Choroidal Hyperreflective Foci in Stargardt Disease Shown by Spectral-Domain Optical Coherence Tomography Imaging Correlation With Disease Severity

Niloofar Piri, MD; Brooke L. W. Nesmith, MD, JD; Shlomit Schaal, MD, PhD

IMPORTANCE The presence of choroidal hyperreflective foci in Stargardt disease is, to our knowledge, a potentially new finding. Evaluation of these foci may aid in better understanding of the disease process.

OBJECTIVES To report the presence of choroidal hyperreflective foci in spectral-domain optical coherence tomography (SD-OCT) images from eyes with Stargardt disease and investigate the relationship between the number of hyperreflective foci and disease severity.

DESIGN, SETTING, AND PARTICIPANTS Twenty-six eyes of 13 patients with a clinical diagnosis of Stargardt disease were evaluated in a retrospective case series. Patient data were collected between January 1, 2009, and August 31, 2014.

MAIN OUTCOMES AND MEASURES The number of choroidal hyperreflective foci in Stargardt disease as well as correlation with visual acuity, central macular thickness (CMT), and disease duration were the main outcomes. A total of 707 macular SD-OCT scans of 13 patients with Stargardt disease were reviewed and evaluated for the presence and number of retinal/choroidal hyperreflective foci, central macular thickness, visual acuity, and disease duration. Enhanced depth imaging with OCT (EDI-OCT) scans available for 2 patients were compared with SD-OCT scans. A PubMed/Google search was performed to identify SD-OCT images in Stargardt disease; these findings were reviewed for the presence of choroidal hyperreflective foci.

RESULTS The mean (SD) numbers of hyperreflective foci in each retinal/choroidal layer in decreasing frequency were as follows: Bruch membrane/retinal pigment epithelial (RPE) complex, 78.22 (24.39); choriocapillaris, 25.77 (17.57); Sattler layer, 18.59 (12.89); outer retina, 16.64 (6.96); inner retina, 0.95 (1.58); and Haller layer, 0.73 (0.87). The number of hyperreflective foci in the Bruch membrane/RPE complex increased exponentially with decreasing CMT ($R^2 = 0.99; P = .008$). The number of hyperreflective foci in the Bruch membrane/RPE complex, choriocapillaris, and Sattler layer increased proportionally with decreasing visual acuity ($R^2 = 0.97, R^2 = 0.95, and R^2 = 0.99$, respectively; and $P = .007, P = .006$, and $P = .008$, respectively). Direct correlation existed between the number of hyperreflective foci in the choriocapillaris and the Sattler layer and disease duration ($R^2 = 0.98 and R^2 = 0.99$, respectively; and $P = .006$ and $P = .009$, respectively). In the 10 studies on Stargardt disease, choroidal hyperreflective foci were present in 51 of 54 SD-OCT images (94%).

CONCLUSIONS AND RELEVANCE Based on the findings of the present study, choroidal hyperreflective foci in Stargardt disease, prominent at the Bruch membrane/RPE complex, choriocapillaris, and Sattler layer, correlate with disease severity in terms of retinal atrophy, decline in vision, and disease duration. Further studies are necessary to assess whether these findings are unique to Stargardt disease.

Published online January 15, 2015.

Copyright 2015 American Medical Association. All rights reserved.
n 1909, Karl Stargardt, a German ophthalmologist, first described a rapidly progressive macular dystrophy of juvenile onset with poor visual outcome. He noted the presence of yellowish-white deposits called retinal flecks, which have become the hallmark feature in Stargardt disease. Stargardt disease is an autosomal recessive retinal degeneration caused by a mutation in the ABCA4 gene, which encodes the retina-specific adenosine triphosphate-binding cassette transporter. A defect in this transporter, which is located in the RPE, results in accumulation of the toxic all-trans-retinal and its derivative, resulting in the death of retinal pigment epithelial cells (RPEs) and their overlying cone and rod photoreceptors.

Fundus flavimaculatus, a variant of Stargardt disease, was first described by Franceschetti, a European ophthalmologist, in 1962. Fundus flavimaculatus tends to be of later onset than Stargardt disease, and its presentation is predominantly one of retinal flecks. In the present study, the term Stargardt disease is used for both entities.

