Evidence for an association of HLA-DRB1*15 and DRB1*09 with leprosy and the impact of DRB1*09 on disease onset in a Chinese Han population

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Abstract

Background: Human leukocyte antigens (HLAs) have been proposed to modulate the immune response to Mycobacterium leprae. The association of HLA-DRB1 with leprosy has been reported in several populations, but not in a Chinese population.

Methods: The polymerase chain reaction-sequence-specific oligonucleotide probe with Luminex100 (PCR-SSOP-Luminex) method was used to genotype HLA-DRB1 alleles in 305 leprosy patients and 527 healthy control individuals.

Results: The HLA-DRB1*15 allele was significantly more prevalent among leprosy patients than healthy controls, whereas the frequency of the HLA-DRB1*09 allele was lower among leprosy patients, especially those with early-onset disease.

Conclusion: HLA-DRB1 alleles are associated with leprosy susceptibility in a Chinese population. The HLA-DRB1*09 allele was found to be protective exclusively in a subset of early-onset leprosy patients.

Background

Leprosy is a chronic infectious disease that occurs in genetically predisposed individuals. It is caused by infection with the intracellular macrophage pathogen Mycobacterium leprae. Leprosy is characterized by a spectrum of disease symptoms that result from interactions between the host immune response and invading M. leprae. At the lepromatous extreme, patients exhibit multibacillary infection and an absence of antigen-specific cellular immunity. At the tuberculoid extreme, patients exhibit paucibacillary infection and a strong cellular immune response.
response. Between these two extremes, borderline leprosy patients show intermediate phenotypes [1].

Leprosy was once prevalent worldwide. However, there has been a steady decline in the prevalence of leprosy since the introduction of multidrug therapy by the World Health Organization (WHO-MDT) in 1982 [2]. The detection rate of new cases, however, remains steady in some countries, including several regions of China [3,4]. Since 2002, nearly 1,600 new cases have been detected annually in China [4].

Previous studies have suggested a strong influence of genetic factors in regulation of the anti-M. leprae immune response [5,6]. Two recent genome-wide linkage analyses have identified chromosome 6p21 as a major leprosy-susceptibility locus [7,8]. This region harbors the HLA gene cluster, which has been extensively studied for its role in leprosy pathogenesis [9-12]. In particular, HLA-DR alleles have been consistently found to be associated with leprosy [13]. Several studies reported an association of the HLA-DR2 alleles HLA-DRB1*15, *16, *04, *10 and *12 with susceptibility or resistance to leprosy in Brazilian, Vietnamese, South Indian, Indonesian, Thai and Argentine populations [14-18]. In addition, HLA-DR3 alleles were also found to be associated with leprosy susceptibility in Surinamese and Mexican populations [19,20]. These studies have not only shown the importance of the HLA-DR locus in leprosy susceptibility, but have also highlighted the fact that different HLA-DR alleles are associated with leprosy in different populations.

Although leprosy remains a major public health concern and one of the infectious diseases most capable of causing disability in China, the genetic susceptibility to leprosy in Chinese populations has received little study. The only such report was an investigation of the association of HLA-DRB1 with leprosy in Southern China; however, due to the small sample used, this study failed to detect this well-established association [21]. In the current study, we investigated the association of HLA-DRB1 with leprosy by genotyping DRB1 alleles in a large Chinese Han sample of 305 leprosy patients and 527 healthy control individuals. We further investigated the association of DRB1 alleles with clinical subtype and age-of-onset of the disease.

### Methods

#### Patients and controls

The study included 305 unrelated individuals with a diagnosis of leprosy and 527 control samples. The diagnosis of leprosy was based on clinical assessment and detection of acid-fast bacilli in skin-slit smears and histopathological assessment of skin lesions. The patients were further classified as multibacillary (MB) or paucibacillary (PB) on the basis of clinical and histological criteria [1]. The MB patient subset (n = 179), which included patients with lepromatous leprosy (LL), borderline lepromatous leprosy (BL) and borderline (BB) leprosy, had a bacterial index (BI) > 0. The PB group (n = 126) had a BI = 0, and included patients with borderline tuberculoid leprosy (BT) and tuberculoid leprosy (TT). Control sample donors were recruited from the Shandong Blood Center. Some characteristics of the 305 cases and 527 controls are summarized in Table 1.

All subjects gave informed consent to participate in the study. The protocol was approved by the Ethical Committee of the Shandong Provincial Institute of Dermatovenerology.

