

Late-onset encephalopathy associated with a C11777A mutation of mitochondrial DNA

M. Deschauer, MD; C. Bamberg, MD; D. Claus, MD; S. Zierz, MD; D.M. Turnbull, FRCP; and R.W. Taylor, PhD

Abstract—A 67-year-old man presented with cognitive deficits, status epilepticus, left hemiparesis, and severe lactic acidosis. Respiratory chain enzyme analysis of skeletal muscle revealed a defect in complex I activity, associated with a heteroplasmic C11777A mutation in the mitochondrial *ND4* gene. This case is remarkable not only because of the late onset of symptoms, but because this mutation affects the identical ND4 codon as the G11778A mutation that causes Leber hereditary optic neuropathy.

NEUROLOGY 2003;60:1357–1359

Mitochondrial encephalomyopathies due to rearrangements and point mutations of mitochondrial DNA (mtDNA) often present in early adult life.¹ Although the majority of pathogenic point mutations have been described in transfer RNA (tRNA) genes, the number of reported mutations within mitochondrial genes encoding structural subunits of the respiratory chain continues to grow.²

Here, we report the identification of a heteroplasmic mtDNA mutation in the gene encoding *ND4* of complex I in a patient with late-onset encephalopathy. This mutation is of particular interest as it affects the identical codon that is disrupted by the primary G11778A mutation associated with Leber hereditary optic neuropathy (LHON).³

Case report. A 67-year-old patient with a 9-month history of left-sided resting tremor was admitted to the hospital because of headache and confusion. Neurologic examination revealed a severe cognitive deficit (Mini-Mental State Examination: 14/30 points) and left-sided upper motor neurone signs but no paresis. Four days after admission, he developed status epilepticus requiring ventilation. This was treated successfully with phenytoin, but he remained somnolent despite the absence of sedation. Additionally, he developed a left hemiparesis. In the following days he had acute pancreatitis and paralytic ileus and died 24 days after admission. Family history revealed that his mother had had maturity-onset diabetes and had died at age 75 years, and his surviving 72-year-old brother had had a myocardial infarction but no neurologic disease.

Following admission, laboratory evaluation revealed a severe lactic acidosis with venous value of 21 mmol/L (normal, <2.4 mmol/L) and elevated lactate in the CSF of 12.5 mmol/L (normal, <2.1 mmol/L); cells and protein of CSF were normal. Brain MRI demonstrated T2-weighted hyperintense lesions in the right frontal and parietal lobe as well as in the left cerebellar hemisphere that did not conform to a vascular territory (figure 1). Histologic examination of a muscle biopsy specimen showed subsarcolemmal

accumulation of mitochondria in a few fibers, but no cytochrome *c* oxidase-deficient fibers. Respiratory chain enzyme analysis of a skeletal muscle homogenate showed a specific defect in the activity of complex I activity (approximately 40% of control values). A limited analysis of the brain was performed, showing infarctions in the frontal lobes and hippocampus that did not conform to a vascular territory. The areas of infarction were associated with loss of cortical architecture, capillary proliferation, and astrogliosis, similar to previous reports.⁴ Loss of the granular layer and Purkinje cells was evident in the cerebellar hemisphere, together with Bergmann cell astrocytosis.

Methods. *Mitochondrial DNA analysis.* Total DNA was extracted from muscle and several paraffin-embedded postmortem brain tissues by standard procedures. Muscle DNA was screened for the A3243G tRNA^{Leu(UUR)} mutation by PCR restriction fragment length polymorphism (RFLP) analysis, and the presence of large-scale mtDNA rearrangements was excluded by Southern blotting. Sequencing of the mitochondrial genome was performed using muscle DNA as described.⁵

The level of heteroplasmy of the C11777A mutation was determined in several tissues by last hot cycle PCR-RFLP analysis, using an M13-tailed (lowercase sequence) forward mismatch primer (mismatch nucleotide shown in boldface) 5' tgtaaacgacg-cgccagtCAAACCTACGAACGCCTACTCAGAG 3' (nt 11754–11775) and a reverse primer (nt 12038–12058). Prior to the last cycle of PCR, 5 μ Ci [α -³²P]deoxycytidine triphosphate (3,000 Ci/mmol) was added. Labeled products were digested with 10 U *Hinf*I, separated through a 12% nondenaturing polyacrylamide gel, and the radioactivity in each fragment was quantified using ImageQuant software (Molecular Dynamics, Eugene, OR). The mismatch in the forward primer creates an additional *Hinf*I restriction site in wild-type mtDNA, cleaving the 323-bp amplicon into fragments of 176, 38, and 109 bp. The presence of the C11777A mutation leads to the loss of a restriction site, cleaving the mutant mtDNA into fragments of 214 and 109 bp (figure 2, B and C).

