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Retinal nerve fiber thickness and MRI white matter abnormalities in healthy relatives of multiple sclerosis patients

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ABSTRACT

Objectives: To compare retinal nerve fiber (RNFL) thickness and conventional and non-conventional MRI characteristics of healthy controls (HCs) from the general population (non-fHC) to healthy relatives of multiple sclerosis (MS) patients (fHC).
Methods: Sixty-eight (68) HCs underwent optical coherence tomography (OCT) and 3T MRI examination. Subjects were classified based on whether or not there was a family history of MS. The study enrolled 40 non-fHC who had no relatives with MS and 28 fHC with at least one relative affected with MS. The associations between OCT parameters and conventional and non-conventional MRI measures were investigated.
Results: There were no significant OCT or conventional and non-conventional MRI measureable differences between the non-fHC and fHC groups. Periventricular localization and total volume of white matter (WM) signal abnormalities (SA) were more common in the fHC group but the differences did not reach a level of significance. A significant association between decreased RNFL thickness with increased volume ($p = 0.001$), number ($p = 0.003$) and frequency of $\geq$9 T2 ($p = 0.003$) WM SAs on MRI was found in the fHC group. No association between OCT and MRI parameters was detected in the non-fHC group.
Conclusion: There is an association between decreased RNFL thickness on OCT and increased WM injury in healthy relatives of MS patients. Further studies should explore the pathophysiology of these findings.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system characterized by demyelination, axonal dysfunction and neuronal loss [1]. The severity of axonal injury has a critical role in the development of permanent disability [2]; and its investigation of using multiple diagnostic techniques is of importance for better understanding the underlying pathobiology of MS.

Magnetic resonance imaging (MRI) is a key diagnostic and monitoring tool in MS. Conventional MRI techniques are limited in their ability to assess axonal loss [3–5] but non-conventional MRI techniques such as diffusion tensor imaging (DTI) can provide additional information about axonal and myelin integrity [5]. DTI reveals that T1 hypointense lesions exhibit increased mean diffusivity (MD) and decreased fractional anisotropy (FA), which are indicative of a more severe underlying destructive pathology, compared to T2 hyperintense lesions [6]. DTI can identify abnormalities in normal appearing white matter (NAWM) and gray matter (GM) in MS patients, even before lesions are visible on conventional MRI [7]. Clinical correlation was observed between DTI changes and progressive stages of the disease [8], increased disability [9] and severity of cognitive impairment. [10,11].

In the last 5–10 years, optical coherence tomography (OCT) has been gaining increased attention in addition to MRI as a promising non-invasive method for the quantification of neurodegeneration in MS [12,13]. The retina is the only place where a tissue layer made up of axons can be imaged directly so quantification of the retinal nerve fiber layer (RNFL) thickness and total macular volume (TMV) have been proposed as potential surrogate markers for the

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assessment of neuroaxonal degeneration in MS [13]. OCT studies have shown thinning of the RNFL both in MS patients with or without a history of optic neuritis [14]. Several studies confirmed a relationship between decreased RNFL thickness and increased disability in MS patients [15–17]. Moreover, decreased RNFL thickness was found to relate to more advanced brain atrophy [18,19], disease burden [20,21] and DTI measures [21,22].

Although MS is predominantly a sporadic disease, there is a genetic predisposition with a familial MS history. O'Gorman et al. estimated the overall contribution of the known MS loci to the genetic rick of MS to be 18–24% [23]. MRI studies in healthy relatives of MS patients showed an increased incidence of white matter (WM) signal abnormalities (SA) consistent with MS [24–26].

Against this background, the aim of this study was to investigate the association between OCT and MRI measures (including DTI) in a cohort of healthy controls (HCs) with and without a familial history of MS.

2. Materials and methods

2.1. Subjects

This was a prospective study in which HC without known CNS and ophthalmologic pathology or neurological complaints were recruited from the following volunteer sources: (1) hospital personnel, (2) respondents to a local newspaper advertisement and (3) relatives of MS patients that are followed in our center. The inclusion criteria required: (1) fulfilling the health screen questionnaire requirements containing information regarding medical history (illnesses, surgeries, medications, family history of MS, etc.), (2) normal physical examination, (3) having at least one MS relative followed in our center (for healthy relatives of MS patients) as well as (4) being able to undergo MRI scanning and OCT examination. Subjects with: (1) pre-existing medical conditions known to be associated with CNS pathology (e.g., neurodegenerative disorder, cerebrovascular disease, cognitive impairment, history of psychiatric disorders, seizures, trauma, etc.), (2) patients with comorbid ocular conditions not related to MS, including advanced glaucoma, significant refractive errors (±8 diopters), and (3) previous known history of retinal pathology (i.e., diabetic retinopathy) as ascertained by a detailed history and examination were excluded from the study [20].

