HLA-DQ A1, -DQB1 AND –DRB1 Gene Polymorphism - In Malay Type 1 Diabetes Mellitus Patients And Their Use For Risk Prediction

A G Rohana*, K C Loh, S K Tin***, C H Soh****, W M Wan Nazaimoon*****, K Y Fong******, K Nor Azmi*******, B A K Khalid********

*Lecturer and Consultant Endocrinologist, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia, **Consultant Endocrinologist, Endocrine Unit, Department of General Medicine; Tan Tock Seng Hospital, Singapore, ***Senior Research Assistant, Autoimmune Research Laboratory; Tan Tock Seng Hospital, Singapore, ****Medical Statistician, Clinical Research Unit, Tan Tock Seng Hospital, Singapore, *****Head, Division of Endocrinology, Institute for Medical Research, Kuala Lumpur, Malaysia, ******Senior Consultant, Autoimmune Research Laboratory; Kuala Lumpur General Hospital, Malaysia, *******Professor & Consultant Endocrinologist, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia, ********Professor of Medicine and Head of the School of Clinical Sciences, Monash University, Malaysia

SUMMARY

HLA-DQA1, -DQB1, and -DRB1 gene polymorphism were analyzed to study type 1 DM susceptibility in Malay patients from Southeast Asia (Malaysia and Singapore). Patients showed significant increases in the occurrence of DQA1*0501 (50.7% vs. 20.4%; RR = 3.97; Pc < 0.01), DQB1*0201 (48% vs. 19.1%; RR = 3.86; Pc < 0.05), and DRB1*0301 (38.7 vs. 6.8%; RR = 8.36; 95% Pc < 0.05). Conversely, significant decreases were noted in the occurrence of DQA1*0601 (14.7% vs. 35.2%; RR = 0.33; Pc = 0.008) and DQB1*0601 (4% vs. 23.5%; RR = 0.16; Pc < 0.05) in type 1 DM patients. Using a logistic regression model, we derived a risk prediction model for type 1 DM in our indigenous Malay population based on the identified HLA genotypes. The RR for type 1 DM increases by a factor of 5.68 for every unit increase in the number of DRB1*0301 allele (P < 0.001), and decreases by a factor of 0.18 per unit increase in the number of DQB1*0601 allele (P < 0.001). After adjusting for these two HLA genotypes, DQA1*0501, DQB1*0201 and DQA1*0601 were not statistically significant as risk predictors. The lower incidence of type 1 DM in the Malay population may be contributed by the genotypic combinations of DR and DQ genes as well as the linkage disequilibria between susceptible and protective alleles.

ABBREVIATIONS

CI, confidence interval; MHC, major histocompatibility complex; Pc, P value corrected for the number of statistical comparisons; PCR, polymerase chain reaction; RR, relative risk; Type 1 DM, type 1 diabetes mellitus.

KEY WORDS:
Type 1 diabetes mellitus; HLA-DR alleles; HLA-DQ alleles; genetics; Malay

INTRODUCTION

Type 1 diabetes mellitus (type 1 DM) is recognized as a disease with heteregenous aetiology, influenced by environmental factors and prevalent autoimmune susceptibility. Predisposition of the autoimmune pancreatic beta cell destruction has been associated with genetic variations on different chromosomes. Accrued data suggest that genes in the class II major histocompatibility complex (MHC) on the short arm of chromosome 6 constitute the major genetic determinant for the development of type 1 DM. Associations between human leucocyte antigen (HLA) genes and type 1 DM were first reported among Caucasians with class I molecules B8 or B15 [1]. Subsequent studies showed stronger associations of type 1 DM with the MHC Class II genes [2] with obvious racial variations, being predominantly DR3 or DR4 in the Caucasians [3], and DR4 or DR9 in the Japanese [4], respectively.

In the mid-1980s, genotyping of HLA alleles provided a more precise definition in susceptibility and protective gene markers [5]. Based on genotyping, *HLA-DQA1, -DQB1*, and -*DRB1* gene polymorphism were found to be most robustly associated with type 1 DM, although variations were observed in the different ethnic populations studied [6]. These *HLA-DR/DQ* alleles acted as either predisposing or protective of Type 1 DM [7]. This discovery allowed better risk prediction for type 1 DM and subsequently provided more information on other aspects of this debilitating disease of the young including the prediction of pancreatic β-cell destruction and ketosis-prone diabetes [8,9].

