BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **238,** 323–325 (1997)
ARTICLE NO. RC977166

Bilateral Striatal Necrosis and MELAS Associated with a New T3308C Mutation in the Mitochondrial ND1 Gene

Yolanda Campos,* Miguel A. Martín,* Juan C. Rubio,* Maria C. Gutiérrez del Olmo,† Ana Cabello,‡ and Joaquín Arenas*,[1]

**Centro de Investigación, †Servicio de Neurología, ‡Servicio de Neuropatología, Hospital Universitario "12 de Octubre," Madrid, Spain*

Received July 11, 1997

We found a novel maternally inherited T3308C mutation in the mtDNA ND1 gene in a patient with bilateral striatal necrosis and stroke-like episodes. Muscle biopsy from the proband showed mitochondrial proliferation in blood vessels and normal respiratory chain activities. The mutation, which was not present in 100 normal controls or in 30 patients with mitochondrial disease, was heteroplasmic in both muscle and blood of the proband and in blood from her asymptomatic mother. This mutation results in a Met → Thr change at the highly conserved amino acid position 1. The T3308C mutation may alter the hydrophobicity and antigenicity of the N-terminal peptide of ND1.

MtDNA mutations are increasingly recognized as a cause of human diseases (1). These mutations fall into two groups: those affecting general mitochondrial protein synthesis, such as point mutations or deletions involving rRNA or tRNA genes, and those that affect specific respiratory chain complexes, such as point mutations in the protein-coding genes (2). Maternal inheritance is commonly observed in diseases associated with mtDNA point mutations (2). Several pathogenic mutations in the tRNAs have been described, almost invariably associated with the presence of ragged-red fibers (RRF) in muscle (1,2). The most common of these mutations are associated with MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes) or MERRF (myoclonus epilepsy with ragged-red fibers) phenotypes (2).

On the other hand, mutations in protein-coding genes have been observed in Leber's hereditary optic neuropathy (LHON), neuropathy, ataxia, and retinitis pigmentosa (NARP), or Leigh's disease, and are tipically not associated with RRF (1,2). Recently, a missense mutation in the mtDNA COIII gene has been documented in a patient with MELAS syndrome (3).

We now report a novel T3308C missense mutation in the ND1 gene of mtDNA in a patient with combined features of bilateral striatal necrosis and MELAS syndrome.

CASE REPORT

A 22-year-old woman developed normally until age 3, when she developed ataxia and dysarthria from which she recovered within a week. Laboratory studies and EEG were normal. At 12 years of age, she presented with unsteadiness and clumsiness. On examination, there was dystonic posturing involving trunk, neck, and left hand. Computed tomography (CT) scan of the brain and laboratory studies were normal. At age 20 years, magnetic resonance imaging of the brain (MRI) showed bilateral T1 and T2 signal increases in basal ganglia. At age 21 years, when she was eight months pregnant, she suffered from generalized tonic-clinic seizures that were difficult to control. The neurological condition worsened. Labor was induced, and after delivery generalized seizures persisted. She presented loss of conciousness and stupor. An EEG showed slow background. A CT scan of the brain revealed multiple bilateral strokes in the territories of middle and posterior cerebral arteries. Several days later she began to slowly improve. On examination a week later, she was inattentive with a halting and hypofluent speech. Her gait was awkward and she had spasticity, hyperreflexia, and generalized marked dystonia. In particular, dystonic tongue movements were apparent. Tone was reduced. Plantar responses were equivocal. Laboratory studies, including blood and CSF lactate were normal. Presently, the patient is confined to a wheelchair due to spasticity. She also has slurred speech and neurological regression. Family members are normal, except for the presence of scoliosis. In particular, her mother, who was genetically tested, was asymptomatic.

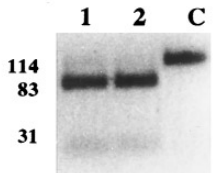
METHODS

Biochemical and morphological studies in muscle and extraction of total DNA were performed as described (4). There were no large-scale rearrangements by Southern blot, and known point mutations in the ATPase 6-8 (7), Nds (7,8) and COX (3,7,8) genes were ruled

[1] To whom correspondence should be addressed at Centro de Investigafción, Hospital 12 de Octubre, Avda de Córdoba km 5.4, 28041 Madrid, Spain. Fax: 1-3908358. E-Mail: jarenas@h12o.es.

