Raising the sensitivity of fetal RhD typing and sex determination from maternal blood

The fact that all currently available methods for tissue sampling carry procedure related risks has been a motivation for many years to develop less invasive techniques for prenatal diagnosis, ideally by studying fetal cells in the maternal circulation. Methods directly detecting fetal DNA by PCR amplification were for the first time performed on fetal cells from maternal blood have been successfully established. However, these methods have so far not reached the precision required for routine application, since rates of false results, about 20% and even higher, are very high. On testing PCR based sex determination and RhD typing in over 100 samples of amniotic cells, chorionic villi, cord blood, or peripheral blood, we obtained no false results.

Chromosomal typing of the relevant chromosome in females had to be used without previous PCR based sex determination, general concerns about discrepancies between serological typing and

PCR derived RhD type have been raised. Recently, a large scale evaluation of the accuracy of PCR based sex determination with using DNA derived from adult blood samples was performed. Two of 632 RhD positive subjects were characterised as RhD negative and seven of the 133 RhD negative subjects were characterised as RhD positive. Therefore, the false positive results might also lead to the interpretation that not all RhD negative subjects exhibit total loss of the RhD gene. Our data do not support this interpretation. There is no indication that avces to avoid sample contamination and by including positive and negative controls, we did not obtain any false results doing PCR analysis of over 100 samples of amniotic cells, chorionic villi, cord blood, or peripheral blood. However, when we analysed fetal sex and RhD status by PCR from maternal blood samples, we obtained a relatively high rate of false positive results. The assumption that these false positive results are not the result of inaccuracy in our PCR technique is further supported by the observation that analysing artificial DNA dilution from X chromosome DNA or the RhD gene with excess of negative DNA did not lead to these rates of false positives. An alternative interpretation might be that these results represent a fundamental biological phenomenon because of fetal cells in the maternal circulation from previous pregnancies or a previous maternal blood transfusion. In the cases presented here we could not explain the observed high rate of false positive results this way.

In summary, the data presented here describe a successful approach to increasing the sensitivity of fetal PCR diagnosis from maternal blood by reducing the rate of false negative results. However, as long as the PCR setting is not optimised to diminish the observed positive results, one might wonder whether this diagnostic approach can routinely be used without previous enrichment of fetal cells.

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