E-ISSN: 2790-6094
P-ISSN: 2957-4587

Journal Biological And Allied Health Sciences

The Forensic Science of the Future: A Journey through Omics Technologies

Hifz Ur Rehman[1,*], Hashim Tufail[1], Amna Arooj[2], Ahsan Raza[1], Ume e Kalsoom[3], Hafiz Muhammad Abbas Malik[4], Muhammad Shahid Cholistani[1], Samiaan Qurban Khan[2], Muhammad Abdullah Nasir[1], Zain Ali[1], Mudassar Irshad[5], Haris Faheem[5]

Original Article

ARTICLE INFORMATION

Corresponding Author:

- **hifzforensic.pk@gmail.com**

Affiliations:

1. Institute of Biological Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Punjab, Pakistan
2. Department of Forensic Science, Islamia University of Bahawalpur, Punjab, Pakistan
3. Department of Forensic Science, Institute of Molecular Biology and Biotechnology, University of Lahore, Punjab, Pakistan
4. The Department of Pharmaceuticals, Faculty of Pharmacy, Islamia University of Bahawalpur, Punjab, Pakistan
5. Punjab Forensic Science Agency, Lahore, Punjab, Pakistan

Keywords: forensic science, omics, microbiomics, genomics, epigenomics, transcriptomics, proteomics, metabolomics.

ABSTRACT

Forensic science has witnessed tremendous advances with the advent of omics technologies. Six of the most important omics technologies (genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiomics) are discussed and explored in this review in the context of forensic science. Genomics identifies individuals based on DNA profiling, ancestry, and prediction of physical characteristics. Epigenomics provides information about DNA modifications that can be used to determine age and body fluid identification. Transcriptomics offers an overview of gene expression profiles in forensic studies. Proteomics facilitates the identification of body fluids, study of post-translational modifications, and discovery of protein biomarkers. Metabolomics offers data on metabolic profiles for toxicology, drug analysis, and post-mortem examination. Microbiomics employs microbial communities as markers of evidence for postmortem interval estimation and person identification. There are possible advantages and opportunities for AI-based microbiome analysis in increasing the accuracy of postmortem interval (PMI) estimation. These technologies greatly improve the limitations of criminal science, enhance the accuracy and effectiveness of investigations, and eventually assist in the quest for justice and truth. Omics technologies hold vast potential for forensic science, and ongoing research is unveiling new ways of applying these technologies to criminal investigation applications.

Introduction:

Fundamentally, forensic science is the use of scientific theory to provide answers to questions of a legal nature. Omics is the description and measurement of groups of biological molecules of importance to an organism's structure, function, and dynamics [1]. Omics technology has revolutionized forensic science, providing new means to solve crimes and uncover new evidence previously undetectable. They enable insights into suspects' identities, the time of crime commission, and the cause of death in complex cases. Forensic investigations have used them more and more.

Traditionally, forensic analysis depended significantly on classical identifiers like blood typing, STR profiling, or physical evidence matching [2]. Although groundbreaking at the time, these techniques have some limitations, especially where there are degraded samples, mixed samples, or where one wants to get more information than just identity. Omics-based methodologies give a three-dimensional picture of the biological sample. For instance, metabolomics is able to reflect biochemical alterations happening right before or immediately after death.

With DNA profiling in forensic analysis becoming more common, scientists are considering how other omics technologies, including proteomics, metabolomics, and transcriptomics, would provide supplementary information and enhance the accuracy of results. An example is proteomics, where proteins and peptides are analyzed, which may yield information regarding a suspect's body fluids, tissue types, and age and sex. The analysis of small molecules and metabolites in human body fluids, or metabolomics, can yield information on a suspect's lifestyle, diet, and state of health. Analysis of RNA molecules, or transcriptomics, can yield information on patterns of gene expression and help identify any relevant genetic change.

While their potential is great, their application in forensics is limited by challenges in standardization, data interpretation, and admissibility as evidence. Inclusion of high-dimensional omics data in the forensic process demands more than technical know-how since it necessitates developing effective analytical pipelines as well as reference databases. In addition, court admissibility requires that such evidence adhere to standards of scientific reliability and relevance by the legal tests like Daubert or Frye [3]. However, ongoing research continues to validate and hone these techniques, bringing them nearer to mainstream forensic practice.

This manuscript explores the revolutionary role of omics in forensic science, with an in-depth analysis of each of the key omics fields and their applications in forensic science. Through critically assessing existing developments, methodological challenges, and possibilities, we hope to give a thorough account of how omics is transforming forensic inquiry and determining the future of forensic evidence interpretation.

Genomics

Genomics, or the examination of an organism's entire DNA set, has emerged as an important weapon within forensic science. Forensic investigations have gained unprecedented abilities for individual identification, ancestry calculation, and

physical feature prediction through the use of the potential of genomics. Criminal investigation insights can be obtained by the analysis of specific genetic markers, including ancestry informative markers (AIMs), single-nucleotide polymorphisms (SNPs), and short tandem repeats (STRs), in DNA samples. [4]

Applications of Genomics in Forensic Science

Estimating Ancestry by Genomics:

By matching an individual's DNA against the DNA of people from various populations, genomics can help ascertain ancestry [5]. This is done by analyzing genetic markers, which are little differences in DNA that occur with high frequency in specific populations. People can figure out their ancestry by comparing the number and type of genetic markers they possess to those of individuals from distinct populations.

Through forensic genomics, one can identify ancestry, determine ancestry forensic genomics accomplishes by using DNA testing. There could be many genetic markers unique to specific groups or geographic regions present throughout the human genome. By examining these markers, scientists can detect genetic connections among certain individuals and different ethnic or ancestral populations. Scientists have found specific genetic markers, or "ancestry informative markers," which vary considerably in groups. AIMs are selected on the basis of their ability to differentiate between ancestry groups. Such markers may comprise single-nucleotide polymorphisms (SNPs) or other types of variations. Forensic genomics can illuminate an individual's ancestry by analyzing their DNA at these AIMs. [6]

In identifying the probability that an individual is from a certain ancestry group, forensic genomics applies statistical methods and concepts of population genetics. Using probabilistic inferences, scientists can estimate a subject's ancestry by comparing the frequencies of AIMs in their genome with those in different groups. This research can uncover the ancestral components and most probable geographic origins of the individual. Forensic genomics can also provide insight into some phenotypic or physical features that are associated with specific ancestral populations. Forensic genomics can forecast an individual's probable appearance by examining particular genetic markers associated with features such as skin color, eye color, hair texture, or facial structure. It can also be identified if an individual possesses unique phenotypic features that are associated with specific ancestral backgrounds.

Forensic Genealogy and Genomics

One of the most successful techniques used to crack cold cases and find criminals is forensic genealogy, which combines DNA analysis with traditional genealogical inquiry. Forensic genealogy has redefined the field of forensic investigation by leveraging genomics advancements by using DNA profiles to pinpoint familial connections and uncover concealed bonds. When ancient records are missing or unavailable, genomics facilitates easier recognition of biological connections such as parent-offspring or sibling relationships. Researchers can determine kinship relations and create family networks through the comparison of genetic markers, e.g., autosomal SNPs or STRs, which can assist in locating missing individuals or resolving familial-related legal issues. [7]

In forensic genealogy, several specific genetic markers are utilized to analyze DNA samples and determine relationships among individuals. These markers are critical in determining ancestry and contain information regarding genetic origin. STRs and autosomal SNPs are commonly utilized in forensic investigations. STR analysis, where the frequency of repeating sequences at a specific locus is counted, may be employed for the identification of family relationships like parent-offspring or sibling relationships.

Although mtDNA is utilized to establish maternal lineages, Y-chromosomal STRs (Y-STRs) are loci on the Y chromosome that follow paternal lines. X-chromosomal markers consider X-chromosome inheritance patterns and come in handy in cases where female DNA is involved or following connections back to the grandmother on the mother's side.

In addition, specific heritage Informative Markers (AIMs) are selected to separate ancestral origins and provide information about an individual's most probable regional heritage. These markers examine the absence or presence of specific genetic variants associated with specific populations or ethnicities in order to identify an individual's ancestral heritage. Forensic genealogy applies the strength of genomics to find family connections and solve cold cases by employing multiple markers. [8]

Individual Identification through Genomics:

The field of forensic science has been totally revolutionized by developments in genomics, particularly identifying individuals from DNA evidence. Genomic techniques allow identification of suspects, victims, and missing persons due to the powerful tools they provide for the retrieval of useful information from DNA. Genomics can also inform us about an individual's phenotype, such as physical features and ancestry.

