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Single nucleotide polymorphism rs2229634 in the ITPR3 gene is associated with the risk of developing coronary artery aneurysm in children with Kawasaki disease

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Single nucleotide polymorphism rs2229634 in the *ITPR3* gene is associated with the risk of developing coronary artery aneurysm in children with Kawasaki disease

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Running title: *ITPR3* polymorphism in Kawasaki disease

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Summary

Kawasaki disease (KD) is the most common form of pediatric vasculitis. Though its etiology is unknown, researchers have suggested that it is related to genetics. The inositol 1,4,5-triphosphate receptor type 3 (*ITPR3*) gene has a strong association with the development of type 1 diabetes and, plays a critical role in the development of autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and Graves' disease. The aim of study is to examine the association of *ITPR3* polymorphisms with KD risk in Taiwanese children. This study evaluates the single nucleotide polymorphisms (SNP) rs2229634 in the *ITPR3* gene with KD in a case-control study involving 93 KD patients and 680 healthy, gender- and age-matched controls. The frequency of the rs2229634 T/T genotype was significantly higher in KD patients with coronary artery aneurysm (CAA) than in patients without CAA [odds ratio (OR) = 2.56, 95% confidence interval (95% CI) = 1.35–4.88, p = 0.004]. In addition, KD patients with the T/T genotype elevated mean serum levels of C-reactive protein compared with patients with the C/C or C/T genotype (12.2 mg/dL vs. 8.5 mg/dL, p = 0.036). In conclusion, the results of this study suggest that the rs2229634 SNP in the *ITPR3* gene is associated with the risk of CAA formation in Taiwanese KD patients.

Keywords: autoimmune, coronary artery aneurysm, *ITPR3*, Kawasaki disease

polymorphism

Introduction

Kawasaki disease (KD) is an acute, self-limited, immune-mediated form of vasculitis that primarily affects infants and young children. Inflammation caused by the disease can lead to coronary artery aneurysm (CAA) and myocardial infarction making KD the most common cause of acquired heart disease in children in developed countries (Taubert and Shulman, 1999; Onouchi et al., 2008). The etiology of KD remains unknown, but is presumably influenced by the interaction between genetic and environmental factors and, possibly, by infection (Rowley et al., 2008). Researchers suspect genetic influences contribute to the development of KD based on the following observations. Children of Asian ethnicity are at higher risk for developing KD, and while the disease has been reported in most ethnic groups, KD is overrepresented in Asian and Asian-American populations (Cook et al., 1989). In addition, the risk of developing KD is higher in the siblings of children with KD, especially twins with KD, than in other children. Moreover, children with a parent who had KD as a child also have a higher risk of developing the disease (Uehara et al., 2003; Fujita et al., 1989; Dergun et al., 2005).

Inositol 1,4,5-triphosphate receptor type 3 (ITPR3) is a Ca^{2+} release channel that responds to the second messenger inositol 1,4,5 trisphosphate (ITP)(Patterson et al., 2004). ITP can be phosphorylated by Inositol 1,4,5-trisphosphate 3-kinase (ITPK) and

serves as a negative regulator of the Ca^{2+}/nuclear factor of activated T-cells signaling pathway (Imboden and Pattison, 1987). Previous reports have indicated that a single-nucleotide polymorphism (SNP) rs28493229 in the *ITPKC* gene is associated with susceptibility to KD and CAAs in both Japanese and American children but not in Taiwanese children (Chi et al., 2010; Onouchi et al., 2008). ITPR3 is thought to play a crucial role in regulating apoptosis signaling in T lymphocytes (Hanson et al., 2004; Blackshaw et al., 2000; Mendes et al., 2005). Activated T lymphocytes are important in the pathogenesis of KD patients with CAA (Onouchi et al., 2008; Brown et al., 2001). In addition, *ITPR3* variants have a strong association with the development of type 1 diabetes in the Swedish population (Roach et al., 2006) and autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, and Graves' disease in the Japanese population (Oishi et al., 2008). Type 1 diabetes is also thought to be an autoimmune disease with cellular and humoral immunological abnormalities (Atkinson and Eisenbarth, 2001).

