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DNA Methylation: Bridging life's experience and genetics within forensic scenario

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Abstract

DNA serving as the "blueprints of life" is a complex found in all biological cells carrying the genetic instructions for development and functioning of the organism. Inheritance of unique combination of genetic polymorphism results in DNA profile. In past few decades, the field of forensic genetics has witnessed several advancements from the radio-labelled DNA probes to the Short Tandem Repeats (STR) and Single Nucleotide Polymorphism (SNP) that have fostered the emergence of biological materials as most reliable evidences at a crime scene for the purpose of human identification but then, are inadequate in distinguishing monozygotic twins. Also, these techniques are of unavailing in providing informations such as tissue-specificity of the DNA source, phenotypic identification, and age estimation. Epigenetics, particularly DNA methylation is evolving as a promising technique to overcome such drawbacks of conventional profiling techniques.

Keywords: Monozygotic Twins; Tissue Source Identification; Ageing; Environmental Factor; DNA Methylation; Epigenetics

1. Introduction

Conventional forensic genetic methods employed for human identification purposes face challenges while dealing with cases of monozygotic twins, tissue source identification and age & gender determination of unknown biological source of DNA. Mixture of several biological fluids at the scene of crime often from multiple individual sources creates a hindrance in linking individuals to the scene of crime. Several studies have unveiled the significance of epigenetic markers over routine technique such as STR, SNP and mt DNA analysis in such impeding cases. Epigenetics include the study of any modification in DNA or associated histone protein that affects the activity of DNA without causing any alteration in its sequence (1,2). Epigenetic mechanisms mediates genetic variations that are adapted as a response of each cell of individual to short-term or long-term environmental changes and individual's lifestyle, allowing cells to develop into functionally and metabolically specialized cell types. This explains the tissue specific differentiation within an individual as well as phenotypic variation, such as disease, age, sex; between individuals, even in case of individuals with identical DNA profile (3,4). Three reported mechanisms – DNA methylation, Histone Modification and RNA associated silencing, either independently or in interaction with each other encourages epigenetic modification.

2. Types of Epigenetic Modifications

Epigenetic modification occurs due to external environment and an individual's lifestyle. Three reported mechanisms – DNA methylation, Histone Modification and RNA associated silencing, either independently or in interaction with each other encourages epigenetic modification.

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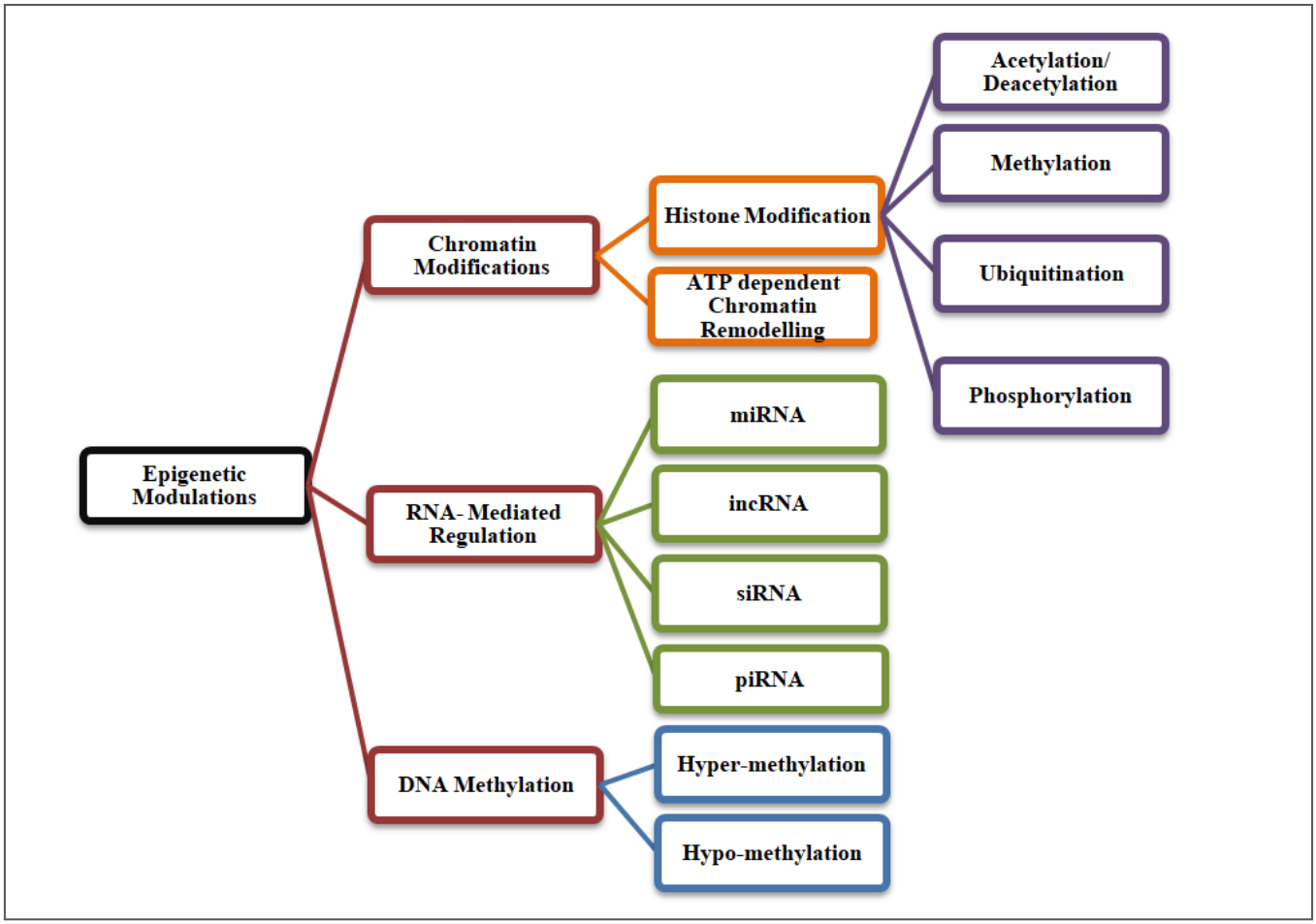

