Brief Genetics Report

"Extended" A1, B8, DR3 Haplotype Shows Remarkable Linkage Disequilibrium but Is Similar to Nonextended Haplotypes in Terms of Diabetes Risk

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To evaluate potential differential diabetes risk of DR3 haplotypes we have evaluated class I alleles as well as two microsatellites previously associated with differential risk associated with DR3 haplotypes. We found that over one-third of patient DR3 chromosomes consisted of an extended DR3 haplotype, from DQ2 to D6S2223 (DQ2, DR3, D6S273-143, MIC-A5.1, HLA-B8, HLA-Cw7, HLA-A1, and D6S2223-177) with an identical extended haplotype in controls. The extended haplotype was present more frequently (35.1% of autoimmune-associated DR3 haplotypes, 39.4% of control DR3 haplotypes) than other haplotypes (no other haplotype >5% of DR3 haplotypes) and remarkably conserved, but it was not transmitted from parents to affected children more frequently than nonconserved DR3-bearing haplotypes. This suggests that if all alleles are truly identical for the major A1, B8, DR3 haplotype (between A1 and DR3), with different alleles on nonconserved haplotypes without differential diabetes risk, then in this region of the genome DR3-DQ2 may be the primary polymorphisms of common haplotypes contributing to diabetes risk. ***Diabetes*** **54:1879–1883, 2005**

A number of studies are now evaluating the development of anti-islet autoantibodies in both children from the general population and first-degree relatives of patients with type 1 diabetes (1–3). It is clear from these studies that anti-islet autoantibodies can develop either in the 1st year of life or, subsequently, that anti-insulin autoantibodies are frequently the first autoantibody expressed, and that the presence of multiple anti-islet autoantibodies and persistent autoantibodies are associated with a high risk of progression to type 1 diabetes in both the general population and in relatives. Many of the current studies have evaluated risk based upon HLA class II alleles (4–7). The highest-risk genotype for early-onset type 1 diabetes and expression of anti-islet autoantibodies consists of DR3-DQ2 (DQA1*0501-DQB1*0201) on one chromosome and DR4-DQ8 (DQA1*0301-DQB1*0302) on the second chromosome (7). Most studies currently evaluate children for the expression of autoantibodies reacting with GAD65, insulinoma-associated protein 2 (ICA512), and insulin autoantibodies. The risk for children with the DR3-DQ2/DR4-DQ8 genotype in general population is <5% for the development of persistent anti-islet autoimmunity by age 5 years versus >20% for offspring and perhaps as high as 40% for siblings (of a patient with type 1 diabetes) with the same DR/DQ genotype (8). Such a remarkably high risk for anti-islet autoimmunity among relatives with DR3/DR4 suggests that other alleles within the major histocompatibility complex (MHC) may be contributing risk, in addition to the class II alleles, in that most often siblings of probands with diabetes who are DR3/DR4 are HLA identical to the proband (two identical haplotypes). It is also likely that non-MHC alleles contribute to this increased risk, but by specifying HLA genotype, only this chromosomal region is "fixed" to usually be identical to the proband and the risk for other HLA genotypes is <5%.

Prior studies of Alper and colleagues (9,10) and Dawkins and colleagues (11,12), more than a decade ago, suggested that extended or ancestral MHC haplotypes provided greater risk of autoimmunity compared with alleles of any single gene, and a DR3 "Basque" haplotype (DR3, C4s°, C4F, BfF1, B18, Cw5, A30) has been associated with extremely high diabetes risk (13). This haplotype is rare in most Caucasian populations outside of France and thus has a somewhat minor overall contribution to diabetes risk in the U.S. A more common ancestral haplotype has the class I alleles A1 and B8 (14). It is also now clear that DR4 subtypes, such as DRB1*0403, alter diabetes risk when associated with high-risk DQ alleles (e.g., DQA1*0301, DQB1*0302) (15,16). In the prospective Diabetes Autoimmunity Study in the Young (DAISY), we have obtained DNA from parents and autoantibody-positive and -negative children to allow the unambiguous assignment of haplotypes (8). Most of the families with autoantibody-positive children are characterized by very early activation

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DAISY, Diabetes Autoimmunity Study in the Young of Denver, Colorado; MHC, major histocompatibility complex.

