Polymorphism of the 3'-ApoB VNTR system in a population sample from the province of Parma.

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INTRODUCTION

DNA polymorphism (Wyman and White, 1980; Jeffreys et al., 1985) analysis has been introduced into routine casework in many forensic haemogenetics laboratories. PCR amplification (Saiki et al., 1985) of target DNA has been shown to be especially useful in typing small or partially degraded specimens.
AMP-FLP's (3'-ApoB, YNZ22, D1S80, Col2A1) (Boerwinkle et al., 1989; Ludwig et al., 1989; Kasai et al., 1990; Horn et al., 1989; Wu et al., 1990) are among the most reliable polymorphisms studied so far, as Mendelian inheritance and balanced allelic distribution have been established in many population studies.
3'-ApoB polymorphism lies on the short arm of chromosome 2 at the 3' end of the Apolipoprotein B gene and consists of tandemly repeated, 15-16 bp long, AT rich sequences (Knott et al., 1986).
A high number of alleles has been observed in previous population studies; a maximum number of 14 alleles has been discovered in sample of 318 individuals from northern Europe (Ludwig et al., op cit), whilst other population studies have shown a number of alleles ranging from 11 to 13 (Giorgetti et al., 1991; Rand et. al., 1992, d'Aloja et al., 1992).
The 3'-ApoB polymorphic system has been analysed on a population sample from the province of Parma (Northern Italy) to obtain database information necessary for practical applications to criminal and paternity caseworks.

MATERIALS AND METHODS

115 blood samples were obtained from healthy blood donors living in the province of Parma and stored at -80 °C until use. DNA was extracted following the protocol developed at the FBI Academy, Quantico (Budowle and Baechtel, 1990). Amplification was carried out in a thermal cycler PTC 100-60 (MJR, Watertown, MA USA) for 29 cycles, at the following conditions: denaturation at 94 °C 1 min, annealing at 58 °C 1 min, extension at 58 °C 5 min, followed by a final extension time of 7 minutes.
The primers used were those proposed by Boerwinkle et al. (op cit): 5'-ATGGAAACGGAGAAATTATG-3', 5'-CCTTCTCACTTGGCAAATAC-3'.
The amplification mix contained 30 ng DNA, 2U Taq DNA-polymerase (Promega, Madison, WI USA), 5 μl buffer (500mM KCl, 100mM Tris-HCl, 15mM MgCl, 1% Triton X-100), 1 mM each primer, 200mM each dNTP up to a total volume of 50 μl.
10 μl of amplified product were separated by electrophoresis in 2,5% agarose gel (NuSieve 3:1 Agarose, FMC, Rockland, ME USA) in 1X TAE buffer at 70V for 16h. After ethidium-bromide staining, bands were identified by comparison with molecular size standards (123 bp ladder, pGem, ϕX174), applying the local reciprocal method of Elder and Southern (1983).
Alleles were pooled into four groups (Brenner and Morris, 1990; Rand et al., op cit) in order to obtain suitable numbers for χ^2 calculation under Hardy-Weinberg law; the heterozigosity rate, allelic diversity, and discriminating power (P_D)were also calculated.

RESULTS AND DISCUSSION

Ten alleles were observed in the population sample studied, ranging in size from about 600 to 900 bp (Fig.1) and differing from each other mostly by multiples of a repeat unit of about 30 bp. A further allele was observed, which differed from contiguous alleles (35 and 37) only by 15 bp (half of the standard repeat unit). The frequency distribution of alleles is shown in Figure 2.

Fig.1. 3'-ApoB alleles after agarose gel electrophoresis and ethidium bromide staining. From left to right: pGem, 49-53, 31-39,33-35,35-43,123 bp, 36-37, 35-37, 33-39, 47-49, pGem.

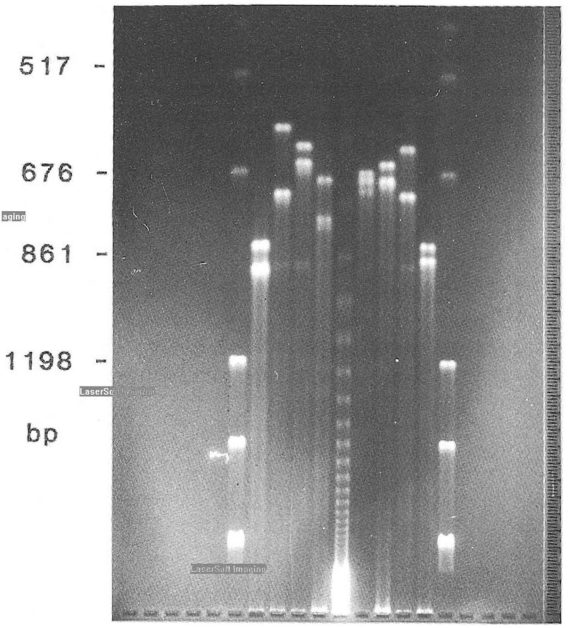
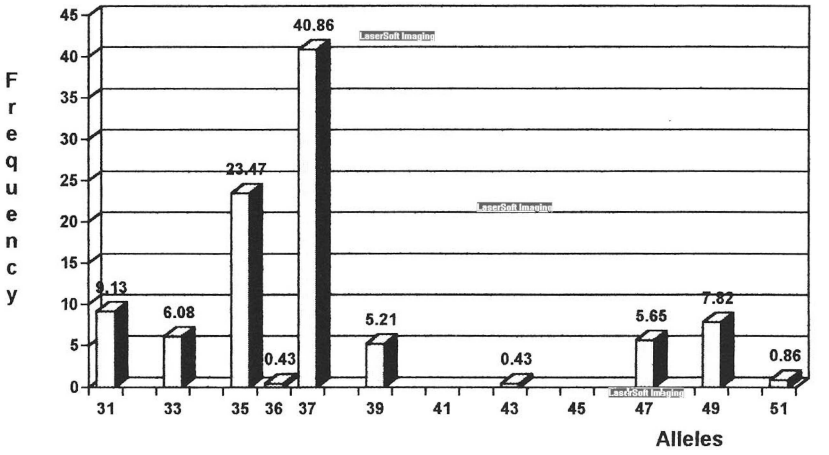


Fig.2. Frequency distribution of 3'ApoB VNTR alleles



Allelic frequency data were found to be similar to those observed in other population studies, both in Italy and in Europe. Allele 36 seems to be a rare variant; a previous observation (Schnee-Griese and Teifel-Greding, 1991) of a similar allele in a larger population sample (n=340) suggested that this mutational event was unlikely to be recent.

The χ^2 test (χ^2=6,9; 0,7<P<0,8; 9 df) showed no significant deviation from Hardy-Weinberg equilibrium. Further evidence of good agreement with Hardy-Weinberg equilibrium was the similarity between the

heterozigosity rate (0,80) and the allelic diversity (0,837±0,0344). On the basis of the allelic frequencies found the (P_D) was calculated as 0,902, and the overall results obtained in the present study show that 3'-ApoB locus is a reliable tool in identification and paternity analysis.

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