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Haplotyping the human T-cell receptor β-chain gene complex by use of restriction fragment length polymorphisms

(*TCRB* locus/linkage disequilibrium)

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ABSTRACT **We have studied the genetic segregation of human T-cell receptor β-chain (TCRβ) genes on chromosome 7q in 40 CEPH (Centre d'Etude du Polymorphisme Humain) families by using restriction fragment length polymorphisms (RFLPs). We constructed haplotypes from eight RFLPs by using variable- and constant-region cDNA probes, which detect polymorphisms that span more than 600 kilobases of the TCRβ gene complex. Analysis of allele distributions between TCRβ genes revealed significant linkage disequilibrium between only 6 of the 28 different pairs of RFLPs. This linkage disequilibrium strongly influences the most efficient order to proceed for typing of these RFLPs in order to achieve maximum genetic informativeness, which in this study revealed a 97.3% level of heterozygosity within the TCRβ gene complex. Our results should provide new insight into recent reports of disease associations with the TCRβ gene complex and should assist in designing future experiments to detect or confirm the existence of disease-susceptibility loci in this region of the human genome.**

The human T-cell receptor β-chain (TCRβ) locus is a gene complex containing variable (V), diversity (D), and joining (J) gene segments that participate in somatic cell rearrangement with a constant (C) gene to encode the β-chain of the T-cell receptor (1). By *in situ* hybridization, the TCRβ locus resides at chromosomal band 7q35 (2), with the C gene telomeric to the V-region genes (3). By current estimates, this complex spans more than 600 kilobases (kb) of DNA and contains 70–80 V-region segments (4–7). Following successful DNA rearrangement of the V, D, and J gene segments, the translated β-chain polypeptide pairs with a T-cell receptor α chain and can be expressed on the surface of the T cell (reviewed in ref. 8). The T-cell receptor then functions in recognizing antigen in the context of a self major histocompatibility molecule (9).

Since T cells play a pivotal role in the differentiation and regulation of effector mechanisms within the immune system (10), it has been postulated that variations in the germ-line TCRβ repertoire might contribute to certain disease susceptibilities. Such theories have recently been tested by studying germ-line DNA polymorphisms of the α and β chain in normal and disease populations (reviewed in refs. 11 and 12). Positive associations have been reported between specific TCRβ restriction fragment length polymorphisms (RFLPs) and human autoimmune diseases (13–20). While these positive associations are based on TCRβ RFLP frequency comparisons between populations, for multiple sclerosis the TCRβ association has been confirmed by a sib-pair analysis (21).

In this report we present our results of tracing the inheritance of eight TCRβ RFLPs through 40 large CEPH (Centre d'Etude du Polymorphisme Humain) pedigrees. As a result of this study, we have determined the usefulness of each of these polymorphisms individually, and in combination, for the purposes of future investigations into the contributing role of the TCRβ genes to disease susceptibility. We have measured the nonrandom association (i.e., linkage disequilibrium) between the eight RFLPs. We have also demonstrated the utility of these TCRβ RFLPs for genetic linkage analyses by constructing a genetic map of this region of the long arm of chromosome 7 with the TCRβ locus in the middle of this map.

MATERIALS AND METHODS

DNA Probes. Cloned cDNA segments for the $V_\beta 1$, $V_\beta 7.2$, $V_\beta 8.2$, and $V_\beta 11$ gene segments (4) as well as the C_β (23) and $V_\beta 15$ (5) gene segments were employed in detecting RFLPs.

Southern Blot Analysis. Southern blot analyses were carried out as described (12).

Pedigrees Analyzed. DNA was isolated from lymphoblastoid B-cell lines from the 40 CEPH families (24). These pedigrees include over 500 individuals (primarily Northern European Caucasians) with approximately 700 potentially informative meioses available for segregation analysis. CEPH genotypes for the probe/enzyme system MET/*Taq* I were submitted to the CEPH data base by the laboratories of R. Williamson (London) and R. White (Salt Lake City). CEPH genotypes for the "CRI" probe/enzyme systems were submitted by Collaborative Research. Typing of all TCRβ RFLPs was performed by us, and the genotypes collected will be part of the CEPH Version 3 and/or Version 4 data bases.

