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MATERNAL CONTAMINATION OF AMNIOTIC FLUID DEMONSTRATED BY DNA ANALYSIS

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SUMMARY

DNA from 16 sets of samples comprising DNA from uncultured amniotic fluid cells, cultured amniotic fluid cells, fetal tissue, and maternal blood was analysed by the polymerase chain reaction (PCR) with AC-repeat primers. The analysis was performed to investigate the presence of contaminating maternal cells in amniotic fluid which would affect the reliability of DNA studies for prenatal diagnosis. In three sets, maternal contamination of uncultured amniotic fluid cells was detected. In one of the three sets, maternal contamination was present in both uncultured and cultured amniotic fluid cells. The use of amniotic fluid cells as a source of DNA for prenatal diagnosis should be limited to cases where the purity of the DNA can be demonstrated prior to the diagnostic test being performed. This limitation in the use of amniotic fluid DNA also extends to other forms of diagnosis relying on the purity of amniotic fluid samples, particularly the new *in situ* hybridization methods currently being developed.

KEY WORDS—Amniotic fluid, prenatal diagnosis, PCR, *in situ* hybridization.

INTRODUCTION

Recent developments in the prenatal diagnosis of chromosome aberrations have been concerned with reducing the gestational age at the time of the test. This has been achieved by the introduction of chorionic villus sampling (CVS) (Teitung Hospital, 1975; Kazy *et al.*, 1982; Rodeck *et al.*, 1983) and, more recently, the development of early amniocentesis (Rooney *et al.*, 1989; Nevin *et al.*, 1990). As part of our continuing research into early amniocentesis, a study of the potential usefulness of DNA extracted from uncultured early amniotic fluid cell samples for molecular genetic diagnosis has been undertaken.

It has been demonstrated that sufficient DNA can be extracted from early amniotic fluid samples for diagnosis by the polymerase chain reaction (PCR) (Rebello *et al.*, 1991). However, an important area for concern is the possibility of maternal contamination of the DNA sample and the risk of misdiagnosis that this may introduce. In order to test the purity of DNA samples extracted from uncultured amniotic fluid samples, we performed an analysis of DNA on a number of samples using PCR for single copy repeat regions (Weber and May, 1989; Litt and Luty, 1989). Our findings have important implications for prenatal diagnosis using PCR or *in situ* hybridization on interphase cells in amniotic fluid.

MATERIALS AND METHODS

Samples

The samples were taken, with the informed consent of the patient, prior to termination of pregnancy. A set of samples was taken from each patient comprising amniotic fluid, maternal blood, and fetal tissue. The amniotic fluid was collected in 5 ml aliquots. Maternal blood was collected in 5 ml EDTA tubes. The fetal tissue was collected in sterile saline immediately after the termination.

The amniotic fluid and fetal tissue samples were prepared for DNA analysis as described previously (Rebello *et al.*, 1991). The maternal blood was freeze-thawed and a 200 μl aliquot was washed in 0·17 M NH_4Cl to remove all the visible haem before being boiled in 500 μl of 50 mM NaOH for

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Table I—Results of amplification with MTC112 primers

Sample reference	Gestational age (weeks)	Amplification with MCT			
		Amino	C-Amino	Fetus	M-Blood
KC	10	E		E	E
KP	10	E		E	E
KK	11	E		E	E
KT	11	M		E	E
KA	12	E		E	E
KL	12	E		E	E
KM	12	E		E	E
KY	12	E		E	E
KB	13	E		E	E
KQ	11	M	M	E	E
KV	11	M	E	E	E
KS	12	E	E	E	E
KU	12	E	E	E	E
KX	12	E	E	E	E
KN	15	E	E	E	E
KW	15	E	E	E	E

E=Amplification of fragments of the expected size; M=amplification of mixed DNA.

15 min to rupture the cells. The samples were neutralized with 100 μl of 1 M Tris (pH 7·5), and 10 μl aliquots were used for the PCRs.

Polymerase chain reactions

The primers used in the PCRs were the MCT112 primers described by Wallis *et al.* (1990) and were used according to the method described by Wallis *et al.* The amplified products were visualized after electrophoresis on a standard sequencing gel and overnight exposure on Kodak X-Omat film.

