

## Forensic Individualization of Suspected Criminals using Apolipoprotein B (APoB) Hyper Variable Region

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### Abstract:

In forensics, DNA has been used to sort out the problem related to personal identification for over three decades with absolute accuracy. In this study an attempt has been made to isolate DNA from exhibits like Cigarette butt, Handkerchief and scalp hair collected from crime scene and subsequently the control samples were taken from the suspects. The isolated DNA was amplified by using universal APoB primer, resulting in the formation of band pattern which was helpful in interlinking the suspects with assassination. The present study concludes that the exhibits collected from crime scene containing sweat and saliva even in trace amount and hair can be used as reliable source for individualization using VNTR based DNA typing.

**Keywords:** Forensic Science, DNA isolation, Polymerase chain Reaction, variable number of tandem repeats, APoB primer, Hyper Variable Region.

### Introduction:

It has been evident that Variable Number of Tandem Repeats (VNTRs) are highly polymorphic markers<sup>[1-3]</sup> Each VNTR locus is associated with a large number of different allele, each allele corresponding to a specific number of repeated sequence in any population. Each individual carries two alleles only – one on each of two homologous chromosome. The important methodological merit of VNTRs is its unique pattern for every individual.

Due to highly polymorphic character VNTRs are used as an important tool in forensic identification and comparison. They also constitute preferred system for ‘DNA Fingerprinting’, the direct practical application of which includes criminal and forensic examination, in solving the problem of identification amongst a large number of suspects. DNA isolated from biological materials can be analyzed by DNA Profiling to help establish the source of deoxyribonucleic acid (DNA) <sup>[7-8]</sup>. It has been demonstrated many time that blood, semen, hair root, tissues, bone are good source of DNA for identity

testing <sup>[9-11]</sup>. Sweat is also a useful material in this respect. These biological materials recovered from crime scene rely primarily upon the identification of blood group, antigens and rarely on iso-enzyme and polymorphic protein. These approaches have several inherent limitations, especially their limited detectability because of the low concentrations of antigens, isoenzymes and proteins in the samples. In addition to these, drying, aging, and contamination associated with the samples limit the number of markers that can be typed. The potential for discrimination using these markers are also less sensitive than DNA analysis.

The study performed by **Tamer et al [12]** estimated the frequency of APoB point mutations in 179 atherosclerotic, 145 hyperlipidaemic Individuals and 272 healthy individuals in the east Mediterranean region of Turkey. Lipid and lipoprotein level were measured with routine biochemical analysis and APoB mutation was detected using realtime PCR. In this region, APoB mutation was observed as rare causes of hyperlipidaemia and atherosclerosis may therefore be unrelated to them. **Dekha et al [13]** reported the allele frequency distribution at the hypervariable locus 3' to the apolipoprotein B gene (ApoB 3' VNTR) in five well-defined human populations by using the PCR technique. A total of 12 segregating alleles were detected in the pooled sample of 319 individuals. A fairly consistent pattern of allele frequency distribution was found apparent in most of these geographically and genetically diverse populations, suggesting that the ApoB 3' VNTR polymorphism predates the geographic dispersal of ancestral human populations. In spite of the observed high degree of polymorphism at this locus, the genotype distributions in all populations confirm to their respective Hardy-Weinberg predictions. The result also shows that alleles that are shared by all populations have the highest average frequency within populations. The APoB gene contains a hypervariable region consist of VNTRs DNA sequences. They suggested a general method for amplification of targeted VNTR region.

These findings demonstrate the potential utility of highly informative hypervariable loci such as the ApoB 3' VNTR locus in population genetic research.

Now a days the criminals are active and aware about the evidence which normally left at the scene of crime during committing a crime, but they unknowingly and hurriedly leave some physical / biological evidence at crime scene. The major challenge for forensic scientist is to isolate DNA from such evidences containing trace amount of biological materials.

It is necessary to use a suitable and appropriate method for extraction and isolation of DNA from different biological sources using reliable methods, because once the sample are utilized wrongly it can't be used again. The DNA sample are very much sensitive to cross contamination, hence the use of appropriate primer for DNA typing is necessary to form a definite interpretation of result.

