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Original articles

Suitability of PCR methods for forensic investigation

Analysis of the 3′apoB VNTR system in an Italian population sample

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Summary. The PCR method has been applied to amplify a Variable Number Tandem Repeat (VNTR) sequence located at the 3′ end of the apolipoprotein B (ApoB) gene. The study was conducted on an Italian population sample and in a 3-generation family of 13 members, whose relationships were previously established using conventional blood systems. The allele frequencies found were compared with those reported in the literature. The results also confirmed the Mendelian inheritance of the alleles and the suitability of the PCR method for forensic purposes.

Key words: DNA polymorphism – VNTR – PCR – Paternity testing

Zusammenfassung. Die PCR Methode wurde angewendet, um eine VNTR-Sequenz zu amplifizieren, welche am 3. Ende des Apolipoprotein B-Gens lokalisiert ist. Die Studie wurde an einer italienischen Populationsstichprobe durchgeführt und in einer 3-Generationen-Familie mit 13 Mitgliedern, deren Verwandtschaftsverhältnisse vorher durch konventionelle Blutgruppensysteme abgesichert wer. Die gefundenen Allelfrequenzen wurden mit jenen aus der Literatur verglichen. Die Resultate bestätigen die Mendel'sche Vererbung der Allele und die Anwendbarkeit der PCR-Methode für forensische Zwecke.

Schlüsselwörter: DNA-Polymorphismus – VNTR – PCR – Vaterschaftstests

Introduction

Non-coding human DNA has been found to contain numerous repeated sequences of short nucleotide fragments, defined as variable number tandem repeats (VNTR) (Nakamura et al. 1987). Unlike Jeffreys' multilocus minisatellites (Jeffreys et al. 1985), which are scattered throughout the genome, VNTRs are present only at a single locus.

VNTR polymorphism is generally high (Gasparini et al. 1990; Pascali et al. 1990), since there is an extremely variable number of repetitions of the basic sequence; the number of alleles at each VNTR locus is in fact an expression of this variability. Allele transmission according to genetic principles and the high polymorphism of the system justify the interest in VNTRs in forensic haemogenetics, especially in paternity testing and in individual identification (Baird et al. 1990).

Most of the information on VNTRs so far acquired has been drawn from Southern blot analysis and subsequent detection using isotopic methods (Southern 1975). The polymerase chain reaction (PCR) system (Saiki et al. 1985) has recently been introduced into VNTR analysis. It permits the in vitro amplification of the target sequence using two oligonucleotide primers corresponding to the flanking region of interest, a suitable buffer, a mixture of dNTPs and a heat-stable polymerase.

The VNTR systems studied so far are numerous, but those whose flanking region sequence is currently known include ApoB (Boerwinkle et al. 1989; Ludwig et al. 1989), MCT118 (Nakamura et al. 1988; Kasai et al. 1990; Budowle et al. 1990), YNZ22 (Horn et al. 1989), COL2A1 (Wu et al. 1990).

The ApoB VNTR locus is located next to the 3′ end of the apolipoprotein B gene and consists of a series of tandemly repeated short A+T-rich DNA sequences exhibiting a high number of alleles: 12 have been identified in a sample of 125 individuals (Boerwinkle et al. 1989) and 14 in a sample of 318 individuals (Ludwig et al. 1989).

In the present study the PCR technique has been applied to the ApoB system to study population sample of 107 unrelated healthy individuals living in Ancona (Central Italy) and a large family. The aim was to assess the allelic frequencies of the system in Italians, to confirm the Mendelian inheritance and, finally, to test the overall suitability of the method for medico-legal purposes.

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Materials and methods

The study was conducted on a population sample consisting of 107 blood donors living in Ancona (Central Italy) and in a 3-generation family of 13 members, whose relationships were previously established using the common tests for blood grouping (red blood cell allotypes, red blood cell enzymes and plasma proteins).

DNA Extraction was performed by the phenol-isoamyl alcohol method following the protocol developed at the FBI Academy, Quantico. (Budowle and Baechtel 1990).

