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Mixed DNA Profiles and Donor DNA Persistence: Forensic Implications and Interpretation Challenges

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ABSTRACT

In forensic DNA analysis, the presence of donor-derived DNA following blood transfusion, hematopoietic stem cell transplantation (HSCT), and chimerism can lead to complex or misleading short tandem repeat (STR) profiles. These biological events may result in mixed or discordant genetic patterns that are not attributable to multiple contributors but rather to underlying physiological mechanisms. As a result, interpreting such profiles presents major challenges in criminal investigations, paternity testing and identifying disaster victims.

Transfusion-associated microchimerism is generally transient, although persistence may extend beyond the expected duration under certain clinical conditions. Allogenic HSCT, on the other hand, can result in full or mixed heamatopoietic chimerism, in which Tetragametic chimerism further complicates forensic interpretation due to the lifelong coexistence of genetically distinct cell lines across different tissues.

These complexities increase the risk of false inclusions, exclusions, and misinterpretation of DNA evidence, particularly when reliance is placed on a single biological sample. The issue is of growing relevance in the Indian context due to the increasing number of transplant procedures and the continued reliance on blood as the primary reference sample in forensic practice.

This review examines the mechanisms underlying donor DNA persistence, its impact on STR profiling, and the associated forensic implications. It further highlights the need for medical-history documentation, multi-tissue sampling strategies, and alignment with international forensic guidelines to improve the accuracy and reliability of DNA-based identification.

Index Terms - Blood transfusion, Chimerism, Donor DNA persistence, hematopoietic stem cell transplantation (HSCT), short tandem repeat (STR).

I. INTRODUCTION

1.1 Forensic DNA Evidence in India and Globally

Forensic DNA profiling has become a cornerstone of modern criminal justice systems, enabling reliable individual identification through the analysis of short tandem repeats (STRs) (Butler, 2015; Gill et al., 2012). The development of standardized STR kits, combined with national DNA databases such as the Combined DNA Index System (CODIS) in the United States and the National DNA Database (NDNAD) in the United Kingdom, has significantly enhanced the accuracy and efficiency of forensic investigations (Jobling & Gill, 2004; Kayser & de Knijff, 2011). International guidelines issued by organizations such as the International Society for Forensic Genetics (ISFG) and the Scientific Working Group on DNA Analysis Methods (SWGDAM) further support standardization in DNA profiling and interpretation (SWGDAM, 2017; ISFG, 2020).

In the Indian context, forensic DNA analysis is increasingly integrated into criminal investigations, particularly following the enactment of the Bharatiya Nagarik Suraksha Sanhita (BNSS), 2023, which permits the collection of biological samples for identification purposes under specified legal provisions. Additionally, the DNA Technology (Use and Application) Regulation Bill 2019, has been proposed to establish a regulatory framework for DNA data banking and forensic usage; however, it remains draft legislation and has not yet been fully enacted (Government of India, 2019). Despite these developments, practical challenges persist in standardizing DNA collection and interpretation protocols across Central and State Forensic Science Laboratories (FSLs) (Kansra et al., 2021).

1.2 Emergence of Non-Criminal Mixed DNA Profiles

Since the early 2000s, forensic scientists have increasingly reported complex DNA profiles that cannot be attributed solely to multiple contributors or laboratory contamination. Instead, these profiles often arise from biological phenomena such as blood transfusion, hematopoietic stem cell transplantation (HSCT), and chimerism (Jacewicz et al., 2013; Tozzo et al., 2021; Kalafut et al., 2022).

Transfusion-associated microchimerism occurs when donor-derived leukocytes persist temporarily in the recipient's circulation. Although typically transient, several studies have demonstrated that donor DNA may persist for extended periods—ranging from weeks to years—particularly in trauma patients or immunocompromised individuals (Utter et al., 2012; Lee et al., 2010; Zhang et al., 2019). In contrast, allogeneic HSCT results in hematopoietic chimerism, where donor stem cells engraft and replace the recipient's

blood-forming system. This may lead to complete or mixed chimerism, with donor-derived DNA predominating in blood while other tissues retain the recipient genotype (Butler, 2015; Chen et al., 2018).

Tetragametic chimerism, a rare congenital condition arising from the fusion of two embryos, results in the coexistence of genetically distinct cell lines within a single individual. This condition can produce tissue-specific genetic variation, complicating forensic interpretation, particularly in kinship analysis and identity testing (Iacona et al., 2020; Madan, 2020). These biological mechanisms may generate additional alleles, peak-height imbalances, or apparent multi-contributor profiles, thereby mimicking contamination or mixed DNA samples (Gill et al., 2012; Bright et al., 2013).

1.3 Research Gap: Limited Indian Forensic Evidence

While a substantial body of international research has examined the forensic implications of donor DNA persistence and chimerism, studies from India remain limited. Most available literature originates from Western clinical and forensic settings, with relatively few studies addressing the Indian forensic context, population diversity, or laboratory practices (Tozzo et al., 2021; Kalafut et al., 2022).

Existing Indian research has primarily focused on isolated case reports or specific applications, such as artificial chimerism in sexual offence investigations (Govindarajan & Bhaskar, 2025). However, comprehensive studies evaluating the persistence of donor DNA across different tissues, its impact on STR profiling, and its implications for Indian forensic workflows are lacking. Given the increasing number of HSCT procedures in India, particularly for conditions such as thalassaemia and leukaemia, this gap presents a significant challenge for forensic interpretation (Swaminathan et al., 2020).

1.4 Aim and Objectives of the Review

The present review aims to examine the impact of donor DNA persistence and chimerism on the interpretation of STR profiles in forensic casework, with particular emphasis on the Indian context.

The specific objectives are:

1. To explain the biological mechanisms underlying transfusion-associated microchimerism, acquired hematopoietic chimerism following HSCT, and Tetragametic chimerism.
2. To evaluate the effects of these conditions on forensic applications, including criminal investigations, paternity testing, DNA database searches, and disaster victim identification.
3. To assess the challenges faced by forensic laboratories in India in detecting and interpreting such complex DNA profiles.
4. To propose evidence-based strategies and guidelines, aligned with ISFG and SWGDAM recommendations, for improving forensic DNA interpretation.

