

# Congenital Megaconial Myopathy Due to a Novel Defect in the Choline Kinase Beta Gene

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**Objectives:** To describe the first American patient with a congenital muscle dystrophy characterized by the presence in muscle of gigantic mitochondria displaced to the periphery of the fibers and to stress the potential origin and effects of the mitochondrial changes.

**Design:** Case report and documentation of a novel mutation in the gene encoding choline kinase beta (*CHKB*).

**Setting:** Collaboration between 2 tertiary care academic institutions.

**Patient:** A 2-year-old African American boy with weakness and psychomotor delay.

**Interventions:** Detailed clinical and laboratory studies, including muscle biopsy, biochemical analysis of the

mitochondrial respiratory chain, and sequencing of the *CHKB* gene.

**Main Outcome Measures:** Definition of unique mitochondrial changes in muscle.

**Results:** This patient had the same clinical and laboratory features reported in the first cohort of patients, but he harbored a novel *CHKB* mutation and had isolated cytochrome *c* oxidase deficiency in muscle.

**Conclusions:** Besides confirming the phenotype of *CHKB* mutations, we propose that this disorder affects the mitochondria-associated membrane and the impaired phospholipid metabolism in the mitochondria-associated membrane causes both the abnormal size and displacement of muscle mitochondria.

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**R**ECENTLY, MITSUHASHI AND coworkers<sup>1</sup> described 15 patients with a new congenital myopathy characterized clinically by early-onset muscle weakness and mental retardation. The hallmark of the disease was the presence in the muscle biopsy specimen of greatly enlarged mitochondria displaced to the periphery of the fibers. Ten of their patients were Turkish; 4, Japanese; and 1, British, and they all harbored a variety of mutations in the gene encoding choline kinase beta (*CHKB*), the enzyme that catalyzes the first step in the de novo biosynthesis of phosphatidyl choline (PtdCho) and phosphatidylethanolamine (PtdEtn) via the Kennedy pathway. We now report an African American boy with the same clinical and pathological features and a novel mutation in *CHKB*.

Although the muscle biopsy specimen in our patient also shows dystrophic features, we think that the striking mitochondrial abnormalities cannot be ignored and

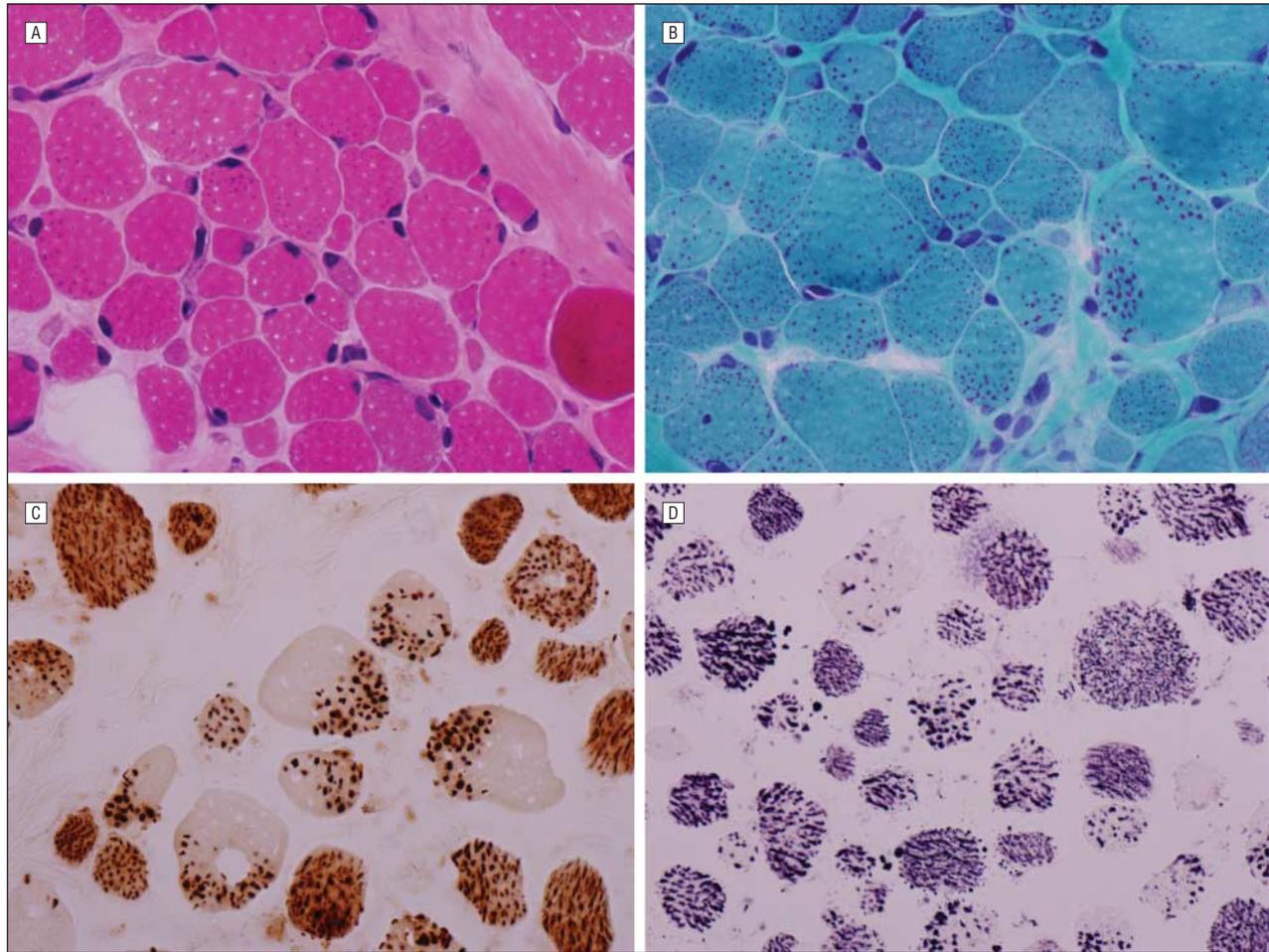
mitochondrial dysfunction may have an important role in pathogenesis.

