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Early Patterns of Macular Degeneration in *ABCA4*-Associated Retinopathy

Kamron N. Khan, PhD, FRCOphth,[1,2,3,*] *Melissa Kasilian, BA,*[2,*] *Omar A.R. Mahroo, PhD, FRCOphth,*[1,2] *Preena Tanna, MD,*[1,2] *Angelos Kalitzeos, PhD,*[1,2] *Anthony G. Robson, PhD,*[1,4] *Kazushige Tsunoda, MD, PhD,*[5] *Takeshi Iwata, PhD,*[6] *Anthony T. Moore, FRCS, FRCOphth,*[1,2,7] *Kaoru Fujinami, MD, PhD,*[1,2,5,8] *Michel Michaelides, MD(Res), FRCOphth*[1,2]

Purpose: To describe the earliest features of *ABCA4*-associated retinopathy.

Design: Case series.

Participants: Children with a clinical and molecular diagnosis of *ABCA4*-associated retinopathy without evidence of macular atrophy.

Methods: The retinal phenotype was characterized by color fundus photography, OCT, fundus autofluorescence (FAF) imaging, electroretinography, and in 2 patients, adaptive optics scanning laser ophthalmoscopy (AOSLO). Sequencing of the *ABCA4* gene was performed in all patients.

Main Outcome Measures: Visual acuity, OCT, FAF, electroretinography, and AOSLO results.

Results: Eight children with *ABCA4*-associated retinopathy without macular atrophy were identified. Biallelic variants in *ABCA4* were identified in all patients. Four children were asymptomatic, and 4 reported loss of VA. Patients were young (median age, 8.5 years; interquartile range, 6.8 years) with good visual acuity (median, 0.155 logarithm of the minimum angle of resolution [logMAR]; interquartile range, 0.29 logMAR). At presentation, the macula appeared normal (n = 3), had a subtly altered foveal reflex (n = 4), or demonstrated manifest fine yellow dots (n = 1). Fundus autofluorescence identified hyperautofluorescent dots in the central macula in 3 patients, 2 of whom showed a normal fundus appearance. Only 1 child had widespread hyperautofluorescent retinal flecks at presentation. OCT imaging identified hyperreflectivity at the base of the outer nuclear layer in all 8 patients. Where loss of outer nuclear volume was evident, this appeared to occur preferentially at a perifoveal locus. Longitudinal split-detector AOSLO imaging in 2 individuals confirmed that the greatest change in cone spacing occurred in the perifoveal, and not foveolar, photoreceptors. Electroretinography showed a reduced B-wave–to–A-wave ratio in 3 of 5 patients tested; in 2 children, recordings clearly showed electronegative results.

Conclusions: In childhood-onset *ABCA4*-associated retinopathy, the earliest stages of macular atrophy involve the parafovea and spare the foveola. In some cases, these changes are predated by tiny, foveal, yellow, hyperautofluorescent dots. Hyperreflectivity at the base of the outer nuclear layer, previously described as thickening of the external limiting membrane, is likely to represent a structural change at the level of the foveal cone nuclei. Electroretinography suggests that the initial site of retinal dysfunction may occur after phototransduction. *Ophthalmology 2017;■:1–12 © 2017 by the American Academy of Ophthalmology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).*

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Stargardt disease (STGD1; Online Mendelian Inheritance in Man identifier, 248200) is an autosomal recessive retinal dystrophy resulting from dysfunction in the photoreceptor-specific ATP-binding cassette transporter *ABCA4*. To date, more than 1000 mutations in *ABCA4* have been reported, associated with phenotypes that include macular, cone, or cone–rod dystrophies.[1] Symptoms may develop as early as the first decade of life, but more commonly occur in the second or third decade. A minority of individuals demonstrate a late-onset form of disease in their forties or fifties that typically spares the central fovea.[2] For the youngest patients, the earliest signs of disease are poorly described, and despite some understanding of the protein function, the precise pathogenic mechanisms are yet to be elucidated. As therapeutic clinical trials either already have commenced (e.g., stem cell transplantation [clinicaltrials.gov identifier, NCT01345006], modified vitamin A preparations [clinicaltrials.gov identifier, NCT02402660], and gene therapy [clinicaltrials.gov identifier, NCT01367444]) or are planned, there is a need to identify the site of retinal dysfunction in early disease and the pattern of progression. Because most reported cases of STGD1 manifest signs of macular atrophy at presentation, there is little information about earlier stages of disease. Herein, we present a series of patients with molecularly confirmed *ABCA4*-associated retinopathy without macular atrophy in which detailed retinal imaging and functional testing have provided new insights into the earliest stages of disease.

