Differential Association between HLA and Diffuse Panbronchiolitis in Northern and Southern Chinese

Yu Chen¹, Jian Kang², Min Wu³, Arata Azuma⁴ and Li Zhao¹

Abstract

Background  Diffuse panbronchiolitis (DPB) is a progressive inflammatory pulmonary disease that predominately affects East Asians. Genetic susceptibility to DPB is correlated with the human leukocyte antigens HLA-B54 in Japanese and HLA-A11 in Koreans. However, no systematic genetic study of DPB pathogenesis has been conducted in the Chinese population. The aim of this study was to investigate the possible association between HLA and disease susceptibility in Chinese patients with DPB.

Methods  A literature review of both Chinese and English language studies on Chinese DPB patients, published between 1983 and 2010, was conducted. Seventy subjects met the inclusion criteria and were retrospectively analyzed for HLA gene frequency according to geographic region.

Results  HLA-B54 frequency was significantly greater in DPB patients than in controls in the Northern Chinese group (35.7% vs. 4.6%, p=7.5×10⁻⁷). Although the HLA-B54 frequency was slightly increased in the Southern Chinese patients, the difference was not significant compared with control subjects (14.3% vs. 5.7%, p=0.28). The HLA-A11 frequency was significantly greater in DPB patients than controls in the Southern Chinese group (54.8% vs. 26.4%, p=0.009). Despite an increase of HLA-A11 frequency in the Northern Chinese group, no significant variation in HLA-A11 frequency was found compared with control subjects (42.9% vs. 30.8%, p=0.535). The HLA-A2 frequency was significantly decreased in DPB patients than in controls in the Southern Chinese group (22.9% vs. 66.0%, p=0.001). However, no significant difference in HLA-A2 frequency was found in the Northern Chinese group (50.0% vs. 46.9%, p=0.872).

Conclusion  HLA-B54 and HLA-A11 were positively associated with DPB in Northern and Southern Chinese, respectively. Population substructure may impact the genetic predisposition of DPB in China.

Key words: Chinese, diffuse panbronchiolitis, genetic susceptibility, human leukocyte antigens

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Introduction

Diffuse panbronchiolitis (DPB) is a chronic disease with obstructive ventilatory impairment with obscure etiology (1, 2). It was previously grouped with other chronic pulmonary diseases, such as chronic obstructive pulmonary disease and bronchiectasis, until Yamanaka and coworkers designated this disease entity as DPB in 1969 (3). Its characteristic pathological features include chronic inflammatory lesions around the respiratory bronchioles. However, the pathogenesis of the disease is still unknown. Although DPB was recognized internationally (4-8), cases of DPB have been noted predominately in East Asians, such as Japanese, Chinese, and Koreans (9). Familial cases of DPB (10) and the disease’s feature of predominantly affecting East Asians suggest that the disease might be associated with genetic and racial susceptibility. In 1990, Sugiyama and co-workers showed that 63.2% of patients with DPB possess the human leukocyte antigen (HLA)-B54, which is known to be unique to East Asian ethnic groups compared with 11.4% of the disease-free Japanese population (11). Thereafter, this asso-

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Diffuse panbronchiolitis is reportedly not rare and widely distributed in China (14). A study of 24 Chinese DPB patients showed that HLA-A11 appeared to have a positive association with the disease (15). However, systemic genetic studies of Chinese DPB patients have been sparse. We reviewed the research literature from 1983 to 2010, which included 70 Chinese DPB cases, to investigate the possible association between HLA and disease susceptibility and to compare the HLA profiles of Chinese patients with those of Japanese and Koreans.

Methods

The study was performed in Shengjing Hospital of China Medical University. Data analysis was approved by Ethical Committee of the hospital. Because data were collected by literature review, the study does not include patient consent.

Search strategy

We searched Medline from 1983 to 2010 using the terms “Chinese” and “diffuse panbronchiolitis”. A systematic review was conducted of English language literature that reported cases of DPB in Chinese patients. In addition, we searched the Chinese Biomedical Literature Database (CBMdisc) from 1988 to 2010 for Chinese language medical literature published in mainland China. Chinese articles concerning DPB studies were carefully reviewed. Two of the investigators searched independently.

For inclusion in the present study, recruited cases were required to meet the diagnostic criteria for DPB (16, 17), and to have undergone HLA examination. Subjects with specific occupational histories or other chronic lung diseases were excluded. Duplicate reports and cases without HLA data were also excluded. HLA information was not the diagnostic prerequisite in all cases. In addition, all articles which met the inclusion criteria were collected in the study in order to avoid publication bias.

