Title:  The association of HLA-DQB1, -DQA1 and -DPB1 alleles with anti-GBM disease in Chinese patients

Running Title:  HLA and anti-GBM disease

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Abstract.

[Background] Human leukocyte antigen (HLA) alleles are associated with many autoimmune diseases, including anti-glomerular basement membrane (GBM) disease. In our previous study, it was demonstrated that HLA-DRB1*1501 was strongly associated with anti-GBM disease in Chinese. However, the association of anti-GBM disease and other HLA class II genes, including HLA-DQB1, -DQA1, -DPB1 alleles, has rarely been investigated in Asian, especially Chinese patients. The present study further analyzed the association between anti-GBM disease and HLA-DQB1, -DQA1, and -DPB1.

[Method] This study included 44 Chinese patients with anti-GBM disease and 200 healthy controls. The clinical and pathological data of the patients were collected and analyzed. Typing of HLA-DQB1, -DQA1 and -DPB1 alleles were performed by bi-directional sequencing of exon 2 using the SeCoreTM Sequencing Kits.

[Results] Compared with normal controls, the prevalence of HLA-DPB1*0401 was significantly lower in patients with anti-GBM disease (p=1.48×10^{-4}, pc=0.005). No other alleles had significant association with this disease. However, no significant difference of gender, age, level of anti-GBM autoantibodies, serum creatinine, other clinical manifestations and pathological parameters were found between anti-GBM patients with and without HLA-DPB1*0401.

[Conclusions] HLA-DPB1*0401 might be a protective allele to anti-GBM disease in Chinese patients.

[Keywords] Anti-GBM disease; HLA-DPB1*0401; Chinese
**Introduction**

Anti-glomerular basement membrane (GBM) disease, defined by the presence of autoantibodies in the circulation and bound to the α3 chain non-collagen 1 domain of type IV collagen [α3(IV)NC1] [1], is a severe autoimmune disease. It manifests as rapidly progressive glomerulonephritis; when accompanied with alveolar hemorrhage, it is termed Goodpasture’s disease.

Human leukocyte antigen (HLA) alleles, located on the short arm of chromosome 6, have been well known associated with most autoimmune diseases [2]. HLA genes encode numerous molecules including the HLA class I and II molecules, which have immunological functions. HLA class I molecules present peptides to CD8-positive cytotoxic T cells, while HLA class II molecules are responsible for presentation of antigenic peptides to CD4-positive T-cells. It was reported that anti-GBM disease was positively associated with HLA-DRB1*1501 and negatively associated with HLA-DRB1*07 [3]. In Asian populations, HLA-DRB1*1501 was also considered as a risk allele for Japanese [4] and Chinese [5]. These data suggested that HLA-DRB1*1501 is a common risk allele for anti-GBM disease in various populations. However, the association of anti-GBM disease and other HLA class II genes, including HLA-DQB1, -DQA1,-DPB1 alleles, has rarely been investigated in Asian, especially Chinese patients. The purpose of the current study was to investigate the distribution and clinical association of these alleles.

**Methods**

**Patients**

44 patients with anti-GBM disease, who were diagnosed at Renal Division, Peking University
First Hospital, from 1996 to 2007, were included in this study. The diagnosis was established in all the cases by the presence of glomerulonephritis and serum anti-GBM autoantibodies, detected by ELISA. All the 44 patients received renal biopsy. Clinical and pathological data were collected at the time of renal biopsy. 200 ethnically matched healthy blood donors were employed as normal controls. The research was in compliance of the declaration of Helsinki and approved by the ethic committee of the local hospital. Informed consent was obtained from each patient.

**Detection of serum anti-GBM autoantibodies**

Anti-GBM autoantibodies were measured by ELISA using bovine α(IV)NC1 as the solid phase antigen, which was described previously [6]. The results were expressed as relative absorbance value to a reference positive serum, that was, percentage of a known positive control, and values greater than 13% were regarded as positive.

**Samples**

Peripheral blood samples (10 ml) from patients with anti-GBM disease and normal controls were collected in EDTA. Genomic DNA was obtained from peripheral blood leukocytes with a salting-out procedure[7]

**Sequence based typing**

Typing of HLA-DQB1, -DQA1 and -DPB1 alleles were performed by bi-directional sequencing of exon 2 using the SeCoreTM Sequencing Kits (Invitrogen, Brown Deer, WI, USA).