## METHODS

### REPORT OF A CASE

A 2-year-old African American boy was born after a normal pregnancy and delivery to a 32-year-old woman, who had chronic hypertension, Wolff-Parkinson-White syndrome, and type 2 diabetes mellitus. He was the only child of this nonconsanguineous couple. Apgar scores were 8 at 1 minute and 9 at 5 minutes; head circumference was 35 cm; length, 47 cm; and weight, 2.94 kg. Soon after he was discharged home, the mother noticed rhythmic jerking of arms with stiff legs, occurring 3 to 4 times per night. These episodes were labeled "muscle spasms" by the general practitioner.

When first seen at age 11 months, the child could roll over both ways, sit without support for 1 minute (if placed), stand holding onto a chair for a few seconds, and speak a few words. At age 22 months, he was still not able to sit up and did not utter any intelligible word.



**Figure 1.** Muscle biopsy specimen, light microscopy. A, Variation in fiber size, occasional basophilic regenerating fibers, and irregularly distributed areas of granular basophilic material (hematoxylin-eosin, original magnification  $\times 200$ ). B, The coarse granules were highlighted by the modified Gomori trichrome stain (original magnification  $\times 200$ ). C and D, The granule also stained with cytochrome *c* oxidase (C) and succinate dehydrogenase (D) (original magnification  $\times 200$ ). Scattered fibers showed absence of staining for cytochrome *c* oxidase and succinate dehydrogenase except in the areas of abnormal mitochondrial accumulation.

At age 25.5 months, physical examination showed an irritable and uncooperative child, who fell asleep intermittently. Head circumference was 51 cm; weight, 12.5 kg; and height, 211 cm. He had a broad flat face, large philtrum, wide nasal bridge, and low-set ears. There was no organomegaly. Neurological examination showed normal cranial nerves but bilateral facial weakness. There was marked hypotonia (on vertical suspension, he slipped through the examiner's hands) and limb weakness, more pronounced in the legs. Sensory examination appeared normal and there was no ataxia, tremor, or nystagmus. He had episodes of unresponsiveness, with staring, drooling, and slow turning of the head and eyes to one side. Although these appeared to be seizures, the electroencephalogram was normal.

Chromosomal analysis was normal. Normal laboratory examinations included serum lactate and pyruvate levels, cardiac evaluation, nerve conduction velocities, and brain auditory evoked responses. The serum creatine kinase level was consistently elevated and varied between 318 and 522 U/L (normal,  $<176$  U/L) (to convert to microkatal per liter, multiply by 0.0167). Electromyography showed polyphasic units with low amplitudes, consistent with myopathy. Brain magnetic resonance imaging at 1 year of age showed prominent cerebrospinal fluid spaces over the frontal convexities and ventricular enlargements of the lateral ventricles.