Statistical Analysis. The extent of nonrandom allelic association, or linkage disequilibrium, between the TCRβ polymorphisms examined was calculated using the test statistic Q, as suggested by Hedrick *et al.* (25).

The percent heterozygosity of every possible grouping of loci for the CEPH parents was tabulated by using a computer program written in dBASEIII. Linkage analysis was performed using the computer program MENDEL (26, 27). Lalouel's least-squares criterion was used to generate 40 candidate orders (28). Maximum multipoint logarithm of odds (lod) scores were then computed for each of the identified candidate orders to find the most likely multipoint order.

RESULTS

DNA Polymorphisms and Haplotype Construction. *Eco*RV restriction digests of DNA from the CEPH parents revealed an RFLP detected with the $V_\beta 7$ probe. This $V_\beta 7$/*Eco*RV RFLP is biallelic with polymorphic restriction fragments of

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Abbreviations: CEPH, Centre d'Etude du Polymorphisme Humain; RFLP, restriction fragment length polymorphism; TCRβ, T-cell receptor β chain; V, variable; D, diversity; J, joining; C, constant; lod, logarithm of odds; cM, centimorgan(s).

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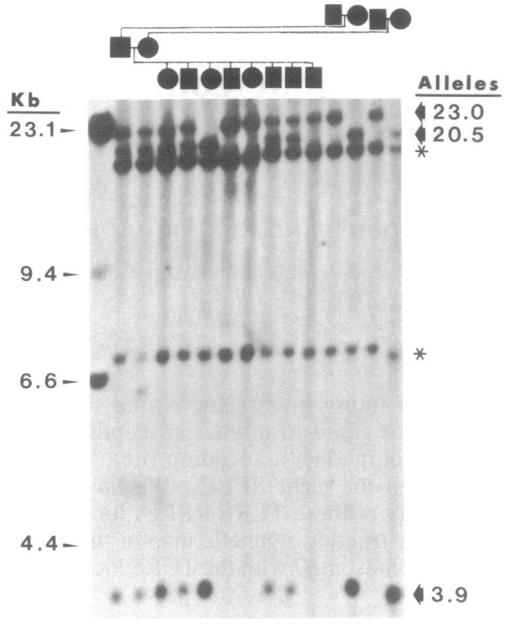

FIG. 1. Demonstration of the codominant segregation of a previously unreported *Eco*RV polymorphism detected with a $V_\beta 7$ probe. Two alleles are detected, (*i*) 23 kb and (*ii*) 20.5 and 3.9 kb, as indicated at right (monomorphic bands are designated with an asterisk). Electrophoretic size standards are in the left-most lane and identified according to molecular size in kilobases.

either 23 kb, or 20.5 and 3.9 kb, as demonstrated by the Mendelian segregation in Fig. 1. Descriptions of the C_β/*Bgl* II (29), C_β/*Kpn* I (30), $V_\beta 8$/*Bam*HI (31), $V_\beta 11$/*Bam*HI (31), $V_\beta 1$/*Taq* I (12), $V_\beta 7$/*Bam*HI (12), and $V_\beta 15$/*Msp* I (22) RFLPs have been reported. These TCRβ RFLPs are summarized in Table 1. The observed genotype distribution of these TCRβ RFLPs in the CEPH pedigrees did not differ significantly from the distribution predicted assuming Hardy–Weinberg equilibrium ($P > 0.01$). Because of the structure of the CEPH pedigrees—i.e., mostly three-generation with large sibships—parental haplotypes were unambiguously determined for all of these probe/enzyme systems.

