Reduction of optic nerve fiber layer thickness in CADASIL

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Our study aims to assess nerve fiber layer (NFL) thickness in patients affected by cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL). Six CADASIL patients (mean age 42 ± 16 years, best corrected visual acuity >20/20 with refractive error between ±3 diopters, intraocular pressure <18 mmHg) were enrolled. They were compared with 16 age-matched controls. In all subjects enrolled, NFL thickness was measured by optical coherence tomography (OCT). Three different measurements were taken in each quadrant (superior, inferior, nasal, and temporal) and averaged. The data from all quadrants (12 values averaged) were identified as NFL overall. In CADASIL eyes there was a reduction of NFL thickness in each quadrant and in the NFL overall evaluation compared with the values observed in control eyes. Our results suggest that in CADASIL patients there is a reduction of NFL thickness evaluated by OCT. This morphological abnormality could be ascribed to an impairment of the retinal vascular supply leading to a global neuroretinal involvement. These anatomical changes may precede the onset of the neurological clinical manifestations.

Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is an hereditary late-onset microangiopathy of the brain due to mutations of Notch3 gene, clinically characterized by recurrent subcortical ischemic lesions, vascular dementia, mood disturbances and migraine with aura [1].

The main pathological hallmark of the disease is degeneration of the muscle cells of small arteries and arterioles walls associated with deposition of granular osmiophilic material. Although the symptoms exclusively involve the CNS, there are some circumstantial evidence that the pathological process also involves vessels in the skeletal muscle, skin, and peripheral nerves [2,3]. Moreover, in CADASIL subjects, a dysfunction of the innermost retinal layers (ganglion cells and their fibers) and of the inner retinal layers (i.e. amacrine cells) has been detected by means of electrophysiological evaluations [4,5], as a part of a wide impairment of the visual system [6–10].

It was recently observed that an impairment of the innermost retinal layers [11,12], like what we have shown in patients with glaucoma [13], multiple sclerosis [14] and Alzheimer's disease [15], was significantly correlated to a decrease in optic nerve fiber layer (NFL) thickness evaluated by optical coherence tomography (OCT). The former represents a non-invasive method allowing eye cross-sectional imaging and, thus, an objective method to quantify the optic nerve fiber layer thickness '*in vivo*' [16–18].

Therefore, the aim of the present study was to evaluate the retinal NFL thickness and morphology '*in vivo*' by mean of OCT in subjects affected by CADASIL and in asymptomatic positive carriers. We reasoned that the NFL thickness would be reduced if CADASIL patients, beside the retina, also have an involvement of the optic nerve as already described in few case reports [7,19].

Subjects and methods

Three patients with full-blown CADASIL and three subjects (patients' sons and daughters) carrying the haplotype at risk were enrolled in this study. None of the subjects had common vascular risk factors. Doppler sonography of cerebro-afferent vessels was normal. Molecular analyses were performed in all enrolled subjects according to the method described elsewhere [20].

Brain MRI was performed in all subjects using a 1.5 T machine (Philips Gyroscan NT-Intera, Philips,

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Eindhoven, The Netherlands). The protocol included axial and coronal spin-echo proton density and T2-weighted images (TR/TE 2295/20,90), axial fluid-attenuated-inversion-recovery (FLAIR) images (TR/TE/TI, 6000/100/2000), sagittal and axial T1-weighted images (TR/TE, 582/15) [5].

Patient description

Subject III-2 (male, age 52), Subject III-4 (female, age 62) and Subject III-5 (female, age 62) showed the Cys146Tyr mutation in the CADASIL gene. Subject III-2 had a history of recurrent migraine-like episodes, occasionally accompanied by right-sided hemiparesis and motor aphasia with complete recovery in a few days. Neuropsychological examinations showed the development of a progressive cognitive impairment. Subject III-4 had recurrent migraine-like episodes associated with sensory-motor aphasia and mental confusion. Neuropsychological examinations showed moderate memory loss and attention deficit. The MRI of these two subjects showed diffuse, bilateral T2-weighted hyperintensity in the cerebral white matter. Subject III-5 had occasional episodes of migraine-like attacks with spatial and temporal disorientation. No deficits were observed during the neuropsychological examinations. T2-weighted MRI images showed symmetrical and bilateral areas of hyperintensity in the white matter, particularly in the fronto-temporal region.

