Objective. Kawasaki disease (KD) is a pediatric systemic vasculitis of unknown cause for which a genetic influence is supposed. The purpose of this study was to identify possible genetic variants in the major histocompatibility complex (MHC) region that are associated with KD and the development of coronary artery aneurysms (CAAs) in a Taiwanese population.

Methods. The 168 genetic variants covering the MHC locus were analyzed in an association study of a Taiwanese cohort of 93 KD patients and 680 unrelated healthy children matched for sex and age with the study patients.

Results. Eleven single-nucleotide polymorphisms (SNPs) were associated with the occurrence of KD. The SNP located at the 3'-untranslated region of HLA–E (rs2844724) was highly associated ($P < 1 \times 10^{-7}$). In addition, the frequency of the C allele was higher in KD patients without CAAs than in controls ($P < 0.001$) due to a significantly increased frequency of the CC and CT genotypes. Plasma levels of soluble HLA–E were significantly higher in KD patients than in controls regardless of the presence of CAAs. Furthermore, there was a trend toward higher plasma levels of soluble HLA–E in KD patients with the CT and TT genotypes of the HLA–E gene polymorphism.

Conclusion. Our results suggest that the HLA–E gene polymorphism may play a role in the pathogenesis of KD.

Kawasaki disease (KD) is an acute, self-limited, and systemic vasculitis that is one of the leading causes of acquired heart disease in children (1–3). The vascular inflammation may cause the development of aneurysms and cardiac complications. Patients with these cardiovascular complications are at increased risk of developing ischemic heart disease, which may lead to myocardial infarction and sudden death (4). Although KD is a mysterious disease of unknown etiology and pathogenesis, it is believed to be caused by infectious agents, host immune dysregulation, and genetic susceptibility (5–8). Moreover, KD is overrepresented in Asian children (1,9–13). The annual incidence of KD in Taiwan is estimated to be 66/100,000 children, the third highest in the world after Japan and Korea (3,14).

During the acute stage of KD, activation of vascular endothelial cells and increased serum levels of pro-inflammatory cytokines are involved in the occurrence of inflamed and injured vessels (15,16). The injured vascular tissues show subendothelial edema, vascular damage, gap formation, and fenestration of endothelial cells and contribute to the pathogenesis of this disorder (17,18). Human vascular endothelial cells process antigens and express class I and class II major histocompatibility complex (MHC) molecules and costimulatory
molecules on their surface for presenting antigenic peptides to T cells and then initiating an acquired immune response (19,20).

The roles of HLAs from the MHC region have been investigated in immune-mediated vascular diseases (21–32). However, HLAs that contribute to the pathogenesis of KD have been less well characterized. Genetic studies of HLA class I genes have demonstrated an association between the MICA gene and KD (30). The association between HLA class II genes and KD has also been investigated (27,28). However, no significant associations between either HLA–DRB1, DRB3, DQA1, DQB1, or DPB1 and KD have been demonstrated and none of them have proved clinically useful in terms of KD susceptibility. Thus, the MHC polymorphism data from case–control studies have not been conclusive.

In the present study, we searched for genes that influence susceptibility to KD in Taiwanese children. A total of 168 polymorphic, evenly spaced common variations (target density 1 single-nucleotide polymorphism [SNP] for every 20 kb) in the MHC region were evaluated in 93 children with KD and in 680 unrelated healthy individuals. We investigated whether the identified gene polymorphisms were associated with KD or with the occurrence of coronary artery aneurysms (CAAs) in a case–control study.

**PATIENTS AND METHODS**

**Study population.** From 1998 to 2005, 93 individuals who attended the Department of Pediatrics, China Medical University Hospital in Taichung, and who fulfilled the diagnostic criteria for KD were identified and enrolled in this study (33–37). Every patient underwent regular echocardiography examinations, beginning during the acute stage of KD, at 2 months and 6 months after disease onset, and once a year thereafter. A CAA was identified when either the right coronary artery or the left coronary artery showed a dilated diameter of ≥3 mm in children younger than 5 years or ≥4 mm in children older than 5 years (38).

The control group consisted of 680 healthy children randomly selected from the Han Chinese Cell and Genome Bank, in which 3,312 unrelated descendants of the Han Chinese were recruited based on their geographic distribution across Taiwan (39). Control subjects were matched for sex and age with the study patients. The estimated prevalence of KD is fewer than 1/1,000 children; therefore, it should be assumed that there were no KD cases in the control group.

