LETTERS TO THE EDITOR

Rapid prenatal diagnosis of myotonic dystrophy in the second trimester using polymerase chain reaction

Close genetic linkage between the myotonic dystrophy (DM) locus and a few polymorphic DNA markers has made feasible presymptomatic and prenatal diagnosis of this disease. However, DNA analysis using conventional Southern blotting methods is time consuming. This is a major limitation which, in most cases, prevents the monitoring of fetuses in those families which have not been analysed before the beginning of pregnancy. To overcome these difficulties we have successfully used the polymerase chain reaction (PCR) technique to amplify DNA obtained by placental biopsy in the second trimester.

A 24 year old woman (II.1, figure) with manifestations of DM, including grip myotonia and myopathic facies, was seen by us at 16 weeks of gestation. Her 54 year old father (I.2) was also affected, presenting with myotonic potentials on EMG and lens opacities on slit lamp examination. Genomic DNA was extracted from blood samples from all relevant family members (figure) and from a placental biopsy performed using an 18 gauge spinal needle and a freehand ultrasound guided technique, with no anaesthesia. DNA was used for PCR analysis.

Oligonucleotide primers flanking the polymorphic restriction enzyme sites TaqI and NcoI for the CKMM locus were synthesised based upon sequence analysis using a 381A DNA synthesiser (Applied Biosystems, Warrington). The reaction was started by incubation at 94°C for five minutes followed by centrifugation in a microfuge for 10 seconds; 2 to 3 units of Taq polymerase (LEP Scientific Limited, Bucks) were then added.

A total of 30 cycles of amplification was performed, each comprising one minute incubation at 60°C, one minute at 72°C, and one minute at 94°C in an automated thermocycler (LEP Scientific Limited, Bucks). Ten μl of amplified DNA (approximately 1 μg) were digested with 25 units NcoI according to the supplier’s instructions and electrophoresed on a 1% agarose gel stained with ethidium bromide.

The results of the analysis are shown in the figure. The mother at risk (II.1) was heterozygous for alleles 1 and 2. Allele 2 was inherited from the homozygous affected father and was marking the disease chromosome. The fetus had inherited allele 2 from the mother and therefore was considered affected. These results were obtained three days after placental biopsy and were then confirmed by Southern blotting analysis using markers D19S51 and D19S22 (data not shown). The parents chose to terminate the pregnancy.

Prenatal diagnosis of DM has been achieved before. Our experience indicates that second trimester placental biopsy, which is a safe and efficient procedure, allows very rapid fetal genotype analysis when used in combination with DNA amplification. Major indications for this protocol are late booking of prenatal diagnosis and lack of previous molecular characterisation of the family.

We thank Dr R G Korneluk (Ottawa) and Dr C Junien (Paris) for generously making data available in advance of publication and for encouragement and helpful collaboration. This work was supported by grants from Italian CNR PF Biotecnologie/Biostromazione and P F Ingegneria Genetica.