SUITABILITY OF THE ACTBP2 (SE33) STR-POLYMORPHISM FOR LEGAL MEDICINE INVESTIGATION IN THE POPULATION OF CATALONIA (NORTH-EAST SPAIN).

Gené M.; Huguet E.; Corbella J.; Mezquita J.

Forensic Haemogenetics Laboratory. Faculty of Medicine. University of Barcelona. Barcelona. Spain.

INTRODUCTION

The human genome contain DNA segments with variable number of short tandemly repeated sequences (STR). The copy number of such core sequences reveals a highly polymorphism larger than that detected by classical serologic and biochemical genetic markers.

Here we describe the genotype and allele frequency distribution at one locus on chromosome 6, the human beta actin pseudogene H-beta-Ac-psi-2 (ACTBP2), the polymorphic $(AAAG)_n$ repeat begins at base pair 176. The polymorphism is typed using the polymerase chain reaction (PCR). Given that this system has been studied only in few populations the objective of this paper is to present data on the frequencies of ACTBP2 alleles and on the distribution of different genotypes in a population sample from Catalonia (Spain).

MATERIAL AND METHODS

The nomenclature system adopted is based in the one recently proposed by Polymeropoulos et al. (1992).

Samples from 149 unrelated individuals were collected. The study was carried out with blood donors samples from the "Hospital Clínic" Blood Bank, and with samples (blood and hairs) of unrelated paternity cases and university employees.

DNA was extracted using phenol-chloroform-isoamyl alcohol (Auxbel et. al.) or Chelex TM 100 (Walsh et al.).

PCR amplification of ACTBP2 was accomplished by the method and primers described by Polymeropoulos M.H. et al. with slight modifications. PCR reactions were carried out in a 25 μl volume containing 10 to 50 ng of genomic DNA template, 2.5 pmol each oligonucleotide, 200 μM each dGTP, dCTP, and dTTP, 2.5 μM α^{35}S-dATP at 500 Ci/mmol., 50 mM KCl, 10 mM Tris (pH8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, and 0.5 units of Taq polymerase. Samples were processed through 30 temperature cycles consisting of 1 min.24 seg. at 94°C, 2 min. at 57°C, and 2 min. at 72°C. The last elongation step was lengthened to 10 min. The PCR products were resolved using denaturing polyacrilamide-urea gel (40x20x0.04 cm.) electrophoresis, along with a ladder of a cocktail sample. Gels were then fixed, dried, and processed for autoradiography.

RESULTS AND DISCUSSION

Table 1 shows the genotype and the allele frequencies obtained. The study demostrated the presence of 25 alleles, 21 of which are identical to the ones observed by Polymeropoulos M.H., et al. (1992). The additional 4 alleles correspond to: R* (326bp) under the allele 1; 8' (286bp) betwen A8 and A9; 22 (230bp) and 23 (226bp). No alleles of size 322 were found.

There is no significant difference between the observed and expected numbers of heterozygous and homozygous individuals in the sample, indicating no departure from Hardy-Weinberg equilibrium ($0.10 > p < 0.05$) of allele frequencies at the VNTR site (Chakraborty et al. 1991).

Heterocigosity (HI) observed= 92.61%
Power of discrimination (PD)= 99.33%
"A priori" Chance Exclusion (CE)= 88.08%
Essen-Möller value (EM)= 9.01

We have observed an homogeneous distribution of allele frequencies, similar to the one described by Wiegand P. et. al., but we have not established any comparison because the allelic nomenclature is different.

Allele	Freq.	Allele	Freq.
R*	0.003	13	0.018
1	0.003	14	0.044
2	0.010	15	0.054
3	0.013	16	0.060
4	0.027	17	0.080
5	0.064	18	0.094
6	0.084	19	0.070
7	0.074	20	0.040
8	0.064	21	0.040
8'	0.048	22	0.003
9	0.030	23	0.007
10	0.020		
11	0.027		
12	0.023		

Table 1. Allele frequencies.

CONCLUSIONS

The high degree of variability of the ACTBP2 polymorphism makes it extremely useful in forensic haemogenetics.

