STUDY OF THE APO B POLYMORPHISM IN TUSCANY (ITALY)

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INTRODUCTION

The APO B VNTR locus is located next to the 3' end of the apolipoprotein B gene and consists of a series of AT rich tandem repeats, each 14-16 bp long (for a review about the genetic relationship between the 3'-VNTR and diallelic apolipoprotein B gene polymorphism, see Renges *et al*, 1992; for a recent proposal of a molecular model, see Desmarais *et al* 1993). Its polymorphism was described by Boerwinkle *et al* (1989) and by Ludwig *et al* (1989) using the PCR technique. Since then, the APO B has become a genetic marker quite frequently used in forensic practice.
In this study we report the distribution of the APO B polymorphism in a population sample from Tuscany.

MATERIALS AND METHODS

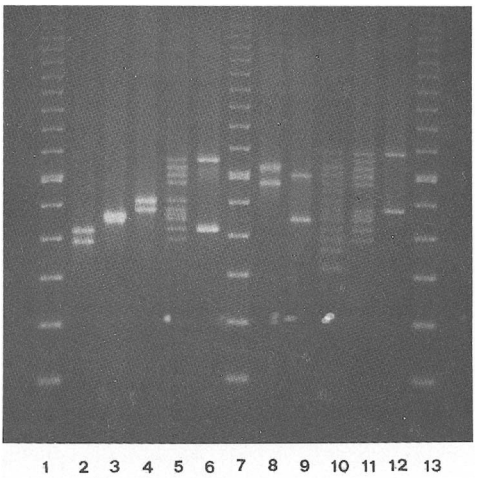
The study was conducted on a population sample of 100 unrelated healthy individuals born in Tuscany (Italy).
DNA extraction was performed by the phenol-isoamyl alchool method following the protocol developed at the FBI Academy, Quantico (Budowle *et al*, 1990).
Genomic DNA samples were amplified according to the method described by Boerwinkle *et al* (1989) with minor modifications. The electrophoretical separation of the amplified fragments was carried out in agarose gel (2% v/w). A home-made allelic ladder was used for classifying phenotypes (fig 1).
Hardy Weinberg equilibrium test was conducted by using the proportion of heterozygous individuals, as well by using a four allele model as suggested by Skowasch *et al* (1992). The allele pools were: group 1 = APOB*31-33, group 2 = APOB*35, group 3 = APOB*36-37, group 4 = APOB*39-51.
In order to test for the heterogeneity between population samples, the G^2 statistics was used, as described by Piazza *et al* (1989); in this case also alleles were pooled in four groups.

RESULTS AND DISCUSSION

In table 1, the distribution of observed and expected APO B phenotypes and of allele frequencies is shown. Eleven different alleles (ranging in size from $\cong$ 600 bp to $\cong$ 900 bp) and twenty seven phenotypes were identified. The allelic designation follows the nomenclature originally suggested by Boerwinkle *et al* (1989).
No significant deviation from Hardy-Weinberg equilibrium could be found in our population sample, both by heterozygosity test (observed 74, expected 77.3, χ^2 = .605, df =1; P = .30-.50) and by four allele model (χ^2 = 4.28, df 6, P = .70-.50).
The allele frequency distribution appears to be bimodal, as in nearly all described Caucasian and not Caucasian populations [see: Schnee-Griese *et al* (1991), Deka *et al* (1992), Renges *et al* (1992), Desmarais *et al* (1993), März *et al* (1993), Hixson *et al* (1993)]. One mode is around APOB*35 and 39 alleles, and a less frequent mode is observed at APOB*47 and 49 alleles (see fig 2).
The heterogeneity test between our gene frequencies and those previously reported by Giorgetti *et al* (1991), d'Aloja *et al* (1992) and Novelli *et al* (1992) for Continental Italy was at the boundary (G^2 = 19.853, df = 9, P $\cong$ 0.02). The favourable phenotype frequency distribution of the APO B system

Type	Observed	Expected	Allele frequencies
31-33	1	0.85	APOB*31 = 0.065
31-35	5	3.77	
31-36	1	0.13	APOB*33 = 0.060
31-37	6	4.49	
33-33	1	0.42	APOB*35 = 0.285
33-35	3	3.77	
33-37	3	4.49	APOB*36 = 0.010
33-49	3	0.85	
35-35	10	8.41	APOB*37 = 0.355
35-36	1	0.58	
35-37	17	20.01	APOB*39 = 0.070
35-39	4	4.06	
35-43	1	1.16	APOB*41 = 0.000
35-45	2	0.57	
35-47	1	2.90	APOB*43 = 0.020
35-49	2	3.71	
35-51	1	0.58	APOB*45 = 0.010
37-37	12	11.90	
37-39	9	4.83	APOB*47 = 0.050
37-43	2	1.38	
37-47	6	3.45	APOB*49 = 0.065
37-49	3	4.49	
37-51	1	0.69	APOB*51 = 0.010
39-49	1	0.91	
43-47	1	0.20	
47-47	1	0.25	
49-49	2	0.42	
Total	100	89.45	

Table 1: phenotypes distribution and allele frequencies in the population sample from Tuscany



Lanes

1,7,13= 100 bp ladder
2= 31-33
3= 35-36
4= 37-39
5= allelic ladder (31-33-35-36-37-39-43-45-47-49)
6= 33-49
8= 43-47
9= 35-45
10= allelic ladder (25-29-31-33-35-36-37-39-41-43-45-47-49-51)
11= allelic ladder (31-33-35-36-37-43-45-47-49-51)
12= 37-51

Figure 1: APO B phenotypes

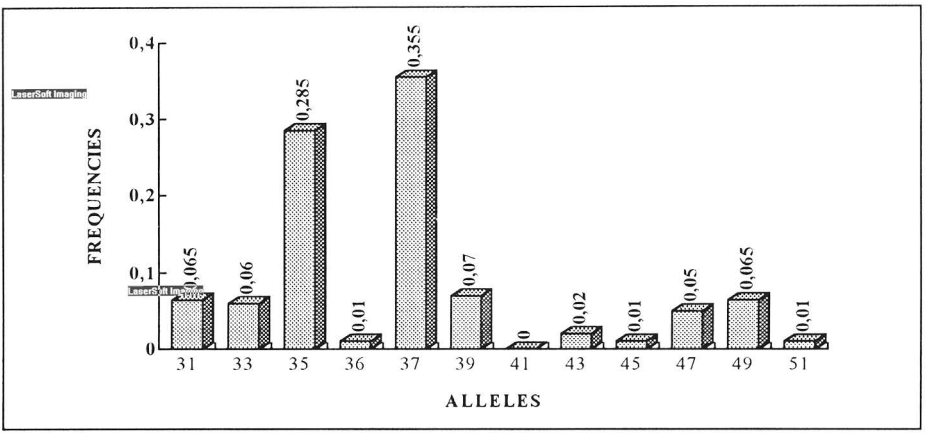


Figure 2: bimodal distribution of APO B allele frequencies

confirms its usefulness in forensic medicine. However, further studies, using larger sample sizes, would needed to provide more accurate estimation of rare allele frequencies.
Although some authors prefer typing by denaturing polyacrylamide gel electrophoresis, we found that agarose gel electrophoresis provided a clear identification of DNA fragments differing by only one repeat unit (14-16 bp).

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