Morphology and function of the retina in children and young adults with Stargardt dystrophy

Morfologija in delovanje mrežnice pri otrocih in mladih odraslih s Stargardtovo distrofijo

Martina Jarc-Vidmar,[1] Darko Perovšek,[1] Damjan Glavač,[2] Jelka Brecelj,[1] Marko Hawlina,[1] Branka Stirn-Kranjc[1]

[1] *Eye Hospital, University Medical Centre Ljubljana, Grabovičeva 46, 1525 Ljubljana, Slovenija*

[2] *Department of Molecular Genetics, Faculty of Medicine, University of Ljubljana, Slovenia*

Korespondenca/ Correspondence:
asist. dr. sc. Martina Jarc-Vidmar, dr. med, University Eye Clinic, Grabovičeva 46, 1000 Ljubljana, Slovenija
E-mail: martina.jarc-vidmar@mf.uni-lj.si

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Abstract

Background: The aim of our study was to evaluate retinal function in children and young adults with Stargardt dystrophy by correlating retinal morphology (autofluorescence, OCT) with functional tests (visual acuity, microperimetry) and electroretinography (full-field ERG, pattern ERG, multifocal ERG).

Patients and methods: 6 patients (3 F, 3 M, VA: 0.2 ± 0.1, average onset of problems at 10 yrs of age) were included in the study. Autofluorescence (AF) was recorded and the central 10 ° visual fields were tested by microperimetry. The morphology of the retina was recorded by optical coherence tomography (OCT). Full-field ERG, pattern ERG (PERG) and multifocal ERG (mfERG) were recorded according to ISCEV standard in all the patients.

Results: AF showed mottled central hypo-hyperautofluorescent areas with or without peripheral hyperautofuorescent flecks in 4 patients and a central hypoautofluorescent region with hyperautofluorescent ring in 2 patients. OCT showed transverse loss of the central photoreceptor layers (average OCT thickness: 38.9 ± 14.8µm, average OCT volume 6.1 ± 0.5 mm^3). Microperimetry showed superior shift of fixation to the preferred retinal locus (PRL). PERG was abnormal in 8 eyes. Full-field ERG was normal in all except two patients with abnormal cone and 30 Hz responses. MfERG showed reduced responses in all five rings (ring 1: 22.6 % of mean normal value, ring 2: 27.3 %, ring 3: 38.9 %, ring 4: 57.9 %, ring 5: 64.2 %).

Conclusions: In young patients with Stargardt dystrophy central retinal atrophy was shown by OCT and AF. The shift of fixation to the PRL was seen in all the patients. MfERG showed central cone dysfunction in all the patients.

Izvleček

Uvod: Namen študije je oceniti delovanje centralne mrežnice pri otrocih in mladih odraslih s Stargardtovo distrofijo s primerjavo morfoloških značilnosti (avtofluorescenca, OCT), s funkcionalnimi (vidna ostrina, mikroperimetrija) in elektroretinografskimi testi (skotopični in fotopični ERG, slikovni ERG, multifokalni ERG).

Bolniki in metode: V študijo je bilo vključenih 6 bolnikov (3 Ž, 3 M, VO: 0,2 ± 0,1, začetek težav z vidom v starosti 10 let). Bolnikom smo posneli avtofluorescenco (AF) ozadja. Opravili so preiskavo centralnih 10° vidnega polja z mikroperimetrijo. Morfologijo mrežnice smo ovrednotili z optično koherenčno tomografijo (OCT). Pri bolnikih smo posneli skotopični in fotopični ERG (SFERG), slikovni ERG (PERG) in multifokalni ERG (mfERG) v skladu s standardi ISCEV.

Rezultati: AF je pokazala lisasto centralno hipo--hiperavtofluorescentne predele s periferno hiperavtofluorescentnimi lisami pri 4 bolnikih in centralno hipoavtofluorescentno regijo z hiperavtofluorescentim obročem pri 2 bolnikih. OCT je pokazal izgubo centralnih fotoreceptorskih plasti (povprečna OCT debelina: 38,9 ± 14,8µm, povprečna OCT prostornina: 6,1 ± 0,5 mm^3). Mikroperimetrija je pokazala premik fiksacije navzgor na nov preferenčni areal mrežnice. PERG je bil abnormen pri 8 očeh. Odgovori SFERG so bili normalni pri vseh, razen pri dveh bolnikih z abnormnim odgovorom čepnic in 30 Hz odgovorom. MfERG je pokazal nižje vrednosti odgovorov v vseh petih obročih (obroč 1: 22,6 % povprečne normalne vrednosti, obroč 2: 27,3 %, obroč 3: 38,9 %, obroč 4: 57,9 %, obroč 5: 64,2 % povprečne normalne vrednosti).

