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Identification of the human mitochondrial FAD transporter and its potential role in multiple acyl-CoA dehydrogenase deficiency

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Abstract

Multiple acyl-CoA dehydrogenase deficiency (MADD) or glutaric aciduria type II (GAII) is most often caused by mutations in the genes encoding the α- or β-subunit of electron transfer flavoprotein (ETF) or electron transfer flavoprotein dehydrogenase (ETF-DH). Since not all patients have mutations in these genes, other as yet unidentified genes are predicted to be involved as well. Because all affected mitochondrial flavoproteins in MADD have FAD as a prosthetic group, the underlying defect in these patients may be due to a thus far undisclosed disturbance in the metabolism of FAD. Since a proper mitochondrial flavin balance is maintained by a mitochondrial FAD transporter, a defect of this transporter could also cause an MADD-like phenotype. In yeast, FAD is transported across the mitochondrial inner membrane by the FLX1 protein. An FLX1-mutated *Saccharomyces cerevisiae* strain exhibits a decreased activity of several mitochondrial flavoproteins. In the present study, we report the identification of the human mitochondrial FAD transporter. Based on sequence similarity to FLX1, we identified two human candidate genes (MFT and N111), which were cloned and characterized by functional expression in an FLX1-mutated yeast strain. Of the two candidate genes, only the previously described mitochondrial folate transporter (MFT) was able to functionally complement the FLX1 mutant. Candidates for mutations in the MFT gene are patients with a clinical suspicion of MADD but without any mutation in the α- or β-subunit of ETF or ETF-DH.

Keywords: Multiple acyl-CoA dehydrogenase deficiency; Glutaric aciduria type II; Flavoprotein; Riboflavin; Vitamin B2; FAD; Mitochondrial FAD transport; FLX1; MFT; Folate

Introduction

Multiple acyl-CoA dehydrogenase deficiency (MADD) or glutaric aciduria type II (GAII) is an inherited disease (MIM: 231680) affecting amino acid, fatty acid, and choline metabolism. The clinical presentation of MADD is very heterogeneous and ranges from neonatal death to late onset myopathy. In the majority of cases, MADD is caused by mutations in the genes encoding the α- or β-subunit of electron transfer flavoprotein (ETF) or electron transfer flavoprotein dehydrogenase (ETF-DH) [1–3]. ETF serves as an electron acceptor for the acyl-CoA dehydrogenases involved in fatty acid oxidation as well as for several dehydrogenases involved in amino acid and choline metabolism. Subsequently, these electrons are transferred via ETF-DH to ubiquinone in the respiratory chain.

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In some patients diagnosed with MADD on the basis of clinical and biochemical abnormalities, no mutations have been found in the genes encoding ETF and ETF-DH [2]. Since both ETF and ETF-DH have FAD as a prosthetic group, it has been hypothesized that the underlying defect in these patients may be due to an as yet unidentified disturbance in the metabolism of FAD [2,4,5].

Riboflavin is a precursor in the synthesis of FMN and FAD. Both products are the prosthetic groups of numerous enzymes (called flavoproteins) that catalyze the various electron-transferring reactions in energy-producing, biosynthetic, detoxifying, and electron-scavenging pathways. Most of these flavoproteins are found in mitochondria; ETF and ETF-DH are among them. There is still no consensus about the origin of flavin cofactors in mitochondria, and contradicting models have been published in literature [6–9].

In rats deprived of riboflavin, a severe impairment in the mitochondrial oxidation of fatty acids and amino acids was observed, caused by decreased activities of various acyl-CoA dehydrogenases and resembling the clinical presentation of human MADD [10]. Furthermore, in riboflavin-deficient mitochondria, all mature acyl-CoA dehydrogenases and ETF proteins lost their stability and were rapidly broken down. The presence of mitochondrial FAD was necessary for catalytic function and stability of the mature flavoproteins. FAD was shown to be imported into mitochondria independently from the flavoproteins [11]. This predicts the existence of a specific mitochondrial transport system for FAD.

