LHON, LEBER'S HEREDITARY OPTIC NEURORETINOPATHY, ASSOCIATED WITH MITOCHONDRIAL DNA POINT MUTATION 11778 G→A IN TWO CROATIAN FAMILIES

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Abstract

Background

LHON is LEBER's hereditary optic neuroretinopathy with bilateral acute or subacute loss of central vision due to optic atrophy. It is linked to point mutations of mitochondrial DNA. Mitochondrial DNA (mtDNA) is a small circular molecule of 16,5 kB, which encodes the enzymes of the respiratory chain in mitochondria. It is inherited maternally. The most common pathogenic mtDNK point mutations associated with LHON are 3460 G→A, 11778 G→A and 14484 T→C. These mutations are linked with the defects of subunits of complex I (NADH-dehydrogenase-ubiquinone reductase) in mitochondria. The 11778 G→A point mutation has the worst optic outcome (blindness).

Methods

Complete ophthalmologic examination including best corrected visual acuity on the both eyes according to Snellen chart, ophthalmoscopy, Goldmann perimetry and colour vision with Ishichara plates were done. mtDNA point mutation 11778 G→A was detected in DNA of peripheral blood lymphocytes using PCR and RFLP method.

Results

This is the first study of Croatian patients with LHON defect associated with mtDNK mutations. Two LHON 11778 G→A families are presented in this paper. The mothers and female relatives are LHON mutants without symtoms, while the sons mutants suffered from blindness.

Conclusions

Molecular diagnosis may help in explanation of the cause of LHON disease. LHON should not be based solely on clinical description

Introduction

Mitochondria are organelles responsible for energy production by respiratory chain, oxidative degradation of carbon substrates into water and oxygen. These reaction are catalysed by respiratory chain complexes I, II, III, IV imbedded in the inner membrane of the mitochondria. The mitochondrial genome is 16569 bp long. It contains double stranded DNA molecule and no introns. It replicates independently of the nuclear genome. Mitochondrial DNA is maternally inherited (3, 4, 17 Zeviani, Bauer, Chinnery). The defects of mitochondrial DNA are large scale rearrangements with single deletions or more rarely duplications. Over 118 point mutations of mitochondrial DNA are known today (16Van W) and are associated with different clinical phenotypes (3, 4, 17 Zeviani, Bauer, Chinnery).
Leber's hereditary optic neuroretinopathy (LHON) has been linked to nearly 20 different point mutations of mitochondrial DNA (14, 17 Zeviani, Torroni). The most common pathogenic point mutations named primary are 3460 G→A 11778 G→A 14484 T→C. Point mutation 3460 G→A is associated with the defect of ND1 subunit of complex I (6Huoponen), 11778 G→A with the defect of ND4 subunit of complex I (16 Wallace), while 14484 T→C with the defect of ND6 subunit of complex I (7, 8 Johns). LHON was first linked to point mutations of mitochondrial DNA in 1988 (16 Wallace 1988). Clinically, LHON is manifested with bilateral acute or subacute loss of central vision due to optic atrophy. The typical funduscopic finding in the acute stage of the disease is a peripapillary microangiopathy. The visual defect is usually the only clinical feature. However, it can be occasionally associated with cardiac conduction abnormalities (pre-excitation syndrome), peripheral neuropathy and/or ataxia (3, 4, 17 Zeviani, Chinnery). Usually, the onset of the disease is in the second and third decade, with male predominance (17 Zeviani). The average onset of the disease among males is 24.3 years, while among females 31.3 years (Leo-Kottler 10). However, there is a case report about a 6 year old girl with LHON (2 Balayre). Point mutation 11778 G→A has poor prognosis for vision, while 14484 T→C is linked to mild course of the disease (4, 17 Zeviani, Chinerry). A multiple sclerosis has been described in some female patients with point mutation 11778 G→A. In multiple sclerosis investigation for oligoclonal bands in cerebrospinal fluid (CSF), evoked potentials and MR brain scan can be supplemented with mitochondrial diagnosis (13 Olsen NK). Association between mitochondrial DNA point mutation 11778 G→A with demylenating disease as a marked female predominance has been reported (5 Flaningan KM). Spontaneous recovery from LHON has been described in 5 out of 136 patients (7, Johns).

