Short reports

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

S Muro, C Pérez-Cerdá, P Rodríguez-Pombo, B Pérez, P Briones, A Ribes, M Ugarte

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

Propionic acidaemia (PA, OMIM, http://www3.nbci.nlm.nih.gov/Omim/) is an inborn error of organic acid metabolism in humans caused by deficiency of propionyl-CoA carboxylase (PCC, EC 6.4.1.3.). Affected patients generally have a life threatening crisis during the neonatal period with severe metabolic acidosis and hyperammonaemia.[1] The disease has an autosomal recessive mode of inheritance and results from mutations in the PCCA or PCCB genes which encode the α and β subunits of PCC, respectively.[2 3] In Spanish PA patients, we have previously described two frequent mutations in the PCCB gene, ins/del and c1170insT, accounting for 42% of the mutant alleles studied.[4 5] Up to now, prenatal diagnosis of PA has been performed by means of biochemical analysis using chorionic villi and amniotic fluid. However, there is no valid

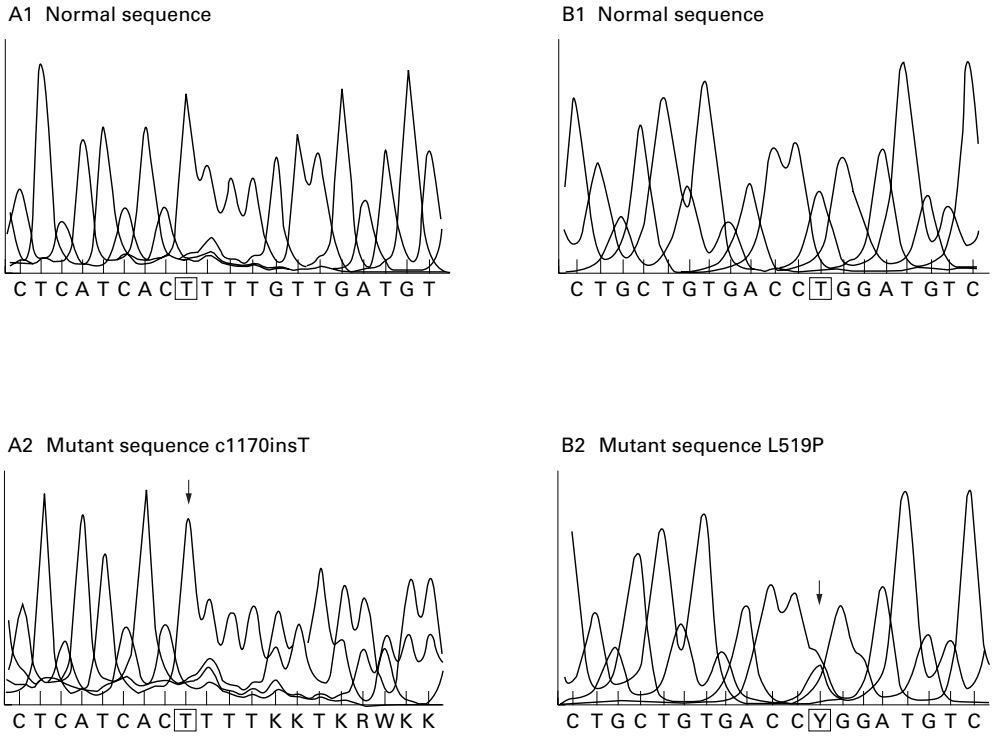

Figure 1 Comparison of gDNA sequences from normal control (A1 and B1) and from the proband heterozygous for the mutations c1170insT (A2) and L519P (B2).

Centro de Diagnóstico de Enfermedades Moleculares, Departamento de Biología Molecular, CBMSO, Universidad Autónoma de Madrid, 28049 Madrid, Spain
S Muro
C Pérez-Cerdá
P Rodríguez-Pombo
B Pérez
M Ugarte

Instituto de Bioquímica Clínica, Corporació Sanitaria y CSIC, Barcelona, Spain
P Briones
A Ribes

Correspondence to:
Dr Ugarte.

