Structural and Genetic Assessment of the *ABCA4*-Associated Optical Gap Phenotype

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PURPOSE. To investigate the developmental stages and genetic etiology of the optical gap phenotype in recessive Stargardt disease (STGD1).

METHODS. Single and longitudinal data points from 15 patients, including four sibling pairs, exhibiting an optical gap phenotype on spectral-domain optical coherence tomography (SD-OCT) with confirmed disease-causing *ABCA4* alleles were retrospectively analyzed. Fundus images with corresponding SD-OCT scans were collected with a confocal scanning laser ophthalmoscope. Structural phenotypes were assigned to three developmental stages according to SD-OCT. The *ABCA4* gene was screened in all patients.

RESULTS. At least two disease-causing *ABCA4* variants were identified in each patient; all except one (91%) were compound heterozygous for the p.G1961E mutation. All patients exhibited structural findings on SD-OCT that grouped into three progressive developmental stages over several years. Stage 1 was characterized by mild disruptions of the ellipsoid zone (EZ) band over the fovea. Stage 2 was a progressive expansion of the EZ band loss resulting in an empty lesion devoid of photoreceptors. Stage 3 observed a structural collapse of the inner retinal layers into the optical gap space leading to involvement and atrophy of the RPE thereafter.

CONCLUSIONS. The optical gap phenotype in STGD1 can be structurally divided into three progressive stages spanning several years. This particular phenotype also appears to be highly associated with the p.G1961E mutation of *ABCA4*. Taken together, it appears that a focal loss of photoreceptors sequentially precedes RPE dysfunction in the early development of *ABCA4*-associated optical gap lesions.

Keywords: *ABCA4*, Stargardt disease, optical gap, p.G1961E mutation, optical coherence tomography

Stargardt disease (STGD1) is an early-onset autosomal recessive macular dystrophy with a reported prevalence between 1:8000 to 1:10,000, making it the most common form of juvenile macular disease.¹ Stargardt disease is caused by mutations in the *ABCA4* gene, which encodes an adenosine triphosphatase (ATP)-binding cassette transporter located in the outer segments of photoreceptors.² *ABCA4* performs an important function in the visual cycle being responsible for flipping of all-*trans*- and 11-*cis*-retinoids from the intradiscal space to the cytoplasm.³ Mutations in *ABCA4* result in the accumulation of protonated N-retinylidene-PE (N-ral-PE) in the photoreceptor outer segments along with a secondary accumulation of N-retinylidene-N-retinyl-ethanolamine (A2E) in the RPE cells during the process of disc shedding and subsequent phagocytosis.^{4,5} The excess of A2E has been associated with a toxic effect on RPE cells resulting in cell death.^{6,7} In addition to phenotypic heterogeneity within the clinical spectrum of STGD1,⁸ mutations in *ABCA4* have been reported in other retinal degenerative diseases such as cone-rod dystrophy,⁹ autosomal recessive retinitis pigmentosa,^{10,11} and AMD.¹² Stargardt disease often initially presents with early atrophic changes in the macula and white-yellow pisciform flecks, but can vary in time from the appearance of bull's eye

maculopathy¹³ to extensive chorioretinal atrophy.^{14,15} Several grading systems have been established to characterize the overall progression of STGD1 phenotype.^{14,16} Functionally, STGD1 can be staged into three groups with respect to electrophysiological findings of the outer retina: Group 1 exhibits pattern electroretinography (pERG) abnormalities, but normal full-field photopic and scotopic responses, group 2 exhibits changes in isolated photopic function and group 3 exhibits significant dysfunction in both the scotopic and photopic systems.¹⁶

A less common previously documented phenotype within the STGD1 clinical spectrum is the optical gap, also referred to as an optical empty lesion or foveal cavitation.^{13,17,18} In addition to STGD1, optical gaps have been described in solar retinopathy, rod monochromatism, and maculopathies associated with *RP11* mutations.^{19–23} The optical gap is exclusively detectable by spectral-domain optical coherence tomography (SD-OCT) and appears to represent a focal loss of ellipsoid zone (EZ) reflectance in the outer fovea.¹⁷ The aim of this study was to characterize the optical gap phenotype according to its developmental stages and to investigate its association with specific *ABCA4* mutations.