Despite the steady global rise in the overall prevalence of Type 1 DM, Asians are generally known to demonstrate a lower prevalence rate compared to the Caucasians. This could well be influenced by the varying susceptibility to type 1 DM conferred by the HLA expressions. As there are no reported local data of the association of genetic polymorphism within the Malay population, we examined *HLA-DQA1, -DQB1*, and *-DRB1* gene polymorphism in our local Malay type 1 DM patients.

MATERIALS AND METHODS

Patients and controls

Seventy-five unrelated Malay patients affected by type 1 DM and 162 unrelated healthy Malay controls were randomly selected for the study. Patients were diagnosed according to

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Corresponding Author: Rohana Abdul Ghani, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia
Email: agrohana@gmail.com

Table I: Characteristics of 75 patients (34 males, 41 females) with type 1 DM

	Median	(range)
Age at evaluation	22.0 yr	(0.2 – 52.0 yr)
Age at diagnosis	14.0 yr	(0.2 – 38.0 yr)
Body mass index (BMI)	19.9 kg/m2	(13.6 – 29.7 kg/m2)
Waist-hip ratio (WHR)	0.8	(0.7 – 0.9)
Basal C-peptide*	133 pmol/L	(0 – 487 pmol/L)
Stimulated C-peptide*	201 pmol/L	(0 – 669 pmol/L)
Proportion of patients with positive autoimmune markers for type 1 DM		
Glutamate decarboxylase antibody (GAD)	0/75 (40.0%)	
Tyrosine phosphatase antibody (IA-2)	4/48 (8.3%)	
Insulin auto-antibodies (IAA)	24/48 (50.0%)	

*Basal and stimulated C-peptide levels refer to basal (fasting) and 30-minute post 1 mg glucagon-stimulated values respectively.

Table II: Distribution of DQA1 alleles among type 1 DM patients and healthy controls

	Patient % (n = 75)	Control % (n = 162)	Relative Risk (95% CI)	P value	Pc value
DQA1*0101	10.7	10.5	1.05 (0.44 – 2.50)	0.968	NS
DQA1*0102	33.3	50.0	0.50 (0.29 – 0.89)	0.016	NS
DQA1*0103	8.0	12.3	0.65 (0.26 – 1.64)	0.319	NS
DQA1*0201	9.3	21.0	0.41 (0.18 – 0.95)	0.027	NS
DQA1*0301	18.7	12.3	1.64 (0.79 – 3.42)	0.197	NS
DQA1*0401	0.0	3.1	0.19 (0.01 – 3.47)	0.182	NS
DQA1*0501	50.7	20.4	3.97 (2.20 - 7.15)	< 0.001	< 0.01
DQA1*0601	14.7	35.2	0.33 (0.16 - 0.66)	0.001	0.008

Pc = corrected P value for the number of different DQA1 alleles tested
NS = not statistically significant

Table III: Distribution of DQB1 alleles among type 1 DM patients and healthy controls

	Patient % (n = 75)	Control % (n = 162)	Relative Risk (95% CI)	P value	Pc value
DQB1*0201	48.0	19.1	3.86 (2.13 - 6.99)	< 0.001	< 0.05
DQB1*0301	26.7	44.4	0.46 (0.25 - 0.83)	0.009	NS
DQB1*0302	12.0	7.4	1.72 (0.71 - 4.20)	0.247	NS
DQB1*0303	10.7	7.4	1.52 (0.61 - 3.79)	0.401	NS
DQB1*0402	0.0	0.6	0.71 (0.03 – 17.71)	1.000	NS
DQB1*0501	22.7	33.3	0.60 (0.32 - 1.11)	0.095	NS
DQB1*0502	17.3	17.9	0.98 (0.48 - 1.99)	0.915	NS
DQB1*0503	6.7	13.0	0.51 (0.19 - 1.37)	0.149	NS
DQB1*0601	4.0	23.5	0.16 (0.05 - 0.48)	< 0.001	< 0.05
DQB1*0602/3	0.0	2.5	0.23 (0.01 - 4.39)	0.311	NS
DQB1*0604	2.7	0.6	3.66 (0.47 – 28.25)	0.236	NS

Pc = corrected P value for the number of different DQB1 alleles tested
NS = not statistically significant

the 1997 American Diabetes Association (ADA) criteria, including a typical history of diabetic ketoacidosis and a reduced glucagon-stimulated C-peptide response. All the patients were on insulin treatment since diagnosis (Table I). The study was approved by Hospital Universiti Kebangsaan Malaysia and Tan Tock Seng Hospital Ethics Committee, respectively; and informed consent was obtained from all participants.

