Asian-Specific HLA Haplotypes Reveal Heterogeneity of the Contribution of HLA-DR and -DQ Haplotypes to Susceptibility to Type 1 Diabetes

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To assess the effect of Asian-specific HLA haplotypes on susceptibility to type 1 diabetes, we investigated the association of genotypic combinations of *DRB1-DQB1* haplotypes with susceptibility to type 1 diabetes. We studied 132 Japanese patients with type 1 diabetes and 157 control subjects, along with 67 Korean patients and 109 control subjects. *DRB1*0405-DQB1*0401* and *DRB1*0901-DQB1*0303* were confirmed to be two major susceptible HLA haplotypes in the Japanese population. The frequencies of heterozygotes and homozygotes with *DRB1*0405-DQB1*0401* were similarly higher in patients than in control subjects (homozygotes, 5.3% vs. 3.8%; heterozygotes, 48.5% vs. 26.1%). In contrast, homozygotes, but not heterozygotes, with *DRB1*0901-DQB1*0303* were more frequent in patients with type 1 diabetes than in control subjects (homozygotes, 12.9% vs. 0.6%; heterozygotes, 22.0% vs. 24.8%). A similar tendency was also observed in the Korean population. In multiple logistic regression analysis, *DRB1*0405-DQB1*0401* fitted a dominant model and *DRB1*0901-DQB1*0303* fitted a recessive model. These data, which indicate that the contribution of HLA haplotypes to the genetic susceptibility to type 1 diabetes differs depending on the genotypic combination of HLA haplotypes, suggest the importance of extensive analysis of genotypes in studies on HLA and disease association in general. *Diabetes* 51:545–551, 2002

Type 1 (insulin-dependent) diabetes is caused by autoimmune destruction of insulin-producing β-cells of the pancreas (1–3). Susceptibility to type 1 diabetes is determined by a combination of genetic and environmental factors. In both animal models and humans, type 1 diabetes is a polygenic trait, with a major locus encoded by the major histocompatibility complex (MHC) and several other loci contributing to disease susceptibility (4–8). In humans, the contribution of HLA to the genetic risk of type 1 diabetes is estimated as ~50% (5). The role of HLA in type 1 diabetes was first indicated by the association with HLA-B8 and -B15, and then with the HLA-DR3 and -DR4 antigens encoded at the DRB1 locus (9–13). Subsequently, the *DQB1* and *DQA1* genes were shown to be more strongly associated with type 1 diabetes (14–22), and the DQ molecule was favored as the primary susceptibility factor. Although several studies have clearly demonstrated that both DQ and DR influence type 1 diabetes susceptibility (23–28), the interpretation of the complex HLA associations with type 1 diabetes is still under extensive discussion.

Population studies have shown that HLA associations may vary depending on geographic and ethnic origin (29). In Caucasian populations, predisposition to type 1 diabetes is mostly associated with the *DRB1*03-DQB1*0201* and/or *DRB1*04-DQB1*0302* haplotypes, whereas the *DRB1*15-DQB1*0602* haplotype confers strong protection against the disease. In the Japanese population, where the prevalence of type 1 diabetes is less than one-tenth that in most Caucasian populations, the *DRB1*03-DQB1*0201* haplotype is absent, and *DRB1*04-DQB1*0302* is not associated with type 1 diabetes (30). Instead, three haplotypes, *DRB1*0405-DQB1*0401*, *DRB1*0802-DQB1*0302*, and *DRB1*0901-DQB1*0303*, which are rare in Caucasian populations, confer susceptibility to type 1 diabetes (30–32). Moreover, the *DRB1*1502-DQB1*0601* haplotype, which is rare in Caucasian populations, is a major protective haplotype, in addition to DRB1*1501*0602, a well-known protective haplotype in almost all ethnic groups (30–32).

*DRB1*03-DQB1*0201* and *DRB1*04-DQB1*0302* haplotypes in Caucasians, in particular in a heterozygous state, are so strong that the contribution of other haplotypes to type 1 diabetes susceptibility is easily masked, making it difficult to assess the effect of other haplotypes on disease susceptibility. In contrast, the absence of highly susceptible haplotypes, *DRB1*03-DQB1*0201* and *DRB1*04-DQB1*0302*, in Japanese, together with the presence of Asian-specific haplotypes, is expected to make it easier in the Japanese than in the Caucasian population to clarify the contribution of genotypic combinations and the dose effect of moderately susceptible HLA haplotypes to susceptibility to type 1 diabetes. We therefore studied the association of HLA haplotypes with type 1 diabetes in Japanese, with special reference to the genotypic combination of haplotypes, in either heterozygotes or homozy-

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AIC, Akaike's information criterion; MHC, major histocompatibility complex; OR, odds ratio; *Pc*, corrected *P* value.

