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ORIGINAL PAPER

Retinal single-layer analysis with optical coherence tomography shows inner retinal layer thinning in Huntington's disease as a potential biomarker

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Abstract
Purpose There have been ongoing clinical trials of therapeutic agents in Huntington's disease (HD) which requires development of reliable biomarkers of disease progression. There have been studies in the literature with conflicting results on the involvement of retina in HD, and up to date there is not a study evaluating the single retinal layers in HD. We aimed to evaluate the specific retinal changes in HD and their usability as potential disease progression markers.
Methods This cross-sectional study used spectral-domain optical coherence tomography with automatic segmentation to measure peripapillary retinal nerve fiber layer (pRNFL) thickness and the thickness and volume of retinal layers in foveal scans of 15 patients with HD and 15 age- and sex-matched controls. Genetic testing results, disease duration, HD disease burden scores and Unified HD Rating Scales motor scores were acquired for the patients.
Results Temporal pRNFL, macular RNFL (mRNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer and outer plexiform layer thicknesses and IPL, retinal pigment epithelium and outer macular volume were found lower in HD compared to controls, while outer nuclear layer and outer retinal layer thickness were increased ($p < 0.05$). We found significant correlations between inner retinal layer thicknesses, most significantly with mRNFL and GCL and disease progression markers.
Conclusion The outcomes of this study points out that retinal layers, most significantly mRNFL and GCL, are strongly correlated with the disease progression in HD and could serve as useful biomarkers for disease progression.

Keywords Huntington's disease · Macula · Optical coherence tomography · Retina · Retinal nerve fiber layer · Retinal single layers

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Background

Huntington disease (HD) is a progressive neurodegenerative disorder with the prevalence of 2.71 in 100,000 and incidence of 0.38 in 100,000 [1]. The disease is inherited in an autosomal dominant pattern and affects movement, cognitive functions and personality. It is caused by an expansion of the cytosine-adenine-guanine (CAG) triplet repeat in the 5′ region of the *HTT* gene on chromosome 4p16.3 [2]. Even though there is no current curative treatment modality for this disease, there has been ongoing research and

therefore a rising need for biomarkers that would objectively show the disease progression. Optical coherence tomography (OCT) has been used to show retinal changes in central nervous system diseases. Various studies have shown segmental peripapillary retinal nerve fiber layer (pRNFL) thinning and decrease in macular volume in the neuropathological course of the disease [3]. Recently thinning of macular choroidal thickness also has been shown in HD [4]. However, in a postmortem human study, no histopathological changes were shown microscopically in HD compared to normal healthy retina, contrary to what had been shown in many mouse models in the literature [5]. Recent studies have suggested that determination of thicknesses and volumes of retinal single layers gives more accurate results in neurodegenerative diseases in terms of outlining retinal pathologies, compared to evaluation of pRNFL or total retinal thickness or volume alone [6]. The objective of this study is to outline the specific patterns of retinal single layer pathologies in HD as a potential marker of neurodegeneration and to determine its usability as a diagnostic and progression marker. To our knowledge, this is the first study in the literature studying retinal single layers' thicknesses and volumes in HD.

Methods

The study was approved by the Institutional Review Board of Medical School of Erciyes University (No: 2017/143, March 3, 2017) and was conducted in accordance to the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study. A total of 15 patients with HD, all of them having confirmed genetic diagnosis, and 15 age- and sex-matched healthy volunteers recruited from the patients admitted to the ophthalmology clinic were enrolled in the study. All healthy volunteers also underwent a complete neurological examination and determined healthy neurologically. All patients with HD had their genetic testing results with CAG repeat length. Calculation of the HD disease burden score (DBS = [CAG − 35.5] × age) was performed for all the patients [7]. Neurological examination of the patients included Unified Huntington's Disease Rating Scale (UHDRS) motor scoring, total functional capacity and Independence Scale by a board-certified neurologist specialized in movement disorders [8]. The exclusion criteria for all the participants were refractive errors of spherical values > 5 Diopters and astigmatic values of > 3 Diopters, previous ocular trauma, intraocular pressure greater than 22 mmHg, coexisting ocular disease (i.e., retinal pathology, glaucoma, narrow anterior chamber, senile cataract resulting in poor-quality images), history of cataract surgery within the last year or retinal surgery at any time, admission of topical/systemic medication use that has been known to have impact on retinal thickness (i.e., diuretics, steroids). A complete ophthalmologic examination was performed to all patients and controls and features of detailed ophthalmologic examination were obtained. All participants were examined through dilated pupils. Best corrected visual acuity (BCVA), color vision assessment with Ishihara color plates, anterior segment examination with slit-lamp biomicroscopy, intraocular pressure measurement with Goldmann applanation tonometry, stereoscopic fundus examination through dilated pupils were obtained for all participants.