Nonrandom Allelic Association (Linkage Disequilibrium). We tested for the presence of significant linkage disequilibrium between each pair of polymorphisms by comparing the overall distribution of the two-locus haplotypes *observed* as compared to the haplotype distribution *expected* assuming random allelic association (Table 2). When the extent of linkage disequilibrium was converted into *P* values and the level of significance ($P \leq 0.01$) was corrected for the 28 pairwise comparisons made, significant linkage disequilibrium was found amongst the RFLPs. We noted very strong disequilibrium ($P \leq 10^{-19}$) between several pairs of loci, including $V_\beta 7$/*Eco*RV with $V_\beta 7$/*Bam*HI, $V_\beta 8$/*Bam*HI with $V_\beta 11$/*Bam*HI, $V_\beta 8$/*Bam*HI with $V_\beta 15$/*Msp* I, and $V_\beta 11$/*Bam*HI with $V_\beta 15$/*Msp* I. We also observed some level of disequilibrium between $V_\beta 7$/*Eco*RV and $V_\beta 1$/*Taq* I and between $V_\beta 7$/*Bam*HI and $V_\beta 8$/*Bam*HI, but these were below the level of uncorrected statistical significance of $P \leq 0.01$ (but were not statistically significant when corrected for the number of comparisons made). Despite the extreme levels of nonrandom allelic association, we noted only one case of "complete" linkage disequilibrium between any pair of markers: with $V_\beta 11$/*Bam*HI and $V_\beta 15$/*Msp* I, the first $V_\beta 11$ allele (25 kb) was always found with the third $V_\beta 15$ allele (5.5 kb), and the second $V_\beta 11$ allele (20 kb) always occurred with the second $V_\beta 15$ allele (6.3 kb) (Table 1). These $V_\beta 11$ and $V_\beta 15$ genes are within 20 kb of one another (unpublished data).

There is a conspicuous absence of linkage disequilibrium between the *Bgl* II and *Kpn* I C_β RFLPs. These sites are only 1.8 kb apart (30). Consistent with this observation, $V_\beta 15$/*Msp* I is the only V_β RFLP in disequilibrium with C_β/*Bgl* II, yet $V_\beta 15$/*Msp* I is not in disequilibrium with the C_β/*Kpn* I polymorphism (Fig. 2).

Strategies for Achieving Maximum Heterozygosity. In attempts to characterize the segregation of the TCRβ complex in many pedigrees, it may not be feasible or necessary to determine genotypes for all eight RFLPs (Table 1). The presence of linkage disequilibrium among these RFLPs implies that there may be overlap in their heterozygosities. We therefore tabulated the level of heterozygosity achieved by every possible set of the six restriction enzymes tested (Table 3). For any single restriction enzyme, *Bam*HI has the best likelihood (82.4%) of distinguishing the two TCRβ chromosomes in any given individual. While *Bam*HI detects three different TCRβ RFLPs ($V_\beta 7$, $V_\beta 8$, and $V_\beta 11$), the 82.4% level of heterozygosity was achieved by probing with only $V_\beta 7$ and $V_\beta 8$. (Fortuitously, the *Bam*HI restriction fragments detected on the Southern blot with $V_\beta 7$ and $V_\beta 8$ probes do not

Table 1. DNA polymorphisms studied within the TCRβ complex

Probe	Enzyme	Allele	Fragment size(s), kb	% allele frequency*	% observed heterozygosity*	No. of individuals typed
$V_\beta 7$	*Eco*RV	1	23.0	38	49	211
		2	20.5, 3.9	62		
$V_\beta 7$	*Bam*HI	1	13.0, 8.4	60	63	404
		2	12.3	26		
		3	7.8	14		
$V_\beta 1$	*Taq* I	1	3.9	18	26	308
		2	3.3	82		
$V_\beta 8.2$	*Bam*HI	1	23.0	51	54	468
		2	2.0	49		
$V_\beta 11$	*Bam*HI	1	25.0	47	51	472
		2	20.0	53		
$V_\beta 15$	*Msp* I	1	8.1	29	64	339
		2	6.3	52		
		3	5.5	19		
C_β	*Bgl* II	1	10.0	57	52	470
		2	9.0	43		
C_β	*Kpn* I	1	6.9	49	47	280
		2	1.9	51		

*Based on the parental genotypes.

Table 2. Linkage disequilibrium between TCRβ polymorphisms

	$V_\beta 7$/*Eco*RV	$V_\beta 7$/*Bam*HI	$V_\beta 1$/*Taq* I	$V_\beta 8$/*Bam*HI	$V_\beta 11$/*Bam*HI	$V_\beta 15$/*Msp* I	C_β/*Bgl* II	C_β/*Kpn* I
$V_\beta 7$/*Eco*RV		10^{-19}	0.0004	0.59	0.85	0.78	0.21	0.70
$V_\beta 7$/*Bam*HI	127		10^{-7}	0.009	0.29	0.15	0.06	0.98
$V_\beta 1$/*Taq* I	132	184		0.68	0.80	0.97	0.51	0.24
$V_\beta 8$/*Bam*HI	145	220	197		10^{-33}	10^{-22}	0.39	0.46
$V_\beta 11$/*Bam*HI	140	217	196	223		10^{-33}	0.04	0.46
$V_\beta 15$/*Msp* I	123	168	158	161	153		10^{-8}	0.39
C_β/*Bgl* II	149	228	203	227	226	180		0.014
C_β/*Kpn* I	122	165	169	178	178	149	167	

