The Effect of Light Deprivation in Patients With Stargardt Disease

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• PURPOSE: To investigate whether long-term protection from light exposure affects the rate of disease progression in patients with autosomal recessive Stargardt disease (STGD1), measured using fundus autofluorescence imaging.

• DESIGN: Longitudinal, retrospective, interventional case series.

• METHODS: Five patients with Stargardt disease protected 1 eye from light exposure by applying a black contact lens during waking hours for ≥12 months. Disease progression was followed by performing autofluorescence imaging at semi-regular intervals. Longitudinal changes in autofluorescence were studied by evaluating areas of decreased autofluorescence and areas of increased autofluorescence as a measure of retinal pigment epithelium damage and lipofuscin accumulation, respectively.

• RESULTS: We observed less progression of decreased autofluorescence in 4 out of 5 light-protected eyes relative to their respective nonprotected eyes. The progression of increased autofluorescence, on the other hand, was highly variable and did not respond consistently to treatment.

• CONCLUSIONS: Areas of decreased autofluorescence may serve as a useful biomarker for measuring the progression of Stargardt disease. The reduced progression of decreased autofluorescence in the light-protected eyes suggests that light deprivation might be beneficial in patients with Stargardt disease. (Am J Ophthalmol 2015;159(5):964–972. © 2015 by Elsevier Inc. All rights reserved.)

Autosomal recessive Stargardt disease (STGD1) is the most common inherited juvenile macular degeneration. Most patients develop bilateral loss of vision in childhood or early adulthood. This subtype of Stargardt disease is caused by mutations in the ABCA4 gene, which encodes a retina-specific transporter protein (ABCR) in the rims of rod and cone photoreceptor outer segment discs. Retinal degeneration in ABCA4-linked Stargardt disease is believed to result from the toxic effects of lipofuscin that accumulates in the retinal pigment epithelium (RPE) and the subsequent degeneration of photoreceptors.

Light can induce photochemical injury at the ocular fundus. Depending on the level and duration of the irradiance, the primary site of damage can be either the photoreceptors or the RPE. In ABCA4-linked retinopathies, products generated by the visual cycle accumulate and contribute to retinal damage via both direct toxic effects and increased photosensitivity. A major fluorophore of lipofuscin, bis-retinoid N-retinylidene-N-retinyl-ethanolamine (A2E), accumulates with other, currently unidentified lipofuscin constituents within the RPE. Thus, an excessive accumulation of A2E has been observed in both Abca4-/- mice and patients with Stargardt disease. Lipofuscins (and A2E in particular) are potent photosensitizers that can induce oxidative damage, thereby accelerating light-induced retinal damage and RPE atrophy. This oxidative damage may affect the rate of disease progression in patients with Stargardt disease.

The total quantity of A2E oxiranes in Abca4-/- mice increases in response to light exposure. In addition, a recent review of light-induced and inherited retinal degenerations suggested that light exposure might modify the disease course of genetically well-defined retinal dystrophies, including Stargardt disease. An early study used functional and ophthalmoscopic examinations to test the effect of 5 years of unilateral light deprivation in a patient with autosomal recessive retinitis pigmentosa and in a patient with autosomal dominant retinitis pigmentosa; the author observed symmetrical disease progression. However, in the absence of a genetic diagnosis, and without clear insight into the pathogenesis of these 2 RP patients, no conclusions can be drawn with respect to patients with Stargardt disease.

Because the aforementioned results suggest that patients with Stargardt disease might be more sensitive to light, we considered using light protection as a means to slow disease progression in patients with Stargardt disease. Therefore, we retrospectively examined the effects of using a single black contact lens in an attempt to spare disease progression in 1 eye in patients with Stargardt disease. We followed disease progression in 5 patients using the fundus autofluorescence images.
autofluorescence imaging data, as this test provides a sensitive, noninvasive measure of RPE abnormalities and lipofuscin accumulation in patients with Stargardt disease.\textsuperscript{20–22}

