Longitudinal Changes of Fixation Location and Stability within 12 Months in Stargardt Disease: ProgStar Report No. 12

Short title: Longitudinal Changes of Fixation in Stargardt Disease

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Introduction

The most common type of juvenile macular degeneration is Stargardt disease (STGD1; OMIM: 248200).[1, 2] Affected individuals start to develop progressive decline in best-corrected visual acuity (BCVA) often within the first or second decades of life.[3] Currently, no treatment to preserve or restore vision is available for STGD1 patients. New therapeutic approaches, such as pharmacotherapy, gene therapy, stem cell therapy, retinal prostheses, and optogenetics are being developed; some of them are already being investigated in clinical trials.[2, 4, 5] Outcome parameters for STGD1 require special considerations with respect to the centrifugal progression of atrophy in STGD1 (initially affecting the macula before the periphery).[4]

The Progression of Atrophy Secondary to Stargardt disease (ProgStar; NCT01977846) study investigates structural and functional outcome measures in the natural history of STGD1.[6] We recently showed that fundus autofluorescence (FAF) as an anatomical measure may serve as a monitoring tool for clinical trials for STGD1.[7, 8] Best-corrected visual acuity (BCVA), which has been the primary functional outcome measure for most treatment trials for retinal diseases in the past, is not a sensitive parameter for STGD1 and may be reduced early in the course of the disease due to foveal involvement.[7, 9, 10] Fundus-tracking microperimetry can establish structure-function correlation between topographical locations of the macula in STGD1[11] and it can also characterize an eye's fixation behavior.[12] Prior research has shown that fixation stability correlates with reading speed,[13-16] and visual search ability[17] in patients with macular disease. We recently demonstrated the complex relationship of fixation location and stability with BCVA in STGD1[12] but little data on the progression of fixation parameters in STGD1 are available to date.[18]

The purpose of this research is to investigate longitudinal changes of fixation location and stability in a large cohort of molecularly confirmed STGD1 from the ProgStar study. These parameters may prove useful to describe the disease progression.

Methods

Microperimetry testing was performed as part of the Progression of atrophy secondary to Stargardt disease (ProgStar) study, a prospective natural history study on the progression of STGD1 (cohort study). ICH GCP Guidelines, the applicable regulatory requirements, and the current Declaration of Helsinki were followed. All participants provided written informed consent prior to enrollment into the study. ProgStar is in compliance with HIPAA and was approved by the Western Institutional Review Board, the local institutional review boards (IRB) and the Human Research Protection Office of the U.S. Army Medical Research & Materiel Command prior to enrollment of the first patient.

Patient eligibility and exclusion criteria

ProgStar report No. 1[6] describes the design, organization, inclusion and exclusion criteria in detail. Key inclusion criteria relevant to this report include a minimum age (aged ≥ 6 years) and the presence of ≥2 pathogenic mutations in the *ABCA4* gene or 1 pathogenic mutation with a typical STGD1 phenotype. At least one well-demarcated area of atrophy as visualized by FAF with a minimum diameter of 300 μm was required for inclusion and all lesions together had to add to less than or equal to 12 mm^2 (equivalent to no more than 5 disc areas in at least one eye) in the primary study eye. We also only included eyes with clear ocular media. BCVA had to be 20 ETDRS letters (20/400 Snellen equivalent) or better. The patient had to be able to cooperate in performing the examinations.

Data collection and management

Visual acuity information, demographic and clinical exam data were entered into REDCap and checked for completeness and consistency by the data-coordinating center (DCC).[19] The central reading center was the Doheny Imaging Reading Center (DIRC) at the Doheny Eye Institute. The coordinating and data management center was the Dana Center for Preventive Ophthalmology, Wilmer Eye Institute, Johns Hopkins University School of Medicine and Johns

Hopkins Bloomberg School of Public Health. The Nidek MP-1 was used for all microperimetry tests according to the study protocol and all machines were certified by the DIRC.[20]

The quality and completeness of the submitted examinations was assessed by the DIRC and in case of poor quality or missing data the centers were notified. Adjudication processes were applied if the two DIRC-certified graders had discordant gradings and a final assessment was given by a DIRC investigator. All processed data were transferred electronically to the DCC.