Figure 1 Types of Epigenetic Modifications

2.1. Chromatin Modifications

Chromatins are the protein-DNA complexes composed of a repeating structure of DNA strand and 8 histone molecules known as nucleosomes (5). Histones are described as alkaline protein components of chromatin that wind around the nulear DNA strands and help them to condense into chromosome, providing them a more compact shape to fit inside the nucleus. Besides histones also play crucial role in gene expression, damaged DNA repair and epigenetic regulation. Chromatin modifications resulting in epigenetic expression develop in two ways- firstly by the covalent alteration in the terminal tail region of histone molecules and secondly, by ATP hydrolysis dependent remodelling in the structure of nucleosomes (6). Histone modifications primarily occur at basic amino acid in the N- terminal region of histone tail (7,8). Epigentic regulatory mechanism involves various post-translational covalent modifications in the histone structure that includes acetylation, deacetylation, phosphorylation, methylation ubiquitination, sumoylation (9,10). Among these acetylation and phosphorylation are flexible, reversible and unstable while methylation is concerned with long term modifications (11).

2.2. RNA Mediated Regulation

Non-coding RNA (ncRNA) are the ones originated from the transcription of intergenic regions of DNA (12) and do not translate into proteins (13). These ncRNA are involved in post transcriptional regulation of expression of mRNA by binding to the untranslated portion of mRNA resulting in mRNA cleavage and ultimately affects protein synthesis and regulation of gene expression (11). These ncRNAs regulate epigenetic gene expression by controlling the process of transcription through polycomb complexes that modify chromatin structure, down regulation of DNA methylation as well as affecting post transcriptional process such as splicing, translation etc. (14,15).

2.3. DNA Methylation

DNA methylation is the only epigenetic mechanism that involves modification of DNA itself. It refers to a post-replicative biochemical process involving covalent addition of methyl (-CH3) group to the DNA strand, particularly, to the fifth

carbon atom of a cytosine ring of CpG dinucleotide (2,16). It is a heritable epigenetic modulation, which can be reversed or altered by several factors throughout the life span of the individual and is associated with processes such as gene silencing through transcription repression, gene imprinting, X chromosomal inactivation of transcription (17,18).

2.3.1. DNA methyltransferases

DNMTs play a pivotal role in epigenetic regulation. DNA Methyltransferases (DNMTs) are concerned with displacement of methyl group from S-adenosyl-L-methionine (SAM) considered as the universal methyl donor to the 5-C position of cytosine (19).

