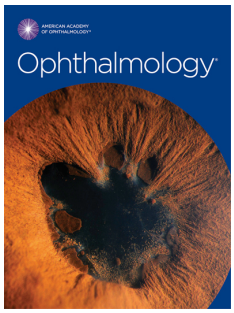
Journal Pre-proof



Prognostication in Stargardt disease using Fundus Autofluorescence: Improving Patient Care

Malena Daich Varela, Yannik Laich, Shaima Awadh Hashem, Omar A. Mahroo, Andrew R. Webster, Michel Michaelides

PII: S0161-6420(23)00428-1

DOI: https://doi.org/10.1016/j.ophtha.2023.06.010

Reference: OPHTHA 12441

To appear in: *Ophthalmology*

Received Date: 6 March 2023

Revised Date: 22 May 2023

Accepted Date: 6 June 2023

Please cite this article as: Varela MD, Laich Y, Hashem SA, Mahroo OA, Webster AR, Michaelides M, Prognostication in Stargardt disease using Fundus Autofluorescence: Improving Patient Care, *Ophthalmology* (2023), doi: https://doi.org/10.1016/j.ophtha.2023.06.010.

Prognostication in Stargardt disease using Fundus Autofluorescence: Improving Patient Care

Malena Daich Varela,[1,2] Yannik Laich,[1,2] Shaima Awadh Hashem,[1,2] Omar A. Mahroo,[1,2] Andrew R. Webster,[1,2] Michel Michaelides[1,2*]

[1] Moorfields Eye Hospital, London, United Kingdom.

[2] UCL Institute of Ophthalmology, University College London, London, United Kingdom.

*Corresponding Author:

Professor Michel Michaelides

UCL Institute of Ophthalmology

11-43 Bath Street, London, EC1V 9EL, UK

michel.michaelides@ucl.ac.uk

Meeting presentations: This work has not been presented in any meeting or conference.

Financial Support: Supported by grants from the National Institute for Health Research Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, and The Wellcome Trust (099173/Z/12/Z and 206619/Z/17/Z).

Conflict of interest: The authors alone are responsible for the content and writing of this article. MM consults for MeiraGTx Ltd.

Running head: Prognostication in STGD using FAF

Keywords: Stargardt; autofluorescence; electroretinogram; genetics; inherited; retina

Abstract

Purpose: To explore fundus autofluorescence (FAF) imaging as an alternative to electroretinogram (ERG), as a non-invasive, quick, and readily interpretable method to predict disease progression in Stargardt disease (STGD).

Design: Retrospective case series of patients who attended Moorfields Eye Hospital (London, UK).

Subjects: Patients with STGD who met the following criteria were included: (i) biallelic disease-causing variants in *ABCA4*, (ii) ERG testing performed inhouse with an unequivocal ERG group classification, and (iii) ultra-widefield (UWF) FAF imaging performed up to 2 years before or after the ERG.

Methods: Patients were divided into three ERG groups based on retinal function and three FAF groups according to the extent of the hypoautofluorescence and their retinal background appearance. FAF imaging of 30 and 55° were also subsequently reviewed.

Main outcome measures: ERG/FAF concordance and its association with baseline visual acuity and genetics.

Results: 234 patients were included in the cohort. 170 patients (73%) had the same ERG and FAF group, 33 (14%) had a milder FAF than ERG group, and 31 (13%) had a more severe FAF than ERG group. Children under the age of 10 (n=23) had the lowest ERG/FAF concordance, 57% (9 out of the 10 with discordant ERG/FAF had milder FAF than ERG), and adults with adult onset had the highest (80%). Missense genotypes were more commonly seen in the mildest phenotypes. In 97% and 98% of the cases, respectively, 30° and 55° FAF imaging matched with the group defined by UWF FAF.

Conclusions: We demonstrate that FAF imaging is an effective modality to determine the extent of retinal involvement and thereby inform prognostication, by comparing FAF to the current gold standard of ERG testing to determine retinal involvement and thereby prognosis. In 80% of patients in our large molecularly proven cohort we were able to predict if the disease was confined to the macula or also affected the peripheral retina. Children assessed at a young age, with at least one null variant, early disease onset, and/or poor initial VA may have wider retinal involvement than predicted by FAF alone and/or progress to a more severe FAF phenotype over time.

Introduction

Stargardt disease (STGD, MIM 248200) is the most common inherited retinal dystrophy (IRD) worldwide, with an estimated prevalence of 1 in 6578 individuals.[1–3] STGD was first described over a century ago, and occurs due to biallelic disease-causing variants in *ABCA4*, with more than 1500 pathogenic variants reported to date.[4,5] *ABCA4* encodes a transmembrane protein located in photoreceptor disks, responsible for translocating all-trans-retinal and its by-products from inside the outer segment disks to the photoreceptor cytoplasm.[6] It is inherited in an autosomal recessive pattern, however, due to up to 10% of the population carrying pathogenic variants in *ABCA4*, pseudodominance can also occur.[7]

STGD has a highly variable phenotype, with an age of onset ranging from under 10 years of age to the sixties, with incidence peaking in childhood, early adulthood and, less frequently, late adulthood.[8] The most common visual complaints are central vision loss, delayed dark adaptation and pericentral scotomas, and patients often become severely visually impaired 5 to 11 years after symptom onset.[9–11]

Retinal examination is typically characterized by macular atrophy and pisciform yellow deposits in the perimacula.[8] Comprehensive investigations are important for early accurate clinical diagnosis and monitoring, including fundus autofluorescence (FAF) imaging, spectral-domain optical coherence tomography (SD-OCT), and electrophysiological assessment.[12] Several clinical classifications have been established to help assess disease severity and correlate with genotype. FAF-based categorization typically consists of three groups: the first with circumscribed decreased

AF at the fovea and a homogeneous background; the second with decreased AF at the macula and a heterogeneous background; and the third with multiple areas of decreased AF at or beyond the posterior pole.[13–16] This classification has been previously used in smaller cohorts to correlate the different FAF groups with functional parameters such as best correct visual acuity (BCVA), visual field, and electroretinogram (ERG) findings.[17]