Received 27 April 1998
Revised version accepted for publication 8 September 1998

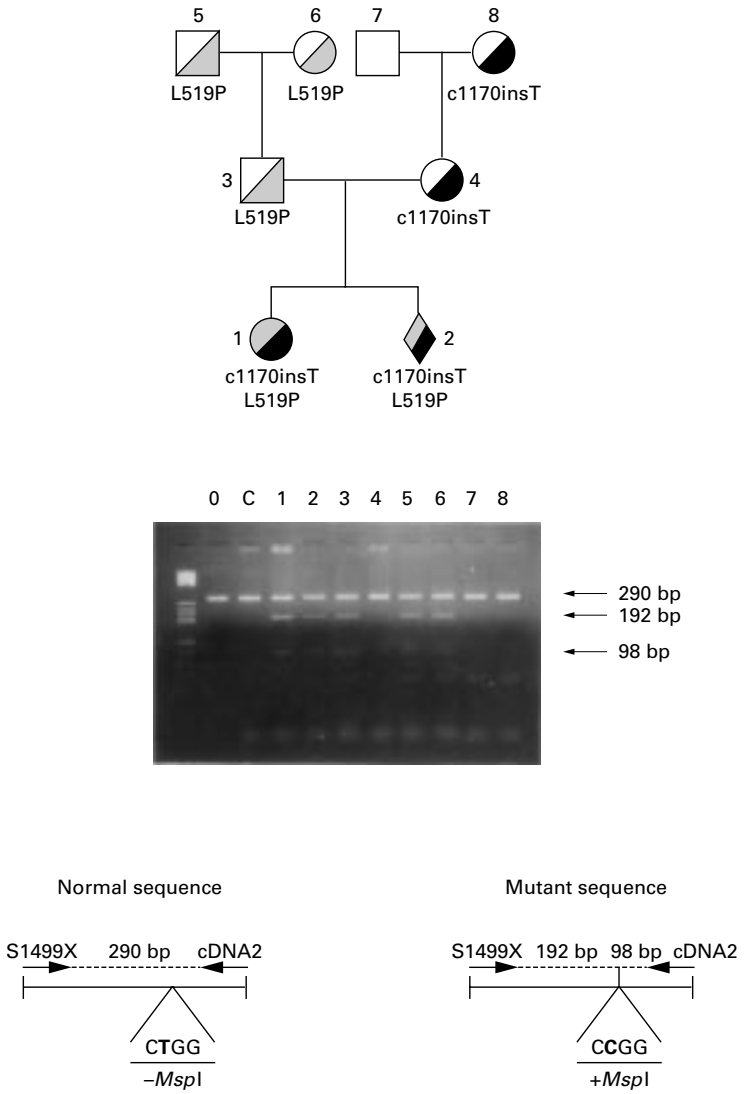

Figure 2 Mendelian segregation of mutations c1170insT and L519P and restriction analysis for L519P by MspI digestion. Lane 0: undigested control; lane C: control; lane 1: proband; lane 2: fetus; lane 3: father; lane 4: mother; lane 5: paternal grandfather; lane 6: paternal grandmother; lane 7: maternal grandfather; lane 8: maternal grandmother.

method for determining carrier status for PA resulting from PCCB gene defects, as PCCB heterozygotes have PCC activities in the control range, in contrast to PCCA heterozygotes who have PCC activities approximately half that of controls.[6] Here we report the feasibility of DNA analysis for prenatal diagnosis and carrier detection in a family at risk for PA with the c1170insT mutation and the missense mutation L519P in the PCCB gene.

