

**THE 3' HYPERVARIABLE VNTR-LOCUS APO B:
THREE DIFFERENT ANALYSING METHODS REVEALING DIFFERENT
ALLELES AND LARGE FAMILY STUDIES.**

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SUMMARY

Separation of polymerase chain reaction (PCR) amplification of the Apo B 3'-VNTR was carried out on horizontal, 4%, denaturing polyacrylamide(PAA)/piperazindiacrylamide(PDA)-gels crosslinked with 3% C using a discontinuous buffer system, providing an efficient typing system. Furthermore we compared three different electrophoretic systems and proved that in several samples, alleles showed a completely different migration velocity in agarose- and PAA-gels. Fifteen different alleles containing 14-53 repeats of the basic 15-bp unit were distinguished in a population study of 220 individuals from 38 northwest-portuguese families. For population studies the Hardy-Weinberg-equilibrium was checked by forming groups of phenotypes and no significant deviation could be found. Family studies confirmed the formal conception of an autosomal codominant type of inheritance. Two children of one family showed a new mutation to allele 49 on the condition of their legitimacy.

1. INTRODUCTION

Genotyping hypervariable regions (HVR) is a widely used method for gene mapping as well as for forensic applications and it has become even more important with the implication of HVRs as a cause of genetic and somatic diseases including e.g. the fragile X syndrome, myotonic dystrophy, Huntington's disease and coronary heart disease.

One of these well-known tandemly repeated sequences is the apolipoprotein 3'-hypervariable region, located 73 bp downstream from the second putative polyadenylation signal at the 3'-end of the human Apo B-gene which maps to chromosome 2p24. It consists of a dimeric AT-rich core repeat sequence of 30bp, respectively of two structurally related sequences "x" and "y" with an average length of 15bp (Knott et al. 1986).

Using PCR and agarose gel electrophoresis Boerwinkle et al. (1989) found 12 different alleles, while Ludwig (1989) used the more rapid and high-resolution denaturing polyacrylamide gel electrophoresis (PAGE) to detect 14 different alleles. To date, 24 alleles have been identified, containing between 14 and 55 repeats.

In fact, VNTR-alleles differing in length may not represent the total allelic polymorphism of the Apo B 3'- Minisatellite due to sequence microheterogeneity of the hypervariable elements (HVE) (Heliö 1991; März et al. 1993; Ellsworth et al. 1995).

2. MATERIALS AND METHODS

DNA-Isolation: genomic DNA samples were extracted from peripheral blood leucocytes according to Miller et al. (1988).

PCR: Amplification was carried out for 31 cycles under the following conditions: 3 cycles 94°C-2', 62°C-3' and 28 cycles 95°C-90", 61°C-3'.

The amplification mix contained 2.5 U Taq Polymerase, 200mM of each dNTP, 1.5mM MgCl₂ and 100µl reaction buffer (all Perkin Elmer-Appl. Biosyst. Weiterstadt, Germany), 10 pmol each primer and 25ng genomic DNA up to a total volume of 100µl, layered with 60µl mineral oil.

Molecular weight markers: 100bp-ladder (Gibco BRL) 100-1500bp, Amplisize standard (BioRad) 50-2000bp.

Allelic ladder: a home made, reamplified allelic ladder was used for classifying phenotypes.

Electrophoresis:

- a) 2% agarose gel (NuSieve 3:1 Agarose, Biozym, Oldendorf, Germany) in 1xTBE-buffer at 70V for 16h.
- b) horizontal denaturing PAA-gel (4%T, 3%C) with PDA as crosslinker, using a discontinuous buffer system (Tris-Formiat/Tris-Borat), at constant 1000V and 5W for 1h, 10W for 1h and 15W for 1.5h (190x250x0.5 mm).
- c) horizontal native PAA-gel (4%T, 3%C), on same conditions as b).

Detection:

- agarose gels were stained with ethidiumbromide and the separated products visualized by means of UV-light.

- PAGE was followed by silver staining.

The alleles were typed by comparing the products with an allelic ladder which was constructed from alleles observed during this study and previous studies by R.Pörtl in the same lab.

Family-studies: The phenotypes of the children were pooled into eight groups in order to obtain a suitable number for X^2 calculation under Hardy-Weinberg law.

Population genetics: Alleles, respectively phenotypes of all unrelated individuals of the population sample were pooled into six groups. Heterozygosity rate and allelic diversity were calculated.

3. RESULTS AND DISCUSSION**3.1 Denaturing PAGE**

Fig.1 shows the fragment pattern of family 22. The allelic ladders run in lanes 1 and 7, the 100bp ladder runs in lane 8. We obtained very good resolution with the described gel formulation which yields sharp bands for almost error-free interpretation. Using this method, we were able to easily distinguish 15bp differences in VNTR allele length.

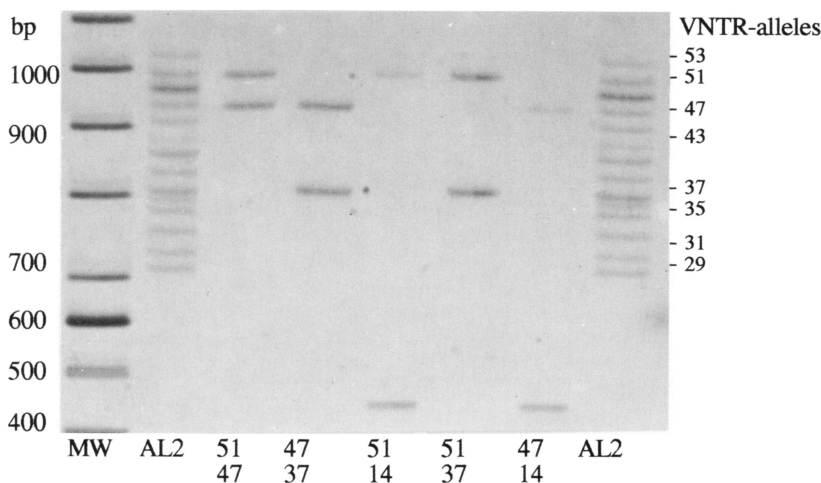


Fig.1 3'- Apo B alleles after denaturing PAGE and silver staining. From left to right, lane: 1= 100bp ladder, 2= allelic ladder 2, 3-5=three children of the family, 6= mother, 7=father, 8=allelic ladder2.

3.2 Comparison of three different analysing methods

It is evident that alleles in certain samples show a completely different migration whether they are separated in agarose or in PAA. An example is given in fig.2. It shows two different allelic ladders separated in agarose and PAA. Both of them seem to be identical in the agarose gel. PAGE reveals a completely different allele composition of both ladders. Exact characterization of the alterations could be obtained by single separation of each sample that contributes to the allelic ladder in both gel types.

One reason for this effect could be the impact of varying secondary structures due to sequence differences within the repeat units. So far unknown correlations between these secondary structures and the both gel types might influence the migration velocity. Moreover, computer analysis of tertiary structures of minisatellites showed varying curvature of repeat sequences, depending on their base composition. This effect may lead to the same observed consequences (Trifonov 1985; Bachmann 1990). In any case it is evident that sequence differences interfere with the base pair number in determining the migration velocity. As this can lead to most profound forensic consequences, agarose gel electrophoreses has to be used with extreme caution in analysing repetitive DNA.

Native and denaturing PAGE produced identical results, except for a more rectilinear migration front and sharper bands in the denaturing system.