Subjects IV-3 (female, age 33), Subjects IV-6 (male, age 28), Subjects IV-7 (male, age 24), sons and daughters of subjects III2 and III4, were positive for the CADASIL gene Cys146Tyr mutation. None of these subjects reported acute neurological manifestations. Neuropsychological and neurological examinations were normal. MRI showed small T2-hyperintense foci in the periventricular white matter, combined, in two cases (IV-6, IV-7), with small bilateral T2/hyperintense areas in the subcortical white matter.

Control subjects

As it is known that there is a reduction of NFL with age [15] and considering the different ages of CADASIL subjects enrolled, we divided the healthy controls in two age-matched groups. Subjects III-2, III-4, and III-5 were compared with a control group consisting of 16 normal subjects with age ranging from 50 to 65 years (mean age 58.2 $\pm$ 3.5 years, Control group 1); subjects IV-3, IV-6, and IV-7 were compared with a control group consisting of 16 normal subjects with age ranging from 20 to 35 years (mean age 27.2 $\pm$ 2.5 years, Control group 2). Inclusion criteria for all normal subjects were: no dismetabolic diseases, no arterial hypertension, no other neurological diseases. Ocular inclusion criteria were: best corrected visual acuity of 20/20 with refractive error between ± 2 spherical equivalent, intraocular pressure < 18 mmHg, absence of present or previous history of optic media opacity, cataract or early lens opacity, glaucoma, retinal detachment, early age-related macular degeneration, or other macular degeneration, optic neuropathy, retinal vascular diseases.

OCT examination

Optical coherence tomography (OCT3; Humphrey, Dublin, CA, USA), including the fiber optic delivery system coupled with slit-biomicroscope, was used in all subjects enrolled according to methods described elsewhere [13–15].

This system provides the operator with a video-camera view of the scanning probe beam on the fundus, and OCT imaging acquired in real time on a computer monitor. After dilatation with 1% tropicamide, each eye was scanned three times using a circle size of 3.4 mm (1.7 mm radius). Near-infrared light (840 nm wavelength) was used. Throughout scanning, the patient kept his/her eyes constantly fixed on an internal target provided by the equipment. The measurements were obtained from three non-consecutive scans (i.e. the patient was allowed to rest for a few seconds before being re-positioned to proceed to the following scan).

As previously reported, the OCT software provides an automated computer algorithm that identifies the anterior and posterior borders of the retina. This has been claimed as offering the possibility of calculating both nerve fiber layer (NFL) and total retinal thicknesses [18]. The software allows the mapping of the thickness data according to both quadrant-by-quadrant and a clock hour analyses. Retinal thickness was determined by computer as the distance between the first reflection at the vitreoretinal interface and the anterior boundary of the second reflective layer, corresponding to the retinal pigment epithelium and the choriocapillaris. As discussed elsewhere, NFL thickness was automatically assessed by computer assuming the correlation with the highly-reflective red layer at the vitreoretinal interface [18].

We considered the average value of three different measurements per quadrant (superior, inferior, nasal and temporal); the overall data obtained in all quadrants (12 values averaged) was identified as NFL Overall. Normal limits were obtained from both group of control subjects by calculating mean values -3 standard deviations for each NFL parameters.

The research followed the tenets of the declaration of Helsinki and the study was approved by the local ethics committee.

Statistics

Normal control limits (NCL) of the NFL thickness were obtained separately from the two groups of age-matched control subjects by calculating mean values minus 3 standard deviations. In both control group 1 and 2, only one eye of each control subject was considered.