TABLE 1
HLA-*DRB1* and *DQB1* phenotype in Japanese patients with type 1 diabetes and control subjects

	Patients (n = 132)	Control subjects (n = 157)	OR	P	Pc
DRB1					
**0405*	79 (59.8)	48 (30.6)	3.38	<0.000001	<0.0001
**0901*	49 (37.1)	40 (25.5)	1.73	0.033	NS
**0802*	20 (15.2)	8 (5.1)	3.32	0.004	NS
**1501*	3 (2.3)	15 (9.6)	0.22	0.011	NS
**1502*	5 (3.8)	45 (28.7)	0.1	<0.0000001	<0.000001
DQB1					
**0401*	72 (54.5)	47 (29.9)	2.81	<0.0001	0.0003
**0303*	52 (39.4)	43 (27.4)	1.72	0.030	NS
**0302*	37 (28.0)	25 (15.9)	2.06	0.013	NS
**0601*	17 (12.9)	62 (39.5)	0.23	<0.000001	<0.00001
**0602*	1 (0.8)	16 (10.2)	0.07	0.0006	0.008

Data are n (%).

gotes, in search for the effect of specific genotypes on disease susceptibility.

RESEARCH DESIGN AND METHODS

Subjects. We studied 132 unrelated Japanese patients (male, 54; female, 78) with type 1 diabetes and 157 unrelated healthy control subjects (male, 89; female, 68). Mean (± SD) age at onset of the disease was 16.1 ± 12.4 years (range, 2–59). The diagnosis of type 1 diabetes was defined by both clinical features and laboratory data. All the patients were ketosis-prone, lacked endogenous insulin secretion as judged by urinary C-peptide levels of <3.3 nmol/day, and needed more than four insulin injections per day. Control subjects consisted of medical staff in our department. They had normal glucose tolerance and no family history of type 1 diabetes or other autoimmune diseases. The age of the control subjects was 42.0 ± 10.9 years. All patients and control subjects were of Japanese origin and resided in the Osaka area (western Japan). Informed consent was obtained from all subjects.

Genomic DNA samples of 67 Korean patients (male, 20; female, 47) with type 1 diabetes and 109 unrelated healthy control subjects (male, 68; female, 41) were obtained courtesy of Dr. Inkyuu Lee. The age at onset of the disease was 11.9 ± 7.2 years (range, 1–32). The criteria for the diagnosis of type 1 diabetes were the same as those of our laboratory. All patients and control subjects were of Korean origin and resided in the Taegu area, located in southeast Korea.

Typing of HLA-DR and -DQ. HLA-*DRB1* and *-DQB1* alleles were determined by PCR-restriction fragment length polymorphism methods as reported previously (33–36). The most probable HLA-DR and -DQ haplotypes were deduced from known linkage disequilibria (37).

Statistical analysis. Allele frequencies were estimated by direct counting. The significance of the difference in distribution of alleles between patients with type 1 diabetes and healthy control subjects was determined by χ^2 method or Fisher's exact probability test. P values were corrected for the number of different alleles tested (Pc). Statistical significance was defined as $P < 0.05$.

The predisposition of individual genotypes to type 1 diabetes was analyzed by multiple logistic regression. Initially, the saturated model was constructed. Then, several models considering the mode of contribution of each HLA *DRB1-DQB1* haplotype were analyzed. For each model, twice the negative logarithmic likelihood ratio between the model under consideration and the saturated model was calculated as a deviance. The deviance is distributed asymptotically as a χ^2 distribution with degrees of freedom equal to the difference in the number of parameters under the null hypothesis (i.e., the saturated model does not fit the data better than the model under consideration). Furthermore, the models were compared in respect of fit to the data using Akaike's information criterion (AIC) (38). Logistic regression analysis was performed with the SPSS for Windows statistics program.

