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 estrogens and androgens and regulate immune responses through modulation of the hormone in mammals. The objective of this study was 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. Genotyping analysis involved 93 KD patients and 680 unrelated healthy children. Our findings indicated frequency of A allele in polymorphisms rs421446 was markedly higher among patient (43.3%) than in controls the group (34.0%; p=0.032). Children with the A allele at rs421446 SNP may show higher risk of developing KD, particularly in whom with AA homozygous genotype. Also, from comparison of haplotype frequencies between case and control, children with AC haplotype appeared more “at-risk” for Kawasaki disease 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.

RUNNING TITLE:

17β-HSD8 SNPs and KD

INTRODUCTION

Kawasaki disease (KD) is an acute febrile vasculitic syndrome of early childhood who present with fever, rash, conjunctival injection, cervical lymphadenitis, inflammation of the lips and oral cavity, and erythema and edema of the hands and feet[1]. The first cases were identified among Japanese Children in the 1967[2]. Cardiac sequela, such as coronary artery lesions (CAL), is one of the most important aspects of this disease[3]. Cause of the disease is a syndrome of unknown etiology, generally believed to be an infectious agent, host immune dysregulation, and genetic susceptibility[4,5]. Moreover, KD is overrepresented in Asian children, with gender differences observed: i.e., more often in boys (ratio of about 1.5:1)[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[8,9].

The immune response is characterized by the accumulation and expansion of T-helper 1 (Th1) lymphocytes and increased amounts of several proinflammatory

58 cytokines, such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and sex
59 hormones[10-13]. Sex hormonal action on the immune system is thought to account for
60 gender differences in immune capability, dispelling the notion that sex steroid
61 hormones exclusively affect sexrelated endocrinologic functions[14]. Among vertebrates,
62 the levels of sex steroids within tissues are regulated by a variety of steroidogenic
63 enzymes. The 17β-hydroxysteroid dehydrogenases (17β-HSDs) enzymes catalyze the
64 oxidoreduction of hydroxyl/keto groups of androgens and estrogens[15] and they are
65 involved in the biosynthesis of estrogens and androgens and modulation of their
66 hormone action in steroidogenic as well as in peripheral tissues in mammals[16].
67 Multiple types of 17β-HSDs (named types 1–12) have been cloned and have been
68 shown to be expressed in several human and animal tissues[15,17,18]. The type 8
69 17β-HSD has been recently identified. It is also known as the product of the Ke 6
70 gene, which is found in the human leukocyte antigen region[19].

71 As well know, KD is thought to be an infectious disease with immunologic
72 expressions and maybe caused by the genetic susceptible individuals. In this study, we
73 hypothesized that *17β-HSD8* genetic variants in the 3'UTR confer KD susceptibility.
74 We examined and compared *17β-HSD8* genotype distribution in a group of
75 Taiwanese KD patients and a non-KD control group. An attempt was also made to
76 clarify the association between *17β-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 (Research Committee on Kawasaki Disease). Patients were treated with intravenous immunoglobulin (IVIG; 2 g/kg infused over 8-12 h) and oral aspirin (80-100 mg/kg/day). Echocardiographs were obtained by the pediatric 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 internal lumen diameter was > 3 mm in a child younger than 5 years or > 4 mm in those older than 5 years, if the internal diameter of a segment measured ≥ 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 to serve as a control group. All blood samples were drawn before IVIG therapy in KD patient groups; 680 control samples had no prior history of KD and were tested in parallel with patient samples. The ethics committee of China Medical University Hospital's Institutional Review Board approved the study, with written informed consent from parents of all subjects

(DMR97-IRB-246).

SNP selection

17β-HSD8 SNPs genotypes information was downloaded in December 2008 from the HapMap CHB + JPT population. HapMap genotypes were analyzed within Haploview and Tag SNPs were selected using the Tagger function by applying the following additional criteria: (i) a threshold minor allele frequency (MAF) in the HapMap CHB + JPT population of 0.05 for"tag SNPs"; and (ii) 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 *17β-HSD8* gene (Figure 1).

Genomic DNA Extraction and Genotyping of *17β-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 6:33174783 and 6:33183730 in 3'UTR of *17β-HSD8* (Figure 1) gene were identified by high-throughput matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass

spectrometry. A detailed description of the procedures is presented by Lin *et al*, 2009[20].