Results

Examples of NFL assessment in one CADASIL eye (III-2) and in one control eye are shown in Fig. 1. All CADASIL subjects had normal visual acuity (20/20) and intraocular pressure less than 18 mmHg. On Table 1 is reported the optic NFL thickness and visual acuity findings of CADASIL subjects

In Control group 1 subjects, the NFL Overall thickness was between 99.8 and 133.2 μm (mean: 119.1 ± 10.7 μm). In Control group 2 subjects, the NFL Overall thickness was between 99.8 and 133.2 μm (mean: 119.1 ± 10.7 μm).

In both eyes of three patients with full-blown CADASIL (III-2, III-4 and III-5) and in three patients' sons and daughters (IV-3, IV-6, IV-7) was observed an NFL Overall thickness between 56.3 and 75.6 μm and therefore reduced when compared with the normal limits obtained in their age-matched control subjects. The NFL thickness evaluated in the individual quadrants (inferior, superior, nasal and temporal) was reduced in CADASIL subjects when compared with the normal limits obtained in their age-matched control subjects.

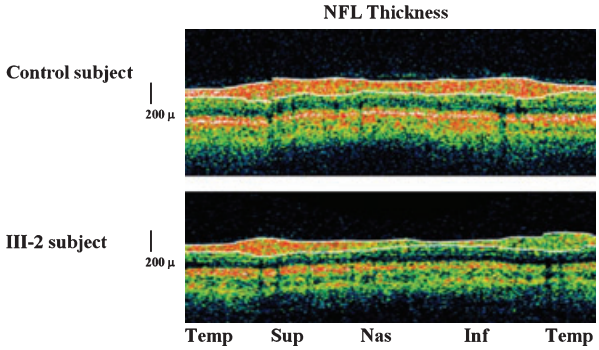

Figure 1 Circular optical coherence tomography (OCT) taken in cylindrical sections of tissue surrounding the optic disc. The most anterior red reflection indicates the nerve fiber layer (NFL). Compared to control eyes, OCT image of subject III-2 (right eye) shows a marked decrease of NFL reflection (lower NFL thickness) in each quadrant.

Table 1 Visual Acuity and Optic NFL thickness findings in three patients with full-blown CADASIL (III-2, III-4 and III-5) and three patients' sons and daughters (IV-3, IV-6, IV-7) carrying the haplotype at risk

	VA	NFL Overall (μm)	NFL inferior (μm)	NFL Nasal (μm)	NFL Superior (μm)	NFL Temporal (μm)
III-2, M (52 years)						
RE	20/20	69.2*	59.6*	70.6*	77.3*	69.6*
LE	20/20	65.1*	67.0*	69.0*	57.0*	67.4*
III-4, F (62 years)						
RE	20/20	56.3*	62.3*	57.3*	48.6*	57.0*
LE	20/20	57.0*	66.3*	56.4*	46.5*	58.5*
III-5, F (58 years)						
RE	20/20	57.7*	68.0*	51.6*	45.0*	66.2*
LE	20/20	61.7*	71.5*	56.5*	49.5*	66.6*
NCL-1 (Mean ± 1SD)	20/20	82.5 (112.2 ± 9.9)	91.0 (119.8 ± 9.6)	82.1 (109.7 ± 9.2)	95.9 (122.6 ± 8.9)	78.7 (102.4 ± 7.9)
IV-3, F (33 years)						
RE	20/20	69.9*	77.0*	64.3*	72.0*	66.5*
LE	20/20	67.3*	82.0*	79.5*	75.0*	63.0*
IV-6, M (28 years)						
RE	20/20	75.6*	88.3*	64.5*	82.0*	67.8*
LE	20/20	68.5*	87.6*	84.6*	41.3*	60.6*
IV-7, M (24 years)						
RE	20/20	70.1*	89.6*	82.2*	45.2*	63.7*
LE	20/20	70.8*	71.8*	77.5*	63.3*	70.6*
NCL-2 (Mean ± 1SD)	20/20	87.0 (119.1 ± 10.7)	97.4 (126.9 ± 9.8)	86.8 (113.4 ± 8.8)	99.2 (128.9 ± 9.9)	81.3 (106.8 ± 8.51)

