Progression of anterograde trans-synaptic degeneration in the human retina is modulated by axonal convergence and divergence

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In the visual pathway of patients with multiple sclerosis (MS), the inner nuclear layer (INL) of the retina is a tight barrier for retrograde trans-synaptic degeneration. In this observational, retrospective cross-sectional study, segmented macular spectral domain optical coherence tomography (OCT) volume scans are reviewed to investigate if this observation also holds true for anterograde trans-synaptic degeneration. Significant thinning was found in all retinal layers in patients with outer retinal diseases compared to the healthy controls, while there was no significant attenuation of the outer retina in patients with MS. In contrast to the tight barrier function observed with retrograde trans-synaptic degeneration, the INL appears to be more permissive for propagation of anterograde trans-synaptic degeneration. We speculate that this may be due to the size of the area affected and be explained by convergence and divergence of axons within the retinal layers. These findings are likely relevant to future restorative stem cell treatment of the outer retinal layers, as time may matter.

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Introduction

Degeneration of the nervous system is driven by a range of mechanisms. One of these is trans-synaptic degeneration (TSD). In TSD axonal loss leads to apoptosis of functionally connected, but anatomically remote neurons. Propagation of this neurodegenerative cascade occurs in two directions: anterograde (Wallerian) and retrograde TSD. In anterograde degeneration, axonal injury gives rise to degeneration towards the distal axon terminal.[1] Retrograde degeneration occurs towards the proximal cell body.[2,3] In addition, TSD describes how neurodegeneration is passed on to the next neuron.

Recent research on retrograde TSD in the visual pathway of patients with multiple sclerosis (MS) suggests a possible physiological barrier at the level of the inner nuclear layer (INL) of the retina. Retrograde degeneration pointed towards the retina and was held at the INL. The outer retinal layers remained undamaged.[1] It was hypothesized that this would be due to the neuroplastic nature of the INL, a retinal layer that consists of a network of multiple closely connected cell types.[4,5] In addition, evidence for bidirectional TSD in the visual system was given for patients with MS. Anterograde TSD (towards the visual cortex) was found as an effect of optic neuritis, thus resulting in atrophy of the visual cortex.[6,7]

However, little is known about the extent of anterograde TSD, originating from the outer retina. This process is hypothesized to start at the photoreceptor level and then proceeding towards the inner retina. Few studies, however, have noted that eye diseases originating from structures in distal parts of the retina (pigment epithelium, photoreceptors and outer nuclear layer) usually remain confined to this section. Progression to severe stages may lead to only mild attenuation of the inner parts of the

retina.[8,9] Quantitative evidence from layer specific optical coherence tomography (OCT) segmentation to support this observation is lacking.

Most research on the subject of TSD in the retina includes the use of OCT, a non-invasive technique that images intraretinal structures.[10] Separate retinal layers can be segmented and analysed individually.[11] Atrophy (thinning) of the retinal layers is directly related to the neuronal degeneration.[4] This makes the retina a suitable model to investigate bidirectional TSD.

The aim of this study is to investigate the existence of a physiological barrier to bidirectional TSD, originating from the direction of outer retinal layer and inner retinal structures. Study objectives are to identify the physiological or physical structure of this barrier and pinpointing structural changes as seen in OCT.

Methods

This study was approved by the medical ethics committee of the VU Medical Center in Amsterdam, The Netherlands (protocol 15.431), and is declared to not be subject to the Medical Research Involving Human Subjects Act.

Patient population and study design

In this observational, retrospective cross-sectional study, findings in segmented and analysed macular OCT volume scans are reviewed. The study population includes patients referred to the VU Medical Center department of Ophthalmology and the VU Medical Center Dutch Expertise Centre Neuro-ophthalmology (ORPHA436691), who previously underwent SD-OCT imaging (Spectralis, Heidelberg Engineering, Heidelberg, Germany). The study population consists of three groups including patients

diagnosed with ocular diseases originating from outer retinal structures, patients with MS and a group of healthy controls (HCs).

