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TNF promoter SNP variation in Amerindians and white-admixed women from Misiones, Argentina

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Summary

The aim of this study is to describe genetic variation in the TNF promoter in the ethnically diverse population of Misiones, north-eastern Argentina. We analysed 210 women including 66 Amerindians of the Mbya-Guarani ethnic group and 144 white-admixed individuals from urban and rural areas of Misiones. Their DNA samples were surveyed for TNF polymorphisms -376 A/G, -308 A/G -244 A/G and -238 A/G by PCR amplification and direct sequencing and for the Amerindian marker -857 C/T by real-time PCR. Our main findings are as follows:(i) a distinctive pattern of Single Nucleotide Polymorphism (SNP) distribution among these groups, (ii) genetic differentiation between the Mbya-Guarani and the white-admixed populations ($P < 0.05$), (iii) lower gene diversity (~0.05) in Mbya-Guarani compared with the white-admixed group (~0.21); and (iv) linkage disequilibrium between the -376A and -238A SNPs in white-admixed populations. These data highlight the principal role of population history in establishing present-day genetic variation at the TNF locus and provide a framework for undertaking ethnographic and disease association studies in Misiones.

Introduction

Tumor necrosis factor (TNF; formerly TNFα) is a key mediator of the inflammatory response and is critical for host defence against a wide variety of pathogenic microbes (Goldfeld & Tsai, 1996). The human TNF gene is located on human chromosome 6 (6p21.3) at the human leucocyte antigen (HLA) region between the Class I HLA-B and the Class II HLA-DR loci (Old, 1985). The analysis of genetic variation in the TNF promoter region has been studied worldwide and revealed the existence of a number of Single Nucleotide Polymorphisms (SNPs), including those located at positions -1031, -863, -857, -376, -308, -244 and -238 from the transcription start point (Uglialoro *et al.*, 1998; Baena *et al.*, 2002).

The analysis of these SNPs in human populations reveals that certain of them show distinct patterns in different ethnic groups (Richardson *et al.*, 2001; Valente *et al.*, 2009a). For example, the -308 SNP, a G-to-A substitution, occurs at a relatively high frequency in European (21%) and African (~12%) groups, but has not been detected in indigenous Amerindian populations. By contrast, the -857 SNP (a C-to-T change) is highly frequent in Amerindians (up to 45%) compared with Africans (2%) and Europeans (12%) (Baena *et al.*, 2002). These differences may be related to the population history of the particular groups being studied and perhaps also to the selective pressures they have experienced in the past.

In this regard, the TNF-308A has been linked to various physiological and pathological conditions, including susceptibility to HPV-induced cervical cancer, leishmaniasis and dengue fever (Cabrera *et al.*, 1995; Perez *et al.*, 2010; Liu *et al.*, 2012; Badano *et al.*, 2012). Interestingly, some of these diseases are highly frequent in Misiones, Argentina. Despite the fact that environmental, socioeconomic and other factors may play a role in the incidence of these diseases, the different genetic backgrounds of Misiones populations may also be important variables that shape disease risk and occurrence.

In a previous paper, we reported an association between TNF-308A and cervical cancer among urban women from Posadas City (Capital of Misiones) (Badano *et al.*, 2012). This work prompted the question about how TNF variation is distributed among other populations of different ethnic background from Misiones.

The Misiones Province is located at the north-eastern tip of Argentina and shares international borders

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with Paraguay and Brazil. Across its geography, the Mbya-Guarani Amerindians are concentrated in small communities inside the rain forest. Until the last decade, the Guarani social units had been self-sustaining, avoiding direct exchanges with the white population of urban areas. However, the loss of ancestral territories has altered dramatically their cultural survival, economic livelihood and food supply and generated an important change in the Guarani–white relationship, compelling male Indians to integrate the rural working force in white running agricultural establishments, where the *colonos* lives (Cebolla Badie, 2000).

