Influence of Interleukin 18 Promoter Polymorphisms in Susceptibility to Kawasaki Disease in Taiwan

KAI-CHUNG HSUEH, YING-JU LIN, JENG-SHENG CHANG, LEI WAN, YU-HSIN TSAI, CHANG-HAI TSAI, and FUU-JEN TSAI

ABSTRACT. Objective. Proinflammatory cytokines such as interferon-γ (IFN-γ) play an important role in the pathogenesis of Kawasaki disease (KD). Interleukin 18 (IL-18) plays a pivotal role in the Th1-type response, principally owing to its ability to induce IFN-γ production. We assessed potential associations between functional IL-18 gene promoter polymorphisms and susceptibility to KD, in addition to clinical features of KD in individuals from Taiwan.

Methods. One hundred forty-six patients with KD and 136 ethnically matched controls from the same geographic area were genotyped for IL-18 –656T/G, –607A/C, and –137C/G promoter polymorphisms.

Results. No significant differences in allele and genotype frequencies were found between KD patients and controls for any of the IL-18 polymorphisms investigated. When we compared the overall distribution of haplotype frequencies between KD patients and controls, a significant difference was observed (p < 0.0001). In addition, the frequency of the GCG haplotype was significantly higher (p = 0.00001, pc = 0.00004; OR 20.8, 95% CI 3.05–142.3), whereas the frequency of the TAG haplotype was significantly lower in KD patients compared with controls (p = 0.0001, pc = 0.0004; OR 0.35, 95% CI 0.19–0.61). No significant associations were found for comparisons of KD patients to those with and without coronary artery lesions.

Conclusion. Our results suggest a potential implication of IL-18 promoter polymorphisms in susceptibility to KD in Taiwan. (First Release May 15 2008; J Rheumatol 2008;35:1408–13)

Key Indexing Terms: INTERLEUKIN 18 KAWASAKI DISEASE PROMOTER POLYMORPHISM

Kawasaki disease (KD) is an acute systemic vasculitis that predominantly affects infants and young children. Coronary artery lesions (CAL) develop in 20%–25% of patients, which makes KD the leading cause of acquired heart disease in children. The cause of this disease is unknown but is generally believed to be an infectious agent. Activation of the immune system, including neutrophils, monocytes/macrophages, and lymphocytes, has been observed in the acute phase of KD. Serum concentrations of various cytokines, including T helper 1 (Th1) and Th2 cytokines, have been shown to be elevated in patients with KD, such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin 1 (IL-1), IL-2, IL-4, IL-6, and IL-10. These cytokines produce the systemic signs of inflammation that characterize the disease and may mediate the endothelial damage that leads to coronary artery disease. It has been proposed that KD is an immunologic reaction to some sort of infection in genetically predisposed individuals, particularly Asians. Reports have shown that blood levels of IL-18 were normal in the acute phase of KD and were elevated during the subacute phase. Patients with CAL showed a significantly higher incidence of elevated subacute IL-18 values than patients without CAL. A positive correlation between IL-18 values in the subacute phase of KD and CAL has also been reported.

IL-18, a member of the IL-1 family, is a pleiotropic proinflammatory cytokine that participates in both innate and acquired immune responses. IL-18 in synergy with IL-12 plays a pivotal role in the Th1-type response, principally owing to its ability to induce IFN-γ production in T cells and natural killer (NK) cells. It has been noted that IL-18 is expressed at chronic inflammatory sites of Th1-type autoimmune diseases in humans, such as rheumatoid arthritis (RA) and Crohn’s disease.
The human IL-18 gene is located on chromosome 11q22.2-22.3, and is composed of 6 exons. Expression is regulated by 2 distinct promoters, promoter 1 upstream of exon 1 and promoter 2 in intron 1. Three single-nucleotide polymorphisms (SNP) at positions –656 (T/G), –607 (A/C), and –137 (C/G) within the promoter 1 region and 2 SNP in the 5' non-translated regions of the IL-18 gene have recently been identified. Of these polymorphisms, the 2 SNP at positions –137 (C/G) and –607 (A/C) have been reported to affect transcription factor binding and gene activity and to be associated with various chronic inflammatory diseases, such as type I diabetes, sarcoidosis, RA, and inflammatory bowel disease. However, no previous study has evaluated the association between KD and polymorphisms in the IL-18 promoter.

We investigated whether common polymorphisms in the IL-18 gene, either individually or as part of a common haplotype, are associated with KD or with the occurrence of CAL in a case-controlled study of patients with KD in Taiwan.

MATERIALS AND METHODS
Subjects. We enrolled patients with KD from the Department of Pediatrics at the China Medical University Hospital. The study group included 146 patients, all of whom met the criteria proposed by the Japanese Kawasaki Disease Research Committee.