TABLE 1. Summary of Demographic and Clinical Data of ABCA4-Associated Optical Gap Patients

Patient #, SEX	Age, y	BCVA Snellen (logMAR)		Age of Onset, y	Optical Gap Staging				ERG Group	Lesion Shape on AF		Fixation	
		OD	OS		Initial		Current			Elliptical	Circular	OD	OS
					OD	OS	OD	OS					
P1, F	22	20/40 (0.3)	20/50 (0.40)	Unknown	1	1	1	1	N/A	-	+	N/A	N/A
P2, F	25	20/40 (0.3)	20/30 (0.18)	24	1	1	1	1	N/A	-	+	Eccentric	Eccentric
P3, M	30	20/30 (0.18)	20/30 (0.18)	25	1	1	1	1	G1	-	+	N/A	N/A
P4, M	30	20/25 (0.10)	20/25 (0.10)	25	1	1	1	1	G1	-	+	N/A	N/A
P5, F	18	20/60 (0.48)	20/60 (0.48)	14	3	0	0	1	G1	+	-	Eccentric	Eccentric
P6, F	24	20/150 (0.88)	20/150 (0.88)	14	2	2	2	2	G1	Speckled	-	N/A	N/A
P7, F	22	20/100 (0.70)	20/100 (0.70)	17	2	2	2	2	G1	+	-	Eccentric	Eccentric
P8, F	19	20/80 (0.60)	20/80 (0.60)	15	2	2	2	2	G1	+	-	Eccentric	Eccentric
P9, F	25	20/100 (0.70)	20/150 (0.88)	15	1	2	2	2	G1	+	-	Eccentric	Eccentric
P10, F	23	20/40 (0.3)	20/30 (0.18)	18	1	2	2	2	G1	+	-	Foveal	Eccentric
P11, M	23	20/40 (0.3)	20/30 (0.18)	22	1	2	2	2	G1	-	+	Foveal	Eccentric
P12, F	22	20/50 (0.40)	20/70 (0.54)	21	3	2	2	2	G1	+	-	N/A	N/A
P13, M	12	20/50 (0.40)	20/50 (0.40)	10	2	2	2	2	G1	+	-	Eccentric	Eccentric
P14, F	30	20/100 (0.70)	20/100 (0.70)	25	2	Atrophy	2	Atrophy	G1 ⁺	-	-	Eccentric	Eccentric
P15, F	28	20/30 (0.18)	20/30 (0.18)	26	3	3	3	Atrophy	G1	+	-	Eccentric	Eccentric

F, female; M, male; N/A, not available.

* Left eye only.

MATERIALS AND METHODS

Patients and Clinical Evaluation

A retrospective review of 437 patients with a clinical diagnosis and genetic confirmation of STGD1 was conducted at the Department of Ophthalmology, Columbia University (New York, NY, USA). Of this cohort, color fundus photos, autofluorescence (AF) imaging, and SD-OCT were available in 179 patients. A single reader (KN) identified the presented optical gap phenotype on SD-OCT within this set of clinical data and confirmed the findings with the referring retina physician (SHT). Fifteen patients with the optical gap phenotype were identified and included in the study. Phenotypic staging of each patient was defined and carried out by two independent graders (KN and WL).

All patients were enrolled in the study after consenting under the protocol #AAAI9906. The protocol was approved by the institutional review board at Columbia University and adhered to tenets set out in the Declaration of Helsinki. Each patient underwent a complete ophthalmic examination by a retinal specialist (SHT), including slit-lamp examination and dilated fundus examination. The function of the retina was assessed with full-field and multifocal ERG, best corrected visual acuity (BCVA) was detected, while the structure was examined using color fundus photography, AF imaging, and SD-OCT. Fixation was assessed using fundus photos with fixation needle. Patients were grouped according to the developmental stages of the optical gap phenotype, which were generated based on SD-OCT images.

Retinal Imaging

Color fundus photos were obtained with a FF 450plus Fundus Camera (Carl Zeiss Meditec AG, Jena, Germany). Spectral-domain OCT scans and corresponding fundus images (488-nm AF and/or near-infrared [IF] reflectance) were acquired using a Spectralis HRA-OCT (Heidelberg Engineering, Heidelberg, Germany). Data from one patient (P14) was acquired with the Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA, USA). Autofluorescence images were acquired by illuminating the fundus with an argon laser source (488 nm) and viewing the resultant fluorescence through a bandpass filter with a short wavelength cutoff at 495 nm.