Zaključki: Pri mladih bolnikih s Stargardtovo distrofijo sta AF in OCT pokazala centralno atrofijo mrežnice s premikom fiksacije na preferenčni areal mrežnice. MfERG je pri vseh bolnikih pokazal disfunkcijo centralnih čepnic.

Introduction

Originally described by Stargardt in 1909, this juvenile macular dystrophy is one of the most common hereditary macular dystrophies, usually starting in the second decade of life, prevalence is estimated between 1 in 8,000 to 1 in 10,000.[1] It is characterized by the presence of bilaterally symmetric atrophic macular lesions associated with white flecks. Patients with this predominanty autosomal recessive disorder characteristically present with progressive loss of central vision. The disease is caused by mutation in the photoreceptor-specific ATP-binding cassette transporter gene (ABCA4) mapped on the short arm of chromosome 1, which is responsible for export of photoisomerised all-trans- retinal from the rod outer segment to the retinal pigment epithelium (RPE).[2] ABCA4 was first reported to be expressed exclusively in rods, later it was shown that it is present in both cones and rods and that visual deterioration arises directly from ABCA4-mediated foveal cone degeneration.[3] Mutations in the ABCA4 cause accumulation of lipofuscin in the RPE, followed by degeneration of photoreceptor layers. In addition, ABCA4 mutations have been identified in autosomal recessive cone-rod dystrophy and retinitis pigmentosa.[4]

Stargardt disease can be clinically very heterogenous; initially, only subtle pigmentary motling within the fovea may be apparent ophthalmoscopically. Even with only these minimal ophthalmoscopic changes, visual acuity may decrease dramatically. Findings of fundus examination do not correlate well with retinal function.[5] Based on electrophysiology results, three patterns of function loss in patients with Stargardt were recognized: there is loss of macular function with or without loss of generalized cone, or cone and rod function.[6]

It is well known that in patients with low vision from central sensory deficit the visual system has adapted by choosing an eccentric preferred retinal locus (PRL) to perform visual tasks that used to be performed by nonfunctioning fovea. The first prospective study about PRL development in 25 patients with macular pathology and recent visual loss showed that new PRL developed in 6 months and that 64 % of patients were not aware of using PRL for seeing.[7] An investigation of fixation behavior in 40 eyes of 21 patients with Stargardt dystrophy revealed central scotoma with eccentric fixation in 19 eyes, where PRL was at the top of the scotoma.[8] Another 8 eyes varied between two fixational areas, central and eccentric. It was shown that centre for fixation chan-

