ANNALS OF HUMAN BIOLOGY, 2002, VOL. 29, NO. 2, 176–191

Taylor & Francis
healthsciences

Microsatellite DNA markers from *HLA* region (*D6S105*, *D6S265* and *TNFa*) in autochthonous Basques from Northern Navarre (Spain)

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Received 23 October 2000; in revised form 23 April 2001; accepted 29 May 2001

Summary. *Background*: The extent of the genetic polymorphism of the *HLA* complex is becoming well characterized in Basque population and their subpopulations. This level of knowledge mainly concerns *HLA* class I loci. However, Basque population surveys dealing with *HLA* class II genes and/or microsatellites in the *HLA* region are still very scarce.
Aim: The population genetics of three highly polymorphic short tandem repeat (STR) loci, *D6S105*, *D6S265* and *TNFa*, from *HLA* region has been analysed in autochthonous (indigenous) Basques from Northern Navarre (Spain). The same blood samples have been typed for *HLA* class II genes from *DQ*/*DR*/*DP* regions and some findings from that information can be found therein.
Subjects and methods: Blood samples were taken from 107 unrelated autochthonous Basques from Northern Navarre. The criterion used to define Northern Navarrese identity was that of three generations of Basque surnames and birthplaces.
Results: The main features observed in Navarrese Basques were the rather high frequencies of alleles *D6S105*4* and *D6S265*7*. A novel allele has been detected at the *D6S265* locus (13: 145 bp). The most frequent haplotype was *D6S105*8-D6S265*4* with a highly significant linkage disequilibrium being presented. The high frequency of allele *TNFa*1* in Basques is noteworthy and this characteristic is not shared by other European populations, where *TNFa*1* is absent or shows negligible values. The multidimensional scaling analysis (MDS) for *TNFa* allele frequencies has shown a high variability among populations and that alleles *TNFa*1* ($F_{ST} = 0.0615$) and *TNFa*12* ($F_{ST} = 0.0424$) seem to have significant influence over the spatial population configuration. *TNFa*2* showed the lowest *FST* value (0.0077) because of its conspicuous homogeneous distribution all over the European populations.
Conclusions: Findings shown here on *HLA* microsatellites and their relationships with other *HLA* class I and class II genes in Basques can be helpful for those studies mainly addressed at detecting associations between *HLA* genes and diseases in the Basque area as a whole, and particularly in its autochthonous population, settled there since remote times.

1. Introduction

The genome of eukaryotic species contains tandemly repeated elements of DNA sequences of various sizes. These monotonously repeated nucleotides are known as minisatellites or VNTRs (variable number tandem repeats) and microsatellites or STRs (short tandem repeats). Microsatellite sequences are distinguished by a different number of repeats (10–50 copies) of a sequence motifs of 2–6 bp, and they usually reach a total length of around 100 bp (Hearne, Ghosh and Todd, 1992).

Because of their high allele polymorphism, STRs are recognized as excellent genetic marker loci and for these reasons they are appropriated, among other applications, for use in population and evolutionary genetics (Zhivotovsky and Feldman 1993) as well as in molecular taxonomy evolution studies (Valdés, Slatkin and Freimer 1993, Bowcock, Ruiz-Linares, Tomfohrde *et al.* 1994), even though the latter approach is

Annals of Human Biology ISSN 0301–4460 print/ISSN 1464–5033 online © 2002 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/0301446011007567 5

under discussion (Coote and Brudford 1996). Microsatellites are also powerful tools in forensic medicine, genetic mapping analysis and linkage disequilibrium with mutations to provoke diseases, which permits population risks to be assessed (Jin, Macaubas, Hallmayer *et al.* 1996, Foissac, Crouau-Roy, Faure *et al.* 1997).

Since the early 1990s there has been a significant increase in the number of human evolution studies based of DNA polymorphisms. In this line, the characterization of microsatellites into major contemporaneous continental human groups, associated with mitochondrial DNA analysis, has been used to reconstruct the evolutionary history of modern humans and to unveil underlying questions about human origins (Vigilant, Stoneking, Harpending *et al.* 1991, Mountain and Cavalli-Sforza 1994). The human major histocompatibility complex (MHC) known as the *HLA* spans around 4000 kb in the short arm of chromosome 6. The main characteristic is its high level of polymorphism, which has been mostly conserved in evolution as a result of biological functions of *HLA* genes (Klein and Takahata 1990, Apanius, Penn, Slev *et al.* 1997). Therefore the *HLA* complex has become recognized as making a major contribution to anthropological studies (Cavalli-Sforza, Menozzi and Piazza 1994, Bodmer, Marsh, Albert *et al.* 1997).

The extent of the genetic polymorphism of the *HLA* complex is becoming well characterized in Basque population and their subpopulations. This level of knowledge mainly concerns *HLA* class I loci (de Mouzon, Ohayon, Ducos *et al.* 1979, García de Masdevall, Ercilla *et al.* 1982, Calderón, Wentzel and Roberts 1993, Martínez-Laso, de Juan, Martínez-Quilez *et al.* 1995, Comas, Calafell, Mateu *et al.* 1998). However, Basque population surveys dealing with *HLA* class II genes and/or microsatellites in the *HLA* region are still very scarce (Esparza, Pestoni, Martin *et al.* 1995, García-Fernández, Arrieta, Rioun *et al.* 1997, Charron 1997, Comas *et al.* 1998).

Microsatellites *D6S105*, *D6S265* and those from locus *TNF* (*TNFa*, *b*, *c*) map together with others in the *HLA* region. *D6S105* (6p22.1–p21.23) is telomeric to locus *HLA-A*, *D6S265* (6p21.3) is between *HLA-A* and *-B* and microsatellite *TNFa* (3.5 kb upstream of *TNF* (gene) is located (6p21.3) between *HLA-B* and complement loci (Thomsen, Alcalay, Barmada *et al.* 1997) (see figure 1). *D6S105* and *D6S265* microsatellite loci contain CA/TG repeats and have been characterized as having at least 13 and 7 alleles, respectively while *TNFa* possesses AC/GT repeats and has a total of 13 observed alleles. The length polymorphism for *D6S105*, *D6S265* and *TNFa* ranges between 139 and 115 bp, 123 and 135 bp, and 97 and 121 bp, respectively. A detailed overview of microsatellites within the *HLA* region can be found in Foissac, Crouau-Roy, Faure *et al.* (1997).

