Forensic science in the DNA technology era

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The ability to detect polymorphic repetitive sequences in vertebrate DNA has opened up the modern era of forensic genetics. Using the Southern blotting technique in combination with oligonucleotide probes, these polymorphisms can produce an individual barcode-like pattern known as a "genetic fingerprint". Current techniques for personal identification and kinship testing are mainly based on autosomal and gonosomal short tandem repeats (STRs). In certain instances, mitochondrial DNA sequencing can be used to complement nuclear DNA typing.

Characterisation, or typing, of DNA for the purposes of criminal investigation and kinship testing can be thought of as an extension of forensic blood typing that has been commonplace over the last century. In the past, all forensic genetic analyses were carried out using serological genetic markers such as blood groups, polymorphic proteins and enzymes, and HLA typing. These genetic markers were rather limited when analysing minimal or degraded material, which is frequently the case in forensic testing. In addition, the analytical techniques suitable for measuring biological materials other than blood were very limited, making it difficult to obtain useful information from saliva, hair or semen.

Following the description of repetitive DNA sequences in the human genome by Jeffreys et al. in 1985 [1], geneticists developed a branch of DNA technology with the potential of being used as a tool in forensic medicine, namely DNA fingerprinting.

**Hypervariable minisatellite DNA**

The human genome consists of 3 x 10^9 base pairs. Approximately 5% of the DNA has a known function and is considered to be the coding region of the DNA. About 95% of DNA is the so-called "junk DNA," that is, it may not have a known function. The process of natural selection eliminates mutations that result in non-viable, or even less-viable phenotypes. The 95% of DNA that does not encode protein is much less subject to natural selection. Non-coding DNA has therefore far more variability.

Jeffreys discovered the so-called hypervariable minisatellites which are repetitive sequences within the DNA. The composition and length of these minisatellite regions varies between individuals, even within the same species. In addition, there may be interspersed polymorphic restriction sites within these minisatellite regions. Treating the DNA with endonucleases cleaves the DNA into fragments to create a pattern that is unique to each individual. Using the Southern blotting technique [2], the patterns of restriction fragments can be viewed following electrophoretic separation and hybridisation with radioactive or alternatively labelled multi-locus probes such as (CAC)_5 and (CAG)_5.

Oligonucleotide probing of restriction fragments produces a barcode-like pattern which is unique to the individual. The probability of the patterns from two randomly selected individuals matching is in the order of more than 1 in 10^10. This multi-locus typing procedure became known as DNA fingerprinting because of its ability to distinguish two individuals [Figure 1]. Nevertheless, this technique has some disadvantages, such as insufficient genetic transparency and an inability to evaluate the results using mathematical analyses.

For these reasons, the oligonucleotide probe method has been replaced by the analysis of variable number tandem repeats (VNTRs) using specific single locus probes [3]. Unlike oligonucleotide probes, the sequence recognised by single locus probes is found in only one position on the genome. However, if several single locus probes are used in parallel, it is possible to approach the same level of individualisation as that achieved with multi-locus fingerprinting. This approach provides a simple restriction fragment length polymorphism (RFLPs) pattern with high discriminatory power. Figure 2 shows an example of a paternity testing analysis using single locus typing.

Both multi-locus and single locus probing require a large amount of sample material for the analyses, which is a disadvantage in criminal applications. In addition, interlaboratory standardisation has been difficult to achieve.

**Short Tandem Repeats (STRs)**

The technique of PCR has overcome these difficulties and has greatly enhanced the role of DNA profiling in forensic science. During the early 1990s, typing of amplifiable VNTRs (i.e. minisatellites) [4] and short tandem repeats (STRs) [5,6] became the main focus of investigation. However, because of the large size of minisatellites, efforts were mainly concentrated on the amplification of STRs. STRs are dispersed throughout the whole genome and can be found in large numbers, even in the gene introns. Dinucleotide polymorphisms are the most common STRs but they are strongly affected by strand slippage during amplification, producing artefactual stutter bands. Tetra and pentanucleotide repeats [Figure 3] are less prone to slippage and are therefore more suitable for forensic analyses.

