Am J Hum Genet 39:751–762, 1986

RFLP Analysis of HLA-DR and -DQ Genes and Their Linkage Relationships in the Pacific

M. R. J. KOHONEN-CORISH[1] AND S. W. SERJEANTSON

SUMMARY

Human genomic DNA samples from Melanesians, Micronesians, and Caucasoids of known HLA-DR type were examined with cDNA probes for *HLA*-DRα, -DRβ, -DQα, and -DQβ chain genes. DRβ hybridizations with *Taq*I-digested DNA did not detect any new DR specificities in the Pacific. However, within the DR5 specificity a common DNA subtype was found in Pacific Islanders that was not seen in Caucasoids. Altogether, four DNA subtypes of DR5 are described. With the DQα and DQβ probes, significantly more variation could be demonstrated between populations. For example, DR2 was associated with a DQβ *Taq*I pattern in the Pacific that was very rare in Caucasoids and additional RFLP analysis with other enzymes showed that this pattern is probably associated with the Dw12 subtype of DR2. DRw8-positive samples showed two different DQα *Taq*I patterns, and these correlated with DQw1 and DQw3 specificities. DRα hybridizations with *Bgl*II-digested DNA also revealed different linkage relationships of the HLA-class II region genes between Pacific and Caucasoid specimens. The different population linkage disequilibrium relationships have permitted tentative assignment of *Taq*I fragments to either the DRβ1 or DRβ2 genes and are highly suggestive that the DQw1 specificity is encoded by the DQα chain gene. This study shows the value of population comparisons in contributing to knowledge of the genetic organization of the genome.

INTRODUCTION

The histocompatibility class II antigens in man, designated HLA-DP, -DQ, and -DR, have been defined by serological [1] and cellular [2] assays. These cell-

Received March 27, 1986; revised July 23, 1986.

This work was supported in part by grant AM25446 from the National Institutes of Health.

[1] Both authors: Department of Human Biology, John Curtin School of Medical Research, The Australian National University, Canberra, Australia.

 0002-9297/86/3906-0010$02.00

surface antigens are heterodimers, comprising alpha and beta polypeptides that are encoded by separate, but closely linked genes. There are two pairs of alpha and beta genes at DQ and DP [3], although not all genes are expressed [4], while at the DR locus there is only a single alpha gene but a variable number of beta genes [5]. HLA-DP, -DQ, and -DRβ as well as DQα genes are markedly polymorphic in contrast to the DPα and DRα genes that show only limited variability [6, 7]. The HLA-DQ and -DR loci are so closely linked on chromosome 6 that linkage disequilibrium relationships ensure a marked correlation between DR and DQ specificities; the DP gene complex also maps to the short arm of chromosome 6 but is not in linkage disequilibrium with DR and DQ.

The production of cloned MHC class II probes [8, 9] has permitted correlation of restriction fragment length polymorphisms (RFLPs) at DR and DQ loci with known serological HLA-DR antigens [10, 11]. Although it may have been expected that variation in restriction sites in flanking regions and introns of the transcribed class II genes could mask simple correlates, we have reported [12] a remarkably consistent association between *Taq*I fragments of DRβ and DR antigen specificities. Where heterogeneity within a DR specificity does occur, as in *Taq*I fragments of DR3, the RFLPs are consistent for given haplotypes, with B8.DR3 bearing a 10-kilobase (kb) *Taq*I DRβ fragment and B18.DR3 bearing the alternative 12-kb fragment [12].

The correlation between DR types and DRβ RFLPs extends also to DQα [13] and DQβ [12], reflecting the strong linkage disequilibrium in this region of the genome. This linkage disequilibrium is also observed using serological techniques, where in Caucasoids, for instance, DR1-, 2-, and w6-positive cells are almost always DQw1 positive, whereas DR3 and 7 cells are usually DQw2 positive [14]. These DR/DQ relationships can vary in different populations, so that whereas DRw8 is associated with DQw3 or a DQ "blank" in Caucasoids [14], it occurs with DQw1 in Pacific populations [15]. These population differences in linkage relations have been important in establishing the genetic organization of the HLA-D region [16]; in the same way, population comparisons of class II RFLPs may assist in assigning the determinants for the HLA-DQ specificities to the DQ alpha or beta chain genes.

Our study examines *Taq*I class II RFLPs in Melanesians and Micronesians of known HLA-DR types and compares DR/DQ linkage relationships with those observed in Caucasoids.

