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REVIEW ARTICLE

AN OVERVIEW OF MOLECULAR MARKER BASED IDENTIFICATION TECHNIQUES AND ADVANCES IN FORENSIC INVESTIGATION

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ABSTRACT

The molecular marker based identification techniques are extremely reliable methodologies that are currently used in forensic crime investigation of human, animals as well as plants. Generally the markers are short tandem repeats (STRs), species specific primers, SNPs (single nucleotide polymorphism) are based on PCR and NGS methods which is still under extreme investigation by FBI for development of more convenient, easy and reliable procedures. The human DNA profile generated through genetic analyzer (Electrophoresis of amplified STR markers) is the most used method which dealt with murder, sexual assault, rape, paternity dispute and physically burnt cases. However, the interpretation of results become difficult in cases of partial profiles generated from challenging forensic samples such as dead cells or mixed DNA profile and sometimes very difficult or impossible in case of monozygotic twin's. The advances in NGS platforms applied in generation of DNA database from different sources, phenotypic analysis or phylogenetic analysis and can be used in analysis of mixed DNA identification, complex paternity cases, monozygotic twin's identification, body fluid analysis, as well as species origin detection in forensic science. Recently, it has been reported protein can be also used in the identification of humans as the variations can be unique to an individual or a community of people. Thus, the advent of unique protein based human identification process might be an asset in forensic investigation which is the major new era challenge in forensic science.

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INTRODUCTION

Markers are characters whose inheritance pattern followed at morphological, biochemical, or DNA level. Morphological markers are not very accurate, limited in numbers, influenced by environmental and genotypic interactions which move the research towards molecular markers which are DNA based. The properties of a good marker should be highly polymorphic, highly reproducible, simple, easy and less technical or complicated. The DNA based molecular markers are expressed as variants of DNA regions or segments or length. All genetic markers occupy specific genomic position within a chromosome known as locus. DNA was first isolated and purified by Friedrich Miescher in 1869 and named it as nuclein. DNA ds-helical structure was solved by James Watson and Francis Crick in 1953. In 1985, Alec Jeffreys while working in the University of Leicester (UK) discovered the use of DNA to identify an individual known as DNA fingerprinting leads to a new area of science and later applied in criminal investigations and court cases. In the 1990s DNA fingerprinting methods based on RFLP, RAPD and AFLP analysis were gradually adapted (Martinez and Yman, 1998; Partis and Wells, 1996; Vos *et al.,* 1995; Edwards *et al.,* 1991). The DNA based markers are RFLP- hybridization based, RAPD, AFLP- PCR-based, SSR, SNP-sequencing and DNA chip based. However, restriction fragment length polymorphism (RFLP) based methods, RAPD and AFLP (PCR-based) methods were limited by the quality and quantity of the DNA and reliability of genetic profiles generated from different sources (Datwyler and Weiblen, 2006; Campbell *et al.,* 2003; Guha and Kashyap, 2006; Kuperus *et al.,* 2006). Therefore, necessity of methods which should be less time consuming, easily compared and easily supported in court leads to new breakthrough in the forensic investigation. Microsatellite which is termed as short tandem repeats (STRs) was found suitable in forensic applications which are more sensitive than RFLP, RAPD and AFLP methods.

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Table 1. DNA marker based methods used in forensic investigation

S. No.	Marker	Limitations
1	Short Tandem Repeats (STRs)	Difficult to interpret the result in cases of degraded DNA samples i.e. partial DNA profile generated from such a sample and analysis of mixed DNA
2	Species specific primers	Discrimination boundaries of the species required
3	SNPs (Single Nucleotide Polymorphism)	Large number of loci needed comparative to STRs
4	Mitochondrial DNA (mtDNA) markers	Presence of more than one type of organelle genome
5	mRNA marker	Discrimination rate and reliability
6	miRNA marker	Discrimination rate and reliability

Table 2. DNA based methods used in forensic investigation

S. No.	Methods	Application/Limitations
1	PCR Based (RAPD, AFLP)	The quality and quantity of the DNA, reproducibility and reliability of genetic profiles generated from different sources
2	DNA microarray or Gene Chip	As a tool in establishing genetic relatedness (Genotyping errors)
3	DNA Bar-coding	Analysis of Phylogenetic and Geographical origin (DNA sequence repository for most of the organisms yet not available for comparison as a reference)
4	Nanotechnology	Incorporated into the process of PCR amplification
5	DNA Methylation	Tissue specific differential methylated regions (tDMRs)-fluid identification in mammals
6	NGS Platforms	High-throughput technique enhances the generation of DNA data base form different organism but the sequence analysis and interpretation of data is difficult

Table 3. Application of NGS platform in forensic

S. No.	Application
1	Construction of DNA database from different sources
2	Mixed DNA identification in case of mixed samples
3	Multiple STR: Multi locus detection on different chromosomes
4	Monozygotic twins identification
5	Ancestry and Phenotypic analysis
6	miRNA used in forensic identification of fluids
7	SNPs and STRs used in forensic identification
8	Analysis of mitochondrial genome in forensic identification