RESULTS

Frequencies of phenotypes and alleles of HLA-DRB1 and HLA-DQB1. The phenotypic frequency of *DRB1*0405* was significantly higher in patients with type 1 diabetes than in control subjects ($Pc < 0.0001$), and that of *DRB1*1502* was significantly lower in patients than in control subjects ($Pc < 0.000001$) (Table 1). The phenotypic frequency of *DQB1*0401* was significantly higher in patients with type 1 diabetes than in control subjects (Pc = 0.0003), and those of *DQB1*0601* and **0602* were significantly lower in patients than in control subjects ($Pc <$ 0.00001 and Pc = 0.008, respectively) (Table 1). The difference in phenotypic frequency of *DRB1*0901* and *DQB1*0303* was not significant ($Pc > 0.05$) (Table 1). The allele frequencies of *DRB1*0405* and *DQB1*0401* were significantly higher in patients with type 1 diabetes than in control subjects (Pc = 0.0001 and 0.003, respectively) (Table 2), and those of *DRB1*1502*, *DQB1*0601*, and **0602* were significantly lower in patients than in control subjects ($Pc < 0.00001$, $Pc < 0.0001$, and Pc = 0.011, respectively). In contrast, the allele frequencies of *DRB1*0901* and *DQB1*0303*, whose phenotypic frequencies did not show a significant difference between patients and control subjects, were significantly higher in patients than in control subjects (Pc = 0.005 and 0.004, respectively).

Frequencies of phenotypes, chromosomes, and genotypes of DRB1-DQB1 haplotypes. The phenotypic frequency of *DRB1*0405-DQB1*0401* haplotype was significantly higher in patients than in control subjects (Pc = 0.0008) (Table 3), and those of *DRB1*1501-DQB1*0602* and *DRB1*1502-DQB1*0601* haplotypes were significantly lower in patients than in control subjects (Pc = 0.023 and $Pc < 0.000001$, respectively) (Table 3).

The chromosome frequency of *DRB1*0405-DQB1*0401* haplotype was significantly higher in patients than in control subjects (Pc = 0.006) (Table 4), and those of *DRB1*1501-DQB1*0602* and *DRB1*1502-DQB1*0601* haplotypes were significantly lower in patients than in control subjects (Pc = 0.027 and $Pc < 0.00001$, respectively) (Table 4). The chromosome frequency of *DRB1*0901-DQB1*0303* haplotype, whose phenotypic frequency did not show a significant difference between patients and control subjects, was significantly higher in patients than in control subjects (Pc = 0.017) (Table 4).

Table 5 shows the genotypic frequency of *DRB1-DQB1* haplotype. The frequencies of *DRB1*0405-DQB1*0401/DRB1*0802-DQB1*0302* and *DRB1*0901-DQB1*0303/DRB1*0901-DQB1*0303* were significantly higher in patients than in control subjects.

Comparison of DR4 and DR9 haplotypes. The differen-

TABLE 2
HLA-*DRB1* and *DQB1* allele in Japanese patients with type 1 diabetes and control subjects

	Patients (n = 264)	Control subjects (n = 314)	OR	P	Pc
DRB1					
**0405*	89 (33.7)	54 (17.2)	2.45	<0.00001	0.0001
**0901*	67 (25.4)	41 (13.1)	2.26	0.0002	0.005
**0802*	20 (7.6)	8 (2.5)	3.14	0.005	NS
**1501*	3 (1.1)	15 (4.8)	0.23	0.012	NS
**1502*	5 (1.9)	46 (14.6)	0.11	<0.0000001	<0.00001
Others	80 (30.3)	150 (47.8)			
DQB1					
**0401*	79 (29.9)	53 (16.9)	2.10	0.0002	0.003
**0303*	69 (26.1)	44 (14.0)	2.17	0.0003	0.004
**0302*	39 (14.8)	26 (8.3)	1.92	0.0139	NS
**0601*	18 (6.8)	65 (20.7)	0.28	<0.00001	<0.0001
**0602*	1 (0.4)	16 (5.1)	0.07	0.0008	0.011
Others	58 (22.0)	110 (35.0)			

Data are n (%).

tial association of phenotypes and genotypes with type 1 diabetes as observed for DR4 and DR9 suggests the importance of the genotypic combination of haplotypes in susceptibility to the disease. To clarify the difference in the genetic contribution of the two major HLA haplotypes, DR4 (*DRB1*0405-DQB1*0401*) and DR9 (*DRB1*0901-DQB1*0303*), to type 1 diabetes, we compared the frequencies of homozygotes and heterozygotes with the DR4 and/or DR9 haplotypes (Table 6). The frequencies of heterozygotes and homozygotes with the DR4 haplotype (*DRB1*0405-DQB1*0401*) were similarly higher in patients than in control subjects. In contrast, homozygotes, but not heterozygotes, with the DR9 haplotype (*DRB1*0901-DQB1*0303*) were more frequent in type 1 diabetic subjects than in control subjects.

