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## A Novel Mutation in the Mitochondrial DNA Cytochrome *b* Gene (*MTCYB*) in a Patient With Mitochondrial Encephalomyopathy, Lactic Acidosis, and Strokelike Episodes Syndrome

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### Abstract

Mutations in the mitochondrial DNA cytochrome *b* gene (*MTCYB*) have been commonly associated with isolated mitochondrial myopathy and exercise intolerance, rarely with multisystem disorders, and only once with a parkinsonism/mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS) overlap syndrome. Here, we describe a novel mutation (m. 14864 T>C) in *MTCYB* in a 15-year-old girl with a clinical history of migraines, epilepsy, sensorimotor neuropathy, and strokelike episodes, a clinical picture reminiscent of MELAS. The mutation, which changes a highly conserved cysteine to arginine at amino acid position 40 of cytochrome *b*, was heteroplasmic in muscle, blood, fibroblasts, and urinary sediment from the patient but absent in accessible tissues from her asymptomatic mother. This case demonstrates that *MTCYB* must be included in the already long list of mitochondrial DNA genes that have been associated with the MELAS phenotype.

### Keywords

cytochrome *b*; *MTCYB*; mtDNA; MELAS; mitochondria

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### Author Contributions

VE and ES conducted the molecular and biochemical diagnosis of the patient. VE helped in the literature research and in drafting the manuscript. PGR and HOA conducted detailed molecular analysis and assessed pathogenicity of the mutation. JG, RI, and ARF were involved in management of the patient. And SDM supervised and coordinated the study and drafted the manuscript.

### Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Ethical Approval

Informed consent for anonymous publication of the patient's clinical features and analyses of biological samples were obtained under a Columbia University Institutional Review Board approved protocol.

Isolated mitochondrial complex III deficiency is a relatively rare cause of respiratory chain dysfunction. Complex III (ubiquinol-cytochrome *c* reductase) catalyzes the electron transfer from ubiquinol to cytochrome *c* and consists of 11 subunits, only 1 of which, cytochrome *b* (cyt *b*), is encoded by mitochondrial DNA. The cyt *b* protein is 380 amino acids long and the *MTCYB* gene encompasses nucleotide positions 14747 to 15887 of mitochondrial DNA.<sup>1</sup>

Isolated complex III deficiency can be due to mutations in the nuclear genome or in *MTCYB*. Mutations in nuclear genes can affect complex III subunits (“direct hits”)<sup>2,3</sup> or assembly proteins (“indirect hits”).<sup>4–6</sup> Mutations in *MTCYB* are most commonly associated with sporadic myopathy dominated by exercise intolerance with or without myoglobinuria,<sup>7–14</sup> but have also been reported in multisystem disorders.<sup>15–18</sup> One young adult patient with a 4-base-pair deletion in *MTCYB* had bilateral strokelike lesions in the parieto-occipital lobes following a 5-year clinical history of parkinsonian symptoms and seizures, a condition termed parkinsonism/mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS) overlap syndrome.<sup>15</sup>

Here, we describe a novel missense heteroplasmic mutation (m.14864T>C) in *MTCYB* in a 15-year-old girl with a 5-year history of migraine, epilepsy, strokelike episodes, and chronic sensorimotor axonal polyneuropathy, a clinical presentation consistent with MELAS.

## Case Report

A 10-year-old girl presented to the emergency room complaining of headache, impaired vision, and unsteady gait. There was a stuttering evolution of her visual symptoms over 2 days in the setting of a febrile respiratory infection, resulting in complete left homonymous hemianopsia. Brain magnetic resonance imaging (MRI) obtained on the third day after onset of symptoms showed an acute infarction in the right occipital and medial temporal lobes (Figure 1). Magnetic resonance angiography demonstrated a diminutive right vertebral artery throughout its entire course, consistent with either a remote dissection or congenital hypoplasia of uncertain clinical significance. Brain magnetic resonance spectroscopy revealed increased lactate peaks over the brain parenchyma and cerebrospinal fluid spaces, but blood tests did not reveal elevated lactate levels. An electroencephalography (EEG) performed on the fifth day after onset of symptoms showed right posterior quadrant slowing but no epileptiform discharges. Studies for stroke risk factors were normal, including magnetic resonance angiography of the neck, echocardiogram with bubble study, and comprehensive thrombophilia studies. Her headache resolved in a week, whereas the hemianopsia persisted at hospital discharge and ultimately resolved by 1 year after onset.