D6S105, *D6265* and *TNFa* microsatellites participate as important components of other MHC allelic associations (Jin, Macaubas, Hallmayer *et al.* 1996) and are involved in disease susceptibilities, for example *D6S265* alleles with haemochromatosis (Camaschella, Roetto, Gasparini *et al.* 1996) and also associations between several *TNF* alleles and multiple sclerosis, insulin-dependent (type I) diabetes mellitus (IDDM) and coeliac disease (Roth, Nogueira, Coppin *et al.* 1994b, García Merino, Alper, Usuku *et al.* 1996, McManus, Maloney, Borton *et al.* 1996) have been detected. These microsatellites are starting to be considered as important genetic markers in human genetic variation studies.

In this investigation we have studied the three dimeric microsatellites *D6S105*, *D6S265* and *TNFa* in autochthonous Basques from Northern Navarre. Allele frequencies, heterozygosity levels and linkage disequilibrium gametic associations are

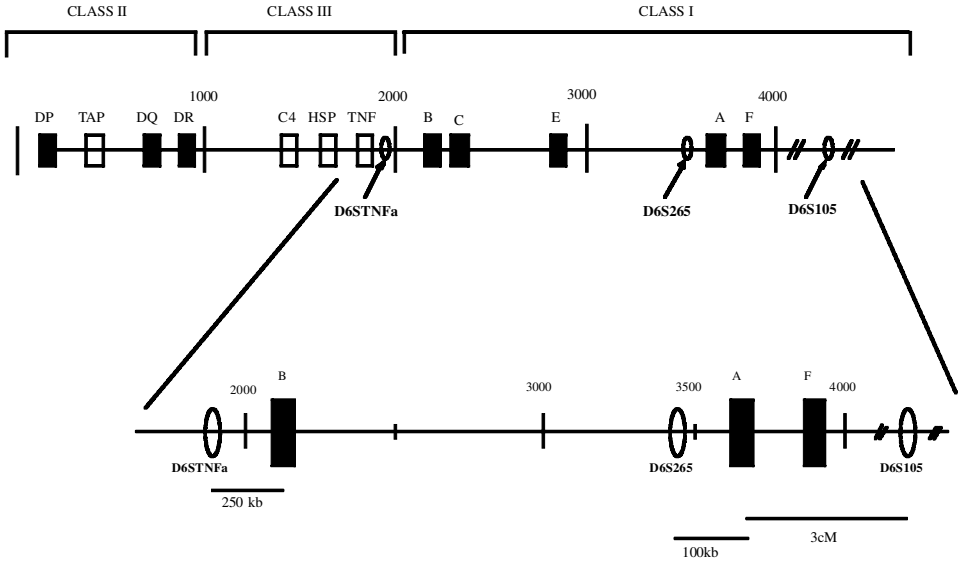

Figure 1. Schematic diagram of the human MHC. The map shows *HLA* loci (black rectangles) and a subset of loci of interest within the *HLA* region. The map positions of the three microsatellite loci analysed are represented as ovals; *D6S105* and *D6S265* are respectively located 3 cM and 100 kb telomeric of *HLA-A* whereas *D6STNFa* is mapped 250 kb centromeric of the *HLA-B* locus.

reported. An analysis of European population relationships based on *TNFa* allele frequencies is also provided. This research has other underlying goals: the same blood samples have been typed for *HLA* class II genes from *DQ*/*DR*/*DP* regions and we are in the process of combining this information with that from *D6S105*, *D6S265* and *TNFa* microsatellite data.

Data shown here represent the first report on the genetic geography for these microsatellite loci in a Basque subpopulation from Spain. Basques from Navarre have traditionally been the most poorly characterized in the Basque area from a genetic and anthropological standpoint, in comparison to the other Basque subpopulations in Spain and France. Recently the genetic position of Navarrese Basques with respect to the other Basque subpopulations of Spain and France has been analysed on the basis of variation pattern of *GM* and *KM* immunoglobulin allotypes (Calderón, Pérez-Miranda, Peña *et al.* 2000).

The analysis of genetic diversity of Basque subpopulations may prove to be an important tool in the study of anthropological populations in order to unveil the main underlying aspects of the evolutionary genetic history of humans.

2. Materials and methods

2.1. Population sampling

Blood samples were taken from unrelated students at *ikastolas* (schools where all lessons are taught in the Basque language) administratively linked to four villages (Elizondo, Bera, Lekaroz and Leitza) distributed throughout the Northern part of Navarre province (see figure 2). Samples were collected between 1996 and 1997 by this research group from the Basque Country University. The criterion used to define Northern Navarrese identity was that of three generations of Basque surnames and birthplaces. Precise information on the geography, history, archaeological sites and