**Statistical analysis**

The difference in the frequencies of HLA alleles in disease samples and controls was compared
using the Chi-square test or Fisher's exact test as appropriate. To compare the HLA alleles of subjects stratified by various demographic and clinical parameters, Chi-square test, Fisher's exact test, or nonparametric test was used as appropriate. Bonferroni correction was applied to correct p-value (p corrected, pc). It was considered significant difference if the pc value was less than 0.05. The statistical analysis was performed in SPSS statistical software package (version 11.0, Chicago, Ill, USA).

Results

Demographic and clinicopathological features

Among the 44 patients with anti-GBM disease, 30 were male and 14 were female. The median age of the 44 patients on diagnosis was 27 (range 13-82) years old. 16 out of 44 patients had pulmonary hemorrhage. All of the patients had hematuria and proteinuria. 17/44 (38.6%) patients had anuria or oliguria. On diagnosis, the level of serum creatinine was 765.4±388.7 µmol/L. Renal biopsy was performed in all the 44 patients. 41/44 (93.8%) patients had crescent formation in more than 50% of the glomeruli and 30 (68.2%) had crescent formation in more than 85% of the glomeruli in the renal specimen. Direct immunofluorescence examination was performed in 35 cases. All of them showed linear or fine granular IgG and/or C3 deposition along glomerular capillary wall. Outcome data were available for 40 out of the 44 patients. At the end of 1 year after diagnosis, only 7/40 (17.5%) patients were dialysis independent, and 33/40 (82.5%) patients were dialysis dependent or died.

HLA-DQB1, -DQA1 and -DPB1 alleles and association to anti-GBM disease

The frequencies of each HLA-DQB1, -DQA1 and -DPB1 allele for the 44 patients with
anti-GBM disease and 200 ethnically matched healthy controls were determined by sequence
based typing. A total of eleven HLA-DQB1 alleles, nine HLA-DQA1 and thirty-eight
HLA-DPB1 alleles typed in our study. Compared with normal controls, the prevalence of
HLA-DPB1*0401 was significantly lower in patients with anti-GBM disease \( (p=4.4 \times 10^{-4},
\) pc=0.025) (Table 1). There was no significant difference detected between these patients and
normal controls on other HLA alleles. Besides, no significant difference of gender, age, level of
anti-GBM autoantibodies, serum creatinine, other clinical manifestations and pathological
parameters were found between anti-GBM patients with and without HLA-DPB1*0401 (Table
2).

**Discussion**

The current study analyzed the distribution of HLA-DQB1, -DQA1 and -DPB1 alleles in
patients with anti-GBM disease. Our typing results indicated that HLA-DPB1*0401 might be
non-predisposing on anti-GBM disease.

We stratified by the presence of DPB1*0401 on patients with anti-GBM disease and tried
to investigate how this allele has its protective influence on clinical and pathological
characteristics of patients. However, no significant difference of gender, age, level of
anti-GBM autoantibodies, serum creatinine, other clinical manifestations and pathological
parameters were found between anti-GBM patients with and without HLA-DPB1*0401.

However, since there were only 3 patients with positive HLA-DRB1*0401, larger sample size
is need to investigate the association between this allele and the disease.

Previous studies have focused on association between HLA-DR and -DQ genes and their
haplotype with anti-GBM disease [3, 8-9]. The single allele DQB1*0302, haplotypes DQB1*0602-DRB1*1501 and DQB1*0201-DRB1*0301 were identified as risk alleles [3, 8-9], while HLA-DQB1*0501 was considered as a protective allele [3, 9]. However, these potential associations were not observed in our study. Actually, no HLA-DQ allele was found significantly associated with patients with anti-GBM disease in our study.

HLA class II alleles have been demonstrated a connection with many autoimmune diseases [10-12]. The mechanisms behind the strong positive association with DRB1*1501 [4-5, 8-9] and negative associations with DRB1*01 and DRB1*07 [3] have been studied [9, 13-14]. The HLA association in anti-GBM disease is believed to reflect the ability of certain class II molecules to bind and present peptides derived from the autoantigen to T helper cells [9]. Phelps et al. [14] suggested that DR1/7 (encoded by DRB1*01/07) could protect by capturing α3(IV)NC1 peptides and preventing their display bound to DR15, which is consistent with the theory above. In our study, we found HLA-DPB1*0401 might have a protective effect on anti-GBM disease. We speculate the protective mechanism of this allele might be similar to DRB1*01/07, i.e., the molecule produced by DPB1*0401 might prevent some risk peptide to bind. Nonetheless, the exact mechanism requires further research to confirm.