The patient presented 1 year later at age 11 years with right visual field obscurations followed by headache evolving over 6 hours to a complete right homonymous hemianopsia associated with confusion and expressive aphasia. Video EEG 12 hours after onset of symptoms revealed focal nonconvulsive status epilepticus involving the left posterior quadrant. Brain MRI 16 hours after onset showed no new infarct. Plasma lactate was increased (3.3 mMol/L) on presentation and returned to normal (1.86 mMol/L) 12 hours later, coinciding with resolution of electrographic seizures. Electrographic seizures stopped within 24 hours of treatment with lorazepam, levetiracetam, and a full loading dose of phenobarbital. Her confusion, aphasia, and hemianopsia resolved by 48 hours of admission. Treatment on discharge included continued levetiracetam and initiation of a mitochondrial cocktail of L-carnitine 1 g and coenzyme Q 90 mg 3 times daily.

As her evolving clinical picture was concerning for MELAS, 20 hours after onset of symptoms she was treated with intravenous L-arginine, 500 mg/kg, infused over 1 hour. This was well tolerated and resulted in cessation of electrographic seizures within 4 hours after

completion of the infusion. The next day, her mental status returned to normal and the hemianopsia and aphasia resolved. Brain MRI at that time showed only a single punctate focus of restricted diffusion in the left medial occipital lobe. In the region of the previously identified right occipital infarct, there was evidence of mild hyperintensity by T2 fluid-attenuated inversion recovery MRI sequences with associated focal volume loss. Perfusion MRI using arterial spin labeling technique<sup>19</sup> showed striking hyperperfusion in the left occipital lobe (Figure 2). She was discharged home on a tapering dose of phenobarbital and maintenance doses of levetiracetam, L-arginine 4 mg daily, carnitine, coenzyme Q, and riboflavin. On this regimen, she has remained clinically stable for 2½ years, free of seizures or recurrent strokelike episodes. She has mild exercise intolerance, recurring mild headaches, and mild neurocognitive impairment for which she has appropriate educational modifications in place and functions well at the ninth-grade level.

The patient had a medical history of recurrent migraine-like headache since 7 years of age. At 9 years, she had 2 generalized tonic-clonic seizures, both of which were preceded by an aura of left visual field loss and headache and had occurred following physical exertion, such as swimming. She also had a history preceding her strokelike episodes of diminished exercise tolerance with weakness and absent tendon reflexes in the legs.

There is no known family history of similar symptoms. In particular, the mother is asymptomatic as are an older and a younger sister.

On neurologic examination at age 15 years, the patient was well developed, with weight and height at 10th percentile for age, appearing cheerful and cooperative. Cranial nerves were intact, including normal-appearing fundi bilaterally, and no appreciable visual field loss on confrontational testing. On neuromuscular examination, there was weakness of the intrinsic hand and plantar flexor muscles (4/5 MRC scale). All other muscle groups had normal strength (5/5 MRC scale). Deep tendon reflexes were absent bilaterally in both arms and legs. Vibratory sensation was mildly decreased in fingers and toes. Examination of cerebellar function revealed large-amplitude tremor most notable during extension of the arms and hands. She had difficulty walking on her heels but could walk on her toes. There were also spontaneous finger movements that appeared somewhat choreiform in nature.

## Methods

### Histochemical and Biochemical Analysis

A right quadriceps muscle biopsy was analyzed with standard histological and histochemical stainings. Respiratory chain enzyme activities were measured spectrophotometrically as previously described in muscle and fibroblast extracts.<sup>20</sup>

### Molecular Studies

Total DNA was extracted from muscle, blood, urinary sediment, and fibroblasts using standard procedures. The most common MELAS mutations of the mitochondrial DNA (3242 A>G, 3256 C>T, 3271 T>C, 3291 T>C, 5814 A>G, 8356 T>C, 13513 G>A) were screened by restriction fragment length polymorphism analysis. Mitochondrial DNA was measured using the 12-S ribosomal TaqMan mitochondrial assay labeled with the fluorochrome 6-carboxyfluorescein (6-FAM) and the primers 5'-CCACGGGAAACAGCAGTGATT-3' and 5'-CTATTGACTTGGGTAAATCGTGTGA-3'. Measurements of the nuclear single-copy gene RNaseP were performed by using the kit PDARs RNaseP (Applied Biosystems, Foster City, California) and a 4,7,2'-trichloro-7'-phenyl-6-carboxyfluorescein (VIC)-labeled probe. The values of mitochondrial DNA levels were normalized by nuclear DNA, and the data were expressed in terms of percentage relative to controls. Direct sequencing of all 22 mitochondrial DNA transfer RNA genes,

