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Complete manuscript title: Subclinical thinning of macular ganglion cell layer in Leber optic neuropathy carriers. Report of a Spanish pedigree.

Running title: Subclinical thinning of macular ganglion cell layer in Leber carriers

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Subclinical thinning of macular ganglion cell layer in Leber optic neuropathy carriers. Report of a Spanish pedigree.

Introduction

Leber optic hereditary neuropathy (LHON) is a rare disease. Despite its low prevalence (1:54,000 in the Danish population), LHON is considered, after dominant optic atrophy, the second most frequent inherited neuropathy (1).

LHON is usually characterized by painless acute or subacute visual loss and it predominantly affects young men, although women can also be affected. Three mitochondrial DNA point mutations are responsible for the large majority of cases reported worldwide.(2) These mutations are located at nucleotide positions 11778 (G to A), 3460 (G to A) and 14484 (T to C), respectively in the ND4, ND1 and ND6 subunit genes of complex I of the oxidative phosphorylation chain in the mitochondria.(3)

The **mitochondria are organelle** found in all the cells of eukaryotic organisms. However LHON affects selectively the ganglion cells of the papillomacular bundle. This specificity is poorly understood. Some authors state that because of their small diameter and the abscense of myelin, the fibers of the papillomacular bundle require higher amounts of energy. (4)

Encouraging treatments currently undergoing investigation include ubiquinone analogs, such as idebenone and epi 743,(5) as well as gene therapy(6) and stem cells to restore

ATP synthesis and provide neuroprotection to surviving retinal ganglion cells.(4) Most authors think that commencing treatment shortly after the onset of symptoms is likely to have the best therapeutic effect.(4, 7-9) Indeed in the observational follow-up study (RHODOS-OFU), and in Carelli et al retrospective study the improvement was more prominent in those patients who had initiated treatment with idebenone earlier. (4, 10, 11) Thereby most studies include patients with a short history of visual loss.(5)

The unproved hypothesis that prognosis is better when treatment is started early,,has increased the interest for biomarkers that could predict conversion to disease status. Major advancements in understanding LHON were possible after the introduction of optical coherence tomography (OCT).(12) Some researchers have suggested that the evaluation of the macular ganglion cell layer (mGCL) is superior to the evaluation of the peripapillary retinal nerve fiber layer (pRNFL), because it avoids the inter-individual variability in the pRNFL distribution and the artefact produced by the presence of edema at the optic nerve.(12) The GCC thickness plunged sharply within 3 months followed by gradual decline until 6 months, thereafter showing a plateau up to 24 months.(13) Akiyama et al in a recent paper, reported that in four of six patients with LHON mGCL thickness was reduced at the initial visit and interpreted this finding as a sign of conversion from carrier to disease status.(14) However, several ocular morphological (3) and functional abnormalities (15) have been reported in LHON carriers, and to to the best of our knowledge, no article has considered that mGCL thinning may be just a marker of carrier status.

Case report

A 19-year-old man was seen in our Department due to visual loss in his right eye (RE). Definite diagnosis of LOHN was not made until six months later when the left eye (LE) was involved. His past medical history was unremarkable and he didn`t smoke or drink. At the time of diagnosis, visual acuity (VA) was counting fingers in his RE and 20/200 in his LE. Intraocular pressure (IOP), anterior segment examination and pupillary reactions were normal. Fundus examination showed small and crowded optic discs with slight blurring of the margins, and visual fields revealed severe cecocentral scotomas in both eyes (Figure 1). Progressive thinning of mGCL, using optic coherence tomography (3D OCT 2000, Topcon) was confirmed during the visual loss period (Figure 2 and 3). Our OCT device does not offer a progression analysis of mGCL, however qualitative observation of the reports shows that thinning on this layer progressed during the two years of follow up. The morphology of the optic nerve head, showed cupping and pallor during this period (Figure 4), and peripapilar RNFL showed also severe thinning during this time (Figure 5).