Since the *ITPR3* gene appears to play a critical role in the development of multiple autoimmune diseases, we hypothesized that genetic variations in *ITPR3* that are involved in innate immune responses would be associated with the development of KD and/or with the development of CAA. Therefore, we examined the association of a marked SNP rs2229634 in the *ITPR3* gene with KD risk in Taiwanese children.

Materials and Methods

Study population

From 1998 to 2005, we enrolled ninety-three individuals who fulfilled the KD diagnostic criteria, according to the Department of Pediatrics, China Medical University Hospital, Taiwan. All the KD individuals in this study were Han-Chinese in Taiwan. The clinical parameters for patients included in the study, which included white blood counts (WBC) and levels of hemoglobin (Hb), platelet (PLT), alanine aminotransferase (AST), aspartate aminotransferase (ALT), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP), were obtained by reviewing the medical records. CAAs were defined when either the right or left coronary arteries with a diameter of ≥3 mm in children younger than five years or ≥4 mm in children older than five years or with a diameter >1.5 times that of an adjacent vessel (Arjunan et al., 1986).

The control group consisted of samples from 680 healthy children randomly selected from the samples of the Taiwan Han-Chinese Cell and Genome Bank (Pan et al., 2006). Control subjects were matched for gender and age with the study patients. The Human Studies Committee of China Medical University Hospital approved the study, and informed consents were obtained from the participants or their parents.

SNP selection and genotyping

This study selected the SNP rs2229634, which locates in the *ITPR3* gene on chromosome 6p21.3. A study by Roach et al reported that the most important SNP rs2296336, and the second most important SNP rs2229634 in *ITPR3,* were associated with type 1 diabetes in Swedish individuals (Roach et al., 2006). However, we selected rs2229634 instead of rs2296336 for the following two reasons: (i) the SNPs rs2229634 and rs2296336 are in strong linkage disequilibrium (LD) with each other; and (ii) rs2229634 is a synonymous polymorphism, but rs2296336 is located in an intronic region of the *ITPR3* gene. For genotyping, genomic DNA was extracted from peripheral blood mononuclear cells using the QIAamp Blood Kit (Qiagen, Chatsworth, CA). The SNP was genotyped using high-throughput matrix-assisted laser desorption ionization time-of-flight mass spectrometry, as described previously by Lin et al. (Lin et al., 2009). Seven control subjects could not be genotyped. This may be attributable to the poor quality of the template DNA used for genotyping.

Statistical analysis

Pearson χ^2 tests were used to assess differences in genotype and allele distribution. Genotype frequency was tested for Hardy–Weinberg equilibrium by goodness of fit test. Student's *t*-test was used to determine the clinical parameters of

KD patients associated with different genotypes. Odds ratios (OR) and 95% confidence intervals (CI) were estimated for associations between risk alleles and genotypes and KD in a logistic regression model. For calculating the risk allele, the trend across the C/C, C/T, and TT genotypes was tested by logistic regression, and a variable with the values 0, 1, and 2, respectively, was used. Statistical analyses were performed using the Statistical Package for the Social Sciences software package, v17.0 (SPSS Inc., Chicago, IL), and a p value of <0.05 was considered significant.

Results

Polymorphism in the *ITPR3* gene Table 1 presents the frequencies of alleles and genotypes of SNP rs2229634 in the *ITPR3* gene. The genotypic frequencies for both KD cases and controls conformed to the Hardy–Weinberg equilibrium. Neither the allele nor the genotype frequencies of SNP rs2229634 differed significantly between the KD patient and control groups.

