A53 Detection of Differentially Methylated Parental Allele in Imprinted Gene SNRPN Using MS-SSCA

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After attending this presentation, attendees will learn a new method to identify differentially methylated parental alleles.

This presentation will impact the forensic science community by using MS-SSCA in dried blood spots to identify differentially methylated parental alleles.

In a classic forensic paternity test, the obligatory gene often cannot be determined in motherless cases, or when the mother and child share the same heterozygous genotype. Recently, the combination of Single Nucleotide Polymorphism (SNP) typing and genomic imprinting has shown promising potential in paternity testing or personal identification. SNPs are the most abundant type of human polymorphism, and became valuable markers for forensic identification and paternal testing. Determining the parental origin of SNPs is helpful to some cases. Genomic imprinting is the phenomenon where the two alleles of some genes are differentially expressed according to their parental origin. DNA methylation pattern of an imprinted locus is unique to each allele. The parental origin of specific methylation status at the imprinted loci provides a new means to detect the obligatory gene without genealogical analysis. Classic techniques to detect the differentially methylated parental alleles are methylation-specific PCR (MSP) and post-digestion PCR. The established procedures; however, often require relatively large amounts of DNA. In daily practice, samples submitted for analysis might contain very small amounts of poor quality material, as is often the case with forensic stain samples, while dried blood spots have become preferred forensic samples in many forensic laboratories, as they are easy to collect, transport, and store. In this study, a new technique was developed that only requires small amount DNA to identify the obligatory gene in forensic paternity testing. First, a modified, more efficient method of bisulfite genomic sequencing in dried blood spots was tested. Briefly, the genomic DNA extracted from 3mm dried blood spots using QIAamp micro kit was treated with sodium bisulfite (EpiTect) followed by methylation-specific PCR (MSP) and Sanger sequencing. The imprinted region the gene, small nuclear ribonucleotide protein N (SNRPN) was examined for differential methylation. The data shows efficient DNA extraction and full conversion of unmethylated cytosine to uracil. Second, the methylation-sensitive single-strand conformation polymorphism (MS-SSCA) method to detect the parental origin of the allele of SNP locus rs220030 (C/T) was used. The SNP locus rs220030 is found in the promoter region of the maternal imprinted gene SNRPN. Rs220030 (C/T) in maternal imprinted gene SNRPN was typed using the TaqMan SNP Genotyping assay. The methylated status of maternal and paternal alleles were determined by MS-SSCA on four family trios with heterozygous children. The results have shown that MS-SSCA has great potential in the discrimination of differentially methylated parental alleles of imprinted genes in forensic paternity testing. This study suggested that bisulfite treatment of genomic DNA, combined with classical non-denaturing polyacrylamide electrophoresis can become a new parental origin determination method in imprinted genes for forensic purposes.

DNA Methylation, Methylated Parental, MS-SSCA

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