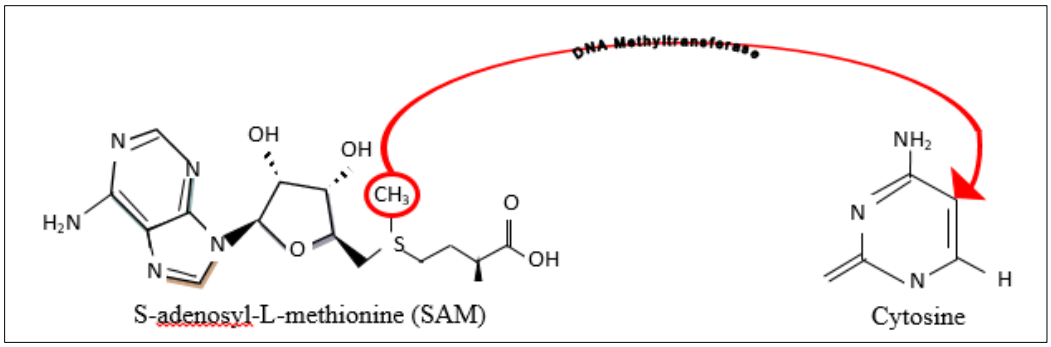

Figure 2 Displacement of Methyl Group from SAM to Cytosine resulting in methylation of Cytosine

In mammals, there are two major groups of DNMTs: De novo methyltransferase and maintainance methyltransferase. De novo methyltransferases consist of DNMT3 family: DNMT3a, DNMT 3b and DNMT 3l. DNMT3a and DNMT3b are concerned with establishment of methylation pattern during the early developmental stage in the stem cells (20) while DNMT 3l lacks independent methyltransferases activity. Dnmt3l acts as a booster to the action of other de-novo DNMTs by binding to their C-terminal, increasing their activity by three times (21). Once the methylation patterns are encoded, maintenance and propagation of these patterns after replication is carried out by DNMT1- the maintenance methyltransferase. DNMT1 functions to set up methylation onto the unmethylated strand in the hemimethylated state of DNA, as observed after the process of DNA Replication thus maintaining the original parental methylation pattern (22).

3. Applications of DNA Methylation in Forensic Science

3.1. Tissue specificity

Existence of tissue-specific differentially methylated regions (tDMRs) has been reported in several studies. Global demethylation during dynamic remodeling of DNA methylation in embryonic stage is then followed by a progressive establishment of methylation pattern, maintaining their lineage specificity. Due to interaction with chromatin modifications, small RNAs and interference of DNA binding factors, the methylome of different cell type exhibit distinct level of methylation that contributes to cellular identity and genomic stability (23). Different cell or tissue type within an organism encase tissue-specific differentially methylated regions (tDMRs) and associated variable DNA methylation profiles, which makes it a suitable marker for body fluid identification and tissue based DNA analysis (24,25). The study conducted by Frumkin et al. (2011) was among the first successful approach towards DNA-methylation based tissue identification. The team obtained 100% tissue identification by analyzing distinct ratios of methylation among 7 loci (L91762, L68346, L50468, L14432, L30139, L15952, and L26688) in blood, saliva, semen, and skin epidermis. Later, Antunes and collegues worked on different body fluids and identified 6 genome locations capable of distinguishing 3 body fluids. Hypermethylation of 7 CpGs of C20orf117 for blood and hypermethylation of BCAS4 in saliva contributed to their discrimination from semen and skin epithelial cells. In semen, hypermethylation of FGF7 and hypomethylation of ZC3H12D allowed discrimination of semen from other body fluids (27). Similar findings were obtained by Madi et al (2012) by using bisulfite modification and pyrosequencing. (25) reported eight novel CpG sites (two sites for each body fluid) using pyrosequencing that are forensically relevant methylation markers for identification of blood, saliva, semen and vaginal secretion. Development of methylation based tissue identification assay- DSI by (Wasserstrom et al. (2013) accounts for further advancement in the field. DNA source identifier (DSI) - semen is an assay comprised of a biochemical process similar to normal DNA profiling followed ny an automation process that results in generation of a specific DNA profile corresponding to the source tissue (semen).