The HLA-DR antigenic profiles have been described for Melanesians from New Caledonia [17] and for Micronesians from Nauru [18]. These populations lack HLA-DR1, 3, 7, and w10, but may have serological subtypes, as described for HLA-DR5 [15], that are not seen in Caucasoid groups. Evidence for these subtypes in RFLPs is examined in the Pacific Islanders.

MATERIALS AND METHODS

Study Populations

Micronesians from Nauru were studied, as described in [19], when they presented with informed consent at a general medical survey. Melanesians from New Caledonia

were studied during the Second Asia-Oceania Histocompatibility Workshop [15]. Peripheral blood lymphocytes were frozen at −70°C in cryoprecipitate within 12 hrs of collection and subsequently shipped in liquid N or dry ice to Canberra. For 75 of these unrelated Pacific Islanders, as well as for 74 Caucasoid cells from our local control series, HLA serological typing and/or DNA-DR typing [12] was available.

DNA-Digestion and Southern Blots

Genomic DNA was prepared from peripheral blood lymphocytes using standard methods [20]. Approximately 10 μg of DNA was digested with *Taq*I (40 U, Biolabs) at 65°C for 2 hrs, with *Bgl*II (20 U, P-L Pharmacia), *Pvu*II (40 U, P-L Pharmacia), and *Eco*RI (50 U, Boehringer) at 37°C overnight, under conditions recommended by the manufacturer.

Fragments were separated in horizontal electrophoresis through 0.8% agarose gels for 16 hrs in TAE-buffer (0.04 M Tris-acetate; 0.001 M EDTA). DNA was then transferred onto GeneScreen Plus membrane according to manufacturer's (New England Nuclear) specifications following the method of Southern [21]. *Taq*I-digested samples were hybridized with the DRβ, DQα, and DQβ probes and *Bgl*II-digested samples with the DRα probe. DQβ hybridizations were also performed with *Eco*RI- and *Pvu*II-digested specimens and DQα hybridizations with *Eco*RI-digested DNA. The DQα probe cross-hybridized with DXα, yielding two allelic *Taq*I fragments [13] of size 2.3 and 2.2 kb, and the DQβ probe similarly cross-hybridized with a monomorphic *Taq*I DXβ fragment [6].

Probes and Hybridization

cDNA clones for HLA-DRβ and HLA-DQβ [8], HLA-DQα [9], and for HLA-DRα [22] were used. Prehybridization was carried out at room temperature and for 1 hr in a solution containing 10% dextran sulphate, 0.6 M NaCl, 0.18 M Na_2HPO_4, 0.06 M EDTA, 1% sodium lauroyl sarcosine, and 60 μg/ml of sonicated denatured salmon sperm DNA, pH 6.2 [23]. Membranes were then hybridized for at least 40 hrs at 65°C in a similar buffer containing the ^{32}P-labeled probe.

Membranes were washed at 60°C in 2× SSC, 0.1% SDS; 1× SSC, 0.1% SDS; 0.5× SSC, 0.1% SDS; about 30 min in each. Sometimes an additional wash in 0.25× SSC, 0.1% SDS was necessary, if radioactivity remained high (1× SSC = 150 mM NaCl, 15 mM trisodium citrate). Membranes were dried and autoradiographed with intensifying screens for 3–7 days.

RESULTS

There was no evidence from *Taq*I fragments hybridizing with the DRβ chain probe for any new, population-specific HLA-DR alleles; that is, apparent homozygotes in the series were confirmed as homozygotes on Southern blots.

Class II Taq*I Fragments in DR1-, 2-, and w6-Positive Cells*

DRβ probe hybridizations showed no differences in DR2 and DRw6 between Caucasoids and the Pacific specimens. DRw6 shows two DNA subtypes in Caucasoids [12], characterized by 12- or 10-kb fragments that are evident also in the Pacific. The subtype with the 10-kb fragment is more common in Caucasoids (67%), whereas the 12-kb fragment is more frequent (83%) in the Pacific specimens. The DQβ probe hybridized with a 5.5-kb fragment in all DR1

TABLE 1

FREQUENCY (%) OF HLA-DQβ *TaqI* FRAGMENTS ACCORDING TO THE HLA-DR TYPE

	RFLP PATTERN	CAUCASOIDS		PACIFIC	
		No.	%	No.	%
DR1	5.5 kb	10	100	...	...
DR2	3.0 kb	20	83	5	16
	5.5 kb	1	4	1	3
	3.0, 5.5 kb -ve	3	13	26	81
DRw6	3.0 kb	10	48	...	...
	5.5 kb	11	52	13	100

and in 52% of DRw6 haplotypes in Caucasoids as shown in table 1 and in figure 1. In the Pacific, all DRw6-positive specimens showed the 5.5-kb band, whereas in Caucasoids, 48% of DRw6 haplotypes carried the allelic 3.0-kb fragment; that is, the DQβ *TaqI* 5.5-kb band correlated with DRw6 with values r = .50 in Caucasoids and r = .96 in the Pacific.

