Patient with multiple acyl-CoA dehydrogenation deficiency disease and FLAD1 mutations benefits from riboflavin therapy

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Abstract

Multiple acyl-CoA dehydrogenation deficiency is genetically heterogenous metabolic disease with mutations in genes involved in electron transfer to the mitochondrial respiratory chain. Disease symptoms vary from severe neonatal form to late-onset presentation with metabolic acidosis, lethargy, vomiting, muscle pain and weakness. Riboflavin therapy has been shown to ameliorate diseases symptoms in some of these patients. Recently, mutations in FAD synthase have been described to cause multiple acyl-CoA dehydrogenation deficiency. We describe here the effect of riboflavin supplementation therapy in a previously reported adult patient with multiple acyl-CoA dehydrogenation deficiency having compound heterozygous gene variations in FLAD1 (MIM: 610595) encoding FAD synthase. We present thorough clinical history including laboratory investigations, muscle MRI, muscle biopsy and spiroergometric analyses comprising of a follow-up of 20 years. Our data suggest that patients with adult-onset multiple acyl-CoA dehydrogenation deficiency with FLAD1 gene mutations also benefit from long-term riboflavin therapy.

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1. Introduction

Multiple acyl-coenzyme A dehydrogenation deficiency (MADD, MIM #231680) is an autosomal recessive inborn error of metabolism that affects the oxidation of fatty acids and also certain amino acids and choline [1]. Neonatal-onset forms are usually fatal, but late-onset MADD is much milder resulting typically in exercise intolerance, muscle weakness and pain during exercise. The majority of the late-onset patients benefit from riboflavin therapy [2,3]. The genetic background is heterogeneous. Most adult cases show a deficiency of electron transfer flavoprotein dehydrogenase (ETFDH) protein, while a minority have mutations in the genes encoding the alpha (ETFα) and beta (ETFβ) subunits of the electron transfer flavoprotein [2]. Also, defects in riboflavin transporter genes (SLC52A1, SLC52A2 and SLC52A3) and in the mitochondrial FAD transporter gene (SLC25A32) have been described to cause MADD like phenotype [4,5].

Vitamin B derivatives take part in a number of cellular processes important in signaling and energy metabolism. Riboflavin or vitamin B2 is a precursor for the cofactor of flavo-enzymes that catalyze crucial steps in cellular oxidation–reduction reactions. Flavin mononucleotide (FMN) is formed from riboflavin by the riboflavin kinase (RFK, EC 2.7.1.26). FAD synthase (FADS, EC 2.7.7.2) [6,7] is responsible for catalyzing FMN adenylation to flavin adenine dinucleotide (FAD). FADS is the product of FLAD1 (MIM: 610595). The
FADS protein contains an N-terminal molybdopterin binding (MPTb) domain and a C-terminal domain sufficient to catalyze FAD synthesis (FADS domain). Also FLAD1 isoforms that code only the FADS domain have been characterized [8]. Recently, different mutations in FLAD1 encoding FAD synthase (FADS) were identified as the cause of MADD in nine patients, including the patient in this study, most presenting with early-onset and fatal course [8]. Clinical picture and benefit of riboflavin therapy was dependent on the FLAD1 genotype [8]. We report here detailed clinical follow-up of a previously reported woman [8] presenting with adult onset MADD and carrying two different FLAD1 mutations: a missense variant affecting the FADS domain, and a frameshift variant in the molybdopterin binding (MPTb) domain.

2. Patient and methods

2.1. Patient

The patient has been followed regularly for over 20 years at Helsinki University Central Hospital (HUCH). The first examination was performed at the onset of symptoms. There is no family history of muscle diseases. Her mother is of Finnish ancestry and her father’s ethnic background comes from Germany and Sweden. Diagnostic procedures including tissue sampling, electromyography (EMG) and muscle MRI were performed according to diagnostic procedures at HUCH. All tissue samples were taken in accordance with the declaration of Helsinki.

2.2. Spiroergometric testing

A work conducted with maximal spiroergometric testing was performed at age 44 years as described earlier [9]. A cannula was inserted in the left cubital vein, and the blood specimen for venous, ammonia and lactate were drawn at nine time points: at rest, low exercise, maximal exercise and 2, 4, 6, 10, 20 and 30 minutes after exercise. The test was started with 30 W work load with increase of 30 W in 3 minutes steps. Maximal subjective level of 19/20 in Borg scale was attained.