Electrophysiological assessment is particularly helpful in providing better-informed advice on prognosis.[18,19] A classification of three functional phenotypes based on ERG findings is well-established: Group 1 - severe pattern electroretinogram (PERG) abnormality (macular dysfunction) with normal full-field ERGs (ffERG); Group 2 - severe PERG abnormality with additional generalised cone dysfunction on ffERGs; and Group 3 - severe PERG abnormality with additional generalised cone and rod dysfunction on ffERGs.[18,19] A longitudinal ERG study has confirmed the prognostic implications of the aforementioned ERG groups, with Group 1 having the best prognosis; Group 2 having an intermediate or variable prognosis; and Group 3 having the worst prognosis.[18,19] All patients with initial rod ERG involvement demonstrated clinically significant electrophysiological deterioration; whereas, only 20% of patients with normal ffERGs at baseline showed clinically significant progression over time. These findings are supported by the association with genotype grouping (e.g., Group 1 harbouring milder variants, whilst group 3 is associated with a greater prevalence of null variants).[13,20,21] Further analysis demonstrated that those with abnormal ffERG also showed decreased BCVA and higher rate of scotoma and atrophy enlargement than those with normal ffERG.[15,22]

Despite its utility in providing advice on prognosis in STGD, ERG testing is not (readily) available in many centers worldwide, is challenging and time consuming to undertake testing reliably and interpret the results, requires highly trained and dedicated personnel to perform testing and provide reports, has a high intersession variability, and is often long and uncomfortable for patients. In direct contrast, FAF imaging, both widefield and posterior pole imaging, has none of these aforementioned limitations. Herein, FAF is explored as an alternative to ERG, as a non-invasive, quick, cheap and readily interpretable method, available in most ophthalmology departments, to predict disease progression.

Methods

This study was a retrospective case series of patients who attended Moorfields Eye hospital (MEH, London, UK) and were diagnosed with STGD disease. Patients were identified through a clinical database search and had to meet the following criteria to be included in this study: (i) have biallelic disease-causing variants in *ABCA4*, (ii) have ERG testing performed at MEH with an unequivocal report that allowed classification into an ERG group, and (iii) have ultra-widefield (UWF) FAF imaging done up to 2 years before or after the ERG testing. UWF FAF was chosen initially in order to be able to compare peripheral retinal imaging with peripheral retinal function (ffERG), given that the ERG prognostic groups are based on the extent of retinal involvement. Informed consent was obtained from all patients. Ethical approval was provided by the local ethics committee and the study honored the tenets of the Declaration of Helsinki.

Patient electronic healthcare records were reviewed to retrieve relevant clinical information. Age of onset was defined as the age at which visual difficulties were first noticed by the patient. Snellen visual acuities were recorded and converted to LogMAR for the purpose of statistical analysis. Count fingers vision was given a value of LogMAR 1.98, hand motion LogMAR 2.28, light perception LogMAR 2.7, and no light perception LogMAR 3.0, respectively.[23,24] When testing associations between groups and visual acuity, only the right eye was considered to avoid clustering effect. Patients were categorized using the World Health Organization (WHO) visual impairment criteria, that defines no or mild visual impairment as BCVA $\leq$ 0.48 (6/18, 20/60), moderate impairment as BCVA > 0.48 and $\leq$ 1.0 (6/60, 20/200), severe as BCVA >1.0 and $\leq$ 1.3 (3/60, 20/400), and blindness as BCVA > 1.3.

UWF (green) FAF photography was taken with Optos (Optos PLC, Dunfermline, UK). A subset of patients also had 30° and 55° (blue) FAF imaging (Heidelberg Spectralis, Heidelberg Engineering, Inc., Heidelberg, Germany). Based on previous work,[13–15] individuals were classified into three FAF groups: group 1 corresponds to an area of hypoAF at the fovea and a homogeneous background; group 2 is characterized as an area(s) of hypoAF at the macula and a heterogeneous background; and group 3 is represented by an area(s) of definitely decreased AF (DDAF) at the posterior pole, extending beyond the vascular arcades, and a heterogeneous background (**Figure 1**).

Both pattern and full-field ERG testing were performed in all cases to determine the ERG group. Testing was done incorporating the International Society for Clinical Electrophysiology of Vision (ISCEV) standards.[25,26] ERG groups correspond to those

described by Lois et al.[18] Patients with ERG reports that were unclear/not definitive regarding ERG group were excluded (n=14).

Genetic testing was performed using panel-based targeted next generation sequencing (NGS), whole exome sequencing, or whole genome sequencing. Where appropriate and when available, blood samples were taken from parents or siblings to confirm segregation of proposed variants. Genotype grouping was performed according to the presence of one or more null variants, that were assumed to result in a loss of function (nonsense, frameshift, splice site alteration, and exon deletion). Deep-intronic variants largely result in protein truncations, hence they were also considered as null.[27]

GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis. The threshold of significance was set at $p < 0.05$. T-tests were used to assess parametric variables, chi-square to test the relationship between categorical variables, and odds ratio to prove the association between two categories. Welch's t-test variation was employed when the sample sizes were significantly different.

Results

The final cohort that met all eligibility criteria consisted of 234 patients who had ERG and FAF testing between 2012 and 2022 (median 2018), at 33.7 $\pm$ 17.1 years old (median 32, range 6 - 83) (**Supplementary Table**). Forty-three patients (18%) had their assessments as children (<17 years of age), and 191 (82%) as adults. One hundred

and forty-four (62%) had follow-up UWF FAF imaging and 43 (18%) had a previous ERG assessment.

