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Macular function and morphological features in juvenile Stargardt disease: Longitudinal study

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Abstract

Purpose—to evaluate disease progression in a cohort of patients with clinical and genetic diagnosis of Stargardt disease.

Design—longitudinal cohort study.

Subjects—56 selected patients with a clinical and molecular diagnosis of Stargardt disease, an early age of onset and a median follow-up length of two years.

Methods—patients underwent routine examination including full-field electroretinography, microperimetry and optical coherence tomography.

Main Outcome Measures—best corrected visual acuity, mean retinal sensitivity, fixation stability, preferred retinal locus, inner-outer segment (IS/OS) junction loss, atrophic lesion area.

Results—56 patients with a mean age of disease onset of 15.3 years (range: 3 - 28 years), a mean disease length of 12.1 years and a mean age at baseline of 27.4 years were analyzed. The median best corrected visual acuity was 20/200 in both eyes. Optical coherence tomography parameters (IS/OS alteration and retinal pigment epithelium lesion area) were obtained in 49 patients because signal quality was poor in the remaining 7 patients. Optical coherence tomography revealed a mean retinal pigment epithelium lesion area of 2.6 mm², preserved foveal IS/OS in 4.1% of patients, loss of foveal IS/OS in 59.2%, and extensive loss of macular IS/OS in 36.7%. Microperimetric findings showed a reduced macular sensitivity (mean 10 dB) and an unstable fixation in half of the patient cohort. The longitudinal analysis showed a significant progressive reduction of best corrected visual acuity and macular sensitivity (at an estimated rate of 0.04 decimals and 1.19 dB per year, respectively) associated with a significant enlargement of retinal pigment epithelium lesion area (0.282 mm² per year). No significant changes in ophthalmoscopic findings and electroretinographic responses were detected.

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Conclusions—this study highlights the importance of microperimetry and optical coherence tomography in monitoring Stargardt patients. In fact, quantifying the decline of visual functionality and detecting morphological macular changes proves useful to evaluate disease progression over a short-term follow-up and should be taken into account for the design of future gene therapy clinical trials to treat retinal dystrophy.

Introduction

Stargardt disease is a bilateral, symmetrical and progressive inherited macular dystrophy characterized by a decrease in central vision and the presence of bilateral atrophic-appearing foveal lesions. These lesions may have a beaten metal appearance with or without yellowish-white fundus flecks at the posterior pole and/or mid-peripheral retina¹⁻³. Genetically, Stargardt disease is a heterogeneous disorder that is usually inherited in an autosomal recessive manner and can rarely present as an autosomal dominant trait. Autosomal recessive Stargardt disease (STGD1) is caused by mutations in photoreceptor specific ATP binding cassette transporter (*ABCA4*) gene^{4, 5}.

Clinical investigation of macular diseases is based on both morphological and functional approaches. To date, clinical diagnosis of STGD1 is based on various examinations: visual acuity, fundus exam, electroretinography, fundus autofluorescence imaging, fluorescein angiography¹⁻³.

The recent introduction of additional exams, such as optical coherence tomography (OCT) and microperimetry (MP), could provide specific clinical information useful for the follow-up of STGD1 patients⁶. In particular, OCT is a specific morphologic exam of retinal layers with micrometer resolution that allows the precise visualization of retinal features, which could be correlated with other functional data, i.e., those derived from microperimetric testing.^{6, 7} MP provides the exact topographic correlation between fundus details and its light sensitivity. The principle of MP relies on the ability to analyze, in real time, the retina under examination and to project a light stimulus over an individual and selected location.⁸ Currently, the use of MP has greatly improved the role of psychophysical tests in the evaluation of macular dystrophy^{7, 9-13}.

Several clinical studies have attempted to identify prognostic factors useful to evaluate the natural history of STGD1. Three different retrospective studies^{2, 14, 15} investigated the progression of visual acuity and its correlation with fundus lesion over a follow-up period ranging from 2 to 12 years. However, these papers reported exclusively ophthalmoscopic data without assessing other functional retinal parameters. Later, other studies¹⁶⁻¹⁸ described the natural history of Stargardt disease by analyzing clinical and electrophysiological features and correlating them with the genotype. Most studies focused only on morphological or functional clinical features, while the few studies, attempting to analyze the correlation between retinal morphology (i.e., assessed by OCT) and functionality (i.e., quantified by MP parameters), did not provide information about disease progression since they had a cross-sectional design. Moreover, different studies showed the usefulness of OCT and MP in cohorts of patients affected by macular diseases, including *ABCA4*-related dystrophy^{7, 9, 12, 19}. Nevertheless, to best of the authors' knowledge, no published report

focused on a large cohort of patients with clinical and genetic diagnosis of STGD1, as the previous studies investigated a heterogeneous patient sample (i.e., including different retinal diseases) or without a complete molecular analysis.