In the present study, DNA extracted from saliva and sweat suspected over cigarette butt and handkerchief, as well as from hair root meshed in the fist of victim. VNTR studied in this report is present at 73 base pair (b.p) 3' to the second polyadenylation signal of the human 'Apolipoprotein B, (APoB) gene (which is located on chromosome 2) and is commonly referred to as APoB 3'HVR (Hyper Variable Region) [4-6]. APoB 3'-HVR alleles and genotype have been much studied in numerous population. The banding pattern obtained has been utilized in identification of culprit involved in the murder case.

#### **Materials and Methodology:**

##### ***Extraction and isolation of DNA from cigarette butt:***

The cigarette butt recovered from the crime scene, which was suspected to contain saliva, left for air dry at room temperature. To isolate DNA from cigarette butt, the filter end was removed and cut into small pieces. The pieces of cigarette butt were soaked in 0.9% saline solution for 10 minutes. The extract was collected into ependroff tube and centrifuged at 6,000rpm for 5 minute. The supernatant was discarded and 1 ml of lysis buffer (10mM tris HCl, 10mM EDTA, and 2% SDS) was added to the pellet. 500 µl of 5 M NaCl was poured to the solution and mixed gently for 1 minute and then centrifuged at 4,000rpm for 10 minutes. The supernatant (500µl) was transferred into clean ependroff tube and 1ml of 95% cold ethanol was added to it. Mixed it well and incubated it at 4°C for overnight.

After overnight incubation it was again centrifuged at 4000rpm for 10 minutes. The supernatant was discarded and 1ml of 70% ethanol was added to the

pellets. The mixture was vortexed for 1 minute and centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded and pellet was allowed to air dry. The pellets contain DNA finally dissolved in 50 µl of TE-Buffer and preserved at 4°C.

##### ***Extraction and isolation of DNA from handkerchief:***

The handkerchief recovered from crime scene, suspected for sweat was allowed to air dry at room temperature. To isolate DNA from handkerchief, 1cm<sup>2</sup> suspected area was removed and cut into fine pieces. The piece of handkerchief was soaked in 0.9% saline solution for 10 minutes. The extract was taken into ependroff tube, 1ml of 1.5% of SDS solution was added and it incubated at 37°C for 1 minute. The mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant (0.5ml) was taken into another clean tube; with an addition of equal volume of 95% ethanol and it allow to incubate at 4°C for overnight.

After overnight the material was centrifuged at 12,000 rpm for 10 minutes, the supernatant was discarded and 650 µl of ethanol was added to the pellets and then centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded and pellet was allowed to air dry. The pellet containing DNA was dissolved in 50 µl TE buffer and stored at 4°C.

##### ***Extraction and isolation of DNA from hair:***

Hair sample collected from fist of victim was taken out with the help of forcep. The posterior region (0.5cm) of hair, contain hair root were cut and macerated in 0.5 ml of aqueous CTAB buffer with the help of pestle mortar. After maceration it was transferred into ependroff tube and then incubated at 65°C for one hour. The contents centrifuged at 10,000rpm for 5 minutes, 0.5 ml of supernatant was transferred into another tube containing 0.5 ml of Iso-amyl alcohol and again centrifuged at 10,000 rpm for 5 minutes. The supernatant was transferred into another tube and an equal amount of ice cold Isopropanol was added to it. The mixture was incubated at 4°C for overnight. The overnight material was centrifuged at 10,000rpm for 10 minutes. The supernatant was discarded and the pellets were allowed to air dry. The pellets containing DNA was dissolved in 1 TE -Buffer and preserved at 4°C.

##### ***Extraction and isolation of DNA from buccal cell taken from five suspects:***

The cotton swabs were soaked in 0.9% saline solution for 10 minutes. The extract was collected into ependroff tube and centrifuged at 6,000rpm for 5 minute. The supernatant was discarded and 1 ml of lysis buffer (10mM tris HCl, 10mM EDTA, and 2%

SDS) was added to the pellet. The mixture was vortexed for 1 minute and then incubated at 65°C for 10 minutes. Poured 500 µl of 5 M NaCl to the solution mixed gently for 1 minute and then centrifuged at 4,000rpm for 10 minutes. The supernatant (500µl) was transferred into clean ependroff tube and 1ml of 95% cold ethanol was added to it. Mixed it well and incubated it at 4°C for overnight.

