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NARATTIVE REVIEW

Single Nucleotide Polymorphism (SNP) Assays for Disaster Victim Identification (DVI)

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Abstract

Disaster victim identification (DVI) is crucial in the aftermath of mass casualty events, necessitating rapid and precise identification methods. Single-nucleotide polymorphisms (SNPs) have gained significant prominence in forensic genetics due to their abundance, stability, and ease of analysis. SNPs are highly valuable genetic markers for DVI, particularly because they are insensitive to DNA degradation and possess high annotation potential, making their underlying biological information invaluable for human identification in molecular forensics. Unlike traditional methods, SNP typing offers a more powerful set of genetic markers, enabling complex analysis and profiling techniques suitable for various genotyping scenarios, from specialized forensic markers to expanded tiling arrays. The small differences in DNA due to polymorphisms, approximately 1 in 1,000 nucleotides, provide sufficient information to uniquely identify a person. SNP assays are particularly effective for analyzing severely damaged DNA samples, a common characteristic of disaster remains, as demonstrated in real-world applications such as the 2004 Indian Ocean tsunami, the 2010 Haiti earthquake, the 2015 Germanwings Flight 9525 crash, and the Yazidi Genocide in Iraq. These assays offer advantages including cost-effectiveness, multiplexing capabilities, and suitability for robotic automation. They also provide valuable information for ancestry inference and the prediction of externally visible characteristics. The continuous evolution of SNP assay technologies and their integration into DVI protocols underscore their vital role in providing accurate identifications and closure for affected families.

Keywords: Single Nucleotide Polymorphism (SNP), Disaster Victim Identification (DVI), forensic genetics

1. Introduction

This review examines the application of SNP assays in DVI, their benefits, challenges, and future potential. Case studies and real-world applications are also examined to highlight the practical benefits of SNP technology in DVI. The ability to carry out specific assays, generating information that has immediate relevance to disaster victim identification, is especially desirable in the circumstances of mass fatality situations. Rapidly screening large numbers of samples for the presence of one or a few key nucleotide variants present in likely victims and their close relatives would allow individuals of the same family to be rapidly excluded from further potential victim identity testing. The use of highly multiplexed direct target genotyping approaches, which directly establish sequence variants of interest under standard laboratory conditions, can provide useful coverage within a single assay [1]

Single nucleotide polymorphism assays are already in use in a wide variety of fields. Assays utilizing bead array or chip technology allow significant parallelization such that many thousands or even millions of polymorphisms can be interrogated at relatively low cost. This high level of multiplexing makes SNP genotyping, using both genome-wide and targeted approaches, increasingly common in both the academic research environment and clinical, forensic, and agricultural applications[2].

2. Definition and Significance of SNPs

Rapid advancements in forensic genetics have not only revolutionized human identity testing but have also made invaluable strides in diversifying the evidence types with which DNA testing can be applied[4]. As single nucleotide polymorphisms (SNPs) are insensitive to DNA degradation and have a high annotation potential, the underlying biological information from a large number of SNP markers is invaluable in the application of molecular forensics for human identification[5]. In particular, SNP typing has the potential to result in a more powerful set of genetic markers, allowing complex analysis and profiling techniques and enabling different industries to converge in their goals towards standardized genotyping[6]. World associations may help future legislative considerations by formulating DVI-specific trait tables against which gene-by-gene SNPs can also be measured [7]. The goal of SNPs in the disaster victim identification (DVI) setting involves identifying an individual and/or the associated phenotypic traits such as eye color, hair color, anatomic features, ancestry, and many others that can be used to aid in the process of identification [8]. Its inherent advantage lies in using a base pair assay performed at thousands of conveniently designed assays for forensic identification, hence reducing the complexity of the search for an NGS approach [9]. The sheer flexibility of SNP-based assays allows them to cater to a wide variety of genotyping scenarios to satisfy the search or query requirements [10], from a specialized list of forensic markers only to an expanded SNP tiling array, including minor variations [11]. In addition, SNPs help lead the way through SNP data exchange standards based on the growing knowledge base of routine forensic marker sets evolving from this standardized approach [12].

