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Polymorphic restriction endonuclease sites linked to the *HLA-DRα* gene: Localization and use as genetic markers of insulin-dependent diabetes

(*HLA* class II loci/restriction fragment length polymorphism)

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ABSTRACT **Polymorphic restriction endonuclease sites within the *HLA-DRα* gene have been defined, localized, and used as genetic markers in the analysis of susceptibility to insulin-dependent diabetes mellitus (IDDM). Hybridization of *Bgl* II-digested human genomic DNA with a cDNA clone for the HLA-DR α chain (pDRα-1) has revealed three allelic restriction fragment lengths: 3.8 kilobase pairs (kb), 4.2 kb, and 4.5 kb. Hybridization of *Eco*RV-digested human genomic DNA with the same probe has revealed two allelic polymorphic restriction fragment lengths: 9.2 kb and 13.0 kb. By analysis of double digests of genomic DNA from individuals homozygous for each of the allelic variants, the polymorphic restriction sites were found to be clustered near the 3′ end of the *HLA-DRα* gene. The observed correlations of *DRα Bgl* II restriction site variants with serologically determined DR specificities suggest linkage disequilibrium between the *DRα* and *DRβ* loci. The 3.8-kb fragment is correlated with the DR1 type ($P_c = 4.4 \times 10^{-4}$); and the 4.2-kb fragment, with a subset (B8,DR3) of the DR3 type ($P_c = 5.1 \times 10^{-4}$) and with the DR6 type. The segregation pattern of *HLA-DRα* polymorphic *Bgl* II restriction fragments was analyzed in six IDDM families. The observed association of IDDM with the *Bgl* II 4.2-kb *DRα* restriction variant is higher than with existing serological markers and supports the utility of this approach in elucidating IDDM inheritance.**

The human major histocompatibility complex (MHC, or the *HLA* region), located on the short arm of chromosome 6, encodes a number of different cell surface glycoproteins that mediate a variety of immunological functions. These glycoproteins have been divided into two classes on the basis of structure, tissue distribution, and function. The class I molecules, the classical transplantation antigens encoded by the *HLA-A*, *-B*, and *-C* loci, are found on the surface of all nucleated cells and function as targets in T-cell recognition. In contrast, the class II molecules, the products of the *HLA-D/DR* region, are found primarily on the surface of B lymphocytes, macrophages, and activated T cells and mediate immune responsiveness due to their role in T- and B-cell cooperation and in antigen presentation (reviewed in refs. 1 and 2). The HLA-DR antigens defined by alloantisera are cell surface heterodimers composed of a relatively nonpolymorphic 34-kDa heavy chain (α) and a polymorphic 29-kDa light chain (β) (reviewed in ref. 1), as well as a 31-kDa invariant chain (I_i or DRγ), encoded on chromosome 5 (unpublished data) that is associated with the α and β chains during biosynthesis (1, 3–5). Genetic studies involving the HLA-DR α chain have been difficult due to the limited polymorphism revealed by current analytic techniques; however, cDNA clones for the HLA-DR α chain have recently been obtained by several groups (6–10). These clones allow the definition of DNA sequence polymorphisms which can serve as valuable markers in genetic analysis.

Genetic susceptibilities to a variety of diseases show significant association with specific serologically defined HLA types (reviewed in ref. 2). Insulin-dependent diabetes mellitus (IDDM) represents one of the most frequent and severe of the *HLA*-associated diseases and is associated with the HLA-DR3 and -DR4 specificities (11). However, the genetic heterogeneity of serologically defined DR types (e.g., DR4) has been demonstrated by cellular typing as well as by two-dimensional gel analysis of DR β chain electrophoretic mobilities (12, 13). DNA polymorphisms in the *HLA* class II loci are potentially capable of subdividing the relevant serologic types (e.g., DR3 and DR4 for IDDM) in the population and offer a new approach to examining the association between disease susceptibility and HLA-DR type.

Here we define several DNA sequence polymorphisms within and closely linked to the DR α-chain gene (*DRα*), detected as restriction fragment length polymorphisms (RFLPs) of human DNA digested with the enzymes *Bgl* II and *Eco*RV. The RFLP distribution in control and IDDM populations and the segregation analysis of polymorphic restriction fragments with IDDM is presented, as well as the association of specific fragments with individual HLA-DR specificities. These studies illustrate the general applicability and utility of RFLPs as markers in genetic analysis, particularly for those loci whose products show limited serological or biochemical polymorphism.