The exact location of the characteristic flecks seen in Stargardt disease, as well as their chemical composition, was controversial for many years. In 1967, mucopolysaccharide deposits (hyaluronic acid) were identified in retinal cells. In 1980, light and electron microscopy were used to demonstrate massive accumulation of lipofuscin in the RPE cells of patients with Stargardt disease. Better understanding of the location of these flecks came about with the introduction of optical coherence tomography (OCT) imaging.

With OCT imaging, a diagnostic modality that has transformed the vitreoretinal field for more than a decade, evaluation of high-resolution cross-sectional images of the retinal layers in patients with Stargardt disease is possible. Correlation of imaging studies to the histology of retinal tissues in vivo can be accomplished with OCT. The introduction of spectral-domain OCT (SD-OCT) with axial resolution as low as 7 μm further advanced the high-resolution imaging of different layers of both the retina and choroid.

In 2006, Querques et al analyzed retinal flecks in patients with Stargardt disease using time-domain OCT (Stratus OCT; axial resolution, 8-10 μm). The investigators defined 2 types of hyperreflective foci: type 1, located in the inner wall of the RPE layer, and type 2, located in the outer nuclear layer and clearly separated from the RPE. The investigators presumed that the 2 types of retinal hyperreflective foci reflect different stages of the same disorder. The authors hypothesized that type 2 hyperreflective deposits could be the residual of type 1 deposits, which would be consistent with progressive degradation of the flecks in the fundus from a well-defined lesion to residual material.

In 2010, retinal flecks in Stargardt disease were evaluated using SD-OCT (axial resolution of 7 μm, faster, and with fewer motion artifacts) and demonstrated 5 different stages of evolution of the hyperreflective foci, which were shown to occur between the Bruch membrane/RPE complex and the outer nuclear layer.

After observing the presence of choroidal hyperreflective foci on SD-OCT in a patient with Stargardt disease, we initiated the present study. To our knowledge, this is the first study to describe the presence of choroidal hyperreflective foci in SD-OCT macular sections in patients with Stargardt disease. This study also demonstrates the correlation between the presence of hyperreflective foci in Stargardt disease with retinal thickness, visual acuity, and disease duration.

Methods

This retrospective study was approved and monitored by the institutional review board of the University of Louisville with waiver of informed consent. Patients with the diagnosis of Stargardt disease were identified through the billing office at Kentucky Lions Eye Center, University of Louisville, between January 1, 2009, and August 31, 2014, by retrieval of the International Classification of Diseases, Ninth Revision, code for sensory retinal dystrophy (362.75). Medical records were then reviewed for the clinical diagnosis of Stargardt disease. Twenty-six eyes of 13 patients were included in this study. Data collected included age at presentation, duration of disease, sex, best-corrected visual acuity (BCVA), central macular thickness (CMT), color fundus photos, fundus autofluorescence images, fluorescein angiography (Heidelberg Retina Angiograph, HRA 2; Heidelberg Engineering), and 18 to 37 macular SD-OCT sections (OCT; Heidelberg Engineering). All OCT cuts were reviewed carefully for the presence and character of retinal and choroidal hyperreflective foci, defined as round or oval hyperreflective areas 10 to 50 μm in diameter. A total of 707 macular SD-OCT scans of 13 patients with Stargardt disease were included in this study. Two observers (N.P. and B.L.W.N.) independently quantified hyperreflective foci according to this definition by counting, on a high-magnification SD-OCT section of the central macula crossing through the fovea (Figure 1), the number of hyperreflective foci in the retinal and choroidal layers as follows: inner retinal layers (between the inner limiting membrane and external limiting membrane), outer retinal layers (between the external limiting membrane and RPE including the external limiting membrane, myoid zone, ellipsoid zone, and interdigiting zone), Bruch membrane/RPE complex, choriocapillaris, Sattler layer of the choroid, and Haller layer of the choroid. Before independently quantifying the hyperreflective foci, we agreed on the identification of each layer in the SD-OCT sections. The interobserver agreement on the number of hyperreflective foci in every layer was subsequently measured and statistical analysis was performed. Enhanced depth imaging-OCT (EDI-OCT) scans, available for only 2 patients, were compared with the corresponding SD-OCT scans to ascertain whether there was a difference in visualization of the choroid and in the number of hyperreflective foci.