METHODS

THIS STUDY WAS A LONGITUDINAL, RETROSPECTIVE, INTERVENTIONAL CASE SERIES OF PATIENTS DIAGNOSED WITH STARGARDT DISEASE. THE INTERVENTION WAS APPLIED IN THE CONTEXT OF CLINICAL CARE AT THE REQUEST OF THE PATIENTS (OR LEGAL GUARDIAN) AND NOT IN THE CONTEXT OF A PROSPECTIVE CLINICAL TRIAL. THEREFORE, NONSTANDARD ANALYSES (EG, ROUTINE IMAGING) WERE PERFORMED TO ENSURE INTERNAL QUALITY CONTROL AND TO DETECT ANY ADVERSE EVENTS IN A TIMELY MANNER. ALL PATIENTS HAD BEEN DIAGNOSED PREVIOUSLY AS HAVING STARGARDT DISEASE WITH AT LEAST 1 ABCA4 MUTATION, AND ALL PATIENTS PRESENTED WITH THE TYPICAL CLINICAL SYMPTOMS ASSOCIATED WITH THIS RETINAL DYSTROPHY.

All genetic analyses were performed by the Department of Human Genetics at Radboudumc (Nijmegen, the Netherlands). Patients were screened for known ABCA4 mutations using the arrayed primer extension microarray (Asper Biotech, Tartu, Estonia), and exon duplications and/or deletions were detected using multiplex ligation-dependent probe amplification (P151 and P152; MRC-Holland, Amsterdam, the Netherlands). If no mutations—or only a single heterozygous mutation—were identified, the exons and intron-exon boundaries were sequenced using the Sanger method to screen for mutations in the other allele. All identified mutations were confirmed using Sanger sequencing.

The study was performed in accordance with the tenets established by the Declaration of Helsinki, and informed consent was obtained from all patients (or from the legal guardians of underage patients) for a retrospective analysis of therapy-related data. Patients were previously advised regarding the potential benefits of wearing sunglasses, avoiding direct light exposure, and limiting their dietary intake of vitamin A.\textsuperscript{23} Complete protection from light exposure was suggested as a treatment option to patients who had a pressing request for any potentially effective treatment, given the current absence of suitable treatments for Stargardt disease. The institutional review board (Commissie Mensegebonden Onderzoek, Region Arnhem-Nijmegen) retrospectively approved this study (2013/062) after the last patient’s final visit and during the study.

Patients were enrolled in the study and followed from January 2006 through December 2009 at the clinical practice of the Department of Ophthalmology, Radboudumc, Nijmegen. After eliminating any medical concerns regarding the use of contact lenses, we suggested that the patients protect their intuitively best eye (determined at the time of enrollment) during waking hours for at least 1 year using a black contact lens. The age of disease onset was defined as the age at which visual loss was first recorded. Best-corrected visual acuity (BCVA) was measured using a Snellen chart or the finger-counting method. To provide the most complete light protection, a customized, black, soft contact lens designed to cover the entire cornea (Ercon, Assen, the Netherlands) was applied; this lens blocked >90% of light in the visible spectrum.

Patients underwent a routine ophthalmologic examination, including BCVA, slit-lamp examination, binocular funduscopy, and confocal fundus autofluorescence imaging of both eyes with a Spectralis device (Heidelberg Engineering, Heidelberg, Germany). After pharmacologic pupil dilation to ≥6 mm with phenylephrine 2.5% and tropicamide 0.5% eye drops, we recorded fundus autofluorescence images centered on the fovea and including part of the optic disc; images were obtained using an excitation wavelength of 488 nm and a barrier filter (2500 nm) in high-resolution mode (30-degree field of view, 1536 pixels × 1536 pixels). Baseline and follow-up images of both eyes were captured with equal system sensitivity settings. Clinical phenotyping was performed in accordance with Fishman and associates (1999)\textsuperscript{24} by an experienced clinician (author R.H.) who was masked with respect to the study results. Fundus autofluorescence was used to phenotype the disease stages as described by Cideciyan and associates.\textsuperscript{25}

Fundus autofluorescence images were acquired using a standard procedure as follows. First, focusing was performed using the near-infrared reflection mode of the Spectralis. To account for chromatic aberrations, slight refocusing in the autofluorescence mode was performed, and 9 separate images were acquired after sensitivity adjustment, thereby covering the entire macular area and including part of the optic disc. To increase the signal-to-noise ratio, all images were aligned and a mean image was calculated using the internal software (without normalization by histogram stretch). All other image processing and analysis was performed using scripts written in MatLab (R2006; Math-Works, Natick, Massachusetts, USA). Initial and final fundus autofluorescence images were automatically registered as reported previously.\textsuperscript{26} The alignment of each image pair was manually confirmed; if necessary, registration was adjusted iteratively until a satisfactory result was obtained. Pixel size was normalized to the fovea-disc margin distance.\textsuperscript{26}

