

## *Clinical Report*

# Progressive Cerebral Vascular Degeneration With Mitochondrial Encephalopathy

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MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes) is a maternally inherited disorder characterized by recurrent cerebral infarctions that do not conform to discrete vascular territories. Here we report on a patient who presented at 7 years of age with loss of consciousness and severe metabolic acidosis following vomiting and dehydration. She developed progressive sensorineural hearing loss, myopathy, ptosis, short stature, and mild developmental delays after normal early development. Biochemical testing identified metabolites characteristic of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency (hexanoylglycine and suberylglycine), but also severe lactic acidemia (10–25 mM) and, in urine, excess of lactic acid, intermediates of the citric cycle, and marked ketonuria, suggesting mitochondrial dysfunction. She progressed rapidly to develop temporary cortical blindness. Brain imaging indicated generalized atrophy, more marked on the left side, in addition to white matter alterations consistent with a mitochondrial disorder. Magnetic resonance angiography indicated occlusion of the left cerebral

artery with development of collateral circulation (Moyamoya syndrome). This process worsened over time to involve the other side of the brain. A muscle biopsy indicated the presence of numerous ragged red fibers. Molecular testing confirmed compound heterozygosity for the common mutation in the MCAD gene (985A>G) and a second pathogenic mutation (233T>C). MtDNA testing indicated that the muscle was almost homoplasmic for the 3243A>T mutation in tRNA<sup>Leu</sup>, with a lower mutant load (about 50% heteroplasmy) in blood and skin fibroblasts. These results indicate that mitochondrial disorders may be associated with severe vascular disease resulting in Moyamoya syndrome. The contribution of the concomitant MCAD deficiency to the development of the phenotype in this case is unclear.

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**Key words:** MELAS; mitochondrial disorder; MCAD deficiency; Moyamoya; lactic acidosis; metabolic acidosis; mitochondrial encephalopathy; vascular disease

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### INTRODUCTION

Mitochondria have several essential functions, which include the generation of significant amounts of energy for the cell using the respiratory chain, the production and removal of reactive oxygen species, a central role in programmed cell death (apoptosis), and the catabolism and oxidation of nutrients including fat and pyruvate [Wallace, 1999]. Mitochondria are essential for aerobic metabolism. Organs highly dependent on aerobic metabolism (such as

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brain, skeletal muscle, heart, the renal and endocrine systems) are in general preferentially affected by mitochondrial disorders [Shoffner, 2001; Wallace et al., 2001; Longo, 2003].

MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes, OMIM #540000) is a progressive neurodegenerative disorder with onset usually between 2 and 10 years of age. Most children have normal early milestones and might present with a history of repeated headaches, recurrent vomiting or with seizures following neurologic deficits resembling strokes (hemianopsia, hemiparesis). Many of these children have easy fatigability, tend to have short stature, and develop sensorineural hearing loss over time [Shoffner, 2001; Wallace et al., 2001; Longo, 2003]. Brain imaging can show abnormal areas in the posterior cerebrum at the time of acute attacks. These abnormal areas might not correspond to the distribution of major arteries. Metabolic testing shows lactic acidosis in most cases. Muscle biopsy can show ragged red fibers, which usually stain positively for cytochrome *c* oxidase. Blood vessels can stain strongly positive for succinyl dehydrogenase. MELAS is caused, in most cases, by mutations in the mitochondrially encoded leucine tRNA (tRNA<sup>Leu</sup>). Mutation 3243A>G accounts for about 80% of cases whereas 3271T>C and 3252A>G account for an additional 15% of cases. The 3291T>C and 3260A>G mutations are more rare [Shoffner, 2001; Wallace et al., 2001]. In one patient, MELAS was associated with a rapid progressive encephalopathy and a 3243A>T change in tRNA<sup>Leu</sup> [Shaag et al., 1997]. 3243A>T leads to a dramatic reduction in aminoacylation efficiency when probed in a cell-free system [Sohm et al., 2003], possibly explaining the rapidly progressive clinical course compared to 3243A>G. Mutations in the tRNA for valine (1642G>A), serine (7512T>C), phenylalanine (583G>A), cysteine (5814A>G) and for mitochondrial proteins (COXIII-9997T>C; ND45-13513G>A; Cytochrome *b*-4 bp deletion) have also been reported in rare patients with MELAS. These mutations may be found in blood, but in some cases a muscle biopsy is needed to confirm or exclude this diagnosis [Shoffner, 2001].