Statistical analysis

Hardy-Weinberg equilibrium was tested for each marker using χ^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 *17β-HSD8* gene was estimated using Haploview version 4.1 based on accelerated EM algorithm[21]. Intergroup differences in haplotype frequency were assessed by χ^2-test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were obtained by logistic regression to define association between *17β-HSD8* alleles/genotypes/haplotypes and KD susceptibility. All data were analyzed with SPSS Version 15.0 software (SPSS Inc., Chicago, IL, USA), p value < 0.05 considered statistically significant.

Results

Table 1 plots 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, results showed A allele frequency in rs421446 polymorphism significantly higher in the patient group (43.3%) than in the control group (34%; p = 0.032, OR = 1.47; 95% CI = 1.03-2.13). Therefore, children with A allele may have higher risk of developing KD. Significant difference in genotype frequency was also found in KD patients and controls (p = 9.557E-06), but none in rs213213 SNP.

Haplotype frequencies were estimated using the rs421446 and rs213213 SNPs (Table 2). Three haplotypes of the *17β-HSD8* were present in the study population. The GC and AC were the common haplotypes both in KD patients (56.3% and 35.6%, respectively) and health control (65.0% and 27.5%, respectively) groups. Data indicated that compared with haplotype frequencies between groups, children with AC haplotype appeared to be a significant "at-risk" haplotype for KD progression (p = 0.022, OR = 1.46; 95% CI = 1.05-2.01). In addition, the GC haplotype appeared a significant "protective" haplotype compared with other haplotypes (p = 0.021, OR =

0.7; 95% CI = 0.51-0.95) (Table 2).

In addition, we analyzed whether certain rs421446 and rs213213 haplotypes are associated with development of coronary artery lesions (CAL) in the KD patients (Table 3). Compared with haplotype frequencies in KD patients with/without CAL, GC and AC were common haplotypes both in the KD patients with (65.0% and 30.0%, respectively) and without CAL (51.5% and 39.0%, respectively). Data also indicated that KD patients with GC haplotype seem to develop CAL (65.0%) more often, but the difference was not statistically significant.

Furthermore, we compared the association between clinical parameters and diplotypes with/without haplotype AC in *17β-HSD8* gene (Table 4). The results showed that higher level of glutamate oxaloacetate transaminase (GOT) was observed in KD patients with AC haplotype compared with non AC haplotype ($p = 0.022$). Likewise, glutamate pyruvate transaminase (GPT) level in KD patients with AC haplotype was statistically significant higher than patients with non AC haplotype ($p = 0.03$).

DISCUSSION

Currently, KD is viewed as an infectious disease with immunologic expressions caused by genetic susceptibility of individuals[4]. Polymorphic gene sequences of

169 cytokines definitely involved in pathogenesis of KD are potential markers of disease
170 susceptibility: e.g., tumor necrosis factor-α, Interleukin-1, 10, and 18[22-25].

171 This study focused on the variant of *17β-HSD8* 3'UTR genetic polymorphisms
172 (rs421446 and rs213213) previously investigated for biosynthesis of estrogens and
173 androgens in mammals[16]. Our data linked the correlation between KD and rs421446
174 of *17β-HSD8* polymorphism, and AA homozygous genotype frequency in KD
175 patients was significantly higher than in controls (Table 1). However, the call rate of
176 rs421446 in KD patients was a little bit low. Therefore, we used TaqMan assay
177 (Applied Biosystems) for validating 10% of all samples and got the concordant results.
178 Since rs421446 is located in the promoter region (-176) of *17β-HSD8* gene. Previous
179 report indicated that sequences from -260 to -75 of *17β-HSD8* promoter region are
180 required for full transcriptional activity[26]. This suggests that the SNP rs421446 located
181 in the important region of *17β-HSD8* gene promoter and may be contributed the
182 different KD develop risk with different transcriptional activity. In addition, our
183 results also indicated haplotypes of *17β-HSD8* gene play a significant role in creating
184 susceptibility to KD in the Taiwanese population. Table 2 shows the AC haplotype
185 present in an estimated 35.6% of KD patients. AC haplotype appeared a susceptibility
186 factor for developing KD in our cohort. We also observed individuals with AC
187 haplotype seemed more “at-risk” for Kawasaki disease progression (OR: 1.46, 95%

188 CI: 1.05-2.01; p = 0.022). Briefly, these haplotypes may be involved in a potential
189 role of *17β-HSD8* gene in KD pathogenesis, although the precise mechanism remains
190 undetermined.

