

EXERCISE INTOLERANCE DUE TO MUTATIONS IN THE CYTOCHROME *b* GENE OF MITOCHONDRIAL DNA

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ABSTRACT

Background The mitochondrial myopathies typically affect many organ systems and are associated with mutations in mitochondrial DNA (mtDNA) that are maternally inherited. However, there is also a sporadic form of mitochondrial myopathy in which exercise intolerance is the predominant symptom. We studied the biochemical and molecular characteristics of this sporadic myopathy.

Methods We sequenced the mtDNA cytochrome *b* gene in blood and muscle specimens from five patients with severe exercise intolerance, lactic acidosis in the resting state (in four patients), and biochemical evidence of complex III deficiency. We compared the clinical and molecular features of these patients with those previously described in four other patients with mutations in the cytochrome *b* gene.

Results We found a total of three different nonsense mutations (G15084A, G15168A, and G15723A), one missense mutation (G14846A), and a 24-bp deletion (nucleotides 15498 to 15521) in the cytochrome *b* gene in the five patients. Each of these mutations impairs the enzymatic function of the cytochrome *b* protein. In these patients and those previously described, the clinical manifestations included progressive exercise intolerance, proximal limb weakness, and in some cases, attacks of myoglobinuria. There was no maternal inheritance and there were no mutations in tissues other than muscle. The absence of these findings suggests that the disorder is due to somatic mutations in myogenic stem cells after germ-layer differentiation. All the point mutations involved the substitution of adenine for guanine, but all were in different locations.

Conclusions The sporadic form of mitochondrial myopathy is associated with somatic mutations in the cytochrome *b* gene of mtDNA. This myopathy is one cause of the common and often elusive syndrome of exercise intolerance. (N Engl J Med 1999; 341:1037-44.)

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EXERCISE intolerance is a common symptom of the encephalomyopathies that are associated with mutations in mitochondrial DNA (mtDNA).^{1,2} These usually multisystemic disorders include several distinct syndromes: the Kearns–Sayre syndrome; mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes

(the MELAS syndrome); myoclonus epilepsy with ragged-red fibers; Leber's hereditary optic neuropathy; and maternally inherited Leigh's syndrome. All these disorders are transmitted by nonmendelian, maternal inheritance, except the Kearns–Sayre syndrome, which is a sporadic condition. Until recently, little was known about the molecular basis of exercise intolerance as the sole manifestation of mitochondrial dysfunction in patients with sporadic cases.

In 1993, Bouzidi et al.³ found low activity of respiratory-chain complex III (ubiquinol–cytochrome *c* reductase) in muscle from a 25-year-old man with exercise intolerance in whom they later identified a missense mutation (G15615A) in the mitochondrial cytochrome *b* gene (the only mtDNA-encoded subunit of complex III).⁴ In 1998, Kennaway et al. described a woman long known to have mitochondrial myopathy with complex III deficiency⁵; she had a nonsense mutation (G15242A) in the cytochrome *b* gene.⁶ We later identified two patients with similar clinical, biochemical, and molecular features: a 38-year-old woman with a missense mutation (G15762A), and a 27-year-old man with a nonsense mutation (G15059A) in the cytochrome *b* gene.^{7,8} These findings prompted us to assess patients with exercise intolerance and biochemical evidence of complex III deficiency in muscle. In rapid succession, we

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identified five more patients with distinct mutations in the cytochrome *b* gene. The mutations were deemed pathogenic because biochemical studies showed complex III deficiency in muscle and because of the deduced effects on the crystal structure of complex III, which was elucidated in 1998.⁹

Here, we describe the five new patients we have identified and review the characteristics of the four patients identified previously. A comparison of clinical, morphologic, biochemical, and genetic features of these patients reveals a remarkable uniformity and suggests that mutations in the cytochrome *b* gene can cause exercise intolerance without evidence of maternal inheritance.

METHODS

Patients

Only one patient will be described in detail since all five patients had similar presentations (Table 1).

