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A highly polymorphic microsatellite marker in the human MHC class III region, close to the *BAT2* gene

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The area usually referred to as the major histocompatibility complex (MHC) class III region lies between the *HLA-B* locus at the telomeric end of 6p21.3 and the *HLA-DR* locus at its centromeric end. The *TNF* gene cluster is at the telomeric end of the MHC class III region (Carroll et al. 1987) and includes the *TNF* gene itself and the two lymphotoxin genes (*LT-a* and *LT-b*, Browning et al. 1993). This cluster lies between two genes, *BAT1* and *BAT2*, whose mRNA transcripts are expressed with that of the *HLA-B* gene (Spies et al. 1989). As shown in Fig. 1, four recent studies have reported the existence of several new genes in the DNA surrounding the *TNF* locus and still lying within the boundaries of the *BAT1* gene (telomeric) and the *BAT2* gene (centromeric). The position of these genes is illustrated in Fig. 1. These genes share the property with those of the *TNF* gene cluster that they are expressed predominantly, if not exclusively, within cells of the immune system and appear to be induced by cytokines.

An understanding of genetic variation within this region may be important in understanding some aspects of the genetic susceptibility to autoimmune disease; the region is likely to contain many genetic variants. Of these, the *TNFA* microsatellite with its 13 common alleles, has been widely used as a highly informative marker within the MHC class III region both in population (Crouau-Roy et al. 1994; Gallagher et al. 1997) and disease-association studies (Monos et al. 1995; Mizuki et al. 1995). To date however, equally informative markers in more centromeric regions of this area have been lacking.

The DNA sequence Z15025 (Iris et al. 1993) contains the *BAT2* gene and a long GT repeat, spanning bases 31004–31039. We examined this GT repeat and found it to be highly polymorphic, with 12 alleles. We now describe the distribution of these alleles of this new *BAT2* microsatellite and demonstrate their close association with those of the *TNFA* microsatellite.

Oligonucleotide primers were designed to flank the *BAT2* GT repeat, thus:

<i>BAT2A</i>	5'	CTC.CAG.CCT.GGA.TAA.CAG	3'
<i>BAT2B</i>	5'	ACA.AGG.GCT.TTA.GGA.GGT.CT	3'

such that they matched and complemented bases 30937–30954 and 31066–31085 of Z15025, respectively (Cruachem Ltd, Glasgow, Scotland). DNA from 84 unrelated individuals (Gallagher et al. 1997) was used. Genotyping at the *BAT2* microsatellite was carried out in a final reaction volume of 25 μ l. After an initial melting time of 5 min, samples were subjected to 30 rounds of 95 °C, 1 min; 62 °C, 1 min; 72 °C, 1 min, all followed by a final extension step at 72 °C for 5 min, in a Biometra (Göttingen, Germany) Uno Thermoblock. In addition to the test DNA, each reaction contained: 1 μ M each primer; 200 μ M

each dATP, dGTP, dTTP, and 20 μ M dCTP (Pharmacia, Milton Keynes, England); 0.5 units Primezyme (Biometra), in 1 \times reaction buffer with 1.5 mM MgCl₂ (Biometra); α -³²P-dCTP (Amersham International, Amersham, England) was added to label the reaction product. Polymerase chain reaction products were denatured in formamide (80 °C, 10 min), and resolved in a 6% acrylamide (Gibco, Paisley, Scotland) sequencing gel (19:1) containing 7 M urea (Appligene, Strasbourg, France). The alleles were visualized on X-ray film. Gels were calibrated with an end-labeled 10 base pair ladder (Gibco) run in two lanes. In addition, samples 057, 062, and 068 were always run as standards, genotyping to *BAT2.3/3*, *1/7*, and *7/12*, respectively. The widely available tissue-typing cell lines OLL, T7527, LZL, and IBW9 typed as *BAT2.2/3*, *9/9*, *2/2* and *4/4*, respectively. The overall allelic distribution is shown in Fig. 2. Allele *BAT2.12* was observed in an individual who was related to a member of our test panel. Thus, we included it as a calibration aid but excluded it from the analysis of allele distribution in unrelated individuals. The most common alleles were *BAT2.3* (31.3%), *BAT2.7* (26.5%), and *BAT2.2* (19.9%). The most common genotype was *BAT2.3/7* (15.5%), while *BAT2.2/3*, *2/7*, *3/3*, and *7/7* were equal at 9.5%. The population showed a total heterozygosity of 77.4%. Typing for the *TNFA* locus was taken from our previous study (Gallagher et al. 1997).