Patients and methods

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), hyperglycinaemia (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.[5] The L519P mutation was also detected by direct sequencing of amplified PCR products from gDNA using primers S1499X (sense) 5'-ctaccatctctgtatcaggttg-3' and cDNA2 (antisense) previously described.[9] 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 *Msp*I 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 *Msp*I 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 *Msp*I digestion in the

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 methylcitrate in amniotic fluid by GC-MS-SIM[10] and by enzymatic determination in fetal tissue.[11] Although a false negative prenatal diagnosis by methylcitrate measurement is rare, discrepant results between metabolite and enzymatic determinations have been reported.[12] 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.[13] 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,[6] 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 c1170insT and the paternal grandparents are both heterozygous 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.[14 15] 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.

1 Fenton WA, Rosenberg LE. Disorders of propionate and methylmalonate metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic and molecular bases of inherited disease*. 17th ed. New York: McGraw-Hill, 1995:1423-99.
2 Gravel RA, Akerman BR, Lamhonwah AM, León del Río A, Italiano I. Mutation participating in interallelic complementation in propionic acidemia. *Am J Hum Genet* 1994;**55**:51-8.
3 Richard E, Desviat LR, Pérez B, Pérez-Cerdá C, Ugarte M. Three novel splice mutations in the PCCA gene causing identical exon skipping in propionic acidemia patients. *Hum Genet* 1997;**101**:93-6.
4 Pérez-Cerdá C, Rodríguez-Pombo P, Ugarte M. Identification of the insertion/deletion mutation in Spanish β-propionyl CoA carboxylase-deficient patients. *J Inherit Metab Dis* 1994;**17**:661-3.
5 Hoenicka J, Rodríguez-Pombo P, Pérez-Cerdá C, Muro S, Richard E, Ugarte M. A new frequent mutation in the PCCB gene in Spanish propionic acidemia patients. *Hum Mutat* 1998;**suppl**:S234-6.
6 Wolf B, Rosenberg LE. Heterozygote expression in propionyl coenzyme A carboxylase deficiency. Differences between major complementation groups. *J Clin Invest* 1978;**62**:931-6.
7 Suormala T, Wick H, Bonjour JP, Baumgartner RE. Rapid differential diagnosis of carboxylase deficiencies and evaluation for biotin-responsiveness in a single blood sample. *Clin Chim Acta* 1985;**145**:151-62.
8 Old J. Fetal DNA analysis. In: Davies K, ed. *Human genetic diseases: a practical approach*. New York: IRL Press, 1986:1-16.
9 Ohura T, Narisawa K, Tada K. Propionic acidaemia: sequence analysis of mutant mRNAs from Japanese β subunit-deficient patients. *J Inherit Metab Dis* 1993;**16**:863-7.
10 Jakobs C. Antenatal diagnosis of inherited metabolic disorders using gas chromatography-mass spectrometry. In: Farriaux JP, Dhont JL, eds. *New horizons in neonatal screening*. Amsterdam: Elsevier Science, 1994:357-64.
11 Pérez-Cerdá C, Merinero B, Sanz P, *et al*. Successful first trimester diagnosis in a pregnancy at risk for propionic acidaemia. *J Inherit Metab Dis* 1989;**12**(suppl 2):274-6.
12 Chadefaux B, Rabier D, Bonnefont JP, Jakobs C, Dumez Y, Kamoun P. Prenatal diagnosis of propionic acidaemia: amniocentesis at the 11th week of pregnancy. *Prenat Diagn* 1989;**9**:448.
13 Rodríguez-Pombo P, Hoenicka J, Muro S, *et al*. Human propionyl-CoA carboxylase β subunit gene: exon-intron definition and mutation spectrum in Spanish and Latin American propionic acidemia patients. *Am J Hum Genet* 1998;**63**:360-9.
14 Surtees RAH, Matthews EE, Leonard JV. Neurologic outcome of propionic acidemia. *Pediatr Neurol* 1992;**8**:333-7.
15 Pérez-Cerdá C, Merinero B, Martí M, *et al*. An unusual late-onset case of propionic acidemia: biochemical investigations, neuroradiological findings and mutation analysis. *Eur J Pediatr* 1998;**157**:50-2.