When the second eye was involved, genetic testing confirmed the presence of a T14484 C point mitochondrial mutation, consistent with LHON. His fraternal twin (who smoked 10 cigarettes/day) and his 52-year-old mother (who suffered type 2 diabetes mellitus, with good metabolic control and without diabetic retinopathy) also underwent genetic testing, and both had the same mutation. These two carriers did not have significant refractive defect, had normal visual examination with 20/20 VA in both eyes, normal visual fields and a pRNFL thickness within normal limits. However, both presented a significant reduction of the mGCL thickness (Figure 6 and 7) compared to the subjects of the same age that form part of the normative database of our OCT. The absolute values of mGCL thickness in both carriers (66/70 microns in the mother and 68/70

micros in the brother) were similar to the values of mGCL in the patient when visual loss began (66 microns in the RE and 67 microns in the LE).

Discussion

Although there is currently no approved therapy for LHON, many promising novel treatment modalities are being evaluated, with several clinical trials underway or in the planning stages.(7) Current knowledge suggests that these therapies should be administered in the early stage of the disease in order to be effective and avoid ganglion cell loss. This requirement has increased the interest for biomarkers that might predict the conversion from carrier to disease status.(4) .

Recent publications have reported changes in the mGCL in patients with LHON during the initial days of visual loss, and have proposed that macular changes in GCL thickness may precede the conversion from carrier status to disease status.(12, 16, 17) These studies suggest that macular changes in GCL may precede and be more specific than the changes that occur in the pRNFL.(16)

However these publications in many cases do not usually include controls (13, 14, 17) or include controls extracted from the general population.(12, 16) These controls are probably not valid, since several ocular morphological (3) and functional abnormalities (15) have been reported in LHON carriers. Sadun et al found that several subclinical fundus abnormalities were common among carriers in a large Brazilian pedigree. These abnormalities included peripapillary microangiopathy, localized swelling of the pRNFL and deficits in the visual fields, often coherent with the fundus abnormalities.(3) Similarly abnormalities in color vision (18) and in pattern electroretinographic responses have also been described in asymptomatic carriers.(15, 19)

Therefore, we believe that to properly determine conversion biomarkers, the appropriate controls would be carriers and not healthy subjects extracted from the general population. In the family we present herein, one of the carriers was the fraternal twin of the proband: he was the same age, although he was not identical from a genetic point of view and he didn´t share potential risk factors. Tobacco and alcohol are considered triggers of visual loss in LHON carriers.(20) Curiously the sibling affected in this pedigree was the non-smoker. Both family carriers showed decreased mGCL, without subjective visual loss or any other abnormalities in ancillary evaluations. The presence of this subtle anatomic changes in the mGCL explains the reduced pattern electroretinographic responses previously reported by Ziccardi et al and Guy et al in carriers.(15, 19) This would suggest that mGCL thinning is not so much a marker of conversion as a sign reflecting carrier status.. Until larger studies are performed, the presence of an area of thinning in the macular GCL in a single examination, should not be taken as a herald of imminent visual loss. The demonstration of progressive thinging in several examinations is necessary to demonstrate conversion from carrier status to disease status.

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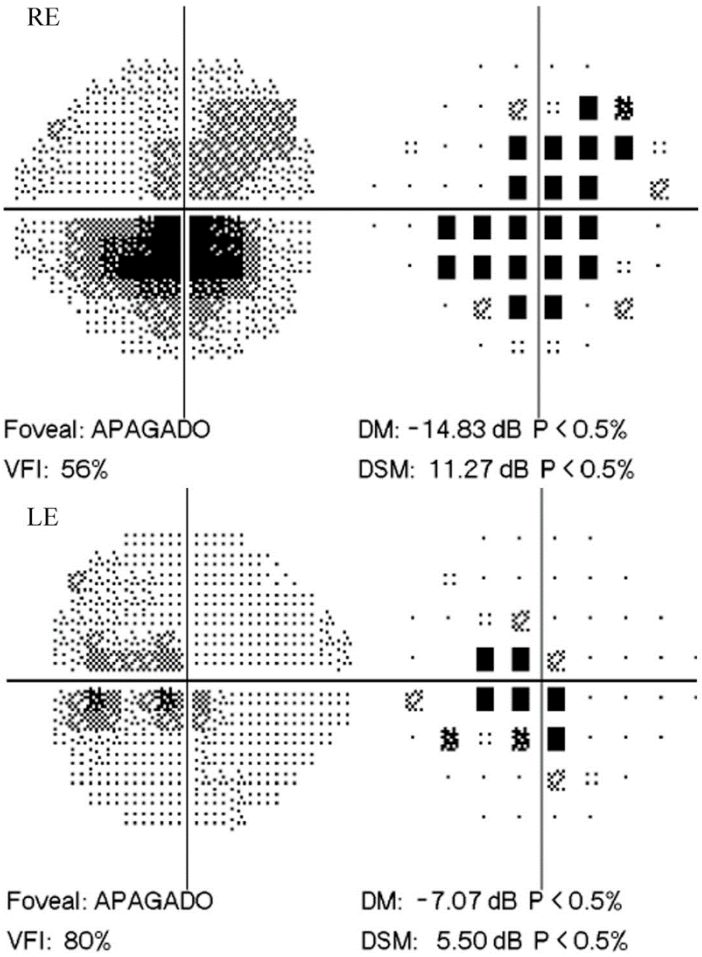