A comprehensive literature search was performed in PubMed and Google for articles from January 1, 2009, through August 31, 2014, using the search terms Stargardt disease, retinal dystrophy, and optical coherence tomography. Articles including high-quality SD-OCT images with all retinal and choroidal layers visible were then reviewed independently to assess the presence or absence (without quantification) of choroidal hyperreflective foci.
Statistical analysis was carried out using SPSS software, version 19.0 (SPSS Inc). The data were categorized in groups according to the retinal or choroidal layer that was analyzed. Correlation was sought between the number of hyperreflective foci in different layers and BCVA, disease duration, and CMT. Multivariate analysis was performed using 1-way analysis of variance.

Results

Demographic findings of the 13 patients are presented in the Table. Mean (SD) age at diagnosis was 35 (19.7) years (range, 9-49 years). Of 13 patients, 7 (54%) were male and 6 (46%) were female. Mean BCVA was 0.67 (0.44), with an approximate Snellen equivalent of 20/100 (6.5 lines) (logMAR range, 0-1.5; Snellen equivalent, 20/600-20/20). The mean duration of disease was 9 (10) years (range, 1-28 years).

For each patient, 18 to 37 SD-OCT cuts were reviewed. Evaluation of the SD-OCT images demonstrated not only retinal hyperreflective foci but also multiple hyperreflective deposits in different layers of the choroid in all patients (Figure 2). The hyperreflective foci in the choroidal layers were adjacent to the vascular borders; none were found within the vessels. The hyperreflective foci in the Bruch membrane/RPE complex were primarily round; however, in deeper layers, they also appeared to be oval.

There was a high degree of interobserver agreement on the number of hyperreflective foci in the different layers in this study (eTable 1 in the Supplement). The highest degree was in the outer retina, the Bruch membrane/RPE complex, and

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Disease Duration, y</th>
<th>Snellen Visual Acuity</th>
<th>Genetic Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/58</td>
<td>12</td>
<td>OD: 20/125 OS: 20/25</td>
<td>ABCA4, exon 46, homozygote; exon 49, heterozygote; RDS exons 1 and 3, homozygote</td>
</tr>
<tr>
<td>2/F/10</td>
<td>1</td>
<td>OD: 20/80 OS: 20/80</td>
<td>NA</td>
</tr>
<tr>
<td>3/F/28</td>
<td>7</td>
<td>OD: 20/160 OS: 20/160</td>
<td>ABCA4, exon 19, heterozygote</td>
</tr>
<tr>
<td>4/F/14</td>
<td>5</td>
<td>OD: 20/160 OS: 20/125</td>
<td>NA</td>
</tr>
<tr>
<td>5/F/17</td>
<td>4</td>
<td>OD: 20/100 OS: 20/100</td>
<td>ABCA4, exon 19, heterozygote</td>
</tr>
<tr>
<td>6/F/16</td>
<td>3</td>
<td>OD: 20/160 OS: 20/160</td>
<td>NA</td>
</tr>
<tr>
<td>7/F/31</td>
<td>21</td>
<td>OD: CF: 2 m OS: CF: 2 m</td>
<td>ABCA4, exons 6 and 22, heterozygote</td>
</tr>
<tr>
<td>8/M/46</td>
<td>23</td>
<td>OD: 20/160 OS: CF</td>
<td>NA</td>
</tr>
<tr>
<td>9/M/50</td>
<td>28</td>
<td>OD: 20/125 OS: 20/125</td>
<td>NA</td>
</tr>
<tr>
<td>10/M/50</td>
<td>1</td>
<td>OD: 20/20 OS: 20/20</td>
<td>NA</td>
</tr>
<tr>
<td>11/M/26</td>
<td>1</td>
<td>OD: 20/20 OS: 20/20</td>
<td>NA</td>
</tr>
<tr>
<td>12/M/18</td>
<td>2</td>
<td>OD: 20/40 OS: 20/40</td>
<td>NA</td>
</tr>
<tr>
<td>13/M/31</td>
<td>3</td>
<td>OD: 20/30 OS: 20/30</td>
<td>Pending*</td>
</tr>
</tbody>
</table>

Abbreviations: CF, counting fingers; NA, not available; RDS, retinal degeneration slow.

*Indicates that laboratory test results had not been returned at the time of this report.
the choriocapillaris (>90% observed agreement). Agreement was less than 90% in the Sattler and Haller layers, possibly because decreased signal intensity in the deeper layers caused more discrepancy between the 2 observers.

The mean number of hyperreflective foci measured in the central cuts crossing through the fovea in the retinal and choroidal layers differed between the layers (P = .03) and, in decreasing frequency, were as follows: Bruch membrane/RPE complex, 78.22 (24.39); choriocapillaris, 25.77 (17.57); Sattler layer, 18.59 (12.89); outer retina, 16.64 (6.96); inner retina, 0.95 (1.58); and Haller layer, 0.73 (0.87).