Spectral-domain optical coherence tomography measurements

Retinal single-layer analysis was performed with commercial spectral-domain (SD)-OCT (Spectralis; Heidelberg Engineering, Heidelberg, Germany) with a ~ 840 nm wavelength. For SD-OCT, only those images with a signal-to-noise score higher than 25 dB were analyzed. Scans with misalignment, segmentation failure, decentration of the measurement circle, and poor illumination or those out of focus were excluded from the analysis. Thus, manual correction of plotting errors of automated segmentation was not performed in this study.

Average thicknesses were calculated for pRNFL, macular RNFL (mRNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), inner and outer segments of the photoreceptors (PR) and retinal pigment epithelium (RPE; Figs. 1, 2). Average layer thicknesses were reported within an annulus centered at the fovea by the software. The annulus covered a 6-mm circle, with the central 1 mm excluded. The results of the paramacular volumetric and different retinal layers measurements were automatically segmented. The measurement of inner

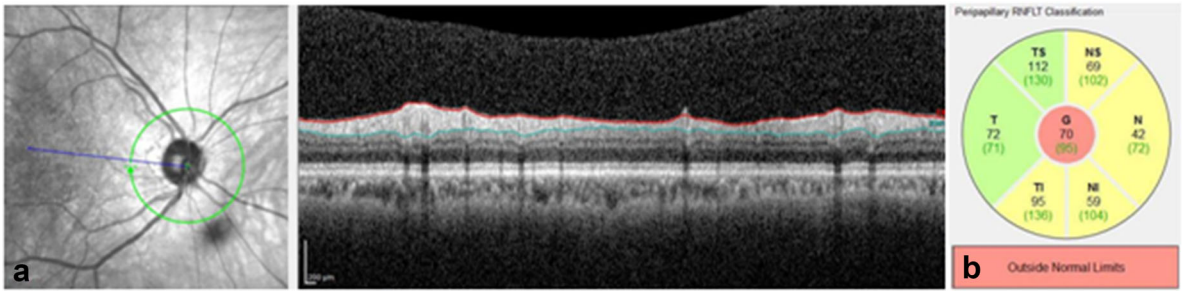

Fig. 1 **a** Peripapillary retinal nerve fiber layer analysis of the right eye of a patient with Huntington's disease in a 3.4-mm circular scan centered on the optic disk and **b** sector distribution

retinal layer (IRL) includes internal limiting membrane (ILM), mRNFL, GCL, IPL and INL, while the outer retinal layer (ORL) consists of OPL, ONL and inner and outer segments of the PRs up to the RPE as defined by the manufacturers of the SD-OCT software [6]. To assess the pRNFL, a circular scan with a diameter of approximately 3.4 mm was performed after manually positioning the center on the middle of the optic disk (Fig. 1). The pRNFL Spectralis protocol generates a map showing the average thickness and maps with 6 sector thicknesses (superonasal, nasal, inferonasal, inferotemporal, temporal and superotemporal) [9]. Macular volumes were quantified using the software of the manufacturer based on the ETDRS protocol (Fig. 3). Three retinal volumes were obtained, centered on the foveola with radii of 1, 3 and 6 mm. Inner macular volume was defined as the average of 5 measurements at foveal center and 3 mm away from the nasal, temporal, superior and inferior directions of the foveal center. Outer macular volume was defined as the average of 4 measurements between 3 and 6 mm ETDRS ring.

Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics 22.0 package program (IBM Corp., Armonk, NY, USA). After verifying that the values did not statistically differ by using a *t* test, only the right eyes from the patients and controls were included in the study. Data are expressed as mean ± standard deviation (SD). Retinal values were reported as microns. Normality and homogeneity of the variances were tested. Parametric values were compared with the Student *t* test. Pearson correlation was used to evaluate the relationship between OCT parameters and HD severity as measured by disease duration, CAG repeats, burden score, total motor score (TMS)-UHDRS, total functional capacity, and Independence Scale. The value of $p < 0.05$ denoted statistical significance.

Results

This is a non-randomized prospective study including 15 eyes of 15 patients (9 males, 6 females) with HD and 15 eyes of 15 healthy controls (9 males, 6 females). The median ages were 48 years in both HD group (31–59 years) and controls (31–60 years). The distributions of age and gender between the groups were similar and there were no significant differences between the groups ($p > 0.05$). The median disease duration of the HD was 5 years (1–16 years). All of the patients were receiving antichorea drug, tetrabenazine; one of the patients was also receiving antipsychotic treatment (risperidone). Mean CAG repeats (larger allele) were 43.3 ± 3.5 long (range 40–50). Mean scores were 9.27 ± 3 for total functional capacity, 82 ± 15 for Independence Scale, 20.7 ± 2 for TMS-UHDRS and 356.3 ± 184.8 for burden score.

