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SLC25A32 Mutations and Riboflavin-Responsive Exercise Intolerance

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TO THE EDITOR

Multiple acyl–coenzyme A dehydrogenation deficiency is an inborn error of metabolism with frequent muscle involvement. This deficiency is due to defects in the electron-transfer flavoprotein genes *ETFA* and *ETFB*[1] or in the electron-transfer flavoprotein ubiquinone oxidoreductase gene *ETFDH*.[2] In patients with this deficiency, all the mitochondrial flavoprotein dehydrogenases are defective with a specific biochemical phenotype for multiple acyl–coenzyme A dehydrogenation deficiency. Yet, in a few patients who have a deficiency that is similar to multiple acyl–coenzyme A dehydrogenation deficiency, no mutations are identified in *ETFA*, *ETFB*, or *ETFDH*.[3]

We report on a 14-year-old girl who presented with recurrent exercise intolerance. She had biochemical features of multiple acyl–coenzyme A dehydrogenation deficiency, but she did not have mutations in *ETFA*, *ETFB*, or *ETFDH*. Oral supplementation with riboflavin led to dramatic improvement in the clinical and biologic abnormalities.

Riboflavin is the precursor of flavin adenine dinucleotide (FAD), the cofactor for electron-transfer flavoprotein and electron-transfer flavoprotein ubiquinone oxidoreductase. We thus suspected a defect in FAD biosynthesis or transport and studied genes involved in riboflavin metabolism.

Two heterozygous mutations, c.425G→A (p.Trp142*) and c.440G→A (p.Arg147His), were identified in solute carrier family 25, member 32 (*SLC25A32*). *SLC25A32* encodes the mitochondrial FAD transporter, an inner mitochondrial membrane carrier that imports FAD from the cytosol into the mitochondria.[3,4] Allelic segregation confirmed autosomal recessive transmission. At the complementary DNA level, the missense mutation appeared homozygous, but the substitution responsible for the nonsense mutation was not identified. Consequently, we speculate that the messenger RNA (mRNA) harboring the nonsense mutation is degraded by the nonsense-mediated mRNA decay machinery.

We performed studies in yeast that showed that the missense mutation introduced in *FLX1,* the homologue of human *SLC25A32* in *Saccharomyces cerevisiae*, resulted in a severe growth defect that was rescued by exogenous expression of wild-type *FLX1* and also by *SLC25A32*. These findings show the deleterious effect of this mutation (Fig. S1A, S1B, and S1C in the Supplementary Appendix, available with the full text of this letter at NEJM.org).

We hypothesized that impaired mitochondrial FAD transport in our patient affected the activities of mitochondrial flavoproteins involved in fatty acid oxidation, as indicated by the biochemical profile of multiple acyl–coenzyme A dehydrogenation deficiency and by impaired fatty acid oxidation flux in lymphocytes (not shown). We also hypothesized that impaired mitochondrial FAD transport affected the activities of mitochondrial respiratory-chain flavoproteins.[3,5] Our hypothesis was supported by faint succinate dehydrogenase staining observed in a skeletal-muscle–biopsy specimen obtained from the patient (Fig. 1) and by a modest effect of *SLC25A32* haploinsufficiency on FAD-dependent mitochondrial enzymes observed in the patient's fibroblasts (Fig. S1D in the Supplementary Appendix).

In conclusion, in our patient with haploinsufficiency of *SLC25A32*, a deficiency in FAD mitochondrial transport caused late-onset exercise intolerance associated with abnormalities in the acylcarnitine profile. The patient's symptoms appeared to be highly responsive to oral supplementation with riboflavin. Our findings underscore the importance of studying genes involved in riboflavin metabolism in such patients and expand knowledge of the spectrum of inborn errors of riboflavin transport.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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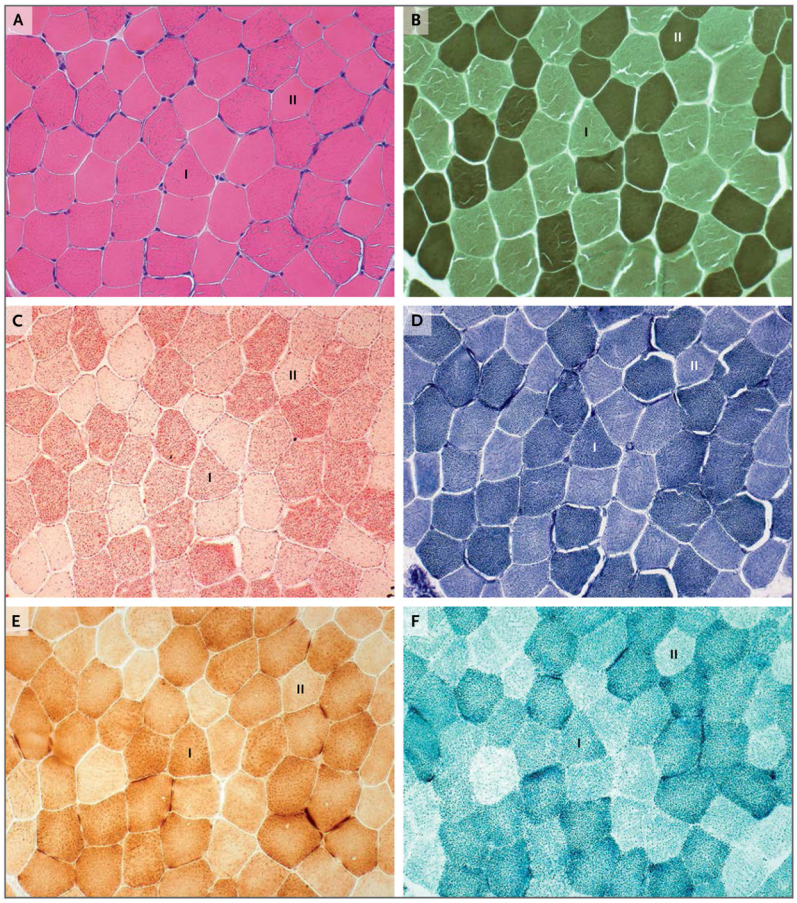

Figure 1. Histochemical Images of Skeletal-Muscle–Biopsy Specimens Obtained from the Patient

Cross sections of skeletal-muscle–biopsy specimens stained with hematoxylin and eosin (Panel A) show some ragged-red fibers observed mainly in type I fibers (I), which have a predominantly oxidative metabolism, rather than in type II fibers (II), which have a predominantly glycolytic metabolism. ATPase staining at pH 9.4 (Panel B) shows type II fibers (dark), which are slightly smaller than type I fibers (clear). Lipid storage was detected, particularly in type I fibers (Panel C). Oxidative enzyme reactions showed the presence of ragged-red fibers in some muscle fibers with NADH–tetrazolium reductase staining (Panel D) and with cytochrome *c* oxidase staining (Panel E). In contrast, the large majority of the fibers (Panel F) stained poorly for succinate dehydrogenase (flavin adenine dinucleotide–dependent mitochondrial respiratory-chain complex II), and some did not appear to stain at all.