Lack of Association between Interleukin-8 Gene +781 C/T Polymorphism and Henoch–Schönlein Purpura in Childhood

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ABSTRACT

Henoch–Schönlein purpura (HSP), a common allergic hemorrhagic disease, occurs frequently in children affecting kidney, joint and skin. While interleukin-8 (IL-8) plays an important role in inflammation, the association between IL-8 gene +781 C/T polymorphism and HSP remains unclear.

Interleukin-8, an important chemokine related to the initiation and amplification of acute inflammatory responses, has been reported to be involved in the pathogenesis of some autoimmune and inflammatory diseases. In this study, we aimed to investigate whether IL-8 gene +781 C/T (rs2227306) polymorphism has an influence on susceptibility and clinical manifestations of patients to HSP.

This hospital-based case-control study comprised 192 patients with HSP and 202 healthy controls. The genotypes of IL-8 gene +781 C/T polymorphism were identified using PCR-TaqMan method.

All genotype frequencies of both groups (patients and controls) conformed to the Hardy–Weinberg equilibrium. No significant differences in allele or genotype frequencies of IL-8 gene +781 C/T polymorphism were observed between patients with HSP and controls ($p=0.98$, $\chi^2=0.000$ and $p=0.49$, $\chi^2=1.432$, respectively). When patients were stratified for the presence of joint, gastrointestinal and renal manifestations, genotype frequencies of IL-8 gene polymorphism were found no statistically significant differences ($p>0.05$).

Our findings do not support that IL-8 gene +781 C/T polymorphism has an effect on the susceptibility to HSP in Chinese children.

Keywords: Case-control study; Henoch–Schönlein purpura; Inflammatory; Interleukin-8; Polymorphism

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INTRODUCTION

Henoch–Schönlein purpura (HSP) is the most common type of systemic small vessel vasculitis in children, pathologically characterized by immunoglobulin A (IgA) which is mainly deposited in the small vessels. Cases mostly occur between the ages of 3 and 10 years and is rarely found in adults. The major clinical features of this disease are non-thrombocytopenic palpable purpura, arthritis, internal organ involvement, such as the gastrointestinal (GI) tract and the kidney. The disease is generally acute and self-limited; however, renal involvement results in the most serious clinical consequences, and long-term prognosis of HSP is dependent on the severity of renal involvement.

The common risk factors of HSP are infection and heredity. Upper respiratory tract infection is the main etiological factor, and winter and autumn are the typical onset seasons. Furthermore, several studies have revealed the role of some relevant genetic variants in both susceptibility and HSP clinical heterogeneity. Recent studies performed in a well characterized cohort of Caucasian HSP patients have described the human leukocyte antigen region as an important genetic factor associated with HSP.

Interleukin-8 (IL-8), one of the most widely studied proinflammatory cytokine to date, belongs to the superfamily of CXC chemokines. This chemokine is a major mediator of inflammation, which can be produced by a variety of different cells, including monocytes, epithelial cells, and endothelial cells. IL-8 can attract neutrophils, basophils and macrophages and exerts a wide range of proinflammatory effects. The ability of IL-8 to mediate the activation of neutrophils and stimulate the migration of neutrophils from the peripheral blood into the tissues suggests that this cytokine may take part in the pathogenesis of many inflammatory diseases.

A role for IL-8 in HSP has been studied. The serum level of IL-8 increased in patients with HSP has been reported. Furthermore, in vitro study has revealed that active sera of patients with HSP can activate human umbilical venous endothelial cells to produce IL-8. Subsequent research further described that IgA anti-endothelial cell antibodies derived from acute stage of HSP may bind to endothelial cells and enhance endothelial cells to produce IL-8 via the mitogen/extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway. These observations suggested that the IL-8 might be a risk factor for HSP.

The IL-8 gene, which is located on chromosome 4q12-q13 in humans, encodes the IL-8 cytokine. It contains four exons, three introns and a proximal promoter region. Polymorphisms of the IL-8 gene were found to be associated with a variety of autoimmune and inflammatory diseases such as systemic lupus erythematosus (SLE), Graves’ disease (GD) and Graves’ ophthalmopathy (GO), rheumatoid arthritis (RA), IgA nephropathy (IgAN), nephritis in cutaneous vasculitis, respiratory syncytial virus bronchiolitis and bronchial asthma, acute respiratory distress syndrome, osteoarthritis, periodontitis.

