APO B POLYMORPHISM IN THE DISTRICT OF BOLOGNA (ITALY)

S.Pelotti*, P. Degli Esposti°, G. Pappalardo*

*Institute of Legal Medicine University of Bologna, Italy
°Institute of Anthropology University of Bologna, Italy

INTRODUCTION

VNTRs are high polymorphic segments, called micro and minisatellites and due to the degree of allelic variability and the Mendelian inheritance they are useful markers in forensic application. Their polymorphism is attribuited to allelic variation in the number of tandemly repeated DNA sequences. One such hypervariable region is APOB 3' HVR, located 75 bp downstream from the second polyadenilation signal at the 3' end of human APOB, wich maps to human chromosome 2 (Knott et al 1986). The polymorphism is due to the repeat of two structurally related sequences "x" and "y" of 15 bp in lengh (Boerwinkle et al. 1989, Ludwig et al 1989).
Initially the polymorphism was studied by Southern blotting with APOB gene probes, recently it is detected by PCR with flankig primers to amplify HVR sequences, and the products were separated according to size by electrophoresis on agarose gels (Boerwinkle et al 1989, Deka et al 1992) or on denaturing and non denaturing polyacrilamide gels (Ludwig et al 1989, Rand et al 1992).
On the basis of different criterion to evaluate repeat elements, Boerwinkle (1989) proposed a nomenclature at odd alleles (29-51) corresponding to even alleles (30-52) of Ludwig et al (1989), who also observed two alleles with an odd number of repeats: 25 and 35. Recently, in a personal communication to Deka (1992), Boerwinkle , sequencing PCR amplified alleles, has modified his first terminology.
Really, HVR alleles differing in length may not represent the total allelic polymorphism of this locus because of sequence microheterogeneity undetected by means of electrophoresis.

MATERIALS AND METHODS

DNA was extracted from blood of 200 blood donors unrelated, residents in the district of Bologna. DNA was isolated using the fast protocol Genomix (Kontron) .
30 ng of genomic DNA were taken for PCR.DNA amplification was performed using the primers described by Boerwinkle et al (1989) : 5' ATGGAAACGGAGAAATTATG-3' and 5' CCTTCTCACTTGGCAAATAC-3'. Genomic DNA was amplified in a total volume of 50 ul containing 10mM Tris-HCl pH 8,8,1,5 mM MgCl2, 50mM KCl, 0,1% Triton X-100 ,100uM each dNTP, 0,2 uM each primer, 2,5 U Taq DNA-polymerase (Promega).Amplification was carried out using the thermal cycler Perkin Elmer 480 with the following condition: denaturation 1' at 95°, annealing and extension 6' at 58°. A total of 26 cycles was carried out. The hot start tecnique was employed before the first thermal cycle.
Gel electrophoresis of amplified DNA fragments was performed using 2% agarose gel or horizontal polyacrilamide gel, 750 um thick, 6% T, 3% C with PDA as cross-linker (Budowle et al 1991). For page a discontinuous buffer system was used (Allen et al 1989) and the separation distance was 18 cm. Sample were applied to the gel surface via a silicone applicator mask (Serva, Heidelberg). Separation was carried out at 500 V and stopped after 1h 30'. DNA bands were visualized by silver stain (Bio Rad) and phenotyping was obtained by comparison with the 100 bp ladder (Pharmacia) and with an internal ladder of some alleles. The gel image was recorded by a video camera and the analysis was performed by UVP's GelBase software using UVP's System 5000.

RESULTS AND DISCUSSION

All DNA samples could be typed for APO B 3' HVR locus. In the present study we have applied to the alleles the nomenclature of Boerwinkle (1989) . According to Chakraborty R. (1992), the sample size of this study may be adequate for estimates of allele and genotype frequencies. The alleles show large differences in size, ranging from 27 to 51 repeats. In table 1 allele designation and frequencies are reported together with the index of heterozigosity . 14 different alleles were observed , 4 of which occuring at a frequency greater than 0.10. To this regard the distribution of alleles shows three peaks at repeats 33,35,37 and another peak at repeat 47. This confirms the bimodal pattern of allele frequency distribution at this locus (Deka et al 1992) (Fig.1). The total sample meets H-W expectations for APO B 3' HVR locus. Any class with less than 4 observations was pooled.

Non denaturing page makes a good separation of the alleles without disturb the migration of the DNA fragments and of the molecular weight marker. Depending on the percentage of T, some first bands of less molecular weight of the marker may be lost, but the resolution is better than agarose gel electrophoresis. This may be important for a correct typing especially when a complete allelic ladder is not available.