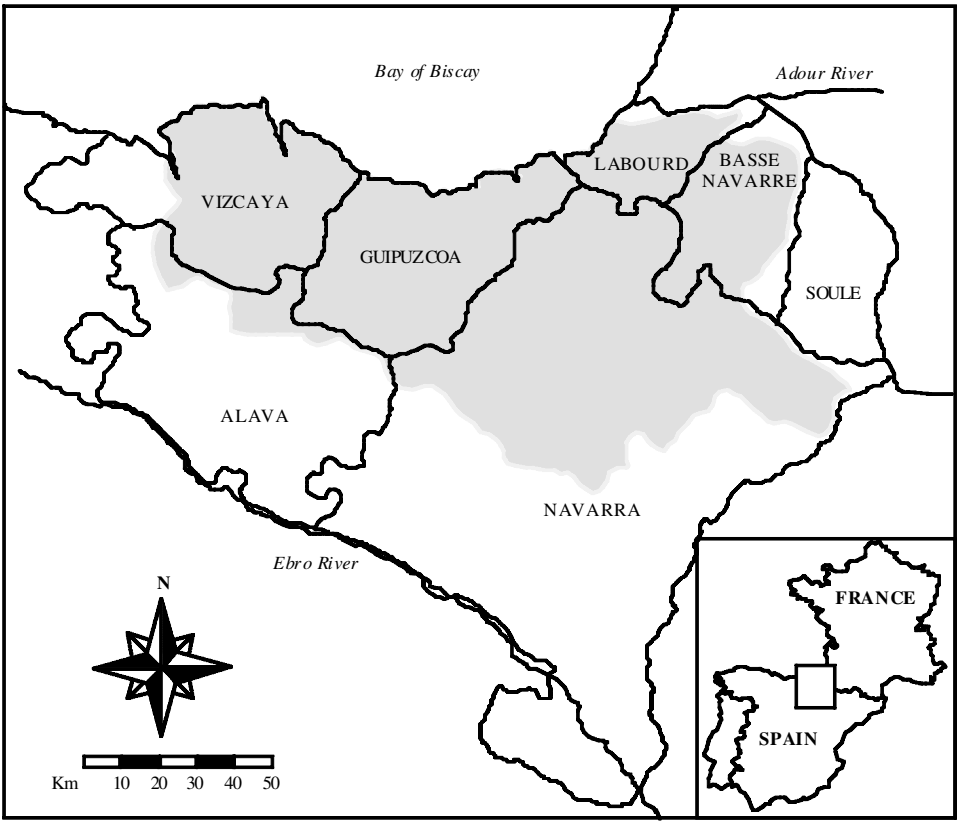

Figure 2. The map of the Basque area showing the linguistic limits of the Basque language or *Euskera* in the 19th century (shaded).

demography of Navarre, together with linguistic particularities, mainly from the Northern Pyrenean Navarrese population, can be found elsewhere (Calderón, Vidales, Peña *et al.* 1998, Calderón *et al.* 2000).

2.2. Genomic DNA extraction, PCR conditions and allele sizes

Blood samples were taken from 107 unrelated autochthonous Basques from Northern Navarre in haemolytic tubes containing EDTA anti-coagulant. Genomic DNA was extracted according to the salting-out method (Miller, Dykes and Polesky 1988). This process was performed at the Laboratory of Physical Anthropology of the Basque Country University (Bilbao, Spain). DNA purity (OD260/OD280 ranges from 1.8 to 2) in each sample was evaluated by spectrophotometry.

The following primers were used to amplify the target DNA: for *D6S105*,

5′-GCCCTATAAAATCCTAATTAAC-3′

and

5′-CCCCTCTTCATCCTCCCTTTCA-3′;

for *D6S265*,

5′-ACGTTCGTACCCATTAACCT-3′

and

5́-ATCGAGGTAAACAGCAGAAA-3́;

and for *TNFa*,

5́-GCCTCTAGATTTCATCCAGCCACA-3́

and 5́-CCTCTCTCCCCTGCAACACACA-3́. A 100 ng sample of genomic DNA was amplified in a total volume of 10 μL containing 10 mM of Tris–HCl, 50 mM of KCl, 1.5 mM of MgCl2, 250 μM of each dNTP, 1 μM of each primer and 0.5 units of *Taq* DNA polymerase. For *D6S105* and *D6S265* initial denaturation was at 94°C for 7 min followed by 23 cycles of 94°C denaturation (1 min), 55°C annealing (1 min), 72°C extension (1 min), with a final extension cycle of 72°C for 10 min. For *TNFa* initial denaturation was at 95°C for 5 min followed by 37 cycles of 95°C denaturation (30 s), 58°C annealing (30 s), 72°C extension (10 s), with a final extension cycle of 72°C for 5 min.

The amplified products were loaded into a 9% polyacrylamide gel with a standard GENESCAN GS-2500 TAMRA marker. Fragment analysis took place in a PE Applied Biosystems 373 automatic sequencer. Allele size was determined using GENESCAN Analysis software. The three microsatellites were typed by personnel from this laboratory at the Unité de Physiopathologie Cellulaire et Moleculaire, CNRS ERS 1590, in Toulouse, France.

2.3. *Statistical and mathematical analysis*

The length variability for alleles of the three study microsatellites was evaluated in a sample ranging from 96 to 107 individuals, depending on the microsatellite locus. Gene frequencies and standard errors (SE) were calculated by the maximum likelihood method.

Guo and Thompson's (1992) exact test procedure and heterozygosity test were used to determine agreement with the Hardy–Weinberg expectations of genotype frequencies. Haplotype frequencies (HF) and allelic association in linkage disequilibrium (LD) were calculated using ARLEQUIN (Schneider, Roessli and Excoffier 2000). The significance of D values was estimated by the chi-square test and the relative linkage disequilibrium (RD) was also calculated.

The polymorphism of a microsatellite depends on variation in number of repeats and its frequency distribution. That polymorphism was quantified by mean estimates of PIC (polymorphism information content) and also from expected heterozygosity, H or gene diversity. The latter parameter proved to be one of the most important measures of genetic variability in a population (Nei 1987).

The PIC value depends on the number of alleles as well as the frequencies registered in a population, and it was estimated by means of the formula displayed below

$$\mathrm{PIC} = 1 - \left(\sum_{i=1}^{n} p_i^2\right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

$$h = 1 - \left(\sum_{i=1}^{n} p_i^2\right)$$

where h is the heterozygosity by locus (H would be the average of that quantity over all loci), n is the number of alleles for each genetic marker and p_i, p_j the frequency of

the *i*th and *j*th alleles in the study population. The standard error for the expected number of heterozygotes, h is

$$\mathrm{SE} = p(1 - -p)/N$$

Microsatellite evolution has been considered under two models of mutation, that so-called infinite-allele model (IAM) proposed by Kimura and Crow (1964) and the charge state or stepwise mutation model (SMM) proposed by Ohta and Kimura (1973), Wehrhann (1975) and Kimura and Ohta (1978). Under a neutral model, the gene diversity of a population at equilibrium between genetic drift and mutation is a simple function of $N_e\nu$ (where N_e is the effective population size and ν is the neutral mutation rate per locus per generation).

