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An intragenic polymorphism in the human tumor necrosis factor alpha (*TNFA*) chain-encoding gene

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A G-A transition was detected at position 4583 [according to the sequence number reported by Nedospasov and coworkers (1986)] in the first intron of the *TNFA* gene by analyzing a polymerase chain reaction (PCR)-amplified DNA fragment spanning positions 4422 and 4820. The variation was first detected by heteroduplex analysis of the PCR products in a non-denaturing 12% polyacrylamide gel. The presence of the polymorphic sequence was tested in the population by dot blot of the PCR-amplified DNA fragment hybridized with sequence-specific oligonucleotides (SSO).

In a panel of 102 *HLA*-typed random healthy Italian individuals the following genotype frequencies were observed: GG (0.74), AG (0.24), AA (0.02). The tested population was in Hardy-Weinberg equilibrium at this locus with *G* and *A* gene frequencies 0.86 and 0.14, respectively.

Linkage disequilibria between the *A/G* alleles and markers of the *HLA* region were calculated in the same panel. Class II, class I, and complement loci were considered plus three microsatellite loci in the *TNF* region (see Figure 1). All the significant associations concerned the less frequent *A* allele and are reported in Table 1.

The strong association with the *TNFa10* allele suggests that all the observed A nucleotides at position 4583 derived from the same or few mutational events. Linkage disequilibrium decreases rapidly with increasing distances. Moreover, the *A*-positive individuals do not share any particular common combination of *HLA* markers. Thus, the less frequent *A* allele is not a marker of conserved *HLA* ancestral haplotypes.

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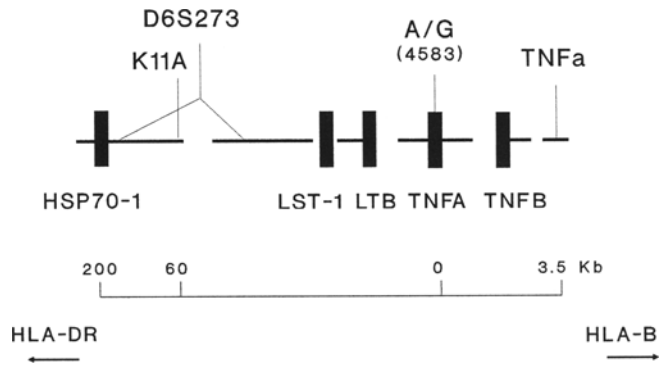

Fig. 1 *TNFa:* microsatellite located in the 5′ region of *TNFB* (Udalova et al. 1993) with 13 alleles (*TNFa1–a13*). The relative positions of the two microsatellite loci *K11A* (ten alleles ranging from 145 to 163 base pairs (bp); Colonna et al. 1992) and *D6S273* (eight alleles: 128–140 bp plus 120 bp; Martin et al. 1995) are not defined

PCR and SSO hybridization conditions

PCR primers: Sense oligo: 5′TGCACTTTGGAGTGATCGGC3′;
Antisense oligo: 5′TTCCCGCTCTTTCTGTCTCA3′
SSOs: 5′AAAAAAACATGGAGAAAG3′ and 5′CTTTCTCCACGTTTTTTTC3′, detecting the *A* and *G* sequences, respectively.

PCR was performed in 25 µl containing 20 ng DNA, 10 pmol each primer, and 0.5 units *Taq* DNA polymerase (Perkin Elmer, Norwalk, CT), 2 mM $MgCl_2$. The PCR profile included: 1′ 95°C, 1′ 55°C, 1′ 72°C for 30 cycles, in a Perkin Elmer 480 Thermal cycler. Two µl of PCR product were spotted on a positively charged nylon membrane and hybridized with 0.5 pm/ml digoxigenin-dUTP (Boehringer Mannheim, Mannheim, Germany) 3′ labeled SSO in hybridizazion buffer containing 0.9 M NaCl, 60 mM Na_2HPO_4, 2 mM ethylenediaminetetraacetate (EDTA), 0.5% sodium dodecyl sulfate (SDS), 5× Denhardt's solution (0.1% polyvinylpyrrolidone, 0.1% ficoll 400, 0.1% bovine serum albumin), at 46°C and 50°C, respectively,

Table 1 Significant associations between the *A* allele and other *HLA* region markers

Marker allele	Associated allele	X^2 (Yates)	P*	Delta
A	*TNFa10*	60.0	$<10^{-4}$	0.0781
A	*K11A-159*	12.08	0.0005	0.0380
A	*D6S273-134*	8.69	0.0032	0.0414
A	*DR14*	7.20	0.0073	0.0203
A	*B18*	5.88	0.015	0.0269

* The P value is not corrected for the number of comparisons (N≈100)

for A- and G-detecting SSOs. Two stringent washings were performed in 0.9 M NaCl, 60 mM Na_2HPO_4, 2 mM EDTA, 1% SDS at 48°C and 52°C, respectively, for the A- and G-detecting SSOs. The membranes were visualized using an anti-digoxigenin Alkaline Phosphatase conjugate antibody and a luminogen Alkaline Phosphatase substrate, according to the manufacturer's instructions (DIG Luminescent Detection Kit, Boehringer).

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