Increased autofluorescence and/or decreased autofluorescence relative to the image background was quantified by an observer (author M.L.; this author was masked with respect to the treated eye) based on non-normalized mean images as published previously.\textsuperscript{20,22,28} In brief, each fundus autofluorescence image was leveled by subtracting a 12-zone quadratic polynomial mathematical model of the image’s background autofluorescence, which was calculated for each image. The threshold for increased or decreased autofluorescence was set at 1.5 × σ above or below, respectively, the mean pixel intensity of the resulting image. Consistent with previous reports,\textsuperscript{20} we found...
that this threshold provided the best detection of visually evident autofluorescence abnormalities. In some cases, the precise location of the fovea could not be determined owing to pathologic changes; in such cases, determining where to place the center of the quadratic polynomial model was questionable. Therefore, we adjusted the model’s position slightly until the selection of any visually evident autofluorescence abnormalities was optimized. For some images, multiple repositioning of the model was required in order to capture all autofluorescence abnormalities; in these cases, we combined the resulting pixels showing increased and/or decreased autofluorescence, respectively. Pixels that were identified incorrectly in vessels and/or bifurcations were removed manually.

The total number of pixels showing increased and/or decreased autofluorescence was measured in each image, and the annual rate of change in decreased autofluorescence (change in decreased AF) in serial image pairs of the right eye (OD) was calculated as follows:

\[
\text{\% Change in decreased AF (OD)/yr} = \frac{(\text{Decreased AF}_{\text{post}}(\text{OD}) - \text{Decreased AF}_{\text{pre}}(\text{OD}))}{(\text{Decreased AF}_{\text{pre}}(\text{OD}) + \text{Decreased AF}_{\text{pre}}(\text{OS}))} \times 100\% \times \frac{1}{\text{Time between images (yr)}}.
\]

The annual rate of change in the left eye (OS) was calculated as follows:

\[
\text{\% Change in decreased AF (OS)/yr} = \frac{(\text{Decreased AF}_{\text{post}}(\text{OS}) - \text{Decreased AF}_{\text{pre}}(\text{OS}))}{(\text{Decreased AF}_{\text{pre}}(\text{OD}) + \text{Decreased AF}_{\text{pre}}(\text{OS}))} \times 100\% \times \frac{1}{\text{Time between images (yr)}}.
\]

Dividing by the total initial number of pixels showing decreased autofluorescence in both eyes minimized potential artificial increases in progression that might have arisen when the initial quantity in 1 eye was low. To evaluate the progression of decreased autofluorescence in the light-protected eye, we normalized its progression to the control (nonprotected) eye as follows:

\[
\text{Progression of decreased AF (normalized)} = \left(1 - \frac{\text{\% Change in decreased AF}_{\text{untreated}}/\text{yr} - \text{\% Change in decreased AF}_{\text{treated}}/\text{yr})}{\text{\% Change in decreased AF}_{\text{untreated}}/\text{yr}}\right) \times 100\%.
\]

All calculations were performed for annual changes in increased autofluorescence. Statistical analyses were not performed owing to the relatively small sample size and the absence of empirical data regarding the progression of altered autofluorescence under conditions of light deprivation.

**RESULTS**

The patients’ demographics and clinical data are summarized in Table 1. Five white patients (3 male, 2 female) with Stargardt disease (mean age at inclusion was 22.6 years; range: 10–46 years) enrolled and completed the treatment with a mean follow-up period of 17.8 months (range: 11–26 months). We identified disease-related mutations in both ABCA4 alleles of 4 patients; only 1 ABCA4 mutation was identified in Patient 5.

Treatment compliance was assumed based on information provided by the patients or their legal guardians, and they reported that they complied. The course of BCVA was similar in both eyes of each patient and was stable in all but 1 patient (Patient 1) throughout the study period. In Patient 2, whose BCVA was stable throughout the study, bilateral nuclear cataract was identified at the final follow-up visit. No geographic atrophy was observed in any patient throughout the study period. None of the study participants developed any contact lens–related adverse events or signs of deprivation amblyopia.