Patient 2 was a 52-year-old woman who had had exercise intolerance since childhood; she remembered rushing to a friend's house at the age of 5 and arriving there "exhausted and nauseous." This symptom had progressed until she was unable to walk more than one block without extreme fatigue. She also had premature fatigue of masticatory muscles while eating. An examination at the age of 39 showed weakness against resistance in the proximal limb muscles. The venous lactate concentration was 5.0 mg per deciliter (0.6 mmol per liter; normal value, <2.2 mg per deciliter [0.2 mmol per liter]) while the patient was at rest. She never had episodes of pigmenturia. Phosphate-31-labeled nuclear magnetic resonance spectroscopy of muscle showed a low ratio of phosphocreatine to inorganic phosphate at rest; the ratio decreased further with exercise and returned slowly to base line after the cessation of exercise. Electromyography of four limb muscles showed a few short-duration motor units in the biceps brachii and deltoid muscles and some polyphasic units in the tibialis anterior muscle, suggesting the presence of a mild myopathic proc-

ess. The patient's mother had died of cancer at the age of 69 but had been an active woman before her illness. The patient's brother and two children were healthy and had no symptoms of premature fatigue.

As shown in Table 1, exercise intolerance was the predominant symptom in Patient 2 as well as in the other four patients. Premature fatigue was induced by normal play or by activities of daily living and worsened with age. All five patients described uncomfortable tightness of masticatory muscles with prolonged chewing. The feeling of fatigue was accompanied by muscle cramps during exercise in four patients. Mild proximal limb weakness developed in three patients, one of whom (Patient 5) also had some facial weakness without ptosis or ophthalmoparesis. Even in these patients, however, early fatigue rather than weakness was the main symptom. Severe myalgia with pigmenturia had occurred in only one patient, who had crouched for several hours in an attic while installing insulation. Although most patients reported shortness of breath with exercise, cardiac function was normal in all but one (Patient 3), who had electrocardiographic evidence of the Wolff-Parkinson-White syndrome. None of the patients had a multisystem disorder.

The results of routine laboratory tests were normal, including serum creatine kinase concentrations in the resting state. Serum lactate concentrations were elevated in the resting state in all but one patient and increased excessively after aerobic exercise in all five patients. Electromyography showed mild myopathy in all five patients.

None of the patients had a family history of such symptoms. The mothers of all five patients were or had been normally active, and neither the siblings nor the children of the patients reported exercise intolerance.

All patients provided written informed consent for the study, and the study was approved by the ethics committees of all participating institutions.

Biochemical Analysis

Muscle specimens obtained by open biopsy were frozen and stored in liquid nitrogen. Biochemical measurements of NADH dehydrogenase (complex I), rotenone-sensitive NADH-cytochrome *c* reductase (complexes I and III), succinate dehydrogenase (complex II), succinate-cytochrome *c* reductase (complexes II and

TABLE 1. CLINICAL AND MOLECULAR CHARACTERISTICS OF THE FIVE PATIENTS.

CHARACTERISTIC	PATIENT 1	PATIENT 2	PATIENT 3	PATIENT 4	PATIENT 5
Sex/age (yr)	M/43	F/52	F/38	M/32	M/51
Family history of syndrome	—	—	—	—	—
Time of onset of disorder	30 yr	Childhood	Childhood	Childhood	Childhood
Exercise intolerance	+	+	+	+	+
Weakness	+	+	—	—	+
Myoglobinuria	+	—	—	—	—
Myopathy on electromyography	+	+	+	+	+
Hyperlactacidemia	—	+	+	+	+
Cytochrome oxidase-positive ragged-red fibers (%)*	11	22	10	5	ND
Cytochrome <i>b</i> gene mutation	Deletion of 24 bp	G14846A	G15168A	G15084A	G15723A
Change in amino acid sequence	Deletion of 8 amino acids	G34S	W141X	W113X	W326X
Mutant mtDNA (heteroplasmy) (%)	50	85	70	87	87

*In Patients 1, 2, 3, and 4, ragged-red fibers were identified by the succinate dehydrogenase staining method. In Patient 5, ragged-red fibers were identified by the modified Gomori trichrome staining method. ND denotes not determined.

