Association study of the HLA-DRB1 locus reveals the first evidence for the association of HLA-DRB1*15 and DRB1*09 with leprosy and the impact of DRB1*09 on the onset of disease in Chinese population

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Abstract:

**Background:** Previous studies have suggested an influence of HLA molecules on the regulation of the anti *Mycobacterium leprae* immune response. The association of HLA-DRB1 with leprosy has been reported in several populations, but not in Chinese population.

**Objectives:** To analyze the association of the HLA-DRB1 locus with leprosy susceptibility and disease subtypes in Chinese Han population.

**Methods:** 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 controls.

**Results:** HLA-DRB1*15 allele was found to be significantly more prevalent in leprosy patients than the healthy controls, whereas a decreased frequency of DRB1*09 allele in the patients, especially the ones with early-onset disease, was found by comparison with the healthy controls.

**Conclusions:** The HLA-DRB1 alleles play an important role in leprosy susceptibility in Chinese population, and the HLA-DRB1*09 allele has an impact on the onset of the disease.

**Keywords:** leprosy; HLA genotyping; HLA-DRB1

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

Leprosy is a chronic infectious disease that occurs in genetically predisposed individuals. And it is caused by the intracellular macrophage pathogen *Mycobacterium leprae*, which also infects Schwann cells and is responsible for nerve injury. Leprosy is characterized by a spectrum of disease symptoms, resulting from interactions between the host immune response and the invading *M. leprae*. At the lepromatous pole patients exhibit multibacillary infection and an absence of antigen-specific cellular immunity. At the tuberculoid pole patients exhibit paucibacillary infection and a strong cellular immune response. Between these two poles borderline leprosy patients show intermediate phenotypes [1].

Leprosy was once prevalent in the worldwide. There has been a steady decline in the prevalence of leprosy since the introduction of multidrug therapy by the *world healthy organization* (WHO-MDT) in 1982 [2]. But the detection rate of new cases remains steady in some countries and in some areas of China [3, 4]. Since 2002, nearly 1,600 new cases have still been detected each year in China [4].

Previous studies have suggested a strong influence of genetic factors on the 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 have reported association of HLA-DR2 alleles, such as HLA-DRB1*15, *16 *04, *10, *12, with susceptibility or resistance to leprosy in Brazil, Vietnam, South India, Indonesia, Thailand and Argentina populations [14-18]. In addition, the
HLA-DR3 alleles were also found to be associated with leprosy susceptibility in Surinam and Mexico populations [19, 20]. These studies have not only shown the importance of the HLA-DR locus in leprosy susceptibility but also highlighted that different alleles of HLA-DR are associated with leprosy in diverse populations.

Although leprosy is still a major concern for public health care and one of the top infectious diseases which could cause disability in China, the genetic susceptibility to leprosy was poorly studied in Chinese population. The only effort was an investigation of the association of HLA-DRB1 with leprosy in Southern China, but the study failed to detect this well-established association, due to the small sample employed [21]. Thus, to investigate the role of the HLA-DRB1 locus in leprosy susceptibility in Chinese Han population, we analyzed DRB1 alleles in a large Chinese Han sample of 305 leprosy patients and 527 healthy controls. We also investigated the association of the DRB1 alleles with the clinical forms of the disease, such as multibacillary (MB) and paucibacillary (PB) subtypes as well as the early- and late-onset leprosy.

2. Materials and methods

Patients and controls: The study included 305 unrelated individuals with a diagnosis of leprosy (278 men and 27 women). The diagnosis was based on clinical assessment and detection of acid-fast bacilli in skin slit smears and skin lesion histopathology. Of the 305 patients, 179 were with multibacillary (MB) disease, and 126 are with paucibacillary (PB) patients were studied, covering the entire clinical spectrum of the disease (polar and borderlines forms). 527 control samples, collected from the Shandong Blood Center, were matched to the patients regarding to ethnic origin, age and the male/female ratio.
All the subjects gave informed consent to participate in the study. The protocol was approved by Ethical Committee of Shandong Provincial Institute of Dermatoverenology.

