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SHORT REPORT

Sequence analysis of Hungarian LHON patients not carrying the common primary mutations

J. Horvath[1]*, *R. Horvath*[2], *V. Karcagi*[3], *S. Komoly*[4] *and D. R. Johns*[5]

[1]Department of Neurology and Neurosciences, Geneva University Hospital, Switzerland; [2]Metabolic Disease Center Munich-Schwabing, Muenchen, Germany; [3]Department of Applied Biochemistry, Fodor Jozsef National Center for Public Health, Budapest, Hungary; [4]Department of Neurology, Jahn Ferenc Hospital, Budapest, Hungary; [5]Department of Neurology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA

*Correspondence: Department of Neurology and Neurosciences, Geneva University Hospital, Rue Micheli-du-Crest 24, 1211 Geneva, Switzerland.
E-mail: Judit.Horvath@hcuge.ch

Summary: We describe sequence analysis of the mitochondrial DNA of five Hungarian patients diagnosed with probable LHON, who do not carry any of the three primary point mutations. We report three novel mutations, one of which might have a pathogenic role.

Leber hereditary optic neuropathy (LHON) is a maternally inherited disease characterized by a subacute, sequential, bilateral loss of central vision. The vast majority of LHON cases are associated with one of the three primary point mutations at nucleotide positions np11778, np3460 and npl4484 of the mitochondrial DNA (mtDNA).

In this report, we describe the sequencing analysis of the mtDNA of five Hungarian patients (H1–H5) who were diagnosed with probable LHON but were tested negative for the three primary point mutations. H1 and H2 are siblings while the three other patients have no family history.

We have sequenced all the complex I genes of the mtDNA of patients H1, H3, H4 and H5. For patients H1 and H5 we extended our sequencing analysis to the cytochrome-*c* oxidase (COX) I, COX II, COX III, and the cytochrome *b* (Cytb) genes (Anderson et al 1981).

The DNAs had been isolated from total blood using a standard protocol of salt precipitation and were amplified using standard PCR conditions. The sequencing was performed on a polyacrylamide gel using an ABI 377 automated sequencer. For the identified novel mutation at np10237 we created a restriction digest test with *AseI*.

We found that patient H3 carries a C-to-A transition at npl3735 of the *ND5* gene leading to a replacement of a nonconserved leucine by an isoleucine (Leu467Ile). Sequencing the complex I genes of patient H4 revealed a few silent mutations only. Patient H5 was found to carry the sequence variations characteristic of the European haplogroup J. In addition, he carries a homoplasmic C-to-T transition at np10192 that results in a replacement of a not very conserved serine by a phenylalanine

residue at codon 45 of the *ND3* gene (Ser45Phe) (Fearnley and Walker 1992). The pathogenic role of these mutations in LHON does not seem to be likely (Huoponen et al 1993).

Our most remarkable finding is a homoplasmic T-to-C transition at np10237 of the *ND3* gene of patients H1 and H2 resulting in a substitution of an isoleucine with a threonine residue (Ile59Thr). It occurs in a very conserved region of the *ND3* gene, in a potential membran-spanning α-helix. The isoleucine at this position is highly conserved: other species carry lle, Leu or Val at this position and these are all amino acids with a hydrophobic side-chain (Fearnley and Walker 1992). Threonine, however, has an aliphatic hydroxyl side-chain. The mutation is also present in homoplasmic form in the mother and maternal aunt of patients H1 and H2 but was not found in the 85 control DNA samples from the same ethnic background, and was also absent in a control group of 100 DNA samples studied recently by R. Horvath (personal communication). Apart from the mutation l0237T>C, patients H1 and H2 carry some previously identified polymorphisms that are not suspected to have a pathogenic role. Since we have no knowledge of other affected family members we cannot rule out the possibility of a recessive optic atrophy in this case. Still the mutation 10237T>C deserves attention. Biochemical studies and further data about its occurrence in LHON and control populations can determine its role in the pathogenesis of LHON.

REFERENCES

Anderson S, Bankier AT, Barrell BG, et al (1981) Sequence and organisation of the human mitochondrial genome. *Nature* **290**: 457–465.

Fearnley IM, Walker JE (1992) Conservation of sequences of subunits of mitochondrial complex I and their relationship with other proteins. *Biochim Biophys Acta* **1140**: 105–134.

Huoponen K, Lamminen T, Juvonen V, Aula P, Nikoskelainen E, Savontaus ML (1993) The spectrum of mitochondrial DNA mutations in families with Leber hereditary optic neuroretinopathy. *Hum Genet* **92**: 379–384.