3.2. Aging and Age-related disease

Aging is a slow, unstoppable deteriorating process involving impairement of functioning of cells, tissues and organs (29) resulting in chronic diseases and death. Various environmental, intrinsic and genetical factors are associated with physiological and mechanical deterioration that generate a difference between the biological age and chronological age of an individual (30). Recent studies reveal association of aging with global hypomethylation as well as promoter-specific hypo- or hyper-methylation. These studies supports a general outlooks of association of global methylation with non-CpG areas while hypermethylation occurs in the CpG islands (31,32). Global or genomic hypomethylation is the progressive loss of the total methylcytosine contents probably due to passive demethylation (33) particularly at the repetitive sequences such as Alu, LINE-1 that are scattered throughout the genome (34,35). In addition to the global methylation, promoter-specific hypermethylation is also observed throughout the aging process. Site-specific hypermethylation of loci is described as the escalated level of methylcytosine contents that are associated with the specific genes (36). Such hypermethylation restrict the expression of genes concerned with various phenotypes including aging and various age-related diseases. Hypermethylation of various polycomb group proteins also overlaps with other epigenetic modification in age-related processes. These polycombs complexes are involved in gene-regulation and transcriptional silencing process through interaction with DNA and histones during cell development and differentiation (37).

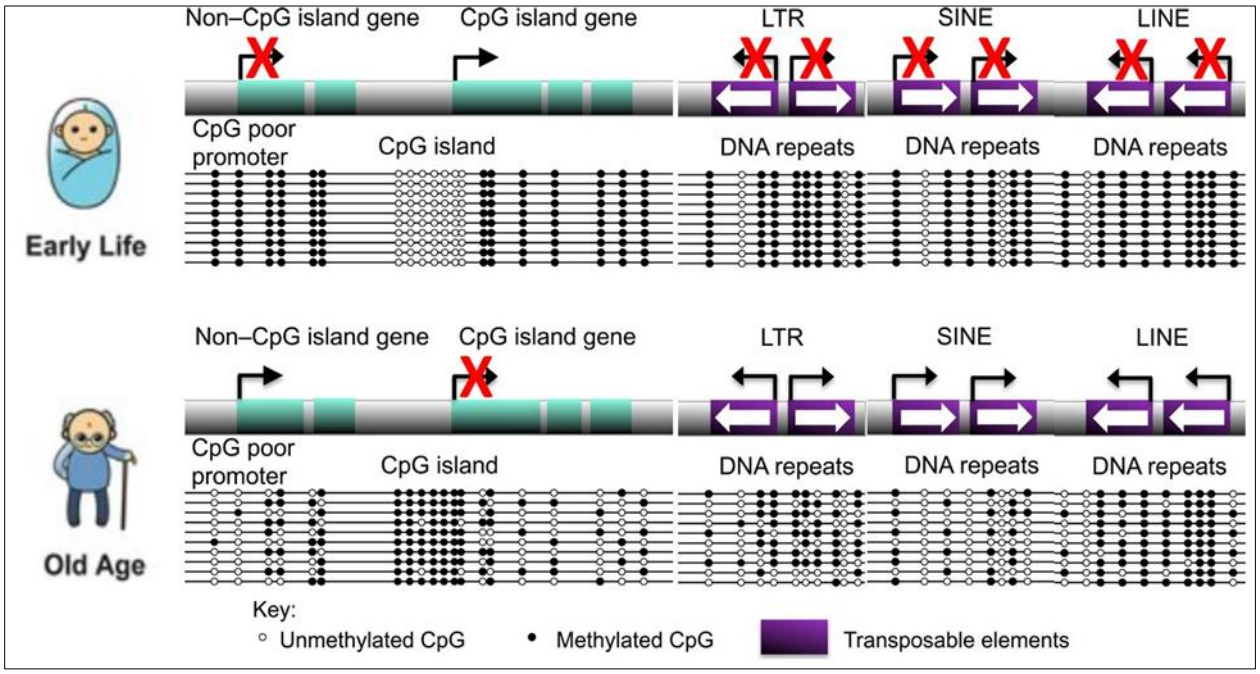

Figure 3 Illustration of modification in Methylation Patterns during Aging

3.3. Distinguishing Monozygotic Twin

