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RETINAL DISORDERS

Insights into autofluorescence patterns in Stargardt macular dystrophy using ultra-wide-field imaging

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Abstract
Purpose To characterize autofluorescence (AF) patterns occurring in Stargardt macular dystrophy (STGD1) using ultra-wide-field (UWF) imaging.
Methods This paper is a cross-sectional observational study of 22 eyes of 11 patients (mean age 23.44 years) with Stargardt disease-fundus flavimaculatus who presented with decrease of vision at a tertiary eye care center. UWF short-wave AF images were obtained from all the patients using an Optos TX200 instrument. The main outcome measures were to assess patterns of AF changes seen on UWF AF imaging.
Results All eyes showed a central area of hypoautofluorescence at the macula along with retinal flecks extending centrifugally as well as to the nasal side of the optic disc. Peripapillary sparing was seen in 100% of the eyes. Flecks were seen to be hypoautofluorescent in the center and hyperautofluorescent in the periphery in 77.8% eyes and were only hyperfluorescent in 27.2%. A background-increased fluorescence was visible in 100% of eyes, the outer boundary of which was marked by distribution of flecks in 81.9% eyes. A characteristic inferonasal vertical line was seen separating the nasal hypoautofluorescent area from the temporal hyperautofluorescent area in all the eyes.
Conclusions UWF AF changes in STGD1 are not limited to the posterior pole and may extend more peripherally. UWF imaging is a useful tool for the assessment of patients with Stargardt macular dystrophy.

This manuscript has not been submitted elsewhere for publication

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Keywords Autofluorescence · Fundus flavimaculatus · Stargardt disease · Ultra-wide field

Introduction

Stargardt macular dystrophy (STGD1) is commonly encountered in clinical practice, being the most common type of juvenile macular degeneration [1, 2]. One theory of STGD1 is that it is caused by lipofuscin (LF) toxicity to the retinal pigment epithelium (RPE) [3]. However, the suggested main toxic component, N-retinylidene-N-retinyethanolamine (A2E), has recently been shown not to be a major LF component in the human macula [4]. Furthermore, LF toxicity in general has been shown to be an unlikely mechanism in age-related macular degeneration (AMD) or STGD1 because areas of high LF do not predict future atrophy [5, 6]. However, quantitative AF (qAF) has shown definitively that LF levels are high in most STGD1 [7], and it is generally agreed that AF patterns of LF are important disease markers. Clinically this is seen in the form of yellowish flecks on RPE that fade away with time leaving behind RPE and photoreceptor atrophy, which leads to slowly progressive vision loss [8].

Fundus autofluorescence (AF) is based on the ability of certain pigments to emit light of longer wavelength when excited by the light of a particular wavelength. When excited by short wavelength, predominant fluorescence in the retina is from the pigment lipofuscin, contained in the RPE [9]. Because LF is an important marker in STGD1, the short wavelength AF (SWAF) provides an excellent means of analyzing the health of RPE in patients with STGD1. SWAF using a confocal scanning laser ophthalmoscope is considered the gold standard [10] and several studies have demonstrated the utility of SWAF in patients with STGD1 [11–13].

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Table 1 Details of eleven patients with STGD1

S. no.	Age	Sex	Eye	BCVA	Central atrophy	Peripapillary sparing	Fleck crops	Peripheral flecks	Background hyper AF	Vertical Line
1	7	M	R	0.16	Y	Y	Y	Y	Y	Yes
			L	0.25	Y	Y	Y	Y	Y	Yes
2	12	M	R	0.33	Y	Y	N	N	Y	Yes
			L	0.33	Y	Y	N	N	Y	Yes
3	30	M	R	0.1	Y	Y	Y	N	Y	Yes
			L	0.1	Y	Y	Y	N	Y	Yes
4	34	M	R	0.08	Y	Y	Y	N	Y	Yes
			L	0.08	Y	Y	Y	N	Y	Yes
5	38	M	R	0.25	Y	Y	Y	N	Y	Yes
			L	0.25	Y	Y	Y	N	Y	Yes
6	18	F	R	0.16	Y	Y	Y	Y	LIMITED	Yes
			L	0.16	Y	Y	Y	Y	LIMITED	Yes
7	13	M	R	0.25	Y	Y	PARTIAL	N	Y	Yes
			L	0.25	Y	Y	PARTIAL	N	Y	Yes
8	36	M	R	0.16	Y	Y	Y	Y	Y	Yes
			L	0.16	Y	Y	Y	Y	Y	Yes
9	23	F	R	0.5	Y	Y	N	N	Y	Yes
			L	0.5	Y	Y	N	N	Y	Yes
10	36	M	R	0.1	Y	Y	Y	Y	LIMITED	No
			L	0.05	Y	Y	Y	Y	LIMITED	Yes
11	16	F	R	0.33	Y	Y	N	N	Y	Yes
			L	0.25	Y	Y	N	N	Y	Yes