III), cytochrome *c* oxidase (complex IV), and citrate synthase activities were performed as described previously.¹⁰

Histochemical Analysis and Single-Fiber Polymerase-Chain-Reaction Assay

Cross-sections of skeletal muscle obtained with a cryostat were stained to assess the activities of cytochrome oxidase and succinate dehydrogenase.¹¹ A single-fiber polymerase-chain-reaction (PCR) assay was performed in Patient 2 according to methods described elsewhere.^{12,13}

Myoblast and Fibroblast Culture

Muscle specimens were cleaned of connective tissue, cut into small pieces, and exposed to trypsin at 37°C for one hour to release muscle satellite cells, as described previously.¹⁴ Satellite cells were allowed to attach to the surface of the culture dishes, and two weeks later, myoblast clones were isolated from the dishes and propagated as individual cultures. Fibroblasts were cultured from skin-punch-biopsy specimens, as described previously.¹⁵

Sequence Analysis of the Cytochrome *b* Gene

Total DNA was extracted from muscle and blood according to conventional methods. A fragment that overlapped the entire sequence of the cytochrome *b* gene was obtained with the use of a PCR assay with CYTB-1F as the forward primer (nucleotides 14680 to 14699) and CYTB-3R as the reverse primer (nucleotides 15973 to 15991). Direct sequencing of the PCR products was performed in an automatic sequencer (model 310, Perkin-Elmer Applied Biosystems, Foster City, Calif.) with these primers plus six internal primers: CYTB-2F (nucleotides 15100 to 15119), CYTB-3F (nucleotides 15481 to 15500), CYTB-5F (nucleotides 14993 to 15512), and CYTB-6F (nucleotides 15400 to 15419) as the forward primers and CYTB-1R (nucleotides 15101 to 15120) and CYTB-2R (nucleotides 15481 to 15500) as the reverse primers. All sequences were numbered according to the L-strand of the Cambridge reference sequence.¹⁶

PCR and Restriction-Fragment–Length Polymorphism Analysis

Patient 1

A 200-bp fragment from a muscle-biopsy specimen obtained from Patient 1 was amplified with use of the 20-mer forward primer (nucleotides 15400 to 15419) and the 20-mer reverse primer (nucleotides 15581 to 15600). These primers produced two distinct bands on an ethidium bromide–stained gel, corresponding to the wild-type and mutant mtDNA molecules. To identify the breakpoint of the deletion, the PCR product was processed in a 2.5 percent agarose gel with a low melting point, in order to separate the wild-type from the mutant mtDNA. Each band was subcloned into a pCR 2.1 vector, then transformed into INV α F'–competent cells (TA cloning kit, Invitrogen, San Diego, Calif.). Direct sequencing of 20 clones was performed with the use of M13 primers. The proportion of mutant mtDNA was calculated by PCR–restriction-fragment–length polymorphism (RFLP) analysis with the use of *Tsp509I*, whose restriction site at nucleotide 15509 is absent in the mutant mtDNA, which has a deletion of 24 bp.

Patient 2

A 440-bp fragment from a muscle-biopsy specimen obtained from Patient 2 was amplified with the use of the CYTB-1F and CYTB-1R primers. The mutation identified (G14846A) creates a new restriction site of *AluI*, which is absent in the wild-type mtDNA.

Patient 3

An 820-bp fragment from a muscle-biopsy specimen obtained from Patient 3 was amplified with the use of CYTB-1F as the for-

ward primer and CYTB-3R as the reverse primer. The mutation identified (G15168A) abolishes a restriction site for *MnI*, which is present in the wild-type mtDNA.

Patient 4

A 427-bp fragment from a muscle-biopsy specimen obtained from Patient 4 was amplified with the use of CYTB-1F as the forward primer and a mismatched 23-mer reverse primer (5'GC-AGTGAGGATAATGCCGATGTCT3'). The mutation identified (G15084A) abolishes a restriction site for *BsmAI*, which is present in the wild-type mtDNA.

