Forensic Identification by DNA Fingerprinting and Mitochondrial DNA Typing

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Abstract

Short tandem repeats (STRs), that represent an important source of highly polymorphic markers in human genome, and mitochondrial DNA (mtDNA) typing, that its sequences were conserved within the same maternal lineage, facilitated by use of the polymerase chain reaction (PCR) provide a powerful tool for forensic identification. We report the analysis of 9 STR loci and mtDNA typing of a muscle biopsied sample with 2 months postmortem by comparison with the genotype of the relative. The DNA profile showed common alleles with that of the relative but only 12 from 20 alleles (60%) were identifiable. Then, we performed mt DNA sequencing of the hypervariable region I (HV I) and obtained 100 per cent homology with that of the relative. In conclusion, personal identification can be performed precisely by the data of DNA profile and mtDNA typing compared to the genotype of the relative.

Key word : Forensic Identification, DNA Fingerprinting, mtDNA

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DNA analysis has become the standard method in forensic stain typing(1). A Jefferys et al introduced DNA fingerprinting technique and mentioned that the technique provided a powerful method for paternity and maternity testing and could be used in forensic application as well(2). Since the application of a variable number of tandem repeats (VNTRs) or minisatellites for personal

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identification has been limited by the low frequency, asymmetric distribution of these repeats in the genome, and the inability to determine alleles precisely with Southern hybridization-based detection schemes. Therefore, trimeric and tetrameric shot tandem repeats (STRs) which represent a rich source of highly polymorphic markers in the human genome become a useful marker for application to personal identification in the medical and forensic sciences[3-5].

Recently, there were reports of human mitochondrial DNA (mtDNA) in forensic application[6-8]. The chief advantage of mtDNA typing over nuclear DNA typing is the high copy number of mtDNA per cell[8]. In the present report, we applied both STR analysis and mtDNA typing to identify an evidentiary sample's siblings.

MATERIAL AND METHOD

Samples

A 2 month postmortem muscle biopsy and fresh EDTA-blood from the suspected person's sister were sent to Human Genetics Unit, Department of Pathology, Ramathibodi Hospital for evidentiary sample analysis. The parents of the suspected person had passed away and the suspected person had only his sister.

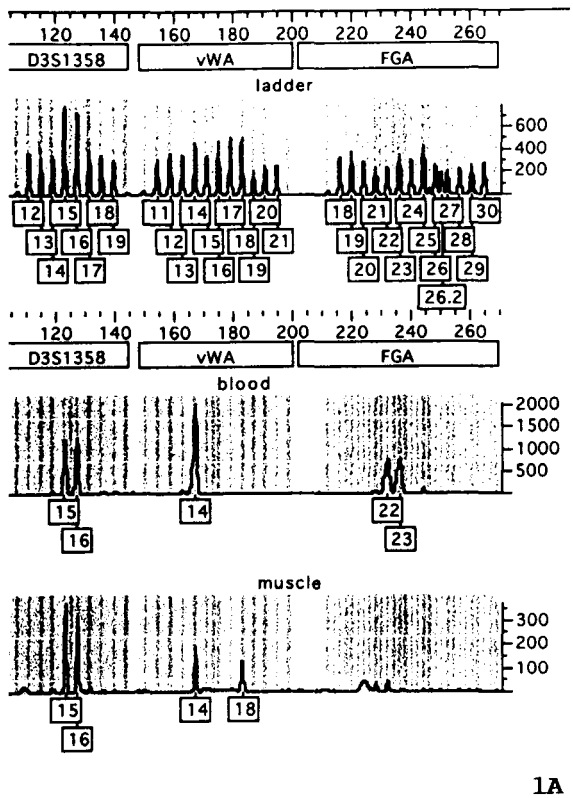
DNA fingerprinting

DNA extraction from the muscle and blood was modified from that previously reported[9,10]. Then, we performed PCR based STR analysis for DNA fingerprinting technique. We co-amplified 9 STR loci: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820 and the segment of the X-Y homologous gene amelogenin in a single tube. This PCR amplification kit was purchased from PE applied Biosystem (Perkin Elmer, USA). In the kit, one primer of each locus-specific primer was labeled with either the 5-FAM, JOE or NED NHS-ester dye. Amplifications were carried out in thin-walled MicroAmp tubes (Perkin Elmer) in a geneAmp PCR System 2400 (Perkin Elmer), using the following conditions: 95°C for 13 min, 94°C for 1 min, 59°C for 1 min, 72°C for 1 min, for 28 cycles and followed by 60°C for 45 min.

