**ITPKC gene SNP rs28493229 and Kawasaki disease in Taiwanese children**

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Kawasaki disease (KD) is a systemic vasculitis caused by unknown infectious agents, host immune dysregulation and genetic susceptibility in children. Coronary artery lesions (CALs) complicate 15–25% of cases of untreated KD. The aim of this study was to investigate if the single-nucleotide polymorphism (SNP) rs28493229 of the *ITPKC* gene is associated with susceptibility to KD or with CALs in Taiwanese children. A total of 385 unrelated Taiwanese children (222 boys and 163 girls) with KD were included, 140 of whom had CALs. Mean age at diagnosis was 1.9 ± 1.7 (0.1–10.2) years. Rs28493229 was genotyped in children with KD and 1158 ethnically matched healthy controls using the TaqMan Allelic Discrimination Assay. In 184 families with KD, both biological parents were available, constituting 184 trios with their children. They were assessed in a family-based study by means of a transmission/disequilibrium test (TDT). No significant differences in genotype (*P* = 0.29 and *P* = 0.29, respectively), allele (*P* = 0.14 and *P* = 0.22, respectively) and carrier (*P* = 0.22 and *P* = 0.25, respectively) frequencies of the SNP were found between healthy controls and children with KD or those with CALs. TDT in the 184 family trios and in 69 trios where the child had CALs did not reveal significant overtransmission of the C allele. In conclusion, we did not find a statistically significant association between the *ITPKC* gene SNP rs28493229 and KD or CALs in Taiwanese children.

**INTRODUCTION**

Kawasaki disease (KD) is an acute febrile illness with considerable geographic and racial variability that predominantly affects infants and young children (1–3). The major clinical features are fever lasting longer than 5 days, bilateral non-purulent conjunctivitis, enlarged cervical lymph nodes, erythematous indurations of hands and feet, inflammation of lips, oropharynx and tongue, polymorphous skin rash and perianal and periungual desquamation (4). Vasculitis is the pathological lesion underlying most of the clinical findings. In 15–25% of untreated cases, coronary artery lesions (CALs) develop (4,5). KD is the number one cause of acquired heart disease in children in the developed world (6,7). It is thought to be an infectious disease, but no pathogen has as yet been identified. Furthermore, the mechanisms involved in the development in some patients of CALs remain unclear. The high incidence of KD in Asians (3,8), coupled with a disproportionally high rate among Asian Americans (9), has led to a search for genetic markers associated with susceptibility to this disease. It is believed that T-cell activation and cytokines play an important role in the pathogenesis of vascular endothelial cell injury by eliciting proinflammatory reactions at the onset of KD (10–13). For this reason, genetic variations in immune modulation, inflammation and cardiovascular disease genes have been investigated, looking for an
association with KD in general or CALs in particular (14–16). Inositol 1,4,5-trisphosphate (IP3) is a second messenger which transduces signals from cell surface receptors in T cells (17). If the T-cell receptor receives a stimulus, phospholipase Cγ is activated, resulting in phospholipid hydrolysis that yields diacylglycerol and IP3. IP3 then binds to IP3 receptors on the endoplasmic reticulum, inducing Ca\textsuperscript{2+} release into the cytoplasm (18). Inositol 1,4,5-trisphosphate 3-kinase (ITPK) phosphorylates IP3 and serves as a negative regulator of the Ca\textsuperscript{2+}/nuclear factor of activated T-cells signaling pathway (19,20). The C allele of the functional single-nucleotide polymorphism (SNP) rs28493229 in the \textit{ITPKC} gene located on chromosome 19q13.2 is associated with susceptibility to KD and CALs in both Japanese and US children (21). The aim of our study was to determine whether this SNP is associated with susceptibility to KD or CALs in Taiwanese children.

RESULTS

The genotype frequencies of the controls and patients were in Hardy–Weinberg equilibrium (Table 1). There were no significant differences in the genotype, allele or carrier frequencies of rs28493229 between controls and children with KD or between controls and children with CALs (Table 2). Neither were there any differences when stratified by gender (data not shown). No significant differences in genotype ($P = 0.11$), allele ($P = 0.19$) and carrier ($P = 0.35$) frequencies of the SNP were found between 385 children with KD and 99 unaffected siblings. These frequencies, however, did differ significantly between our Taiwanese controls and Japanese historical controls (20). TDT for the families of children with KD (184 trios) and those with CALs (69 trios) did not reveal significant overtransmission of the C allele (Table 3).

**DISCUSSION**

We investigated the \textit{ITPKC} gene SNP rs28493229 of in a Taiwanese population and studied its correlation with KD. There was no significant difference in genotype, allele or carrier frequencies of the polymorphism between controls and children with KD or with CALs. Study of family trios also showed no significant overtransmission of any allele in children with KD or CALs.