Here we report on an unusual patient with MELAS whose clinical course was complicated by severe vascular involvement in the brain, leading to rapid deterioration. Biochemical and molecular testing indicated that this patient not only had the rare 3243A>T mutation in the mitochondrially encoded tRNA<sup>Leu</sup>, but also had medium chain acyl-CoA dehydrogenase (MCAD) deficiency.

### CLINICAL REPORT

A 7-year-old white female presented with loss of consciousness and severe metabolic acidosis following vomiting and dehydration. Past medical history

indicated that, after normal initial growth and development, she developed progressive sensorineural hearing loss, myopathy, ptosis, short stature, decreased stamina with fatigue after brief walks, headaches, and mild developmental delays. She was adopted and no family history was available. Initial laboratory testing indicated metabolic acidosis, for which biochemical testing was obtained. This was reported consistent with MCAD deficiency. She received intravenous fluids containing glucose and was discharged after stabilization.

At the time of subsequent referral, she was short (height <1st centile) with correspondingly low weight (<1st centile), but with normal weight for height (11th centile). Her head size was normal. She had normal speech with well-formed sentences. However, she was slow in processing information and required extra time to complete tasks. She attended school for only a few hours per day since she became fatigued and had to rest. She wore bilateral hearing aids, had severe ptosis (bilateral) and had a Cushingoid appearance with hirsutism. Muscle mass and muscle tone were decreased. She had mild ataxia, but was able to walk independently. Urine organic acids (Fig. 1A) identified hexanoylglycine ( $9.6 \pm 5.8$  mmol/mol creatinine,  $n = 8$ ), suberylglycine ( $9.5 \pm 6.0$  mmol/mol creatinine,  $n = 8$ ), and phenylpropionylglycine (indicative of MCAD deficiency), but also severe lactic/pyruvic aciduria, excess intermediates of the citric cycle, and marked ketonuria. Acetoacetic acid was  $45 \pm 24$  mmol/mol creatinine ( $n = 11$ ) when stable and reached levels  $>4,000$  when sick (normal  $<4$  mmol/mol creatinine); 3-OH-butyric acid was  $333 \pm 192$  mmol/mol creatinine ( $n = 11$ ) when stable and increased to  $>10,000$  mmol/mol creatinine when sick (normal  $<4$  mmol/mol creatinine). These findings were interpreted as suggestive of mitochondrial dysfunction. Ketonuria and lactic aciduria were always present, even at times when she was metabolically well compensated and apparently healthy. Free carnitine levels were mildly reduced ( $15 \mu\text{M}$ , normal  $26\text{--}81 \mu\text{M}$ ) with normal levels of esterified carnitine ( $17 \mu\text{M}$ , normal  $0\text{--}19 \mu\text{M}$ ). Plasma acylcarnitine profile (Fig. 1B) indicated severe elevation of C6, C8 ( $2.61\text{--}4.34 \mu\text{M}$ , normal  $<0.23 \mu\text{M}$ ), and C10:1 acylcarnitines, consistent with MCAD deficiency. She had elevated lactic acid levels in plasma (average  $9.2 \pm 2.5$  mM ( $n = 38$ ), range  $4.6\text{--}14.5$  mM) with an increased lactate/pyruvate ratio ( $49.3 \pm 14.5$  ( $n = 8$ ), range  $26.5\text{--}69.7$ ). CSF lactate was also elevated ( $9\text{--}11.7$  mM). Plasma amino acids indicated severe hyperalaninemia (up to  $1.081$  mM, normal  $0.24\text{--}0.60$  mM). Urine amino acids were normal, with the exception of increased excretion of alanine. Testing of multiple hormones (cortisol, ACTH, 17-hydroxyprogesterone, testosterone, free T4, TSH, IGF-I, IGF-BP3) on multiple occasions failed to detect any abnormality. Liver function