Linkage disequilibrium among the unrelated CEPH Caucasian founders as measured by the Q statistic described by Hedrick *et al.* (30). Uncorrected P values are shown above the diagonal and are based on the χ^2 distribution of Q. The extent of linkage disequilibrium between adjacent RFLPs can be read immediately above the diagonal and are underlined. Degrees of freedom for each pair of loci are calculated by multiplying the number of alleles minus one that are present at each locus (Table 1). The P level for significant disequilibrium should be corrected for the number of comparisons made—i.e., $P = 0.01 \div 28 = 0.00036$. P values below this level of significance are in italic type. The numbers of haplotypes examined are shown below the diagonal.

overlap with one another and can be hybridized simultaneously in a single experiment.) When all 15 possible sets of two restriction enzymes were surveyed for heterozygosity, a maximum of 91.9% informativeness was achieved by hybridizing the *Bam*HI and *Bgl* II Southern blots (Table 3); these blots would thus be probed with $V_\beta 7$ and $V_\beta 8$, and with C_β. Though not listed, the combination of *Bam*HI and *Kpn* I digests yielded 90.5% heterozygosity. When each of the 20 possible combinations of three restriction digests were examined, *Bam*HI, *Bgl* II, and *Kpn* I blots yielded a maximal level of heterozygosity, 97.3%. No additional heterozygosity was achieved using the remaining restriction digests.

Genetic Linkage Analysis Using the TCRβ Complex. The TCRβ haplotypes constructed from the eight probe/enzyme systems in Table 1 were used for analysis of linkage with 39 markers on chromosome 7 in the CEPH Version 2 genotype data base. Because of the informativeness of the TCRβ haplotypes, statistically significant lod scores (>3.0) resulted from linkage with genetic markers located more than 25 centimorgans (cM) away (by pairwise analyses), e.g., *MET* and *D7S104*. Segregation data from seven 7q markers on CEPH families were also subjected to multipoint analysis. The resulting "most likely map" extended in both directions from the TCRβ complex for a total distance of more than 60 cM (Fig. 3). The map is oriented with the *met* protooncogene homolog centromeric based on its tight linkage with the cystic fibrosis locus (0.4% recombination) (33), which has recently been localized to chromosome 7q31 (34). The TCRβ complex is telomeric to the cystic fibrosis locus, having been physically mapped by *in situ* hybridization to band 7q35 (2).

DISCUSSION

While calculation of linkage disequilibrium cannot always be used to establish the physical distance between markers due to recombination hotspots in the human genome (22), linkage disequilibrium can be helpful in establishing the relative order of markers. Our estimates of linkage disequilibrium between the eight RFLPs roughly correlate with the physical order of these RFLPs as seen in Fig. 2, and as listed in order across Table 2. Perhaps the most surprising pairwise comparison measured is the lack of disequilibrium between the adjacent C_β RFLPs detected with *Bgl* II and *Kpn* I. Despite a known physical separation of 1.8 kb (30), these RFLPs are not in significant linkage disequilibrium. This observation could be explained if a recombination hotspot were located between these two RFLPs. In contrast, the $V_\beta 8$/*Bam*HI and $V_\beta 11$/*Bam*HI polymorphisms showed very significant disequilibrium and are physically separated by approximately 180 kb (7).

While significant disequilibrium occurs between five of the seven adjacent pairs of RFLPs, we noted only one instance of significant disequilibrium between a nonadjacent pair ($V_\beta 8$/*Bam*HI with $V_\beta 15$/*Msp* I) out of 21 comparisons made between nonadjacent pairs. This indicates that while there is indeed measurable linkage disequilibrium within the TCRβ complex, the disequilibrium becomes quite reduced over greater distances within the gene complex, presumably due to hotspots for meiotic recombination. It is likely that careful analysis of any chromosomal region will similarly reveal hotspots for recombination, as revealed by linkage disequilibrium measurements.