Multiple logistic regression analysis. To further investigate the differential association of the DR4 (*DRB1*0405-DQB1*0401*) and DR9 (*DRB1*0901-DQB1*0303*) haplotypes with type 1 diabetes, the predisposition to the disease expected by DR4- and/or DR9-containing genotypes was analyzed by multiple logistic regression (Table 7). In multiple logistic regression analysis, two models (dominant model and recessive model) were generated for the DR4 and DR9 haplotypes; therefore, four models were initially examined. Two models, the "DR4 recessive" model and "DR9 dominant" model, were rejected, while the other two models were not rejected (Table 7). We also made a combined "DR4 dominant, DR9 recessive" model. The combined model was not rejected (Table 7) and showed the smallest AIC value, indicating that it is the best fit for the data.

DRB1 genotype in the Korean population. To confirm that the observation in Japanese is applicable to other ethnic groups, in particular those with the susceptible DR3 haplotype, the Korean population was studied (Table 8). The Korean population is unique in that the DR3 haplotype

TABLE 3
Phenotype frequency of *DRB1-DQB1* haplotype in Japanese patients with type 1 diabetes and control subjects

	Patients (n = 132)	Control subjects (n = 157)	OR	P	Pc
*DRB1*0101-DQB1*0501*	12 (9.1)	26 (16.6)	—	—	—
*DRB1*0403-DQB1*0302*	4 (3.0)	6 (3.8)	—	—	—
*DRB1*0405-DQB1*0302*	5 (3.8)	0 (0.0)	13.59	0.019	NS
*DRB1*0405-DQB1*0401*	71 (53.8)	47 (29.9)	2.72	<0.0001	0.0008
*DRB1*0406-DQB1*0302*	1 (0.8)	9 (5.7)	0.13	0.024	NS
*DRB1*0407-DQB1*0302*	4 (3.0)	3 (1.9)	—	—	—
*DRB1*0410-DQB1*0402*	1 (0.8)	4 (2.5)	—	—	—
*DRB1*0802-DQB1*0302*	18 (13.6)	4 (2.5)	6.04	0.0004	0.0084
*DRB1*0802-DQB1*0402*	2 (1.5)	4 (2.5)	—	—	—
*DRB1*0803-DQB1*0601*	10 (7.6)	18 (11.5)	—	—	—
*DRB1*0901-DQB1*0303*	46 (34.8)	40 (25.5)	—	—	—
*DRB1*1001-DQB1*0501*	2 (1.5)	6 (3.8)	—	—	—
*DRB1*1101-DQB1*0301*	1 (0.8)	7 (4.5)	—	—	—
*DRB1*1201-DQB1*0301*	5 (3.8)	12 (7.6)	—	—	—
*DRB1*1201-DQB1*0303*	5 (3.8)	2 (1.3)	—	—	—
*DRB1*1202-DQB1*0301*	3 (2.3)	5 (3.2)	—	—	—
*DRB1*1302-DQB1*0604*	18 (13.6)	15 (9.6)	—	—	—
*DRB1*1401-DQB1*0502*	1 (0.8)	5 (3.2)	—	—	—
*DRB1*1401-DQB1*0503*	0 (0.0)	6 (3.8)	0.09	0.033	NS
*DRB1*1501-DQB1*0602*	1 (0.8)	15 (9.6)	0.07	0.001	0.023
*DRB1*1502-DQB1*0601*	5 (3.8)	45 (28.7)	0.10	<0.0000001	<0.000001

Data are n (%). Genotypes, whose total frequencies in both patients and control subjects were five or more than five, are listed in this table.

TABLE 4
Chromosome frequency of *DRB1-DQB1* haplotype in Japanese patients with type 1 diabetes and control subjects