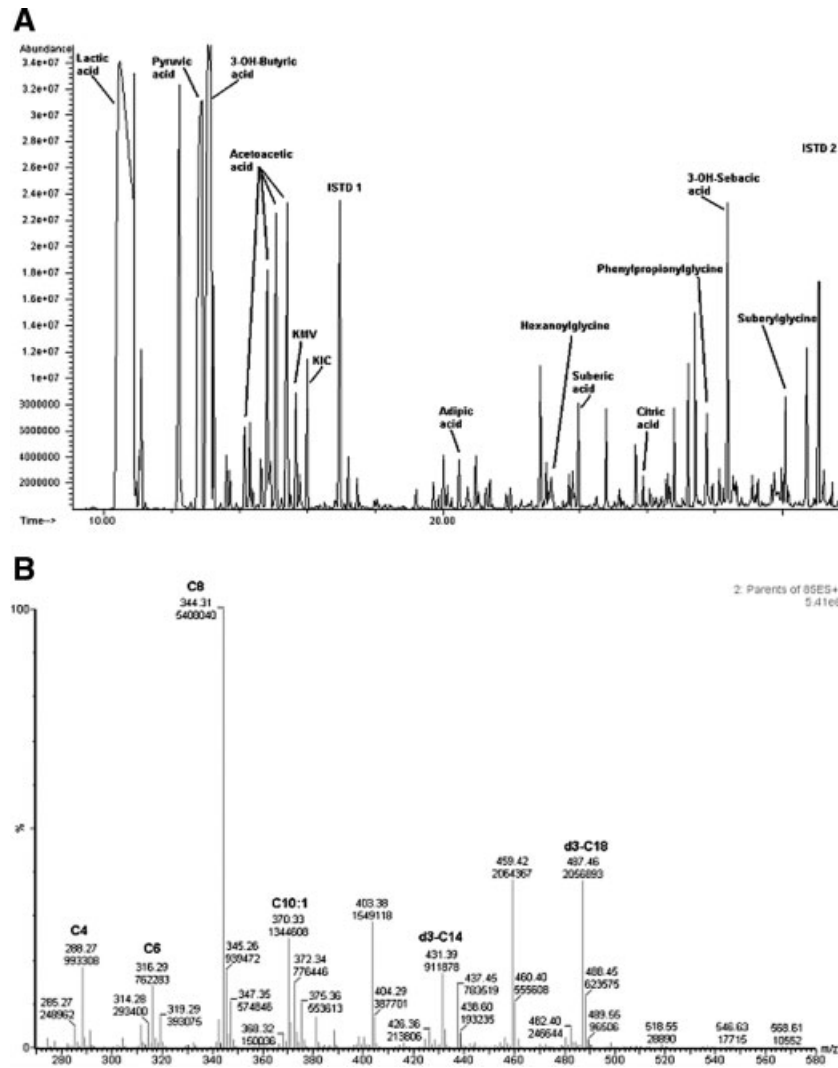


FIG. 1. Urine organic acids (A) and plasma acylcarnitine profile (B) in a patient with MELAS and concomitant MCAD deficiency. A: Urine organic acids were analyzed by GC/MS. They were collected while the patient was clinically stable. Note marked lactic/pyruvic aciduria, severe ketonuria, and presence of metabolites characteristic of MCAD deficiency. B: The plasma acylcarnitine profile shows a marked elevation of C6, C8, and C10:1 characteristic of MCAD deficiency.

tests always indicated low alkaline phosphatase (66–91, normal 175–420 U/L). Measurement of serum zinc confirmed zinc deficiency (40–45  $\mu\text{g}/\text{dl}$ , normal 66–144  $\mu\text{g}/\text{dl}$ ). Zinc supplements normalized zinc levels and improved alkaline phosphatase activity (up to 147 U/L). Transaminases became mildly increased (ALT 46–139, normal 10–35 U/L; AST 56–183, normal 15–40 U/L) after episodes of vision loss and apparent seizures. CK was normal with normal isozyme fractions. A low fat diet without MCT oil, carnitine supplements, Coenzyme Q10 200 mg per day, and citrate supplements was initiated in view of chronic metabolic acidosis ( $\text{CO}_2=8\text{--}18$  mmol/L with elevated anion gap). There was no evidence of chronic hypoglycemia, and she had one glucose level mildly below the normal range (54 mg/dl, normal 60–115 mg/dl) only once. She required continuous feedings at night with Ensure with fibers

using a nasogastric tube to improve failure to thrive. Additional medications and supplements included zinc sulfate (due to low levels of serum zinc), arginine, biotin, famotidine, and erythromycin (to favor intestinal motility), phenytoin (for apparent seizures/vision loss), and polyethylene glycol as needed for constipation.

