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Leber's hereditary optic neuropathy mitochondrial DNA mutations in normal-tension glaucoma

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Abstract *Background:* in Leber's hereditary optic neuropathy, increased optic nerve cupping has been reported by several authors. Recently, a mitochondrial DNA (mtDNA) mutation at nucleotide 11778 typically associated with Leber's hereditary optic neuropathy (LHON) was identified in a patient treated for glaucoma but lacking typical signs of LHON. The question arises: should all normal-tension glaucoma patients be further evaluated for LHON? Methods: we screened 54 unselected patients with normal-tension glaucoma (age range 20–96 years, 16 men and 38 women) for the primary mtDNA LHON mutations at nucleotides 3460, 11778 and 14484. Results: none of the patients harboured the mtDNA mutations at nucleotides 3460, 11778 or 14484 (95% confidence intervals for each mutation ranged from 0% to 5.3%). *Conclusions:* primary LHON mtDNA mutations are rare or absent in unselected normal-tension glaucoma patients. Therefore, unselected normal-tension glaucoma patients should not be screened for these mutations. It is probable that only normal-tension glaucoma patients with atypical features (rapid progression, early deep central scotoma, pallor of neuroretinal rim, elevated disc, peripapillary teleangiectasia) or a positive family history of visual loss compatible with a matrilinear transmission should be further evaluated.

Keywords Leber's hereditary optic neuropathy · Mitochondrial DNA · Normal-tension glaucoma

Introduction

About 20–30% of patients with open-angle glaucoma have intraocular pressures within the normal range [8, 17]. This form of open-angle glaucoma is known as low-tension glaucoma or normal-tension glaucoma and is usually defined by the clinical features of glaucomatous optic disc cupping, glaucomatous visual field defects, open and otherwise normal anterior chamber angles, and intraocular pressures always below 22 mmHg without treatment [5]. Because glaucomatous optic nerve damage occurs without elevated intraocular pressures in normal-tension glaucoma, the following main pathogenic mechanisms have been proposed: systemic vascular problems, decreased optic disc resistance, immune disorders, excitatory amino acid toxicity, nitric oxide, and programmed

cell death. However, the exact cause(s) remain(s) unknown [4, 5, 7].

Leber's hereditary optic neuropathy predominantly affects young men, causing acute optic neuropathy usually in one eye, followed within weeks to months, by optic neuropathy in the other eye. Typically, the visual impairment is severe with poor recovery [13]. Atypical courses can be difficult to distinguish from other forms of optic neuropathy [14]. Sometimes a characteristic peripapillary angiopathy is seen in Leber's hereditary optic neuropathy patients [15]. About half of the Leber's hereditary optic neuropathy patients have a G-to-A mutation at nucleotide (nt) 11778 in the mitochondrial DNA (mtDNA) resulting in an arginine-to-histidine amino acid substitution in subunit 4 of the mitochondrial oxidative phosphorylation enzyme complex I [NADH dehydrogenase (ND) 4] [13, 19]. A second mutation at nt-3460 (ND1 gene) is found in about 15% of Leber's hereditary optic neuropathy patients [6, 13]. A third mutation at nt-14,484 (ND6 gene) seems to be associated with a relatively good prognosis for visual recovery [9]. The mutations at nt-3460, nt-11778 and nt-14484 are called primary, because their association with Leber's hereditary optic neuropathy seems pathogenic. Other, secondary, mutations have been associated with Leber's hereditary optic neuropathy but their significance remains unclear [13].

Some cases of Leber's hereditary optic neuropathy show delayed visual field loss [13] and increased optic nerve cupping [16, 18]. In a study by Trobe et al. stereoscopic optic disc photographs of 16 eyes harbouring Leber's hereditary optic neuropathy were assessed by masked observers. Four eyes (25%) were found to have pathologic optic disc cupping [18]. In a large family harbouring the mitochondrial nt-11778 Leber's hereditary optic neuropathy mutation, a larger optic disc cupping was observed in affected compared with nonaffected members and compared with nonaffected mutation carriers [16]. In 1993, the Leber's hereditary optic neuropathy mtDNA mutation at nt-11778 was identified in a patient with a family history of normal-tension glaucoma having bilateral visual acuity and visual field deterioration over several months [20]. Examination revealed in both eyes glaucomatous optic nerve cupping with notching, only questionable rim pallor, and a peripapillary haemorrhage in the left eye. Elevated intraocular pressures were noted only on one occasion. The patient lacked other typical signs of Leber's hereditary optic neuropathy [20].

In order to study the association of Leber's hereditary optic neuropathy and normal-tension glaucoma and to better understand which patients with normal-tension glaucoma should be further evaluated for Leber's hereditary optic neuropathy mutations, we screened unselected patients with clinically defined normal-tension glaucoma for the primary Leber's hereditary optic neuropathy nt-3460, nt-11778, and nt-14484 mtDNA mutations using polymerase chain reaction (PCR) and restriction enzyme fragment length polymorphism (RFLP) techniques.

Materials and methods

Patients

The study protocol was approved by the Review Boards of the Universities of Bern, Basel and Lausanne, Switzerland and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients after explanation of the nature and possible consequences of the study. Fifty-four unselected patients with normal-tension glaucoma seen at the Departments of Ophthalmology of the Universities of Bern, Basel, and Lausanne between April 1997 and October 1998 agreed to participate.

For the diagnosis of normal-tension glaucoma, the following criteria had to be fulfilled: typical glaucomatous optic nerve cupping, glaucomatous visual field defects, open and otherwise normal anterior chamber angles, and untreated intraocular pressures below 22 mmHg during at least two diurnal assessments.

PCR and RFLP techniques

Ten millilitres of blood was collected in EDTA tubes. Total leukocyte DNA was extracted using standard protocols.

