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Epigenetic Markers in Criminal Investigation: A Narrative Synthesis of Recent Evidence

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Abstract

Forensic epigenetics has become a rapidly expanding frontier in molecular forensics, offering analytical capabilities that surpass those of traditional genetic markers. Advances in high-resolution methylome profiling, small-RNA analysis, and multi-omics approaches now support increasingly precise inferences about biological age, tissue origin, lifestyle exposures, and, in some cases, individual differentiation. Despite this progress, the field remains transitional, and its adoption in routine forensic practice requires methodological standardization, analytical validation, and legal evaluation. This narrative review synthesizes findings from studies indexed in PubMed, Scopus, and Web of Science, of which 61 met the eligibility criteria after duplicate removal and full-text assessment. These studies were categorized according to epigenetic mechanism, forensic application, methodological design, technological innovation, and legal or operational considerations. DNA methylation emerged as the most extensively explored mechanism, particularly for age estimation, body-fluid identification, and differentiation of monozygotic twins. MicroRNAs constituted the second most frequently examined biomarker class, demonstrating high stability in degraded samples and strong tissue specificity, with applications in postmortem interval estimation and cause-of-death assessment. Histone modifications were investigated less often due to technical challenges related to protein instability and environmental degradation. Across mechanisms, limited standardization in sampling, extraction, sequencing, and computational modeling remains a major barrier to reproducibility. Nevertheless, recent advances—including third-generation sequencing, refined methylation assays, and machine-learning frameworks integrating complex epigenomic and transcriptomic signals—suggest substantial future potential. Persistent le-

gal challenges, particularly related to validation, error-rate determination, and privacy concerns, highlight the need for caution. Overall, forensic epigenetics is progressing toward broader applicability, provided ethical, legal, and methodological frameworks evolve in parallel.

Subject Areas

Genetics

Keywords

Epigenomics, DNA Methylation, microRNAs, Forensic Genetics, Biomarkers

1. Introduction

Epigenetics encompasses a set of molecular mechanisms that regulate gene expression without altering the DNA sequence, including DNA methylation, post-translational histone modifications, and the activity of non-coding RNAs [1]-[3]. These processes play essential roles in development, cellular differentiation, and homeostasis, in addition to mediating adaptive responses to environmental, physiological, and pathological stimuli [4] [5].

Recent advances in sequencing technologies, bioinformatic analysis, and methylation-quantification methods have expanded our understanding of these mechanisms, enabling their application in translational fields, including forensic genetics [6]-[8]. In the forensic context, epigenetic markers stand out for providing dynamic information that cannot be obtained through traditional genetics, which relies on STRs and SNPs, whose sequences remain stable throughout life [9] [10]. In contrast, epigenetic alterations accumulate over time and are influenced by environmental factors, allowing inference of characteristics such as biological age, exposure to drugs or pollutants, behavioral patterns, and stress responses [11]-[13].

DNA methylation is the most extensively studied mechanism for forensic purposes, particularly in the development of age-prediction models known as "epigenetic clocks," which can estimate age with high accuracy across different tissues, such as blood, saliva, teeth, and bones [14] [15]. Another application derives from the identification of biological fluids, since both methylation signatures and microRNA profiles exhibit tissue specificity, enabling the differentiation of blood, semen, urine, skin, and others [16] [17]. Furthermore, studies show that epigenetic signatures can distinguish monozygotic twins, a differentiation that is not feasible using conventional genetic markers [18]. MicroRNAs have gained prominence due to their high postmortem stability and their usefulness in estimating the postmortem interval and indicating specific causes of death, such as cardiac injury or hypoxia [19] [20].

Despite the rapid advancement of forensic epigenetics, important challenges

remain, such as the lack of pre-analytical standardization, tissue-specific influences, interindividual variability, sample degradation, and the need for multicenter validation for routine implementation. Nevertheless, the field is moving quickly toward consolidation, driven by high-throughput technologies, artificial intelligence models, and the growing integration of genomics, epigenomics, and forensic science. Understanding these advances and limitations is essential to transforming epigenetics into a robust operational tool in modern criminal investigation.