1.5 Scope of the Study

This review focuses on three major biological phenomena relevant to forensic DNA interpretation:

(i) Transfusion-associated microchimerism,

(ii) Acquired hematopoietic chimerism following allogeneic HSCT, and

(iii) Tetragametic chimerism.

Other forms of chimerism, including pregnancy-related microchimerism and organ transplantation, are excluded to maintain a focused analysis on mechanisms most directly relevant to routine forensic casework. This scope allows for a detailed examination of persistence patterns, tissue-specific DNA variation, and their implications for STR-based identification.

II. REVIEW OF LITERATURE

The methodology adopted for selecting and analysing the included studies is described in Section 4.

Over the past two decades, advances in forensic DNA analysis have substantially improved the interpretation of complex genetic evidence. Short tandem repeat (STR) profiling remains the primary method for individual identification; however, challenges arise when DNA profiles deviate from expected single-source patterns (Butler, 2015; Gill et al., 2012). Traditionally, such complexities were attributed to mixed samples or laboratory contamination. More recent research, however, indicates that biological phenomena—including chimerism and donor DNA persistence—can also generate similar patterns, thereby complicating forensic interpretation (Bright et al., 2013; Tozzo et al., 2021).

Earlier forensic studies largely focused on mixture interpretation and stochastic effects associated with low-template DNA, including allele drop-in, drop-out, and peak imbalance (Gill et al., 2006; Balding & Buckleton, 2009). Subsequent work has expanded this perspective by demonstrating that non-criminal biological processes can produce apparent mixed profiles, highlighting the need for more cautious and context-aware interpretation of DNA evidence (Butler, 2015).

2.1 Transfusion-Associated Microchimerism

Blood transfusion is a well-recognised source of transient donor DNA introduction into the recipient's body. Transfusion-associated microchimerism occurs due to the persistence of donor leukocytes, even in leukoreduced blood products (Lee et al., 2010; Utter et al., 2012). While most studies indicate that donor DNA is typically cleared within days to weeks, evidence from trauma and critically ill patients suggests that microchimerism may persist for extended periods, sometimes months or even years (Utter et al., 2012; Zhang et al., 2019; Reed et al., 2007).

From a forensic perspective, the presence of low-level donor DNA may contribute to minor allelic peaks in STR profiles, potentially mimicking low-level mixtures (Gill et al., 2012; Bright et al., 2013). However, compared to other forms of chimerism, transfusion-related effects are generally transient and of limited long-term forensic impact (Tozzo et al., 2021).

2.2 Hematopoietic Stem Cell Transplantation and Acquired Chimerism

Allogeneic hematopoietic stem cell transplantation (HSCT) represents a more significant source of donor DNA persistence. Following transplantation, donor stem cells engraft in the recipient's bone marrow, resulting in hematopoietic chimerism, which may be complete or mixed depending on engraftment success (Jacewicz et al., 2013; Butler, 2015).

Numerous studies have demonstrated that, after successful HSCT, blood-derived DNA profiles often reflect the donor genotype, whereas other tissues such as hair follicles and fingernails retain the recipient's original DNA (Chen et al., 2018; Iacona et al., 2020). Buccal cells may exhibit mixed profiles due to the presence of donor-derived leukocytes, while rare reports indicate partial donor DNA contribution in semen (Chen et al., 2018; Thiede, 2004).

These tissue-specific differences create substantial forensic challenges. Blood samples may incorrectly identify the donor rather than the recipient, potentially leading to false inclusions or exclusions in criminal investigations and database searches (Butler, 2015; Tozzo et al., 2021). Case studies have further demonstrated that such discrepancies can complicate kinship analysis and paternity testing, particularly when only a single tissue type is analyzed (Jacewicz et al., 2013; Wenk et al., 2001).

2.3 Tetragametic Chimerism

Tetragametic chimerism is a rare congenital condition resulting from the fusion of two embryos during early development, leading to the coexistence of genetically distinct cell lines within one individual (Madan, 2020; Iacona et al., 2020). Unlike acquired chimerism, this condition is lifelong and may affect different tissues to varying extents.

The forensic implications of tetragametic chimerism are particularly significant in identity and kinship testing. Several documented cases have demonstrated false exclusions in maternity or paternity testing when DNA samples were obtained from tissues that did not represent the germline genotype (Yu et al., 2013; Ramírez et al., 2009). These findings highlight the importance of multi-tissue sampling and careful interpretation in suspected cases of chimerism.

2.4 Other Forms of Microchimerism

In addition to transfusion and HSCT, other forms of microchimerism have been reported, including fetal and maternal microchimerism, which involve bidirectional cell exchange during pregnancy (Kinder et al., 2017; Bianchi, 2012). Solid organ transplantation may also introduce low levels of donor DNA into recipient tissues (Kościelniak et al., 2014).

Although these forms typically result in lower levels of donor DNA, advances in analytical techniques such as next-generation sequencing (NGS) have increased the sensitivity of detection, raising the possibility of detecting minor donor contributions in forensic samples (Blouin et al., 2021; Phillips et al., 2012). However, their overall forensic significance remains limited compared to HSCT-related chimerism.