Thirteen publications that reported 70 DPB cases were included in the study. All of the patients were unrelated Chinese individuals. The corresponding authors of included articles were contacted if additional information or clarification was required. A total of 183 subjects were collected as the normal control group, of which 83 cases were from the above publications and 100 healthy subjects were from another publication (18).

Study profiles

Data of HLA profiles regarding DPB patients and controls are summarized in Table 1. Among the 13 included articles which reported DPB cases, two of them were case-control studies (15, 19). The remaining 11 articles were case reports. HLA-B54 was examined in all 70 patients with DPB, whereas HLA-A11, HLA-A2, and HLA-A33 were examined in 45, 45 and 44 patients, respectively. Among the 70 patients, 24 had directly identifiable individual HLA alleles at the nucleotide sequence level using polymerase

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Table 1. HLA Profiles of Chinese Diffuse Panbronchiolitis Patients and Controls

<table>
<thead>
<tr>
<th>Author</th>
<th>Geographic location (North or South)</th>
<th>DPB subjects (n)</th>
<th>HLA types tested (Positive/tested)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DPB patients</td>
<td>Control subjects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B54</td>
<td>A11</td>
</tr>
<tr>
<td>Chen XP(20)</td>
<td>Tianjin (N)</td>
<td>1</td>
<td>0/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Sun JZ(21)</td>
<td>Shandong (N)</td>
<td>1</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Yuan XL(22)</td>
<td>Liaoning (N)</td>
<td>1</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Zhang L(23)</td>
<td>Beijing (N)</td>
<td>1</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Zhang JM(24)</td>
<td>Tianjin (N)</td>
<td>1</td>
<td>0/1</td>
<td>1/1</td>
</tr>
<tr>
<td>He ZY(25)</td>
<td>Beijing (N)</td>
<td>1</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Wang HD(26)</td>
<td>Beijing (N)</td>
<td>1</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Liu JG(27)</td>
<td>Beijing (N)</td>
<td>3</td>
<td>3/3</td>
<td></td>
</tr>
<tr>
<td>Liu YN(28)</td>
<td>Beijing (N)</td>
<td>5</td>
<td>1/5</td>
<td></td>
</tr>
<tr>
<td>Li HP(29)</td>
<td>Shanghai (S)</td>
<td>11</td>
<td>3/11</td>
<td></td>
</tr>
<tr>
<td>Tsang KW(8)</td>
<td>Hong Kong (S)</td>
<td>7</td>
<td>0/7</td>
<td>3/7</td>
</tr>
<tr>
<td>Wang S(18)</td>
<td>Liaoning (N)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
chain reaction-based techniques (15). The remaining 46 cases were examined serologically for HLA antigens. According to the subjects’ regions of residence, the 70 patients were classified as either Northern Chinese or Southern Chinese, with the Yangtze River serving as the geographical boundary, which has been proposed by previous studies (30, 31). The HLA frequencies in the different groups were compared between the patients and control subjects.

**Statistical analysis**

Disease association was assessed using the Pearson $\chi^2$ test (SPSS version 15.0; SPSS Inc., Chicago, IL). When any expected number in the 2×2 contingency table was less than 5 and more than or equal to 1, the p value was calculated using a continuity correction test. A value of $p<0.05$ was considered statistically significant. The odds ratio (OR) was defined as the cross-product ratio of the numbers shown in the 2×2 table.

**Results**

**Association between HLA-B54 and DPB**

The frequencies of HLA-B54 in different groups of Chinese DPB patients and normal control subjects are shown in Table 2. The remarkable finding was the frequencies of HLA-B54 in the subpopulation groups, which showed different associations. A positive association with DPB was found in Northern Chinese patients (35.7% vs. 4.6%, $p=7.5 \times 10^{-7}$, OR=11.5). Although the HLA-B54 frequency was slightly increased in the Southern Chinese patients, the difference was not significant compared with control subjects (14.3% vs. 5.7%, $p=0.28$, OR=2.8).

**Association between HLA-A11 and DPB**

The distributions of HLA-A11 in the different groups of Chinese DPB patients and normal control subjects are shown in Table 3. Different frequencies of HLA-A11 were observed in Southern and Northern Chinese DPB patients. In Southern Chinese patients, HLA-A11 had a positive association with the disease (54.8% vs. 26.4%, $p=0.009$, OR=3.4). However, although an increase of HLA-A11 frequency in the Northern Chinese group was observed, no significant variation in HLA-A11 frequency was found compared with control subjects (42.9% vs. 30.8%, $p=0.535$, OR=1.7).

