Association between 17β-HSD8 polymorphisms and Kawasaki disease among Han Chinese children in Taiwan

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ABSTRACT: Kawasaki disease (KD) is considered infectious, with immunologic expressions caused by genetic susceptibility of individuals. The 17β-hydroxysteroid dehydrogenase type 8 (17β-HSD8) enzymes are involved in the biosynthesis of oestrogens and androgens and regulate immune responses through hormonal modulation in mammals. To clarify the relationship between 17β-HSD8 gene single nucleotide polymorphisms (SNPs) and the pathogenesis of KD, we investigated association between 17β-HSD8 SNPs (rs421446, rs213213) and KD in Taiwanese children. Genotype analysis involved 93 KD patients and 680 unrelated healthy children. Our findings indicated the frequency of A allele in polymorphisms rs421446 was markedly higher among the patient group (43.3%) than in the control group (34.0%; \( p = 0.032 \)). Children with the A allele at rs421446 SNP may show higher risk of developing KD, particularly those with AA homozygous genotype. From comparison of haplotype frequencies between case and control, children with AC haplotype appeared more “at-risk” for KD progression (\( p = 0.022 \)). Our results suggest that rs421446 polymorphism and the haplotypes in 17β-HSD8 gene are associated with the risk of KD in Taiwanese children.

KEYWORDS: coronary artery lesions

INTRODUCTION

Kawasaki disease (KD) is an acute febrile vasculitic syndrome of early childhood that presents with fever, rash, conjunctival injection, cervical lymphadenitis, inflammation of the lips and oral cavity, and erythema and oedema of the hands and feet\textsuperscript{1}. The first cases were identified among Japanese Children in 1967\textsuperscript{2}. Cardiac sequelae, such as coronary artery lesions (CAL), are one of the most important aspects of this disease\textsuperscript{3}. The causes of the disease are unknown, but are generally believed to be an infectious agent, host immune dysregulation, and genetic susceptibility\textsuperscript{4,5}. Moreover, KD is overrepresented in Asian children, with gender differences observed (boy:girl ratio of about 1.5:1)\textsuperscript{6,7}. Annual incidence of KD in Taiwan is estimated to be 69/100,000 children, the third highest in the world after Japan and Korea\textsuperscript{8,9}.

The immune response is characterized by the accumulation and expansion of T-helper 1 (Th1) lymphocytes and increased amounts of several proinflammatory cytokines, such as interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) and sex hormones\textsuperscript{10–13}. Sex hormonal action on the immune system is thought to account for the gender differences in immune capability, dispelling the notion that sex steroid hormones exclusively affect sex related endocrine functions\textsuperscript{14}. Among vertebrates, the levels of sex steroids within tissues are regulated by a variety of steroidalogenic enzymes. The 17β-hydroxysteroid dehydrogenases (17β-HSDs) enzymes catalyse the oxidoreduction of hydroxyl/keto groups of androgens and oestrogens\textsuperscript{15} and are involved in the biosynthesis of oestrogens and androgens and modulation of their hormone action in steriodogenic as well as in peripheral tissues in mammals\textsuperscript{16}. Multiple types of 17β-HSDs (named types 1–12) have been cloned and have been shown to be expressed in several human and animal tissues\textsuperscript{15,17,18}. The type 8 17β-HSD has been recently identified as the product of the Ke 6 gene, which is found in the human leukocyte antigen region\textsuperscript{19}.  

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In this study, we hypothesized that $17\beta$-HSD8 genetic variants in the 3’UTR confer KD susceptibility. We examined and compared $17\beta$-HSD8 genotype distribution in a group of Taiwanese KD patients and a non-KD control group. An attempt was also made to clarify the association between $17\beta$-HSD8 and KD severity.