She improved with this therapy and had good weight gain for about 1 year until she reached the 15th centile for weight and the 5th centile for height. She improved academically and started to attend school at her grade level. She learned to read well, but continued to have some problems with writing, possibly related to her muscle weakness. At the age of 8.5 years, however, she started a rapidly progressive course with episodes of complete vision loss and neurological deterioration following minor episodes of fever or dehydration. These resolved in part

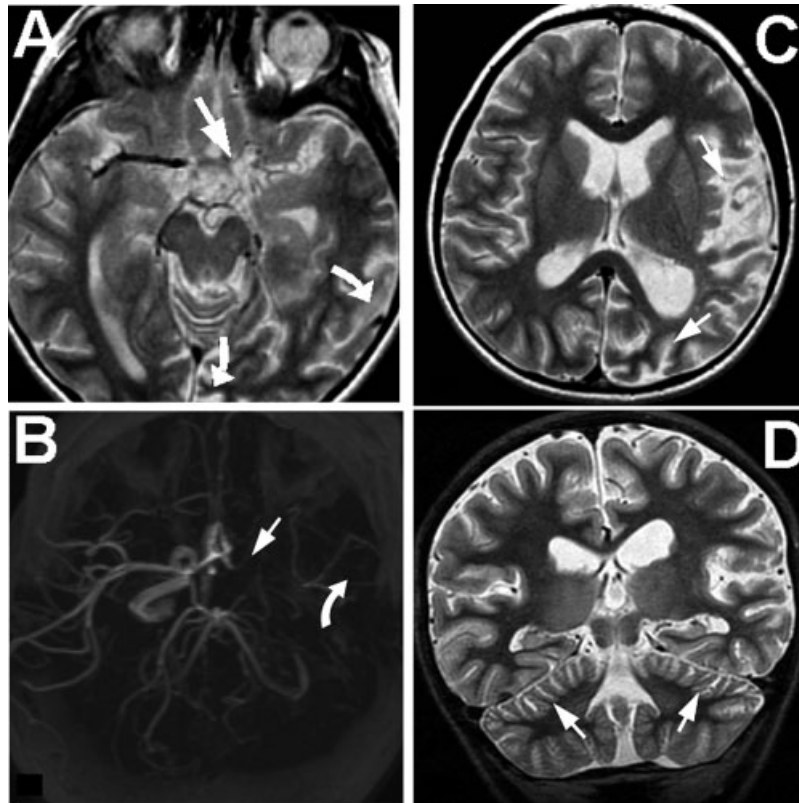


FIG. 2. Brain MRI and MR angiography in a patient with MELAS. **A:** The axial T2-weighted image shows absence of flow-signal within the left middle cerebral artery (arrow). Additionally, atrophy of the left temporal and occipital lobes is noted (curved arrows). **B:** The 3D time-of-flight MRA demonstrates an absence of flow signal within the left internal carotid artery (arrow). Note the lack of flow within the distal branches of the left middle cerebral artery (curved arrow). **C:** This axial T2-weighted MR image illustrates atrophy of the left temporal and occipital regions (arrows). **D:** Coronal T2-weighted MR image, indicating mild atrophy of the superior cerebellar hemispheres (arrows).