Figure 1. Visual fields at the time of diagnosis (24-2, SITA-Standard with III stimulus).

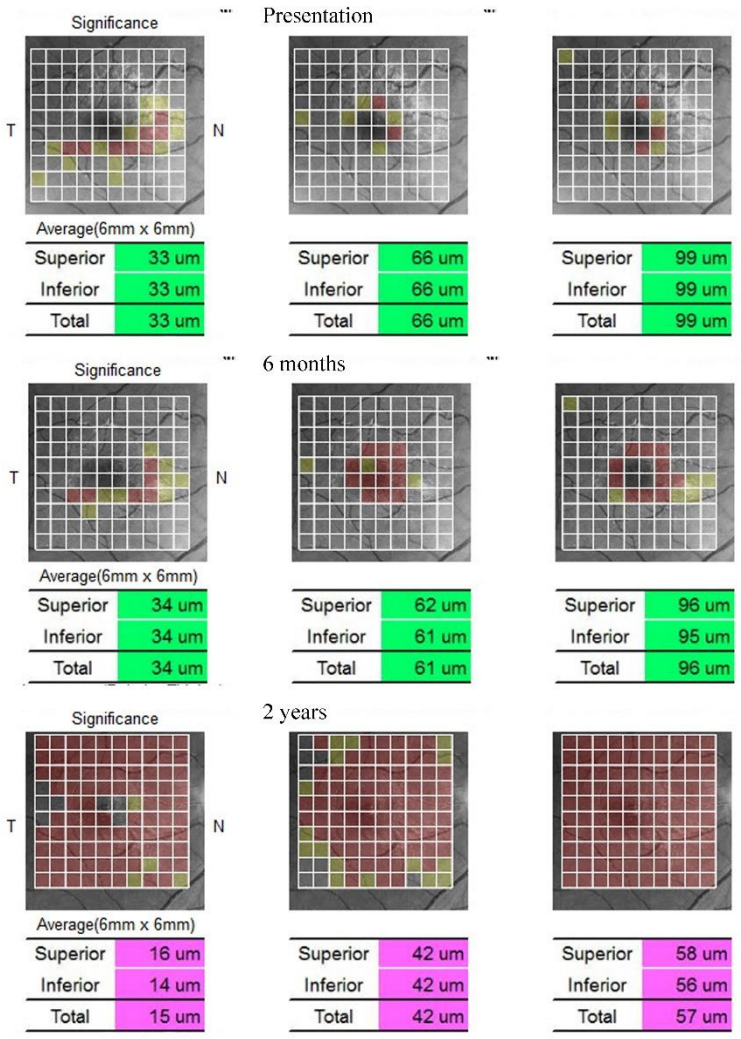

Figure 2. Evolution of macular ganglion cell layer (mGCL) in the right eye (RE) of the proband. Examinations were performed at the time of presentation (three days after the patient referred visual loss in his RE), one month, and eight months later. In the last examination, there was also visual loss in the left eye.