Y Yes, *N* No, Fleck crops – central flecks hypoautofluorescent, peripheral hyperautofluorescent

With the introduction of Optos (Optos Tx200, Optos PLC, Dunfermline, Scotland, UK), it is possible to capture up to 200 degrees of retina (approximately 82% of retinal area) in a single image [14]. It utilizes a confocal scanning laser ophthalmoscope (CSLO) for obtaining the images and can provide excellent ultra-wide-field SWAF images (using a green laser for excitation) despite minimal patient cooperation. The purpose of this study is to report the patterns of ultra-wide-field SWAF in patients with STGD1, some of which have not been reported before in the literature.

Methods

This was a cross-sectional observational study of 22 eyes of 11 patients with STGD1 who were imaged with the help of an Optos Tx200 device between the period January 2015 to March 2016 at a tertiary eye care center. The study was conducted in accordance with institutional guidelines and adhered to the 1964 Declaration of Helsinki. Informed consent was obtained from all the patients (guardians in case of minors).

The patients who presented with bilateral symmetric macular lesions with peripheral flecks (Stargardt disease and fundus flavimaculatus) were labeled as STGD1. The patients with macular lesion (Stargardt disease) or peripheral flecks (fundus flavimaculatus) only were not included in the study since molecular diagnosis was not available. Patients with signs of intraocular inflammation, peripheral bony spicules or nyctalopia were also excluded from the study. The patients with hazy media and uncooperative patients were excluded.

After detailed ophthalmic examination including best-corrected visual acuity (BCVA, Snellen's fraction), slit lamp examination including intraocular pressure and dilated fundus examination, all the patients underwent ultra-wide-field