Color vision scores were significantly poorer in HD compared to controls (11 ± 3 vs 13 ± 1 out of 14 Ishihara plates; $p < 0.001$). Although not found to be statistically significant, a decreasing trend toward color vision between CAG repeats ($\rho = -0.26$, $p = 0.16$), burden score ($\rho = -0.237$, $p = 0.21$) and TMS-UHDRS ($\rho = -0.209$, $p = 0.26$) was observed. As for the retinal anatomic parameters, color vision was found to be significantly related to ONL thickness ($\rho = -0.65$, $p < 0.001$), ONL

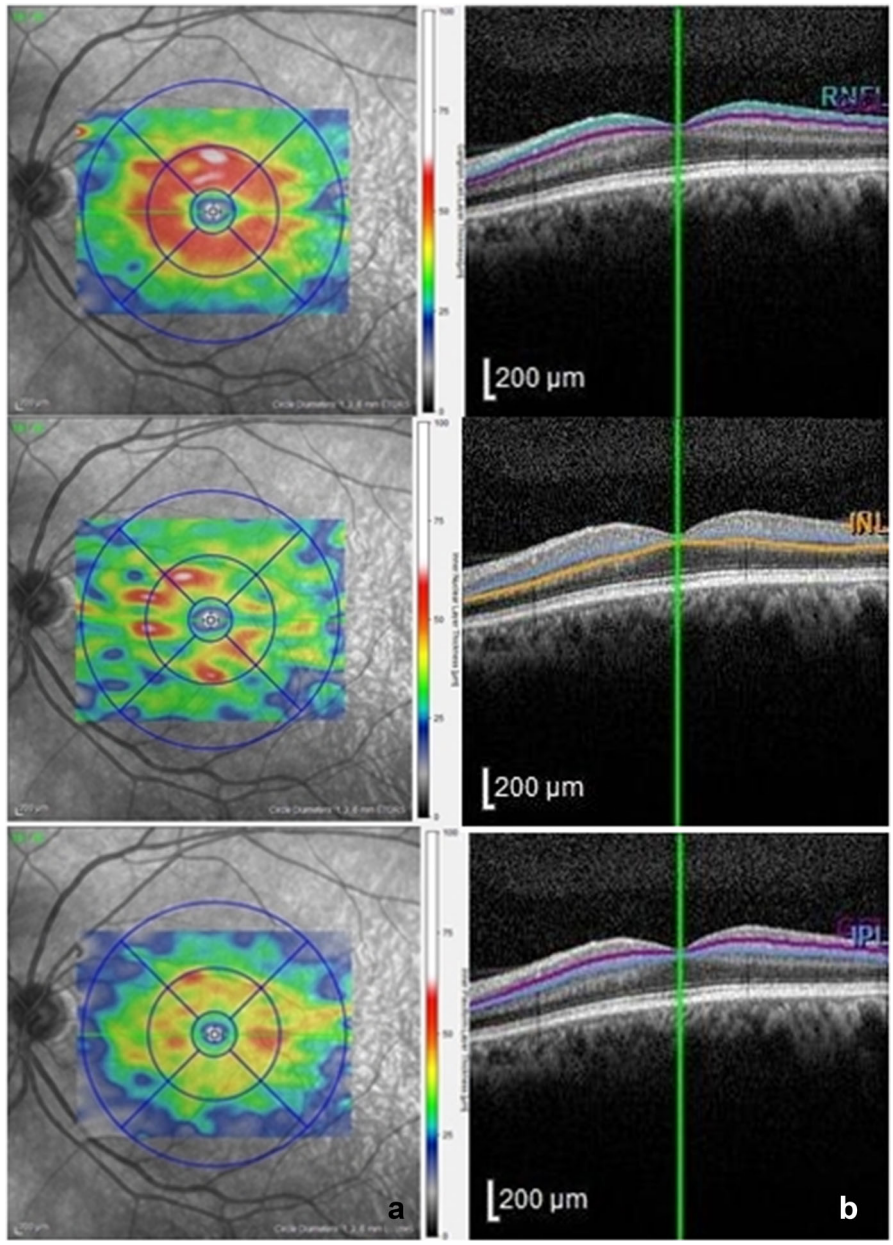

Fig. 2 **a** Macular ganglion cell layer, inner nuclear layer and inner plexiform layer thickness maps of the left eye of a patient in the control group and **b** horizontal B-scans centered in the fovea, showing the automated segmentation of the layers

volume ($\rho = -0.39$, $p = 0.032$), IRL thickness ($\rho = 0.41$, $p < 0.023$) and PR volume ($\rho = 0.43$, $p = 0.017$).