3. Basic Principles of SNP Analysis

A single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide - A, T, C, or G (as shown in figure 1) - in the genome differs between members of a species or paired chromosomes in an individual [13]. Each human cell carries about 6 billion bits of information in the nucleus in the form of DNA molecules [14]. Every time a cell divides, these 6 billion bits must be copied so that each daughter cell can have identical genetic material [15]. The small differences in the DNA, about 1 in 1,000 nucleotides due to polymorphisms [16], allow for the genetic variation that exists within the human gene pool (as shown in figure 2), and they provide enough information to uniquely identify a person [17]. The scientific technique by which polymorphisms and other variations in the human genome are identified and located is called DNA fingerprinting, genotyping, or mapping [18]. SNP typing is a newer technique, but it has proven to be increasingly useful for a wide variety of genome scanning applications and is now widely performed for a variety of mapping purposes, including linkage analysis, association studies, haplotyping, and segregation of new mutations [19].

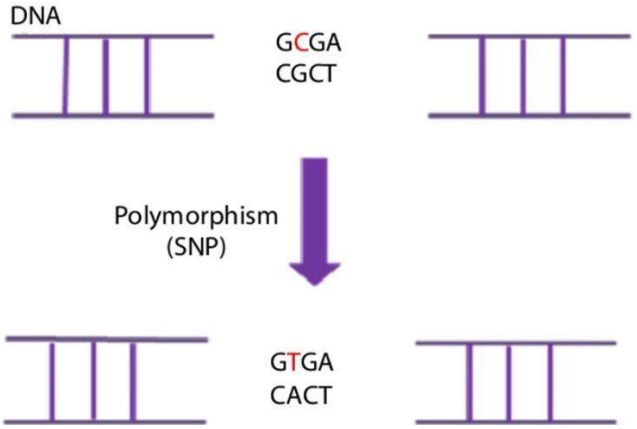

Figure 1: Single nucleotide polymorphisms (SNPs),

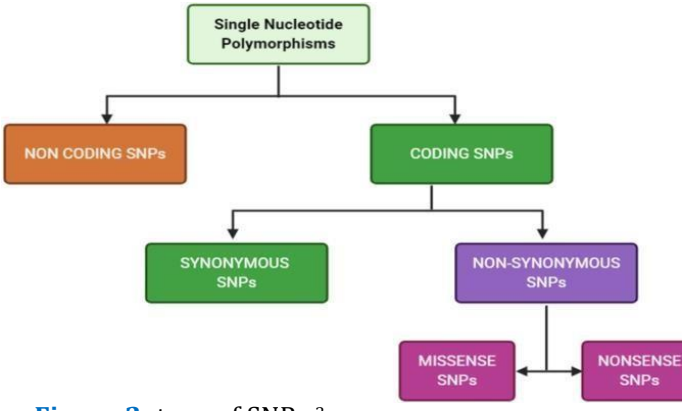

Figure 2: type of SNPs [3]

4. Disaster Victim Identification (DVI) Process

Disaster Victim Identification (DVI) is a complex process that typically involves multiple organizations and includes tasks such as identifying and documenting personal effects, recovering and examining human remains, and cross-referencing ante- and post-mortem data [20,21]. DNA analysis plays a significant role in the DVI process, especially when ante-mortem and post- mortem data are used to establish familial relationships [22]. In particular, DNA-based human identification typically uses the amelogenin locus (The amelogenin locus is a widely utilized genetic marker in forensic DNA analysis, primarily for determining the biological sex of an individual. This gene, which encodes for the amelogenin protein involved in tooth enamel formation, is present on both the X (AMELX) and Y (AMELY) sex) [23]. Here, we develop eleven single-nucleotide polymorphism (SNP) assays on autosomal STRs, compare them with previously published multiplex STRs, and ultimately utilize the developed system to perform a successful DNA analysis and identification of several human remains from a disaster [24].

5. Disaster Victim Identification in Iraq and Neighboring Countries

Iraq faces a significant challenge with a large number of missing persons due to prolonged conflicts, human rights violations, and atrocities committed during various periods, including the Ba'ath Party regime, by Da'esh, and during wars with neighboring countries. Since 2003, over 250 mass or clandestine graves have been excavated by relevant government agencies. The International Commission on Missing Persons (ICMP) has been actively involved in Iraq since 2003, establishing an office in Baghdad in 2008 and Erbil in 2010. ICMP has trained over 550 Iraqi professionals from various institutions in effective identification methodologies, including DNA matching between recovered bone samples and blood samples from surviving family members. The overarching goal is to build Iraq's institutional capacity for a sustainable process of accounting for all missing persons and securing the rights of families. While DNA matching is mentioned, the specific percentage of reliance on SNP assays within this framework for Iraq or its immediate neighbors is not detailed in the available sources [25].