TABLE 1
List of DR3 haplotypes which are present more than twice

	Number haplotypes	DR3	D6S273	MIC-A	HLA-B	HLA-C	HLA-A	D6S2223
Diabetes/Ab+	13 (35)	3	143	5.1	8	7	1	177
CONT	13 (39)							
Diabetes/Ab+	2 (4)	3	143	5.1	8	7	1	179
CONT	2 (2)							
Diabetes/Ab+	2 (4)	3	143	5.1	8	7	2	175
CONT	0 (0)							
Diabetes/Ab+	0 (0)	3	143	5.1	8	7	3	177
CONT	3 (8)							
Diabetes/Ab+	2 (4)	3	143	5.1	8	4	11	177
CONT	0 (0)							
Diabetes/Ab+	2 (4)	3	132	4	18	5	30	177
CONT	0 (0)							

Upper line of each row tabulates n (%) of diabetic and/or autoantibody-positive haplotypes, and lower line shows control haplotypes. Ab+, autoantibody-positive children; CONT, autoantibody-negative nondiabetic children.

of anti-islet autoimmunity given only 8 years of prospective follow-up for DAISY, with many children followed from birth. We hypothesized that if alleles of genes in addition to DR3-DQ2 in the MHC between A1 and DR3 have a frequent contribution to diabetes, then a major "conserved" haplotype will have different diabetes association and transmission compared with multiple nonconserved haplotypes.

RESEARCH DESIGN AND METHODS

We studied 43 DAISY families with DR3 or DR4 and autoantibody-positive children and 15 control families from the DAISY population with DR3 or DR4 and autoantibody-negative children. Informed consent and institutional review board approval were obtained.

HLA and microsatellite typing. HLA-A and -B, DRB1, and DQB1 genotyping was performed using the Dynal RELI SSO HLA-A and -B, DRB1, and DQB1 Typing Kits (Dynal Biotech, Oslo, Norway).

HLA-linked microsatellite markers D6S273, MIC-A, and D6S2223 were determined with a fluorescence-based method as reported previously (17,18). Analysis of family members allowed determination of haplotypes. The primers for the microsatellites were used as previously (17,18). D6S2223 is located ~4 Mb telomeric from DQB1.

Statistical analysis. Linkage disequilibrium between alleles at different loci was estimated using the method described by Lewontin (19). The linkage disequilibrium coefficient D was calculated as $D\text{ij} = p\text{ij} - pi\,pj$ and then standardized by the maximum value it can take ($D_{\max}$), given the allele frequencies, as $D' = D\text{ij}_{\max}$, where $p$ij is the frequency of the haplotype carrying alleles i and j, pi and pj are the frequencies of alleles i and j, respectively, and ($D\text{ij}_{\max}$) is either min [$pipj$,$(1 - pi)(1 - pj)$] if Dij <0 or min[$(1 - pi)pj$, $pi(1 - pj)$] if Dij >0 (19,20). The transmission/disequilibrium test of Spielman was used to assess differential transmission of parental haplotypes to affected children (21). The statistical significance of differences in allele frequencies and of linkage disequilibrium was calculated using Fisher's exact two-tailed test.

RESULTS

We have created family-specific haplotypes for eight loci of the HLA region (DQB1, DRB1, D6S273, MIC-A, HLA-B, HLA-C, HLA-A, and D6S2223). Of the 102 haplotypes in a diabetic and/or autoantibody-positive child, 37 of 102 (36.3%) had DR3, DQ2. Of the 37 DR3, DQ2 haplotypes in a diabetic or autoantibody-positive child, 13 of 37 (35.1%) had the conserved haplotype DQ2, DR3, D6S273-143, MIC-A5.1, HLA-B8, HLA-Cw7, HLA-A1, and D6S2223-177. As shown in Table 1, no other DR3 haplotype was present more than four times. Of the 111 control haplotypes (not present in diabetic and autoantibody-negative child or parent from both the control families and the nontransmitted haplotypes of the autoantibody-positive families), 33 of 111 (29.7%) had DR3, DQ2. (Note control families were selected to have DR3, DQ2 to compare with the DR3, DQ2 haplotypes of autoantibody-positive families.) Of the 33 control DR3, DQ2 haplotypes, 13 of 33 (39.4%) were the conserved DR3, DQ2 haplotype.

