ALLELIC HETEROGENEITY OF SINGLE LOCUS PROBES IN THE POPULATIONS OF THE BASQUE COUNTRY, SPAIN: APPLICATION IN PATERNITY TESTING

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Both for genetic and anthropological studies, the Basque population is one of the most interesting in Europe. Anthropologically, the Basques are generally regarded as the relict population, the last representative of an early people who were thought to occupy much of western Europe, whose descendents are now found only in the Northern Pyrenes Over the long span of their micro evolution, the Basques have maintained their unique language, structure, and they are distinct from other western Europeans in a number of cultural and social features. Genetically the Basques have maintained the purity of their gene pool. In spite of the successive waves of invasions from the Alpine and Mediterranean races, the Basques have resisted to assimilate any foreign gene pool in the past. However in their recent history there is a greater mixing with the local Non Basque population. Genetic differentiation, increased endogamy and only the recent mixing with Non-Basques suggest that the present day populations of the Basque Country (Basques and Non Basques) represents different genetic subgroups. The present investigation aims to understand the magnitude of the differences of polymorphisms at four variable number of tandem repeat loci among two main subgroups of the Basque country. The effect of the variation in allele frequencies on paternity testing is also evaluated.

Material and Methods

In Spain the Basques live in the provinces of Vizcaya, Guiptazcoa, Alava and Navara. In our recent field investigation in the region of Vizcaya and Guiptazcoa, 10ml EDTA blood samples were collected from individuals with both the grandparents of Basque ancestry. The Non Basque (Spanish) blood samples were also collected from the same region.

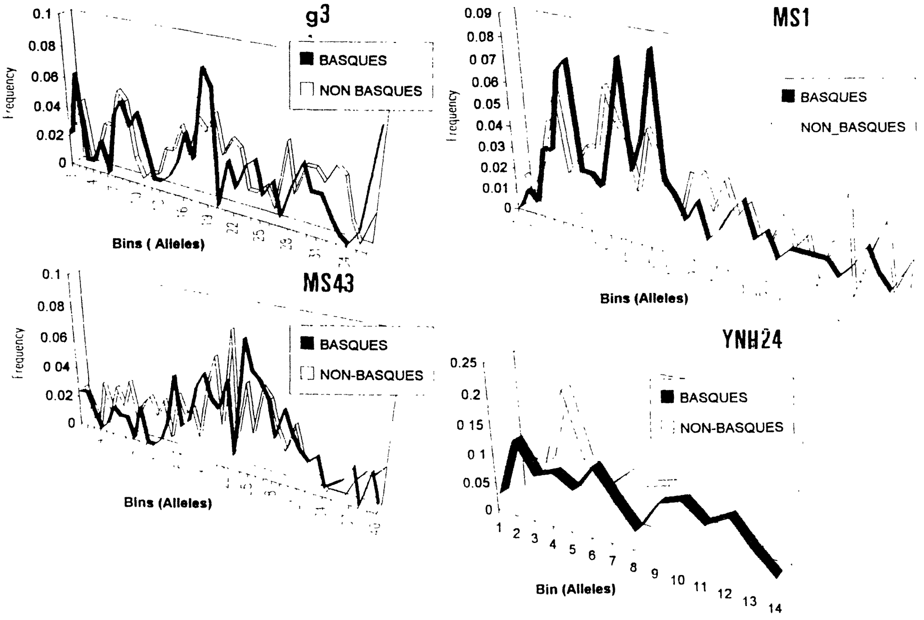
Blood samples were frozen and transported to Newcastle where DNA extraction was carried out. Four VNTR loci were studied, D1S7, D7S22 and D12S11 using g3, MS1 and MS43 probes from Cellmark Diagnostic, and the D2S44 locus was studied using the probe YNH24 from Promega. The DNA samples were digested with Hinf I and analysed by standard technique used for RFLP analysis.