6. Global DVI and the Mechanism of SNP Reliance

Globally, Disaster Victim Identification follows international standards, such as those developed by INTERPOL. The DVI process typically involves several phases [26].

1) Scene examination: Recovery of victims and evidence.
2) Post-mortem (PM) data collection: Examination of human remains by specialists to gather biometric data (fingerprints, dental records, DNA profiling, physical indications).
3) Ante-mortem (AM) data collection: Interviewing next of kin to collect information about the missing person (detailed descriptions, personal items, dental and medical records, X-rays, fingerprints, and DNA samples).
4) Reconciliation: Comparison of PM and AM data by a team of specialists to establish identity.

7. Mechanism for Relying on SNP Assays in DVI:

I. Robustness with Degraded DNA: SNPs are highly valuable for analyzing degraded or low-quantity DNA samples, which are common in mass fatality incidents. Their smaller amplicon sizes make them more amenable to analysis when DNA is compromised [27].

II. High Discriminatory Power: SNP assays, utilizing technologies like multiplex PCR, primer extension, and next-generation sequencing (NGS), allow for the simultaneous analysis of numerous markers. This provides a high degree of discriminatory power, crucial for complex kinship analysis and identifying victims through comparison with reference samples from relatives [28].

III. Kinship Analysis: Carefully selected SNP panels are particularly effective for establishing kinship relationships, aiding in identifying victims when direct matching is not possible.

IV. Ancestry and Phenotypic Prediction: Beyond identity, NGS-based SNP analysis can provide valuable investigative leads by inferring biogeographical ancestry and predicting externally visible characteristics.

V. Cost-Effectiveness and Automation: SNP assays offer benefits such as cost-effectiveness, multiplexing capabilities, and suitability for robotic automation, which can enhance the efficiency of DVI efforts.

VI. Real-world Applications: The practical benefits of SNP technology in DVI have been highlighted in various major incidents, including the 2004 Indian Ocean tsunami, the 2010 Haiti earthquake, the 2015 Germanwings Flight 9525 crash, and the Yazidi Genocide in Iraq. These cases demonstrate SNP's utility in situations with highly degraded DNA and the need for robust identification methods [29].

While the available information does not provide specific percentages of DVI cases where SNP assays are the primary identification method, it strongly indicates that SNPs are a critical and increasingly integrated tool in forensic genetics for DVI worldwide due to their unique advantages in challenging conditions [30].

8. Role of SNP Assays in DVI

Single-nucleotide polymorphisms (SNPs) are the most informative of available human genetic markers [31]. There are millions of human SNPs in coding regions and in the 98% of the genome that does not encode proteins. SNPs can be used for all genetic identification testing, including the identification of disaster victims, if an assay format targeted to the mass disaster scenario is applied [32]. Assays targeted for disaster victim identification (DVI) require specificity, sensitivity, reproducibility, and the ability to accurately genotype degraded DNA in multiple samples in parallel. For example, specific PCR-free SNP genotyping by hybridization to bi-allelic probes can accurately genotype DNA from thousands of samples, including input DNA derived from one or two human cells [33]. An assay that converts single nucleotide differences to a detectable output such as a fluorescent signal can be rapidly converted into a massive parallel format, and additional multiplexing can be accomplished with minimal impact on the single to low copy DNA level input samples used in DVI testing [34].In addition, since the goal of DVI is not the statistical exclusion of a victim but rather confirmation of the identity of a presumed victim or identification of presumed victim family members, the panel can include well-validated, statistically powerful markers such as ancestry informative SNPs [35]. These SNPs are also valuable for residual victim tracking applications. In fact, multiplexing these ancestry-informative SNPs with bi-allelic structure-informative SNPs into the same procedure reduces the net effect of laboratory work required to conduct the analysis while providing valuable information [36]. Use of informative SNPs to confirm suspected relationships between individuals involved in mass disasters and their intimate family groups is a recommended standard for DVI assays [37].Disaster Victim Identification (DVI) is a critical process globally, especially in regions affected by prolonged conflict or natural disasters. While specific percentages regarding the use of Single Nucleotide Polymorphism (SNP) assays in DVI for Iraq, its neighboring countries, and globally are not readily available in the provided information, the general mechanisms and the importance of SNP technology are well-documented [38].