Considering ERG groups, 145 patients (62%) belonged to group 1 (ERG1), 23 (10%) to group 2 (ERG2), and 66 (28%) to group 3 (ERG3) (**Table 1** and **Figure 2**). Assessing UWF FAF, 126 (54%) belonged to group 1 (FAF1), 69 (29%) to group 2 (FAF2), and 39 (17%) to group 3 (FAF3) (**Table 1** and **Figure 2**). There were no significant differences in the age of the patients at the time of the ERG and FAF between ERG groups (p 0.49 – 0.96), however patients in FAF3 were significantly older than those in FAF1 (<0.0001) and FAF2 (0.02). One hundred and seventy patients (73%) had the same ERG and FAF group, 33 (14%) had a milder FAF than ERG group, and 31 (13%) had a more severe FAF than ERG group. It is of note that those with milder FAF than ERG were significantly younger at the time of the assessment than those with worse FAF than ERG and those with the same FAF/ERG grouping (mean age 19.9 years old versus 34.4 and 31.9, p 0.001).

If ERG groups 2 and 3 are combined to compare to 1, thereby to compare generalized retinal involvement versus isolated macular disease respectively, 82% had matching ERG and FAF pattern; 78 out of 89 (88%) patients in ERG group 2&3, and 114 out of 145 (79%) patients in ERG group 1.

There was a significant association between the three ERG and FAF groups (p <0.0001). Patients in ERG1 had 51 times the odds of being in FAF1 compared to those with ERG3, and 18 times the odds compared to patients with ERG2. Patients in ERG2 had 18 times the odds to be in FAF2 compared to ERG1, and 10 times the odds of

someone in ERG3. Patients in ERG3 had 195 times the odds to be in FAF3 compared to those in ERG1, and 31 times the odds compared to ERG2.

Age and disease onset

Age of onset was available for 206 patients (88%), with a mean of 21.9 ± 14.9 years old (median 18, range 4 – 68). Forty-three patients were pediatric (21%) with childhood-onset, 58 were adults (28%) who were symptomatic before age 17, and 105 were adults (51%) with symptoms onset ≥ 17 years old.

The most frequent groups in children (n=43) were FAF1 (70%), ERG1 (56%), ERG3 (33%), and FAF2 (23%). In adults with childhood-onset, the most common groups were ERG3 (48%), ERG1 (41%), FAF2 (36%), and FAF3 (33%). Lastly, for adults with adulthood-onset the most common findings were ERG1 (81%), FAF1 (66%), FAF2 (28%), and ERG3 (11%).

Children in ERG3 were significantly younger compared to ERG1 (9 versus 11 years old, p 0.04). Children under the age of 10 (n=23) had the lowest ERG/FAF match, 57% (9 out of the10 with discordant ERG/FAF had milder FAF than ERG), and adults with adult onset had the highest, 80% match. The highest mismatch was in ERG3 in children (4.6 times less FAF3 than expected), followed by ERG2 in adults (3.6 times more FAF2 than expected).

Patients in ERG3 had a significantly earlier onset than those in ERG1 (14.6 versus 24.5, p <0.0001), and patients in FAF3 also had a significantly earlier onset when compared

to FAF1 (14.8 versus 22.5, p 0.001), and FAF2 (14.8 versus 24.2, p 0.003). Those with milder FAF than ERG group also had significantly earlier onset compared to those with the same ERG/FAF grouping and those with a worse ERG than FAF group (13.9 versus 22.1 and 28.9, 0.002 and 0.006). This pattern suggests that this discrepancy between FAF and ERG can be a potential feature of childhood-onset disease, where functional impairment detectable by ERG precedes structural loss detectable by FAF.

ERG group 1

Of the 145 patients in ERG1, 114 (79%) were in FAF1, 30 (21%) in FAF2, and 1 (1%) in FAF3; with an overall ERG/FAF match of 79%. Twenty-four (17%) were paediatric patients, 24 (17%) were adults with childhood onset, and 85 (59%) were adults with adult onset. There were no significant differences in age of onset (p 0.18) or age at the assessment (p 0.07) between the matching (ERG1 & FAF1) and discordant groups. No differences were found regarding genotype, with 52% of the discordant group having at least one null variant, versus 49% of the matching group; and 48% of the discordant group having missense genotypes versus 50% of the matching group.

Twenty-one had a previous ERG assessment, 9 $\pm$ 4.6 (1-17) years before, 20 of these reported group 1 and one reported group 2, 10 years before the assessment included in the study. Ninety-two individuals had follow-up FAF after 3.6 $\pm$ 1.8 years (1-10), and 7 (8%) progressed to a more severe FAF group over time; 4 of the latter being children under the age of 10 at baseline visit for this study.

204

205 ***ERG group 2***

206 Among the 23 individuals in ERG2, 19 (83%) were in FAF2 and 4 (17%) in FAF1; with
207 an ERG/FAF match of 82%. Three of the 4 discordant patients had their assessments
208 under the age of 10. The remaining adult stayed in FAF group 1 until his latest follow
209 up, 6 years after the ERG. Five (22%) were paediatric patients, 6 (26%) were adults
210 with childhood onset, and 8 (35%) were adults with adult onset.

211 Thirteen had follow up FAF after 4 $\pm$ 2 years (1-7) and 2 adults progressed to a more
212 severe FAF group. Five had a previous ERG assessment (5 to 16 years before), with no
213 change between groups.

214

215 ***ERG group 3***

216 Of the 66 individuals in ERG3, 7 (11%) were in FAF1, 21 (32%) in FAF2, and 38 (58%)
217 in FAF3; with an ERG/FAF match of 58%. Six of the 7 patients in FAF1 had their
218 assessments under the age of 10, and 2 of them had follow up imaging at 12 and 14
219 years old, showing progression to FAF 2 and 3, respectively. Fourteen (21%) were
220 paediatric patients, 28 (42%) were adults with childhood onset, and 12 (18%) were
221 adults with adult onset.

222 Thirty-nine had follow up FAF after 3.5 $\pm$ 1.8 years (1-7) and 5 patients progressed to a
223 more severe FAF group. Fifteen patients had a previous ERG assessment (2 to 17

years before), 10 remained in the same group, 4 changed from group 2 to 3 (1 child and 3 adults), one adult from group 1 to 3. One adult had a second ERG 3 years after the ERG assessment used for this study and changed from ERG3 to ERG2 (and belonged in FAF2).

