F24 Is Epigenetics Ready for Prime Time? The Potential of Using DNA Methylation Patterns to Differentiate Monozygotic Twins and to Estimate DNA Donor Age

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Learning Overview: After attending this presentation, attendees will understand the potential for using epigenetic science in the form of DNA methylation patterns as forensic evidence.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by summarizing the scientific literature on DNA methylation studies as it relates to distinguishing monozygotic twins and determining DNA donor age and addressing whether the present state of the research is sufficient to meet *Daubert* or *Frye* standards for courtroom admissibility.

Forensic DNA testing was first used in the 1980s and has since revolutionized forensic science. There have been tremendous advances in DNA technology through the decades, including Polymerase Chain Reaction (PCR) amplification and probabilistic genotyping, yet there remains limits to what traditional DNA testing can do. Among those are: (1) differentiating between monozygotic twins, and (2) estimating the age of a DNA donor.

The discipline of epigenetics offers a potential answer to these questions that may be on the cusp of qualifying for courtroom admissibility. The *Oxford Dictionary* defines epigenetics as "[t]he study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself." "Epi-" is a Greek prefix meaning "above," "upon," or "in addition to." Here, this presentation will be looking at some of the potential information that can be found in a DNA sample "in addition to" the autosomal DNA profile.

The focus of this presentation will be on one area of epigenetics—DNA methylation. DNA methylation means the addition of methyl groups (CH_3) to the DNA molecule. The methyl groups do not change the DNA code or sequence, but they change the way the DNA is expressed. Stated another way, DNA methylation results in gene silencing. This is necessary for normal growth and development, but can also affect disease progression.[1,2]

Of the four DNA bases, traditional dogma holds that only Cytosine (C) can be methylated in mammals—recent research suggests that Adenine (A) can also be methylated; thus nearly all of the scientific research has involved cytosine.[3,4] When cytosine is methylated it becomes 5-methylcytosine (5-mC). Most 5-mCs are found adjacent to Guanine (G) bases, which are called "CpG sites." Clusters of CpG sites are called "CpG islands." A test called "bisulfite sequencing" can be used to develop a DNA methylation profile. When DNA is processed using sodium bisulfite, methylated cytosine remains cytosine, while unmethylated cytosine changes to uracil, thus making it relatively easy to map DNA methylation patterns via conventional sequencing methods.[1,2,5]

The scientific literature on DNA methylation is extensive and goes back more than a half century. In 1945, scientist Rollin Hotchkiss described his findings on the methylation of cytosine into 5-methylcytosine. In the 1970s, Sir Adrian Bird described the role DNA methylation plays in gene transcription. The bisulfite sequencing test was invented in the 1990s by Marianne Frommer and Susan Clark and is considered the gold standard of DNA methylation analysis.[1,5] Over the decades, numerous studies, many of them involving twins, have reported findings based upon DNA methylation. Therefore, there exists a mature, extensive body of scientific research involving DNA methylation and, in recent years, some studies have specifically discussed the potential of DNA methylation science as forensic evidence.[6,7]

Several research papers reporting the ability to differentiate monozygotic twins using DNA methylation patterns will be discussed.[8-17] The extent and quality of the research will be examined with an eye toward whether they appear sufficient to meet *Dauber* or *Frye* standards.[18,19]

The case of *Commonwealth v Dwayne McNair* will also be reviewed.[20] In *McNair*, the prosecution attempted to use a different type of DNA test to distinguish monozygotic twins.[20] Specifically, the prosecution attempted to use ultra-deep next generation sequencing to search for somatic mutations.[21] The cost of the test was reportedly $120,000. In April 2017, the trial judge ruled that such testing does not meet *Daubert* standards for admissibility. The decision was not appealed, but the prosecution prevailed at trial based on other evidence.

A significant number of recent research papers report the ability to estimate DNA donor age to a range of about 3.5 to 7 years based on DNA methylation patterns.[22-33] Such evidence could be used as investigative leads or settle questions of possible lab contamination in certain DNA-based cold cases.

Finally, this presentation will conclude by discussing a 2018 paper by Vidaki and Kayser that discusses in detail the state of DNA methylation research and the authors' recommendations for further validation testing.[34] Further reference will be made to a similar Webinar by Vidaki and Lee recorded in 2017.[35]

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Epigenetics, DNA Methylation, DNA Donor Age

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