



Genetic Polymorphisms at TNF Microsatellites from the HLA CLASS III Region in a South Tunisian Population

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Abstract

To evaluate the tumor necrosis factor (TNF) a-b-c microsatellite polymorphism in South Tunisia, we studied 123 healthy unrelated controls from South Tunisia. TNF microsatellite alleles were typed using genomic DNA amplified with specific primers. Statistical analyses were performed using ARLEQUIN software. The TNFa6 (18.9%), TNFa2 (16.4%), TNFb5 (41.9%), and TNFc1 (68.7%) were the most frequent alleles in our population, whereas the TNFa8 and TNFb2 were the least frequent alleles. To investigate the association among the three microsatellites, a detailed haplotype analysis was performed. 236 different haplotypes were possible but there was only 15 haplotypes had frequencies greater than 2%. In our population the most frequent haplotype TNFcab were "TNFc1a6b5" (10.03%). These results may be informative for studies of the associations of individual TNF region markers with secretion levels, immunity, and disease.

Keywords

TNF, Microsatellite, Haplotype, Population

Subject Areas: Genetics, Immunology

1. Introduction

One group of microsatellites in the HLA class III region is TNF a, b and c. The TNF a and b microsatellites are located 3.5 kilobases upstream of the Lymphotoxin- α (LTA) gene (**Figure 1**).

The three microsatellite regions span around the TNF region, exhibiting 13 alleles for TNFa (a1-a13), 8 for TNFb (b0-b7), 2 for TNFc (c1-c2).

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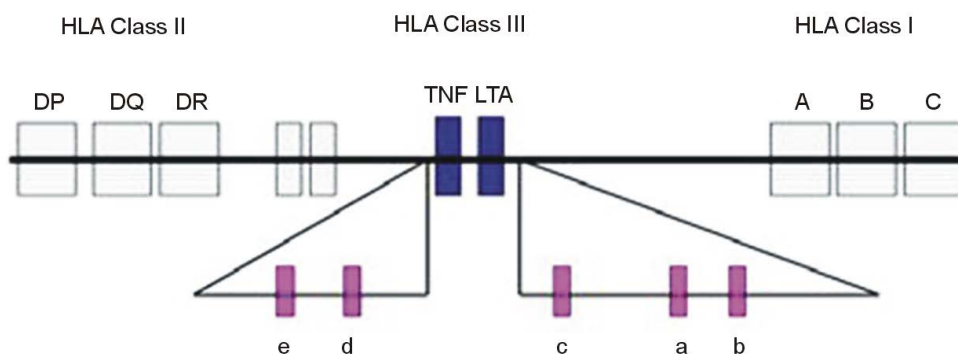


Figure 1. Location and order of 5 TNF microsatellites within the HLA complex on chromosome 6 [1].

TNF α and TNF β are composed of (GT) n and (GA) n repeats, respectively.

The TNF γ microsatellite is a (GA) n repeat located within the first intron of the TNF α gene [2].

Hence, the present study is carried out in a Tunisian population to investigate the frequency of these TNF microsatellite alleles and their relationships with five European populations (French, Basque, Greek, Danish and Italian) and Asian Orientals populations (Indian, Japanese and Chinese).

2. Methods

1) Sample population

The study was accomplished on a sample of 123 healthy unrelated from south Tunisia. The population sample is the same as the one tested for HLA in the paper published in *Ann. Hum. Biol.* 2013, 40, 41-47.

The samples of population studied were from different regions of south Tunisia.

2) Microsatellites genotyping

PCR amplification of the polymorphic TNF α , β and γ microsatellite was carried out with primers. The forward primer was labeled at the 5'-end with the fluorescent VIC and Pet reagent respectively TNF α , TNF β and TNF γ .

Table 1 (<http://www.ncbi.nlm.nih.gov/gv/mhc/xslcgi.cgi?cmd=mssearch>) [3].

To determine the number of the repeat in the region of the TNF gene, the amplified products were denatured for 3 min at 96°C, mixed with formamide and electrophoresed in an automated DNA sequencer (Applied Biosystems model 310 sequencer). The number of microsatellite repeat was estimated automatically using the GENESCAN 3.1 software (Applied Biosystems) with a size standard (Applied Biosystems). The different alleles of the TNF α microsatellites correspond to fragment sizes as follows in **Table 2**.