***ITPR3* polymorphism and occurrence of CAAs** Table 2 presents the distribution of KD patients and genotypic frequencies according to the presence or absence of CAA. The T allele occurred more frequently in KD patients who had CAA than in patients without CAA (60.0% vs. 35.7%, p = 0.003). This association remained significant after calculating the *p*-value using 10^6 permutations (p=0.0038). When compared with KD patients without CAA, the T allele was significantly associated with a 2.56-fold increase in CAA formation in KD patients (95% CI = 1.35–4.88; p = 0.004).

Clinical parameters in KD patients We analyzed the clinical parameters of KD, including WBC, Hb, PLT, AST, ALT, ESR, and CRP in KD patients with different rs2229634 genotypes. As Table 3 shows, there were no significant differences in the

mean levels of the clinical parameters mentioned earlier between KD patients with the C/C or C/T genotypes and patients with the T/T genotype, except for the mean level of CRP. In KD patients, the mean level of CRP in T/T genotype was significantly higher than in patients with C/C or C/T genotypes (12.2 mg/dL vs. 8.5 mg/dL). This finding suggests that the T/T genotype is likely associated with increased serum levels of CRP.

Discussion

This study found evidence of a link between the SNP rs2229634 in the *ITPR3* gene and the susceptibility to the development of CAAs in Taiwanese KD patients (OR = 2.56, 95% CI = 1.35–4.88; Table 2). However, we did not find this link in children with KD (Table 1). Several KD candidate genes, such as Fc gamma receptors (Taniuchi et al., 2005), vascular endothelial growth factor receptor (Kariyazono et al., 2004), interleukin 10 (Jin et al., 2007), CD14 antigen (Nishimura et al., 2003), tumor necrosis factor-α (Quasney et al., 2001), matrix metallopeptidase 13 (Ikeda et al., 2008), as well as tissue inhibitor of metalloproteinase 2 (Furuno et al., 2007), have reported this kind of association.

The SNP rs2229634 is a synonymous polymorphism located in exon 20 of the *ITPR3* gene. New evidence indicates that even some synonymous mutations have constrained effects, often because they affect splicing and/or mRNA stability (Chamary et al., 2006). Many synonymous mutations cause disease by disrupting the splicing process (Pagani and Baralle, 2004; Cartegni et al., 2002). To determine whether this SNP modifies the expression level of ITPR3 requires further study. Since ITPR3 may play a crucial role in regulating apoptosis signaling in T-cells suggests ITPR3 can via activate T-cells to play an important role in the pathogenesis of CAA in KD (Onouchi et al., 2008; Mendes et al., 2005; Hanson et al., 2004; Brown et al.,

2001; Blackshaw et al., 2000). However, the role of ITPR3 in the pathogenesis of CAA in KD awaits further characterization.

Coronary artery aneurysm frequently results from vasculitis in KD patients. Since inflammation is believed to play a role in the pathogenesis of cardiovascular events, researchers have proposed measuring markers of inflammation as a method to improve risk prediction for these events (Danesh et al., 2004). Several previous studies suggested that evaluating CRP levels may provide a useful marker for assessing the risk of cardiovascular disease in apparently healthy individuals (Ridker et al., 2001; Visser et al., 1999). The present study observed that KD patients with the T/T genotype had a higher frequency of CAA (33.3%) and had elevated CRP levels compared with KD patients without the T/T genotype (Table 3). This finding suggests that SNP rs2229634 in the *ITPR3* gene may affect CRP levels in KD patients.

In addition, ethnic differences are present in the SNP rs2229634. Table 4 reports the C and T allele frequencies, respectively, to be 0.523 and 0.477 in Chinese populations, 0.572 and 0.428 in Japanese populations, 0.633 and 0.367 in European populations, and 0.816 and 0.184 in sub-Saharan African populations (http://www.ncbi.nlm.gov/SNP/). In the Taiwanese population, the allele frequencies were similar to those seen in Asian populations. Europeans have a slightly lower frequency than Japanese do. Africans have the lowest T allele frequency. This finding

suggests ethnic backgrounds are a worthy topic for future investigation.