*MTND1–MTND6* genes, and *MTCYB* gene was performed in an ABI Prism 310 Genetic Analyzer using Big Dye Terminator Cycle Sequencing Reaction Kits (Perkin–Elmer Applied Biosystems, Foster City, California), using appropriate sets of primers (see supporting material). For restriction fragment length polymorphism analysis, mitochondrial DNA was amplified by polymerase chain reaction (PCR) using forward and reverse primers at nucleotide positions 14850 to 14862 and 15030 to 14998. In the presence of the mutation, the mismatched forward primer creates a restriction site for the BanII endonuclease. Digestion of the 183-bp fragment harboring the mutation yields 2 fragments of 159 bp and 24 bp, whereas the wild-type fragment remains uncut. The PCR products were run through a 15% nondenaturing polyacrylamide gel and subjected to SYBR Gold Nucleic Acid Stain (Invitrogen) to quantify the proportion of mutant mitochondrial DNA.

## Results

Histochemical analysis demonstrated mild variation in fiber size and shape, but no ragged-red fibers, ragged-blue fibers, or cytochrome *c* oxidase–negative fibers. However, mitochondrial enzyme reactions revealed increased subsarcolemmal staining in scattered fibers.

Biochemical studies of muscle extracts showed mild deficiencies (about 70% of normal mean values) in the activities of complexes I and III (Table 1). Respiratory chain enzyme activities in fibroblasts were normal (data not shown).

Quantitative PCR failed to detect any mitochondrial DNA depletion (data not shown). Screening for the most common mitochondrial DNA mutations associated with MELAS was negative, and direct sequencing of all 22 transfer RNAs and the *MTND1–6* genes did not detect any pathogenic point mutations.

Sequencing of the *MTCYB* gene showed a novel m.14864T>C transition (Figure 3A, B). PCR–restriction fragment length polymorphism showed that the mutation load was 39% in muscle, 32% in blood, 57% in urinary sediment, and 42% in fibroblasts (Figure 4). The mutation was undetectable in DNA from blood and urine of the patient’s mother.

## Discussion

The first mutation in *MTCYB* was described in 1996, in a patient presenting with exercise intolerance and reduced complex III activity in muscle.<sup>21</sup> Since then, many more patients have been reported with *MTCYB* mutations and a pure myopathic phenotype involving exercise intolerance, weakness, and myoglobinuria.<sup>8–14</sup> Although less frequently, *MTCYB* mutations have also been associated with multisystem disorders, including hypertrophic cardiomyopathy,<sup>16,22</sup> neonatal polyvisceral failure,<sup>23</sup> septo-optic dysplasia,<sup>24</sup> and encephalomyopathy<sup>15,17,18,25</sup> (Table 2).

Our patient presented at age 7 years with migraine-like headaches and early signs of sensorimotor neuropathy, followed at age 9 years by generalized convulsions heralded by headache and transient visual aura, and at age 10 years by her first strokelike event with hemianopsia and unilateral occipital infarct. Similar strokelike episodes recurred at 1-year intervals, during which it became evident that her acute-onset hemianopsia and related mental status changes were symptomatic of focal nonconvulsive status epilepticus arising from the occipital lobe. Her focal deficits resolved promptly when electrographic status epilepticus was identified and treated early. This constellation of clinical and radiographic findings suggested the diagnosis of MELAS. After excluding the most common MELAS mutation (m.3243A>G in the transfer RNA<sup>Leu(UUR)</sup> gene), mutations in other transfer RNA

genes, and in the *MTND* genes, the biochemical finding of partial complex III deficiency in muscle prompted us to sequence *MTCYB*.

We found a novel m.14864 T>C transition (Figure 3A) that fulfills canonic criteria for pathogenicity. First, it has not been reported as a polymorphism in the mitochondrial genome databases (<http://www.mitomap.org>, <http://www.genpat.uu.se/mtDB>) and was not found by our screening of 100 healthy individuals with the same ethnic background.

Second, this change was heteroplasmic in several tissues (Figure 4), a common feature of pathogenic mitochondrial DNA mutations.

Third, it caused the substitution of a highly conserved cysteine with an arginine at position 40 (C40R) of the cytochrome *b* molecule (Figure 3B), which was considered probably damaging with a score of 1.0 by the PolyPhen program (<http://genetics.bwh.harvard.edu/pph2/index.shtml>).