MATERIALS AND METHODS

Study population

We enrolled 93 patients from the Department of Pediatrics at China Medical University Hospital from 1998 to 2005. All met the criteria proposed by the Japanese Kawasaki Disease Research Committee. Patients were treated with intravenous immunoglobulin (IVIG, 2 g/kg infused over 8–12 h) and oral aspirin (80–100 mg/kg/day). Echocardiograms were obtained by the paediatric cardiologist before or within two weeks of IVIG administration. CAL were diagnosed from echocardiograms, using criteria proposed by the Japanese Kawasaki Disease Research Committee: coronary arteries were classified as abnormal if the internal lumen diameter was $>3$ mm in a child younger than 5 years or $>4$ mm in a child older than 5 years, if the internal diameter of a segment measured $\geq 1.5$ times that of an adjacent segment, or if the coronary lumen was clearly irregular. We also studied 680 gender-age-matched unrelated healthy children with no prior history of KD to serve as a control group. All blood samples were drawn before IVIG therapy in KD patient groups and were tested in parallel with control samples. The ethics committee of China Medical University Hospital Institutional Review Board approved the study, with written informed consent from parents of all subjects (DMR97-IRB-246).

SNP selection

$17\beta$-HSD8 SNPs genotypes information was downloaded in December 2008 from the HapMap CHB + JPT population. HapMap genotypes were analysed within HAPLOVIEW and Tag SNPs were selected using the Tagger function by applying the following additional criteria: (1) a threshold minor allele frequency in the HapMap CHB + JPT population of 0.05 for “tag SNPs”; and (2) probe/primers that pass the qualification as recommended by the manufacturer (Applied Biosystems), to ensure a high genotyping success rate. Two polymorphisms met the criteria and were selected, including SNP rs421446 (A/G) and rs213213 (C/T) in 3’UTR of $17\beta$-HSD8 gene (Fig. 1).

Genomic DNA extraction and genotyping of $17\beta$-HSD8 polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Genomic DNA kit; Qiagen, Valencia, CA, USA). Genotypes of SNPs rs421446 and rs213213 at chromosome positions 6:33,174,783 and 6:33,183,730 in 3’UTR of $17\beta$-HSD8 (Fig. 1) gene were identified by high-throughput matrix-assisted laser desorption ionization time-of-flight mass spectrometry. A detailed description of the procedures is presented by Lin et al.

Statistical analysis

Hardy-Weinberg equilibrium was tested for each marker using a $\chi^2$-test. Chi-square test or Fisher’s exact tests determined statistically significant differences in allele/genotype frequencies between case and control groups. Allelic frequencies were expressed as percentage of aggregate alleles. Haplotype combination at rs421446 and rs213213 in $17\beta$-HSD8 gene was estimated using HAPLOVIEW version 4.1 based on an accelerated EM algorithm. Intergroup differences in haplotype frequency were assessed by $\chi^2$-test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were obtained by logistic regression to define association between $17\beta$-HSD8 alleles/genotypes/haplotypes and KD susceptibility. All data were analysed with SPSS Version 15.0 software (SPSS Inc., Chicago, IL, USA), $p$ value < 0.05 was considered statistically significant.

RESULTS

Table 1 shows genotypic and allelic frequencies of rs421446 and rs213213, A allele frequencies at rs421446 polymorphism in KD patients and controls are 43.3% (58/134) and 34% (441/1296), respectively. G allele frequencies in KD patients and controls are 56.7% (76/134) and 66% (855/1296), respectively. When we compared frequencies between case and control groups, we found the A allele frequency in rs421446 polymorphism significantly higher in the...
Table 1 Genotypic and allelic frequencies of 17β-HSD8 gene genetic polymorphism in KD patients and controls.