## HISTOLOGY AND BIOCHEMISTRY

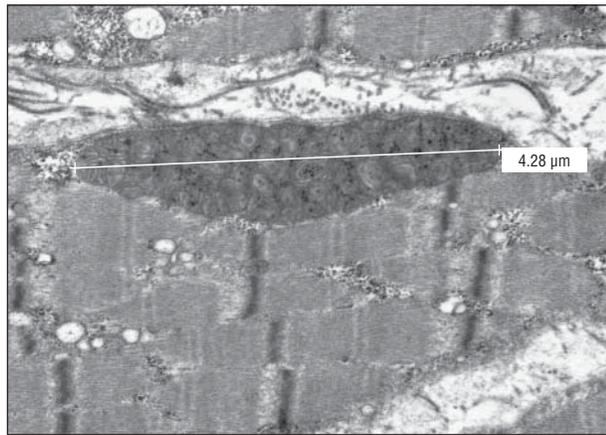
Histochemical studies of muscle using 8- $\mu$ m-thick sections were carried out as described.<sup>2</sup> The activities of respiratory chain enzymes were measured in 10% muscle extracts as previously described.<sup>3</sup>

## MOLECULAR ANALYSIS

Total DNA was extracted from muscle by standard procedures (Purogene; Gentra System Inc) according to the manufacturer's instructions. The entire coding sequence of *CHKB* was amplified by polymerase chain reaction using specific primers described by Mitsuhashi et al.<sup>1</sup> The polymerase chain reaction fragments were sequenced with an ABI 3130XL genetic analyzer (Applied Biosystems).

## RESULTS

A left quadriceps muscle biopsy specimen was snap-frozen in isopentane-liquid nitrogen and cryosections were stained with a standard battery of histological and histochemical reactions (**Figure 1**). Dystrophic fea-



**Figure 2.** Ultrastructural study demonstrated the presence of markedly enlarged mitochondria (“megaconia”) with abnormal cristae (electron microscopy, original magnification  $\times 25\,000$ ). No paracrystalline inclusions were noted.

tures, evident in the hematoxylin-eosin stain (Figure 1A), included extreme variation in fiber size, excessive interstitial fibrosis, and necrotic “hyaline” fibers. The most striking changes, however, were seen in the modified Gomori trichrome stain (Figure 1B) and in the histochemical reaction for cytochrome *c* oxidase (Figure 1C). Greatly enlarged mitochondria (looking like dabs rather than points) were apparent at the periphery of most fibers, leaving the central areas empty.

Electron microscopy confirmed the presence of giant mitochondria (megaconia<sup>4</sup>), containing densely packed and whorled cristae (**Figure 2**).

Biochemical analysis of mitochondrial enzymes showed slightly increased activity of citrate synthase, a matrix protein and a good marker of mitochondrial mass, suggesting that the increased volume of mitochondria made up for the lack of organelles in the empty areas. When the activities of respiratory chain enzymes were corrected for citrate synthase activity, complexes I, I+III, and II+III were essentially normal, whereas cytochrome *c* oxidase activity was only 30% of normal and succinate dehydrogenase activity was 48% of normal (**Table**).

Our patient had a homozygous *CHKB* mutation (p.E292X) in exon 8 (**Figure 3**), which introduces a premature stop codon and results in a truncated *CHKB* protein (292 instead of 395 amino acids). Although 3 of the 15 patients reported by Mitsuhashi et al<sup>1</sup> harbored mutations in exon 8, this particular mutation was not encountered by them.

## COMMENT

In 1964 and 1966, Shy and Gonatas<sup>5</sup> and Shy et al<sup>4</sup> described children with myopathy and giant mitochondria and dubbed this condition “megaconial myopathy.” Although it was initially believed that giant mitochondria would be the hallmark of specific diseases, it soon became apparent that enlarged mitochondria are a common feature of diverse mitochondrial myopathies, including Luft syndrome.<sup>6</sup>

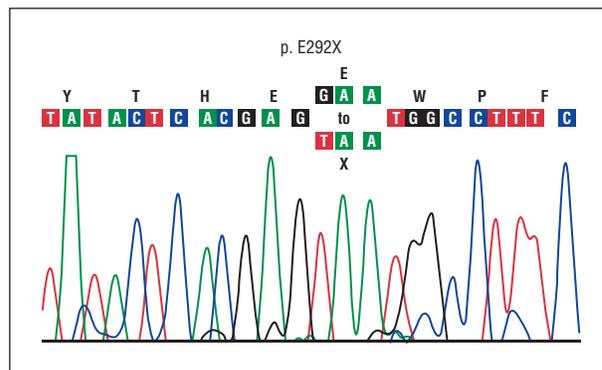
However, in 1998, Nishino and coworkers<sup>7</sup> described 4 patients with congenital muscular dystrophy and muscle