MATERIALS AND METHODS

Genomic Blotting. High molecular weight DNA prepared from B-lymphoblastoid cell lines was digested with *Bgl* II or *Eco*RV under conditions suggested by the supplier, electrophoresed in a 0.6% agarose gel, and transferred to Genatran 45 (Plasco). The filters were hybridized as described with pDRα-1 DNA (6) (labeled *in vitro* by nick-translation to a specific activity of 4×10^8 cpm/μg), washed as described (6), and analyzed by autoradiography. The hybridization probe pDRα-1 contains a 1070-base-pair (bp) insert encoding 13 amino acids of the HLA-DR α-chain leader peptide, the entire mature protein (229 amino acids), and 344 bp of 3′ untranslated sequence.

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Abbreviations: IDDM, insulin-dependent diabetes mellitus; RFL, restriction fragment length; RFLP, RFL polymorphism; bp, base pair(s); kb, kilobase pair(s).

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RESULTS

To identify RFLPs associated with the *HLA-DRα* gene, genomic DNA from a variety of individuals was digested with nine different endonucleases, electrophoresed, and analyzed by blot hybridization with the pDRα-1 probe. The number of different individual DNAs tested by a given restriction endonuclease varied from 2 to 10. The enzymes *Hin*dIII, *Eco*RI, *Kpn* I, *Msp* I, *Xho* I, *Pst* I, and *Pvu* II failed to reveal any RFLPs, but digestion with *Bgl* II and *Eco*RV revealed the presence of three polymorphic restriction fragments [3.8 kilobase pairs (kb), 4.2 kb, and 4.5 kb] and two polymorphic restriction fragments (9.2 kb and 13.0 kb), respectively. The genomic-blotting analysis of *HLA* deletion variants, homozygous typing cells, and families indicated that each polymorphic fragment length represents an allele of the single-locus *HLA-DRα* gene.

Bgl II RFLP analysis of a representative family, indicating cosegregation of specific polymorphic restriction fragments with unique parental *HLA* haplotypes, is shown in Fig. 1*A*. The intrafamilial distribution of restriction fragments shows that the paternal 3.8-kb fragment segregates with the *b* haplotype, and the maternal 4.5-kb fragment, with the *d* haplotype; the maternal 4.2-kb fragment segregates with *c*, and the paternal 4.2-kb fragment, with *a*. In 20 other families similarly analyzed (Fig. 4 and data not shown), the *Bgl* II restriction fragments segregate with unique parental *HLA* haplotypes. The results of Southern blot analysis of DNA from a heterozygous cell line and its derived mutant with a hemizygous deletion of the MHC are also consistent with both the allelic nature and *HLA* linkage of the polymorphic *Bgl* II fragments (14).

Localization of Polymorphic Restriction Sites. *Pst* I digestion of the pDRα-1 cDNA insert yields a 5′ fragment of 525 bp and a 3′ fragment of 565 bp. To define the arrangement of the polymorphic *Bgl* II restriction sites, genomic DNA from individuals homozygous for each of the three restriction fragment length (RFL) allelic variants (see above) was digested with *Bgl* II and *Pst* I. Genomic blots of the *Bgl* II/*Pst* I double digests were hybridized with ^{32}P-labeled intact pDRα-1, 5′ *Pst* I fragment of pDRα-1, and 3′ *Pst* I fragment of pDRα-1. DNA from all three RFL types yielded a 2.6-kb *Bgl* II–*Pst* I genomic fragment, which hybridized to the 5′ probe, and another genomic fragment, which varies in size with each RFL type and which hybridized to the 3′ probe (Fig. 2). Thus, a *Bgl* II site at the 5′ end of the *DRα* gene is conserved, in contrast to polymorphic *Bgl* II sites at the 3′ end of the gene.