Even though the average pRNFL thickness value was the same among the groups, temporal, superotemporal and inferotemporal sector pRNFL thicknesses were significantly lower in HD group compared to controls ($p = 0.006, p = 0.010, p = 0.028$, respectively; Table 1). Also even though the overall central foveal thickness (CFT) was not found to be significantly reduced in HD, each one of the inner single retinal layers were found to be significantly thinner in HD compared to controls (Table 2). However, the sum of the overall mean inner retinal layer thickness difference did not reach a statistical significance ($p = 0.104$). Contrary to all other retinal single layers, ONL and outer retinal layer thicknesses were found to

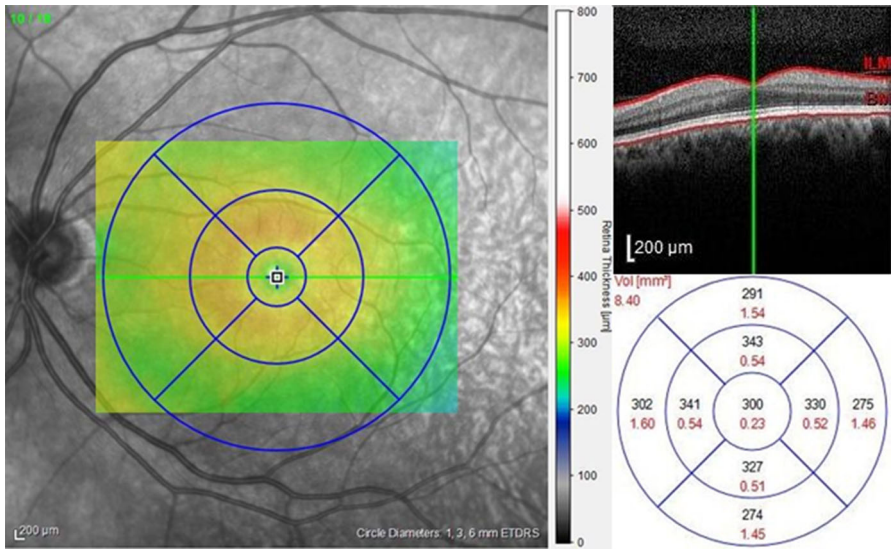

Fig. 3 Macular volume analysis of the left eye of a patient in the control group in circle diameters of 1.5, 3 and 6 mm centered on the foveola

Table 1 pRNFL thickness (µm) of the subjects

pRNFL sector	HD ($n = 30$)	Control ($n = 30$)	p
Average	96.00 ± 7.86	99.23 ± 10.40	0.180
Temporal	64.00 ± 9.51	74.83 ± 10.57	0.006*
Nasal	78.00 ± 14.42	77.80 ± 15.89	0.824
Inferonasal	100.20 ± 14.90	104.26 ± 24.11	0.790
Inferotemporal	134.43 ± 18.85	144.03 ± 20.78	0.028*
Superotemporal	123.76 ± 20.84	137.50 ± 15.34	0.010*
Superonasal	103.50 ± 21.86	113.63 ± 12.71	0.646

The values of the parameters were shown as mean ± standard deviation

pRNFL peripapillary retinal nerve fiber layer and *HD* Huntington disease

*$p < 0.05$

be significantly higher in HD group compared to controls ($p = 0.007$ and $p = 0.013$, respectively).

We found significant correlations between disease progression markers and some retinal single layer thicknesses (Table 3). None of the retinal parameters were found to be associated with functional capacity. We found moderate correlations between mRNFL and GCL thicknesses and progression markers at the 0.01 level and demonstrated with scatter plots in Fig. 4.

We also analyzed the volumes of different retinal layers (Table 4). Volumes of IPL and RPE layer and outer macula were significantly reduced in HD ($p = 0.022$, $p = 0.009$ and $p = 0.020$, respectively). Correlations between progression markers and retinal layer volumes are outlined in Table 5.

Conclusion

This is the first study in the literature that has studied retinal single layers in HD. Recent studies have shown that measurement and analysis of retinal single layers show more accurate information in neurodegenerative conditions rather than examining total retinal thickness or pRNFL thickness alone [6]. This is explained by the theory that retinal ganglion cell bodies are 10–20 times the diameter of their axons, and GCL is more than one cell thick in the macula [10].