Microperimetry

Microperimetry and fixation measures were performed using the Nidek MP-1 microperimeter (Navis Software 1.7.0 or higher; Nidek Technologies Srl) using a modified Humphrey 10-2 grid as described in ProgStar Report No. 1.[6] Dynamic quantification of fixation was performed during the sensitivity test. Fixation was also determined during a separate fixation task when the patients were asked to fixate their gaze steadily on the center of a red single cross on a white, monochromatic background. The extension of this cross was set as 2°, the thickness was 1° as default but both could be increased if necessary. All tests were performed monocularly with the contralateral eye patched. The duration for the fixation test was roughly 30 seconds after the patient had located the red cross. At a frequency of 25 Hz, real-time fundus images were acquired and the locations of fixation were registered for each image. In order to reduce effects of learning, patients who never had an MP-1 exam before and for whom no scotopic examination was planned underwent a training exam before doing microperimetry. This training exam employed a different test pattern that included 8 test locations (white Goldmann III stimuli on a white background of 200 ms duration; 4-2 automatic strategy while fixating a red single cross of at least two degrees extension and one degree thickness).

Description of the PRL

The PRL was defined as the distance in degrees between the center of gravity of all recorded fixation events and the foveal center. A concentric grid was automatically centered on the center

of gravity of all recorded fixation points using Nidek's NAVIS software (Figure 1, Navis Software 1.7.0 or higher; Nidek Technologies Srl). Two graders at the central reading center manually determined the location of the anatomic fovea. For difficult cases with foveal atrophy, the graders looked for the point of maximal inner retinal layer convergence on optical coherence tomography and used the adjoining B-scans immediately superior and inferior to the approximate the foveal center to determine this center as precisely as possible. In a preliminary subgroup analysis, 45 eyes were randomly selected and the PRL distance from the fovea was determined for the full macular sensitivity test and for the separate fixation exam using the described methods. The PRL eccentricity was not significantly different (ρ_{RPL}= 0.87; p<0.001; N= 45). We used fixation recordings from the macular sensitivity test to determine the PRL because this examination was available in a larger number of patients.

Description of the BCEA

The raw data containing the coordinates of each fixation event during the separate fixation task were extracted and the BCEA (Bivariate Contour Ellipse Area) was calculated using Microsoft Excel (Redmond, WA). For this analysis, we used data from a separate fixation task as in most of the available literature on fixation stability. In this study, we calculated the global BCEA, which encompasses 1 standard deviation (68.3 %- BCEA) of all fixation points (Figure 1, left). The following equation was used to describe fixation stability:

$$BCEA = 2k\pi\,\sigma_H\sigma_V\,(1 - \rho^2)^{\frac{1}{2}}.$$

σ_H and σ_V are the standard deviations of the horizontal and vertical eye movements, ρ is the Pearson product-moment correlation coefficient of eye movements in the horizontal and vertical meridian. k is dependent on the chosen probability area:

$$P = 1 - e^{-k}$$

And e is the base of the natural logarithm. For a probability of 68.3 %, k must be about 1.15 for the equation to be true.

Statistical Analyses

Outcome measures for this manuscript include the eccentricity of the preferred retinal locus (PRL) from the anatomical fovea in degrees and the BCEA in deg^2. Linear models were used to estimate the association between the one-year change in PRL eccentricity and the one-year change in BCEA, to account for the correlation between eyes of the same subject. Standard errors were corrected using the generalized estimated equation (GEE) approach. Linear mixed models with time as the independent variable were used to estimate the yearly change for each outcome.

Results

At baseline, MP-1 was graded for a total of 238 patients (444 eyes). The PRL was determined for 396 eyes and was ungradable for 48 eyes (12 eyes with the test terminated before completion, 26 with missing color fundus images, 3 eyes with multiple PRLs, 7 with other problems). The BCEA was determined for 427 eyes at baseline and 17 eyes have missing values (13 without a fixation test performed, 3 eyes with technical problems, and 1 eye with another reason). At the month 12 visit, MP-1 was graded for 218 patients (408 eyes). For the month 12 visit, the PRL was determined for 392 eyes and was ungradable for 16 eyes (6 eyes with the test terminated before completion, 3 with missing color fundus images, 6 where the grader was unable to determine the foveal center, 1 eye with other problems). The BCEA was determined for 344 eyes at baseline with 64 ungradable BCEAs (fixation exam not performed in 60 eyes and 4 eyes with technical problems). Both the baseline and month 12 visits were gradable for the PRL in 79 % of eye and for the BCEA in 74 % of eyes. The complete enrollment count is detailed in supplement table 1 (Supplemental Material at AJO.com).