Table 1. Characteristics of the patients with Kawasaki disease in this study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>146</td>
</tr>
<tr>
<td>Age, yrs at time of diagnosis</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.1</td>
</tr>
<tr>
<td>Median (range)</td>
<td>1.82 (0.2–7.5)</td>
</tr>
<tr>
<td>Sex, n</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>87</td>
</tr>
<tr>
<td>Female</td>
<td>59</td>
</tr>
<tr>
<td>Coronary artery lesions, n</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>101</td>
</tr>
<tr>
<td>Present</td>
<td>45</td>
</tr>
</tbody>
</table>

The sex distribution of KD patients overall was a male/female ratio of 1.5:1, comparable to previous reports of ratios ranging from 1.5:1 to 1.7:1. Forty-five patients (30%) were identified as having coronary artery lesions. All KD patients were enrolled in the study on a voluntary basis; therefore, the study population is not representative of the true incidence of either KD or CAL in Taiwan.

Allele and genotype frequencies of IL-18 promoter polymorphisms. The genotypes of the 3 promoter polymorphisms including –656T/G, –607A/C, and –137C/G were identified by the probe hybridization method employing the LightCycler 480 instrument (Roche Diagnostics, Indianapolis, IN, USA) with corresponding primers and probes (Table 2).

RESULTS
Characteristics of the study patients. The characteristics of the 146 patients with KD are shown in Table 1. The sex distribution of KD patients overall was a male/female ratio of 1.5:1, comparable to previous reports of ratios ranging from 1.5:1 to 1.7:1.

Allele and genotype frequencies of IL-18 promoter polymorphisms. The genotypes of the 3 promoter polymorphisms including –656T/G, –607A/C, and –137C/G were identified by the probe hybridization method in KD patients and controls with corresponding primers and probes (Table 2). However, that the total numbers for KD patients or controls may lead to PCR failure. Therefore, the total numbers for KD patients or controls were considered in order to have enough successful PCR reactions for analysis.

The allele and genotype frequencies of the 3 IL-18 promoter polymorphisms in the KD and control subjects are shown in Table 3. Both populations were in Hardy–Weinberg equilibrium for all polymorphisms tested. No differences in allele and genotype frequencies between KD patients and controls were found for any of the IL-18 polymorphisms.
–137C/G and –656T/G. Thus, the –607A/C was in strong linkage disequilibrium with the –656T/G.

Haplotype frequencies were estimated using the 3 promoter polymorphisms with an allele frequency > 5% (Table 4). Four main haplotypes of the IL-18 promoter were present in the study population. The most common haplotype in healthy controls was GCC. In the KD patients, TAC was the most common haplotype. When we compared the overall distribution of haplotype frequencies between KD patients and healthy controls, a significant difference was observed (p < 0.0001, by chi-square test from a 4 × 2 contingency table). In addition, the GCC haplotype appeared to be a significant “at-risk” haplotype (p = 0.0001, p_c = 0.00004; OR 20.8, 95% CI 3.05–142.3). The TAG haplotype appeared to be a significant “protective” haplotype (p = 0.0001, p_c = 0.0004; OR 0.35, 95% CI 0.19–0.61). Although the frequency of the GCC haplotype was slightly decreased in KD patients compared with controls (p = 0.013, OR 0.65, 95% CI 0.46–0.90), this difference was not significant when Bonferroni adjustment was applied (p_c = 0.052).

Influence of IL-18 promoter polymorphisms in the clinical spectrum of KD. As a further step, we assessed whether there were differences in genotype, allele, or haplotype frequencies of the IL-18 promoter polymorphisms between KD patients with and without coronary artery lesions. There was no significant association between the CAL and any of the polymorphisms.
SNP we studied. In the haplotype analysis, none of the estimated haplotype frequencies in KD patients with CAL differed from those without CAL (data not shown).

Comparison of allele and haplotype frequencies of IL-18 promoter polymorphisms between Taiwanese and Caucasians. Frequency data on the 3 IL-18 promoter polymorphisms were compared with data from Dutch Caucasian subjects (Table 5). The allele and haplotype frequencies found in our Taiwanese controls were significantly different from those reported in Caucasian populations.

DISCUSSION
KD is currently thought to be an infectious disease with immunologic manifestations that occur only in genetically susceptible individuals. Polymorphic gene sequences of cytokines known to be involved in pathogenesis of KD are potential markers of disease susceptibility. Many studies have examined the relationship between cytokine gene polymorphisms and the incidence of KD, including TNF-α, IL-1 RA, IL-10, and vascular endothelial growth factor (VEGF). Our results provide evidence that polymorphisms of the IL-18 promoter are associated with susceptibility to KD in the Taiwanese population.