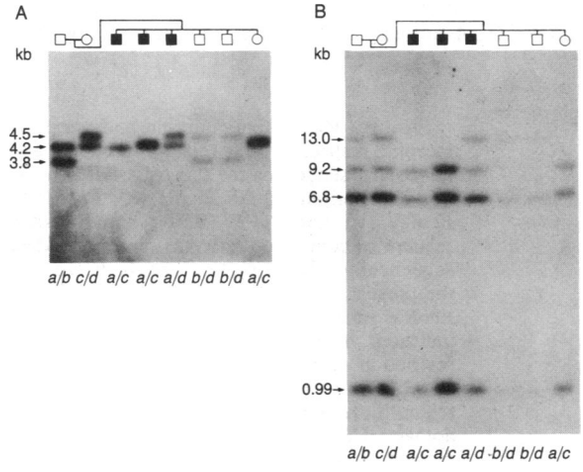

FIG. 1. RFLPs linked to the *HLA-DRα* gene. DNA was prepared from Epstein–Barr virus-transformed lymphocytes from IDDM family 4 (17), digested with *Bgl* II (*A*) or *Eco*RV (*B*) and analyzed by blot hybridization with the pDRα-1 probe. IDDM-affected individuals are denoted by filled boxes. Haplotypes are given below the lanes: *a* = *HLA-A1,B8,DR3*; *b* = *HLA-A2,Bw35,DR*$^-$; *c* = *HLA-A9-(24),B18, DR6*; *d* = *HLA-A2,B7,DR4*.

The precise location of the polymorphic *Bgl* II sites relative to coding and noncoding sequences was determined by Southern blot analysis of *Bgl* II and *Bgl* II/*Eco*RI-digested DNA of the 3.8-kb RFL type (from the homozygous typing cell 1BW4 and the B-lymphoblastoid line CA-SC) and comparison of this genomic blot pattern with published sequence data from a *DRα* genomic clone (15, 16). The *Bgl* II digestion yielded a 3.8-kb fragment and a 760-bp fragment; combined *Bgl* II/*Eco*RI digestion yielded a 2.5-kb fragment and a 600-bp fragment (Fig. 2). This result defines an *Eco*RI site within the 760-bp *Bgl* II fragment and located 600 bp from one end of the fragment. Inspection of the nucleotide sequence of a *DRα* genomic clone (16) reveals two sites at which a single nucleotide substitution could generate *Bgl* II recognition sequences, resulting in the observed 3.8- and 4.2-kb variants. The sequence of a DR α-chain cDNA clone from the cell line JY was, in fact, reported to contain a *Bgl* II recognition sequence in the site that would generate the 4.2-kb variant (15). The location of the 3′ *Bgl* II sites for the three RFL types relative to coding and noncoding sequences and *Eco*RI sites is shown in Fig. 3. Thus, all the polymorphic *Bgl* II sites are clustered in a region of ≈800 bp near the 3′ end of the *DRα* gene.

Digestion of genomic DNAs with *Eco*RV revealed two nonpolymorphic restriction fragments (0.99 kb and 6.8 kb) and two polymorphic restriction fragments (13.0 kb and 9.2 kb) that hybridized to pDRα-1. Family studies and Southern blot analysis of the hemizygous deletion mutant 6.3.6 and its parental line T5-1 (14) established that the 13.0-kb and 9.2-kb fragments represented allelic variants, since they segregated with unique parental haplotypes (Fig. 1*B* and data not shown). The relationship of polymorphic and nonpolymorphic *Eco*RV sites to *Bgl* II sites and to coding and noncoding sequences was determined by hybridization with 5′ and 3′ pDRα-1 probes and is shown in Fig. 3, with the polymorphic *Eco*RV site localized near the 3′ end of the *DRα* gene, as are the polymorphic *Bgl* II sites.

Use of Polymorphic Restriction Sites as Genetic Markers. Table 1 shows the frequency of the *Bgl* II and *Eco*RV alleles separately as well as that of *Bgl* II/*Eco*RV haplotypes in a small panel of homozygous typing cells (nine cell lines) and a larger population of unrelated control individuals. The *Bgl* II 3.8-kb,*Eco*RV 13.0-kb type and the *Bgl* II 4.2-kb,*Eco*RV 9.2-kb type are more frequent than would be expected assuming random association, demonstrating that these combinations of alleles are in positive linkage disequilibrium. Similarly, the *Bgl* II 3.8-kb,*Eco*RV 9.2-kb type and the *Bgl* II 4.2-kb,*Eco*RV 13.0-kb type are less frequent than would be expected by random association.