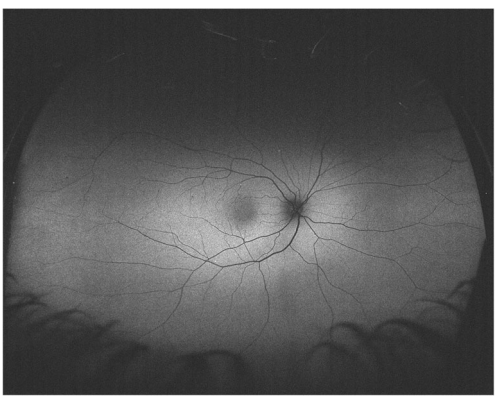

Fig. 1 UWF SWAF image of a normal eye

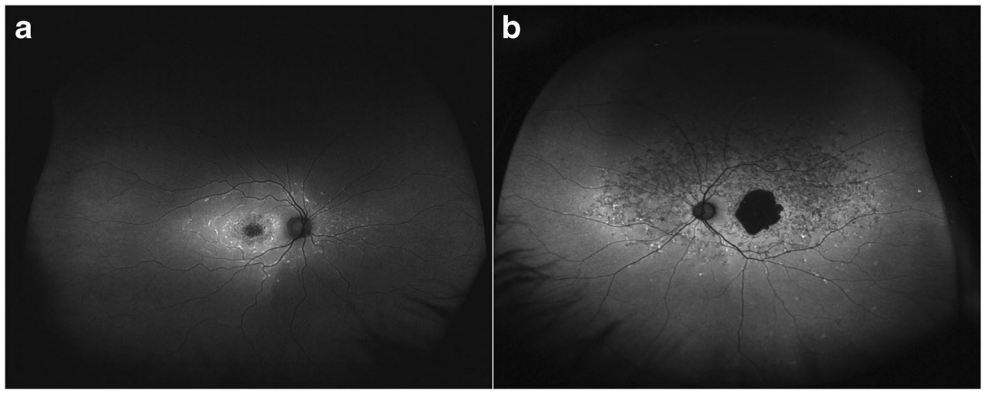

Fig. 2 **a** UWF SWAF of the right eye of 23-year-old female (patient 9) showing a small central area of hypoautofluorescence; BCVA was 0.5. All the flecks are hyperautofluorescent and background AF is increased. **b** UWF SWAF of the left eye of 36-year-old male (patient 8) showing a large central area of hypofluorescence; BCVA was 0.16. A large area of the retina is showing flecks. While the central flecks are hypoautofluorescent, the peripheral flecks are hyperautofluorescent. Flecks extend to the inferotemporal periphery

(UWF) imaging with an Optos Tx200 device. Pseudo-color and SWAF images were obtained and analyzed without any digital alteration of the images. The primary objective of the study was to qualitative analysis (patterns of SWAF changes) of UWF SWAF images.

Results

Ultra-widefield SWAF images of 22 eyes of 11 patients were obtained. The details of each patient are presented in Table 1. Eight patients were males while three were females. The mean age of the patients was 23.9 years (range 7–38 years). All the patients presented with decreased visual acuity. The mean visual acuity was 0.22 (range 0.05 to 0.33). An UWF SWAF image of a normal eye is shown in Fig. 1. All 18 eyes showed a central oval-shaped area of hypoautofluorescence corresponding to the central RPE atrophy (Fig. 2). A greater size and amount of hypoautofluorescence was seen in more advanced stages and was associated with poorer visual acuity (Fig. 2a and b).

All eyes had retinal flecks, which extended centrifugally from the area of central RPE atrophy for a variable distance (Fig. 3). The flecks extended to the nasal side of the optic disc in 100% of the eyes, sparing the peripapillary retina (Fig. 4). On SWAF images, the retinal flecks were hypo- or hyperautofluorescent. In 16 out of 22 eyes (72.8%), the retinal flecks closer to the center of the macula were hypoautofluorescent, while the ones towards the retinal periphery were hyperautofluorescent (Figs. 2b, 3a, b and 4). In 4 eyes (27.2%), all the retinal flecks were hyperautofluorescent (2a, 5). In 6 eyes (33.3%), the retinal flecks were seen extending more peripherally (Figs. 2b, 3a, b, 7). This was more prominent on UWF SWAF images rather than clinical examination.

A characteristic pattern of diffuse background increase of AF was noted in 18 out of 22 eyes (81.8%). The spared peripapillary retina lined the area of this background hyperautofluorescence centrally, while its outer boundary corresponded to the outer extent of retinal flecks (Figs. 5, 6). Only in 4 out of 22 eyes (18.2%), was the area of background hyperautofluorescence limited only to the posterior pole and

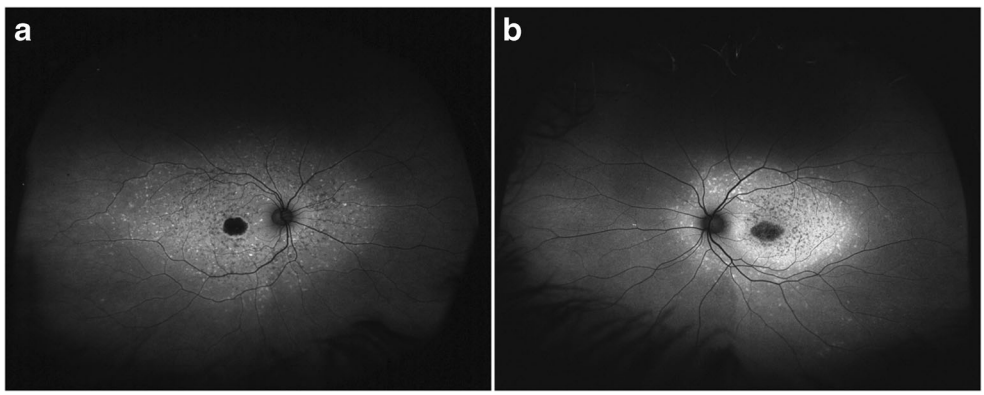

Fig. 3 UWF SWAF of the right eye of (**a**) a 30-year-old male (patient 3) and left eye (**b**) of a 7-year-old male (patient 1) showing retinal flecks extending for a variable distance from the macula. The background increased AF is apparent with vertical line separating the nasal area of hypoautofluorescence from temporal area of hyperautofluorescence. In picture b, the flecks extend to the periphery in the inferotemporal quadrant