From the point of view of population genetics it would be advisable to extend the study of this polymorphism to other population groups.

REFERENCES

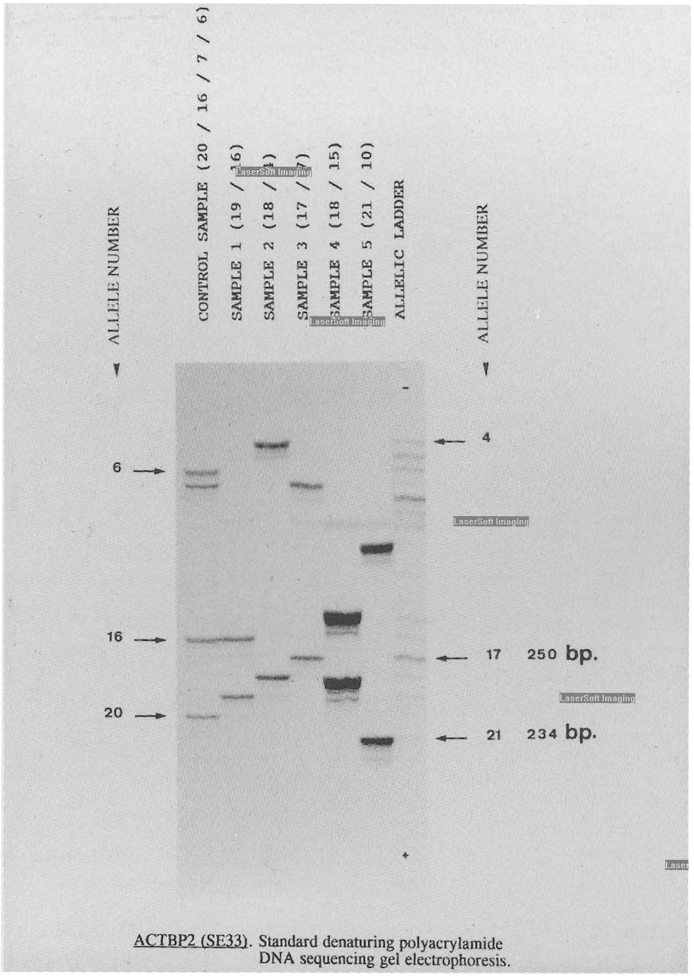
Auxbel FM. et al. (1987/88) Current Protocols in Molecular Biology. New York. Wiley.

Chakraborty R. et al. (1991) Population Genetics of Hipervariable Loci: Analysis of PCR Based VNTR Polymorphism Within a Population. In Burke T. et al. DNA Fingerprinting: Aproaches and Applications. Birkhaüser Verlag. Basel.

Polymeropoulos M.H. et al. (1992) Nucleic Acids Res. 20,6:1432.

Walsh P.S. et al. Biotechniques. (1991); 10:506-513.

Wiegand P. et al. (1993) Int. J. Leg. Med. 105;6:315-320.



ACTBP2 (SE33). Standard denaturing polyacrylamide DNA sequencing gel electrophoresis.

STUDY OF THREE MINISATELLITES (D1S80, YNZ22, 3'ApoB) PERFORMED BY P.C.R. IN THE POPULATION OF CATALONIA (NORTH-EAST SPAIN).

Gené M.*; Huguet E.*; Sánchez-García C.*; Moreno P.**; Corbella J.*; Mezquita J.*

* Faculty of Medicine. University of Barcelona. Spain.
** Faculty of Biology. University of Barcelona. Spain.

The polymerase chain reaction (PCR) method has been applied to amplify three minisatellites: D1S80 (pMCT118), 3'ApoB, and YNZ22 (D17S5), variable number of tandem repeats (VNTRs) on a random population sample from Catalonia.

Loci studied:	D1S80 (n=386)	3'ApoB (n=126)	YNZ22 (n=177)
Allele sizes (bp):	±300-1000	±500-1000	±170-1200

METHODS:

DNA was extracted using phenol-chloroform-isoamyl alcohol or Chelex TM 100.