Patient 5

A 443-bp fragment from a muscle-biopsy specimen obtained from Patient 5 was amplified with the use of a forward primer (nucleotides 15553 to 15572) and a reverse primer (nucleotides 15977 to 15996). The mutation identified (G15723A) creates a new restriction site for *MseI*, which is absent in the wild-type DNA.

For all five patients, variations from the consensus sequence¹⁶ were compared with the information contained in our data base and with the MITOMAP data bases for human mtDNA variants.¹⁷

RESULTS

Histochemical and Biochemical Analysis

In all five patients, histochemical analysis of muscle-biopsy specimens showed abundant ragged-red fibers, which stained intensely with both succinate dehydrogenase and cytochrome *c* oxidase (Fig. 1). The proportion of ragged-red fibers ranged from 5 percent in Patient 4 to 22 percent in Patient 2.

The results of biochemical studies of respiratory-chain enzymes in muscle extracts from Patients 1, 2, and 3 are summarized in Table 2. We compared the levels of activities to that of citrate synthase, a matrix enzyme and a reliable marker of mitochondrial abundance, and found abnormally low values for rotenone-sensitive NADH–cytochrome *c* reductase (complexes I and III) and succinate–cytochrome *c* reductase (complexes II and III), in contrast to the virtually normal activities of NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), and cytochrome *c* oxidase (complex IV). These results indicated a marked defect in complex III in all three patients.

In an earlier study, complex III activity was 19 percent of the normal value in muscle extracts and 12 percent of the normal value in isolated mitochondria from Patient 4, with normal levels of reducible cytochrome *b*.¹⁸ In Patient 5, individual enzymes were not measured, but polarographic analysis of freshly isolated muscle mitochondria showed markedly depressed respiratory rates with both nicotinamide adenine dinucleotide–dependent and flavoprotein-dependent substrates, indicative of complex III deficiency, as well as low levels of cytochrome *b* as compared with values in the literature.¹⁹

Molecular Genetic Analysis

Patient 1

Sequence analysis of the subcloned PCR products in Patient 1 revealed a 24-bp in-frame deletion from

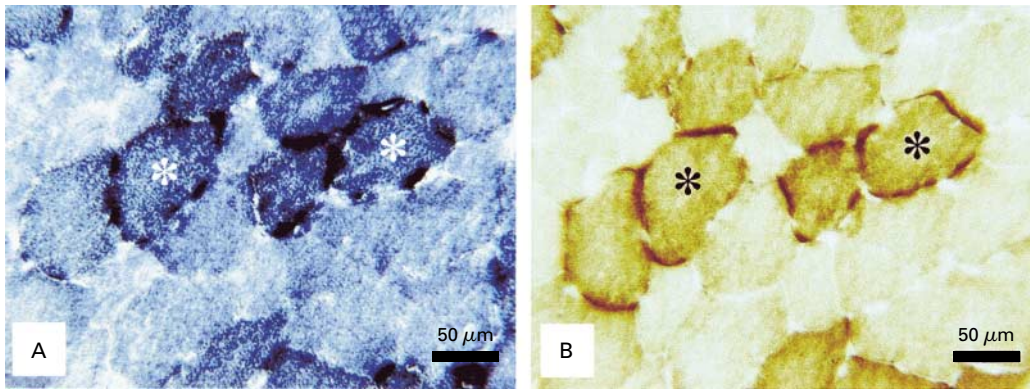


Figure 1. Serial Sections of Muscle-Biopsy Specimens from Patient 2, Stained with Succinate Dehydrogenase (Panel A) and Cytochrome *c* Oxidase (Panel B). Fibers that are intensely positive for succinate dehydrogenase (indicative of ragged-red fibers) are also intensely positive for cytochrome *c* oxidase (asterisks).

TABLE 2. ACTIVITIES OF RESPIRATORY-CHAIN ENZYMES IN MUSCLE EXTRACTS FROM PATIENTS 1, 2, AND 3.