This work represents the first study of Croatian families with LHON using molecular diagnosis. DNA from peripheral blood lymphocytes was tested, as a more convenient tissue, than muscle or skin fibroblasts.

Materials and methods

Patients

23 pateints from five families were included in this study. Loss of vision was the criteria for LHON testing. Maternal line of inheritance was used for testing the relatives. DNA analysis was done by consent of patients and their relatives.

Ophthalmologic examinations

A complete ophthalmologic examination was performed for patients with visual impairment:

1. Best corrected visual acuity using Snellen charts
2. Colour vision was tested with Ishichara plates
3. Ophthalmoscopy
4. Goldmanns kinetic and/or automated perimetry

DNA testing from peripheral blood lymphocytes using PCR-RFLP method

There is no LHON specific laboratory findings except DNA analysis. DNA was isolated from peripheral blood lymphocytes (11 Miller SA). PCR reaction was performed for each point mutations separately using specific set of primers for each point mutation. The position of primers in the sequence of the mitochondrial genome (1 Anderson) was for 3460 G→A point mutation fw 5'3150 and 3'3600 rv, for 11778 G→A point mutation fw 5'11680 and rv 3' 12000 and for 14484 T→C fw 5'14450 and rv 3' 14608. PCR reaction was performed using the PCR Core Kit (Roche, Germany). PCR products were verified on 1.5% agarose gels. Specific PCR reaction was followed by RFLP (restriction lenght fragment polymorphism) method. PCR products were digested overnight at 37^{o}C with restrictive enzymes, for each point mutation a specific restrictive enzyme was used: BsaH1 for 3460, SfaN1 for 11778 (17 Wallace) and Mbo for 14484 (Johns 8) (Bio Labs, New England). Restrictive fragments were tested on 1.5 % agarose gel using ethidium-bromide for UV visualisation of DNA (17 Zeviani M). Positive mutant DNA was included as control in testings.

Results

mtDNA point mutation 11778 G→A was detected in ten DNA samples out of twenty three tested. Point mutations 3460 G→A and 14484 T→C were not found in any of DNA samples tested. 2 LHON family cases are presented in this paper (Figure 1, Figure 2). The diagnosis of other patients was neuroopticopathia.

Family case No. 1.

The 4 persons positive for LHON 11778 G→A mitochondrial point mutations are family members, two female cousins (aged 50 and 53), their two sons (aged 27 and 25) (Figure 1). The mothers were LHON mutants, with no LHON symptoms. However, their sons are both 11778 G→A mutants, with visual acuity 0.01 blindness.

The male patient (26 years old) was hospitalized firstly on the Neurological clinic, Osijek, because of unilateral loss of the right eye vision and papiloedema. Laboratory testing showed no pathological values, CAT scan and MRI with and without contrast was performed and revealed no intracranial process but only sferoidal and ethmoidal sinusitis of the right side. He was transferred to the Ophthalmology Department, Clinical Hospital Osijek. A month later from the onset of the disease he lost the other eye vision. Ophthalmoscopy revealed teleangiectatic microangiopathy in carrier and nerve fiber layer swelling (papiloedema). Goldmans kinetic perimetry revealed centroceal scotoma in affected person. As he already had a male cousin in second degree, who suffered from sudden visual loss of both eyes few years ago, connected through maternal inheritance line (their mothers were cousins) he was suspected for LHON disease. Indeed, DNA testing turned to be LHON positive for 11778G→A mitochondrial point mutation for him, his cousin and their mothers. A month later from the onset of the disease he lost the other eye vision. His male cousin was diagnosed LHON 11778G→A at the age of 17, also lost both eyesight having visual acuity 0.01.

Family case No. 2.