Figure 1 shows the characteristic fundus autofluorescence appearance in 1 patient during the study period. All 5 patients presented with a complex textural fundus...
### TABLE 1. Demographics, Genetics, and Clinical Appearance of 5 Patients With Stargardt Disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at Inclusion (y)</th>
<th>Age at Onset (y)</th>
<th>Disease Duration (mo)</th>
<th>Follow-up Period (mo)</th>
<th>Eye</th>
<th>Initial BCVA</th>
<th>Final BCVA</th>
<th>Mutation Allele 1</th>
<th>Mutation Allele 2</th>
<th>Ophthalmoscopy</th>
<th>Fluorescence Angiography</th>
<th>Electroretinography</th>
<th>Stargardt Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>20</td>
<td>19</td>
<td>41</td>
<td>28</td>
<td>OD</td>
<td>OS* 20/40</td>
<td>20/100</td>
<td>c.768G&gt;T</td>
<td>c.872C&gt;T</td>
<td>Yellowish white flecks in parafoveal area, central parafoveal pigment alterations, bullseye-like appearance</td>
<td>No dark choroid</td>
<td>Within normal limits</td>
<td>PF</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>46</td>
<td>46</td>
<td>32</td>
<td>27</td>
<td>OD</td>
<td>OS* 20/16</td>
<td>20/16</td>
<td>c.2588G&gt;C</td>
<td>c.2828G&gt;A</td>
<td>Yellowish flecks throughout the posterior pole extending anterior to the vascular arcades and peripapillary region</td>
<td>No dark choroid, but cSLO recording, which may mask the dark choroid sign</td>
<td>Within normal limits</td>
<td>FF</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>10</td>
<td>10</td>
<td>22</td>
<td>13</td>
<td>OD</td>
<td>OS* 20/40</td>
<td>20/400</td>
<td>c.3335C&gt;A</td>
<td>c.5461 -10T&gt;C</td>
<td>Yellowish white flecks in para- and perifoveal area, central RPE alterations</td>
<td>No dark choroid</td>
<td>OD photopic: moderately reduced, OS pathologically reduced (lowered amplitude)</td>
<td>PF</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>13</td>
<td>11</td>
<td>53</td>
<td>29</td>
<td>OD</td>
<td>OS* 20/125</td>
<td>20/160</td>
<td>c.2588G&gt;C</td>
<td>c.4539 +1G&gt;T</td>
<td>Central RPE alterations and widespread atrophic lesions throughout the posterior pole extending anterior to the vascular arcades</td>
<td>Diffuse hyperfluorescent lesions</td>
<td>Photopic moderately reduced</td>
<td>DA</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>24</td>
<td>12.3</td>
<td>284</td>
<td>12</td>
<td>OD</td>
<td>OS* 20/400</td>
<td>CF</td>
<td>c.IVS39&gt;-10T&gt;C</td>
<td>NF</td>
<td>Central RPE alterations with intraretinal pigmentations; small atrophic lesions (presumably resorbed yellow flecks) scattered throughout the posterior pole extending anterior to the vascular arcades</td>
<td>Not performed</td>
<td>OD and OS photopic moderately reduced, OD scotopic moderately reduced, OS scotopic pathologically reduced</td>
<td>DA</td>
</tr>
</tbody>
</table>

Mean 23 18 86 22
SD 14 17 111 9

BCVA = best-corrected Snellen visual acuity; CF = finger counting; cSLO = confocal scanning laser ophthalmoscopy; DA = diffuse atrophic type; FF = fundus flavimaculatus type; NF = not found; OD = right eye; OS = left eye; PF = perifoveal type; RPE = retinal pigment epithelium.

* indicates treated/light-protected eyes.

† indicates age at onset of the disease and the final image.

Mutations in the ABCA4 gene.

As defined by Fishman et al (1999).24

Allele unknown.
autofluorescence appearance (ie, lesions of increased or decreased fundus autofluorescence). Central diffuse hypoautofluorescent areas were present in the initial images of 4 patients; however, these areas were not sharply demarcated and had stronger signals than would be expected in areas of geographic atrophy in Stargardt disease.20

During the study period, pixels showing increased and/or decreased autofluorescence emerged, disappeared, and/or changed (ie, from an increase to a decrease or vice versa). We observed that the temporal sequence of autofluorescence changes was heterogeneous, both between patients and between a given patient’s eyes (eg, some hypoautofluorescent areas expanded, but also regressed partially into the background autofluorescence). This heterogeneity was observed in visually evident lesions as well as in lesion-free areas. Progression analysis was performed in accordance with Cideciyan and associates15 and revealed that all 5 patients progressed during the study period; 1, 2, and 2 patients progressed to stage 3, stage 4, and stage 5, respectively.