Full-Field and Multifocal Electroretinography

Ganzfeld full-field ERGs for each patient were recorded using the Diagnosys Espion Electrophysiology System (Diagnosys LLC, Littleton, MA, USA). For each recording, the pupils were maximally dilated and measured before testing using guttate Tropicamide (1%) and Phenylephrine Hydrochloride (2.5%); and the corneas were anesthetized with guttate Proparacaine 0.5%. Silver impregnated fiber electrodes (DTL; Diagnosys LLC, Littleton, MA, USA) were used with a ground electrode on the forehead. Full-field ERGs to test generalized retinal function were performed using extended testing protocols incorporating the International Society for Clinical Electrophysiology of Vision standard.²⁴ Multifocal (mf) ERG was recorded and analyzed with the VERIS system (VERIS EDI, San Mateo, CA, USA) using a Burian-Allen contact electrode. Test was performed according to International Society for Clinical Electrophysiology of Vision standards and guidelines.²⁵

ABCA4 Screening and Analysis

All patients were screened for ABCA4 variants by complete sequencing of all coding and intron/exon boundaries of the

TABLE 2. Summary of Genetic Data of *ABCA4*-Associated Optical Gap Patients

Patient #, Sex	<i>ABCA4</i> Mutations	
	DNA Level	Protein Level
1, F*†	c. [286A > G]; [5882G > A]	p. [(N96D)]; [(G1961E)]
P2, F*†	c. [286A > G]; [5882G > A]	p. [(N96D)]; [(G1961E)]
P3, M*	c. [1622T > C(;) 3113C > T(;) 5882G > A]	p. [(L541P(;) A1038V(;) G1961E)]
P4, M*	c. [1622T > C(;) 3113C > T(;) 5882G > A]	p. [(L541P(;) A1038V(;) G1961E)]
P5, F*†	c. [1622T > C; 3113C > T]; [5882G > A]	p. [(L541P; A1038V)]; [(G1961E)]
P6, F*†	c. [1622T > C; 3113C > T]; [5882G > A]	p. [(L541P; A1038V)]; [(G1961E)]
P7, F*†	c. [1622T > C]; [5882G > A]	p. [(L541P)]; [(G1961E)]
P8, F*†	c. [1622T > C]; [5882G > A]	p. [(L541P)]; [(G1961E)]
P9, F†	c. [5882G > A]; [6448T > C]	p. [(G1961E)]; [(C2150R)]
P10, F†	c. [4139C > T]; [5882G > A]	p. [(P1380L)]; [(G1961E)]
P11, M	c. [5318C > T(;) 5882G > A]	p. [(A1773V(;) G1961E)]
P12, F†	c. [5196+1056A > G]; [5882G > A]	p. [?]; [(G1961E)]
P13, M†	c. [2461T > A]; [6449G > A]	p. [(W821R)]; [(C2150Y)]
P14, F	c. [5882G > A(;) 6229C > T]	p. [(G1961E(;) R2077W)]
P15, F†	c. [1622T > C; 4328G > A]; [5882G > A]	p. [(L541P; R1443H)]; [(G1961E)]

* Sibling pairs: P1 and P2, P3 and P4, P5 and P6, P7 and P8.

† The variants are confirmed on different chromosomes.

gene by either Sanger sequencing or by next-generation sequencing (NGS) as described before,²⁶ or with the Illumina TruSeq Custom Amplicon protocol (Illumina, San Diego, CA, USA), followed by sequencing on Illumina MiSeq platform. The NGS reads were analyzed and compared with the reference genome GRCh37/hg19, using the variant discovery software NextGENe (SoftGenetics LLC, State College, PA, USA). All detected possibly disease-associated variants were confirmed by Sanger sequencing and analyzed with Alamut software (in the public domain at <http://www.interactive-biosoftware.com>). Segregation of the variants with the disease was analyzed if family members were available. The allele frequencies of all variants were compared to the Exome Variant Server (EVS) dataset, NHLBI Exome Sequencing Project, Seattle, Washington, United States (in the public domain at <http://snp.gs.washington.edu/EVS/>; accessed March 2014).