Amplification was carried out in a DNA Amplifier (Violet) with 30 cycles at a denaturing temperature of 94°C for 1 min, annealing and extension at 58°C for 6 min, according to Boerwinkle et al. (1989). The reaction mix was made up as recommended by Perkin Elmer Cetus (USA) with minor modifications in a final volume of 50 µl. The amount of DNA sample used ranged between 0.5 µg and 0.2 µg.

The primer sequences were those suggested by Boerwinkle et al. (1989):

5′-ATGGAAACGGAGAAATTATG-3′
5′-CCTTCTCACTTGGCAAATAC-3′,

corresponding to the flanking region of the target sequence located on the 3′ side of the ApoB gene.

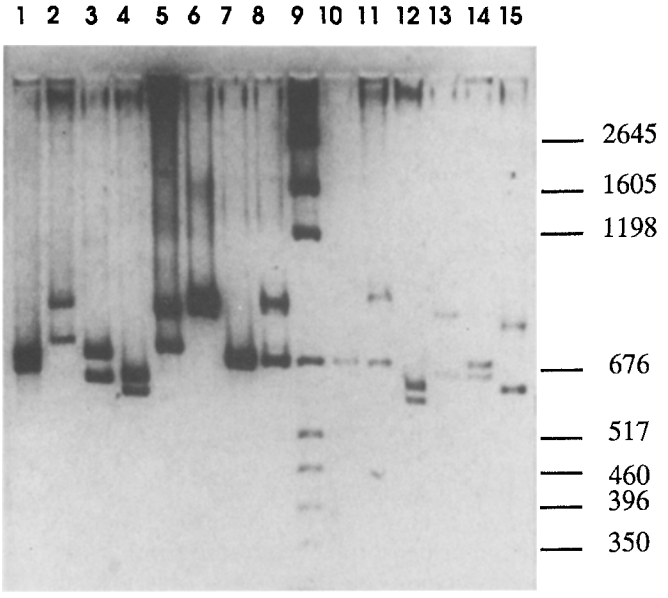

Fig. 1. Amplification products of the hypervariable region on the 3′ side of the ApoB gene after 3% agarose gel electrophoresis for 18 h at 70 v. The digitalized image has been processed to enhance the faint bands. Lane 1: 35/39; lane 2: 41/49; lane 3: 33/39; lane 4: 31/33; lane 5: 39/47; lane 6: 47/47; lane 7: 37/37; lane 8: 37/49; lane 9: pGEM marker; lane 10: 37/37; lane 11: 37/51; lane 12: 29/31; lane 13: 33/45; lane 14: 33/35; lane 15: 31/43. The size (bp) is indicated on the right

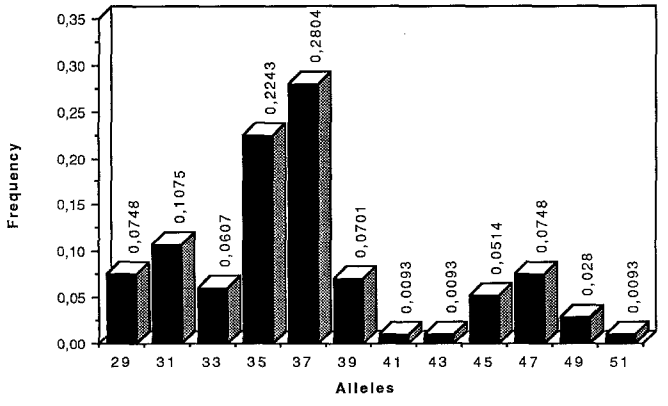

Fig. 2. Frequency distribution of the apoB alleles in a population sample of 107 unrelated adults living in Central Italy. Alleles are defined according to Boerwinkle (1989)