Six persons LHON 11778 G→A mutants are family members: a young men, his mother, sister, the mother's sister and her daughter and grand-daughter (Figures 2, 3). The male patient (26 years old) was hospitalized on the Department of Ophthalmology, Clinical Hospital Center Zagreb due to unilateral visual loss on the right eye and papiloedema. Two months later he had loss of vision on the left eye. Best corrected visual acuity on the both eyes was 0.08 according to Snellen chart and ophthalmoscopy revealed papilloedema. Goldmann

perimetry showed the presence of central scotomatas with absence of inner isopters. Laboratory testing showed no pathological values. CAT scan revealed no intracranial process. Pulse steroid therapy was administrated. Since there was no satisfactory recovery of visual function, we suspected to LHON disease. In next several years he was hospitalized both on Department of Ophthalmology, as well on Department of Neurology due to deterioation of vision, where he was administrated pulse steroid therapy. Despite of therapy, on the last ophthalmological examination best corrected visual acuity is 0.08 according to Snellen charts, ophthalmoscopy reveals temporal palor of optic disc. Automated Octopus perimetry showed general depression with the presence of central scotomatas. DNA testing turned to be positive for LHON 11778G→A mitochondrial point mutation. His sister, mother, mother's sister-aunt, her daughter and grandaughter were also LHON 11778G→A mutants, but without visual impairement.

Discussion

This work is the first Croatian pilot study of LHON linked to mitochondrial DNA point mutations. The most common three mitochondrial DNA point mutations associated with LHON 3460 G→A, 11778 G→A and 14484 T→C were tested in twenty three patients with LHON symptoms. Mitochondrial DNA point mutation LHON 11778 G→A was detected in ten patients, four and six in two different families. In this study, the onset of the disease was in one patient at the age of 16, in his cousin at the age of 26, and in the third patient at the age of 26. These data are in line with literature about the onset of LHON in young males (4, 16, 17 Wallace, Zeviani, Chinnery). LHON 11778 G→A has poor diagnosis, these young men almost completely lost their eyesight bilaterally (17 Zeviani). The mothers and female relatives were LHON 11778 G→A mutants, carriers, having no clinical symptoms (17 Zeviani). These findings are in-line with literature data about male predominance of the the clinically manifested disease.

The other patients tested were negative for these three most common LHON mutations and were diagnosed as neuroopticopathia. However, LHON may not be completely excluded, since over 20 mtDNA point mutations linked to LHON have been described in the literature untill now (17 Zeviani, 4 Chinnery).

Identification of the disease should not be based solely on clinical description, because it may be an entity other than LHON in some individuals or even in entire pedigrees. Adjunct tests apart from genetic analysis (9, 12) generally are of a limited value in the evaluation of LHON.

Molecular diagnosis accurately explains the cause of LEBER'S optical defect as maternally inherited mtDNK point mutation. The role of genetic counseling in this disease should not be underestimated either (9 Kjer, 12 Nikoskelainen). LHON prognosis is better for females, mutants without symptoms.

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Figure 1.

Presentation of a LHON 11778 G→A family No. 1

Legend
Female, not tested
Male, not tested
LHON mutant, tested, mother without clinical symptoms
LHON mutant, tested, son without clinical symptoms

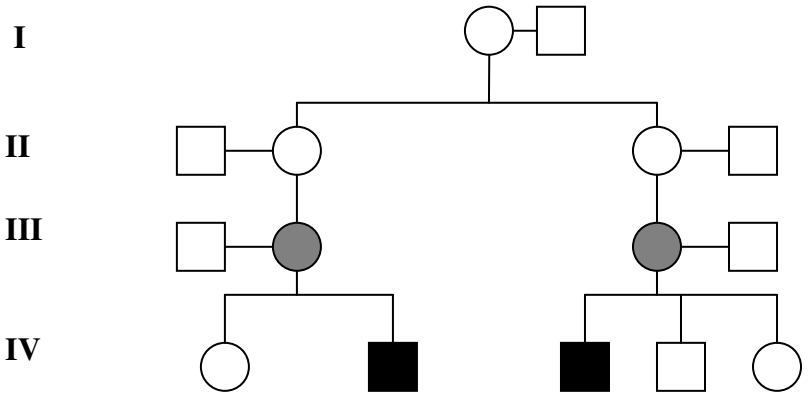


Figure 2.

