Leber’s hereditary optic neuropathy:
clinical and molecular genetic findings
in a patient with a new mutation in the ND6 gene

Abstract  ● Background: Leber’s hereditary optic neuropathy (LHON) is a maternally inherited ocular disease associated with mutations in the mitochondrial DNA (mtDNA). We describe the clinical and molecular genetic findings in a LHON patient and his family with a new mtDNA mutation at np14568 in the ND6 gene. ● Methods: Ophthalmological examination was performed in one affected male and two maternal relatives. Direct sequence analysis of the complete mtDNA protein coding region was initiated in the affected patient. Four unaffected maternal relatives also underwent molecular genetic evaluation. ● Results: Clinical examination of the affected male showed typical features of LHON. In his unaffected mother slight peripapillary microangiopathy was found. Molecular analysis did not show any of the common LHON mutations. A nucleotide exchange was detected at position 14568 replacing a glycine by serine in the ND6 gene. This mutation was the only new mutation found within the entire protein and tRNA coding region of the patient’s mitochondrial genome. This novel mutation was also present in four non-affected maternal family members, but absent in 60 other LHON lineages and 175 unrelated controls. ● Conclusion: The new mutation at nucleotide position 14568 lies in the close vicinity of other LHON-related mutations (np14459, np14484, np14498, np14596) within the evolutionarily most conserved region of the ND6 gene. Since no other mutation was detected throughout the mtDNA coding region and the new alteration was excluded in controls, our clinical and molecular genetic findings suggest that the novel point mutation at np14568 is responsible for LHON in this family.

Introduction

Leber’s hereditary optic neuropathy (LHON) is characterized by rapid bilateral loss of central vision. Primarily male patients are affected in early adult life. Optic atrophy commonly appears within 3 months after the onset of the disease. Relatives in the maternal line often show a peripapillary microangiopathy typical for LHON [15, 20].

LHON is a rare maternally inherited disease associated with mitochondrial DNA (mtDNA) mutations (Fig. 1; see also http://www.gen.emory.edu/mitomap.html). So far three point mutations at nucleotide positions 11778 in the ND4 gene [21], 3460 in the ND1 gene [7] and 14484 in the ND6 gene [8] are undoubtedly associated with LHON and account for the vast majority of all LHON cases. The pathogenic role of other mtDNA mutations found at increased frequencies in LHON patients is still controversial, since they can also be identified in unrelated healthy controls or act generally in association with one of the other major missense mutations [5]. In some LHON families which do not carry any of the three primary point mutations, new mtDNA missense mutations have been proposed to cause LHON, suggesting that there
is further genetic heterogeneity. A Finnish family with a sporadic mutation at np9101 of the ATP6 gene has been reported which is associated with reduced oligomycin-sensitive ATPase activity and decreased oxidative phosphorylation [13]. Recently new mutations in the ND6 gene at np14459 [11] and np14596 [2] have been associated with LHON and dystonia. Another novel point mutation has been described at nucleotide position 14498 in the ND6 gene [14, 23].

In this study we describe the clinical and molecular genetic findings in one affected patient who does not carry any of the common LHON mutations although presenting typical clinical features of LHON. We have found a new point mutation in the ND6 gene of respiratory complex I in this patient and his unaffected maternal relatives which is likely to cause LHON in this family.

**Patients**

Two generations of one family were examined. The pedigree of this family is given in Fig. 2. The affected patient (III:1) and his unaffected mother (II:3) and brother (III:2) were clinically examined. No eye problems were known in the maternal line of the index patient, and the family history was negative for ophthalmic and neurological disease.

Ophthalmological evaluation included visual acuity, visual field examination (Tübingen Automated Perimeter 30’), color vision testing (Farnsworth D15) and ophthalmoscopic findings. The affected male (III:1) also underwent fluorescence angiography and detailed neurological examination (cerebrospinal fluid analysis, computed tomography of the brain). Pattern and flash VEPs were recorded according to the standard of clinical VEP recording. A Ganzfeld ERG following the International Society for Clinical Electrophysiology of Vision (ISCEV) standard protocol and multifocal ERG responses were also obtained. Blood cell count, blood chemistry and serological tests were also performed.

Molecular genetic examination was initiated in the affected patient (III:1) and four non-affected maternal relatives (II:1, II:2, II:3 and III:2). The panel of controls used in molecular analysis consisted of a random collection of 175 healthy subjects and of 60 LHON patients, all of similar racial origin.

**Molecular genetic analysis**

Venous blood samples for molecular genetic analysis were obtained from the family members following informed consent. DNA was extracted from total blood employing the method of Miller et al. [18]. Common primary and secondary LHON mutations (np11778, np3460, np14484, np14459, np15257, np13708, np4917 and np4216) were tested by polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism (RFLP) analysis [23].

DNA sequence analysis was initiated for the complete mtDNA protein and tRNA coding region (np3126-np16104) of patient III:1 (Fig. 2). The mitochondrial DNA was amplified as a series of 18 overlapping PCR fragments of average length 800 bp. Single-strand templates were generated by magnetic Dynabead technology. For the ND6 gene, overlapping mtDNA fragments were amplified and sequencing was performed using both strands as template [23].

Screening for the np14568 mutation was performed by PCR and RFLP analysis (BcgI) in four maternal members of the family (II:1, II:2, II:3 and III:2) and in 235 control subjects. Additional methodological information has been published elsewhere [23]. A complete list of primers used for PCR amplification and DNA sequencing is available on request from the authors.