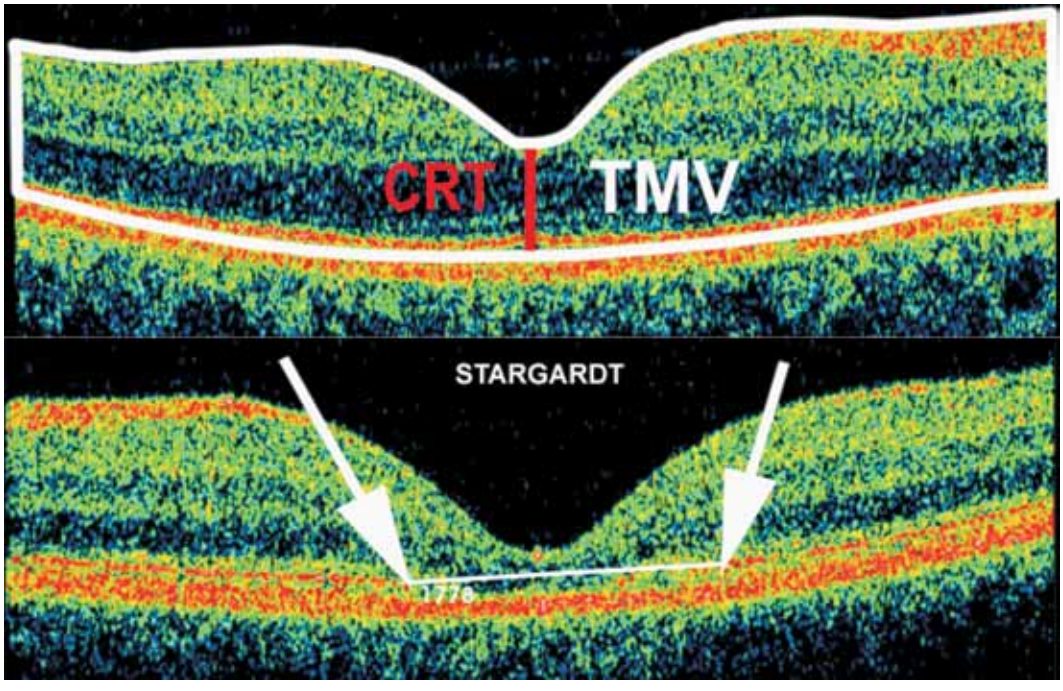


Figure 1: Central retinal thickness (CRT) and total macular volume (TMV) as seen by OCT. Note loss of central photoreceptor layers in patient with Stargardt dystrophy.

Table 1: Clinical characteristics, genetic data, microperimetry testing, SD-OCT imaging and electrophysiology results in Stargardt patients.

Patient	Sex	Age	Age	Mutation	Mutation	Eye	Vis ac	F AF	MP	OCT	OCT	f,sERG	PERG	mfERG
		Exam (y)	Onset (y)	Allele 1	Allele 2				fixation	CRT (mm)	TMV (mm3)			
1	M	28	8	P143L	IVS38–10T>C	R	0.1	SMD	eksc			Abn cone,30Hz	Abn	Abn
						L	0.1		eksc			Abn cone,30Hz	Abn	Abn
2	M	20	9	IVS49–3T>C	C230S	R	0.3	SMD	eksc	39	6.34		Abn	Abn
						L	0.2		eksc	26	6.3		Abn	Abn
3	F	14	13	G1091E	G1961E	R	0.1	ring	eksc	37	5.89	N	Abn	Abn
						L	0.1		eksc	34	5.94	N	Abn	Abn
4	F	17	15			R	0.3	ring	eksc	42	6.1	N	N	Abn
						L	0.3		eksc	50	6	N	N	Abn
5	F	10	8	R2149X	R2149X	R	0.3	FF	eksc	44	5.45	Abn cone,30Hz	Abn	Abn
						L	0.1		eksc	70	5.4	Abn cone,30Hz	Abn	Abn
6	M	21	10	IVS38–10T>C	IVS40+5G>A	R	0.1	FF	eksc	14	6.88	N	N	Abn
						L	0.2		eksc	33	6.84	N	N	Abn

Vis ac, visual acuity; FAF, fundus autofluorescence; SMD, stargardt macular dystrophy; FF, fundus flavimaculatus; MP, microperimetry; OCT CRT, central retinal thickness; OCT TMV, total macular volume; f,s ERG, full-field ERG; PERG, pattern electroretinography; mfERG, multifocal electroretinography.