To establish a distinctive genetic profile, DNA profiling examines specific regions of an individual's DNA. Genomics is central to this process. In DNA profiling, STRs or SNPs are frequently utilized as markers. Forensic experts can determine matches or exclusions by comparing DNA profiles obtained from crime scene evidence with known individuals or databases to help identify suspects or victims. With great effort and accuracy, massively parallel sequencing, also known as next-generation sequencing, allows for the analysis of numerous DNA markers simultaneously.

The technique enhances the chances of detecting individuals even from degraded or contaminated DNA samples through the collection of more comprehensive genetic data from DNA samples [8]. An individual's phenotype, encompassing observable characteristics such as eye color, hair color, and ancestry, can be uncovered through genome analysis. Genomic analysis applies in forensic DNA phenotyping to predict these physical traits from DNA samples. Computer algorithms can generate composite views of the appearance of an unknown individual by examining specific genetic markers that are associated with phenotypic traits, aiding in suspect identification and generating leads for investigation. Candidate gene strategy involves the exploration of specific genetic markers which have been proven to be linked with

E-ISSN: 2790-6094
P-ISSN: 2957-4587

specific phenotypic traits. For example, HERC2, OCA2, and SLC24A4 gene changes are linked with eye colour variation, while MC1R gene variation is linked with hair colour variation. Computational algorithms are able to predict the likelihood of specific physical characteristics by examining these specific markers on a person's DNA. [9]

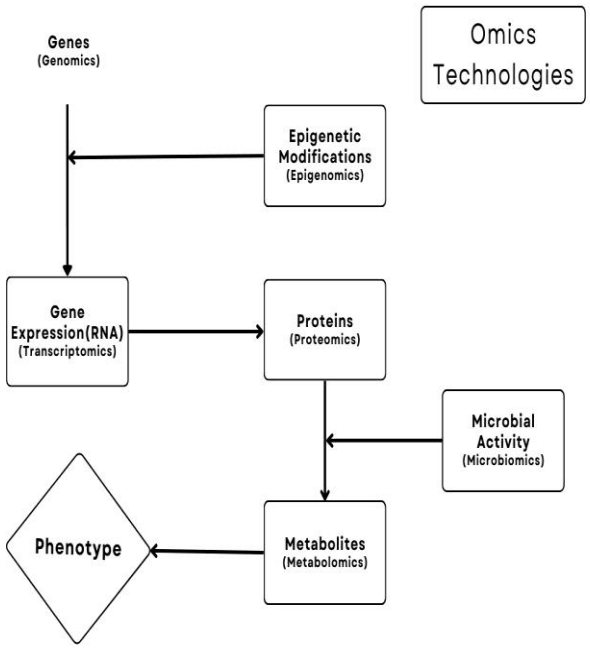

Figure. 1. Relation of Omics to Phenotype

Epigenomics

Epigenomics is the term used for the investigation of chemical modifications that are made to DNA and histone proteins, which create an intricate regulatory network that affects chromatin structure and genome function. These modifications have the capacity to be inherited in the next generation and can exert strong effects on gene regulation and expression. The comprehensive description of these heritable changes potentially throughout the genome is known as the epigenome. The epigenome in a cell is composed by a number of factors including genetic determinants, lineage, and environment. [10]

Epigenomics is a new area of study that seeks to comprehend regulation of gene expression without changing the sequence of DNA. It involves the study of chemical modifications to DNA and its associated proteins, which can have significant impacts on gene function. These modifications are known as epigenetic marks, and they play a vital role in a range of biological processes, including development, aging, and disease. [11]

Non-coding RNA in Forensics:

Forensic analysis frequently involves the determination of the cellular or tissue-type source of biological traces recovered at crime scenes. Determining such sources is essential in establishing the significance of the traces to the crime and reconstructing the sequence of events. Messenger RNA and microRNA markers have been handy when it comes to tissue identification but are problematic in terms of correlating the identified cell or tissue types with DNA-identified trace donors, especially with mixed traces. DNA methylation markers have been a way out, but technical challenges come with the DNA treatment needed by most of the analysis techniques.

A novel DNA markers for forensic tissue identification has been presented that are assessed without previous DNA treatment. These are referred to as copy number variations (CNV). CNV markers were discovered via genome-wide screening with subsequent targeted qPCR validation and qPCR screening of other CNV-like candidate DNA markers in samples of all widely encountered forensically-relevant tissue types. The researchers were able to identify DNA markers respective of blood and semen, respectively.

The qPCR assays developed were shown to be sensitive, specific, and robust in the presence of degraded DNA, thereby qualifying for forensic uses. Moreover, the CNV qPCR products served as input materials for downstream forensic STR analysis, which provided complete STR profiles, creating new potential for utilizing a single DNA aliquot for both tissue and individual identification.

CNV markers are highly advantageous for tissue identification, especially in cold/old cases when aged/old DNA extracts and no RNA are present. With further forensic validation experiments, CNV markers can be used in forensic tissue identification in future forensic casework [12].

Forensic Applications of Epigenomics

Age Estimation

Studies have been conducted to determine a method to accurately estimate the age of an individual using DNA methylation patterns. DNA methylation is a physiological process that alters DNA and is subject to aging. The feasibility of using DNA methylation-based age estimation models in forensic investigations, especially where the conventional methods of age estimation cannot be applied, was illustrated by [13]. They developed an expanded age prediction model from 895 Spanish DNA blood samples evenly distributed between 2 and 104 years old. Levels of DNA methylation in seven CpG sites of seven genomic regions were identified by Agena Bioscience EpiTYPER® technology.

Vidaki & Ballard [14] had found 45 precise DNA sites that were associated with age and used a technique known as Illumina's genome-wide methylation platform to analyze them. They applied statistical approaches to find the most significant DNA sites in age prediction and found that a machine learning model known as a generalized regression neural network model enhanced their performance in age prediction. They validated the performance of their model on various types of samples, such as blood and saliva, and discovered that it performed reasonably in all instances. They then created a new technique based on next-generation sequencing to measure the levels of DNA methylation at the individual DNA sites they discovered and discovered that this could also be used to predict age with reasonable accuracy.

Ambroa-Conde et al. [15] applied tissue-specific and age-correlated CpG sites from publicly accessible data to create tissue-dependent and age-prediction models from epigenetic markers, which is a DNA tool applied for criminal identification and age-prediction of physical traits. Methylation patterns in the various tissue samples, including

saliva and buccal cells, were depended upon for this approach.

dentification of Twins

MZ twins cannot be identified using routine forensic DNA examination because they have identical DNA profiles. This is a difficulty for the police. Rare somatic mutations can be detected using ultra-deep whole-genome sequencing, which is not always a success, and this is costly. Epigenetic contribution to some discordant phenotypes between genetically identical MZ twins has been suggested by a number of studies, pointing towards significant epigenetic heterogeneity within MZ twin pairs. While the forensic utility of epigenetic profiling in discriminating MZ twins has been discussed in a few studies, it still remains unclear as to whether twin-to-twin differences are twin pair-specific or global and applicable to all twin pairs. In recent years, forensic epigenetics has been applied to distinguish between MZ twins, and the majority of twin-distinguishing CpG sites are reproducible by targeted approaches in trace-type DNA from bloodstains, though there are technical hurdles to overcome. The number of epigenetic marks that are required for statistically valid identification of individual MZ twins is not clear, considering that current screening technologies are not applicable for trace investigation. Further studies of DNA methylation stability across time and tissues, technologies, and methodologies will reveal if differential DNA methylation proves to be an adequate method for answering this forensic question. [16]

Drug Identification

The potential of predicting drinking habits of individuals through forensic epigenomics has been demonstrated, with differential DNA methylation observed in regular alcohol consumers compared to non-drinkers. Investigative guidance can be provided by this information. Traditional forensic toxicological tests for alcohol metabolite detection exist, but they cannot provide information on regular drinking habits. Numerous epigenetic markers associated with alcohol metabolism were identified in the first epigenome-wide association study (EWAS) for alcohol dependency, the majority of which were found to be hypomethylated in alcoholics versus non-drinkers. A meta-analysis of several studies suggested five CpGs as biomarkers for heavy alcohol drinking, which explained a substantial proportion of interindividual variance in alcohol consumption. Based on 144 CpGs, a preliminary prediction study achieved AUC > 0.90. However, robust markers for a forensically suitable prediction tool need to be identified through further research. Additionally, maternal alcohol intake during pregnancy can alter gene-specific methylation in placental cord blood, potentially leading to false-positive predictions [16].