Using *DR* haplotype information from homozygous typing cells, family segregation patterns, *HLA*-deletion variants, and informative individuals, we examined the correlation between a particular *Bgl* II type and *HLA-DR* type among 40 unrelated chromosomes (Table 2). The 3.8-kb fragment was associated with the DR1 specificity ($P_c = 4.4 \times 10^{-4}$); and the 4.2-kb fragment, with a specific subset of the DR3 specificity [the *B8,DR3* haplotype ($P_c = 5.1 \times 10^{-4}$)] and with the DRw6 specificity. The higher-frequency ($f = 0.68$), 4.5-kb fragment failed to show significant association with any particular DR type. For several HLA-DR types, the RFL type is capable of subdividing the serological specificity. For example, for HLA-DR3 in normal individuals, 6/15 chromosomes are the 4.2-kb type, whereas 9/15 are the 4.5-kb type.

The use of RFLPs as genetic markers in family studies is illustrated in Fig. 1 *A* and *B*, as previously discussed. This pedigree is particularly informative because siblings 1, 2, and 3 are all affected with IDDM. Fig. 1*A* shows that all affected individuals have received the 4.2-kb fragment from one or both

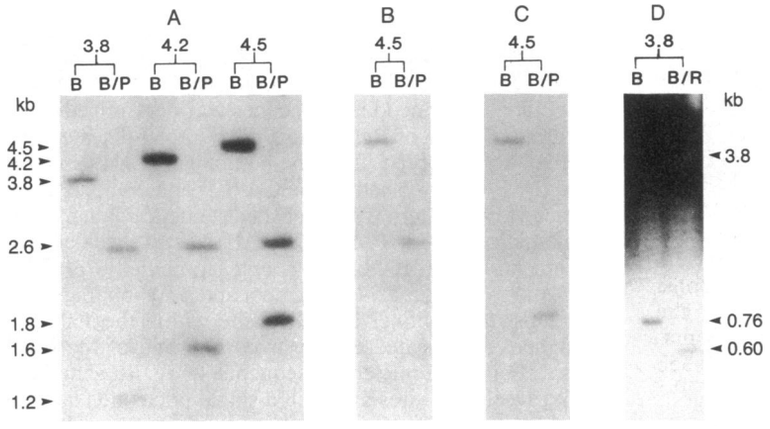

FIG. 2. Localization of *Bgl* II *DRα* polymorphism. (*A–C*) Genomic DNA from the 3.8-, 4.2-, and 4.5-kb *Bgl* II *HLA-DRα* types was digested with *Bgl* II and *Pst* I (lanes B/P) or *Bgl* II alone (lanes B) and analyzed by Southern blotting. Either intact ^{32}P-labeled pDRα-1 (*A*), the 5′ *Pst* I fragment of pDRα-1 (*B*), or the 3′ *Pst* I fragment of pDRα-1 (*C*) were used as hybridization probes. (*D*) Genomic DNA from the 3.8-kb *Bgl* II *HLA-DRα* type was digested with *Bgl* II and *Eco*RI (lane B/R) or *Bgl* II alone (lane B) and analyzed by blotting and hybridization to a ^{32}P-labeled 3.2-kb *Eco*RI genomic fragment containing all but the first exon of the *HLA-DRα* gene (16).

parents. The paternal chromosome containing the 4.2-kb fragment is *HLA-DR3*; the maternal chromosome with the 4.2-kb fragment is *HLA-DRw6*. The presence of the 4.2-kb fragment in all affected individuals suggests that this fragment may be linked to a disease susceptibility gene for IDDM; however, not all individuals with the fragment are affected (e.g., sibling 6). In a similar manner, segregation analysis of this family and five additional IDDM families (ref. 17 and Fig. 4) was used to calculate the restriction fragment frequencies and *DR* haplotype assignments shown in Tables 1 and 2, respectively. The allele frequency of the *Bgl* II 4.2-kb fragment is increased in the IDDM probands relative to the control population (Table 1) and is increased in *DR3* chromosomes of IDDM patients (4/5) relative to control *DR3* chromosomes (6/15) (Fig. 4). The four IDDM *DR3* haplotypes with the 4.2-kb marker are *B8,DR3* while the exceptional IDDM *DR3* haplotype lacking the 4.2-kb marker (family 7, Fig. 4) is *B18,DR3*. The frequency of IDDM probands containing the 4.2-kb *Bgl* II fragment (0.83) is also greater than the frequency of control individuals with the 4.2-kb fragment (0.29) (Table 1). The relative risk (RR) (2) calculated from these data (RR for 4.2 kb = 11.8; RR for *DR3* = 5.5) is higher than any other single gene marker for IDDM and is consistent with the preliminary results from a larger study (data not shown).