RESULTS

The results are summarized in Table I. Complete sets of samples were taken from 16 cases with a gestational age range of 10–15 weeks. Seven of these sets included DNA from cultured and uncultured amniotic fluid cells, while the DNA in the remaining nine sets was extracted from uncultured amniotic fluid cells only. Of the 16 sets of samples, three sets—KT, KQ, and KV—showed clear evidence of maternal contamination of the amniotic fluid. Two of these sets, KQ and KV, included DNA from cultured amniotic fluid samples. Set KQ showed contamination of both the cultured and the uncultured samples, and set KV showed contamination of the uncultured sample only.

Figure 1 comprises three sets of samples showing the allelic segregation and demonstrating the presence of contamination.

DISCUSSION

The primers used in the PCR generate variable length fragments which are inherited in a Mendelian fashion and can be used to observe patterns of inheritance in related individuals, and detect maternal contamination of fetal DNA.

There was evidence of maternal contamination of the amniotic fluid in three of the 16 sets amplified with the MCT112 primers. This is clearly shown in lane D of sets KV and KT of Fig. 1. Our interest in amniotic fluid DNA was to find a safer alternative to CVS for early prenatal diagnosis. This finding, however, leads us to conclude that DNA extracted from amniotic fluid, even after culture, is unsuitable for diagnostic genetic prenatal diagnosis using PCR unless precautions are taken to ensure the purity of the DNA sample prior to testing. This could be achieved by analysing the samples with PCR primers as described here.

In an earlier report (Rebello *et al.*, 1991), we analysed 14 sets of samples, comprising amniotic

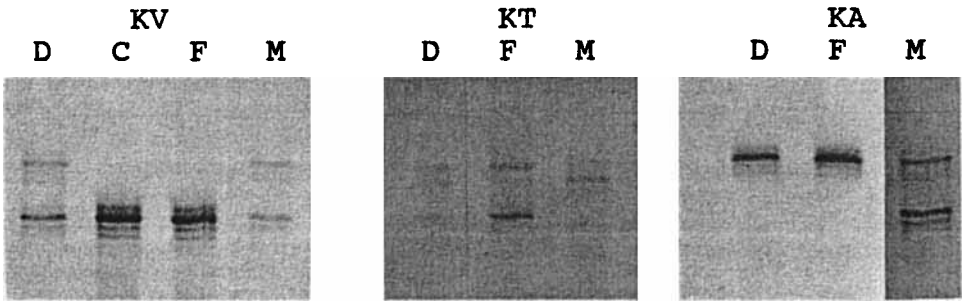

Fig. 1—PCR products after MCT amplification. Set KV demonstrates the banding patterns from a contaminated direct amniotic fluid DNa in lane D; lane C shows the cultured amniotic fluid DNA which has no maternal contamination. Set KT demonstrates the three-band pattern predicted for detection of maternal contamination in lane D, and the bands can be correlated with the fetal and maternal bands in lanes F and M. Set KA demonstrates an uncontaminated sample in lane D with the maternal alleles distinguishable in lane M. D=Direct amniotic fluid DNA; C=cultured amniotic fluid DNA; F=fetal tissue DNA; M=maternal blood DNA

fluid DNA and fetal DNA, with Mfd5 primers (Weber and May, 1989). No evidence of maternal contamination was noted in this study; this is probably due to the fact that the Mfd5 primers are less polymorphic than the MCT primers and there is therefore a greater chance that the mother will have the same alleles as the fetus. In this event, detection of maternal contamination would be impossible. Since maternal typing was not included in the initial study, this potential error was not appreciated at the time.

The factors mentioned above highlight the importance of using informative primers for this analysis.

The fact that contaminating maternal cells in amniotic fluid may affect the results of prenatal diagnosis by *in situ* hybridization has been acknowledged by several authors (Guyot *et al.*, 1988; Christensen *et al.*, 1992; Bryndorf *et al.*, 1992). Consequently, scientists in this field exclude visibly blood-stained samples for this very reason. Our results suggest that even microscopic levels of maternal contamination may give erroneous results.