Table 1 shows the demographic features of the cohort at baseline. Out of a total of 238 patients at baseline, 105 were male (44 %), 203 were white (85 %) and all had a molecularly confirmed diagnosis of STGD1. The median age of the participant cohort was 32 years (mean± SD, 33.8± 15.2years; range, 7- 69 years). Out of 238 patients, 100 (42 %) had first symptoms of macular disease at an age of 18 years or earlier and 121 patients (51 %) developed symptoms later. Only 2 participants (1 %) were diagnosed with STGD1 without symptoms and 15 patients (6 %) did not recall the beginning of their symptoms. Overall, there were 444 eligible eyes, 206 (86.6%) participants contributed with 2 eyes, and 322 (13.4%) participants with one eye. Median BCVA, evaluated in the 444 eyes at baseline, was 42 ETDRS letters (mean, 46.2 ± 16.3 ETDRS letters; range, 20-88 letters; the lower limit of 20 ETDRS letters for the BCVA is based on the inclusion criteria of the ProgStar study).

The distance of the PRL, which is defined as the gravitational center of all fixation points from the fovea, was graded in 396 eyes at baseline, in 386 eyes at 6 months and in 392 eyes at 12 months. Data from the separate fixation test to evaluate the stability of fixation are available for 412 eyes at baseline, for 336 eyes at 6 months, and for 333 eyes at 12 months. The measurements for the eccentricity of the PRL and the BCEA for the exams at baseline and at 6 and 12 months of follow-up are presented in table 2. After 12 months of follow-up, the change in the eccentricity of the PRL from the anatomical fovea was -0.0014 deg (95 % CI, - 0.27deg - 0.27 deg; p = 0.99). The deterioration in the stability of fixation as expressed by a larger BCEA encompassing 1 SD of all fixation points was 1.21 deg^2 (95 % CI, -1.23 deg^2, 3.65 deg^2; p = 0.33). The estimates of the yearly rate of change of fixation parameters are presented in table 3. One degree of additional PRL eccentricity after 12 months was associated with an increase of 0.89 deg^2 in BCEA after 12 months but this correlation did not reach statistical significance (p = 0.14, N= 276, Figure 2).

There were individual cases where the fixation parameters delivered interesting information about an eye's visual function that could not be captured with other imaging or functional equipment. Figure 3 shows the example of an eye where fixation first turned to a more central location (from 4.5 deg to 3.0 deg distance from the fovea) before it began to fixate with a more eccentric location (6.0 deg from the fovea). Against the trend in the overall cohort, fixation also became more stable this case ($BCEA_{baseline}$= 4.82 deg^2; $BCEA_{Month12}$= 2.21deg^2).

Discussion

This research provides data on the longitudinal changes of fixation parameters assessed with the Nidek MP-1 microperimeter in a large cohort of patients with molecularly confirmed STGD1. Using continuous parameters to quantify fixation location and stability in cross-sectional data, we recently showed that an earlier age of onset of STGD1 symptoms and poor BCVA are associated with more unstable and more eccentric fixation.[12] Hence, our previous results suggested that fixation may be a useful visual function biomarker. However, there is only little data on longitudinal changes using microperimetry.[18] Furthermore, most of the available literature on fixation assessment used non-continuous, less accurate categories, or they simply localized the fixation cloud to a certain quadrant of the posterior pole[14, 21-24], e.g. foveal, parafoveal, and extrafoveal.[18] To the best of our knowledge, this is the first report on prospective longitudinal changes of the PRL eccentricity and fixation stability using the BCEA in STGD1.

Month 12 changes in fixation location

The distance of the center of all fixation events from the anatomical fovea in degrees was determined during the macular sensitivity test for all eyes and a median of 6.0° was found at baseline. In a preparatory study comparing fixation location in 45 eyes with STGD1 obtained during a separate fixation task with data from the macular threshold test, we found no difference in eccentricity from the fovea (ρ_{RPL}= 0.87; p<0.001; N= 45). Therefore, we used the data from the macular threshold test to determine the eccentricity of the PRL. After 12 months of follow-up, the statistical analysis showed no change in the eccentricity of fixation of -0.0014° (95% CI: -0.27 °, 0.27; p=0.99). Based on the association of longer disease duration with more eccentric fixation in our own cohort[12] and in a report by Testa et al.,[18] we expected the fixation location to represent the progressive nature of the disease and to become more eccentric during follow-up. Multiple reasons may account for the observed null change. Firstly, 12 months of follow-up may

not be enough to detect a significant change using this method. Secondly, there is a large heterogeneity in the pattern of the development of fixation location with eyes where fixation turned from a very eccentric location towards the fovea and eyes where fixation became more eccentric, overall outweighing the change. Certain subgroups based on baseline fixation may show a unidirectional change. Thirdly, some eyes may actually have multiple PRLs with an unknown effect on the analysis. Four, there may be adaptation processes with an improvement of fixation that allow more central fixation.

