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Megaconial muscular dystrophy caused by mitochondrial membrane homeostasis defect, new insights from skeletal and heart muscle analyses

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ABSTRACT

Megaconial congenital muscular dystrophy is a disease caused by pathogenic mutations in the gene encoding choline kinase beta (*CHKB*). Microscopically, the disease is hallmarked by the presence of enlarged mitochondria at the periphery of skeletal muscle fibres leaving the centre devoid of mitochondria. Clinical characteristics are delayed motor development, intellectual disability and dilated cardiomyopathy in half of reported cases. This study describes a patient presenting with the cardinal clinical features, in whom a homozygous nonsense mutation (c.248_249insT; p.Arg84Profs*209) was identified in *CHKB* and who was treated by heart transplantation. Microscopic evaluation of skeletal and heart muscles typically showed enlarged mitochondria. Spectrophotometric evaluation in both tissues revealed a mild decrease of all OXPHOS complexes. Using BN-PAGE analysis followed by activity staining subcomplexes of complex V were detected in both tissues, indicating incomplete complex V assembly. Mitochondrial DNA content was not depleted in analysed tissues. This is the first report describing the microscopic and biochemical abnormalities in the heart from an affected patient. A likely hypothesis is that the biochemical findings are caused by an abnormal lipid profile in the inner mitochondrial membrane resulting from a defective choline kinase B activity.

1. Background

Megaconial congenital muscular dystrophy (OMIM #602541) is an autosomal recessive disorder characterised by muscle weakness and intellectual disability. Besides skeletal muscle weakness, around 50% of patients also suffer from cardiomyopathy. The condition is caused by loss-of-function mutations in *choline kinase beta* (*CHKB*) (OMIM *612395). Twenty-two patients have been reported until now (Mitsuhashi et al., 2011a; Gutiérrez Ríos et al., 2012; Quinlivan et al., 2013; Castro-Gago et al., 2014; Cabrera-Serrano et al., 2015; Oliveira et al., 2015). Most characteristic phenotypic features are delayed gross motor function, delay of speech and language and autism spectrum disorder (Mitsuhashi and Nishino, 2013). In the patients who initially presented with congenital hypotonia, at a later stage a progressive proximal muscular weakness was seen. Creatine kinase (CK) activity was increased in serum (range: 190–2676 IU/l) (Quinlivan et al., 2013). Ichthyosis-like skin changes were noticed in seven out of twenty-two patients. Structural brain abnormalities are not a typical finding. MR spectroscopy, however, performed in one patient revealed a reduced choline:N-acetyl aspartate and choline:creatine ratio (Quinlivan et al., 2013).

Cardiac involvement occurs relatively frequently in the patients with megaconial congenital muscular dystrophy. Nine out of twenty-two patients developed dilated cardiomyopathy (DCMP). The age of onset and degree of clinical impairment were variable. One patient presented at the age of 14 years with mild DCMP which remained stable over time (Quinlivan et al., 2013). Another patient died at the age of eight years due to acute heart failure not responding to medical therapy (Quinlivan et al., 2013). Three patients died at the age of 2 1/2, 13 and 23 years but it is not clear to which extent the cardiac involvement contributed to the fatal outcome.

Histological findings in skeletal muscle showed pathognomonic abnormalities regardless of the clinical presentation. Light microscopic

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(LM) and electron microscopic (EM) examination in muscle fibres revealed enlarged and peripherally displaced mitochondria, acknowledging the original denomination of the syndrome as 'megaconial myopathy'. Enzyme deficiency of choline kinase (CHK) was first reported by Mitsuhashi et al. (2011a) in five patients and later confirmed in other patients (Quinlivan et al., 2013). In these patients, phosphatidylcholine levels were decreased. Oxidative phosphorylation (OXPHOS) complex activity analyses in skeletal muscle from affected patients did not show consistent abnormalities. Normal (Quinlivan et al., 2013; Nishino et al., 1998) as well as decreased activities have been reported. The latter group encompasses isolated complex deficiencies of complex I (Quinlivan et al., 2013) and complex IV (Gutiérrez Ríos et al., 2012) as well as a combined OXPHOS deficiencies involving complexes I, III and IV (Castro-Gago et al., 2014). The profile of combined deficiencies has been attributed to mitochondrial DNA (mtDNA) depletion in skeletal muscle. OXPHOS analyses in heart muscle from CHKB patients have not been reported so far. In skeletal muscle from mice, abnormal findings in the mitochondria were reported by Mitsuhashi et al. (2011b) such as decreased ATP synthesis, decreased amount of coenzyme Q, increased superoxide production and excessive activation of mitophagy.

In previously reported patients, 15 different mutations were detected in the *CHKB* gene, many of these are clear loss-of-function mutations (nonsense or frameshift) others are missense mutations. These were located throughout the whole coding region comprising 11 exons (for review see Mitsuhashi and Nishino, 2013).

The current study adds crucial biochemical, molecular and clinical insights to the existing knowledge of this rare disease as in particular due to the first description of OXPHOS activities and microscopic findings of heart tissue and report on a new pathogenic variant. Furthermore detailed clinical description is given and the feasibility of heart transplantation is described as possible treatment.