STR methods can be easily standardised and are suitable for multiplexing. Fluorescence-based technologies and the use of gene scanners have enabled the measurement of amplicon length to be automated.
STR multiplex kits including up to 16 loci are now commercially available. Each capillary electrophoresis run contains amplicons in three (or four) colour channels, with one additional channel for an internal size standard.

The allele type is assigned by counting the number of repeats within the STR; this is closely related to the amplicon length. Therefore, a series of figures can be given as a genetic fingerprint for personal identification purposes. As an example, the Interpol Working Party on DNA Profiling recommend a common European Standard Set of 7 loci per genetic fingerprint, namely VWAFA31/A, TH01, D21S11, FGA, D8S1179, D3S1358, D18S51 together with amelogenin. The latter is a gonosomal (i.e. Y and X chromosomal) dimorphism which serves as a marker of gender. The combined discriminatory power of such a set of loci is enormous and the probability of two unrelated individuals matching by chance (Perfect Match, PM) is lower than 10\(^{-10}\). In the USA, a core set of loci is enormous and the probability of two individuals sharing an exact STR profile is lower than 10\(^{-14}\), which is sufficient for forensic purposes.

Highly-developed countries operate databases which contain the genetic profiles of all known perpetrators of sexual crimes as well as trace samples from serious unsolved criminal cases. These can be used to link serial crimes and unsolved cases involving repeat offenders nationwide. The Forensic Science Service in the UK launched the world’s first criminal intelligence DNA database in 1995. This now contains more than 2000 profiles and currently links approximately 4000 scenes of crime to known individuals every month. Furthermore, if a semen trace has been identified in connection with a sexually-motivated murder, screening the male population in the appropriate region of the country can help to identify the perpetrator if the STR pattern matches the semen profile.

STR typing is also the method of choice for kinship testing [Figure 4]. Forensic DNA laboratories carry out a large number of simple kinship tests at the request of the courts or private individuals.

**Gonosomal STRs**

DNA typing procedures to identify markers located on the 22 autosomal pairs, which do not differ between the sexes, are suitable for most forensic requirements. However, there are some good reasons to involve polymorphic markers located on the sex chromosomes (gonosomes). The homologue gonosomes are called chromosome Y (ChrY) and chromosome X (ChrX). Gonosomal markers do not principally differ in structure, but rather in their inheritance. Roewer, et al. [7] began ChrY testing following his description of the DYS19 STR which can be used in combination with autosomal STRs. Female individuals normally lack a ChrY so the ability to identify male-specific DNA renders Y chromosomal STR systems an invaluable addition to the standard panel of autosomal loci. It is particularly useful in cases of rape and other sexual assaults, as well as for paternity testing where Y-STR haplotyping can help to close information gaps. The individuality of the male-specific part of the Y-chromosome can be analysed using a set of short tandem repeat markers approved by the forensic and scientific community. Roewer’s group have suggested that at least eight STRs should be used to obtain a Y-haplotyping.

When a sexual crime takes place, it may be necessary to analyse a male trace on a female background, for example, sperm on a vaginal swab or male saliva on female skin. ChrY STR typing does not produce mixed stains, but rather a proper male ChrY marker pattern. As ChrY markers cannot recombine during meiosis, the process of calculating the matching probability of the Y chromosomal pattern requires a special mathematical approach which is based on the haplotype frequencies found in the appropriate population group. Roewer’s group initiated a world-wide search by the Y-user scientific community which resulted in the development of a freely accessible international YSTR database [8]. This database comprises 22,872 haplotypes from 200 different population groups (last entry 30/1/2004). Combined with a new mathematical approach [9], it allows ChrY typing to be used with confidence as evidence in court.