We analyzed linkage disequilibrium between each pair of MHC loci. DR3 is in linkage disequilibrium with D6S273-143 ($D' = 0.87$), MIC-A5.1($D' = 0.46$), HLA-B8 ($D' = 0.85$), and HLA-A1 ($D' = 0.67$). In the same way, the allele D6S273-143 is in linkage disequilibrium with MIC-A5.1 ($D' = 0.94$), HLA-B8 ($D' = 0.89$), HLA-Cw7 ($D' = 0.77$), and HLA-A1 ($D' = 0.62$), and HLA-B8 is in linkage disequilibrium with HLA-Cw7 ($D' = 0.87$) and HLA-A1 ($D' = 0.73$) and less linkage disequilibrium between D6S2223-177, HLA-A1 ($D' = 0.44$), and MIC-A5.1 ($D' = 0.33$).

As shown in Fig. 1, of the diabetic haplotypes, 65% of the DR3 haplotypes had the D6S273-143 allele. Of non-DR3 haplotypes <5% had D6S273-143 ($P < 0.0001$). Of haplotypes with DR3 and D6S273-143, 100% had MIC-A5.1. Of the haplotypes with all three alleles (DR3, D6S273-143, and MIC-A5.1), 92% had both HLA-B8 and HLA-Cw7. Of the haplotypes that had all five alleles of the conserved haplotype, 75% had HLA-A1. Furthermore, of the haplotypes which had all six alleles, 87% had D6S2223-177.

We obtained very similar results in control DR3 haplotypes. There were no significant differences between the diabetic haplotypes and the control haplotypes in linkage disequilibrium pattern. The MIC-A5.1 frequency on non-DR3 haplotypes was less than for DR3 haplotypes (24.6 vs. 75.7%, respectively). The frequencies of HLA-B8 and HLA-A1 were also decreased on non-DR3 haplotypes. The D6S2223-177 allele frequency was relatively high for both DR3 and non-DR3 haplotypes.

We compared allele frequencies between haplotypes with DR3 and DR4. The frequencies of D6S273-143, MIC-A5.1, HLA-B8, HLA-Cw7, and HLA-A1 were significantly higher on haplotypes with DR3 than on haplotypes with DR4; however, there were no significant differences between the diabetic haplotypes and the control haplotypes (Fig. 2).

The transmission disequilibrium test was utilized to assess the transmission of the DR3-DQ2 haplotypes from parents to diabetic and/or autoantibody-positive children (Table 2). Overall DR3-DQ2 haplotypes were transmitted 70.0% (56 of 80, $P < 0.001$) of the time from heterozygous

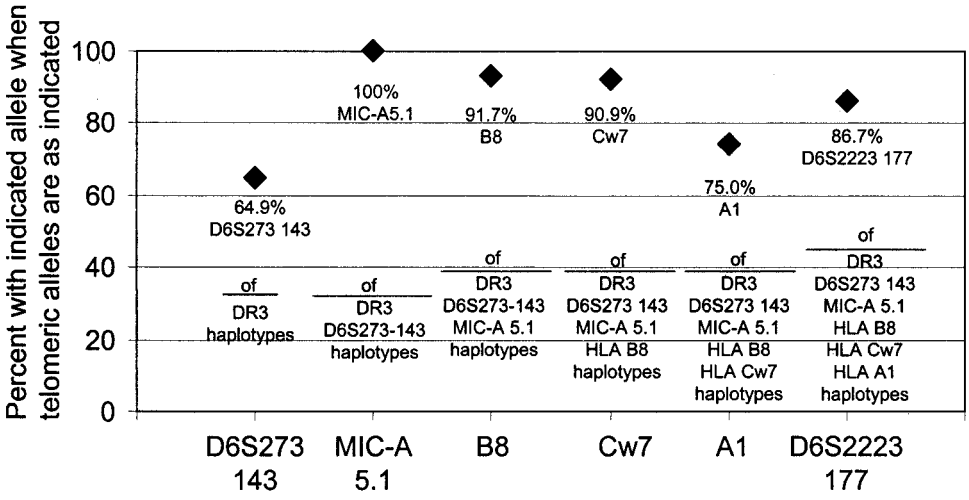

FIG. 1. Linkage disequilibrium in DR3 haplotypes for autoantibody-positive or diabetic individuals. The *y*-axis shows proportion with indicated allele when telomeric alleles are as indicated.