Given this scenario of technical progress and emerging challenges, it is essential to critically synthesize the available knowledge on the role of epigenetics in forensic practice. Thus, this narrative review aims to compile and analyze recent scientific evidence on the main epigenetic mechanisms—DNA methylation, histone modifications, and non-coding RNAs—and their applications in criminal investigation, highlighting the potential of these markers for estimating biological age, identifying fluids and tissues, distinguishing genetically similar individuals, and inferring environmental exposures. Additionally, it seeks to discuss the technical limitations that still restrict their applicability, as well as identify knowledge gaps and future perspectives for developing robust, reproducible, and operationally feasible epigenetic approaches suitable for modern forensic routines.

2. Materials and Methods

This narrative review was conducted with the purpose of identifying, selecting, and critically synthesizing recent scientific literature related to epigenetic mechanisms and their applications in the context of forensic genetics. Although it does not fully follow the structure of a systematic review, a structured approach was adopted to ensure transparency, reproducibility, and methodological coherence.

1) Information sources and databases consulted

The bibliographic search was carried out on PubMed, Scopus, and Web of Science platforms, recognized for their breadth and relevance in the biomedical field. The selection of these databases was based on the need to capture original studies, critical reviews, and methodological advances published in high-quality scientific journals. Searches were conducted between May and October 2025 and included only articles published in the last five years (2020-2025) to reflect the state of the art in forensic epigenetics research.

2) Keywords and search strategies

To identify relevant studies, controlled and uncontrolled keywords in English, Portuguese, and Spanish were used, combined with Boolean operators. Key terms included: *epigenetics*, *DNA methylation*, *histone modifications*, *microRNA*, *non-coding RNA*, *forensic genetics*, *forensic science*, and *epigenetic biomarkers*. Search expressions were combined using operators such as AND and OR to expand search sensitivity without compromising specificity. Examples of search combinations include: "DNA methylation AND forensic science," "microRNA AND forensic genetics," and "epigenetic biomarkers AND forensic identification."

3) Inclusion criteria

Included works comprised original articles, narrative reviews, and systematic reviews published between January 2020 and 2025, available as full-text PDFs and written in English, Portuguese, or Spanish. Studies were considered eligible if they directly addressed epigenetic mechanisms—such as DNA methylation, histone modifications, and non-coding RNAs—with demonstrated or potential application in the forensic field, including age estimation, identification of biological fluids, differentiation of genetically similar individuals, and inference of environmental or physiological exposures.

4) Exclusion criteria

Studies published before 2020, articles without access to the full PDF text, works that did not explicitly address the relationship between epigenetics and forensic applications, and those that discussed epigenetic mechanisms solely from clinical or biological perspectives without forensic implications were excluded. Editorials, letters to the editor, and opinion pieces lacking substantive data or structured syntheses were also excluded.

5) Study selection and screening process

After conducting searches in the selected databases, the results were exported to a reference manager, where duplicates were identified and removed. Next, an initial screening was performed through title and abstract reading, during which studies clearly irrelevant or outside the thematic scope were excluded. Potentially eligible articles were obtained in full text and assessed in detail according to the inclusion criteria. At the end of this process, 61 studies were selected to compose the qualitative synthesis of the review.

6) Data extraction, organization, and synthesis

The included articles were analyzed qualitatively, and relevant information was extracted and organized according to key variables such as type of epigenetic mechanism studied, type of biological sample, forensic application investigated, main findings, and methodological limitations. The synthesis was structured into thematic axes, allowing an integrated discussion of the roles of DNA methylation, microRNAs, and histone modifications in contemporary forensic practice.

7) Ethical considerations and methodological notes

Although this is a narrative review, efforts were made to approximate the adopted method to widely accepted models such as PRISMA, using an adapted flowchart to describe the stages of identification, screening, eligibility, and inclusion of studies. However, narrative reviews do not aim to exhaust all available literature or to conduct statistical meta-analyses, which represents a limitation inherent to the method. Even so, the adopted approach enables a deep and updated critical synthesis, appropriate for an emerging field such as forensic epigenetics.