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Methods

Individuals with features suggestive of early *ABCA4*-associated retinopathy were recruited prospectively from the pediatric retinal genetics clinics at Moorfields Eye Hospital (MEH), London, United Kingdom, and Tokyo Medical Center, Tokyo, Japan. In addition, a retrospective case note review was performed at MEH to identify further participants. Those with macular atrophy at presentation were excluded from this study. Each patient underwent a full clinical examination, including Snellen or logarithm of the minimum angle of resolution (logMAR) visual acuity, color vision testing (Hardy Rand Rittler pseudoisochromatic color plate test, fourth edition), and dilated fundus examination. Retinal imaging included 35° color fundus photography (TRC-50DX; Topcon Corp, Tokyo, Japan) and 30° or 55° fundus autofluorescence (FAF)[3] imaging (HRA2; Heidelberg Engineering, Ltd, Heidelberg, Germany). Spectral-domain OCT imaging was acquired using a Heidelberg HRA2 or Cirrus HD-OCT 500 (Cirrus; Carl Zeiss Meditec, Ltd, Dublin, CA) system. Adaptive optics scanning laser ophthalmoscopy (AOSLO) was carried out on 2 individuals using a custom-built instrument as previously described.[4] Confocal and split-detector (nonconfocal) image sequences were acquired simultaneously.[4–6] Parafoveal locations were imaged using either a 1° or 1.5° field of view up to 5° eccentricity from the fovea. The images were processed and a montage was created to illustrate a continuous photoreceptor mosaic. Retinal function was tested by full-field electroretinography and pattern electroretinography (PERG), obtained using either gold-foil electrodes, incorporating the current International Society for Clinical Electrophysiology of Vision (ISCEV) standards in 3 patients, or with Ganzfeld stimulation and lower eyelid skin electrodes in the 2 youngest children, according to pediatric protocols previously described.[7–9] Genetic testing was performed by targeted exome sequencing for individuals at MEH (Stargardt/Macular dystrophy panel, version 3; Casey Eye Institute Molecular Diagnostics Laboratory, Portland, Oregon), or by whole exome sequencing for patients at Tokyo Medical Center as reported previously.[10] Pathogenic variants were confirmed by Sanger sequencing. This study received institutional review board approval at Moorfields Eye Hospital, London, and adhered to the tenets of the Declaration of Helsinki. Patients or their legal guardians consented to the use of their clinical data for research purposes.

Results

Eight individuals were identified who fulfilled the study criteria. Six were recruited prospectively and 1 was recruited retrospectively from MEH. Some clinical information for patient 7 has been published previously.[11] The eighth patient was identified from the Inherited Eye Disease Service, National Hospital Organization, Tokyo Medical Center, Tokyo, Japan. The clinical and molecular genetic characteristics for all participants are presented in Table S1 (available at www.aaojournal.org).

Individuals were young (median age, 8.5 years; interquartile range, 6.8 years; range, 5−13 years) with good visual acuity (median, 0.15 logMAR; interquartile range, 0.29 logMAR; range, −0.14 to 0.3 logMAR) at their initial visit. Four patients showed symptoms at presentation, reporting a subjective decline in the quality of their vision. Four children with the best acuity were asymptomatic. These individuals were identified because of the presence of peripheral flecks (patient 3) or as a result of having a more severely affected elder sibling (patients 4, 6, and 8). Color vision was normal in all individuals tested. In most patients (6/8), the central macula either appeared normal or showed subtle alteration in the foveal reflex. One patient demonstrated yellow dots at the fovea (patient 6), whereas a further 2 children demonstrated these during follow-up (patients 7 and 8). In 7 of 8 patients, the peripheral retina appeared normal on slit-lamp biomicroscopy. Patient 3 showed widespread pisciform outer retinal flecks at presentation that spared the macula.

Autofluorescence Imaging

Fundus autofluorescence abnormalities were evident in all patients, even when funduscopy results appeared normal. All patients retained a variable degree of physiologic hypoautofluorescence at the foveola that qualitatively appeared to be reduced in size when compared with normal parameters (Fig 1A). The perifoveal retina was mildly hyperautofluorescent (Fig 1B). With increasing retinal eccentricity, a further band of hyperautofluorescence was evident in patients 1, 4, and 5, but less convincingly in patient 2 (Fig 1C). This was broader and more diffuse than the well-defined ring often observed in patients with other retinal dystrophies. At their first visit, numerous discrete hyperautofluorescent dots were present at the fovea in patients 3, 6, and 7 (Fig 2C). Patient 3 was the only patient to manifest hyperautofluorescent peripheral retinal flecks at presentation (Fig 2A). No patient demonstrated a complete loss of foveal autofluorescence, suggesting that the retinal pigment epithelium (RPE) remained intact at this early stage.

OCT

OCT imaging confirmed that the fourth highly reflective outer retinal band, thought to represent the RPE−Bruch's membrane[12] complex, was well preserved in all individuals (Fig 3). Moreover, no areas of increased signal penetrance into the choroid were observed, in keeping with the absence of RPE atrophy or disease. The most prevalent finding, observed in all 8 patients, was a marked increase in reflectivity observed in the outer retina, commencing at the first highly reflective outer retinal band, thought to represent the external limiting membrane (ELM), and extending part way through the outer nuclear layer (ONL; Fig 3). This characteristic pattern of hyperreflectivity was highly reproducible and was evident in all patients, with a maximum thickness at the foveola, reducing nasally and temporally symmetrically, extending into the peripheral fovea. In most cases, the second highly reflective line thought to represent the ellipsoid zone was visible below this abnormally reflective band, but with reduced clarity, suggesting possible subtle disturbance to the photoreceptor outer segments (Fig 3). In all patients, focal collapse of the inner retinal layers was visible because of loss of outer retinal structures (Fig 4). This appeared to occur at perifoveal loci, rather than at the foveola. The first sign of outer retinal atrophy seen on OCT imaging appeared to spare the fovea. In keeping with this, sequential imaging over a 35- and 58-month period in 2 individuals (patients 8 and 7, respectively), demonstrated that the foveolar photoreceptors degenerated last (Fig 5). For patient 5, progressive loss of foveal outer segments occurred over a 19-month period, resulting in a broad outer retinal cavity (Fig 4B and C). Importantly, an intact ELM of physiologic proportions also was visible. Where the evolution of outer retinal degeneration was captured on OCT, the temporal perifovea always seemed to be affected first.

Adaptive Optics Scanning Laser Ophthalmoscopy

Patients 5 and 6 underwent further retinal imaging of the photoreceptor layer of their right eyes using a custom-build AOSLO.