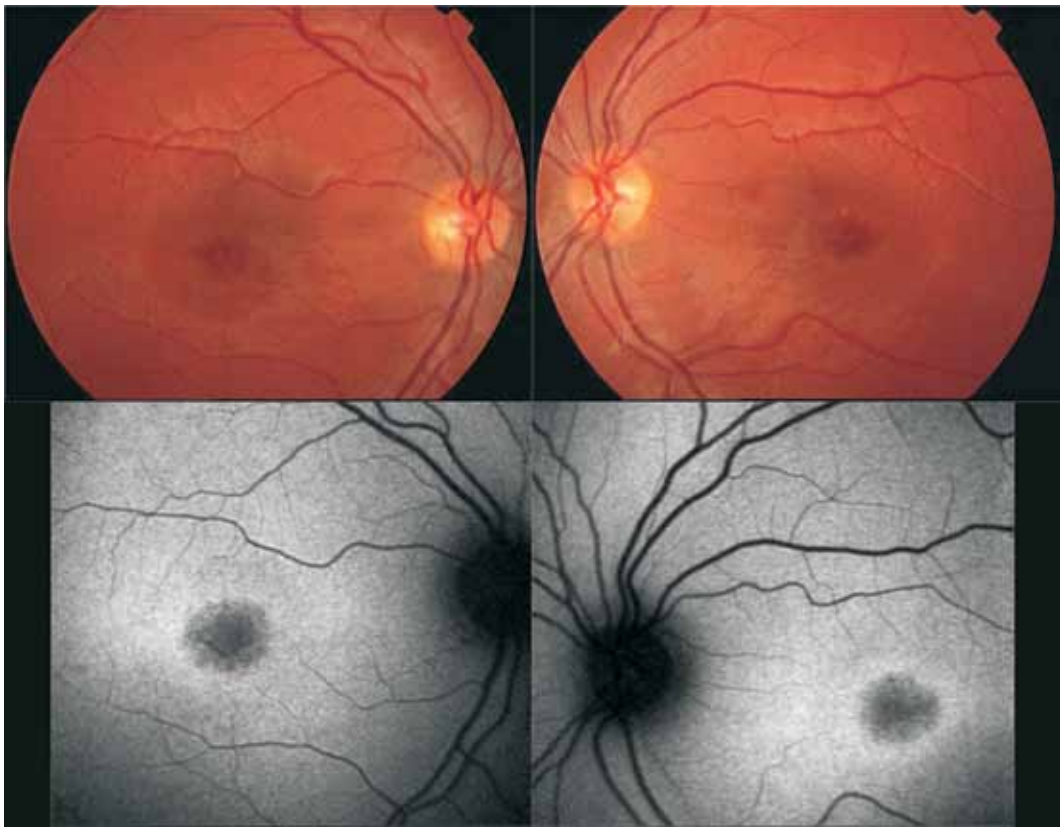


Figure 2: Hyperautofluorescent parafoveal ring with central hypoautofluorescence (Table 1, patient 3).

ges for different fixation targets – fixation for small targets is central and stable, while larger objects will lead to PRL located more peripherally, mostly straight upwards of the fovea, more seldom to the left.

Spectral domain optical coherence tomography (OCT) is a non-invasive technique that provides in vivo morphological information of the retina. In patients with Stargardt disease extensive foveolar thinning as well as macular volume loss and transverse photoreceptor loss were found.[9-11]

Purpose of the study

The purpose of the present study was to evaluate central retinal function in children and young adults with Stargardt dystrophy by correlating retinal morphology (autofluorescence and OCT) with functional tests (microperimetry and visual function) and with electrophysiological tests (full-field ERG, PERG, mfERG).

Patients and methods

The research followed the tenets of declaration of Helsinki, and was approved by the Ethics committee at the Ministry of Health of Slovenia. 6 patients (3 females, 3 males) with visual acuity (VA): 0.2 ± 0.1, VA range from 0.3 to 0.1 were included in the study. All were referred as children and were folllowed clinically and electrophysiologically at the children's department of the Eye clinic. In the patient group, the mean age of onset was 10.5 ± 2.9 years (range 8–15 years), and the mean duration of disease was 7.8 ± 7.5 years (range 1–20 years). At presentation the mean range of patients was 18 ± 6.2 years (range 10–28 years). Their blood was taken for genetic analysis, in 5 patients the mutation on both alleles was already confirmed. Autofluorescence was recorded by Heidelberg retina angiograph, Heidelberg Engineering (HRA). Autofluorescence is a noninvasive method, enabling in vivo detection of lipofuscin accumulation in the retina. Excessive lipofuscin accumulation is due to abnormal photoreceptor or retinal pigment epithelium function. Abnormal autofluorescence was defined either as an increased or decreased autofluorescence signal compared with the autofluorescence outside such lesions, the latter being referred to as normal autofluo-

