Association of Optic Disc Size with Development and Prognosis of Leber’s Hereditary Optic Neuropathy

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PURPOSE. To study the optic nerve head (ONH) morphology of patients with Leber’s hereditary optic neuropathy (LHON) in a large family from Brazil carrying the 11778/ND4 mutation and in a case series of unrelated Italian families bearing different mitochondrial DNA (mtDNA) pathogenic mutations.

METHODS. Enrolled in the study were 15 LHON-affected patients (LHON-affected) and 45 LHON unaffected mutation carriers (LHON carriers) belonging to the previously reported Brazilian SOA-BR LHON pedigree and 56 LHON-affected and 101 LHON carriers from 45 unrelated LHON Italian pedigrees molecularly defined. The LHON-affected were subgrouped according to the extent of visual recovery. All individuals underwent optic nerve head (ONH) analysis by optical coherence tomography.

RESULTS. In the Brazilian sample, the mean optic disc area was significantly larger in LHON carriers than in the control group ($P = 0.002$). In the Italian sample, the mean optic disc area and vertical disc diameter were significantly higher in LHON carriers than in both LHON-affected (respectively, $P = 0.008$ and $P < 0.001$) and control subjects ($P < 0.001$ in both cases). The LHON-affected with visual recovery had a significantly larger vertical disc diameter when compared with those without visual recovery ($P = 0.03$).

CONCLUSIONS. The results, revealing that the ONH size is larger in LHON carriers than in LHON-affected, suggest a protective role for this anatomic trait. Such a hypothesis is reinforced by the observation that, among the LHON-affected, larger discs correlated with visual recovery and better visual outcome. The findings may be relevant for prognosis and provide a mechanism for identifying nuclear-modifying genes implicated in the variability of penetrance in LHON. (Invest Ophthalmol Vis Sci. 2009;50:1666–1674) DOI:10.1167/iovs.08-2695

Optic nerve head (ONH) anatomic conformation presents a well-known variability in the general population in terms of optic disc area and shape, as seen at the fundus examination or measured by different techniques.1 The configuration of the human ONH is determined by a variety of factors including the diameter and length of the scleral canal and the number of retinal ganglion cell (RGC) axons that form the optic nerve.2 Structural and glial connective tissues also contribute significant portions of the optic nerve.3 Histomorphometric studies have shown a positive correlation of optic disc area with total axon count,4 but this correlation remains somewhat controversial.1 Optic nerve axon crowding characterizes eyes with a small optic disc.4 Racial differences in optic disc structure comparing Caucasians with African-Americans, Hispanics, and Asians indicate that Caucasians have smaller optic discs.1,5,6 Several studies have reported that small optic disc size is a risk factor for nonarteritic ischemic optic neuropathy (NAION).7–12 ONH conformation and size have also been implicated as an influential factor for the pathogenesis of other optic nerve diseases such as optic nerve head drusen and glaucoma.1,3,13–15

Leber’s hereditary optic neuropathy (LHON), a blinding disease inherited through females and affecting young males more often, is due to three frequent mitochondrial (mt) DNA mutations at positions 11778/ND4, 3460/ND1, and 14484/ND6 all affecting complex I, the first site of the mitochondrial respiratory chain.16–17 LHON is characterized by incomplete penetrance: most maternally related individuals along a given maternal lineage and carrying a homoplasmic mtDNA pathogenic mutation do not develop the optic neuropathy.16,17 Clinically, the unaffected mutation carriers frequently show fundus abnormalities including swelling of the retinal nerve fiber layer (RNFL) and microangiopathic vascular changes.18 Only a few of these individuals develop subacutely the disease-manifesting dyschromatopsia, loss of visual acuity, and a central scotoma. Within a year, the disease usually stabilizes, leaving the patient with diffuse optic atrophy and significant reduction of visual acuity.19 In a subset of patients a variable recovery of visual acuity occurs, even years after disease onset.16,17–21