Transcriptomics

Transcriptomics is the study of the transcriptome, the complete set of RNA transcripts including mRNA, tRNA, rRNA, and ncRNAs that are produced by the genome, under specific circumstances or in a specific cell, using high-throughput methods, such as microarray analysis. Understanding the genome's expression at the transcriptional level through transcriptome analysis gives us knowledge of the structure of the genes, how they are regulated, how their products work, and how the genome changes over time. [17]

Applications of Transcriptomics in Forensic Science

Identification of Body Fluids

Conventional techniques for identifying body fluids require an assortment of time- and money-consuming, labor-intensive, and technologically diverse procedures that are carried out sequentially rather than simultaneously. These techniques are based on protein-based, or enzyme-facilitated, tests that are not always conclusive. In recent years, advancements in genomic studies have revealed the diversity in transcriptome and its potential in identifying body fluids. Methods based on mRNA make it simple to identify bodily fluids and replace the existing array of biochemical and serological testing.

It was believed that RNA is less in quantity and degrades over time, and thus is not a suitable candidate for forensic analysis, but recent studies have proved that RNA can be retrieved in a quantity and quality suitable for analysis and has been demonstrated to be stable in biological stains. [18][19]

From biological stains, RNA can be extracted in sufficient amount and quality for analysis, several potential tissue-specific candidate genes that might aid in the accurate identification of body fluids. The targeted RNA test is based on the identification of up-regulated biomarkers in certain bodily fluids, and intra-class heterogeneity in biomarker expression within each class is seen.

Several candidate tissue-specific genes biomarkers have high expression, as ALAS2, hemoglobin alpha (HBA) and hemoglobin beta (HBB) in blood, and for semen, protamine 1 (PRM1) and protamine 2 (PRM2) as sperm markers and semenogelin 1 (SEMG1) as seminal plasma marker, for saliva, histatin 3 (HTN3) and statherin (STATH), in vaginal secretions (CYP2B7P1), in menstrual blood (MMP10), and LCE1C in skin cells. [18][20][21].

mRNAs are used widely for forensic purposes, but the large size of mRNA makes it prone to degradation, and this can hinder forensic investigations, where samples are degraded in most cases. To cope with this challenge, miRNAs (microRNAs) are being studied as a potential alternative to mRNA-based identification. miRNAs are small noncoding RNAs (ncRNAs) that play a regulatory role in cells. miRNAs are between 16-24 nucleotides in length. Many studies over the years have found miRNAs as potential biomarkers for the identification of body fluids in forensic investigations. [22]

miRNAs have been demonstrated to be extremely tissue-specific; the expression patterns of miRNAs in various bodily fluids vary. Weber et al. [23] investigated the miRNA spectrum in 12 body fluids and mentioned a number of tissue-specific miRNA markers that can be utilized for the identification of body fluids. miR-585, miR-187 and miR-192 are blood plasma specific, miR-1, miR-197, miR-588, miR-617 and miR-20b are seminal fluid specific, miR27a, miR-492, let-7a are saliva specific. The research also reported miRNA markers for several other body fluids, such as amniotic fluid, tears, etc.

These markers could be employed to identify body fluids when standard methods do not work, due to degradation of the sample or poor amount of material. The analysis of miRNA expression profiles in different body fluids can

provide a more sensitive and specific method to ascertain the type of body fluid. miRNA analysis can provide a more accurate and reliable method of detecting body fluids in forensic examinations that can assist in crime investigation and close victims' rights.

Detection of Drug Abuse

Criminal investigations are based on the proper and timely identification of drug use. New drugs with masking capacity are quickly adopted as a result of the desire to avoid detection. Drug identification via standard tests is challenging at a certain point because of drug elimination from the system. Transcriptomic biomarkers have become a viable method for drug detection in forensic cases in recent years. Transcriptomic microarrays' capacity to identify unique changes in gene expression following blood manipulation has been supported by several studies.

Recombinant human erythropoietin (rHuEPO) is a synthetic form of the hormone erythropoietin (EPO), widely used for sports doping. Red blood cell synthesis is primarily regulated by the hormone erythropoietin (EPO). Recombinant EPO has emerged as the most effective medication for treating anemia due to several reasons; nonetheless, it is occasionally abused in sports to enhance performance and endurance. [24]

The Athlete Blood Passport employs a range of blood and urine tests to check for rHuEPO; however, it was discovered by (Ashenden et al. 2011) that conventional methods of testing in ABP did not flag any participants that received micro doses of rHuEPO. Micro doses of rHuEPO are widely used in doping because they are not easily detected; however, in recent years, transcriptomic approaches have been studied to detect rHuEPO use, as use of rHuEPO will alter the indigenous transcriptome. Wang et al. [25] study identified numerous transcriptomic biomarkers such as ALAS2, BCL2L1, DCAF12, EPB42, GMPR, SELENBP1, SLC4A1, TMOD1, and TRIM58 that exhibited differential expression during and across the post phase of micro dose rHuEPO administration. This indicates that there is a possibility of transcriptomics being utilized as a consistent approach to detection of doping.

Sports doping involves the use of autologous blood transfusions (ABTs). ABTs can help athletes perform better by raising their red blood cell count. Researchers in Leuenberger et al. [26] analyzed the potential of circulating miRNAs as biomarkers for the detection of autologous blood transfusion, a significant but still undetected doping method. Ten miRNAs were discovered to be elevated in transfusion samples, including miR-26b, miR-30b, and miR-30c.

While investigating transcriptomic biomarkers for ABTs, Kannan and Atreya [27] found that by differential profiling of red blood cell (RBC) miRNAs, 4 out of 52 selected miRNAs expression changed. These were miR-96, miR-150, miR-196a, miR-197.

Gene doping refers to the use of gene therapy for non-therapeutic goals, such as improving sports performance or beauty. It involves the deliberate alteration of a person's DNA to enhance their physical or mental capabilities beyond what is seen as normal or natural.

Transcriptomics has a potential use in the detection of gene doping. Transcriptomics can be used to identify changes in gene expression of genes that have been doped. Upregulation in gene expression of a particular gene can potentially identify a doped gene; however, the inability to discriminate between naturally occurring intra-individual and technical variance and the influence of doping on gene expression appears to be a key issue with mRNA profiling, especially when using the blood transcriptome. [28]

Estimation of Post-Mortem Interval

The period since the time of death is known as the post-mortem interval (PMI). In criminal, civil, and forensic cases, the postmortem interval (PMI) must be established. Many methods for evaluating PMI have been developed over time, but their accuracy and scope of application are frequently constrained. Transcriptomic methods can be used to examine changes in gene expression patterns that occur in the body's tissues and fluids after death. The PMI can be determined by RNA degradation, which depends on environmental factors such as temperature, moisture, and microbial activity.

Transcriptomic analysis may provide a rapid and non-destructive method to establish the PMI.

Ye-Hui Lv et al. [29] examined if there were chances for the estimation of PMI based on mRNA abundances, 18S rRNA, U6 snRNA, and microRNA transcript abundances. They removed the rat spleen tissues at different PMIs at 4°C or 25°C and quantified the gene transcript abundance in the samples using RT-qPCR. The study said GAPDH2 and ACTB2 mRNAs degraded quickly after death, so they were suitable for estimating early PMI. Under altered ambient temperatures and PMIs, 18S rRNA transcripts showed a range of degradation patterns and therefore could be used to estimate longer PMIs.

microRNAs (miRNAs) and circular RNAs (circRNAs) were also more stable as reference genes in cadavers than other types of RNAs, since research [30] has demonstrated the stability of multi-RNA markers in mouse heart, liver, and skeletal muscles 8 days after death. They identified the reference genes for three types of tissues based on their tissue-specific expression: miR-122, miR-133a, and 18S for heart tissues; LC-Ogdh, circAFF1, and miR-122 for liver tissues; and miR-133a, circ-AFF1 for skeletal muscle tissues. They also chose suitable biomarkers for post-mortem interval (PMI) estimation, which was most related to PMI. Gapdh, Rps18, U6, and -actin were found to be unstable and chosen as candidate target biomarkers.

Na et al. [31] studied the possibility of PMI estimation through the study of RNA expression in bone tissues. Autopsy of 71 individuals with PMIs of one day to two years yielded 71 bones (patella). On the basis of existing literature, let-7e and miR-16 miRNAs were used as internal controls for bone tissue. To correct the target miRNA levels to normal, the internal spike-in control miRNA Ce_miR-39_1 was utilized

. let-7e and miR-16 expression levels were compared with rising PMI using real-time quantitative reverse transcription polymerase chain reaction. The results imply that PMI might be calculated using the amount of expression of certain miRNAs (let-7e and miR-16) in bone tissue.

A mathematical model to estimate PMI was developed by [35], and they suggest that utilizing a multi-temperature and multi-index mathematical model will significantly improve the precision of PMI estimation by RNA. They showed that the PMI had a substantial correlation with ACTB and

E-ISSN: 2790-6094
P-ISSN: 2957-4587

GAPDH. The study's low error rate (7.4% of 15 rats and 12.5% of 8 people) indicates the mathematical model's dependability, which represents fresh development in improving the precision of PMI estimates and offers a useful tool for forensic pathologists.