Table 1: Comparison of Mechanisms of Donor DNA Persistence

Mechanism	Source of Donor DNA	Duration of Persistence	Affected Tissues	Forensic Impact	Key References
Blood Transfusion (Microchimerism)	Donor leukocytes	Hours to weeks; may extend to months or years in trauma cases	Blood	Low to moderate; may produce minor STR peaks resembling mixtures	Utter et al., 2012; Lee et al., 2010; Zhang et al., 2019
Allogeneic HSCT (Acquired Chimerism)	Donor stem cells	Long-term or lifelong	Blood (donor), other tissues (recipient ± mixed)	High; may cause false inclusions/exclusions and database mismatches	Jacewicz et al., 2013; Chen et al., 2018; Butler, 2015
Tetragametic Chimerism (Congenital)	Fusion of two embryos	Lifelong	Tissue-specific (dual genomes)	High; may cause discordant STR profiles and false parentage results	Madan, 2020; Iacona et al., 2020
Other Microchimerism (Pregnancy, Organ Transplant)	Fetal/maternal cells or transplanted tissue	Variable (low-level persistence)	Various tissues	Low; may affect highly sensitive analysis methods	Kinder et al., 2017; Kościelniak et al., 2014

Donor DNA persistence varies depending on the underlying biological mechanism, with transfusion-associated microchimerism typically being transient, while hematopoietic stem cell transplantation (HSCT) and tetragametic chimerism may result in long-term or lifelong persistence (Butler, 2015; Jacewicz et al., 2013; Iacona et al., 2020; Tozzo et al., 2021).

2.5 Impact on STR Profiling and Interpretation

Donor DNA persistence can significantly affect STR profiling by introducing additional alleles, peak height imbalances, and apparent multi-contributor profiles (Gill et al., 2012; Bright et al., 2013). These features may be misinterpreted as contamination or true mixtures, particularly in the absence of relevant medical history.

The use of probabilistic genotyping software has improved mixture interpretation; however, such systems may still misinterpret donor DNA contributions as separate contributors if chimerism is not considered (Taylor et al., 2013; Cowell et al., 2015). Consequently,

forensic guidelines emphasize the importance of contextual information, including medical history and sample origin, in interpreting DNA profiles (ISFG, 2020; SWGDAM, 2017).

2.6 Limitations of Current Research

Despite extensive research, several limitations persist in the literature. Many studies are based on case reports or clinical observations rather than controlled forensic investigations, and there is limited standardization in methodologies used to assess donor DNA persistence (Tozzo et al., 2021). Additionally, most research originates from Western populations, with limited data available from other regions, including India.

This lack of region-specific data is particularly important given differences in healthcare systems, population genetics, and forensic laboratory practices. Further research is needed to evaluate donor DNA persistence in diverse populations and to develop standardized protocols for forensic interpretation.

III. METHODOLOGY

3.1 Study Design

The present study is structured as a narrative literature review aimed at examining the forensic implications of donor DNA persistence arising from blood transfusion, hematopoietic stem cell transplantation (HSCT), and chimerism. A narrative approach was considered appropriate due to the diverse nature of available literature, which includes case reports, clinical studies, forensic analyses, and guideline documents. This approach allows for the integration of findings from multiple domains to develop a comprehensive understanding of the topic (Green et al., 2006; Ferrari, 2015).

3.2 Literature Search Strategy

Relevant literature was identified through a systematic search of major academic databases, including PubMed, Google Scholar, ScienceDirect, SpringerLink, and Wiley Online Library. These databases were selected to ensure broad coverage of biomedical, forensic, and genetic research relevant to donor DNA persistence.

The search was conducted using combinations of the following keywords:

- “Donor DNA persistence”
- “micro chimerism AND forensic DNA”
- “Hematopoietic stem cell transplantation AND STR profiling”
- “tetra gametic chimerism AND forensic genetics”
- “Mixed DNA profiles AND interpretation”
- “Forensic DNA chimerism”

Boolean operators such as AND and OR were used to refine the search and improve the relevance of retrieved studies (Bramer et al., 2018).

3.3 Inclusion Criteria

Studies were included in the review based on the following criteria:

- Peer-reviewed journal articles, review papers, and relevant case reports
- Publications written in English
- Studies focusing on forensic DNA analysis, STR profiling, or chimerism
- Research addressing transfusion-associated microchimerism, HSCT, or congenital chimerism
- Articles discussing forensic implications such as mixture interpretation, kinship analysis, and DNA database applications

3.4 Exclusion Criteria

The following types of studies were excluded:

- Non-English publications
- Abstract-only articles without accessible full text
- Animal studies not directly relevant to forensic applications
- Clinical studies lacking forensic relevance
- Studies focusing exclusively on pregnancy-related microchimerism or organ transplantation, unless directly linked to forensic interpretation

3.5 Study Selection Process

The initial search yielded a broad set of articles, which were screened based on titles and abstracts for relevance to the research topic. Duplicate records were removed, and full-text articles were evaluated against the inclusion and exclusion criteria.

Only studies that provided meaningful insights into donor DNA persistence, tissue-specific distribution, STR profile interpretation, or forensic case implications were selected for detailed analysis. Emphasis was placed on studies that demonstrated clear forensic relevance or practical application.

3.6 Data Extraction and Thematic Analysis

Relevant data from the selected studies were extracted and organized into thematic categories, including:

- Mechanisms of donor DNA introduction
- Duration and persistence patterns

- Tissue-specific distribution of donor and recipient DNA
- Effects on STR profiling and electropherogram interpretation
- Forensic implications in criminal investigations, kinship testing, and disaster victim identification

The extracted information was qualitatively synthesized to identify recurring patterns, inconsistencies, and research gaps. A thematic approach was adopted to structure the findings across different sections of the study (Ferrari, 2015).

3.7 Scope and Delimitations

This review focuses specifically on three major mechanisms relevant to forensic DNA interpretation:

1. Transfusion-associated microchimerism
2. Acquired hematopoietic chimerism following HSCT
3. Tetragametic chimerism

Other forms of chimerism, including pregnancy-related microchimerism and organ transplantation, were not explored in detail in order to maintain a focused analysis on mechanisms most relevant to routine forensic casework.

3.8 Limitations of the Study

As a narrative literature review, this study is subject to certain limitations. The selection of literature may be influenced by database availability and search strategy, and the absence of quantitative meta-analysis limits statistical comparison between studies (Green et al., 2006).

Additionally, much of the available literature consists of case reports and clinical studies rather than controlled forensic investigations. The limited availability of region-specific data, particularly from India, further restricts the generalizability of findings.