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The pathologic mechanism leading to LHON is under investigation.\textsuperscript{16,22} A biochemical dysfunction of complex I is assumed, with a documented loss of energetic efficiency, increased oxidative stress, and propensity to apoptotic cell death.\textsuperscript{16,22} Altered axonal transport of organelles and axoplastic stasis with swelling of axons has been proposed as a key feature leading to the threshold for the acute phase of the disease.\textsuperscript{16,22} It is believed that the mtDNA pathogenic mutation is a necessary but not sufficient condition for development of LHON, and further environmental or genetic factors are needed to trigger the pathologic process.\textsuperscript{35} Recent studies suggest that both environmental triggers, such as tobacco smoking, alcohol drinking, or exposure to toxins,\textsuperscript{24,25} and genetic factors, such as mtDNA haplogroups\textsuperscript{26,27} and putative nuclear modifying genes on the X-chromosome,\textsuperscript{28,29} may be relevant. The anatomic conformation of the ONH has also been suggested as a factor influencing both penetrance and clinical expressivity,\textsuperscript{30} analogous to the “disc-at-risk” hypothesis in NAION.

This study was undertaken to investigate whether the ONH size may be associated with the different clinical expressions of LHON, by comparing unaffected carriers (LHON carriers) and affected patients (LHON-affected) with and without visual recovery (VR).

**METHODS**

**Subjects**

Two independent samples, one from Brazil and one from Italy (all subjects with a molecularly confirmed diagnosis of LHON), were sequentially studied with the same protocol.

The first sample was enrolled during the Fifth International Field Investigation in Colatina, Brazil (October 2005), of a large Brazilian family of Italian ancestry coded as SOA-BR.\textsuperscript{24,26} This follow-up study, which is still ongoing, was started in 2001 when the family was identified and diagnosed by our multinational team, and part of the results have been reported in numerous publications.\textsuperscript{34,26,25,51,53} For the current investigation, we have collected and included 60 individuals, of whom 15 were LHON-affected and 45 were LHON carriers (Table 1), all belonging to the maternal lineage of the SOA-BR family and invariably carrying the homoplasmic 11778/ND4 mtDNA mutation on a haplogroup J background.\textsuperscript{24,26}

Since the preliminary analysis on the Brazilian sample suggested that the ONH anatomic conformation may be of relevance for LHON (described later), we sought to replicate these results on a larger cohort of LHON patients from Italy belonging to multiple unrelated pedigrees with different pathogenic mutations. Therefore, the second sample was enrolled by inviting all the patients to participate who were referred to the Department of Neurologic Sciences at the University of Bologna between 1990 and 2005. Between September and December 2006, 56 LHON-affected patients and 77 LHON carriers from 45 unrelated pedigrees were examined. Twenty-eight pedigrees carried the 11778/ND4 mutation, nine the 3460/ND1 mutation, six the 14484/ND6 mutation, and two the rare 3733/ND1 mutation.\textsuperscript{35} LHON-affected patients were further stratified into those without or with VR (Table 1), was defined as any improvement of at least three lines of visual acuity from the nadir. All pedigrees were also molecularly characterized for the mtDNA background (haplogroup), in previous studies, certifying that they were unrelated.\textsuperscript{26,27}

To avoid possible bias related to ethnic differences, we compared the first sample to a control group from Brazil and the second to a control group from Italy. The Brazilian control group (n = 78, Table 1) included 26 subjects married into the family, who tested negative for the mtDNA mutation (Off-maternal). Another 52 subjects were descendants of affected or carrier males who did not inherit the mutant mtDNA (Male-descendant); of these, 39 subjects were first-degree Male-descendant and 13 were second-degree Male-descendent. The Italian control group (n = 81) was composed of volunteers without evidence of either optic disc or retinal disease. All subjects had a complete ophthalmic examination, including best corrected visual acuity measurement, slit lamp biomicroscopy, intraocular pressure measurement, indirect ophthalmoscopy, and optic nerve head photography. For both samples exclusion criteria were: (1) presence in one or both eyes of any retinal and/or optic nerve disease other than LHON, (2) follow-up of less than 1 year in LHON-affected patients, (3) unusual presentation of LHON, such as childhood onset and a subclinical or slowly progressive disease course.\textsuperscript{56}

All participants gave their informed consent according to the Declaration of Helsinki and the study was approved by the internal review board of the Department of Neurologic Sciences at the University of Bologna and the Institutional Review board of the Federal University of Sao Paulo.