The MS cohort includes a group with existing multiple sclerosis associated optical neuritis (MSON) and a group with MS absent of optic neuritis (MSNON), divided into three subtypes of MS, relapsing remitting (RR), secondary progressive (SP) and primary progressive (PP). This cohort as well as the HC cohort have been described in previous research.[4]

Patients diagnosed with outer retinal diseases were manually selected and enrolled from an outpatient database in which diagnosis was stated from September 1st 2011 through September 15th 2015. Physicians of the department of Ophthalmology were able to refer additional patients from October 26th 2015 through the 4th of January 2016, in case potential eligible patients were not present in the outpatient database. In order for patients to be eligible to participate in this study, diagnosis with current outer retinal originated disease was necessary. The outer retinal diseases used in this research include Fundus Flavimaculatus/ Stargardt Disease, Rod-cone-/Cone-rod dystrophies, Geographic Atrophy (GA), Central Areolar Choroidal Dystrophy (CACD), Vitelliform macular dystrophy/ Best disease, Acute Zonal Outer Occult Retinopathy (AZOOR) and Bull's-eye maculopathy (BEM). The key pathological features of these diseases are summarised in Table 1.[12-24]

The exclusion criteria for this study are based on the pathology of the retina to be considered in OSCAR-IB criteria and were modified from the original table in the OSCAR-IB publication.[25] They include patients with retinal detachments with macular

involvement, an epiretinal membrane, a history of ocular photocoalgulation or retinal surgery, vascular ocular disease (e.g. anterior optic neuritis, posterior optic neuritis, central retinal occlusion or central retinal vein occlusion), >6 dioptre of myopia or hyperopia, diabetic retinopathy, or exudative macular degeneration. These criteria concerning the overall retinal integrity as well as criteria which presumably influence OCT quality and segmentation quality. Patients meeting any of the exclusion criteria were excluded alongside patients in whom no definite diagnosis could be made.

Patients had to have macular OCT volume scans of good quality in order to be eligible to participate in this study. The availability and quality of macular OCT volume scans was examined in all patients. Patients in whom there were no macular OCT volume scans were excluded. Macular OCT volume scans of poor quality due to vitreoretinal interface problems were excluded as well, before commencing OCT segmentation.

OCT segmentation

The available macular OCT volume scans were acquired with a SD-OCT device (Spectralis, Heidelberg Engineering, Heidelberg, Germany, Software version 1.7.1.0). Due to the retrospective nature of this study, the settings of the OCT machine were not standardized. Segmentation of the retinal layers was acquired using Heidelberg segmentation software (Heidelberg Engineering, Heidelberg, Germany, Software version 5.9.0.3.), a reliable segmentation technique.[11] After the automated segmentation by the software, manual quality control took place. When an error due to algorithm failure was observed, manual reconstruction of the deviated lines was in order. This was done by using the same Heidelberg software. When manual construction was not achievable due to low OCT quality, the OCT scans were excluded. For each retinal

layer, a mean thickness in μm is determined on a 1 mm, 2.22 mm and 3.45 mm grid, which is shown in Figure 1.

The thicknesses of the RNFL, the GCL, the IPL and the INL were exported from the software for the perimacular area, including the middle four grid areas, thus excluding the foveolar area, in which the INL is not present. The outer retinal thickness was calculated by subtracting the exported retinal thicknesses from the total retinal thickness. The OCTs of the MS patients and healthy controls were previously quality controlled and now used in the same smaller (1 mm, 2.22 mm and 3.45 mm) grid.

Patient records

Various variables were collected to maintain homogeneity between the three main patient groups. Table 2 shows the collected variables from patient records of the included patients.

Statistical analysis

Demographic data was analyzed using a general linear model (GLM). GLM was chosen because there were more than two groups, the data distribution of which violated ANOVA asumptions. The outer retinal diseases were put together in one group, the so called outer retinal diseases group. The other groups were MS patients and HCs.