**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 PCR-SSOP-Luminex method included PCR amplification, hybridization, streptavidin-phycoerythrin (SA-PE) reaction, and the measurement of the analysis [22]. Target DNA was PCR-amplified using 5’ biotin-labeled primers that were highly specific to certain sequences of HLA-DRB1 genes. PCR was carried out using a 20ul reaction system, containing Lifecodes mixture 6 µ L, Taq polymerase 0.2 µ L, nuclease-free water 11.8 µ L and genomic DNA 3 µ L. After denaturization, amplified DNA is allowed to hybridize to complementary DNA probes coupled to microbeads. The hybridized PCR product on the oligobeads is labeled with Streptavidin-phycoerythrin. Luminex apparatus identified the fluorescent intensity of phycoerythrin on each coded oligobead that had hybridized with the biotin-labeled PCR product. Genosearch typing software (Quick-Type for LifeMatch 2.0, Luminex Corporation) assisted in determining the HLA-DRB1* genotype of the sample DNA.

**Statistical analysis:** Power calculations carried out using PS software (www.biostat.mc.vanderbilt.edu/twiki/bin/view) showed that our sample size of 315 patients and 527 controls had more than 95% (P>0.05) power to detect an OR of 2.0 when the relevant allele has frequency >0.10. Allele frequencies were calculated by direct counting. The significance of the allele frequency difference between the patients and controls was analyzed by using Fisher’s exact probability test. The nominal P values were then corrected for
multiple testing (Pc value) by using the Bonferroni correction, this is, by multiplying the nominal P values by the number of HLA alleles being tested. The level of Pc<0.05 was accepted as statistically significant. The strength of association was estimated by calculating the OR. Statistical analysis was carried out by using the SPSS software (version 11.0).

3. Results

Table 1 summarizes the allele frequencies of HLA-DRB1* alleles in the leprosy patients and controls and the results of statistical analysis. Of the 13 HLA-DRB1 alleles determined by the PCR-SSOP-Luminex method in the Chinese Han patients with leprosy, three alleles showed significant difference in allele frequency (P<0.05) between the patients and controls. The frequency of the DRB1*15 allele was 0.32 (195 / 610) in the patients, which was significantly higher than 0.18 (185/1054) observed in the healthy controls (P<0.001, OR=2.207). The evidence for association remains significant after correction for multiple testing (Pc<0.001). In contrast, the frequency of HLA-DRB1*09 allele was significantly lower in the patients than in the controls (P= 0.001 Pc=0.013, OR=0.57). The frequency of HLA-DRB1*07 allele was also lower in the patients than in the healthy controls, but the evidence was only suggestive because its significance could not survive the correction for multiple testing (P=0.049, Pc=0.637, OR=0.738).

We further investigated the role of the HLA-DRB1* alleles in different clinical subtypes of leprosy. We first compared the allele frequencies between MB and PB patients of leprosy, but no statistically significant difference in allele frequency was observed (data not shown). We then investigated the association of HLA-DRB1* alleles with early-onset (age-onset ≤16 years old, 141 individuals) and late-onset leprosy (age-onset >16 years old, 164 individuals).
Interestingly, while the DRB1*15 allele show significant association with both early- and late-onset leprosy, the DRB1*09 only show the significant association with the early-onset leprosy.

4. Discussion

Leprosy is a chronic infectious disease caused by the organism *Mycobacterium leprae*. But it occurs in only a small percentage (1-3%) of infected people [23], which supports the important participation of host genetic factors in the development of leprosy disease. Many studies have detected associations of HLA-DR2 with leprosy, which is consistent with the finding that HLA-DR antigens are associated with disease and indicating that the majority of restriction determinants for *M. leprae* reside on DR, and not DP or DQ molecules [24]. Functional study about 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 [25]. Thus, HLA-DR antigens play a major role in the presentation of *M. leprae* antigens to T cells in leprosy patients.