3. Results

The search conducted in the PubMed, Scopus, and Web of Science databases initially yielded 548 publications related to the use of epigenetics in the forensic context. Additionally, 12 further articles were identified through manual reference

screening of key studies, totaling 560 records before screening. After removing duplicates, 421 unique articles remained and were subjected to title and abstract screening for preliminary evaluation.

During this screening stage, 289 studies were excluded for lacking direct relevance to forensic epigenetics, addressing exclusively clinical epigenetics, or failing to examine epigenetic mechanisms applicable to criminal investigation. Thus, 132 articles proceeded to full-text evaluation. After thorough assessment, 87 studies were excluded for not meeting the inclusion criteria: absence of a clear forensic application, lack of concrete epigenetic data, unavailability of full-text PDF, methodological inadequacy, or publication prior to the defined period (2020-2025).

At the end of this process, 61 studies were deemed eligible and included in the qualitative synthesis. These articles comprise original investigations, structured reviews, and methodological studies exploring forensic applications of DNA methylation, histone modifications, microRNAs, and other non-coding RNAs (Figure 1).

Analysis of the 61 studies revealed four major thematic axes that encompass the principal contributions of the recent literature:

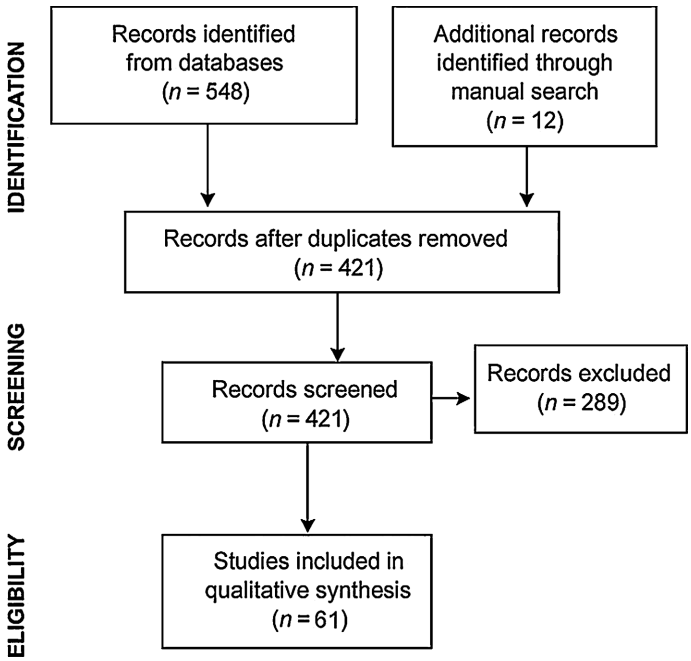

Figure 1. Stages of study selection for the narrative review.

3.1. DNA Methylation as a Forensic Tool

DNA methylation was the most extensively investigated epigenetic mechanism, representing approximately 70% of the included studies. Findings consistently demonstrate that methylation patterns at CpG sites provide excellent performance for estimating biological age, with high accuracy in samples such as blood, saliva, teeth, and bones. Models employing machine learning, penalized regression, and neural networks exhibited strong predictive capacity. Furthermore, studies show

that methylation signatures enable the identification of biological fluids, distinguishing semen, blood, urine, saliva, and other tissues with high specificity. Another relevant finding concerns the ability to differentiate monozygotic twins, as environmental exposures accumulated over the lifespan generate divergent epigenetic profiles, overcoming limitations of traditional STR markers.

