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Gene-antigen register

Polymorphic markers related to a single *Tcrb-V6* gene segment

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The central role of the antigen-specific alpha/beta T-cell receptor (Tcr) in immune recognition has led to a search for *Tcr* gene polymorphism relevant to autoimmune diseases. Previous reports primarily emphasized associations with constant (*C*)-region (Millward et al. 1987; Demaine et al. 1989; Freimark et al. 1987) restriction fragment lenght polymorphisms (RFLPs) as opposed to polymorphisms of variable (*V*)-region genes, which determine the specificity of antigen-MHC recognition by the Tcr. Although *Tcr-V-* and *C*-region genes are linked, recent family studies have reported a lack of linkage disequilibrium between *V-* and *C*-region polymorphisms (Robinson and Kindt, 1987; Charmley et al. 1988; Charmley et al. 1990) indicating that *C*-region polymorphisms alone may be of limited value in studying *Tcr* disease association (Nivens et al. 1990; Charmley et al. 1990). Further study of disease associations has been hampered by the paucity of data regarding the extent of polymorphism of *Tcr-V*-region genes in normal Caucasian populations.

In the present study, we evaluated RFLPs related to *Tcrb-V* genes in 100 normal, unrelated, Caucasoid individuals using five *Tcrb-V* gene specific cDNA probes, V4, V5, V6.1, V8.1 and V18 (Leiden and Strominger 1986; Yanagi et al. 1984). Southern blot analysis of genomic DNA digested with the restriction enzymes *Bgl*-II, *Bam* HI, *Eco RI, and Taq* I, was carried out as described (Southern 1975).

Polymorphic restriction enzyme sites were detected by two restriction enzymes, *Taq* I and *Bgl* II, with the Tcrb-V6.1 cDNA probe. Each probe/enzyme combination defines a bi-allelic polymorphism. Hybridization of the Tcrb-V6.1 probe to blots containing *Bgl* II digested DNA revealed a variant band of 5.7 kilobases (kb) whose intensity varied in a reciprocal fashion with the intensity of a ubiquitous 12.5 kb fragment (Fig. 1). Three hybridization patterns were observed. The ubiquitous presence of the 12.5 kb fragment suggests the existence of at least two cross-hybridizing 12.5 kb fragments per haplotype, only one of which contains a polymorphic *Bgl* II restriction site permitting the assignment of genotypes as indicated in Figure 1. The less intense staining of 12.5 kb fragment as compared to the 5.7 kb fragment observed in the 5.7 kb homozygotes indicates that the *Tcrb-V* gene segment within the ubiquitous cross-hybridizing 12.5 kb fragment displays a lesser degree of homology with the Tcrb-V6.1 cDNA probe than the *Tcrb-V* gene segment within the polymorphic *Bgl* II fragments. It was possible to assign one of these genotypes to each individual, consistent with the presence of a biallelic locus. Genotype assignments were verified by blinded evaluation of autoradiograms. In addition, double digest of 25 DNA samples with *Eco* RI and *Bgl* II were performed which permitted discrimination of 5.7/5.7 kb and 5.7/12.5 kb genotypes on a basis other than intensity of the 12.5 kb and 5.7 kb bands. A 4 kb band is consistently present in individuals assigned the 12.5/5.7 as well as the 12.5/12.5 genotypes and absent in those assigned the 5.7/5.7 genotype (Fig. 2).