DISCUSSION

Polymorphic restriction sites within or linked to the 3′ end of the *HLA-DRα* gene, detected as RFLPs, have been identified and localized for the enzymes *Bgl* II and *Eco*RV. The observed frequency of some of the *Bgl* II, *Eco*RV RFLP haplotypes reflects strong linkage disequilibrium between these markers. Analysis of *DRα* RFLP in a population of *HLA-DR*-typed individuals has also revealed correlations between RFL variants and serologically defined *HLA-DR* types. The *Bgl* II allelic variant 3.8 kb is associated with *HLA-DR1* and the *Bgl* II allelic variant 4.2 kb is associated with *HLA-DR3* and *DRw6* (Table 2). Furthermore, *Bgl* II RFLPs are capable of subdividing *HLA* haplotypes bearing a particular DR specificity. The strength of the association between particular DR specificities and the polymorphic *HLA-DRα* restriction fragment presumably reflects the linkage disequilibrium between the *DRα* marker and the loci encoding the *DRβ* chains. In fact, a recently identified *DRβ* *Taq* I RFLP is in strong linkage disequilibrium with the 4.2-kb *DRα* fragment, with a subset of *DR3 (B8,DR3)* and *DRw6* specificities, and with IDDM (unpublished data).

It is likely that genetic markers capable of subdividing the DR3 and DR4 types, associated with IDDM, should prove to be better predictors of disease susceptibility. The observation that the majority of DR3 and DR4 individuals do not develop IDDM, and the lack of complete penetrance in monozygotic twin studies (18), indicates that *HLA*-linked genes predispose to, rather than cause, IDDM. In addition, the relatively low proportion of DR3 and DR4 individuals who develop IDDM may reflect the genetic heterogeneity of the DR types. In two recent studies (19, 20), incorporating the results of *HLA*-linkage analysis, *HLA*-haplotype distribution

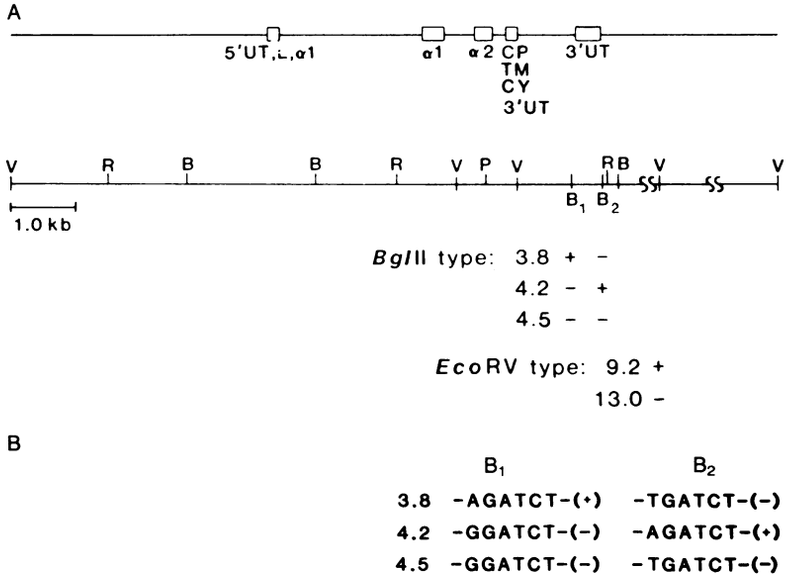

FIG. 3. Map of polymorphic and nonpolymorphic restriction sites relative to coding and noncoding sequences for the *HLA-DRα* locus. (*A*) The position of the *Eco*RI (R), *Pst* I (P), *Eco*RV (V), and *Bgl* II (B, B_1, and B_2) sites relative to coding (open boxes) and noncoding sequences for the *HLA-DRα* locus is shown with the presence (+) or absence (−) of *Bgl* II and *Eco*RV sites in each restriction fragment type indicated. The two pairs of wavy lines on the restriction map represent a compression (7 kb and 2 kb) of the map. 5′ UT, 5′ untranslated region; L, leader peptide; α1, α1 domain; α2, α2 domain; CP, connecting peptide; TM, transmembrane region; CY, cytoplasmic tail; 3′UT, 3′ untranslated region. (*B*) The polymorphic *Bgl* II sites B_1, located within an intron 41 bp from the start of the 3′UT exon, and B_2, located 9 bp 3′ of the polyadenylylation original, are expanded, with the proposed DNA sequence at each site for each restriction fragment type shown and the presence (+) or absence (−) of *Bgl* II sites indicated.

