C>T						Weakens the donor by 14%
30	c.4537dup	p.Q1513fs					
IVS30	c.4540-2A>G						Eliminates the acceptor
35	c.4918C>T	p.R1640W	Probably damaging	C65	Deleterious	Disease causing	
35	c.4925C>A	p.S1642I[a]	Benign	C35	Deleterious	Polymorphism	
36	c.5056G>A	p.V1686M	Probably damaging	C15	Deleterious	Disease causing	
36	c.5077G>A	p.V1693I[a]	Benign	C0	Tolerated	Polymorphism	
36	c.5186T>C	p.L1729P	Possibly damaging	C0	Deleterious	Disease causing	
37	**c.5289del**	**p.V1764fs**					
IVS38	c.5461-10T>C						Effect unknown
42	c.5882G>A	p.G1961E	Probably damaging	C65	Deleterious	Disease causing	
43	**c.5932A>G**	**p.K1978E**	Probably damaging	C55	Deleterious	Disease causing	
44	c.6119G>A	p.R2040Q	Probably damaging	C35	Deleterious	Disease causing	
45	c.6229C>G	p.R2077G	Probably damaging	C65	Deleterious	Disease causing	
46	c.6316C>T	p.R2106C	Probably damaging	C65	Deleterious	Disease causing	
46	c.6320G>A	p.R2107H	Probably damaging	C25	Deleterious	Disease causing	

Nucleotide positions and protein translation correspond to CCDS747.1 and NP_000341.2, respectively. Nucleotide numbering uses the A of the ATG translation initiation start site as nucleotide 1.
Novel *ABCA4* variants are indicated **in bold**.
Prediction programs (except for Polyphen-2) were accessed via Alamut software version 2.2 (http://www.interactive-biosoftware.com), using automated computation of this software version. Polyphen-2 results were retrieved from the single entry Web form (http://genetics.bwh.harvard.edu/pph2/) with the HumDiv-model and version 2.2.2 of the software.
[a]Benign variants.

(p.S1642I). Several known frequent missense or silent variants, including c.635G>A (p.R212H), c.1268A>G (p.H423R), c.1269C>T, c.2828G>A (p.R943Q), c.4203C>A, c.5603A>T (p.N1868I), c.5814A>G, c.5843C>T (p.P1948L), c.5844A>G, c.6069C>T, c.6249C>T, c.6285T>C, c.6732G>A, and c.6764G>T (p.S2255I), which have been previously defined as benign, were not included in the analysis. Although the allele frequencies of some of these variants are very different between the general populations of European and African descent, these are not different between the affected and unaffected individuals in each ethnic group. Seven *ABCA4* variants, c.6320G>A (p.(R2107H)), c.2966T>C (p.(V989A)), c.2971G>C (p.(G991R)), c.5461–10T>C, c.6119G>A (p.(R2040Q)), c.926C>G (p.(P309R)), and c.5882G>A (p.(G1961E)), were identified in two or more patients, 32 *ABCA4* variants were each detected only once (Tables 1 and 3).

Five mutations, c.1208A>T (p.(D403V)), c.2608C>T (p.(P870S)), c.3043T>C (p.(F1015L)), c.52089del (p.(V1764fs)), and c.5932A>G (p.(K1978E)), had not been detected before, and two mutations, c.1592A>G (p.(E531G)) and c.2925C>G (p.(I975M)), had not been described as associated with the disease. Interestingly, no large (i.e., those undetectable with PCR-based sequencing methods, usually one exon or larger) copy number variants in the *ABCA4* locus were identified in any patient.