(Probability maps of the 6 x 6 mm central area displayed. The first column represents macular RNFL, the second column represents macular GCL and the third column represents macular GCL + inner plexiform layer).

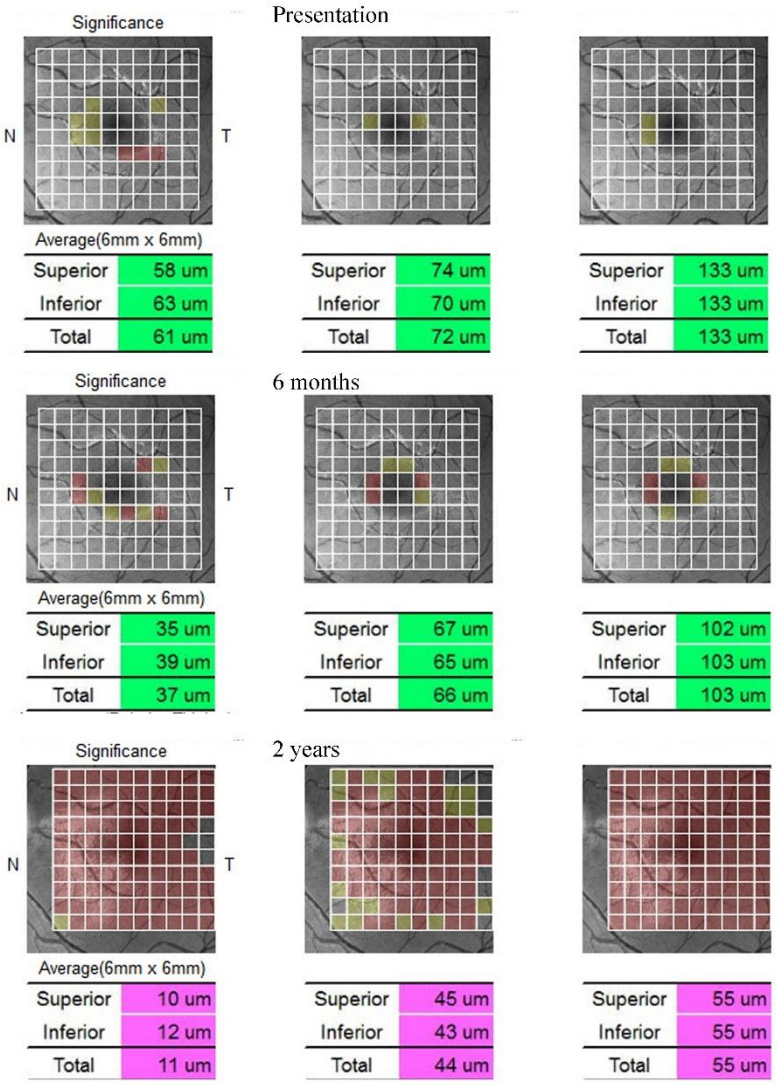

Figure 3. Evolution of macular ganglion cell layer (mGCL) in the left eye of the proband. Examinations were taken at the time of presentation (three days after the patient referred visual loss in his right eye), one month, and eight months later. In the last examination, there was also visual loss in the left eye.

(Probability maps of the 6 x 6 mm central area displayed. The first column represents macular RNFL, the second column represents macular GCL and the third column represents macular GCL + inner plexiform layer).

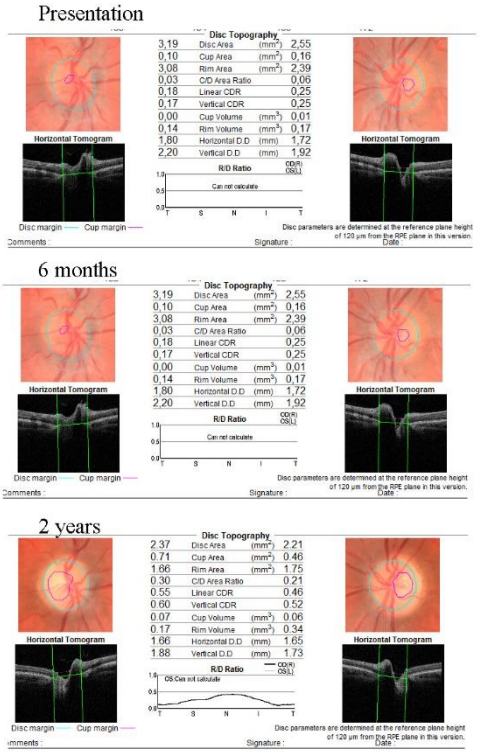

Figure 4. Evolution of the optic disc, during the 2 years of follow up. Severe pallor and cupping can be observed in the last examination.