PATIENT	NADH-CYTOCHROME <i>c</i> REDUCTASE	NADH DEHYDROGENASE	SUCCINATE CYTOCHROME <i>c</i> REDUCTASE	CYTOCHROME <i>c</i> OXIDASE (COMPLEX IV)	SUCCINATE DEHYDROGENASE
<i>μ</i> mol/min/unit of citrate synthase activity (% of control value)					
Patient 1	0.05 (43)	2.87 (76)	0.038 (54)	0.286 (101)	0.083 (82)
Patient 2	0.023 (20)	2.88 (77)	0.015 (21)	0.240 (85)	0.105 (104)
Patient 3	0.006 (5)	3.26 (87)	0.006 (9)	0.283 (100)	0.108 (107)
Control*	0.115±0.038	3.760±0.715	0.070±0.023	0.283±0.052	0.101±0.053

*Plus-minus values are means ±SD.

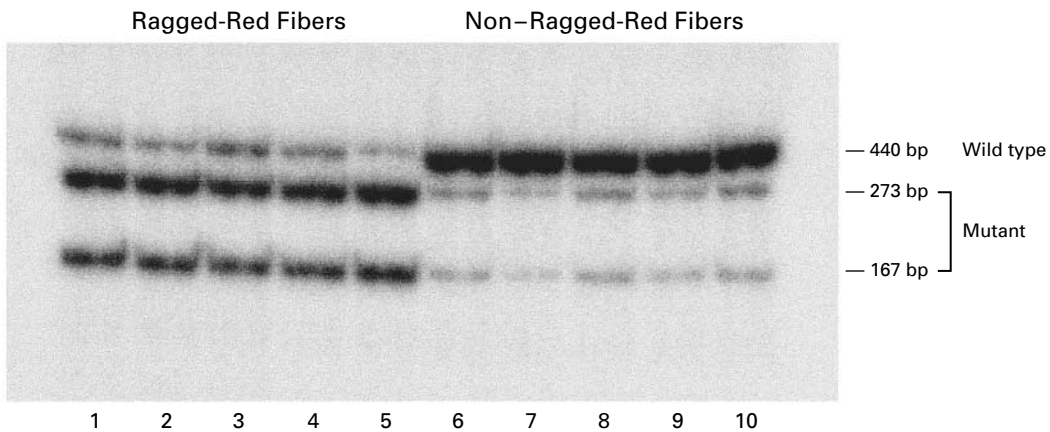


Figure 2. Single-Fiber PCR Analysis of a Muscle-Biopsy Specimen from Patient 2. In the presence of the mutation (G14846A), the 440-bp PCR-amplified DNA fragment is digested by *A**lu*I into two fragments (273 bp and 167 bp). The autoradiogram shows much higher proportions of mutant mtDNA in ragged-red fibers (lanes 1 through 5) than in non-ragged-red fibers (lanes 6 through 10).

nucleotides 15498 to 15521, resulting in the loss of eight amino acids (251 to 258: GDPDNYTL) from the cytochrome *b* protein. The mutant mtDNA had a direct-repeat sequence of five nucleotides at the breakpoint. The proportion of mutant mtDNA in muscle, quantitated by PCR-RFLP analysis with *Tsp509I*, was 50 percent. We did not detect this mutation in DNA from peripheral blood, cultured myoblasts, or skin fibroblasts, even in early passages.

Patient 2

Sequence analysis of mtDNA isolated from a muscle-biopsy specimen obtained from Patient 2 showed the substitution of adenine for guanine at nucleotide 14846, which changed glycine to serine (G34S) in the amino acid sequence. RFLP analysis confirmed that the mutation was heteroplasmic in muscle mtDNA (frequency, 85 percent) but was absent in DNA isolated from peripheral-blood cells, cultured myoblasts, and skin fibroblasts. The mutation was also absent in DNA from blood, cultured myoblasts, and skin fibroblasts from the patient's two children. Single-fiber PCR analysis showed a much higher proportion of the mutation in ragged-red fibers (91 percent) than in non-ragged-red fibers (17 percent) (Fig. 2).

Patient 3

Sequence analysis of muscle DNA from Patient 3 showed the substitution of adenine for guanine at nucleotide 15168, which changed tryptophan (UGA) to a stop codon (UAA) at position 141 (W141X). PCR-RFLP analysis showed that the mutation was heteroplasmic in muscle (frequency, 70 percent) but was undetectable in blood.

