Transcomplementation of HLA DQA1-DQB1 in DR3/DR4 and DR3/DR9 Heterozygotes and IDDM in Taiwanese Families

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OBJECTIVE — To study the human leukocyte antigen (HLA)-DQ heterodimers in the susceptible DR haplotypes for patients with insulin-dependent diabetes mellitus (IDDM) in Taiwan.

RESEARCH DESIGN AND METHODS — Extended class II HLA haplotypes were studied in 57 unrelated IDDM patients, 31 simplex IDDM families, and 105 unrelated control subjects recruited from the same area in Taiwan. Class II HLA genotyping was based on PCR-SSO DNA typing techniques. Extended class II HLA haplotypes were deduced unequivocally by the Taiwanese pedigree studies.

RESULTS — DR3/DR3, DR3/DR4, and DR3/DR9 genotypes were strongly associated with IDDM susceptibility in this population. In addition to the reported DR3/DR4 in Caucasians, the heterozygotic effect of DR3/DR9 for IDDM was remarkable in the Taiwanese population. Extended HLA haplotypes studies revealed that DRB1*0301/DQA1*0501/DQB1*0201, DRB1*0405/DQA1*0301/DQB1*0302, and DRB1*0405/DQA1*0301/DQB1*0401 were the susceptible haplotypes in this population. There were several hypothetical ways to produce susceptible HLA-DQ heterodimers to explain the susceptibility carried by DR3/DR4 and DR3/DR9 genotypes. Among all DR4 subtypes, only DRB1*0405 was associated with the increased risk of IDDM.

CONCLUSIONS — These data strongly suggest that the HLA-DR–associated IDDM susceptibility is most likely explained by the formation of the susceptible DQ heterodimers encoded by the DQA1/DQB1 either in *cis* or in *trans*.

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Received for publication 19 April 1995 and accepted in revised form 13 July 1995.

EF, etiologic fraction; HLA, human leukocyte antigen; IDDM, insulin-dependent diabetes mellitus; PF, prevented fraction; RR, relative risk.

The major gene factors associated with insulin-dependent diabetes mellitus (IDDM) are human leukocyte antigen (HLA) genes within the major histocompatibility complex on human chromosome 6 (1). Specific HLA haplotypes identified from the studies in different ethnic groups greatly improve our understanding of the possible pathogenic roles of the HLA genes in IDDM by transracial comparison (2,3). Previously, the protective or susceptible effect of HLA genes had been localized to the 57th amino acid residue of the DQ β-chain (4,5). This hypothesis cannot hold true to explain the overall data from various populations, including the Taiwanese population (6). As cell surface DQ molecules are composed of $\alpha\beta$ heterodimers (7,8), both DQA1 and DQB1 genes are implicated in the susceptibility of IDDM in a dose-dependent manner (9,10).

In Chinese populations, the reported distribution of the class II HLAs is not always consistent because of the huge territory and the ethnic heterogeneity (6,11,12). However, there is no published pedigree information from the Chinese so far to support reliable haplotype analysis. Here, we studied simplex IDDM families and unrelated individuals selected in the Chinese population in Taiwan for class II HLA haplotype analysis, with emphasis on the DR-associated DQA1/DQB1 susceptible haplotypes and genotypes in the pathogenesis of IDDM.

RESEARCH DESIGN AND METHODS

— There were 31 pedigrees of simplex IDDM families, 57 unrelated individuals with IDDM, and 105 unrelated normal control subjects recruited in this study. All individuals were Han Chinese living in Taiwan. IDDM was diagnosed using the criteria of the National Diabetes Data Group, including a typical history of diabetic ketoacidosis and a reduced glucagon-stimulated C-peptide response (6). Class II HLA–DNA typing of DRB1, DQA1, and DQB1 was performed using PCR-SSO DNA typing

Table 1—*Distribution of HLA-DR among Taiwanese IDDM patients and control subjects*

HLA-DR	IDDM patients	Control subjects	RR (95% confidence interval)	Corrected *P* value (for χ^2)	EF (%)	PF (%)
DR1	1 (0.6)	1 (0.5)	—	NS	—	—
DR2	13 (7.4)	37 (17.6)	0.37 (0.20–0.74)	0.07	—	11
DR3	54 (30.7)	11 (5.2)	8.01 (3.93–15.2)	0.00	27	—
DR4	48 (27.3)	23 (11.0)	3.05 (1.75–5.18)	0.01	18	—
DR5	8 (4.5)	38 (18.1)	0.22 (0.10–0.49)	0.00	—	14
DR6	16 (9.1)	23 (11.0)	—	NS	—	—
DR7	3 (1.7)	3 (1.4)	—	NS	—	—
DR8	5 (2.8)	31 (14.8)	0.17 (0.07–0.46)	0.01	—	12
DR9	26 (14.8)	37 (1.76)	—	NS	—	—
DR10	2 (1.1)	2 (1.0)	—	NS	—	—

Data are *n*(%) of 176 IDDM patients and 210 control subjects.

techniques according to the previous reports (6,13,14).