The *Tcrb*-V6.1 *Taq* I probe/enzyme combination defines another biallelic polymorphism with variant bands at 6.5 kb and 5.3 kb permitting genotype assignments as indicated in Figure 3. The polymorphic 5.3 kb *Taq* I band constitutes the lower band of the doublet visible on Southern blots, whilst the upper band represents a weakly cross-hybridizing fragment. Verification of genotype assignments, particularly descrimination of the 5.3/6.5 genotype from the 6.5/6.5 genotype, was confirmed by performing double digests of 17 DNA samples with *Taq* I and *Bgl* II. The polymorphic 6.5 kb and 5.3 kb *Taq* I bands were unaltered by additional digestion with *Bgl* II whilst the upper band of the 5.3 kb doublet was no longer

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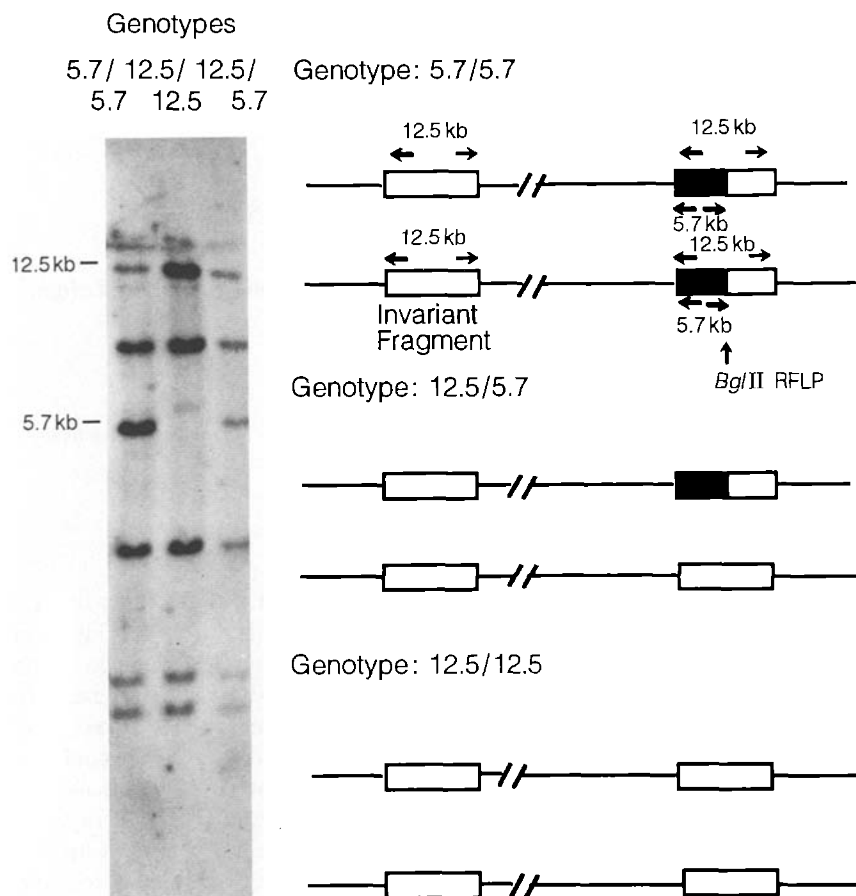

Fig. 1. Bi-allelic polymorphism defined by *Tcrb-V6.1/BflII*. The *Bgl* II RFLP has been provisionally localized to a 12.5 kb fragment cross-hybridizing with an invariant 12.5 kb fragment.

visible (see below and Figure 4, lanes A, B, and E). To our knowledge, these particular polymorphic restriction sizes have not been described previously. Table 1 summarizes the distribution of *Tcrb* V6.1/*Bgl* II and *Tcrb* V6.1/*Taq* I RFLP genotypes in 174 normal, unrelated, Caucasoid individuals. The bi-allelism of both markers was confirmed by the finding that genotypes met expectations based on Hardy-Weinberg equilibrium conditions (data not shown).

Calculations based on phenotypic frequencies of alleles of the two *Tcrb* V6 related loci also revealed evidence for strong linkage disequilibrium indicating close linkage between the two loci. In particular, the presence of *Bgl* II polymorphic restriction enzyme sites on both the *Tcrb* haplotypes of 87 individuals is absolutely associated with the presence of *Taq* I polymorphic restriction sites (genotype 5.3/5.3); conversely, all 43 individuals with an absent *Taq* I polymorphic restriction site (genotype 5.3/6.5 or 6.5/6.5) also have an absent *Bgl* II polymorphic restriction site on at least one *Tcrb* haplotype (genotype 5.7/12.5). Thus, the presence of a polymorphic 6.5 kb *Taq* I fragment is always associated with the presence of at least one polymorphic 12.5 kb *Bgl* II fragment.