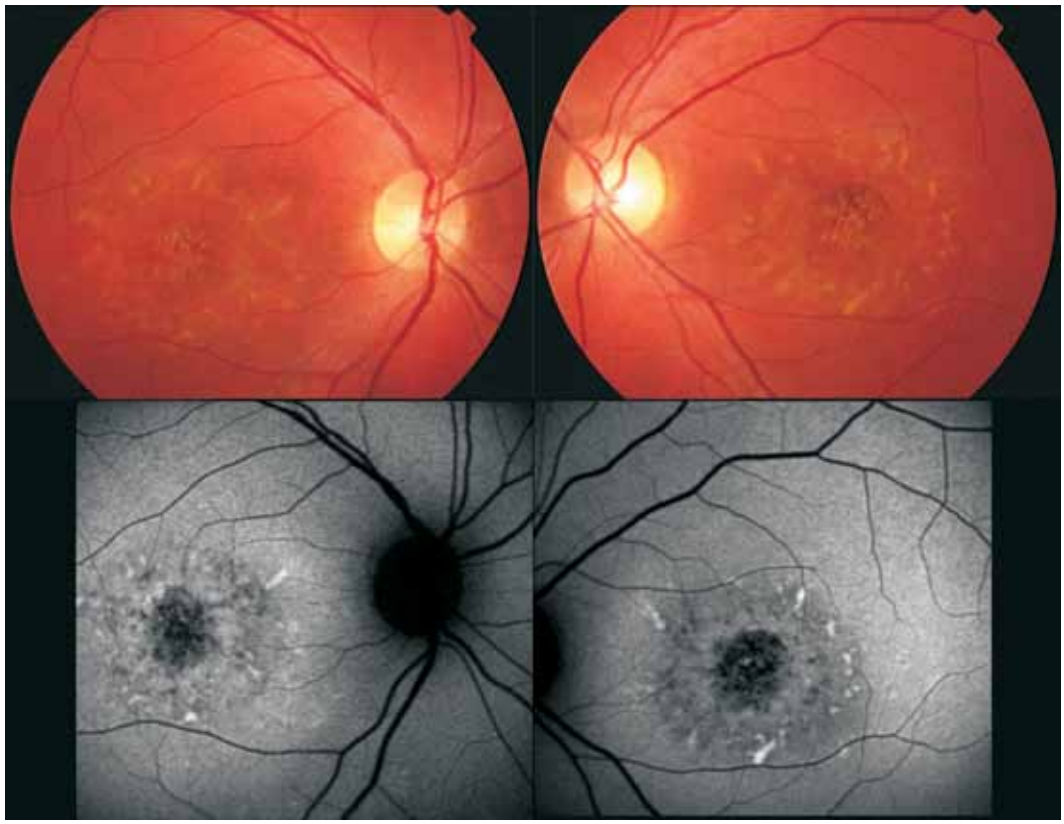


Figure 3: Hypo-hyperautofluorescent flecks centrally in the macula–Stargardt macular dystrophy (SMD; Table 1, patient 1).

rescence.[12] Fundus autofluorescence images in normal eyes have been published previously, with the autofluorescence being higher at the posterior pole, with a localized dip in the fovea, decreasing toward the periphery.

The central 10° visual fields were tested with microperimetry (MP1, Nidek technologies), that enables one to compare central retinal sensitivity and fixation patterns in relation to the fundus image. The central 10° visual field was tested with Humphrey 10–2°16 dB56s or Humphrey 10–2°4dB56s program (fast strategy, background illumination: 1.27cd/m^2, stimulation time 200ms, stimulation spot size: Goldmann III). In all the 0 dB, equivalent to 1.27cd/m^2 represented the brightest luminance, and the stimulus intensity varied from 0 to 20dB. Each eye was tested separately. The red cross was used for fixation and the stability of fixation was determined by MP1.

The morphology of the retina was recorded by SD OCT (3D OCT – 1000, Topcon, Tokyo, Japan). Scans were covering 6mm (horizontal) x 6mm (vertical) x 1.7mm (axial) block of the macular region. Central retinal thickness (CRT) and total macular volume (TMV) were automatically computed by the OCT software. CRT is defined as the point of thickness in the centre of the fovea and is measured in μm. TMV is defined as the sum of the volume of the neurosensory retina in the central 6mm and is measured in mm^3 (Figure 1).

Full-field, multifocal and pattern electroretinograms (ERGs) were recorded simultaneously from both eyes following the standards of International Society of Clinical Electrophysiology of Vision (ISCEV).[13-15] The recording electrode was a HK-loop, placed in the fornix of the lower eyelid.[16] The silver-chloride reference electrode was placed on the ipsilateral temple, and the ground electrode was positioned on the forehead. For full-field and multifocal ERGs, pupils were dilated with 1 % tropicamide (Mydriacyl®). Full-field electroretinogram was performed using a Ganzfeld stimulator of the RETI port unit (Roland Consult, Wiesbaden, Germany). Dark adapted ERG responses (rod response, maximal response and oscillatory potentials) were obtained after 20 minutes of dark adaptation and light adapted responses (cone response and 30-Hz flicker) after 10 minutes of light adaptation to the background luminance of 22 cd/m^2. The intensity

Figure 4: Central hypoautofluorescent region with extensive hyperautofluorescent flecks all over the fundus – Fundus flavimaculatus (FF; Table 1, patient 6).

of standard flash (maximal response, oscillatory potentials, cone response and 30-Hz flicker) was 2.4 cd s/m² and the intensity of attenuated dark-adapted flash (rod response) was 0.03 cd s/m². Multifocal electroretinogram (mfERG) was recorded with a 30° stimulus size (61 hexagons) using the RETI scan system (Roland Consult,Wiesbaden, Germany). The stimulus was at a distance of 260 mm and refractive errors were corrected due to dilated pupils with +3.50 diopters. Recording session consisted of 8 trials of 50 seconds duration. The pattern ERG (PERG) was elicited with a 0.8° checkerboard pattern with 99 % contrast that reversed 1.8 times per second, which was presented on a 21.6° x 27.8° screen stimulator. The PERG recordings were obtained from non-dilated eyes, and 100 responses were averaged.