Thus, STRs profiling became a more reliable technique as a molecular method for identification in forensic. Newer techniques such as DNA sequencing can help in identification process with great accuracy and reliability. Recently, Centre for DNA Finger-printing and Diagnostics (CDFD) have developed a set of 70 genetic markers from 462 samples of Indian populations from different locations that can be especially used to generate the DNA profile of Indian Population. DNA based methods used in forensic are summarizes in table 1 and table 2. In present scenario limitations of STR typing leading towards the new methodology that is development of hair protein based marker. New methodologies are regularly introduced for increasing the reliability of techniques with improved sensitivity in forensic investigations.

Importance of forensic investigation

The forensic investigation using DNA samples from different sources has immense impact on justice. Forensic investigation play key role in the identification of suspects, victims, criminal or serial killers, rapist and accused thus exclude the possibility of arrest or imprisionment of innocent people. It helps in mitigation of several crimes including crimes concern with human and illegal trades of wildlife crimes.

Biological sample collection and DNA analysis

In the forensic investigation process lifting and collection of biological samples are very important. DNA for DNA profiling is extracted from the nucleated cells in biological exhibits (Kuperus *et al.,* 2003) which includes wet or dry blood stains (vascular or menstrual blood), secretions released in sexual contacts, semen in liquid or dry form, body fluid secretions, bone, teeth, cytological smears and hairs. After extraction of DNA the more complex process occurred is the DNA quantification, DNA amplification, detection of the DNA amplified products their analysis and data interpretation. In the forensic laboratories most widely used source of DNA is blood which is utilized in the identification of suspects, reference samples for comparision of DNA profiles generated during interpretation of results and paternity dispute. In autopsy blood can be harvested from blood vessels, heart chambers, heart muscle, long bones, skeletal muscles and other tissues. Blood collected by doctors can be conserved at 4°C for a 3-4 days or stored at -20°C for a week or at -80°C for longer periods of time (Bond and Hammond, 2008). Biological samples collected from the crime scenes are often most challenged mixed with different types of inhibitors due to which PCR inhibitors creates problem during simple preparation for DNA profiling.

STRs methods used in forensic

The DNA source being utilized for human identification in forensic investigation of sexual assault cases, paternity dispute, burnt and murder cases are bone (Burn case, buried dead body), blood (murder case, accidental cases), saliva (cigarette, chewed tobacco), semen (rape cases) and hair. Markers used in STRs profiling of human in forensic investigation are Short tandem repeats (STRs) markers, Y-STR markers and Mini-STR markers. The human genome is full of highly repetitive DNA sequences especially in the cetromeric region of the chromosome. The DNA regions with short repeat units (usually 2-6 bp in length) are called short tandem repeats (STRs) which is mainly found around the cetromeric region.

An individual inherits one copy of an STR from one parent and second copy from next parent. In CODIS (Combined DNA Index System) the national database standard of STR loci for human identification has been well established for forensic investigation. The STR loci accepted internationally for human identification are D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA.

- The analysis of STRs is easy because polymerase chain reaction (PCR) used for amplification.
- Inspite of degraded DNA which is obtained from challenging forensic evidences small STRs are able to discriminate as it is only 2-6 bp in size. Thus, small size of STRs is suitable for human identification in forensic (Minifiler).
- Data generated from small STRs is relatively more stable because these have very low chance of mutation.

Mini-STR marker

In cases of highly degraded samples obtained from challenged exhibits which are not sufficient in quantity as well as quality. The mini-STR target to the larger STR loci therefore, gives the amplification of the larger loci. Thus, sensitivity of DNA detection increases in mini-STR and helps to resolves the cases where some locus are missing in DNA profiles generated from STRs marker for human identification in forensic investigation. The mini-STR loci accepted internationally for human identification are D13S317, D7S820, Amelogenin, D2S1338, D21S11, D16S539, D18S51, CSF1PO and FGA.

Y-STR marker

Y-STRs utilized for the male sex determination are present on the short arm of the Y chromosome. The Y-STRs locus is inherited from male parents to sibling generations. Since in YSTRs only part of male DNA is amplified hence can be easily detected in sexual assault case. However, it can be utilized in case of mixed forensic samples for non-sexual assault cases. The Y-STR loci accepted internationally for human identification are DYS456, DYS3891, DYS390, DYS389II, DYS458, DYS19, DYS385, DYS393, DYS391, DYS439, DYS635, DYS392, GATAH4, DYS437, DYS438 and DYS448.