2.3. Molecular genetic studies

Sequencing of the ETFA, ETFB and ETFDH genes were performed essentially as previously described [10]. Subsequently, genes involved in conversion of riboflavin into the co-factors FMN and FAD were studied: hRFT1 (SLC52A1), hRFT2 (SLC52A3), hRFT3 (SLC52A2), riboflavin kinase (RFK), and FAD synthase (FLAD1) as described elsewhere [8].

3. Results

3.1. Clinical picture of the patient

The patient’s developmental milestones were normal. During childhood she was able to run but was slower than her peers and experienced symptoms of illness during long-lasting exercise. First evident muscle weakness was observed at age 20 years as she carried heavy bags. At that time she had continuously elevated creatine kinase (CK over 1000 U/L, reference range 35–210 U/L), and symptoms of tachycardia. Cardiac function analyses showed normal results. Thereafter she experienced muscle discomfort and weakness during physical activity, and after strenuous exercise she had occasional muscle pain, vomiting and loss of weight.

During her pregnancy at age 30 years, one month before the delivery, her muscle weakness drastically deteriorated with a raise in CK values ranging between 5000 and 6612 U/L (reference range 35–210 U/L). At that point, she was unable to control her head. Swallowing, talking as well as walking were difficult, and she needed hospital care for over three months. She was advised to follow a high carbohydrate, moderate protein and low fat diet (dietary fat max. 20 g/day), which ameliorated her symptoms. At age 33 years she could walk 2 kilometres on low pace, and had difficulties in walking the stairs. At that instant, riboflavin therapy (100 mg daily) was introduced and she continued the dietary therapy. This resulted in drastic amelioration of muscle symptoms, and increase in muscle strength. She was able to cycle and perform low pace exercise 6 hours per week, and walk distances up to 10 kilometres. At age 44 years, the patient still experienced marked benefit from riboflavin. However, her dietary fat intake had risen to 30–40 g/day, and the patient was now obese (BMI 31.6 kg/m²). She showed normal muscle strength in her lower legs and neck flexors, and a mild weakness in shoulder girdle muscles. Tendon reflexes, speech and eye movements were normal. Her 6-min-walking test was within control range according to age and sex (525m). Stomach musculature strength was reduced. Grip strength was 31 kg and 34 kg for right and left side respectively, and within control range. Needle EMG showed chronic myopathy changes with some dystrophic features but minimal active progression. No increased neuromuscular jitter due to instability of neuromuscular junction was present. In follow-up muscle biopsy, there was remarkable reduction of the lipid droplets (Fig. 1). In muscle MRI after 20 years of timespan, there were signs of fatty infiltration of the medial gastrocnemius musculature (Fig. 2).

3.2. Laboratory examinations

The urine analysis showed increased amount of lactate, mildly increased ethylmalonate, an increased 2-hydroxyglutarate combined with a low/normal 2-ketoglutarate excretion that was consistent with multiple acyl-CoA dehydrogenation deficiency. In line, the acylcarnitines in dried blood spot demonstrated slightly elevated concentration of various short-, medium-, and long-chain acylcarnitines. After 11 years of riboflavin therapy, there was no detectable and pathological excretion of organic acids in the urine. In laboratory analyses, her CK values (CK 125 U/L, reference range 35–210 U/L), lactate (0.9 mmol l/L, 0.5–2.2 mmol/l) and liver function tests (ALAT 12 U/L and GT 6 U/L, reference ranges <35 U/L and <40 U/L respectively) were within control limits. No signs of hypoglycemia, myoglobinuria, or hyperammonimia were observed. In spiroergometric testing, low ammonia response during and after exercise was found (Fig. 3).
Fig. 1. All muscle biopsies before (C) and after (A, B and D) riboflavin therapy showed findings consistent with mild metabolic myopathy. Some COX negative fibers were seen representing 2% of all fibers. The most remarkable change was the reduction of lipid droplets after riboflavin therapy. A) HE staining showing myopathic changes in the form of increased fiber size variation. B) COX-SDH staining showing three COX negative fibers present in this field. C) Oil Red O staining in muscle biopsy during time of MADD diagnosis suggesting lipid myopathy with increased amount of lipid droplets. D) Oil Red O staining after riboflavin and dietary therapy shows less extensive lipid accumulation. Muscle biopsy from tibialis anterior (A, B and D) and vastus lateralis (C), frozen sections; HE (A), COX-SDH (B), Oil Red O (C-D); original magnification x200 (A, B, D) and x400 (C).

