Feasibility of DNA based methods for prenatal diagnosis and carrier detection of propionic acidaemia

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Abstract
Propionic acidaemia (PA) is an autosomal recessive disease caused by a genetic deficiency of propionyl-CoA carboxylase (PCC). Defects in the PCCA and PCCB genes that code for the α and β subunits of PCC, respectively, are responsible for PA. A proband with PA was previously shown to carry the c1170insT mutation and the private L519P mutation in the PCCB gene. Here we report the prenatal diagnosis of an affected fetus based on DNA analysis in chorionic villus tissue. We have also assessed the carrier status in this PCCB deficient family, which was not possible with biochemical analysis. (J Med Genet 1999;36:412–414)

Keywords: DNA; prenatal diagnosis; propionic acidaemia; PCCB

Figure 1 Comparison of gDNA sequences from normal control (A1 and B1) and from the proband heterozygous for the mutations c1170insT (A2) and L519P (B2).
The proband was a female born to non-consanguineous parents who presented with refusal to feed, vomiting, failure to thrive, lethargy, and hypotonia at 3 days of age. Abnormal laboratory findings included hyperammonaemia (228 μmol/l), hyperglycaemia (1222 μmol/l), and high urinary excretion of 3-hydroxy-propionic (62 mmol/mol creat) and methylcitric (128 mmol/μmol creat) acids. Undetectable PCC activity in fibroblasts confirmed the diagnosis of PA. PCC activity in the parents' fibroblasts was indistinguishable from a control (father 0.96, mother 0.78, parallel control 0.83 nmol/min/mg protein), consistent with a PCCB gene defect. The paternal grandparents were first cousins and had had three miscarriages and twins who died neonatally with similar clinical features to the proband. After genetic counselling, the parents requested prenatal examination in the next pregnancy, which ended in a spontaneous abortion at 8 weeks of gestation.

Chorionic villi from the aborted fetus, skin fibroblasts from the patient and her parents, and blood spots from her grandparents were obtained to perform enzymatic or molecular studies or both. Skin fibroblasts and chorionic villi were cultured according to standard procedures. PCC activity was assayed according to Suormala et al.\(^7\) Isolation of genomic DNA (gDNA) was carried out as described previously.\(^8\) Samples were screened for the c1170insT mutation by direct sequencing of PCR products amplified from gDNA of cultured cells of the patients and parents and directly from dried blood spots of the grandparents using the primers and conditions previously described.\(^7\) The L519P mutation was also detected by direct sequencing of amplified PCR products from gDNA using primers S1499X (sense) 5'-ctcaccatctcctgtatcaggttg-3' and cDNA2 (antisense) previously described.\(^7\) The PCR fragments were sequenced using the fmol DNA sequencing system (Promega) and analysed on an automated ALFexpress DNA sequencer (Pharmacia). The L519P mutation was confirmed by MspI restriction digest analysis.

**Results and discussion**

The proband of this family was found to be a compound heterozygote for two mutations in the PCCB gene, resulting in lack of PCC activity in fibroblasts. One allele carries the mutation c1170insT (fig 1), a very frequent change found in the Spanish population that alters the open reading frame generating a TGA stop signal three triplets downstream in the mRNA. The other mutation, L519P, consists of a substitution of leucine for proline owing to a 1556T→C transition (fig 1), which creates an MspI restriction site and therefore can easily be detected by direct restriction analysis of the corresponding amplified PCR products. This mutation has not been detected elsewhere and results in a change of the secondary structure of the β polypeptide of the PCC protein according to “in silico” predictions. Sequencing all exons including intron-exon junctions showed no other mutations. DNA isolated from cultured chorionic villi of the fetus showed the same genotype as the proband, indicating an affected fetus, consistent with the undetectable PCC activity found. Karyotype analysis showed a severe anomaly in the fetus (46,XX/46,XX,r(18),45,XX,−18), which might explain the spontaneous abortion. Screening for the c1170insT mutation in the family showed that the mother and maternal grandmother were carriers for this mutation. Restriction analysis by MspI digestion in the

![Diagram](http://jmg.bmj.com/10.1136/jmg.36.5.412)
family members showed that the father and both paternal grandparents were heterozygous for the L519P mutation (fig 2).

Previously, prenatal diagnosis for PA was performed by measurement of methylocitrinate in amniotic fluid by GC-MS-SIM and by enzymatic determination in fetal tissue. Although a false negative prenatal diagnosis by methylocitrinate measurement is rare, discrepant results between metabolite and enzymatic determinations have been reported. Therefore, once the mutation(s) in a family at risk for PA has been precisely identified, DNA tests are the most rapid and reliable procedure for prenatal diagnosis of a metabolic disorder in which the results of biochemical analysis are often ambiguous and require considerable experience for proper interpretation. We have recently described the genomic structure of the PCCB gene and characterised the disease causing mutations in 21 unrelated Spanish patients. The mutational spectrum includes 16 mutations, accounting for 98% of the total mutant alleles, with four prevalent ones (ins/del, c1170insT, E168K, and A497V). In this way, amplification of the corresponding exons by polymerase chain reaction (PCR) followed by restriction enzyme digestion or direct sequence analysis or both will facilitate prenatal diagnosis in a large number of Spanish PA families. In the family reported here, after the identification of PA causing mutations in the proband, we successfully diagnosed PA in the aborted fetal tissue, which showed an affected genotype. As heterozygotes for PA caused by a defect in the β subunit of PCC show PCC activity values indistinguishable from controls, the only reliable way to determine carrier status is at the molecular level. By direct mutation analysis on blood spots, we verified that the maternal grandmother is a carrier for the L519P mutation. This fact could explain the reported deaths of twins during the neonatal period, who were probably affected by PA.

PA is one of the most frequent organic acidurias. The outcome is generally poor with a high incidence of mental retardation and movement disorder. Therefore, prenatal diagnosis plays an important role in genetic counselling in families with PCC deficiency.

In this paper we report for the first time the use of DNA analysis in chorionic villus tissue for diagnosis of PA. Our results show that DNA analysis provides accurate and reliable prenatal detection and is the only way to determine carrier status in PCCB deficient families.

We thank Dr Hernández for providing samples of the family and C Hernández and A Sánchez for their excellent technical assistance. This work was supported by grants from Fundación Areces and Fondo de Investigaciones Sanitarias (95/0477). The institutional grant of “Fundación Ramón Areces” to the Centro de Biología Molecular “Severo Ochoa” is gratefully acknowledged.