191 Since inflammation is believed to play a role in pathogenesis of cardiovascular
192 events, measuring inflammation markers has been proposed to enhance prediction of
193 risk of these events[27]. And several studies suggest that C-reactive protein (CRP) level
194 evaluation may provide a useful method of assessing risk of cardiovascular disease in
195 apparently healthy persons[28,29]. In our study, high level of CRP (> 5 mg/dL) was
196 observed both in KD patients with/without AC haplotype. We also found total fever
197 duration in KD patients with AC haplotype elongated compared with non AC
198 haplotype, though the difference was not significant (Table 3). In addition, GOT and
199 GPT levels in KD patients with AC haplotype were statistically significant higher
200 than patients with non AC haplotype (p = 0.022 and 0.03, respectively) (Table 4).
201 Previous study indicated that liver function impairment is common in acute stage of
202 KD patients[30]. Briefly, our data indicated individuals with AC haplotype appeared to
203 be a significant "at-risk" haplotype for Kawasaki disease progression maybe due to
204 the retardations observed in clinical parameters of GOT and GTP which compared
205 with non AC haplotype.

206 Previously study indicated that CAL will be occurred in about 25% of KD

patients without therapy, and death may result from coronary artery aneurysm (CAA) rupture or thrombosis, myocardial infarction, or myocarditis[3]. In this study, we observed 29.9% (20/67) of KD patients with CAL and we analyzed 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).

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.

Acknowledgements: This work was supported by China Medical University (CMU98-N1-18), China Medical University Hospital (DMR-98-042 and DMR-98-144), National Science Council in Taiwan (NSC 98-2314-B-039-004-MY2) and Asia University (CMU-asia-05) in Taiwan.

REFERENCES

1 Taubert KA, Shulman ST (1999) Kawasaki disease. *Am Fam Physician* **59,** 3093-102, 3107-8.

2 Kawasaki T (1967) Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. *Arerugi* **16,** 178-222.

3 Pahlavan PS, Niroomand F (2006) Coronary artery aneurysm: a review. *Clin Cardiol* **29,** 439-43.

4 Wang CL, Wu YT, Liu CA, et al (2005) Kawasaki disease: infection, immunity and genetics. *Pediatr Infect Dis J* **24,** 998-1004.

5 Tse SM, Silverman ED, McCrindle BW, Yeung RS (2002) Early treatment with intravenous immunoglobulin in patients with Kawasaki disease. *J Pediatr* **140,** 450-5.

6 Burns JC, Glode MP (2004) Kawasaki syndrome. *Lancet* **364,** 533–44.

7 Juan CC, Hwang B, Lee PC, et al (2007) The clinical manifestations and risk factors of a delayed diagnosis of Kawasaki disease. *J Chin Med Assoc* **70,** 374-9.

8 Chang LY, Chang IS, Lu CY, et al (2004) Epidemiologic features of Kawasaki disease in Taiwan, 1996-2002. *Pediatrics* **114,** e678-82.

9 Park YW, Han JW, Park IS, et al (2005) Epidemiologic picture of Kawasaki disease in Korea, 2000-2002. *Pediatr Int* **47,** 382-7.

10 Sugerman PB, Savage NW, Walsh LJ, et al (2002) The pathogenesis of oral lichen planus. *Crit Rev Oral Biol Med* **13,** 350-65.

11 Simark Mattsson C, Jontell M, Bergenholtz G, et al (1998) Distribution of interferon-gamma mRNA-positive cell in oral lichen planus lesions. *J Oral Pathol Med* **27,** 483-8.

12 Simark-Mattsson C, Bergenholtz G, Jontell M, et al (1999) Distribution of interleukin-2-4-10, tumor necrosis factor-alpha and transforming growth factor-beta mRNA in oral lichen planus. *Arch Oral Biol* **44,** 499-507.