We have identified that although the average pRNFL is not seem to be significantly affected, thicknesses of the various sectors of the pRNFL is impaired in HD. We also showed that central foveal thickness and total inner retinal layer thickness alone

Table 2 Retinal single layer thickness analysis of the subjects

Retinal layer thickness (μm)	HD ($n = 30$)	Control ($n = 30$)	p
Central fovea	266.43 ± 18.87	270.03 ± 16.09	0.370
mRNFL	25.95 ± 3.69	30.02 ± 2.34	< 0.001*
GCL	34.92 ± 3.29	44.83 ± 3.03	< 0.001*
IPL	32.08 ± 2.67	36.11 ± 1.97	0.049*
INL	27.44 ± 2.14	36.77 ± 2.44	< 0.001*
OPL	24.17 ± 2.51	30.91 ± 1.42	< 0.001*
ONL	67.59 ± 6.15	60.32 ± 4.69	0.007*
PR	78.40 ± 4.07	87.06 ± 4.91	0.068
RPE	14.22 ± 0.88	14.69 ± 1.14	0.085
Inner retinal layers	227.87 ± 8.99	239.05 ± 8.71	0.104
Outer retinal layers	81.88 ± 1.53	78.35 ± 2.53	0.013*

The values of the parameters were shown as mean ± standard deviation

mRNFL retinal nerve fiber layer, *GCL* ganglion cell layer, *IPL* inner plexiform layer, *INL* inner nuclear layer, *OPL* outer plexiform layer, *ONL* outer nuclear layer, *PR* photoreceptor, *RPE* retinal pigment epithelium and *HD* Huntington disease

*$p < 0.05$

Table 3 Correlation of retinal layer thicknesses in Huntington disease subjects with disease progression markers

Variables	Disease duration		CAG repeats		Burden score		TMS-UHDRS score		Independence Scale	
	r	p	r	p	r	p	r	p	r	p
mRNFL	− 0.406	0.026*	− 0.392	0.032*	− 0.436	0.016*	− 0.443	0.014*	0.527	0.003*
GCL	− 0.556	0.001*	− 0.613	< 0.001*	− 0.656	< 0.001*	− 0.648	< 0.001*	0.442	0.014*
IPL	− 0.420	0.021*	− 0.580	0.001*	− 0.590	0.001*	− 0.443	0.014*	0.182	0.335
INL	− 0.353	0.103	− 0.446	0.013*	− 0.166	0.381	− 0.439	0.015*	0.292	0.127
OPL	− 0.215	0.253	− 0.197	0.297	− 0.171	0.366	− 0.175	0.354	0.107	0.572
ONL	0.086	0.652	0.067	0.724	0.021	0.914	0.031	0.871	− 0.149	0.432
PR	− 0.502	0.005*	− 0.258	0.168	− 0.322	0.082	− 0.413	0.023*	0.416	0.022*
RPE	− 0.276	0.122	− 0.407	0.026*	− 0.375	0.041*	− 0.248	0.192	0.255	0.181
Inner retinal layers	− 0.499	0.05*	− 0.239	0.204	− 0.314	0.091	− 0.437	0.016*	0.395	0.031*
Outer retinal layers	0.289	0.121	0.026	0.893	0.153	0.421	0.054	0.776	− 0.134	0.482

mRNFL macular retinal nerve fiber layer, *GCL* ganglion cell layer, *IPL* inner plexiform layer, *INL* inner nuclear layer, *OPL* outer plexiform layer, *ONL* outer nuclear layer, *PR* photoreceptor, *RPE* retinal pigment epithelium and r spearman correlation coefficient

*$p < 0.05$

are not statistically different in HD compared to controls, yet all the inner retinal single layers plus OPL is reduced in HD when studied by single retinal segmentation. We also found ONL and outer retinal layers were significantly increased in thickness in HD. We also studied volumes and revealed that outer macular volume and various single retinal layer volumes were significantly reduced in HD.

The retina is unique in the way that it shows axonal damage as an accurate model for the study of neurodegeneration as it contains only the demyelinated nerves. Thinning of the pRNFL layer correlates with the retrograde degeneration associated with the damage to the optic nerve or optic tract, and it is shown that the greater the brain atrophy the more is the retinal involvement measured as the loss of pRNFL [11].

