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SHORT COMMUNICATION

First prenatal molecular diagnosis in a family with holocarboxylase synthetase deficiency

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Objectives We report on the first prenatal molecular diagnosis of holocarboxylase synthetase (HLCS) deficiency in the fourth pregnancy of an at-risk family. This disorder is a rare autosomal recessive inborn error of metabolism, leading to a multiple carboxylase defect (MCD). HLCSD diagnosis was performed postmortem in the proband on DNA from autoptic biological material. Molecular analysis of the proband's entire HLCS gene by direct sequencing identified the R508W amino acid change, at the homozygous status.

Methods Fetal DNA was isolated from chorionic villus sampling at 11 weeks of gestation. Direct sequencing of exon 6 of the fetal HLCS gene was performed.

Results The R508W mutation was identified in the fetal DNA at the homozygous level. The genetic lesion was confirmed on abortive tissue.

Conclusion Molecular diagnosis has several advantages over enzymatic activity assay of carboxylases in chorionic villi or amniocytes. It can be performed earlier, is faster, and the response time is shorter. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: holocarboxylase synthetase; prenatal diagnosis; molecular diagnosis

INTRODUCTION

Holocarboxylase synthetase (HLCS) deficiency (MIM 253270) is a rare autosomal recessive inborn error of metabolism leading, as with biotinidase deficiency (MIM 253260), to a multiple carboxylase defect (MCD). Nutritional biotin deficiency (Sweetman *et al*., 1981) and biotin transport defects can also cause MCD (Mardach *et al*., 2002).

Most patients with holocarboxylase synthetase deficiency (HLCSD) present early in life with feeding and respiratory difficulties, vomiting, hypotonia, seizures, lethargy, and coma (Narisawa *et al*., 1982; Wolf, 2000). In these patients, rapid diagnosis and treatment is necessary to avoid death. However, HLCSD can also be late onset. Some patients also exhibit skin lesions and erythematous rash, hair loss, as well as developmental delay (Wolf, 2000). The main laboratory findings are metabolic acidosis, lactic acidosis, ketosis, hyperammonemia, and organic aciduria showing a typical urinary excretion pattern (Nyhan and Ozand, 1998). Clinical and biochemical findings usually improve markedly following treatment with pharmacological doses of biotin, but some patients have been reported as only partially biotin responsive (Wolf *et al*., 1981; Suormala *et al*., 1997; Morrone *et al*., 2002).

Determination of the HLCS gene's genomic organization (Morrone *et al*., 2002) makes molecular and prenatal diagnosis easy.

We report on a prenatal diagnosis in an Iranian family. Two children had previously died in a local hospital without being diagnosed. HLCSD diagnosis was made postmortem in the second affected child using autoptic biological material. The R508W amino acid change, a common mutation in several ethnic groups (Dupuis *et al*., 1996; Sakamoto *et al*., 1998; Hwu *et al*., 2000; Tang *et al*., 2003), was found at the homozygous status in the sample (Morrone *et al*., 2002).

FAMILY HISTORY

The patient, a male, was born to first-cousin Iranian parents after an uneventful pregnancy and delivery (Figure 1). His sister was normal. The second child of the family died at 6 months of age without diagnosis, but with the same symptoms as the proband. The patient was well until 6 months after birth, when he was admitted to a local hospital for respiratory distress, drowsiness, lethargy, and, subsequently, coma with severe metabolic acidosis (pH 7.09) and a very high anion Gap (32). He died a few days later. Biological fluids and autoptic material were collected and shipped to our center. GC/MS analysis, performed postmortem, showed a typical MCD urinary organic acid pattern with elevated

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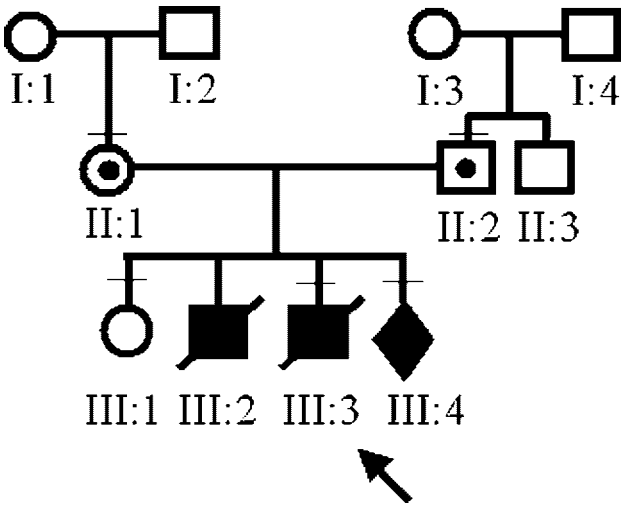

Figure 1—Family pedigree. The symbols without a line on top represent family members not available for biochemical or genetic testing. Black symbols represent affected individuals. Shaded figures indicate a possibly affected individual. Carriers are represented with a single dot inside the symbol. An arrow indicates the proband

excretions of 3-hydroxypropionate 448 mmol/molcreat (n.v. <10), 3-methylcrotonylglycine 333 mmol/molcreat (n.v. <2), 3-hydroxyisovalerato 61 mmol/molcreat (n.v. <46), methylcitrate 15 mmol/molcreat (n.v. trace), propionylglycine 131 mmol/molcreat (n.v. <2), tiglylglycine 198 mmol/molcreat (n.v. <2) and lactate 4948 mmol/molcreat (n.v. <259). Plasma biotinidase activity was a normal 6.2 nmol/min/mL (n.v. 6.3–10.54).

