HLA-DQB1 Codon 57 and IDDM in Chinese Living in Taiwan

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OBJECTIVE — To study the human leukocyte antigen (HLA)-DQB1 genetic background in the Chinese population in Taiwan and its association with the low incidence of insulin-dependent diabetes mellitus (IDDM) in this population.

RESEARCH DESIGN AND METHODS — Forty-eight IDDM patients and 59 nondiabetic unrelated control subjects were recruited from the population in Taiwan. HLA-DQB1 exon 2 was enzymatically amplified by polymerase chain reaction. HLA-DQB1 alleles were diagnosed by dot blotting and hybridization with 16 sequence-specific oligonucleotide probes.

RESULTS — DQB1*0201 and DQB1*0302 alleles were more frequent and DQB1*0301 and DQB1*0601 were less frequent in Chinese with IDDM than in control subjects. Genotypes for homozygous non–aspartic acid residue (NA/NA) at position 57 were positively associated with IDDM at a relative risk of 4.34 ($P < 0.001$), and those for homozygous aspartic acid (A/A) were negatively associated with IDDM at a relative risk of 0.14 ($P < 0.001$). Among the NA/A heterozygotes, only DQB1*0201/DQB1*0303 was significantly increased in IDDM subjects.

CONCLUSIONS — The amino acid residue at position 57 of HLA-DQ β-chain is significantly associated with the development or prevention of IDDM in Chinese subjects living in Taiwan. Other genetic and environmental factors may also play important roles in pathogenesis of IDDM.

There is a wide range of difference in the incidence of insulin-dependent diabetes mellitus (IDDM) in the world (1–3). The incidence of IDDM in the population in Taipei was 1.35/100,000 person-years in the years 1980–1990 (Taipei IDDM Registry Group, unpublished observations), which is much lower than incidences reported from the Western world. However, there is a strong association between the human leukocyte antigen (HLA) and IDDM in almost all populations, including the Chinese (4–7). Detailed molecular studies have revealed that a substitution of aspartic acid (Asp) by a non-Asp, such as alanine, valine, and serine, at the 57th position of the HLA-DQ β-chain is highly associated with IDDM susceptibility (8,9). Although this association is very strong in the Caucasian population (10,11), the association between DQβ-57 and IDDM in Oriental racial groups has not been confirmed. No association was found in Japanese subjects (12,13), and controversial results have been reported in Chinese subjects (14,15). One reason for these discrepancies could be the very small size of the sample studied by Bao et al. (14). Alternatively, because of the huge territory, heterogeneity is expected among Chinese populations of different geographical areas. The notion that the frequency of non-Asp alleles of DQB1 gene in different populations might correlate with the incidence of IDDM is still poorly documented (11). Therefore, in this study, we determined HLA-DQB1 typing by a polymerase chain reaction and sequence-specific oligonucleotide hybridization technique in a population of Chinese living in Taiwan to examine the role of DQβ-57 Asp in Chinese IDDM patients.

RESEARCH DESIGN AND METHODS

— Forty-eight subjects with IDDM, with a mean age of 18.4 ± 7.6 (SD) years at diagnosis, were selected at the Diabetic Clinic of the National Taiwan University Hospital (Taipei, Taiwan). IDDM was diagnosed according to the criteria published by the National Diabetes Data Group (16), the clinical history of proneness to diabetic ketoacidosis, and the C-peptide determinations in glucagon stimulation test. Subjects' mean body mass index was 20.3 ± 4.8 kg/m^2, basal C-peptide levels were 0.06 ± 0.28,

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IDDM, insulin-dependent diabetes mellitus; HLA, human leukocyte antigen; Asp, aspartic acid; RR, relative risk; CI, confidence interval; EF, etiological fraction; PF, prevented fraction; NA, non–aspartic acid; A, aspartic-acid allele.