**Table 1. Clinical and Genetic Characteristics of the Study Groups**

<table>
<thead>
<tr>
<th></th>
<th>LHON-Affected</th>
<th>Without Visual Recovery</th>
<th>With Visual Recovery</th>
<th>LHON Carrier</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brazilian Sample</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects</td>
<td>(n = 15)</td>
<td>(n = 15)</td>
<td>(n = 45)</td>
<td>(n = 78)</td>
<td></td>
</tr>
<tr>
<td>Mean age (SD)</td>
<td>44.6 (14.7)</td>
<td>35.9 (15.4)</td>
<td>28.3 (12.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range age</td>
<td>17–72</td>
<td>10–60</td>
<td>10–54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>13/2</td>
<td>25/20</td>
<td>39/39</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Italian Sample</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects</td>
<td>(n = 26)</td>
<td>(n = 30)</td>
<td>(n = 101)</td>
<td>(n = 81)</td>
<td></td>
</tr>
<tr>
<td>Mean age (SD)</td>
<td>35.7 (15.0)</td>
<td>33.3 (12.6)</td>
<td>41.4 (16.1)</td>
<td>33.5 (9.8)</td>
<td></td>
</tr>
<tr>
<td>Range age</td>
<td>16–68</td>
<td>14–67</td>
<td>10–85</td>
<td>10–54</td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>19/7</td>
<td>22/8</td>
<td>27/74</td>
<td>29/52</td>
<td></td>
</tr>
<tr>
<td>Mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11778 (28 pedigrees)</td>
<td>13</td>
<td>17</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3460 (9 pedigrees)</td>
<td>8</td>
<td>5</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14484 (6 pedigrees)</td>
<td>3</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3733 (2 pedigrees)</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Instrumentation and Procedures

All measurements were obtained by a commercially available optical coherence tomographer (Stratus OCT; software version 4.0.1; Carl Zeiss Ophthalmic System Inc., Humphrey Division, Dublin, CA). Optic nerve head (ONH) analysis was performed with the Fast Optic Disc acquisition protocol, which has been reported to provide reproducible measurements of the optic disc that correlated highly with those acquired by means of other technologies such as confocal scanning laser ophthalmoscopy. The examination was performed under mydriasis by two experienced operators (CdVF and PB), who were masked regarding the clinical status of each subject. At the beginning of the examination, the OCT lenses were adjusted for the patient’s refractive error. Polarization was optimized to maximize the reflective signal and the best centralization of the scan with respect to the optic disc was always looked for. The inclusion criteria for scans were as follows: signal strength ≥ 6; no images with artifacts, missing parts, or showing seemingly distorted anatomy; and a fundus image clear enough to see the optic disc and the scan circle. The oval pattern of the optic disc was evaluated by means of the tilt ratio to exclude tilted discs (all patients, carriers, and control subjects had a ratio > 0.77).

As regards ONH analysis, the automatically defined ONH boundaries were manually repositioned if the RPE edges were not correctly identified by the software, so that the resulting ONH profile did not fit with that observed by fundus photographs (this method has been reported to be more realistic in view of frequent failed disc margin recognition; Fig. 1). In the event of peripapillary atrophy of the RPE, the optic disc edge was no longer identified on the basis of the RPE edge, but on the basis of the neuroretinal rim, as seen on the fundus photograph.

Among the OCT measurements, the following parameters were examined: optic disc area and vertical and horizontal disc diameters.

Statistical Analysis

For statistical purposes, only one eye, randomly chosen, was considered for each LHON-affected, LHON carrier, and control subject. All statistical analyses were performed with commercial software (SPSS, ver. 12.0; SPSS Sciences, Chicago, IL). The equality of variance of the considered OCT parameters was verified by the Levene’s test (which showed equal variances across all samples). Optic nerve head parameters were compared by analysis of variance (ANOVA) with Bonferroni multiple comparison post hoc test. In the Italian sample, an unpaired t-test was used to compare ONH parameters after stratifying LHON-affected patients and LHON carriers by the mtDNA mutation.