The relation between the two polymorphic restriction sites was further analyzed by performing *Bgl* II/*Taq* I double digests on 17 DNA samples whose *Tcrb* V6.1 *Bgl* II and *Taq* I RFLP genotypes included the various combinations observed in the general population. The *Taq* I polymorphic bands were unaltered by additional digestion with *Bgl* II (Fig. 5) suggesting that, in view of the linkage disequilibrium between these RFLP alleles, these *Taq* I restriction sites are situated within the polymorphic *Bgl* II fragments. The finding of the original 5.3 kb *Taq* I band in double digests (*Taq* I/*Bgl* II) of DNA from individuals homozygous for the 5.3 kb *Taq* I and 5.7 kb *Bgl* II bands (Figure 4, lane C) is in agreement with this view.

This relationship of the *Bgl* II and *Taq* I sites to each other was also investigated further by the isolation of the 12.5 kb and 5.7 kb fragments from Southern gels of *Bgl* II digested DNA followed by DNA extraction and digestion with *Taq* I. The presence, still, of the original 5.3 kb and/or 6.5 kb *Taq* 1 bands confirms that the *Taq* I restriction sites are situated within the polymorphic *Bgl* II fragments (Fig. 5). Thus, isolation of the 5.7 kb *Bgl* II fragment from three double heterozygotes (genotype 5.7/12.5, 5.3/6.5) followed by *Taq* I digestion resulted in the appearance of 5.3 kb *Taq* I bands only (Fig. 5a)

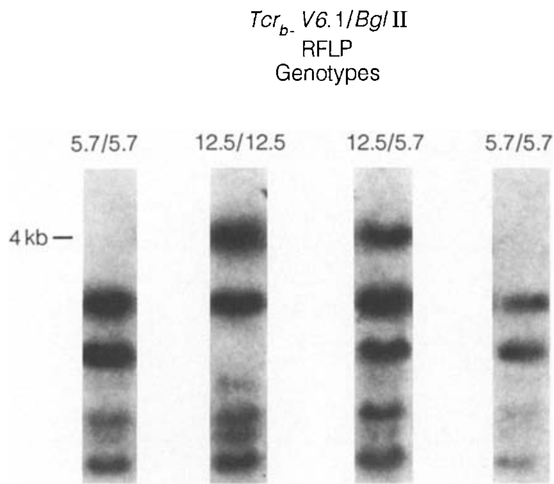

Fig. 2. *Bgl* II/*Eco* RI double digests of genomic DNA probed with a *Tcrb-V6.1* cDNA. A 4 kb band is present in genotypes 5.7/12.5, 12.5/12.5 and absent in genotype 5.7/5.7.

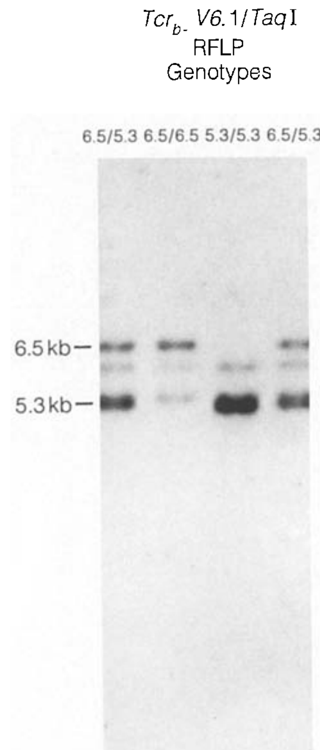

Fig. 3. Bi-allelic polymorphism defined by *Tcrb-V6.1/Taq* I.

