Variants in the FAD Synthase Gene as a New Cause of Combined Respiratory Chain and Multiple Acyl-CoA Dehydrogenation Deficiency Including Riboflavin-Responsive Mutants

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Riboflavin, or vitamin B2, is the precursor of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), essential cofactors of numerous dehydrogenases involved in essential cellular mechanisms such as antioxidant defence, protein folding, apoptosis, and mitochondrial electron transport.
Riboflavin must be obtained as a nutrient via intestinal absorption. It is transported into the bloodstream and taken up by target cells by the riboflavin transporters. Within cells riboflavin is converted into FMN by the riboflavin kinase, and FMN is then converted into FAD by FAD synthase (FADS). FADS is the product of the *FLAD1* gene.
Here, we identified clinically relevant variants in *FLAD1* as a cause of multiple acyl-CoA dehydrogenase deficiencies (MADD) and respiratory chain dysfunction in nine cases derived from metabolic centers in six countries. In most cases we identified bi-allelic frameshift variants in the molybdopterin binding domain located upstream of the FAD synthase domain. Using RNAseq analysis combined with protein mass spectrometry, we discovered *FLAD1* isoforms explaining the residual FADS activity associated with frameshift variants. A subgroup of cases with a milder clinical phenotype responsive to riboflavin was shown to have single amino acid changes in the FAD synthase domain. When overexpressed in *E. coli* these variants of FADS proteins resulted in impaired but detectable FADS activity. Moreover, flavinylation significantly improved protein stability, arguing for a chaperone-like action.
In this study we describe a novel disease associated gene causing a disturbed riboflavin metabolism.