The term *colonos* is one usually applied to farmers of immigrant origin. The colonization of the province by European immigrants (Poles, Ukrainians, Germans, Swiss, British, Finns, Swedes, Norwegians) began in the last decades of the nineteenth century, with many of them settling in accordance with their ethnic and national origin in the rural areas of Misiones. In the text, we will refer to them as the Rural population. On the other hand, the peopling of Posadas, the capital of Misiones, was different. Here, a combination of '*mestizos*' (individuals of mixed Native American and European descent), people coming from internal rural migration within the province and the country, and immigrants from neighbouring Republic of Paraguay contributed to the current ethnic make-up of the city (Bartolome, 1990). Moreover, the important influence of Paraguay to the region can be traced through history, mainly during the War of the Triple Alliance (1865–1870), when the territory of Posadas was lost and officially incorporated to Paraguay more than once (Cambas, 1960). We will refer to this group in the text as the urban population. Together, we will group the Rural and Urban populations into the larger white-admixed population.

The complex population dynamics of Misiones has been described from a social, historical and cultural point of view (Bartolomé, 1969; Bartolome, 1990; Cebolla Badie, 2000), but remains largely unknown from a genetic perspective. Only two studies have addressed genetic variation in Mbya-Guarani populations through the analysis of mtDNA, Y-chromosome and autosomal markers (AIMs) (Altuna *et al.*, 2006; Sala *et al.*, 2010). However, none of the white-admixed populations of the province have been similarly analysed or surveyed for immunogenetic markers, such as cytokines. Therefore, the goal of this project was to analyse genetic variation in the TNF promoter among individuals who are representative of Misiones' history and provide a framework for future ethnographic and disease-association studies.

Materials and methods

Population and bioethics

We analysed TNF variation in 210 women from three major populations of Misiones. The first group included 66 Mbya-Guarani Amerindians (age range 15–70, median 28), who are the southernmost representatives of the Tupí-Guarani language group and inhabit the rainforest of Misiones. They largely follow a traditional lifestyle and are not a part of the urban economy. The second group included 144 white-admixed women. It was further subdivided according to the geographic location and the ethnic origin of the participants into two subgroups. The first included 104 Urban women from Posadas (age range 18–83, median 31), the capital of Misiones (27° 21′ 59″ S; 55° 53′ 39″ W), representing European-Amerindian descendants from the Spanish colonization starting in the sixteenth century. The second consisted of 40 Rural women (age range 19–76, median 39) from the village Ruiz de Montoya (26° 58′ 7″ S; 55° 3′ 27″ W), representing European descendants from the immigration process sponsored by the government in the nineteenth century.

Genomic DNA was obtained from epithelial cells of healthy unrelated women from these three populations. Field research was carried out as part of a clinical and epidemiological investigation related to cervical cancer as previously described in Tonon *et al.* (2003) and Badano *et al.* (2011). The Research Ethics Committee of the Madariaga City Hospital approved the study design (Aval #04/04/08), and the University of Pennsylvania IRB approved the analysis of genetic variation in these populations under protocol #803744. All procedures were carried out in accordance with the Helsinki Declaration.

TNF Promoter SNPs

Polymorphisms in the promoter region of the TNF gene were determined through PCR amplification and direct sequencing of 727 bp of the 5′ UTR (position -678 from the transcription start site to position +49 of the gene), as described in Badano *et al.* (2012). This region encompasses four reported SNPs, including -376 A/G, -308 A/G, -244 A/G and -238 A/G. A subset of samples (~72% of the total population) was assayed for the -857 C/T SNP using a Custom TaqMan assay and Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on a ABI 7900HT real-time PCR machine. To test the accuracy of genotyping, we randomly repeated the genotyping of 61 sequences (29.0%) and obtained identical results.

TNF Nomenclature

When discussing TNF variants, the gene name is typically given and followed by a number (nucleotide position of the SNPs from the transcriptional start point) and a letter (allele), for example, TNF-308A. To describe the nucleotide changes at these positions, we indicate the wild-type and then the variant alleles (SNPs), for example, TNF-244G/A. According to the SNP database polymorphism identification numbers

(IDs), TNF-238 (G/A) is rs361525; TNF-308 (G/A) is rs1800629; TNF-376 (G/A) is rs1800750 and TNF-857 (C/T) is rs1799724. Those SNPs are also reported in the literature as -237, -307, -375 and -857, respectively.