Normal confidence limits (NCL) obtained by calculating mean values of control subject minus 3 SDWe considered two groups of age-matched controls: control group 1 with mean age of 58.2 ± 3.5 years (NCL-1) and control group 2 with mean age of 27.2 ± 2.5 years (NCL-2).
*reduced value respect to normal confidence limits.
VA: best corrected Snellen Visual acuity; NFL, nerve fiber layer. C1: Control group 1; C2: Control Group 2; RE, right eye. LE, left eye.

Discussion

Our OCT results reveal a reduced NFL thickness in all examined quadrants of CADASIL patients, indicating the presence of a global involvement of the neuroretinal tissue. This reduction in NFL thickness was also observed in younger CADASIL patients (IV-3, IV-6, IV-7), without neurological symptoms, but with MRI evidence of abnormal signal in the white matter.

Our data seem not in agreement with a recent study showing not statistical difference in the optic nerve head topographic parameters between CADASIL patients and controls [10]. The authors wrote that it could be related to the fact that the patients enrolled were too young for any changes to have occurred.

Optical coherence tomography has been widely employed to assess the reduction of NFL thickness in glaucoma [13,16] multiple sclerosis [14] and in Alzheimer's disease [15]. Chauhan and Marshall [21] raised some doubts regarding the accuracy of OCT in measuring NFL thickness. However, they demonstrated a good correlation between excimer-laser induced ablation of the inner retina and the signal recorded by OCT, stating that '…the thickness of the inner band was reduced by the same amount as the ablation step height'. Therefore, as previously suggested [12–15], the accuracy of OCT in quantifying NFL thickness is still a matter of debate, but we can assume that progressive changes in the OCT signal coming from the inner retina (including optic nerve fiber layer, inner plexiform layer and ganglion cell layer) are paralleled by similar changes occurring in the tissue.

Our CADASIL subjects, in whom a reduction of NFL thickness was observed, have been previously tested by electrophysiological methods [4,5]. The abnormal responses found by means of oscillatory potentials and pattern electroretinogram recordings indicate respectively a dysfunction of the inner retinal layers (preganglionic elements: amacrine cells) [22] and of the innermost retinal layers (ganglion cells and their fibers) [11]. These retinal dysfunctions were attributed to the well-described clinically retinal vascular alterations [6,8,23], the same as documented by postmortem ultrastructural findings in the central retinal vessels [24], resembling those shown in specimens of nervous tissue involvement of brain arteries [25].

Considering our present data, it is possible that the same vascular abnormalities may induce not only the retinal dysfunction [4,5], but also a reduction in ganglion cells and their fibers as suggested by our OCT results. Optic nerve morphological findings in the autopsy material of a CADASIL patient showed diffuse myelin pallor and rarefaction of optic nerve fibers [19]. These evidence and the indication of the anatomopathological retinal involvement in its different layers [26], are in favor of a global involvement of the neuroretinal tissue in CADASIL patients.

Notwithstanding this loss of neuroretinal elements, all our CADASIL subjects showed preserved normal visual acuity. This observation are in agreement with the data reported in the literature [6,8,27]. It is worth noting that a normal visual acuity can be observed in other neuroophthalmologic conditions such as glaucoma, in which the dysfunction of the innermost retinal layers leads to visual field and PERG impairment in the presence of initially preserved visual acuity [12,13].

Finally, the '*in vivo*' evaluation of the neuroretinal tissue of CADASIL subjects shows the presence of an abnormal morphology of optic NFL. We believe that the typical CADASIL vascular pathophysiological abnormalities could induce a global neuroretinal involvement and, therefore, could explain the anatomical changes seen in our patients, which may precede the onset of neurological clinical manifestations.

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