Presentation of the LHON 11778 G→A family No. 2

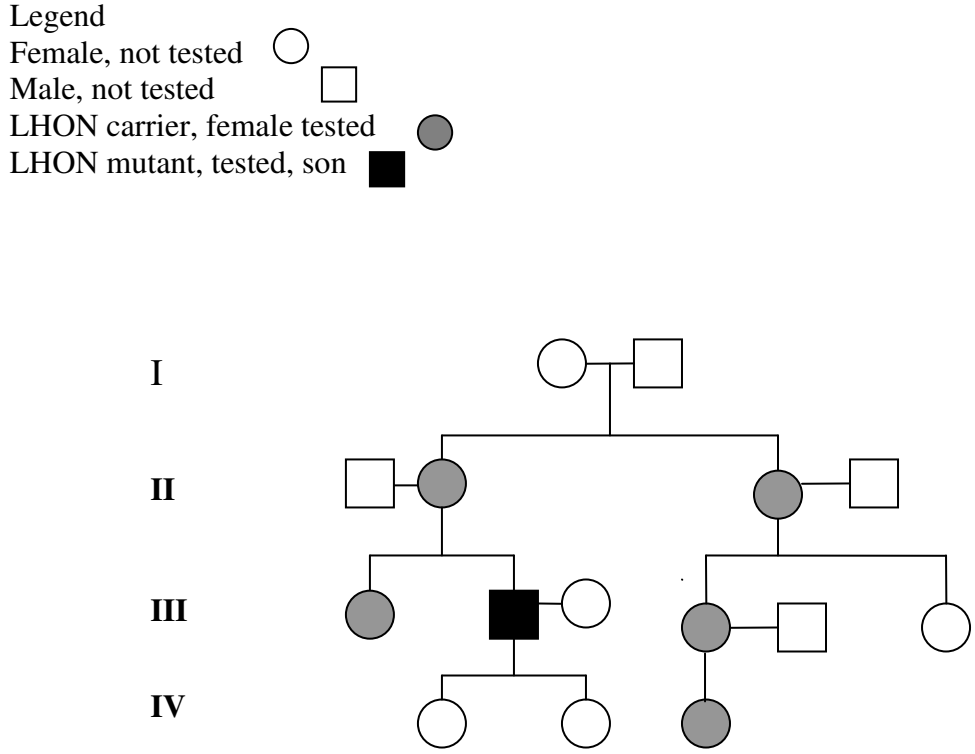
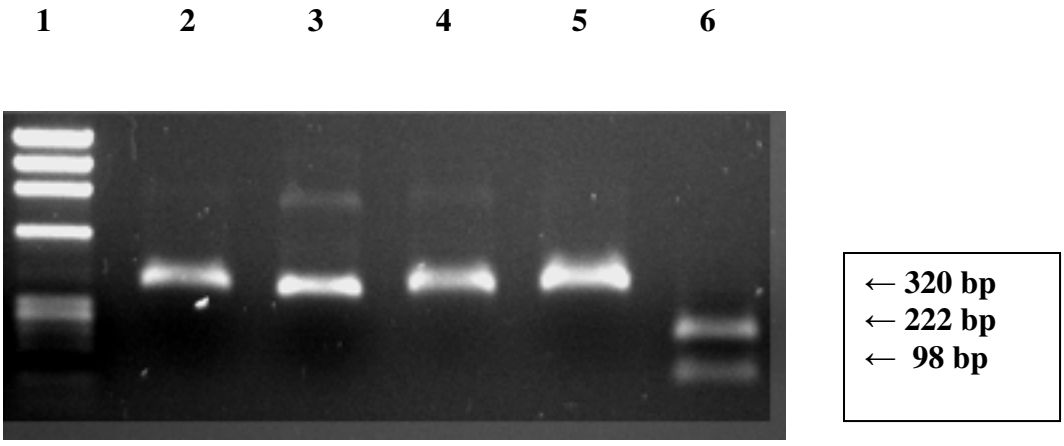


Figure 3.

DNA testing of the LHON family with mitochondrial DNA point mutation 11778 G→A. RFLP analysis in 1.5% agarose gel is presented.



Lane 1. DNA standard

Lane 2. patient, LHON mutant 11778 G→A (no restriction, 320 bp)

Lane 3. the mutant's mother, LHON mutant 11778 G→A

Lane 4. the mutant's aunt (mother's sister), LHON mutant 11778 G→A

Lane 5. positive control, LHON mutant 11778 G→A

Lane 6. negative control (two restriction fragments 222 and 98 bp)