13 Strober W, Kelsall B, Fuss I, et al (1997) Reciprocal IFN-gamma and TGF-beta responses regulate the occurrence of mucosal inflammation. *Immunol Today* **18,** 61-4.

14 Ahmed SA, Hissong BD, Verthelyi D, et al (1999) Gender and risk of autoimmune diseases: possible role of estrogenic compounds. *Environ Health Perspect* **107,** 681-6.

15 Mindnich R, Müller G, Adamski J (2004) The role of 17betahydroxysteroid dehydrogenases. *Mol Cell Endocrinol* **218,** 7–20.

16 Pletnev VZ, Duax WL (2005) Rational proteomics IV: modeling the primary function of the mammalian 17beta-hydroxysteroid dehydrogenase type 8. *J Steroid Biochem Mol Biol* **94,** 327-35.

17 Luu-The V (2001) Analysis and characteristics of multiple types of human 17beta-hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* **76,** 143–151.

18 Peltoketo H, Luu-The V, Simard J, Adamski J (1999) 17beta-hydroxysteroid dehydrogenase (HSD)/17-ketosteroid reductase (KSR) family; nomenclature and main characteristics of the 17HSD/KSR enzymes. *J Mol Endocrinol* **23,** 1-11.

19 Kikuti YY, Tamiya G, Ando A, et al (1997) Physical mapping 220 kb centromeric of the human MHC and DNA sequence analysis of the 43-kb segment including the RING1, HKE6, and HKE4 genes. *Genomics* **42,** 422-35.

20 Lin YJ, Wan L, Wu JY, et al (2009) HLA-E gene polymorphism associated with susceptibility to Kawasaki disease and formation of coronary artery aneurysms. *Arthritis Rheum* **60,** 604-10.

21 Barrett JC, Fry B, Maller J, Daly MJ et al (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21,** 263-5.

22 Quasney MW, Bronstein DE, Cantor RM, et al (2001) Increased frequency of alleles associated with elevated tumor necrosis factor-α levels in children with Kawasaki disease. *Pediatr Res* **49,** 686-90.

282 23 Wu SF, Chang JS, Wan L, et al (2005) Association of IL-1Ra gene polymorphism,
283 but no association of IL-1ß and IL-4 gene polymorphisms, with Kawasaki disease.
284 *J Clin Lab Anal* **19,** 99-102.
285 24 Jin HS, Kim HB, Kim BS, et al (2007) The IL-10 (-627 A/C) promoter
286 polymorphism may be associated with coronary aneurysms and low serum albumin
287 in Korean children with Kawasaki disease. *Pediatr Res* **61,** 584-7.
288 25 Chen SY, Wan L, Huang YC, et al (2009) Interleukin-18 gene 105A/C genetic
289 polymorphism is associated with the susceptibility of Kawasaki disease. *J Clin Lab*
290 *Anal* **23,** 71-6.
291 26 Villar J, Celay J, Alonso MM, et al (2007) Transcriptional regulation of the human
292 type 8 17beta-hydroxysteroid dehydrogenase gene by C/EBP. *J Steroid Biochem*
293 *Mol Biol* **105,** 131-9.
294 27 Ridker PM, Hennekens CH, Buring JE, Rifai N (2000) C-reactive protein and
295 other markers of inflammation in the prediction of cardiovascular disease in
296 women. *N Engl J Med* **342,** 836-43.
297 28 Ridker PM, Rifai N, Clearfield M, et al (2001) Measurement of C-reactive protein
298 for the targeting of statin therapy in the primary prevention of acute coronary
299 events. *N Engl J Med* **344,** 1959-65.
300 29 Visser M, Bouter LM, McQuillan GM, et al (1999) Elevated C-reactive protein

levels in overweight and obese adults. *JAMA* **282,** 2131-5.

30 Eladawy M, Dominguez SR, Anderson MS, Glodé MP (2011) Abnormal liver panel in acute kawasaki disease. *Pediatr Infect Dis J* **30**,141-4.