After assessing that ONH area was not significantly different between Brazilian control groups (Off-maternal, first-degree Male-descendant and second-degree Male-descendant) we pooled all the individuals (n = 78) in a single large control group.

In the Italian sample, normal distribution curves (using Origin-Pro7.5; OriginLab Corp. Northampton, MA) of vertical disc diameter were separately obtained from the LHON-affected patients stratifying them into with and without VR groups. The cutoff value of the intercept between the curves was found. The incidence of VR in patients with vertical disc diameter under and over the cutoff value was examined by χ² test. P < 0.05 was accepted as statistically significant in all analyses.

RESULTS

Brazilian Sample

The Brazilian sample included 15 LHON-affected (all without VR) and 45 LHON carriers; 78 individuals negative for the 11778/ND4 mutation (Off-maternal plus Male-descendant) from the same family were used as control subjects. Demographic data (mean age and sex) of patients and control subjects are provided in Table 1.

Optic nerve head topographic values, as measured by OCT, are reported in Table 2 and Figures 2 and 3. Analysis of variance showed significant differences in the disc area, which was larger in the LHON carrier subgroup (P = 0.002), but not in the vertical and horizontal disc diameters. The post hoc test confirmed that LHON carriers had significantly larger mean optic disc area than did the control group (2.28 ± 0.37 vs. 2.08 ± 0.27 mm², P = 0.002), whereas the vertical and horizontal disc diameters were increased without reaching statistical significance. When comparing the LHON carriers to LHON-affected groups, we detected a tendency toward increased values of the disc area and vertical and horizontal diameters, although statistical significance was not reached. Finally, the LHON-affected and control groups had comparable mean values for the disc area and vertical and horizontal disc diameter.

Italian Sample

We collected 56 LHON-affected patients, 26 without and 30 with VR. The demographic data, as well as stratifications by sex

FIGURE 1. Representative ONH color pictures and OCT analysis (DA: optic disc area and VDD: vertical disc diameter) of a control subject (A, E, D), an LHON-affected patient with VR (B, F, J), an LHONaffected patient without VR (C, G, K), and an LHON carrier (D, H, I). All individuals are from the Italian sample.
Table 1. Topographic ONH Measurements Comparison between Brazilian LHON-Affected, LHON-Carrier, and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Brazilian Control* (n = 78 eyes)</th>
<th>LHON-Affected* (n = 15 eyes)</th>
<th>LHON-Carrier* (n = 45 eyes)</th>
<th>ANOVA P</th>
<th>Post Hoc Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disc area, mm²</td>
<td>2.08 (0.27)</td>
<td>2.09 (0.26)</td>
<td>2.28 (0.57)</td>
<td>0.002</td>
<td>1.0</td>
</tr>
<tr>
<td>Vertical disc diameter, mm</td>
<td>1.72 (0.12)</td>
<td>1.71 (0.15)</td>
<td>1.77 (0.20)</td>
<td>0.159</td>
<td>1.0</td>
</tr>
<tr>
<td>Horizontal disc diameter, mm</td>
<td>1.50 (0.14)</td>
<td>1.49 (0.16)</td>
<td>1.57 (0.17)</td>
<td>0.053</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Data are expressed as the mean (SD).
† Bonferroni multiple comparison post hoc test between LHON-affected and control.
‡ Bonferroni multiple comparison post hoc test between LHON-carriers and control.
§ Bonferroni multiple comparison post hoc test between LHON-affected and carrier.
|| Statistically significant.

and mtDNA mutation, are reported in Table 1. Most patients had the 11778/ND4 mutation (53.6%), whereas 3460/ND1, 14484/ND6, and 3733/ND1 mutations represented 23.2%, 14.3%, and 8.9% of the sample, respectively. Table 1 also reports the data from 101 LHON carriers. Similar to the LHON-affected group, most carriers had the 11778/ND4 mutation (43.5%), and the 3460/ND1, 14484/ND6, and 3733/ND1 mutations represented 38.6%, 9.9%, and 7.9% of the carrier population, respectively. The control group consisted of 81 subjects (29 males and 52 females).