The molecular weight of the DNA fragments was estimated using the molmatch program supplied by UVP. The sizes of separated DNA fragments formed 36 classes of fixed bins for D7S22 and D1S7 loci, 40 and 14 bins for D12S11 and D2S44 loci respectively. The frequencies of these bins were used to calculate the theoretical power of exclusion, probability of match, and the discriminate probability of each loci using the methods of Garber and Morris (1983) and Jones (1972) respectively.

Results and Discussion

The distribution of the allele frequencies detected by the single locus probes g3, MS1, MS43 and YNH24 are given in Figure 1 (a-d). For g3 probe there were three major peaks in Basques at bins 2 (1.6-1.8kb), 18 (6.1-6.4kb), and 19 (6.4-6.7kb), and a greater number of large DNA fragments were found in Basques (>12Kb). In Non Basques several alleles showed similar values. For the MS1 probe, the Basques showed three alleles of high values (bin 7, 3.2-3.5Kb; bin 12, 4.7-5.0Kb; and bin 15, 5.6-5.9Kb) while in the Non Basque population none of the alleles showed such a high frequency and the highest frequency was found for bin 10, 4.1-4.4Kb. The distribution of the MS43 alleles gave a considerable overlap between the Basque and the Non-Basque populations. Both populations showed a greater number of the fragments between 8.5 and 9.1Kb. The YNH24 probe showed considerable variation between these two populations. The Basques show the highest frequency of allele with low molecular weight DNA fragments (Bin 2, 2.5-2.7Kb; f=13%) while the highest frequency of the allele in Non-Basques was 21% for Bin 4, 2.7-2.9Kb.

Figure 1: Distribution pattern of allele frequencies for g3, MS1, MS43 and YNH probes in Basques and Non Basques



The heterozygosity (H) of each locus, the most common allele, the range of DNA fragments found for each locus, power of exclusion of the system, match probability, and the discriminant probablity, are all given in Table 2. The H in the Non-Basques for YNH24 and g3 are the lowest (83% and 85% respectively) perhaps due to the outbred nature of these populations. However the Basques with all four probes and the Non Basques for probes MS43 and MS1 gave the H values near or above 90%. The theoretical exclusion probability is lowest for probe YNH24 (Basques 82% and Non Basques 78%) while for all other probes the PE is above 90%. The cumulative PE is 99.99%. The discriminant probability for these loci ranges from 88-90% and similar values were also observed for the Non-Basque populations.

These results indicate that the distribution patterns of various VNTR loci are considerably different for both the Basque and Non-Basque populations, but all four loci are extremely useful in paternity and forensic testing.

Reference

Garber, B.A. and Morris, J.W. (1983) General equation for average power of exclusion for genetic systems of n codominant allele in one parent and no parent case of disputed parentage. In: Walker, R.H. (ed.) *Inclusion probabilities in parentage testing.* American Association Blood Bank. pp. 277-280.

Jones, D.A. (1972) Blood sample: probability of discrimination. *J.For.Sci.Soc.*, **12**, 355-7.

Table 1: Heterozygosity (H), Frequency (F) of most common allele, power of exclusion (PE), probability of match (PM) of four VNTR probes in Basque and Non-Basque populations

Probe	Population	H %	Range of fragments Kb	Common allele	% F	PE	PM
g3	Basques	89.0	1.5-18.5	6.1-6.4	8.4	0.9084	0.0354
	Non-Basques	85.0	1.5-16.1	2.8-3.0	5.7	0.9196	0.0270
MS1	Basques	96.8	1.7-1.9	5.6-5.9	8.6	0.9050	0.0400
	Non-Basques	98.5	1.3-1.9	4.1-4.4	6.5	0.9266	0.0298
MS43	Basques	87.9	3.0-14.3	7.8-8.0	6.0	0.9145	0.0382
	Non-Basques	89.0	3.0-15.4	8.4-8.6	8.4	0.9235	0.0285
YNH24	Basques	90.5	2.2-7.2	2.5-2.7	13.0	0.8241	0.0860
	Non-Basques	82.9	1.9-5.4	2.7-2.9	21.3	0.7765	0.1114