The amplified products were separated by automated capillary electrophoresis (Applied Biosystem automated DNA sequencer model 310). DNA profiles were generated using Genescan and Genotyper solfware.

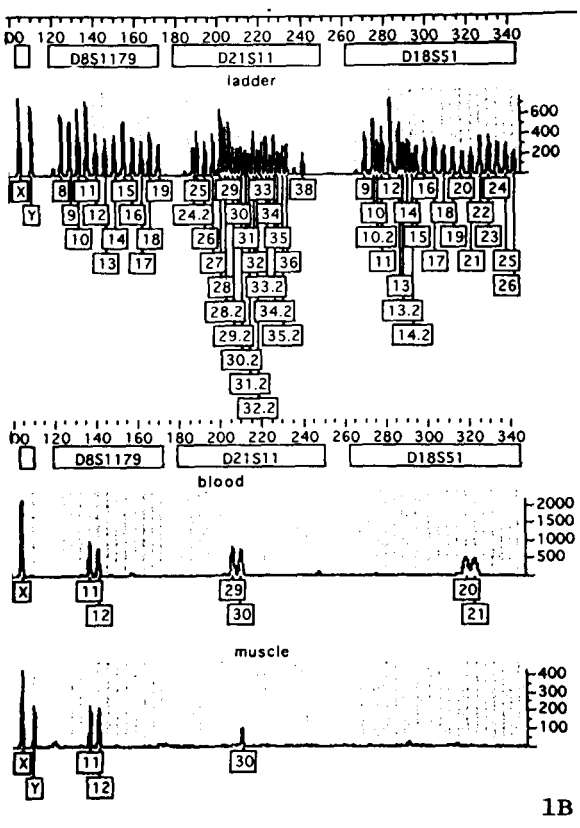
Mitochondrial DNA typing

Mitochondrial DNA typing was performed following that previously described[11]. The mtDNA region subjected to analysis was HV I (hypervariable region I) from position 16024 to 16365[12]. Two regions of mtDNA were amplified by using 2 pairs of primers, L15971 (5'-TTAACTC CACCA T T AGCACC-3')-H16258 (5'-TGGC TTTGGAGTTGCAGTTG-3') and L16140 (5'-TACTTGACCACCTGTAGTAC-3') - H16410 (5'-CACGGAGGATGGTGGTC AAG-3'). Then, the amplified products were subjected for DNA sequencing by using automated sequencer as mentioned earlier.



1A

Fig. 1A. DNA profile of suspected person's sister (blood) and 2 month postmortem muscle sample (muscle). A) STR alleles of loci D3S1358, vWA and FGA, respectively.