Genetics

Dividing the cohort into FAF groups, there was a significantly higher proportion of missense genotypes versus at least one null in the FAF1 and 2 groups, compared to FAF3 (p 0.009 and 0.005). Patients in FAF1 and FAF2 had 3 and 4 times the odds of having a missense genotype compared to FAF3, respectively. Considering ERG groups, there were significantly more missense genotypes versus two or more null in ERG1 than ERG2 (0.02) and ERG3 (0.003). Patients in ERG1 had nearly twice (1.84) the odds of having a missense genotype compared to patients in ERG2 and ERG3.

Regarding genotypes, there were no significant differences in the percentage of missense and null variants between the matching FAF/ERG, milder FAF than ERG, and worse FAF than ERG groups (p 0.15).

The mild variant p.Gly1961Glu was primarily seen in patients with matching ERG1 and FAF1 (49 patients), being seen only once in ERG1 and FAF2, once in ERG2, and 3 times in ERG3.[28] The intronic variant c.5461-10T>C (previously associated with a more severe phenotype) was seen in 13 patients in ERG 1, 11 in ERG 3, and 2 in ERG 2.[19]

Baseline visual acuity

Patients in FAF3 had significantly worse initial VA compared to FAF1 (p <0.0001) and FAF2 (p <0.0001). Similarly, ERG3 had significantly worse initial VA compared to ERG1 (p <0.0001) and ERG2 (p 0.005). Focusing on children, those with ERG3 had significantly worse VA compared to ERG1 (p 0.005), despite being younger.

The group with milder FAF than ERG group had significantly worse initial VA compared to those with worse FAF than ERG and compared to those with the same ERG and FAF grouping (mean 0.9 versus 0.7 and 0.6, p 0.002 and 0.03). The group of those with milder FAF than ERG group had the smallest proportion of patients with no or mild visual impairment (15% versus 42 and 52% in those with the matching ERG/FAF group and more severe FAF than ERG, respectively), and consequently the largest proportion of patients with blindness (9% versus 8 and 1%), severe (12% versus 6 and 0%) and moderate visual impairment (64% versus 44 and 45%).

30- and 55-degree autofluorescence

One hundred and forty-eight patients (63%) had both 30° and 55° FAF imaging concurrently with UWF FAF, 37 (16%) had only 55° and UWF, 41 (18%) only 30° and UWF, and 8 (3%) had UWF imaging only.

In 97% and 98% of the cases, respectively, 30° and 55° FAF imaging matched with the FAF group defined by UWF FAF. Namely that when compared with UWF groups, FAF

groups 2 and 3 were not fully captured in 6 cases (3%) with 30° imaging, and in 3 (2%) with 55° imaging.

Discussion

This study evaluated the largest cohort of patients from a single tertiary referral centre with molecularly confirmed STGD and concurrent electrophysiological assessment and FAF imaging (both UWF and 30/55-degree). The primary purpose was to assess if FAF imaging could be used to provide reliable information on disease extent and thereby inform prognostication, by comparing it to the current gold standard of ERG testing, and thereby inform patient management. We also explored any potential associations with various clinical and genetic parameters.

ERG and FAF groups were significantly associated, with more than 70% of patients having the same ERG and FAF group. If further simplified into isolated macular versus widespread retinal involvement, more than 80% of patients had matching ERG and FAF pattern. There was a similar likelihood of under and over estimating severity of prognosis with FAF, based on ERG data. A high correlation between ERG and FAF was also previously described in a smaller cohort by Abalem *et al*.[17] More than half of our cohort consisted of adults with adult onset STGD, and belonged in ERG1 and FAF1, which was in keeping with previous reports.[18,29]

Only 10% of the cohort progressed to a more severe FAF phenotype during follow-up; this percentage is smaller than a previous study that analysed fewer patients. Our study

of a larger cohort may be more reflective of STGD behaviour, but differences in cohort characteristics cannot be excluded.[14] Previous reports have also described a progression in ERG groups over time, with 20% of patients in ERG1 and 40% in ERG2 progressing to more severe ERG groups.[19] This was not captured in our cohort, but we found that 21% of the patients in ERG1 had a more severe FAF involvement. One possibility is that generalised ERG involvement (ERG2 and ERG3) may occur in these patients over time, and thereby FAF abnormalities have preceded functional changes in these cases; or that this represents a true disconnect between these evaluations in a minority of patients. On the other hand, we also found that 17% of patients in ERG2 and 43% of ERG3 had a less severe FAF phenotype, and patients in FAF3 were significantly older than those in FAF1 and FAF2. This, in direct contrast, illustrates that functional changes may manifest before structural changes are visible, which would be the most common observation in inherited retinal disease.[30]

Children in ERG2 and ERG3 groups were younger than those in ERG1 and had poorer FAF correlation. This may be due to possible technical difficulties affecting this age group, as well as FAF changes indeed manifesting at an older age (4 out of 7 children progressed to a more severe FAF group after turning 10 years old). Childhood-onset STGD has been reported to be characterised by a greater rate of progression than adult onset.[31–34] FAF 'catching up' with ERG testing, with a high rate of atrophy development/enlargement, would thereby be in keeping.[14]

Patients with milder FAF severity than ERG, were significantly younger at the time of assessment, had earlier onset, and the largest baseline proportion of visually impaired patients when compared to those with the same and worse ERG/FAF. Initial VA has

been reported to have an impact on the rate of VA loss, with better baseline VA correlating with slowest change over time.[35,36] Taken together, we observed that young patients in the FAF1 group, with at least one null variant, with early disease onset and poor initial VA, often develop wider retinal involvement and progress to a more severe phenotype over time.

Missense genotypes were seen more often in milder phenotypes, as previously reported.[14,27] The variant p.Gly1961Glu was the most common amongst patients with the least severe phenotype (ERG1 and FAF1), agreeing with previous reports that locate it at the milder end of the disease spectrum.[37]

Even though peripheral retinal changes can occur in STGD and may change the FAF group in a minority of patients, we found that in 97% and 98% of patients, 30° and 55° FAF imaging matched with the FAF group defined by UWF FAF. This supports the potential use of Heidelberg FAF imaging not only for diagnosis/characterization of STGD, but moreover for prognostication and counselling.