with hospital admission, hydration and administration of intravenous glucose, but led to progressive neurological decline, which was attributed to metabolic strokes. Brain MR imaging demonstrated generalized cerebral atrophy, more marked on the left side (Fig. 2). Magnetic resonance angiography indicated occlusion of the left cerebral artery with development of collateral circulation, compatible with Moyamoya syndrome. Coagulation factor studies excluded a thrombophilic diathesis. Hydrogen magnetic resonance spectroscopy (MRS) showed a characteristic lactate doublet within the occipital poles, but not in the basal ganglia (Fig. 3). Involvement of brain blood vessels worsened over time affecting the other (right) side of the brain with narrowing of the end of the carotid artery and the origin of cerebral arteries (Fig. 4). In view of the rapid progression of symptoms, revascularization of the brain by left superficial temporal artery to middle cerebral artery anastomosis was performed at age 9 years. The bypass procedure was difficult because of thin arterial diameters. In an attempt to provide additional supplemental blood flow, an encephaloduromyosynangiosis with the posterior aspect of the temporalis muscle was also performed. Post-operatively her condition continued to worsen;

she developed increased cortical infarcts, as well as cerebellar infarcts, renal failure with fluid overload, and apnea requiring intubation. She died 10 days after surgery.

## METHODS

The mitochondrial genome was investigated by denaturing high performance liquid chromatography (DHPLC) and direct DNA sequencing. First, the mitochondrial DNA was amplified in 19 overlapping sections. The larger amplicons were then digested with restriction enzymes to create fragments of the correct size for DHPLC analysis. Each DNA sample was mixed with the ion pairing reagent triethylammonium acetate to facilitate binding of the DNA to a hydrophobic DNASep cartridge. Samples were eluted based on size and on percentage of helicity using a DHPLC approach with a WAVE Nucleic Acid Fragment Analysis System (Transgenomic, Inc., Omaha, NE). Heteroduplex DNA species indicative of heteroplasmic mutations eluted earlier from the column than homoduplex DNA and were detected by comparison of the chromatogram of the patient sample with that from a control. Because homoplasmic changes cannot be

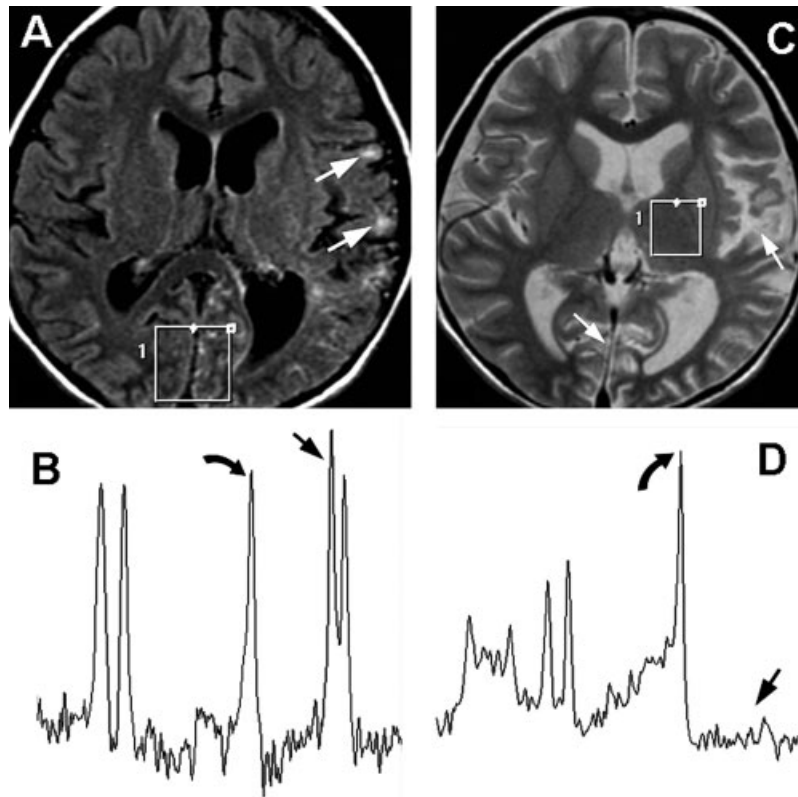


FIG. 3. Brain MR spectroscopy in a patient with MELAS. **A:** Axial fluid attenuated inversion recovery image (FLAIR) shows subtle peripheral left temporal lobe bright signal indicative of gliosis (arrows). Note the occipital gray matter voxel placement for MRS. **B:** MRS single voxel sampling of the occipital poles demonstrates a large lactate doublet at 1.3 ppm (arrow). Note the decline in N-acetyl aspartate (NAA) (curved arrow). **C:** Axial T2 weighted image reveals atrophy of the left temporal and occipital regions (arrows). Note the placement of a left basal ganglia voxel for MRS. **D:** Single voxel proton spectroscopy demonstrates normal preservation of NAA (curved arrow) and absence of a lactate peak at 1.3 ppm (arrow).