The ONH measurements for LHON-affected, LHON carrier, and control subjects are summarized in Table 3 and Figures 2 and 3. Analysis of variance showed highly significant differences for the optic disc area and vertical disc diameter, which were larger in the LHON carriers (P < 0.001 in both cases), but not for the horizontal disc diameter. The Bonferroni post hoc test confirmed that the mean values for disc area and vertical disc diameter were significantly higher in LHON carriers than in both the LHON-affected (respectively, P = 0.008 and P < 0.001) and control groups (P < 0.001 in both cases). It must be noted that LHON-affected and control groups had comparable mean values for disc area and vertical and horizontal disc diameters.

In Table 4, as well as in Figures 2 and 3, the LHON-affected patients were stratified by the occurrence of VR. The analysis of variance among the two LHON-affected subgroups and the control group showed significant differences in optic disc area and vertical disc diameter, which were larger in the VR subgroup (respectively, P = 0.042 and P = 0.004), but not for the horizontal disc diameter. The post hoc test between LHON-affected patients with and without VR confirmed that the mean value of the vertical disc diameter was higher in patients with VR (1.82 ± 0.19 vs. 1.66 ± 0.18 mm, P = 0.03), and that a tendency toward increased values of disc area was also present in patients with VR, just short of reaching statistical significance (P = 0.065). Although the difference was not statistically significant, LHON-affected subjects with VR had a mean disc area and vertical and horizontal disc diameters larger than those in control subjects, whereas the ONH size of LHON-affected without VR was almost identical with that of control subjects.

**Figure 2.** Optic disc area of control subjects, LHON-affected and LHON carriers in Brazilian and Italian samples. Box: median, quartiles, and extreme values; circles: outliers.

**Figure 3.** Vertical disc diameter of control subjects, LHON-affected and LHON carriers in Brazilian and Italian samples. Box: median, quartiles, and extreme values; circles: outliers.
Table 3. Optic Nerve Head Parameters Comparison between Italian LHON-Affected, LHON-Carrier, and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Controls* (n = 81 eyes)</th>
<th>LHON-Affected* (n = 56 eyes)</th>
<th>LHON-Carrier* (n = 101 eyes)</th>
<th>ANOVA</th>
<th>Post Hoc Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disc area, mm²</td>
<td>2.05 (0.32)</td>
<td>2.11 (0.39)</td>
<td>2.29 (0.39)</td>
<td>0.008</td>
<td>1.0 &gt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.008</td>
</tr>
<tr>
<td>Vertical disc diameter, mm</td>
<td>1.66 (0.14)</td>
<td>1.66 (0.18)</td>
<td>1.82 (0.19)</td>
<td>&lt;0.001</td>
<td>1.0 &gt;0.001</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Horizontal disc diameter, mm</td>
<td>1.54 (0.15)</td>
<td>1.57 (0.16)</td>
<td>1.59 (0.17)</td>
<td>0.088</td>
<td>1.0 0.086</td>
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<td></td>
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<td></td>
<td>0.796</td>
</tr>
</tbody>
</table>

* Data are expressed as the mean (SD).
† Bonferroni multiple comparison post hoc test between LHON-affected and control.
‡ Bonferroni multiple comparison post hoc test between LHON-carriers and control.
§ Bonferroni multiple comparison post hoc test between LHON-affected and carrier.
|| Statistically significant.

Table 4. ONH Parameters Comparison between Italian Patients with LHON with and without VR with Respect to the Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Control* (n = 81 eyes)</th>
<th>LHON with VR* (n = 30 eyes)</th>
<th>LHON without VR* (n = 26 eyes)</th>
<th>ANOVA</th>
<th>Post Hoc Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disc area, mm²</td>
<td>2.05 (0.32)</td>
<td>2.21 (0.38)</td>
<td>1.99 (0.38)</td>
<td>0.042</td>
<td>0.09 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.063</td>
</tr>
<tr>
<td>Vertical disc diameter, mm</td>
<td>1.66 (0.14)</td>
<td>1.73 (0.17)</td>
<td>1.60 (0.16)</td>
<td>0.004</td>
<td>0.15 0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Horizontal disc diameter, mm</td>
<td>1.54 (0.15)</td>
<td>1.60 (0.14)</td>
<td>1.53 (0.17)</td>
<td>0.20</td>
<td>0.32 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.36</td>
</tr>
</tbody>
</table>