Table 1. Genotypes observed

Genotypes	*N*	%
35–37	13	12.14
37–37	9	8.40
35–35	8	7.46
31–37	7	6.53
37–47	5	4.67
29–35	4	3.72
29–37	4	3.72
31–31	3	2.80
31–35	3	2.80
33–37	3	2.80
35–45	3	2.80
35–47	3	2.80
37–39	3	2.80
29–33	2	1.87
29–39	2	1.87
31–47	2	1.87
33–35	2	1.87
35–39	2	1.87
37–45	2	1.87
37–49	2	1.87
37–51	2	1.87
39–39	2	1.87
39–47	2	1.87
29–29	1	0.94
29–31	1	0.94
29–47	1	0.94
31–33	1	0.94
31–43	1	0.94
31–45	1	0.94
31–49	1	0.94
33–33	1	0.94
33–39	1	0.94
33–45	1	0.94
33–49	1	0.94
35–41	1	0.94
35–49	1	0.94
37–43	1	0.94
39–45	1	0.94
41–49	1	0.94
45–45	1	0.94
45–47	1	0.94
47–47	1	0.94
Total	107	100.00

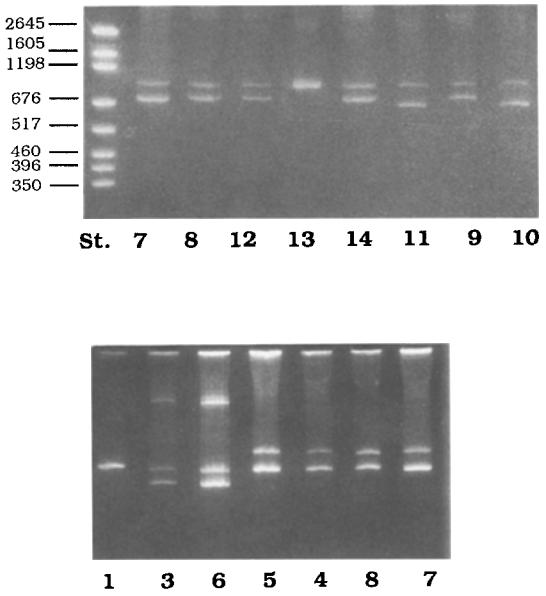

Fig. 3. Patterns from the 13 members of one family after 5% polyacrylamide gel electrophoresis for 45′ at 120 v. The Pedigree is shown on the right. Number 2 represents a member whose blood was unavailable

Electrophoresis of 10 µl of the amplified product with 2 µl loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol in water) was performed on 3% agarose gels (18 h at 50 V) and on 5% polyacrylamide gels (45 min at 120 V) in TBE buffer with direct visualization of the amplification products with ethidium bromide under UV light at 300 nm (Fig. 1). PGEM (Promega) was used as marker.

The gel image was recorded by a video camera and computerised (Macintosh IIx) using a digitizing card (Color Capture). Band mobility was measured in pixel units after image processing and enhancement (Optilab software).

Results and discussion

Twelve different bands (alleles) ranging in size from ≈ 570 bp to ≈ 900 bp have been observed in our study. These alleles correspond to those detected by Boerwinkle et al. (1989) in Caucasians of French ancestry and the same nomenclature has been used.

The estimation of the fragment size has been computed by applying the local reciprocal method of Elder and Southern (1983) to the digitized and enhanced image. The band positions were determined by fitting a polynomial curve to the profile of the marker.

The allele frequency shows a multimodal distribution (Fig. 2). The comparison of our data with those drawn from the histograms given by Ludwig and Boerwinkle using the chi-square test shows no significant differences ($P > 0.05$) from Ludwig's data, whereas significant differences ($P < 0.05$) have been found from the data of Boerwinkle, essentially due to a greater occurrence of smaller alleles in our study and a lower frequency of the 690 bp allele (indicated by Boerwinkle as 37 and by Ludwig as 36).

We have obtained a heterozygosity index of 0.76, very similar to that found by Boerwinkle (0.75) and slightly lower than that reported by Ludwig (0.78). Genotypes observed are shown in Table 1.

The number of homozygotes observed here is not significantly greater than expected under the assumption of Hardy-Weinberg equilibrium ($0.30 > P > 0.50$). In the family study each band scored in a sibling was also present either in the maternal or paternal pattern (Fig. 3). The alleles were regularly transmitted through all 3 generations, confirming the Mendelian inheritance of the alleles in the Apo B system. No mutations were observed.

The digitalized image system used enables the mobility of the fragments to be calculated accurately, the patterns to be stored and handled easily, and the faint bands can be enhanced, thus facilitating their identification.

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