Figure 3A shows the number of hyperreflective foci in the different layers of the retina and choroid as a correlation of CMT. There was no correlation between the mean number of hyperreflective foci in the inner retina (0.95 [1.58]; P = .29), the outer retina (16.64 [6.96]; P = .38), choriocapillaris (25.77 [17.57]; P = .29), Sattler layer (18.59 [12.89]; P = .08), or Haller layer (0.73 [0.87]; P = .18) and CMT. The mean number of hyperreflective foci in the Bruch membrane/RPE complex (78.22 [24.39]) increased exponentially with decreasing retinal thickness according to the following formula: number of hyperreflective foci = 88 − 40 • (1 − exp [0.03 • CMT]); R² = 0.99; P = .008.

Figure 3B shows the number of hyperreflective foci in the different layers of the retina and choroid as a correlation of BCVA. There was no correlation between the number of hyperreflective foci in the outer retina, inner retina, or Haller layer with BCVA (P = .06).

The mean number of hyperreflective foci in the Bruch membrane/RPE complex increased proportionally with decreasing visual acuity according to the following formula: number of hyperreflective foci = 171 + 3 • (logMAR)³; R² = 0.97; P = .007.
The mean number of hyperreflective foci in the choriocapillaris increased proportionally with decreasing visual acuity according to the following formula: number of hyperreflective foci = 21 + 3 \cdot (\log\text{MAR})^2; \ R^2 = 0.95; \ P = .006.

The mean number of hyperreflective foci in the Sattler layer increased proportionally with decreasing visual acuity according to the following formula: number of hyperreflective foci = 24 - 1 \cdot (\log\text{MAR})^3; \ R^2 = 0.99; \ P = .008.

Figure 3C shows the number of hyperreflective foci in the different layers of the retina and choroid as a correlation of duration of the disease. There was no correlation between the number of hyperreflective foci in the outer retina, inner retina, Bruch membrane/RPE complex, or Haller layer with the duration of the disease (\(P = .07\)).

The mean number of hyperreflective foci in the choriocapillaris increased proportionally with disease duration according to the following formula: number of hyperreflective foci = 15 + 2 \cdot \text{years}; \ R^2 = 0.98; \ P = .006.

The mean number of hyperreflective foci in the Sattler layer increased proportionally with disease duration according to the following formula: number of hyperreflective foci = 10 + 3 \cdot \text{years}; \ R^2 = 0.99; \ P = .009.

Figure 4 shows EDI-OCT and SD-OCT scans of the same foveal cut for 2 patients. There was no difference in the visualization of the choroid or the number of hyperreflective foci between the 2 imaging methods. Enhanced depth imaging–OCT demonstrated a mean of 4.5 (4.5) (range, 1-8) more hyperreflective foci than were demonstrated by the SD-OCT.

The literature review identified 47 studies with high-quality SD-OCT images. In the 10 studies on Stargardt disease, choroidal hyperreflective foci were present in 51 of 54 (94%) SD-OCT images. The remaining 37 studies discussed other retinal dystrophies, in which 16 of 195 (0.08%) SD-OCT scans demonstrated the presence of choroidal hyperreflective foci (eTable 2 in the Supplement). The hyperreflective foci in these studies were of the same shape and size as those in the present study. The number of hyperreflective foci was not quantified.

**Discussion**

Spectral-domain OCT has contributed substantially to our understanding of the disease process of various vitreoretinal disorders. In the case of Stargardt disease, which is characterized by the clinical appearance of yellow flocks throughout the fundus, SD-OCT enables the identification of the location and distribution of hyperreflective foci within the retinal and choroidal layers. The flocks seen clinically in Stargardt disease were previously shown to occur between the Bruch membrane/RPE complex and the outer nuclear layer. The present study clearly demonstrates that hyperreflective foci are not only present in the retinal layers of patients with Stargardt disease but are more prevalent in the choroidal layers, specifically in the Bruch membrane/RPE complex, choriocapillaris, and Sattler layer.

