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


M ATERIALS 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 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 B151. Subsequent studies showed stronger associations of type 1 DM with the MHC Class II genes1 with obvious racial variations, being predominantly DR3 or DR4 in the Caucasians1, and DR4 or DR9 in the Japanese2, respectively.

In the mid-1980s, genotyping of HLA alleles provided a more precise definition in susceptibility and protective gene markers1. 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 studied3. These HLA-DR/DQ alleles acted as either predisposing or protective of Type 1 DM4. 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 ß-cell destruction and ketosis-prone diabetes5.

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.

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.

INTRODUCTION

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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.

**Blood and DNA extraction**

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. Confidentiality was assured by coding the DNA samples, and the master list made available only to the investigators.

**Gene amplification by polymerase chain reaction (PCR)**

About 50 – 100 ng 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.
H LA Genotypes in Malay Type 1 Diabetes Patients

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

<table>
<thead>
<tr>
<th>DRB1* Allele</th>
<th>Patient % (n = 75)</th>
<th>Control % (n = 162)</th>
<th>Relative Risk (95% CI)</th>
<th>P value</th>
<th>Pc value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*0103</td>
<td>1.3</td>
<td>1.2</td>
<td>1.29 (0.17 - 9.97)</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*0301</td>
<td>38.7</td>
<td>6.8</td>
<td>6.36 (0.92 - 17.31)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>DRB1*0302</td>
<td>0.0</td>
<td>1.2</td>
<td>0.43 (0.03 - 8.97)</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*0401</td>
<td>0.0</td>
<td>0.6</td>
<td>0.71 (0.03 - 17.71)</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*0402</td>
<td>1.3</td>
<td>0.6</td>
<td>2.17 (0.22 - 21.19)</td>
<td>0.534</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*0405</td>
<td>10.7</td>
<td>3.1</td>
<td>3.61 (1.19 - 10.94)</td>
<td>0.028</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*0406</td>
<td>4.0</td>
<td>4.3</td>
<td>1.00 (0.27 - 3.67)</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*0701</td>
<td>5.3</td>
<td>12.3</td>
<td>0.44 (0.15 - 1.26)</td>
<td>0.096</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*0802</td>
<td>0.0</td>
<td>1.2</td>
<td>0.43 (0.02 - 8.97)</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*0803</td>
<td>5.3</td>
<td>8.6</td>
<td>0.64 (0.22 - 1.93)</td>
<td>0.371</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*0901</td>
<td>9.3</td>
<td>13.0</td>
<td>0.72 (0.30 - 1.74)</td>
<td>0.421</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1001</td>
<td>5.3</td>
<td>4.9</td>
<td>1.14 (0.35 - 3.71)</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1101</td>
<td>2.7</td>
<td>1.9</td>
<td>1.55 (0.30 - 8.04)</td>
<td>0.653</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1201</td>
<td>1.3</td>
<td>0.0</td>
<td>6.54 (0.26 - 162.53)</td>
<td>0.316</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1202</td>
<td>30.7</td>
<td>50.6</td>
<td>0.44 (0.25 - 0.78)</td>
<td>0.004</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1302</td>
<td>0.0</td>
<td>0.6</td>
<td>0.71 (0.03 - 17.71)</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1304</td>
<td>0.0</td>
<td>0.6</td>
<td>0.71 (0.03 - 17.71)</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1305</td>
<td>0.0</td>
<td>3.7</td>
<td>0.16 (0.01 - 2.87)</td>
<td>0.181</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1401</td>
<td>2.7</td>
<td>1.2</td>
<td>2.18 (0.37 - 12.88)</td>
<td>0.593</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1404</td>
<td>4.0</td>
<td>6.2</td>
<td>0.70 (0.20 - 2.43)</td>
<td>0.760</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1405</td>
<td>0.0</td>
<td>0.6</td>
<td>0.71 (0.03 - 17.71)</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1501</td>
<td>13.3</td>
<td>16.0</td>
<td>0.83 (0.38 - 1.79)</td>
<td>0.588</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1502</td>
<td>22.7</td>
<td>37.7</td>
<td>0.49 (0.27 - 0.92)</td>
<td>0.022</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1601</td>
<td>1.3</td>
<td>1.2</td>
<td>1.29 (0.17 - 9.97)</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1*1602</td>
<td>2.7</td>
<td>0.0</td>
<td>11.05 (0.52 - 233.16)</td>
<td>0.099</td>
<td>NS</td>
</tr>
</tbody>
</table>

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

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:

\[ \text{Odds of Type 1 DM} = 0.39 \times 5.68^{\text{DRB1*0301}} \times 0.18^{\text{DQB1*0601}} \]

Where \( \text{DRB1*0301} = 0 \), 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 \( \text{DQB1*0601} \) 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 \(^1\)\(^-\)\(^3\), Blacks \(^4\), and Chinese \(^5\)\(^-\)\(^7\), except for the Japanese \(^8\)\(^-\)\(^10\). 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 \(^9\)\(^-\)\(^11\). 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 \(^12\). 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 \(^13\)\(^-\)\(^15\).

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 \(^16\), no significant associations has been noted between HLA-DQB1*0601 and type 1 DM in several other studies on Caucasian subjects from Norway \(^17\), Italy (2723) and northern Spain \(^18\). The lack of association between HLA-DQB1*0601 and type 1 DM was also evident in studies on other ethnic groups including the Hispanics \(^19\), Blacks \(^20\), Japanese \(^21\) and Chinese \(^22\)\(^-\)\(^24\). Conversely, we did not find the protective effect of HLA-DQB1*0602/3 against type 1 DM as observed in the Caucasians \(^25\), Blacks \(^26\), northern Indians \(^27\) and Japanese \(^28\). 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 \(^26\)\(^-\)\(^28\), Blacks \(^29\)\(^-\)\(^30\), Japanese \(^31\) and northern Indians \(^32\) which implicate 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 \(^33\)\(^-\)\(^35\). 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 haplotypes \(^36\)\(^-\)\(^38\). 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 \(^39\).

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 \(^40\)\(^-\)\(^42\), Black \(^43\) and Japanese populations \(^44\); whereas HLA-DQB1*0201 was associated variously with the Caucasian \(^45\)\(^-\)\(^47\), Black \(^48\) and Chinese populations \(^49\). 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. 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 \(^50\)\(^-\)\(^54\).

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.
ACKNOWLEDGEMENTS

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REFERENCES