9. SNP Assay Technologies

SNP assays can be classified according to a number of different criteria, including their method of genotyping and detection, their potential for high-throughput analysis, and their potential for robotic automation [40]. These details are important to consider in the context of a forensic application such as human DVI because, for the purpose of human identification, good technologies should be amenable to singleplex detection (diagnostic or analytical method designed to detect only one specific target analyte or pathogen per reaction. This is in contrast to multiplex detection, which aims to detect multiple targets simultaneously in a single reaction)[39]. high- throughput analysis, and robotic platforms. They should exhibit high discriminating statistical power and low sequencing error, and should demonstrate a high bi-allelic nature, low mutation rate, and an even distribution throughout the genome [41]. While many SNP genotyping technologies are too low-throughput or non- automatable for practical use in a forensic application such as human DVI, the following SNP genotyping technologies are all high-throughput, amenable to singleplex assays [42], suitable for use with robotic automation platforms, have been widely used in DVI applications with a diverse range of species, and can also be useful or readily adaptable for use in human identity testing
[43].Single base extension (SBE). A typical SBE assay includes a locus-specific single base extension primer annealed near a SNP site, a polymerase with a 5' to 3' exonuclease activity, and di-deoxy terminators that are only labeled in the DNP position [44]. After conventional PCR amplification using biotinylated primers, excess

primers are removed from the reaction in preparation for the minisequencing step. SBE primer extension is followed by depletion of unincorporated di-deoxy terminators by shrimp alkaline phosphatase treatment, and then a magnetic bead or membrane separation step to purify the products [45]. After photodetection or fluorescence detection, alleles are called based on size differences between the biotinylated ends of the extended and unreacted primers[46]. The formally best performing singleplex SNP allele discrimination method at low coverage Whole Genome Sequencing sites is the original protocol, which has large differences between calls for long insertions compared with errors, but this method is incompatible with SNPs with large insertions nearby. Other indirect methods' data are also of interest: the calls with medium coverage data and the calls with low coverage data. No SNP calling method provides completely accurate calls for all SNPs [47].

10. PCR-Based SNP Genotyping

DNA analysis is an essential tool in the successful identification of disaster victims [48]. Tremendous scientific and technological advances in forensic and molecular medicine have been made in the last few years [49]. This collaboration allowed the identification of thousands of missing people, helped the justice system by clarifying criminal cases using biological evidence, hindered several diseases through the use of predictive markers of high risk, and is used in several other fields of expertise 50. Human identification by DNA is the application of DNA technology in forensics to assign an identity to a person or a sample [51].

11. Microarray-Based SNP Genotyping

Several studies have validated the use of microarray systems for SNP genotyping. There is a large body of literature that describes the use of microarray technology to genotype hundreds to thousands of SNPs in many diverse species [52]. The discovery of SNPs that have potential uses for forensic analyses of human remains has resulted in efforts to develop new and faster ways to genotype these small loci[53]. With the availability of genomic information and the design of microarrays, it is feasible today to exploit this content to develop optimized forensic SNP-typing assays that are highly informative, cost-effective, easy to use, and well characterized for multiple potential applications [54].

12. Applications of SNP Assays in Forensics

Several real-world examples demonstrate the use of SNP analysis for victim identification following major disasters. The 2004 Indian Ocean tsunami and the 2010 Haiti earthquake are notable cases where SNPs were successfully employed. In these instances, SNP information proved crucial for identifying victims, working in conjunction with other identification techniques (54).

- Indian Ocean Tsunami (2004)

The 2004 Indian Ocean tsunami was a catastrophic event, resulting in the loss of over 230,000 lives across fourteen countries. Many bodies were so severely damaged that traditional identification methods were inadequate. This is where SNP analysis proved essential. By providing detailed genetic profiles, scientists were able to assist in identifying numerous victims. (55,57).

- Haiti Earthquake (2010)

The 2010 Haiti earthquake was a catastrophic disaster that led to a significant loss of life and widespread destruction. The resulting chaos made it incredibly difficult to identify victims. Scientists utilized SNP analysis to examine severely damaged DNA samples, aiding in the identification of bodies and facilitating the reunification of families (56).