After overnight incubation it was again centrifuged at 4000rpm for 10 minutes. The supernatant was discarded and 1ml of 70% ethanol was added to the pellets. The mixture was vortexed for 1 minute and centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded and pellet was allowed to air dry. The pellets contain DNA finally dissolved in 50 µl of TE-Buffer and preserved at 4°C.

**Amplification of VNTR region (Apo B) sequence by PCR:**

For amplification, PCR was done using universal APoB-VNTR primers (5'ATGGAAACGGAGAAATTATG3' and 3'CCTTCTCACTTGGCAAATAC5'). Each DNA amplification reaction used a total reaction volume of 35.013 µl (genomic DNA with TE-Buffer=2 µl,10X PCR Buffer=4µl,25mM MgCl<sub>2</sub>=1µl,2.5 mM dNTP = 2µl, 10pm/ µl 20 strand forward primer =2µl,10pm/ µl ,20 strand reverse primer =2µl, 1µg/ml Taq = 0.013 µl, Nuclease free water =22µl. ) The reactions were done in a Labnet DNA thermal cycler (model: Multigene II) and involved pre-denaturation for 5 min at 95°C, Denaturation for 1 min at 95°C,Annealing for 1 min at 55°C ,Amplification for 2 min at 72°C and proof reading for 5 min at 72°C. The number of cycle used for the purpose was 32.

**Agarose Gel Electrophoresis:**

The amplification products were analyzed by electrophoresis using 0.8% agarose gels in TE-Buffer (4 hrs. at 100 V) and detected by Ethidium Bromide staining. The fragment patterns of individuals were analyzed on the basis of presence of bands at particular distance.

**Results and Discussion:**

Samples were collected from crime exhibits i.e. cigarette butt (for saliva), handkerchief (for sweat) and hair from the fist of victim. For forensic identification of DNA the exhibits of the samples were extracted and isolated properly with unique labeling A1(from saliva), A2(from Sweat) ,A3 (from Hair)along with five control sample (buccal swab) from suspects S1,S2,S3,S4, and S5 respectively.

A speedy method of aprecisely typing a VNTR on human APoB gene was performed. The amplification products were directly visualized by staining on

agarose gel electrophoresis. The method described here enables one to distinguish alleles that are indistinguishable by traditional Southern blot analysis. PCR required microgram of genomic DNA, which were lesser of magnitude than typing by Southern blot analysis. PCR was successfully performed on traced DNA on exhibits had accurately typed the APoB VNTR from their amplification products, which would have been impossible with Southern blot analysis. The method was simplified with a greater accuracy.

After examination of the bands under UV transilluminator it was found that The VNTR band pattern of A1 matched with that of S2, band pattern of A2 matched with that of S5, the band pattern of A3 matched with that of S1 while the band pattern of S3 and S4 did not matched with the band pattern of A1, A2, or A3.

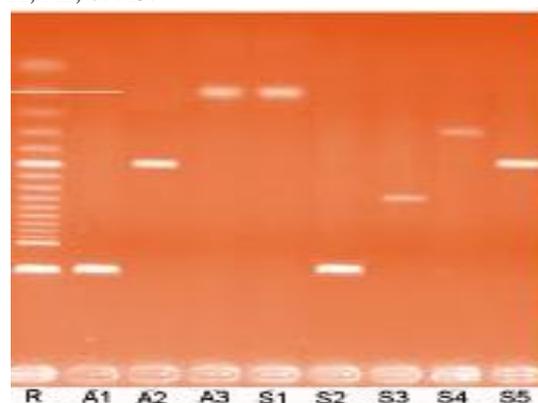


Fig.1 Band pattern of suspect and evidence samples

**Conclusion:**

APoB is a universal marker found in almost every individual but with different base pair length .In the present study APoB marker was used to solve a murder case which gave a trustworthy result. From this study it was concluded that APoB locus can be used in forensic science for identification of criminals in almost every cases.

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