The major limitation of this study is the sample size of KD patients who had CAAs. Our relatively small sample size reflects the difficulties in recruiting young patients for a relatively rare disease. Future studies with a larger number of subjects are needed to confirm these findings. The other limitation is that we only tested rs22296234 as a tag SNP of the *ITPR3* gene. Whether the polymorphisms we tested cause KD or are merely in linkage disequilibrium (LD) with other functional polymorphic sites within or flanking chromosome 6p21 is unclear. The *ITPR3* gene is located about 500 kb centromeric to the *HLA* class II genes *DQ* and *DR*; this region contains genes encoding molecules that either confer protection or are risk factors in a variety of infections and autoimmune diseases (Singh et al., 1997; Hill, 1998). Roach et al reported that *ITPR3* and *HLA* class II molecules have independent effects in subjects with type 1 diabetes (Roach et al., 2006), but Qu et al reported that the association between type 1 diabetes and the *ITPR3* gene polymorphism is because of LD with *HLA* class II (Qu et al., 2008). No one has reported such an LD between *HLA* class II and *ITPR3* in KD patients. Recently Kim et al. examined the entire MHC region and the results suggest that SNPs in the *HLA-G* locus are associated with KD in Korean (Kim et al., 2008). Furthermore, we reported that an SNP (rs2844724) located in the *HLA-E* gene was significantly associated with KD susceptibility and

CAA development (Lin et al., 2009). Since the chromosomal distance between the *HLA-G* or *HLA-E* and rs2229634 (*ITPR3*) is more than 3000 kb, the association between *ITPR3* and KD susceptibility and CAA development is not likely due to LD with the *HLA-G* or *HLA-E* gene. In addition, because of the high variation in the frequencies of *HLA* alleles among different ethnic populations, the same association may not necessarily be observed in all populations. Possibly, the effect of ITPR3 is independent to HLA in Taiwanese children with KD.

In conclusion, we have shown that the T allele of the rs2229634 SNP in the *ITPR3* gene is associated with increased risk of developing CAA in Taiwanese KD patients.

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Table 1. Allelic and genotypic frequency of SNP rs2229634 in KD patients and controls

rs2229634	KD cases n=93	Controls n=680[a]	*p* value	OR (95% CI)
Allelic frequency				
C	105 (56.5)	689 (51.2)	0.205	1.00 (Ref)
T	81 (43.5)	657 (48.8)		0.81 (0.59-1.10)
Genotypic frequency				
C/C	32 (34.4)	176 (26.2)	0.244	1.00 (Ref)
C/T	41 (44.1)	337 (50.1)		0.67 (0.41-1.10)
T/T	20 (21.5)	160 (23.8)		0.69 (0.38-1.25)

CI: confidence interval; KD: Kawasaki disease; OR: odds ratio.

[a]Total number of controls does not add up to 680 because of failed to genotyping.

Table 2. Genotypic frequency of rs2229634 in KD patients according to the presense or absence of CAA

KD patients presence or absence CAA	rs2229634				
	n (%)				
	C/C	C/T	T/T	OR (95% CI)	*p* value
Absence (n=63)	28 (44.4)	25 (39.7)	10 (15.9)	1.00 (Ref)	
Presence (n=30)	4 (13.3)	16 (53.3)	10 (33.3)	2.56 (1.35-4.88)	0.004

CAA: coronary artery aneurysm; CI: confidence interval; KD: Kawasaki disease; OR: odds ratio