DR2-positive specimens were associated with three different DQβ *TaqI* patterns, characterized by a 3.0-kb or 5.5-kb fragment or the absence of both of these (table 1 and fig. 1). In Caucasoids, 83% of DR2-positive haplotypes showed the 3.0-kb band; some 50% of the Melanesian DR2 haplotypes but none of the Micronesian samples were associated with this fragment. One DR2-positive Nauruan with European ancestry showed the 5.5-kb band, but other Micronesians as well as half of the Melanesians had neither fragment on the DR2 haplotype. The DQβ *TaqI* 3.0-kb fragment correlated with DR2 with

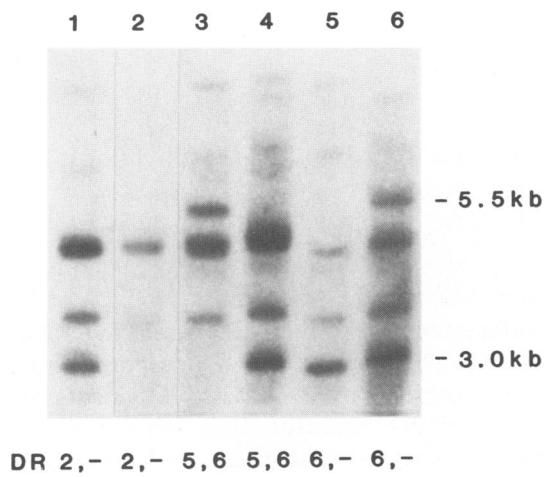

FIG. 1.—HLA-DQβ hybridization of *TaqI*-digested genomic DNA showing the different RFLP patterns detected within DR2 and w6 specificities.

TABLE 2

FREQUENCY (%) OF HLA-DQα *TaqI* FRAGMENTS ACCORDING TO THE HLA-DR TYPE

		CAUCASOIDS		PACIFIC	
	FRAGMENT (kb)	No.	%	No.	%
DR1	3.8, 2.9	10	100	...	...
DR2	6.8	3	13	...	...
	6.2	21	87	36	97
	3.8, 2.9	...	...	1	3
DRw6	6.8	10	48	...	...
	6.2	6	28	...	...
	3.8, 2.9	5	24	13	100
DRw8	6.8	...	...	12	86
	6.2	5	100	2	14
DR3	6.2	1	4	...	...
	5.0	23	96		
DR5	6.2	...	...	25	60
	5.0	13	100	17	40

values r = .76 in Caucasoids and r = .34 in the Pacific. A similar distinction was made by another two restriction enzymes, *Pvu*II and *Eco*RI, in conjunction with the DQβ probe. The 3.0-kb *Taq*I band coincided with a 2.2-kb *Eco*RI band, whereas DR2 haplotypes that were not associated with the 3.0-kb or 5.5-kb *Taq*I bands showed an 18-kb *Eco*RI band.

DQα variants in DR1, 2, and w6 are shown in table 2. Within the DRw6 specificity, the DQα and β patterns are associated so that the DQβ 3.0-kb fragment is always found with the DQα 6.8-kb fragment and the DQβ 5.5-kb fragment with the other two DQα patterns. All known Caucasoid DRw14-positive cells showed the 3.8-kb, 2.9-kb DQα RFLP pattern, thus confirming previous findings [6]. In the Pacific, all DRw6-positive haplotypes showed this RFLP pattern, indicating that DRw14 is more common in these populations than in Caucasoids.