Limitations of DNA based human identification

- Mutational events in human populations in the particular selected loci make the analysis very difficult. Though, mutational events are very rare.
- Some time DNA samples are so challenging that it provides only a partial DNA profile of human which is not sufficient to interpret the result. The major reason is the exposure of DNA to biological, environmental and chemical effect that results in the loss of DNA in samples (Lindahl, 1993; Ottoni *et al.,* 2009).
- The apoptosis process during the hair biogenesis is the major reason behind the poor DNA extraction from hair. In the biogenesis process of hair most of the DNA degraded results trace DNA which is beyond the PCR limitations (Bengtsson *et al.,* 2012; McNevin *et al.,* 2005).
- The discrimination of the monozygotic twins becomes very challenging in DNA based human identification.
- DNA is not extractable for DNA profiling specifically in case of human hair that is another challenge of STR based human identification in forensic.
- In a sexual assault case, when both female and male DNA fractions are present. In such cases in PCR amplification of male DNA sample, the female DNA component is also amplified which masking the male DNA hence make the result analysis difficult.

Limitations of SNPs marker

- In the SNP based identification the power of discrimination is very low due to less variation as compared to STRs based identification.
- Large numbers of SNPs are required to identify an individual as compared to standard 13 STRs with the same reliability. Thus, use of SNPs is not universal or remains specific.

Next Generation Sequencing (NGS) Platform in forensic

The limitations of first-generation sequencing to analyze degraded DNA, mtDNA and mixed DNA samples lead towards to explore the NGS technology in forensic science. NGS technology not only can be used to solve the problems such as analysis of multiple loci on different chromosomes in mitochondrial genome, mixed DNA identification and complex paternity cases but also can be applied in generation of DNA database from different sources, phenotypic analysis or phylogenetic analysis, monozygotic twin's identification, body fluid analysis and species origin and detection (Hawkins *et al.,* 2002; Kwok, 2002; Wells *et al.,* 1999). Thus, NGS platform resolves the problems with STR genotyping strategies. The application of NGS in forensic science are summarizes in table 3.

RNA (mRNA and miRNA) marker used in forensic

In forensic science investigation new methods are incorporated because of technical development. In the past years, European DNA Profiling Group (EDNAP) has established mRNA as a new tool for the identification of blood, semen, saliva, skin (Cao *et al.,* 2006; Li *et al.,* 2010; Tang and Huang, 2010). Another tool is miRNA which used in identification of body fluids (Hansen-Goos and Mecke, 2009). In forensic cases, advances of the knowledge help to determine the nature of biological samples (vascular blood or menstrual blood, saliva, skin and vaginal secretion).

Mitochondrial DNA marker used in forensic

Mitochondrial DNA **(**mtDNA) used in phylo-genetic analysis of biological samples. The cells with little DNA like hair shafts mtDNA markers is used for analysis. Thus, Mitochondrial DNA (mtDNA) can be useful for identifying degraded DNA where chance of DNA occurrence is very little. The mtDNA marker 16S rRNA, 12S rRNA, cytochrome b and Cytochrome Oxidase are utilized in identification of phylogenetic and geographical origin.

Limitations of mitochondrial marker

- Less variability as compared to nuclear DNA. As mtDNA is present in both male and female but inheritance pattern is only maternal.
- It doesn't have the exclusion power like standard STRs.

Protein based human identification

The hair protein is consists mainly of such a peptides which is highly coiled-coil in structure and covalently linked peptides that's why these proteins are robust and flexible in structure (Coulomb *et al.,* 2002; Lee *et al.,* 2012). In crime scene investigation hair is forensically important and environmentally stable component compare to DNA (Thompson *et al.,* 2014; Van *et al.,* 2010; Wilson, 2008; Wilson *et al.,* 2007). However, the extraction of DNA from hair is difficult. So the alternative can be a hair protein (Poinar and Stankiewicz, 1999; Bada *et al.,* 1999; Orlando *et al.,* 2013; Allentoft *et al.,* 2012; Wadsworth and Buckley, 2014). So, the protein marker could be an asset in the identification of human from hair. Therefore, there much needed to focus on screening of a unique protein marker which provide unique discrimination like provided by DNA based markers. Glendon parker and his team tried to develop the feasibility of protein based human identification of European and African human population by development of protein marker. Still, there is need to optimize protein based marker of human populations.

Limitations of protein based human identification

- The major limitation is the amount of hair protein that is not sufficient in challenging samples used in forensic investigations. Thus, the major challenge to the new era human identification based on hair protein marker is to develop such a unique hair protein marker which could be as reliable as DNA based human identification.
- Discrimination of gender has not been established till date in case of protein based human identification.
- Range of discrimination also has not been established till date.

Conclusion

The molecular markers and advances in technology becomes important tool in resolving the cases in forensic science. Identifications and detection processes has became easy and reliable due to the recent advances and adaptation of molecular tools, biomarkers and methods in forensic while study the body fluids, mixed stains, multiple loci on different chromosomes, complicated paternity or maternity case, identification of individuals in crimes or disasters, establishment of sexual assault and rape. In future advances in tools techniques and new methodologies might be useful to resolve the major limitations of the forensic science. Thus, advances in forensics provides creative tools for solving crimes.

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