The choroidal hyperreflective foci seen on SD-OCT imaging in the present study were identified in the choroidal layers of...
all patients. Using polarization-sensitive OCT, Ritter et al\textsuperscript{57} described increased depolarizing material in the choroid of patients with Stargardt disease, which may be related to the findings in the present study. The present study also shows that, in contrast to previous beliefs, the hyperreflective foci are not confined to the outer retina; they can also be seen in the more superficial retinal layers. In the choroid of patients with RPE atrophy, such as age-related macular degeneration with geographic atrophy, similar hyperreflective foci were not found.

In this study, we identified an inverse correlation between the number of hyperreflective foci in the Bruch membrane/RPE complex and CMT as well as an inverse correlation between the number of hyperreflective foci in the Bruch membrane/RPE complex, choriocapillaris, and Sattler layer and BCVA. In addition, the number of hyperreflective foci increased in the choriocapillaris and Sattler layer with increasing duration of disease. This latter finding may reflect a downward migration from the outer retina with time. Furthermore, as apparent in Figure 2, the hyperreflective foci in the choroidal layers increase as vision declines.

The reason for the presence of hyperreflective foci in the choroid is uncertain. The present understanding of the underlying pathologic mechanism in Stargardt disease is concentrated in the outer retinal layers. The adenosine triphosphate-binding cassette transporter protein that is defective in Stargardt disease is present in the outer segments of photoreceptors, specifically in the rim of the photoreceptor discs.\textsuperscript{3} Thus, one would expect to see an accumulation of these hyperreflective foci, or lipofuscin deposits, in the outer retinal layers. The presence of hyperreflective foci mainly in choroidal layers, as well as in superficial retinal layers, might suggest migration of the lipofuscin deposits from the outer retina toward both the inner retina and choroid, possibly following a degradation process. The significance of this migratory process in either disease progression or its relationship to visual outcome is yet to be determined. This discussion of underlying mechanisms is contingent upon the demonstration that the hyperreflective foci are composed of lipofuscin deposits and are unique to Stargardt disease, which was not demonstrated in this study and presents an area for further investigation. No attempt to pursue information on the clinical correlates of the hyperreflective foci seen using OCT was made in this study. Therefore, the possibility of a correlation between OCT findings and those noted in color fundus photographs, fluoresent...
cein angiography, or fundus autofluorescence images from the same eye should be investigated. In the absence of such correlation analysis, no direct conclusion regarding the origin, nature, and significance of the choroidal lesions can be drawn.

The small number of cases available for evaluation owing to the rarity of the disease is one limitation of our study. Further limitations include the lack of serial OCTs to monitor possible changes in the hyperreflective foci over time, including changes in the number and position of the foci in the retina and choroid. In addition, dark adaptation or microperimetry was not available in this study population; thus, only correlations between BCVA and CMT could be made with disease severity. The use of SD-OCT scans instead of EDI-OCT scans, which would arguably allow for better visualization of the choroidal layers, may also be a limitation; however, EDI-OCT scans were available for 2 patients in this cohort and were compared with each other (Figure 4). When SD-OCT and EDI-OCT were compared on the same cuts, it was apparent that choroidal hyperreflective foci were visible to the same degree in both imaging techniques in these 2 patients. This visibility may be secondary to the atrophy of retinal layers in Stargardt disease. Indeed, the CMT of both patients who underwent EDI-OCT imaging was less than 200 μm. Thus, there is better penetration of infrared wavelength to deeper choroidal layers and better visualization of choroidal layers in the absence of enhanced depth imaging. Chun et al58 recently described choroidal atrophic changes with infrared scanning laser ophthalmoscopic imaging, demonstrating that both retinal and choroidal atrophy allow better visualization of deeper layers even without EDI-OCT. We assume that if EDI-OCT imaging were available for all our patients, the results would not differ substantially.

Conclusions

To our knowledge, this study demonstrated for the first time that the characteristic hyperreflective foci clinically observed in the fundus of patients with Stargardt disease are present not only in the outer retinal layers, as previously noted, but also in the choroid. These hyperreflective foci correlate with disease severity in terms of degree of retinal atrophy and visual acuity as well as disease duration. Studies using serial, longitudinal SD-OCT and EDI-OCT will aid in better understanding of the disease process in Stargardt disease.
25. Mutations in the structure in retinal degeneration associated with...
32. Ophthalmol technique of enhanced depth imaging optical imaging in inherited retinal disease using the...
2010;51(7):3673-3679.
26. PROM1 mutation p.R373C causes an autosomal...
37. Michaelides M, Webster AR, Moore AT. High-resolution optical coherence tomography measures of outer segment layer characterizations.