Screening of high-effective DNA methylation markers for discriminating monozygotic twins

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The ability to distinguish monozygotic (MZ) twins is an unresolved issue in forensic investigations. We and others have revealed the potential of DNA methylation as a marker for forensic MZ twin discrimination. We screened hundreds of differentially methylated regions (tDMRs) from the whole genomes of newborn MZ twin pairs using MeDIP sequencing. Here, we selected several tDMRs and assessed their potential as markers to differentiate MZ twins.

We assessed 120 MZ twin pairs. The methylation of nine candidate regions including 3 genic-sequences (GSs) and 6 intergenic-sequences (IGSs) were detected through bisulphite-pyrosequencing. Using thresholds of 5%, 10%, 20% and 30%, we identified that all regions except for GS2 represented distinguishable MZ twin pairs, accumulatively reaching 97.50%, 53.33%, 22.50% and 13.33% respectively. IGSs were significantly more powerful than GSs as a whole. IGS2 most strongly discriminated the majority of MZ twins whilst IGS4 discriminated one pair of MZ twins with the largest methylation difference (≥ 70%). We found that the degree of methylation in mouthwash samples was lower than that of blood samples, but methylation differences were more distinguishable in mouthwash samples, indicating that reference and trace samples should originate from the same tissues for forensic applications.

In conclusion, we recommend 10% as the minimum threshold for differentiating MZ twins using bisulphite pyrosequencing and that IGSs are the preferred sites for discriminating MZ twins. More effective regions like IGS2 or IGS4 would be identified to enhance the discrimination power.

Keywords: Monozygotic twins; DNA methylation; Bisulphite conversion; Pyrosequencing; Intergenic region

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