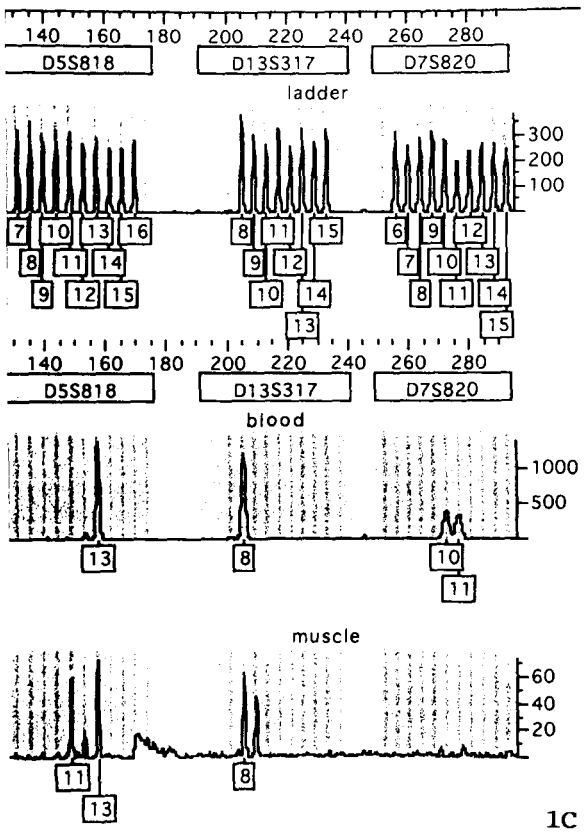


Fig. 1B. DNA profile of suspected person's sister (blood) and 2 month postmortem muscle sample (muscle). B) STR alleles of loci Amelogenin, D8S1179, D21S11 and D18S51, respectively.

Fig. 1C. DNA profile of suspected person's sister (blood) and 2 month postmortem muscle sample (muscle). C) STR alleles of loci D5S818, D13S317 and D7S820, respectively.

RESULTS

Genotyping of 9 STR loci and the X-Y homologous gene amelogenin were performed using the muscle and blood samples. The DNA profile of the blood sample was completely identified, whereas, that from the muscle sample was partly identified. The unsuccessful STR loci were FGA, D18S51, D7S820 (Fig. 1A-C). However, the analysis of identifiable STR alleles revealed that they, at least, shared one common allele for each allelic genotype.

Mitochondrial DNA typing

For mtDNA typing, the amplified products were subjected to do sequencing by an automated capillary electrophoresis. In order to determine the mtDNA sequence, information from both strands which were separately sequenced were aligned together. The edited sequence is shown in Fig. 2. The sequence of muscle and blood samples was identical.

DISCUSSION

DNA analysis is becoming an integral part of forensic investigation. Genetic information from samples with degraded DNA or from samples which do not contain much DNA may be obtained by *in vitro* amplification of the DNA using the PCR[13]. PCR based assays included the amplification of the HLA DQA1 locus, various VNTR

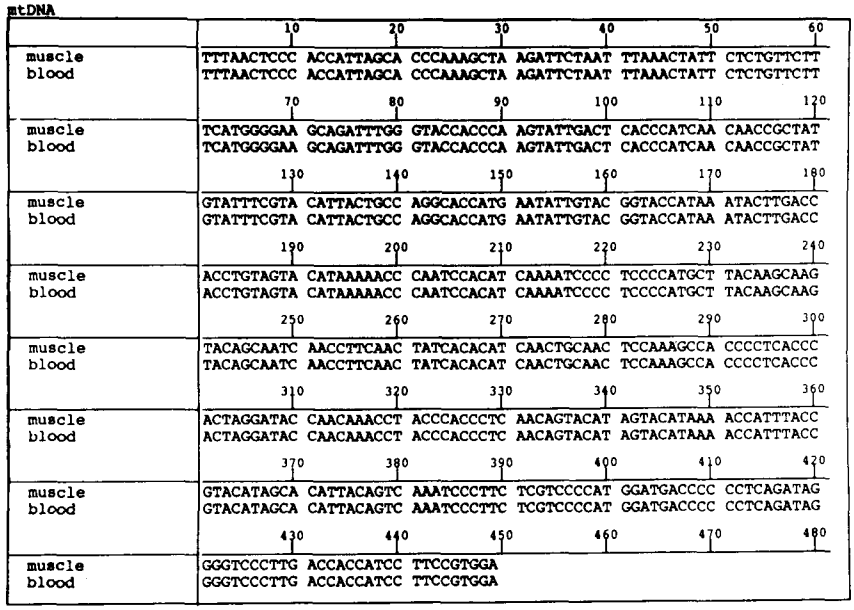


Fig. 2. Mitochondrial DNA typing of hypervariable region I of suspected person's sister (blood) and 2 month postmortem muscle sample (muscle).

loci, many STR loci, and mtDNA typing[13]. We used multiplx PCR amplification typing kit which is commercially available to simultaneously amplify specific regions of 9 genetic loci. Since the sizes of these 9 STR loci in this multiplex PCR assay do not overlap, unequivocal typing of all loci is possible.