Collection of blood and extraction of DNA

Ten ml of EDTA peripheral venous blood was obtained from each subject under aseptic conditions. Genomic DNA (1-2 µg) was then extracted from peripheral blood leucocytes using DNAzol and stored at 4°C until ready for typing [10]. Confidentiality was assured by coding the DNA samples, and the master list made available only to the investigators.

Gene amplication by polymerase chain reaction (PCR)

About 50 – 100 ηg of genomic DNA was used as template for HLA class II gene amplification by the PCR method. Amplification was started with initial denaturation at 94°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 10 seconds, annealing at 60°C (except for DRB1 whereby the annealing temperature was 55°C) for 30 seconds, and extension at 72°C for 30 seconds. This was followed by final extension at 72°C for 5 minutes. DNA polymerase DyNAzyme (Finnzymes Oy) was used and thermal cycling was performed on PTC-200 thermal cycler (MJ Research, USA). Positive and negative controls as well as blank controls were used in every PCR amplification.

DQA1, DQB1, DRB1 gene polymorphism analysis

After amplification, 7 µl of PCR products were cleaved

Table IV: Distribution of DRB1 alleles among IDDM patients and healthy controls

	Patient % (n = 75)	Control % (n = 162)	Relative Risk (95% CI)	P value	Pc value
DRB1*0103	1.3	1.2	1.29 (0.17 - 9.97)	1.000	NS
DRB1*0301	38.7	6.8	8.36 (3.92 - 17.81)	< 0.001	< 0.05
DRB1*0302	0.0	1.2	0.43 (0.02 - 8.97)	1.000	NS
DRB1*0401	0.0	0.6	0.71 (0.03 - 17.71)	1.000	NS
DRB1*0402	1.3	0.6	2.17 (0.22 - 21.19)	0.534	NS
DRB1*0405	10.7	3.1	3.61 (1.19 - 10.94)	0.028	NS
DRB1*0406	4.0	4.3	1.00 (0.27 - 3.67)	1.000	NS
DRB1*0701	5.3	12.3	0.44 (0.15 - 1.26)	0.096	NS
DRB1*0802	0.0	1.2	0.43 (0.02 - 8.97)	1.000	NS
DRB1*0803	5.3	8.6	0.64 (0.22 - 1.93)	0.371	NS
DRB1*0901	9.3	13.0	0.72 (0.30 - 1.74)	0.421	NS
DRB1*1001	5.3	4.9	1.14 (0.35 - 3.71)	1.000	NS
DRB1*1101	2.7	1.9	1.55 (0.30 - 8.04)	0.653	NS
DRB1*1201	1.3	0.0	6.54 (0.26 - 162.53)	0.316	NS
DRB1*1202	30.7	50.6	0.44 (0.25 - 0.78)	0.004	NS
DRB1*1302	0.0	0.6	0.71 (0.03 - 17.71)	1.000	NS
DRB1*1304	0.0	0.6	0.71 (0.03 - 17.71)	1.000	NS
DRB1*1305	0.0	3.7	0.16 (0.01 - 2.87)	0.181	NS
DRB1*1401	2.7	1.2	2.18 (0.37 - 12.88)	0.593	NS
DRB1*1404	4.0	6.2	0.70 (0.20 - 2.43)	0.760	NS
DRB1*1405	0.0	0.6	0.71 (0.03 - 17.71)	1.000	NS
DRB1*1501	13.3	16.0	0.83 (0.38 - 1.79)	0.588	NS
DRB1*1502	22.7	37.7	0.49 (0.27 - 0.92)	0.022	NS
DRB1*1601	1.3	1.2	1.29 (0.17 - 9.97)	1.000	NS
DRB1*1602	2.7	0.0	11.05 (0.52 - 233.16)	0.099	NS

Pc = corrected P value for the number of different DRB1 alleles tested
NS = not statistically significant

overnight with respective restriction enzymes as described according to the manufacturers' instructions. A total of 22 different restriction enzymes were used in *HLA class II DRB1, DQA1* and *DQB1* typing [5].

Gel electrophoresis
Samples of restriction enzyme-cleaved amplified DNAs were subjected to electrophoresis using ethidium-bromide stained 3% agarose gel or 4% NuSieve GTG agarose gel, depending on the sizes of the cleaved fragments. All results have been examined under ultraviolet light and documented by photography.