<table>
<thead>
<tr>
<th>dbSNP ID</th>
<th>Patient with KD (n = 93)</th>
<th>Control (n = 680)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs421446</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>n = 67 (%) n = 648 (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>24 (35.8) 15 (91.9)</td>
<td>528 (78.5)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>10 (14.9) 251 (38.7)</td>
<td>1256 (18.5)</td>
<td>0.93</td>
<td>0.79</td>
</tr>
<tr>
<td>GG</td>
<td>33 (49.3) 302 (46.6)</td>
<td>655 (95.3)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>GG + GA</td>
<td>43 (64.2) 553 (85.3)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele frequency</td>
<td>A 58 (43.3) 441 (34.0)</td>
<td>1.47</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G 76 (56.7) 855 (66.0)</td>
<td>(1.03–2.13)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

rs213213

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patient with KD (n = 93)</th>
<th>Control (n = 679)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>79 (84.9) 582 (85.7)</td>
<td>1.06</td>
<td>0.84b</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>13 (14.0) 92 (13.6)</td>
<td>(0.58–1.95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>1 (1.1) 5 (0.7)</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT + TC</td>
<td>14 (15.1) 97 (14.3)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele frequency</td>
<td>C 171 (91.9) 1256 (92.5)</td>
<td>0.93</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T 15 (8.1) 102 (7.5)</td>
<td>(0.53–1.64)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio.

Table 2 Distribution of 17β-HSD8 gene haplotype frequencies in patients with KD and controls.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Patient with KD (n = 67)</th>
<th>Control (n = 648)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC</td>
<td>56.3% 65.0% 0.70 (0.51–0.95)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>35.6% 27.5% 1.46 (1.05–2.01)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>6.5% 6.6% 0.99 (0.53–1.83)</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio.

Table 3 Distribution of 17β-HSD8 haplotype frequencies in KD patients with/without coronary artery lesions (CAL).

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Patient with KD (n = 20)</th>
<th>CAL(+) CAL(−)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC</td>
<td>65.0% 51.5% 1.75 (0.93–3.31)</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>30.0% 39.0% 0.67 (0.35–1.29)</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>0.0% 9.2% 0.06 (0.00–19.04)</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio.

Table 4 Distribution of cytokines levels in KD patients with/without CAL.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Patient with KD (n = 67)</th>
<th>Control (n = 648)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate oxaloacetate transaminase (GOT)</td>
<td>1.05–2.01</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate pyruvate transaminase (GPT)</td>
<td>1.85–5.60</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio.

DISCUSSION

Currently, KD is viewed as an infectious disease with immunologic expressions caused by genetic susceptibility of individuals. Polymorphic gene sequences of cytokines definitely involved in the pathogenesis of KD are potential markers of disease susceptibility: e.g., tumour necrosis factor-α, Interleukin-1, 10, and...
Inflammation markers may facilitate predicting the risk of these events. For example, C-reactive protein (CRP) level evaluation may provide a useful method to assess risk of cardiovascular diseases in apparently healthy persons. In our study, high level of CRP (>5 mg/dl) was observed both in KD patients with/without AC haplotype. We also found fever lasted longer in KD patients with AC haplotype compared with non AC haplotype, although the difference was not significant (Table 3). CAL is likely to occur in about 25% of untreated KD patients, and death may result from coronary artery aneurysm rupture or thrombosis, myocardial infarction, or myocarditis. In this study, we observed 29.9% (20/67) of KD patients with CAL and we analysed the relationship between rs421446 and rs213213 polymorphisms and CAL development in the KD patients. Our data showed that compared with the KD CAL(−) patients, the KD patients with GC haplotype seem to have higher frequency of CAL (65.0%), though the difference was not significant (Table 3).

Transaminases levels are indicators of liver function, which is impaired in the acute stage of KD. Accordingly, GOT and GPT levels in KD patients with AC haplotype were statistically significant higher than patients with non AC haplotype (p = 0.022 and 0.03, respectively) (Table 4). The AC haplotype appeared to be a significant "at-risk" haplotype for KD progression maybe due to the retardations observed in clinical parameters of GOT and GPT compared to non AC haplotype.

Our data provide a new information of rs421446 and rs213213 SNPs in disease progression of KD patients which may have important implications in the development of strategies for the prevention, diagnosis and treatment of KD. In conclusion, our study showed variant genotype distribution of 17β-HSD8 gene between controls and KD patients. And data also suggested that 17β-HSD8 gene (rs421446 and rs213213) SNPs may be the underlying cause of KD. Polymorphism revealed by this study warrants further investigation.

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### REFERENCES


