A MELAS-Associated ND1 Mutation Causing Leber Hereditary Optic Neuropathy and Spastic Dystonia

Liesbeth Spruijt, MD; Hubert J. Smeets, PhD; Alexandra Hendrickx, BA PlatSc; Marijke Wefers Bettink-Remeijer, MD; A. Maat-Kievit, MD, PhD; Kees C. Schoonderwoerd, PhD; Wim Sluiter, PhD; Ireneaus F. de Coo, MD, PhD; Rogier Q. Hintzen, MD, PhD

Objective: To report a novel mutation that is associated with Leber hereditary optic neuropathy (LHON) within the same family affected by spastic dystonia.

Design: Leber hereditary optic neuropathy is a mitochondrial disorder characterized by isolated central visual loss. Of patients with LHON, 95% carry a mutation in 1 of 3 mitochondrial DNA–encoded complex I genes. The complete mitochondrial DNA was screened for mutations in a patient with LHON without 1 of these 3 primary mutations. The heteroplasmy level and biochemical consequence of the mutation were determined.

Results: A pathogenic 3697G\textsuperscript{H11022}A/ND1 mutation was detected and seemed associated with an isolated complex I deficiency. This family has similar clinical characteristics as the previously described families with LHON and dystonia with an ND6 mutation.

Conclusions: The 3697G\textsuperscript{H11022}A/ND1 mitochondrial DNA mutation causes the LHON and spastic dystonia phenotype in the same family. This mutation can also cause MELAS syndrome (which encompasses mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke), and other genetic factors may contribute to the clinical expression.

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EBER HEREDITARY OPTIC NEUROPATHY (LHON) is a mitochondrial inherited optic atrophy, characteristically presenting with (sub)acute bilateral vision loss.\textsuperscript{1} More than 95% of all cases are caused by 1 of 3 primary mitochondrial DNA mutations (11778G\textsuperscript{H11022}A, 3460G\textsuperscript{H11022}A, or 14484T\textsuperscript{H11022}C) that are all encoding a subunit of complex I.

The association of LHON with spastic dystonia is rare (Online Mendelian Inheritance in Man 500001), and all mutations thus far have occurred in the ND6 subunit of complex I. The common characteristics are optic atrophy and spastic dystonia, asymptomatic basal ganglia lesions, and nearly absent complex I activity.

In this study, we sequenced the complete mitochondrial DNA (mtDNA) of a female patient with LHON in whom the primary mutations were absent. This search for a novel mtDNA mutation was also motivated by the fact that the brother of the patient had childhood-onset spastic dystonia with unknown cause.

We found a rare pathogenic 3697G\textsuperscript{H11022}A/ND1 mutation in both patients, which has only once been reported in association with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS).\textsuperscript{2} To our knowledge, this report is the first of an ND1 mutation causing LHON and spastic dystonia. The family had a presentation similar to the previously described families with LHON and spastic dystonia who had ND6 mutations.

Methods

REPORT OF A CASE

The proband is a 35-year-old white woman with sequential left and right vision loss, reaching a level of bilateral visual acuity of counting fingers in 14 months. Ophthalmoscopy revealed no abnormalities at the start of the symptoms; after 6 months, optic nerve atrophy became apparent. Color vision was disturbed, and visual fields showed a bilateral central scotoma. Visual evoked potentials and contrast vision were disturbed in both eyes. The results of a neurological examination were normal. Magnetic resonance imaging of the brain showed only a few aspecific punctuated white matter abnormalities in both hemispheres. Serum and cerebrospinal fluid lactate levels were increased to 19.8 mg/dL (2.2 mmol/L) (normal, <16.2 mg/dL [<1.8 mmol/L]) and 33.3 mg/dL (3.7 mmol/L) (normal, <18.0 mg/dL [<2.0 mmol/L]).
mmol/L), respectively. The results of a muscle biopsy showed a discrete increase of fiber type diameter variation, without ragged red fibers or other signs of mitochondrial myopathy. The patient smoked 10 cigarettes per day and did not abuse alcohol. She has 2 healthy children, aged 2 and 4 years. Three years after the initial onset of symptoms, the visual acuity of her left eye improved to 0.05% and the scotoma in the left visual field became smaller.