parents to diabetic and/or autoantibody-positive children versus 55.4% (41 of 74) to autoantibody-negative, nondiabetic control subjects. The conserved DR3-DQ2-D6S273-143-MIC-A5.1-B8-Cw7-A1 haplotype was not more often transmitted (64.5%, 20/31) to affected children compared with nonconserved DR3-DQ2 haplotypes (63.0%, 17 of 27).

DISCUSSION

Prior studies have demonstrated a few remarkably conserved HLA haplotypes, conserved between HLA-B and DR (9,12,22). These extended haplotypes can be utilized as markers for multiple polymorphisms within the MHC region given the degree of linkage disequilibrium. Extended haplotypes have aided in the analysis of the contribution of specific genes in this region to disease susceptibility and immune function.

Raum et al. (9) reported that the extended haplotypes occur with different frequencies among patient and normal chromosomes and allow the differentiation of more specific associations of certain extended haplotypes with susceptibility genes of type 1 diabetes than is possible for individual alleles.

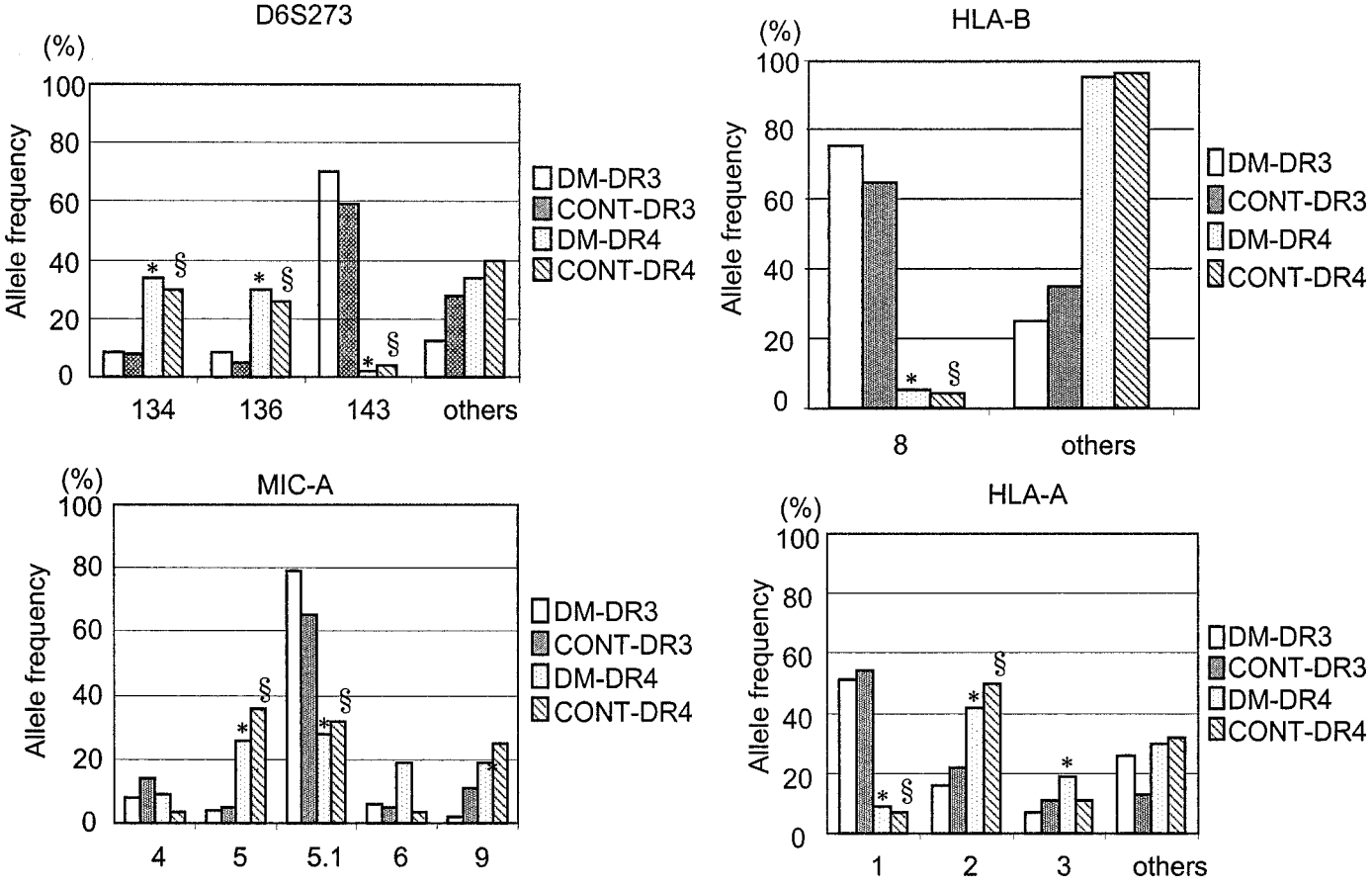

FIG. 2. Allele frequencies of DR3 or DR4 haplotypes.

TABLE 2
The transmission disequilibrium test of haplotype with DR3 and extended halotype to diabetic and/or autoantibody-positive children or control children

	All DR3 haplotypes				DR3 extended haplotype			
	T	NT	%T	*P*	T	NT	%T	*P*
Ab+/ nondiabetic children	23	14	0.62	0.18	8	5	0.62	0.41
Diabetic children	33	10	0.77	<0.001	12	6	0.67	0.16
Ab+/diabetic children	56	24	0.70	<0.001	20	11	0.65	0.11
CONT	41	33	0.55	0.12	17	10	0.63	0.18

Ab+, autoantibody-positive children without diabetes; CONT, autoantibody negative nondiabetic children.

Previous studies showed that HLA-B8, B18, B15, HLA-DW3/DR3, and HLA-DW4/DR4 were increased in Caucasian type 1 diabetic patients and largely the result of the increased frequencies of a few extended haplotypes that bear the corresponding alleles (10). These extended haplotypes have been identified in populations of normal Caucasian chromosomes and are defined as specific combinations of HLA-B, DR, and complotype alleles in significant linkage disequilibrium.