Results

The main clinical, morphological and electrophysiological data of Stargardt patients are reported in Table 1. The AF showed three patterns of fundus changes: hyperautofluorescent parafoveal ring with central hypoautofluorescence in two patients (Figure 2), hypo-hyperautofluorescent flecks centrally in the macula in two patients – Stargardt macular dystrophy (SMD) (Figure 3) and central hypoautofluorescent region with extensive hyperautofluorescent flecks all over the fundus in 2 patients – fundus flavimaculatus (FF) (Figure 4).

A progression of Stargardt diasese is shown in Figure 5. The patient first visited University Eye clinic at the age of 13 years, with already poor visual acuity (RE: 0.3, LE: 0.2), and poor colour vision (Ishihara 1/11 ou), but very discrete fundus changes and almost normal AF image. The visual acuity in the next five years dropped to 0.1 in both eyes, yellowish flecks were clearly seen on his fundus and AF image showed well defined hypo-hyperautofluorescent flecks centrally in the macula.

Microperimetry showed shift of fixation to the preferential retinal locus (superior or nasal to the lesion) with relatively unstable or unstable fixation in all the patients (Figure 6).

OCT showed transverse loss of the central photoreceptor layers, with statistically significant thinner retina (average central retinal thickness CRT: 38.9 ± 14.8 µm, average total macular volume TMV: 6.1 ± 0.5 mm³, compared to the values of normal age mat-

Figure 5: Stargardt disease progression (Table 1, patient 2).

ched controls CRT: 181 ± 15.2µm, TMV: 7.55 ± 0.27 mm³ (Figure 7).

Full-field ERG was normal in all except two patients with abnormal cone and 30 Hz responses (Stargardt type 2). MfERG showed reduced responses in all five rings (P1 amplitudes ring 1: 22.6 % of mean normal value, ring 2: 27.3 %, ring 3: 38.9 %, ring 4: 57.9 %, ring 5: 64.2 %). Pattern ERG P50 and N95 waves were not abnormal in all patients (PERG was abnormal in 8 out of 12 eyes), because a larger stimulus field size was used. Electrophysiology recording in 15 years old patient is shown in Figure 8.

Conclusions

Our study demonstrates significant phenotypic variability in patients with Stargardt disease. Three different autofluorescent patterns were seen by AF: hyperautofluorescent parafoveal ring, hypo-hyperautofluorescent flecks centrally (SMD) and central hypoautofluorescent region with extensive hyperautofluorescent flecks all over the fundus (FF). Fundus autofluorescence images in early stages of Stargardt disease typically show a central oval area of reduced signal, surrounded by small disseminated spots of reduced and increased intensity.[17,18]

Transverse loss of photoreceptor layers was observed by OCT in all our patients, which is in accordance with other studies.[9-11] The values of central retinal thickness and total macular volume were significantly lower than expected for the age (CRT: 21.5 % of the normal controls, TMV: 81.3 % of the normal controls).

Multifocal ERG was shown to be of considerable use in these children with Stargardt disease, also under the age of 15. Abnormal mfERG were found in all our patients, which confirmed mfERG a precise test to detect central cone dysfunction especially in early stages of Stargardt dystrophy, as previously shown.[19] It is well known that diseases such as Stargardt disease produce focal regions in the macula which correlates well with undetectable mfERG. The amplitudes of mfERG responses in our patients were under 40 % of mean normal values in the inner three rings, whereas they ranged from 57.9 to 64.2 % of mean normal values in the outer two rings of mfERG. It was shown by another study that at more peripheral loci, mfERG responses approach those of normal subjects, and implicit times are not markedly delayed.[20]

It has already been shown that full-field ERG can have a prognostic value in Stargardt's disease, where early peripheral cone and rod involvement indicates higher risk of development of peripheral visual loss and thus a more severe disease.[21] Full-field ERG responses were normal except in two patients (Table 1, patient 1 and 5), with abnormal 30 Hz and cone responses (electrophysiologically Stargardt type 2). Both patients with abnormal 30 Hz and cone responses had typical clinical picture of Stargardt disease with clearly visible typical yellowish flecks seen on the fundus. Therefore it is sometimes impossible to distinguish between cone-rod dystrophy and Stargardt dy-