Her younger brother (by 1 year) had developed a slowly progressive (later stationary) generalized spastic dystonia that had appeared at the age of 3 years. He has been wheelchair bound since the age of 27 years and has mental retardation, scoliosis, dysarthria, and bilateral divergent strabismus without ophthalmoplegia. His serum lactate level was increased (27.0 mg/dL [3.0 mmol/L]), and there was accumulation of swollen mitochondria on electron microscopic analysis of a sural nerve biopsy specimen. Magnetic resonance imaging of the brain showed bilateral hyperintensities in the putamen. The family history was unremarkable, except for frequent occurrence of migraine. The mother of both siblings is healthy.

mtDNA ANALYSIS

The entire mtDNA coding region was amplified with two 25-µL polymerase chain reactions,3 using a polymerase chain reaction system (Expand Long Template PCR System; Roche Diagnostics GmbH, Mannheim, Germany). After purification, fragmentation, and labeling, the products were hybridized on a prehybridized oligonucleotide microarray (MitoChip), as described in a resequencing array protocol (CustomSeq Resequencing Array Protocol) (both available from Affymetrix Inc, Santa Clara, Calif). The chips were washed and stained on a fluidics station (GeneChip Fluidics Station 400; Affymetrix Inc) using the preprogrammed resequencing wash-and-stain protocol (DNA ARRAY-WS2). The oligonucleotide microarrays (Mi
tochips) were scanned (GeneChip Scanner 3000; Affymetrix Inc), creating CEL files for subsequent batch analysis. DNA analysis software (GeneChip DNA Analysis Software, version 3.0.1.3 beta; Affymetrix Inc) was used for the analysis. The presence and percentage of a mutation were determined by mutation-specific restriction digestion of a fluorescently labeled polymerase chain reaction product and gene scan analysis on an analyzer (ABI3100 Genetic Analyzer; Applied Biosystems, Foster City, Calif).3

BIOCHEMISTRY

Tissue homogenates were prepared from frozen muscle in 0.25M sucrose, 10mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, and 1mM EDTA, pH 7.4. Citrate synthetase was mea-

The 3697G>A/ND1 (G131S) mutation was detected (Figure 1) with an oligonucleotide microarray (MitoChip). The sequence can be deduced from the oligonucleotide with the strongest signal that hybridizes best to the template. The mutation load was greater than 97% in the muscle of the patient with LHON. The mutation load was 56% in her blood and 88% and 19% in the blood of her severely affected brother and healthy mother, respectively (Figure 2).


Respiration and enzyme studies in muscle tissue of the patient with LHON and her brother revealed an isolated complex I residual activity to 8% and 16%, respectively, compared with the control subject (Figure 2). The other complexes were normal. The level of citrate synthetase was increased in the sister (168%) and brother (216%), compared with the citrate synthetase activity of 50 controls.

COMMENT

The 3697G>A/ND1 mutation was reported once before, in a young boy with a typical MELAS phenotype.2 The mutation changes an evolutionary highly conserved amino acid (G131S) within the hydrophilic transmembrane loop of the mitochondrial ND1 protein. Pathogenicity was confirmed by cybrid studies.2 The mutation causes an isolated complex I defect as well, in our controls.

Figure 1. Mitochondrial DNA analysis showing the wild-type sequence (A) and the 3697G>A mutation (B). Each column shows the 4 alternative nucleotides in the middle position of the oligonucleotide. The sequence can be deduced from the oligonucleotide with the strongest signal that hybridizes best to the template.

Figure 2. Pedigree reflecting the differences in mutation load and complex I residual activity in correlation with the clinical presentation. Circles indicate females; square, male; unshaded symbol, unaffected subject; and shaded symbol, affected subjects. The activities of the complexes of the mitochondrial respiratory chain were measured in muscle homogenates, as previously described.2 7
LHON and spastic dystonia (H1 and T, respectively); however, although specific mtDNA haplotypes may confer a higher risk for the expression of certain complex diseases, it is unlikely that this is the case and the difference merely indicated that the 3697G>A mutation occurred independently and is not caused by a common founder. The mutation load (in blood, muscle, and fibroblasts) was comparable in the presently described patients and in the previously described patient with MELAS and, therefore, is unlikely to explain the clinically distinct presentations.