IV. DONOR DNA PERSISTENCE AND TEMPORAL–TISSUE DYNAMICS

Donor DNA persistence is a critical factor influencing the interpretation of mixed or discordant genetic profiles in forensic casework. The extent and duration of donor-derived DNA within an individual depend on the underlying biological mechanism, including blood transfusion, hematopoietic stem cell transplantation (HSCT), and congenital chimerism. These mechanisms exhibit distinct temporal patterns and tissue-specific distributions, which must be considered during forensic analysis (Butler, 2015; Tozzo et al., 2021).

4.1 Temporal Persistence and Clearance Patterns

The persistence of donor-derived DNA varies significantly depending on the source of introduction. In blood transfusion, donor leukocytes are typically transient and are gradually cleared by the recipient's immune system. Most studies report detectability ranging from hours to several weeks; however, prolonged microchimerism has been documented in trauma patients and immunocompromised individuals, where donor DNA may persist for months or longer (Utter et al., 2012; Lee et al., 2010; Zhang et al., 2019).

In contrast, allogeneic HSCT results in long-term or permanent persistence of donor DNA in hematopoietic tissues due to successful engraftment of donor stem cells. Depending on engraftment efficiency, individuals may exhibit complete donor chimerism or mixed chimerism, with both donor and recipient DNA detectable in varying proportions (Jacewicz et al., 2013; Thiede, 2004).

Tetragametic chimerism differs fundamentally in that it is not a transient phenomenon. Instead, it involves the lifelong coexistence of genetically distinct cell lines, with no temporal clearance of either genome (Madan, 2020; Iacona et al., 2020).

4.2 Tissue-Specific Distribution of Donor DNA

The distribution of donor DNA across different tissues plays a central role in forensic interpretation. Following HSCT, blood-derived DNA typically reflects the donor genotype, whereas non-hematopoietic tissues such as hair follicles and fingernails retain the recipient's original genetic profile (Chen et al., 2018; Butler, 2015).

Buccal epithelial samples present additional complexity, as they may contain both epithelial cells and donor-derived leukocytes, resulting in mixed or intermediate DNA profiles (Jacewicz et al., 2013). Although germline tissues generally preserve the recipient genotype, rare cases have reported partial donor DNA contribution in semen following HSCT, indicating that tissue-specific assumptions must be made cautiously (Chen et al., 2018; Tozzo et al., 2021).

In transfusion-associated microchimerism, donor DNA is primarily confined to circulating blood cells and does not typically integrate into other tissues, further emphasizing its transient and limited forensic impact (Utter et al., 2012).

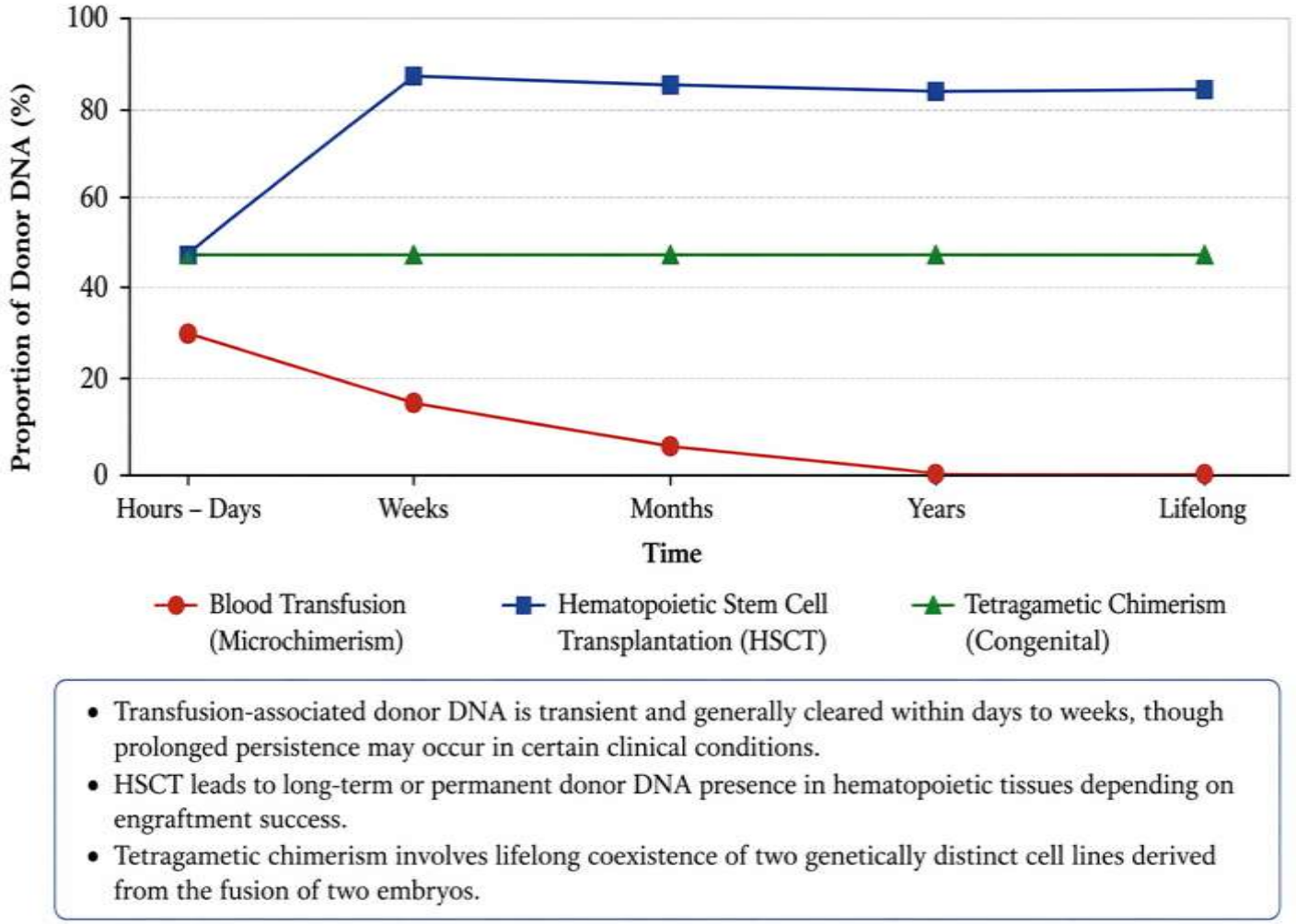

Figure 1. *Temporal Dynamics of Donor DNA Persistence Across Different Mechanisms*

Temporal dynamics of donor DNA persistence following blood transfusion, hematopoietic stem cell transplantation (HSCT), and tetragametic chimerism. Transfusion-associated microchimerism demonstrates a gradual decline in donor DNA over time, whereas HSCT results in long-term or permanent donor DNA presence in hematopoietic tissues depending on engraftment. In contrast, tetragametic chimerism represents lifelong coexistence of genetically distinct cell lines (Butler, 2015; Jacewicz et al., 2013; Madan, 2020; Utter et al., 2012; Zhang et al., 2019).

