

STR - Analysis of maternal and foetal DNA in human placenta: a preliminary study

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Abstract

The aim of this study was to evaluate the possibility of obtaining maternal and foetal genetic profiles analysing the most common polymorphic markers (microsatellites - STR) in the forensic field, with extraction and typing of human genomic DNA at different times from placenta expulsion using different conservation methods.

Although the DNA extracted from all the samples was useful in the analysis of the STR polymorphisms, only mixed profiles were obtained. This suggests that sampling with the naked eye or the stereomicroscope does not allow for complete separation of the maternal side from the foetal side and, therefore, the characterization of the single genetic profiles.

The authors propose that the use of alternative isolation methods, such as the laser microdissection may be of help in solving this problem.

Introduction

As both in medical and forensic settings, it may be useful to be able to identify, who the placenta belongs to, in case of abortion or delivery, or to make paternity testing without the foetus or newborn, or even to resolve issues of professional liability related to diagnostic examinations.

A study was carried out to evaluate the feasibility of obtaining maternal and foetal genetic profiles from placenta by the analysis of the most common *short tandem repeat* (STR) at various sampling times after placenta expulsion. Sampling was performed on both fresh and stored tissue.

Materials and Methods

Sampling was carried out from a human placenta after eutocic delivery. Part of each specimen was used to prepare histological samples for microscopic observation, whilst the remaining sections were analysed both fresh and stored at room temperature, at -20°C or fixed in Complucad[®] (Muniz et al. 2000).

Sections from different parts of the placenta were taken: i.e. three cotyledons, which included both the foetal (chorionic plate) and the maternal side (decidua basalis), were isolated. Two samples (one from each side of placenta) were collected at time zero, from one of the cotyledons, the rest was stored at room temperature; a second cotyledon was then stored at -20°C and the third one fixed in Complucad[®].

Further sampling was done on each cotyledon, at one week followed by one, six and ten months. The NucleoMag 96 Blood Kit (Macherey-Nagel, Düren, Germany) was used to isolate DNA from the samples which, after cell lysis with chaotropic reagents and proteinase-K, was bound to silica-coated magnetic beads. Washing and elution steps were performed using the automatic magnetic separator KingFisher mL (Thermo LabSystem, Vantaa, Finland). Each sample was amplified for the single F13B locus (Nishimura & Murray, 1992) so as to establish the presence of any human genomic DNA for the STR analysis.

The isolated DNA was then amplified by the multiplex PCR of 15 STR loci and the Amelogenin gender-determinating marker (Nakahori et al, 1991) using the "AmpFISTR Identifiler PCR Amplification Kit" (Applied Biosystems), according to the manufacture's recommendations.

The PCR products were separated by capillary electrophoresis on the ABI PRISM[™] 310 Genetic Analyser (Applied Biosystems), electrophoretic data were analysed using Genotyper 3.7 software (Applied Biosystems).

Results and Discussion

Although it was possible to extract adequate DNA, in terms of both quality and quantity, for STR analysis, from all the samples, at different sampling times and under the various storage conditions, only mixed profiles (mother-

foetus) were obtained. This led to the conclusion that sampling with either the naked eye or the stereomicroscope does not allow for the complete separation of the maternal from the foetal side, and, therefore, the characterization of the single genetic profiles.

The authors propose the adoption of alternative isolation methods, such as the laser microdissection (Robino et al, 2006), in their ongoing study in an effort to solve this problem.

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