Previous studies have indicated that genetic variants in the major histocompatibility complex and inflammatory molecules are important in KD in terms of modulating susceptibility to the disease, outcome, progression and complications (14–16,22–24). In the acute phase of the disease, serum cytokines such as interleukin 1 (IL1), IL2, IL4, IL6, IL10 and tumor necrosis factor-α (TNF-α)—substances that regulate the interaction of T cells, B cells and macrophages—are abnormally increased (12,13,25,26). The IP3-induced release of Ca\textsuperscript{2+} (17,18,28) allows the ion to bind to calmodulin, which in turn activates the calmodulin-dependent phosphatase calcineurin in a process that enhances T-cell activation (20,27,28). The C allele of rs28493229, located in intron 1 of the \textit{ITPKC} gene, reduces the gene expression by 30% by altering splicing efficiency (21). It has been suggested that this reduction in ITPKC activity allows for enhanced T-cell activation that is presumably triggered by an unknown infectious agent, with KD being the end result. It is conceivable that the SNP also alters the function of other immune (e.g. macrophages, B cells and neutrophils) and non-immune cells (e.g. endothelial cells and myocardium) that may be involved in pathogenesis of KD.

The incidence of KD in Taiwanese children younger than 5 years is 69/100 000, one of the highest in the world (29). Despite the association of rs28493229 with KD and particularly with the risk for CALs in Japanese and American children (21), we did not find a similar association in our

**Table 1. Polymorphism of rs28493229 of the ITPKC gene in children with KD and controls**

<table>
<thead>
<tr>
<th>Genotype, $n = 385$ (%)</th>
<th>Taiwanese controls, $n=1158$ (%)</th>
<th>Japanese controls, $n=1034$ (%)</th>
<th>OR $^b$</th>
<th>95% CI</th>
<th>$\chi^2$</th>
<th>$P$-value</th>
<th>$P_{\text{value}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>1 (0.3)</td>
<td>3 (0.3)</td>
<td>29 (2.8)</td>
<td>1.00</td>
<td>0.10–9.67</td>
<td>0.00</td>
<td>0.99</td>
</tr>
<tr>
<td>C/G</td>
<td>61 (15.8)</td>
<td>147 (12.7)</td>
<td>249 (24.1)</td>
<td>1.29</td>
<td>0.94–1.79</td>
<td>2.46</td>
<td>0.12</td>
</tr>
<tr>
<td>G/G</td>
<td>323 (83.9)</td>
<td>1008 (87.0)</td>
<td>756 (73.1)</td>
<td>0.78</td>
<td>0.56–1.07</td>
<td>2.42</td>
<td>0.12</td>
</tr>
<tr>
<td>Allele $^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>63 (8.2)</td>
<td>153 (6.6)</td>
<td>307 (14.8)</td>
<td>1.26</td>
<td>0.93–1.71</td>
<td>2.20</td>
<td>0.14</td>
</tr>
<tr>
<td>G</td>
<td>707 (91.8)</td>
<td>2163 (93.4)</td>
<td>1761 (85.2)</td>
<td>0.79</td>
<td>0.59–1.08</td>
<td>2.20</td>
<td>0.14</td>
</tr>
<tr>
<td>Carrier $^d$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>61 (16.1)</td>
<td>150 (13.0)</td>
<td>278 (26.9)</td>
<td>1.22</td>
<td>0.89–1.68</td>
<td>1.53</td>
<td>0.22</td>
</tr>
<tr>
<td>G</td>
<td>384 (99.7)</td>
<td>1155 (99.7)</td>
<td>1005 (97.2)</td>
<td>0.82</td>
<td>0.59–1.12</td>
<td>1.53</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Data reported as $n$ (%). KD, Kawasaki disease; ns, not significant. Hardy–Weinberg test for patients with KD: $P = 0.56$; for Taiwanese controls: $P = 0.62$; for Japanese controls: $P = 0.54$.

$^a$Genotype: KD versus controls: $\chi^2 = 2.46, P = 0.29$ (3 × 2 contingency table). Controls: Taiwanese versus Japanese. $\chi^2 = 73.29, P = 1.21E−16$ (3 × 2 contingency table).

$^b$Allele: KD versus controls: $\chi^2 = 2.20, P = 0.14$; Fisher’s exact test, $P = 0.16$ (2 × 2 contingency table). Controls: Taiwanese versus Japanese. $\chi^2 = 75.34, P = 3.97E−18$ (2 × 2 contingency table).