In human, there is still much unknown about the metabolism of riboflavin and FAD. In yeast, all involved genes have been identified and characterized. FAD is transported across the mitochondrial membrane by a specific transporter protein, called FLX1 [7]. A yeast mutant deleted for the *FLX1* gene showed a respiratory deficient phenotype and was no longer able to utilize glycerol as a substrate. Mitochondria of this yeast FLX1 mutant contained three times less FAD compared to the wild-type strain, while the mitochondrial FAD/FMN ratio was decreased seven times. Furthermore, the activity of the mitochondrial flavoprotein lipoamide dehydrogenase in the mitochondria of the FLX1 mutant strain was virtually fully deficient.

Using an FLX1 mutant strain in another genetic background, Bafunno et al. [12] did not detect any abnormality in the mitochondrial FAD content, although the activities of both lipoamide dehydrogenase and succinate dehydrogenase were reduced by 50–65%.

Combined, both studies indicate that FLX1 functions as a mitochondrial FAD transporter. In the present study, we report the identification of the human orthologue of FLX1 and demonstrate by functional complementation of an FLX1 mutant strain that it functions as a mitochondrial FAD transporter.

Materials and methods

Identification of human FLX1 orthologues

Potential human orthologues for yeast FLX1 were identified by searching a philogenetic tree created by D.R. Nelson (University of Tennessee, TE, USA; http://drnelson.utmem.edu/mitocarriers.html) [13], using the FLX1 amino acid sequence as query. The amino acid sequences of potential orthologues and FLX1 were aligned with Clustal W multiple sequence alignment software (http://www.ch.embnet.org/software/ClustalW.html). Homology analysis was performed using Boxshade software (http://bioweb.pasteur.fr/seqanal/interfaces/boxshade.html).

Strains of Saccharomyces cerevisiae and growth media

The FLX1 mutant (aE536/LU1) and wild-type (W303-1A) *S. cerevisiae* strains used in this study were described and kindly provided by Dr. A. Tzagoloff (Columbia State University, New York, NY, USA) [7].

The strains were cultured in the following media: rich glucose (YPD): glucose 20 g L^{-1}, peptone 20 g L^{-1}, and yeast extract 10 g L^{-1}. Rich glycerol (YPG): glycerol 30 g L^{-1}, peptone 20 g L^{-1}, and yeast extract 10 g L^{-1}. Minimal glucose (YND): glucose 3 or 20 g L^{-1}, yeast nitrogen base (without amino acids) 6.7 g L^{-1}. For plates, agar 20 g L^{-1} was added. Peptone, yeast extract, yeast nitrogen base, and agar were Difco (Sparks, MD, USA) products. Amino acids were added as needed to final concentrations of 0.16 mM. Folinic acid was added to a final concentration of 0.1 mM.

Cloning of MFT, N111, and FLX1

The open reading frames of the cDNAs for MFT (GenBank Accession No. gi:21314738) and N111 (Hypothetical protein FLJ10618, GenBank Accession No. gi:15559392) were amplified by PCR using human fibroblast cDNA as template, while for FLX1 (GenBank Accession No. gi:762845) yeast genomic DNA was used as template. The PCR was performed with the following primers: MFT: 1MFTCfKpn 5′-TAT AGG TAC CAT GAC GGG CCA GGG CCA-3′ and 961MFTCrSal 5′-TAT AGT CGA CGT CCT CTT TGA GCT TAC TTT C-3′; N111: 1N111fKpn 5′-TAT AGG TAC CAT GAG CCA GAG GGA CAC GC-3′ and 941N111rSal 5′-ATA TGT CGA CTT CGT GCT GCT ATC CAT TGA G-3′; FLX1: 1FLX1fKpn 5′-TAT AGG TAC CAT GGT CGA TCA CCA GTG GAC GCC-3′ and 1317FLX1rSal 5′-TAT AGT CGA CAG CTA AAG CCT ATG CTT AAG GTT CTC-3′. The primers contain the restriction sites *Kpn*I and *Sal*I, which are underlined.