0006-291X/97 $25.00

a)



b)

Amino-Terminal Sequences of ND1

	1				5				10				15					20
Patient	T	P	M	A	N	L	L	L	L	I	V	P	I	L	I	A	M	A	F	L
Human	M	P	M	A	N	L	L	L	L	I	V	P	I	L	I	A	M	A	F	L
Cow	M	F	M	I	N	I	L	M	L	I	I	P	I	L	L	A	V	A	F	L
Rat	M	Y	F	I	N	I	L	T	L	L	I	P	I	L	I	A	G	L	F	L
X. laevis	M	L	T	I	I	T	H	L	I	N	P	L	L	Y	M	I	P	I	L	L
D. melanogaster	M	E	F	I	L	S	L	I	G	S	L	L	L	I	I	C	V	L	V	S
P. tetraurelia	M	L	I	Y	S	I	V	L	M	L	V	V	T	L	I	I	A	S	I	T
N. tabacum	M	I	I	D	T	T	E	I	E	T	I	N	S	F	S	K	L	E	S	L
Rb. capsulatus	M	A	D	F	W	A	T	S	L	G	Q	T	L	I	L	L	A	Q	G	L

FIG. 1. (a) RFLP analysis of the T3308C mutation.Mutant mtDNA is cleaved into two fragments of 83 bp and 31 bp, while wild-type mtDNA, sized 114 bp, remains uncut. 1, proband's muscle; 2, proband's blood; c, control. (b) Interspecies homology of the amino terminal sequence of the ND1 gene of mtDNA.

out. Direct sequencing of the tRNALeu$^{(UUR)}$ and NDs regions were done by using fluorescent dye dideoxy nucleotide terminators in a DNA sequencer model 373A (Applied Biosystems).

For RFLP analysis we amplified a 114 bp fragment by PCR, using a forward primer (5′-3′) 3225-3250, and a mistmatched bacward primer (5′-3′) 3339-3309, according to the published Cambridge sequence (5). Mistmachs involved a dinucleotide sustitution CC at positions 3313 and 312. In combination with the T3308C mutation, the mismatchs create a new site for the restriction enzyme SgrAI that is absent in the wild-type sequence. The PCR product was digested with SgrAI and the digestion products electrophoresed through a 2% agarose gel (Fig. 1). The relative abundance of mutant genomes was quantitated as described (6).

RESULTS

Muscle biopsy at age 22 showed a irregular oxidative pattern. Blood vessels in muscle frequently showed abnormal proliferation of mitochondria (SSV). No cytochrome c oxidase deficiency was found in muscle by histochemical staining. Respiratory chain enzymes in muscle homogenates were normal.

We found a T-to-C transversion at mtDNA position nt-3308, in the ND1 gene. This mutation results in a Met → Thr change at aminoacid position 1 of ND1 The mutation was heteroplasmic, the patient had 85% mutated mtDNA in muscle and 80% in blood. The mother had 80% of mutant genomes in blood. This mutation was not found in 100 normal controls, nor in 30 patients with other mitochondrial disorders.

DISCUSSION

We found a maternally transmitted novel missense point mutation in the mitochondrial ND1 gene in a patient with a progressive encephalopathy chracterized by combined features of bilateral striatal necrosis and MELAS syndrome. The proband shared with MELAS patients the presence of SSV in blood vessels and the stroke-like episodes (2,7). In addition, she had features in common with patients harboring point mutations in the ATPase 6-8 gene or in the ND genes, such as dystonia, ataxia and bilateral basal ganglia abnormalities (2,7,8).

Several lines of evidence support the etiologic role of this mutation. First, evolutionary comparison indicates that the metionine at amino acid position 1 in human ND1 is highly conserved throughout evolution (9) (Fig 1b). Moreover, this aminoacid is part of a highly conserved hydrophobic domain that seems to play a key role in anchoring ND1 in the lipid membrane (9). The change observed may impede the initiation of the translation of ND1. Also, although the translation process of ND1 worked to some degree, the shift towards a less hydrophobic peptide would alter its anchoring to the mitochondrial membrane. Second, the mutation was heteroplasmic both in the patient and in her mother. Heteroplasmy is regarded as an indicator of pathogenicity, and it usually correlates with the severity of

the phenotype (2). Third, the mutation has never been reported and was not detected in 100 normal subjects or in 30 disease controls. These data indicate that this mutation does not occur commonly in the general population or in association with other mutations which are known to be pathogenic per se.

