Diagnosis of complete molar pregnancy by microsatellites in archival material

S A Lane, G R Taylor, B Ozols, P Quirke

Abstract

Aims—To develop an assay which would determine the parentage of hydatidiform molar pregnancies.

Methods—DNA was extracted from formalin fixed, paraffin wax embedded tissue from hydatidiform molar pregnancies and spontaneous abortions after separation of chorionic villi and decidua. PCR amplification of dinucleotide repeat sequences (“microsatellites”) was performed using three different primers. Products were radioactively labelled and visualised by autoradiography of dried polyacrylamide gels.

Results—With informative microsatellites, diagnostic patterns of amplification were obtained. Complete moles yielded either one or two microsatellites which differed from both maternal (decidual) microsatellites. Complete mole could be excluded by all the microsatellites showing alleles identical with those in maternal DNA.

Conclusions—This technique offers a method of determining the presence of entirely paternal alleles in a molar pregnancy and thus confirming a complete hydatidiform mole.

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0.97. Trace amounts of 35S labelled ATP were added to the PCR mixture to label the amplified products. In each reaction a negative (distilled water) and positive (normal human DNA) control were included in place of test DNA. The PCR mix comprised 50 mM KCl, 10 mM TRIS, 1.5 mM MgCl2, 0.01% gelatin, 0.4 mM each of dTTP, dCTP, and dGTP, 0.2 mM dATP, 0.1 μM of each oligonucleotide primer, 1.5 U super-Taq polymerase pH 8.3 (HT Biotechnology Ltd).

The thermal cycling sequence was as follows: denaturing at 95°C for 5 minutes, 35 cycles at 95°C for 30 seconds and 58°C for 2 minutes, final extension at 72°C for 5 minutes.

Products were denatured at 95°C for 3 minutes and electrophoresed at 2000 V in an 8% polyacrylamide gel in TBE (45 mM TRIS-borate, 1 mM EDTA). The gels were dried and autoradiography performed using high affinity Kodak x ray film for 3 to 10 days.

**Discussion**

These results provide a qualitative method of determining paternal or maternal origin of samples of hydatidiform mole tissue, as compared with maternal decidual tissue from archival material.

Using our panel of three microsatellite primers, an unambiguous result was found in all four cases of complete mole; complete moles yielded either a single or two microsatellite bands which differed from both maternal microsatellites with at least two primer sets. This pattern was not seen with the partial moles or spontaneous abortion tissues. A similar finding with complete moles was reported by Fukuyama et al. using the VNTR primer Apo2B on fresh molar tissue. Two of four of our cases, however, gave equivocal results with one primer and it is possible that, using only one primer, cases of complete mole may have been missed.

In the cases of partial mole only two alleles were identified, one of which was the same as a maternal allele: this did not exclude or confirm partial molar pregnancy, and a similar pattern was seen with four of five of the spontaneous abortion samples (table).

Microsatellite alleles may be as close as two base pairs from each other. Band laddering made readings of genotypes with allele sizes which were very close to each other problematic. In some cases of partial mole one allele

![Figure 1](http://jcp.bmj.com/)

**Figure 1** Products from the complete molar tissue (CM) differ from those of maternal decidua (D) with all three primer sets.

![Figure 2](http://jcp.bmj.com/)

**Figure 2** The staining intensity of one product from a partial mole (PM) is greater than the other and could indicate the presence of an identical third allele.
showed a much greater intensity. This may indicate the presence of the expected third allele in a triploid partial mole.

Determining which alleles are maternal or paternal would be easier if a blood sample was available from each parent and could confirm that the non-decidual alleles were indeed paternal. The use of a microsatellite on the Y chromosome would also confirm the paternal contribution in male moles or abortuses.

Work is ongoing to address these problems and to permit actual quantitative measurement of the PCR products by fluorescence. This will result in the determination of ploidy and the possible presence of a “double dose” of one allele from the same parent at the same time as paternity is determined.

Three cases of partial mole did not generate PCR products with any of the three primer sets. This may reflect the limited amount of molar tissue available in a case of partial mole which is a patchy process, although failure of PCR on archival material has been reported before.

In conclusion, this technique offers a confirmation of complete molar pregnancy with the presence of entirely paternal alleles in the molar tissue. Combined with flow cytometry to measure ploidy, a definitive diagnosis may be given and both techniques may be used on fresh or archival material.

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