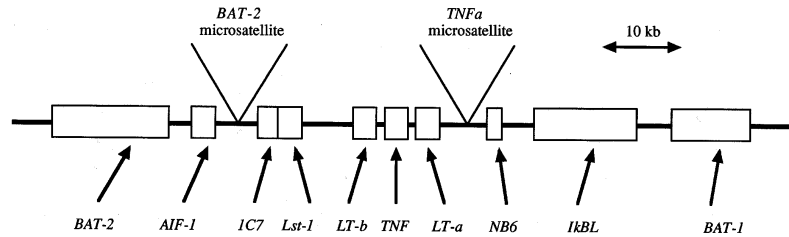
The proportion of each allele present was calculated assuming no null alleles. Associations between loci were estimated using a series of 2 \times 2 tables, analyzed by the Chi-square test. The probability (*P*) obtained was corrected for multiple comparisons (PC) according to the number of alleles observed. Associations between alleles (*P* < 0.05) were considered as "strong" where PC < 0.05.

As expected, a number of associations were observed between *BAT2* alleles and *TNFA* alleles: *BAT2.1* with *TNFA2* ($\chi^2 = 21.094$, PC = 0.00058), *BAT2.2* with *TNFA7* ($\chi^2 = 13.025$, PC = 0.0114), *BAT2.3* with *TNFA11* ($\chi^2 = 20.002$, PC = 0.00058), *BAT2.4* with *TNFA4* ($\chi^2 = 7.419$, *P* = 0.0065, PC = not significant), *BAT2.7* with *TNFA10* ($\chi^2 = 13.241$, PC = 0.0174), and with *TNFA6* ($\chi^2 = 10.388$, PC = 0.0754). Of these, those between *BAT2.1* and *TNFA2* alleles, and the *BAT2.3* and *TNFA11* alleles were particularly strong, suggesting their incorporation into the recognized *HLA A1.B8.DR3* and *A3.B7.DR2* extended haplotypes, respectively.

The MHC region is characterized both by a very high degree of polymorphism and by the strong linkage disequilibria which exist over long distances. That these have not been disrupted by recombination demonstrates the importance to the immune system of combinations of antigen presenting molecules such as *HLA-A1*, *B8*, and *DR3*. The *BAT2* and *TNFA* microsatellites are in a very useful location with regards to a part of the MHC which contains an important set of inducible, inflammatory genes (Fig. 1), many of whose functions are unknown. A close examination of the association between their alleles in various autoimmune diseases may yield much new information.

In conclusion, we present the characterization of a new microsatellite locus in the human MHC class III region, adjacent to the *BAT2*

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gene. We present further data on its allelic associations with those of the *TNFA* microsatellite and suggest that the relationship between these two microsatellite loci (*BAT2* and *TNFA*) may be useful in allowing wider relationships across the human MHC to be defined.

Fig. 1 Position of the *BAT2* microsatellite in relation to the *TNFA* microsatellite and those genes known to lie between them and adjacent to them. The two microsatellites are 30 kilobases apart. Genes are positioned from published information: the *IKB* gene (Iris et al. 1993; Albertella and Campbell 1994), the *NB6* gene (Albertella et al. 1996), the *Lst-1* gene (Holzinger et al. 1995), the *IC7* gene (Nalabolu et al. 1996), and the *AIF-1* gene (Utans et al. 1995)

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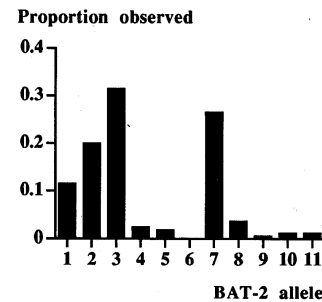


Fig. 2 The polymorphic nature of the *BAT2* microsatellite. The proportion of each allele observed in our panel of 84 unrelated individuals is shown

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