Clinical and Molecular Analysis of Stargardt Disease With Preserved Foveal Structure and Function

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• PURPOSE: To describe a cohort of patients with Stargardt disease who show a foveal-sparing phenotype.
• DESIGN: Retrospective case series.
• METHODS: The foveal-sparing phenotype was defined as foveal preservation on autofluorescence imaging, despite a retinopathy otherwise consistent with Stargardt disease. Forty such individuals were ascertained and a full ophthalmic examination was undertaken. Following mutation screening of *ABCA4*, the molecular findings were compared with those of patients with Stargardt disease but no foveal sparing.
• RESULTS: The median age of onset and age at examination of 40 patients with the foveal-sparing phenotype were 43.5 and 46.5 years. The median logMAR visual acuity was 0.18. Twenty-two patients (22/40, 55%) had patchy parafoveal atrophy and flecks; 8 (20%) had numerous flecks at the posterior pole without atrophy; 7 (17.5%) had mottled retinal pigment epithelial changes; 2 (5%) had multiple atrophic lesions, extending beyond the arcades; and 1 (2.5%) had a bull's-eye appearance. The median central foveal thickness assessed with spectral-domain optical coherence tomographic images was 183.0 μm (n = 33), with outer retinal tubulation observed in 15 (45%). Twenty-two of 33 subjects (67%) had electrophysiological evidence of macular dysfunction without generalized retinal dysfunction. Disease-causing variants were found in 31 patients (31/40, 78%). There was a higher prevalence of the variant p.Arg2030Gln in the cohort with foveal sparing compared to the group with foveal atrophy (6.45% vs 1.07%).
• CONCLUSIONS: The distinct clinical and molecular characteristics of patients with the foveal-sparing phenotype are described. The presence of 2 distinct phenotypes of Stargardt disease (foveal sparing and foveal atrophy) suggests that there may be more than 1 disease mechanism in *ABCA4* retinopathy. (Am J Ophthalmol 2013;156: 487–501. © 2013 by Elsevier Inc. All rights reserved.)

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STARGARDT DISEASE IS AN AUTOSOMAL RECESSIVE disorder caused by mutations in the *ABCA4* gene.[1,2] It is the most common single-gene retinal degeneration, with a reported prevalence of 1:10 000.[3,4] Most cases typically present with central visual loss within the first 2 decades of life, and during the course of the disorder there is macular atrophy with yellow-white flecks in the posterior pole, at the level of the retinal pigment epithelium (RPE).[4,5] However, Stargardt disease is associated with a variable phenotype and severity.[4–13] Autofluorescence (AF) imaging and electroretinography may assist the diagnosis, and parameters such as age of onset, visual acuity, AF pattern, and the nature of the electrophysiological findings assist both in the determination of disease severity and in the provision of prognostic information.[5,8,9,11,14] Increasingly, high-resolution imaging using spectral-domain optical coherence tomography (SDOCT) is providing insights into the retinal architectural changes that occur in Stargardt disease.[12,14–19]

There is wide phenotypic variability in *ABCA4* retinopathy[3,4,6,8,10,12,20–24] and sequence variants in *ABCA4* have also been implicated in cone dystrophy, cone-rod dystrophy, and "retinitis pigmentosa" in addition to Stargardt disease.[2,21,25–28] There is also extensive allelic heterogeneity, with more than 700 sequences in the *ABCA4* gene having been reported to date.[2,3,6,8,10,12,20–22,25–27,29,30-33] The phenotypic variability and the high genetic heterogeneity have confounded attempts to examine genotype-phenotype correlations comprehensively.[4,31,32]

A cohort of Stargardt disease patients who had better visual acuity than "typical" Stargardt disease patients, and who showed sparing of the fovea on funduscopy, has been described.[5] There are also reports of patients with "late-onset" Stargardt disease, including individuals with a foveal-sparing phenotype, who harbor *ABCA4* variants.[6,12,22] In those reports, SDOCT images demonstrated a well-preserved foveal structure including the neurosensory retina,[6,12,22] which differed from previous observations of early foveal photoreceptor damage in "typical" Stargardt disease with

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central retinal atrophy.[13,15] Foveal-sparing forms of Stargardt disease may thus reflect a distinct pathogenesis.

The present study describes the clinical findings and molecular genetic characteristics of "foveal-sparing" Stargardt disease in a large cohort from a single center. The molecular data of the cohort with the foveal-sparing phenotype are compared with *ABCA4* variants observed in patients with Stargardt disease but without foveal sparing.

METHODS

• **PATIENTS:** AF images of the right eyes of 438 individuals with a clinical diagnosis of retinopathy compatible with Stargardt disease were surveyed and 40 patients were identified with an apparently normal AF signal at the fovea (foveal sparing). After informed consent, blood samples were collected and genomic DNA was extracted from the peripheral blood leukocytes. The protocol of the study adhered to the provisions of the Declaration of Helsinki and was approved by the local Ethics Committee of Moorfields Eye Hospital.