The total number of pixels showing decreased autofluorescence generally increased in all but 1 eye. However, in 4 of the 5 patients, the normalized annual progression rate in the light-protected eye was less severe compared to the respective untreated eye (Figure 2). In 1 patient, we observed the opposite pattern; in Patient 3, the progression of decreased autofluorescence was slightly higher in the light-protected eye than in the control eye. On the other hand, the progression of increased autofluorescence was more variable than the progression of decreased autofluorescence; increased autofluorescence developed symmetrically (ie, with similar progression in both the light-protected and untreated eye) in all patients. The results of our autofluorescence analysis are summarized in Table 2.

**DISCUSSION**

HERE, WE REPORT THE EFFECT OF USING A BLACK CONTACT lens to protect the eye from light exposure in patients with Stargardt disease. We observed less progression of decreased autofluorescence in 4 out of 5 light-protected eyes compared to the patients’ unprotected eyes. In contrast, we found no effect of light protection on the progression of increased autofluorescence. Despite an
extensive literature search (for details see Supplemental Material, available at AJO.com), we were unable to find any published reports regarding the effect of light protection in patients with Stargardt disease.

A recent study using the Abca4−/− mouse model found that the accumulation of lipofuscin in the retina makes the retina more vulnerable to the effects of light with respect to disease progression.29 How increased levels of lipofuscin lead to photoreceptor degeneration and visual loss in patients with Stargardt disease is poorly understood. Photo-oxidative processes appear to accelerate cell damage in retinas that have increased lipofuscin levels following light exposure.29,30 The primary site of this light-induced damage in patients with Stargardt disease could be photoreceptor outer segments, the RPE, or both. For instance, prolonged light exposure may cause primary photoreceptor cell damage by allowing photopigment regeneration and subsequent repeated bleaching, which may promote the formation of bisretinoids in photoreceptor cells. Owing to outer segment shedding, however, bisretinoids in photoreceptor cells are kept to a minimum and instead accumulate in the RPE. Although some studies of light damage have reported the greatest damage in the RPE,31–36 others have reported damage in both the RPE and photoreceptors.29

Patients with Stargardt disease can develop increased or decreased autofluorescence compared to background autofluorescence. In Stargardt disease, pixels showing increased and/or decreased autofluorescence tend to be unstable and can vary with respect to the temporal sequence of autofluorescence changes.28 Whether a given fundus area with background, increased, or decreased autofluorescence will develop a focal increase and/or decrease in autofluorescence cannot be predicted at baseline; such a change depends on the stage of the disease in that specific area.17 In our study, we quantified the increased and/or decreased autofluorescence relative to background autofluorescence. Because we did not measure autofluorescence quantitatively,38 we cannot conclude whether background autofluorescence—and by extension, lipofuscin—is equal to, higher than, or lower than normal in a given patient. Therefore, in contrast to well-defined areas of geographic atrophy, pixels showing decreased autofluorescence may not necessarily reflect total RPE atrophy in that region. Pixels showing decreased autofluorescence may therefore represent decreased, normal, or even increased autofluorescence compared to age-matched healthy subjects. Nevertheless, pixels showing decreased autofluorescence (which was referred to in 2009 by Smith and associates20 as “focally decreased autofluorescence”) suggest RPE cell damage.20 Because recent quantitative measurements showed that background autofluorescence is nearly always increased in Stargardt disease (compared to age-matched controls),39 pixels showing increased autofluorescence should indicate increased autofluorescence values in general. Pixels showing increased autofluorescence may therefore be interpreted as an increase in lipofuscin, even when precise quantification is not available.

We observed that a pixel showing increased autofluorescence could change to decreased autofluorescence; however, it could also remain increased or even return to background autofluorescence levels. On the other hand, pixels showing decreased autofluorescence could emerge from background autofluorescence or increased autofluorescence. As discussed above, a pixel showing increased autofluorescence suggests a focal area of increased levels of fluorophores such as lipofuscin and lipofuscin bisretinoids. Thus, our data suggest that light protection does not affect the progression of focal lipofuscin accumulation.

