Fatal cardiomyopathy and hepatic dysfunction with acute onset due to mitochondrial trifunctional protein deficiency diagnosed by whole exome sequencing

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Abstract

In this paper we report the postmortem identification of a rare disorder of mitochondrial fatty acid β-oxidation in a neonate patient which presented with clinical manifestations of cardiomyopathy and hepatic dysfunction which caused his death. The baby, born by caesarean section at term, presented at the age of eight months following a respiratory viral infection with a severely impaired general condition culminating with multiple organ failure and subsequent death in 10 days. Next-generation-sequencing of genomic deoxyribonucleic acid (DNA) isolated from an archived umbilical cord blood sample revealed two heterozygous point mutations within exons 14 and 15 of the 3-hydroxyacyl-CoA-dehydrogenase (HADHA), encoding the α-subunit of the mitochondrial trifunctional protein (MTP), which were subsequently confirmed by targeted polymerase chain reaction and conventional DNA capillary sequencing. MTP is an enzyme complex which catalyzes three steps in mitochondrial β-oxidation of fatty acids: enoyl-CoA hydratase, HADHA/HADHB, and 3-ketoacyl-CoA thiolase. Deficiency of this heterocomplex containing 4α and 4β subunits causes sudden infant and neonate death, Reye-like syndrome, cardiomyopathy, hepatic dysfunction and skeletal myopathy. We determined the molecular basis of the deficiency in our patient with neonatal presentation and later sudden death, and also tested the patient's parents for the same defect, which turned out to be both heterozygous carriers of a mutation within the HADHA gene, associated with the autosomal recessively inherited fatty acid oxidation disorder called MTP deficiency. After the death of their first infant they gave birth to a second baby, which was also tested for this deficiency and was diagnosed also as a heterozygous carrier for the same HADHA deficiency.
Keywords: *mitochondrial trifunctional protein deficiency, infant death, cardiomyopathy, hepatic dysfunction*

Introduction

Mitochondrial β-oxidation of fatty acids represents the main source of energy for the heart and other high energy requiring organs, like the liver or skeletal muscles. Trough β-oxidation long chain fatty acyl-CoA substrates are transformed into acetyl-CoA and a fatty acyl substrate reduced in length by two carbon atoms. That is why, because of the wide range of fatty acyl substrate lengths, multiple enzymes with partially overlapping substrate specificity catalyze each step[(1)]. Mitochondrial trifunctional protein (MTP) deficiency is a rare condition, with an unknown incidence, that prevents the body from converting certain fats to energy, particularly during periods of fasting[(2)].

Signs and symptoms of MTP deficiency may begin during infancy or later in life. Features that occur during infancy include feeding difficulties, lethargy, hypoglycemia, hypotonia and liver problems. Infants with this disorder are also at high risk for serious heart problems, breathing difficulties, coma, and sudden death[(3)]. Problems related to MTP deficiency can be triggered by periods of fasting or by illnesses such as viral infections.

This disorder is sometimes mistaken for Reye syndrome, a severe disorder that may develop in children while they appear to be recovering from viral infections such as chicken pox or flu, most of these cases being associated with the use of Aspirin during these viral infections[(3)].

Clinically there are three distinct classic phenotypes of MTP deficiency depending on the moment of the clinical onset and its severity: early, childhood, and mild[(4)]. Early MTP deficiency starts manifesting clinical signs between birth and age two, consisting usually in

a neonatal onset of a severe, lethal condition resulting in sudden unexplained infant death. Individuals with childhood MTP deficiency have clinical signs during and after infancy, with frequent infantile onset of a hepatic Reye-like syndrome, and late-adolescent onset of primarily a skeletal myopathy. Prognosis for the severe neonatal form of MTP deficiency is very poor. The later onset mild form has a far more favorable prognosis[5].

Signs of early MTP deficiency are nonspecific and include poor appetite, fatigue, longer or more often sleeping periods, hypotonia, fever, vomiting, diarrhea, hypoglycemia, irritability, hyporeflexia, developmental delays or behavioral changes. These symptoms can be caused by many other pathologies, which makes the diagnostic more difficult. A thorough medical examination can diagnose small for gestational age babies, pathologic amniotic fluid, generalized muscle weakness, myalgia, failure to thrive, pigmentary retinopathy, muscular hypotonia, global developmental delay, congestive heart failure, dilated cardiomyopathy, hydrops fetalis, hepatomegaly, respiratory failure, and peripheral neuropathy[6,7].