Results. Initial analysis of the muscle DNA failed to detect either the A3243G tRNA^{Leu(UUR)} mutation or large-scale mtDNA rearrangements. Direct sequencing of the mitochondrial genome revealed a C11777A transversion in the ND4 subunit. The mutation was clearly heteroplasmic on the electropherogram (see figure

From the Department of Neurology (Drs. Deschauer, Turnbull, and Taylor), The Medical School, University of Newcastle upon Tyne, UK; Department of Neurology (Drs. Deschauer and Zierz), Martin-Luther-Universitaet Halle-Wittenberg, Halle/Saale, Germany; and Department of Neurology (Drs. Bamberg and Claus), Klinikum Darmstadt, Teaching Hospital University of Frankfurt, Germany.

Supported by a fellowship from the European Neurological Society (M.D.) and the Wellcome Trust (D.M.T. and R.W.T.).

Received August 23, 2002. Accepted in final form December 24, 2002.

Address correspondence and reprint requests to Professor D.M. Turnbull, Department of Neurology, The Medical School, Framlington Place, University of Newcastle upon Tyne, NE2 4HH, UK; e-mail: d.m.turnbull@ncl.ac.uk

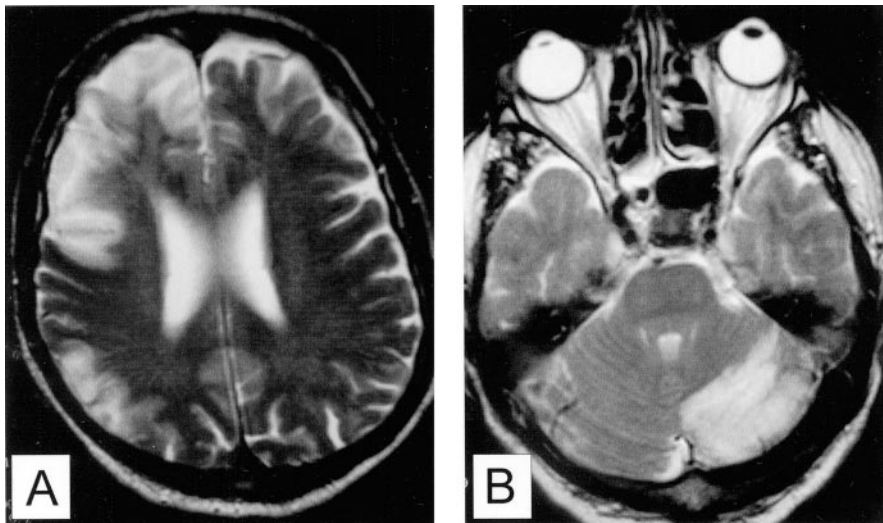


Figure 1. Brain MRI with T2-weighted hyperintense lesions in the right frontal and parietal lobe (A) as well as in the left cerebellar hemisphere (B).

2A) and is predicted to cause a change in the amino acid sequence (Arg340Ser) of the ND4 protein.

PCR-RFLP analysis of the C11777A mutation confirmed mtDNA heteroplasmy, with mutant levels in several brain regions consistently lower (67% in cerebellum and midbrain, 81% in hippocampus, and 69% in gyrus frontalis) than skeletal muscle (93%) (see figure 2C). Furthermore, the C11777A mutation was not represented in a database of 754 human mitochondrial genomes (MitoKor Mitochondrial SNP Database, personal communication).

Discussion. We have identified an mtDNA mutation in a 67-year-old patient presenting with encephalopathy and acute onset. The first feature suggesting the clinical diagnosis of a mitochondrial disorder was the elevated lactate in both blood and CSF. Our patient had stroke-like symptoms, seizures, and a cognitive deficit, similar to those observed in MELAS (mitochondrial myopathy, encephalomyopathy, lactic acidosis, and stroke-like symptoms). Late onset of encephalopathy is not typical for MELAS, however, and additional symptoms such as vomiting or hearing loss were not observed.⁶ Brain MRI revealed stroke-like lesions that did not conform to a vascular territory, as seen in MELAS, although cerebellar lesions are very rare.⁶