All subjects underwent physical, neurological, OCT and MRI examinations and were assessed with a structured questionnaire administered in-person by a trained interviewer unaware of the subjects' HC status. The questionnaire contained information related to demographic characteristics, presence of autoimmune and other concomitant diseases, vascular risk factors and environmental factors, as well as a family history of MS, as previously described [27]. Race/ethnicity was determined using the classifications of the US Census Bureau. Subjects were divided into two groups depending on the presence of relatives with MS: (1) non-familial HC (non-fHC) – subjects without known relatives affected with MS, or (2) healthy relatives of MS patients (fHC) – subjects with at least one relative (first, second, third degree) affected with MS. Classification of first, second and third degree relatives was used as described previously [28].

2.2. MRI acquisition

All subjects were examined on a 3T GE Signa Excite HD 12.0 TwinSpeed 8-channel scanner (General Electric, Milwaukee, WI), using an 8-channel head and neck (HDNV) coil. MRI sequences included multi-planar dual fast spin-echo (FSE) proton density (PD) and T2-weighted image (WI) as well as Fluid-Attenuated Inversion-Recovery (FLAIR). Pulse sequence characteristics were as follows: All scans were acquired with a 256×256 matrix and a 25.6 cm field of view (FOV) for an in-plane resolution of $1 \times 1\ mm^2$ with a phase FOV (pFOV) of 75% and one average. Sequence-specific parameters were as follows: for the PD/T2: 3-mm-thick slices with no gap, echo time (TE)1/TE2/repetition time (TR) = 12/95/3000 ms, echo train length (ETL) = 14, and for the FLAIR scans, 3-mm-thick slices with no gap, TE/inversion time (TI)/TR = 120/2100/8500 ms.

DTI sequence was also acquired as part of the MRI protocol with 3-mm thick slices, no gap, a 96×96 matrix, a 32 cm FOV and a 75% pFOV, resulting in a voxel size of $3.33\ mm \times 2.50\ mm \times 3.00\ mm$. The sequence used a TE/TR of 81.8/8200 ms, 1 average and an ASSET (parallel imaging) factor of 2, resulting in a total acquisition time of 2:28. DTI-specific parameters were 15 directions with a *b*-value of $800\ s/mm^2$.

2.3. MRI analysis

The MRI analyses were blinded to the subjects' demographic and group characteristics.

The WM SA number and volume (WM-SAV) were outlined using a semi-automated edge detection contouring/thresholding technique as previously described [29]. The regional localization of WM SAs was determined based on their presence in the juxtracortical, periventricular, infratentorial and deep WM regions.

In addition, we outlined areas of dirty appearing WM (DAWM). The DAWM was defined as a uniform, non-focal area of signal increase on the FLAIR/T2/PD-WI, with a subtly increased signal intensity compared with the signal intensity of NAWM, as previously proposed [30]. The DAWM showed a relatively diffusely defined border of DAWM areas compared with focal WM lesions and was tapered off toward the NAWM.

A fully automated processing pipeline was used to calculate MD, radial diffusivity (RD) and axial diffusivity (AD) in the WM SAs. First, the DTI image was corrected for eddy currents and deskulled. Next, a diffusion tensor model was fit at each voxel, using DTIfit, part of the FSL package [31]. The resulting MD, RD and AD maps were then masked to calculate mean values.

In order to focus our study on axonal and myelin integrity and decrease number of comparisons, only these DTI measures were included into study.

2.4. Optical coherence tomography (OCT)

Subjects underwent OCT examination (Spectralis, Heidelberg, Germany) to measure RNFL thickness and TMV of both eyes. All scans were obtained by an experienced technician trained in OCT scan capture and in the recognition of common artifacts and errors in OCT imaging. The fast RNFL thickness scan protocol was used for OCT (computes the average of 3 circumferential scans for 360° around the optic disk; 256 axial scans; diameter, 3.4 mm). The optic disk was centered in all scans by the scanning technician and scanning was performed without the use of pharmacologic dilation. The technician ensured that the retinal sections were centered within the scanning window and that the target signal strength was ≥7 (7–10), ensuring high quality of OCT scans. Analysis of the retinal images was performed using the Heidelberg Spectralis software. All scans were reviewed for sufficient signal strength, correct centering and beam placement as well as segmentation.

2.5. Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS), version 16.0 (SPSS, Inc., Chicago IL). Demographic, OCT and MRI differences between groups were