On the basis of observed heterozygosity and sample size, we have estimated the expected number of alleles, n_e for each microsatellite locus from formulation under IAM model ($n_e = 1 + 4N_e\nu$) and simulation under SMM model ($n_e = \sqrt{1} + 8N_e\nu$) and compared the results with the observed number of alleles in the study population. Formulations for IAM and SMM mutation models have been taken out from Chakraborty and Weiss (1991) and Shriver, Jin, Chakraborty *et al.* (1993), respectively.

Genetic distances between populations were computed from the R dispersion matrix (Harpending and Jenkins 1973) and a non-metric multidimensional scaling (Lalouel 1980) was constructed from that genetic distance matrix. Recently a new genetic distance $(\delta\mu)^2$ for microsatellites has been devised by Goldstein, Ruiz-Linares, Cavalli-Sforza *et al.* (1995); however traditional genetic distance methods remain valid (Takezaki and Nei 1996). The degree of inter-population genetic differentiation was estimated by F_{ST} parameter (Wright 1951), and is defined by

$$F_{ST} = \frac{\sigma_p^2}{\bar{p}(1-\bar{p})}$$

where σ_p^2 is the variance of gene frequency p among populations, and $\bar{p}$ is the mean allele frequency.

3. Results

Table 1 shows allele frequencies at the *D6S105*, *D6S265* and *TNFa* microsatellites in the Basque subpopulation from Northern Navarre. Sample sizes were 107, 96 and 106, respectively. A total of 10 alleles were identified for *D6S105*, 8 for *D6S265* and 11 for *TNFa*. At the *D6S265* microsatellite two novel alleles (13: 145 bp) and (12: 143 bp) have been observed. The former was detected in three individuals from our analysed Basque sample while the latter has been observed in Basques from France (B. Crouau-Roy, personal communication). Until present they had not been registered in other European populations. However, samples from other geographical surrounded Iberian populations need to be tested, because they have been shown to be close to Basques when *HLA* markers have been used (Martinez-Laso *et al.* 1995, Sanchez-Velasco, Escribano de Diego, Paz-Miguel *et al.* 1998).

Graphic representations of the observed allele size–distribution frequencies for the microsatellite loci studied are displayed in figure 3. For *D6S105* and *D6S265* microsatellites a trimodal pattern seems to be clear. The most common alleles were *D6S105*6* (0.425), *D6S105*8* (0.173) and *D6S105*4* (0.154) for *D6S105* and *D6S265*4* (0.344), *D6S265*7* (0.271) and *D6S265*6* (0.177) for locus *D6S265*. The same pattern has been observed recently by Crouau-Roy (personal communication)

Table 1. Allele frequencies for *D6S105*, *D6S265* and *TNFa* microsatellites in Basques from Navarre.

D6S105 allele[a]	Frequency ± SE	*D6S265* allele[b]	Frequency ± SE	*TNFa* allele[c]	Frequency ± SE
2	0.070 ± 0.018	2	0.109 ± 0.022	1	0.118 ± 0.023
3	0.009 ± 0.006	3	0.005 ± 0.005	2	0.212 ± 0.028
4	0.154 ± 0.025	4	0.344 ± 0.035	4	0.132 ± 0.022
5	0.103 ± 0.019	5	0.063 ± 0.018	5	0.009 ± 0.007
6	0.425 ± 0.033	6	0.177 ± 0.029	6	0.057 ± 0.015
7	0.037 ± 0.012	7	0.271 ± 0.033	7	0.137 ± 0.025
8	0.173 ± 0.027	8	0.016 ± 0.009	8	0.024 ± 0.010
9	0.009 ± 0.006	13	0.016 ± 0.009	9	0.014 ± 0.008
11	0.009 ± 0.006			10	0.052 ± 0.016
12	0.009 ± 0.009			11	0.231 ± 0.029
				13	0.014 ± 0.008

[a] Alleles decrease in size by 2 bp from allele 1 = 139 bp.
[b] Alleles increase in size by 2 bp from allele 1 = 121 bp.
[c] Alleles increase in size by 2 bp from allele 1 = 97 bp.

in French Basques. Worwood, Raha-Chowdhury and Darke (1994) found similar allelic distribution patterns when analysing *D6S105* and *D6S265* microsatellites in blood donors from Wales (UK). In comparative terms, the main differences registered in Northern Basque Navarrese is the relatively high frequencies of alleles *D6S105*4* ($\chi^2 = 3.84$; d.f. = 1; $p < 0.05$) and *D6S265*7* ($\chi^2 = 10.34$; d.f. = 1; $p < 0.01$). For microsatellite *TNFa* the histogram gave a rather irregular distribution.

A large number of microsatellites from the human MHC region present a polymorphism information content (PIC) of around 0.75 (Foissac *et al.* 1997). For *TNFa* microsatellite locus PIC values around 0.85 have been observed (Jongeneel, Briant, Udalova *et al.* 1991, Crouau-Roy, Briant, Bouissou *et al.* 1993). In the Basque population the PIC for *D6S105* and *D6S265* was 0.72 and for *TNFa* was 0.83. The heterozygosity level for *TNFa* was particularly high (0.85), and greater than 0.70 for *D6S105* and *D6S265* loci (see table 2). Nevertheless, Maliarik, Kost, Harrington *et al.* (1995), characterizing several microsatellites located on chromosome 6 in a sample of Afro-Americans, found a heterozygosity value for *D6S105* even higher (87%) than those reported for European populations.

Table 3 includes results of the two tests for Hardy–Weinberg equilibrium. None of the three microsatellites studied showed a significant departure from panmixia, even though certain heterozygote deficiencies were observed.