	Patients (n = 264)	Control subjects (n = 314)	OR	P	Pc
*DRB1*0101-DQB1*0501*	12 (4.5)	29 (9.2)	0.47	0.029	NS
*DRB1*0403-DQB1*0302*	4 (1.5)	6 (1.9)	—	—	—
*DRB1*0405-DQB1*0302*	5 (1.9)	0 (0.0)	13.33	0.020	NS
*DRB1*0405-DQB1*0401*	78 (29.5)	53 (16.9)	2.07	0.0003	0.006
*DRB1*0406-DQB1*0302*	1 (0.4)	10 (3.2)	0.12	0.014	NS
*DRB1*0407-DQB1*0302*	4 (1.5)	3 (1.0)	—	—	—
*DRB1*0410-DQB1*0402*	1 (0.4)	4 (1.3)	—	—	—
*DRB1*0802-DQB1*0302*	18 (6.8)	4 (1.3)	5.67	0.0005	0.011
*DRB1*0802-DQB1*0402*	2 (0.8)	4 (1.3)	—	—	—
*DRB1*0803-DQB1*0601*	11 (4.2)	18 (5.7)	—	—	—
*DRB1*0901-DQB1*0303*	63 (23.9)	41 (13.1)	2.09	0.0008	0.017
*DRB1*1001-DQB1*0501*	2 (0.8)	6 (1.9)	—	—	—
*DRB1*1101-DQB1*0301*	1 (0.4)	7 (2.2)	—	—	—
*DRB1*1201-DQB1*0301*	5 (1.9)	12 (3.8)	—	—	—
*DRB1*1201-DQB1*0303*	5 (1.9)	2 (0.6)	—	—	—
*DRB1*1202-DQB1*0301*	3 (1.1)	5 (1.6)	—	—	—
*DRB1*1302-DQB1*0604*	18 (6.8)	16 (5.1)	—	—	—
*DRB1*1401-DQB1*0502*	1 (0.4)	5 (1.6)	—	—	—
*DRB1*1401-DQB1*0503*	0 (0.0)	6 (1.9)	0.09	0.034	NS
*DRB1*1501-DQB1*0602*	1 (0.4)	15 (4.8)	0.08	0.001	0.027
*DRB1*1502-DQB1*0601*	5 (1.9)	46 (14.6)	0.11	<0.0000001	<0.00001
Others	24 (9.1)	22 (7.0)			

Data are n (%). "Others" contain rare genotypes, whose total frequencies in both patients and control subjects were less than five.

(*DRB1*0301-DQB1*0201*), which is absent in the Japanese population, is present in addition to Asian-specific DR4 (*DRB1*0405-DQB1*0401*) and DR9 (*DRB1*0901-DQB1*0303*) haplotypes. Therefore, the contribution of Asian-specific DR4 and DR9 haplotypes to type 1 diabetes susceptibility can be tested in the presence of the DR3 haplotype, a well-known susceptible haplotype in Caucasians. The frequencies of *DRB1*0301*, **0405*, and **0901* alleles were significantly higher in patients with type 1 diabetes than in control subjects (Pc = 0.006, 0.03, and 0.0007, respectively). The frequencies of *DRB1*0301/0405* (odds ratio [OR] 8.6), *0301/0901* (OR 15.2), *0405/0802* (OR 15.2), *0405/0901* (OR 18.9), and *0901/0901* (OR 10.4) were higher in patients than in control subjects. As in the

TABLE 5
Genotypic combination of *DRB1-DQB1* haplotype in Japanese patients with type 1 diabetes and control subjects