Patient 4

Sequence analysis of muscle DNA from Patient 4 showed the substitution of adenine for guanine at nucleotide 15084, which changed tryptophan (UGA) to a stop codon (UAA) at amino acid 113 (W113X). This mutation results in the loss of 240 amino acids at the C-terminal of cytochrome *b*, which represents 68 percent of the polypeptide's length. PCR-RFLP analysis revealed that the G15084A mutation was heteroplasmic in muscle (frequency, 87 percent) and was not detectable in DNA from peripheral-blood cells.

Patient 5

Sequence analysis of muscle DNA from Patient 5 showed the substitution of adenine for guanine at nucleotide 15723, which changed tryptophan (UGA) to a stop codon (UAA) at position 326 (W326X) and resulted in the loss of 55 amino acids at the C-terminal of cytochrome *b*. PCR-RFLP analysis showed that the mutation was heteroplasmic in muscle (frequency, 87 percent). Blood samples were not available from this patient.

None of these mutations were found in muscle DNA extracted from 80 normal subjects or from 40 patients with other known pathogenic mutations of mtDNA.

DISCUSSION

The term "mitochondrial encephalomyopathies" refers to a heterogeneous group of clinical disorders due to defects in the mitochondrial respiratory chain. Of the approximately 80 polypeptides that make up the five complexes of the respiratory chain, 13 are encoded by mtDNA and all the others are encoded by nuclear DNA (nDNA). Therefore, mitochondrial encephalomyopathies can be caused by mutations in either genome. Disorders due to mutations in nDNA are transmitted by traditional mendelian inheritance, whereas disorders due to point mutations in mtDNA are usually transmitted by maternal inheritance.

During the past 11 years, more than 60 pathogenic mutations of mtDNA have been associated with a variety of maternally inherited syndromes, most of which are multisystemic and affect virtually every tissue in the body.^{1,2} Most of these mutations are in transfer RNA (tRNA) genes: they impair the overall synthesis of mitochondrial protein and are accompanied by massive proliferation of mitochondria in skeletal muscle. Muscle fibers with excessive numbers of mitochondria have increased peripheral red staining with the modified Gomori trichrome histochemical technique and have therefore been dubbed "ragged-red fibers."²⁰ Ragged-red fibers also stain intensely with succinate dehydrogenase, a mitochondrial enzyme encoded entirely by nDNA. In contrast, staining for cytochrome *c* oxidase, an enzyme that contains three mtDNA-encoded subunits, is usually absent when the synthesis of mitochondrial protein is defective. Thus, cytochrome *c* oxidase-negative ragged-red fibers are characteristic of tRNA mutations, except for the A3243G mutation in the tRNA^{Leu(UUR)} gene that is associated with the MELAS syndrome.

Mutations in protein-coding mtDNA genes have been associated with two major syndromes, both maternally inherited: Leber's hereditary optic neuropathy, an important cause of blindness at a young age, and Leigh's syndrome, a severe encephalopathy of infancy or childhood. Leber's hereditary optic neuropathy is usually due to mutations in one or more of the seven genes encoding subunits of complex I, whereas Leigh's syndrome is usually due to mutations in ATPase 6, one of the two mtDNA genes encoding subunits of complex V (ATP synthetase). For reasons that are not clear, mitochondrial proliferation is not prominent in these two conditions, and ragged-red fibers are not seen.^{1,2}

In the past several years, nine unrelated patients — four of whom are described here — were found