Class II TaqI *Fragments in DR3-, 5-, and w8-Positive Cells*

The DRβ probe hybridized with only one 8.5-kb fragment in DRw8-positive cells both in the Caucasoid and Pacific specimens. Within the DR5 specificity, four DNA subtypes were seen, one of which was clearly population specific. The pattern seen in figure 2 in lane 5 comprised 64% of DR5-positive haplotypes in the Pacific cells but was not seen at all in Caucasoids. We have designated this variant DR5*NAURU and its segregation is shown in a Nauruan family in figure 2. Some 36% of Pacific DR5-positive cells and 85% of Caucasoid haplotypes showed the pattern seen in a heterozygous individual in figure 2, lane 6 (type I). DRβ *Taq*I fragments of the four DNA subtypes of DR5 are summarized in table 3. This heterogeneity in DR5 RFLPs in Caucasoids was not detected in a previous study [6]. The DR5 DNA subtypes resemble the

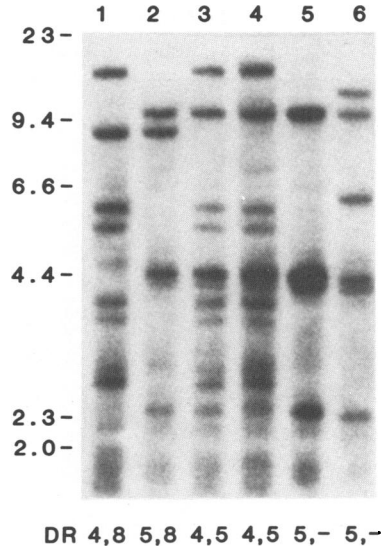

FIG. 2.—HLA-DRβ hybridization of *Taq*I-digested genomic DNA showing segregation of the HLA-DR5*NAURU RFLP pattern in a Micronesian family (*lanes 1* and *4:* parents; *lanes 2* and *3:* children). *Lane 5* shows a homozygote, and *lane 6*, a heterozygote, for this RFLP pattern.

two subtypes of DR3 and w6 with 10-kb and 12-kb fragments. With the DQβ probe, no additional fragments were associated with the DR5 or DRw8 specificity.

DQα variants in DR3-, 5- and w8-positive cells are shown in table 2. Patterns are clearly population specific for DR5 and w8. DR5*NAURU was associated with the 6.2-kb DQα fragment, whereas other DR5 patterns were associated with the 5.0-kb fragment. Some 86% of the Pacific DRw8 haplotypes showed a 6.8-kb DQα fragment, whereas 14% showed the 6.2-kb band as in Caucasoids. The same difference could be demonstrated with another enzyme, *Eco*RI. The 6.8-kb *Taq*I fragment coincided with a 15.5-kb *Eco*RI band, whereas the 6.2-kb *Taq*I band corresponded with a 12.5-kb *Eco*RI fragment, in both DRw8 and DR5*NAURU positive individuals.

TABLE 3

*Taq*I RFLP SUBTYPES OF THE HLA-DR5 SPECIFICITY DETECTED WITH THE HLA-DRβ PROBE

	FRAGMENTS (kb)				
TYPE	12	10	6.0	4.4	4.2
I	+		+		+
II	+			+	
III		+	+		+
5*NAURU		+		+	

TABLE 4

FREQUENCY (%) OF HLA-DRα *Bgl*II FRAGMENTS IN CAUCASOID AND PACIFIC POPULATIONS

Fragment (kb)	Caucasoid (N = 132)	Melanesian (N = 140)	Micronesian (N = 48)
4.5	70	79	94
4.4	18	21	6
3.9, 0.7	12	...	...

NOTE: N = chromosomes.

Class II Taq*I Fragments in DR4-, 7- and w9-Positive Cells*

DRβ hybridization did not reveal any heterogeneity within the DR4 specificity in either the Caucasoid or Pacific cells. However, in DR7, at least four DNA subtypes were seen in Caucasoids. They all shared 15-kb, 5.8-kb, and 4.2-kb fragments, but other fragments varied in size. One variant was similar to the DRw9 pattern. However, with the DQβ probe, a clear difference was seen as this DR7 variant was associated with a 6.7-kb fragment that did not occur in DRw9. One DNA variant was not associated with the 6.7-kb DQβ fragment but showed a 2.9-kb band instead.

With the DQα probe, DR4-, 7-, and w9-positive specimens showed a 5.7-kb fragment and no differences were seen between Caucasoid and Pacific cells. In addition to the DQα fragments mentioned above, two small fragments of 2.3 kb and 2.2 kb were seen in all populations, representing the DXα gene [13].