Table 1 Genotypic and allelic frequencies of *17β-HSD8* gene genetic polymorphism in KD patients and controls.

dbSNP ID		Patient with KD (N= 93)	Control (N= 680)	OR (95% CI)	*p* value
rs421446					
	Genotype	n=67 (%)	n=648 (%)		
	AA	24 (35.8)	95 (14.7)	3.25 (1.85-5.60)	9.56E-06[a]
	GA	10 (14.9)	251 (38.7)	---	
	GG	33 (49.3)	302 (46.6)	---	
	GG + GA	43 (64.2)	553 (85.3)	Ref	
	Allele frequency				
	A	58 (43.3)	441 (34.0)	1.47 (1.03-2.13)	0.03
	G	76 (56.7)	855 (66.0)	Ref	
rs213213					
	Genotype	n=93 (%)	n=679 (%)		
	CC	79 (84.9)	582 (85.7)	1.06 (0.58-1.95)	0.84[b]
	TC	13 (14.0)	92 (13.6)	---	
	TT	1 (1.1)	5 (0.7)	---	
	TT + TC	14 (15.1)	97 (14.3)	Ref	
	Allele frequency				
	C	171 (91.9)	1256 (92.5)	0.93 (0.53-1.64)	0.79
	T	15 (8.1)	102 (7.5)	Ref	

CI, confidence interval; OR, odds ratio.

[a]Compared with rs421446 AA and AG+GG genotype.

[b]Compared with rs213213 CC and TC+TT genotype.

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

Haplotype[a]	Patient with KD (%)[b] (n= 67)	Control (%) (n= 648)	OR (95% CI)	*p* value
GC	56.3%	65.0%	0.70 (0.51-0.95)	0.02
AC	35.6%	27.5%	1.46 (1,05-2.01)	0.02
AT	6.5%	6.6%	0.99 (0.53-1.83)	0.99

CI, confidence interval; OR, odds ratio.

[a] Order of single nucleotide polymorphisms comprising the *17β-HSD8* gene haplotypes: rs421446 and rs213213.

[b] Percentages may not sum to 100% because of the presence of the presence of rare haplotypes (<5%) not presented here.

Table 3 Distribution of *17β-HSD8* SNPs (rs421446, rs213213) frequencies in KD patients with/without coronary artery lesions (CAL).

Haplotype[a]	Patient with KD (%)[b]		KD CAL(+) vs KD CAL (-)	
	CAL (+) (n=20)	CAL (-) (n= 47)	OR (95% CI)	*p* value
GC	65.0%	51.5%	1.75 (0.93-3.31)	0.08
AC	30.0%	39.0%	0.67 (0.35-1.29)	0.23
AT	0.6%	9.2%	0.06 (0.00-19.04)	0.45

CI, confidence interval; OR, odds ratio; KD CAL(+)/CAL(-), KD patients with/without CAL.

[a] Order of single nucleotide polymorphisms comprising the *17β-HSD8* SNPs haplotypes: rs421446 and rs213213.

[b] Percentages may not sum to 100% because of the presence of the presence of rare haplotypes not presented here.

Table 4 Association between *17β-HSD8* gene diplotypes[a] and clinical parameters in KD patients.

Clinical Parameters[b]	Patient with KD		*p* value[c]
	AC (n=32)	non AC (n=35)	
Age, year	1.52 ± 0.87	2.01 ± 2.01	0.21
WBC, $x10^3/mm^3$	14.86 ± 5.97	14.55 ± 5.71	0.85
Hemoglobin, g/dL	11.50 ± 1.00	11.16 ± 0.95	0.28
Platelet, $x10^3/mm^3$	360.24 ± 118.82	415.06 ± 122.68	0.17
ESR, mm/h	79.71 ± 33.45	78.36 ± 33.89	0.89
CRP, mg/dL	9.64 ± 7.39	9.17 ± 5.66	0.80
GOT, IU/L	127.26 ± 158.64	44.87 ± 31.44	0.02
GPT, IU/L	109.87 ± 136.85	42.05 ± 35.43	0.03
Fever duration (day) (before IVIG)	6.14 ± 1.55	5.61 ± 1.47	0.22
Fever duration (day) (after IVIG)	1.68 ± 2.28	1.40 ± 1.56	0.63
Total fever duration (day)	7.82 ± 2.22	6.84 ± 2.12	0.13

[a] *17β-HSD8* 3'UTR diplotypes contain haplotype AC.

[b] Data for each group are expressed as mean ± SD.

[c] Student t-test.