In the OCT research, primary study parameters were perimacular layer thicknesses of several layers. Apart from the total retinal thickness, the thickness of the RNFL, GCIP (GCL+IPL), INL and ORL2 (Outer retinal layers: Retina-(RNFL+GCIP+INL)) were used. These mean thicknesses were compared between three groups: patients who suffer

from outer retina layer (anterograde) or ganglion cell layer (retrograde) loss, and in healthy subjects. In addition, analysis within subgroups of patients with anterograde loss was performed to assess whether variance within groups was be present. General estimating equations (GEE) will be used for group comparisons, which permits to correct for inter-eye interactions and is therefore regarded as the current standard in the field.[26,27]

Results

Patients

Figure 2 shows the exclusion and inclusion of the outer retinal disease patients. As a total of outpatient database and physicians references, information on 513 patients (753 eyes) was examined. A total of 469 patients (682 eyes) were excluded due to a wrong diagnosis or as a result of the patient meeting the exclusion criteria. Forty-four patients (71 eyes) were subsequently investigated on available macular OCT volume scans. Four patients (seven eyes) were excluded due to absence of macular OCT volume scan or poor quality scans due vitreoretinal interface problems. As a following step, the macular OCT volume scans of 40 patients (64 eyes) were then segmented and corrected with the Heidelberg software. In this process, three patients (four eyes) were excluded due to low quality OCT. The data on the macular OCT volume scans of 37 patients (60 eyes) was then used for statistics. Table 3 shows the distribution within this group, as well as information on the MS and HC subjects.

Demographic variables

The GLM analysis on the demographic dependent variable age shows a significant (p=0.0196) difference between the three groups. This procedure also shows a significant

(p<0.0001) difference between ORD and MS patients when looking at the BCVA on the date of the OCT.

Within the group of ORD, the groups were distributed non-Gaussian and were not comparable in size. As for age, patients with AZOOR (p=0.0034), Best (p=0.0027), Stargardt (p=0.0002) and rod-cone dystrophy (p=0.0043) were significantly younger than patients with geographic atrophy. When looking at BCVA, patients with AZOOR (p=0.0196; p=0.0105) and Best (p=0.0096; p=0.0066) had a significantly higher BCVA compared to patients with geographic atrophy or rod-cone dystrophy. The follow-up in patients with AZOOR (p=0.0417), areolar atrophy (p=0.0485), Best (p=0.0285) and Stargardt (p=0.0488) were reported to be significantly shorter than in patients with geographic atrophy and rod-cone dystrophy.

OCT data analysis

Table 4 shows OCT data of the three groups and their comparison corrected for inter-eye correlation within subjects. Significant thinning is visible of all retinal layers in patients with ORD compared to the healthy controls. The information also shows significant thinning of the RNFL, GCIP and retina in patients with MS, compared to healthy controls. There is no significant thinning of the INL or outer retina in patients with MS, compared to healthy controls. Additionally, the INL and ORL2 of patients with ORD is significantly thinner than in patients with MS.

Discussion

The single most striking observation to emerge from the data comparison was that the RNFL, GCIPL and INL in patients with outer retinal diseases is significantly thinner than in healthy controls. In other words, anterograde TSD which emerges form the outer

retina seems to propagate beyond the level of inner nuclear layer. These results may indicate a limited barrier function of the inner nuclear layer when it comes to anterograde TSD. This observation is relevant for restorative stem cell therapy of the outer retina, as time may matter.[28] Early treatment, prior to loss of RNFL/GCIPL will have a higher likelihood of functional success, than later treatment, once TSD of the GCIPL/RNFL has started. In Figure 4 we summarise our interpretation of these data graphically.

Mild attenuation of the inner parts of the retina in severe cases of outer retinal disease were previously described by Fujiwara *et al* and Shields *et al*.[8,9] Two other studies by Lim *et al* and Ohta *et al* also described inner retinal thinning in progressive outer retinal diseases.[29,30] These results are consistent with present data. Because of the broader spectrum of diseases studied at various levels of progression it seems reasonable to extrapolate from our observation to other retinal disorders. Future studies should try to obtain longitudinal data to better investigate the time relationship between disease duration and propagation of TSD. In addition, other neurodegenerative mechanisms playing a role thinning of the retinal layer should be taken into account. Such data will be needed to explain the variation of inner retinal layer atrophy observed in Figure 3.