Table 1—*HLA-DQB1 allele frequencies in Chinese IDDM patients and control subjects*

Allele	IDDM group (n = 96)		Control group (n = 118)		RR (95% CI)	χ^2	P value	EF (%)	PF (%)
	n	%	n	%					
NA alleles	64	66.67	38	32.20	4.15 (2.35–7.34)	25.2	<0.001	50.8	—
DQB1*0201	35	36.46	9	7.63	6.65 (3.00–14.74)	26.9	<0.001	31.2	—
DQB1*0302	19	19.79	12	10.17	2.14 (0.99–4.62)	4.0	<0.05	10.7	—
DQB1*0501	2	2.08	4	3.39	—	—	—	—	—
DQB1*0502	6	6.25	12	10.17	—	—	—	—	—
DQB1*0604	1	1.04	0	0.00	—	—	—	—	—
DQB1*0605	1	1.04	1	0.85	—	—	—	—	—
A alleles	32	33.33	80	67.80	0.24 (0.14–0.43)	25.2	<0.001	—	51.5
DQB1*0301	8	8.33	29	24.58	0.29 (0.13–0.66)	9.8	<0.01	—	17.7
DQB1*0303	10	10.42	22	18.64	—	—	—	—	—
DQB1*0401	10	10.42	9	7.63	—	—	—	—	—
DQB1*0503	3	3.12	6	5.08	—	—	—	—	—
DQB1*0601	0	0.00	9	7.63	0.06 (0.003–1.04)	EXACT	0.004	—	7.2
DQB1*0602	1	1.41	4	3.39	—	—	—	—	—
DQB1*0603	0	0.00	1	0.85	—	—	—	—	—

EXACT, Fisher's exact test.

and postglucagon stimulated levels were 0.12 ± 0.55 ng/ml at 6 min. Fifty-nine unrelated healthy subjects served as control subjects. They had no family history of diabetes and were recruited from among those who had been hospitalized for a physical checkup.

Genomic DNA was extracted from peripheral blood leukocytes (17). Exon 2 of the HLA-DQB1 gene was enzymatically amplified by the polymerase chain reaction (18) using sequence-specific primers (DB130 and DB131) (19). An amplification without target DNA was always included to check for contamination. DNA from cells with known HLA-DQB1 alleles was also amplified and used as a control in typing. Aliquots of the amplified DNA were checked to see if the amplification worked well. Nonradioactive oligotyping for HLA-DQB1 was performed by using 16 horseradish perioxidase-labeled sequence-specific oligoprobes. The detailed hybridization and detection protocols followed the methods published by Bugawan and Erlich (19). The stringency of hybridization and washing was adjusted to obtain accurate signal patterns for the DNA from the control cells. The hybridized probes were detected by the ECL gene detection system (Amersham, Little Chalfont, U.K.). Signals could be captured on X-ray film after 30 s to 5 min of exposure. For reprobing, membranes were treated according to the manufacturer's recommendation.

Statistical analysis

For each allele or genotype, no matter whether structural or functional, a χ^2 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) using confidence interval (CI) inference conferred by each of the different markers in the two groups was calculated according to methods published previously (20,21). The etiological fraction (EF) and prevented fraction (PF), the attributable risk percentage of each marker, were also analyzed (22). Regression analysis with the transformation given in Table 3 was applied for IDDM incidence inferred with the frequency of the functional gene DQB1 codon 57 (23). All analyses were not for multiple comparisons; a 0.05 significance level was applied, with no need for any correction.