This report is an example of successful confirmation of the identity of degraded muscle by DNA analysis. The multilocus genotype data were studied with regard to the relationship between the observed alleles. For the STR loci which are highly polymorphic, fragments are stably inherited and segregate in a Mendalien fashion. We identified such forensic samples that were both degraded muscle and fresh blood and shared a common genotype but not all STR loci of muscle sample were identified (12 from 20 alleles or 60 per cent of all alleles tested). The degraded muscle DNA could not amplify large size STR alleles, FGA (219-267 bp), D18S51(273-341 bp) and D7S820 (258-294 bp). The reason was that the amplification for the loci with alleles more than 200 bp would be easily inhibited, but that for loci with small size alleles was not affected so much[14]. Therefore, mtDNA typing was done. Mitocondrial DNA is high copy number, and more importantly, it is maternally inherited, so sequence variations can be used to determine the maternal lineage of individuals and for forensic purposes. The DNA sequence of mtDNA was successfully obtained and showed 100 per cent homology. Hence, this evidentiary sample was confirmed to be the muscle of the suspected undoubtly *via* mtDNA data and DNA profile.

In conclusion, this study demonstrates that PCR-based STR analysis, which enables the amplification of specific region of a total 9 genetic marker and mtDNA typing which is useful for maternal lineage identification, provides a powerful tool for forensic identity investigation. In addition, automation of DNA profiling and mtDNA sequencing allow dramatic increase in sample throughput as well as computerized on-line recording of the results.

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การพิสูจน์พยานวัตถุโดยการตรวจลายพิมพ์ดีเอ็นเอและการหาลำดับสารพันธุกรรมจากไมโตคอนเดรีย

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การวิเคราะห์ดีเอ็นเอท่อนสั้นที่มีลำดับซ้ำ ๆ กัน หรือ STR เป็นสารพันธุกรรมที่มีความหลากหลายมาก และการหาลำดับสารพันธุกรรมของไมโตคอนเดรียซึ่งเป็นข้อมูลทางพันธุกรรมที่ถูกอนุรักษ์ในมารดาสายพันธุ์เดียวกัน เป็นวิธีการที่สำคัญในการพิสูจน์พยานวัตถุ รายงานนี้เป็นการวิเคราะห์ตำแหน่งของ STR จำนวน 9 ตำแหน่ง เพื่อตรวจลายพิมพ์ดีเอ็นเอของพยานวัตถุซึ่งเป็นชิ้นเนื้อตัวอย่างจากศพที่เสียชีวิตมาแล้ว 2 เดือน โดยเปรียบเทียบกับลายพิมพ์ดีเอ็นเอ ของเลือดของบุคคลที่สงสัยว่าเป็นน้องสาวของผู้ที่เสียชีวิตนี้พบว่า ลายพิมพ์ดีเอ็นเออัลลิลย์ที่เกี่ยวข้องกัน เนื่องจากมีปัญหาการเน่าสลายของชิ้นเนื้อทำให้สามารถจำแนกอัลลิลย์ได้เพียง 12 จาก 20 อัลลิลย์ (60 เปอร์เซ็นต์) ดังนั้นจึงทำการหาลำดับสารพันธุกรรมของไมโตคอนเดรียและพบว่าลำดับสารพันธุกรรมในไมโตคอนเดรียของชิ้นเนื้อพยานวัตถุและของเลือดเหมือนกันทุกประการ สรุปได้ว่าการตรวจลายพิมพ์ดีเอ็นเอและการหาลำดับสารพันธุกรรมจากไมโตคอนเดรียเป็นเครื่องมือที่มีประโยชน์ในการพิสูจน์บุคคล

คำสำคัญ : การพิสูจน์ทางนิติเวชศาสตร์, การตรวจลายพิมพ์ดีเอ็นเอ, ไมโตคอนเดรีย

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