4.3 Factors Influencing Donor DNA Persistence

Several biological and clinical factors influence the persistence and detectability of donor DNA. These include the volume and type of transfused blood, degree of leukoreduction, recipient immune response, and presence of immunosuppression (Lee et al., 2010; Utter et al., 2012).

In HSCT cases, graft-versus-host disease (GVHD) and engraftment success significantly affect the proportion of donor-derived cells in circulation. Higher levels of donor chimerism are typically associated with stable engraftment, whereas mixed chimerism may persist in cases of partial engraftment or relapse (Thiede, 2004; Butler, 2015).

Additionally, methodological factors such as sensitivity of detection techniques, including quantitative PCR and next-generation sequencing (NGS), can influence the ability to detect low-level donor DNA, further complicating interpretation (Phillips et al., 2012; Blouin et al., 2021).

Tissue Type	Predominant DNA Source	Profile Pattern	Forensic Implications
Blood (Leukocytes)	Donor	Donor profile (complete or majority)	High risk of misidentification as donor if medical history is unknown
Buccal (Epithelial cells)	Mixed (Donor + Recipient)	Mixed or intermediate profile	May produce apparent mixtures; careful interpretation required
Hair Follicles (Root)	Recipient	Recipient profile	Useful for determining recipient identity
Fingernails	Recipient	Recipient profile	Reliable source for recipient genotype
Semen	Recipient (± Donor in rare cases)	Predominantly recipient; donor DNA possible	Rare donor contribution reported; interpretation with caution

- Hematopoietic tissues such as blood reflect donor genotype after successful HSCT.
- Non-hematopoietic tissues (hair, nails) retain recipient genotype.
- Buccal and semen samples may show mixed or variable profiles due to the presence of donor-derived leukocytes.

Figure 2: *Tissue-Specific Distribution of Donor and Recipient DNA Following Hematopoietic Stem Cell Transplantation (HSCT)*
Tissue-specific distribution of donor and recipient DNA in individuals following hematopoietic stem cell transplantation (HSCT). Blood-derived samples typically reflect donor genotype due to hematopoietic replacement, whereas non-hematopoietic tissues such as hair follicles and fingernails retain the recipient genotype. Buccal epithelial samples may show mixed profiles due to the presence of donor-derived leukocytes, and semen samples generally reflect the recipient genotype with rare donor contribution (Butler, 2015; Chen et al., 2018; Jacewicz et al., 2013; Tozzo et al., 2021).

4.4 Empirical Evidence from Forensic and Clinical Studies

Empirical studies provide strong evidence for tissue-specific and mechanism-dependent persistence patterns. For example, Jacewicz et al. (2013) demonstrated complete donor DNA replacement in blood following HSCT, while recipient DNA was retained in hair and epithelial tissues. Similarly, Chen et al. (2018) reported mixed DNA profiles in buccal samples and occasional donor DNA presence in semen, highlighting intra-individual variability.

Studies on transfusion-associated microchimerism have confirmed that donor DNA persistence is generally short-lived but may vary depending on clinical conditions, particularly in critically ill patients (Utter et al., 2012; Zhang et al., 2019). These findings collectively indicate that donor DNA persistence follows predictable patterns, but the extent of persistence is influenced by both biological and methodological factors (Tozzo et al., 2021).

4.5 Forensic Interpretation Considerations

Understanding donor DNA persistence dynamics is essential for accurate forensic interpretation. The presence of donor-derived DNA can result in additional alleles, peak height imbalances, and apparent mixed profiles, which may be misinterpreted as contamination or multiple contributors (Gill et al., 2012; Bright et al., 2013).

Failure to account for these biological factors may lead to false inclusions or exclusions in criminal investigations, kinship analysis, and DNA database comparisons. Therefore, interpretation of STR profiles must consider both temporal persistence and tissue-specific distribution of DNA, supported by relevant medical history and appropriate sample selection (ISFG, 2020; SWGDAM, 2017).

V. FORENSIC AND LEGAL IMPLICATIONS OF DONOR DNA PERSISTENCE

Donor DNA persistence introduces a set of interpretational challenges in forensic genetics, particularly when mixed or discordant DNA profiles arise from biological processes rather than multiple contributors. These complexities can affect the reliability of short tandem repeat (STR) profiling, potentially leading to misinterpretation if underlying medical conditions are not considered (Butler, 2015; Gill et al., 2012; Tozzo et al., 2021).

5.1 Impact on STR Profiling and DNA Databases

The presence of donor-derived DNA may result in additional alleles, peak height imbalance, or apparent multi-contributor profiles, which can resemble contamination or true mixtures (Gill et al., 2012; Bright et al., 2013). In individuals who have undergone hematopoietic stem cell transplantation (HSCT), blood-derived DNA profiles may reflect the donor genotype rather than that of the individual, potentially leading to discrepancies in reference profiles stored in DNA databases (Butler, 2015; Jacewicz et al., 2013).

Such discrepancies may result in false-positive database matches or misleading investigative leads, particularly when medical history is unknown. Studies have highlighted that failure to account for chimerism may compromise the accuracy of database-driven identifications (Tozzo et al., 2021).