The purpose of our study is to show longitudinal data of a cohort of patients with clinical and genetic diagnosis of STGD1, including both the clinical tests required for diagnosis and the most advanced morphological and functional techniques to evaluate disease progression. These findings could be useful for the clinical management of patients and for the design of future clinical trials, i.e., for gene therapy evaluation.

Material and Methods

Patients

The medical records of all patients, who had previously been examined at the Referral Center for Hereditary Retinopathies of the Second University of Naples from January 2009 to January 2012, were reviewed retrospectively by the authors. Patient with a clinical and molecular diagnosis of STGD1 with an age of onset younger than 30 years were included. Patients were excluded if no follow-up data was available and if no or only one mutation in *ABCA4* gene was found.

The clinical diagnosis of STGD1 was based on a recorded family history compatible with autosomal recessive inheritance, presence of bilateral impairment of central vision, atrophic macular lesions (a beaten metal appearance or large patches of atrophy) with or without the appearance of perimacular and/or peripheral white-yellow flecks and normal to subnormal electroretinogram.

These patients underwent a complete ophthalmological examination, which included the following tests: best corrected visual acuity (BCVA) with manifested refraction by Snellen visual chart, slit-lamp biomicroscopy of anterior segment and fundus examination, full-field electroretinogram (ERG), MP, and OCT.

Fundus lesions were classified according to Fishman et al.²⁰ as follows: phenotype I included patients with a small atrophic-appearing foveal lesions and localized perifoveal yellowish-white flecks; phenotype II included patients with numerous yellowish-white fundus lesions throughout the posterior pole; and phenotype III included patients with extensive atrophic-appearing retinal pigment epithelium (RPE) changes.

Full-field ERG was recorded by corneal contact lens electrodes with a Ganzfeld stimulator (EREV 2000 Electrophysiology system; LACE Elettronica, Pisa, Italy) according to the recommendation of the International Society for Clinical Electrophysiology of Vision (ISCEV).²¹ ERG abnormalities were classified into three groups based on the following criteria proposed by Lois et al.²²: group I had normal full-field ERG amplitudes; group II had normal scotopic rod ERG but reduced photopic b-wave amplitudes; and group III had ERG abnormalities involving both scotopic and photopic b-wave amplitudes.

OCT was performed with Cirrus HD-OCT (Carl Zeiss, Dublin, CA). The acquisition protocol comprised both a five-line raster scan and a macular cube scan pattern (512x128

pixels) in which a 6 x 6-mm region of the retina was scanned within a scan time of 2.4 seconds. Based on the morphology of the inner-outer segment (IS/OS) junction layer of the photoreceptors (a band of high-reflectance, inner to the RPE layer)²³, we classified patients' OCT scans into three categories: category I IS/OS junction preserved in the fovea, category II IS/OS junction loss in the foveal area, and category III extensive loss of IS/OS junction (more than 1 disc diameter from the fovea)⁶. The IS/OS in the OCT images was evaluated in each patient on the same horizontal scan through the centre of the fovea.⁶ Moreover, a recently developed and validated algorithm was used to quantify the area of disease in the RPE, referred to as the RPE lesion area²⁴. The lesion area was defined as the site presenting a significant deviation in RPE contour. This algorithm has been investigated regarding accuracy compared with hand-drawn segmentation (Invest Ophthalmol Vis Sci 49:ARVO E-Abstract 4240, 2008), reproducibility (Invest Ophthalmol Vis Sci 48:ARVO E-Abstract 153, 2007), and clinical applicability^{25, 26}.