Conventional methods of human identification applied in forensics, such as STR, SNP and mtDNA profiling fail to distinguish between monozygotic (MZ) twins as they carry concordant sequences throughout their inherited genome and therefore, possessing a serious hindrance for forensic purpose in such situation of identification. Various studies revealed that DNA methylation patterns in such pairs of MZ twins are indistinguishable during early phase of life, however, with aging life and exposure to external environmental conditions, the patterns of methylation between such MZ twins varies in term of content and genomic distribution (38,39). Park et al. (2017) have suggested six recurrent CpG sites (cg00211609, cg26287080, cg01558909, cg21036194, cg01419577, cg04620228) based on the study of 12 pairs of MZ twins and analyzing their methylation patterns using Illumina's Human Methylation 450 K array. Several studies on methylation level at ALU and LINE-1 regions have significantly applied for distinguishing MZ twins. Xu et al. (2015) evalutated such highly repetitive sequences, as a representative of whole genome for analyzing the methyaltion level of monozygotic twins and revealed 12.61% differentiation between MZ twin pairs. However, there is further requirement of methylation markers with higher discrimination power.

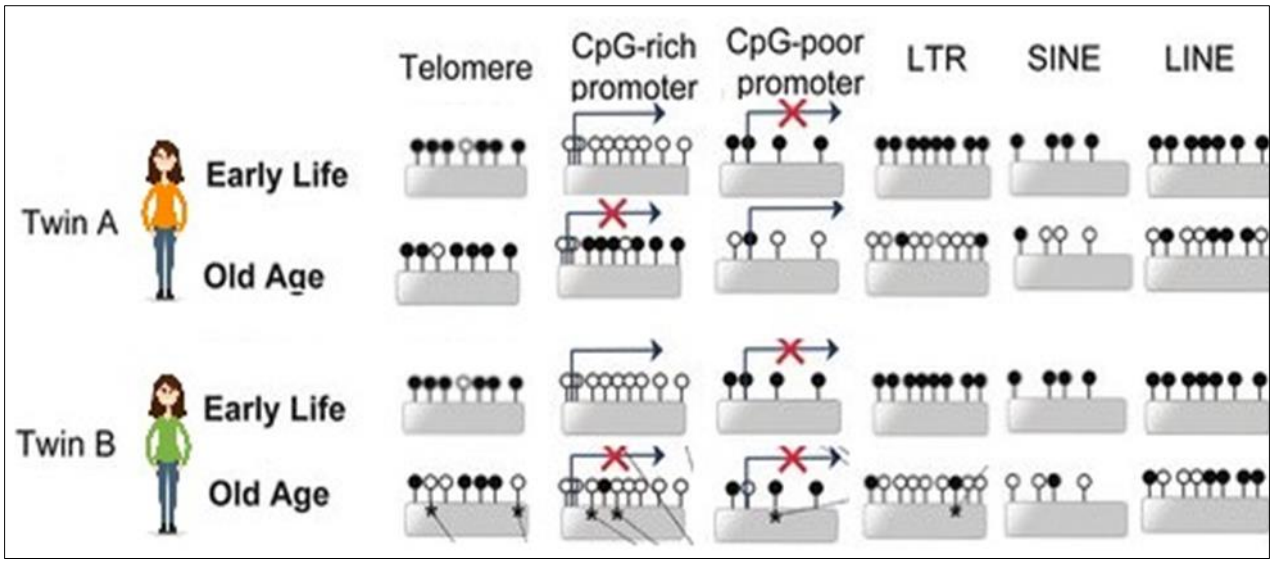

Figure 4 Illustration of divergence in the Methylation Patterns between two Monozygotic Twins during Aging

3.4. Ethinic Diversification

Humans have clustered themselves into distinct ethinic bio-geographical groups by adapting different location, unique lifestyle, distinct culture and tradition, different costumes and eating habits and many more aspects (42)s. Various cases of ethnic origin and immigration have been reported within criminal justice system. DNA methylation pattern analysis can be remarkably utilized in identification and segregation of such ethnic and biogeographical population (43). Elliott et al. (2014) exposed hypomethylation pattern at AHRR locus in European population and hypermethylation in South Assian population at the same marker due to variation in trends of tobacco consumption and smoking. A recent study on Japanese or European American women in age group of 60-65 revealed hypermethylation of 174 differentially methylated CpG markers in Japanese Americans as compared to European Americans (45).