RESULTS

HLA-DQB1 allele frequencies

The oligotyping of the HLA-DQB1 gene was done by hybridization with a combination of 16 nonradioactive oligonucleotide probes according to Bugawan and Erlich (19). Table 1 shows the HLA-DQB1 allele frequencies of the IDDM patients and the control subjects. The comparison of frequency of each allele revealed four different alleles (0201, 0302, 0301, and 0601) that were different among the normal subjects and IDDM patients. The frequency of alleles carrying a non–aspartic acid (NA) residue at position 57 was significantly higher in the IDDM patients compared with the control group (66.7 vs. 32.2%, $P < 0.001$). Among all the NA alleles, only DQB1*0201 and DQB1*0302 were positively associated with IDDM (for 0201, RR = 6.95, $P < 0.001$; for 0302, RR = 2.18, $P < 0.05$). The frequency of Asp alleles (A) was negatively

Table 2—*Genotypic frequencies at the HLA-DQB1 gene in the IDDM patients and control subjects*

Genotype	IDDM group (n = 48) n	%	Control group (n = 59) n	%	RR (95% CI)	χ^2	P value	EF (%)	PF (%)
NA/NA	23	47.9	10	16.9	4.34 (1.82–5.70)	11.9	<0.001	33.9	—
0201/0201	7	14.6	2	3.4	4.16 (0.94–18.37)	EXACT	0.035	11.6	—
0201/0302	4	8.3	0	0.0	12.03 (0.63–1.69)	EXACT	0.038	8.1	—
0302/0302	6	12.5	3	5.1	—	—	—	—	—
Others	6	12.5	5	8.5	—	—	—	—	—
NA/A	19	39.6	18	30.5	—	—	—	—	—
0201/0301	2	4.2	1	1.7	—	—	—	—	—
0201/0303	7	14.6	2	3.4	4.16 (0.94–18.37)	EXACT	0.035	11.6	—
0502/0301	1	2.1	6	10.2	—	—	—	—	—
Others	9	18.8	9	15.3	—	—	—	—	—
A/A	6	12.5	31	52.5	0.14 (0.05–0.36)	18.8	<0.001	—	45.7
0301/0301	1	2.1	4	6.8	—	—	—	—	—
0301/0303	0	0.0	5	8.5	0.10 (0.01–1.90)	EXACT	0.047	—	7.6
Others	5	10.4	22	37.3	—	—	—	—	—

EXACT, Fisher's exact test. Others, other genotypes without significant difference between two groups.

associated with IDDM (RR = 0.24, P < 0.001). Among them, DQB1*0301 and DQB1*0601 were protective against IDDM (for 0301, RR = 0.28, P < 0.01; for 0601, RR = 0.06, P = 0.004).

Distribution of HLA-DQB1 genotypes

DQB1 genotypic frequencies in these two groups are shown in Table 2. The NA/NA genotypes were positively associated with the disease (RR = 4.34, P < 0.001), and A/A genotypes were negatively associated with the disease (RR = 0.14, P < 0.001). Among the NA/NA combinations, only DQB1*0201/NA (at least one of two NA alleles is 0201) was strongly associated with IDDM (RR = 12.95, P < 0.001; for 0201/0201, RR = 4.16, P = 0.035; for 0201/0302, RR = 12.03, P = 0.038). Even when DQB1*0201 was in combination with the Asp allele 0303, this genotype was also susceptible to IDDM (RR = 4.16, P = 0.035). Among the A/A combinations, DQB1*0301/0303 was negatively associated with IDDM (RR = 0.10, P = 0.047).

Prediction of the incidence of IDDM from the frequency of NA alleles in different races

The incidence of IDDM was found to correlate with the NA allele frequency of HLA-DQβ-57 in Sardinians, Norwegians, U.S. whites, U.S. blacks, and Chinese in Tienjin (11). Since then, there have been four more studies on HLA-DQβ-57 in populations with known incidence of IDDM (13,24,25, and this study). The results from these four populations, namely, Japanese, French, Finnish, and Taiwanese, seem to lie outside the regression line based on the original five study populations (11). To perform regression analysis, we transformed the reported incidence values to a nearly normally distributed variable and recalculated the regression band with 95% CI as shown in Fig. 1. The calculated mean expected values (and the respective 95% CI) of the incidence of IDDM for Japanese, French, Finnish, and Taiwanese rejected the null hypothesis that these four populations were on the transformed regression line (Table 3, Fig. 1), suggesting that HLA-DQB1–57 is not the only determinant for predicting the incidence of IDDM given a known frequency of NA allele in different populations.