5.2 Implications in Criminal Investigations

In forensic casework, the interpretation of biological evidence such as blood or semen may be affected by donor DNA persistence. For example, in HSCT recipients, blood samples collected from a crime scene may correspond to the donor genotype, potentially implicating an unrelated individual (Butler, 2015; Tozzo et al., 2021).

Similarly, transfusion-associated microchimerism may produce low-level mixed DNA profiles that resemble multi-contributor samples, complicating interpretation and increasing the risk of erroneous conclusions (Utter et al., 2012; Zhang et al., 2019). These challenges emphasize the importance of considering biological explanations alongside traditional forensic interpretations.

5.3 Parentage Testing and Kinship Analysis

Donor DNA persistence can also affect parentage testing and kinship analysis. In cases of acquired hematopoietic chimerism, blood-based DNA profiles may not match the individual's germline genotype, leading to false exclusions in paternity or maternity testing (Wenk et al., 2001; Madan, 2020).

Tetragametic chimerism presents an additional challenge, as different tissues within the same individual may yield distinct genetic profiles. This may result in apparent inconsistencies in genetic relationships if only a single tissue sample is analyzed (Iacona et al., 2020; Yu et al., 2013). These findings underscore the importance of multi-tissue sampling in complex cases.

5.4 Disaster Victim Identification (DVI)

In disaster victim identification (DVI), accurate matching of DNA profiles is essential for identification. Donor DNA persistence may complicate this process, particularly when reference samples are derived from blood in individuals with a history of HSCT (Tozzo et al., 2021).

International guidelines recommend the use of multiple tissue types and corroborative evidence to ensure accurate identification in such cases (ISFG, 2020). Failure to account for chimerism may result in misidentification, especially in mass fatality scenarios where rapid identification is required.

5.5 Indian Forensic Context and Legal Considerations

In India, forensic DNA analysis is increasingly used in criminal investigations; however, challenges remain in standardizing protocols for complex cases involving donor DNA persistence. Biological samples, particularly blood, are commonly used as reference material, which may not always reflect the true genetic identity in individuals with a history of HSCT.

The Bharatiya Nagarik Suraksha Sanhita (BNSS), 2023 permits the collection of biological samples for forensic purposes under specified conditions. Additionally, the DNA Technology (Use and Application) Regulation Bill, 2019 has been proposed to regulate DNA profiling and data banking; however, it remains draft legislation and has not been fully implemented (Government of India, 2019; Kansra et al., 2021).

Given the increasing number of transplant procedures in India, particularly for conditions such as thalassaemia and leukaemia, the likelihood of encountering donor DNA persistence in forensic casework is expected to rise (Swaminathan et al., 2020). This highlights the need for updated forensic protocols, including medical-history documentation and multi-tissue sampling strategies.

5.6 Risk of Miscarriage of Justice

Failure to recognize donor DNA persistence may lead to serious legal consequences, including false inclusions, wrongful exclusions, or incorrect identifications. Such errors may affect criminal prosecutions, civil disputes, and disaster victim identification, ultimately undermining the credibility of forensic DNA evidence (Butler, 2015; Gill et al., 2012).

Maintaining the reliability of forensic DNA analysis therefore requires careful consideration of biological variability, adherence to established guidelines, and integration of medical context into interpretation processes (ISFG, 2020; SWGDAM, 2017).

VI. DETECTION, INTERPRETATION, AND MITIGATION STRATEGIES FOR DONOR DNA PERSISTENCE

Donor DNA persistence presents a complex challenge in forensic genetics, requiring a multidisciplinary approach that integrates biological understanding, laboratory practice, and interpretational frameworks. The accurate identification of donor-derived DNA and its distinction from genuine mixed profiles depend on careful sample selection, contextual information, and the application of advanced analytical methods. Without appropriate mitigation strategies, donor DNA may lead to erroneous conclusions in forensic casework (Butler, 2015; Gill et al., 2012; Tozzo et al., 2021).

6.1 Selection of Appropriate Reference Samples

The selection of appropriate biological samples is fundamental to accurate DNA profiling. In individuals who have undergone hematopoietic stem cell transplantation (HSCT), blood samples often reflect the donor genotype due to complete or partial hematopoietic replacement. Consequently, reliance on blood as a primary reference sample may result in incorrect identification or database mismatches (Jacewicz et al., 2013; Butler, 2015; Thiede, 2004).

Alternative tissues such as hair follicles, fingernails, and epithelial cells are generally more reliable for representing the recipient's original genotype, as they are derived from non-hematopoietic lineages (Chen et al., 2018; Iacona et al., 2020). Buccal swabs, although

commonly used in forensic practice, may contain both epithelial cells and donor-derived leukocytes, leading to mixed or intermediate DNA profiles (Jacewicz et al., 2013).

The selection of sample type should therefore be guided by both biological considerations and case context. In situations involving suspected chimerism, a hierarchy of sample reliability may be established, prioritizing non-hematopoietic tissues over blood-derived samples (Tozzo et al., 2021).

6.2 Importance of Medical History Documentation

The interpretation of complex DNA profiles cannot rely solely on laboratory data; it must also incorporate relevant contextual information. Medical history, including prior blood transfusions, HSCT, immunosuppressive therapy, or known chimerism, plays a crucial role in identifying cases where donor DNA persistence may influence results (Tozzo et al., 2021; Butler, 2015).

The absence of such information may lead forensic analysts to interpret donor-derived alleles as evidence of multiple contributors or contamination. Therefore, the integration of structured medical-history questionnaires into sample collection protocols is strongly recommended (ISFG, 2020).

In addition, awareness of clinical conditions such as trauma-related microchimerism, which may prolong donor DNA persistence, is important for accurate interpretation (Utter et al., 2012; Lee et al., 2010). Incorporating medical context into forensic workflows enhances both accuracy and interpretational confidence.

The recommended reference sample types and their forensic relevance in cases of donor DNA persistence are summarised in Table 2.