Statistical analysis
Data were analysed using SPSS 8.0 for Windows (SPSS Inc.) and Microsoft Excel (Microsoft Corporation) software. The significance of the differences in allelic frequencies between the patients and controls was determined by Pearson chi-square test or Fisher's exact test where appropriate. Relative risks (RR) and confidence intervals (CI) were computed by the method of Woolf [11] with the Haldane modification for small numbers [12]. Corrected *P values (Pc)* were calculated by multiplying the P value by the number of statistical comparisons performed at each locus. Genotypes found to be significantly associated with type 1 DM by these tests were evaluated as risk predictors by logistic regression using forward selection [13]. The criterion for inclusion of predictor variables in the logistic regression model is $Pc < 0.05$.

RESULTS

Frequency of DQA1 and DQB1 alleles
Table II shows the frequency of DQA1 alleles in type 1 DM patients compared with healthy controls. Correcting for the number of different DQA1 alleles tested, the occurrence of DQA1*0501 was significantly increased in the type 1 DM group compared to controls (50.7% vs. 20.4%; RR = 3.97; $Pc < 0.01$). Conversely, DQA1*0601 was significantly decreased in type 1 DM patients (14.7% vs. 35.2%; RR = 0.33; Pc = 0.008). The frequency of DQB1 alleles in type 1 DM patients compared with healthy controls is shown in Table III. The frequency of DQB1*0201 was significantly higher in the type 1 DM group compared to controls (48% vs. 19.1%; RR = 3.86; $Pc < 0.05$) whereas that of DQB1*0601 was significantly reduced in type 1 DM patients (4% vs. 23.5%; RR = 0.16; $Pc < 0.05$).

Frequency of DRB1 alleles
The frequency of DRB1 alleles in type 1 DM patients compared with healthy controls is shown in Table IV. Among the *DRB1* alleles tested, only the frequency of *DRB1*0301* was significantly different between the type 1 DM group and the controls (38.7 vs. 6.8%; RR = 8.36; 95% $Pc < 0.05$).

Risk Prediction based on HLA Genotypes
Using the trend test [14], a dosing effect was detected for *HLA-DQA1*0501, DQB1*0201, DRB1*0301, DQA1*0601 and DQB1*0601* alleles (highest P value = 0.015; data not shown). For the former three alleles, a positive association was found between the presence of type 1 DM and the number of a particular allele. For the *HLA-DQA1*0601* and *DQB1*0601*

alleles, a negative association between the presence of type 1 DM and the number of a particular allele was detected. The dosing effect was taken into account in the logistic regression model. The selection model is given by:

Odds of Type 1 DM = $0.39 \times 5.68^{N_{DRB1*0301}} \times 0.18^{N_{DQB1*0601}}$

Where $N_{DRB1*0301}$ = 0, if the subject has no DRB1*0301 allele
1, if the subject is heterozygous in DRB1*0301
2, if the subject is homozygous in DRB1*0301

and $N_{DQB1*0601}$ is similarly defined.

The relative risk (RR) for type 1 DM increases by a factor of 5.68 for every unit increase in the number of DRB1*0301 allele ($P < 0.001$). For example, other things being equal, the RR of type 1 DM for homozygotes is 5.68 times that of heterozygotes for *DRB1*0301*. On the other hand, the RR of type 1 DM decreases by a factor of 0.18 per unit increase in the number of *DQB1*0601* allele *($P < 0.001$)*. After adjusting for these two HLA genotypes, DQA1*0501, DQB1*0201 and *DQA1*0601* were not statistically significant as risk predictors at *P* values of 0.063, 0.890 and 0.217, respectively.

The model correctly classified 76.8% of the subjects into their disease status groups (patient or control). This correct classification rate was found to be greater than due to chance alone *($P < 0.001$)*. However, a breakdown showed that the model achieved a correct classification of 94.4% among controls and 38.7% among patients.

DISCUSSION

This study reported both *HLA-DQ* and *HLA-DR* allele frequencies in a large number of Malay patients, with type 1 DM and control subjects. It further defined the influence of the HLA complexes in type 1 DM susceptibility within the Malays. Results obtained supported the data from current literature that the HLA-markers for type 1 DM and the degree of risk they confer, vary between different racial and ethnic groups.