Table 3. ABCA4 Variants Found in Patients of African American Origin, Ranked by Allele Frequency and Compared with the Frequency in the Patients of European Descent

	African origin cohort (44)			European origin cohort (244)		
Variant	Alleles	Allele frequency	Frequency by EVS	Alleles	Allele frequency	Frequency by EVS
p.R2107H	17	19.32	2.04	5	1.02	0.01
p.L1201R[a]	12	13.64	9.35	1	0.20	0.05
p.V989A	6	6.82	0.25	1	0.20	
p.R1300Q[a]	5	5.68	6.17	2	0.41	0.05
p.V643M[a]	3	3.41	1.82			
p.G991R	3	3.41	0.64	2	0.41	
p.S1642I[a]	3	3.41	1.23			
p.R2040Q	3	3.41	0.05			0.01
c.5461-10T>C	3	3.41		23	4.71	0.03
p.P309R	2	2.27	0.16			
p.G1961E	2	2.27	0.11	55	11.27	0.42
p.P62S	1	1.14				
c.302+1G>A	1	1.14				
c.768G>T	1	1.14				
p.R220C	1	1.14				
p.W339G	1	1.14				
p.D403V	1	1.14				
p.V513A[a]	1	1.14				
p.E531G	1	1.14	0.09			
p.R537C	1	1.14	0.02			
p.G607R	1	1.14	0.02			
p.R681*	1	1.14				
p.V849A[a]	1	1.14	1.23			
p.P870S	1	1.14				
p.N965S	1	1.14		3	0.61	0.01
p.V931M[a]	1	1.14	0.41			
p.F1015L	1	1.14				
p.I975M	1	1.14	0.02			
p.T983A	1	1.14		1	0.20	
p.L1126P	1	1.14				
p.L1138P	1	1.14				
p.Y1139C[a]	1	1.14	0.09			
c.3523-1G>A	1	1.14				
p.R1300*	1	1.14				
p.P1380L	1	1.14		26	5.33	0.02
p.W1408L	1	1.14				
c.4253+4C>T	1	1.14		1	0.20	
c.4537dup	1	1.14		1	0.20	
c.4540-2A>G	1	1.14		3	0.61	
p.R1640W	1	1.14		3	0.61	
p.L1729P	1	1.14				
p.V1686M	1	1.14	0.07	1	0.20	0.03
p.V1693I[a]	1	1.14	0.18			
c.5289del	1	1.14				
p.K1978E	1	1.14				
p.R2077G	1	1.14		1	0.20	
p.R2106C	1	1.14	0.05	1	0.20	

Frequency by EVS—frequency of the variant in the general populations of, respectively, African American and European descent as determined in the Exome Variant Server (http://snp.gs.washington.edu/EVS/; accessed March 2014).
[a]Benign variants.

Analysis of Frequent Variants

From more frequent (i.e., represented in three or more patients) variants from both ethnic groups, the intronic disease-associated variant with yet unknown function, c.5461–10T>C, was the only variant detected in both groups with similar allele frequencies, 3.4% in the African American and 4.71% in the European patient group (Table 3). It was also the only frequent variant not seen in the general population of West African descent. The other six frequent variants in African American patients—p.R2107H, p.V989A, p.G991R, p.R2040Q, p.P309R, and p.G1961E—were seen in the matched general population at frequencies between 0.05% and 2% (Table 3). However, their respective frequencies in the patient cohort were at least 10× higher (Tables 2 and 3), therefore strongly suggesting their association with the disease.

The most frequent *ABCA4* mutation in patients of West African descent was p.R2107H, accounting for 19.3% of all disease-associated alleles in this cohort, which is much higher than for any other known *ABCA4* disease-associated variant. By comparison, the most frequent *ABCA4* mutation in patients of European ancestry—p.G1961E—has an average allele frequency of ∼11% [Burke et al., 2012]. Another, somewhat surprising, observation is that the frequency of the p.R2107H in the general population of African Americans is 2% (Table 3), which is much higher than the suggested and observed frequency for highly penetrant disease-associated alleles in general. It also suggests that one in 2,500 African Americans