Several research efforts are ongoing currently, with multiple therapeutic approaches under development; for example drugs targeting lipofuscin formation, antisense oligonucleotides that rescue splice defects, gene supplementation, and stem-cell-derived retinal pigment epithelium transplantation.[8,27] FAF imaging represents a faster, cheaper and widely available method of characterizing and stratifying patients which can be useful when assessing a patient's suitability for a clinical trial and targeting patients most likely to respond.

Electrophysiological testing is associated with notable inter-session variability and low repeatability, which is why it is rarely used in clinical practice to monitor disease progression or in clinical trials to determine treatment response.[38–40] In contrast, FAF imaging has proven to be a useful clinical monitoring tool, providing various quantitative parameters to assess longitudinally (including area of DDAF and questionably DAF, and their respective rate of change), and also functioning as an approved outcome measure for interventional clinical trials.[41,42] UWF FAF imaging does not entail discomfort for the patients, not even needing dilating drops to acquire useful images. Heidelberg FAF ideally needs dilation and testing can be uncomfortable. However, current techniques with reduced illuminance have showed good concordance with conventional FAF, thereby potentially avoiding patient discomfort.[43] Current FAF limitations include the potential benefit of a standardized approach to quantify the spatial distribution of AF (i.e., quantitative FAF), not directly imaging retinal architecture (compared to OCT), and lack of availability of widefield FAF imaging devices.

This study limitations include its retrospective nature and data being acquired in a large-scale clinical context, not suitable for AF quantification. These are largely offset by the large number of genetically confirmed individuals and the thorough multimodal evaluation.

In conclusion, UWF and 30°/55° FAF imaging are excellent instruments from which we can infer to what extent the patient's retina is affected. In the majority of patients, particularly adults, this imaging will enable us to accurately advise the patient regarding their disease prognosis, primarily in terms of whether it will remain confined to the macula or progressively affect the peripheral retina. Patients assessed in early

childhood (especially 10 years and younger), that harbour at least one null variant and/or poor initial VA may have wider retinal involvement or progress to a more severe phenotype over time, than suggested by their baseline FAF imaging; and therefore, careful counselling is required and ideally where possible ISCEV ERGs, if the most accurate advice on prognosis is desired at the earliest opportunity.

References

1. Rahman N, Georgiou M, Khan KN, Michaelides M. Macular dystrophies: clinical and imaging features, molecular genetics and therapeutic options. *Br J Ophthalmol*. 2020;104(4):451-460. doi:10.1136/bjophthalmol-2019-315086

2. Pontikos N, Arno G, Jurkute N, et al. Genetic Basis of Inherited Retinal Disease in a Molecularly Characterized Cohort of More Than 3000 Families from the United Kingdom. *Ophthalmology*. 2020;127(10):1384-1394. doi:10.1016/j.ophtha.2020.04.008

3. Hanany M, Rivolta C, Sharon D. Worldwide carrier frequency and genetic prevalence of autosomal recessive inherited retinal diseases. *Proc Natl Acad Sci U S A*. 2020;117(5):2710-2716. doi:10.1073/pnas.1913179117

4. Stargardt K. Über familiäre, progressive Degeneration in der Maculagegend des Auges. *Albr von Graefes Arch für Ophthalmol*. 1909;71(3):534-550.

5. Cornelis SS, Runhart EH, Bauwens M, et al. Personalized genetic counseling for Stargardt disease: Offspring risk estimates based on variant severity. *Am J Hum Genet*. 2022;109(3):498-507. doi:https://doi.org/10.1016/j.ajhg.2022.01.008

6. Allikmets R, Singh N, Sun H, et al. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Starqardt macular dystrophy. *Nat Genet*. 1997;15(3):236-246. doi:10.1038/ng0397-236

7. Huckfeldt RM, East JS, Stone EM, Sohn EH. Phenotypic Variation in a Family

With Pseudodominant Stargardt Disease. *JAMA Ophthalmol*. 2016;134(5):580-583. doi:10.1001/jamaophthalmol.2015.5471

8. Tanna P, Strauss RW, Fujinami K, Michaelides M. Stargardt disease: clinical features, molecular genetics, animal models and therapeutic options. *Br J Ophthalmol*. 2017;101(1):25-30.

9. Lambertus S, Lindner M, Bax NM, et al. Progression of Late-Onset Stargardt Disease. *Invest Ophthalmol Vis Sci*. 2016;57(13):5186-5191. doi:10.1167/iovs.16-19833

10. Lambertus S, van Huet RAC, Bax NM, et al. Early-Onset Stargardt Disease: Phenotypic and Genotypic Characteristics. *Ophthalmology*. 2015;122(2):335-344. doi:https://doi.org/10.1016/j.ophtha.2014.08.032

11. Kohli P, Kaur K. *Stargardt Disease*. StatPearls Publishing, Treasure Island (FL); 2022. http://europepmc.org/abstract/MED/36508525

12. Michaelides M, Hunt DM, Moore AT. The genetics of inherited macular dystrophies. *J Med Genet*. 2003;40(9):641 LP - 650. doi:10.1136/jmg.40.9.641

13. Fujinami K, Sergouniotis PI, Davidson AE, et al. The clinical effect of homozygous ABCA4 alleles in 18 patients. *Ophthalmology*. 2013;120(11):2324-2331. doi:10.1016/j.ophtha.2013.04.016

14. Fujinami K, Lois N, Mukherjee R, et al. A Longitudinal Study of Stargardt Disease: Quantitative Assessment of Fundus Autofluorescence, Progression, and

Genotype Correlations. *Invest Ophthalmol Vis Sci*. 2013;54(13):8181-8190. doi:10.1167/iovs.13-12104

15. McBain VA, Townend J, Lois N. Progression of Retinal Pigment Epithelial Atrophy in Stargardt Disease. *Am J Ophthalmol*. 2012;154(1):146-154. doi:https://doi.org/10.1016/j.ajo.2012.01.019

