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RESEARCH REPORT

Mitochondrial Trifunctional Protein Deficiency: Severe Cardiomyopathy and Cardiac Transplantation

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Abstract We describe mitochondrial trifunctional protein deficiency (MTPD) in two male siblings who presented with severe cardiomyopathy in infancy. The first sibling presented in severe cardiac failure at 6 months of age and succumbed soon after. The second sibling came to attention after newborn screening identified a possible fatty acid oxidation defect. Dietary therapy and carnitine supplementation commenced in the neonatal period. Despite this the second child required cardiac transplantation at 3 years of age after a sudden and rapid decline in cardiac function. The outcome has been excellent, with no apparent extracardiac manifestations of a fatty acid oxidation disorder at the age of 7. Pathogenic *HADHA* mutations were subsequently identified via genome wide exome sequencing. This is the first reported case of MTPD to undergo cardiac transplantation. We suggest that cardiac transplantation could be considered in the treatment of cardiomyopathy in MTPD.

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Introduction

The mitochondrial trifunctional protein (MTP, OMIM 609015) is an enzyme complex which catalyses the last 3 steps in the long chain fatty acid β-oxidation cycle (Houten and Wanders 2010). This protein complex comprises 4 α-subunits with enoyl CoA hydratase (LCEH) and 3-hydroxyacyl CoA dehydrogenase (LCHAD) activity and 4 β-subunits with 3 ketoacylCoA thiolase (LKAT) activity (Uchida et al. 1992). The α and β subunits are encoded by the *HADHA* (OMIM 600890) and *HADHB* (MIM 143450) genes, respectively (Kamijo et al. 1994), which both map to 2p22.3 (Yang et al. 1996).

MTP deficiency demonstrates a heterogeneous clinical spectrum including a severe neonatal form with cardiomyopathy, Reye-like features and early death; a hepatic phenotype with recurrent hypoketotic hypoglycaemia; and a milder later onset neuromyopathic type with episodic rhabdomyolysis (Boutron et al. 2011; den Boer et al. 2003). Mortality remains high, reported at 39% (LCHAD deficiency) to 76% (MTP deficiency) in the largest case series (Boutron et al. 2011; den Boer et al. 2002, 2003).

Cardiac involvement is common in long chain fatty acid oxidation defects (LC-FAOD) and is often a cause for

mortality (Vockley et al. 2015; Baruteau et al. 2014). Cardiac presentations include arrhythmias, hypertrophic cardiomyopathy, dilated cardiomyopathy, left ventricular non-compaction cardiomyopathy, and even severe in utero hypertrophic cardiomyopathy (den Boer et al. 2003; Baruteau et al. 2014; Spiekerkoetter et al. 2008; Emura and Usuda 2003; Ojala et al. 2015). These clinical phenotypes bear resemblance to the cardiac manifestations of the mitochondrial respiratory chain defects (Yaplito-Lee et al. 2007) and represent a significant cause of morbidity.

Improvements in the care of children with cardiomyopathy, congenital heart disease and acquired heart disease have led to an increased number of children surviving with advanced heart failure (Alexander et al. 2014; Kindel and Everitt 2016). Key improvements include the development of left ventricular assist devices (LAD) and a clearer understanding of immunology in the prevention of transplant rejection (Zangwill 2017). Donor availability and thus suitable candidate selection remain challenges. Herein we describe the first case of MTPD to undergo cardiac transplantation.

Case Reports

These siblings are the product of a non-consanguineous union with two older healthy children. The ultimate diagnosis of MTP deficiency came via genome wide exome sequencing after sibling 2 had received a cardiac transplant.

Sibling 1 This previously well male infant presented at 6 months of age with an intercurrent viral respiratory illness, in cardiac failure secondary to severe dilated cardiomyopathy. He required intensive support including extracorporeal membrane oxygenation (ECMO). There were no other manifestations to suggest a multi-system disease or an infective process. Plasma acylcarnitine profile demonstrated persistently elevated long and medium chain fatty acylcarnitine species, i.e. tetradecenoylcarnitine C14 1.9 μmol/l (reference range $<$ 0.7 μmol/l), tetradecanoylcarnitine C14:1 1.1 μmol/l (RR $<$ 0.3), hexadecanoylcarnitine C16 1.1 μmol/l (RR $<$ 0.6), decanoylcarnitine C10 0.8 μmol/l (RR $<$ 0.4), octanoylcarnitine C8 0.3 μmol/l (RR $<$ 0.2) and hexanoylcarnitine C6 0.3 μmol/l (RR $<$ 0.2). The urine organic acids consistently demonstrated significantly raised levels of 3-hydroxydicarboxylic acids (C10 $>$ C12, C8 and C6) with moderate dicarboxylic acids. Extended newborn screening (ENBS) was normal. ENBS was collected at 52 h of age while the child was clinically well and breast feeding in the maternity ward. Very long chain acyl-CoA dehydrogenase enzyme assay was normal, as were acylcarnitine studies performed on cultured fibroblasts were normal (performed in New South Wales Biochemical Genetic Service, Lehman et al. 1990). This screening assay studies the acylcarnitine profile produced by intact cells in culture medium with added palmitate and carnitine, with the butylated acylcarnitine species detected by electrospray ionization tandem mass spectrometry. The latter result appeared inconsistent with the plasma and urine results. A cardiac biopsy demonstrated interstitial oedema and fibrosis, and mitochondrial respiratory chain analysis on a muscle biopsy demonstrated mildly reduced complex IV activity 2.16 (3.3–9.1/min/mg, performed in MCRI Mitochondrial laboratory). A long chain fatty acid oxidation defect was suspected, the patient was managed with carnitine supplementation (50–75 mg/kg/day), avoidance of prolonged fasting, and trialled triheptanoin at 1 g/kg/day which was not well tolerated due to palatability and diarrhoea. The child succumbed to cardiac failure at 9 months of age prior to a final diagnosis being forthcoming.