**Table. Respiratory Chain Enzyme Activities in the Patient’s Muscle<sup>a</sup>**

Enzyme/Complex	Controls, Activity, Mean $\pm$ SD	Patient, Activity (% of Normal)
NADH–cytochrome <i>c</i> reductase	0.319 $\pm$ 0.11	0.407 (128)
NADH–CoQ reductase	0.119 $\pm$ 0.01	0.099 (83)
Succinate–cytochrome <i>c</i> reductase	0.071 $\pm$ 0.02	0.066 (93)
Cytochrome <i>c</i> oxidase	0.283 $\pm$ 0.05	0.086 (30)
SDH	0.101 $\pm$ 0.05	0.048 (48)

Abbreviations: CoQ, coenzyme Q<sub>10</sub>; NADH, reduced nicotinamide adenine dinucleotide; SDH, succinate dehydrogenase.

<sup>a</sup>Activities are micromoles substrate used/minute/gram fresh tissue. Values are corrected for citrate synthase activity. Normal values are the mean of 78 samples  $\pm$  SD.

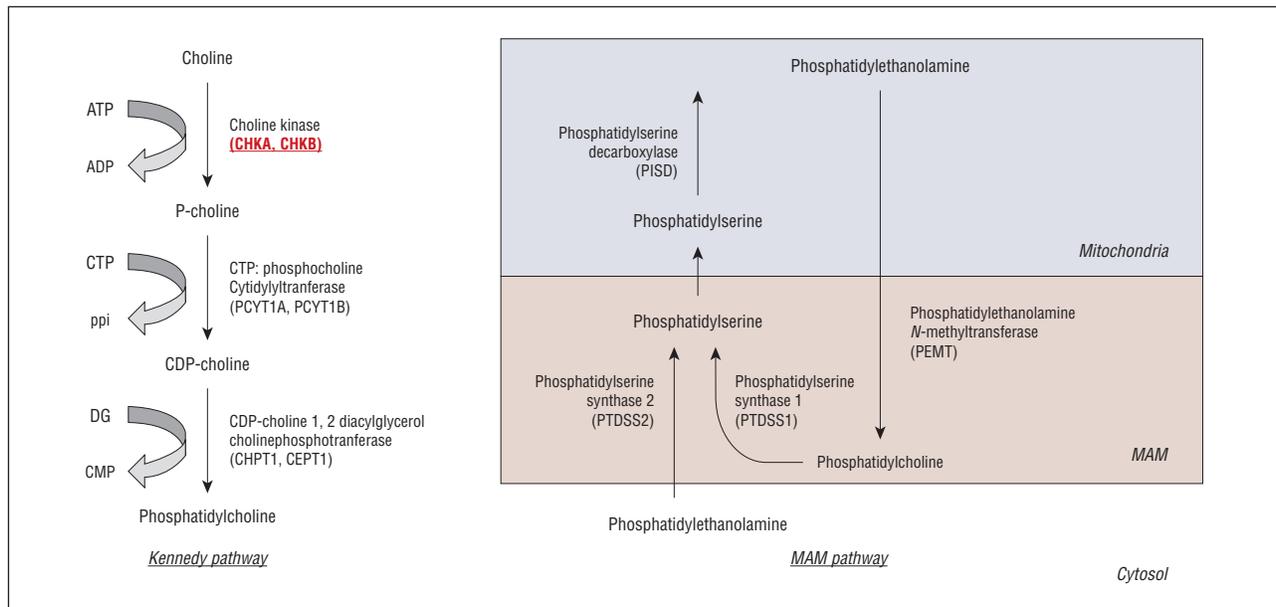


**Figure 3.** Electropherogram showing the p.E292X homozygous mutation detected in the eighth exon of the patient’s choline kinase beta gene (*CHKB*). Nucleotides are denoted by the colors of the corresponding peaks. Each codon is labeled with the single letter identifying the encoded amino acid.

mitochondria that were not only gigantic but also peculiarly displaced toward the periphery of muscle fibers, leaving the center devoid of organelles. Recently, the similarities of these clinical and morphological features with those of a spontaneous mutant mouse harboring a loss-of-function mutation in the choline kinase beta gene (*Chkb*) prompted the Nishino et al group to sequence *CHKB* in their 4 original patients and in 11 more patients, 10 from Turkey and 1 from the United Kingdom.<sup>1</sup> They found deleterious mutations in all patients and defined the molecular basis of this congenital megaconial muscular dystrophy.