Interestingly, the 3 youngest patients in our cohort (Patients 1, 3, and 4) had a decrease in the number of pixels showing increased autofluorescence even in the light-protected eye. It should be noted that Patients 1, 3, 4, and 5 were classified as having early-onset Stargardt disease and were included in a recent study conducted by our department.40 This decrease—in combination with an increase in the number of pixels showing decreased autofluorescence in Patients 1, 3, and 4—is likely associated with a trend of decreasing overall autofluorescence.20 This trend may be more pronounced when the disease progresses rapidly, as occurs in early-onset Stargardt disease.40 Patients 3 and 5, however, had an extremely large central diffuse hypoautofluorescent lesion and a large number of pixels showing decreased autofluorescence. This finding suggests more destructive disease, as suggested by Lamber-
tus and associates.40

On the other hand, decreased autofluorescence appeared to be associated with light exposure in our cohort; in other words, light deprivation reduced the progression of decreased autofluorescence in 4 of 5 patients. Irradiance

Figure 2. Summary of the effect of light exposure on the progression of decreased autofluorescence in 5 patients with Stargardt disease. Normalized progression of decreased autofluorescence (AF) measured in the light-protected eyes (black bars) and the untreated eyes (gray bars) of 5 patients. The progression of decreased autofluorescence was reduced in Patients 1, 2, 4, and 5; in contrast, Patient 3 had more progression in the light-protected eye compared to the untreated eye. The value below each patient indicates the treatment duration (in months).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Treatment period</th>
<th>Normalized progression of decreased AF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.9</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>24.4</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>10.9</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>25</td>
</tr>
</tbody>
</table>
with visible light of sufficient intensity photobleaches lipofuscin autofluorescence in RPE cells, and primary RPE damage with no anatomic changes in the retinal photoreceptors can occur following exposure to visible light. In Abca4−/- mice, light damage in the RPE can also be caused by toxicity attributable to increased bisretinoids and their oxidation products. Therefore, it is likely that excessive light exposure is harmful to patients with Stargardt disease. Other lines of evidence suggest that blue light may be primarily responsible for this damage. Therefore, eyeglasses or contact lenses that filter out short-wavelength light might be a viable alternative to total light deprivation.

The advantage of our study is that we compared each light-protected eye to the untreated eye in the same patient, providing each treated eye with its own internal control. By comparing the light-protected eye to the untreated eye in the same patient, both genetic and environmental effects (aside from the use of the contact lens) were minimized.

On the other hand, this study also has several potential limitations. First, we studied a relatively small number of patients with a range of ages and in various stages of Stargardt disease. Therefore, our cohort included eyes in different disease stages and eyes with different degrees of lipofuscin accumulation. However, we included only patients with recent subjective and objective progression, strong motivation to participate in this lengthy treatment, and no contraindication to contact lens use. Moreover, our approach to light-deprive the subjectively better eye may have influenced our results. However, we believe that any selection bias was unlikely, as the disease was symmetrical at the start of treatment with respect to both functional and anatomic examination (shown using BCVA, funduscoppy, and the number of pixels showing increased autofluorescence and decreased autofluorescence). In addition, randomization was not possible, as this was not a prospective clinical trial. The treating physician (author C.H.) felt that any treatment—if effective—should be applied to the subjectively better eye in an attempt to preserve function in that eye.

Furthermore, because this study was not performed prospectively, it relied on retrospective data analysis. Therefore, autofluorescence imaging was not performed using a standardized protocol, nor were the images obtained at predetermined time points. Moreover, media opacity and pupil dilation—2 potential confounders of autofluorescence—were not routinely measured before imaging. Taken together, our study was subject to 2 specific forms of selection bias: (1) the strong motivation of the patients to participate in this study, and (2) our retrospective quality selection of the imaging data.

Consistent with previous studies, our study was designed to observe the progression of increased autofluorescence and/or decreased autofluorescence during a specific period of time and to identify any difference in progression between the untreated and treated eyes in the same patient. However, our study did not follow the continuous development of changes in autofluorescence over a prolonged interval (ie, until geographic atrophy and vision loss occurred). A long-term prospective study including more frequent and regular measurements using standardized autofluorescence imaging over several years might help address the important question of whether light protection can delay vision loss—rather than merely delaying focal RPE damage—in patients with Stargardt disease.

