Investigating DNA Methylation Analysis for the Individualization of Monozygotic Twins

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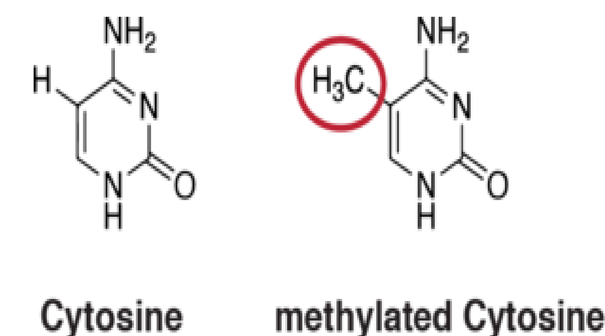
INTRODUCTION

A known issue that can arise in forensic investigations is the differentiation between monozygotic (identical) twin DNA. Monozygotic twins share the exact same DNA sequence, therefore identifying them with traditional STR profiling is not possible.

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).



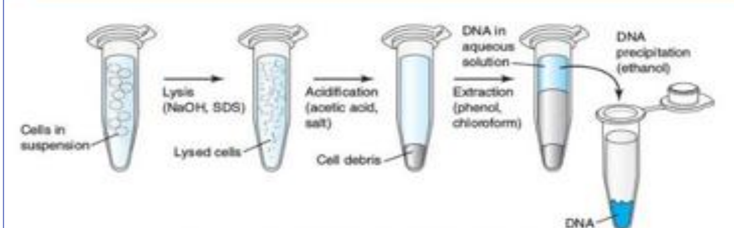
DNA methylation patterns can be unique for each individual and for identical twins [1]. This field of research is at the forefront of forensic science for various purposes, including body fluid identification, age estimation, and more recently, monozygotic twin identification [2]. Several commercial kits have been created for the purpose of DNA methylation profiling, and many studies have been performed to investigate different CpG sites and genes in an attempt to identify potential DNA methylation markers that could be used to individualize monozygotic twins. It is crucial to investigate the ideal methodology and candidate CpG sites that provides individualization in order to implement this novel method into forensic science laboratories.

RESEARCH AIM

- 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.

METHODOLOGY

1. DNA Extraction



QIAmp DNA Investigator Extraction Kit (Qiagen®)
4 pairs of twins: total 8 samples

2. DNA Quantitation

Nanodrop One UV-Vis Spectrophotometer
Qubit 3.0 Fluorometer

3. Bisulfite Conversion

4. qPCR of Targets

Targets: KIFC3 & EF1α

RESULTS

- Quantifiable amounts of DNA were recovered from each sample.
- Several samples for the KIFC3 gene marker were undetected.
- The gene marker EF1α was undetected in all samples.

