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Identification of a Novel Mutation in the mtDNA ND5 Gene Associated with MELAS

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We report a novel G13513A mutation in the mitochondrial ND5 gene in a patient who had morphologically and biochemically abnormal muscle mitochondria and died at age 45 with a diagnosis of MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes). The mutation affects an evolutionarily conserved nucleotide and was heteroplasmic in muscle, leukocytes, and several autopsy tissues, including brain. The mutation was less abundant (<5%) in leukocytes from an asymptomatic sister and was not found in over 100 controls, thus satisfying accepted criteria for pathogenicity. Our report reinforces the concept of genetic heterogeneity in MELAS and confirms that MELAS can be due to mutations in polypeptide-coding mtDNA genes. © 1997 Academic Press

MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke like episodes) is a maternally-inherited disorder belonging to a clinically diverse group of syndromes (mitochondrial encephalopathies) that also includes Kearns-Sayre syndrome (KSS) and MERRF (myoclonic epilepsy with ragged-red fibers) (1). Typical clinical features include onset before age 40, lactic acidosis, episodic vomiting, seizures, migraine-like headaches, and recurrent cerebral insults resembling strokes and causing hemiparesis, hemianopia or cortical blindness (2). Muscle biopsy is characterized by subsarcolemmal proliferation of mitochondria in ragged-red fibers (RRF), which, however, in contrast to other mitochondrial myopathies, generally stain positively for cytochrome *c* oxidase (COX) activity. Biochemical studies of muscle often show decreased activities of oxidative enzymes, especially complexes I and IV.

Genetic heterogeneity is well documented in MELAS. A point mutation (A3243G) in the $tRNA^{Leu(UUR)}$ gene of mtDNA is present in more than 80% of typical MELAS patients (3). About 10% of patients harbor different mutations, some in the $tRNA^{Leu(UUR)}$ gene (T3271C; 3271DelT; T3291C) (1), one in the $tRNA^{Cys}$ gene (T5814C) (4,5), and one in a polypeptide-coding gene, COX III (T9957C) (6). We now report a novel mutation (G13513A) in yet another polypeptide-coding gene - subunit 5 of complex I (ND5) - in a patient with typical MELAS and none of the previously reported MELAS mutations. The new mutation meets accepted criteria for pathogenicity.

CASE REPORT

A 43-year-old man, with bilateral hearing loss since childhood and a history of bilateral cataract removal at age 42, presented with acute right-sided weakness and seizures. Following this episode, weakness and decreased sensation persisted on the right side. Electroencephalography showed diffuse alteration of cerebral activity without epileptiform foci. A CT scan showed a lucency in the high left parietal region consistent with a cerebral infarction. Successively, he had several other strokes resulting in right-sided motor and sensory symptoms, generalized myoclonus and seizures, aphasia, left hemianopsia, cortical blindness, and headaches.

Lactic acid levels were increased on several occasions in both blood (range 2.9-3.2 mM/L, normal < 1.8 mM/L) and CSF (2.5 times normal). Brain biopsy at age 45 years showed reactive glial tissue with neuronal loss consistent with ischemic injury. A muscle biopsy showed RRF by both the modified Gomori trichrome and succinate dehydrogenase (SDH) stains. Most RRF were cytochrome *c* oxidase (COX) positive by histochemistry. A diagnosis of MELAS was made.

Confined to a rehabilitation institute, he suffered additional strokes and died of aspiration pneumonia at age 45. Autopsy showed ischemia-type lesions in multiple areas of the cortex.

METHODS

Standard techniques were used for histological and histochemical studies of autopsy tissues from the proband (7). Spectrophotometric measurements of respiratory chain enzymes activities and citrate

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synthase (CS) were carried out in brain, heart, kidney, liver, and muscle specimens, as reported (8).

There were no large-scale rearrangements by Southern blot, and the most common pathogenic mtDNA mutations were ruled out by described PCR methodologies (9-11). The whole mtDNA was PCR-amplified in 20 overlapping fragments and directly sequenced with a 373A DNA sequencer and the cycle dye terminator DNA sequencing kit of Applied Biosystems.

To screen for the novel G13513A mutation, we used a mismatched forward primer (5′-3′) at nt 13491-13512 (with G at nt 13510) and a consensus backward primer (5′-3′) at nt 13610-13593 (12), to PCR-amplify a 119-bp fragment. In combination with the wild-type base at nt 13513, the PCR-amplified fragment contained a unique restriction site for the endonuclease *Bbs*I ($GAAGACN_2/N_6$) which was absent in the mutated genomes. Assessment of abundance of mutant mtDNAs was as reported (5).

RESULTS AND DISCUSSION

We performed histopathological examination of several autopsy tissues from a MELAS patient who had tested negative for all mtDNA pathogenic mutations described thus far (1). The pathology in this patient was typical of MELAS. Multiple cortical areas of the brain showed changes resembling ischemic injuries, such as vascular proliferation with reactive-appearing endothelial cells, neuronal loss, and mild-to-moderate astrogliosis. These changes were especially evident in the deep cortical layers, while the subcortical white matter showed gliosis and spongiosis.