MP was performed by an automatic fundus-related perimeter (MP1 Microperimeter, Nidek Technologies, Padova, Italy). The following parameters were used: a fixation target of 2° in diameter consisting of a red ring; a white, monochromatic background with a luminance of 1.27 cd/m²; a Goldman III-size stimulus with a projection time of 200 ms; and predefined automatic macular test pattern covering 6° centred onto the gravitational centre of all the fixation points with 43 stimuli.

The following MP parameters were computed: mean sensitivity (MS), the percentage of fixation points within 2° (Fixation Stability - FS2°) and within 4° diameter circle (Fixation Stability - FS4°) centred at the gravitational centre of all the fixation points. The percentage values were used to define three grades of fixation stability: (I) "stable", when more than 75% of fixations fall within the 2° circle; (II) "relatively stable", when more than 75% of fixations fall within the 4° circle; and (III) "unstable", when less than 75% of fixations fall within the 4° circle. Finally, the eccentricity of the preferred retinal locus (PRL) was assessed as the distance between the gravitation centre of all the fixation points and the fovea. Following the classification proposed by Cideciyan et al.⁷ who distinguished between foveal and extrafoveal fixation, we introduced a classification according to PRL eccentricity in three stages: (I) foveal (no ectopic PRL or PRL eccentricity not higher than 2°); (II) parafoveal (PRL within 8°); (III) extrafoveal fixation (PRL outside 8°).

The study adhered to the tenets of the Declaration of Helsinki and was approved by Local Ethics Committees. Moreover, each patient gave written informed consent for his or her involvement.

Statistics

Our set of data is described by continuous (e.g. BCVA, MS, age) and categorical (Fishman phenotype, Lois group, IS/OS) variables. Continuous variable normality was assessed by Jarque-Bera test ($p > 0.01$). The relationships between clinical variables, disease duration and age of onset were assessed by linear or multinomial regression for continuous and categorical variables, respectively. Repeated measure regression models were adopted to analyse the change of the clinical variables over follow-up time and the relationship between

them. All the regression models were estimated using Generalized Estimating Equations, which enable to include correlated data, such as the value from both eyes of the same patients, and repeated measurements over the time (longitudinal data), by adopting an appropriate covariance structure, as described by Zeger et al²⁷. A p-value less than 5% was considered statistically significant.

Results

The study cohort consisted of 318 patients with clinical diagnosis of STGD1, initially identified for possible study inclusion. We excluded 262 patients for the following reasons: no mutation in *ABCA4* (41 patients), mutations in only one allele (72 patients), late onset, i.e., after 30 years (20 patients), no follow-up data (129 patients).

The selected patients are 56 (33 females and 23 males) with a median follow-up length of two years ($2.3 \text{ years} \pm 1 \text{ SD}$). All the patients complained of central visual loss with mean age of onset of 15.3 years (range: 3 - 28 years) and mean disease duration of 12.1 years (range: 1-44 years). Mean age at baseline was 27.4 years (range: 9 - 62 years).

Median BCVA, evaluated in all patients, was 20/200, range 20/2000 to 20/20 for the right eye, and 20/200, range 20/1000 to 20/20 for the left eye. According to Fishman classification, ophthalmoscopic lesions were consistent with Fishman phenotype I in 36 patients (64.3%), phenotype II in 4 patients (7.1%), and phenotype III in 16 patients (28.6%). ERG responses of the selected patients were consistent with Lois group I in 33 patients (58.9%), group II in 4 patients (7.1%), group III in 19 patients (33.9%). Evaluation of OCT parameters (IS/OS alteration and RPE lesion area) was obtained in 49/56 patients because signal quality was poor in 7 patients. OCT analysis revealed a mean RPE lesion area of 2.6 mm^2 , a foveal preserved IS/OS in 4.1% of patients, a foveal IS/OS loss in 59.2%, and extensive macular IS/OS loss in 36.7%. Microperimetric findings showed reduced MS (mean 10 dB) and unstable fixation in half of the patient cohort. All OCT and MP parameters at baseline, for right and left eyes, are reported in Table 1.

In order to investigate disease progression, we analysed the relationship between retinal features and age of onset, disease length or age, as reported in Table 2. An early disease onset (i.e., in the first decade) was associated with a higher risk to develop: extensive loss of IS-OS (category III), extinguished ERG responses (Lois group III), extrafoveal PRL (stage III) and reduced MS. With the increasing of disease duration, the patients showed decline of BCVA and a progression toward extensive loss of IS-OS (category III), extrafoveal PRL (stage III), and unstable fixation (grade III). Moreover, the RPE lesion area enlarged with increasing age.