Several studies reported that the IL-8 gene polymorphisms at positions -251 and +781 can affect IL-8 expression. To date, two studies evaluating the association between IL-8 gene polymorphism at position +2767 (A/G) and HSP have been performed, and the same result was obtained from the two studies which described that HSP patients with renal involvement had a significantly higher “A” allele frequency.

To the best of our knowledge, there is currently no published study regarding the role of IL-8 gene polymorphisms at positions +781 in HSP. The aim of the present study was to research whether the IL-8 gene polymorphism at position +781 is associated with the risk of HSP.

MATERIALS AND METHODS

Patients and Methods

The case-control study involving 192 patients with HSP and 202 healthy controls was conducted between June 2011 to June 2013 in the Zhejiang University of Children's Hospital (Hangzhou, China). All cases were fulfilled the American College of Rheumatology criteria for the classification of HSP. Patients who had other systemic vasculitis, thrombocytopenic purura, systemic lupus erythematosis, juvenile dermatomyositis, diabetes, and skin, or kidney biopsy findings not compatible with HSP were excluded from the study. All patients who had been newly diagnosed with HSP and had a minimum follow-up of 6 months were included in this study. Two hundred and two healthy children without a...
IL-8 Polymorphism and HSP

A history of allergic purpura were randomly selected to enroll in the study. The demographics of patients and controls together with detailed clinical information are provided in Table 1.

We reviewed the following variables determined for HSP patients in the acute stage from the hospital records: leucocyte, serum levels of creatinine, albumin, complement 3(C3), IgA, and proteinuria. Estimated glomerular filtration rate (GFR) was calculated with the Schwartz formula.

The research was approved by the hospital ethics committee, and informed consent was obtained from all the families of the patients and controls.

Clinical Definitions

Nephritis was defined as the presence of any hematuria and/or proteinuria. Joint manifestations were defined if arthralgia or peripheral arthritis was observed on examination. For gastrointestinal (GI) manifestations, bowel angina was considered present if there was diffuse abdominal pain that worsened after meals or bowel ischemia usually with bloody diarrhea. GI bleeding was defined as the presence of melena, hematochezia, or a positive test for occult blood in the stool.

Analysis of IL-8 Gene Polymorphism

Leukocytes were withdrawn by centrifugation from EDTA-anticoagulated blood samples. DNA of the leukocytes was extracted by using a standard proteinase K digestion and phenol-chloroform method. The TaqMan SNP genotyping assay was performed by using the LightCycler 480 II Real-Time PCR System (Roche Applied Science, Switzerland). The primers and TaqMan probe sequences were Rs2227306-FP: TGACCAGATAAAAATACCATGAAG; Rs2227306-RP : TTCTCCTAGCCCTTGACCTC; rs2227306-P-C : FAM-CATTGAACGACTTCC-MGB; rs2227306-P-T : VIC-CATTGAACACTTCC-MGB; TaqMan PCR reaction volume was 20μl, including: genotype qPCR Master Mix (1X) 10 μl, PCR forward primer (500nM) 1μl, PCR reverse primer (500nM) 1μl, TaqMan probe T (250nM) 0.5μl, TaqMan probe C (250nM) 0.5μl, genomic DNA 1μl (100ng), dH2O 6μl. We followed the instructions provided with the assay kit (Huirui Biotechnology Co, Ltd, Shanghai, China). Briefly, the reaction procedures consisted of a hot start DNA polymerase at 95°C for 5 minutes, followed by 40 cycles of denaturing at 95°C for 10 seconds and annealing at 55°C for 45 seconds. Deionized water was used as the negative control.

Table 1. Demographic and clinical characteristics of HSP patients and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients, n(%) or mean±SD</th>
<th>Controls,n(%) or mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>192</td>
<td>202</td>
</tr>
<tr>
<td>Age at disease onset</td>
<td>7.2±2.5</td>
<td>7.0±2.1</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>112(58.3)</td>
<td>108(53.5)</td>
</tr>
<tr>
<td>female</td>
<td>80(41.7)</td>
<td>94(46.5)</td>
</tr>
<tr>
<td>Purpuric rash</td>
<td>192(100)</td>
<td></td>
</tr>
<tr>
<td>Arthralgias and/or arthritis</td>
<td>113(58.9)</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal manifestations</td>
<td>140(72.9)</td>
<td></td>
</tr>
<tr>
<td>Renal involvement</td>
<td>69(35.9)</td>
<td></td>
</tr>
<tr>
<td>White blood cell (×10^9/L)</td>
<td>10.46±5.18</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.48±0.23</td>
<td></td>
</tr>
<tr>
<td>C3 (g/l)</td>
<td>1.20±0.25</td>
<td></td>
</tr>
<tr>
<td>IgA (g/l)</td>
<td>1.98±0.82</td>
<td></td>
</tr>
<tr>
<td>ALB (g/l)</td>
<td>40.6±6.1</td>
<td></td>
</tr>
<tr>
<td>Proteinuria(mg/Kg/day)</td>
<td>16.5±29.8</td>
<td></td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td>156.3±32.8</td>
<td></td>
</tr>
</tbody>
</table>