3.2. MicroRNAs and Other Non-Coding RNAs

MicroRNAs have emerged as highly promising biomarkers due to their postmortem stability, resistance to environmental variations, and tissue-specific expression. The included studies show that microRNAs are useful for identifying bodily fluids and characterizing tissues in degraded samples. In addition, several articles report that microRNA profiles can estimate the postmortem interval (PMI) with greater accuracy than classical biochemical methods, particularly in cardiac and hepatic tissues. Evidence also indicates their usefulness in identifying specific causes of death, including acute myocardial infarction, trauma, and hypoxia.

3.3. Histone Modifications:

Investigations into post-translational histone modifications were less numerous, reflecting an early stage of development in this area. Although biologically relevant, marks such as *H3K4me3* and *H3K27ac* demonstrated limited applicability in real forensic contexts due to protein instability, susceptibility to environmental degradation, and the lack of standardized methods for recovering and analyzing these proteins in compromised samples. However, some experimental studies show that certain histone modifications may provide information about physiological state and pre-mortem gene activity, potentially contributing in the future to inferences related to cause of death.

3.4. Environmental Influences and Epigenetic Signatures

A significant portion of the studies highlighted that environmental factors—such as smoking, air pollution, chronic stress, drug use, diet, and exposure to pesticides—generate detectable epigenetic signatures capable of reflecting recent or chronic exposures. Although promising, these approaches still lack robust population-level validation and rigorous control of confounding variables, which limits their immediate adoption in forensic practice. Nevertheless, the ability to capture biological markers of exposure positions epigenetics as a potential tool for reconstructing pre-crime events or physiological conditions preceding death.

Overall, the results indicate that forensic epigenetics is an expanding field, with greater maturity in research involving DNA methylation and microRNAs, while histone modifications and integrative analyses represent emerging frontiers. The literature highlights the need for standardization, multicenter validation, pre-analytical protocols, and population-based databases so that these techniques can be applied safely and reproducibly in routine criminal investigations.

4. Discussion

The narrative synthesis of the 61 studies included in this review demonstrates that forensic epigenetics is a rapidly expanding field, driven by advances in sequencing, bioinformatics, and molecular biomarker analysis [7] [21]-[23]. The results reflect the rigorous criteria used in selecting the evidence, which prioritized recent publications (2020-2025) with an explicit focus on epigenetic mechanisms applicable to the forensic context [8] [16] [24]-[26]. Thus, the discussion presented here is supported by updated, methodologically consistent literature aligned with the state of the art in molecular epigenetics [6] [7] [21] [22].

4.1. Consolidation of DNA Methylation as the Main Forensic Epigenetic Tool

The predominance of studies involving DNA methylation reinforces its role as the most established epigenetic mechanism in contemporary forensic practice [7] [14] [16] [21] [26]. The most mature and widely validated application is biological age estimation, with models based on CpG sites demonstrating errors of less than 5 years across various biological tissues [11]-[15]. However, relevant methodological limitations were identified, particularly the lack of standardization in CpG panels and the heterogeneity of statistical models, which complicate comparisons between studies [8] [24] [27] [33]. The lack of multicenter validation further highlights the need to expand sample sizes and experimental settings before these biomarkers can be fully implemented in forensic routine practice [25] [28].

Differentiation of monozygotic twins represents one of the most compelling advantages of epigenetic analysis over conventional STR-based profiling. While traditional DNA markers are identical between monozygotic twins, accumulating evidence shows that environmentally influenced DNA methylation patterns, microRNA profiles, and other epigenomic features diverge progressively across the lifespan [17] [18] [20] [22].

Recent studies demonstrate that these epigenetic signatures may enable the identification of twin-specific molecular differences with increasing accuracy, particularly when high-resolution methylation arrays or next-generation bisulfite sequencing are used [14] [21] [23] [29]. Although routine forensic adoption will require standardized panels, robust validation, and clearly defined discriminatory thresholds, the ability to distinguish monozygotic twins highlights a transformative application of forensic epigenetics—one not achievable with any current genetic profiling method.