16. Klufas MA, Tsui I, Sadda SR, Hosseini H, Schwartz SD. ULTRAWIDEFIELD AUTOFLUORESENCE IN ABCA4 STARGARDT DISEASE. *Retina*. 2018;38(2):403-415. doi:10.1097/IAE.0000000000001567

17. Abalem MF, Otte B, Andrews C, et al. Peripheral Visual Fields in ABCA4 Stargardt Disease and Correlation With Disease Extent on Ultra-widefield Fundus Autofluorescence. *Am J Ophthalmol*. 2017;184:181-188. doi:10.1016/j.ajo.2017.10.006

18. Lois N, Holder GE, Bunce C, Fitzke FW, Bird AC. Phenotypic Subtypes of Stargardt Macular Dystrophy–Fundus Flavimaculatus. *Arch Ophthalmol*. 2001;119(3):359-369. doi:10.1001/archopht.119.3.359

19. Fujinami K, Lois N, Davidson AE, et al. A longitudinal study of stargardt disease: clinical and electrophysiologic assessment, progression, and genotype correlations. *Am J Ophthalmol*. 2013;155(6):1075-1088.e13. doi:10.1016/j.ajo.2013.01.018

20. Starace V, Battista M, Brambati M, et al. Genotypic and phenotypic factors influencing the rate of progression in ABCA-4-related Stargardt disease. *Expert*

Rev Ophthalmol. 2021;16(2):67-79. doi:10.1080/17469899.2021.1860753

21. Glinton SL, Calcagni A, Lilaonitkul W, et al. Phenotyping of ABCA4 Retinopathy by Machine Learning Analysis of Full-Field Electroretinography. *Transl Vis Sci Technol*. 2022;11(9):34. doi:10.1167/tvst.11.9.34

22. Zahid S, Jayasundera T, Rhoades W, et al. Clinical phenotypes and prognostic full-field electroretinographic findings in Stargardt disease. *Am J Ophthalmol*. 2013;155(3):465-473.e3. doi:10.1016/j.ajo.2012.09.011

23. Lange C, Feltgen N, Junker B, Schulze-Bonsel K, Bach M. Resolving the clinical acuity categories "hand motion" and "counting fingers" using the Freiburg Visual Acuity Test (FrACT). *Graefe's Arch Clin Exp Ophthalmol = Albr von Graefes Arch fur Klin und Exp Ophthalmol*. 2009;247(1):137-142. doi:10.1007/s00417-008-0926-0

24. Day AC, Donachie PHJ, Sparrow JM, Johnston RL. The Royal College of Ophthalmologists' National Ophthalmology Database study of cataract surgery: report 1, visual outcomes and complications. *Eye (Lond)*. 2015;29(4):552-560. doi:10.1038/eye.2015.3

25. Bach M, Brigell MG, Hawlina M, et al. ISCEV standard for clinical pattern electroretinography (PERG): 2012 update. *Doc Ophthalmol*. 2013;126(1):1-7. doi:10.1007/s10633-012-9353-y

26. Robson AG, Frishman LJ, Grigg J, et al. ISCEV Standard for full-field clinical electroretinography (2022 update). *Doc Ophthalmol*. 2022;144(3):165-177.

doi:10.1007/s10633-022-09872-0

27. Khan M, Cremers FPM. ABCA4-associated Stargardt disease. *Klin Monbl Augenheilkd*. 2020;237(03):267-274.

28. Cella W, Greenstein VC, Zernant-Rajang J, et al. G1961E mutant allele in the Stargardt disease gene ABCA4 causes bull's eye maculopathy. *Exp Eye Res*. 2009;89(1):16-24. doi:10.1016/j.exer.2009.02.001

29. Testa F, Rossi S, Sodi A, et al. Correlation between Photoreceptor Layer Integrity and Visual Function in Patients with Stargardt Disease: Implications for Gene Therapy. *Invest Ophthalmol Vis Sci*. 2012;53(8):4409-4415. doi:10.1167/iovs.11-8201

30. Daich Varela M, Georgiou M, Hashem SA, Weleber RG, Michaelides M. Functional evaluation in inherited retinal disease. *Br J Ophthalmol*. Published online November 25, 2021:bjophthalmol-2021-319994. doi:10.1136/bjophthalmol-2021-319994

31. Georgiou M, Kane T, Tanna P, et al. Prospective Cohort Study of Childhood-Onset Stargardt Disease: Fundus Autofluorescence Imaging, Progression, Comparison with Adult-Onset Disease, and Disease Symmetry. *Am J Ophthalmol*. 2020;211:159-175. doi:10.1016/j.ajo.2019.11.008

32. Tanna P, Georgiou M, Aboshiha J, et al. Cross-Sectional and Longitudinal Assessment of Retinal Sensitivity in Patients With Childhood-Onset Stargardt Disease. *Transl Vis Sci Technol*. 2018;7(6):10. doi:10.1167/tvst.7.6.10

33. Tanna P, Georgiou M, Strauss RW, et al. Cross-Sectional and Longitudinal Assessment of the Ellipsoid Zone in Childhood-Onset Stargardt Disease. *Transl Vis Sci Technol*. 2019;8(2):1. doi:10.1167/tvst.8.2.1

34. Fujinami K, Zernant J, Chana RK, et al. Clinical and molecular characteristics of childhood-onset Stargardt disease. *Ophthalmology*. 2015;122(2):326-334. doi:10.1016/j.ophtha.2014.08.012

35. Kong X, Fujinami K, Strauss RW, et al. Visual Acuity Change Over 24 Months and Its Association With Foveal Phenotype and Genotype in Individuals With Stargardt Disease: ProgStar Study Report No. 10. *JAMA Ophthalmol*. 2018;136(8):920-928. doi:10.1001/jamaophthalmol.2018.2198

36. Kong X, Strauss RW, Cideciyan A V, et al. Visual Acuity Change over 12 Months in the Prospective Progression of Atrophy Secondary to Stargardt Disease (ProgStar) Study: ProgStar Report Number 6. *Ophthalmology*. 2017;124(11):1640-1651. doi:10.1016/j.ophtha.2017.04.026