There are several lines of evidence supporting the etiologic role of the C11777A mutation. First, it was heteroplasmic in several tissues. Second, it changes the charged amino acid arginine 340 to an uncharged serine residue. The C11777A mutation occurs at the nucleotide directly beside the G11778A mutation, the most common mutation associated with LHON.³ Indeed, the C11777A mutation affects the same codon, although in LHON the arginine 340 is replaced by histidine. Third, it was associated with a biochemical defect in muscle complex I activity, similar to patients with the G11778A mutation.⁷ Fourth, it was not detected in a database of 754 human mitochondrial genomes, which argues against it being a polymorphic variant. Finally, evolutionary comparison of mitochondrial ND4 sequences (Protein Information Resource, <http://pir.georgetown.edu/>) indicates that this residue is

invariant in all homologous genes, in agreement with previous reports.³

Further evidence to support the pathogenic nature of the C11777A mutation is provided by the recent description of the same mtDNA mutation in two children presenting with a relatively mild clinical course of Leigh syndrome.⁸ Moreover, the pathogenic role of the mutation was confirmed by cybrid studies, which have shown complex I deficiency in transmitochondrial cell lines containing the C11777A mutation (Goto Y, unpublished data). Age at onset, clinical phenotype, and clinical course in our patient are clearly different, indicating phenotypical variability of the C11777A mutation. The level of mutant DNA is known to influence the phenotypic expression of other coding region mutations, and a similar mechanism could be operating if the level of mutant mtDNA in the brains of the patients presenting with Leigh syndrome was higher than that observed in our patient. Interestingly, the levels of mutant mtDNA in several brain regions of our patient were consistently lower than those observed in skeletal muscle, although a similar phenomenon has been reported previously in a patient with the A3243G mutation.⁹

A further fascinating aspect of the C11777A mutation is the remarkable difference in the clinical phenotype between these patients, none of whom presented with signs of optic neuropathy, and patients with the G11778A LHON mutation, in which this is the main clinical abnormality. The increase in the severity of the clinical features in patients with C11777A compared with G11778A probably reflects the amino acid change at residue 340. The G11778A mutation alters arginine 340 to an amino acid of similar charge (histidine), whereas the C11777A mutation predicts a loss of charge at this position.

Although reports of late-onset mitochondrial disorders are rare, our investigations emphasize that mtDNA abnormalities can also present in older patients. The reason for this late presentation is not

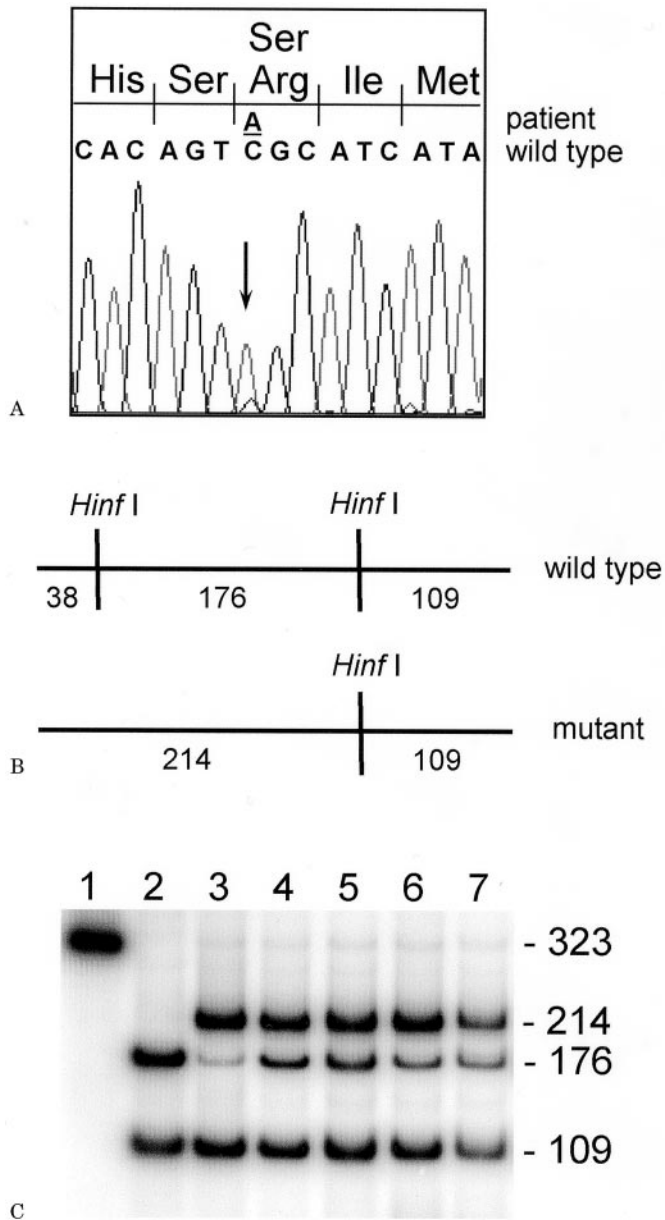


Figure 2. Identification and characterization of the C11777A mutation. Sequencing electropherogram (A) showing the heteroplasmic C to A transversion at nucleotide 11777, which predicts an amino acid substitution (Arg340Ser) at this site. Schematic (B) of the PCR restriction fragment length polymorphism (RFLP) showing the sizes of expected restriction products following digestion with *HinfI*. Quantitation (C) of the relative amounts of mutant and wild-type mtDNA by last hot cycle PCR-RFLP analysis. Lane 1, uncut control; lane 2, control; lane 3, skeletal muscle; lane 4, left cerebellum; lane 5, midbrain; lane 6, left hippocampus; lane 7, right gyrus frontalis.