4.2. MicroRNAs as Emerging Biomarkers: Stability and Specificity

MicroRNAs emerged as the second most investigated group and proved to be highly stable biomarkers, even in degraded samples [17] [18]. Their robustness is further supported by broader epigenetic studies demonstrating the resilience of molecular markers under environmental and physiological stress conditions [19]-[21]. Their tissue specificity allows the identification of biological fluids—such as

blood, semen, and saliva—complementing traditional protein-based methods and reflecting advances in forensic tissue discrimination and epigenomic profiling [22]-[24]. In addition, microRNA-based approaches have shown promising usefulness in estimating the postmortem interval (PMI) [17] [25], and in identifying specific causes of death, including hypoxic and cardiovascular events—phenomena mechanistically linked to epigenetic responses to cellular stress and injury [26] [27]. Despite their potential, universally validated panels are still lacking, and further studies applied to real forensic scenarios are needed—an issue aligned with ongoing discussions on methodological standardization and precision epigenomics [17] [20] [25].

4.3. Histone Modifications: Conceptual Potential Limited by Technical Challenges

Post-translational histone modifications were less frequently investigated due to protein instability and the difficulty of recovering them from degraded samples [26]. Although biologically relevant, these marks depend on highly controlled laboratory conditions, which limits their practical usefulness in criminal contexts [27]. Recent proteomic technologies, however, suggest potential advances that may support their future forensic application [28].

4.4. Epigenetic Signatures and Reconstruction of Environmental Exposures

The included studies indicate that environmental exposures—such as smoking, pollution, stress, and pesticides—leave detectable epigenetic signatures [5] [29]-[31]. Such markers may assist in reconstructing previous biological events relevant to criminal investigations [7]. However, interindividual variability and behavioral influences limit their immediate application [31] [32]. Additionally, most studies originate from clinical rather than forensic contexts, highlighting important gaps for future research [32].

Many environmentally induced epigenetic signatures are inherently limited by their biological dynamics. Several methylation changes, particularly those associated with short-term exposures, are transient and may revert once the stimulus is removed, while others require prolonged or repeated exposure to stabilize sufficiently for forensic interpretation [1] [32]. As a result, most validated exposure-associated markers reflect long-term lifestyle factors—such as smoking or chronic pollution exposure—rather than discrete, acute events. This distinction reinforces why environmental epigenetic profiles are more informative for reconstructing cumulative biological histories than for pinpointing a single momentary exposure relevant to a specific crime [5] [32]-[36].

4.5. Need for Standardization, Validation, and Methodological Integration

The lack of standardization in the collection, storage, and analysis of epigenetic material remains one of the principal barriers to operational implementation [7]

[8]. Likewise, the scarcity of multicenter studies with broad population diversity limits the establishment of universal recommendations that ensure reproducibility across laboratories [32] [32]. In parallel, the integration of multiple epigenomic platforms—supported by advances in sequencing technologies and artificial intelligence—is emerging as a powerful direction for forensic science [21] [23] [37]. This multi-omics strategy enables the extraction of complementary layers of biological information from a single, often limited, evidentiary sample. For instance, DNA methylation markers can provide highly accurate age estimates [14] [15], while microRNA profiles offer tissue-specific signatures capable of identifying the biological source of trace material [17] [26]. When these modalities are combined, they allow investigators to infer both who the sample may have originated from and what type of tissue was deposited, substantially increasing evidentiary value in degraded or low-quantity samples. Such integrated approaches enhance discriminatory power, improve robustness under challenging forensic conditions, and align with emerging analytical pipelines that merge methylation sequencing, small-RNA profiling, and AI-based classification models [23] [37]-[39].

4.6. Practical and Legal Challenges for Courtroom Admissibility of Epigenetic Evidence

Although epigenetic biomarkers increasingly demonstrate scientific value, their admissibility in judicial proceedings faces substantial practical and legal constraints. Courts applying either the Frye standard ("general acceptance") or Daubert criteria ("testability, peer review, error rates, standards, and known limitations") require forensic methods to demonstrate analytical validity, reproducibility, and transparent quality-control procedures—requirements that remain only partially fulfilled for DNA methylation panels, microRNA signatures, histone-based assays, and other emerging platforms [7] [28] [39]. Recent reviews emphasize that, unlike conventional genetic markers, epigenetic assays are subject to additional biochemical, environmental, and computational sources of variability, complicating claims of stability and reliability [1] [2] [32].