*HLA-DR*α Bgl*II Fragments in the Pacific*

Distribution of the *Bgl*II fragments hybridizing with the HLA-DRα probe is shown in table 4. The 4.5-kb fragment was the most common in the Caucasoid as well as in the two Pacific populations. In Caucasoids, the 4.4-kb fragment occurred on the A1.B8.DR3 haplotype and on approximately half of the DRw6-positive haplotypes coinciding with the 6.8-kb DQα and 3.0-kb DQβ *Taq*I fragments. The 3.9-kb and 0.7-kb fragments were associated with DR1 and the Bw57.DR7 haplotype, as noted previously [12]. In the Pacific specimens, HLA-DR1 and DR7 antigens as well as the 3.9-kb and 0.7-kb fragments are absent. However, although DR3 is also absent from Pacific populations, the frequency of the 4.4-kb fragment was not reduced in Melanesians, where it was found associated with other DR antigens including examples of DRw6 and w8. In the Micronesian specimens, the 4.4-kb fragment was rare and it was found only in DRw8-positive cells or in specimens showing evidence of foreign admixture in their HLA phenotypes. The DRα probe subdivided the DRw8 specificity so that some showed a 4.4-kb band and others a 4.5-kb band. These RFLP patterns were associated with the two DQα patterns within DRw8 (table 2). The 4.4-kb DRα *Bgl*II fragment coincided with the 6.8-kb DQα *Taq*I fragment, whereas the 4.5-kb DRα *Bgl*II band and the 6.2-kb DQα *Taq*I band co-occurred.

DISCUSSION

The *Taq*I HLA-DRβ RFLPs observed in Melanesians and Micronesians are remarkably similar to those seen in Caucasoids. In the HLA-D region, there is extreme sequence divergence between alleles of DRβ, DQα, and DQβ chain genes, in the noncoding as well as the coding regions, and it may have been expected that at least some of this allelic variability had accumulated after divergence of the races. Admittedly, this study was largely dependent on use of a single restriction endonuclease, *Taq*I, but this enzyme has consistently proved one of the most informative in detecting RFLPs in man [24], since sites containing CpG are the most polymorphic [25]. Sequence data in Caucasoids for DQα-associated alleles DQw1 [26] and DQw2 [27] show 23 differences in amino acids [6], and, in general, as many as 10%–20% of the amino acid residues in the α_1 and β_1 domains of class II polypeptides vary between alleles [28]. These regions of extreme variability are accompanied, in the MHC, by extensive variability in noncoding regions, as has been shown for class II genes in laboratory and wild mice [29].

Thus, the correlation of HLA-DR types with class II RFLPs is largely dependent on linkage disequilibrium between restriction sites and the DNA coding for the DR and DQ antigens. It follows that the correlation between HLA-DR antigens and RFLPs is expected to be more homogeneous between populations when using the DRβ chain probes, compared with DRα, DQα, and DQβ probes. This has proved true in our comparison of RFLPs in Caucasoids and Pacific Islanders, where at DRβ, only one antigen, DR5, shows a variant *Taq*I fragment pattern that is not seen in Caucasoids. This DR5 subtype, DR5*NAURU, shows the same RFLP pattern as a Chinese DR5/Dw blank-typing cell [12], and our unpublished data suggest this variant is widespread in Chinese.

At DQα and DQβ, no new *Taq*I fragments were detected in Micronesians or Melanesians, but the correlation of these fragments with DR type varied markedly between populations, reflecting different linkage disequilibrium relationships between DRβ and DQα, DQβ. These different linkage arrangements had been suggested earlier by mixed lymphocyte cultures (MLC) where MLC heterogeneity was seen within DR2 and DR5 in Melanesians [30]. For instance, HLA-Dw2 and -Dw12 cells are mutually stimulatory in MLCs, for although they share HLA-DR2 antigenic determinants, there are antigenic differences coded by the second DR beta chain gene [31] and possibly also by the DQ locus. These MLC subtypes of DR2 have different population distributions, with Dw12 rare in Caucasoids but common in Oriental populations [32] and in coastal Melanesians [33]. The *Taq*I DQβ RFLPs reflect these different DR2 associations with DQ. Whereas most Caucasoid DR2-positive cells showed a 3.0-kb DQβ *Taq*I fragment, most Pacific DR2 cells were missing this as well as the allelic 5.5-kb fragment. Several restriction enzymes detect DQβ RFLP patterns characteristic for Dw subtypes of DR2 [34]. For example, *Eco*RI fragments 2.2 kb and 18 kb correspond with Dw2 and Dw12, respectively. We

found this difference can also be demonstrated with *Taq*I. Although the appropriate MLC typings have not been done, our data show that all DR2-positive Micronesians have the *Taq*I DQβ fragments associated with Dw12, while island Melanesians have both Dw2- and Dw12-associated patterns.