Table 1. Various Techniques used in Omics Analysis (I)

Omics Technology	Techniques	Uses	References:
Genomics	Next-Generation Sequencing (NGS)	Provide large-scale genomic sequencing data	[4][7][33]
	Genome Assembly	piecing together short DNA sequence reads generated by sequencing technologies to reconstruct the complete genome sequence	
	Structural Variation Analysis	Detect and characterize structural variations in the genome	
Epigenomics	Chromatin Immunoprecipitation (ChIP)	Investigation of protein-DNA interactions and the mapping of histone modifications or transcription factor binding sites	[12][10][16]
	Epigenetic Profiling Arrays	comprehensive view of the epigenetic landscape and facilitate comparative epigenomic studies	
	Histone Modification Analysis	Detect and quantify the presence of specific histone marks.	
Transcriptomics	RT-PCR	amplification and quantification of specific RNA molecules	[27][28][32]
	qPCR	Validate and quantify gene expression data.	
	RNA-Seq	identification of known and novel transcripts, detection of alternative splicing events, and accurate measurement of gene expression levels.	

Proteomics

The extensive study of proteomes is known as proteomics. An organism, system, or biological setting produces a set of proteins known as a proteome. The proteome of a species (like Homo sapiens) or an organ (for example, the liver). The proteome is dynamic; it varies from cell to cell, and alterations occur over time [34]. The proteome varies periodically from cell to cell and in response to environmental factors. [35]

Proteomics in Forensics

Forensic science involves handling, transferring, and analyzing biological evidence. With a few notable exceptions, protein makes up most of the evidence. Several biomolecules that are relevant for forensic purposes, including DNA, but also other compounds, are found in the matrix. A prerequisite for further investigation is the breakdown of the protein matrix and the isolation of these components. Many forensic scientists view the use of proteins as historical. [36]

Human genome sequencing was a recent innovation brought about by the revolution in proteomics. It also made it possible to map out the entire human proteome, hastening the DNA revolution. Even though each peptide's fragmentation pattern is distinct, isolating the spectrum that results from matching one amino acid sequence to another can be extremely difficult and imprecise. The matching procedure becomes easier and more certain when the number of predicted peptide sequences that can be present in a sample is constrained in a procedure known as PSM (peptide spectral matching). Each match between a theoretical mass from a database and a fragmentation mass spectrometry spectrum mass from sample data gets a score, which rises with more precise alignments. It is possible to generate statistical scores to determine the likelihood and degree of uncertainty that a specific score would occur randomly. [36]

The high specificity of proteome detection and analysis yields a wide range of data on various topics, including the species to which a particular sample belongs, a person's health, and even, via bioindicators, environmental conditions. There are remnants of accidents, crimes, natural disasters, and armed conflicts across the area where the forensic examination is being conducted. A large portion of the evidence that is found at the crime scenes and that can be studied is biological (blood, skin fragments, fluids), non-biological (textile fibers or soils), or both. These materials contain protein molecules that, after being isolated and characterized, can provide information that goes beyond human identification or ancestry determination. [37] The most comprehensive and adaptable tool in large-scale proteomics is mass spectrometry (MS), which employs mass analysis to characterize proteins. [38]

Applications of Forensic Proteomics for Human Samples

Hair Proteomics

Frequently, hair can be found at crime scenes. Keratinization of epidermal keratinocytes results in its formation. Because of its constituents, which are mainly coiled-coil proteins with intermolecular disulfide bonds, hair is physically flexible and strong. It often endures a variety of environmental circumstances. However, because DNA can be severely degraded as a result of the keratinization process, hair may not be a good sample for DNA analysis. Hair can be used to identify specific individuals using a proteomic technique. Interestingly, employing a combined process with excellent compatibility, mitochondrial genome and proteome profiles were found in hair samples. An analysis of hair samples with great sensitivity was suggested in a recent work, and a library including all detected peptides generated from hair was developed. [39]

Bone Proteomics

The extracellular matrix contains proteins that make up bones, and approximately 90% of these proteins are collagenous. Due to their cross-linked structure and the protection provided by the bone matrix, bone collagens are incredibly stable. As a result, Buckley et al. identified a 33 amino acid peptide produced from collagen that may be utilized to differentiate between sheep and goat bone because it differs between the two species at two places. While many low-abundance proteins in bone deteriorated with time, Wadsworth et al. observed that serum albumin and alpha-2-HS-glycoprotein (AHSG) could be readily retrieved in ancient bone. Due to their interactions with bone collagen, other proteins such as lumican, chondroadherin, and biglycan also survive well. They can help classify species and research phylogenetic conclusions in bones found in archaeological and paleontological specimens. [39]

Blood Proteomics

Since blood constitutes one of the primary markers of a violent incident and is difficult to remove from the scene of the forensic inquiry, its traces can persist for a considerable amount of time, making it one of the most significant pieces of biological evidence. Also, this fluid contains vital biological data about the victim and the offender, like their sex, blood type, and medical condition, among other things. Since each biological fluid serves a particular purpose, the kind and quantity of proteins that are present in each fluid are distinctive and can be used as a signature to distinguish one fluid from another. It is feasible to discover fluid-specific markers, such as amylase in saliva, prostatic antigen in semen, or β-spectrine and haemoglobin in the case of blood, using proteomic techniques. Most importantly, mass spectrometry is an important technique that allows us the identify protein markers accurately. [37]

Any sample containing proteins can be characterized using proteomics. Protein sequences include a lot of the same information (both functional and phylogenetic) as DNA, since the base sequence of DNA encodes the amino acid sequence of proteins. The identity and quantity of each organ, tissue, or cell type's proteome are unique, in contrast to DNA, which is the same in every cell of an organism. Hence, forensic techniques can make use of both abundance and sequencing information. It may only be necessary to use sequences to identify the organism(s) responsible for a proteinaceous sample or to distinguish between genetically separate members of the same species. Information on protein abundance may be needed to distinguish between different tissues, organs, or biofluids from the same organism. [40]

Metabolomics

Metabolomics focuses on the comprehensive analysis of metabolites within a biological system. It is accurately defined as a discipline that offers systematic and comprehensive insights into the temporal fluctuations of metabolite levels in biofluids and tissues [41]. Among the various branches of omics, such as genomics, transcriptomics, and proteomics, metabolomics is the most recent addition and considered the youngest member.

Analytical Approaches used in Metabolomics

Various methodologies, such as targeted analysis, untargeted analysis, metabolomics fingerprinting, and metabolomics footprinting, are employed as approaches for metabolite analysis [41]. Among these, targeted and untargeted analyses are the most prevalent methods in current practice. The targeted approach involves the examination of a specific metabolite or a group of metabolites sharing similar chemical structures. On the other hand, the untargeted approach aims to comprehensively analyze all the metabolites present in each sample [42]

Untargeted metabolomics analysis can be carried out using either NMR or MS in combination with one of the separation techniques, such as liquid chromatography (LC), gas chromatography (GC), or capillary electrophoresis (CE). GC-MS is suitable for the measurement of volatile metabolites or those that can be converted into a volatile form. CE-MS is effective in detecting polar and ionogenic metabolites, while LC-MS can be employed for measuring both polar and non-polar metabolites. High-resolution MS analyzers, such as Orbitrap or time of flight (TOF), are typically used for untargeted analysis. Similar techniques can be utilized for targeted analysis as well, although the protocols may differ. [42]

Applications of Metabolomics in Forensic Science

Metabolomics, a burgeoning field that delves into the intricate study of small molecules or metabolites within biological systems, has emerged as a valuable asset in the realm of forensic science [43]. Through metabolomic analysis, crucial information can be gleaned for forensic investigations, including but not limited to identification of body fluids, determination of postmortem interval (PMI), detection of drug or toxin exposure, and exploration of the cause and manner of death. By scrutinizing the distinct metabolic profiles associated with various biological samples or forensic scenarios, metabolomics can provide valuable insights in unraveling intricate forensic cases and enhancing the comprehension of forensic evidence. Nevertheless, akin to any analytical tool, metabolomics in forensic science possesses certain limitations, such as sample stability, standardization of analytical methods, and interpretation of results. [44]