Allelic associations between *D6S105*, *D6S265* and *TNFa* microsatellite loci were analysed. Table 4 presents the only two point haplotypes whose frequencies were higher than $2/N$ (for N = sample size) and indeed showed positive linkage disequilibrium (LD) values at significant levels ($p \leqslant 0.05$). The most frequently (HF = 0.121) observed haplotype was the *D6S105*8-D6S265*4*, which showed a highly significant linkage disequilibrium ($LD = 0.061; \chi^2 = 14.9, p < 0.01$). In addition, both alleles were associated with *TNFa*1*, with haplotype *D6S105*8-D6S265*4-TNFa*1* being the most commonly registered (HF = 0.091) of those allelic associations involving three locus genotype. Also, strong allelic associations ($p < 0.01$) were detected between *D6S105*4-TNFa*7* and *D6S265*2-TNFa*11*. In a study of *D6S105* and *D6S265* loci in blood donors in Wales, Worwood *et al.* (1994) found five haplotypes in linkage disequilibrium at a significant level, with *D6S105*8-D6S265*4* being the most frequently recorded (HF = 0.165; LD = 0.060, $p < 0.001$). That survey also

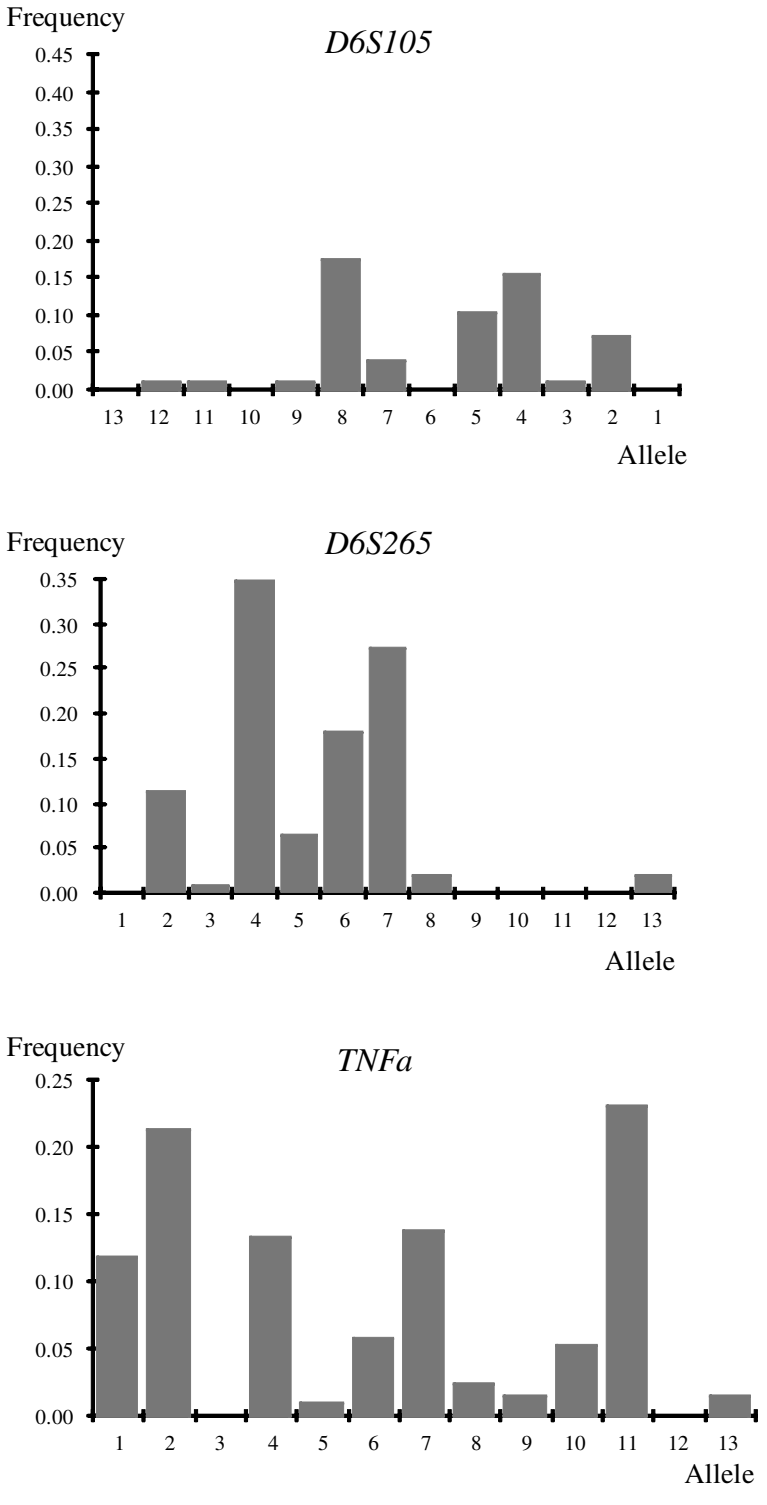

Figure 3. Allele size distributions for each microsatellite loci *D6S105*, *D6S265* and *TNFa* in Basques from Northern Navarre.

Table 2. Heterozygosity and PIC values for *D6S105*, *D6S265* and *TNFa* microsatellites in Basques from Navarre.

Locus	No. of chromosomes	No. of observed alleles	Observed heterozygosity	PIC
D6S105	214	10	0.748	0.719
D6S265	192	8	0.761	0.724
TNFa	212	11	0.845	0.826

Table 3. Tests for Hardy–Weinberg expectations of genotype frequencies.

Locus	*n*	Exact test $p \pm$ SE	Heterozygosity test			
			Observed	Expected	χ^2	*p*
D6S105	107	0.18 ± 0.03	77	80.4	0.58	0.45
D6S265	96	0.61 ± 0.03	67	73.4	2.37	0.12
TNFa	106	0.56 ± 0.03	86	89.9	1.11	0.29

Table 4. Two loci haplotypes showing significant positive *D* values ($p < 0.05$).