For the purposes of this report, patients presenting to the hospital with signs of atrophy within the macula, bilaterally, with or without surrounding flecks were potentially included as having Stargardt disease. Patients with a stationary visual dysfunction were excluded. A careful drug history was taken to allow exclusion of those with retinotoxic maculopathy. Patients with a dominant family history were excluded. Where a family history was not extensive, or whenever 2 generations were affected, the *RDS/PRPH2* gene, with its coding region and intron-exon boundaries, was sequenced. In those patients over 50 years of age, care was taken not to include cases of atrophic age-related macular degeneration in which there were soft drusen, or patients with maternally inherited diabetes and deafness in whom the distribution of atrophy and autofluorescence appearance had a distinctive appearance. The m.3243A>G variant was assayed if this phenotype was in any way suggested.

• **CLINICAL ASSESSMENT:** A detailed medical history was obtained and a comprehensive ophthalmologic examination was performed for all 40 patients. The age of onset was defined as the age at which visual loss was first noted by the patient or as the age at the latest examination for asymptomatic patients. The duration of the disease was calculated as the difference between age at onset and age at the latest examination. Assessment included best-corrected visual acuity, dilated ophthalmoscopy, color fundus photography, AF imaging, SDOCT imaging, and electrophysiological assessment. Best-corrected Snellen visual acuity was converted to equivalent logMAR visual acuity.

Color fundus photography was performed with a TRC-50IA retinal fundus camera (Topcon, Tokyo, Japan). AF images before 2009 were obtained with an HRA 2 (Heidelberg Engineering, Heidelberg, Germany; excitation light 488 nm; barrier filter 500 nm; field of view 30 × 30 degrees),[34] and images after 2009 were undertaken using the Spectralis with viewing module version 5.1.2.0 (Heidelberg Engineering; excitation light 488 nm; barrier filter 500 nm; fields of view 30 × 30 degrees and 55 × 55 degrees).[35]

SDOCT was undertaken with the Spectralis with viewing module version 5.1.2.0. The SDOCT protocol included a horizontal linear scan (100 B-scans averaged to improve the signal-to-noise ratio) centered on the fovea, where possible, and a volume scan (minimum of 19 B-scan slices, 20 × 20 degrees). The HEYEX software interface (version 1.6.2.0; Heidelberg Engineering) was used for retinal thickness measurement.[36] Central foveal thickness was defined as the distance between the inner retinal surface and the inner border of the RPE.[36,37] Evidence of outer retinal tubulation was assessed from all the B-scan slices of each eye by 2 authors (K.F. and A.R.W.).[35,38]

• **ELECTROPHYSIOLOGY:** Electrophysiological assessment included full-field electroretinogram (ERG) and pattern electroretinogram (PERG) recorded with gold foil electrodes that incorporated the standards of the International Society for Clinical Electrophysiology of Vision.[39–41] The full-field ERGs were used to assess generalized rod and cone system function and included: (1) dark-adapted dim flash 0.01 candela-seconds per square meter ($cd \cdot s \cdot m^{-2}$); (2) dark-adapted bright flash 11.0 $cd \cdot s \cdot m^{-2}$; (3) light-adapted 3.0 $cd \cdot s \cdot m^{-2}$ 30 Hz flicker; and (4) light-adapted 3.0 $cd \cdot s \cdot m^{-2}$ at 2 Hz. The PERG P50 component and multifocal electroretinogram (mfERG) were used to assess macular function. Some patients had mfERG recording (RETIscan System; Roland Consult, Wiesbaden, Germany) with a stimulus consisting of 61 scaled hexagons covering in total a visual field of 55 degrees, at a viewing distance of 33 cm.[42] All main components of the ERG and the PERG P50 component were used to classify patients into 3 groups; this is a partial modification of a previous report[9]: (1) patients with normal ERGs with or without a PERG P50 abnormality; (2) subjects with a PERG P50 abnormality and additional generalized cone-mediated ERG abnormality (assessed with photopic ERGs); and (3) individuals with a PERG P50 abnormality and generalized cone system electrophysiological abnormality and additional generalized rod-mediated ERG abnormality (assessed using scotopic ERGs).

• **MUTATION SCREENING AND MOLECULAR GENETIC ANALYSIS:** Blood samples were collected in EDTA tubes and DNA was extracted with a Nucleon Genomic DNA extraction kit (BACC2; Tepnel Life Sciences, Manchester, United Kingdom), or the Qiagen Gentra Puregene blood kit (Qiagen, Venlo, Netherlands). Mutation screening of *ABCA4* was performed with the arrayed primer extension (APEX) microarray (ABCR400 chip, Asper Ophthalmics,