#### DNA extraction and HLA-DRB1* typing

Genomic DNA was extracted from peripheral blood using a commercially available DNAzol extraction kit (E.Z.N.A Blood DNA KIT, Omega Bio-tek, Inc).

The procedure for HLA genotyping by the polymerase chain reaction-sequence-specific oligonucleotide probe with Luminex100 (PCR-SSOP-Luminex) method included PCR amplification, hybridization, a streptavidin-phycocerythrin (SA-PE) reaction, and analytical measurements [22]. Target DNA was amplified by PCR using 5’ biotin-labeled primers that were highly specific to certain sequences of HLA-DRB1 genes. PCR was carried out in a 20 μL reaction containing Lifecodes mixture (6 μL), Taq polymerase (0.2 μL), nuclease-free water (11.8 μL) and genomic DNA (3 μL). After denaturation, amplified DNA was allowed to hybridize to complementary DNA probes coupled to microbeads. The oligobead-coupled, hybridized PCR product was labeled with streptavidin-phycocerythrin. The fluorescent intensity of phycocerythrin on each coded oligobead that had hybridized with the

| Population | Sample size (LL/BL/BB/BT/TT) (MB/PB) | Male/Female | Mean age | Mean age onset |
|------------|-------------------------------------|-------------|----------|---------------|
| Case       | Chinese Han                         | 305 (158/15/6/6/120) (179/126) | 278/27   | 67.5          | 19.0          |
| Control    | Chinese Han                         | 527         | 451/76   | 50.2          | NA            |

NA: no onset age for controls
biotin-labeled PCR product was measured using a Luminex apparatus, and the HLA-DRB1* genotype was determined with the assistance of Genosearch typing software (Quick-Type for LifeMatch 2.0, Luminex Corporation). This HLA-typing analysis was done at the Shandong blood center.

**Statistical analysis**

Power calculations, carried out using PS software [http://www.power-analysis.com/home.htm](http://www.power-analysis.com/home.htm), showed that our sample size of 305 patients and 527 controls had greater than 95% power to detect an odds ratio (OR) of 2.0 at a significance level of 5% when the frequency of the allele of interest was greater than 0.10. Allele frequencies were calculated by direct counting. The differences in allele frequency between patients and controls and between early onset and late-onset patients were tested using logistic regression analyses with adjustments for age and gender. The significance of the allele frequency difference between the MB and PB cases was analyzed using Pearson Chi-square tests. The nominal P-values were corrected for multiple testing (Pc-value) using the Bonferroni correction (i.e., by multiplying the nominal P-values by the number of HLA alleles being tested). A Pc-value < 0.05 was accepted as statistically significant. The strength of associations was estimated by calculating ORs. Statistical analyses were carried out using SPSS software (version 11.0).

**Results**

Table 2 summarizes the allele frequencies of HLA-DRB1* alleles in the leprosy patient and control populations and the results of association analyses. Of the 13 HLA-DRB1 alleles determined by the PCR-SSOP-Luminex method, two alleles showed a significant difference in frequency (P < 0.05) between patients and controls. The frequency of the DRB1*15 allele among patients was 0.32 (195/610), which was significantly higher than the frequency of 0.18 (185/1054) observed in the healthy controls (P < 0.001). This difference remained significant after correcting for multiple testing (Pc < 0.001). In contrast, the frequency of the HLA-DRB1*09 allele was significantly lower in the patient population than among controls (P = 0.002, Pc = 0.026).

We further investigated the association of HLA-DRB1* alleles with the clinical subtypes of leprosy. No significant differences in the allele frequencies were observed between the MB and PB forms of the disease (Table 2). We then investigated the association of HLA-DRB1* alleles with early-onset (age-at-onset ≤ 16 years; n = 141) and late-onset (age-at-onset > 16 years; n = 164) leprosy. Interestingly, whereas the DRB1*15 allele was significantly associated with both early- and late-onset leprosy, the DRB1*09 was only significantly associated with early-onset leprosy (Table 3).

All markers with allele frequency above 0.01 have been tested and were in HW equilibrium.