By analyzing a large Chinese sample of leprosy, the present study has identified the first evidence for the association of HLA-DRB1 with leprosy in Chinese population and provided further supporting evidence for the important role of HLA in pathogenesis of leprosy. HLA-DRB1*15 has been demonstrated to be a susceptible allele to leprosy in Brazilians [14] and Indians [15]. In our study, HLA-DRB1*15 was also found to be associated with an increased risk in Chinese population, and its allele frequency in the cases (32%) is similar to the one observed in Indian populations and higher than the one observed in Brazilians (15%). The HLA-DRB1*09 was found to be associated with protection against leprosy. The frequency
was significantly lower in leprosy patients than in controls when corrected for multiple testing (P= 0.001, Pc=0.013). This allele had also showed a protective effect against the disease in Southern India and the allele frequency of cases was 0.017 [15], lower than in Chinese patients (AF of cases =0.08).

It has been reported that some HLA locus (LTA+80) in 6p21 chromosomal region was a major risk factor for early-onset (16 years old was used as a cut-off value) leprosy [26]. In order to investigate whether the effect of HLA-DRB1* on leprosy risk was age dependent, we stratified leprosy patients into early-onset (≤16 years) and late-onset (>16 years) groups in association analysis. Interestingly, we found that HLA-DRB1*09 is only associated with protection effect against the early-onset leprosy (P<0.001.), but not the late-onset leprosy (P=0.128). In contrast, HLA-DRB1*15 didn’t show age-onset dependent effect. To our knowledge, this is the first evidence for HLA-DRB1*09 to have an impact on the onset of the disease. Furthermore, it has also been reported that certain HLA-DRB1 alleles are associated with MB or PB forms of leprosy [27-29]. However, when the two forms of the disease were analyzed in our study, the frequencies of the HLA-DRB1 alleles were found to be very similar between the MB and PB patient groups. Taking together, our association analysis in the stratified patient groups suggest that the HLA-DRB1 locus is largely associated with leprosy per se, and only the HLA-DRB1*09 allele show an age-onset dependent effect.

5. Conclusion

In summary, by performing a genetic association study of the HLA-DRB1 locus in a large Chinese Han sample of leprosy, we have identified a strong evidence for the association of HLA-DRB1*15 (as susceptibility alleles) and DRB1*09 (as protective alleles) with leprosy in
Chinese population. Furthermore, for the first time, we have identified the evidence that the HLA-DRB1*09 has an impact on the onset of disease by only providing protective effect for early-onset leprosy. These alleles could act alone or in combination with other genes to confer differential susceptibility and also protection to leprosy disease in Chinese Hans population.
References


Table 1 Occurrence of *HLA-DRB1* alleles in leprosy patients and controls

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Patients (n=305)</th>
<th>Controls (n=527)</th>
<th>P value</th>
<th>P_c value</th>
<th>OR</th>
<th>OR (95% CI)</th>
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<td>23</td>
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<td>0.738</td>
<td>0.549-0.992</td>
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<td>&lt;0.001</td>
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</table>

*n*: the number of individuals; AF, allelic frequencies; OR, odds ratio; CI, confidence interval

*P* values are from allele-based test.
<table>
<thead>
<tr>
<th>Alleles</th>
<th>Controls (n=527)</th>
<th>Early-onset (n=141)</th>
<th>Late-onset (n=164)</th>
<th>AF</th>
<th>AF</th>
<th>P value</th>
<th>Pc value</th>
<th>AF</th>
<th>P value</th>
<th>Pc value</th>
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<tr>
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<td>0.896</td>
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<tr>
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<td>&lt;0.001</td>
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</table>

*n: the number of individuals; AF, allelic frequencies; OR, odds ratio; CI, confidence interval

*P* values are from allele-based test.