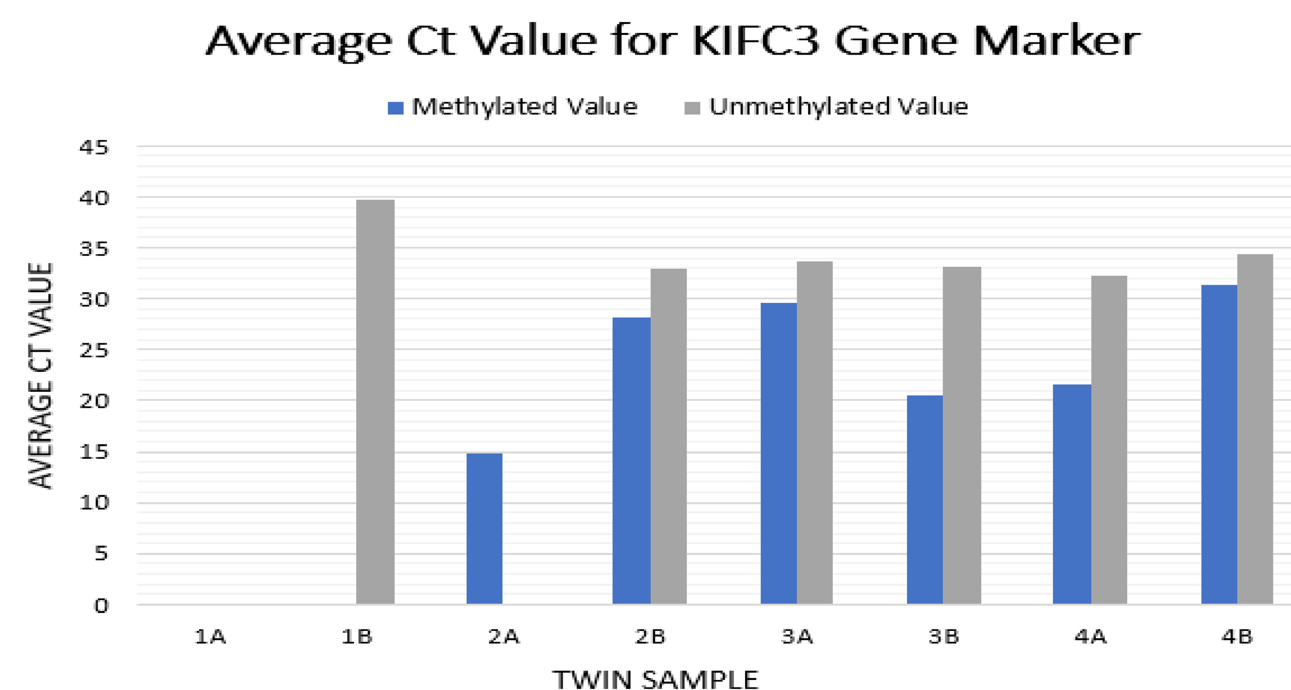


Figure 1: Average Methylated and Unmethylated Ct Values for the KIFC3 Gene Marker

- Twin pairs 3 and 4 obtained several Ct (cycle threshold) values and the twins were further compared to determine if the methylation and unmethylation levels were significantly different.

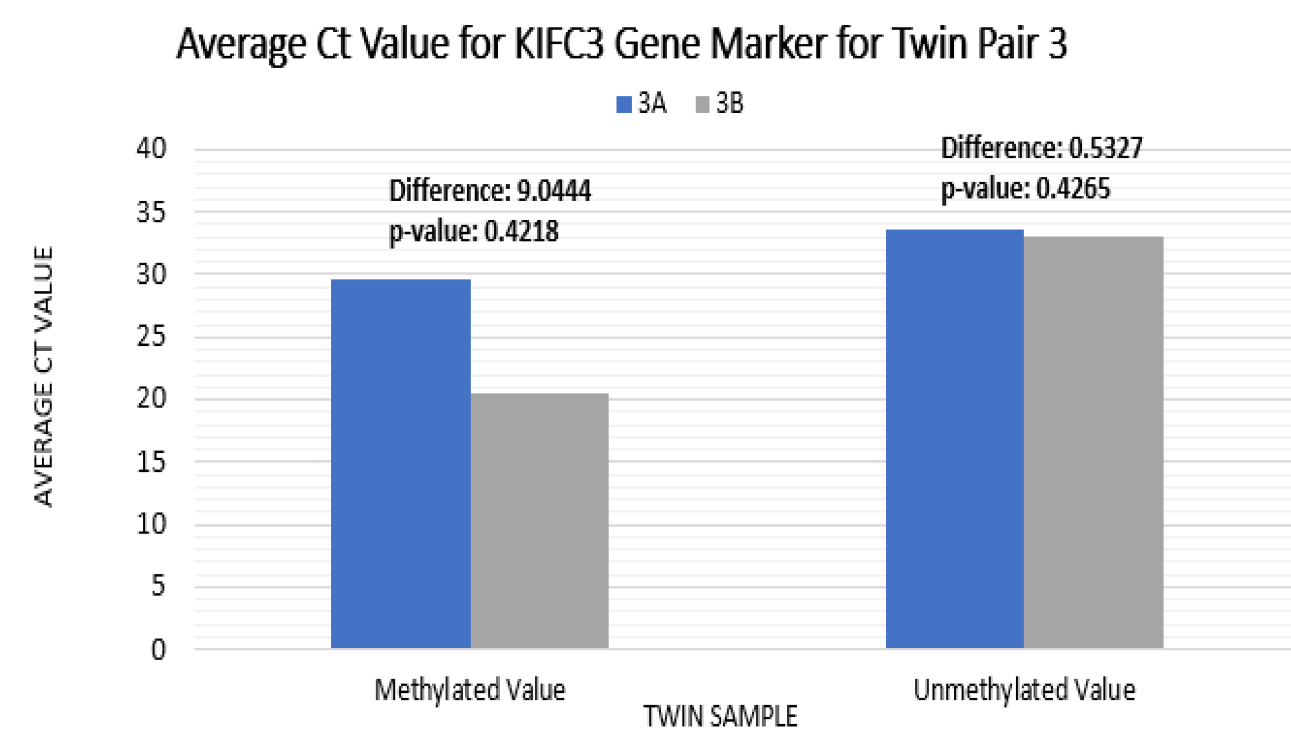


Figure 2: Average Methylated and Unmethylated Ct Value for the KIFC3 gene marker for twins 3A and 3B