Our observations of random association between the majority of the nonadjacent pairs of RFLPs examined herein has important implications for interpreting TCRβ/disease association studies. First, disease association studies that only examine RFLPs within the C region of the TCRβ complex cannot draw conclusions regarding the possibility of susceptibility loci that might lie within all but the most 3′ (i.e., the most proximal) V-region genes. An examination of Table 2 indicates that there has been sufficient shuffling of genes to randomly associate nearly all of the V-region segments with the C-region genes. It is therefore logical to expect that a disease-susceptibility locus located amongst the V-region genes would also be randomly associated with C-region

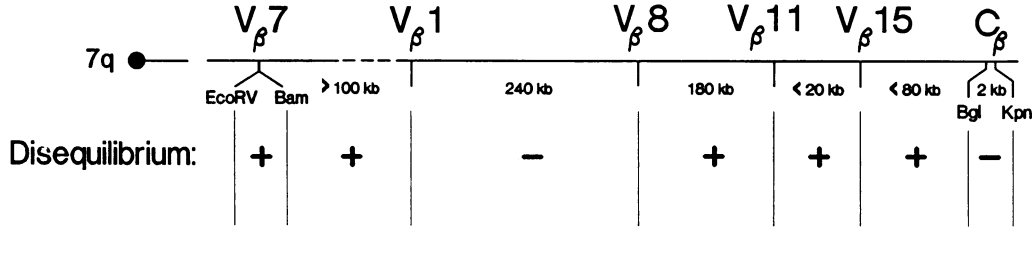

FIG. 2. Organization of TCRβ polymorphisms used in this study as determined by Lai *et al.* (7), Perl *et al.* (30), and unpublished data. The conclusions of linkage disequilibrium between adjacent RFLPs (based on Table 2) are summarized as presence (+) or absence (−) of significant linkage disequilibrium (see Table 2 legend). The $V_\beta 8$/*Bam*HI polymorphism is located near the $V_\beta 8.1$–$V_\beta 8.3$ gene cluster as opposed to the $V_\beta 8.4$–$V_\beta 8.5$ gene cluster, based on relative hybridization strengths (data not shown). The order of the $V_\beta 7$/*Bam*HI and *Eco*RV polymorphisms has not been determined by physical mapping techniques.

Table 3. Most efficient strategy for achieving maximum heterozygosity at the TCRβ locus, based on empirical data

No. of enzymes examined	% observed heterozygosity	Best combination of restriction enzymes
1	82.4	*Bam*HI
2	91.9	*Bam*HI/*Bgl* II
3	97.3	*Bam*HI/*Bgl* II/*Kpn* I
4	97.3	*Bam*HI/*Bgl* II/*Kpn* I/*Eco*RV or *Bam*HI/*Bgl* II/*Kpn* I/*Taq* I or *Bam*HI/*Bgl* II/*Kpn* I/*Msp* I
5	97.3	*Bam*HI/*Bgl* II/*Kpn* I/*Eco*RV/*Taq* I or *Bam*HI/*Bgl* II/*Kpn* I/*Eco*RV/*Msp* I or *Bam*HI/*Bgl* II/*Kpn* I/*Taq* I/*Msp* I
6	97.3	*Bam*HI/*Bgl* II/*Kpn* I/*Eco*RV/*Taq* I/*Msp* I

All possible sets of restriction enzymes noted to detect RFLPs (Table 1) were tested by a computer program for the frequency of heterozygosity in the CEPH parents. Note that *Bam*HI restriction enzyme digests can reveal heterozygosity at $V_\beta 7$, $V_\beta 8$, or $V_\beta 11$, while all other enzyme digests could only be scored as heterozygous with one TCRβ probe tested (Table 1).