Table 1. APO B 3' HVR genotypes

Genotype	observed	(expected)	frequency
27-35	1	(0.27)	0.005
29-29	1	(0.08)	0.005
29-33	1	(1.28)	0.005
29-35	2	(2.16)	0.01
29-37	1	(1.62)	0.005
29-41	1	(0.04)	0.005
29-47	1	(0.90)	0.005
31-31	3	(1.62)	0.015
31-33	4	(5.76)	0.02
31-35	13	(9.72)	0.065
31-37	6	(7.29)	0.03
31-41	1	(0.18)	0.05
31-47	6	(4.05)	0.03
33-33	8	(5.12)	0.04
33-35	19	(17.28)	0.095
33-37	13	(12.96)	0.065
33-39	2	(1.44)	0.01
33-45	4	(2.24)	0.02
33-47	3	(7.20)	0.015
33-49	2	(3.36)	0.01
34-35	3	(1.89)	0.015
34-47	3	(0.78)	0.015
34-49	1	(0.37)	0.005
35-35	10	(14.58)	0.05
35-37	23	(21.87)	0.115
35-39	3	(2.43)	0.015
35-45	4	(3.78)	0.02
35-47	15	(12.15)	0.075
35-49	5	(5.67)	0.025
37-37	10	(8.20)	0.05
37-39	3	(1.82)	0.015
37-45	1	(2.83)	0.005
37-47	7	(9.11)	0.035
37-49	5	(4.25)	0.025
37-51	2	(0.60)	0.01
39-47	1	(1.01)	0.005
43-47	1	(0.11)	0.005
45-47	2	(2.83)	0.01
45-49	3	(0.73)	0.015
47-47	2	(2.53)	0.01
47-49	1	(2.36)	0.005
47-51	1	(0.33)	0.005
49-49	2	(0.55)	0.01

Allele frequency

Allele	Frequency
27	0.0025
29	0.02
31	0.09
33	0.16
34	0.0175
35	0.27
37	0.2025
39	0.0225
41	0.005
43	0.0025
45	0.035
47	0.1125
49	0.0525
51	0.0075

Heterozigosity (n=164) = 0.82

Hardy Weinberg equilibrium (chi square 10.61, df =9, $0.20 < P < 0.30$)

Advances in Forensic Haemogenetics 5 (c) Springer-Verlag Berlin Heidelberg

REFERENCES

Allen RC,Graves G, Budowle B (1989) Polymerase chain reaction amplification products separated on rehydratable polyacrilamide gels and stained with silver. Biotechniques 7:736-744
Boerwinkle E, Xiong W, Fourest E, Chan L (1989) Rapid typing of tandemly repeated hypervariable loci by the polymerase chain reaction: application to the apolipoprotein B 3' hypervariable region. Proc Natl Acad Sci USA 86: 212-216
Chakraborty R (1992) Sample size requirements for addressing the population genetic issue of forensic use of DNA typing. Hum Bio 64, 2: 141-159
Deka R, Chakraborty R, DeCroo S, Rothhammer F Barton SA, Ferrel RE (1992) Characteristics of polymorphism at a VNTR locus 3' to the apolipoprotein B gene in five human populations. Am J Hum Genet 51: 1325-1333
Ludwig EH, Friedl W, McCharty BJ (1989) High resolution analysis of a hypervariable region in the human apolipoprotein B gene. Am J Hum Genet 45: 458-464
Rand S, Puers C, Skowasch K, Wiegand P, Budowle B, Brinkmann B (1992) Population genetics and forensic efficiency data of 4 AMPFLP's . Int J Leg Med 104:329-333

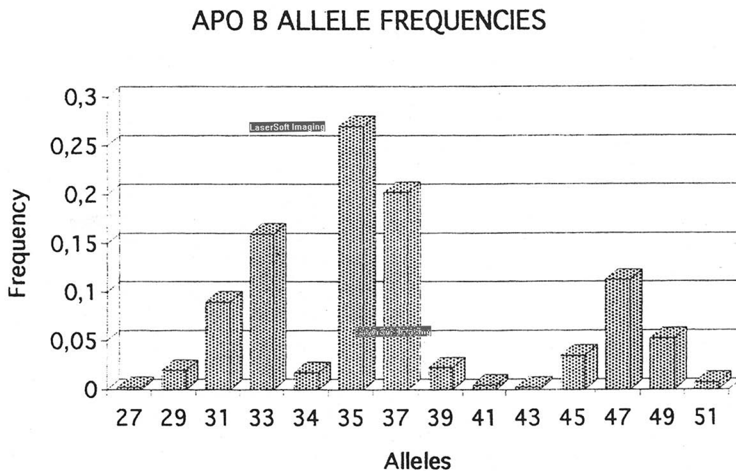

Fig. 1