Herein, we describe a 2-year-old African American child with the same rather stereotypical clinical and laboratory features reported in the cohort of patients with *CHKB* mutations: profound weakness affecting the legs more than the arms and severe psychomotor delay. The presence in his muscle biopsy specimen of mitochondria that were both gigantic and displaced to the periphery of the fibers led us to sequence the *CHKB* gene and we documented a novel homozygous nonsense mutation (E292X) in exon 8.

In their seminal article, Mitsuhashi et al<sup>1</sup> documented the defect of choline kinase activity in muscle of patients and the decreased concentrations of PtdCho and PtdEtn in muscle of 3 patients; they ascribed the predominant involvement of skeletal muscle and brain to the different distribution of the 2 choline kinase iso-



**Figure 4.** Schematic representation of phospholipid metabolism in the cytosol (Kennedy pathway) and phospholipid trafficking between mitochondria and mitochondria-associated membranes (MAMs). ADP indicates adenosine diphosphate; ATP, adenosine triphosphate; CDP, cytidine diphosphate; CMP, cytidine monophosphate; CTP, cytidine triphosphate; DG, diacylglycerol; and ppi, inorganic phosphate.

forms, alpha and beta, and proposed that the severity of muscle weakness was proportional to the degree of choline kinase activity.

In a second article, Mitsuhashi et al<sup>8</sup> described mitochondrial dysfunction in muscle from mutant mice, including decreased adenosine triphosphate synthesis, decreased coenzyme Q, increased superoxide production, and excessive activation of mitophagy.

The de novo synthesis of PtdCho and PtdEtn takes place in the cytoplasm and endoplasmic reticulum (or, in muscle, the sarcoplasmic reticulum) via the relatively minor Kennedy pathway, in which choline and ethanolamine are initially converted to phosphocholine and phosphoethanolamine, respectively, via the CHKB, with further enzymatic reactions generating PtdCho and PtdEtn (**Figure 4**). However, there is a second phospholipid biosynthetic pathway that involves a specialized compartment of mitochondria-associated membranes (MAMs).<sup>9</sup> In the MAM, both PtdCho and PtdEtn are converted to phosphatidylserine via exchange reactions with serine, catalyzed by MAM-located phosphatidylserine synthases. Phosphatidylserine then enters the mitochondria, where it is converted to PtdEtn by phosphatidylserine decarboxylase, which is located in the mitochondrial matrix. The PtdEtn then travels back to the MAM, where it is converted to PtdCho by the MAM-localized enzyme phosphatidylethanolamine methyltransferase.<sup>10</sup>

This alternative pathway to generate PtdCho may be up-regulated in a compensatory way and may explain the muscle mitochondrial “hypertrophy” observed in CHKB deficiency. Also, a number of proteins involved in mitochondrial dynamics, including mitofusin 2,<sup>11</sup> Rab32,<sup>12,13</sup> and Fis1,<sup>14</sup> are an integral part of MAM. Thus, if the congenital megaconial myopathy is a MAM disease, altered mitochondrial dynamics could explain both the increased size and the cellular displacement of mitochondria.

Is there any evidence of mitochondrial respiratory chain

dysfunction? None of the 4 original Japanese patients had lactic acidosis and the activities of respiratory chain complexes were normal in the muscle from 1 patient.<sup>7</sup> Our patient had no lactic acidosis and structurally normal brain magnetic resonance imaging but a pronounced defect of cytochrome *c* oxidase activity in muscle.

In this regard *CHKB*, which is located on chromosome 22q13.33, is immediately upstream of *CPT1B* (carnitine palmitoyltransferase 1B), a key lipid transport enzyme located in the mitochondrial outer membrane. Interestingly, there is evidence of transcription of a *CHKB-CPT1B* bicistronic read-through transcript (eg, GenBank NR\_027928.2). Thus, besides the potential effect of the loss of *CHKB* function on MAM-mediated mitochondrial structure and function, there may also be a secondary, downstream effect on mitochondria resulting from alterations in overall fatty acid metabolism due to the effects on *CPT1B*, ultimately affecting mitochondrial respiratory chain activity.

Irrespective of whether the respiratory chain is affected, this mitochondrial myopathy is secondary to a defect in phospholipid biosynthesis, and in this sense, it is reminiscent of Barth syndrome, a mitochondrial cardiomyopathy due to altered synthesis of cardiolipin.<sup>15</sup>

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