Population differences in DR/DQ linkage relationships have been recognized previously in serological assays. For instance, HLA-DR5 is associated with DQw3 in Caucasoids but with DQw1 in African blacks [35]. Similarly, HLA-DRw8 is associated with DQw3 or a DQ "blank" in Caucasoids, with DQ "blank" in Amerindians [36] but with DQw1 in Melanesians [15], Micronesians, and Australian Aborigines [37]. Examination of serological patterns in populations with different linkage relations has permitted assignment of class II antigenic specificities to DR or DQ loci. As yet, it has not been possible to discriminate between the DQα and β chain genes as the determinants of DQ serological specificities. The RFLPs in Pacific cells, however, are highly suggestive that the DQα gene encodes DQw1 and DQw3. For instance, the 6.2-kb *Taq*I fragment in DQα is found in Caucasoid DRw8-positive cells that are DQw3 positive, but not in Pacific DRw8 cells that are DQw1 positive. Instead, these DRw8 cells have a 6.8-kb fragment. Similarly, Spielman et al. [13] described an HLA-DR5 cell line, FpF, that is DQw1 positive and shows the characteristic 6.8-kb fragment. The DR5*NAURU variant, known from our serological studies to be linked with DQw3, has the 6.2-kb fragment associated with Caucasoid DQw3, DRw8-positive cells.

Assignment of the DQw1 and DQw3 specificities of the DQα chain is enforced further by the *Eco*RI hybridization results. DRw8 in Caucasoids shows a characteristic 12.5-kb fragment, but in the Pacific, has the 15.5-kb fragment found in DQw1-positive cells. Clearly, further studies with additional restriction enzymes and with additional populations are required to unravel the correlations between DQ serological specificities and DQα, DQβ RFLPs.

In the same way that population comparisons have permitted tentative assignment of DQ specificities to the DQα chain, our population comparisons of DR5 RFLPs suggest tentative assignment of DRβ *Taq*I fragments to the β1 and β2 DR genes. In Caucasoids and Pacific Islanders, a 15-kb *Taq*I fragment invariably occurs in DRw53-positive cells. However, assignment of fragments to DRw52-positive cells (usually DR3-, 5-, w6-, or w8-positive) has proved more difficult because of *Taq*I heterogeneity in DR3 and DRw6 cells. Comparison of DR5 RFLPs in the Pacific with those in Caucasoids clearly shows two characteristic patterns for DR5 (6.0, 4.2, or 4.4 kb) that occur with either a 10- or 12-kb fragment. Similarly, the characteristic DR3 and DRw6 RFLPs occur in conjunction with either a 10- or 12-kb *Taq*I DRβ fragment, suggesting that these larger fragments correlate with DRw52.

In contrast to the extensive polymorphism associated with DQα, DRα is highly conserved. The murine equivalent, I-Eα, also shows only minor allelic variation and is associated with very little variation in noncoding DNA [29]. The same situation is true in man as Trowsdale et al. [6] point out, for otherwise DRα probes would detect as much variation as DQα probes. This study

confirms that DRα and associated regions are highly conserved, by finding only limited polymorphism in Melanesians and Micronesians even at the *Bgl*II sites that are known to occur in the 3'UT region [38].

Finally, this study has described DNA correlates of HLA-DR, DQ, and Dw phenotypes in populations where the logistics of HLA typing has been formidable in the past, since viable lymphocytes are required for serological and MLC typing. Use of a single restriction endonuclease, *Taq*I, and three cDNA probes has revealed greater genetic diversity in the populations studied than would be possible using currently available serological reagents. Study of additional populations in Asia and the Pacific using HLA class II RFLPs will permit more detailed analysis of the complexities of the genetic interrelationships in the region.

ACKNOWLEDGMENTS

We thank Dr. B. Mach, University of Geneva, for providing the cDNA probes for DRβ and DQβ; Dr. D. Larhammar, University of Uppsala, for the cDNA DQα probe; and Dr. H. Erlich, Cetus Corporation, for the cDNA DRα probe.

