

Carriers of Mutations in Calpain Genes May Increase the Risk of HyperCKemia in Mitochondrial Disease Patients [P06-084]

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Background

Mitochondrial diseases exhibit a broad array of phenotypes, but only a small proportion of patients have significant increases in creatine phosphokinase (CPK). Most patients with mitochondrial disease have a normal CPK. The mechanism for significant CPK increases in patients with mitochondrial disease is unknown. Other genes contribute to the muscle fiber degeneration. In a patient with mitochondrial disease plus significant increases in CPK, we identified a single calpain 3 (CAPN3) mutation.

Calpains are a large family of proteins (calcium-activated neutral proteases) that are nonlysosomal cysteine proteases. Mutations in the CAPN3 gene produce variable phenotypes that include limb girdle dystrophy (LGD2A) and asymptomatic hyperCKemia. Inheritance is autosomal recessive with a carrier frequency of 1:103. Calpains are thought to be involved in a multitude of physiological and pathological events, but their precise functions are poorly understood.

Interestingly, Calpain 10 (CAPN10) is localized to the mitochondria and is a mediator of mitochondrial dysfunction. CAPN10 has been shown to specifically inhibit two Complex I proteins, NDUFB2 and ND6 and to activate the mitochondrial permeability transition pore. (*Am J Physiol Cell Phys.* 291:C1159-c1171, 2006.)

Clinical Description

- 46 year old male: Myoclonus, increased CPK, prior pontine stroke.
1. Participated in Gulf War in 1984 -1985.
 2. Myoclonus began after pontine infarction. Frequency of myoclonus increased over time. (Initially nocturnal with increases to 4-5x per day)
 3. Increased CPK (>2000). The CPK gradually increased from 500-600 10 years ago to ~1000 a year ago and now > 500-2000 depending on activity level.
 4. Patient has been on no statins and no myotoxic medications or supplements.
 5. Patient has experienced hamstring cramping since adolescence. No myalgias. Mild right hemiplegia.
 6. Family history: Maternal half brother chronic inflammatory demyelinating polyneuropathy diagnosis. This brother had a child who died with severe hypotonia at 4 months of age. Otherwise noncontributory.

Muscle Histology

Histology: Nonspecific changes with increased myofiber size variation affecting Type I and Type II fibers. Scattered fibers with internal nuclei. No necrotic fibers. No inflammatory infiltrates. Occasional cytochrome c oxidase deficient fibers were observed.

Immunohistochemistry: No evidence of inflammatory myopathy: No inflammatory infiltrates, normal Major Histocompatibility Complex I expression, no membrane attack complex (C5b9) binding.

OXPHOS Immunohistochemistry/ Immunofluorescence: Normal distribution of selected OXPHOS subunits from Complexes I-V making defects in OXPHOS enzyme assembly and defects in protein synthesis unlikely.

Metabolic Labs

Urine Organic Acids, Urine Amino Acids, Plasma Lactate, Blood Pyruvate, Plasma amino acids, Blood carnitine, Acylcarnitines and Muscle Coenzyme Q10 were normal.

Most patients with mitochondrial disease (particularly adults) will have negative metabolic testing.

Muscle (OXPHOS) Enzymology Complex I defect

Abnormal oxidative phosphorylation enzymology on mitochondria isolated from fresh muscle testing displayed a Complex I Defect.

OXPHOS Enzyme Assay	Patient Activity (<5% level)	Mean±SD [5%-95%]
Complex I Assay (n-decyl CoQ electron acceptor)	32	85 ± 34 [47-160]
Complex I Assay (CoQ1 electron acceptor)	80	246 ± 118 [97-438]

OXPHOS enzymology was performed on mitochondria isolated from fresh muscle. Defects were diagnosed by comparison of activity with the 5%-95% reference intervals calculated from 254 controls. Activity is expressed as nanomoles substrate/minute/mg of mitochondrial protein.

Abnormal Respirometry Live Muscle

	Patient [<5% level]	Mean [5%-95%]
Uncoupling Ratio	2.21	3.61 [2.26-5.40]
Net Routine Control Ratio	0.45	0.19-0.44
Respiratory Control Ratio	1.50	1.48-3.24
Leak Flux Control Ratio	0.68	0.31-0.68

The respiratory reserve capacity of OXPHOS to supply energy (ATP) for myofiber function was abnormal (decreased Uncoupling Ratio and Respiratory Control Ratio; increased Net Routine Flux Control Ratio). This appeared in part due to an inability of the myofibers to use the proton gradient generated by Complexes I-IV for ATP synthesis by Complex V (Increased Leak Flux Control Ratio).

Abnormal OXPHOS Subunit Protein Chemistry

OXPHOS Enzyme Subunit Tested (Normalized to GAPDH)	Patient [<5% level]	Mean [5%-95%]
Complex I (ND6 subunit; mtDNA coded)	26%	71.8% [40.0%-103.6%]
Complex II (30 kDa subunit; nuclear DNA coded)	19%	40.0% [27.0%-53.0%]
Complex III (core 2 subunit; nuclear DNA coded)	31%	81.6% [48.2%-118.1%]
Complex IV (COX II subunit; mtDNA coded)	50%	136.3% [87.8%-184.7%]
Complex V (F1 alpha subunit; nuclear DNA coded)	53%	97.5% [64.7%-130.3%]

Patient OXPHOS subunit levels are lower than concurrently assayed patient harboring mtDNA deletions.

Gene Sequencing

Mitochondrial DNA	NEGATIVE
Caveolin-3	NEGATIVE
Calpain 3 (CAPN3)	Single Heterozygous Mutation Valine (GTT) 605 Serine (ATT); c.1813G>A

The valine at this position is highly conserved from humans to fruit flies. Mutation of the adjacent AA at position 605 is described to produce calpainopathy. Identification of a single CAPN3 mutation does not exclude the diagnosis of calpainopathy. (*Brain.* 2007 Dec;130(Pt 12):3237-49)

Summary

1. Diagnosis of mitochondrial disease requires a multi-faceted approach. This patient has mitochondrial disease with significantly increased CPK, particularly with activity.
2. Increased CPK and even rhabdomyolysis occurs in many classes of mitochondrial disease including muscle CoQ10 deficiencies, and mtDNA mutations in the cytochrome b gene. The cause for the increased CPK is unknown.
3. Mutations in other genes may be important in the increased CPK and may increase the risk of rhabdomyolysis in some patients.
4. We have identified only one mutation. The patient may be a carrier of a CAPN3 mutation or a second mutation is present that was not identified by sequencing.
5. Since mitochondrial dysfunction interferes with intracellular calcium regulation, the effects of a CAPN3 mutation, even in the carrier state, may be enhanced.