In contrast to anterograde TSD, retrograde TSD appears to stop at the level of the INL. These results are consistent with data obtained in previous studies in which the same cohort was used and a detailed recent meta-analysis of the existing literature.[4,7,31]

One important and time consuming limitation of the automated proprietary segmentation software of macular OCT scans was the high number of individual layer

algorithm failures. This was likely caused by loss of contrast between layers in outer retinal disease. There was need to manually correct individual B-scan in about 80% of the macular volume scans. As the outer retina decreases in size in these patients, the proprietary software struggles to place the correct lines. Focal disruptions and deletions of the outer retinal tissue made the macular OCTs seemingly unsuitable to automated segmentation. This problem has also been described with the use of other types of OCT segmentation software.[32] Our manual correction was guided by the results of a previous multi-rater study.[11] So one limitation of the present study, use of a small EDTRS grid only is explained by the poor inter-rater reliability of manual segmentation in the outermost sectors.[11] Future studies should also consider a region specific approach to further optimise data analysis.[33] Another technical limitation of this study is that is is not yet possible to permit for well defined intra-individual comparisons of matched region specific individual layer segmentation data. Future methodological developments should consider to permit for selection of geographic areas for analyses to be matched between subjects.

The most important shortcoming of this study is the retrospective design of an electronic database search. In 469 of 513 cases a careful review of the notes did reveal alternative diagnoses, co-morbidity or flagged exclusion criteria which substantially reduced the numbers of patients to be included. Likewise, the cross-sectional nature of the study did not permit to describe the time course of TSD. Therefore, longitudinal prospective data with rigorous inclusion and exclusion criteria are needed. Only this will permit for correlation of progression of degeneration to several other research parameters such as disease progression and changes in visual acuity.

It is likely that propagation of TSD varies between diseases and will be influenced by genetic (Table 1) and epigenetic factors. Straightforward Mendelian conditions will be different to spectrum disease. Contemporary understanding of the aetiology of AZOOR and BEM suggests spectrum disease. For example, AZOOR has been linked to a 28% incidence of one or more autoimmune diseases. The involvement of viral or infectious aetiologies has not been excluded either.[22] Likewise, the aetiology of BEM is thought to be multifactorial.[23,24] All of these likely add to variation of propagation of TSD in retinal pathology. Finally, one needs to consider the possibility of a wheel range of other neurodegenerative   factors, vascular aetiologies, systemic diseases, nutritional and toxic issues, all of which we   are unable to address with this retrospective study.

The INL is known to be capable of high neuroplasticity, mostly due to the presence of a network of amacrine, bipolar and horizontal cells.[4] The understanding of the literature on neuroplasticity (in the visual pathway) is limited. Therefore, these cells' high synaptic plasticity and dense network make for an interesting field of research.[5,34] This study contributes to the understanding of neuroplasticity as a possible dam for (anterograde) TSD. Previously, neuroplasticity was found to be a possible dam in (retrograde) TSD.[4] Creating or inducing neuroplasticity may therefore be a promising strategy to limit propagation of neurodegeneration by TSD.

In summary, this study is consistent with previous research on the capacity of the inner nuclear layer to represent a physiological barrier in retrograde TSD. In contrast, there is a limited barrier function of the inner nuclear layer when it comes to anterograde TSD.

We have discussed factors likely to modulate propagation of anterograde TSD through the INL, such as size of the area affected, disease duration, genetic and epigenetic factors. Our findings are likely relevant for planning of future retinal stem cell treatments building on the success of recent trials.[28] We anticipate that such vision restorative treatments will only have a chance of success if initiated in time to prevent anterograde propagation of TSD through the INL.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Acknowledgements

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Table 1 Pathogeneses of included ocular diseases. N/A= not available