REFERENCES

1. Tosi R, Nobuyuki T, Centis D, Ferrara GB, Pressman D: Immunological dissection of human Ia molecules. *J Exp Med* 148:1592–1611, 1978
2. Shaw S, Kavathas P, Pollack MS, Charmot D, Mawas C: Family studies define a new histocompatibility locus, SB, between HLA-DR and GLO. *Nature* 293:745–749, 1981
3. Bodmer WF: The HLA system, 1984, in *Histocompatibility Testing 1984*, edited by Albert ED, Baur MP, Mayr WR, Berlin, Springer-Verlag, 1984, pp 11–22
4. Servenius B, Gustafsson K, Widmark E, et al.: Molecular map of the human HLA-SB (HLA-DP) region and sequence of an SBα (DPα) pseudogene. *EMBO J* 3:3209–3214, 1984
5. Böhme J, Andersson M, Andersson G, Möller E, Peterson PA, Rask L: HLA-DRβ genes vary in number between different DR specificities, whereas the number of DQβ genes is constant. *J Immunol* 135:2149–2155, 1985
6. Trowsdale J, Young JAT, Kelly AP, et al.: Structure, sequence and polymorphism in the HLA-D region. *Immunol Rev* 85:5–43, 1985
7. Cohen D, Paul P, Le Gall I, et al.: DNA polymorphism of HLA class I and class II regions. *Immunol Rev* 85:87–105, 1985
8. Long EO, Wake CT, Strubin M, et al.: Isolation of distinct cDNA clones encoding HLA-DRβ chains by use of an expression assay. *Proc Natl Acad Sci USA* 79:7465–7469, 1982
9. Schenning L, Larhammar D, Bill P, et al.: Both α and β chains of HLA-DC class II histocompatibility antigens display extensive polymorphism in their amino-terminal domains. *EMBO J* 3:447–452, 1984
10. Owerbach D, Lernmark Å, Rask L, Peterson PA, Platz P, Svejgaard A: Detection of HLA-D/DR-related DNA polymorphism in HLA-D homozygous typing cells. *Proc Natl Acad Sci USA* 80:3758–3761, 1983
11. Hui K, Festenstein H, de Klein A, Grosveld G, Grosveld F: HLA-DR genotyping by restriction fragment length polymorphism analyses. *Immunogenetics* 22:231–239, 1985
12. Kohonen-Corish MRJ, Serjeantson SW: HLA-DRβ gene DNA polymorphisms revealed by TaqI correlate with HLA-DR specificities. *Hum Immunol* 15:263–271, 1986