Sibling 2 The younger male sibling came to attention in the neonatal period after an abnormal ENBS result, with elevated long chain acylcarnitine species, i.e. elevated C14 1.29 (RR $<$ 0.63 μM), C14:1 1.36 (RR $<$ 0.6 μmol/l), 3-hyrdoxypalmitoylcarnitine (C16-OH), 3.2 (RR $<$ 0.2 μmol/l). On this basis, as well as the family history, he was managed for a presumed fatty acid oxidation disorder with avoidance of fasting, carnitine supplementation (50–75 mg/kg/day) and medium chain triglyceride-based formula (Monogen 50 g twice daily). Urine organic acids and repeat plasma acylcarnitine profiles were normal. Mitochondrial respiratory chain studies performed on the explanted cardiac tissues were normal (performed in MCRI Mitochondrial laboratory). Sequencing of the *ACAD9* gene (Mater Pathology Brisbane), the *ACADVL* gene (Department of Biochemistry and Molecular Biology, Arhus University Hospital, Denmark), the common *HADHA* mutation c.1528G>C and a next generation sequencing cardiomyopathy panel of 69 genes (performed in Victorian Clinical Genetics Pathology Service, Victoria), all returned normal results.

At 3 years of age he developed severe dilated cardiomyopathy detected on routine monitoring, the left ventricle had dilated significantly to 51 mm, shortening fraction 21% and biplane ejection fraction 41%. Over the ensuing weeks he rapidly progressed toward congestive cardiac failure. Medical management including the use of Lisinopril and carvedilol. D-βeta-hydroxybutyrate (300 mg/kg/day) was attempted and while this generated a measurable ketoacidosis on urine testing, there was no appreciable improvement in cardiac function. A cardiac transplant was considered the only long-term option for survival. This was facilitated by the implantation of a left ventricular assist device followed by conversion to a Berlin heart. He

required multiple explorations for bleeding and removal of thrombus from the cannula. He had a brief generalized tonic clonic seizure triggered by hypoxia in the context of pericardial tamponade. Neuroimaging at this time was normal. Orthotopic heart transplantation occurred 3 months after initiation of augmented circulatory supports, when suitable donor was available. Our recipient had become sensitized and was mismatched for Class I and II antigens by Luminex Single Antigen testing, as well as being CMV mismatched on serology (donor positive – recipient negative). The post-transplant course was complicated by lymphopenia secondary to mycophenalate motefil, mild rejection on endocardial biopsies, gastric bleeding due to a gastric ulcer, adrenal suppression secondary to steroid immune suppression and medical procedure anxiety. The patient is doing well at the age of 7. He is intellectually normal and has no signs of a multisystem disease process. Rather than repeating specific FAOD enzyme assays on cultured fibroblasts, we proceeded to whole exome sequencing. He is not on any specific metabolic management currently.

Whole Exome Sequencing

A trio-based clinical exome, and subsequent sanger sequencing, was performed in the Macrogen laboratories (http://www.macrogen.com/eng/). After enrichment of all the coding and flanking intronic regions of the genes mentioned above, sequencing analysis was performed using an Illumina HiSeq platform. 97.7% of targeted regions achieved ×100 coverage and 99.7% achieved ×10 coverage. The only clinically relevant sequence variations with an allele frequency <0.1% were HADHA NM_000182 c.1712T>C; p.Leu571Pro. (maternal), and HADHA NM_000182 c.446G>T; p. Gly149Val (paternal). The variants have not been previously reported on dbSNP. Minor/alternative allele frequencies are not reported in the 1000 genome or the NHLBI GO Exome Sequencing Project data sets at either of these loci. *HADHA* NM_000182 c.1712T>C; p.Leu571Pro, overlaps with evolutionary constrained element (detected using SiPhy-ω and SiPhy-π statistics). The conservation across 28 species is described with PhyloP (score: 2.33). GERP identifies constrained elements in multiple alignments by quantifying substitution deficits (score: 6.07). The BLOSUM62 substitution matrix reports a score of −3 for this alteration, with a PhyloP score of 2.33 and aGERP score of 6.07. HADHA NM_000182 c.446G>T; p. Gly149Val, variant overlaps with evolutionary constrained element (detected using SiPhy-ω and SiPhy-π statistics). The BLOSUM62 substitution matrix reports a score of −3 for this alteration. The conservation across 28 species is described with a PhyloP score of 1.47 and GERP score of 4.94. Both are predicted to be missense mutations.