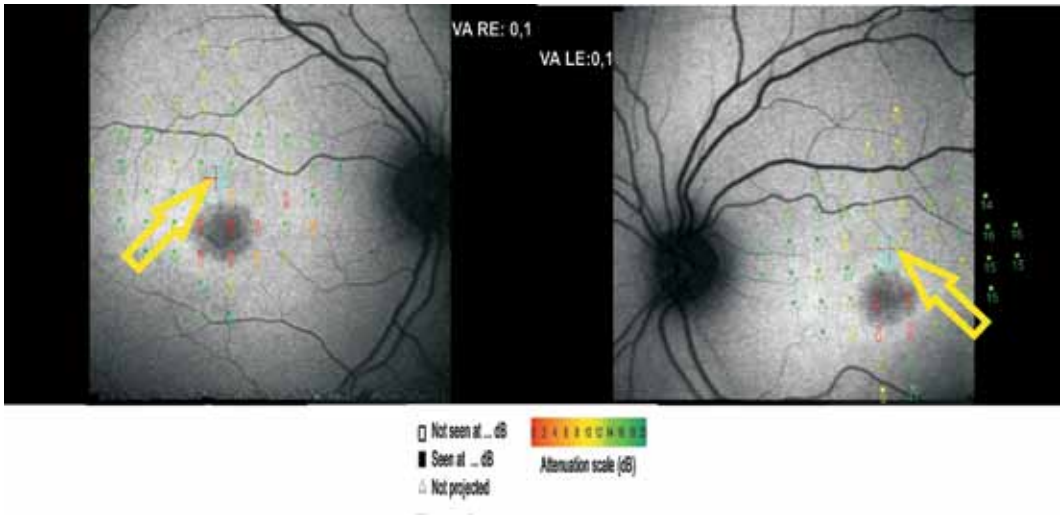


Figure 6: Microperimetry showed reduced central sensitivity and shift of fixation to the PRL superior to the lesion in both eyes (Table 1, patient 3).

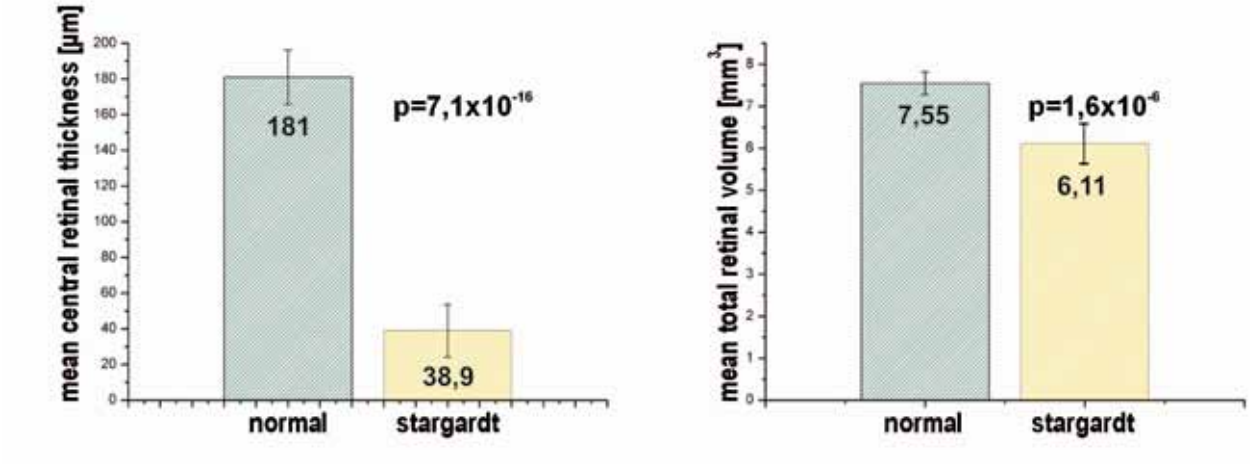
strophy based on electrophysiology results only, especially if the fundus shows no typical yellowish white flecks. At the begining cone-rod dystrophy and Stargardt disesase may exhibit comparable symptoms because both may be caused by the same genetic defect.[4]

PERG was not abnormal in all the patients, which can be explained by using larger stimulus field for stimulation (21.6 ° x 27.8 ° screen stimulator). PERG amplitude dependance on stimulus field size was shown by our previous study done by Lenassi et al,[11] showing that standard stimulus field size allows detection of early maculopathy in patients with Stargardt disease, whereas larger stimulus field provides better evaluation of the degree of retinal involvement and quantification of progression of photoreceptor damage.

Microperimetry showed absolute central scotomas with shift of fixation to the preferred retinal locus mostly superior, rarely nasal to the lesion in all our patients, which is in accordance with other studies. All our patients had unstable or relatively unstable fixation. In other studies reduced stability of fixation has been found even in patients with Stargardt dystrophy and still central fixation.[8]

Children with Stargardt disease can have very poor visual acuity and colour vison without clearly visible fundus changes. Beside genetic testing, autofluorescence imaging of the retina together with ERG recording can help confirming the diagnosis. OCT recordings clearly showed morphological changes in the central retina with photoreceptor layers loss already in children.

