Haplotype of IL-8 -251T and 781C is associated with the Susceptibility to Respiratory Syncytial Virus

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Summary

Objective: To study the association of haplotypes of IL-8 -251T/A and 781 C/T single nucleotide polymorphisms (SNPs) with the susceptibility of respiratory syncytial virus (RSV).

Methods: This study included 101 hospitalized patients under 2 years who suffered from RSV pneumonia and 108 hospitalized patients under 2 years who suffered only from pneumonia without RSV infection. Genotypes of two SNP loci in all enrolled persons were defined by allele-specific polymerase chain reaction. The allele’s frequencies of SNPs were analyzed with case–control study, linkage of two loci and haplotypes composed by the two loci were also studied.

Results: (i) The frequency of IL-8 -251T in cases was dramatically high (OR = 2.08, p = 0.0002). (ii) Haplotype of TC was significantly high in cases (p = 0.01).

Conclusion: These findings support that haplotype of TC composed by IL-8 -251T and 781C is associated with the susceptibility of RSV.

Key words: respiratory syncytial virus, association study, single nucleotide polymorphism, IL-8, allele-specific polymerase chain reaction.

Introduction

Respiratory syncytial virus (RSV) is a common cause of respiratory tract infection in infants and young children during winter and spring endemics. Almost all infants and young children are infected with RSV by the age of 2 years [1], but differ in the clinical severities that range from asymptomatic infection, slightly upper respiratory tract infection, lower respiratory tract infection, respiratory failure and even to death. The cause that leads to the different severities after RSV infection is so far unclear. The inflammation of RSV bronchiolitis is characterized by neutrophil infiltration [2, 3]. Studies show that high levels of IL-8 is secreted by the epithelial cells of the airways after RSV infections [4, 5], and elevated IL-8 is also detected from both the nasal secretions and serum of infants and young children [6, 7]. IL-8 is a major chemotactic factor of neutrophils, and thus the individual variation of IL-8 gene expression might play an important role in the process of RSV infection. IL-8 -251A/T is located in one of the exons of the promotor region. Hull et al. [8] suggested that the IL-8 -251A allele tended to be associated with increased IL-8 production. IL-8 781C/T is located in the intron, CAAT box is located in downstream of IL-8 781C/T and could bind to CAAT/enhancer-binding protein β (C/EBPβ), so that it promotes the IL-8 gene transcription and regulation, and thus participates in the inflammation response [9]. We aim to detect IL-8 -251A/T and IL-8 781C/T single nucleotide polymorphism (SNP) and to explore the association of SNP with the susceptibility to RSV.

Materials and Methods

Study subjects

A total of 209 children under 2 years old, admitted to a local children’s hospital from September 2005 to March 2006 with the diagnosis of pneumonia, were enrolled in this study. The reason we chose hospitalized children as subjects was that the cases were relatively severe. Informed consent was acquired
from guardians and the study was agreed by the ethics committee of the hospital. Information including gender, age and nation were collected, and all patients were reported to be Han Chinese. The pharynx secretions were aspirated the next morning after admission and RSV antigen was detected by using direct immunofluorescence assay (Kit from JinMei Corporation, Shanghai, China; Item NO. 3110). Of the 209 subjects, 101 children with RSV-infected pneumonia were assigned to the case group and 108 children with pneumonia but not RSV infected were assigned to the comparison group. Mycoplasma, adenovirus, influenza virus detection and sputum culture in all patients were found to be negative.

**Methods**

Genomic DNA samples were collected from peripheral blood. Allele-specific polymerase chain reaction (AS-PCR) amplification of target gene was carried out [10, 11]. Human β-actin gene was used as internal control. Characteristics of each gene, AS-PCR primers, product sizes and PCR reaction conditions are listed in Table 1. The hot-start gold taq polymerase was chosen to increase the reaction specificity. AS-PCR products were electrophoresed on 2% agarose gels and observed under viltalight lamp (Figs 1 and 2).

**Statistical analysis**

Logistic regression analysis of the case–control study were performed using SPSS 13.0 for windows (School
of Public Health). Genotypes of IL-8 -251 T/A and IL-8 781 C/T loci were evaluated for Hardy–Weinberg equilibrium and linkage analysis with LDA1.0 software [12]. PHASE 2.0 program [13] was used to examine the haplotyping of the 2 loci.