Statistical analysis

Allele and genotype frequencies were calculated by direct counting. Their distribution between populations was compared by chi-squared test or two-tailed Fisher exact test. Genotype proportions were tested for Hardy–Weinberg equilibrium using Arlequin 3.11 (Excoffier *et al.*, 2005). The population genetic package DNAsp version 5.00.04 (Rozas, 2009) was used for haplotype construction and estimation of linkage disequilibrium (LD). The haplotype data were used to calculate the haplotypic diversity within populations (H) and deviations from neutrality (Fu & Li, 1993). Pairwise comparisons of population samples were made by computing conventional F-statistics from haplotype frequencies (pairwise F_{ST} statistics), using Arlequin 3.11 (Excoffier *et al.*, 2005). We considered haplotype analysis more informative than a single locus-by-locus test due to the LD between the SNPs.

Results and discussion

We analysed 210 healthy women for TNF promoter variation, including 66 Amerindians of the Mbya-Guarani ethnic group that inhabits the rainforest of Misiones, and 144 white-admixed women with different European background living in urban and rural areas of Misiones. The allele frequencies of TNF promoter SNPs are shown in Table 1, and the haplotypes inferred from sequencing data are shown in Table 2.

Tumor necrosis factor-244A was absent in all of the populations. The Amerindian group was characterized by the absence of TNF-308A and the higher frequency of TNF-857T (30%), with both differences being statistically significant compared with the two white-admixed populations ($P < 0.05$). This is an important finding, as the TNF-857T allele has been shown to be nonrandomly associated with the HLA haplotypes HLA-Cw*0102, -B*1522, -DRB1*0407 and HLACw*03041, B*4002 in other indigenous populations from South America (Baena *et al.*, 2002). The absence of -308A has also been reported in other Native American communities, such as the Terena Indians from Brazil and the Quechua from Peru and Paez from Colombia (Albuquerque *et al.*, 2004; Baena *et al.*, 2002).

On the other hand, the white-admixed populations had a similar frequency of TNF-308A (about 0.05 in Urban and Rural groups), which is included in, and is marker of, an extended Caucasian HLA haplotype A1, B8 and DR3 (Wilson *et al.*, 1993). In addition, in the Urban population, TNF-376A always occurred in association with the TNF-238A SNP, indicating linkage disequilibrium between them (LD coefficient of $D' = 1$; $P = 0.000$). This haplotype has been previously described in 1.38% of European-derived populations from Australia and is linked to the haplotypes HLA DRB103, B18 and A30 in this population (Valente *et al.*, 2009b). Based on these observations, it will be necessary to extend our study to the evaluation of HLA alleles in linkage disequilibrium with the described TNF SNPs.

Allele frequencies vary among human populations, and these differences may be related to population-specific histories or due to the effects of natural selection. Application of the F_{ST} statistics to all pairs of population revealed significant differences between Amerindians and white-admixed populations, but not within the Urban and Rural groups (Table 2). We also investigated whether positive selection was operating on these loci by using the Fu and Li test of neutrality (Fu & Li, 1993). The resulting values were not significant, indicating that the polymorphisms under study were evolving neutrally. Furthermore, we also found no deviations from HWE expectations for the genotype data. We thus believe that the population history of the study groups is primarily responsible for establishing the observed genetic variation at the TNF locus in Misiones. This information is important for ethnographic and disease-association studies when considering the outcome of parasitic and viral diseases that are affecting the region.

