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"Investigating DNA Methylation Analysis for the Individualization of Monozygotic Twins"
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A known issue that arises in forensic cases is the differentiation of monozygotic (identical) twin DNA. Monozygotic twins share the same exact DNA sequence, making it difficult to identify an individual who is a monozygotic twin. Epigenetics, and more specifically, DNA methylation, is novel field that has gained the interest of forensic scientists. DNA methylation is an epigenetic process of the addition of a methyl group to a cytosine base of DNA, occurring at cytosine-guanine dinucleotides (CpG sites). DNA methylation patterns are unique for every individual, and therefore could also be unique for monozygotic twins [1]. It is crucial to investigate the ideal methodology and candidate CpG sites that provide individualization in order to implement this novel method into forensic science laboratories. An extensive search of published literature revealed that the KIFC3 gene marker could be a potential candidate target for the individualization of monozygotic twins, and that the EF1α gene marker is an endogenous control gene. The aim of this research was to investigate, using real-time quantitative-PCR (q-PCR), the DNA methylation patterns of the KIFC3 and EF1α gene markers, reported to be associated with differentiation of monozygotic twins.

Following approval from the Institutional Review Board with written informed consent, buccal swabs were collected from four pairs of monozygotic twins. The QIAamp DNA Investigator kit (Qiagen®) was used to extract DNA from the collected samples following the manufacturer's protocol. Following extraction, the samples were quantified using the Qubit 3.0 Fluorometer (ThermoFisher Scientific) using the double stranded DNA High Sensitivity assay. The EpiTect Bisulfite Kit (Qiagen®) was utilized to perform bisulfite conversion on the DNA extracts to prepare the samples for quantitative-PCR, following manufacturer protocol. The Epitect MethylLight PCR +ROX Vial Kit (Qiagen®) was used for quantitative methylation analysis with methylation-specific primers and probes, targeting KIFC3, and EF1α as the housekeeping gene. MethyLight specific primers and Taqman™ probes for the KIFC3 and EF1α markers were designed using the MethPrimer 2.0 software. The DNA samples were quantified using the Applied Biosystems™ 7500 Real Time-PCR Instrument according to the Epitect MethylLight PCR +ROX Vial Kit (Qiagen®) protocol. Following quantitative-PCR, the cycle threshold (Ct) values of the methylated and unmethylated regions were obtained. Several samples for the KIFC3 gene marker were undetected, and the gene marker EF1α was not detected in any sample. Both twins in twin pairs 3 and 4 obtained Ct values for the methylated and unmethylated KIFC3 markers; however, these values yielded statistically insignificant results. Conclusively, the EpiTect MethyLight PCR +ROX Vial Kit (Qiagen®) is not an optimal method for DNA methylation analysis. The gene marker EF1α, while reported to be endogenous in human samples, was not detected in any sample after bisulfite conversion and the KIFC3 gene marker did not produce statistically significant results. It is suggested that the bisulfite conversion step is deleterious to the samples therefore impacting the ability to obtain usable data. Further analyses need to be performed in order to determine an optimal method and gene markers for the purpose of individualizing monozygotic twin DNA.

References

1. Rawat, Suchita, and K. P. S. Kushwaha. 2016. “Application of DNA Methylation in Forensic Science: A Review.” Indian Journal of Forensic Medicine & Toxicology 10 (2): 108–12. doi:10.5958/0973- 9130.2016.00073.6.