Haplotype	HF[a]	LD[b]	Chi-square[c]	RLD[d]
*D6S105*4-D6S265*7*	0.074	0.032	5.2*	0.285
*D6S105*5-D6S265*2*	0.028	0.017	3.9*	0.183
*D6S105*7-D6S265*7*	0.027	0.017	5.2*	0.622
*D6S105*8-D6S265*4*	0.121	0.061	14.9**	0.542
*D6S105*4-TNFa*7*	0.058	0.037	12.2**	0.321
*D6S105*7-TNFa*2*	0.026	0.018	6.1*	0.603
*D6S105*8-TNFa*1*	0.080	0.060	33.1**	0.611
*D6S265*2-TNFa*11*	0.058	0.033	7.9**	0.388
*D6S265*4-TNFa*1*	0.072	0.031	5.3*	0.404
*D6S265*7-TNFa*2*	0.091	0.033	4.2*	0.216

[a] Haplotype frequency.
[b] Linkage disequilibrium values.
[c] Significance of the LD value (*$p < 0.05$, **$p < 0.01$).
[d] Relative linkage disequilibrium values.

showed that 83% of individuals possessing *HLA A1-B8* also had alleles *D6S105*8* and *D6S265*4*. Associations between *TNFa*2* and *HLA* alleles (*A1*, *B8*, *DR3*) has also been observed in a population from West Scotland (Gallagher, Eskdale, Oh *et al.* 1997). The haplotype *A1-B8* is one of the most common *HLA* allelic associations in Europeans, and the Basque population shares this characteristic (Cambon-de Mouzon, Ohayon, Hauptman *et al.* 1982, Martinez-Laso *et al.* 1995, Comas *et al.* 1998).

Table 5 presents the number of observed and those expected alleles under IAM and SMM mutation models. When using the IAM model the number of estimated alleles was higher than predicted from SMM, especially in the case of microsatellite *TNFa*. Parallel analysis carried out by Shriver *et al.* (1993) for *TNFa* closely agrees with our results. *D6S105* was the only to show deviations in the direction of the IAM mutation model.

Population allele frequencies in microsatellites are still very scarce and the available information mostly come from Caucasian populations. Population data on locus *TNFa* are modest but more abundant than those for microsatellites *D6S105*

Table 5. Fit of neutral mutation models to allele frequency data.

Locus	Expected heterozygosity	No. of alleles observed	Expected no. of alleles	
			IAM	SMM*
D6S105	0.752	10	11.2	7.5 (6.0, 9.0)
D6S265	0.765	8	11.5	8.1 (6.1, 10.2)
TNFa	0.849	11	18.2	11.9 (10.4, 13.3)

* The numbers in parentheses represent the 95% confidence limits.

and *D6S265*. This prevents us from working out any global population relationship analysis based on these three markers. Microsatellites at locus *TNF* (*TNFa*, *b*, *c*) were the first STRs from the *HLA* region used in population genetic studies in Europeans. *TNFa* allele frequencies have been analysed in French (FRA), Basques from France (BFR), Greeks (GRE) and Danes (DAN) (Crouau-Roy *et al.* 1993); also in Irish (IRE) (McManus *et al.* 1996), Italians (ITA) (Ciusani, Salmaggi, Pociot *et al.* 1997), Scots (SCT) (Gallagher *et al.* 1997) and Croatians (CRT) (Grubic, Moghaddam, Giphart *et al.* 1999). This data set has been used to evaluate differences in gene diversity patterns in population relationships. With this approach a non-metric multidimensional scaling (MDS) analysis was applied to the Euclidean genetic distance matrix emerging from Harpending's *R* dispersion matrix.

Figure 4 presents a plot of the two first dimensions, which explain 97.5% of the total variation (stress value = 0.070). As shown, populations are grouped into two main clusters. The first cluster contains the two Basque subpopulations (*BFR* and

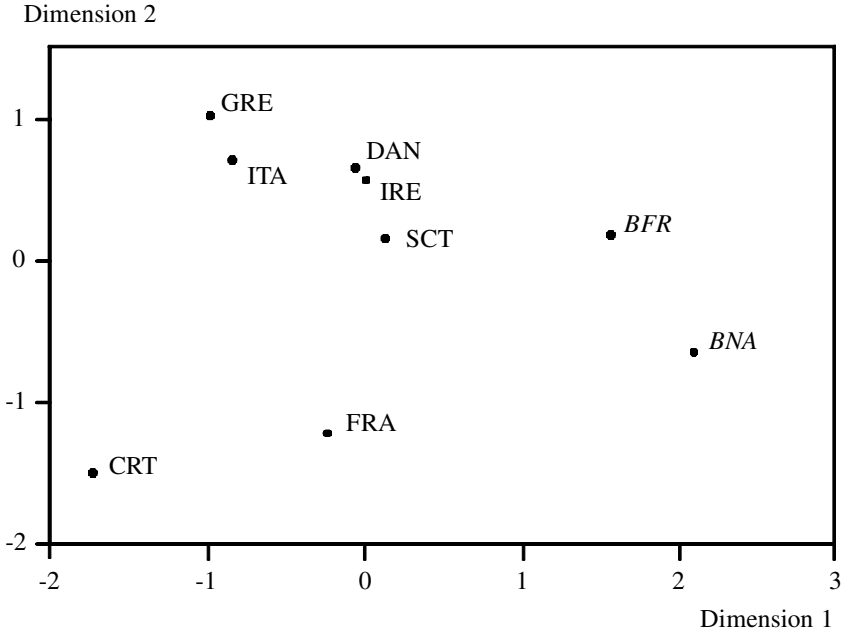

Figure 4. Non-metrical multidimensional scaling (MDS) applied on *R* matrix for *TNFa* allele frequencies registered in nine European populations. The Basque populations (in italic) are: *BNA*, Navarre Basques; *BFR*, French Basques. The other populations are: CRT, Croatians; DAN, Danish; FRA, French; GRE, Greeks; IRE, Irish; ITA, Italians; SCT, Scots.

BNA) and their co-ordinate scores place them significantly apart from the rest of European population groups. In contrast, the Mediterranean (ITA and GRE) and north-western Europeans seem to form a second large, close cluster on the negative side of the first dimension; French and Croatian samples appear isolated in the lower-left quadrant of the diagram.