Serial sections of a psoas muscle biopsy showed mild variation in fiber size with scattered RRF, which were strongly reactive with the SDH stain. The smooth musculature of blood vessels also showed increased SDH staining. Increased activity was also present in muscle fibers and blood vessels with the COX stain, and there were only scattered COX deficient fibers. Autopsy revealed panlobar broncopneumonia, bilateral renal cortical scars, and chronic pyelonephritis. Heart and liver architectures were spared.

Biochemically, when values were referred to CS, the activities of respiratory chain complexes were partially reduced. Complex I was 46% of normal in brain and 52% in liver; complex I+III was 40% and 27% of normal in the same tissues; complex IV was normal.

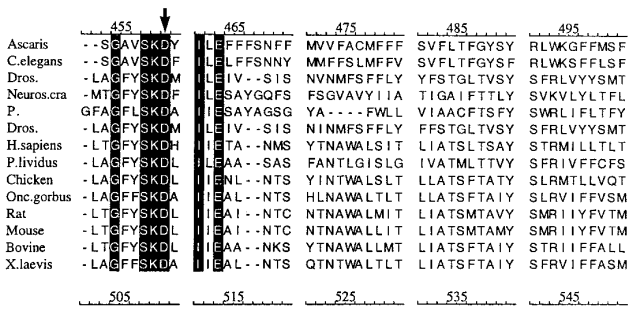

FIG. 1. Position of the novel heteroplasmic G13513A mutation (arrow) and homology with other species.

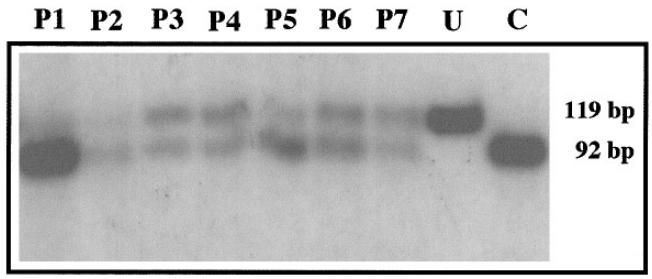

FIG. 2. Restriction fragment length polymorphism analysis. The amplified 119-bp fragment was cleaved with the endonuclease *Bbs*I ($GAAGACN_2/N_6$) into two fragments sized 27-bp and 92-bp (only the 92-bp fragment is shown here). The G13513A mutation abolishes this unique site of cut. P1, patient's blood (10%); P2, patient's kidney (34%); P3, patient's muscle (51%); P4, patient's heart (53%); P5, patient's liver (25%); P6, patient's cerebellum (57%); P7, patient's cerebrum, frontal lobe (54%); U, uncut; C, normal control.

Not surprisingly, given the high mtDNA mutation rate, sequence analysis of the proband's total mitochondrial genome revealed several differences from the reference Cambridge sequence (12). We assessed the potential pathogenicity of each mutation according to the following criteria. The mutation should: (i) affect an evolutionarily conserved nucleotide; (ii) be heteroplasmic; (iii) be absent in a large series of controls. These prerequisites excluded from further consideration all but one mutation, a novel G13513A transition in the ND5 gene.

This mutation changes a conserved aspartic acid to asparagine at amino acid position 393 in the ND5 peptide (D393N, Figure 1). The mutation was present in various proportions in multiple tissues from the proband, and the highest percentages were found in brain (73%) and muscle (68%), two clinically affected tissues. Less abundant mutated genomes (<5%) were found in blood from one of the three sisters studied (Figure 2). None of 132 control individuals, including 40 patients with known mtDNA mutations, harbored the G13513A mutation. Hence, canonical criteria for the definition of a disease-related nucleotide base change were satisfied. The mutation was also absent in 18 patients with symptoms and signs of MELAS in whom the molecular defect remains unknown: this suggests that the G13513A mutation is an infrequent cause of MELAS.

We found a good correlation between distribution of the mutation and tissue involvement: the highest proportions of mutated genomes were in brain and muscle, and the lowest (<5%) were in leukocytes from an asymptomatic sister.

Our proband's clinical features were those of a severe encephalopathy with recurrent strokes, similar to that seen in patients with typical MELAS and the A3243G mutation (1,2). However, onset was later than in typical MELAS patients. Increased metabolic demands associated with the onset of seizures may have overtaxed

an already defective cerebral metabolism and accelerated the fatal outcome.

The partial defect in complex I activity that we observed in brain and liver is in keeping with the identified ND5 mutation. Although the amino acid change (D393N) is not structurally dramatic, it alters the charge in the peptide and this might impair the activity of the whole complex. Isolated partial complex I deficiency has been documented in Leber hereditary optic neuropathy (LHON), another maternally-inherited disorder associated with several distinct mutations in mtDNA-encoded complex I subunits (12-14). It remains to be understood why different mutations in genes encoding subunits of the same respiratory chain complex should cause such dramatically different clinical phenotypes as LHON and MELAS. It is also unusual for a mutation in a structural gene to be associated with RRF, which, for example, are not seen in patients with LHON. While these and other conundrums remain to be explained, it is important to remember that rare cases of MELAS can be due to mutations in mtDNA genes encoding subunits of respiratory chain complexes, including COX (6) and Complex I.

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