37. Burke TR, Fishman GA, Zernant J, et al. Retinal Phenotypes in Patients Homozygous for the G1961E Mutation in the ABCA4 Gene. *Invest Ophthalmol Vis Sci*. 2012;53(8):4458-4467. doi:10.1167/iovs.11-9166

38. Otto T, Bach M. Retest variability and diurnal effects in the pattern electroretinogram. *Doc Ophthalmol*. 1996;92(4):311-323. doi:10.1007/BF02584085

39. Grover S, Fishman GA, Birch DG, Locke KG, Rosner B. Variability of full-field

electroretinogram responses in subjects without diffuse photoreceptor cell disease. *Ophthalmology*. 2003;110(6):1159-1163. doi:10.1016/S0161-6420(03)00253-7

40. Holopigian K, Snow J, Seiple W, Siegel I. Variability of the pattern electroretinogram. *Doc Ophthalmol*. 1988;70(1):103-115. doi:10.1007/BF00154741

41. Strauss RW, Kong X, Ho A, et al. Progression of Stargardt Disease as Determined by Fundus Autofluorescence Over a 12-Month Period: ProgStar Report No. 11. *JAMA Ophthalmol*. 2019;137(10):1134-1145. doi:10.1001/jamaophthalmol.2019.2885

42. Strauss RW, Muñoz B, Ho A, et al. Incidence of Atrophic Lesions in Stargardt Disease in the Progression of Atrophy Secondary to Stargardt Disease (ProgStar) Study: Report No. 5. *JAMA Ophthalmol*. 2017;135(7):687-695. doi:10.1001/jamaophthalmol.2017.1121

43. Strauss RW, Muñoz B, Jha A, et al. Comparison of Short-Wavelength Reduced-Illuminance and Conventional Autofluorescence Imaging in Stargardt Macular Dystrophy. *Am J Ophthalmol*. 2016;168:269-278. doi:10.1016/j.ajo.2016.06.003