The MTP is a hetero-octamer protein attached to the inner mitochondrial membrane composed of 4 alpha 3-hydroxyacyl-CoA-dehydrogenase (HADHA) and 4 beta 3-hydroxyacyl-CoA-dehydrogenase (HADHB), catalyzes three out of four steps in the mitochondrial beta-oxidation of fatty acids: long-chain 3-hydroxyacyl-CoA dehydrogenase, long-chain enoyl-CoA hydratase, and long-chain thiolase activities[8].

Mutations in the HADHA and HADHB genes are the cause of this deficiency and are autosomal recessively inherited. These genes provide the genetic information for coding the enzyme complex called MTP. MTP activates in the mitochondria, which is the energy-producing center within the cells. As the name suggests, MTP contains three enzymes that each perform a different function[9]. During periods of fasting, fatty acids are also an important energy source for the liver and other tissues[10,11]. There is no known cure for this disease. Current management of patients with MTP deficiency include long-term dietary therapy of avoiding long fasting periods, low fat diet, restricting the amount of long chain fatty acid intake and substituting them with medium chain fatty acids and administrating L-carnitine, which is a source of energy[12,13,14].

The diagnosis of MTP can be established by biochemical tests such as analytic determination of the level of enzymes from the complex and through enzyme assay, or by genetic tests such as the sequence analysis of the entire coding region or the selected exon and the identification of deletion or duplication in the involved genes[15,16].

We report here the postnatal diagnosis of an infant that presented sudden death following a respiratory viral infection at the age of eight months, whose parents turned out to be bot heterozygous carriers of the point mutations within exons 14 and 15 of the HADHA encoding the α-subunit of the MTP.

Case report

The baby in case was born by elective caesarian section at 39 weeks of gestation, after a normal medically supervised pregnancy. Neither of his parents did not present in their medical history any significant pathologies. The mother's obstetrical history did not include any other child births or abortions. At the age of eight months the infant developed an initially mild respiratory viral infection. The baby's general status soon became critical and culminated with the development of myocarditis which quickly leads to cardiac failure. The disease progressed rapidly, generating multiple organ failure. On 21 November 2013, at day ten of disease, the baby developed a cardio-respiratory arrest which could not be resuscitated.

Results

After the infant's death an autopsy was being performed, and the results were not conclusive, the pathologist's report showing myocarditis and hepatic steatosis. The conclusion drawn was that the infant suffered from a possibly hereditary cardyomyopathy and hepatic dysfunction syndrome with neonatal onset. The parents decided to pursue genetic testing in order to discover the disorder causing the baby's death. Molecular genetic analysis (i.e. whole-exome sequencing) was performed at Neuromuscular Research Department from the Center of Anatomy & Cell Biology at Medical University of Vienna in March 2014. The material analysed consisted in deoxyribonucleic acid (DNA) from 1 ml of umbilical cord blood, extracted at birth and deposited at a Stem Cell Cord Blood Bank in Cluj-Napoca. The methods used for investigation were next-generation-sequencing (construction of a DNA library), bioinformatic analysis focusing on genes with causal relationship to the presumptive clinical diagnosisaccording to the literature and the OMIM database entries. Unidirectional DNA sequencing of the HADHA gene (exons 14 and 15 and exon-intron boundaries) of genomic DNA from an archived umbilical cord blood sample revealed two heterozygous point mutations within exons 14 and 15 of the HADHA gene, encoding the alpha subunit of the MTP, which were subsequently confirmed by targeted polymerase chain reaction (PCR) and conventional DNA capillary sequencing.

The genetic analysis of whole genome sequencing included a screening technique which allowed the study of a multitude of human genes at the same time. Data analysis was performed by short read mapping to the human reference genome, variant detection annotation and of these variants followed by a comparison to the literature database entries. The bioinformatic analysis was limited to genes causally linked to the presumptive clinical diagnosis, and detected mutations we confirmed by targeted PCR followed by conventional DNA capillary sequencing.

In subsequent carrier analyses, both unaffected parents of the patient were analyzed for these mutations. The mother turned out to be a heterozygous carrier of

the mutation c.1430C>G p.(Ser477Cys) within exon 14, while the father a heterozygous carrier of the mutation c.1528G>C p.(Glu510Gln) within exon 15.

These results prove the genetic transmission of both mutations from exon 14 inherited from the carrier mother and exon 15 inherited from the carrier father in the deceased baby. The mutation detected in the mother's DNA was not showed to determine HADHA-related MTP dysfunction until now. The segregation of both messiness lead to a beta oxidation mithocondrial disease, compatible with the clinical manifestations of congenital cardiomyopathy with hepatic failure. These findings support the idea of prenatal genetic testing for HADHA mutations in cases with suspicion of an inherited mithocondrial disease.