### TABLE 2. Course of Fundus Autofluorescence in 5 Patients With Stargardt Disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>Eye</th>
<th>Treatment Period (mo)</th>
<th>Initial FIAF (Pixels)</th>
<th>Final FIAF (Pixels)</th>
<th>FIAF Change %/Year</th>
<th>Initial FDAF (Pixels)</th>
<th>Final FDAF (Pixels)</th>
<th>FDAF Change %/Year</th>
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<tr>
<td>1</td>
<td>OD</td>
<td>1664</td>
<td>326</td>
<td>−16.6</td>
<td>88</td>
<td>199</td>
<td>22.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OS'</td>
<td>25.9</td>
<td>1897</td>
<td>431</td>
<td>−18.2</td>
<td>129</td>
<td>129</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>OD</td>
<td>3894</td>
<td>6160</td>
<td>11.6</td>
<td>4231</td>
<td>4342</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OS'</td>
<td>24.4</td>
<td>4650</td>
<td>5745</td>
<td>5.6</td>
<td>4117</td>
<td>4214</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>OD</td>
<td>10.9</td>
<td>1464</td>
<td>750</td>
<td>−15.4</td>
<td>12172</td>
<td>14395</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>OS</td>
<td>2720</td>
<td>1859</td>
<td>−18.6</td>
<td>17665</td>
<td>19394</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>OD</td>
<td>2946</td>
<td>1302</td>
<td>−16.9</td>
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</tr>
<tr>
<td></td>
<td>OS'</td>
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<td>1127</td>
<td>329</td>
<td>−8.2</td>
<td>4098</td>
<td>4153</td>
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</tr>
<tr>
<td>5</td>
<td>OD</td>
<td>1613</td>
<td>2663</td>
<td>34.7</td>
<td>24383</td>
<td>27187</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OS'</td>
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<td>2284</td>
<td>3636</td>
<td>29546</td>
<td>35152</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>17.8</td>
<td>2426</td>
<td>2320</td>
<td>−1.5</td>
<td>10099</td>
<td>11415</td>
<td>5.4</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>7.0</td>
<td>1136</td>
<td>2200</td>
<td>20.1</td>
<td>10432</td>
<td>12174</td>
<td>7.0</td>
</tr>
</tbody>
</table>

FDAF = focal areas of decreased autofluorescence; FIAF = focal areas of increased autofluorescence; OD = right eye; OS = left eye.

Images were registered and adjusted for pixel size (ie, the fovea-disc distance had identical pixel numbers in follow-up series). Total numbers of pixels showing increased and/or decreased autofluorescence in initial and final images were measured, and yearly changes in the numbers of pixels showing increased and/or decreased autofluorescence were calculated as a percentage of the total number in the initial images of both eyes (as % FIAF/year and % FDAF/year, respectively).

*indicates the study eyes.
Moreover, adding quantitative autofluorescence imaging to future study protocols would be highly informative. Unfortunately, appropriate research tools for normalizing autofluorescence grayscale values to an internal reference were not available at the time of our study.

In conclusion, our results suggest that decreased autofluorescence may serve as a biomarker for disease progression in patients with Stargardt disease. Under light-deprivation conditions, we found reduced overall progression of decreased autofluorescence compared with the untreated eye, suggesting a reduction in the rate of RPE damage in the treated eye. Our data suggest that light deprivation may confer a protective effect in patients with Stargardt disease and therefore merits further investigation.

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REFERENCES


SUPPLEMENTAL MATERIAL

AN EXTENSIVE LITERATURE SEARCH WAS PERFORMED USING THE following search string in the PubMed database (available at http://www.ncbi.nlm.nih.gov/). The search was performed on November 2, 2014.


Biosketch

Michel M. Teussink, MSc, received his Masters degree in Medical Biology from the Radboud University Nijmegen with a specialty in biomedical imaging. He is experienced in advanced microscopical techniques and analyses, and is currently completing his PhD project on functional optical coherence tomography and imaging biomarkers of retinal diseases. He has co-authored 3 publications in peer reviewed journals. Mr. Teussink is PhD student at the Department of Ophthalmology, Radboudumc, Nijmegen, The Netherlands.