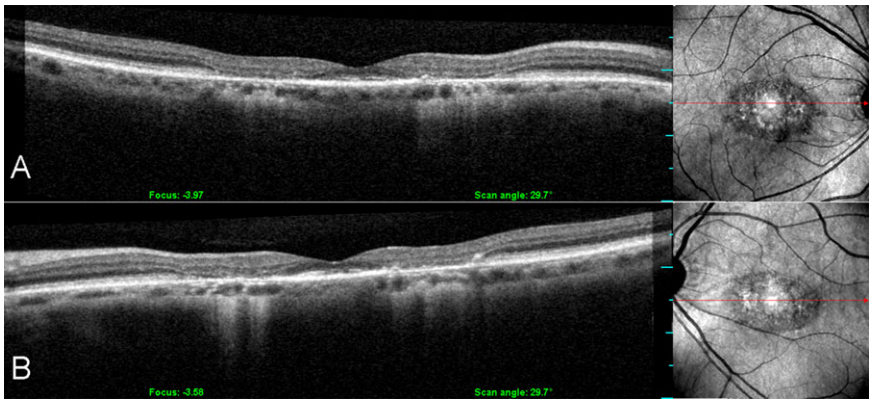

Figure 1. Clinical presentation of STGD1 in patient 40. Spectral-domain optical coherence tomography horizontal line scans through the fovea of the right **(A)** and left **(B)** eyes of patient 40, who was homozygous for the p.R2107H mutation. The inner-segment ellipsoid and outer nuclear layers are absent in areas of the parafovea, but both layers are present in the fovea. Hyper-reflectance seen in the parafovea on the infrared scanning laser ophthalmoscopy images (right side of **A** and **B**) corresponds to increased reflectance from the choroid and sclera on SD-OCT, which is suggestive of RPE and choriocapillaris atrophy in those areas.

is homozygous for the p.R2107H variant, implicating a much higher than the suggested prevalence (1:10,000) of *ABCA4*-associated disease [Blacharski, 1988]. Furthermore, if the variant is fully penetrant, 4% of STGD patients of African descent should be homozygous for the variant; one out of 44 (2.3%) patients was in our cohort. Finally, the p.R2107H variant was called "disease-causing" by all predictive programs (Table 2) and has been previously shown to alter ATP hydrolysis in vitro [Sun et al., 2000]. Altogether, these data suggest that the p.R2107H variant is a highly penetrant disease-associated allele in patients of West African descent presenting with *ABCA4*-associated diseases.

Analysis of Rare Variants

Among 36 variants detected only once, 32 were classified as disease-associated by predictive programs and/or segregation analyses (Table 2). Variants c.1538T>C (p.(V513A)), c.2546T>C (p.(V849A)), c.2791G>A (p.(V931M)), c.5077G>A (p.(V1693I)), and possibly, c.3416T>C (p.(Y1139C)) were deemed benign. Interestingly, of the 32 variants only 10 had been seen in large patient cohorts of European descent suggesting that 22 (more than 2/3) variants are specific for patients of West African descent. The clinical expression of the disease in patients with rare mutations was very heterogeneous, as expected. The onset, expression and progression depend, as in patients of European descent, on the specific combinations of severe and mild *ABCA4* disease-associated alleles. Since most mutations were detected only once in this group, no specific genotype/phenotype correlations were possible. Seven patients carried definitely deleterious mutation (~8% from all alleles), which is two times less than, on average, seen in cohorts of European descent (~15%). This may also explain the milder, later onset disease in African American patients, as discussed below.

Patient Phenotypes

Patient #40 was homozygous for the p.R2107 variant. He was seen at the age of 61 years, at which point he had noted subtle color vision changes for the past year. Best corrected Snellen visual acuity was $20/20^{-3}$ in each eye. Fundus examination showed a bull's eye-appearing macular lesion, with a notable absence of fundus flecks and relative foveal sparing. SD-OCT (Fig. 1) showed loss of the inner-segment ellipsoid layer (ISe) and outer nuclear layer (ONL) in the parafoveal area, with relative sparing of those layers in the fovea.

Patient #25 was homozygous for the p.V989A variant. When seen at the age of 39 years, she reported blurred central vision, mild color vision changes, and some difficulty seeing in dim illumination. Her best corrected visual acuities at the most recent examination, when she was 42 years of age, were $20/30^{+2}$ OD and $20/40^{-2}$ OS. On fundus examination, she had bull's eye-appearing macular lesions with a ring of flecks confined to the macula (stage 1) and relative foveal sparing (Fig. 2) in both eyes. SD-OCT showed ISe and ONL loss in the parafoveal area, with relative sparing of those layers in the fovea (Fig. 2). Short wavelength FAF showed a ring of hypo-AF, a few highly hypo-autofluorescent areas of atrophy, and hyper-autofluorescent flecks in the macula. Full-field ERG scotopic and photopic amplitudes were within normal limits.