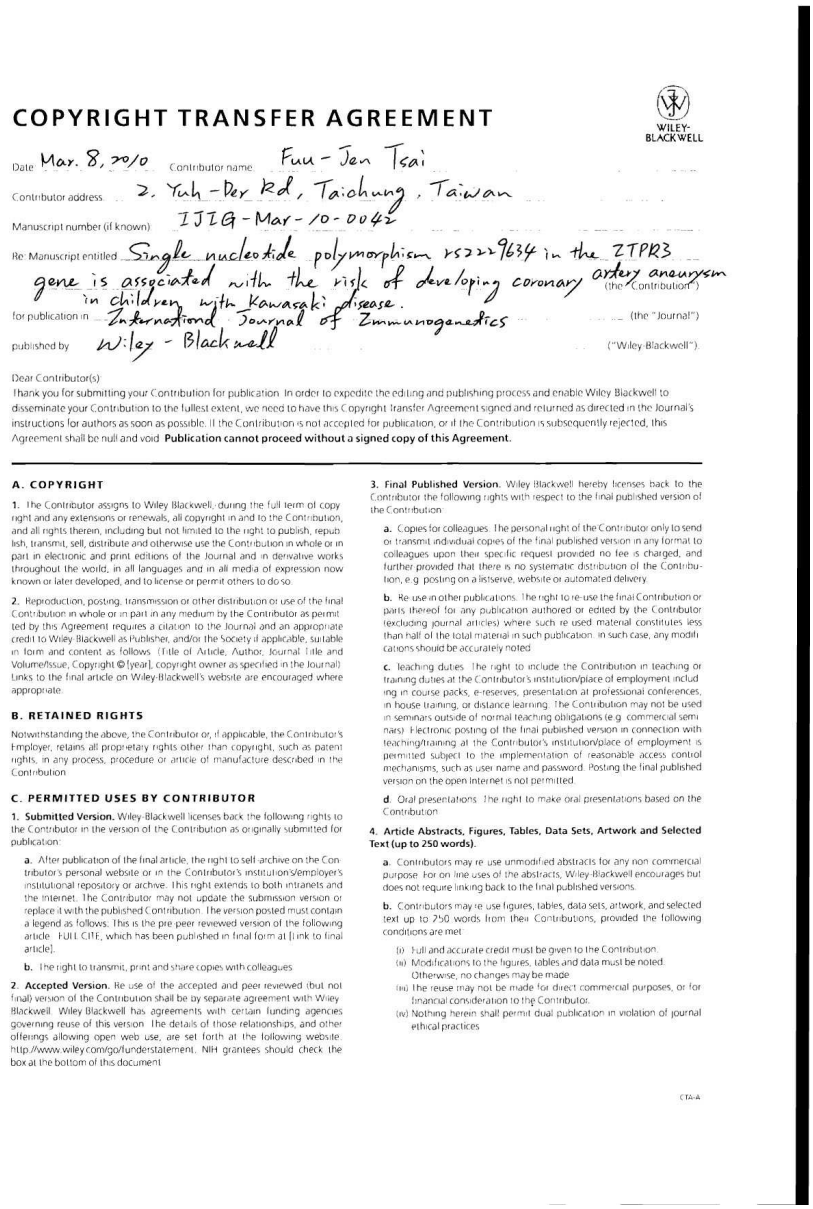
Table 3. Clinical parameters of KD patients according to rs2229634 genotypes

Clinical parameters [normal range]	Genotype in KD patients		*p* value
	C/C+CT	TT	
WBC [4-10] ($\times 10^3/mm^3$)	14.5 ± 5.5	14.6 ± 5.5	0.906
Hb [male, 14-18; female, 12-16] (g/dL)	11.3 ± 1.2	11.1 ± 1.0	0.528
PLT [130-400] ($\times 10^3/mm^3$)	426.9 ± 158.1	387.2 ± 179.2	0.438
AST [5-34] (IU/L)	78.9 ± 103.4	75.1 ± 113.9	0.899
ALT [5-40] (IU/L)	74.0 ± 100.8	61.4 ± 63.2	0.641
ESR [male<15; female<20] (mm/h)	77.1 ± 33.2	93.9 ± 30.8	0.071
CRP [<0.8] (mg/dL)	8.5 ± 6.6.	12.2 ± 5.8	0.036