Table 1. Frequency of *HLA-DRα Bgl* II and *Eco*RV alleles, haplotypes, and selected genotypes in control and IDDM populations

	Control subjects					
	f_{obs}	Δ	χ^2	f_{obs} in IDDM probands	P	P_c
Allele						
Bgl II	(n = 55)			(n = 12)		
3.8 kb	0.164			0.083		
4.2 kb	0.164			0.5	0.034	NS
4.5 kb	0.673			0.417		
*Eco*RV	(n = 53)			(n = 12)		
9.2 kb	0.377			0.50		
13.0 kb	0.623			0.50		
Haplotype						
Bgl II/*Eco*RV	(n = 47)			(n = 12)		
3.8,9.2 kb	0	−0.061	3.74	0		
3.8,13.0 kb	0.170	0.062	3.74	0.083		
4.2,9.2 kb	0.106	0.060	4.49	0.5	0.007	<0.05
4.2,13.0 kb	0.021	−0.060	4.49	0		
4.5,9.2 kb	0.255	0.002	0.08	0		
4.5,13.0 kb	0.447	−0.001	0.08	0.417		
Genotype						
Bgl II	(n = 35)			(n = 6)		
4.2/X	0.29			0.83	0.035	NS
Bgl II/*Eco*RV	(n = 25)			(n = 6)		
4.2,9.2/X	0.24			0.83	0.024	NS

Allele and haplotype frequencies were derived from family analyses, homozygous typing cells (most of which are from consanguineous matings), hemizygous deletion variants, and informative individuals. P and P_c (i.e., P corrected for the number of comparisons performed) values are shown for control vs. IDDM frequencies; NS denotes not significant. Δ is the difference between observed and expected haplotype frequencies ($f_{obs} - f_{calc}$). The expected frequency of *Bgl* II/*Eco*RV haplotypes assuming random association was calculated by multiplying the frequency of the *Bgl* II allele by the frequency of the *Eco*RV allele in the group of 47 chromosomes for which haplotypes could be determined. The significance of each Δ is given by the χ^2 value. The genotypes of individuals containing at least one copy of the 4.2-kb allele or the 4.2,9.2-kb haplotype are designated 4.2/X and 4.2,9.2/X, respectively.

among affected siblings, and antigen-genotype frequencies, a model was proposed in which two copies of *HLA*-linked susceptibility genes are required. Moreover, the increase in the relative risk of *DR3/DR4* heterozygotes over *DR3/DR3* and *DR4/DR4* homozygotes (2, 11) suggests that proposed disease-susceptibility alleles in positive linkage disequilibrium with *DR3* and with *DR4* are different and that they interact at the molecular, cellular, or physiological level.

Table 2. Correlation of *DRα Bgl* II alleles with *HLA-DR* type

	No. with *Bgl* II allele		
	3.8 kb	4.2 kb	4.5 kb
DR type			
1	4 (1)		1 (1)
2			4 (2)
3*		6 (4)	9 (1)
4	(1)		4 (7)
5			4
6		2 (3)	2
7	(1)		4
Haplotype			
B8,DR3		5 (4)	0 (0)
All other *DR3*		0 (0)	8 (1)

Data for the 40 randomly selected haplotypes were derived from family analyses, homozygous typing cells, hemizygous deletion variants, and informative unrelated individuals. Additional independent haplotype data from the six IDDM families (Figs. 1*A* and 4) are in parentheses. Significant P and P_c values for the 40 randomly selected haplotypes are as follows: *DR1* vs. 3.8 kb, $P = 5.5 \times 10^{-5}$ ($P_c = 4.4 \times 10^{-4}$); *B8,DR3* vs. 4.2 kb, $P = 6.4 \times 10^{-5}$ ($P_c = 5.1 \times 10^{-4}$).