RESULTS

Clinical and Genetic Evaluation

A comprehensive review of clinically diagnosed and genetically confirmed STGD1 patients with well-documented SD-OCT and corresponding AF images was undertaken. In this cohort, 15 (8 of whom represent sibling pairs) patients were observed to exhibit the optical gap phenotype. A summary of the clinical and demographic findings in each of these patients is presented in Table 1. The mean age of the patients was 23.5 years (range, 12–30 years) and predominantly consisted of women (73.3%). The reported age of the disease onset was in the second and third decades of life (mean age of onset of 19.4 years) corresponding to a mean symptomatic disease duration of 4 years. One patient (P1) was reportedly asymptomatic at the time of presentation.

All except two patients (P1, P2) had undergone full-field ERG at an initial visit and were categorized to group 1 in STGD1 disease as described earlier.¹⁶ Scotopic and maximal responses were within age-matched normal limits and 30-Hz flicker responses were relatively spared with minor implicit delays in P6 and P10. Multifocal ERG data were collected in six patients, while one-half of them had data available only for the right eye. Each had decreased responses in the central 5° to 15°

on mfERG, showing much larger affected area than SD-OCT and AF images predicted.

Thirteen patients exhibited a bull's eye-like phenotype on color fundus photos. Parafoveal flecks were observed in 6 patients (12 eyes). Fixation was assessed at first visit in 10 patients. Eighteen eyes from 10 patients had extrafoveal fixation, except for P10 and P11, who had preserved central fixation in the right eye only.

Results of the *ABCA4* screening are summarized in Table 2. The cohort of 15 patients included 11 unrelated cases and four sibling pairs. Segregation analysis was available for 11 patients. At least two (expected) disease-causing variants were identified in each patient. Interestingly, 91% of unrelated cases were compound heterozygous for the p.G1961E variant. Therefore, the allele frequency of p.G1961E in patients with the optical gap phenotype in this study was 46.7% and in our entire STGD1 cohort it is 13.4% (see below and Supplementary Tables S1, S2), which results in a highly statistically significant difference ($\chi^2 = 20.9$; $P = 5 \times 10^{-6}$).

Structural Staging and Assessment of the Optical Gap Phenotype With SD-OCT and AF Imaging

A thorough analysis of each patient indicated that the optical gap phenotype may be structurally divided into three developmental stages on SD-OCT over several years.

Stage 1: Moderate Ellipsoid Zone Disruptions. Five patients (10 eyes) from the cohort presented at the initial stage of optical gap. An apparent thinning of the outer nuclear layer (ONL) of the macula was present; however, a relative sparing of laminar structure was noted. Distinctive structural breaks in the EZ band and degradation of photoreceptors in the foveal outer retina were observed on SD-OCT. Photoreceptor debris and remnants of the ellipsoid were present in the gap while the RPE and external limiting membrane were intact (Fig. 1, P2 and P3). A small, dark roundish lesion with reduced AF signal was seen on AF images of four patients (Fig. 1, P2 and P3), while P5 exhibited a more horizontally-elongated lesion. Small parafoveal flecks within close proximity to the lesion were apparent in P3 and P4. Best corrected visual acuity was relatively mildly affected in patients from this group, ranging from 20/25 to 20/60 (mean 20/40) and appeared to be dependent on the degree of EZ band loss.

FIGURE 1. Structural staging of the *ABCA4*-associated optical gap phenotype. Stage 1: Early subfoveal ellipsoid zone (EZ) band disruption and lysis. Note photoreceptor debris in the optical gap (P2 and P3 SD-OCT, *white arrows*) and early flecks in the parafovea (P3, AF and SD-OCT, *red arrows*) with apparent thinning of the outer nuclear layer. Stage 2: Gap expansion and total absence of the EZ in the subfoveal area (P12 and P7, SD-OCT). Note residual granular deposits attached to the ELM (P7, SD-OCT, *small arrow*). A central, ellipsoidal lesion with a hyperautofluorescent border is detected on AF imaging of the macula. Stage 3: A structural collapse of the neurosensory retina into the cavity with residual gaps along the edge of the lesion (P14 and P15, SD-OCT, *white arrows*) correlated with darkening of the lesion on AF imaging. Note the small area of relative outer retina layer sparing in the central fovea in P15 (P15, SD-OCT).