CONCLUSIONS — The short gene segment around amino acid codon 57 of

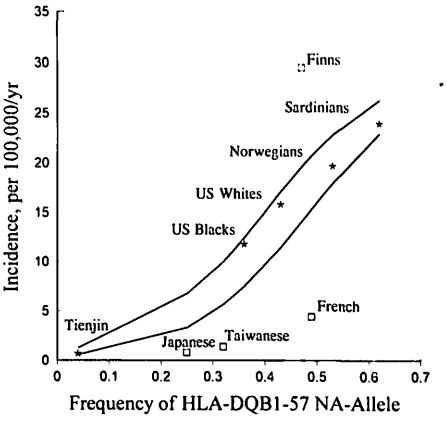

Figure 1—*Relationship between the frequencies of NA-alleles of the HLA-DQB1 and the incidence of IDDM. The 95% CI band was derived from Sardinians, Norwegians, U.S. whites, U.S. blacks, and Chinese in Tienjin (11).*

Table 3—*Regressional inference for population incidence on the HLA-DQβ-57 NA-allele frequency*

P: population name	*Io(P)*: observed incidence	*I(P)*: expected incidence	*I(P)*: 95% CI
Taiwanese	1.35	7.62	5.65–10.02
French	4.40	18.15	15.32–26.77
Japanese	0.80	4.65	3.33–6.74
Finns	29.50	16.86	14.03–19.57

Testing hypothesis: I(P) on the regression line of Sardinians, Norwegians, U.S. whites, U.S. blacks, and Chinese in Tienjin. Population incidence, $I(P) = 0.0003/[1 + \exp(-y)]$, where $y = y(P) = -3.91 + 8.85\ P \pm 0.85[0.20 + 0.25(P - 0.40)^2]^{1/2}$, ($n = 5$), and P for the NA allele frequency of the population. CI indicates statistical significance at the level of 95%.

the HLA-DQ β-chain may involve the process of immune recognition (26). A strong association between IDDM and the genotypes of this region has been observed in various ethnic groups (Table 4). Absence of this association is found only in the Japanese population (12,13). Most people in Taiwan are descendants of Han Chinese who immigrated from the southeast coast of mainland China as early as 400 years ago. The island is located far from the northern port city of Tienjin in mainland China (Fig. 2). The incidence of IDDM is low in the Oriental countries compared with incidences in the Western countries (Fig. 2). Although the incidence of IDDM in this island is low, it was found to be about twice that in Tienjin area (2). Meanwhile, the frequency of the NA alleles in Taiwanese was higher than that in Tienjin (32 vs. 4%). This increased frequency of NA alleles may account for the twofold higher incidence of IDDM in Taiwan than in Tienjin.

Our data support the DQβ-57-Asp hypothesis in general. However, there were quantitative differences for the strength of association for various alleles. In this population, the alleles DQB1*0201 and DQB1*0302 were strongly associated with IDDM. DQB1*0201 had the most powerful risk effect (RR = 6.95, EF = 31.2%), and the genotypes containing DQB1*0201 (DQB1*0201/0201 and 0201/0303) were associated with increased risk of IDDM. The haplotypes containing DQB1*0201 with risk of IDDM included DR3/DQA1*0501/DQB1*0201 in whites (8) and DR7/DQA1*3/DQB1*0201 and DR9/DQA1*3/DQB1*0201 in blacks (27). Our data may support the previous finding of the excess risk conferred by DR3/DR4 heterozygosity found in IDDM because of the associated DQB1*0201/0302 (28). The increase of DQB1*0201/0303 heterozygosity in our IDDM subjects might be related to the susceptible DR3/DR9 genotype, which had been reported in a Chinese population (7), and a susceptible DR9 haplotype containing DQA1*03/DQB1*0303 was also found in Japanese (29). Interestingly, the homozygosity of DQB1*0201/0201 was frequent among IDDM patients in this population. The reason for this result is not clear. However, homozygosity of DQB1*0201/0201 might be functionally heterozygous in terms of DQαβ heterodimer formation with respective DQA1 molecules and different DR association.