4. Discussion

Basques have been recognized as a notable and singular worldwide anthropological population (Cavalli-Sforza *et al.* 1994) and they have been thoroughly analysed for almost all traditional genetic polymorphisms. The recent characterization of the Basque population for other classical but highly polymorphic and geographically informative genetic markers (i.e. *GM* immunoglobulin allotypes) has led to establishing the extent of their intra-population genetic differentiation (Calderón *et al.* 1998, 2000) but has also provided a novel view of the evolutionary history of the Basques as a peculiar population within the European genetic map. However, a recent paper by Simoni, Calafell, Pettener *et al.* (2000) on mtDNA did not detect significant differences in most European populations.

Knowledge of geographical diversity patterns of DNA polymorphisms in many human populations around the world is attaining a rapid development and completeness. This research strategy will permit reliable relationships between genes and geography to be identified, and will be helpful in the reconstruction of human evolutionary history. Meanwhile the results we show here represent merely a small step in that direction, and the best achievements are yet to come.

Population genetic studies have repeatedly displayed alleles that are significantly represented in an area in peculiar patterns in relation to other local or continental population groups. An example in the present study would be *TNFa*2*, which seems to be the most frequent allele (20–32%) at the *TNFa* locus in many European populations. Basques from Navarre also place *TNFa*2* by itself as the second most represented type (21%), preceded only by *TNFa*11* (23%). These observations are consistent with the hypothesis that *TNFa*2* may be considered a genetic marker of Caucasians (Roth, Dolbois, Borot *et al.* 1994a).

The high frequency of allele *TNFa*1* in Basques is noteworthy and this characteristic is not shared by other European populations, where *TNFa*1* is absent or shows negligible values. In Basques from Spain (present study) the frequency of *TNFa*1* (0.127) was very similar to that observed (0.116) in French Basques (Crouau-Roy *et al.* 1993). The lowest value of *TNFa*6* is that observed in our study sample, closely followed by that of French Basques.

In this regard it is interesting to point out the particularly strong associations between *TNFa*1* and alleles from *HLA* class I, class II regions such as have been reported in different surveys. *TNFa*1* is associated with the haplotype *HLA B18-DR3*, which is also common in south-western European populations, particularly in Basques and Sardinians (Mouzon *et al.* 1979, Jongeneel *et al.* 1991) and it is also observed in high frequencies in Spaniards (Martinez-Laso *et al.* 1995). This haplotype *B18-DR3* is associated with an increased risk of insulin-dependent (type I) diabetes mellitus (IDDM) (Cambon-de Mouzon *et al.* 1982, Contu, Deschamps, Lestradet *et al.* 1982). Further, recent studies have provided more evidence for these observations. Crouau-Roy, Bouzekri, Carcassi *et al.* (1996), analysing healthy and diabetic Basques and Sardinians, showed that *B18-DR3* was significantly associated with haplotype *TNFa*1-b5-c2*, and indeed this association was the same in both

IDDM and normal individuals. This finding closely agrees with the population genetics *TNF* microsatellite results reported earlier by the same French team. In that line, Moghaddam, de Kniff, Schipper *et al.* (1998) analysed linkage disequilibrium (LD) patterns between *HLA* class I (*HLA-A*, *-B*), class II (*HLA-DR*, *-DQ*) and microsatellite loci within and close to the major histocompatibility complex. The study sample was healthy, unrelated Dutch Caucasoid individuals. Again, the linkage disequilibrium analysis provided strong evidence that only the alleles of the *D6STNFa* locus (not other flanking loci, *D6S273*, *D6S248* and *D6S265*) were in LD with *HLA-B* and *HLA-DR* loci. This fact has been interpreted in terms of a possible functional interdependence (and not an structural relationship) between TNF-(gene and class I and class II alleles in accomplishing their biological functions.

As mentioned above, the present Basque sample has also been typed for *HLA* class II genes at DNA level (R. Calderón, personal communication). *DR* polymorphism data analyses have shown a highly significant linkage disequilibrium between *DRB1*03* and *TNFa*1* ($D = 0.075; \chi^2 = 45.1, p < 0.01$). This observation could be interpreted in terms of high frequencies of both alleles in the population studied. In fact, in Basques from Navarre *DRB1*03* was the third most represented allele (15%), with *DRB1*07* being the most frequent (19%) at the *HLA-DRB1* locus. Unfortunately, frequencies of *HLA* class I antigens are not currently available from Navarrese. However, other *HLA* surveys performed on Spanish Basques or geographically well-defined Basque subpopulations in Spain have evidenced that allele *HLA-B*18* presents frequencies higher than 5% (Calderón *et al.* 1993, Comas *et al.* 1998).

MDS analysis for *TNFa* allele frequencies has shown high variability among populations and it seems be evident that *TNFa*1* and *TNFa*12* alleles have had a significant influence over the spatial population configuration. In fact, both alleles show the highest F_{ST} values, *TNFa*1* ($F_{ST} = 0.0615$) and *TNFa*12* ($F_{ST} = 0.0424$), while *TNFa*2* presented the lowest F_{ST} value (0.0077) as a consequence of its conspicuously homogeneous distribution over all European populations.

The observed mean F_{ST} value for locus *TNFa* was compared with the one calculated for *GM* immunoglobin (Ig) haplotypes (data not shown) in the same group of European populations. The results of this genetic heterogeneity analysis surprisingly showed that the F_{ST} value for *TNFa* microsatellite yielded a figure 7.4 times higher than that estimated for *GM* marker. It is clear that the lack of correspondence between genetic and geographical distances for *TNFa*, as shown in the MDS analysis, could be interpreted as a consequence of studying a single locus, albeit one possessing a high level of allelic polymorphism. However, our results could also suggest that the less extensive population genetic heterogeneity displayed for *GM* allotypes with regard to microsatellites could be attributed to coding genes at the *GM* loci, which would show a more conservative evolution determined by selective pressures. In this context it is remarkable that all three *HLA* microsatellites studied in our Basque Navarrese subpopulation fit well with IAM or SMM mutation models, which could suggest a major effect of genetic drift in shaping spatial distribution of gene frequencies.