In conclusion, HLA-DPB1*0401 might be a protective allele to anti-GBM disease in Chinese patients.

Acknowledgments

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Conflict of interest statement
None declared.

Reference


Table 1. Distribution of HLA-DQB1, -DQA1 and DPB1 alleles in patients with anti-GBM disease and normal controls

<table>
<thead>
<tr>
<th>HLA alleles</th>
<th>Anti-GBM disease</th>
<th>Normal controls</th>
<th>p</th>
<th>pc</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQB1*0201</td>
<td>17</td>
<td>95</td>
<td>0.37</td>
<td>0.77</td>
<td>0.43-1.37</td>
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<tr>
<td>DQB1*0302</td>
<td>2</td>
<td>13</td>
<td>1.00</td>
<td>0.69</td>
<td>0.15-3.12</td>
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<tr>
<td>DQB1*0602</td>
<td>21</td>
<td>94</td>
<td>0.94</td>
<td>1.02</td>
<td>0.59-1.75</td>
<td></td>
</tr>
<tr>
<td>DQA1*0102</td>
<td>19</td>
<td>148</td>
<td>0.0060</td>
<td>0.47</td>
<td>0.27-0.81</td>
<td></td>
</tr>
<tr>
<td>DQA1*0201</td>
<td>16</td>
<td>36</td>
<td>0.011</td>
<td>2.25</td>
<td>1.18-4.27</td>
<td></td>
</tr>
<tr>
<td>DPB1*0401</td>
<td>3</td>
<td>74</td>
<td>0.00044</td>
<td>0.16</td>
<td>0.048-0.51</td>
<td></td>
</tr>
<tr>
<td>DPB1*0801</td>
<td>3</td>
<td>1</td>
<td>0.020</td>
<td>14.08</td>
<td>1.45-137.03</td>
<td></td>
</tr>
<tr>
<td>DPB1*0901</td>
<td>4</td>
<td>3</td>
<td>0.022</td>
<td>6.30</td>
<td>1.39-28.68</td>
<td></td>
</tr>
</tbody>
</table>

[Abbreviations] Pc, P corrected; OR, odds ratio; CI, confident interval.
<table>
<thead>
<tr>
<th></th>
<th>DPB1*0401 positive</th>
<th>DPB1*0401 negative</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>1/2</td>
<td>29/12</td>
<td>0.234</td>
</tr>
<tr>
<td>Age, median (1st and 3rd quartile)</td>
<td>24 (21, 26)</td>
<td>27 ( 22.5, 45.0)</td>
<td>0.221</td>
</tr>
<tr>
<td>Level of anti-GBM antibodies (%) median (1st and 3rd quartile)</td>
<td>47(26, 141.6)</td>
<td>76 (42.0, 110.5)</td>
<td>0.659</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>3/3</td>
<td>13/41</td>
<td>0.042</td>
</tr>
<tr>
<td>Oliguria or anuria</td>
<td>1/3</td>
<td>16/41</td>
<td>1</td>
</tr>
<tr>
<td>Interval between onset and diagnosis (days) median (1st and 3rd quartile)</td>
<td>60 (42, 103)</td>
<td>49 (24.2, 83.0)</td>
<td>0.595</td>
</tr>
<tr>
<td>Scr (µmol/L), median (1st and 3rd quartile)</td>
<td>838 (793, 1442)</td>
<td>767 (409, 1000)</td>
<td>0.299</td>
</tr>
<tr>
<td>Percentage of crescents in glomeruli median (1st and 3rd quartile)</td>
<td>100 (87.5, 100)</td>
<td>94.87 (67.7, 100)</td>
<td>0.448</td>
</tr>
<tr>
<td>Dialysis dependent or died</td>
<td>3/3</td>
<td>31/37</td>
<td>1</td>
</tr>
</tbody>
</table>