Fifteen patients were heterozygous for the p.R2107H allele; in four patients, this was the only *ABCA4* allele found, 11 patients were compound heterozygous for another disease-associated variant. The clinical presentation of the disease varied widely in these patients most likely depending on the severity of the other allele. For example, patients with clearly deleterious splicing mutations on the other chromosome (c.3523–1G>A in patient #11, c.768G>T in patient #14, and c.4540–2A>G in patient #27) had an earlier disease onset and rapid progression, whereas patients with a "milder" second mutation had later onset and slower disease progression. As an example from this group, patient #15 was compound heterozygous for p.R2107H and p.V989A mutations and also had a third variant, p.P870S. She was seen at the age of 49 years, at which time she reported decreased central vision for the past year, as well as difficulty with night driving for the past 5 years. Her best corrected visual acuity was $20/50^{-2}$ OD and 20/40 OS. Fundus examination showed bull's eye-appearing lesions in both eyes, with a ring of flecks in the macula, and mild temporal optic disc pallor, but otherwise normal optic nerves and retinal vessels.

Comparison with Patients of European Descent

Of the six most frequent disease-associated variants (p.R2107H, p.V989A, p.G991R, c.5461–10T>C, p.R2040Q, and p.P309R) in African American patients, only the intronic c.5461–10T>C