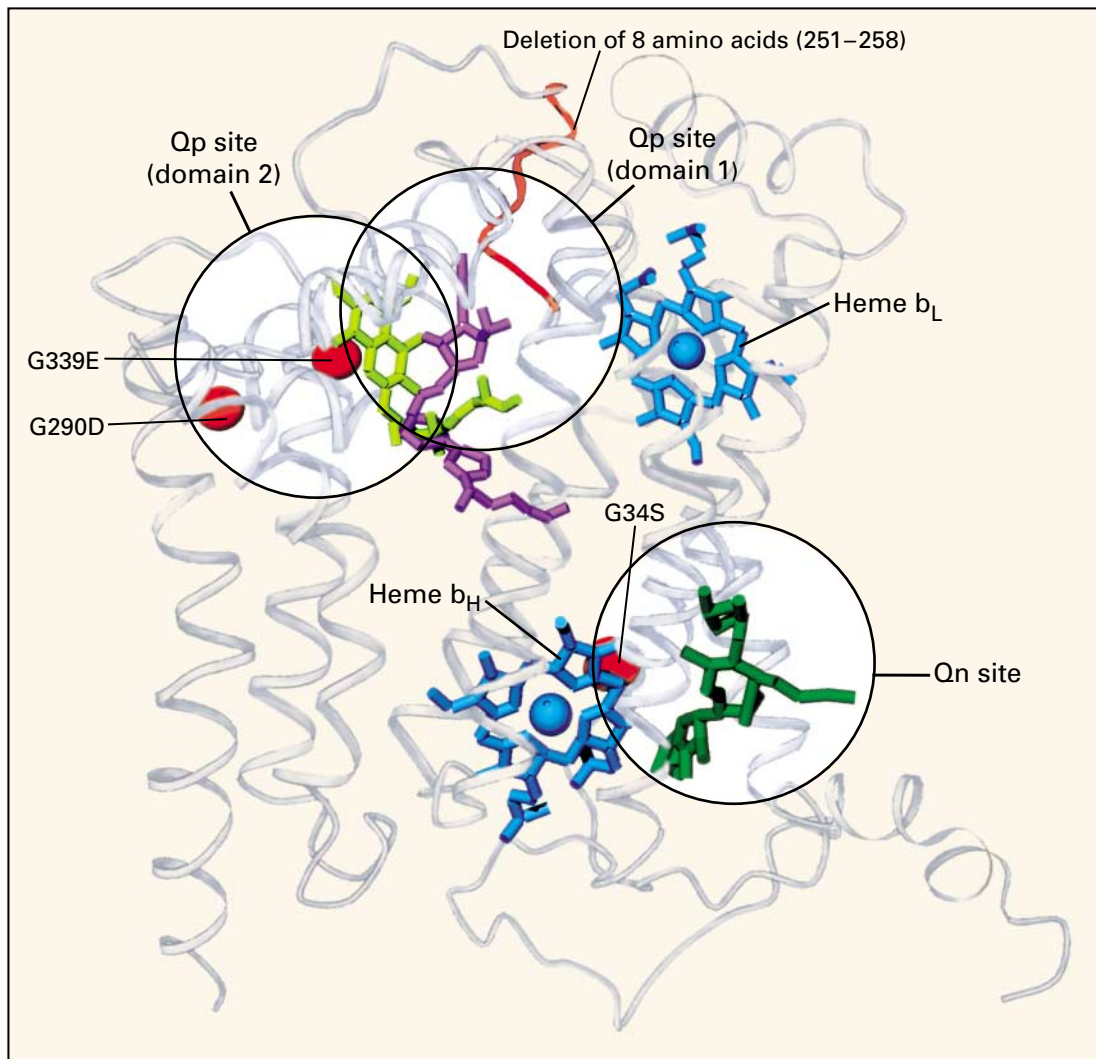


Figure 3. Locations of Deletions or Missense Mutations on the Cytochrome *b* Molecule.