Blood Samples and Metabolomics

Blood, a dynamic fluid that serves as a conduit for diverse molecules, including metabolites, within the human body. It consists of erythrocytes, plasma, leukocytes, and platelets, each playing a unique role. The plasma component of blood encompasses an array of proteins and metabolites, including those that seep into the bloodstream from damaged cells post-cellular injury. After death, blood chemistry undergoes notable changes, with pH plummeting from 7.35-7.45 to a range of 5-5.5, and hematocrit surging from 40-45% to 47-78%. Several factors contribute to the increased acidity of blood, such as CO_2 accumulation and a drastic rise in lactic acid levels from 0.5-2.5mM to 50-60mM after 24 hours of death, accompanied by a 20% surge in acid phosphatase compared to ante-mortem levels after 48 hours of death [41]. Post-mortem serum concentrations of sodium and chloride, on average, decrease by 1 mmol/L per hour for the initial 3–52 hours post-mortem, while serum potassium levels rapidly surge within the first 1–2 hours after death. Plasma calcium concentration also witnesses a swift rise within an hour post-mortem, from 4 mmol/L to peak at 9.4 mmol/L five hours

later, before gradually declining to ante-mortem levels by 11 hours post-mortem. Inorganic phosphate, which is present at ante-mortem concentrations of 0.6–0.9 mmol/L, escalates within one hour post-mortem and reaches concentrations of 6.6 mmol/L by 18 hours after death (Donaldson and Lamont). These metabolites have been explored as potential postmortem interval (PMI) indicators; however, it should be noted that universal readings are elusive, as different studies report varied values influenced by diverse factors. In cases of death by poisoning, such as cyanide poisoning, the metabolite of hydrogen cyanide known as 2-aminothiazoline-4-carboxylic acid (ATCA) can be detected and quantified using an LC-MS/MS method. [45]

Pericardial Fluid and Metabolomics

The pericardium, a fluid-filled sac-like layer that envelops the heart, acts as a protective barrier. Within the pericardial cavity, a virtual space that typically encases the heart, lies the pericardial fluid (PF), a pale-yellow serous fluid with a volume ranging from approximately 15 to 60 mL under normal physiological conditions. The ^{1}H NMR technique was employed to analyze pericardial fluid samples obtained from 24 autopsies to assess metabolite activities. Choline, glycine, ethanolamine, and hypoxanthine were determined to be the most important metabolites potentially to be used for the estimation of postmortem interval (PMI) in forensic practice. [46]

Hair/Nail Samples and Metabolomics

Hair samples are an important type of trace evidence because they have the capacity to be found with DNA, which is useful in cases where other primary DNA sources are not available. Hairs also provide an extended period during which metabolites can be detected compared to other biological samples. Metabolites are usually cleared from the body shortly after intake of drugs, yet they can stay within hair shafts for a long time. Likewise, nails also grow slowly, and thus a longer window for detecting metabolites exists. Zolazepam and tiletamine drugs were incorporated into the hair of Long-Evans rats, with limits of quantification ranging from 20 to 50 pg in 10 mg of hair. The drug incorporation rates were found to be 0.03±0.01% for zolazepam and 2.09±0.51% for tiletamine in pigmented hair. This method was also successfully applied in human cases [47].

Nails have been employed to identify intoxication of drugs like arsenic and metal exposure of cadmium, copper, lead, zinc, iron, and magnesium through nail analysis. Liquid chromatography quadrupole time-of-flight mass spectrometry was utilized for the analysis of hair and nail samples of 70 postmortem cases. Results derived from nail clippings and complete nail samples were similar to those derived from hair analysis. The research identified 89 various analytes, such as antidepressants, drugs of abuse, and antihypertensives, confirming the inclusion of multiple substances in the nail matrix. [48]

New Psychoactive Substances/Drugs of Abuse and Metabolomics

Older analytical techniques might not be enough to effectively screen for New Psychoactive Substances (NPS) anymore. Detection of drug use in screening methods could be feasible only by detection of one or more specific metabolites, especially when the parent drug itself is undetectable in samples. For example, an untargeted strategy was employed on the urine samples of 20 subjects 4.5 hours post-gamma-hydroxybutyric acid (GHB) ingestion, which identified GHB carnitine, GHB glycine, and GHB glutamate as metabolites [49].

Liquid chromatography with high-resolution mass spectrometry (LC-HRMS) was used to detect markers of valproic acid in blood and reported the identification of sodium adducts of C7H14O3 and C8H14O3, and 3-hydroxy-4-en-VPA. [50]

Biomarkers for methamphetamine intoxication were identified through urinary and plasma samples, revealing the presence of 5-oxoproline, saccharic acid, uracil, 3-hydroxybutyrate, adipic acid, glucose, glucose 6-phosphate, fructose 1,6-bisphosphate, and tricarboxylic acid cycle intermediates (fumarate), as reported by Shima et al.

It was found that crack users exhibited a reduction in carnitine and acylcarnitine levels, along with an accumulation of histidine in their serum. [51]

Furthermore, levels of benzoylecgonine, a major cocaine metabolite from ester hydrolysis, were comparable in urine samples, but the levels of cocaine metabolites from oxidative metabolism, such as N-hydroxybenzoylnorecgonine and hydroxybenzoylecgonine, differed significantly between rat and mouse species, indicating species-dependent cocaine metabolism. [52]

Post-Mortem Interval and Metabolomics

Researchers are actively seeking a more precise method for estimating the time of death, which includes considering compounds involved in metabolism. One such promising candidate is free trimethylammonium (fTMA), which has been proposed as a viable marker. [42]

Metabolites obtained from the livers of rats at various time intervals (0, 24, and 48 hours) after sacrifice were subjected to analysis using UPLC/Q-TOF MS. The findings revealed that polysaccharides, steroids, and amino acids could potentially serve as biomarkers that enable the estimation of Postmortem Interval. [53]

It was observed that concentrations of hypoxanthine, ammonia, NADH, and formic acid all exhibited an increase over time after death, suggesting their potential as indicators for estimating the time of death. [54]

Microbiomics

The term "microbiome" refers to the community of microorganisms that inhabit both the external and internal environments of a particular species, along with their collective genome. Microorganisms are present on the epidermis, within the gastrointestinal tract, on other mucosal membranes, and on rare occasions, inside the cellular structure of the human body. The microbiota predominantly comprises bacteria, although it is also composed of fungi, archaea, and viruses. [55]

In recent years, microbiomics has emerged as a powerful tool for studying microbial communities in unprecedented detail. Several methods and techniques are utilized to study complex microbial communities, including DNA sequencing, RNA sequencing (transcriptomics), proteomics, metabolomics, metagenomics, bioinformatics, computational tools, and traditional culturing methods. [55]

DNA sequencing represents the pivotal tool in microbiomics, as it facilitates the identification of the genetic composition of microorganisms within a designated microbiome. RNA sequencing enables researchers to identify specifically which genes are being actively expressed. The elucidation of this information can offer significant knowledge pertaining to the functional roles that microbes undertake in their respective habitats, as well as their interactions with host organisms. Proteomics and metabolomics techniques aid in elucidating the functional capacity and metabolic activities of microbiomes at a deeper level. Through the correlation of gene expression with protein and metabolite synthesis, scholars can attain a more comprehensive comprehension of the functional significance of microorganisms residing in a microbiome.

The study of microbiomes is being comprehensively investigated through the amalgamation of high-throughput sequencing, multi-omics techniques, bioinformatics tools, and conventional culturing practices. This approach enables intricate characterization of the microbiome at an unparalleled level of detail. The utilization of said techniques has facilitated the understanding of intricate microbial communities among researchers, ultimately leading to the formulation of methodologies to optimize the utilization of such communities in the enhancement of both human and environmental health. The field of microbiomics is currently experiencing rapid development and presents substantial prospects for interdisciplinary research and innovative advancements. [55]

Applications of Microbiomics in Forensic Science

Forensic Microbiomics is a nascent discipline that employs the distinctive microbial DNA found within the human microbiome for forensic objectives. The microbiota evidence presents a novel level of evidence within a legal context, and the potential implications of Microbiomics in the field of forensic science are considerable. The investigative capabilities of microbial communities have been the focus of forensic microbiomics research, which has endeavored to address a variety of forensic concerns, including but not limited to the facilitation of crime resolution, identification of potential suspects, and the monitoring of postmortem interval and environmental exposure alterations.

Microbial Signatures on Personal Items:

The emerging field of forensic microbiomics has demonstrated that individual-specific microbial signatures are harbored by personal articles, including but not limited to cellular devices, computer keyboards, and clothing. In a particular investigation, researchers procured specimens from the keyboards utilized by a triad of distinct individuals and could effectively distinguish between the varied samples based on discernible variances within their microbiological constituencies. The findings suggest the potential application of microbiomics techniques to determine culprits based on the microbial profiles that they leave on their objects.