This study was approved by the Human Studies Committee of China Medical University Hospital, and informed consent was obtained from either the participants or their parents.
SNP genotyping. A total of 201 SNPs from the dbSNP database at the National Center for Biotechnology Information were considered (40,41). The 201 SNPs were located in 4 Mb of the MHC region on chromosome 6p21.3; they included 9 classic HLA loci, 2 TAP genes, and 18 microsatellites (42). After excluding SNPs with a genotype call rate of 0.85, a total of 168 of the 201 SNPs remained, and these were used in our study. The mean intermarker spacing was 21.5 kb, with a median of 15.7 kb, and a standard deviation of 21.3 kb. A summary of the SNP information, including the rs number, position, corresponding gene, and allele frequency, is available upon request from the corresponding author.

Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Genomic DNA kit; Qiagen, Chatsworth, CA). SNPs were genotyped using high-throughput matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) mass spectrometry. Briefly, primers and probes were designed by using SpectroDesigner software (Sequenom, San Diego, CA). Multiplex polymerase chain reactions were performed, and unincorporated dNTPs were dephosphorylated with shrimp alkaline phosphatase (Hoffman-La Roche, Basel, Switzerland), followed by primer extension. The purified primer extension reaction product was spotted onto a 384-element silicon chip (SpectroChip; Sequenom) and analyzed in a Bruker Biflex III MALDI-TOF SpectroReader mass spectrometer (Sequenom). The resulting spectra were processed with SpectroTyper (Sequenom) software.

Analysis of haplotype blocks. Based on the Haploview software, we used the Lewontin D measure to estimate the intermarker coefficient of linkage disequilibrium in both controls and KD patients (43). The confidence interval for linkage disequilibrium was estimated using a resampling procedure and was used to construct the haplotype blocks (44).

Enzyme-linked immunosorbent assay (ELISA) for detection of soluble HLA–E. Concentrations of soluble HLA–E were quantified using an ELISA kit from USCN Life Science & Technology (Missouri City, TX; online at http://www.uscnlife.com/elisa/1257023105.html). ELISA was performed according to the manufacturer’s instructions. Briefly, plasma samples (100 μl) were applied directly to wells. After 2 hours of incubation, the plasma samples were removed. Detection Reagent A (100 μl) was then added and incubated for another 2 hours. After wash treatments, Detection Reagent B (100 μl) was applied and incubated for 1 hour. After the final wash treatments, soluble HLA–E was then detected with the substrate and stop solutions.
Statistical analysis. Categorical data were compared between groups using Fisher’s exact test, and continuous data (presented as the median and range) were compared with the use of 2-sample \( t \)-tests. Allelic association screening was performed using the Cochran-Armitage trend test for each SNP (45).

RESULTS

Association study of the MHC region. To identify KD susceptibility genes, a total of 168 SNPs within the MHC region (from 29,900,000 to 33,900,000 bp on chromosome 6) were genotyped in 93 Taiwanese patients with KD and in 680 healthy individuals from the general population of Taiwan who were of Han Chinese ethnic background for the SNP association study (Figure 1). Haplotype block profiles for the controls and the KD patients, as determined using Haploview software (43), are shown at the bottom of Figure 1. There were apparent differences in haplotype block structures.

As shown in Tables 1 and 2, the genotype distributions and allele frequencies of 11 SNPs in the MHC region were statistically different in KD patients as compared with controls (\( P < 0.05 \)). These SNPs were rs1611750, rs410909, rs2844724, rs2517523, rs1064190, rs2844476, rs2269425, rs1555115, rs2395161, rs1383267, and rs2076311. Among these 11 SNPs, the SNP located at the 3'-untranslated region (3'-UTR) of HLA–E (rs2844724) was found to be highly significantly associated with KD (\( P = 3.84 \times 10^{-7} \)) (Tables 1 and 2). A statistically significant difference in genotype frequency distribution was found in the KD patients as compared with the controls (\( P = 4.26 \times 10^{-5} \)) (Table 1). The frequencies of the CC and the CT genotypes were significantly higher in KD patients than in controls, with an odds ratio (OR) of 7.11 (95% confidence interval [95% CI] 2.90–17.38) for the CC genotype and an OR of 6.98 (95% CI 3.28–14.88) for the CT genotype. The C allele frequency was significantly higher in KD patients as compared with controls (OR 2.31 [95% CI 1.66–3.21], \( P = 3.84 \times 10^{-7} \)) (Table 2).