The χ^2 test or Fisher's exact probability test was used where appropriate to compare the differences in the frequencies between the IDDM and control groups. Relative risk (RR), etiologic fraction (EF), prevented fraction (PF), and the attributable risk percent of each marker were analyzed. A 0.05 significance level was applied to calculate 95% confidence interval of RR in each comparison. *P* values were corrected wherever appropriate.

RESULTS — Table 1 gives distribution of 10 DR types in the 88 IDDM patients and 105 healthy nondiabetic control subjects. HLA-DR3 and DR4 were increased while DR2, DR5, and DR8 were decreased in the IDDM subjects as compared with those of the control subjects. Among the DR genotypes (Table 2), DR3/DR4, DR3/DR9, and DR3/DR3 were the most significantly susceptible HLA-DR genotypes for IDDM. The heterozygotes of DR3/DR4 were associated with the highest risk (RR = 38.48, EF = 15%) along with DR3/DR9 (RR = 5.89, EF = 12%), while homozygous DR3/DR3 contributed to IDDM to a lesser extent (RR = 22.7, EF = 9%).

A total of 43 different DRB1-DQA1-DQB1 haplotypes were constructed from 31 Taiwanese families. Among the nonrelated individuals, 21 were homozygous for DRB1-DQA1-DQB1. Based on this information, a total of 67 different haplotypes were available for analysis. Among the various haplotypes (Table 3), only DRB1*0301/DQA1*0501/DQB1*0201, DRB1*0405/DQA1*0301/DQB1*0302, and DRB1*0405/DQA1*0301/DQB1*0401 were significantly increased in IDDM. It is interesting to note that the DRB1*0901/DQA1*0301/DQB1*0303 haplotype alone was neutral in IDDM susceptibility.

There are several ways to produce diabetogenic DQ heterodimers either in *cis* or in *trans*, as shown in Table 4. Type 2 and 3 diabetogenic DQ dimers encoded in *cis* and type 4 and 5 dimers encoded in *trans* could be produced to explain the excess risk of DR3/DR4 heterozygotes in the Taiwanese. In DR3/DR9 heterozygotes, DQ heterodimer of DQA1*0301/DQB1*0303 (formed in *cis*) was not associated with risk of IDDM in our population. However, the DQ molecule of DQA1*0301/DQB1*0201 (type 6 dimer) could be formed in *trans* to confer susceptibility to IDDM.

CONCLUSIONS — In this study, extensive DNA typing for HLA-DR, DQA1, and DQB1 were performed in Taiwanese simplex IDDM families as well as IDDM and control individuals. This work has allowed us to define the HLA DR-DQ haplotypes unequivocally in the Taiwanese population. Among them, DRB1*0301/DQA1*0501/DQB1*0201, DRB1*0405/DQA1*0301/DQB1*0302, and DRB1*

Table 2—*Distribution of HLA-DR genotypes among IDDM patients and normal controls subjects in Taiwanese*

HLA-DR genotypes	IDDM patients	Control subjects	RR (95% confidence interval)	Corrected *P* value (for χ^2)	EF (%)	PF (%)
DRX/DRX	16 (18.2)	75 (71.4)	0.09 (0.05–0.18)	0.000	—	65
DR9/DRY	6 (6.8)	23 (21.9)	0.26 (0.11–0.69)	NS	—	—
DR9/DR9	2 (2.3)	4 (3.8)	—	NS	—	—
DRY/DRY	8 (9.1)	48 (45.7)	0.12 (0.06–0.28)	0.000	—	—
DR3/DRX	24 (27.3)	10 (9.5)	3.56 (1.57–7.61)	0.001	20	—
DR3/DR9	13 (14.8)	3 (2.9)	5.89 (1.56–17.6)	0.048	12	—
DR3/DRY	11 (12.5)	7 (6.7)	—	NS	—	—
DR4/DRX	18 (20.5)	17 (16.2)	—	NS	—	—
DR4/DR9	3 (3.4)	3 (2.9)	—	NS	—	—
DR4/DRY	15 (17.0)	14 (13.3)	—	NS	—	—
DR3/DR3	8 (9.1)	0 (0)	22.70 (2.85–81.9)	0.012*	9	—
DR4/DR4	8 (9.1)	3 (2.9)	—	NS	—	—
DR3/DR4	14 (15.9)	0 (0)	38.48 (4.99–136)	0.00*	15	—

Data are *n*(%) of 88 IDDM patients and 105 control subjects. DRX = non-DR3 or DR4; DRY = non-DR3, DR4, or DR9. *Fisher's exact test.