* Data are expressed as the mean (SD).
† Bonferroni multiple comparison post hoc test between LHON with visual recovery and control.
‡ Bonferroni multiple comparison post hoc test between LHON without visual recovery and control.
§ Bonferroni multiple comparison post hoc test between LHON with and without VR.
|| Statistically significant.

**DISCUSSION**

One of the main unexplained features of LHON remains the variability in penetrance. A number of factors are assumed to be influential on the risk of becoming affected when an individual carries one LHON mutation in homoplasmic fashion (100% of the mtDNA molecules are mutant). Our seminal investigation of the same Brazilian family in this study showed that the LHON-affected subgroup had a striking recurrence of exposure to environmental risk factors, which included tobacco, alcohol, and agricultural pesticides. On the other hand there is a strong indication that genetic modifiers in the nuclear genome may also be relevant for disease penetrance in LHON. This study was conducted to investigate whether the ONH size may also be involved in the penetrance variability of the disease.

We used OCT to measure the ONH size in two samples of LHON patients: a large LHON pedigree from Brazil and a large cohort of Italian LHON families with different pathogenic mutations. In both samples the optic discs, as measured by different morphologic parameters, were significantly larger in LHON carriers compared with LHON-affected and control groups. Moreover, Italian LHON-affected patients with VR showed larger ONHs than patients without VR.

Howell hypothesized that the so-called disc at risk, well studied in NAION, may also be relevant in LHON pathogenesis. Our data support his hypothesis, in that a larger ONH size, probably associated with less crowding of RGC axons, may be a protective factor preserving LHON carriers from developing the acute phase of the disease. In accord with this, the LHON-affected subgroup of patients with VR also had a significantly larger ONH, supporting the idea that this anatomic feature is not only a protective factor in LHON carriers, but also influences the final visual outcome in those individuals who develop the optic neuropathy. These findings are mirrored by another observation recently reported by our group on childhood LHON cases, as defined by disease onset before 10 years of age. Among the cases studied, those with acute onset showed a significantly smaller optic disc area and vertical diameter of the ONH, suggesting that small optic discs in these cases represented an unfavorable prognostic factor.

Other results of interest concern the stratification by mtDNA pathogenic mutation, which confirms the general finding of larger ONH in LHON carriers, except for the 14484/ND6 LHON-affected group. This may depend on the limited number of 14484/ND6 cases investigated. Alternatively, we must assume that this mutation may follow a slightly different pathomechanism to induce the disease. It is of note that the 14484/
ND6 mutation is associated with the highest rate of VR\(^{16,17}\) and has a very strict association with a specific mtDNA background, the so-called haplogroup J, to be penetrant and express the clinical phenotype.\(^ {26,27}\)

These observations may have multiple implications, both for the clinical management of LHON patients and for the search of putative genetic modifiers involved in the incomplete penetrance of LHON. The notion that ONH anatomic conformation contributes to disease risk in LHON may have important implications on prognosis and clinical surveillance of LHON carriers in the critical age at highest risk for developing LHON. Therefore, further work is needed to refine an algorithm predicting the subjects who are approaching the threshold for clinical conversion to the acute phase.