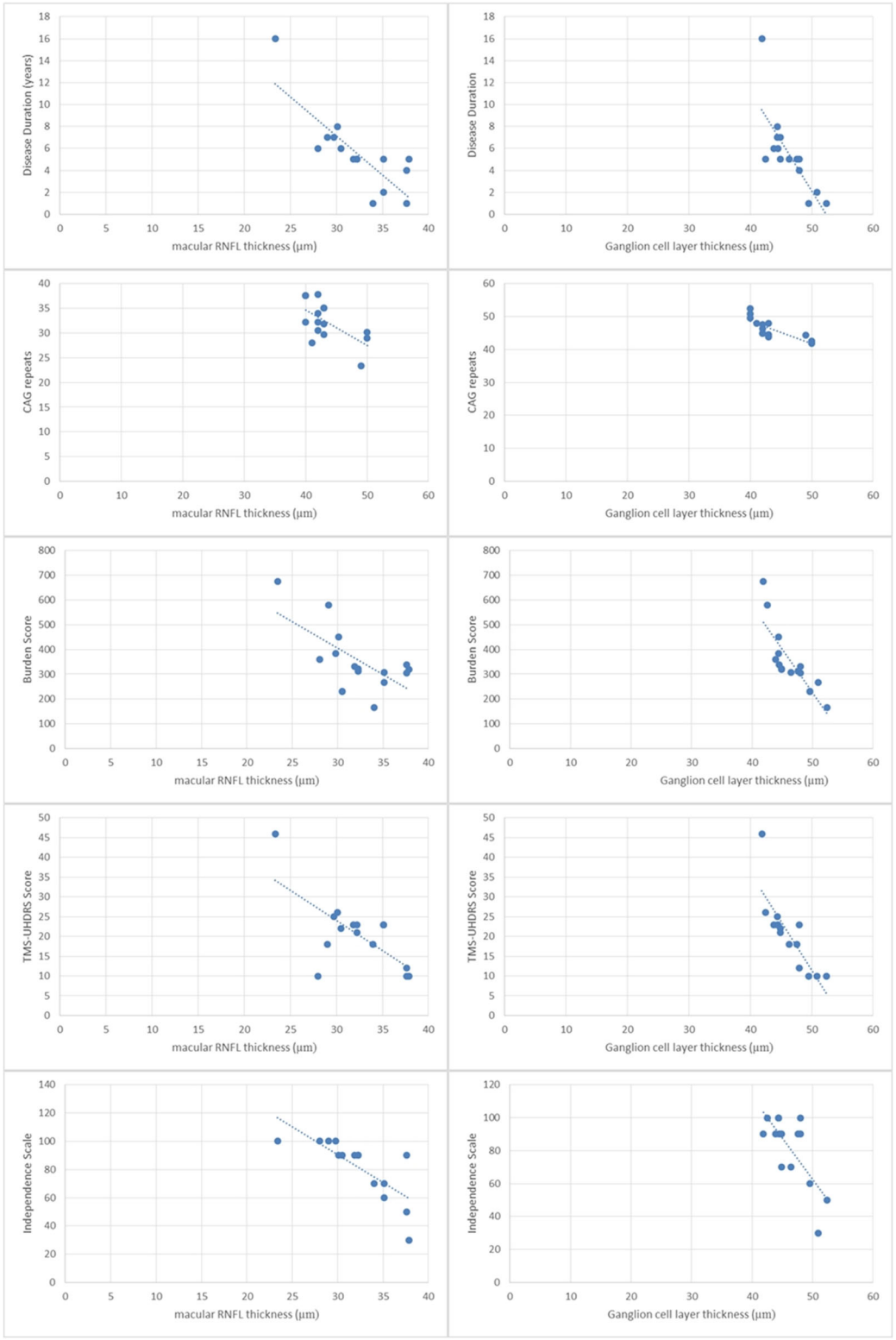

Fig. 4 Scatter plots showing relationships between disease progression markers and optical coherence tomography parameters

Table 4 Retinal single layer volume analysis of the subjects

Retinal layer volumes (mm^3)	HD ($n = 30$)	Control ($n = 30$)	p
Central fovea	0.20 ± 0.01	0.21 ± 0.01	0.158
mRNFL	11.53 ± 2.28	13.43 ± 1.22	0.923
GCL	1.06 ± 0.13	1.12 ± 0.07	0.192
IPL	0.87 ± 0.09	0.92 ± 0.04	0.022*
INL	0.95 ± 0.07	0.97 ± 0.04	0.523
OPL	0.83 ± 0.08	0.83 ± 0.06	0.275
ONL	1.67 ± 0.15	1.62 ± 0.15	0.057
PR	2.18 ± 0.09	2.20 ± 0.09	0.172
RPE	0.38 ± 0.02	0.40 ± 0.03	0.009*
Inner retinal layers	6.32 ± 0.42	6.42 ± 0.29	0.830
Outer retinal layers	2.18 ± 0.09	2.20 ± 0.09	0.238
Macula	8.50 ± 0.49	8.62 ± 0.33	0.558
Inner macula	2.35 ± 0.07	2.32 ± 0.10	0.103
Outer macula	6.14 ± 0.43	6.30 ± 0.30	0.020*