Month 12 changes in fixation stability

We used the BCEA as a continuous parameter to describe how close the fixation points are together, as opposed to other study groups that used the percentage of fixation points within a 2 or 4-degree circle or the Fujii categorization.[18, 23] The BCEA as a continuous parameter has been shown to have multiple advantages over the semi-quantitative Fujii method such as a better correlation with reading abilities, the absence of arbitrary categorizations, and the recognition of the elliptical distribution of the registered fixation events.[25, 26] Using the Nidek MP-1, the repeatability of the estimated BCEA has been shown to be acceptable.[27] The ability to deliberately fixate a target was tested during a short fixation task because longer testing durations (as during the sensitivity test) may destabilize fixation.[28] For the baseline cohort, we found a mean±SD of the 1SD-BCEA of 4.5 ± 9.1 deg^2 (Median (IQR), 2.3 deg^2 (0.8, 4.7 deg^2); range, 0.01, 134.9 deg^2). Values of the 1SD-BCEA between 0.08 deg^2 and 0.45 deg^2 can be found in the literature for undiseased eyes.[28-30] We tested again after 6 and 12 months of follow-up and calculated a yearly change of 1.21 deg^2 in 1SD-BCEA (95% CI: $- 1.23$ deg^2, 3.65 deg^2; p=0.33). We recently reported an association of longer disease duration with more unstable fixation in STGD1.[12] We therefore expected an increase in BCEA during follow-up. The direction of the change is as predicted and may turn statistically significant in the month 24 data of the ProgStar study. Many eyes demonstrated a diminished size of the BCEA after 12 months,

suggesting possible neuronal adaptation processes with an unknown magnitude of effect. The literature provides some evidence for improvement of fixation through eccentric viewing training in patients with macular disease.[31, 32] Similar processes may have taken place in our study population but although many eyes showed more stable fixation after 12 months, the overall mean BCEA increased and remained far larger than for undiseased eyes.[28-30] Moreover, the presence of multiple PRLs, which are present in about 5 % of eyes with STGD1,[21] may have altered our measurements because we used one global BCEA to describe fixation stability. One limitation to our results is the fact that the separate fixation test was not performed in a number of cases. This led to a loss of BCEA values on follow up.

Conclusions

This is the first prospective longitudinal analysis of continuous fixation parameters in a large cohort of genetically confirmed cases of *ABCA4-related* STGD1. The results may help better understand the complex nature of fixation measures and how they change over time. Based on the presented results, a follow-up period of 12 months does not provide statistically significant changes for a large cohort of patients that could be used to compare effects of treatments in clinical trials. Underlying reasons may be the complex heterogeneity of the changes of fixation parameters that include both deteriorations and improvements. Neuronal adaptation processes may be involved in cases where fixation becomes more central or more stable over time. It is also possible that a follow up period of 12 months is too short a period to demonstrate significant centrifugal displacement and destabilization of fixation despite the large number of study participants. However, fixation parameters may serve as useful secondary outcome parameters in selected cases and for counseling patients to explain changes to their visual functionality. In addition the observed changes may explain cases of improvement in BCVA.

Acknowledgement

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Figures

Figure 1. Grading of the Bivariate Contour Ellipse Area (BCEA), the Preferred Retinal Locus, and analysis of fixation on fundus autofluorescence images in the example of the right eye of an 18-year-old female who had been diagnosed with Stargardt disease 5 years before. Left, the BCEA is the smallest ellipse that encompasses 1, 2, or 3 standard deviations of all fixation events. The MP-1 can fully automatically draw this ellipse around the recorded fixation points and calculate the area as shown in the image. A smaller BCEA corresponds to more stable fixation. Center, a grid was automatically placed on the center of gravity of all fixation events in blue color. The distance between the center of the grid and the fovea could then be determined. Right, the recorded fixation events can be overlaid on a fundus autofluorescence image which allows for clearer identification of atrophic macula and analysis of fixation in relation to atrophy.

Figure 2. Correlation of the change in eccentricity of the preferred retinal locus (PRL) with the change in 1 SD-BCEA (Bivariate Contour Ellipse Area using 1 standard deviation). Each degree of PRL eccentricity was associated with a 1SD-BCEA which is 0.089 deg^2 larger (p= 0.14; N= 276).

Figure 3. Example of a left eye of a 45-year-old male 14 years after diagnosis that first developed more central, then more eccentric fixation over the course of 12 months. In this example, fixation becomes more stable on each follow up visit. This case demonstrates the complex nature of the course of fixation parameters over time and how they may provide useful additional information on top of structural or other functional parameters.