3. Statistics

Allele frequencies were estimated by direct counting.

Hardy-Weinberg equilibrium, haplotype frequencies, linkage disequilibrium and their significance as determined by chi 2 test were calculated using the Arlequin version 3.0.

Some statistical parameters of forensic interests such as heterozygosity (Hz), power of discrimination (PD), mean exclusion chance (MEC) and polymorphic information content (PIC) were calculated using the software package Power Stats (Promega).

A linkage disequilibrium study was performed using an online software SHEsis (<http://202.120.7.14/analysis/myAnalysis.php>) [4].

Two LD coefficients, Lewontin's D' and Hill's r^2 were evaluated by the same software.

4. Results

1) TNF microsatellites alleles

Allele frequencies were shown in **Table 3**.

A total of 13 alleles at TNF α , 8 alleles at TNF β , and 2 alleles at TNF γ microsatellite were observed among South Tunisian population.

For TNF α microsatellite locus PIC values around 0.87 have been observed.

Table 1. Characteristics of TNF microsatellites.

Microsatellites	Primer
TNFa	F: VIC 5'GCCTCTAGATTTTCATCCAGCCACA3' R: 5'CCTCTCCCCCTGCAACACACA3'
TNFb	F: VIC 5'GCACTCCAGCCTAGGCCACAGA3' R: 5'tgtgtgtgcaggggagagagg3'
TNFc	F: PET 5'gggaggtgtgtttccgccc3' R: 5'cgttcaggtggtgcatggg3'

VIC: fluorescent label; PET: fluorescent label.

Table 2. The corresponding length en pb and allele of TNF a, b, c STR.

TNF allele	Length en pb	TNF allele	Length en pb
a1	98	b0	116
a2	100	b1	118
a3	102	b2	120
a4	104	b3	122
a5	106	b4	124
a6	108	b5	126
a7	110	b6	128
a8	112	B7	130
a9	114		
a10	116		
a11	118		
a12	120	c1	97
a13	122	c2	99

Table 3. Allele frequencies of TNF microsatellite markers in controls.

Allele Number	Allele frequency (%)		
	TNFa (n = 246)	TNFb (n = 246)	TNFc (n = 246)
0	-	1.20	-
1	5.30	11.40	68.70
2	16.40	1.60	31.30
3	1.60	13.80	
4	9.40	20.70	
5	12.70	41.90	
6	18.90	2.80	
7	9.40	6.50	
8	0.40		
9	3.70		
10	11.10		
11	4.90		
12	3.70		
13	2.80		
Ho	0.813	0.577	0.447
PIC	0.870	0.890	0.340
PD	0.968	0.710	0.577

n, number of chromosomes; Ho, observed heterozygosity; PIC, polymorphic information content; PD, power of discrimination.

Likewise, PIC oscillated from 0.34 at TNFc to 0.87 at TNFa, whereas PD varied from 0.57 at TNFc to 0.96 at TNFa. The heterozygosity level for TNFa was particularly high (0.81), and greater than 0.57 for TNFb and 0.44 for TNFc loci.

The present study with a South Tunisian population shows that among TNFa alleles, TNFa6 allele was the most frequent (18.9%) and TNFa2 (16.4%) is the second most frequent allele. However TNFa8 had a low frequency in our population (0.4%).

Among TNFb alleles, TNFb4 and b5 were the most frequent (20.7%, 41.9% respectively), whereas TNFb0, TNFb2 and TNFb6 were very low in our population (1.2%, 1.6%, 2.8% respectively).

For TNFc, the most frequent allele was TNFc1 (68.7%).

2) TNF microsatellites Haplotypes

To investigate the association among the three microsatellites, a detailed haplotype analysis was performed in all samples.

Given the observed polymorphism at TNFa, b, and c microsatellites in our population (13, 7, 2 alleles, respectively), 236 different haplotypes were possible but there were only 69 haplotypes observed in our population.

The 15 most frequent haplotypes with frequency greater than 2% were shown in **Table 4**.

In our population the most frequent haplotypes TNFc, a, b were **TNFC1a6b5** (10%), **TNFC1a10b4** (5.6%). Two rare haplotypes with frequencies lower than 0.1% were observed: **TNFC2a12b3** and **TNFC2a3b1**.