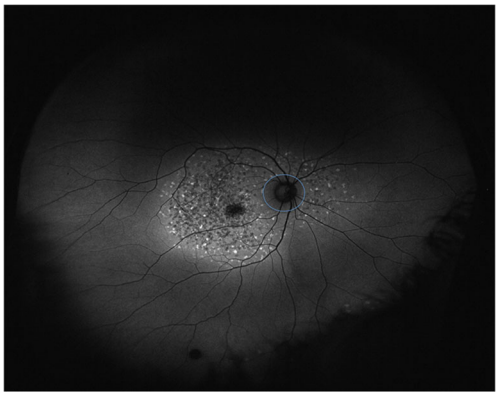

Fig. 4 UWF SWAF of the right eye of 38-year-old male (patient 5) showing a central oval area of hypoautofluorescence and hypo- and hyperautofluorescent flecks sparing the peripapillary retina (*blue circle)*

did not correspond to the extent of the retinal flecks (Fig. 7). This background hyperfluorescence extended inferiorly in all the eyes and had a sharp near-vertical border below the disc. Nasal to this vertical border, it continued as normal background fluorescence of the peripheral retina.

Discussion

This study shows the utility of UWF SWAF imaging in the assessment of LF deposition in STGD1 and highlights several characteristics of A2E (Lipofuscin) deposition in STGD1 that could help us understand the pathogenesis of this condition better.

Earlier microscopic studies of RPE abnormalities in cadaveric eyes with fundus flavimaculatus showed that RPE cells were packed with granular substance that had ultra-structural, autofluorescent and histochemical properties consistent with an abnormal form of lipofuscin and this deposition was maximum in the posterior fundus [15]. The high levels of lipofuscin in RPE were later shown by spectrophotometry in Stargardt disease-fundus flavimaculatus (SD-FF) having an angiographic dark choroid sign [16]. von Ruckmann et al. demonstrated high levels of AF using a confocal scanning laser ophthalmoscope in eyes with SD-FF with or without a dark choroid [17]. Subsequent studies, however, mentioned that AF is not universally high in all SD-FF cases and it could be normal or low [18]. A characteristic background increase in AF was seen in all the eyes in this series, which corresponded with the outer boundary of the retinal flecks. This was apparent because direct comparison with the relatively normal peripheral retina was possible due to UWF images.

The background increased AF was noted to be more on the temporal side with a near-vertical line separating it from the lower AF on the nasal side. Duncker et al. [19] demonstrated a similar pattern of inferonasal AF in eyes with Stargardt macular dystrophy. Duncker et al. also demonstrated the similar pattern of inferonasal AF in healthy eyes but the contrast was enhanced in eyes with STGD1 [20]. The authors hypothesized that this

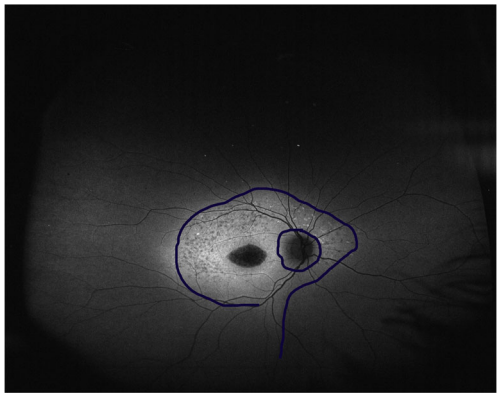

Fig. 6 UWF SWAF of the right eye of 13-year-old male (patient 7) showing increased background AF. *Blue lines* mark the boundaries of increased background AF corresponding to the distribution of retinal flecks. An infero-nasally vertical line is seen separating the areas of hypo- and hyperautofluorescence