Genetic analysis of DNA from autoptic liver tissue demonstrated that the patient was homozygous for the known R508W mutation due to the transition c1522C > T in exon 6 of the HLCS gene (Morrone *et al*., 2002). Enzymatic restriction analysis confirmed the mutation at a heterozygous level in both parents, while his healthy sister was not a carrier.

With the aim of making a prenatal diagnosis in the couple's fourth pregnancy, DNA was isolated from chorionic villus sampling at 11 weeks of gestation. Direct sequencing of exon 6 in the fetal HLCS gene identified the R508W mutation at the homozygous level. Organic acid analysis of methylcitric and 3-hydroxyisovaleric acid in the selected ion monitoring (SIM) mode using, for methylcitric acid, the stable isotope dilution method, was also performed on 0.5 mL of amniotic fluid obtained during villocentesis. The results were not informative (methylcitric acid 0.49 μM, normal control 0.55 μM; 3-hydroxyisovaleric acid not detected).

We advised the parents that in all reported cases this mutation has been associated with biotin responsiveness, but they decided to interrupt the pregnancy.

The genetic lesion was confirmed on abortive tissue at the homozygous level.

DISCUSSION

The R508W mutation, mapped within the biotin-binding domain, has been reported in several other patients at the homozygous level (Dupuis *et al*., 1996; Hwu *et al*., 2000; Tang *et al*., 2003). It has been associated with the biotin-responsive and late-onset form of HLCSD, according to the age of our patient and his brother at presentation (6 months). Unfortunately, both died before HLCS diagnosis was made and biotin therapy started. It is probable that the severity of their clinical manifestations and early death were due to a lack of appropriate therapy (Thuy *et al*., 1999a).

Prenatal diagnosis of HLCS deficiency can be made by activity assay of carboxylases (propionyl CoA carboxylase, 3-methylcrotonyl CoA carboxylase, and pyruvate carboxylase) or of HLCS in cultured amniocytes or in chorionic villous (Thuy *et al*., 1999a, b). The determination of methylcitric acid and/or 3-hydroxyisovaleric acid in amniotic fluid at the 16th week of pregnancy by stable isotope dilution techniques has also been used (Jakobs *et al*., 1984). However, organic acid analysis of amniotic fluid may be inconclusive in an affected fetus (Suormala *et al*., 1998; Thuy *et al*., 1999a, b) and carboxylases activity assays in cultured villus or amniocytes are subsequently required to confirm diagnosis. Enzymatic activity assay is time consuming and results take a long time. Moreover, cells must be cultured in a biotin-low medium to ensure that the deficiency is detected (Suormala *et al*., 1997; Thuy *et al*., 1999a, b; Touma *et al*., 1999).

As expected, the determination of methylcitric acid and 3-hydroxyisovaleric acid levels at the 11th week of gestation, performed significantly earlier than the traditional 16–19 weeks, did not yield informative results.

Knowledge of the HLCS gene's genomic organization has lead to the development of a much faster and more certain means of diagnosis via chorionic villus sampling at 10–11 weeks of pregnancy. Up to now (to our knowledge), only a few HLCS prenatal diagnosis have been described in the literature, all performed at a biochemical level. This is the first prenatal molecular diagnosis reported in a case of HLCSD. Clearly, prenatal molecular diagnosis has advantages over both the activity assay of carboxylases and the determination of organic acids in amniotic fluid as it can be performed earlier, is quicker, and leads to an accurate diagnosis in a very short time. Mutation detection in this disorder is a good diagnostic means. To our knowledge, only one genetic lesion (Glu42Asp) identified in the gene could complicate the diagnosis, as an expression study showed the same activity as the wild type (Yang *et al*., 2001). In our proband, no polymorphism was detected.

The parents decided to interrupt the pregnancy despite the known association of the R508W mutation with biotin responsiveness. They could have decided to continue the pregnancy, undergoing biotin therapy. However, reported data about prenatal treatment of HLCS deficiency is controversial (Packman *et al*., 1982; Roth *et al*., 1982). It may be that biotin treatment immediately after birth in affected newborns avoids metabolic decompensation (Wolf, 2000).

We stress the importance of storing any biological fluids, tissues, and/or autoptic materials in order to make a postmortem biochemical and/or molecular diagnosis.

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The latter is necessary for carrier detection and, possibly, prenatal diagnosis.

In the case of HLCSD, it is also very important to keep cultured fibroblasts that can be used for testing biotin responsiveness *in vitro* which could lead to more accurate genetic counseling.

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