Table 2. Recommended Reference Samples for Individuals with Donor DNA Persistence

Sample Type	Reliability for Recipient DNA	Ease of Collection	Forensic Utility	Limitations
Hair follicles (with root)	High	Moderate	Reliable non-blood reference; useful in identity testing	Requires intact root; may be degraded
Fingernails	High	High	Non-invasive; reflects recipient genotype	Limited DNA quantity
Buccal swab	Moderate	High	Commonly used; easy collection	May contain donor leukocytes → mixed profiles
Blood	Low (post-HSCT)	High	Routine sample in forensic practice	May reflect donor genotype → misleading results
Semen (where applicable)	High (generally)	Low	Useful in sexual offence cases	Rare donor DNA contribution possible
Tissue biopsy	Very High	Low	Most accurate for confirming genotype	Invasive; ethical constraints

Note. Adapted from forensic and clinical studies on donor DNA persistence and chimerism (Butler, 2015; Chen et al., 2018; Jacewicz et al., 2013; Tozzo et al., 2021).

As shown in Table 2, non-hematopoietic tissues such as hair follicles and fingernails provide a more reliable representation of the recipient genotype compared to blood samples in individuals with HSCT.

6.3 Multi-Tissue Sampling Approach

A multi-tissue sampling strategy is one of the most effective approaches for addressing donor DNA persistence. By analysing multiple biological samples from the same individual—such as blood, buccal cells, hair follicles, and fingernails—it is possible to identify discrepancies between donor and recipient DNA profiles (Iacona et al., 2020; Chen et al., 2018).

This approach enables forensic practitioners to distinguish between true mixed samples and biologically induced chimerism. For example, concordance between hair and nail samples alongside discordance with blood-derived DNA strongly suggests HSCT-related chimerism rather than multiple contributors.

International guidelines emphasize the importance of multi-sample analysis in complex cases, particularly when standard STR interpretation yields unexpected results (ISFG, 2020; SWGDAM, 2017). However, implementation requires careful handling to prevent cross-contamination and ensure proper chain-of-custody documentation.

6.4 Advanced Analytical Techniques

Advances in molecular biology have significantly enhanced the ability to detect and quantify donor DNA. Quantitative PCR (qPCR) and digital PCR techniques allow for precise estimation of donor-to-recipient DNA ratios, providing valuable information on the extent of chimerism (Thiede, 2004; Butler, 2015).

Next-generation sequencing (NGS) technologies offer improved sensitivity and resolution compared to conventional STR analysis, enabling the detection of minor donor DNA components that may be present at very low levels (Phillips et al., 2012; Blouin et al., 2021). These techniques are particularly useful in cases involving low-level microchimerism or mixed DNA profiles.

Y-chromosome STR (Y-STR) analysis can be applied in specific forensic scenarios, such as sexual assault cases, to isolate male-specific DNA profiles in the presence of mixed samples. However, interpretation must consider the potential influence of donor DNA, particularly in transplant recipients (Kayser, 2017).

6.5 Interpretation Strategies and Probabilistic Approaches

The interpretation of DNA profiles in the presence of donor DNA requires a cautious and context-aware approach. Traditional interpretation methods based on allele counting and peak height analysis may not adequately account for biological complexities such as chimerism (Gill et al., 2012; Bright et al., 2013).

Probabilistic genotyping software has improved the interpretation of complex mixtures by incorporating statistical models; however, these systems may misinterpret donor DNA as a separate contributor if the underlying biological context is not considered (Taylor et al., 2013; Cowell et al., 2015). Therefore, interpretation should integrate both quantitative data and contextual information.

Laboratories should avoid applying fixed analytical thresholds without validation, as the detection of minor donor DNA components may vary depending on the sensitivity of the method used (SWGDAM, 2017). Instead, interpretation should be guided by validated laboratory protocols and case-specific considerations.

6.6 Recommendations for Forensic Laboratories in India

To effectively address donor DNA persistence in forensic casework, laboratories in India should adopt a combination of procedural and analytical measures:

- Implementation of standardized medical-history questionnaires during sample collection
- Adoption of multi-tissue sampling protocols in cases involving suspected chimerism or transplantation history
- Training of forensic analysts in recognizing and interpreting chimerism-related DNA profiles
- Integration of ISFG and SWGDAM guidelines into standard operating procedures
- Documentation of sample type and clinical history in forensic reports

Given the increasing number of HSCT procedures in India, these measures are essential to ensure the accuracy and reliability of DNA evidence in both criminal and civil contexts (Swaminathan et al., 2020; Kansra et al., 2021).

6.7 Limitations and Practical Considerations

Despite the availability of advanced analytical techniques, several practical limitations must be considered. Access to technologies such as NGS and digital PCR may be limited in resource-constrained laboratories, and the implementation of multi-tissue sampling protocols may require additional training and infrastructure (Tozzo et al., 2021).

Furthermore, the interpretation of complex DNA profiles remains dependent on the expertise of the analyst, highlighting the need for continuous training and standardization. A balanced approach that considers both scientific accuracy and practical feasibility is therefore essential.

VII. DISCUSSION

Donor DNA persistence represents a complex and often under-recognized factor in forensic DNA interpretation. While conventional forensic frameworks attribute mixed or discordant short tandem repeat (STR) profiles to multiple contributors or contamination, the findings synthesized in this review demonstrate that biological mechanisms such as transfusion-associated microchimerism, hematopoietic stem cell transplantation (HSCT), and tetragametic chimerism can produce similar patterns (Butler, 2015; Gill et al., 2012; Tozzo et al., 2021). Recognizing these mechanisms is therefore essential for accurate forensic interpretation.

7.1 Interpretation Challenges and Biological Complexity

One of the key challenges highlighted in this review is the **tissue-specific nature of donor DNA persistence**, particularly in individuals who have undergone HSCT. In such cases, blood-derived DNA may reflect the donor genotype, whereas non-hematopoietic tissues retain the recipient's original genetic profile (Jacewicz et al., 2013; Chen et al., 2018). This discordance complicates forensic analysis, especially when only a single tissue sample is examined.