FIGURE 2. Eccentric localization of the optical gap can spare foveal function and central vision. (A) A color fundus photo of 23-year-old patient with central fixation (pin) and relatively preserved visual acuity (20/40). Early parafoveal flecks (white arrows) were noted on AF imaging (B), in addition to a dark elliptical lesion. (C) Infrared (IR) reflectance imaging reveals a more extensive view of local changes. (D) A corresponding SD-OCT scan identified the off-set optical gap (white arrow) and the resulting area of ellipsoid preservation under the fixation point (red arrow).

Stage 2: Expanded Foveal Cavitation. A widened empty cavity characterized by a total absence of the EZ band was observed across the fovea on SD-OCT in eight patients. Some granular deposits of residual debris were visibly attached to the concavely arched ELM of the lesion. The increased reflectivity of the ELM above the cavitation was seen in some cases (Fig. 1, P12 and P7). A relatively larger, horizontally-elongated lesion resembling an elliptical bull's eye lesion within a hyperautofluorescent halo was observed on AF imaging in six patients (Fig. 1, P7). Patient 6 had speckled macular appearance on AF images, which corresponded with fleck-like deposits in the central macula. P6 and P10 presented with parafoveal flecks in the central macula. Mean visual acuity in Stage 2 was 20/80, but individual visual acuities appear to span a larger spectrum. Six patients (P6, P7, P8, P9, P12, and P13) had measured BCVA ranging from 20/50 to 20/150 while two patients (P10, P11) had BCVA of 20/40 and 20/30 in the right and left eyes, respectively, and preserved central fixation in the right eye. The optical gap lesion in the latter two patients was atypically eccentrically off-centered resulting in a relatively spared EZ at the fixation point (Fig. 2).

Stage 3: Inner Retinal Collapse. A structural collapse of the inner retinal layers into the vacant ellipsoid space can be

observed with residual spaces along the edge of the previously-occupied gap lesion in two patients (Fig. 1, P14 and P15). The collapsed layers including the ELM and subsequent inner retinal layers appeared to be present but structurally frail. A horizontally elongated lesion of hypoautofluorescence is seen in P14 while P15 exhibits hypoautofluorescent lesion with preserved AF over the center (Fig. 1, P14 and P15). Visual acuities appeared similar to stage 2 patients. The BCVA of P15 was relatively preserved at 20/30 in both eyes, which correlated with a small area of relative outer retina layer sparing in the foveal center seen on AF and SD-OCT images (Fig. 1, P15).

Stage Duration and Progression. Longitudinal AF and SD-OCT documentation for eight patients (P5, P6, P7, P8, P12, P13, P14, and P15) were analyzed. Initial and current stage of the optical gap phenotype for each patient is presented in Table 1. Patients P6, P7, P8 and P13 were at stage 2 at initial presentation and remained at stage 2 after 1.5 to 2 years. Patient P12 remained at stage 2 within 3 years follow up. Interstage progression was observed in patients P5, P14, and P15. P5 initially presented with mild photoreceptor disorganization with EZ granularity at the foveal center and later progressed to stage 1 after 1 year during which an apparent EZ

FIGURE 3. Optical gap development from early structural changes to stage 1. *P5a*: Fundus images of the left macula with corresponding AF image to the right. A small focal loss of AF (*white arrow*) corresponds to area of photoreceptor disruption and debris accumulation seen on an SD-OCT scan over the fovea. Best corrected visual acuity was 20/25. *P5b*: After 1 year, palpable disruptions (*white arrow*) of the EZ and optical gap formation with residual debris were seen in the foveal area. Progression was accompanied by a decrease in vision (20/60) and darkening of the central lesion on AF. *P6*: The sibling of P5, 6 years older, presented with a more progressed disease stage (stage 2). Note an expanded empty cavity devoid of photoreceptors (EZ band) on SD-OCT (*white arrow*). The structural loss corresponded to a larger area of hypoautofluorescence (*white arrow*) and the presence of nascent parafoveal flecks.

break and gap had occurred (Fig. 3, P5a and P5b). Her visual acuity progressed from 20/30 and 20/25 to 20/60 in both eyes. Interestingly, P6, the older sibling of P5, exhibited a more advanced stage 2 optical gap (Fig. 3, P6). P14 progressed from stage 2 to stage 3 within 2 years; however, retained a stable BCVA of 20/100 in both eyes (Fig. 4, P14a and P14b). Progression within stage 2 prior to converting into stage 3 is seen in P8 after 1 year follow-up (Fig. 4, P8a and P8b). Progression of stage 3 gap to progressive atrophy within 1 year is seen in P15. Best corrected visual acuity progressively decreased from 20/30 to 20/50 in both eyes within 2 years (Fig. 5, P15a and P15b). Summary of stage duration and transition is schematically presented in Figure 6.