It has been shown that IL-18 concentrations are markedly elevated during the subacute phase of KD, but that they are not increased during the acute phase of the disease. Normal IL-18 values in the acute phase of KD might be due to a negative feedback mechanism of the IL-18 pathway. In the study by Nomura, et al, IL-18 values in KD patients in the subacute phase showed a significant positive correlation with the presence of coronary artery abnormalities and also with the duration of fever. This suggests that IL-18 levels in the subacute phase may reflect the severity of KD.

Several studies have shown that the level of IL-18 production is related to the IL-18 promoter gene, and 2 SNP at positions −607 and −137 of the IL-18 promoter region have been found to be associated with transcription activity of the IL-18 gene promoter. It has been shown that a change from C to A at position −607 disrupts a potential cAMP responsive element binding protein (CREB) site, resulting in low IL-18 production. Similarly, a change at position −137 from G to C has been reported to affect the H4TF-1 nuclear factor binding site. These studies showed low promoter activity for A and C alleles at position −607 and −137, respectively, and higher promoter activity for C and G alleles in those positions.

IL-18 promoter polymorphisms have been found to be associated with many immune-mediated inflammatory diseases including type I diabetes, sarcoidosis, RA, and inflammatory bowel disease. In a Chinese population, the

### Table 4. Distribution of IL-18 promoter haplotype frequencies in patients with KD and controls.

<table>
<thead>
<tr>
<th>Haplotype*</th>
<th>KD Patients, %†</th>
<th>Controls, %†</th>
<th>p (p&lt;sub&gt;c&lt;/sub&gt;)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCC</td>
<td>31.7</td>
<td>41.8</td>
<td>0.013 (0.052)</td>
<td>0.65 (0.46–0.90)</td>
</tr>
<tr>
<td>TAC</td>
<td>42.8</td>
<td>39.3</td>
<td>NS</td>
<td>1.15 (0.82–1.61)</td>
</tr>
<tr>
<td>TAG</td>
<td>6.4</td>
<td>16.5</td>
<td>0.0001 (0.0004)</td>
<td>0.35 (0.19–0.61)</td>
</tr>
<tr>
<td>GCG</td>
<td>7.8</td>
<td>0.4</td>
<td>0.00001 (0.00004)</td>
<td>20.8 (3.05–142.3)</td>
</tr>
</tbody>
</table>

* Order of single-nucleotide polymorphisms comprising the IL-18 haplotypes: −656T/G, −607A/C, −137C/G. Haplotypes were identified by Bayesian computation from Phase 2.1. † Percentages may not sum to 100% because of the presence of rare haplotypes (< 5%) not presented here. p<sub>c</sub>: p corrected for number of alleles compared. NS: not significant.

### Table 5. Frequencies of alleles and haplotypes of interleukin 18 promoter in Taiwanese and Caucasians.

<table>
<thead>
<tr>
<th>Allele/Haplotype</th>
<th>Taiwanese, n = 136</th>
<th>Caucasian, n = 103</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−656T</td>
<td>0.56</td>
<td>0.37</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>−656G</td>
<td>0.44</td>
<td>0.63</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>−607A</td>
<td>0.58</td>
<td>0.37</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>−607C</td>
<td>0.42</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>−137C</td>
<td>0.84</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>−137G</td>
<td>0.16</td>
<td>0.76</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Haplotype (−656−607−137)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCC</td>
<td>0.42</td>
<td>0.00</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>TAC</td>
<td>0.39</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>TAG</td>
<td>0.17</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>GCG</td>
<td>0.004</td>
<td>0.63</td>
<td></td>
</tr>
</tbody>
</table>

p values by chi-square test.
AA genotype at position –607, but not at –137, is associated with a protective effect against development of RA. However, in Caucasian patients with RA, the conclusions are inconsistent. In German and Scottish populations, both SNP at position –607 and –137 contribute to the genetic background of pathogenesis of RA. In contrast, the 2 polymorphisms were found to play no major role in RA susceptibility in a Spanish population. Although these data of various case–controlled studies are not absolutely conclusive and require confirmation, the overall impression indicates a functional importance for IL-18 promoter polymorphisms in the development of inflammatory diseases.

In our study, there were no differences in genetic frequencies of the investigated IL-18 promoter polymorphisms between KD patients and controls. However, the distribution of various genotypes and haplotypes of the polymorphic sites of the IL-18 promoter differed significantly between our Taiwanese controls and some Caucasian populations, suggesting that IL-18 promoter polymorphisms may be useful anthropologic genetic markers. Further studies of IL-18 promoter haplotypes have similar activities. Further studies of IL-18 promoter polymorphisms in or close to the IL-18 gene, or those located in other genes that are related to angiogenic and inflammatory processes.

Our results suggest a potential implication of IL-18 promoter polymorphisms in creating susceptibility to KD in Taiwanese, but the contribution of these polymorphisms to disease susceptibility or severity in other populations remains unclear. Further studies in patients with KD from different ethnic backgrounds are required to confirm a role for this gene within the complex genetics of KD.

REFERENCES