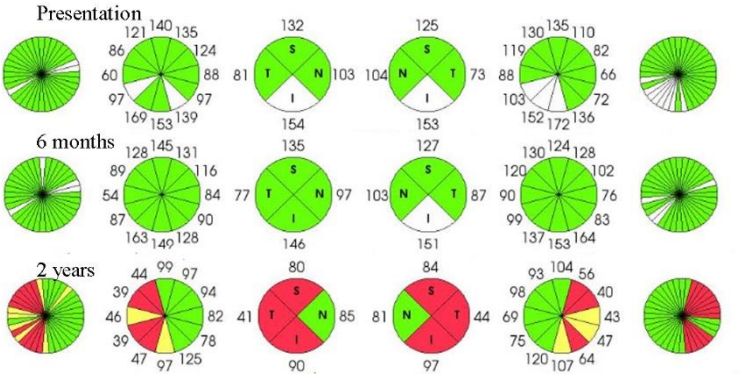

Figure 5. Evolution of the retinal nerve fiber layer. Sever thinning can be observed in the last examination.

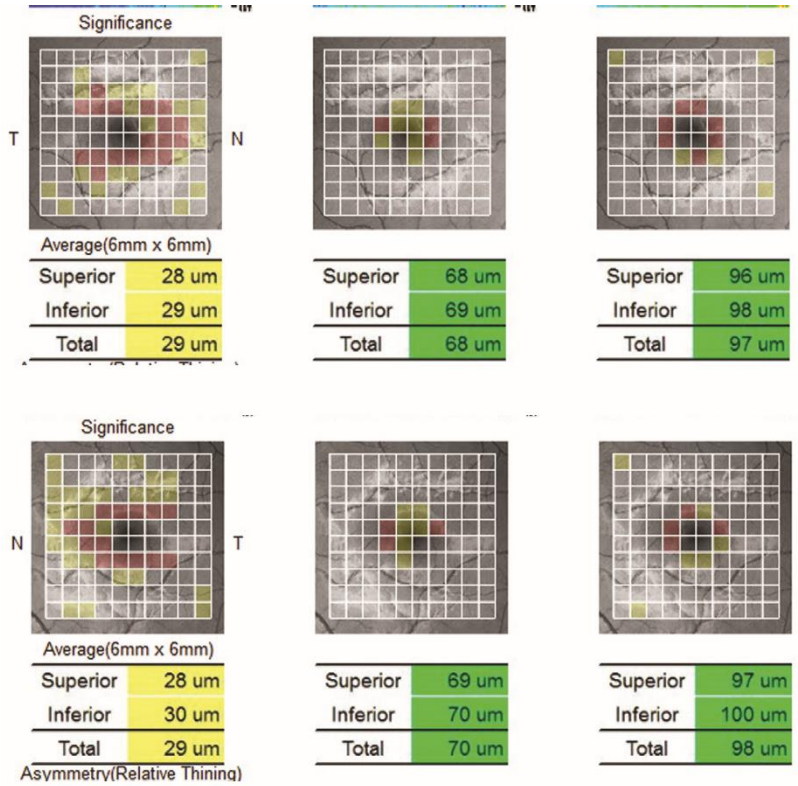

Figure 6. Report of the macular ganglion cell layer (mGCL) of the unaffected brother of the proband. Decreased thickness in the macular region can be observed in both eyes.

(Probability maps of the 6 x 6 mm central area displayed. The first column represents macular RNFL, the second column represents macular GCL and the third column represents macular GCL + inner plexiform layer).