detected by this method and for definitive identification and confirmation of heteroplasmic changes, amplicons representative of many mDHPLC spectral patterns were reamplified. PCR purification was performed by QIAquick PCR purification (Qiagen, Valencia, CA). Purified samples were sequenced directly with fluorescent di-deoxy terminators (ABI)

on an ABI 3100 sequencing instrument (Applied Biosystems, Foster City, CA). Identified sequence changes were confirmed by direct DNA sequencing in the reverse direction. Several mitochondrial databases were consulted for clinical interpretation of the results, including MitoDat and Mitomap: <http://www.nlm.nih.gov/ncicrf.gov/mitodat/>, and <http://www.mitomap.org/>.



FIG. 4. Progression of arterial stenosis in MELAS. **A:** Axial cerebral FLAIR image shows chronic bilateral cortical high intensity abnormalities. **B:** Frontal reprojected right hemispheric "cut-away" time-of-flight MRA image shows a tight stenosis involving the terminus of the right supraclinoid internal carotid artery, the first branch of middle cerebral artery and the first branch of anterior cerebral artery (arrow). This study was obtained 19 months after the first angiographic study shown in Figure 2.

## RESULTS

In view of the abnormal urine organic acids and plasma acylcarnitine profile, DNA testing was obtained to confirm the diagnosis of MCAD deficiency. Molecular testing for MCAD deficiency indicated that the patient had three different mutations in the corresponding gene: the common mutation c.985A>G (p.K304E), c.233T>C (p.I53T), and a novel c.631C>T (p.P186S) (MCAD 18 in McKinney et al. [2004]). Due to the lack of parental availability, the phase of these mutations could not be established. However, the presence of at least two pathogenic mutations and the unequivocal biochemical findings (Fig. 1) fully confirmed a diagnosis of MCAD deficiency.

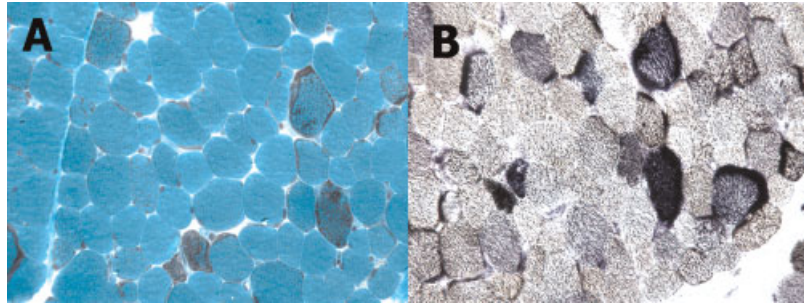


FIG. 5. Muscle histochemistry in a patient with MELAS. **A:** Cryostat sections of the thigh muscle biopsy showing several ragged red fibers by modified Gomori trichrome staining. **B:** The same region contains numerous cytochrome oxidase negative fibers highlighted by their strong blue staining in the combined cytochrome oxidase and succinic acid dehydrogenase enzyme histochemistry preparation (original magnifications 400 $\times$ ). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

A muscle biopsy indicated the presence of numerous ragged red fibers (Fig. 5A). Muscle histochemistry indicated relative sparing of succinate dehydrogenase (SDH) staining with decreased cytochrome *c* oxidase (COX) staining (Fig. 5B, fibers in dark blue). MtDNA testing excluded a number of mutations associated with MELAS (3243A>G, 3271T>C, 3252A>G, 3291T>C, and 3260 A>G), mtDNA duplications, deletions and rearrangements. Denaturing high performance liquid chromatography (DHPLC) screening and subsequent sequencing of mtDNA indicated that the muscle sample was almost homoplasmic for the 3243A>T mutation in tRNA<sup>Leu</sup>, with a lower mutant load of approximately 50% heteroplasmy in peripheral blood cells and cultured skin fibroblasts.