The effect of contamination of amniotic fluid with even a small amount of maternal blood is best illustrated by example. In amniotic fluid from a normal fetus where all the cells are diploid, the proportions of cells having one, two, three, or four signals may be 30, 60, 5, and 5 per cent, respectively; whereas in a trisomic fetus, the proportions may be 5, 30, 60, and 5 per cent, respectively (Christensen *et al.*, 1992). We have shown that the cell counts in amniotic fluid samples range from 400 cells/ml at 8 weeks' gestation to 4000 cells/ml at 17 weeks' gestation (Rebello, M. T., unpublished data), and since the average leucocyte count in adult blood is 7×10^6 cells/ml of blood, the number of cells contributed by maternal blood will be at least 1000 times more, per ml, than the number in amniotic fluid. Therefore, in the case of the trisomy described above, contamination of a 10 ml amniotic fluid sample by even a small volume (10 μl) of maternal blood will add the same number of leucocytes as there are cells in the original amniotic fluid sample. Thus, the proportion of nuclei with two signals will increase and the new ratio of signal numbers will be 17, 45, 33, and 5 per cent. The proportion of nuclei with two signals is now greater than the proportion of nuclei with three signals and interpretation of the result becomes much more difficult.

There is, at present, no method for detecting or excluding maternal contamination in all samples prepared for *in situ* hybridization, and until that is achieved, the analysis of diagnostic samples by these methods should be undertaken with extreme caution.

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REFERENCES

Bryndorf, T., Christensen, B., Philip, J., Hansen, W., Yokobata, K., Bui, N., Gaiser, C. (1992). New rapid test for prenatal detection of trisomy 21 (Down's

syndrome): preliminary report, *Br. Med. J.*, **304**, 1536–1539.

Christensen, B., Bryndorf, T., Philip, J., Lundsteen, C., Hansen, W. (1992). Rapid prenatal diagnosis of trisomy 18 and triploidy in interphase nuclei of uncultured amniocytes by non-radioactive *in situ* hybridisation, *Prenat. Diagn.*, **12**, 241–250.

Guyot, B., Bazin, A., Sole, Y., Julien, C., Dafos, F., Forestier, F. (1988). Prenatal diagnosis with biotinylated chromosome specific probes, *Prenat. Diagn.*, **8**, 485–493.

Kazy, Z., Rozovsky, I.S., Bakharev, V.A. (1982). Chorion biopsy in early pregnancy: a method of early prenatal diagnosis for inherited disorders, *Prenat. Diagn.*, **2**, 39–45.

Litt, M., Luty, J.A. (1989). A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene, *Am. J. Hum. Genet.*, **44**, 397.

Nevin, J., Nevin, N.C., Dornan, J.C., Sim, D., Armstrong, M.J. (1990). Early amniocentesis: experience of 222 consecutive patients, *Prenat. Diagn.*, **10**, 79–83.

Rebello, M.T., Hackett, G., Smith, J., Loeffler, F.E., Robson, S., MacLachlan, N., Beard, R.W., Rodeck, C.H., Williamson, R., Coleman, D.V., Williams, C. (1991). Extraction of DNA from amniotic fluid cells for the early prenatal diagnosis of genetic disease, *Prenat. Diagn.*, **11**, 41–46.

Rodeck, C.H., Morsman, J.M., Gosden, C.M., Gosden, J.R. (1983). Development of an improved technique for first-trimester microsampling of chorion, *Br. J. Obstet. Gynaecol.*, **90**, 1113–1983.

Rooney, D.E., MacLachlan, N., Smith, J., Rebello, M.T., Loeffler, F.E., Beard, R.W., Rodeck, C., Coleman, D.V. (1989). Early amniocentesis: a cytogenetic evaluation, *Br. Med. J.*, **299**, 25.

Teitung Hospital of Anshan Iron and Steel Company, Department of Obstetrics and Gynaecology, Anshan (1975). Fetal sex prediction by sex chromatin or chorionic villus cells during early pregnancy, *Chin. Med. J.*, **1**, 117.

Wallis, J., Williamson, R., Chamberlain, S. (1990). Identification of a hypervariable microsatellite polymorphism within D9S15 tightly linked to Friedreich's ataxia, *Hum. Genet.*, **85**, 98.

Weber, J.L., May, P.E. (1988). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction, *Am. J. Hum. Genet.*, **44**, 388–396.