SHORT COMMUNICATION

DNA-based prenatal diagnosis for very-long-chain acyl-CoA dehydrogenase deficiency

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Very-long-chain acyl-CoA dehydrogenase (VLCAD) (EC 1.3.99.13) deficiency (McKusick 201475) is a clinically heterogeneous disease of mitochondrial fatty acid oxidation, which often has a rather severe outcome. Although the disease was only discovered recently, and fewer than 40 families have been reported in the literature, there have already been reports of seven prenatal diagnoses performed in six unrelated families (Nada et al. 1996; Vianey-Saban et al. 1998), clearly illustrating that prenatal diagnosis is warranted. So far, prenatal diagnosis in VLCAD deficiency has been carried out by traditional enzyme assays (Vianey-Saban et al. 1998) or by the in vitro probe assay performed on cultured cells (Nada et al. 1996).

We have recently established a fast and reliable method for PCR amplification and direct sequencing of the entire VLCAD coding region from genomic DNA and used this method to identify 58 different disease-causing mutations in 55 unrelated families (Andresen et al. 1996a,b, 1999), thereby enabling DNA-based prenatal diagnosis.

MATERIALS AND METHODS

Chorionic villus biopsies were taken by standard methods when the mothers were 10–11 weeks pregnant. Following visual inspection for maternal contamination, and
dependent on the size of the biopsy, it was divided in two pieces and DNA was extracted by standard methods from each of the two samples and analysed in parallel. Duplicate PCR amplifications of the relevant exons of the human VLCAD gene were carried out using M13 (−21) and M13 reverse-tagged intron-located primers under standard conditions in an automated thermal cycler (Thermal Cycler 480, Perkin Elmer). The PCR products were subjected to direct bidirectional cycle sequencing using a DNA dye primer sequencing kit (Perkin-Elmer) in an ABI Catalyst 800 Molecular Biology LabStation (Applied Biosystems). Sequence reactions were run on semi-automated ABI 373A and ABI 377 sequencers (Applied Biosystems). Information on all primers and conditions used for PCR amplification is available upon request (brage@biobase.dk). β-Oxidation activity in intact cultured chorionic villus cells was measured using [9,10-3H2]oleic, [9,10-3H2]palmitic and [9,10-3H2]myristic acids as substrates as previously described by Olpin et al (1997).

RESULTS

We used our knowledge of the mutational status in six families who had previously had a child suffering from VLCAD deficiency to perform diagnosis using DNA extracted directly from uncultured chorionic villus biopsies (CVS) as template for PCR followed by direct sequence analysis (Table 1). We detected one normal fetus (family 2); three fetuses were carriers for one disease-causing mutation (families 1, 3 and 5) and two fetuses (families 4 and 6) were compound heterozygous for two disease-causing mutations and thus were affected. In the normal fetus and in the two fetuses who were carriers for the paternal mutation, maternal contamination of the CVS DNA could be excluded, as the maternal mutation was absent. In the fetus from family 1, who had the same genotype as the mother, maternal contamination was excluded by DNA analysis for seven highly polymorphic markers in DNA samples from the CVS and the parents. Both affected fetuses (families 4 and 6) were aborted. In families 2, 3 and 5 the diagnosis was confirmed when the child was born. In family 6 the diagnosis was confirmed in a DNA sample from the aborted fetus, but in family 4 the parents refused sampling from the aborted fetus. In families 3 and 5 the chorionic villus biopsy was cultured and β-oxidation activity was measured in intact cells using [9,10-3H2]oleic, [9,10-3H2]palmitic and [9,10-3H2]myristic acids as substrates. In both fetuses the β-oxidation activity was consistent with the carrier status shown by DNA analysis. None of the six prenatal diagnoses took more than a week to perform.

DISCUSSION

We have recently investigated the molecular genetic basis for VLCAD deficiency in 55 unrelated families and identified 58 different disease-associated mutations in the VLCAD gene (Andresen et al 1996a,b, 1999). In about half of the families, one or more children had died from the disease. There were no asymptomatic genetically affected siblings in the investigated families, indicating a high penetrance of the

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