Figure 7: OCT showed transverse loss of central photoreceptor layers with lower values of central retinal thickness (CRT) and total macular volume (TMV) compared to controls.



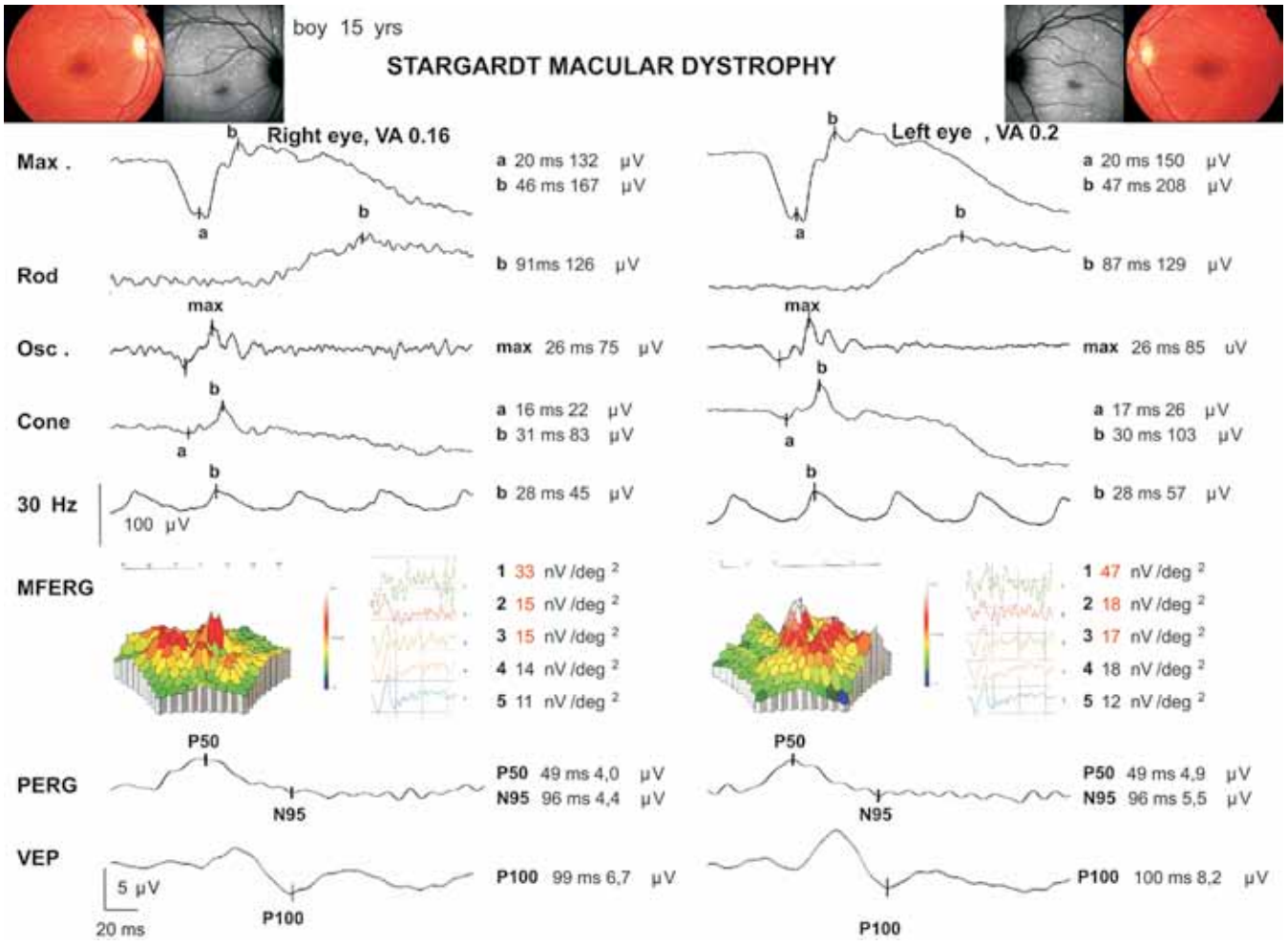


Figure 8:
Electrophysiology results of 15 year old boy with Stargardt dystrophy, note abnormal mfERG, normal PERG and normal VEP (Table 1, patient 6).

Acknowledgments

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