The longitudinal analysis showed a significant progressive reduction of BCVA and MS (at an estimated rate of 0.04 decimals and 1.19 dB per year, respectively) associated with a significant enlargement of RPE lesion area (0.282 mm^2 per year). Moreover, the PRL become more eccentric (0.498° per year). Fundus phenotype and ERG responses remained unchanged over follow-up except in four patients: one patient (1.78%) showed a progression of ERG abnormalities from Lois I to Lois II while in three patients (5.35%) the ERG

responses worsened from Lois II to Lois III. OCT data shows that the IS/OS category remained stable in all but four patients (not the same patients who showed progression of ERG abnormalities): in two patients there was a change from IS/OS I to IS/OS II (4.08%), in the other two from IS/OS II to IS/OS III (4.08%).

Furthermore, we analysed the progression of clinical parameters by stratifying the patients according to Fishman phenotype, Lois group, IS-OS category, fixation stability grade and PRL stage, as reported in Table 3.

Fishman I phenotype and Lois I group was significantly associated with higher values of MS and a smaller RPE lesion area; however, in these patients, disease progression, in terms of BCVA worsening, RPE lesion area enlargement and MS reduction was observed over follow-up.

The analysis of morphological features by OCT revealed that IS-OS category II was associated with a smaller RPE lesion area and preserved MS. Data on IS-OS category I was not reported due to the small number of patients. Over follow-up, BCVA reduction, associated with an increase of PRL eccentricity, was observed in both categories (II and III), while a significant RPE lesion area enlargement occurred only in category II and MS reduction was evident only in category III.

As for MP classification, we observed that patients with stable fixation showed better BCVA and MS, which significantly decreased over follow-up, on the contrary, in patients with unstable fixation all clinical parameters appeared unchanged over follow-up, with the exception of increased PRL eccentricity.

Finally, according to the PRL staging, we observed that patients with foveal fixation (stage I) at baseline showed: better BCVA, Fishmann I phenotype, and Lois I group. Moreover, the longitudinal data of these patients showed a progressive decline of BCVA and MS, and a significant enlargement of RPE lesion area. In patients with parafoveal PRL, disease progression was observed in terms of MS reduction and RPE lesion area enlargement but with no decline in BCVA. Finally, in patients with extrafoveal PRL (stage 3) all clinical parameters (BCVA, Fishmann phenotype, Lois group) appeared unchanged with the exception of MS, which significantly decreased over follow-up.

As shown in Table 4, the 112 mutant *ABCA4* alleles included 69 missense mutations, 27 site splicing mutations, 3 insertions, 9 stop codon, 2 deletions and 2 frame shift mutations.

Discussion

The present study retrospectively investigates a large cohort of STGD1 patients with a short-term follow-up (up to three years) reporting clinical, morphological and functional data.

The study was designed including patients that satisfy the inclusion criteria required in gene therapy clinical trials, such as the StarGenTM study (ClinicalTrial.gov NCT01367444), which enrolled patients with at least two mutations in the *ABCA4* gene. Moreover, Braun et al.²⁸ recently recommended not to enrol subjects without two detectable mutations into

clinical trials of invasive therapies such as gene replacement because if their disease is caused by some other gene or non-genetic phenocopy, the treatment would not help them and could harm them.

Our data highlight the importance of MP and OCT for the study of retinal function and the evaluation of the lesion area in order to accurately define disease progression.

The comparison with literature is limited by the fact that previous studies investigated MP parameters in heterogeneous cohorts of patients with macular diseases including only small subgroups of STGD cases^{7, 9, 10, 12, 13}. Moreover, few data about the OCT lesion area are available since the algorithm is of recent introduction²⁴.