Cytochrome *b* contains two hemes (blue) and two quinone-binding sites, identified with the use of specific inhibitors: antimycin A (dark green) binds to the Qn site (ubiquinone-reduction site), myxothiazol (purple) binds to the Qp site in domain 1, and stigmatellin (light green) binds to the Qp site in domain 2. The sites of the mutations are indicated in red.

to have different pathogenic mutations in still another mtDNA protein-coding gene, that for cytochrome *b*.⁴⁻⁸ All nine patients had severe exercise intolerance that began at various ages and became progressively worse. Mild proximal limb weakness was present in all but two patients. There was no ophthalmoplegia or other neurologic abnormality. Episodes of pigmenturia, almost certainly myoglobinuria, followed unusually intense and prolonged exercise in two patients (Patient 1 and another patient who was described previously⁸). All but one patient had lactic acidosis. Muscle biopsy showed mitochondrial myopathy with ragged-red fibers in all but one patient.⁷ The ragged-red fibers stained

intensely for cytochrome *c* oxidase, in contrast to the cytochrome *c* oxidase-negative ragged-red fibers usually found in patients with mitochondrial myopathies or encephalomyopathies due to mtDNA rearrangements or to mutations in tRNA genes. Biochemical studies showed isolated complex III deficiency in all nine patients, which led to the molecular diagnosis.

The genetic heterogeneity of this condition is striking: all nine patients had different mutations. The mutations were deemed pathogenic on the basis of several findings. All were heteroplasmic, a characteristic that is associated with deleterious mutations rather than neutral polymorphisms. Five were non-

sense mutations, resulting in truncated cytochrome *b* molecules; of the other four mutations, one was an in-frame deletion that removed eight amino acids from the protein, and three were substitutions of highly conserved nucleotides. In two patients, one described previously⁸ and Patient 2 in our study, the results of a single-fiber PCR assay showed a strong correlation between pathologic changes (ragged-red fibers) and the frequency of mutant mtDNA. The cytochrome *b* protein contains two ubiquinone-binding sites, Qp and Qn.⁹ The Qp site is needed for ubihydroquinone oxidation, and the Qn site for ubiquinone reduction. Both sites must be functional for proton transfer to occur. All the missense mutations and the 24-bp deletion are located within or close to these ubiquinone-binding sites and are likely to prevent ubiquinone binding, with loss of enzyme activity (Fig. 3). Although the nonsense mutations are obviously pathogenic and the missense mutations are very likely so, functional effects have not been verified in cultured cells expressing these genetic errors.

The high frequency of somatic mutations in the cytochrome *b* gene may seem surprising but is in keeping with the large number of polymorphisms observed in the same gene in normal persons.²¹ The fact that all pathogenic mutations identified in our patients consisted of the substitution of adenine for guanine further suggests that they may result from oxidative damage.^{22,23}

The molecular basis of the syndrome identified in our patients may have been overlooked previously because it contradicts two prevailing concepts about mitochondrial genetics: mtDNA point mutations are maternally transmitted and cause multisystem disorders. In contrast, all five of our patients had sporadic cases, none had a family history of neuromuscular disorders, all five had isolated skeletal-muscle involvement, and none had mutations in peripheral-blood samples that were analyzed. Furthermore, in the three patients for whom muscle-biopsy samples were available for culture (one described previously³ and Patients 1 and 2 in our study), the mutations were not detected in the limited number of myoblast cell lines studied, presumably because the mutation arose in myoblasts (myogenic stem cells) or myoblast precursors after germ-layer differentiation. For all these reasons, the mutations in the cytochrome *b* gene in our patients are likely to have been somatic — that is, spontaneous events that occurred in muscle and did not affect germ-line cells. The apparent restriction of these mutations to skeletal muscle is interesting but not unique; a similar restriction has been reported with other point mutations of mtDNA that affect both tRNA and protein-coding genes.²⁴⁻²⁷

The syndrome we describe has probably been underdiagnosed, because reports of exercise intoler-

ance are common, difficult to document objectively, and often dismissed as psychogenic. The finding of lactic acidosis in a patient with exercise intolerance and no family history of exercise intolerance should alert the clinician to the possibility of a cytochrome *b* gene mutation. Confirmation of the diagnosis requires muscle biopsy to document complex III deficiency and to identify the molecular defect.

Exercise intolerance is as frustrating to clinicians, who frequently have trouble finding a satisfactory diagnosis, as it is to patients. Having now identified cytochrome *b* gene mutations in seven patients, we believe that somatic mutations in this mitochondrial gene may be common and should be included in the differential diagnosis when patients present with the often elusive symptoms of aches, pain, and cramps.

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