## DISCUSSION

MELAS is a severe mitochondrial disorder with a progressive neurodegenerative course. The patient presented in this paper had an unusually rapid progression of this disease that was most likely due to vascular involvement of the brain (Figs. 2–4). In its typical description, MELAS is characterized by metabolic strokes that do not follow the distribution of a particular artery. However, in this case, brain atrophy paralleled vascular involvement and neurodegeneration progressed along with the progressive stenosis of cerebral arteries (Fig. 4). The molecular basis of her disorder was a single point mutation in the mitochondrial tRNA for leucine (3243A>T tRNA<sup>Leu</sup>). This mutation had been previously associated with MELAS and severe encephalopathy [Shaag et al., 1997]. The only other patient reported had ketosis and lactic acidosis and mild brain ventriculomegaly detected by CT scan (brain MRI or MR angiography were not performed) and suffered a cardiac arrest at 9 years of age due to central apnea [Shaag et al., 1997]. This patient remained alive in a vegetative state, requiring mechanical ventilation and tube feeding. Our patient had a similarly rapidly progressive course that was

dictated by the progressive restriction of blood flow to the brain (Figs. 2 and 4).

MELAS has been associated with temporary arterial stenosis [Noguchi et al., 2005] and with blood-vessel pathology [Betts et al., 2006; Koga et al., 2006], but not with the degree and expression of vascular involvement described in this manuscript. Part of the severity of our patient could be ascribed to the almost complete homoplasmy for the 3243A>T tRNA<sup>Leu</sup> mutation in muscle and probably other tissues and to the dramatic effect that this mutation has on function. In fact, the 3243A>T tRNA<sup>Leu</sup> mutation associated with MELAS is the one that most severely affects aminoacylation efficiency, as it reduces this process to approximately 1% of normal [Sohm et al., 2003]. By contrast, the most common tRNA<sup>Leu</sup> mutation (3243A>G) retains a 10-fold higher aminoacylation efficiency [Sohm et al., 2003]. The combination of a high mutation load with functional severity were probably responsible for the severe and rapid deterioration of this patient.

This patient was unique in that she was also affected by an unrelated autosomal recessive disorder of mitochondrial fatty acid oxidation, MCAD deficiency (Fig. 1). This diagnosis was supported by both biochemical and molecular findings. MCAD deficiency is usually associated with hypoketotic hypoglycemia and sudden death [McKinney et al., 2004]. On the other hand, certain mutations in the MCAD gene (such as 199T>C) have been detected exclusively in patients identified by prospective newborn, but not in symptomatic patients [Andresen et al., 2001]. The mutations identified in our patient (c.985A>G (p.K304E), c.233T>C (p.I53T)) have been previously described in symptomatic patients with MCAD deficiency [McKinney et al., 2004], correlating with the elevated levels of C8-carnitine in plasma (Fig. 1) [Waddell et al., 2006]. In our patient, the severe mitochondrial dysfunction prevented the oxidation of residual ketone bodies that accumulated in excessive amounts even when the child was clinically well (Fig. 1). This observation confirms that at least some amounts of ketones can be produced in MCAD deficiency and that when a

mitochondrial dysfunction is present ketones can become extremely elevated. MCAD deficiency in this patient was biochemically evident but clinically silent, since none of her symptoms appeared related to the typical presentation of this disease. Nevertheless, since both diseases were present simultaneously, we cannot exclude a contribution of MCAD deficiency to the development of the phenotype in this patient.

Blood vessels are altered in MELAS with a striking increase in number of mitochondria in the smooth muscle and endothelial cells [Ohama et al., 1987; Sakuta and Nonaka, 1989; Forster et al., 1992]. Despite histological alterations, there are few reports in which vascular abnormalities have been demonstrated in patients with MELAS or mitochondrial disorders. Our patient shows that vascular stenosis occurs not only temporarily [Noguchi et al., 2005], but can be an important factor in determining progression of mitochondrial disorders. It is interesting that recent evidence suggests that the use of arginine might improve endothelial function in MELAS [Koga et al., 2006]. The clinical course of our patient supports measures aimed at maintaining vascular function and points out the necessity of vascular studies (by angiography or MRA) in patients with MELAS, particularly since some cases of Moyamoya syndrome may be improved with surgery.

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