Previous studies attempted to describe STGD1 disease progression analyzing BCVA data both at baseline and during follow-up. In particular, it has been noticed that patients with STGD1 showed a large variability of BCVA; in fact, cross-sectional studies showed that the range of STGD patients with BCVA of 20/40 or better varied from 14% to 37% and for BCVA higher than 20/200 the range varied from 41% to 80%^{22, 29, 30}. In accordance with these studies our data demonstrate that 43% of eyes had BCVA higher than 20/200. Moreover, recently, Yatsenko et al.³¹ reported that cases with an adult onset showed a better BCVA compared to typical cohort. We also observed that BCVA was 20/40 in 40% of our patients with adult onset (Simonelli F, unpublished data April, 2014), compared to 6.2% of the present cohort, hence confirming these findings. This observation motivated the exclusion of patients with an onset after the first three decades of life, even if this clinical difference was not considered in most previous studies.

Longitudinal studies with a long follow-up (range 5-35 years) suggested that STGD1 patients tend to plateau approximately to 20/200^{1, 15, 20}. In particular, Oh et al.¹⁵ observed that the progression of BCVA was different according to the Fishman phenotype: the patients with Fishman phenotype I demonstrate stability at the level of 20/200 after their initial loss of vision over follow-up (20 years of follow-up); whereas patients with Fishman phenotype II and III follow a more severe long-term course with continued visual loss beyond the 20/200 level (after 10 years of follow-up). Literature supports our results showing that visual acuity data alone are not able to detect STGD disease progression, in particular in short-term follow-up cases (<5 years).

Ophthalmoscopic and electrophysiological data showed that the most frequent clinical phenotype was Fishman I, usually with a normal full-field ERG (Lois group 1) consistently with the findings by Oh et al.¹⁵ and Fujinami et al.¹⁶, and only few cases of progression in Lois group were observed, consistently with the fact that these progressions were usually noticed in a longer follow-up (median 7 years)¹⁴. On the contrary, the MP enabled to detect a sharp decline of retinal functionality with an estimated MS decrease of 1.19 dB per year, and OCT revealed an enlargement of RPE lesion area (0.28 mm²/year). These data are particularly interesting since the classifications of ophthalmoscopic lesions, ERG abnormalities and IS-OS alterations were not able to show disease progression over short-term follow-up.

PRL eccentricity allowed us to identify three groups, which showed different clinical characteristics at baseline and disease progression. Patients with foveal fixation, although they showed a better visual functionality at the baseline, underwent a progressive decline of visual acuity and microperimetric parameters, associated with an enlargement of RPE lesion area. On the contrary, patients with extramacular fixation showed higher values of MS, unchanged BCVA but a progressive decline of MS.

Furthermore, the subgroup analyses pointed out that disease progression was more evident in patients that showed better morphological and functional features at baseline (i.e., Fishman I, Lois I, stable fixation and foveal fixation) compared to those with worse residual visual function and more altered morphology. This clinical behaviour seems to be due to the fact patients with better residual function and more preserved retinal morphology are most likely to show a progressive decline due to retinal degeneration, compared to patients with an advanced disease stage, who had minimal residual function, which appeared to be unchanged. In these patients with stable BCVA, a progressive decrease of MS was observed during follow-up, confirming the ability of MP to detect subtle decline of visual function in STGD¹².

In conclusion, the current study suggests that MP and OCT are useful tools to evaluate progressive decline of visual function in STGD1 patients during a short-term follow-up. In particular, visual function could be quantified by MS and position of PRL, assessed by MP, and morphological macular changes could be revealed by the enlargement of the lesion area evaluated by OCT, even if further reproducibility studies on OCT are needed to confirm our findings. In any case, our results strongly suggest that these parameters, particularly MS, can detect visual functionality variation and can therefore be taken in account for the design of future gene therapy clinical trials, both as inclusion criteria and as outcome measures. For instance, patients with extrafoveal fixation, often associated with worse visual function and macular atrophy, could be enrolled for safety assessment, while patients with foveal fixation, often associated with a small RPE lesion area and with preserved visual function, could benefit more from future trial treatment.