ND1 is a component of the large hydrophobic domain of complex I (9). ND1 contains the rotenone-binding site, and may be involved in electron transfer to ubiquinone (9). Thus, mutations in the ND1 subunit could impair enzyme activity. Abnormal complex I activity has been observed in individuals harboring the point mutation at nt 3460 of mtDNA(8). However, our data failed to show a defect of complexI in muscle of the patient. Further studies at the biochemical level in tissues other than muscle need to be addressed to clarify this point.

Moreover, histochemical staining for COX was normal in muscle fibers, suggesting a relatively homogeneous distribution of the mutation among the fibers, none of which contained sufficient levels of mutant mtDNA to cause a morphologically observable respiratory chain defect. Accordingly, the patient had an encephalopathy, but no myopathy. It is unknown whether the brain of the patient might harbor higher levels of mutant mtDNA, exceeding the treshold for the biochemical and clinical phenotypes. Also, SSV in muscle likely contain great proportions of mutant mtDNAs that may be similar in the blood vessels of the brain (6). This assumption may support the essentially vascular nature of brain involvement in MELAS. We cannot rule out that other mitochondrial, nuclear or environmental factors may play a role in the predominant brain involvement.

Several other missense point mutations in the ND1 gene have been reported, all associated with LHON (1,8). The G3460A mutation is the second most common cause of LHON and can be heteroplasmic or homoplasmic. Interestingly, the clinical manifestations associated with the T4160C mutation have features in common with the MELAS syndrome (8).

On the other hand, there is evidence that mitochondrial peptides encoded by mtDNA play a part of the immune response in rodents, in the form of a maternally transmitted minor murine histocompatibility antigen (MTF) (10). MTF has been identified as the N-terminal peptide of ND1,formed by the first 23 hydrophobic aminoacids (10). This highly conserved hydrophobic residue is involved in cytotoxic responses (10). It is also known that only the protein starting at position 1 is processed to yield MTF (10). The T3308C mutation may alter the hidrophobicity and, therefore, the antigenicity of the N-terminal peptide of ND1. We suggest that this ND1 mutation may create novel antigens that cause autoimmune responses leading to neurological symptoms.

ACKNOWLEDGMENTS

This work was supported by a grant from FIS 95/0658, Ministerio de Sanidad, Spain. Y. Campos was supported by Sigma-Tau, M. A. Martín by the FIS, and J. C. Rubio by the Instituto Carlos III.

REFERENCES

1. Larsson, N. G., and Clayton, D. (1995) *Annu Rev Genetics* **29,** 151–178.
2. DiMauro, S., and Moraes, C. T. (1993) *Arch Neurol* **50,** 1197–1208.
3. Manfredi, G., Schon, E. A., Moraes, C. T., Bonilla, E., Berry, G. T., Sladky, J. T., and DiMauro, S. (1995) *Neuromusc Disord* **5,** 391–398.
4. Campos, Y., García-Silva, T., Barrionuevo, C. R., Cabello, A., Muley, R., and Arenas, J. (1995) *Pediatr Neurol* **13,** 69–72.
5. Anderson, S., Bankier, A. T., Barrell, B. G., De Bruijn, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Shreier, P. H., Smith, A. J. H., Staden, R., and Young, I. G. (1981) *Nature* **290,** 457–465.
6. Moraes, C. T., Ricci, E., Bonilla, E., DiMauro, S., and Schon, E. A. (1992) *Am J Hum Genet* **50,** 934–949.
7. DiMauro, S., and Bonilla, E. (1997) *in* The Molecular and Genetic Basis of Neurological Disease (Rosenberg, S. N., Prusiner, S. B., DiMauro, S., and Barchi, R. L., Eds.), pp. 389–446, Butterworth-Heinemann, Boston, MA.
8. Shoffner, J. M., and Wallace, D. C. (1995) *in* The Metabolic and Molecular Basis of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., Eds.), pp. 1535–1610, McGraw-Hill, New York.
9. Fearnley, I. M., and Walker, J. E. (1992) *Biochim Biophys Acta* **1140,** 105–134.