clear although levels of mutated mtDNA can increase with time in postmitotic tissues.¹⁰ Alternatively, the clinical features may have been triggered by environmental factors or age-related changes in cerebral blood flow. Whatever the cause, it is important to consider possible mitochondrial involvement in older patients with late-onset encephalopathy.

Acknowledgment

The authors thank Dr. Heyny-von Haussen (Klinikum Darmstadt) for providing postmortem tissue and autopsy data, Dr. Gellerich (University of Halle) for biochemical analysis, Prof. Schlote (University of Frankfurt) for histologic analysis, Dr. Huppert (Klinikum Darmstadt) for providing MRI scans, and Geoff Taylor (University of Newcastle, Upon Tyne) for help with the sequencing.

References

- Chinnery PF, Turnbull DM. Clinical features, investigation, and management of patients with defects of mitochondrial DNA. *J Neurol Neurosurg Psychiatry* 1997;63:559–563.
- Taylor RW, Singh-Kler R, Hayes CM, Smith PE, Turnbull DM. Progressive mitochondrial disease due to a novel missense mutation in the mitochondrial DNA ND3 gene. *Ann Neurol* 2001;50:104–107.
- Wallace DC, Singh G, Lott MT, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* 1988;242:1427–1430.
- Tanahashi C, Nakayama A, Yoshida M, Ito M, Mori N, Hashizume Y. MELAS with mitochondrial DNA 3243 point mutation: a neuropathological study. *Acta Neuropathol* 2000;99:31–38.
- Taylor RW, Taylor GA, Durham SE, Turnbull DM. The determination of complete human mitochondrial DNA sequences in single cells: implications for the study of somatic mitochondrial DNA point mutations. *Nucleic Acids Res* 2001;29:E74.
- Hirano M, Ricci E, Koenigsberger MR, et al. MELAS: an original case and clinical criteria for diagnosis. *Neuromuscul Disord* 1992;2:125–135.
- Larsson NG, Andersen O, Holme E, Oldfors A, Wahlstrom J. Leber's hereditary optic neuropathy and complex I deficiency in muscle. *Ann Neurol* 1991;30:701–708.
- Komaki H, Iwata H, Takahashi T, Akanuma J, Nonaka I, Goto Y. Novel mtDNA C11777A mutation in Leigh syndrome changes the same acid as G11778A mutation in LHON. *Am J Hum Genet* 2001;69(suppl):578.
- Macmillan C, Lach B, Shoubridge EA. Variable distribution of mutant mitochondrial DNAs (tRNA(Leu)[3243]) in tissues of symptomatic relatives with MELAS: the role of mitotic segregation. *Neurology* 1993;43:1586–1590.
- Weber K, Wilson JN, Taylor L, et al. A new mtDNA mutation showing accumulation with time and restriction to skeletal muscle. *Am J Hum Genet* 1997;60:373–380.

Neurology[®]

Late-onset encephalopathy associated with a C11777A mutation of mitochondrial DNA

M. Deschauer, C. Bamberg, D. Claus, et al.

Neurology 2003;60;1357-1359

DOI 10.1212/01.WNL.0000055869.99975.4B

This information is current as of April 22, 2003

Updated Information & Services	including high resolution figures, can be found at: http://www.neurology.org/content/60/8/1357.full.html
References	This article cites 9 articles, 3 of which you can access for free at: http://www.neurology.org/content/60/8/1357.full.html#ref-list-1
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): All Cognitive Disorders/Dementia http://www.neurology.org/cgi/collection/all_cognitive_disorders_dementia Mitochondrial disorders http://www.neurology.org/cgi/collection/mitochondrial_disorders Optic nerve http://www.neurology.org/cgi/collection/optic_nerve Status epilepticus http://www.neurology.org/cgi/collection/status_epilepticus
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://www.neurology.org/misc/about.xhtml#permissions
Reprints	Information about ordering reprints can be found online: http://www.neurology.org/misc/addir.xhtml#reprintsus

Neurology® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright . All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.