	Patients (n = 132)	Control subjects (n = 157)	OR	P	Pc
*DRB1*0101-DQB1*0501/DRB1*0101-DQB1*0501*	0 (0.0)	3 (1.9)	—	—	—
*DRB1*0405-DQB1*0401/DRB1*0101-DQB1*0501*	9 (6.8)	6 (3.8)	—	—	—
*DRB1*0405-DQB1*0401/DRB1*0405-DQB1*0401*	7 (5.3)	6 (3.8)	—	—	—
*DRB1*0405-DQB1*0401/DRB1*0407-DQB1*0302*	4 (3.0)	0 (0.0)	11.03	0.042	NS
*DRB1*0405-DQB1*0401/DRB1*0802-DQB1*0302*	9 (6.8)	0 (0.0)	24.23	0.0007	0.017
*DRB1*0405-DQB1*0401/DRB1*0803-DQB1*0601*	6 (4.5)	2 (1.3)	—	—	—
*DRB1*0405-DQB1*0401/DRB1*0901-DQB1*0303*	12 (9.1)	6 (3.8)	—	—	—
*DRB1*0405-DQB1*0401/DRB1*1001-DQB1*0501*	0 (0.0)	3 (1.9)	—	—	—
*DRB1*0405-DQB1*0401/DRB1*1101-DQB1*0301*	0 (0.0)	3 (1.9)	—	—	—
*DRB1*0405-DQB1*0401/DRB1*1201-DQB1*0301*	1 (0.8)	2 (1.3)	—	—	—
*DRB1*0405-DQB1*0401/DRB1*1302-DQB1*0604*	7 (5.3)	1 (0.6)	8.74	0.026	NS
*DRB1*0405-DQB1*0401/DRB1*1501-DQB1*0602*	0 (0.0)	3 (1.9)	—	—	—
*DRB1*0405-DQB1*0401/DRB1*1502-DQB1*0601*	3 (2.3)	5 (3.2)	—	—	—
*DRB1*0405-DQB1*0401/DRB1*1602-DQB1*0502*	1 (0.8)	2 (1.3)	—	—	—
*DRB1*0802-DQB1*0302/DRB1*0901-DQB1*0303*	4 (3.0)	1 (0.6)	—	—	—
*DRB1*0901-DQB1*0303/DRB1*0101-DQB1*0501*	1 (0.8)	3 (1.9)	—	—	—
*DRB1*0901-DQB1*0303/DRB1*0803-DQB1*0601*	1 (0.8)	3 (1.9)	—	—	—
*DRB1*0901-DQB1*0303/DRB1*0901-DQB1*0303*	17 (12.9)	1 (0.6)	23.06	<0.0001	0.0005
*DRB1*0901-DQB1*0303/DRB1*1201-DQB1*0301*	3 (2.3)	0 (0.0)	—	—	—
*DRB1*0901-DQB1*0303/DRB1*1302-DQB1*0604*	1 (0.8)	4 (2.5)	—	—	—
*DRB1*0901-DQB1*0303/DRB1*1502-DQB1*0601*	0 (0.0)	10 (6.4)	0.05	0.0023	NS
*DRB1*1502-DQB1*0601/DRB1*0101-DQB1*0501*	0 (0.0)	5 (3.2)	—	—	—
*DRB1*1502-DQB1*0601/DRB1*1302-DQB1*0604*	0 (0.0)	4 (2.5)	—	—	—
*DRB1*1502-DQB1*0601/DRB1*1401-DQB1*0502*	1 (0.8)	3 (1.9)	—	—	—
Others	45 (34.1)	56 (35.7)			

Data are n (%). "Others" contain rare genotypes, whose total frequencies in both patients and control were less than three.

TABLE 6
Frequencies of homozygotes and heterozygotes with DR4 and/or DR9 haplotypes in Japanese patients with type 1 diabetes and control subjects

	Patients (n = 132)	Control (n = 157)	OR
*DRB1*0405-DQB1*0401* /*DRB1*0405-DQB1*0401*	7 (5.3)	6 (3.8)	2.10
*DRB1*0405-DQB1*0401* /X	64 (48.5)	41 (26.1)	2.81
X/X	61 (46.2)	110 (70.1)	
*DRB1*0901-DQB1*0303* /*DRB1*0901-DQB1*0303*	17 (12.9)	1 (0.6)	23.1
*DRB1*0901-DQB1*0303* /Y	29 (22.0)	39 (24.8)	1.01
Y/Y	86 (65.2)	117 (74.5)	

Data are n (%). X does not contain *DRB1*0405-DQB1*0401*. Y does not contain *DRB1*0901-DQB1*0303*.

case of Japanese, the frequencies of heterozygotes and homozygotes with *DRB1*0405* were similarly higher in patients than in control subjects (OR 2.21 vs. 3.64), whereas homozygotes with *DRB1*0901* were more strongly associated with type 1 diabetes than were heterozygotes (OR 13.3 vs. 3.06).

DISCUSSION

The results clearly demonstrated that the contribution of HLA haplotypes to the genetic susceptibility to type 1 diabetes differs depending on the combination of HLA haplotypes; i.e., DR4 haplotype (*DRB1*0405-DQB1*0401*) showed the best fit in a dominant model, whereas DR9 haplotype (*DRB1*0901-DQB1*0303*) fitted a recessive model. The recessive-like effect of the DR9 haplotype on disease susceptibility is supported by the observation that the DR9 haplotype confers much stronger susceptibility to type 1 diabetes when present in a homozygous state (OR 23.1) than in a heterozygous state (OR 1.0). High susceptibility conferred by a DR9 homozygous state was also observed in the Korean population in this study (OR 13.3) and in a previous study (OR 14.0) (39), indicating that susceptibility to type 1 diabetes in DR9 homozygotes is universal and that the DR9 haplotype is in itself susceptible to type 1 diabetes in the absence of other susceptible haplotypes. Although a DR9 heterozygous state in itself does not confer susceptibility to type 1 diabetes, DR9 heterozygotes with limited haplotypes, *DRB1*0405-DQB1*0401* and *DRB1*0802-DQB1*0302*, are positively associated with type 1 diabetes. Thus, two doses of susceptible haplotypes are necessary for DR9-containing genotypes to confer susceptibility to type 1 diabetes.