As derived from the crystal structure of cytochrome *b*,<sup>10</sup> the altered amino acid is located within the Qn ubiquinone-binding site, which is needed for ubiquinone reduction. Both ubiquinone-binding sites (Qp and Qn) must be functional for proton transfer to occur. The mutation resides in a putative antimycin A binding pocket, whose functional importance was documented in *Leishmania tarentolae* by a mutation in a nearby position (S35I), which made the protozoan antimycin A-resistant.<sup>26</sup>

Fourth, the mutation is consistent with the complex III deficiency observed in our patient's muscle. The mild biochemical defect is probably due to the relatively low mutation load in muscle (39%) and the association of a comparably mild defect in complex I has been reported in other patients with *MTCYB* mutations<sup>12,25</sup> and attributed to the functional interdependence of the 2 complexes.<sup>27</sup>

A review of the literature on *MTCYB* mutations reveals a few recurrent themes that are worth discussing vis-à-vis our patient.

First, despite the extremely high variability of the *MTCYB* sequence,<sup>8,28</sup> isolated complex III deficiency is a relatively rare cause of mitochondrial disease,<sup>8,29,30</sup> implying that pathogenic mutations both in the *MTCYB* gene and in the 10 nuclear genes that comprise the complex are uncommon.

Second, the selective involvement of skeletal muscle in 61% of reported patients is striking. In most adult myopathic patients, symptoms have been confined to exercise intolerance and exercise-induced myalgia since childhood and the pathogenic mutations have not been encountered in accessible non-muscle tissues, which led us to propose that they had arisen de novo in muscle progenitor cells after differentiation of the primary germ layers.<sup>10</sup>

An alternative explanation was offered by Keightley et al,<sup>18</sup> namely, that a sporadic mutation early in embryogenesis or in the maternal germ line segregated preferentially to skeletal muscle either because of skewed mitotic segregation or because of negative selection in nonmuscle tissues. Skewed mitotic segregation could explain the unusual clinical course of their patient, a 34-year-old woman who suffered from exercise intolerance since childhood and did not develop seizures and psychiatric problems until she was 19 years of age.<sup>18,31</sup> Careful analysis of nonmuscle tissues showed barely detectable levels of mutation (0.7%) in blood, low levels (4%–5%) in the ovary, high levels (61%) in the uterus, and extremely variable levels (<0.4%–66%) in individual hair bulbs, which derive from the clonal expansion of one or a few stem cells.<sup>32</sup>

All other cases with multisystem involvement harbored the pathogenic mutation in nonmuscle tissues, including blood, urinary or buccal epithelium, hair follicles, and cultured skin fibroblasts, whereas the mutation was never detected in blood or other accessible tissues from mothers. Even in multisystem cases, muscle was generally the tissue with the highest mutation load, although homoplasmy was reached only in the 1 child with fatal infantile multivisceral presentation. In 2 children with hypertrophic cardiomyopathy, the mutation load was not measured in skeletal muscle but was predictably high (90%) in cardiac muscle from one<sup>16</sup> and in liver (90%) and kidney (82%) from the other.<sup>22</sup>

In contrast with the stereotypical myopathic presentation of most patients, the clinical phenotypes of the 9 sporadic patients with multisystem involvement are extremely varied (Table 2). One additional phenotype not included in the table is Leber hereditary optic neuropathy (LHON) because changes reported in individual patients, such as the m.14894T>C mutation,<sup>33</sup> have not yet been confirmed in a second patient.

Our patient had symptoms strongly suggestive of MELAS, with migraines, seizures, and 3 strokelike episodes affecting the parietal and occipital lobes. This patient returned to her baseline after each strokelike episode. If the similarly low mutation levels found in skeletal muscle, blood, urinary epithelium, and skin fibroblasts extend to the brain, it is conceivable that her relatively benign course and normal blood lactate relate to her unusually low mutation load. Interestingly, her last episode of focal nonconvulsive status epilepticus was refractory to multiple anticonvulsant agents, but appeared to respond to L-arginine administration, thus confirming several, though still anecdotal, previous observations.<sup>34,35</sup> She has been free of recurrences for 2½ years with the addition of arginine to her maintenance regimen. A further novel observation in her case is the striking hyperperfusion on arterial spin labeled perfusion MRI in the affected occipital-parietal lobe during the acute phase of the illness. This may be useful as a diagnostic clue to mitochondrial disease as it is not typically seen in patients with acute ischemic infarction because of vascular occlusive mechanisms, in whom ischemia is usually associated with impaired perfusion.<sup>36</sup>

Mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes was associated with early-onset parkinsonism in a 20-year-old man who had a stroke at age 19 years, after a 13-year history of parkinsonian symptoms, seizures, and behavioral problems.<sup>15</sup> The patient of Keightley et al<sup>18</sup> suffered from a left-sided stroke before age 30 years and the patient of Blakely et al<sup>25</sup> had suffered from migraines and had an episode of “mild hemiparesis” at age 34 years. Thus, strokelike episodes may not be unusual in patients with generalized *MTCYB* mutation, although our patient is the only one thus far presenting with typical MELAS. It is repeatedly stated in the literature that the m.3243A>G mutation in the transfer RNA<sup>Leu(UUR)</sup> gene accounts for 80% of all cases of MELAS, but the precise frequency of the many mitochondrial DNA mutations that have been associated with MELAS remains unknown. In practical terms, after excluding mutations in mitochondrial DNA transfer RNA genes and in the *MTND5* gene, we suggest to consider the possible involvement of the gene encoding cytochrome *b*.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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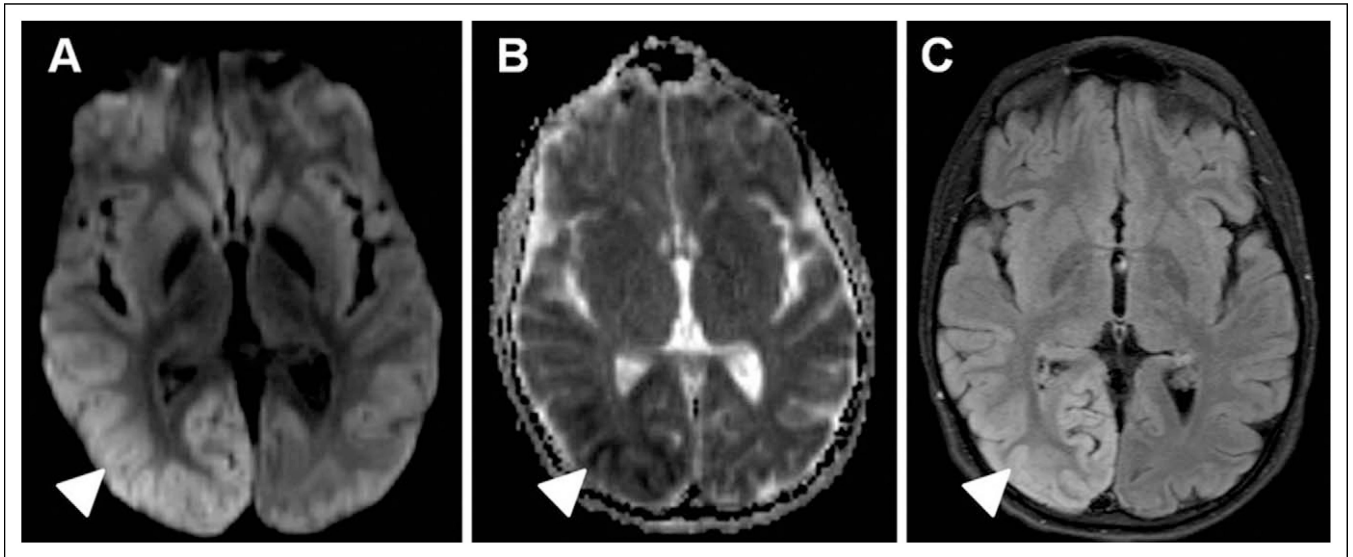
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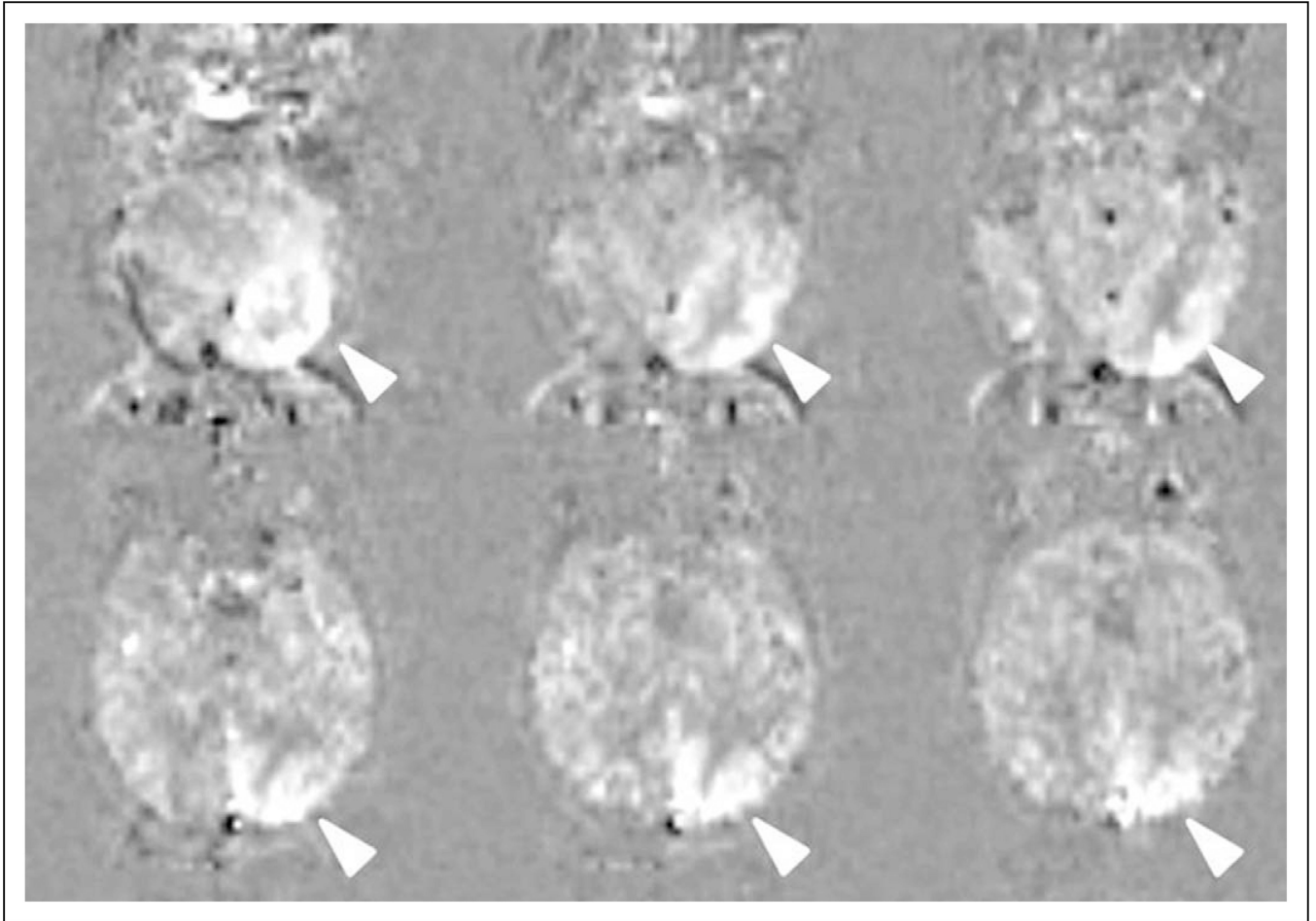
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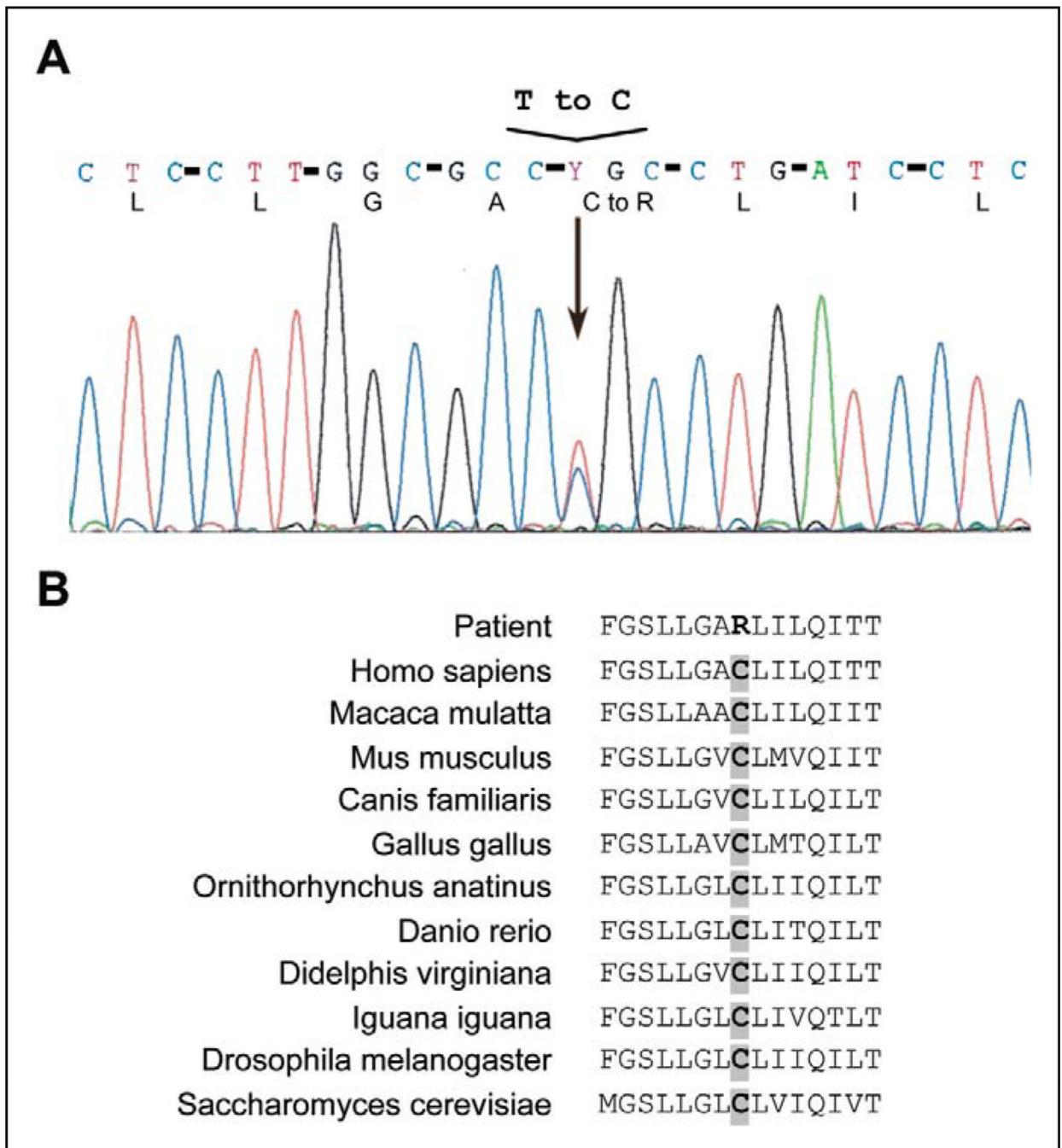