Procopio et al. [56] conducted a pilot study that explores the transferability and persistence of the "touch microbiome" on a surface once the fingerprint deposition has occurred, and its stability over a period of 30 days at room temperature. The study involved a group of 11 subjects who were asked to gently put a glass microscope slide to leave their unique fingerprints. Later, these impressions were then very carefully picked up with swabs. Human and microbial DNA were put to isolation and quantization. The results show that the microbial population on our skin, often known as the 'touch microbiome', can act as forensic markers similar to the already proven application of 'touch DNA'. The research results show that an exploration of the "touch microbiome" has promise for personal identification activities and could provide useful forensic intelligence information in the case of "touch DNA" failures. Additional studies incorporating increased numbers of surfaces, more time points, and bigger datasets are required to clarify the potential utility of "touch microbiome" research in real forensic contexts.

.The acquisition of skin microbiome samples from volunteers entails the collection of epithelial cells from the palmar surface of their hands via swabbing. The study employs the methodology of amplifying the Amylogenin locus and 16 human Short Tandem Repeats (STRs), in addition to sequencing the V4 region of the 16S ribosomal RNA (rRNA) gene using the Illumina MiSeq platform. [56]

Post-Mortem Interval Estimation

The microbiome of a cadaver that is decomposing experiences temporal changes, and this has been used in the field of forensic science to enable estimation of time since death. One such research effort compared changes occurring within the microbial communities that inhabit the skin of a decomposing swine cadaver, showing an expected, recognizable change in microbial community composition throughout decomposition. The researchers hypothesized that this technique has the capability to estimate postmortem interval through the observation of microbial communities present on the corpse, if an in-depth understanding of the subject is attained.

A recent study article discusses the possible use of artificial intelligence (AI) techniques and next-generation sequencing (NGS) in augmenting the dataset of microbial communities in cadavers and surrounding areas. The current research offers a significant contribution within the field of forensic science by showing the promise of AI-based analysis of microbiome to increase the accuracy of postmortem interval (PMI) estimation. The last decade has seen evidence of the promise of using the microbiome in estimating postmortem interval (PMI). The manuscript explains postmortem microbiome succession in corpses and their immediate surroundings. Moreover, it gives an overall idea of the possible advantages, pitfalls, and opportunities related to AI-based microbiome analyses for precise estimation of the Postmortem Interval (PMI). [57]

Soil Microbiomics

Specially, soil samples have been utilized in forensic science to enable the recovery of missing corpses and connecting the offender to the crime scene. In a particular research, some researchers collected soil samples from different geographical locations around a decaying mouse carcass. These people were able to find out through analysis that the microbial populations present in the soil showed consistency with the order of decomposition. The results of this research suggest the potential of soil microbiomics as a useful tool in forensic science to assist in the recovery of deceased bodies and identify possible offenders. [58]

Future of Microbiome in Forensics

E-ISSN: 2790-6094
P-ISSN: 2957-4587

The current research findings assume the need for future investigation to fully understand the patterns of microbial community succession in different environments and hosts, with the potential to maximize the accuracy of postmortem interval (PMI) estimation and other forensic uses. The paper further suggests that future research should aim to explore the potential use of microbiome analysis in other alternative forensic scenarios, beyond the identification of individuals, inference of geospatial location, and postmortem interval estimation. [59]

The utilization of microbiomics in forensic science presents inherent prospects, but also encounters certain constraints calling for resolutions to be sought. Microbial communities are constricted by numerous variables, including dietary patterns, geographical locations, and therapeutic interventions, thereby impeding the identification of "universal" microbial imprints for forensic applications. In addition, microbiomics analyses yield intricate data that may present challenges in interpretation and, consequently, may pose difficulties in the admissibility of such evidence in the court of law. Hence, it is imperative to conduct additional investigations to overcome the limitations and establish the dependability and precision of microbiomics methodologies for forensic applications. [59]

Table 2. Techniques used in Various Omics Analysis (II)

Omics Technology:	Techniques:	Uses	References
Proteomics	Gel Electrophoresis	visualization and comparison of protein patterns	[36][37][39]
	Mass Spectrometry (MS)	protein identification and peptide mass fingerprinting, protein sequencing, identification of post-translational modifications, and protein structure analysis	
	Protein Structural Analysis	Determine the three-dimensional structures of proteins	
Metabolomics	Mass Spectrometry (MS)	Identification and quantification of metabolites based on their mass-to-charge ratios	[42][44][53]
	Nuclear Magnetic Resonance (NMR) Spectroscopy	analyze metabolites based on their characteristic chemical shifts and coupling patterns	
	Gas Chromatography (GC)	separate and detect volatile and semi-volatile metabolites	
Microbiomics	Fluorescent In Situ Hybridization (FISH)	Visualize specific microbial groups or species within a sample	[56][58][59]
	PCR-Based Techniques	accurate quantification of low-abundance microbes or genes	
	DNA Sequencing	Information about the active genes and functional activities of the microbiome	

Discussion

In forensic sciences, genomics has become a potent tool, notably in human identification. DNA profiling and forensic ancestry analysis have been transformed by the examination of DNA markers such as short tandem repeats (STRs) and single-nucleotide polymorphisms (SNPs). Genomic sequencing techniques offer high-resolution data for person identification and can provide insightful genetic information in forensic cases. Investigating heritable variations in gene expression without affecting the DNA sequence is known as epigenomics

In forensic analysis, detection of the tissue origin, age estimation, and identification of bodily fluids are important.Internally, the most significant epigenetic markers utilized in forensic epigenomics are DNA methylation profiling and histone modification. Transcriptomics is the examination of RNA molecules to determine the gene expression levels and identify specific patterns in different tissues or bodily fluids. Transcriptomics could be applied to forensic sciences to identify the time of death, origin of tissues, and gain more information about the individual's physiological state when the event occurred. Proteins in biological samples can be identified and characterized through proteomics. It can be applied in forensic sciences to identify body fluids, profile protein biomarkers for human identification, and search for post-translational modifications. Forensic science can be greatly aided by the proper analysis of proteins. Metabolomics is defined as the study of metabolites, or small molecules, in biological samples by analyzing them. Metabolomics in forensic sciences can detect drugs, poisons, and other small molecules present in body fluids or tissues. It may be applied for calculation of postmortem interval, the identification of metabolic signs associated with some diseases or drugs, and toxicological research. Microbiomics analyzes microbial populations that are present in most environments, including the microbiomes associated with humans. Microbiomics, which gives information regarding an individual's specific microbial composition, is able to assist human identification in forensic science. It can also be employed in microbial forensics for the analysis of microbial signatures within crime scenes or on forensic samples. For instance, our ability to retrieve useful information from biological materials has significantly improved with the integration of these omics technologies with forensic sciences. However, obstacles and restrictions characterize this, such as the need for special equipment, problems with sample deterioration, and strict quality control methods that are required. Subsequent research would need to focus on protocol optimization, standardization of methodologies, and ethical concerns related to privacy and data protection. The integration of genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiomics sequentially in forensic sciences is greatly promising to enhance human identification, identify the source of a tissue sample, ascertain the time of death, examine protein biomarkers, detect small molecules, and explore microbial signatures. In order to harness the potential of these omics technologies in forensic examinations, more research and an interface between the forensic scientists, technologists, and policymakers are needed.

Conclusion

The application of omics technologies in forensic science is a revolutionary leap, broadening the reach of forensic analysis beyond conventional identification processes. With the power of genomics, transcriptomics, proteomics, metabolomics, epigenomics, and microbiomics, forensic experts are now able to retrieve multidimensional data from biological evidence, spanning from identity and ancestry to age, tissue origin, physiological state, time since death, and environmental exposure. These technologies provide increased sensitivity and resolution, even with degraded or limited samples, and are especially useful in tough or unsolved cases. In spite of their vast potential, the use of omics in forensics is still in its developmental phase and struggles with issues of standardization, data interpretation, legal admissibility, and ethical control. Solving these will need to involve cooperation across scientific, legal, and regulatory communities.

Author Contributions

Hifz Ur Rehman: writing—original draft, writing—review & editing, project administration. **Hashim Tufail**: conceptualization, validation, writing—original draft, project administration. **Amna Arooj**: writing—review & editing. **Ahsan Raza**: writing—original draft. **Ume Kalsoom**: writing—review & editing. **Hafiz Muhammad Abbas Malik**: supervision. **Muhammad Shahid Cholistani**: supervision. **Samiaan Qurban Khan**: writing—review & editing. **Muhammad Abdullah Nasir**: writing—original draft. **Zain Ali**: writing—original draft. **Mudassar Irshad**: validation. **Haris Faheem**: validation.