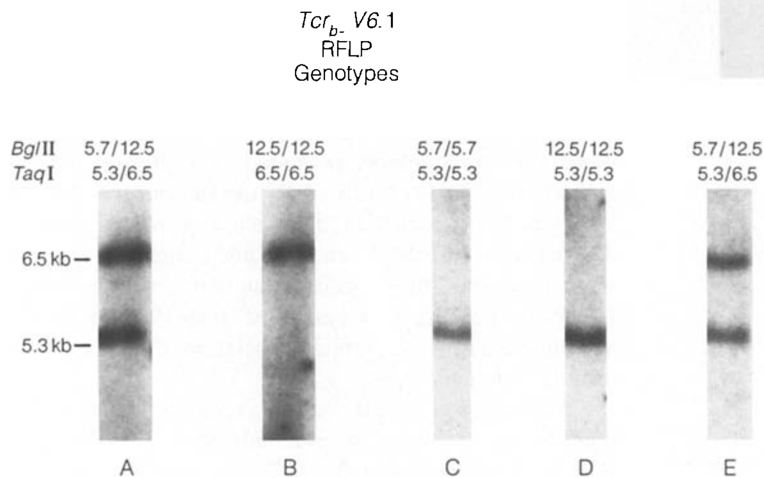

Fig. 4. *Bgl* II/*Taq* I double digests of genomic DNA derived from individuals with different *Tcrb-V6.1/Taq* I and *Bgl* II RFLP genotypic combinations.

whereas *Taq* I digestion of the 12.5 kb *Bgl* II fragment from the same individuals resulted in the 6.5 kb *Taq* I band only (Fig. 5b) illustrating that in these individuals the *Taq* I and *Bgl* II polymorphic restriction sites occur together on one Tcr haplotype and are both absent on the other. These results are consistent with the *Bgl* II/*Taq* I double digests of genomic DNA derived from such individuals (Fig. 4, lane A and E). A schematic illustration of the relationship between these RFLPs is given in Figure 6. The molecular and population genetic data are therefore consistent with the preferential occurence of 5.7 kb *Bgl* II and 5.3 kb *Taq* I fragments on one *Tcrb* haplotype and 12.5 kb *Bgl* II and 6.5 kb *Taq* I fragments on another *Tcrb* haplotype.

The only other bi-allelic polymorphism demonstrated with the probe/enzyme combinations used was the *Tcrb* V8.1 cDNA probe which hybridized to polymorphic bands of either 23 kb or 2 kb on *Bam* HI blots. This RFLP has been described previously (Concannon et al. 1987). The *Tcrb* V4, V5, and V18 cDNA probes revealed no bi-allelic polymorphic restriction sites, although infrequently occurring and often weakly hybridizing polymorphic bands were evident.

A previous study has reported polymorphic restriction enzyme sites in the vicinity of the *Tcrb* V6.7 gene using restriction enzymes *Pvu* II, *Hind* III and *Bam* HI (Li et al. 1990). We did not observe allelic RFLPs using the *Tcrb* V6.1 probe and restriction enzymes *Bam* HI and *Pvu* II (unreported observations) suggesting that the polymorphic restriction sites described in this report map to a *Tcrb* V6 family member other than *Tcrb* V6.7. The limited polymorphism observed in the 5V-region subfamilies in the present analysis of outbred individuals concurs with an earlier finding of limited *Tcrb-V*-region polymorphism

Table 1. Distribution of *Tcrb-V6.1/Bgl* II and *Tcrb-V6.1/Taq* I RFLP genotypes in a healthy Caucasian population.

		Tcrb-V6.1/Taq I RFLP genotypes		
		5.3/5.3	5.3/6.5	6.5/6.5
Tcrb-V6.1/Bgl II RFLP genotypes	5.7/ 5.7	87	0	0
	5.7/12.5	39	34	0
	12.5/12.5	5	8	1

Linkage disequilibrium values for *Tcrb-V6.1* RFLP alleles: 5.7/5.3 (+) 0.461; 12.5/5.3 (−) 0.164; 5.7/6.5 (−) 0.472; 12.5/6.5 (+) 0.472.