ChrY typing cannot distinguish between males belonging to the same family (i.e. brothers, father/son/paternal grandfather, etc.). This can be a disadvantage in criminal investigations, but is actually an advantage in kinship testing: ChrY typing can produce strong evidence for paternity within a male line spanning many generations [10]. Hence, ChrY typing is a useful tool in paternity testing whenever the alleged father is deceased but one of his male relatives can be tested instead. This procedure is called deficiency paternity testing [Figure 5).

ChrX STR typing contributes minimal information to criminal investigations, but may be extremely useful for solving complicated kinship cases. Initially, ChrX testing was only employed sporadically but the use of ChrX markers has been enforced in recent years [11]. Males inherit their single ChrX from their mother, and fathers transmit their ChrX obligate to each daughter, but not to sons. Therefore, when an alleged father is not available for testing, his mother can be investigated instead. Furthermore, all sisters fathered by the same male share an identical paternal ChrX. Of course, ChrX marker testing cannot be used for father-son testing. However, in skeletal identification procedures, X-chromosomal mother-son testing is a very powerful approach. A closely linked ChrX STR can be carried as a stable chain (haplotype) which survives a number of meioses. Hence, in appropriate circumstances, ChrX typing can provide strong evidence of kinship spanning several generations [Figure 6].

**Mitochondrial DNA**

Mitochondrial DNA (mtDNA) testing is a great challenge in forensic genetics. In most cell types there are several thousand mitochondria, each containing 10-20 copies of a circular DNA molecule. In contrast a cell contains only one or two copies of a nuclear DNA (nDNA) locus. MtDNA can therefore reach copy numbers of ten thousand or more. In criminal case-work, traces can therefore be typed with a much better detection rate when mtDNA is investigated instead of nDNA. The main field for typing mtDNA is in the investigation of telegene hairs and putrefied bones.

The mitochondrial genome contains about 16,300 bps but only three regions spanning about 800 bps show considerable variability. These are called the hypervariable regions 1-3. The most frequent mitochondrial polymorphisms are single nucleotide polymorphisms (SNPs). Forensic mt-typing has become more widely used since the introduction of the cycle sequencing method for this application [12]. Mitochondria are only inherited from the mother and in this regard, mt-genetics can be seen as the female counterpart to male ChrY inheritance. Like ChrY analyses, calculation of the mtDNA matching probability requires mtDNA databases [13] and special mathematical approaches. MtDNA evidence will never reach the same certainty as nDNA results.
Nevertheless, in difficult cases, mtDNA testing provides useful additional information and can indicate relationships in the maternal line [Figure 7]. Furthermore, the special inheritance of mtDNA is free from recombination events and has led to the evolution of several mtDNA strains [14], named the haplogroups A-X. The global distribution of the mt-haplogroups is known to the scientific community, therefore forensic mtDNA typing can provide information on the ethnic origin of a stain or skeleton. The same applies to ChrY haplotypes [16].

Summary

Over the past 20 years, many spectacular cases have been solved in criminology and identification throughout the world by use of DNA typing techniques. Since stain investigation and kinship testing employ identical methods, some laboratories now offer confidential paternity tests. These can be carried out using some hairs or stains from the child without the mother’s knowledge. In Germany there are now efforts to prohibit such secret DNA investigations.

Considerable changes have occurred over the period covered in this article with respect to both the structural targets in DNA and the methodologies used for forensic testing. There is a strong trend to investigate simpler polymorphisms characterised by transparent genetic features; this can be combined with the more complex gonosomal haplotyping. It is therefore not surprising that forensic science is focussing on SNPs [17]. Investigation of these markers could enable the use of DNA chips thus allowing automation and improvement of the success rate whenever the DNA is highly degraded. Recent forensic DNA techniques are exclusively used for human identification and kinship evidence. Apart from the information about gender and ethnic origin, these techniques do not reveal any personal attributes. Investigation of SNPs offers the possibility of detecting iris and hair colour, body height, skin type and other features, which may be useful in drawing up arrest warrants for suspected criminals. However, such a change would require a broad discussion and social consent, and is unlikely to become reality in the near future.

References


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