DISCUSSION

The optical gap phenotype has been indiscriminately associated with several hereditary retinal dystrophies including rod monochromatism,^{19,20,23} maculopathies caused by mutations in *KCVN2*²⁷ and *RPILI*²² genes, and recessive Stargardt disease.^{13,17,18} Sporadic cases and nonhereditary occurrences, such as solar retinopathy or isolated occurrences of laser-pointer injury or drug-induced (tamoxifen) toxicity, have also

been reported with optical gap-like lesions.^{21,28,29} Although each condition is etiologically distinct, these conditions comprise a group of disorders that predominately affect foveal cones. STGD1 is a predominantly juvenile-onset condition characterized by early RPE changes leading to macular atrophy.

Phenotypic staging can provide descriptive insight into the natural history of a particular disease. Greenberg et al.²⁰ recently reported such a system in assessing the structurally analogous optical gap phenotype in patients with rod monochromatism. One of the aims of assessment of the *ABCA4*-associated phenotype in this study was to understand its systematic progression to possibly decipher the early effects of *ABCA4* dysfunction in this phenotypic subgroup of STGD1 patients. The analysis of this patient cohort suggests that the development of optical gap can be subdivided into three structural stages over several years. Stage 1 patients present with disorganization of photoreceptors and intermittent breaks in EZ band forming gap lesion in the subfoveal region. The visibility of the EZ band (also known as the inner/outer segment junction) seen on the SD-OCT, has been attributed to the scattering of light by mitochondria in the ellipsoid zone of photoreceptor inner segments.³⁰ Its disappearance has thus been an indicator of photoreceptor loss and an explanation for declines in visual acuity. Central vision was relatively spared in

FIGURE 4. Neurosensory layer collapse of the retina during transition through stage 2 and into stage 3. *P8a*: A 19-year-old female patient presenting with stage 2 disease with the characteristic concave cavity toward the retinal ganglion layers on SD-OCT with corresponding IR reflectance images to the left exhibiting the “fried egg” appearance rather than the central bulge reflex. *P8b*: After 1 year, a loss of space concavity was observed amid the collapsing of the neurosensory retina into the optical gap space. *P14a*: A 30-year-old female with macular RPE atrophy in the right eye and stage 2 optical gap in the left eye, with “bulge reflex” on the red-free image. *P14b*: After 1 year, an apparent collapse is observed with residual spaces along the edge of the prior gap space. Note the loss of “bulge reflex” on the red-free image.

FIGURE 5. Advanced stage 3 disease and subsequent atrophy in a 28-year-old female patient. *P15a*: Color fundus image of the foveal lesion of the right eye with corresponding AF images of the right and left eyes to the right. A dark area of hypoautofluorescence surrounding a central patch of preserved AF was noted. Spectral-domain OCT shows neurosensory collapse, filling the previous empty gap space. *P15b*: Ensuing concentric RPE atrophy 1 year later eliminated residual patterns of optical gap pathology such as EZ band spaces (*white arrows* show previous location of EZ band space). Photoreceptor atrophy and RPE thinning progressed concurrently.

stage 1 patients in our cohort and was also largely dependent on EZ band disruption. A further spatial depletion of the EZ band is seen in stage 2 patients where an expansive subfoveal empty cavity is apparent with an accompanying decline in visual acuity. In some patients residual debris attached to ELM was present, which is hypothesized to represent the degenerative remains of photoreceptor outer segments secondary to loss of direct apposition and contact with RPE cells disturbing their phagocytosis by RPE cells.^{17,20} Optical gaps within these two stages are distinct in that structural changes appear to be restricted to losses of the EZ (photoreceptors) within the subfoveal region, while the RPE seems to stay relatively intact. Stage 3 depicts what appears to be a structural collapse of the inner retina into the gap lesion, which then sets forth the inevitable involvement of other retinal layers. Events following this stage appear to lead to generalized RPE atrophy throughout the macula, which is indistinguishable from STGD1

cases that present with early centralized atrophy, suggesting that optical gaps are likely more prevalent among STGD1 patients than currently estimated, especially if patients are initially examined at a later stage of the disease.