Furthermore, low-level donor DNA may produce minor allelic peaks that resemble stochastic effects or minor contributors in STR profiles (Gill et al., 2012; Bright et al., 2013). Even with the use of probabilistic genotyping approaches, interpretation remains challenging if the underlying biological context is not considered (Taylor et al., 2013; Cowell et al., 2015).

7.2 Implications for the Indian Forensic Context

In the Indian forensic system, DNA profiling is increasingly used in criminal investigations; however, standard practices often rely heavily on blood samples as primary reference material. This approach may be problematic in individuals with a history of HSCT, where blood-derived DNA does not accurately represent the individual's original genotype (Butler, 2015).

Additionally, there is limited implementation of standardized protocols for documenting medical history in forensic workflows. Given the rising number of stem cell transplant procedures in India, particularly for conditions such as thalassaemia and leukaemia, the likelihood of encountering chimerism-related DNA profiles is expected to increase (Swaminathan et al., 2020; Kansra et al., 2021). This underscores the need for updated forensic practices that incorporate clinical context.

7.3 Practical Implementation and Feasibility

Addressing donor DNA persistence in forensic casework requires practical and feasible solutions. While advanced analytical techniques such as next-generation sequencing (NGS) and digital PCR offer improved sensitivity, their implementation may be limited in resource-constrained settings (Phillips et al., 2012; Blouin et al., 2021).

However, several low-cost and high-impact measures can be adopted, including the integration of medical-history documentation, multi-tissue sampling, and training of forensic analysts. These measures can significantly improve the accuracy of DNA interpretation without requiring substantial infrastructural investment (ISFG, 2020; SWGDAM, 2017).

The practical implications of implementing chimerism-aware forensic protocols are summarized in Table 3.

Table 3. Practical Implications of Implementing Chimerism-Aware Protocols in Forensic Laboratories.

Component	Estimated Effort/Cost	Expected Benefit	Overall Impact
Medical history documentation	Low	Identifies chimerism cases early; prevents misinterpretation	High
Multi-tissue sampling	Moderate	Improves the accuracy of DNA profiling	Very High
Analyst training	Moderate	Enhances interpretation of complex profiles	High
Advanced techniques (NGS/qPCR)	High	Detects low-level donor DNA accurately	High (resource-dependent)
SOP updates (ISFG/SWGDAM alignment)	Low to Moderate	Standardises interpretation and reduces errors	Very High

Note. The cost and impact estimates are qualitative and based on general laboratory practices and published forensic guidelines rather than specific economic studies (ISFG, 2020; SWGDAM, 2017; Tozzo et al., 2021).

As shown in Table 3, relatively low-cost interventions such as medical-history documentation and SOP updates can provide substantial improvements in forensic accuracy.

7.4 Role of Medical History in Forensic Interpretation

The importance of medical history in forensic DNA analysis cannot be overstated. Information regarding prior blood transfusions, HSCT, or other medical interventions can provide critical context for interpreting complex DNA profiles (Tozzo et al., 2021; Butler, 2015).

Incorporating structured medical-history questionnaires into forensic workflows can help identify cases where donor DNA persistence may influence results. This approach enhances interpretational accuracy and reduces the likelihood of misclassification of DNA profiles.

A structured medical-history questionnaire can assist in identifying individuals with potential donor DNA persistence, as outlined in Table 4.

Table 4. Recommended Medical-History Questions for DNA Reference Collection

Question No.	Question	Purpose
1	Have you received a blood transfusion in the last 2 years?	Identify possible transient microchimerism
2	Have you undergone a bone marrow or stem cell transplant?	Detect potential hematopoietic chimerism
3	Are you aware of any condition involving chimerism or twin-related anomalies?	Identify congenital chimerism
4	Have you undergone any major medical procedures involving blood products or immunosuppressive therapy?	Assess risk of donor DNA persistence
5	Please provide details of any recent medical treatments involving transfusion or transplantation	Support comprehensive forensic interpretation

Note. These questions are proposed as part of a standardized pre-sampling protocol to identify cases where donor DNA persistence may influence forensic DNA interpretation (ISFG, 2020; Tozzo et al., 2021).

Incorporation of such questionnaires into forensic workflows can significantly reduce the risk of misinterpretation associated with chimerism-related DNA profiles.

7.5 Limitations and Future Directions

Despite growing research in this area, several limitations remain. Much of the available literature is based on case reports and clinical observations, with limited large-scale forensic studies (Tozzo et al., 2021). Additionally, region-specific data, particularly from India, are scarce.

Future research should focus on developing standardized protocols for multi-tissue sampling, validating interpretation frameworks for chimerism-related cases, and generating population-specific data to support forensic practice in diverse settings. Collaborative efforts between forensic laboratories, clinicians, and research institutions will be essential to address these gaps.

VIII. CONCLUSION

Donor DNA persistence arising from blood transfusion, hematopoietic stem cell transplantation, and chimerism presents a critical challenge in forensic DNA analysis. These biological processes can produce mixed or discordant STR profiles that may be misinterpreted as evidence of multiple contributors or contamination if not properly recognized.

This review demonstrates that the persistence and distribution of donor DNA are highly dependent on both temporal and tissue-specific factors. While transfusion-associated microchimerism is generally transient, HSCT can result in long-term or permanent alteration of blood-derived DNA, and tetragametic chimerism leads to lifelong coexistence of distinct genetic lineages.

The forensic implications of these phenomena are substantial, affecting criminal investigations, kinship analysis, DNA database searches, and disaster victim identification. Misinterpretation of such profiles may lead to false inclusions, exclusions, or identification errors, highlighting the need for careful evaluation.

To address these challenges, this review emphasises the importance of:

- Appropriate sample selection
- Integration of medical history
- Multi-tissue sampling strategies
- Use of advanced analytical techniques
- Adherence to international forensic guidelines

In the Indian context, where forensic DNA analysis is increasingly utilized, the development of standardized protocols and increased awareness of chimerism-related effects are essential to ensure the reliability of DNA evidence. Donor DNA persistence represents an important consideration in modern forensic science, and its proper recognition is crucial for maintaining the accuracy, integrity, and credibility of forensic DNA interpretation.

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