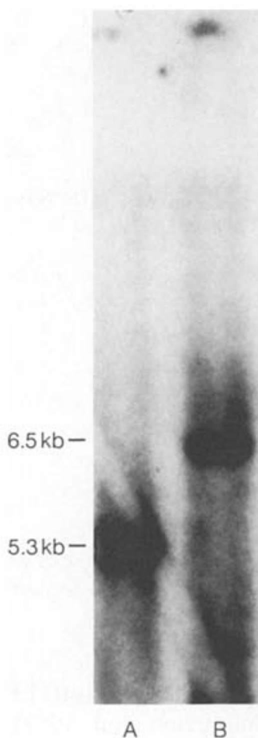

Fig. 5. *Taq* I digests of 5.7 kb (A) and 12.5 kb (B) *Bgl* II restriction fragments derived from an individual heterozygous at both *Tcrb-V6.1* RFLP loci.

in consanguineous individuals (Concannon et al. 1987), in which only two examples of bi-allelic polymorphism were evident.

Although a number of reports have described RFLPs using a particular *Tcrb* V gene probe and several different restriction enzymes, most involve *V* genes belonging to a multimembered *V* gene family so that it is unclear if these RFLPs are associated with the same *V* gene. This is particularly relevant to the evaluation of RFLP markers associated with members of the larger human *Tcrb-V* families like *V6* in which at least nine different V genes have been identified (Toyonaga and Mak 1987). In addition, wide interspersal of gene segments belonging to different *Tcrb-V* families (Lai et al. 1988) as well as recent family studies implicating frequent recombination events in both *Tcr-a* (Robinson and Kindt 1987) and *-b* (Seboun et al. 1989b) haplotypes potentially complicates the examination of *Tcrb-V6*-related RFLP markers for disease associations in unrelated individuals. In contrast, the data derived from population analysis and restriction enzyme mapping in this study localizes the two newly described RFLPs to the same member of the *Tcrb-V6* gene family and illustrates the preferential association of alleles on two distinct *Tcr* haplotypes.

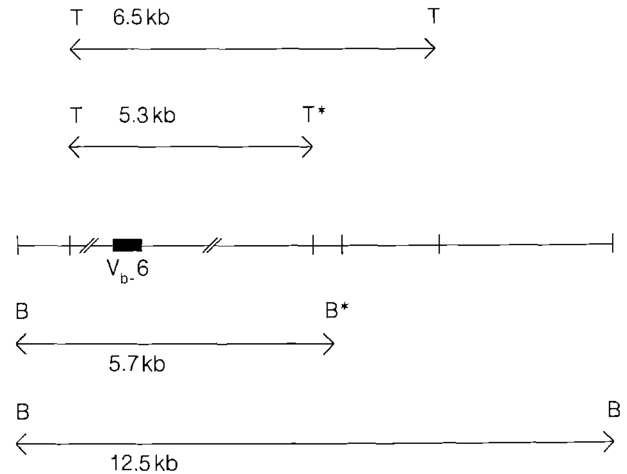

Fig. 6. Schematic illustration showing the relationship between the *Tcrb-V6.1/Taq* I and *Bgl* II defined RFLPs.

Although a recent study reported linkage disequilibrium between several adjacent *Tcrb-V*-region RFLPs which included the RFLP defined by the Tcrb V8/*Bam* HI probe/enzyme combinations (Charmley et al. 1990), RFLPs related to V6 family members were not evaluated. In view of the dominant role played by T-cells expressing particular *Tcrb-V*-region genes in certain murine models of autoimmune disease (Urban et al. 1988; Acha-Orbea et al. 1988; Vandenbark et al. 1989); the findings outlined in this report, namely two RFLP markers related to a particular *Tcrb-V6* gene in the context of two distinct *Tcrb* haplotypes, facilitate the evaluation of this *Tcrb-V*-region in human autoimmune disease.

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