**Association between HLA-A33 and DPB**

The frequencies of HLA-A33 in different groups of Chinese DPB patients and normal control subjects are shown in Table 5. Compared with controls, no positive or negative associations between HLA-A33 and DPB disease were detected in the Northern group (23.1% vs. 13.3%, $p=0.73$, OR=1.95) and Southern group (16.1% vs. 7.5%, $p=0.389$, OR=2.4).

**Discussion**

Diffuse panbronchiolitis is a progressive, inflammatory small airway disease with an unknown pathogenesis. The genetic mechanism of the disease is associated with HLA
class I antigens, including HLA-B54 in Japanese (11, 12), and HLA-A11 in Koreans (13). The Chinese population is also susceptible to the disease. However, no systematic study of the genetic association has been conducted in Chinese DPB patients. In the present study, we reviewed the literature and analyzed the associations between HLA and DPB in different groups of Chinese patients. The most interesting finding was a distinction of HLA frequencies between Northern and Southern Chinese DPB patients, which suggested the possible impact of Chinese substructure on the disease.

Sample homogeneity is a major prerequisite in studies of genetic associations with diseases (32). Han Chinese is the largest ethnic group in the world, comprising 20% of the entire global human population. Two important recent studies revealed that Han Chinese, a seemingly homogeneous population, actually have a complicated substructure. Using over 350,000 genome-wide autosomal single-nucleotide polymorphisms (SNPs) in over 6000 Han Chinese subjects, Chen and coworkers revealed a one-dimensional “North-South” population structure and a close correlation between geography and the genetic structure of the Han Chinese (33). In another study, Xu and co-workers examined SNPs in a diverse group of over 1700 Han Chinese (31). They also observed similar substructure clusters in the Han Chinese population. Based on analyses of archeological, anatomical, linguistic, and genetic data, previous studies consistently suggested the presence of a significant boundary between the Northern and Southern Chinese populations (34). Differences between the two clusters have been found in classic genetic markers (35), nuclear microsatellites (36), mtDNA (37, 38), and Y chromosome SNP markers (39). A distinct genetic difference was observed between the Northern and Southern populations, despite millennia of common history and migrations. Using classic markers, Xiao et al proposed a genetic boundary located approximately along the Yangtze River (30), which runs across China from west to east. Most researchers attributed such a distinction to the presence of geographic barriers and isolation (31, 40).

In the present study, the HLA-B54 frequency was significantly increased in Northern Chinese DPB patients compared with control subjects, suggesting a stronger positive association compared with Southern Chinese patients. Although the HLA-B54 frequency was slightly increased in the Southern Chinese patients, the difference was not significant compared with control subjects. HLA-A11 was positively associated with DPB in Southern Chinese patients. However, no significant association was found in Northern patients, although a slight increase of HLA-A11 frequency was observed. Collectively, Chinese DPB patients may exhibit different HLA predispositions to the disease, which appeared to correlate with geographic region.

A comparative study found marked regional variations in HLA class I markers between Northern and Southern Chinese (41). The study also suggested differential predispositions to disease between Northern and Southern Chinese, including increased susceptibility to nasopharyngeal carcinoma in Southern Chinese. Our data indicate that the heterogeneity of the Chinese population may impact the genetic association with HLA alleles in DPB patients. A recent simulated case-control analysis demonstrated genetic differentiation among the subpopulations. Although the differences were small, they were sufficient to cause an increased rate of false-positive results, even with moderate sample sizes (31).

Compared with their counterparts in Japan and Korea, the Northern Chinese DPB patients had HLA susceptibility that was similar to Japanese, whereas the Southern Chinese DPB patients had HLA susceptibility that was similar to Koreans. Genome-wide association studies demonstrated smaller genetic differences between Northern Chinese and Japanese than between either of the Chinese clusters and Japanese, indicating that the Japanese population is more related to Northern Chinese and suggesting that the Japanese popula-

### Table 4. HLA-A2 in Chinese Patients with Diffuse Panbronchiolitis and Control Subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>DPB Patients</th>
<th>Control Subjects</th>
<th>p values</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n)</td>
<td>Positive (n) (%)</td>
<td>Total (n)</td>
<td>Positive (n) (%)</td>
</tr>
<tr>
<td>Northern Chinese</td>
<td>14</td>
<td>7</td>
<td>50.0</td>
<td>130</td>
</tr>
<tr>
<td>Southern Chinese</td>
<td>31</td>
<td>9</td>
<td>22.9</td>
<td>53</td>
</tr>
</tbody>
</table>

Definition of abbreviation: OR (95%CI) = odds ratio (95% confidence interval).