PCR amplification of D1S80 locus was achieved by the method of Budowle et al. (1991), using the primers described by Kasai et al. (1990). The methods of Boerwinkle E. et al. (1989), and Batanian et al. (1990), with slight modifications, were used for the amplification of 3'ApoB, and YNZ22 loci respectively.

Detection of the different alleles, from PCR amplified products, was carried out by ethidium bromide agarose gel electrophoresis. We tried too, horizontal polyacrilamide gel electrophoresis, but, giving no much better resolution, we used routinately agarose gels.

RESULTS:

Alleles Observed:

D1S80: 26 alleles (25 common and one rare variant) and 82 genotypes.
3'ApoB: 15 alleles (11 common and 4 rare variant) and 35 genotypes.
YNZ22: 14 alleles and 45 genotypes.

Allele frequencies are shown in table 1. The distribution of the genotypes were in Hardy-Weimberg equilibrium for the three loci.

D1S80: Chi square = 16.469;	d.f. = 9;	0.10 > P > 0.05
3'ApoB: Chi square = 5.9368;	d.f. = 5;	0.50 > P > 0.30
YNZ22: Chi square = 10.6869;	d.f. = 6;	0.10 > P > 0.05

	D1S80	3'ApoB	YNZ22
Heterocigosity index (HI):	78.24%	77.70%	81.93%
Power of discrimination (PD):	94.03%	92.36%	95.92%
"A priori" Chance Exclusion (CE):	63.67%	58.86%	69.77%
Essen-Möller value (EM):	9.33	9.47	9.35

D1S80 was examined in 105 paternity testing cases. Of these, 75 cases of practically proved paternity (W≥ 99.73) using conventional markers were confirmed, supporting the assumed autosomal codominant type of inheritance. In the other 30 cases of known exclusion, there are 21 incompatibilities, meaning an exclusion of 70%

3'ApoB was examined in 17 paternity testing cases. Of these, 16 cases of practically proved paternity (W≥ 99.73) using conventional markers were confirmed.

3'Apo B	D1S80		YNZ22
29 = 0	14 = 0	29 = 0.057	1 = 0.065
31 = 0.109	15 = 0.003	30 = 0.008	2 = 0.213
33 = 0.036	16 = 0.001	31 = 0.006	3 = 0.090
35 = 0.198	17 = 0.004	32 = 0.008	4 = 0.271
37 = 0.395	18 = 0.227	33 = 0.003	5 = 0.055
39 = 0.020	19 = 0.001	34 = 0.004	6 = 0.042
41 = 0.008	20 = 0.022	35 = 0.001	7 = 0.016
43 = 0	21 = 0.026	36 = 0.001	8 = 0.035
45 = 0.020	22 = 0.038	37 = 0.003	9 = 0.116
47 = 0.085	23 = 0.017	38 = 0.003	10 = 0.068
49 = 0.097	24 = 0.363	39 = 0	11 = 0.010
51 = 0.024	25 = 0.048	40 = 0.001	12 = 0.013
53 = 0.004	26 = 0.025	R* = 0.006	13 = 0.003
55 = 0	27 = 0.012		14 = 0
57 = 0.004	28 = 0.063		15 = 0.003

Table 1. Allele frequencies.

CONCLUSIONS

The high degree of variability of each PCR polymorphism makes these markers extremely useful in forensic haemogenetics.

In paternity testing the accumulated "a priori" chance exclusion of the three loci is 95.48 %, and the accumulated Essen-Möller value is 8.27

From the point of view of population genetics it would be advisable to extend the study of this polymorphisms to other population groups.

REFERENCES:

Budowle B. et al. (1991) Am. J. Hum. Genet. 48:137-144.

Kasai K. et al. (1990) J.Forensic Sci. 35:1196-1200.

Batanian J.R. et al. (1990) Hum Genet 85:555-559.

Boerwinkle E. et al. (1989) Proc.Natl.Acad.Sci.USA. Vol.86. pp.212-216.