Rapid diagnosis of infantile spinal muscular atrophy by direct amplification of amniocyte and CVS DNA

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Our laboratory, like those of Daniels *et al*¹ and Melki *et al*,² is conducting prenatal diagnoses of type 1 spinal muscular atrophy (SMA) by means of linkage analysis with 5q13 polymorphisms. In our experience, a decision for pregnancy termination is taken if a diagnosis of SMA is made. Consequently, in the interests of generating a rapid prenatal SMA diagnosis, we have recently opted for a solely PCR based methodology using amniocyte or chorionic villus DNA as template.

A 1 year old infant girl who had been noted by her parents to be 'floppy and weak' from approximately 7 months was diagnosed as having type 1 SMA on the basis of EMG and muscle biopsy.³ The mother was 16 weeks pregnant at the time of diagnosis and consequently requested prenatal diagnosis. Amniocentesis was conducted the next day; 15 ml of amniotic fluid were drawn for cell culturing and eventual DNA extraction and an additional 9 ml were taken for direct 5q13 microsatellite CA repeat genotyping. Various approaches were used in the preparation of the amniotic fluid for PCR. The most effective was

found to be a Chelex (Bio-Rad) based approach⁴: 100 µl of amniotic fluid was mixed with an equal volume of 5% Chelex, heated at 56°C (30 minutes), vortexed, heated at 100°C (eight minutes), vortexed, and centrifuged (three minutes, 10 000 g). Optimal results were obtained using 5 to 10 µl of this supernatant in 25 µl PCR reaction volumes. Primer sequences and reaction conditions were as described¹⁻⁵⁻⁷ except that a two minute extension time was used for MAPIB microsatellite repeats. Reaction products were run on an 8% polyacrylamide gel, the gel dried, and autoradiographs developed.

In a second case, a 9 month old infant boy was admitted to paediatric ICU in respiratory failure. A diagnosis of type 1 SMA was made after EMG and muscle biopsy. The infant died at 10 months. The parents conceived again and a chorionic villus sampling was performed at 11 weeks. Two CVS strands were placed in 100 µl of dH₂O and then added to an equal volume of 5% Chelex. Treatment thereafter was identical to that used for amniocyte DNA.

The physical disposition and approximate

Figure 1 Pedigree of family 1.

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interloci genetic distances of the five 5q13 microsatellite loci used in this analysis are (% figures are recombinant fractions)⁵⁸⁹:

D5S76—(4%)—*D5S125*—(1%)—*SMA*—(1%)—*MAP1B*, *MAP2B*—(2.5%)—*D5S39*.

Total informativity was achieved for flanking markers, no marker-marker recombinations were observed, and, unfortunately, in both cases a positive diagnosis of SMA was made (figs 1 and 2).

The diagnosis was communicated to the first family five days after the amniocentesis and the pregnancy was terminated at 17 weeks of gestation. The second family was informed of the diagnosis at 11 weeks' gestation; the pregnancy was terminated at that stage.

We believe with subsequent refinement of this method that the time from amniocentesis to diagnosis, given informativity, will be three days. Moreover, the small amount of amniotic fluid (100 µl) required for amplification of microsatellite diagnosis by this methodology may permit amniocentesis at early stages of pregnancy. This approach to prenatal diagnosis is obviously applicable to other monogenic dis-

orders which are closely linked to microsatellite repeats.

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