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Morpho-functional study in juvenile Stargardt disease

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Table 1

OCT and MP parameters at baseline

Parameter	Left eye	Right eye
RPE lesion area (mm ²)	2.7 ± 3.0	2.4 ± 2.5
IS-OS Category I (IS-OS preserved in the fovea)	2 (4.1%)	2 (4.1%)
IS-OS Category II (IS-OS lost in foveal area)	29 (59.2%)	29 (59.2%)
IS-OS Category III (IS-OS extensive lost in macular area)	18 (36.7%)	18 (36.7%)
MS (dB)	10.6 ± 6.3	9.1 ± 6.1
FS2° (%)	41.1 ± 29.2	36.6 ± 30.0
FS4° (%)	70.0 ± 27.2	63.1 ± 31.8
PRL eccentricity (deg.)	4.3 ± 3.7	3.9 ± 3.4
Fixation Grade I (Stable fixation)	13 (23%)	8 (14%)
Fixation Grade II (Relatively stable fixation)	17 (30%)	18 (32%)
Fixation Grade III (Unstable fixation)	26 (46%)	30 (53%)
PRL Stage I (Foveal fixation)	20 (35%)	23 (41%)
PRL Stage II (Parafoveal PRL)	28 (50%)	25 (44%)
PRL Stage III (Extrafoveal PRL)	8 (14%)	8 (14%)

The values are expressed as mean ± standard deviation for continuous variables and as count (percentage) for categorical variables.

RPE: retinal pigment epithelium, IS/OS: inner - outer segment junction, MS: mean macular sensitivity, FS2°: fixation stability within 2°, FS4°: fixation stability within 4°, PRL: preferred retinal locus.

Table 2

Statistical analyses of the dependency of clinical features by age of onset and disease length.

Independent variable	Parameter	β	p value	Exp(β)	Lower	Upper
95% CI						
Fishmann Phenotype	Age of onset	-.036	.236	.964	.908	1.024
	Disease length	.014	.331	1.014	.986	1.042
Lois Group	Age of onset	-.132	<.001	.876	.823	.932
	Disease length	-.006	.743	.994	.960	1.030
Fixation Grade	Age of onset	.019	.447	1.019	.971	1.069
	Disease length	.048	.007	1.050	1.013	1.087
IS/OS Category	Age of onset	-.078	.012	.925	.871	.983
	Disease length	.056	.010	1.058	1.014	1.103
PRL Stage	Age of onset	-.061	.021	.941	.893	.991
	Disease length	.082	<.001	1.086	1.054	1.119
BCVA	Age of onset	-.002	.552	.998	.992	1.004
	Disease length	-.007	<.001	.993	.989	.996
RPE lesion area	Age	.148	<.001	1.160	1.105	1.218
MS	Age of onset	.202	.036	1.224	1.013	1.477
	Disease length	.012	.840	1.012	.898	1.141
PRL eccentricity	Age of onset	-.108	.087	.897	.792	1.016
	Disease length	.140	<.001	1.150	1.076	1.229

β : beta coefficient, Exp: exponential function, CI: confidence interval, IS/OS: inner - outer segment junction, PRL: preferred retinal locus, BCVA: best corrected visual acuity, RPE: retinal pigment epithelium, MS: mean macular sensitivity

Table 3

Repeated measures regression models for longitudinal analysis of all the clinical features. Significant differences are in bold