Discussion

The pathophysiology of severe, early onset cardiac phenotypes in MTPD is unclear, but provide an indication that the heart is exquisitely sensitive to impaired LC-FAOD, either due to direct toxicity from metabolic accumulation, or from substrate deficiency. The heart undergoes a switch in energy substrate preference from glucose in the foetal period to fatty acids following birth (Spiekerkoetter et al. 2008; Lehman and Kelly 2002). However; the in utero onset of cardiac manifestations in some MTPD cases suggests a pathogenic role in mitochondrial respiratory chain (MRC) function or permeability (Ojala et al. 2015; Tonin et al. 2013; Nsiah-Sefaa and McKenzie 2016).

The beta-oxidation pathway and the MRC share substrates and are linked biochemically. Reduced NAD and FADH2 produced during fatty acid oxidation pass their electrons to the MRC complexes. Primary disorders of one of these pathways have been shown to have deleterious effects on the other (Nsiah-Sefaa and McKenzie 2016), from the build-up of toxic intermediates (Sakai et al. 2015) or physical links between beta-oxidation and MRC protein complexes (Taylor et al. 2012; Nouws et al. 2014). MTP is bound to MRC complex 1 (Sumegi and Srere 1984), suggesting that beta-oxidation-MRC super-complexes are metabolically active structures (Nsiah-Sefaa and McKenzie 2016). Patients with LCHAD deficiency frequently exhibit secondary MRC complex 1 deficiencies (Tyni et al. 1996; Das et al. 2000; Wang et al. 2010), either via physical interaction (Wang et al. 2010), or altered stability via cardiolipin (Taylor et al. 2012). The extreme severity of the neonatal mitochondrial cardiomyopathies, rapidly fatal in a majority of cases, clearly illustrates the major role of myocardial MRC function in the adaptation to extrauterine life (Schiff et al. 2011). The heart relies heavily on oxidative metabolism and is particularly vulnerable to MRC dysfunction (Yaplito-Lee et al. 2007). The consequences of MRC dysfunction include ATP deficiency, aberrant calcium handling, excessive reactive oxygen species production, apoptosis dysregulation and nitric oxide deficiency (Yaplito-Lee et al. 2007).

Subject one demonstrated normal ENBS results despite being collected in appropriate physiological conditions. German experience with newborn screening for MTP defects in 1.2 million infants reports 11 true positives, 10 false positive but no known false negative results (Sander et al. 2005). However, two false negatives were reported in Austrian LCHAD deficient twins who were born prematurely (29 weeks gestation) and supplemented with L-carnitine (Karall et al. 2015). Intermittently normal

acylcarnitine profiles have been reported in cases of later onset neuromyopathic MTPD deficiency (Yagi et al. 2011).

Though a diagnosis of fatty acid oxidation was strongly suspected based on the clinical and biochemical parameters, the diagnosis of MTPD was not formalized when decision-making was required regarding the suitability of sibling 2 as a cardiac transplantation candidate. He demonstrated single organ disease and was of normal intellectual and developmental capabilities. While concerns of cardiac dysfunction secondary to "toxic metabolites" are a possibility in the LC-FAOD, we proposed that the LC-FAOD cardiac clinical phenotypes maybe secondary to substrate deficiency as outlined above, and recurrence in a transplanted heart would not be expected. Possible evidence of substrate depletion being causative is demonstrated by sibling 2's different clinical trajectory after management from birth with metabolic supportive therapy and anaplerotic treatments consequent to his abnormal ENBS. The role of anaplerotic therapy in the LC-FAOD, specifically triheptanoin, is under ongoing investigation (Vockley et al. 2015).

Our patient remains metabolically stable 4 years post cardiac transplantation with no apparent MTPD-related extra-cardiac manifestations such as retinitis pigmentosa, peripheral neuropathy, hepatic disease or neurological disease. However, long-term follow-up will be required as these complications may occur later in life.

Conclusion

In summary, we present the first case of cardiac transplantation in a defect of the mitochondrial trifunctional protein. The outcome in this case has been excellent, and while long-term complications related to the underlying fatty acid oxidation defect may occur despite dietary therapy, our experience suggests that transplantation could be considered to treat severe cardiomyopathy in this disorder.

Synopsis

Cardiac transplantation could be considered in the treatment of cardiomyopathy in mitochondrial trifunction protein deficiency.

Contributors' Statements

Dr. Carolyn Bursle is a metabolic fellow involved in patient care and development of the manuscript.

Drs. David Weintraub, Cameron Ward and Robert Justo are paediatric cardiologists involved in patient care and manuscript development.

Dr. John Cardinal is a medical scientist involved in manuscript development.

Professor David Coman is a metabolic physician involved in patient care and has driven the manuscript design and development.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Conflict of Interest

The other authors have no conflicts of interest to disclose.

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Ethics Approval

N/A.

Patient Consent

The patients' parents consent to publication of this case report.

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