The reported distribution of TNF SNPs in populations from Misiones is consistent with the history of modern human populations. *Homo sapiens* evolved in Africa around 160 000–200 000 years ago and migrated across Asia ~70 000–50 000 years ago (Gonder *et al.*, 2007; Hill *et al.*, 2007; Zhang *et al.*, 2007; Behar *et al.*, 2008). Their spread into Europe occurred independently at a later date (~40 000 years ago) (Richards *et al.*, 2000). The Americas were later populated by humans of North-East Asian ancestry who moved into Beringia some 25 000–20 000 years ago and then expanded throughout the continental region beginning around 15 000–20 000 years ago (Bortolini *et al.*, 2003; Schurr & Sherry, 2004; Zegura *et al.*, 2004; Tamm *et al.*, 2007; Dulik *et al.*, 2012). However, the genetic diversity of Amerindian populations has increased due to historical gene flow from Europeans and Africans over the last 500 years, leading to increasingly admixed populations (Corach *et al.*, 2010; Seldin *et al.*, 2007; Catelli *et al.*, 2011). This history is supported by our TNF data, where the genetic diversity in the Mbya-Guarani ($H = 0.0448$) was about four times lower compared with that estimated for Urban ($H = 0.2085$) or Rural ($H = 0.2134$) groups. The low genetic diversity of cytokine genes has been reported in other Amerindian communities from South America (Xavante from Brazil) and explained by a founder effect or by high inbreeding associated with isolation (Zembrzuski *et al.*, 2010).

According to Baena *et al.* (2002), the five mutations analysed in this study (including the haplotype TNF-376A/-238A) arose in Africa before human popula-

Table 1. Allele and genotype frequencies

SNPs	Genotype	Amerindians *n*	%	Rural *n*	%	Urban *n*	%	HWE[1] *P* value	Population comparisons[2] *P* value
-376	GG	66	100	40	100	98	94.2	Monomorphic[(a)]	1.000[(d)]
	AG	0	0	0	0	6	5.8	Monomorphic[(b)]	0.083[(e)]
	AA	0	0	0	0	0	0	0.7619[(c)]	0.187[(f)]
Allele	A	0.000		0.000		0.029			
-308	GG	66	100	35	87.5	92	88.5	Monomorphic[(a)]	**0.006[(d)]**
	AG	0	0	5	12.5	12	11.5	0.6733[(b)]	**0.004[(e)]**
	AA	0	0	0	0	0	0	0.5324[(c)]	1.000[(f)]
Allele	A	0.000		0.062		0.058			
-238	GG	63	95.5	36	90	92	88.5	0.8501[(a)]	0.422[(d)]
	AG	3	4.5	4	10	12	11.5	0.7392[(b)]	0.166[(e)]
	AA	0	0	0	0	0	0	0.5324[(c)]	1.000[(f)]
Allele	A	0.023		0.050		0.058			
-857[1]	CC	11	44	26	76.5	71	76.3	0.2338[(a)]	**0.018[(d)]**
	CT	13	52	8	23.5	21	22.6	0.4369[(b)]	**0.005[(e)]**
	TT	1	4	0	0.0	1	1.1	0.6863[(c)]	1.000[(f)]
Allele	T	0.300		0.118		0.124			

[1]Genotype proportions were tested for Hardy–Weinberg equilibrium. All SNPs were found to be in HWE within the populations in which they were detected. *P* values for: (a) Amerindians, (b) Rural, (c) Urban.
[2]Comparison of populations *P* values: (d) Amerindians vs Rural; (e) Amerindians vs Urban; (f) Rural vs. Urban. The values shown in boldface are statistically significant ($P < 0.05$).

Table 2. Haplotype analysis, gene diversity and genetic differentiation among populations

Haplotype	Sequence -376, -308, -238	Amerindians 132		Rural 80		Urban 208	
H1	GGG	129	0.977	71	0.8875	184	0.884
H2	GGA	3	0.023	4	0.050	7	0.034
H3	GAG	0	0	5	0.0625	12	0.058
H4	AGA	0	0	0	0	5	0.024
Haplotype diversity		0.045 ± 0.025		0.209 ± 0.059		0.213 ± 0.037	
Fu and Li test D*		0.476 ($P > 0.10$)		0.704 ($P > 0.10$)		0.769 ($P > 0.10$)	
Pairwise analysis of F_{ST}s	Rural	**0.047 ($P = 0.004$)**					
	Urban	**0.035 ($P = 0.002$)**		−0.007 ($P = 0.821$)			