**Results**

**IL-8 -251T/A SNP**

Logistic regression analysis. Logistic regression analysis was performed including gender, age and genotype of the enrolled subjects. The gender, age and genotype of both groups were shown in Table 2. Logistic regression analysis showed that neither gender nor age contributed to RSV infection ($p > 0.05$, respectively, Table 3). The regression coefficient of TT genotype was 1.799 and OR was 6.043 by Wald test, which means that the probability for RSV infection with TT genotype was as 6.043 times as that with AA genotype after the variation including gender and age were excluded.

Case–control study for IL-8 -251T/A. Genotype of IL-8 -251 locus in 108 control subjects followed Hardy–Weinberg equilibrium ($p > 0.05$). The frequency of T allele was significantly higher in the RSV-infected group than in the RSV non-infected group by case–control study (OR = 2.08, $P = 0.0002$, Table 4).

Linkage analysis and haplotype for IL-8 -251T/A and 781 C/T

Linkage of IL-8 -251T/A and 781C/T was analyzed using LDA software, which indicated that IL-8 -251T and 781C was linked ($D' = 0.607 ± 0.03$, $r^2 = 0.2861$, $p = 0.0000$). The haplotypes of these two loci were further studied with PHASE 2.0 program, which showed that haplotype of TC was significantly higher

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**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Male/Female</th>
<th>Age (day) ($\bar{x} \pm s$)</th>
<th>-251 locus genotype, N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TT</td>
</tr>
<tr>
<td>Case group</td>
<td>101</td>
<td>78/23</td>
<td>180±173</td>
<td>49</td>
</tr>
<tr>
<td>Comparison group</td>
<td>108</td>
<td>73/35</td>
<td>205±224</td>
<td>27</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>Variation</th>
<th>Regression coefficient ($\beta$)</th>
<th>Wald value</th>
<th>$p$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>0.518</td>
<td>2.942</td>
<td>0.086</td>
<td>1.679 (0.929–3.037)</td>
</tr>
<tr>
<td>Age</td>
<td>−0.01</td>
<td>1.017</td>
<td>0.313</td>
<td>0.999 (0.998–1.001)</td>
</tr>
<tr>
<td>Genotype</td>
<td>TT</td>
<td>1.799</td>
<td>0.004</td>
<td>6.043 (1.788–20.427)</td>
</tr>
<tr>
<td></td>
<td>TA</td>
<td>0.698</td>
<td>0.269</td>
<td>2.010 (0.583–6.934)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>0.902</td>
<td>0.146</td>
<td>2.463 (0.732–8.295)</td>
</tr>
</tbody>
</table>

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**Fig. 2.** 781C/T AS-PCR electrophoretogram.
in RSV-infected group than in RSV non-infected group (p = 0.01, Table 5).

**Discussion**

Our study found that there is an association between IL-8 -251T and susceptibility with RSV after variables including gender and age were excluded. Hull et al. [8] found an association between IL-8 -251A and susceptibility to RSV in UK infants. However, Heinzmann et al. [14] showed no association of this locus with the susceptibility to RSV in German infants and young children. Our result was different from both of these studies. This disconformity could be caused by several reasons. First, there might be the disparity in different nations. Second, sample sizes were also accounted for this disparity. In addition, statistical results might be different because of the chosen control subjects and its quantity.

Additionally, our study for the linkage and haplotypes of IL-8 -251T/A and 781C/T indicated some linkage between these two loci (D’ = 0.607 ± 0.03, \( r^2 = 0.2861 \), \( p = 0.0000 \)). This result was identical to that of Hull et al. [8] and Heinzman, et al. [14]. Haplotype of TC was associated with the susceptibility to RSV (p = 0.01), which suggested that some RSV predisposing gene could be located in the gene fragment containing haplotype of TC or linked tightly to this fragment.

In conclusion, IL-8 gene polymorphism is associated with the susceptibility to RSV, and some RSV predisposing gene might be located in the gene containing haplotype composed by IL-8 -251T and 781C or linked tightly to this gene fragment. RSV infection is a complex multigenic disease, and could only be partly explained by the existence of predisposing gene in IL-8 gene. The host immune response to RSV infection is a complicated process, and RSV predisposing gene might also be located in the genes of other biological molecules participating into the immune response other than IL-8.

**References**