Gene frequencies of APOB alleles in a sample of random Italian individuals (Central and Southern Italy)

E.d'Aloja, M.Dobosz, M.Pescarmona, A.Moscetti, and V.L.Pascali

Immunohematology laboratory, Institute of Forensic Medicine, Catholic University, Rome, Italy

INTRODUCTION

The region situated downstream of the apolipoprotein B exon contains a hypervariable sequence (APO B HVR, APO B) organized in tandem repeat units. Its polymorphism was described by Boherwinkle et al (1989) using the polymerase chain reaction technique (PCR). These authors reported the existence of 12 allelomorphic fragments in Caucasians with a heterozygosity index amounting to 0.75. Since then, APOB has become a widespread marker, frequently used in forensics.
In view of the application of this polymorphism to our routine casework, we have undertaken a population survey on the distribution of its alleles in the population of Central and Southern Italy. Here we report data referring to a first share of typed samples (109 unrelated individuals) from our reference population.

MATERIALS AND METHODS

Genomic DNA was obtained by phenol-chloroform extraction. Samples of 50 to 100 Ng were amplified by a standard PCR procedure, using a couple of flanking oligonucleotide primers (Boerwinkle et al, 1989) (final ratio 0.1 fM w/v). Amplification conditions were: denaturation: 94°C, 60 sec; annealing: 58°C, 60 sec; extension: 59°C, 300 sec (overall 30 cycles; with a 600 sec extension in the final cycle). Amplified products were separated by agarose (NuSieve, FMC, 2.5% w/v) submarine electrophoresis (80 V, 6h). Phenotypes were classified with the help of a commercial MW standard ladder (123 bp, BRL). In cases of controversial allele classification, a home - made ladder formed by admixture of the commonest allele fragments was used as reference.

RESULTS AND DISCUSSION

In Table I the distribution of observed and expected APOB phenotypes is shown. APOB allele frequencies are sketched in a bar histogram in Fig.1.

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Individual alleles apparently originated from iteration (29 to 47 repeat) of the expected 14-16 bp long repeat unit. Eleven common alleles and 28 phenotypes were identified. We found a favorable distribution of fragments sizes in the system, which helped to achieve a safe amplification of the alleles and minimized the risks of false homozygosity. Mistyping was also avoided by the use of the self-established marker of common APOB types. As a consequence, observed and expected types were consistent with the assumption of population equilibrium (chisquare = 19.61, 11 df, $0.10 > p > 0.05$, compliance with the Hardy Weinberg rule).
The average heterozygosity of the system amounted to 0.74. APOB polymorphism is now used in our laboratory as a routine marker for solving dubious paternity cases. A trial is also under way for the introduction of this system in the analysis of criminal cases.

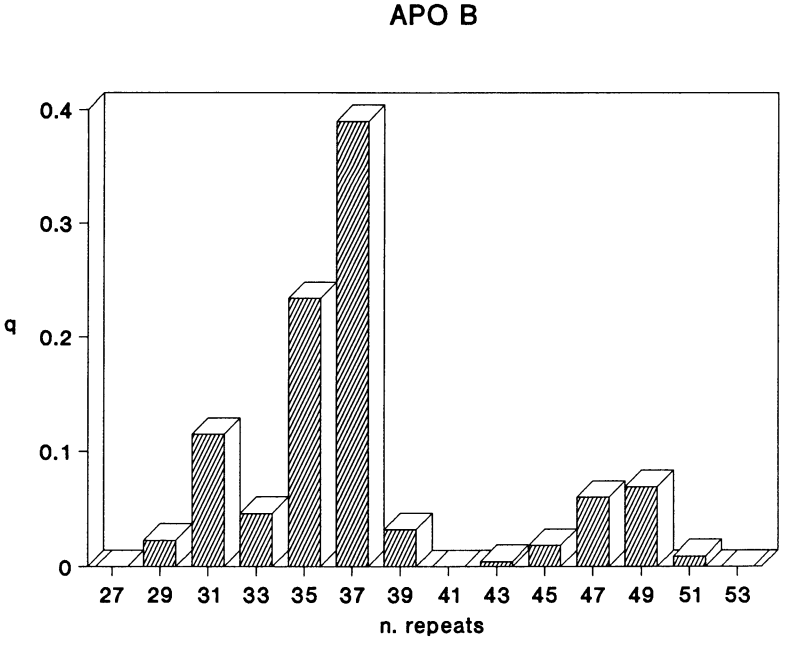

Fig. 1. APO B gene frequencies in 109 unrelated Italians

Table 1. Observed and expected APO B phenotypes

TYPE	OBS	EXP	%
31-31	4	1.43	3.67
35-35	6	5.97	5.50
37-37	18	16.57	16.51
29-47	1	0.30	0.92
31-43	1	0.11	0.92
31-47	1	1.49	0.92
31-49	1	1.72	0.92
33-49	2	0.69	1.83
35-29	3	1.17	2.75
35-31	1	5.85	0.92
35-33	3	2.34	2.75
35-39	2	1.64	1.83
35-45	1	0.94	0.92
35-47	4	3.04	3.67
35-49	7	3.51	6.42
35-51	1	0.47	0.92
39-31	2	0.80	1.83
39-33	1	0.32	0.92
37-29	1	1.95	0.92
37-31	11	9.75	10.09
37-33	4	3.90	3.67
37-35	17	19.89	15.60
37-39	2	2.73	1.83
37-45	2	1.56	1.83
37-47	6	5.07	5.50
37-49	5	5.85	4.59
37-51	1	0.78	0.92
45-47	1	0.24	0.92
sum	109	100.06	100.00

REFERENCES

Boerwinkle E, Xijong W, Fourest E, Chan L (1989) Rapid typing of tandemly repeated hypervariable loci by the Polymerse chain reaction: Application to the apolipoprotein B 3'hypervariable region. Proc Natl Acad Sci USA 86:212-216