Table 3—*HLA DRB1/DQA1/DQB1 haplotypes analysis in the subjects bearing DR3, DR4, and DR9*

HLA-DR	DRB1/DQA1/DQB1	IDDM patients	Control subjects	RR (95% confidence interval
DR3	0301/0501/0201	50 (28.4)	10 (4.8)	7.9 (3.78–15.4)
	0301/0501/0303	2 (1.1)	0 (0)	—
	0302/0501/0201	2 (1.1)	1 (0.5)	—
DR4	0401/0301/0302	4 (2.3)	0 (0)	—
	0402/0301/0302	1 (0.6)	0 (0)	—
	0403,6/0301/0302	7 (4.0)	11 (5.2)	—
	0404/0301/0302	5 (2.8)	3 (1.4)	—
	0405/0301/0302	9 (5.6)	0 (0)	23.9 (3.08–86.5)
	0405/0301/0401	18 (10.2)	7 (3.3)	3.30 (1.32–7.58)
	0405/0301/0501	1 (0.6)	0 (0)	—
	0407/0301/0302	1 (0.6)	0 (0)	—
	0408/0301/0302	1 (0.6)	1 (0.5)	—
	0410/0301/0302	1 (0.6)	0 (0)	—
	0411/0301/0401	0 (0)	1 (0.5)	—
DR9	0901/0103/0301	1 (0.6)	0 (0)	—
	0901/0301/0201	2 (1.1)	0 (0)	—
	0901/0301/0301	1 (0.6)	0 (0)	—
	0901/0301/0303	21 (11.9)	34 (16.2)	—
	0901/0301/0401	1 (0.6)	0 (0)	—
	0901/0301/0504	0 (0)	1 (0.5)	—
	0901/0601/0301	0 (0)	2 (1.0)	—

Data are *n*(%) of 176 IDDM patients and 210 control subjects.

0405/DQA1*0301/DQB1*0401 were the three susceptible haplotypes for IDDM.

It is interesting to note that the three susceptible DR genotypes found in Taiwanese IDDM were DR3-containing, i.e., DR3/DR3, DR3/DR4, and DR3/DR9, suggesting that DR3 is necessary for IDDM susceptibility. Our findings in Taiwanese are similar to those reported in Mexican-Americans (15), where a recessive effect of DR3 requiring another susceptibility haplotype (DR3, DR4, or DR9 in our case) is postulated. The heterozygote effect of DR3/DR4 is well-documented (3) and may be explained by transcomplementation of DQ molecules (7) or through different mechanisms associated with DR3 and DR4 haplotypes. In DR3/DR9 heterozygotes, although DR9-coupled DQA1*0301/DQB1*0303 (formed in *cis*) was reported to be associated with IDDM in Japanese and Chinese living in the northern part of Mainland China (16,17), our study in Taiwanese suggested that susceptibility of DR3/DR9 to IDDM was most likely explained by the DQ heterodimers formed of DQA1*0301/DQB1*0201 (type 6 dimers in Table 4) in addition to DQA1*0501/DQB1*0201 encoded in *cis* (type 1 dimer).

The role of DQB1*0302 in DR4-associated susceptibility is found in almost all populations except Japanese (18). In the Taiwanese population, both DQB1*0302 and DQB1*0401 were associated with DR4 haplotypes. Although DQ molecules of DQA1*0301/DQB1*0401 encoded in *cis* might explain IDDM susceptibility (18), this is not the case in Taiwanese because DR4/DR4 homozygotes in Taiwan were not associated with increased risk of IDDM. These data also argued that DQA1*0301 (previously denoted as A3 allele) alone is not sufficient to account for IDDM susceptibility (19). The DR4-associated susceptibility occurred only when transcomplementing DQ heterodimers (type 4 and 5 dimers) were formed.

The attempt to explain HLA susceptibility with the formation of susceptible DQ molecules may not be fully adequate. For instance, among the DR4-associated haplotypes, only DRB1*0405 was statistically significantly associated with IDDM susceptibility. This DR4 subtype (DRB1*0405) is also found to be associated with IDDM in many other populations (15,20). Taken together, these data suggest that DRB1 allele plays an additional role in determining IDDM susceptibility.

Acknowledgments— This work was supported by the grants NSC-81–0412-B002–42

Table 4—*Different types of susceptible DQ molecules associated with IDDM in Taiwanese as compared with other races*

Type	DR	DQA1	DQB1	Position	Race	Reference
1	DR3	0501	0201	*cis*	White, black, Algerian, Taiwanese	3, 20, present
2	DR4	0301	0302	*cis*	White, black, Algerian, Taiwanese*	3, 20, present
3	DR4	0301	0401	*cis*	Japanese, Taiwanese*	18
4	DR3	—	0201	—	—	—
	DR4	0301	—	*trans*	White, Taiwanese	3, present
5	DR3	0501	—	—	—	—
	DR4	—	0302	*trans*	White, Taiwanese	3, present
6	DR3	—	0201	—	—	—
	DR9	0301	—	*trans*	Taiwanese	Present

*Contradictary results.

and NSC-82-0412-B002-H12 from the National Science Council of the Republic of China.

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