### Table 5. HLA-A33 in Chinese Patients with Diffuse Panbronchiolitis and Control Subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>DPB Patients</th>
<th>Control Subjects</th>
<th>p values</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n)</td>
<td>Positive (n) (%)</td>
<td>Total (n)</td>
<td>Positive (n) (%)</td>
</tr>
<tr>
<td>Northern Chinese</td>
<td>13</td>
<td>3</td>
<td>23.1</td>
<td>30</td>
</tr>
<tr>
<td>Southern Chinese</td>
<td>31</td>
<td>5</td>
<td>16.1</td>
<td>53</td>
</tr>
</tbody>
</table>

Definition of abbreviation: OR (95%CI) = odds ratio (95% confidence interval).
tion either initially migrated from Northeast Asia or was more affected by immigrants from the northern populations of East Asia (31). This finding may explain why the Northern Chinese DPB subjects showed the same genetic susceptibility as the Japanese. The genetic relationship between Southern Chinese and Koreans has not been disclosed. One speculation may be the effect of immigration from Southern China to Korea.

HLA-A2 is considered protective against DPB in Chinese (15), and a tendency toward a decreased frequency of this gene was found in Japanese DPB patients (29.0% vs. 40.0%) (12) and Korean DPB patients (36.7% vs. 55.0%) (13). In the present study, a negative association was found between HLA-A2 and DPB in the Southern Chinese patients. However, no association was found between HLA-A2 and DPB in Northern Chinese patients. The results suggest that HLA-A2 may be negatively associated with the disease only in Southern Chinese.

HLA-A33 had a negative association with DPB in Japanese subjects (12). In contrast to the Japanese data, the HLA-A33 frequencies in Chinese patients were increased in all groups compared with controls. However, we did not detect a significant positive association between HLA-A33 and disease in any Chinese patient groups. The reason for the contrasting associations between HLA-A33 and DPB in Chinese and Japanese patients remains unclear. Our relatively small sample group may account for our inability to identify a significant association. Further investigation is warranted.

Keicho et al analyzed 14 polymorphic marker alleles in 92 Japanese and 20 Korean DPB patients (32). A major disease-susceptibility gene for DPB was located between the HLA-B and HLA-A loci on chromosome 6p21.3. Different historical recombination events around the disease locus may have resulted in DPB associations with HLA-B54 in Japanese and HLA-A11 in Koreans (9). Hijikata et al cloned two novel mucin-like genes, panbronchiolitis-related mucin-like 1 and 2 (PBMUCL1 and PBMUCL2, respectively), which are only 200 kb genes located in the MHC I locus (42). Hence, HLA associations may be proximity artifacts, with mucin-like genes functioning as the true susceptibility genes. Chinese population substructure and variable patterns of linkage disequilibrium, which are pronounced across the HLA region, may account for the variable associations across different Chinese populations. In short, genetic susceptibility may be the same across Chinese populations, (i.e., mucin-like genes PBMUCL1 and PBMUCL2), but the ability of HLA-B54, HLA-A11, or other HLA variants to track mucin-like genes may vary among populations. Different mutations may exist in the mucin-like genes in Northern and Southern Chinese populations. These mutations may have arisen at different times and in different genetic backgrounds, resulting in variable associations with HLA alleles. More studies are needed to define the function of these mucin-like genes and to characterize the differences attributable to their genetic polymorphisms.

There are several limitations to the present study. Because the study was a retrospective analysis and it was not a real case-control study, we could only collect existing information on the subjects. We could not perform a meta-analysis due to the limited data. Furthermore, the number of the subjects was small, especially when subjects were divided into two populations (Northern and Southern Chinese). We confirmed that recruited samples were of local and non-migrating residents of China, but the impact of genetic drift on the ancestries of a few patients is unavoidable. Since the Japanese Professor Shoji Kudoh diagnosed the first case of DPB in China in 1996, Chinese doctors have become cognizant of DPB and its putative association with HLA antigens. However, the reported cases originate disproportionately from large cities because of the different diagnostic levels of Chinese doctors. Additional case-control studies using larger-scale samples with better characterized genetic backgrounds are needed.

This is the first report, to our knowledge, to address the issue of the impact of population substructure on the genetic predisposition to DPB in China. The present findings indicate that the genetic association of DPB with HLA alleles may differ between Chinese subpopulations. HLA-B54 and HLA-A11 were positively associated with DPB in Northern and Southern Chinese, respectively. If population substructure is not considered or local subjects serve as samples for the entire population, then spurious results might be obtained in DPB studies in China.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

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