13. SPIELMAN RS, LEE J, BODMER WF, BODMER JG, TROWSDALE J: Six HLA-D region α-chain genes on human chromosome 6: polymorphisms and associations of DCα-related sequences with DR types. *Proc Natl Acad Sci USA* 81:3461–3465, 1984
14. BAUR MP, NEUGEBAUER M, ALBERT ED: Reference tables of two-locus haplotype frequencies for all MHC marker loci, in *Histocompatibility Testing 1984,* edited by ALBERT ED, BAUR MP, MAYR WR, Berlin, Springer-Verlag, 1984, pp 677–755
15. SERJEANTSON SW, HELIAS M, LE GONIDEC G, NEWLANDS R: Melanesians from New Caledonia, in *Proceedings of the Second Asia and Oceanic Histocompatibility Workshop Conference,* edited by SIMONS MJ, TAIT BD, Toorak, Australia, Immunopublishing, 1983, pp 299–309
16. ALBERT ED, BAUR MP, MAYR WR: Introductory remarks, in *Histocompatibility Testing 1984,* edited by ALBERT ED, BAUR MP, MAYR WR, Berlin, Springer-Verlag, 1984, pp 1–3
17. SERJEANTSON SW, RYAN DP, ZIMMET P, ET AL.: HLA antigens in four Pacific populations with non-insulin-dependent diabetes mellitus. *Ann Hum Biol* 9:69–84, 1982
18. SERJEANTSON SW, KIRK RL, RANFORD P, BEAUCHAMP M: HLA antigens and non-HLA chromosome 6 markers in Micronesians from Nauru. *Tissue Antigens* 22:49–58, 1983
19. SERJEANTSON SW, OWERBACH D, ZIMMET P, NERUP J, THOMA K: Genetics of diabetes in Nauru: effects of foreign admixture, HLA antigens and the insulin-gene-linked polymorphism. *Diabetologia* 25:13–17, 1983
20. MANIATIS T, FRITSCH EF, SAMBROOK J: *Molecular Cloning. A Laboratory Manual.* Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory, 1982
21. SOUTHERN EM: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98:503–517, 1975
22. STETLER D, DAS H, NUNBERG JH, ET AL.: Isolation of a cDNA clone for the human HLA-DR antigen α chain by using a synthetic oligonucleotide as a hybridization probe. *Proc Natl Acad Sci USA* 79:5966–5970, 1982
23. NASMYTH KA: The regulation of yeast mating-type chromatin structure by *SIR:* an action at a distance affecting both transcription and transposition. *Cell* 30:567–578, 1982
24. FEDER J, YEN L, WIJSMAN E, ET AL.: A systematic approach for detecting high-frequency restriction fragment length polymorphisms using large genomic probes. *Am J Hum Genet* 37:635–649, 1985
25. BARKER D, SCHAFER M, WHITE R: Restriction sites containing CpG show a higher frequency of polymorphism in human DNA. *Cell* 36:131–138, 1984
26. AUFFRAY C, LILLIE JW, ARNOT D, GROSSBERGER D, KAPPES D, STROMINGER JL: Isotypic and allotypic variation of human class II histocompatibility antigen α-chain genes. *Nature* 308:327–333, 1984
27. CHANG HC, MORIUCHI T, SILVER J: The heavy chain of human β-cell alloantigen HLA-DS has a variable N-terminal region and a constant immunoglobulin-like region. *Nature* 305:813–815, 1983
28. STEINMETZ M, HOOD L: Genes of the major histocompatibility complex in mouse and man. *Science* 222:727–733, 1983
29. STEINMETZ M, MALISSEN M, HOOD L, ET AL.: Tracts of high or low sequence divergence in the mouse major histocompatibility complex. *EMBO J* 3:2995–3003, 1984
30. HONEYMAN M, BASHIR H, GRIMM P: HLA-D workshop: cellular typing, in *Proceedings of the Second Asia and Oceania Histocompatibility Workshop Conference,* edited by SIMONS MJ, TAIT BD, Toorak, Australia, Immunopublishing, 1983, pp 231–260
31. SONE T, TSUKAMOTO K, HIRAYAMA K, ET AL.: Two distinct class II molecules encoded by the genes within the HLA-DR subregion of HLA-Dw2 and Dw12 can act as stimulating and restriction molecules. *J Immunol* 135:1288–1298, 1985
32. OHTA N, MUTOH M, KIKUCHI I, EGAMI M, NISHIMURA YK, SASAZUKI T: HLA-DR2 related HLA-D specificities, Dw2 and Dw12, in the Japanese population, in *Pro-*

ceedings of the Second Asia and Oceania Histocompatibility Workshop Conference, edited by SIMONS MJ, TAIT BD, Toorak, Australia, Immunopublishing, 1983, pp 457–458

33. CRANE G, BHATIA K, HONEYMAN M, ET AL.: HLA studies of highland and coastal New Guineans. *Hum Immunol* 12:247–260, 1985
34. NOREEN H, SCHLUENDER L, BACH FH, SEGALL M: Patterns of DNA RFLP characteristic for Dw subtypes of DR2 (abstr.). *Hum Immunol* 14:194, 1985
35. OUDSHOORN M, SCHREUDER GMT, CAMPBELL EM, DU TOIT ED: Segregation of DQ and DR: exceptions to the rule, in *Histocompatibility Testing 1984,* edited by ALBERT ED, BAUR MP, MAYR WR, Berlin, Springer-Verlag, 1984, pp 419–420
36. LAYRISSE Z, CARREÑO B, SVEJGAARD A, PLATZ P, RYDER LP, KRISTENSEN T: First level testing of Dw8 HTC, in ibid., pp 278–281
37. TERMIJTELEN A, BOETTCHER B, BRADLEY BA, D'AMARO J, VAN LEEUWEN A, VAN ROOD JJ: DR typing in Australian Aborigines. An indication for a second locus in the HLA-D region defined by serology. *Tissue Antigens* 16:140–146, 1980
38. ERLICH H, STETLER D: HLA class II DNA polymorphisms: markers for genetic predisposition to insulin-dependent diabetes, in *Genetic Variability in Responses to Chemical Exposure,* Banbury Report 16, edited by OMENN G, GELBOIN H, Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory, 1984, pp 321–331