Based on our current results, the pool of genes involved in ONH anatomic conformation may be a source for selecting candidate modifying genes, able to influence the variability of penetrance in LHON.\(^23\) The search for such nuclear-modifying factors is currently an active area of investigation, mostly oriented around chromosome X genes. In fact, linkage analysis has shown the existence of a locus at Xp21-Xq21 in a subset of LHON families from northern Europe and Italy,\(^26\) whereas a different locus at Xq25-28 was found in the SOA-BR family.\(^29\)

Eye development is a highly complex process that is dependent on the coordinated interaction of multiple genes at different times of embryonic development. For example, genes regulating the physiological activation of apoptosis during embryogenesis are very relevant in eye development. Consider the \(Bax^{-/-}\) mouse model, which has been related with a \(>50\%\) increase in RGCs, as well the opposite \(Bcl2^{+/+}\) mouse model, which is associated with the loss of one third of GCs.\(^43\) Genetic variation in these two genes and the many others affecting ocular and optic nerve development may give rise to the observed variability in ONH anatomic conformation in the general population. The same genetic variation could also be associated with penetrance in LHON. Further families of proteins with a pivotal role in eye development are homeobox genes such as \(Vax1\) and \(Vax2\), or the highly conserved transcription factors \(Pax6\) and \(Pax2\).\(^44,45\)

The role of ONH anatomic conformation as a protective or risk factor for development of LHON should be related to our previous results on RNFL thickness in presymptomatic carriers and patients with acute or chronic LHON.\(^6,7\) We propose that the small papillomacular bundle-forming fibers on the temporal side of the optic disc are the first and most severely affected by the LHON pathophysiological process, starting during the sub- and preclinical stages. These fibers show an in-

![FIGURE 4](image_url)

**FIGURE 4.** The normal distribution curves of vertical disc diameter in LHON-affected patients with and without VR. A cutoff value for VR obtained from the intercept of these curves is 1.63 mm.

<table>
<thead>
<tr>
<th>Table 5. ONH Parameters Comparison between LHON-Affected and LHON-Carrier after Stratifying by mtDNA Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disc area, mm(^2)</td>
</tr>
<tr>
<td>Vertical disc diameter, mm</td>
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* Data are expressed as the mean (SD).
† Test between groups.
‡ Statistically significant.
creased thickness, which is currently interpreted as swelling. The subsequent swelling of the superior and inferior arcades, which becomes characteristic of the early stage after conversion to the acute phase of the disease, may trigger dysfunction and axonal death in the papillomacular bundle. The current studies support a mechanical factor, in the presence of increasing pseudoedematous swelling of the axons, as the mediator of these events. In this scenario, the anatomic conformation of ONH may represent a relevant element that prompts or mitigates the progression toward the threshold for a catastrophic and synchronous degeneration of groups of fibers, starting with the most vulnerable papillomacular bundle temporal to the optic disc. It has been documented that smaller optic discs are associated with a higher axonal density, as well as that larger optic discs have a larger total lamina cribrosa area and more lamina pores. However, the nature of axonal swelling in LHON is not fully understood. A currently discussed hypothesis is that impaired axoplasmic transport and altered mitochondrial network organization within portions of the RGC axons cause progressive swelling and increased thickness of the RNFL. This process is not accompanied by altered blood–brain barrier and/or inflammation, as demonstrated by lack of fluorescein leakage at fluorangiography and helps differentiate LHON pathophysiology from NAION and optic neuritis. A further component in the pathogenic cascade of acute LHON may be vascular, as indicated by accompanying microangiopathy, hyperemia, and vessel tortuosity. These vascular features are not fully understood and disappear once the optic atrophy is established. A compensatory role in the mitochondrial dysfunction and energy shortage at the ONH has been proposed. Thus, a reduced blood flow in small optic discs may contribute to crossing the critical threshold for precipitating the neurodegenerative process starting from the small fibers of the papillomacular bundle, known to be the most vulnerable to energy deficiency.

In conclusion, the present study strongly suggests that ONH anatomic conformation correlates with the risk of conversion...
from carrier to affected. Indeed, ONH size may be a significant factor in determining whether unaffected carriers convert to LHON and also a mitigating factor affecting visual prognosis once conversion has occurred. These observations were concordantly derived from a within-family study of a large LHON pedigree and from a study of an extensive cohort of individuals from unrelated families carrying different mtDNA defects. We propose to integrate different objective clinical measures with environmental and genetic factors to predict the risk of developing LHON, anticipate conversion to active disease, and foresee the prognosis on visual outcome. Furthermore, the current observations may be useful in selecting genes to be screened for their possible role as modifiers on the variability of penentration in LHON.

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References