HSP: Henoch–Schönlein purpura; SD: Standard Deviation; C3: complement 3; IgA: immunoglobulin A; GFR: glomerular filtration rate
### Table 2. IL-8 gene polymorphism in HSP and healthy control

<table>
<thead>
<tr>
<th>IL-8 gene +781 C/T polymorphism</th>
<th>Controls (n=202)</th>
<th>HSP (n=192)</th>
<th>$\chi^2$</th>
<th>p-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>74 (36.6%)</td>
<td>76 (39.6%)</td>
<td>1.432</td>
<td>0.49</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>CT</td>
<td>100 (49.5%)</td>
<td>84 (43.8%)</td>
<td>0.82</td>
<td>0.53-1.26</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>28 (13.9%)</td>
<td>32 (16.7%)</td>
<td>1.11</td>
<td>0.61-2.03</td>
<td></td>
</tr>
<tr>
<td><strong>Dominant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>74 (36.6%)</td>
<td>76 (39.6%)</td>
<td>0.363</td>
<td>0.55</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>CT+TT</td>
<td>128 (63.4%)</td>
<td>116 (60.4%)</td>
<td>0.88</td>
<td>0.59-1.33</td>
<td></td>
</tr>
<tr>
<td><strong>Allele</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>248 (61.4%)</td>
<td>236 (61.5%)</td>
<td>0.000</td>
<td>0.98</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>T</td>
<td>156 (38.6%)</td>
<td>148 (38.5%)</td>
<td></td>
<td></td>
<td>1.00 (0.75-1.33)</td>
</tr>
</tbody>
</table>

HSP: Henoch–Schönlein purpura; OR: odds ratio; CI: confidence interval.

About 10% of the samples were randomly selected for repeated genotyping for confirmation, and the results were concordant.

### Statistical Analysis

The genotype distributions of IL-8 gene were tested for the Hardy–Weinberg equilibrium in both patients and controls by using the Chi-square goodness-of-fit test. The allele and genotype frequencies of IL-8 gene between the two groups were analyzed by using Chi-square ($\chi^2$) test. The odds ratios (OR) and the significant of OR were calculated to estimate the risk of developing HSP. The Mean values were compared between the groups by the unpaired student’s t-test. The differences between the groups were considered to be significant if p-values were <0.05. Statistical analysis was performed by using the SPSS software package, revision 17.0.

### RESULTS

The demographic and clinical characteristics of study participants are given in Table 1. Mean age at disease onset of the patients who had been on follow-up for at least 6 months was 7.2±2.5 years and did not differ from healthy subjects (7.0±2.1 years). No statistical differences in age and gender were found between the HSP group and the control group.

Laboratory characteristics (leucocyte, GFR, proteinuria, serum levels of creatinine, albumin, C3, and IgA) in the acute stage of HSP were not statistically different between patients with CC genotype and Non-CC genotype (CT and TT) (data not shown).

The genotype and allele frequencies of the IL-8 gene +781C/T polymorphism in HSP group and the control group are given in Table 2. The observed genotype distributions of the IL-8 gene +781 C/T polymorphism in both groups (control and HSP) accorded with Hardy–Weinberg equilibrium ($\chi^2$=0.396, p=0.53) and ($\chi^2$=1.124, p=0.29, respectively).

No significant differences for IL-8 gene +781C/T polymorphism were observed in the genotype and allele frequencies between patients and controls (p=0.49, $\chi^2$=1.432 and p=0.98, $\chi^2$=0.000, respectively). The CC genotype frequency of IL-8 gene +781C/T polymorphism was insignificantly higher in HSP patients than in controls (39.6% vs. 39.6%). The frequency of mutant allele T in HSP patients was quite similar to that in controls (38.5% vs. 38.6%; T vs. C OR: 1.00, 95% CI: 0.75-1.33, $\chi^2$=0.000, p=0.98). Risk estimates making use of dominant mode showed that patients with CT+TT genotypes did not have a higher risk of developing HSP compared to patients with CC genotype (OR: 0.88, 95% CI: 0.59-1.33, $\chi^2$=0.363, p=0.55).

