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Fetal sex determination from maternal plasma by nested PCR of the amelogenin gene

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Abstract. Fetal sex determination from circulating DNA in maternal plasma has been reported to be a reliable way to avoid invasive prenatal tests. The aim of this work is to reduce the length of the fragments to be amplified with two sucesive PCRs: amelogenin amplification for chromosomes X and Y, and a nested PCR reamplifying only Y chromosome amelogenin fragment. © 2005 Elsevier B.V. All rights reserved.

Keywords: Fetal DNA; Maternal plasma; Amelogenin; Nested PCR

1. Introduction

Fetal sex determination from circulating DNA in maternal plasma has been reported to be a reliable way to avoid invasive prenatal tests, such as amniocentesis or chorionic villus sampling, in pregnancies with high risk of X-linked disorders. This plasma-circulating DNA is highly degraded and makes its detection very difficult. In forensic casework, the analysis of cell free fetal DNA in maternal blood is of great interest for the development prenatal paternity testing. For this purpose, we have started the study of the Y chromosome amelogenin gene in circulating DNA as an initial approach to the analysis of Y chromosome markers with interest in forensic genetics.

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2. Methodology

2.1. Blood sample collection and plasma separation

Twenty-two samples from second- and third-trimester pregnancies were analyzed. Maternal blood samples were collected in Vacutainer EDTA containing tubes and preserved at 4 °C until DNA purification was performed.

Plasma was obtained by centrifugation of maternal blood samples at 2000 rpm for 10 min in a Heraeus Multifuge 3 S-R and aliquoted in 2-ml Eppendorf tubes.

2.2. Plasma DNA purification

DNA purification was carried out from 600 μl plasma aliquots. Approximately 8 aliquots were obtained from each plasma sample. Lysis was performed only adding with Proteinase K (60 mg) and SDS (0.2%) and DNA was purified by phenol–chloroform method and precipitated with ethanol [1]. Purified DNA was resuspended in 10 μl of sterile water.

Once DNA was obtained from the different aliquots of the same sample, they were all mixed in the same Eppendorf, obtaining approximately 80 μl of DNA from each plasma sample. This DNA was then concentrated in Microcon YM-100 columns (Millipore).

2.3. Amelogenin gene amplification and nested PCR

Plasma DNA was amplified for the amelogenin gene [2], obtaining two fragments of 112 bp (Y chromosome amelogenin) and 106 bp (X chromosome amelogenin) in male-carrying pregnancies and only a 106-bp fragment in female-carrying pregnancies. PCR was carried out in an iCycler (BioRad) during 40 cycles with an annealing temperature of 57 °C. Amelogenin amplification was visualized in 2% agarose gels and UV illumination.

Nested PCR of the amelogenin gene was carried out with specific primers designed to reamplify only Y chromosome fragment from previous PCR. A touchdown PCR program was performed during 35 cycles with an annealing temperature of 65 and 67 °C. Reamplification was visualized as described before.

3. Results and conclusions

Twenty-two samples of maternal plasma from second and third trimester of pregnancy were collected. The results of PCR amelogenin amplification are shown in Fig. 1.

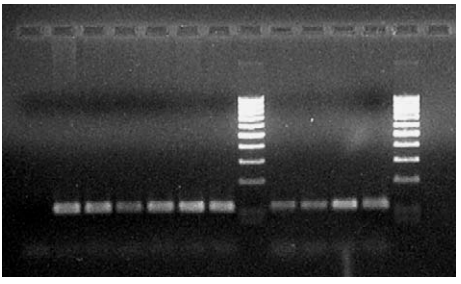

Fig. 1. Amelogenin amplification of some of the samples within the study.

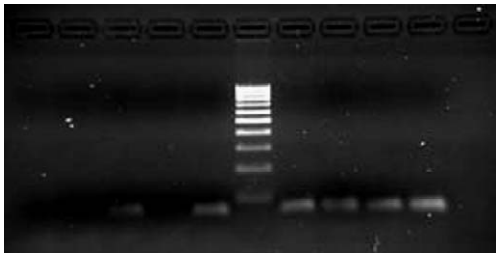

Fig. 2. Amelogenin gene nested PCR amplification. Sample in lane 3 is the amelogenin false-positive.

The nested PCR specific for Y chromosome detected 10 male- and 11 female-carrying pregnancies (Fig. 2). After child birth, only one female pregnancy resulted in nested PCR positive amplification. The percentage of false positive results is 4.5%. However, in prenatal diagnosis of X chromosome linked diseases this false-positive value is not significant because it will never result in an erroneous diagnosis of the disease, but only in a mutation screening.

In conclusion, the method described here has detected one false positive, but no false negative, so it could be considered a reliable approach to fetal sex determination from maternal plasma.

The results obtained in this study are very promising for future analysis of markers currently used in forensic genetics.

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