### Table 2. Allele frequencies of significant SNPs in the MHC region in Taiwanese KD patients and controls*

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Gene</th>
<th>No. (%) of controls</th>
<th>No. (%) of KD patients</th>
<th>( P )</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1611750</td>
<td>29922757</td>
<td>–</td>
<td>G</td>
<td>115 (8.5)</td>
<td>6 (3.3)</td>
<td>0.015</td>
</tr>
<tr>
<td>rs410909</td>
<td>30057147</td>
<td>HCG9</td>
<td>T</td>
<td>1233 (91.5)</td>
<td>174 (96.7)</td>
<td>0.024</td>
</tr>
<tr>
<td>rs2844724</td>
<td>30577169</td>
<td>HLA–E</td>
<td>C</td>
<td>1127 (83.5)</td>
<td>162 (90.0)</td>
<td>3.84 \times 10^{-7}</td>
</tr>
<tr>
<td>rs2517523</td>
<td>31134413</td>
<td>–</td>
<td>T</td>
<td>875 (66.0)</td>
<td>74 (45.7)</td>
<td>0.097</td>
</tr>
<tr>
<td>rs1064190</td>
<td>31183094</td>
<td>C6orf15;CDSN;PSORS1C1</td>
<td>T</td>
<td>594 (45.1)</td>
<td>100 (53.8)</td>
<td>0.026</td>
</tr>
<tr>
<td>rs2844476</td>
<td>31689875</td>
<td>BAT2;AIF1</td>
<td>T</td>
<td>724 (54.9)</td>
<td>86 (46.2)</td>
<td>0.0237</td>
</tr>
<tr>
<td>rs2269425</td>
<td>32231617</td>
<td>C6orf31;PPT2</td>
<td>G</td>
<td>1303 (97.4)</td>
<td>179 (96.2)</td>
<td>0.0306</td>
</tr>
<tr>
<td>rs1555115</td>
<td>32462498</td>
<td>BTNL2</td>
<td>G</td>
<td>25 (2.6)</td>
<td>7 (3.8)</td>
<td>0.37</td>
</tr>
<tr>
<td>rs2395161</td>
<td>32495730</td>
<td>–</td>
<td>C</td>
<td>46 (3.4)</td>
<td>8 (4.3)</td>
<td>0.55</td>
</tr>
<tr>
<td>rs1383267</td>
<td>32941624</td>
<td>PSMB9</td>
<td>T</td>
<td>512 (37.8)</td>
<td>56 (30.1)</td>
<td>0.0425</td>
</tr>
<tr>
<td>rs2076311</td>
<td>33253347</td>
<td>COL11A2</td>
<td>A</td>
<td>295 (22.4)</td>
<td>54 (29.0)</td>
<td>0.044</td>
</tr>
</tbody>
</table>

* Allele frequencies were determined by chi-square test using 2 \( \times \) 2 contingency tables. \( P \) values less than 0.05 were considered significant. SNPs = single-nucleotide polymorphisms; MHC = major histocompatibility complex; KD = Kawasaki disease; OR = odds ratio; 95% CI = 95% confidence interval.
out CAA than in controls (OR 18.95 [95% CI 5.35–67.14] for the CC genotype and OR 15.41 [95% CI 4.75–50.03] for the CT genotype). The allele and genotype frequencies were not statistically different in KD patients with CAA as compared with the controls.

Plasma levels of soluble HLA–E. Plasma concentrations of soluble HLA–E were measured by ELISA in 96 patients with KD and in 93 healthy controls (Figure 2A). Levels of soluble HLA–E in plasma samples from KD patients were significantly higher than those in plasma samples from healthy controls (mean ± SD 209.9 ± 85.19 ng/ml versus 63.63 ± 13.25 ng/ml; P < 0.0001). Plasma levels of soluble HLA–E were also analyzed in KD patients in relation to CAA formation because CAA have been predicted to have possible functional correlations (Figure 2B). No significant difference between KD patients with CAA and those without CAA was observed (107.1 ± 66.3 ng/ml versus 247.1 ± 95.26 ng/ml; P = 0.412). Furthermore, plasma levels of soluble HLA–E in both groups of KD patients were significantly higher than those in the healthy controls.

We also analyzed plasma levels of soluble HLA–E in relation to genotypes (Figure 2C). KD patients with the CT or TT genotype had significantly higher plasma levels of soluble HLA–E than the controls (227.2 ± 79.45 ng/ml in KD patients with the CT genotype [P < 0.001] and 227.2 ± 133.8 ng/ml in KD patients with the TT genotype [P < 0.005]). No significant difference between KD patients with the CC genotype and the control subjects was observed (P = 0.131). The number of KD patients with CAA was insufficient to compare plasma levels of soluble HLA–E with the genotype data.