From a practical standpoint, the absence of harmonized pre-analytical protocols, standardized bisulfite-conversion methods, sequencing workflows, and validated machine-learning models remains a major barrier to operationalization [8] [10] [23]. The Scientific Working Group on DNA Analysis Methods (SWGDAM) underscores the need for rigorous validation, interlaboratory concordance studies, and full transparency in analytical reporting before any technique can be introduced in court [40]. Moreover, because epigenetic states are dynamic and influenced by environmental exposures, lifestyle, medications, aging, and stochastic variation [30] [31] [41], defense arguments may question whether these biomarkers possess adequate temporal stability for individual attribution—a challenge observed in debates over early STR interpretation, mitochondrial DNA sequencing, and probabilistic genotyping models [42] [43]. Additional evidence demonstrating methylation variability under environmental stress, pollution, or

sample degradation further reinforces the need for strict control of analytical conditions [44]-[46].

Legally, admissibility also requires clear articulation of error rates, confidence intervals, and methodological limitations—elements increasingly scrutinized in forensic surveys and judicial analyses [47] [48]. Yet, most epigenetic applications, including age estimation, tissue identification, and lifestyle inference, still lack population-scale validation and defined statistical thresholds for uncertainty [13] [15] [49]. Ethical and privacy concerns introduce an additional dimension of legal scrutiny, as methylation or microRNA profiles may reveal sensitive health, behavioral, or environmental exposure information not directly relevant to the forensic question, paralleling debates in forensic phenotyping and polygenic risk inference [20] [30] [43].

To progress toward courtroom admissibility, forensic epigenetics must therefore meet several criteria: 1) development of standardized, forensic-appropriate protocols endorsed by accrediting bodies [7] [8]; 2) robust multicenter validation using diverse population datasets to establish generalizability and reproducibility [7] [32] [45]; 3) formal determination of error rates, decision thresholds, and confidence intervals for each specific application [48]; 4) implementation of transparent reporting frameworks that clearly communicate assumptions, analytical steps, and limitations [48] [49]; and 5) full compliance with legal disclosure standards to support cross-examination and judicial review. By aligning methodological development with these scientific and legal expectations, epigenetic evidence may progressively advance toward meeting admissibility criteria and supporting responsible, proportionate integration into modern forensic practice [7] [8].

4.7. Critical Synthesis and Future Perspectives

The literature consistently highlights that forensic epigenetics holds substantial potential to transform criminal investigation, offering analytical advantages that surpass those of traditional genetic markers, including greater sensitivity, tissue specificity, and the ability to infer biological age, tissue origin, and lifestyle exposures [50]-[52]. Consolidation of these applications, however, depends on achieving rigorous methodological standardization, multicenter validation, and the development of robust population-level reference databases capable of supporting reproducible interpretation across laboratories [53] [54]. Recent technological advances are expected to accelerate this maturation process: third-generation sequencing and high-resolution methylome profiling are expanding analytical precision [55]-[57], while artificial intelligence and machine-learning models increasingly enable integration of complex epigenomic, transcriptomic, and exposure-related signals into predictive forensic frameworks [58]-[61]. Together, these developments suggest that epigenetics is moving steadily toward operational feasibility, provided that foundational validation and standardization challenges are effectively addressed.

Thus, this review demonstrates that forensic epigenetics is in a transitional

stage, evolving from a promising experimental framework into a scientifically grounded approach with growing potential for integration into routine forensic practice. At the same time, as epigenetic analyses increasingly allow inferences about sensitive lifestyle and health-related information, future applications must carefully address the ethical and privacy implications to ensure that these technologies are implemented responsibly, transparently, and proportionately within the justice system.

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Conflicts of Interest

The authors declare no conflicts of interest.

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