- Germanwings Flight 9525 (2015)

The 2015 crash of Germanwings Flight 9525 in the French Alps was a devastating event that resulted in the loss of all passengers and crew. The impact of the crash obliterated the plane and the bodies, significantly complicating identification efforts. Scientists utilized SNP analysis to examine the severely damaged DNA, assisting in the identification of the victim (58)

- Yazidi Genocide in Iraq

In 2014, the Islamic State group perpetrated horrific acts against the Yazidi people in northern Iraq, resulting in mass graves filled with unidentified victims. The harsh climate and the passage of time severely damaged the remains, rendering traditional identification methods extremely difficult. (59).

13. Forensic DNA Centers and General Forensic Practices in Iraq:

1- FDNA Center – Al-Nahrain University: This is directly referenced as a key institution for DNA analysis in Iraq. Their website outlines their objectives in advancing DNA analysis, training, and research.

2- Department of Forensic Techniques – Al-Manara University: This newer department explicitly aims to provide expertise in forensic evidence, including biological and chemical evidence and DNA methylation, highlighting a modern approach to forensic science in Iraq.

3- Department of Pathology & Forensic Medicine – Al-Nahrain University: This department's activities include teaching and postgraduate studies in forensic medicine, indicating their involvement in various forensic examinations.

4- Iraqi forensic police cooperation with international bodies: Training initiatives like those with the Spanish National Police emphasize strengthening knowledge in forensic examination, including crime scene evidence collection, preservation, and analysis, which implicitly involves the use of various detection methods.

14. Advantages and Limitations of SNP Assays in Forensic Applications

Multiplex SNaPshot panels are cost-effective methods for SNP detection and enable the rapid typing of multiple targets with low DNA requirements (60). SNP assays are particularly useful in ancestry inference and for the prediction of externally visible characteristics. Moreover, many SNPs can be genotyped in degraded DNA samples due to the limitations in template quantity when detecting autosomes, chromosome X, and Y samples (61). There are only two sex- determining SNPs and an amelogenin SNP that have been detected in human samples. SNP assays also have the flexibility to carry out various higher multiplexed samples with higher accuracy and sensitivity for mtDNA and X-chromosomal SNPs (62). This paper describes the 98-SNP multiplex SNaPshot panel that we have developed and validated for the purposes of disaster victim identification using a Malay population (63).

15. Future Directions and Innovations in SNP Assays

Several advances in genetic diversity testing may have a meaningful impact and potential advantages for the use of SNPs in human identification. These advances may represent future directions for SNP genotyping as applied to DVI, as well as ongoing research initiatives that will improve SNP assay performance. The advantages and drawbacks described here may guide researchers in selecting future SNPs for human identification work (64). Multiple recent examples have employed alternative genotyping chemistries to commonly used probe-based methods. Among these, the Luminex detection system and the bi-allelic ligation assay are methods previously targeted at forensics applications (65). Alternative SNP genotyping platforms have important potential advantages, including lower per-sample costs, multiplex capabilities amenable to high-throughput automation, probe designs that may simplify assay selection and execution, and enhanced sensitivity and specificity for a subset of SNPs (66). Upcoming advances in microarray analysis and next-generation technologies may substantially expand the range of assayable SNPs. These recent observations and developing technologies imply that laboratories seeking SNP information may find a range of powerful and specific methods to choose from, increasing the utility of SNPs for casework in the near future (67).

16. Conclusion

In conclusion, SNP analysis has become a vital tool for identifying victims, especially in challenging disaster situations. These tiny genetic variations are abundant, stable, and can be processed rapidly, making them ideal for cases where traditional methods might fail. As technology continues to evolve, the importance of SNP analysis in victim identification is only set to increase.

By analyzing these genetic markers, scientists can effectively identify individuals, even when bodies are severely damaged. When combined with other evidence, SNP data provides a powerful tool for identifying victims, offering closure to families and assisting in legal processes.

The effectiveness of SNP analysis is particularly evident in regions like Iraq, where mass graves resulting from conflicts like the Yazidi genocide pose significant challenges. By integrating SNP data with other forensic methods, investigators can overcome the obstacles presented by severely damaged remains and accurately identify victims in a timely manner.

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The authors declare that they have no conflict of interest.

4- Ethical Approval:

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