**Discussion**

Leprosy is a chronic infectious disease caused by M. leprae. The disease only occurs in a small percentage (1-3%) of infected individuals [23], an observation that supports the important role of host genetic factors in the development of leprosy. The reported male-to-female patient ratio is 2:1 [24], consistent with the known role of gender as an

| Alleles    | Patients (n = 305) | Controls (n = 527) | MB (n = 179) | PB (n = 126) |
|------------|-------------------|-------------------|-------------|--------------|
|            | 2 n   | AF   | 2 n   | AF   | P-value | Pc-value | AF   | AF   | P-value |
| HLA-DRB1*01 | 19    | 0.03  | 23    | 0.02  | 0.246    |          | 0.02  | 0.04  | 0.136    |
| HLA-DRB1*03 | 14    | 0.02  | 40    | 0.04  | 0.520    |          | 0.04  | 0.02  | 0.224    |
| HLA-DRB1*04 | 44    | 0.07  | 98    | 0.09  | 0.387    |          | 0.09  | 0.09  | 0.471    |
| HLA-DRB1*07 | 73    | 0.12  | 164   | 0.16  | 0.427    |          | 0.16  | 0.13  | 0.197    |
| HLA-DRB1*08 | 30    | 0.05  | 53    | 0.05  | 0.965    |          | 0.05  | 0.04  | 0.497    |
| HLA-DRB1*09 | 49    | 0.08  | 140   | 0.13  | **0.002** | **0.026** | 0.13  | 0.07  | 0.06     |
| HLA-DRB1*10 | 5     | 0.01  | 13    | 0.01  | 0.564    |          | 0.01  | 0.00  | 0.571    |
| HLA-DRB1*11 | 43    | 0.07  | 76    | 0.07  | 0.585    |          | 0.07  | 0.06  | 0.188    |
| HLA-DRB1*12 | 61    | 0.10  | 114   | 0.11  | 0.879    |          | 0.11  | 0.12  | 0.705    |
| HLA-DRB1*13 | 29    | 0.05  | 63    | 0.06  | 0.058    |          | 0.06  | 0.04  | 0.232    |
| HLA-DRB1*14 | 31    | 0.05  | 52    | 0.05  | 0.522    |          | 0.05  | 0.06  | 0.183    |
| HLA-DRB1*15 | 195   | 0.32  | 185   | 0.18  | **< 0.001** | **< 0.001** | 0.18  | 0.29  | 0.991    |
| HLA-DRB1*16 | 17    | 0.03  | 33    | 0.03  | 0.844    |          | 0.03  | 0.03  | 0.838    |

n, number of individuals; AF, allelic frequency. P-values are from allele-based tests.
independent risk factor for leprosy susceptibility. In our
study, more male cases were collected than female cases;
thus, the observed association of the \( \text{HLA-DRB1} \)
allele with leprosy was based on a mostly male population.

Many studies have reported an association of \( \text{HLA-DR2} \)
with leprosy, a linkage that is consistent with the finding
that HLA-DR antigens are associated with disease and the
suggestion that a majority of restriction determinants for \( M. \text{leprae} \) reside on DR, and not DP or DQ molecules [25].
A functional study of Ag-specific T-cell responses within
the context of HLA-DR has shown that HLA-DR-associated
immunity may be crucial in the adaptive immune
response to infection [26]. Thus, HLA-DR plays a major
role in the presentation of \( M. \text{leprae} \) antigens to T cells in
leprosy patients.

By analyzing a large number of Chinese leprosy patients,
our study has provided the first evidence for an associa-
tion of \( \text{HLA-DRB1} \) with leprosy in a Chinese population
and provided additional support for the important role of
HLA in the pathogenesis of leprosy. \( \text{HLA-DRB1*15} \) has
been demonstrated to be a leprosy-susceptibility allele
among Brazilian [14] and Indian [15] populations. In our
study, we found that \( \text{HLA-DRB1*15} \) was also associated
with an increased risk of leprosy in a Chinese population,
and exhibited an allele frequency (32%) similar to that
observed in Indian populations (31%) but higher than
that observed among Brazilians (15%). The \( \text{HLA-DRB1*09} \) allele was found to be associated with protec-
tion against leprosy in our study; its frequency was signif-
ically lower in leprosy patients than in controls, and the
association remained significant after correcting for mul-
tiple testing (\( P = 0.002, \text{Pc} = 0.026 \)). This allele also
showed a protective effect against the disease in Southern
India, but the allele frequency among Indian patients was
0.017 [15], much lower than that observed in the Chinese
population studied here (0.08).