*Only *DR3* haplotypes typed as *B8* had the 4.2-kb fragment. Because the only nonconsanguineous *DR3* homozygous typing cell, MWF, was *B8* and *B18*, haplotype assignments could not be made; therefore, this cell was not included in the haplotype analysis.

The ability of RFLPs to reveal heterogeneity within serologically defined *HLA-DR* types and to identify non-*DR3*- or -*DR4* IDDM-susceptible chromosomes may provide new informative markers for IDDM predisposition. For example, segregation of the 4.2-kb allelic variant in IDDM families (Fig. 1*A*, ref. 4, and unpublished data) suggests that this marker may be linked to an IDDM disease-susceptibility locus on both *HLA-B8,DR3* and *HLA-DRw6* chromosomes. In the family represented in Fig. 1, all affected individuals inherited the 4.2-kb fragment from one or both parents. One affected individual (haplotype *a/d*) has one copy of the 4.2-kb *Bgl* II fragment from the *DR3* chromosome (haplotype *a*, which is *B8,DR3*) and a 4.5-kb fragment from the *DR4* chromosome (haplotype *d*). Based on the model requiring two sets of a predisposing gene, we assume that the *DR4* chromosome also bears a disease-susceptibility allele that is not associated with the 4.2-kb fragment, an observation consistent with the idea that the *DR3*- and *DR4*-associated susceptibility alleles may be different. One individual (haplotype *a/c*) in this family is homozygous for the 4.2-kb fragment, yet unaffected, consistent with incomplete penetrance of IDDM susceptibility.

The increase in the frequencies of the *Bgl* II 4.2-kb allele and of the *Bgl* II 4.2-kb,*Eco*RV 9.2-kb haplotype in IDDM patients (Table 1 and unpublished results) and in the frequency of individuals with these markers among IDDM patients suggests that these *DRα* sequence variants are in linkage disequilibrium with IDDM susceptibility alleles. These markers are increased in frequency among *DR3* and *DRw6* chromosomes implicated by segregation analysis as IDDM susceptibility haplotypes, relative to control *DR3* and *DRw6* chromosomes (Table 2), but are absent from *DR4* IDDM

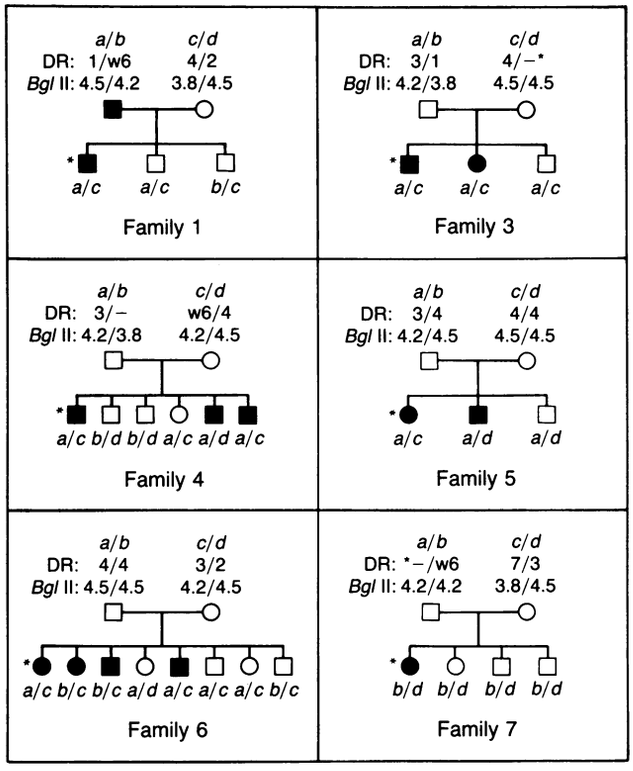

FIG. 4. Segregation of *DRα Bgl* II RFLP in six IDDM families. Genomic DNA was prepared from cell lines derived from six HLA-typed IDDM families (14) and analyzed as described for Fig. 1. IDDM-affected individuals are denoted by filled boxes or circles, and the probands are marked by small asterisks. The large asterisks denote individuals who could not be unequivocally *DR*-typed for both haplotypes (i.e., *DR4/DR4* vs. *DR4/DR*$^-$ in family 3, and *DRw6/DRw6* vs. *DRw6/DR*$^-$ in family 7.

