POLYMORPHISM OF HLA-DRB3 AND DRB4 GENES DETECTED BY RFLPs

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INTRODUCTION

All the characterized human HLA class II genes are encoded within the major histocompatibility complex (MHC) on the short arm of chromosome six. Among the five subregions of the class II region, HLA-DR is unique, in that the grouping of the beta chain genes is not the same for all haplotypes. The DRBI gene codes for the serologically detected DR1 to DRw18 antigens, while the DRB2 gene is probably a pseudogene in most haplotypes. The DRw52 and DRw53 supertypic antigens are coded for by two genes, DRB3 and DRB4, which are not allelic and are not present on all haplotypes. There is only one DRA gene which is not polymorphic.

The alleles of the DRB3 locus of the DRw52 family of haplotypes have been extensively analysed and are closely associated with the DRB1 alleles DRw11(5), DRw12(5), DRw13(6), DRw14(6), DRw17(3) and DRw18(3). Three DRB3 alleles have been recognized by a variety of reagents at the DNA, protein, and cellular level (Tiercy et al. 1988, Termijtelen et al. 1988). These alleles are referred to as DRw52a(Dw24), DRw52b(Dw25) and DRw52c(Dw26). The DRB4 gene, which is supertypic to the DR4, DR7 and DR9 antigens, has until now not shown any polymorphism.

In this study we have analysed further the polymorphism of the DRB3 and DRB4 genes using a full-length DRB cDNA probe and the restriction enzyme TaqI.

MATERIALS AND METHODS

Subjects

We studied 92 DRw52 and 33 DRw53 haplotypes in South African (SA) blacks, whites and individuals of mixed ancestry (Cape Coloureds) (du Toit et al. 1988). ALL subjects were HLA typed using standard serological techniques.

Southern Blot Analysis

The Southern Blot technique involved the digestion of genomic DNA with TaqI, electrophoresis in agarose gel followed by

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transfer to a nylon membrane. The membranes were hybridized with the Tenth Histocompatibility Workshop DRB probe, radiolabelled with [α-^{32}P]dCTP (Marcadet et al. 1989).

RESULTS

The DRw52 related haplotypes DR3, DR5, and DRw6, showed the expected DRB1 RFLPs. Table 1 demonstrates the TaqI restriction fragments associated with the DRB3 gene on the various DRw52 haplotypes. In addition to the well documented DRB3 fragments, 10.3 kb (DRw52a or c) and 12.2 kb (DRw52b), a DRB3 fragment of 11.0 kb, which identified a new allele provisionally called DRw52d, was seen to segregate in three families (Martell et al. 1989). This novel fragment was only seen in SA blacks and individuals of mixed ancestry and was always associated with DRw11(5).

Table 1. The TaqI restriction fragments seen with the various DRw52 haplotypes.

Allele	TaqI RFLPs
DRw52a or c	10.3 kb
DRw52b	12.2 kb
DRw52d	11.0 kb

Table 2. Restriction fragments associated with the DRw53 haplotypes, using the DRB probe and TaqI.

	TaqI RFLPs (kb)					
	DRw53		DRw53a		DRw53b	
HLA-DR	14.5	2.8	7.5	2.6	5.8	2.7
4	+	+	+	+	-	-
7	+	+	+	+	-	-
9	+	+	+	+	-	-
4	+	+	-	-	+	+
7	+	+	-	-	+	+
9	+	+	-	-	+	+

Table 2 demonstrates the pattern of TaqI DRB RFLPs seen with the 33 different DRw53 haplotypes. These haplotypes consisted of 19 DR4, 10 DR7 and 4 DR9 haplotypes. All the subjects studied were SA black or of mixed ancestry, with the exception of 4 SA white individuals. The usual DRB1 fragments correlating with DR4 (5.5 kb), DR7 (4.0 kb) and DR9 (4.1 kb)

were observed for the various DRw53 haplotypes. The 14.5 and 2.8 kb fragments were seen in all DRw53 haplotypes and are associated with the DRB4 gene. In addition, four restriction fragments associated only with DRw53 haplotypes occurred in an allelic pattern (Table 2). The first pattern, provisionally designated DRw53a, was characterized by the 7.5 and 2.6 kb fragments and the second, DRw53b, was characterized by 5.8 and 2.7 kb fragments. The patterns clearly segregated in several families (Martell et al. 1989).

CONCLUSION

This study has demonstrated the existence of another allele of the DRB3 gene provisionally designated DRw52d as well as polymorphism of the HLA-DRw53 specificity which is encoded by the DRB4 gene. We have provisionally called these alleles DRw53a and DRw53b. The two allelic DRw53 patterns have previously been described in association only with DR7, and were used to split this specificity into two subtypes (Bidwell 1988). We have observed the same two RFLP patterns in DR4, DR7 and DR9 haplotypes of Negroid origin, indicating these to be as a result of a split of DRw53. It should, however, be noted that several published sequences of DRw53 have not shown polymorphism, and it may be that the RFLPs defining the DRw53 alleles in this study are due to sequence variations in a non-coding portion of the gene, and would thus not be detectable at a product level.

This study demonstrates the importance of the new histocompatibility typing techniques, in order to reveal polymorphic variants not previously detectable and which appear to be specific to populations of SA Negroid origin. The identification of new variants will improve the degree of HLA class II matching in organ transplantation, particularly when genetically unrelated donor-recipient pairs are considered. These new variants may also provide more precise disease susceptibility markers, and will increase the usefulness of the HLA system as a forensic tool.

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