2. Methods

2.1. Case report

We report on a ten-year-old Moroccan male patient born from consanguineous parents (first cousins) after uncomplicated pregnancy and delivery with a birth weight of 3.0 kg. Neurological development during the first year of life was normal. He was able to sit without support at the age of six months and to walk unaided at the age of 16 months. However, from this young age on it was clear that he walked slower than other children and had difficulties in climbing stairs. His cognitive functions were impaired when tested at the age of eight years (total IQ 53, performal IQ 60, verbal IQ 51, using Wechsler Intelligence Scale for Children III). He received special education for his learning disabilities. When first seen at the age of nine years he was able to walk independently although slower than children of his age. Toe walking was difficult. Mild proximal weakness was observed as evidenced by a positive Gowers' sign. Proximal muscle strength in upper and lower limbs was reduced (motor scale 3 −/5), as opposed to normal muscle strength in distal muscles. Obvious signs of amyotrophy or *scapulae alatae* were not observed neither were signs of pyramidal tract dysfunction. Tactile function was normal. CK in serum was increased (up to 5773 IU/l, normal 36–219 IU/l). A skeletal muscle biopsy was performed. One year later, he was admitted to the paediatric intensive care unit (PICU) due to progressive lethargy and vomiting. Clinical examination was suggestive for congestive heart failure. Echocardiography was performed at the moment of admission and showed a dilated cardiomyopathy (left ventricle end diastolic diameter-LVEDD 58 mm, normal range: 35.5–48 mm) and fractional shortening (FS) of 7% (normal value >30%). The concentration of the N-terminal of the prohormone brain natriuretic peptide (NT-ProBNP) was 87.927 pg/ml (normal value: <84,00 pg/ml). Evidence was found of severe liver and kidney failure secondary to heart failure. Shortly after admission he needed mechanical ventilation and intravenous inotropic support. His clinical condition deteriorated rapidly and one day after admission a left ventricular assist device (LVAD) was placed and he was listed for heart transplantation. Following the placement of the LVAD his condition stabilised and he was extubated after seven days. Liver and kidney function fully recuperated after ten and seven days respectively. During the period of cardiac assistance, he had an episode of sustained ventricular tachycardia and hyponatraemia which was successfully corrected by administration of amiodarone and sodium supplementation. During this period he also received rehabilitation and his motor capacities returned to pre-admission levels. He was transplanted 3,5 months after placement of the LVAD. A sample of the explanted heart was used for microscopic and biochemical evaluation. Immediately after transplantation, he suffered from secondary multi-organ failure due to myocardial stunning and pulmonary hypertension. Fortunately, his condition improved and he was able to leave the PICU 25 days after transplantation. At that moment, he had severe swallowing and speech difficulties, and was limited in his motor skills as he could not roll over nor stand from a sitting position. Intensive rehabilitation was started and three months later he was able to walk with support. He was able to swallow solids and fluids without difficulties. His language skills had returned to previous level. Echocardiography revealed a normal left ventricular function (FS 34%, LVEDD 38 mm). Myocardial biopsy showed no signs of rejection. One year after transplantation, his clinical condition was stable and echocardiography showed a normal systolic and diastolic function, without sings of left ventricular hypertrophy.

2.2. Ethics, consent and permissions

The Ethical Review Board (Ethisch Comité, Universitair Ziekenhuis Gent) of the participating institution approved this study. The patient's legal representatives signed informed consent prior to enrolment.

2.3. Molecular analysis

Genomic DNA was extracted from blood obtained from the patient and from his parents using standard methods. All coding regions and exon–intron boundaries of *CHKB* were PCR-amplified using primer oligonucleotides designed with Primer3 (Rozen and Skaletsky, 2000). Mutation screening was performed using direct sequencing of the purified PCR-fragments with the BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Fragments were separated on an ABI 3730 automated capillary DNA sequencer (Applied Biosystems). The resulting sequences were aligned and analysed with the novoSNP (Weckx et al., 2005) and the SeqManII (DNASTAR Inc., Madison, WI, USA) programme. Mutation nomenclature was applied according to the HGVS nomenclature rules, using NM_005198.4 as RefSeq with the A from the ATG-start codon as +1 (http://www.hgvs.org/mutnomen). Sequence variants were confirmed by repeated PCR and bidirectional sequencing on stock DNA.

Relative quantification of total mtDNA to nuclear DNA was measured by a mitochondrial transfer RNA leucine 1/b-2-microglobulin real-time PCR quantification method using an ABI Prism 7500 Sequence Detection System (Applied Biosystems). The PCR was performed separately for mtDNA and the reference b-2-microglobulin amplification with the TaqMan Universal PCR Mastermix System, according to the manufacturer's instructions (Applied Biosystems).

2.4. Genotyping and paternity testing

Paternity was tested using 15 highly informative STR (short tandem repeat) markers, distributed throughout the genome. STRs were PCR-amplified and PCR fragments were loaded on an ABI 3730 automated DNA sequencer (Applied Biosystems).