Ethics Statement

Not applicable

Informed Consent Statement

Not applicable

Funding

This work received no external funding

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Dai, X., & Shen, L. (2022). Advances and Trends in Omics Technology Development. Frontiers in Medicine, 9, 911861. https://doi.org/10.3389/fmed.2022.911861
2. Cholistani, M. S., Raza, M. H., Farhan, M., Ashraf, B., Ur Rehman, H., Arjamand, M., Luqman, M., Basit, A., Shakoor, S., Saleem, A., & Ashraf, M. A. (2024). Forensic Genealogy: A Powerful Investigation Tool for Resolving the Cold Cases. Journal of Forensic Science & Criminology, 12(3). https://www.annexpublishers.com/articles/JFSC/12301-Forensic-Genealogy-A-Powerful.pdf
3. Cappellino, A. (2018, July 17). Daubert vs. Frye: Navigating the Standards of Admissibility for Expert Testimony. Expert Institute. https://www.expertinstitute.com/resources/insights/daubert-vs-frye-navigating-the-standards-of-admissibility-for-expert-testimony/
4. Budowle, B., & Chakraborty, R. (2015). Forensic genomics: Applications to human identification. Annual Review of Genomics and Human Genetics, 16, 49-71. https://doi.org/10.1146/annurev.genom.4.070802.110352
5. Rehman, H. U., Arooj, A., Aslam, M. A., Cholistani, M. S., Farhan, M., Kareem, K., Ashraf, M. A., & Pervaiz, M. (2025). Methods of extraction of genetic material from hard tissues: A review of the 21st century advancements. Forensic Science International, 367, 112382. https://doi.org/10.1016/j.forsciint.2025.112382
6. Kayser, M., & de Knijff, P. (2011). Improving human forensics through advances in genetics, genomics and molecular biology. Nature Reviews Genetics, 12(3), 179–192. https://doi.org/10.1038/nrg2952
7. Kayser, M. (2015). Forensic DNA Phenotyping: Predicting human appearance from crime scene material for investigative purposes. Forensic Science International: Genetics, 18, 33-48. doi: 10.1016/j.fsigen.2015.02.003
8. Walsh, S. (2019). Forensic human identification using skin DNA: Considerations on the development of a DNA-based forensic phenotype database. Forensic Science International: Genetics, 43, 102152. doi: 10.1016/j.fsigen.2019.102152
9. Liu, F., Chen, H., Han, Y., You, Y., Liu, L., & Wu, H. (2019). Facial prediction from DNA using face-to-DNA classifiers. Forensic Science International: Genetics, 41, 92-101. doi: 10.1016/j.fsigen.2019.04.001
10. Bernstein, B. E., Meissner, A., & Lander, E. S. (2007). The Mammalian Epigenome. Cell, 128(4), 669–681. https://doi.org/10.1016/j.cell.2007.01.033
11. Kundaje, A., Meuleman, W., Ernst, J., Bilenky, M., Yen, A., Heravi-Moussavi, A., … Pfenning, A. R. (2015). Integrative analysis of 111 reference human epigenomes. Nature, 518(7539), 317–330. https://doi.org/10.1038/nature14248
12. Zubakov, D., Chamier-Ciemińska, J., Kokmeijer, I., Maciejewska, A., Martínez, P., Pawłowski, R., … Kayser, M. (2018). Introducing novel type of human DNA markers for forensic tissue identification: DNA copy number variation allows the detection of blood and semen. Forensic Science International: Genetics, 36, 112–118. https://doi.org/10.1016/j.fsigen.2018.06.021
13. Freire-Aradas, A., Girón-Santamaría, L., Mosquera-Miguel, A., Ambroa-Conde, A., Phillips, C., Casares de Cal, M., … Lareu, M. V. (2022). A common epigenetic clock from childhood to old age. Forensic Science International: Genetics, 60, 102743. https://doi.org/10.1016/j.fsigen.2022.102743
14. Vidaki, A., & Ballard, D. (2017). DNA methylation-based forensic age prediction using artificial neural networks and next generation sequencing. Forensic Science International: Genetics, 28, 225–236. https://doi.org/10.1016/j.fsigen.2017.02.009
15. Ambroa-Conde, A., Girón-Santamaría, L., Mosquera-Miguel, A., Phillips, C., Casares de Cal, M. A., Gómez-Tato, A., … Freire-Aradas, A. (2022). Epigenetic age estimation in saliva and in buccal cells. Forensic Science International. Genetics, 61, 102770. https://doi.org/10.1016/j.fsigen.2022.102770
16. Vidaki, A., &Kayser, M. (2017). From forensic epigenetics to forensic epigenomics: broadening DNA

investigative intelligence. Genome Biology, 18(1). https://doi.org/10.1186/s13059-017-1373-1
17. Dong, Z., & Chen, Y. (2013). Transcriptomics: Advances and approaches. Science China Life Sciences, 56(10), 960–967. https://doi.org/10.1007/s11427-013-4557-2.
18. Juusola, J., & Ballantyne, J. (2003). Messenger RNA profiling: a prototype method to supplant conventional methods for body fluid identification. Forensic Science International, 135(2), 85–96. https://doi.org/10.1016/s0379-0738(03)00197-x
19. Li, Z., Chen, D., Wang, Q., Tian, H., Tan, M., Peng, D., Tan, Y., Zhu, J., Liang, W., & Zhang, L. (2021). mRNA and microRNA stability validation of blood samples under different environmental conditions. Forensic Science International: Genetics, 55, 102567. https://doi.org/10.1016/j.fsigen.2021.102567
20. Albani, P. P., & Fleming, R. (2018). Novel messenger RNAs for body fluid identification. Science & Justice, 58(2), 145–152. https://doi.org/10.1016/j.scijus.2017.09.002.
21. Hanson, E., Ingold, S., Haas, C., & Ballantyne, J. (2018). Messenger RNA biomarker signatures for forensic body fluid identification revealed by targeted RNA sequencing. Forensic Science International: Genetics, 34, 206–221. https://doi.org/10.1016/j.fsigen.2018.02.020.
22. Silva, S. S., Lopes, C., Teixeira, A. L., Sousa, M. J. C. de, & Medeiros, R. (2015). Forensic miRNA: Potential biomarker for body fluids? Forensic Science International: Genetics, 14, 1–10. https://doi.org/10.1016/j.fsigen.2014.09.002
23. Weber, J. A., Baxter, D. H., Zhang, S., Huang, D. Y., How Huang, K., Jen Lee, M., Galas, D. J., & Wang, K. (2010). The MicroRNA Spectrum in 12 Body Fluids. Clinical Chemistry, 56(11), 1733–1741. https://doi.org/10.1373/clinchem.2010.147405.
24. Debeljak, N., & Sytkowski, A. J. (2012). Erythropoietin and erythropoiesis stimulating agents. Drug Testing and Analysis, 4(11), 805–812. https://doi.org/10.1002/dta.1341
25. Wang, G., Durussel, J., Shurlock, J., Mooses, M., Fuku, N., Bruinvels, G., Pedlar, C., Burden, R., Murray, A., Yee, B., Keenan, A., McClure, J. D., Sottas, P.-E., & Pitsiladis, Y. P. (2017). Validation of whole-blood transcriptome signature during microdose recombinant human erythropoietin (rHuEpo) administration. BMC Genomics, 18(Suppl 8). https://doi.org/10.1186/s12864-017-4191-7.
26. Leuenberger, N., Schumacher, Y. O., Pradervand, S., Sander, T., Saugy, M., & Pottgiesser, T. (2013). Circulating microRNAs as Biomarkers for Detection of Autologous Blood Transfusion. PLoS ONE, 8(6), e66309. https://doi.org/10.1371/journal.pone.0066309
27. Kannan, M., & Atreya, C. (2010). Differential profiling of human red blood cells during storage for 52 selected microRNAs. Transfusion, 50(7), 1581–1588. https://doi.org/10.1111/j.1537-2995.2010.02585.x
28. Neuberger, E. W. I., Jurkiewicz, M., Moser, D. A., & Simon, P. (2012). Detection of EPO gene doping in blood. Drug Testing and Analysis, 4(11), 859–869. https://doi.org/10.1002/dta.1347
29. Lv, Y.-H., Ma, K., Zhang, H., He, M., Zhang, P., Shen, Y., Jiang, N., Ma, D., & Chen, L. (2014). A Time Course Study Demonstrating mRNA, microRNA, 18S rRNA, and U6 snRNA Changes to Estimate PMI in Deceased Rat's Spleen. Journal of Forensic Sciences, 59(5), 1286–1294. https://doi.org/10.1111/1556-4029.12447
30. Tu, C., Du, T., Ye, X., Shao, C., Xie, J., & Shen, Y. (2019). Using miRNAs and circRNAs to estimate PMI in advanced stage. Legal Medicine (Tokyo, Japan), 38, 51–57. https://doi.org/10.1016/j.legalmed.2019.04.002
31. Na, J.-Y. (2020). Estimation of the post-mortem interval using microRNA in the bones. Journal of Forensic and Legal Medicine, 75, 102049. https://doi.org/10.1016/j.jflm.2020.102049.
32. Lv, Y.-H., Ma, J.-L., Pan, H., Zhang, H., Li, W.-C., Xue, A.-M., Wang, H.-J., Ma, K.-J., & Chen, L. (2016). RNA degradation as described by a mathematical model for postmortem interval determination. Journal of Forensic and Legal Medicine, 44, 43–52. https://doi.org/10.1016/j.jflm.2016.08.015.
33. Patterson, N., Moorjani, P., Luo, J., Mallick, S., Reich, D. (2012). Ancient human genomes provide new insights into human evolution. Nature, 488(7412), 476-481.
34. EMBL-EBI. (2023). What is proteomics? | Proteomics. EMBL-EBI. https://www.ebi.ac.uk/training/online/courses/proteomics-an-introduction/what-is-proteomics/.
35. Aslam, B., Basit, M., Nisar, M. A., Khurshid, M., & Rasool, M. H. (2016). Proteomics: Technologies and Their Applications. Journal of Chromatographic Science, 55(2), 182–196. https://doi.org/10.1093/chromsci/bmw167
36. Parker, G. J., McKiernan, H. E., Legg, K. M., & Goecker, Z. C. (2021). Forensic proteomics. Forensic Science International: Genetics, 54, 102529. https://doi.org/10.1016/j.fsigen.2021.102529.
37. Díaz Martín, R. D., Camacho-Martínez, Z., Ambrosio Hernández, J. R., & Valencia-Caballero, L. (2019). Proteomics as a new tool in forensic sciences. Spanish Journal of Legal Medicine, 45(3), 114–122. https://doi.org/10.1016/j.remle.2019.08.001
38. Yates, J. R., Ruse, C. I., & Nakorchevsky, A. (2009). Proteomics by Mass Spectrometry: Approaches, Advances, and Applications. Annual Review of Biomedical Engineering, 11(1), 49–79. https://doi.org/10.1146/annurev-bioeng-061008-124934.
39. Duong, V-A, Park, J-M, Lim, H-J & Lee, H 2021, 'Proteomics in Forensic Analysis: Applications for Human Samples', Applied Sciences, vol. 11, no. 8, p. 3393. https://doi.org/10.3390/app11083393
40. Merkley, E. D., Wunschel, D. S., Wahl, K. L., & Jarman, K. H. (2019). Applications and challenges of forensic proteomics. Forensic Science International, 297, 350–363. https://doi.org/10.1016/j.forsciint.2019.01.022.
41. Castillo-Peinado, L.S., and M.D. Luque de Castro.