Fig. 2. MRI of distal lower limb musculature at age 22 years, before riboflavin treatment (A) and at age 44 years, on riboflavin treatment (B). Leg MRI at age 44 years showed atrophy and near complete fatty degeneration of soleus muscles and fatty degeneration of the medial head of gastrocnemius muscles. The abnormal finding was quite symmetrical, with signs of progression when compared to the previous MRI at age 22 years. In the thighs there was no fatty conversion in the muscles (data not shown).

Fig. 3. Lactate and ammonium (NH4+ ion) profiles during riboflavin therapy at age 44. A maximal load of 120 W (76% of predicted value) was reached with maximal oxygen uptake of 21 ml/min/kg (72% of predicted value) and maximal heart rate of 179/min, 98% of age maximum. The respiratory quotient (RQ) was 1.2 indicating that anaerobic exercise level was attained. The lactate level increased 4 fold from the rest level, but the ammonia increase was low as related to the reached exercise load and the level of aerobic maximum. The findings were indicative of a muscle metabolic disorder. For comparison, the mean values of 15 healthy control women are presented schematically. The venous blood samples have been studied at rest, low exercise, maximal exercise, 2, 4, 6, 10, 20, 30 and 40 minutes after exercise.
3.3. Genetic analyses

Genetic studies have been reported previously [8]. Briefly, the sequence analysis of the six exons making up the FLAD1 gene revealed two heterozygous and most likely disease-causing variations: a c.568_569dupGC duplication causing a shifted reading frame and introduction of a premature termination codon in exon 2 (p.Val191Glnfs*10) of the molybdopterin binding (MPTb) domain, and a c.1588C>T (p.Arg530Cys) missense variation in the FAD synthase (FADS) domain [8]. The patient’s mother is heterozygous for the c.1588C>T variation and the father is heterozygous for the c.568_569dupGC duplication showing that the patient is compound heterozygous with the two variations located on different alleles.

4. Discussion

We describe here a female patient, who is compound heterozygous for two disease-causing mutations in the FLAD1 gene: a c.568_569dupGC frameshift mutation in the MPTb domain and a c.1588C>T (p.Arg530Cys) missense mutation in the FADS domain. It has been shown that the FADS domain alone is able to mediate FADS activity. In cultured patient fibroblasts, the combination of these mutations resulted in FADS activity of only 38% of that of control individuals, and in vitro overexpression studies in E. coli cells confirmed the pathogenic nature of the p.Arg530Cys variant [8]. Recessive FLAD1 gene defects resulting in sufficient FADS activity can cause a mild, late-onset MADD diseases. Based on the previous report of nine patients with FLAD1 mutations, patients carrying at least one allelic variant in the FADS domain that either changes of deletes an amino acid present with a milder phenotype that is probably more receptive to riboflavin therapy [8]. Accordingly, a p.Ser495del variant, located in the FADS domain, demonstrated improved protein stability when overexpressed and exposed to FAD in vitro. In vitro exposure of FAD did, however, not improve protein stability of the p.Arg530Cys variant [8].

In our patient, defective cellular flavin homeostasis and dysfunction of FAD-containing flavoproteins, of which many are mitochondrial acyl-CoA dehydrogenases, explain the patient’s MADD-like profile of urine organic acids and plasma carnitine conjugates and her response to riboflavin treatment. The patient presented with mild myopathy that had been aggravated by metabolically stressful situations such as fasting and pregnancy. During riboflavin therapy she did not experience any disease progression, and in the muscle biopsy the signs of lipid-storage myopathy had ameliorated during riboflavin therapy. Also, in the laboratory examination there were no detectable excretion of diagnostic organic acids in the urine. However, during spiroergometric testing there was clear evidence of defective amino acid metabolism, as the ammonia increase during and after exercise was very low compared to age and sex matched controls. The clinical picture of our patient resembles that of late onset MADD caused by mutations in the ETFDH gene [3]. Secondary respiratory chain deficiency is observed in both patient groups, and is suggested to be caused by disturbances in supercomplex formation by the deficient respiratory chain flavoenzymes, such as complex II or ETFDH [11] or by a more global metabolic reprogramming of mitochondrial activity [12]. Also, in both diseases the patients’ muscle symptoms and biochemical abnormalities observed in plasma and urine may be ameliorated by riboflavin therapy. In both diseases, a chaperone like action of FAD has been proposed to improve folding/stability properties of the mutated proteins [8,12]. These patients are important to recognize for the possible treatment available. Taken together, riboflavin supplementation is recommended to patients with MADD and FLAD1 gene mutations, but regular clinical follow-up and dietary consultation is warranted.

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References