**Figure 1.** Brain MRI at initial presentation with headache and left hemiparesis (age 10 years). Diffusion-weighted sequence shows (arrowheads) (A) hyperintensity in the right occipital-parietal cortex; (B) patchy restricted diffusion on apparent diffusion coefficient (ADC) map; and (C) subtle hyperintensity on T2 fluid-attenuated inversion recovery.

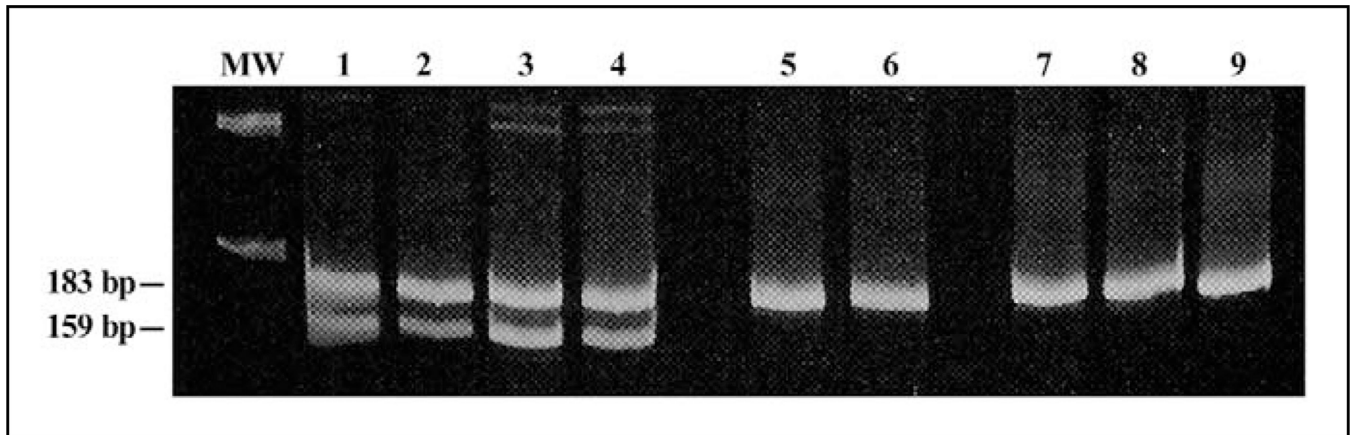


**Figure 2.** Arterial spin label perfusion MRI shows marked hyperemia in the left occipital cortex (arrowheads) coincidental with new acute right hemianopsia and focal nonconvulsive status epilepticus from the left posterior quadrant.



**Figure 3.**

(A) Electropherogram of the *MTCYB* DNA sequence from muscle showing the heteroplasmic T>C transition (arrow). (B) Evolutionary conservation of the cytochrome *b* amino acid sequence in the vicinity of the C40R mutation. The gray shadowing shows the interspecies preservation of the nucleotide that is mutated in the patient.



**Figure 4.**

Polymerase chain reaction–restriction fragment length polymorphism analysis of the 14864T>C mutation using a mismatch primer. The mutant sequence introduces a *Ban*II site yielding an additional 159-bp fragment, whereas the wild-type sequence lacks the *Ban*II site and yields only a 183-bp fragment. MW, molecular weight marker. Lane 1, patient's muscle; lane 2, patient's blood; lane 3, patient's urine sediment; lane 4, patient's fibroblasts; lane 5, blood from the patient's mother; lane 6, urinary sediment from patient's mother; lanes 7 and 8, control DNA samples; lane 9, uncut PCR product.

**Table 1**

## Mitochondrial Enzyme Activities in Muscle.

<b>Enzyme (complex)</b>	<b>Controls (mean <math>\pm</math> SD)</b>	<b>Patient (% of controls)</b>
NADH-CoQ reductase (I)	1.75 $\pm$ 0.65	1.19 (68)
CoQ-cyt. <i>c</i> reductase (III)	1.53 $\pm$ 0.60	1.06 (69)
Cytochrome <i>c</i> oxidase (IV)	2.80 $\pm$ 0.52	2.32 (83)
Succinate dehydrogenase (II)	1.00 $\pm$ 0.53	1.12 (112)
Citrate synthase (CS)	9.88 $\pm$ 2.5	9.54

I, II, III, and IV denote complexes of the respiratory chain; NADH, nicotinamide adenine dinucleotide; CoQ, Coenzyme Q10; cyt *c*, cytochrome *c*.

Table 2

Mutation load in muscle.

Clinical	Age, y, Sex	Mutation	Amino Acid Change	Mutation Load	Inheritance	Reference
Myopathy	Nd	c.15150G>A	p.W135X	60	S	8
	Nd	c.15197T>C	p.G165X	80	S	8
	18, M	c.14849T>C	p.S35P	85	S	14
	38, F	c.15762G>A	p.G339E	85	S	7
	27, M	c.15059G>A	p.G190X	63	S	9
	43, M	c.15498_15522del	p.G251_A259delinsA	50	S	10
	52, F	c.14846G>A	p.G34S	85	S	10
	38, F	c.15168G>A	p.W141X	70	S	10
	32, M	c.15084G>A	p.W113X	87	S	10
	51, M	c.15723G>A	p.W326X	87	S	10
	40, F	c.15170G>A	p.G142X	>99	S	11
	24, F	c.15800C>T	p.Q352X	45	S	12
	19, F	c.15761G>A	p.G339X	73	S	13
	29, M	c.15615G>A	p.G290D	80	S	21
	25, M	c.14849T>C	p.S35P	69	S	24
Septo-optic dysplasia	infancy	c.15498G>A	p.G251D	Nd	S	28
	19, F	c.15579A>G	p.Y278C	88	S	17
Histiocytoid cardiomyopathy	infancy	c.15635T>C	p.S297P	100	S	23
	20, M	c.14787_14798del	p.114AfsX26	>95	S	15
Multisystem disorder	34, F	c.15242G>A	p.G166X	87	S	18
	38, F	c.15699G>C	p.R318P	88	S	25
Neonatal polyvisceral failure	3, F	c.15243G>A	p.G166E	Nd	S	16
	15, F	c.14864 T>C	p.C40R	39	S	this paper

Abbreviations: MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; Nd, not described; S, sporadic.