**Table 3.** Association of the HLA–E gene polymorphism in KD patients according to the presence or absence of CAA*

<table>
<thead>
<tr>
<th>Gene polymorphism</th>
<th>No. (%) of controls</th>
<th>No. (%)</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>No. (%)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA–E (rs2844724, at 3’-UTR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>451 (34.0)</td>
<td>10 (33.3)</td>
<td>0.938</td>
<td>0.97 (0.45–2.09)</td>
<td>78 (59.1)</td>
<td>&lt;0.001</td>
<td>2.80 (1.95–4.04)</td>
</tr>
<tr>
<td>T</td>
<td>875 (66.0)</td>
<td>20 (66.7)</td>
<td>1</td>
<td></td>
<td>54 (40.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>76 (11.5)</td>
<td>0 (0.0)</td>
<td>–</td>
<td></td>
<td>15 (22.7)</td>
<td>18.95</td>
<td>(5.35–67.14)</td>
</tr>
<tr>
<td>CT</td>
<td>299 (45.1)</td>
<td>10 (66.7)</td>
<td>0.168</td>
<td>1.93 (0.65–5.70)</td>
<td>48 (72.7)</td>
<td>&lt;0.001</td>
<td>15.41 (4.75–50.03)</td>
</tr>
<tr>
<td>TT</td>
<td>288 (43.4)</td>
<td>5 (33.3)</td>
<td>1</td>
<td></td>
<td>3 (4.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Allele frequencies were determined by chi-square test using 2 × 2 contingency tables. Genotype frequencies were determined by chi-square test using 2 × 3 contingency tables. P values less than 0.05 were considered significant. KD = Kawasaki disease; CAA = coronary artery aneurysm; OR = odds ratio; 95% CI = 95% confidence interval; 3’-UTR = 3’-untranslated region.
In this study, we used a mapping strategy focusing on the MHC region and identified a SNP that contributes to KD susceptibility in Taiwanese children of Han Chinese ethnic background. We observed a significant association between the HLA-E gene polymorphism and the occurrence of cardiac artery aneurysm in KD patients. We further showed that plasma levels of soluble HLA-E were significantly higher in patients with KD and that there was a trend toward higher plasma levels of soluble HLA-E in both CAA subgroups of KD patients than in the healthy controls. Furthermore, higher plasma levels of soluble HLA-E in KD patients with CT and TT genotypes of the HLA-E gene polymorphism were also noted. Our results suggest that polymorphism of the HLA-E gene may play a role in the pathogenesis of KD.

Our genetic association study showed that the frequencies of alleles and genotypes with 1 or 2 copies of the C allele were significantly higher in KD patients than in controls. Furthermore, this polymorphism was associated with KD patients without CAA. These results suggest that the HLA-E gene polymorphism is involved in disease susceptibility and progression. Individuals with KD who have 1 or 2 copies of the C allele tend not to develop CAA. Therefore, it is logical to assume that the CC or CT genotype of the 3’-UTR of the HLA-E gene polymorphism may affect serum/plasma levels of soluble HLA-E or the development of CAA in KD patients, although the influence of this SNP on the production of soluble HLA-E by vascular endothelial cells remains unknown. A possible explanation for the influence of the 3’-UTR may be because specific sequences in the 3’-UTR of RNA, together with stabilizing and destabilizing proteins, determine the messenger RNA stability and, consequently, the level of expression of proteins (46–48).

Our studies showed that regardless of the presence of CAA, KD patients had significantly higher levels of soluble HLA-E than did healthy controls. Furthermore, KD patients with the CT and TT genotypes of this gene polymorphism appeared to have higher soluble HLA-E levels. HLA-E is a known ligand of CD94/NKG2-C, which are expressed on natural killer cells, a subset of T cells, and vascular endothelial cells (49–51). Recent studies have shown that the expression of soluble HLA-E may have important implications in the pathogenesis of immune-mediated vascular diseases (51). It is also believed that HLA-E has regulatory functions in both the innate and adaptive immune responses. KD is a multisystemic disorder with a possible underlying pathology of immune-mediated vasculitis (1,52). The vascular endothelium is a functional barrier between the vessel wall and the bloodstream, and endothelial cell damage or vascular injury leads to the expression and release of HLA-E molecules (51). Taken together, these data suggest that HLA-E molecules may be involved in the pathogenesis of KD.

In conclusion, we have shown that susceptibility to the development of KD is associated with genetic predisposition in Taiwanese children of Han Chinese ethnic background. Genetic polymorphism in the MHC region, particularly the HLA-E gene, is associated with susceptibility to KD.

**REFERENCES**