TABLE 8
Frequencies of homozygotes and heterozygotes with *DRB1*0405* and/or *DRB1*0901* in Korean patients with type 1 diabetes and control subjects

	Patients (n = 67)	Control (n = 109)	OR
*DRB1*0405/DRB1*0405*	2 (3.0)	2 (1.8)	2.21
*DRB1*0405*/X	23 (34.3)	14 (12.8)	3.64
*DRB1*0901/DRB1*0901*	6 (9.0)	1 (0.9)	13.3
*DRB1*0901*/Y	18 (26.9)	13 (11.9)	3.06

Data are n (%). X does not contain *DRB1*0405*; Y does not contain *DRB1*0901*.

In contrast to the strong susceptibility conferred by a DR9 homozygous state, homozygotes for Asian-specific DR4 haplotype (*DRB1*0405-DQB1*0401*) were only weakly associated with type 1 diabetes (OR 2.1), but the association with the disease was stronger in a heterozygous state (OR 2.8) (Table 6). When individual genotypes were considered, the *DRB1*0405* heterozygous state conferred susceptibility to type 1 diabetes not only in combination with other susceptible haplotypes, such as *DRB1*0802-DQB1*0302* (OR 24.2) and *DRB1*0901-DQB1*0303* (OR 2.52), but also with neutral or even weakly protective haplotypes, such as *DRB1*0101-DQB1*0501* (OR 1.84), *DRB1*0803-DQB1*0601* (OR 3.69), and *DRB1*1302-DQB1*0604* (OR 8.74), which were all negatively associated with the disease in DR9 heterozygotes (OR 0.39, 0.39, and 0.29, respectively). These data suggest the interaction of the *DRB1*0405* haplotype with other haplotypes in conferring susceptibility to type 1 diabetes.

HLA class II DR and DQ molecules bind and present antigen peptides, which are derived from intracellular proteolysis of both foreign proteins and self proteins, to lymphocytes of the immune system. Molecular interactions between HLA-peptide complexes and the T-cell

TABLE 7
Results of multiple regression analysis

Model	Deviance	df	P	AIC
Saturated model				357.75
DR4 dominant	0.54	2	NS	354.30
DR4 recessive	28.93	2	<0.000001	382.68
DR9 dominant	24.06	2	<0.00001	377.81
DR9 recessive	1.48	2	NS	355.23
DR4 dominant, DR9 recessive	1.72	3	NS	353.47

DR4: *DRB1*0405-DQB1*0401*; DR9: *DRB1*0901-DQB1*0303*. df, degrees of freedom. The saturated model is as follows:
$\log\{p/(1-p)\} = b_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_5x_5$
x_1: DR4/D R4, x_2: DR9/DR9, x_3: DR4/DR9, x_4: DR4/DRX, x_5: DR9/DRX
(DRX: DR other than DR4 or DR9)
DR4 dominant: $a_1 = a_3 = a_4$
DR4 recessive: $a_3 = a_5$, $a_4 = 0$
DR9 dominant: $a_2 = a_3 = a_5$
DR9 recessive: $a_3 = a_4$, $a_5 = 0$
Dr4 dominant, DR9 recessive: $a_1 = a_3 = a_4$, $a_5 = 0$

receptor are involved in selection of potentially autoreactive T-cell specificities, peripheral amplification of the potentially autoreactive T-cells, and specific antigen recognition in target organs. The interactions between peptides and HLA-DR/DQ molecules are determined by polymorphism in both the antigen peptide and the HLA molecule. Different HLA-DR/DQ molecules might have different binding affinity to disease-associated peptides. The overall effect of DR and DQ molecules on type 1 diabetes was suggested to be determined through binding competition of β-cell antigens among MHC molecules (40). This hypothesis may explain the molecular basis of the differential effect of DR4 and DR9 haplotypes on type 1 diabetes susceptibility observed in the present study. If class II molecules encoded by the DR4 haplotype have higher affinity to a diabetogenic peptide than those encoded by other haplotypes, then they may effectively bind and present the peptide to induce autoimmunity even in the presence of other class II molecules, and thus fit best in a dominant model. In contrast, if class II molecules encoded by the DR9 haplotype have lower affinity, they might show the strong susceptibility only in a homozygous state.