The values shown in boldface are statistically significant ($P \leq 0.050$).

tions diverged some 70 000–100 000 years ago. Certain SNPs remained in Africa, such as TNF-244A, which is very rare outside of Africa, while others were taken outside of the continent. For example, the TNF-308A became more frequent in European populations (21%) but diminished in frequency in Asians (8%) and was lost in Amerindians. However, this polymorphism later entered the Americas after the Spanish conquest (16th century) and with European immigration (19th century) and became common in the resulting *mestizo* and *colonos* populations (up to 16%).

This scenario is consistent with the results of this study, where the frequency of the TNF-308A SNP is absent in Guarani, but increased with increasing percentage of European ancestry in the population. Although the extent of admixture in the study populations would be revealed by an analysis of ancestry informative markers, unpublished data from our group indicate a European maternal contribution (mitochondrial DNA) of 0.5% in the Mbya-Guarani, 20% in the Urban population and 50% in the Rural population (Schurr *et al.*, in preparation). Moreover, if we compare TNF-308A frequency to worldwide women recruited from similar studies in Africa, Asia, India and Europe (Duarte *et al.*, 2005; Jang *et al.*, 2001; Kohaar *et al.*, 2007; Stanczuk *et al.*, 2003), F_{ST} analysis indicates that (i) the Guarani population is different from other major ethnic groups and has the lowest F_{ST} value with Asians, with whom they share more recent common ancestor, and (ii) the white-admixed groups did not show significant differences with populations from Africa, Asia, India and Europe (Table 3). Unlike Guarani, the genetic make-up of the Urban and Rural populations results from a complex set of demographic changes, migrations and intergroup contacts. Therefore, to understand specific differences within these admixed populations, we will need to examine a large set of polymorphic markers.

Table 3. Allele frequencies of Tumor necrosis factor (TNF)-308A SNPs among different world populations[1]

Population data	Comparisons of pairs of population samples (Population pairwise FSTs)[2]							
Population	TNF-308A	Africa	India	Asia	Europe	Rural	Urban	Amerindian
Africa	0.1089	0.0000						
India	0.0454	**0.0267**	0.0000					
Asia	0.0380	**0.0305**	−0.0036	0.0000				
Europe	0.0984	−0.0029	**0.0170**	**0.0202**	0.0000			
Rural	0.0625	0.0037	−0.0047	−0.0021	0.0003	0.0000		
Urban	0.0577	0.0123	−0.0024	−0.0010	0.0070	−0.0085	0.0000	
Amerindian	0.0000	**0.0861**	**0.0271**	**0.0265**	**0.0604**	**0.0719**	**0.0417**	**0.0000**

[1]From these studies, data are reported for only female samples, with sample sizes <300 and frequencies in HWE: Africa (Stanczuk *et al.*, 2003); India (Kohaar *et al.*, 2007); Asia (Jang *et al.*, 2001); Europe (Duarte *et al.*, 2005); Misiones: Urban, Rural and Guarani (this study).
[2]Computed conventional *F*-statistics from haplotype frequencies. Significant F_{ST} *P* values are shown in boldface (Significance Level = 0.0500, number of permutations = 3024).

As a final point, we acknowledge that this study involved only women because of its focus on women's health (ovarian cancer, cervical cancer, etc.), and, as a result, may have some limitations when extrapolating the patterns of TNF SNP diversity to populations as a whole. Nevertheless, it should be noted that (theoretically) allele frequencies in men and women should be equal if HWE conditions are met. Future work with men from Misiones will verify this assumption. Until then, comparisons with other populations need to be made cautiously. In fact, the statistical conclusions presented in this article came from standardized populations (only healthy women, with the same recruitment strategy).

In summary, these data have provided new information about the genetic differences among Misiones populations. Expanded studies will be needed to more fully characterize the immunogenetic diversity in Argentinean populations.

Conflict of interest

The authors declare no conflict of interest.

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