3.5. Phenotypic Variation and Social Behaviour

Phenotypic variation is considered as an essential foundation of individualization and speciation. Traditional evolutionary concepts define phenotype as the set of observable traits each formed as an expression of the locus specific genetic code the organism carries. For decades, phenotypic variations are regarded as the outcome of genetic mutation together with natural selection, genetic drift and gene flow (46). Recent works favour the involvement of other factors such as multi-gene effect, environmental effect as well as epigenetic modification in phenotypic alteration. Influence of epigenetic variation on the heritable variation of phenotypic traits is picking up steam in the study of phenotypic variation (47). The exclusive influence of epigenetic on phenotypic variation is vogue and debateable and can be justified by relating it to genetics and environment (48). A positive co-relation between elevated level of inflammatory cytokine interleukin-6 (IL-6) in case of anxiety and expression level of DNA Methyltranseferases and Enhancer of Zeste Homolog 2 (EZH2) gene was reported by performing a comparison of gene expression levels of the DNMT (DNMT1, DNMT3A, DNMT3B, DMNT3L) and EZH2 genes between anxious (n=25) and nonanxious individuals (n=22) using quantitative Real Time PCR (49). Evidence of Glucocorticoid receptor *NR3C1* promoter hypomethylation in female Chronic Fatigue Syndrome patients was observed in a study comprising of 76 female patients (46 with no/mild and 30 with moderate/severe childhood trauma) and 19 healthy controls and re-confirmed in a new and independent sample of 80 female CFS patients and 91 female controls. DNA methylation is also associated with severity of fatigue as well as with childhood emotional abuse in CFS patients (50). Epigenetic regulation of GR gene (NR3C1) promoter expression (51), effect of maternal distress during pregenancy on epigenetic changes in placental genes – HSD11B2, FKBP5 (Monk et.al., 2016) and NR3C1, *11β-HSD-2* (52) and is associated with stress response and neurodevelopmental behaviour in human infants.

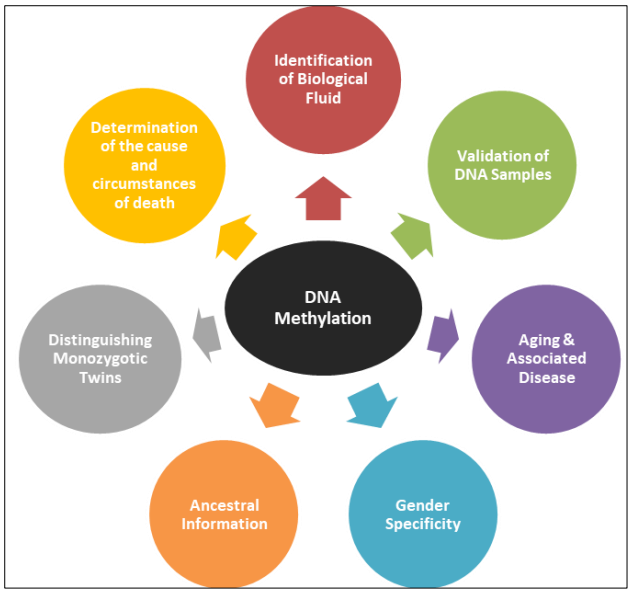

Figure 5 Application of DNA Methylation in Forensic Science

4. Conclusion

The application of epigenetics is constantly expanding in the field of forensic science for examination of critical cases. Among various epigenetic techniques, variation at CpG dinucleotide sequence resulting in variation of methylation patterns without disturbing the actual DNA sequence has been significantly employed for distinguishing monozygotic twins, that genetically possess identical sequence. Various studies have been carried out for age determination, gender identification and tissue source identification by analyzing DNA methylation patterns, that will be efficacious in solving various complicated forensic caseworks. Presence of trace or degraded DNA is another commonly encountered issue in forensic cases that can be appreciably overcome by employing various DNA methylation analytical techniques such as Bisulfite conversion, or methylated DNA immunoprecipitation.

Multitudious in-depth studies of DNA methylation patterns, their relations with various environmental, social and behavioural factors and advancemens in analytical technique for Methylation patterns are encouraging the proliferation of epigenetic markers, profiling of tDMRs and level of methylation as an efficient, reliable and rapid assay for various forensic applications. Further advancement in analytical techniques and identification of additional epigenetic marker will surely escalate the positive disposal of complicated forensic caseworks.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors report no conflicts of interest in this work.

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