RFLPs. Second, because of the random association between most of the V-region gene segments, disease association studies performed on a population-versus-population basis should measure several TCRβ RFLPs dispersed throughout the V-region genes for which linkage disequilibrium relationships are known before conclusions are reached about whether a disease-associated locus exists within this region. In this regard, it would be helpful to identify a novel RFLP located between the polymorphic $V_\beta 1$ and $V_\beta 8$ genes that is in linkage disequilibrium with both of these polymorphisms (see Fig. 2). This would create a contiguous set of polymorphisms in linkage disequilibrium that span more than 600 kb of the TCRβ gene complex, a valuable research tool for investigating the role of the TCRβ genes in human disease. Lastly, the presence of linkage equilibrium between some TCRβ markers predicts that portions of the TCRβ gene complex to either side of an established susceptibility locus may be excluded from containing the susceptibility locus by noting linkage equilibrium between the disease and a DNA polymorphism. We have recently taken this approach for further localizing a multiple sclerosis-susceptibility gene within the TCRβ gene complex (ref. 16 and unpublished data).

For studies that compare the proportion of affected and unaffected siblings sharing TCRβ haplotypes (i.e., sib-pair analysis), as well as for genetic linkage studies, it is imperative that at least one of the two parents of the affected individuals be heterozygous. For this purpose, we have used our extensive TCRβ data base to empirically determine the most efficient experimental design for achieving heterozygosity. Hybridizing three blots (*Bam*HI, *Bgl* II, and *Kpn* I digests) in addition to using probes that span more than 600 kb of the TCRβ gene complex, provided maximum heterozygosity (97.3%). The optimum choice of these three restriction enzyme digests to detect four RFLPs is accounted for by our observation that each of the remaining three polymorphisms is in significant linkage disequilibrium with one of the four RFLPs studied. However, as discussed above, all of these polymorphisms may be useful for population-based disease association studies, for example, after it has been established by sib-pair analysis that there is evidence for a disease-susceptibility locus within the TCRβ complex.

The high level of heterozygosity achieved with RFLPs also allows detection of unmapped genes within or beyond the TCRβ gene complex. Through the use of the TCRβ haplotypes in a genetic linkage analysis, we obtained significant lod scores of linkage between TCRβ and DNA markers up to approximately 30 cM to either side of the TCRβ complex. By virtue of the informativeness of the TCRβ haplotype, it is also possible to statistically exclude (lod < -2.0) unlinked markers from the region surrounding the TCRβ locus. The TCRβ RFLPs will therefore be useful in the continuing effort to construct a high-resolution genetic map in humans.

To summarize, this report on RFLPs within the TCRβ gene complex should be useful for other studies that hypothesize disease-susceptibility loci in the vicinity of this gene complex. The identification of specific regions of linkage disequilibrium among these RFLPs should assist in the design of population-versus-population-based TCRβ/disease association studies. These results point out the necessity of using polymorphisms across the entire TCRβ complex, due to linkage equilibrium within the gene complex. Our recommendations regarding how to most efficiently detect TCRβ heterozygosity should also hasten sib-pair analyses of TCRβ involvement in disease pathogenesis. Based on the growing number of recent reports suggesting that TCRβ germ-line

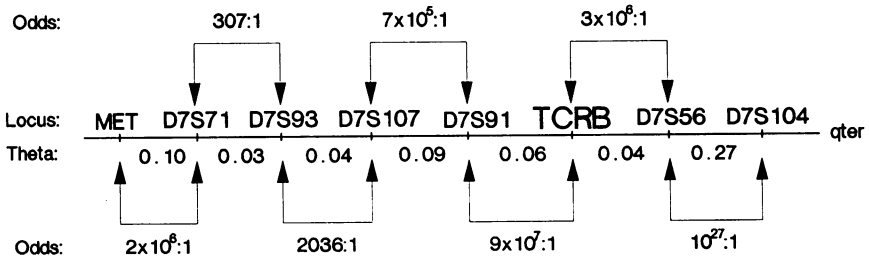

FIG. 3. Genetic map of markers surrounding the TCRβ complex on chromosome 7q, showing the "most likely" order and the sex-independent recombination rates between DNA markers that showed significant linkage (lod > 3) with the TCRβ gene complex (ranging from 6.8 to 29.4). Recombination fraction estimates (theta) are shown between adjacent loci. The odds against inversion of adjacent loci are shown above or below the brackets. The names of the loci detected with anonymous single-copy DNA segments are listed according to their official symbol names (32).

DNA polymorphisms may be associated with human autoimmune diseases (13–20), our observations concerning haplotyping the TCRβ complex by use of RFLPs will hopefully provide a timely source reference.

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