Screening for the nt-3460 mutation was performed by amplifying a 1233-base pair (bp) fragment of mtDNA spanning nt-3116 to nt-4349 using PCR (primers 5'-CCTCCCTGTACCAAAGGAC-3' and 5'-GTTCGATTCTCATAGTCCTAG-3'). PCRs were performed in a Perkin-Elmer Thermocycler for 25 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. Approximately 300 ng of PCR product was digested with 0.5 units of *Acy*I (New England Biolabs, Beverly, Mass.) at 37°C overnight. After digestion, the wild-type mtDNA produces two bands, 889 bp and 344 bp in length. MtDNA with the G-to-A mutation lacks the enzyme site.

For the nt-11778 mutation, screening was performed by amplifying a 746-bp fragment of mtDNA spanning nt-11668 to 12413 using PCR (primers 5'-CCAAACCCCCTGAAGCTTCACCGG-CGCAAG-3' and 5'-GGGTTAACGAGGGTGGTAAGG-3'). PCRs were performed in a Perkin-Elmer Thermocycler for 30 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C. Approximately 300 ng of PCR product was digested with 0.5 units of *Sfa*NI (New England Biolabs) at 37°C overnight. After digestion, the wild-type mtDNA produces two bands, 121 bp and 625 bp in length. MtDNA with the G-to-A mutation lacks the enzyme site.

Screening for the nt-14484 mutation was performed by amplifying a 75-bp fragment of mtDNA spanning nt-14463 to 14538 by PCR (primers 5'-TAGTATATCCAAAGACAACGA-3' and 5'-TTTGGGGGAGGTTATATGGG-3'). PCRs were performed in a Perkin-Elmer Thermocycler for 30 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C. Approximately 150 ng of PCR product was amplified under the same conditions with α-[^{32}P]-radiolabelled dATP for 1 cycle of 2 min at 94°C, 2 min at 55°C, and 5 min at 72°C. Approximately 15 ng of PCR product was digested with 0.5 units of *Dpn*II (New England Biolabs) at 37°C overnight. After digestion, the wild-type mtDNA produces two bands, 54 bp and 21 bp in length. MtDNA with the T-to-C mutation lacks the enzyme site.

We visualized the bands by electrophoresis through a 2% agarose gel stained with ethidium bromide. Radiolabelled bands were visualized on X-ray films.

For each set of PCR amplification and digestion we used positive controls containing the primary DNA mutations.

Statistical methods

A power analysis showed that in case of a negative study result a sample size of about 55 is needed to obtain a 95% confidence interval ranging from 0% to 5%. The exact 95% confidence intervals were calculated according to Diem [3].

Results

The 54 patients included in the study ranged in age from 20 to 96 years; 16 were men and 38 were women.

None of the 54 patients with normal-tension glaucoma harboured the primary Leber's hereditary optic neuropathy nt-3460, nt-11778 or nt-14484 mtDNA mutation (95% confidence interval for each of the mutations ranged from 0% to 5.3%).

Discussion

For two decades, an association between Leber's hereditary optic neuropathy and glaucoma has been suspected [18]. In patients with intraocular pressures within the normal range, the increase of optic nerve cupping associated with Leber's hereditary optic neuropathy [16, 18] might be mistaken for normal-tension glaucoma.

In 1996, Brierley et al. [2] did not find any of the primary mtDNA mutations associated with Leber's hereditary optic neuropathy in eight patients with normal-tension glaucoma. In all eight skeletal muscle biopsy samples, respiratory chain function was normal [2]. Since this study only excludes, with 95% confidence, the possibility that more than one-third of all normal-tension glaucoma patients harbour a systemic defect of the respiratory chain or primary Leber's hereditary optic neuropathy mtDNA mutations, we have investigated this association further by screening a large number of patients with clinically defined normal-tension glaucoma.

In our study, none of the 54 patients harboured any of the primary Leber's hereditary optic neuropathy mtDNA mutation. Because of the limited number of patients, especially males, screened by us, we cannot exclude the possibility that some normal-tension patients might harbour primary mtDNA mutations. Our report suggests with 95% confidence that not more than 5.3% of unselected normal-tension glaucoma patients harbour primary mtDNA mutations. Our study can not exclude the possibility that other mtDNA mutations may be associated with normal tension-glaucoma.

Even if no association exists between Leber's hereditary optic neuropathy and normal-tension glaucoma patients, both conditions may occur together rarely. The prevalence of normal-tension glaucoma in a white population is estimated to be about 0.5% [1, 8, 17]. The prevalence of Leber's hereditary optic neuropathy can only be estimated indirectly using data from Australia. In 1992, an extensive genealogic research identified 3,537 Australians harbouring a Leber's hereditary optic neuropathy mtDNA mutation; 291 individuals had visual loss [10]. Assuming a total population of 16 million in 1992, the prevalence for a mutation would be 0.022% and the prevalence for visual loss about 2 in 100,000. If no association exists, each population of one million would be expected to contain about two normal-tension glaucoma patients harbouring Leber's hereditary optic neuropathy mutations. Patients with normal-tension glaucoma and a visual loss from Leber's hereditary optic neuropathy would be rarer; about 2 per 10 million.

Which patients with normal-tension glaucoma should be screened for Leber's hereditary optic neuropathy mutations? Review of the current literature and our data suggest that only normal-tension glaucoma patients with one or more of the following features need to be screened for Leber's hereditary optic neuropathy mutations: peripapillary teleangiectasia, pallor of neuroretinal rim, optic disc elevation, rapid progression of the condition, early deep central scotoma, patients' relatives manifesting Leber's hereditary optic neuropathy or a family history of visual loss compatible with a matrilinear transmission [11, 12, 13, 20].

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