In the present study we demonstrate that the conserved haplotype (DQ2, DR3, D6S273-143, MIC-A5.1, B8, Cw7, A1, and D6S2223-177) is the only common extended haplotype in the DAISY population among both diabetic and control chromosomes. The transmission from parents to affected children of the high frequency of the A1, B8, DR3 extended haplotype does not differ from nonconserved DR3-bearing haplotypes. A much larger study would be needed to evaluate less common extended haplotypes such as B18 DR3, where there is evidence of increased risk.

The haplotypes with DR3 were significantly transmitted to affected children in diabetic and/or autoimmune antibody-positive haplotypes independent of whether they were extended DR3 haplotypes. This suggests that if all alleles on the major A1, B8, DR3 haplotype are truly identical between A1 and DR3 but differ on nonconserved DR3 haplotypes without differential diabetes risk, then in this region for the major extended haplotype DR3-DQ2 may be the major causative polymorphism. An alternative hypothesis is that there is another polymorphism in linkage disequilibrium with DR3-DQ2 that has similar allele frequencies identically expressed on the extended and nonextended haplotypes that determines risk despite dramatic differences for all loci studied between the extended versus nonextended haplotypes and or non-DR3 haplotypes. These data suggest that the remarkable risk of DR3/DR4-DQ8 relatives of the DAISY study compared with general population DR3/DR4-DQ8 individuals is unlikely to be determined by alleles between A1 and DR for the DR3 haplotypes. Loci linked to and telomeric to HLA-A and centromeric to DR (e.g., DP) may provide additional risk. At present, the very high risk of HLA identical siblings with the genotype DR3/DR4-DQ8 compared with the general population with the same DR and DQ alleles is not explained. As the single nucleotide polymorphism haplotype map of the MHC (23) is developed, a more detailed analysis of the centromeric and telomeric loci of these haplotypes should be feasible.

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