Table 4. Allele frequencies of rs2229634 in different populations

	Allele frequency of rs2229634	
Population[a]	**C allele**	**T allele**
Taiwanese controls in the present study	0.512	0.487
Chinese (HapMap HCB)	0.523	0.477
Japanese (HapMap JPT)	0.572	0.428
European (HapMap CEU)	0.633	0.367
Sub-Saharan (HapMap YRT)	0.816	0.184

[a]Data from the SNP database (http://www.ncbi.nlm.gov/SNP/)



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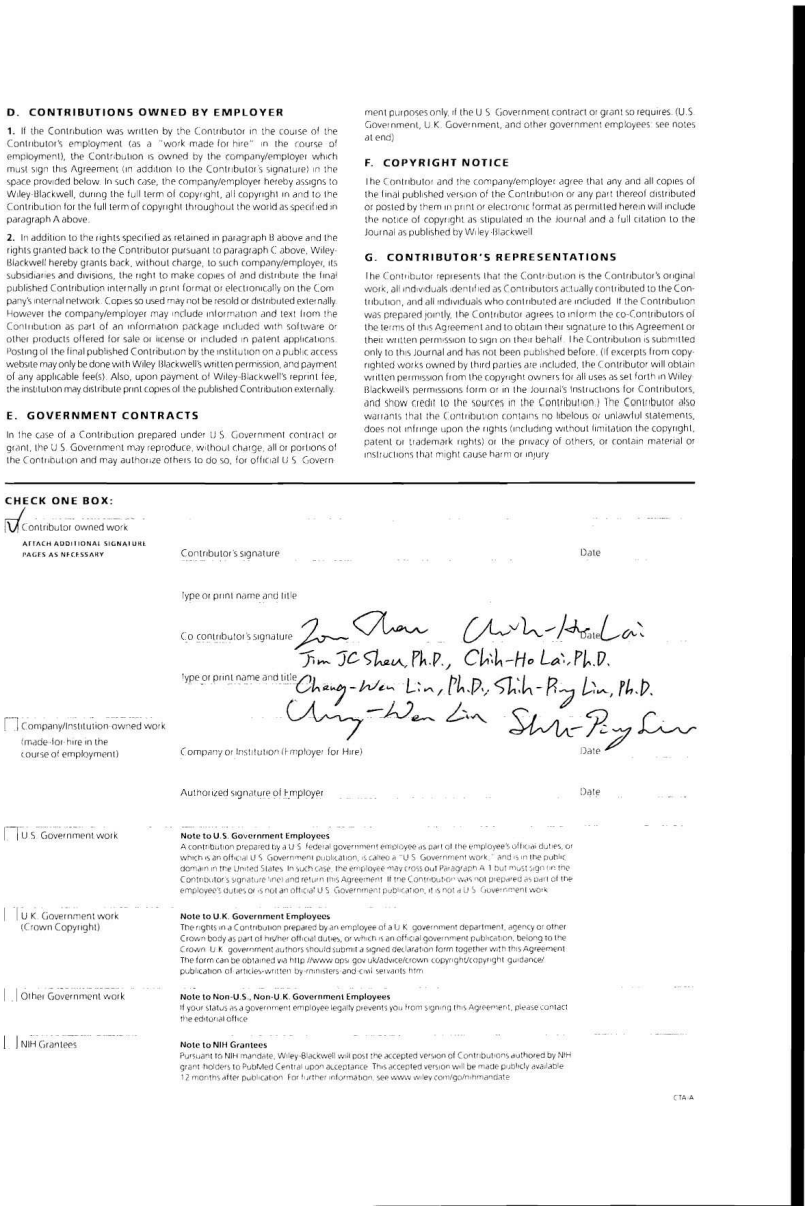

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