The values of the parameters were shown as mean ± standard deviation

mRNFL macular retinal nerve fiber layer, *GCL* ganglion cell layer, *IPL* inner plexiform layer, *INL* inner nuclear layer, *OPL* outer plexiform layer, *ONL* outer nuclear layer, *PR* photoreceptor, *RPE* retinal pigment epithelium and *HD* Huntington disease

*$p < 0.05$

Table 5 Correlation of retinal layer volumes in Huntington disease subjects with disease progression markers

Variables	Disease duration		CAG repeats		Burden score		TMS-UHDRS score		Independence Scale	
	r	p	r	p	r	p	r	p	r	p
mRNFL	− 0.183	0.333	− 0.349	0.059	− 0.436*	0.016*	− 0.589	0.001*	0.298	0.110
GCL	− 0.572	0.001*	− 0.714	< 0.001*	− 0.676	< 0.001*	− 0.613	< 0.001*	0.454	0.012*
IPL	− 0.545	0.002*	− 0.562	0.001*	− 0.577	0.001*	− 0.531	0.003*	0.350	0.058
INL	− 0.373	0.051	− 0.528	0.003*	− 0.447	0.013*	− 0.452	0.012*	0.373	0.063
OPL	− 0.344	0.063	− 0.483	0.007*	− 0.430	0.018*	− 0.365	0.048*	0.261	0.163
ONL	0.279	0.135	0.267	0.154	0.306	0.100	0.263	0.161	0.383	0.057
PR	− 0.490	0.006*	− 0.224	0.233	− 0.243	0.195	− 0.262	0.161	0.249	0.185
RPE	− 0.444	0.014*	− 0.359	0.051	− 0.332	0.073	− 0.430	0.018*	0.415	0.022*
Inner macula	0.197	0.296	− 0.045	0.812	− 0.288	0.123	− 0.168	0.374	0.260	0.165
Outer macula	− 0.507	0.004*	− 0.142	0.455	− 0.346	0.061	− 0.302	0.105	0.261	0.163
Total macular volume	− 0.368	0.052	− 0.426	0.019*	− 0.439	0.015*	− 0.181	0.338	0.312	0.093

mRNFL macular retinal nerve fiber layer, *GCL* ganglion cell layer, *IPL* inner plexiform layer, *INL* inner nuclear layer, *OPL* outer plexiform layer, *ONL* outer nuclear layer, *PR* photoreceptor, *RPE* retinal pigment epithelium and *r* spearman correlation coefficient

*$p < 0.05$

There are very few studies in the literature evaluating the use of SD-OCT in HD with conflicting results. In a study recruited by Kersten et al. [3], they also found significant thinning of temporal pRNFL in HD significantly correlated with the disease duration. They interpreted these findings with the hypothesis that the progression of the disease causes the atrophy of the small retinal ganglion cells in macula which consequently affects the papillo-macular bundle which is located on the temporal aspect of the optic nerve that is affected by the disease either directly or as a result of damage to their cell bodies in macula. We analyzed this hypothesis and showed that along with the mRNFL, the GCL thickness in macula is strongly reduced in HD compared to controls, and GCL seems to be the most affected retinal layer as it is strongly

correlated with the progression markers of the disease. Disadvantageous energy conditions of the small parvocellular axons are thought to be the cause of the loss of temporal retinal ganglion cells and their axons in mitochondrial diseases [12]. Contrary to the latter studies and our findings, Andrade et al. [4] found no difference in average pRNFL thickness or in the different quadrants in their study. However, in eight patients with HD, their sample size is relatively small and that could be the reason for the differences not reaching clinical significance in their results. In patients with MS, studies have shown that GCL and IPL were altered more frequently than average pRNFL as we also found no alteration of average pRNFL in HD [9]. It was also shown that ganglion cell inner plexiform layer complex thickness had a better sensitivity than temporal pRNFL thickness for detecting retinal thickness changes and also was a potential predictor of axonal damage better than pRNFL [13, 14]. These findings are interpreted as the probable fact of neuronal cell bodies suffering an earlier affection than the retinal axons [15]. We also found that GCL was the most significant parameter as a retinal biomarker for progression.