Discussion

This paper reports the identification of a rare disorder of fatty acid oxidation in a neonate patient which presented with clear clinical manifestations of a mitochondrial β-oxidation disorder, which rapidly determined his death. The recent identification of a multifunctional, membrane-bound 9-oxidation enzyme protein catalyzing three enzyme activities suggested an underlying basis for this particular combination of enzyme deficiencies[2].

Clinical manifestations of the infant started at eight months of age and have been precipitated by a common upper respiratory tract viral infection. Rapid progression of the disease and the severe alteration of the baby's general status lead to myocarditis and in a period of days multi-organ failure, which generated an irresuscitable cardiac arrest on day ten. Unlike other young patients affected by beta oxidation enzymatic defects, this infant was born by caesarean section at term after a normal medically supervised pregnancy, and had a normal intrauterine, neonatal and pediatric development being an apparently healthy baby, until eight months of age when a presumably common viral upper respiratory tract infection caused a rapid degradation of his clinical condition and lead to his death.

Next-generation-DNA sequencing is a technique for sequencing all the protein-coding genes in a genome, forming the exome[17]. It first selects only the subset of DNA that encodes proteins, the exons, and then sequencing that DNA using any high throughput DNA sequencing technology. There are 180,000 exons, which form 1% of the human genome, or approximately 30 million base pairs. Mutations in these sequences usually have severe consequences compared to the remaining 99%. Exome sequencing is mainly effective in the identification of rare Mendelian diseases, because it is the most efficient method to identify the genetic variants in all of a patient's genes. These diseases are often caused by very rare genetic variants that are only present in a small number of individuals[18,19]. On the other hand, techniques such as single nucleotide polymorphism arrays can only detect shared genetic variants that are common to many individuals in the wider population. Untill now genetic tests were chosen based on the clinical manifestations of the patient, focused on one gene or a few known to be associated with a particular disease, or surveyed only certain types of variation, like comparative genomic hybridization and provided clear genetic diagnose in fewer than fifty percent of all patients. Massively parallel sequencing technologies, used for exome sequencing, make it now possible to identify the underlining defect of many diseases, with previously unknown causes, by screening thousands of loci at once[20].

The identified heterozygous "missense" mutation within exon 14 (c.1430C>G) causes the exchange of a highly conserved serine residue within HADHA. The mutation is unknown in literature or mutation databases until present[18,19]. Other missenses affecting amino acid positions located closely (i.e. Ala478Val) within the 3-hydroxyacyl-CoA dehydrogenase domain are causatively involved in MTP deficiency. The heterozygous missense mutation within exon 15 (c.1528G>C) identified causes the exchange of a highly conserved glutamate residue (p.Glu510Gln) within the same alpha domain of the HADHA and is known to cause MTP, being listed in the actual mutation databases[21].

Thus, this finding formally proves compound heterozygosity for two HADHA-mutations in the deceased neonate patient and the observed segregation supports a pathogenic role for the unknown until now mutation within exon 14[22,23]. The results of these molecular genetic analyses support the clinical diagnosis of a congenital cardiomyopathy with hepatic failure and are compatible with HADHA-related MTP, which enable prenatal testing of this gene.

Conclusions

The case presented is very rare due to its particularities. Unlike other patients affected by beta oxidation enzymatic defects, the infant in case was born at term after a normal uncomplicated pregnancy, and developed normally as apparently healthy baby until eight months of age when a presumably common viral upper respiratory tract infection caused a rapid degradation of his clinical condition and lead to his death. Both parents of the deceased infant are unaffected carriers of different heterozygous mutations in the HADHA gene, from the MTP complex, unaware of this fact until their first child presented with symptoms of cardiomyopathy and liver failure precipitated by a respiratory virosis. The mutation identified in the mother on exon 14 has not been described in literature or mutation databases until present, but the experiments conducted showed that is highly compatible with a probably pathogenic role in MTP. Although exome sequencing technique used in this case is an expensive method relative to other technologies like hybridization-based technologies currently available, we consider it an efficient strategy to find genetic defects that cause rare Mendelian diseases. By sequencing the exomes of individuals a large quantity of data and sequence information is generated which

requires a significant amount of data analysis. That is why, due to the unusual coincidence of beta oxidation mithocondrial enzyme deficiency detected in this family, we suggest that antenatal genetic testing should be considered as an option. ■

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