Mitochondrial Encephalomyopathy Due to a Novel Mutation in \textit{ACAD9}

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**Complex I** or NADH:ubiquinone oxidoreductase (Enzyme Commission no. 1.6.5.3) is the largest of the 5 oxidative phosphorylation complexes that catalyze electron transfer from NADH along the respiratory chain, using the lipid-soluble ubiquinone as an electron acceptor.\(^1\) Complex I is composed of 45 structural subunits, 14 of which have catalytic functions and 31 of which probably contribute to the assembly, stabilization, and functional regulation of the complex.\(^2\) Complex I deficiency is the most common respiratory chain defect associated with early-onset fatal encephalomyopathy. Although many molecular defects have been described both in mitochondrial and nuclear structural subunits and in assembly factors, the molecular diagnosis remains unknown for many patients.\(^3\) MitoExome sequencing (deep sequencing of the entire mitochondrial genome and the coding exons of >1000 nuclear genes encoding the mitochondrial proteome) has proven extremely useful in identifying new genetic variants associated with infantile oxidative phosphorylation defects.\(^4\) Using MitoExome sequencing, we identified a novel pathogenic \textit{ACAD9} gene variant in a patient with infantile-onset and slowly progressive encephalomyopathy, thus enlarging the clinical spectrum of \textit{ACAD9}-related disorders.

**Report of a Case**

A 13-year-old boy was referred to us because of exercise intolerance, weakness, and mild psychomotor delay. Muscle histochemistry showed mitochondrial proliferation, and biochemical analysis revealed severe complex I deficiency (15% of normal). The level of complex I holoprotein was reduced as determined by use of Western blot both in muscle (54%) and in fibroblasts (57%).
The patient at 13 years of age shows scoliosis and truncal obesity (A); Gomori trichrome (B [original magnification ×20]) and cytochrome c oxidase (C [original magnification ×20]) histochemical stains show mitochondrial proliferation. Images of Western blot analysis of oxidative phosphorylation proteins (D) in the fibroblasts (left) and muscle (right) of the patient (P) and controls (C) are shown. I indicates complex I, II, complex II, III, complex III; IV, complex IV, and V, complex V. The electropherogram of the patient’s DNA shows the variant (c.1240C>T) and evolutionary conservation of the mutated amino acid (E).
downstream to respiratory chain complexes III and IV. Thus, primary complex I deficiency can affect secondarily the activities of complex III and complex IV. The biochemical defect is usually associated with early-onset fatal encephalomyopathy. Molecular defects in the structural genes of the complex explain less than the 40% of cases. In the past decade, an increasing number of mutations have been identified in non-structural genes with the powerful combined use of high-throughput exome sequencing, homzygosity mapping, and proteomic analysis.

Mutations in ACAD9 were first described in 2010 in 4 patients with early-onset and rapidly progressive hypertrophic cardiomyopathy, encephalopathy, and lactic acidosis, leading to death in childhood (46 days–12 years). A profound reduction in complex I activity and a milder reduction in complex IV were identified in the muscle, liver, and fibroblasts. The role of ACAD9 in the assembly or stability of complex I was suggested by studies in fibroblasts that showed a reduction in the level of complex I holenzyme in blue-native gel electrophoresis; this reduction was completely reversed by transduction of wild-type ACAD9. Moreover, complex I activity was increased by supplementing a patient’s fibroblasts with high-dose riboflavin, a known catalytic cofactor of ACADs. Recently, Gerards et al. described a milder childhood-onset clinical form with myalgia and premature fatigue in a doubly consanguineous Dutch family. These patients had peculiar episodes of fatigue relieved by vomiting and difficulty with cognitive activities during these events, which lasted hours. Although there was no reduction in the level of complex I holenzyme, Gerards et al. proposed that ACAD9 might play a role in stabilizing the supercomplexes. Our case adds to the clinical heterogeneity of ACAD9 deficiency, which spans from infantile, rapidly progressive encephalomyopathy and hypertrophic cardiomyopathy to pure myopathy with exercise intolerance and lactic acidosis. Our patient had severe complex I and moderate complex III deficiencies in muscle and fibroblasts. Combined defects of complex I and complex III had been described in patients with mutations in the MTCYB gene, and studies of human and mouse cell lines carrying mutations in MTCYB demonstrated the interdependence of the 2 enzymes. We hypothesize that a reduction the number of electrons transported downstream to the complexes and a lack of complex I assembly can cause secondary complex III deficiency. However, we did not confirm in our patient the previously described complex IV deficiency.

ACAD9 is a β-oxidation component, and the mechanism by which mutations in this pathway impair oxidative phosphorylation remains poorly understood. Our report confirms that ACAD9 has an important role to play in maintaining complex I activity and protein level, thus bolstering the role of ACAD9 as an assembly factor, although further studies are required to fully understand its function. Because ACAD9 is a flavin adenine dinucleotide-containing flavoprotein, it makes sense that patients would benefit from riboflavin supplementation. This was documented both by in vitro studies showing increased complex I activity in patients’ fibroblasts and by clinical experience in all reported patients. Therefore, ACAD9 deficiency is a potentially treatable mitochondrial disorder, and screening for the ACAD9 gene is indicated for all patients with isolated complex I deficiency.

References