The measured visual acuity of stage 2 and 3 patients appeared, at times, inconsistent with their structural disease stage. A plausible explanation may suggest somewhat preserved photoreceptors under the fixation point. Two patients (P10 and P11) at stage 2 had minimally eccentric gap lesion with relatively preserved ellipsoid under the fixation point (Fig. 2), while P15 at stage 3 possibly had some foveal sparing suggested by SD-OCT images and relatively spared visual acuity. Full-field ERG findings with normal retinal scotopic and photopic mass responses suggest a localized disease, while mfERG from nine eyes showed decreased responses in the 5° to 15° of retina in the posterior pole, showing mainly cone dysfunction. Similar to other mfERG studies in STGD1,^{31,32} the

ABCA4 Optical Gap Progression

FIGURE 6. Gantt chart summarizing *ABCA4*-associated optical gap stage progression and duration. Longitudinal OCT imaging was available for eight patients in the study cohort. Patient 5 presented with minor EZ changes (stage 0) and progressed bilaterally to stage 1 optical gap a year after initial examination. Patients 6, 7, 8, and 13 initially presented and remained in stage 2 over a range of 1.5 to 2 years; P12 remained in this stage over a 3-year period. Patients 15 progressed to an atrophic stage following stage 3, while P14 exhibited unilateral progression from stage 2 to stage 3 in the left eye.

functionally affected areas were much larger than structural changes on SD-OCT or AF, suggesting that functional loss precedes the structural changes in these patients. Additionally, given that decreases in mfERG response have been attributed to the influence of the EZ band, a case can be made for early photoreceptor dysfunction preceding structural RPE loss.³³

Genetic screening confirmed that all patients were compound heterozygous for *ABCA4* mutations. Interestingly, the p.G1961E variant was present in 10 of 11 unrelated cases (91%). The p.G1961E mutation is the most frequent disease-associated *ABCA4* allele seen in approximately 10% of STGD1 patients of European origin.³⁴ This fraction was almost the same in our cohort of 179 patients, including 157 unrelated individuals (42/157; 13.4%), but strikingly higher in patients with the optical gap phenotype (46.7% vs. 13.4%, $P < 0.0001$). It has to be noted, however, that while the optical gap phenotype is definitely associated with the p.G1961E variant, the reverse is not the case since a larger fraction (32 unrelated individuals) who harbored the p.G1961E allele did not present with optical gap. Fourteen of these individuals were clinically characterized at the same age after onset as the optical gap group. Of the other disease-associated *ABCA4* alleles compound heterozygous with p.G1961E, the p.L541P mutation, presenting alone or as a complex allele with the p.A1038V variant, was observed in seven cases (four unrelated) with

optical gap (Table 2 and Supplementary Table S1) while only once in patients without the phenotype (Supplementary Table S1). However, due to a relatively small size of the optical gap cohort we cannot make an unequivocal conclusion about the association of this allele with the optical gap phenotype.

With the exception of P13, the optical gap was not observed in the SD-OCT scans of any other non-p.G1961E patients ($n = 131$) whose age at time of examination, age of onset and estimated disease duration were not statistically different from those of p.G1961E ($n = 48$) patients (Supplementary Table S2). Therefore, we can conclude that the p.G1961E variant, maybe sometimes together with the p.L541P or p.(L541P; A1038V) allele, is currently the only *ABCA4* mutation associated with the optical gap phenotype.

In summary, the *ABCA4*-associated optical gap phenotype may be a more prevalent occurrence preceding central macular atrophy in patients with STGD1 than previously thought. A striking association of the optical gap phenotype with the p.G1961E mutant allele in the *ABCA4* gene was observed; however, further studies on larger patient cohorts are needed to validate this phenotype-genotype correlation and determine the functional association. STGD1 patients with the optical gap phenotype present with a localized disease which can be structurally divided into three developmental stages initiated by photoreceptor loss and subsequent RPE involvement.

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