It has been reported that the HLA lymphotoxin-alpha
(\( \text{LTA}+80 \)) locus within the 6p21 chromosomal region is a
major risk factor for early-onset (i.e., < 16 years old) lep-
rosy [27]. To investigate whether the effect of \( \text{HLA-DRB1} \)
on leprosy risk was age-onset dependent, we stratified lep-
rosy patients into early-onset (\( \leq 16 \) years) and late-onset
(> 16 years) groups in association analyses. Interestingly,
we found that \( \text{HLA-DRB1*09} \) was associated with protec-
tion effect against early-onset leprosy (\( P = 0.003 \)), but not
late-onset leprosy (\( P = 0.285 \)). In contrast, the \( \text{HLA-}
\text{DRB1*15} \) allele showed no age-onset-dependent effects.
To our knowledge, this is the first report that \( \text{HLA-}
\text{DRB1*09} \) has an impact on disease onset. Further studies
will be needed to investigate the association of the
LTA+80 locus with leprosy in Chinese populations and
determine whether the association at the \( \text{HLA-DRB1} \) locus
is independent of the LTA+80 locus.

De Vries et al. have proposed a two-step model for the
development of leprosy, proposing that successful infec-
tion of \( M. \text{leprae} \) is first established in genetically vulnera-
ble individuals, and subsequent clinical manifestation of
the disease is influenced by additional host and environ-
mental factors [28]. In our study, the frequencies of \( \text{HLA-}
\text{DRB1} \) alleles were similar in the MB and PB patient
groups. This is consistent with the two-step model, sug-
gesting that the \( \text{HLA-DRB1} \) locus may influence the over-
all susceptibility to leprosy per se. It has also been reported
that certain \( \text{HLA-DRB1} \) alleles are associated with MB or

### Table 3: Allele frequency distribution of \( \text{HLA-DRB1*} \) in early versus late-onset leprosy groups

| Alleles       | Controls (n = 527) | Early onset (n = 141) | Late-onset (n = 164) |
|--------------|------------------|----------------------|----------------------|
|              | AF               | P-value              | Pc-value             |
|              | AF               | P-value              | Pc-value             |
| HLA-DRB1*01  | 0.02             | 0.924                | 0.04                 |
| HLA-DRB1*03  | 0.04             | 0.848                | 0.02                 |
| HLA-DRB1*04  | 0.09             | 0.685                | 0.06                 |
| HLA-DRB1*07  | 0.16             | 0.130                | 0.14                 |
| HLA-DRB1*08  | 0.05             | 0.936                | 0.05                 |
| HLA-DRB1*09  | 0.13             | 0.003                | 0.003                |
| HLA-DRB1*10  | 0.01             | 0.168                | 0.01                 |
| HLA-DRB1*11  | 0.07             | 0.688                | 0.07                 |
| HLA-DRB1*12  | 0.11             | 0.480                | 0.09                 |
| HLA-DRB1*13  | 0.06             | 0.017                | 0.05                 |
| HLA-DRB1*14  | 0.05             | 0.607                | 0.05                 |
| HLA-DRB1*15  | 0.18             | < 0.001              | < 0.001              |
| HLA-DRB1*16  | 0.03             | 0.758                | 0.02                 |

\( n \), number of individuals; AF, allelic frequency.
P-values are from allele-based tests.
PB forms of leprosy [29-31], suggesting that HLA-DRB1 may also be involved in the clinical manifestation of the disease.

Taken together, our association analysis of stratified patient groups indicates that the HLA-DRB1 locus is largely associated with leprosy per se, and only HLA-DRB1*09 shows an age-onset-dependent effect.

Conclusion
In summary, by analyzing a large sample of Chinese Han leprosy patients, our genetic association study of the HLA-DRB1 locus provides strong evidence for an association of HLA-DRB1*15, as a susceptibility allele, and DRB1*09, as a protective allele, with leprosy in a Chinese population. Furthermore, we demonstrate for the first time that HLA-DRB1*09 has an impact on the onset of disease, exerting a protective effect only against early-onset leprosy. These alleles could act alone or in combination with other genes to confer susceptibility or resistance to leprosy in Chinese Han populations.

Abbreviations
HLA: human leukocyte antigen; MB: multibacillary; PB: paucibacillary; PCR: polymerase chain reaction; SSOP: sequence-specific oligonucleotide probe.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
FZ conceived the study, participated in its design and collection of samples. CZ was involved in per- writing the first draft of the paper. SC was involved in study design and interpretation of results, and finalized the manuscript. All authors read and approved the final manuscript.

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