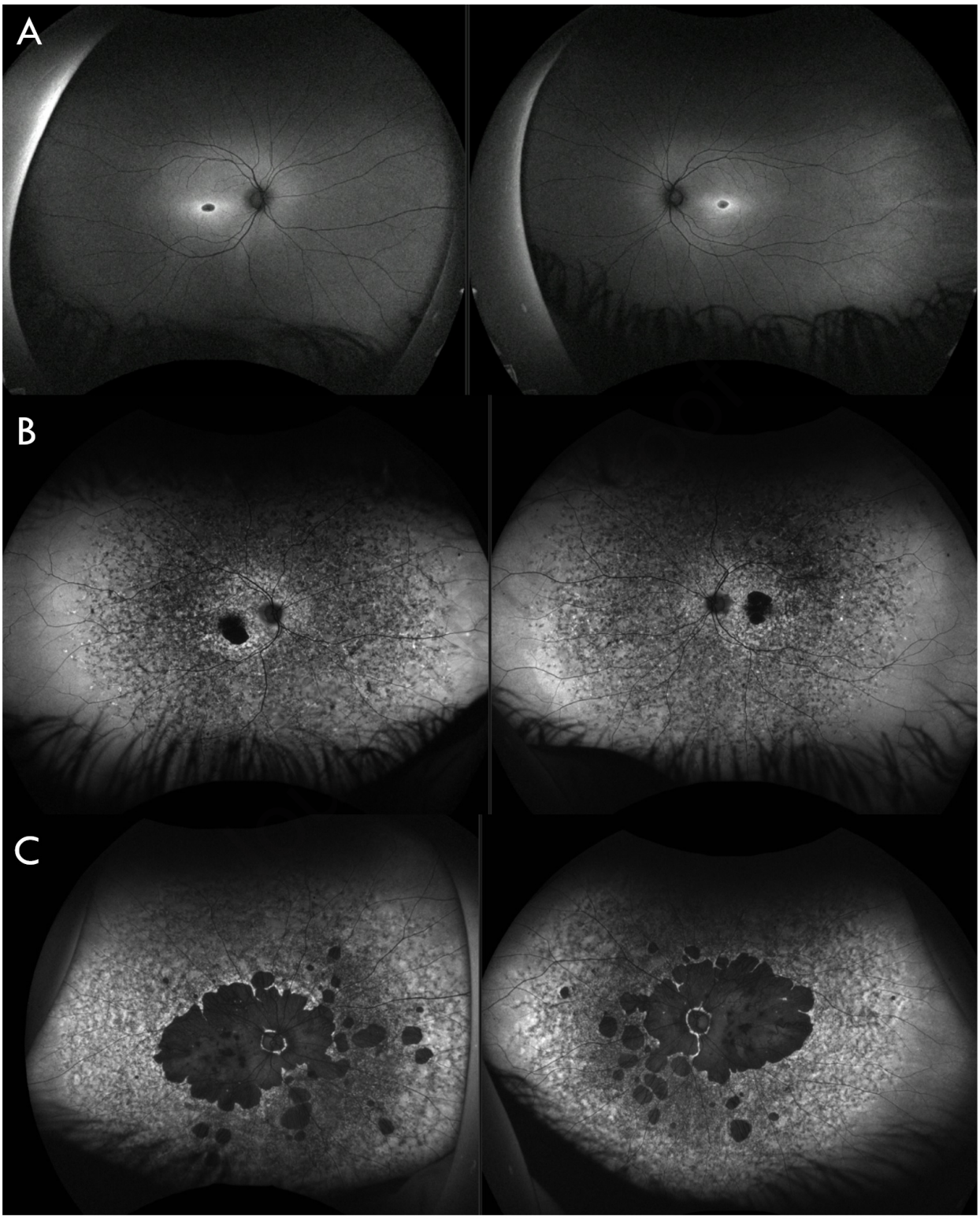
Figure legends

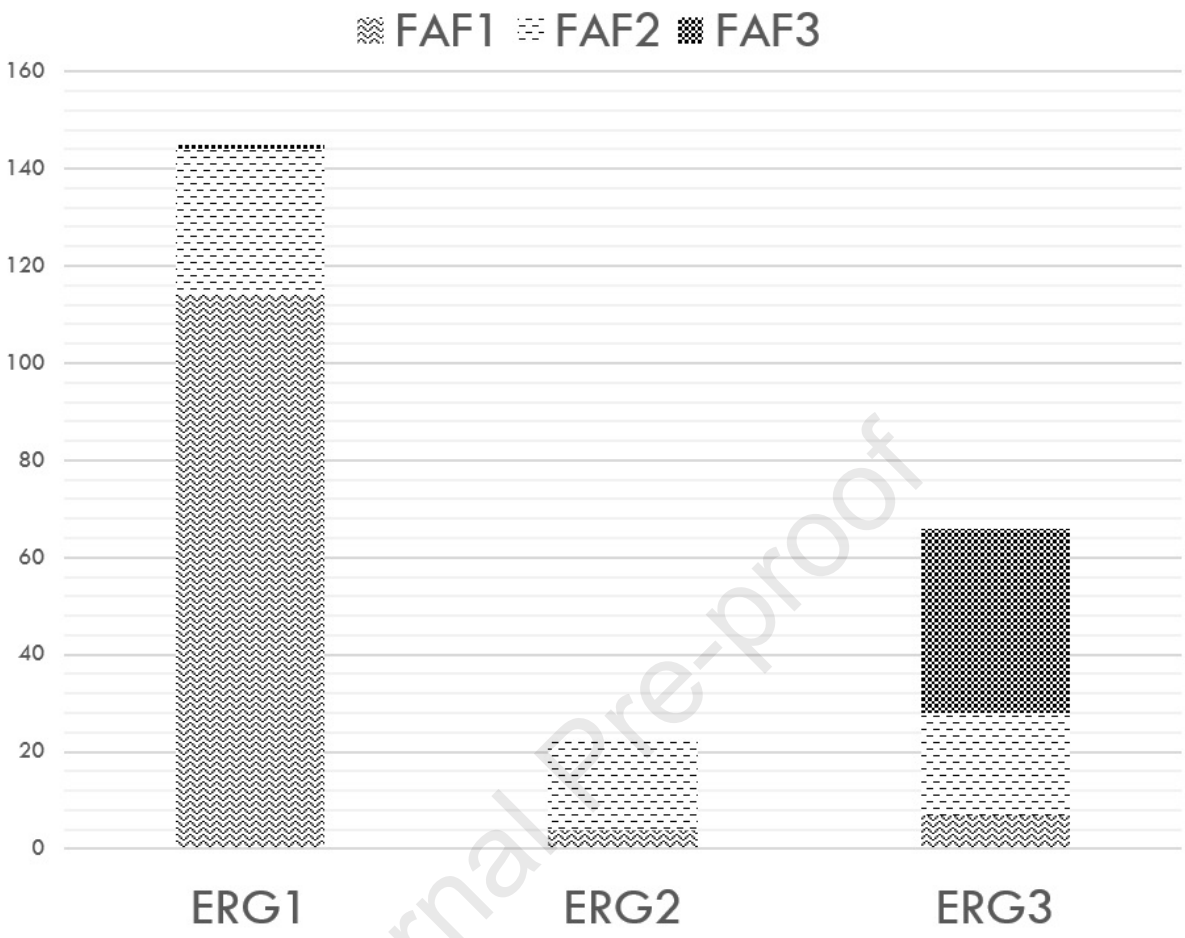
Figure 1: Classification of ultra-widefield fundus autofluorescence (AF) images into three severity groups. A) Group 1 corresponds to an area of hypoAF at the fovea and a homogeneous background; B) Group 2 is characterized by an area(s) of hypoAF at the macula and a heterogeneous background; C) Group 3 is represented by multiple areas of definitely decreased AF at the posterior pole, extending beyond the vascular arcades, and a heterogeneous background.

Figure 2: Electroretinogram (ERG) and fundus autofluorescence (FAF) groups in our cohort. Out of the 234 patients included in total, 145 (62%) had an ERG group 1 (ERG1), 23 (10%) group 2 (ERG2), and 66 (28%) group 3 (ERG3). Of the 145 patients in ERG1, 114 (79%) were in FAF1, 30 (21%) in FAF2, and 1 (1%) in FAF3; with an overall ERG/FAF match of 79%. Among the 23 patients in ERG2, 19 (83%) were in FAF2 and 4 (17%) in FAF1; with an ERG/FAF match of 82%. Of the 66 individuals in ERG3, 7 (11%) were in FAF1, 21 (32%) in FAF2, and 38 (58%) in FAF3; with an ERG/FAF match of 58%.

	FAF1 (n)	FAF2 (n)	FAF3 (n)	Age at assessment (mean ± SD)	Age of onset (mean ± SD)	Children (n)	Adults w/ childhood onset <17 (n)	Adults w/ adult onset ≥17 (n)	Missense genotype (n)	1 null genotype (n)	≥ 2 nulls (n)	Baseline VA (mean ± SD)
ERG1	114	30	1	33.7 ± 16.9	24.5 ± 14.5	24	24	85	72	64	9	0.6 ± 0.4
ERG2	4	19	0	34.8 ± 19.6	24.4 ± 19.1	5	6	8	8	9	6	0.7 ± 0.4
ERG3	7	21	38	33.5 ± 16.3	14.6 ± 11.7	14	28	12	23	30	13	1.1 ± 0.5
FAF1				30.6 ± 16.4	22.5 ± 13.4	30	18	69	61	53	12	0.6 ± 0.4
FAF2				34.7 ± 18.1	24.2 ± 18.3	10	21	29	33	28	8	0.7 ± 0.5
FAF3				42 ± 13.9	14.8 ± 10.1	3	19	7	9	23	7	1.2 ± 0.6

Table 1: Cohort Characteristics. ERG: electroretinogram; FAF: fundus autofluorescence; SD: standard deviation; VA: visual acuity; n: number.


A
B
C



Fundus autofluorescence imaging is an excellent alternative to the electroretinogram, as a non-invasive, quick, and readily interpretable method to predict disease progression in Stargardt disease.