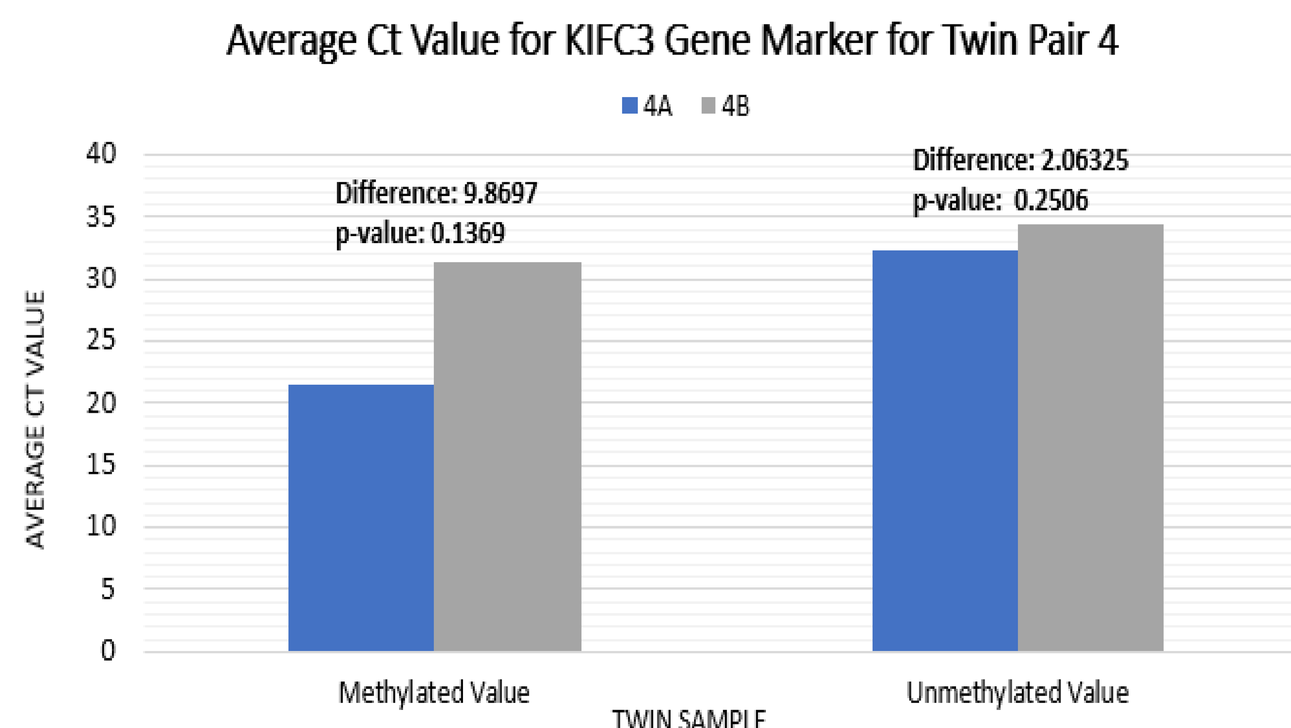


Figure 3: Average Methylated and Unmethylated Ct Value for the KIFC3 gene marker for twins 4A and 4B

DISCUSSION

- All samples obtained quantifiable amounts of DNA.
- Several samples for the KIFC3 gene marker were undetected. 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.
- The Ct values comparing the methylated and unmethylated levels for twins 3A and 3B and 4A and 4B yielded statistically insignificant results.
- We suggest that bisulfite conversion is destructive to the genetic material, which therefore could be detrimental to the results obtained.
- This suggests that q-PCR may not be an ideal approach for analyzing DNA methylation patterns. Pyrosequencing may be a more promising methodology for this application.

CONCLUSION

It was shown that 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. Few samples were detected from the KIFC3 gene marker, and the 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.
2. Vidaki A, Kayser M, Recent progress, methods and perspectives in forensic epigenetics, Forensic Science International: Genetics (2018), https://doi.org/10.1016/j.fsigen.2018.08.008

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