susceptibility haplotypes. The subset of *DR3* chromosomes that contain the 4.2-kb marker are of the *B8,DR3* haplotype, suggesting the possibility that the *DR* region of *B8,DR3* chromosomes may differ from that of other *DR3* chromosomes. This observation is consistent with the implication of the *B8,DR3* "supratype" in a variety of autoimmune diseases (21). The relative risk calculated from these initial data for the *DRα Bgl* II 4.2-kb marker and from a larger study (unpublished results) is at least 2-fold higher than the relative risk obtained with the serologic DR3 marker. The precise localization of informative polymorphic restriction sites within a genomic nucleotide sequence, like that reported here, makes possible oligonucleotide-based approaches (22, 23) to detection of *HLA* polymorphisms and provides well-defined markers for the genetic analysis of *HLA* association with disease.

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1. Shackelford, D. A., Kaufman, J. F., Korman, A. J. & Strominger, J. L. (1982) *Immunol. Rev.* **66,** 133–187.
2. Ryder, L. P., Svejgaard, A. & Dausset, J. (1982) *Annu. Rev. Genet.* **15,** 169–187.
3. Charron, D. J., Aellen-Schulz, M., St. Geme, T., Erlich, H. A. & McDevitt, H. O. (1983) *Mol. Immunol.* **20,** 21–32.
4. Charron, D. J. & McDevitt, H. O. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6567–6571.
5. DeKretser, T. A., Crumpton, M. S., Bodmer, J. G. & Bodmer, W. F. (1982) *Eur. J. Immunol.* **12,** 600–606.
6. Stetler, D., Das, H., Nunberg, J. H., Saiki, R., Sheng-Dong, R., Mullis, K. B., Weissman, S. M. & Erlich, H. A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5966–5970.
7. Lee, J. S., Trowsdale, J. & Bodmer, W. F. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 545–549.
8. Korman, A. S., Knudsen, P. S., Kaufman, J. F. & Strominger, J. L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1844–1848.
9. Larhammer, D., Gustafsson, K., Claesson, L., Brill, P., Wiman, K., Schenning, L., Sundelin, J., Widemark, E., Peterson, P. & Rask, L. (1982) *Cell* **30,** 153–161.
10. Wake, C. T., Long, E. O., Strubin, M., Grass, N., Accolla, R., Carrel, S. & Mach, B. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6979–6983.
11. Platz, P., Jakobsen, B. K., Morling, N., Ryder, L. P., Svejgaard, A., Thomsen, M., Christy, M., Kromann, H., Benn, J., Nerup, J., Green, A. & Hange, M. (1981) *Diabetologia* **21,** 108–115.
12. Nepom, B. S., Nepom, G. T., Mickelson, E., Antonelli, P. & Hansen, J. A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6962–6966.
13. Grover, J. P., Watson, A. J. & Bach, F. H. (1983) *J. Exp. Med.* **157,** 1687–1693.
14. Erlich, H. A., Stetler, D., Saiki, R., Gladstone, P. & Pious, D. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2300–2304.
15. Korman, A. J., Auffray, C., Schamboeck, A. & Strominger, J. L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6013–6017.
16. Das, H., Lawrence, S. & Weissman, S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3543–3547.
17. Buck, D., Greene, A. E., Coriell, L. L. & Mulivor, R. A. (1980) *Cytogenet. Cell Genet.* **28,** 213–216.
18. Donaich, D., Bottazzo, G. F. & Cudworth, A. G. (1983) *Annu. Rev. Med.* **34,** 13–20.
19. Thomson, G. (1981) *Theor. Popul. Biol.* **20,** 168–208.
20. Thomson, G. (1983) *Tissue Antigens* **21,** 81–104.
21. Dawkins, R. L., Christiansen, F. T., Kay, P. H., Garlepp, M., McCluskey, J., Hollingsworth, P. N. & Zilko, P. J. (1983) *Immunol. Rev.* **70,** 5–22.
22. Conner, B. J., Reyes, A. A., Morin, C., Itakura, K., Tepilitz, R. L. & Wallace, R. B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 272–282.
23. Saiki, R., Arnheim, N. & Erlich, H. (1985) *Biotechnology*, in press.