In Caucasians, the DR3 (*DRB1*0301-DQB1*0201*) and DR4 (*DRB1*04-DQB1*0302*) haplotypes are strongly associated with type 1 diabetes, with DR3/4 heterozygotes showing particularly strong susceptibility. Extensive analysis of genotypic combinations of HLA haplotypes in this study revealed that a synergistic effect of genotypes comparable to that in Caucasian DR3/4 was absent in the Japanese population. Although a combination of two susceptible haplotypes, such as DR4 and DR9, was positively associated with type 1 diabetes, it was no more susceptible than expected from two doses of susceptible allele (observed versus expected, 9.1 vs. 14.1%). The lack of synergistic effect in DR4/9 heterozygotes in Japanese can be explained by the *trans* complementation of DQ αβ heterodimer (41,42). *DQA1* allele on both the DR4 and DR9 haplotypes in Japanese is the same *DQA1*0302*, and therefore the DQαβ *trans*-dimer in DR4/9 individuals is the same as the *cis*-dimer. In Caucasian DR3/4 heterozygotes, in contrast, new *trans*-dimers, *DQ*α/β 0301/0201 and *DQ*α/β 0501/0302, are formed by *trans* complementation of the DQα chain encoded by DR3 and the DQβ chain encoded by DR4 haplotype, or vice versa (41,42), which is likely to contribute to the strong susceptibility conferred by a DR3/4 heterozygous state.

In contrast to the strong association of the *DQB1*0302* allele with type 1 diabetes in Caucasian populations, *DQB1*0302* in itself is not associated with type 1 diabetes in Japanese (Tables 1 and 2). Investigation of individual *DRB1-DQB1* haplotypes, however, revealed that *DQB1*0302* is as susceptible in Japanese as in Caucasians when present with susceptible *DRB1* alleles, such as *DRB1*0405* (OR 13.6), *DRB1*0407* (OR 1.60), and *DRB1*0802* (OR 6.04). The apparent lack of association between *DQB1*0302* and type 1 diabetes in Japanese can be explained by the presence of *DQB1*0302* haplotypes containing protective *DRB1* alleles, such as *DRB1*0403* and *DRB1*0406*, in the population (Tables 3 and 4). Previous studies in Chinese (26) and Korean (39) as well as Japanese (30) populations support this hypothesis.

Previous studies suggested that the *DRB1*0405-DQB1*0401* haplotype might confer a strong predisposing effect to type 1 diabetes in Japanese (OR 3.4–4.4), while *DRB1*0901-DQB1*0303* might confer a neutral or weak predisposing effect (OR 1.3–2.39) (30,32,43). In those reports, the association of haplotypes with type 1 diabetes was studied by the frequency of phenotypes, alleles, and/or haplotypes, but not by genotypic combinations, except for one study (30). In the present study, we analyzed the association by genotypic combination of haplotypes in addition to phenotypes, alleles, and haplotypes and found a differential effect of the DR4 (*DRB1*0405-DQB1*0401*) and DR9 (*DRB1*0901-DQB1*0303*) haplotypes in genotypic combination, conferring susceptibility to type 1 diabetes. Multiple logistic regression analysis of these data suggested that the DR4 haplotype predisposes in a dominant fashion, whereas the DR9 haplotype predisposes in a recessive fashion. These results emphasize the importance of genotypic combinations rather than alleles or haplotypes in determining the susceptibility to type 1 diabetes, and extensive analysis of genotypes, in addition to phenotypes and alleles, is therefore recommended for studies on HLA and disease association in general. The data further suggest the importance of low-incidence populations in the analysis of the genotypic contribution of susceptible haplotypes, whose effect can be masked by highly susceptible genotypes, such as DR 3/4 in Caucasian populations.

Although the responsible haplotypes were completely different from those in the present study in Japanese, a previous study in Caucasians reported that DR3 predisposes in a recessive-like fashion and DR4 in a dominant-like or intermediate fashion, after allowing for the DR 3/4 synergistic effect (44). Thus, when highly susceptible DR 3/4 heterozygotes are removed from the analysis, the different mode of inheritance as observed in Japanese can also be observed in high-incidence Caucasian populations.

In summary, the present study indicated that the contribution of HLA haplotypes to the genetic susceptibility to type 1 diabetes differs depending on the genotypic combination of HLA haplotypes: the *DRB1*0405-DQB1*0401* haplotype showed best fit in a dominant model, whereas the *DRB1*0901-DQB1*0303* haplotype fitted a recessive model. These results emphasize the importance of genotypic combination in addition to alleles and haplotypes in determining susceptibility to type 1 diabetes and suggest that extensive analysis of genotypes is important in studies on HLA and disease association in general.

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