Among the Asp alleles, only DQB1*0301 and DQB1*0601 were associated with IDDM protection in our population. The preventive role of DQB1*0301 (PF = 17.7%) was similar to those reported in Caucasians (30). However, to our knowledge, the preventive role of DQB1*0601 (PF = 7.2%) for IDDM was

Table 4—*Distribution of HLA-DQB1 genotypes and non-Asp-57 allele frequencies among the IDDM patients and normal subjects in the world*

	IDDM patients					Normal subjects							
Population	*n*	N/N	N/A	A/A	N	*n*	N/N	N/A	A/A	N	χ^2 (d.f. = 2)	*P* value	Reference
Sardinians	30	1.00	0.00	0.00	1.00	60	0.38	0.47	0.15	0.62	31.39	<0.0005	11
Norwegians	52	0.82	0.16	0.02	0.90	187	0.27	0.51	0.22	0.53	52.90	<0.0005	11
French	112	0.72	0.24	0.05	0.84	405	0.26	0.47	0.27	0.49	81.95	<0.0005	24
Finns	86	0.75	0.22	0.04	0.85	115	0.18	0.57	0.25	0.47	67.38	<0.0005	25
U.S. whites	49	0.61	0.39	0.00	0.81	123	0.20	0.46	0.34	0.43	35.50	<0.0005	11
U.S. blacks	30	0.73	0.27	0.00	0.87	51	0.18	0.37	0.45	0.36	29.84	<0.0005	11
Chinese (T)	48	0.48	0.40	0.12	0.67	59	0.17	0.31	0.53	0.32	21.14	<0.005	This study
Chinese (H)	48	0.23	0.50	0.27	0.48	95	0.11	0.40	0.50	0.31	7.88	<0.025	15
Japanese	72	0.03	0.49	0.49	0.27	85	0.08	0.34	0.58	0.25	4.63	>0.05	13
Chinese (M)	18	0.06	0.72	0.22	0.42	25	0.00	0.08	0.92	0.04	21.86	<0.005	11, 14

N, non-Asp-57 alleles; T, Taiwan; H, Hong Kong; M, Mainland China.

Figure 2—*World map of the incidence of IDDM. The numbers indicate the order of incidence of IDDM in each of our study populations: 1, Finns; 2, Sardinians; 3, Norwegians; 4, U.S. whites; 5, U.S. blacks; 6, French; 7, Taiwanese; 8, Japanese; 9, Chinese in Tienjin.*

only found in Taiwanese. In addition, 12% of IDDM patients in Taiwan had the A/A genotype, in contrast with nearly 0% in the Western countries (Table 4). This discrepancy raises the dispute that the 57th position of the DQβ chain is the only locus that is important for IDDM development.

In both high and low incidence areas, exceptions were found in many populations to support the linear relationship between DQβ-57-NA and the incidence of IDDM, such as Finns and French in high incidence areas and the Japanese and Taiwanese in low incidence areas. If we accept the transformation regression analysis between the frequency alleles in each population and the incidence figures of IDDM in the study of Dorman et al. (11), our data in this area would predict a incidence rate of 5.65–10.02 (versus the observed 1.35/100,000 person-years). This discrepancy might be explained by the different environmental factors involved in the pathogenesis of IDDM in different cultures or by other genetic factors that were not examined in this study. There have been reports of the rising rate of IDDM in several different countries (31–33), suggesting that environmental factors may play a role in the pathogenesis of IDDM. These speculations should be further addressed by a migration study of this population to other areas of high IDDM epidemics and the studies of other genetic loci.

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