Disease	Frequently reported cause	Origin	Results in	OMIM entry (+hyperlink)
Stargardt Disease/Fundus Flavimaculatus	ATP-binding cassette (ABCA4) gene mutation.	Photoreceptor (PR) discs.	Loss of retinal pigment epithelial (RPE), rods and cones.	248200
Rod-cone-/Cone-rod dystrophies	ABCA4 gene mutation.	PR discs.	Loss of RPE and PR.	601777, 600624, 603649, 604116, 600977
Geographic Atrophy (GA)	Incompletely understood, advanced stage of age-related macular degeneration (ARMD).	RPE, outer neurosensory retina and the choriocapillaris.	Dysfunction RPE, loss of PR.	ARMD: 603075
Central Areolar Choroidal Dystrophy (CACD)	Broad variation of mutations in the peripherin/RDS gene.	PR outer segments/discs.	Loss of PR, RPE and choriocapillaris.	215500, 613144, 613105
Vitelliform macular dystrophy/Best disease	Bestrophin 1 (BEST1) gene mutation.	RPE.	RPE atrophy, deposition of fibrillary material beneath RPE.	153700, 153840
Acute Zonal Outer Occult Retinopathy (AZOOR)	Not known.	PR outer segments.	PR outer segment dysfunction.	N/A
Bull's-eye maculopathy (BEM)	Prominin-1 (PROM1) gene mutationABCA4 other.	Varies.	Varies.	608051

Table 2 Patient variables to be collected

Parameter	Definition
Age	Age in years at date of OCT
Gender	Male or female
Ocular disease	See Table
Diagnostic certainty	Confirmed by genetic testing or clinical diagnosis
Disease duration	As reported at time of performed OCT (not known, 1-3mo, 3-12mo, >12mo)
Visual acuity	Best corrected visual acuity (BCVA) measured in both eyes. Right eye-Left eye (RE-LE)
Refraction	Dioptre (D)
Date of OCT	Date
Date last follow-up	Date
Findings of media and fundus examination	The appearance of the media and fundus of the eye

Table 3 Patient characteristics Age in years (median (Inter quartile range (IQR))). BCVA in decimal scale (median (IQR)). Follow-up in months from last OCT to most recent contact (median (IQR)). Disease duration in the scale [0=not known. 1=1-3 months, 2=3-12 months, 3=>1 year] (median (IQR)). Gender in male to female ratio (M:F). N/A: Not available.

	Patients (N)	Age	BCVA	Follow-up	Disease duration	Gender (M:F)
AZOOR	2	39 (33-44)	0.8 (0.6-1.0)	0 (0-0)	3 (3-3)	2:0
Best	10	62 (38-73)	0.7 (0.3-0.8)	0 (0-0)	3 (3-3)	6:4
Bull's Eye Maculopathy	1	53	0.1	15	3 (3-3)	1:0
Central Areolar Choroidal Dystrophy	2	61 (46-76)	0.3 (0.2-0.5)	1 (0-1)	3 (3-3)	2:0
Cone-rod Dystrophy	4	50 (33-66)	0.1 (0.0-0.1)	4 (0-12)	3 (3-3)	2:2
Geographic Atrophy	8	84 (80-87)	0.1 (0.0-0.3)	0 (0-14)	3 (3-3)	3:5
Healthy controls	39	50 (47-55)	N/A	N/A	N/A	27:12
MS patients	40	56 (52-65)	0.9 (0.7-1.0)	N/A	N/A	25:15
Stargardt	10	51 (34-59)	0.2 (0.0-0.4)	1 (0-1)	3 (3-3)	4:6

Table 4 OCT data of included eyes at the perimacular area (1mm to 2.22mm from the foveola). The OCT data represents the absolute (µm, mean±SD) data. *P values from the GEE analyses, corrected for inter-eye correlation within the included subjects. Retinal nerve fibre layer = RNFL, ganglion cell layer + inner plexiform layer = GCIP, inner nuclear layer = INL, outer retinal layers (retina-(RNFL+GCIP+INL)) = ORL2, outer retinal disease eyes = ORD eyes.

Layer	HC eyes (N)	ORD eyes	MS eyes	GEE p Value
	N_{eyes}=78	N_{eyes} =60	N_{eyes} =79	
Retina	344.90 (13.9)	276.8 (55.7)	325.5 (22.4)	<0.0001*
RNFL	19.3 (1.1)	18.3 (4.7)	18.6 (1.4)	0.7010*
GCIP	91.6 (8.2)	74.4 (19.4)	75.2 (17.5)	0.8405*
INL	39.4 (3.1)	34.9 (7.2)	40.8 (3.9)	<0.0001*
ORL2	194.6 (8.6)	149.2 (46.7)	191.9 (9.3)	<0.0001*