In this report, we show that the 3697G>A mutation can cause LHON or infantile spastic dystonia in siblings, which is different from the previously reported MELAS phenotype. Optic atrophy and spastic dystonia are uncommon features for MELAS. In the present patients, there were no indications of stroke-like events or mitochondrial accumulation in muscle tissue. Ragged red fibers, which are associated with MELAS, were absent. Moreover, brain magnetic resonance imaging findings of a patient with MELAS showed several lesions of the right side of the thalamus, the basal ganglia, and the bifrontal cortical region, while, in our patients, there were a few punctuate hyperintensities in one and specific lesions in the putamen in the other. Lactic acidosis, in particular in the cerebrospinal fluid, and isolated complex I deficiency, however, are features that are shared among the 2 families. We conclude that the 3697G>A mutation causes an isolated severe complex I defect, leading to lactate acidosis and functional defects in the central nervous system and variable clinical phenotypes.

A pathogenic point mutation (3376G>A) in the mitochondrial MTND1 gene (the mitochondrial gene encoding the ND1 protein of complex I) has been previously described in association with an overlap syndrome comprising the clinical features of LHON and MELAS in a patient with an isolated complex I deficiency. The combination of spastic dystonia and LHON (Online Mendelian Inheritance in Man 500001) is extremely rare and has been reported in only a few unconnected families with a 14459G>A/ND6 mutation. The present family with LHON and spastic dystonia shares a remarkable similar presentation with those families. The reported clinical presentation was LHON only, spastic dystonia only, or LHON and spastic dystonia. When comparing this present patient with the other 9 described families with LHON and dystonia, the first striking similarities are the bilateral lesions in the basal ganglia, in particular in the putamen; the isolated and severe complex I defect; and the extreme clinical variable phenotype within a family. Spastic dystonia has consistently been observed as associated with basal ganglia lesions, mainly in the nucleus lentiformis (putamen and globus pallidus). Spastic dystonia develops universally before the age of 5 years, and causes a moderate to severe generalized movement disorder sometimes associated with mental retardation. The mutation load might correlate with the phenotype (Figure 2), which has also been reported in most, but not in all, families with the 14459 mutation. The 14459G>A mutation occurs in the hydrophilic extramembrane loop of the ND6 protein, between the transmembrane helixes C and D, facing the intermembrane space, and the present 3697G>A mutation is also situated in the extramembrane loop between the same transmembrane helixes C and D in the ND1 protein; however, this mutation is facing the mitochondrial matrix.

The intrafamilial variation in clinical expression indicates that modifying nuclear factors are likely involved, playing a role in the clinical expression of mitochondrial disease. A recent report of a family with LHON and dystonia without an mtDNA mutation but with a severe isolated complex I defect is another indication for this. Patients with sporadic focal dystonia who have an isolated complex I defect have been identified. Cybrid studies in these families showed that in these patients the complex I defect was not caused by an mtDNA mutation. These data indicate that prognosis or counseling based on the mtDNA mutation and percentage only is troublesome and should be done with great care. It also means that female carriers have no reliable reproductive option to prevent the birth of a severely affected child. Despite this, we advise that patients with LHON or with LHON without the common LHON mutations but with a complex I deficiency be screened for additional mutations in the mtDNA, preferably the ND genes.

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Correspondence: Hubert J. Smeets, PhD, Department of Genetics and Cell Biology, University of Maastricht, PO Box 616, 6200 MD Maastricht, the Netherlands (bert.smeets@molcelb.unimaas.nl).

Author Contributions: Study concept and design: Spruijt, Sluiter, and Hintzen. Acquisition of data: Spruijt, Smeets, Bettink-Remeijer, Maat-Kievit, Schoonderwoerd, de Coo, and Hintzen. Analysis and interpretation of data: Spruijt, Smeets, Sluiter, de Coo, and Hintzen. Drafting of the manuscript: Spruijt, Smeets, Hendrickx, Bettink-Remeijer, Schoonderwoerd, de Coo, and Hintzen. Critical revision of the manuscript for important intellectual content: Spruijt, Smeets, Maat-Kievit, Sluiter, de Coo, and Hintzen. Statistical analysis: Spruijt. Obtained funding: Spruijt and Smeets. Administrative, technical, and material support: Spruijt, Maat-Kievit, and Hintzen. Study supervision: Spruijt, Smeets, de Coo, and Hintzen.

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