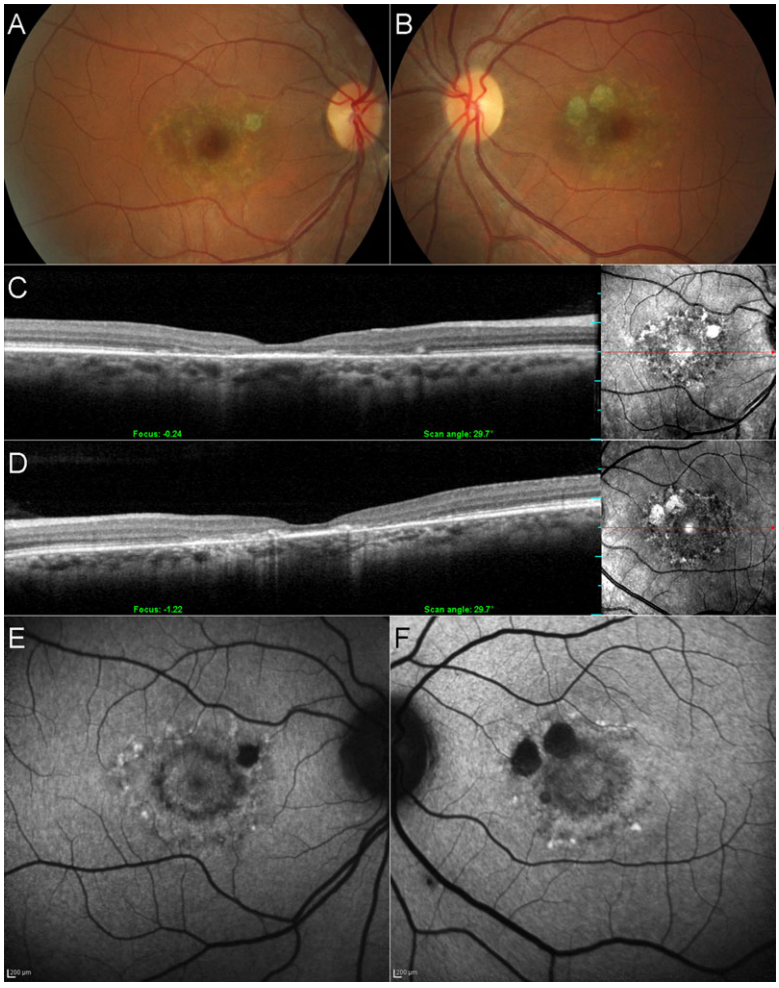

Figure 2. Clinical presentation of STGD1 in patient 25. Fundus photos of the right **(A)** and left **(B)** eyes of patient 25 (homozygous for p.V989A mutations), show bull's eye-appearing macular lesions with macular flecks and well-demarcated atrophic areas in both eyes. Horizontal spectral-domain optical coherence tomography **(C and D)** line scans show loss of inner-segment ellipsoid and outer nuclear layers in the parafovea of the each eye. Corresponding infrared scanning laser ophthalmoscopy images (right side of **C and D**) shows some hypo-reflectance in the parafoveal areas, with hyper-reflectance primarily in the areas of the demarcated atrophic lesions. Short-wavelength fundus autofluorescence **(E and F)** highlights bull's eye-appearing macular lesions in each eye. Macular flecks are hyper-autofluorescent, while localized loss of **RPE** cells are evidenced by the well demarcated hypo-autofluorescent regions.

variant was seen at equal frequency in patients of European descent (Table 3). The p.R2107H, p.V989A, and p.G991R variants were seen in Caucasian patients at much lower frequencies; in fact, the allele frequencies for these variants were lower in patients of European descent than in the general population of West African descent (Table 3). Although these variants were proven to represent disease-associated mutations, their presence in Caucasian patients most likely suggests admixture between ethnic groups. The same is, conversely, true for the two most frequent mutations in Caucasian patients, c.5882G>A (p.(G1961E)) and c.4138C>T (p.(P1380L)), which are (almost) absent in the general population of West African origin (Table 3). These mutations were detected in two and one African American patients, respectively, suggesting admixture. All other most prevalent disease-associated *ABCA4* variants in the cohort of European descent, including c.[1622T>C; 3113C>T] (p.[L541P;A1038V]), c.2588G>C (p.G863A), c.5714+5G>A, c.3322C>T (p.R1108C), c.6079C>T (p.L2027F), and c.161G>A (p.C54Y), were not seen in African American patients at all.

The clinical presentation of the disease differed significantly between the two ethnic groups with patients of West African descent having later onset of the disease. When compared with randomly selected 100 STGD1/CRD patients of Caucasian descent from our database, only 14% of patients of West African descent had the disease onset in the 1st decade, compared with 25% of white patients. Most interestingly, close to 30% of African American patients had the disease onset in the 5th decade or later, whereas this fraction in the Caucasian cohort was only 12% ($P = 0.03$). Detailed analysis of the disease progression was available in only a limited number of patients precluding statistical analysis.

Discussion

Genetic analysis of 44 African American patients revealed that *ABCA4* mutations in patients of this ethnic background differ to a large extent from patients with *ABCA4*-associated diseases of European descent. The mutation spectrum was different on

several levels: First, most mutations in patients of West African descent were unique, or more specific, for this ethnic group. Very different distribution of *ABCA4* disease-associated alleles was true for both frequent and rare variants. Second, several *ABCA4* variants that had been previously classified as disease-causing in patients of European descent were deemed benign since they represent ancestral alleles as determined by evolutionary analyses, and by comparison of allele frequencies in African American patients with *ABCA4*-associated diseases and the general population. Conversely, several variants that were previously considered not associated with the disease, since these were very frequent (up to 2%) in the general population of African American origin, were proven to be causal in *ABCA4*-associated diseases since the allele frequencies for these variants were at least 10× higher in affected individuals than in the general population. This observation was further confirmed by segregation analyses in families and predictive programs.

The differences in genetic causality were also clearly reflected in the clinical phenotypes. The disease in African American patients had a statistically significantly later onset and a trend toward milder expression. Further studies are necessary to establish possible differences in the rate of disease progression. In summary, the spectrum of *ABCA4* mutations in patients of West African descent differs significantly from that in patients of European origin. In addition, the phenotypic presentation of *ABCA4* alleles in African Americans is also different from that in patients of European ancestry. This study confirms extensive genetic and a degree of clinical heterogeneity of *ABCA4*-associated diseases in patients of West African descent and offers guidelines for interpretation of both clinical and, especially, genetic analyses to correctly diagnose African American patients with *ABCA4*-associated diseases.

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