$^c$Carrier: KD versus controls: $\chi^2 = 1.53, P = 0.22$; Fisher’s exact test, $P = 0.24$ (2 × 2 contingency table). Controls: Taiwanese versus Japanese. $\chi^2 = 46.06, P = 1.15E−11$ (2 × 2 contingency table).

$^d$Japanese data from the study done by Onouchi et al. (21).

$^e$ORs in the table are KD versus Taiwanese controls.
In conclusion, this study did not provide evidence to support an association between the ITPKC gene SNP rs28493229 and KD or CALs in Taiwanese children. However, this result does not exclude a possibility of a contribution to the pathogenesis of KD from other polymorphisms in the ITPKC gene. A more extensive array of SNPs may need to be assessed to find such an association, particularly when subjects with different ethnic background are tested.

### MATERIALS AND METHODS

#### Study population

The study sample included 385 unrelated Taiwanese children (222 boys and 163 girls) with KD. Their mean age at diagnosis was 1.9 ± 1.7 (0.1–10.2) years. Among them 140 children (88 boys and 52 girls) were with CALs. The diagnosis of KD was based on the diagnostic criteria of the American Heart Association (37). Oral aspirin (80 mg kg⁻¹ day⁻¹) and intravenous gamma globulin (2 gm kg⁻¹ infused over 8–12 h) were given as soon as the diagnosis was made. All children were examined with two-dimensional echocardiography during the febrile stage and again after hospital discharge. The echocardiograms were blindly interpreted by a pediatric cardiologist (M.R. Chen). CALs were defined as coronary arteries with a diameter (inner border to inner border) greater than or equal to 3 mm in children less than 5 years old or >4 mm in children 5 older or if the diameter was >1.5 times that of the adjacent vessel (38,39).

The control group consisted of 1158 (565 males and 593 females) ethnically matched unrelated healthy Taiwanese subjects with no history of KD, autoimmune or allergic diseases. Ninety-nine unaffected siblings of the patients were also available for comparison. Both biological parents were available in 184 families of the 385 study subjects, constituting 184 trios with their affected children. Similarly, 69 trios in families with CALs were also available. Trios were assessed in a family-based study by means of a transmission/disequilibrium test (TDT). The Institutional Review Board approved...
the study and all participants gave the written informed consent.

SNP association analysis

Genomic DNA was extracted from whole-blood samples using standard methods. The *ITPKC* gene SNP rs28493229 was determined using TaqMan Allelic Discrimination Assay (Applied Biosystems, Foster City, CA, USA). Polymerase chain reaction (PCR) was carried out by using a 96-well GeneAmp PCR System 9700 (Applied Biosystems) with solutions consisting of 10 ng of genomic DNA, 5 µl of TaqMan Universal PCR Master Mix, 0.5 µl of 20 × Assay Mix and double-distilled H₂O up to a final volume of 10 µl. Thermal cycle conditions were as follows: denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s, and annealing and extension at 60°C for 1 min. After PCR, the TaqMan assay plates were transferred to an ABI PRISM 7000 Sequence Detection System (Applied Biosystems) where the endpoint fluorescence intensity in each well was read and analyzed using SDS v1.1 software (Applied Biosystems) to determine each sample’s genotype of.

Statistical analysis

Genotype, allele and carrier frequencies of SNP rs28493229 were determined by direct counting. Hardy–Weinberg equilibrium was assessed using a χ² goodness-of-fit test (40). Statistical differences in the genotype, allele and carrier distributions between groups were assessed using a χ² test with Yates correction where appropriate. Odds ratios and 95% confidence intervals were also calculated (41). Corrected P (Pc) values were derived using the Bonferroni correction for multiple comparisons where appropriate. TDT was performed using Haplovie 4.1 (42). Two-tailed Pc values of <0.05 were considered statistically significant.

Statistical power

Statistical power to detect the effect of rs28493229 on susceptibility to KD was calculated using the Genetic Power Calculator (35). We designed the study to have 98.9% power in the case–control portion and 74.7% in the TDT portion at a 5% significance level to determine a relative risk of 1.89 and statistical differences in the genotype, allele and carrier frequencies of SNP rs28493229 were assessed using a χ² goodness-of-fit test (40). Statistical differences in the genotype, allele and carrier distributions between groups were assessed using a χ² test with Yates correction where appropriate. Odds ratios and 95% confidence intervals were also calculated (41). Corrected P (Pc) values were derived using the Bonferroni correction for multiple comparisons where appropriate. TDT was performed using Haplovie 4.1 (42). Two-tailed Pc values of <0.05 were considered statistically significant.

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Conflict of Interest statement. None declared.

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