NULL ALLELES DETECTION IN LOCI D1S7, D7S21 AND D12S11 BY PCR

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INTRODUCTION

The assumption of HWE is essential to estimate the genotype frequencies, particularly in systems with a large number of alleles, as it is the case of VNTR loci. HWE can be checked by comparing the observed and expected number of homocygotes and heterozygotes. Some authors claim that VNTR loci show an excess of homocygotes due mainly to population heterogeneity (Lander, 1989), that is, the existence of two or more groups within the population whose individuals show limited intergroup mating and different allele frequencies (Devlin et al., 1990).

Devlin et al (1990) proposed a mathematical test to demonstrate that this excess of homozygotes is not necessarily real, because many heterozygotes with similar allele sizes are misclassified as homozygotes. Similarly, Chakraborty et al. (1992) mathematically demostrate that non-detected alleles can explain this defect of heterozygosity. Small sized alleles, with few repeat units, can run off the gel during the electrophoresis or simple pass undetected due to their characteristic low hybridization efficiency. In this sense, Budowle (1991) demonstrated that single banded profiles were heterozygotes when digested with another restriction enzyme, but up to now, no study on population databases has been performed to reveal these pseudo-homozygotes.

With this aim, based on previous experience of Jeffreys et al. (1988), Armour et al (1989) and Gray and Jeffreys (1991), we propose a method employing PCR amplification (Mullis *et al.,* 1986) of homozygous individuals for loci D1S7, D7S21 and D12S11 for the screening of population databases in search of null-alleles that can explain the possible departure of HWE.

MATERIALS AND METHODS

The population analized consisted of those individuals detected as homozygotes with probes MS1, MS31 or MS43A and restriction enzyme Hinf I in our databases of about 350 individuals for each locus. 16 individuals were analyzed for locus D1S7, 20 for D7S21 and 23 for D12S11.

Reaction mixes

D1S7: 50 mM KCl, 10 mM Tris-HCl pH 9, 0.1% Triton X-100, 0.5 mg/ml BSA, 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.3 μM each primer, and 1.5 U Taq polimerase in 20 μl final Volume.

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D7S21: 50 mM KCl, 10 mM Tris-HCl pH 9, 0.5 mg/ml BSA, 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.15 μM each primer , and 1.5 U Taq polimerase in 20 μl final Volume.

D12S11: 50 mM KCl, 10 mM Tris-HCl pH 9, 0.1% Triton X-100. 0.5 mg/ml BSA, 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.1 μM each primer, and 1.5 U Taq polimerase in 20 μl final Volume.

Ten ng of DNA were amplified for D1S7 and D7S21 whilst 100ng were employed for D12S11.

Primers' sequences

locus D1S7	primer A (24 mer)	5'-GCTTTTCTGTGATGAGCCTTGATG-3'
	primer B (24 mer)	5'-AAGAAGCATATGCAACCCATGAGG-3'
locus D7S21	primer A (24 mer)	5'-CCCTTTGCACGCTGGACGGTGGCG-3'
	primer B (24 mer)	5'-ACACCGTCCCCACACGCCCATCCG-3'
locus D12S11	primer A (23 mer)	5'-CTATACATGTTTACACACATGCC-3'
	primer B (23 mer)	5'-GCGGGAGAAATAGAAATAGAACT-3'

Amplification parameteres

Samples were amplified using a capillary thermocycler (Linus) using the following parameters

	1X		**4X**		**20X**		**1X**	
D1S7	96°C	20"	97°C	20"	95°C	20"	50°C	5"
			58°C	5"	50°C	5"	70°C	5'
			70°C	2'	70°C	2'		
	1X		**5X**		**31X**		**1X**	
D7S21	96°C	20"	97°C	15"	95°C	15"	57°C	5"
			61°C	5"	57°C	5"	70°C	5'
			70°C	1'30"	70°C	1'30"		
	1X		**3X**		**22X**		**1X**	
D12S11	96°C	20"	96°C	20"	94°C	10"	48°C	5"
			50°C	5"	48°C	5"	70°C	5'
			70°C	2'	70°C	2'		

After amplification, 10 μl were loaded in an 1% agarose gel and run in similar conditions as conventional VNTR agarose typing gels, until the 2.3 Kb λHind III fragment reached 10 cm from the origin. After Southern transfer, membranes were hybridized with NICE probes MS1, MS31 and MS43A respectively following the manufacturer´s protocol.

RESULTS AND DISCUSSION

Ten out 16 individuals presented a second short-sized D1S7 allele, non detected previously with restriction enzime Hinf I. Only one individual presented positive amplification of a D7S21 null-allele, and no individual showed amplification of null alleles by PCR for D12S11. In all systems, Hinf I alleles shorter than 4-6 Kb could also be amplified. The high number of pseudohomozygotes observed with D1S7 is in

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concordance with the deviation found previously with only Hinf I alleles, from HWE proportions in favor to a excess of homocygotes, while the systems with no significant deviation from HWE, D7S21 and D12S11 show only one or none null alleles. Colalescence of similar size alleles seems not to be a major cause in deviations from HWE given the grouping of alleles necessary to estimate allele frequencies in these systems. Instead, the lack of signal of short alleles appears to be a problem for the detection of these alleles, particularly with re-hybridized membranes and non-isotopically labeled probes. These results also suggest that care should be taken specially when establishing exclusions of paternity by Landsteiner's second rule with these systems.

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