In our study, we found that although the central foveal thickness measurement was not different from controls, all inner retinal layers were thinner in HD particularly. We also found significant thinning in OPL of the outer retina in HD, while the ONL was thicker. We think that the thickening of ONL could be due to a state arising from the swelling of cone cells and enlargement of the nuclei which was also shown in retinal histological examinations of human eyes, even when there was no stated photoreceptor loss with chronic glaucoma, which was also noted in OCT studies as ONL thickening in fovea of patients with glaucoma [16, 17]. Also, Paulus et al. [18] documented electrophysiological changes in 19 patients with HD as higher thresholds during adaptation which suggested abnormal cone function. A recent R6/2 mice study also revealed significant decrease in the cone nuclei count and a decline in cone-mediated ERG responses [19]. In this study, Li et al. stated that the ONL showed a waxy appearance and displayed disorganization in the progressed state. These findings correlates with our findings of the increase in the ONL and outer retinal layers thickness in HD compared to controls. We also found that there was a positive correlation with outer retinal layer thickness with the duration of the disease. However, the correlations of the outer retinal layer volumes were inverse with the disease duration and functional capacity. Another study with R6/1 mouse line showed similar retinal pathologies in HD such as cone-specific dysregulation and functional deficit, and eventual retinal remodeling, Müller cell gliosis and cell death but with later onset and slower progression [20]. Müller cell bodies sit in the inner nuclear layer which we also found to have decreased in thickness in HD. In spite of the findings of these experimental models, a postmortem human microscopic study revealed no histopathological differences in the retina of a patient with a 28-year history of HD compared to normal healthy retina [5]. To date, the histological studies mainly have been on drosophila and mouse models. A report from R6/2 transgenic mice revealed mutant *Htt* expression levels being similar in their retinas as their brains with the aggregates being found in all three nuclear layers most profoundly in GCL neurons [21]. In our study, our findings support these findings and suggest that GCL thickness is the strongest available OCT marker useful for determining the disease progression as it was found significantly correlated to all the disease progression markers of HD most of them being at the 0.01 level.

A very recent study reported that the average macular choroidal thickness was reduced in HD [4]. It is also possible that choroidal dysfunction could affect the retinal layers, leading to a thickening of ONL due to the accumulation of disorganized or apoptotic cells. Very recently, immunohistochemistry showed *HTT* accumulation in horizontal cells and suggested loss of amacrine cells on an experimental study on rats, which suggested that HD appears to selectively affect retinal interneurons causing inner retinal degeneration that supports our findings of inner retinal layer thinning with OCT [22]. Through volume analysis we also found that although the central foveal and overall macular volume was not reduced significantly in HD, the outer macula in between the 3- and 6-mm ETDRS ring was found to be significantly reduced in HD. It is known that inner retinal layers are absent in the very macular center (foveola) and gradually emerge toward the macular periphery [4]. Andrade et al. [4] stated that because of this known fact rather than assessing the macula by quadrants, the radial distance to the foveola may be more significant which supports the results of our findings. We think that because of the more

selective loss in inner retinal layers our results confirmed a thinning in outer macular volume.

We found that all inner retinal layers had significant inverse correlations with disease progression scores, mostly with CAG repeats, burden score and TMS-UHDRS. In another study, the authors also observed a decline in macular retinal thickness as the disease progresses [4].

Our study has some limitations: Taking into account HD is rare, the sample size of our study is small, more so for the number of parameters analyzed. We performed normality testing and have used parametric tests. However, with the small sample size in each group, the power of the data distribution tests might have been low and nonparametric tests in this situation would have reduced the chance of detecting a significant difference when one exists. Also in the case of correlations between parameters, even though the p values showed strong significance, the r values were only able to show moderate correlations which could be due to the small sample size in the study. Unfortunately, we had not performed visual field testing to the participants. Narrowing or decreasing sensitivity in the HD group with visual field testing would have supported the finding of GCL loss in the patients we have shown with OCT and would be very valuable. Future studies combining visual field testing with OCT in patients with HD should be carried out. With the new Heidelberg Spectralis software that allows automated segmentation of the retinal layers, we have confirmed and extended our understanding of the effects of HD on intra-retinal layer thicknesses and volumes and assessed the relationship of those changes to disease progression markers. Further, through comparison of HD eyes with age- and sex-matched controls, we have discovered some new findings in the inner intra-retinal layers of HD eyes. In the inner retina, each one of the retinal layers was thinner in HD eyes. We also found significant thinning of OPL of outer retinal layers in HD eyes, in contrast, the ONL was thicker. This is the first report in the literature investigating the retinal single layers and their relation with progression markers and points out that retinal layers, most significantly mRNFL and GCL, are correlated with the disease progression in HD and could serve as useful biomarkers for disease progression in HD.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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