We found *HLA-DRB1*0301* to be an independent genetic marker for type 1 DM susceptibility in the Malays (RR = 8.36). This is consistent with findings from trans-racial studies on the Caucasians [15-17], Blacks [18] and Chinese [19-22], except for the Japanese [23,24]. In contrast with the Caucasian population, however, we found no association between HLA-DR4 haplotypes and type 1 DM in the Malays; similar to reports in various Chinese populations [19,22,25]. Although there are no reported estimates of the incidence of type 1 DM among the indigenous Malay population, it is believed to be low and probably comparable with that in the Chinese and the Japanese populations. Of note, the frequency of individuals with *DR 3* and/or *DR 4* haplotypes was only 16.6% in our Malay controls, as compared with about 50% in the Caucasian population [26]. Therefore, the low prevalence of type 1 DM in various Asian populations could likely be attributed to the relatively lower frequencies of the HLA-DR associated susceptibility alleles among these ethnic groups [19,24,25].

However, our finding of *HLA-DQB1*0601* as an independent genetic marker against type 1 DM occurrence (RR = 0.16) appears rather peculiar to the Malay population. Besides another study showing a decreased frequency of HLA-*DQB1*0601/0603* in non-Hispanic White children with type 1 DM from Colorado [26], no significant associations has been noted between *HLA-DQB1*0601* and type 1 DM in several other studies on Caucasian subjects from Norway [15], Italy (2723) and northern Spain [16]. The lack of association between *HLA-DQB1*0601* and type 1 DM was also evident in studies on other ethnic groups including the Hispanics [26], Blacks [18], Japanese [24] and Chinese [20,25]. Conversely, we did not find the protective effect of *HLA-DQB1*0602/3* against type 1 DM as observed in the Caucasians [17, 28], Blacks [29], northern Indians [30] and Japanese [31]. It is likely that the low frequency of *HLA-DQB1*0602/3* in our Malay population (as observed in only 2.5% of Malay controls) masks its negative association with type 1 DM.

In contrast with findings from the Hispanic and non-Hispanic Whites [15-17, 26-28], Blacks [18,29], Japanese [24,32,33] and northern Indians [30] which implicated *HLA-DQA1*0301* as a primary allele for type 1 DM susceptibility, we did not find similar association in our Malay subjects. Interestingly, studies in the Chinese populations showed a similar deviation from other races [21,22,25]. In studies from Taiwan, *HLA-DQA1*0301* allele was found to be associated with Type 1 DM only in individuals bearing the DR4 or DR9 haplotyes [21,25]. However, neither the DR4- nor DR9-associated susceptibility was found in our Malay population. Of note, an increased frequency of DR3 but not DR4 or DR 9 haplotypes was similarly reported among Chinese type 1 DM children in Singapore [19].

Our study concurred with most Caucasian populations that HLA-DQA1*0501 had been reported as a risk marker for type 1 DM in the Caucasian [16,30], Black [18] and Japanese populations[33]; whereas HLA-DQB1*0201 was associated variously with the Caucasian [16,30], Black [14] and Chinese populations [20]. A recent North Indian data demonstrated major susceptibility alleles of *HLA- DQA1*0501* and *HLA-DQB1*0201* which observed a highest risk of type 1 DM with the combination of *HLA-DQA*0501-DQB1*0201* [34]. Unfortunately, within our cohort these susceptible markers, *HLA-DQA1*0501* and *HLA-DQB1*0201,* were no longer significant as independent risk factors in the logistic regression model. These observations were likely contributed by linkage disequilibria as adjustments for the influence of other HLA alleles were not performed by the investigators. This is borne out by studies from various ethnic groups demonstrating that the DR3 haplotypes have the allelic constitution *DRB1*0301-DQA1*0501-DQB1*0201* [6,17,21,24,28-30].

In conclusion, we report the unique HLA-DR and DQ markers for type 1 DM in our indigenous Malay population. The disparities of HLA associations between ethnic groups are likely contributed by different linkage disequilibria. Using a logistic regression model, we have also derived a risk prediction model for type 1 DM in this population based on the quantitative presence or absence of a susceptible *HLA-DRB1*0301* allele and a protective *HLA-DQB1*0601* allele, respectively. Recent attention on the influence of these susceptibility genes on various aspects of type 1 DM including disease onset, complete beta cell destruction and ketosis-prone patients promises invaluable information towards early anticipation and recognition of the disease among our patients.

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