	BCVA		RPE lesion area		MS		PRL eccentricity	
	β	p	β	p	β	p	β	p
Time	-.039	<0.001	.282	.034	-1.191	<0.001	.498	<0.001
Fishman 1	.078	.060	-2.209	.005	4.711	.003	-.399	.662
Fishman 2	-.068	.384	-.787	.579	7.914	.007	1.657	.324
Fishman 3	0 ^a		0 ^a		0 ^a		0 ^a	
Fishman 1 * Time	-.051	<0.001	.778	<0.001	-1.203	<0.001	.499	<0.001
Fishman 2 * Time	-.002	.774	.224	.174	.697	.012	.646	<0.001
Fishman 3 * Time	-.039	<0.001	.447	.004	-1.391	<0.001	.767	<0.001
Lois 1	.054	.172	-5.797	<0.001	9.290	<0.001	3.608	<0.001
Lois 2	.041	.247	-1.779	.284	.381	.800	-1.153	.001
Lois 3	0 ^a		0 ^a		0 ^a		0 ^a	
Lois 1 * Time	-.043	<0.001	.108	.287	-.768	<0.001	.450	<0.001
Lois 2 * Time	.004	.773	.447	.488	1.435	<0.001	2.135	<0.001
Lois 3 * Time	-.022	<0.001	-.020	.888	-.969	<0.001	.561	<0.001
IS-OS 1	.445	<0.001	-5.294	<0.001	-2.440	.035	1.243	.033
IS-OS 2	-.011	.470	-4.617	<0.001	5.383	<0.001	1.581	<0.001
IS-OS 3	0 ^a		0 ^a		0 ^a		0 ^a	
IS-OS 1 * Time	-.081	.065	.403	.471	10.26	<0.001	-.345	.675
IS-OS 2 * Time	-.024	<0.001	.403	.004	-.210	.226	.474	<0.001
IS-OS 3 * Time	-.013	<0.001	-.171	.407	-1.259	<0.001	.632	<0.001
Stable fixation	.075	<0.001	.311	.510	3.488	<0.001	-.856	.008
Relatively stable fixation	.111	<0.001	-1.731	<0.001	1.668	.001	-.546	.065
Unstable fixation	0 ^a		0 ^a		0 ^a		0 ^a	
Stable fixation * Time	-.082	<0.001	-.200	.241	-2.049	<0.001	.445	<0.001
Rel. stable fix. * Time	-.026	<0.001	.949	<0.001	-.711	.001	.398	<0.001
Unstable fixation * Time	0.000	.976	.028	.862	-.329	.160	.885	<0.001
Foveal Fixation	.266	<0.001	-.229	.776	-1.543	.128	-7.032	<0.001
Parafoveal PRL	-.021	.360	-.828	.286	-1.908	.027	-4.567	<0.001
Extrafoveal PRL	0 ^a		0 ^a		0 ^a		0 ^a	
Foveal Fixation * time	-.057	<0.001	.238	.041	-.772	.002	.287	<0.001
Parafoveal PRL * time	0.001	.937	.632	<0.001	-.527	.007	.223	<0.001
Extrafoveal PRL * time	.013	.067	.031	.850	-1.585	<0.001	-.086	.215
BCVA			.314	.725	-1.152	.452	-1.977	.001
Time			.259	.049	1.379	.041	.508	.015

	BCVA		RPE lesion area		MS		PRL eccentricity	
	β	p	β	p	β	p	β	p
RPE	.003	.141			-.298	.013	-.159	.025
Time	-.035	<0.001			-.074	.068	.124	<0.001
MS	.000	.950	-.133	.001			-.053	.055
Time	-.037	<0.001	.274	<0.001			.036	<0.001
PRL eccentricity	-.007	.007	.002	.977	.333	.002		
Time	-.035	<0.001	.151	.282	-.177	<0.001		

β : beta coefficient, IS/OS: inner - outer segment junction, PRL: preferred retinal locus, BCVA: best corrected visual acuity, RPE: retinal pigment epithelium, MS=macular sensitivity

^a set to 0, since it is redundant

Table 4*ABCA4* mutations in the study cohort

Mutation	N° of alleles	N° of patients
4709-4711delA	1	1
4736del6bpins2bp	1	1
A1598D	1	1
A1881V	1	1
A60V	1	1
c.5018+1g>a	1	1
c.5196+1G>A	1	1
c.571-1G>T	1	1
C1490Y	1	1
E1087K	1	1
E1271D	1	1
G1844C	1	1
G1977S	1	1
G618R	1	1
G618W	1	1
GIVS+5a	1	1
IVS15-8g>a	1	1
IVS30+1g>t	1	1
IVS6-2a>t	1	1
L1201R	1	1
L2027F	1	1
N865S	1	1
N96D	1	1
N96K	1	1
P143L	1	1
P1486L	1	1
P402A	1	1
Q1513PfsX42	1	1
R18W	1	1
R653C	1	1
S1099P	1	1
S1696N	1	1
S84TfsX16	1	1
V256splice	1	1
Y245X	1	1
A1038V	2	2
C1488R	2	2
c.6282+1g>c	2	2
E207K	2	1

Mutation	N° of alleles	N° of patients
F1015I	2	2
IVS45+1g>c	2	2
IVS5-2a>t	2	1
L541P	2	2
R1443H	2	2
R212C	2	1
T970P	2	2
250InsCAAA	3	3
N96H	4	2
R2030X	4	3
R2149X	4	4
V767D	4	4
IVS40+5g>a	5	5
IVS35+2t>c	8	7
G1961E	23	22

Short-term follow-up data (up to 3 years) on 56 patients with juvenile Stargardt disease showed that microperimetry and optical coherence tomography are helpful to evaluate macular function and morphology.