The clinical characteristics of HSP can be stratified into three subtypes: Kidney group, GI tract group and Joint group. The distributions of the genotype frequencies of IL-8 gene +781C/T polymorphism associated with clinical characteristics of the HSP...
patients were shown in Table 3. However, no significant differences in genotype frequencies among any groups were found \((p>0.05)\).

**DISCUSSION**

HSP is a common systemic childhood vasculitis, characterized by peripheral leucocytosis and polymorphonuclear neutrophils (PMNs) infiltration around the blood vessel\(^3\). Although the etiology of HSP is not fully understood, genetic factors and activation of cellular immune response might play fundamental roles in HSP pathogenesis. IL-8, as an important chemokine that can both initiate and amplify the acute inflammatory responses, has been reported to be involved in the pathogenesis of some autoimmune and inflammatory diseases. In this regard, it has been demonstrated that the serum level of IL-8 in HSP group was significantly higher than controls\(^9,15\) and this in part contributed to leucocytosis and PMNs infiltration around vessels in HSP\(^3\). Furthermore, CAAT/enhancer binding protein \(\beta\) preferentially binds in the presence of the IL-8 gene +781T allele, which can promote IL-8 gene transcription and regulation\(^29,36\). Thus, we speculate that individuals genetically producing more IL-8 may somehow predispose them to a higher risk of developing HSP.

The results from two studies about the possible role of IL-8 gene +2767 G/A in pathogenesis of HSP suggest that allele A is associated with the increased risk of HSP with nephritis\(^21,31\). Of the known IL-8 genetic variants, the role of +781 C/T polymorphism in HSP remains unclear. Due to this, it is entirely plausible that we investigated the possible role of the IL-8 gene +781 C/T polymorphism in the etiology and pathogenesis of HSP.

Association between the IL-8 gene +781 C/T polymorphism and autoimmune and inflammation diseases has been shown. Gu LQ et al.\(^18\) reported IL-8 gene +781 allele C was associated with an increased risk of GD (OR: 1.21, 95% CI: 1.03–1.42) and GO (OR: 1.47, 95% CI: 1.13–1.91) in Chinese cohorts. A study from Netherlands observed that IL-8 gene +781 CC genotype in the RA patients was associated with early onset of disease \((p<0.0001)\).\(^19\) Suh JS et al.\(^20\) indicated that IL-8 gene +781 C/T polymorphism had a positive association with the risk of IgAN in a Korean cohort. Puthothu B et al.\(^24\) previously found that IL-8 gene +781C/T polymorphism and IL-8 haplotypes were associated with asthma in a German cohort \((p=0.011\) and \(p=0.036\), respectively). However, in present study, no association between IL-8 gene +781C/T polymorphism and the susceptibility to HSP was detected.

In the cohort of our study, TT genotype and T allele frequencies were not significantly different in the HSP patients and the controls. Risk assessment found that there was not a significant risk for HSP if the patient is with TT genotype \((OR: 1.11, 95\%\ CI: 0.61-2.03)\) or with CT genotype \((OR: 0.82, 95\%\ CI: 0.53-1.26)\). Patients with the 2767 A allele showed higher proteinuria and creatinine levels at follow-up.\(^31\) Yet, proteinuria and the serum level of creatinine in the acute stage of HSP had no relationship with IL-8 gene +781C/T polymorphism in this study. The TT genotype frequencies of the IL-8 gene +781C/T polymorphism in HSP patients with renal, GI and arthritis manifestations were 17.4%, 15.7%, and 18.6%, respectively. No significant differences in the distribution of genotype frequencies of IL-8 gene +781 C/T polymorphism were found between the controls and HSP patient subgroups. IL-8 gene +781 C/T polymorphism did not seem to have an impact on the development of renal, GI and arthritis manifestations of the disease.
In conclusion, our findings did not provide supportive evidence for IL-8 gene +781 C/T polymorphism associated with HSP disease. To our knowledge, this is the first study to detect the IL-8 gene +781 C/T polymorphism with HSP in Chinese children. In the future, further studies about more other loci at IL-8 gene in a larger sample population will be necessary. Therefore, researchers may understand the precise role of IL-8 gene polymorphism in the pathogenesis of HSP.

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