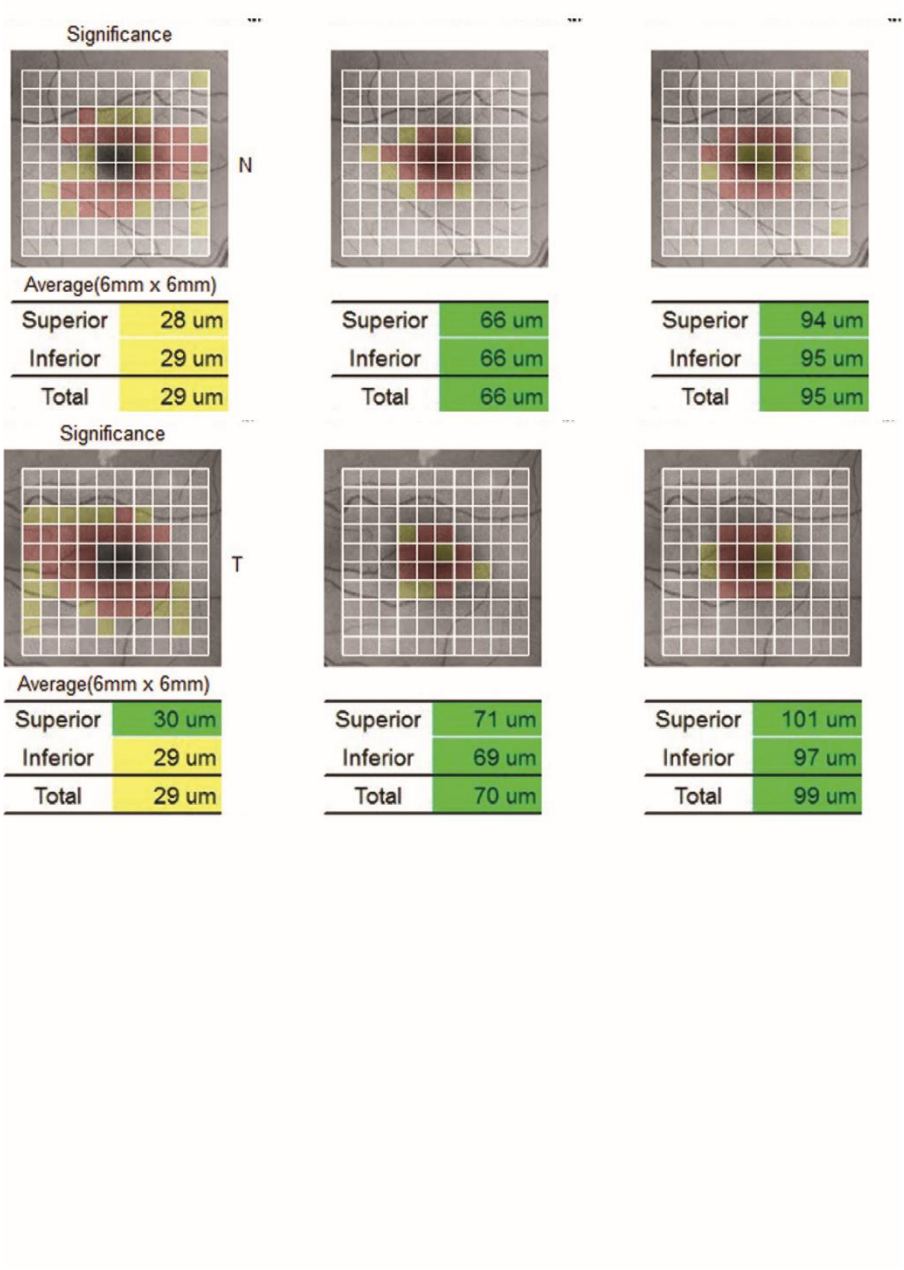

Figure 7. Report of the macular ganglion cell layer (mGCL) of the unaffected mother of the proband. Decreased thickness in the macular region can be observed in both eyes.

(Probability maps of the 6 x 6 mm central area displayed. The first column represents macular RNFL, the second column represents macular GCL and the third column represents macular GCL + inner plexiform layer).