In summary, the findings shown here on the population genetics of some *HLA* microsatellites and their relationships with other *HLA* class I and class II genes in Basques from Northern Navarre should not be confined merely to furthering knowledge of their genetic structure characteristics. This approach can also be helpful for

those researchers mainly addressed at detecting associations between *HLA* genes and diseases in the Basque area as a whole, and particularly in its autochthonous population, settled there since remote times.

Acknowledgements

We would like to thank Pilar Giraldo for excellent technical assistance in this study. This work was supported by the Basque Country University (Research Project UPV 154.310-EA082/98) and the Basque Government (Research Project GV PI 95/66).

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Zusammenfassung. Das Ausmaß eines genetischen Polymorphismus des HLA-Komplexes ist in der Baskischen Population und deren Subpopulationen gut charakterisiert worden. Dieser Stand des Wissens betrifft hauptsächlich die HLA Klasse I Loci. Demgegenüber sind die Baskischen Populationsstudien, die sich mit HLA-Genen der Klasse II und/oder Mikrosatelliten in der HLA-Region beschäftigen, sehr selten.

Die Populationsgenetik von 3 hochpolymorphen Short tandem repeat (STR) - Loci (D6S105, D6S265 und TNFa von der HLA-Region) wurde analysiert bei autochthonen (eingeborenen) Basken aus Nord-Navarra (Spanien). Dieselben Blutproben wurden typisiert hinsichtlich der HLA Klasse II Gene aus den DQ/DR/DP-Regionen und weitere Erkenntnisse wurden daraus abgeleitet.

Die Blutproben von 107 nicht verwandten autochthonen Basken von Nord-Navarra wurden genommen. Das Kriterium für die Identifikation, dass es sich um Basken aus Nord-Navarra handelt, waren die Familiennamen und Geburtsorte von 3 Generationen von Basken.

Die hauptsächlichen Merkmale, die bei den Basken aus Navarra beobachtet wurden, waren die ziemlich hohen Allelfrequenzen D6S105*4 und D6S265*7. Ein neuartiges Allel konnte am D6S265 Locus entdeckt werden (13:145 bp). Der häufigste Haplotyp war D6S105*8-D6S265*4 mit einem vorhandenen hochsignifikanten Koppelungsungleichgewicht. Die hohe Frequenz von Allel TNFa*1 bei Basken ist bemerkenswert. Diese Eigenschaft teilen die Basken nicht mit anderen Europäischen Populationen, da TNFa*1 bei ihnen entweder nicht vorhanden ist oder nur unwesentliche Vorkommen aufweist. Die multidimensionale Skalenanalyse (MDS) für TNFa Allelenfrequenzen hat eine hohe Variabilität zwischen den Populationen gezeigt und dass die Allele TNFa*1 ($F_{ST} = 0.0615$) und TNFa*12 ($F_{ST} = 0.0424$) anscheinend einen signifikanten Einfluss auf die räumlichen Populationsstrukturen haben. TNFa*2 zeigte den niedrigsten F_{ST}-Wert (0.0077) wegen seiner auffallend homogenen Verteilung bei allen Europäischen Populationen.

Die Ergebnisse, welche hier an HLA-Mikrosatelliten und deren Beziehungen mit anderen HLA-Genen der Klasse I und II bei Basken gezeigt werden, können hilfreich sein für solche Studien, die hauptsächlich darauf gerichtet sind, Beziehungen zwischen HLA-Genen und Krankheiten in der Baskenregion als

ganzes, aber auch besonders bei seinen antochthonen Gruppen, die hier seit Vorzeiten angesiedelt sind, aufzudecken.

Résumé. *Arrière-plan:* L'étendue de la variabilité du polymorphisme génétique du complexe *HLA* commence à être bien connue pour la population basque et ses sous populations. Ce niveau de connaissance concerne principalement les loci de classe I, mais les enquêtes qui s'intéressent aux loci de classe II ou aux microsatellites, sont encore très rares.
Objectif: La génétique des populations de trois loci très polymorphes en "short tandem repeat" (STR) *D6S105*, *D6S265* et *TNFa* de la région *HLA*, ont été analysés chez des basques autochtones (indigènes) du nord de la Navarre (Espagne). Les mêmes échantillons sanguins ont été typés pour les gènes de classe II des régions *DQ*/*DR*/*DP* et quelques résultats de cette information combinée sont présentés ici.
Sujets et méthodes: les échantillons de sang ont été prélevés chez 107 basques autochtones non apparentés du nord de la Navarre, identifiés sur la base de trois générations de noms et de lieux de naissance basques.
Résultats: Les principales caractéristiques observées sont les fréquences plutôt élevées des allèles *D6S105*4* et *D6S265*7*. Un nouvel allèle a été détecté au locus *D6S265* (13: 145 bp). L'haplotype le plus fréquent est *D6S105*8-D6S265*4* , associé à un très fort déséquilibre de linkage. La haute fréquence de l'allèle *TNFa*1* chez les basques est notable et cette caractéristique n'est pas partagée par les autres populations européennes, chez lesquelles *TNFa*1* est absent ou présente des valeurs négligeables..L'analyse des fréquences alléliques *TNFa* par multidimensional scaling (MDS), indique une haute variation interpopulationnelle et que les allèles *TNFa*1* (F_{ST} = 0,0615) et *TNFa*12* (F_{ST} = 0,0424) paraissent avoir une influence significative sur la configuration spatiale des populations. *TNFa*2* présente les plus basses valeurs de F_{ST} (0,0077) suite à l'évidence de l'homogénéité de sa distribution dans l'ensemble des populations européennes.
Conclusion: Ces résultats obtenus sur les microsatellites *HLA* et leurs relations avec les autres gènes *HLA* de classe I ou II des basques peuvent être utiles aux études qui cherchent à détecter des associations entre gènes *HLA* et maladies dans l'ensemble de la région basque et plus particulièrement dans sa population autochtone, dont l'établissement local est très ancien.