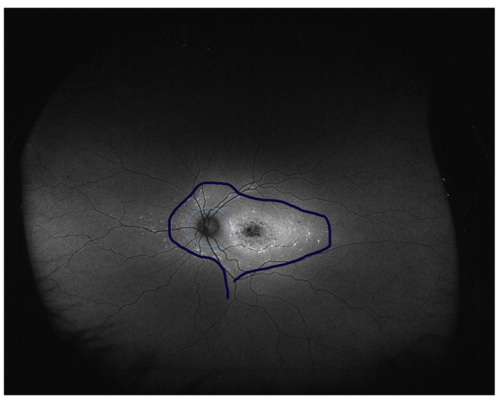

Fig. 5 UWF SWAF of the left eye (**a**) of 23-year-old female (patient 9) showing that all the flecks are hyperautofluorescent. The *light blue circle* marks the peripapillary spare area and inner boundary of background increased AF. The *dark blue line* marks the area of increased background AF corresponding to the distribution of retinal flecks

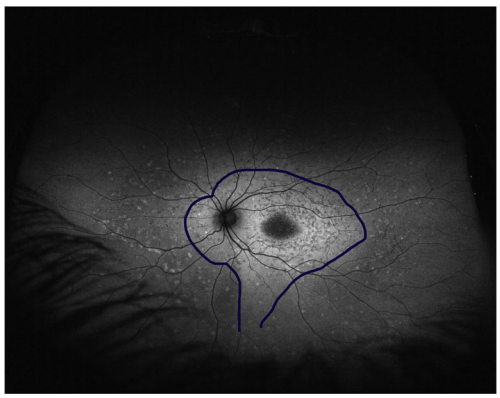

Fig. 7 UWF SWAF of the left eye (**a**) of 18-year-old female (patient 6) showing widespread flecks. The boundary of background increased AF (*blue line*) is smaller than the extent of flecks. Infero-nasally vertical line is seen separating the areas of hypo- and hyperautofluorescence

vertical line may correspond to the embryonic optic fissure. In the present study, the vertical line corresponding to the AF difference was prominently seen but could not be traced to the periphery of the retina. Retrospective reviewing of UWF SWAF images in healthy subjects in our database revealed that this boundary was much less conspicuous in healthy eyes. Though the exact significance of this boundary is difficult to comment on, two patients (four eyes) in this series had flecks extending more peripherally in the inferotemporal area only. Since the background hyperautofluorescence is seen to correlate with retinal flecks, inferotemporal hyperautofluorescence may indicate the inferotemporal retina as a preferential peripheral site of future flecks.

The retinal flecks were both hypofluorescent and hyperautofluorescent as documented in the previous studies [20]. The eyes with posteriorly limited disease had only hyperfluorescent flecks. But the eyes with more extensive flecks had posterior flecks, which were hypofluorescent while more anteriorly placed flecks were hyperfluorescent. This indicates that the newly formed flecks are hyperfluorescent and they appear first at the posterior pole. As they age, they fade away and a hypoautofluorescent area is seen near them. The new flecks appear more peripherally. Typical peripapillary sparing was seen as well [21]. UWF SWAF images additionally revealed that retinal flecks might extend to the retinal periphery as well (Figs. 2b, 3b, 7).

Klufas et al. recently described UWF SWAF in molecularly confirmed cases of Stargardt disease and concluded that peripheral changes were seen in a majority of the patients in Stargardt disease [22]. Depending on the presence or absence of peripheral changes, they classified the AF patterns in to three types. However, they did not mention about the novel SWAF findings described in this study.

Lastly, UWF imaging (Optos Tx200) is an excellent tool to provide quick, high-quality CSLO-based SWAF images without much patient cooperation. Various montaging techniques can be used to increase the imaged area. This is, however, quite cumbersome, especially in conditions with poor central vision and fixation like STGD1. Acquiring multiple images of the same eye for making a montage can be a source of significant discomfort to the patient as well.

There are several limitations of this study. The sample size is relatively small and molecular diagnosis was not made. Longitudinal studies with larger sample size would provide more insight into the STGD1.

To conclude, UWF AF is a useful tool for the assessment of patients with Stargardt macular dystrophy even though it is considered a disorder of posterior fundus. UWF AF demonstrates clearly and for the first time the striking coincidence of a well-defined border of elevated background fluorescence with the farthest extent of hyperautofluorescent flecks. These findings deserve further investigation for insight on the STGD1 disease mechanism.

Compliance with ethical standards

Funding No funding was received for this research.

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institution and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent For this type of study, formal consent is not required.

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Disclosure None.

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