Regarding linkage disequilibrium (LD) between microsatellite markers (TNFa, b and c), our results showed a weak LD between TNFa and TNFc ($D' = 0.263$, $r^2 = 0.007$), TNFa and TNFb ($D' = 0.424$, $r^2 = 0.02$), TNFb and TNFc ($D' = 0.328$, $r^2 = 0.03$) (**Figure 2**).

5. Discussion

In a Tunisian study assessing TNFa association with Graves disease [5], 14 alleles were typed in the Tunisian Healthy controls and TNFa2 was the most frequent allele (19.26%) followed by TNFa7 (14.53%) and TNFa6 (13.85%).

Comparing our findings with the studies done on other populations, it was observed that alleles' distribution in our population was different from Europeans [6].

The most common TNFa microsatellite in the European population was TNFa2 with a frequency varying between 20.8% in the French sample and 32% in a Danish study [7].

Table 4. Haplotype frequency distribution in samples.

Haplotype TNFcab	Frequency (%)
c1a6b5	10.03
c1a10b4	5.64
c1a2b3	5.58
c2a2b1	5.03
c1a5b5	4.35
c1a5b7	3.66
c1a6b4	3.64
c1a7b5	3.03
c1a4b7	2.85
c1a12b5	2.73
c1a4b5	2.54
c1a10b5	2.38
c2a4b5	2.17
c2a7b1	2.10
c2a11b5	2.08

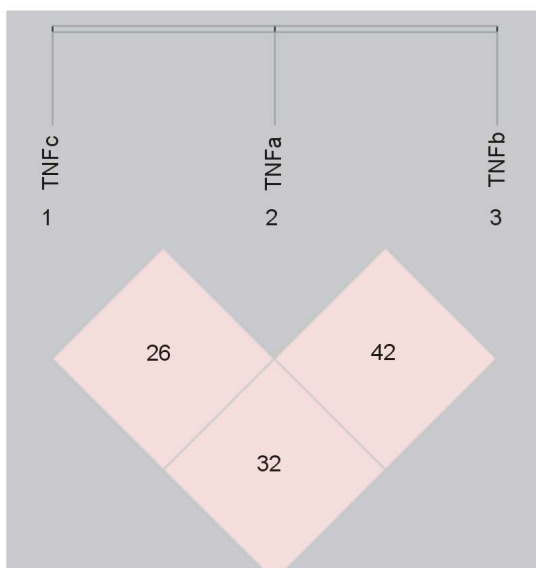


Figure 2. Pairwise linkage disequilibrium (LD) analysis of TNFc-a-b loci.

However our study placed TNFa2 by itself as the second most represented type (16.4%), preceded only by TNFa6 (18.9%).

Comparing our findings with the studies done on Asian Orientals, a different pattern of allele's distribution was observed: In the Japanese population, TNFa13 was the most frequent allele; In Indians and in Chinese, the most frequent TNFa alleles were TNFa10 and TNFa6.

TNFa1 distribution differed among populations. In Basques, it was relatively frequent (11.6%) but low in the French population as well as in our study (3.1%, 5.3% respectively), very low in Danish samples (0.6%), and nonexistent in the Greeks.

Among TNFb alleles, TNFb5 was the most frequent (41.6%), whereas TNFb2 and TNFb6 were very low in our study. This pattern of TNFb allele distribution was quite similar to that in the Basque, Danish and Chinese populations [8] but quite different from the French population where TNFb5 was found to have a low frequency (6.7%).

Analysis of TNFc polymorphism revealed that the observed frequency of TNFc1 allele in our population (68.7 %) was quite similar to those found in Caucasoid originating from Germany (70.6%) and from France (77.1%).

Haplotype studies were powerful than gene frequency studies and can be a useful tool to characterize populations. The most frequent TNFcab haplotype was **TNFc1a6b5** (10%) in our study, **TNFc1a12b4** in French (7.3%) and **TNFc1a4b5** in Danish (4.6%).

These data suggest that the TNF microsatellite haplotypes constitute a highly polymorphic system and that will provide useful information on the association between the TNF marker and the immune diseases.

Acknowledgements

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