"Present and Foreseeable Future of Metabolomics in Forensic Analysis." Analytica Chimica Acta, vol. 925, June 2016, pp. 1–15, https://doi.org/10.1016/j.aca.2016.04.040
42. Szeremeta, Michal, et al. "Applications of Metabolomics in Forensic Toxicology and Forensic Medicine." International Journal of Molecular Sciences, vol. 22, no. 6, 16 Mar. 2021, p. 3010, https://doi.org/10.3390/ijms22063010
43. Soltysik S, van Beek TA, Zadlo A. Metabolomics in forensic science: Current trends, challenges, and future prospects. Forensic Sci Int. 2018 May;286:140-159. doi: 10.1016/j.forsciint.2018.02.038
44. Schoenmakers PJ, Weyermann C. Forensic metabolomics: applications of high-resolution mass spectrometry in forensic urine drug testing. Bioanalysis. 2017 Oct;9(19):1479-1496. doi: 10.4155/bio-2017-0097.
45. Giebułtowicz, Joanna, et al. "LC-MS/MS Method Development and Validation for Quantitative Analyses of 2-Aminothiazoline-4-Carboxylic Acid – a New Cyanide Exposure Marker in Post Mortem Blood." Talanta, vol. 150, Apr. 2016, pp. 586–592, https://doi.org/10.1016/j.talanta.2015.12.076.
46. Chighine, Alberto, et al. "Metabolomics Investigation of Post-Mortem Human Pericardial Fluid." BioRxiv (Cold Spring Harbor Laboratory), 1 Mar. 2023, https://doi.org/10.1101/2023.02.28.530436
47. Kim, Jihyun, et al. "A Comprehensive and Sensitive Method for Hair Analysis in Drug-Facilitated Crimes and Incorporation of Zolazepam and Tiletamine into Hair after a Single Exposure." Analytical and Bioanalytical Chemistry, vol. 408, no. 1, 10 Oct. 2015, pp. 251–263, https://doi.org/10.1007/s00216-015-9099-y
48. Krumbiegel, Franziska, et al. "Nails Are a Potential Alternative Matrix to Hair for Drug Analysis in General Unknown Screenings by Liquid-Chromatography Quadrupole Time-of-Flight Mass Spectrometry." Forensic Science, Medicine, and Pathology, vol. 10, no. 4, 19 July 2014, pp. 496–503, https://doi.org/10.1007/s12024-014-9588-x.
49. Steuer, Andrea E., et al. "Identification of New Urinary Gamma-Hydroxybutyric Acid Markers Applying Untargeted Metabolomics Analysis Following Placebo-Controlled Administration to Humans." Drug Testing and Analysis, vol. 11, no. 6, 16 Jan. 2019, pp. 813–823, https://doi.org/10.1002/dta.2558
50. Christian Brinch Mollerup, et al. "Retrospective Analysis for Valproate Screening Targets with Liquid Chromatography–High Resolution Mass Spectrometry with Positive Electrospray Ionization: An Omics-Based Approach." Drug Testing and Analysis, vol. 11, no. 5, 1 May 2019, pp. 730–738, https://doi.org/10.1002/dta.2543
51. Costa, Tássia R, et al. "Insights into the Effects of Crack Abuse on the Human Metabolome Using a NMR Approach." Journal of Proteome Research, 2 Nov. 2018, https://doi.org/10.1021/acs.jproteome.8b00646.
52. Yao, Dan, et al. "Characterization of Differential Cocaine Metabolism in Mouse and Rat through Metabolomics-Guided Metabolite Profiling." Drug Metabolism and Disposition: The Biological Fate of Chemicals, vol. 41, no. 1, 1 Jan. 2013, pp. 79–88, pubmed.ncbi.nlm.nih.gov/23034697/, https://doi.org/10.1124/dmd.112.048678. Accessed 7 Apr. 2021.
53. Kang, Yi-Rae, et al. "UPLC/Q-TOF MS Based Metabolomics Approach to Post-Mortem-Interval Discrimination: Mass Spectrometry Based Metabolomics Approach." Journal of Pharmaceutical Investigation, vol. 42, no. 1, 27 Jan. 2012, pp. 41–46, https://doi.org/10.1007/s40005-012-0006-7
54. Donaldson, Andrea Evelyn, and Iain L Lamont. "Estimation of Post-Mortem Interval Using Biochemical Markers." Australian Journal of Forensic Sciences, vol. 46, no. 1, 29 Apr. 2013, pp. 8–26, https://doi.org/10.1080/00450618.2013.784356.
55. Kumari, P., Prakash, P., Yadav, S., & Saran, V. (2022). Microbiome analysis: An emerging forensic investigative tool. Forensic Science International, 340, 111462. https://doi.org/10.1016/j.forsciint.2022.111462.
56. Procopio, N., Lovisolo, F., Sguazzi, G., Ghignone, S., Voyron, S., Migliario, M., Renò, F., Sellitto, F., D'Angiolella, G., Tozzo, P., Caenazzo, L., & Gino, S. (2021). "Touch microbiome" as a potential tool for forensic investigation: A pilot study. Journal of Forensic and Legal Medicine, 82, 102223. https://doi.org/10.1016/j.jflm.2021.102223
57. Wang, Z., Zhang, F., Wang, L., Yuan, H., Guan, D., & Zhao, R. (2022). Advances in artificial intelligence-based microbiome for PMI estimation. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.1034051
58. Cui, C., Song, Y., Mao, D., Cao, Y., Qiu, B., Gui, P., Wang, H., Zhao, X., Huang, Z., Sun, L., & Zhong, Z. (2022). Predicting the Postmortem Interval Based on Gravesoil Microbiome Data and a Random Forest Model. Microorganisms, 11(1), 56. https://doi.org/10.3390/microorganisms11010056
Zhang, J., Liu, W., Simayijiang, H., Hu, P., & Yan, J. (2022). Application of Microbiome in Forensics. Genomics, Proteomics & Bioinformatics, 21(1). https://doi.org/10.1016/j.gpb.2022.07.007

CONFLICT OF INTEREST

Authors declared no conflict of interest, whether financial or otherwise, that could influence the integrity, objectivity, or validity of their research work.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

Authors declared no specific grant for this research from any funding agency in the public, commercial or non-profit sectors

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

JBAHS web address: www.jbahs.pk
Email address: editor.jbahs@superior.edu.pk