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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[1]. The first cases were identified among Japanese Children in 1967[2]. Cardiac sequelae, such as coronary artery lesions (CAL), are one of the most important aspects of this disease[3]. The causes of the disease are unknown, but are 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 (boy:girl 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 cytokines, such as interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) and sex hormones[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[14]. Among vertebrates, the levels of sex steroids within tissues are regulated by a variety of steroidogenic enzymes. The 17β-hydroxysteroid dehydrogenases (17β-HSDs) enzymes catalyse the oxidoreduction of hydroxyl/keto groups of androgens and oestrogens[15] and are involved in the biosynthesis of oestrogens and androgens and modulation of their hormone action in steroidogenic as well as in peripheral tissues in mammals[16]. Multiple types of *17β-HSD*s (named types 1–12) have been cloned and have been shown to be expressed in several human and animal tissues[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[19].

In this study, we hypothesized that *17β-HSD8* genetic variants in the 3′UTR confer KD susceptibility. We examined and compared *17β-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 *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. 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 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 $\geqslant$ 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

17β-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 *17β-HSD8* gene (Fig. 1).

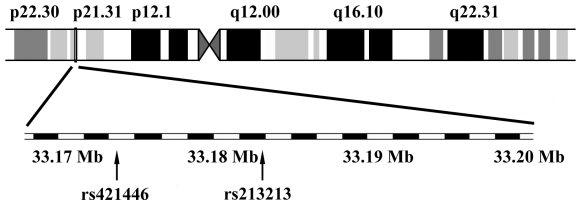

Fig. 1 Map of *17β-HSD8* (rs421446 and rs213213) located within Chromosome 6p21.31 region (33 172 088–33 174 932 bp).

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 6:33 174 783 and 6:33 183 730 in 3′UTR of *17β-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[20].

Statistical analysis

Hardy-Weinberg equilibrium was tested for each marker using a 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 an 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 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.

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)	0.00001[a]
GA	10 (14.9)	251 (38.7)	–	
GG	33 (49.3)	302 (46.6)	–	
GG + GA	43 (64.2)	553 (85.3)	1.00	
Allele frequency				
A	58 (43.3)	441 (34.0)	1.47 (1.03–2.13)	0.03
G	76 (56.7)	855 (66.0)	1.00	
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)	1.00	
Allele frequency				
C	171 (91.9)	1256 (92.5)	0.93 (0.53–1.64)	0.79
T	15 (8.1)	102 (7.5)	1.00	

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 rare haplotypes ($< 5\%$) not presented here.

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

Haplotype[a]	Patient with KD[b]		CAL(+) vs 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.

CAL(+)/CAL(−): KD patients with/without CAL.

[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 rare haplotypes not presented here.

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 ($p = 1 \times 10^{-5}$), 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 healthy control (65.0% and 27.5%, respectively) groups. 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 to be 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 analysed 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.

We also compared the association between clinical parameters and diplotypes with/without haplotype AC in *17β-HSD8* gene (Table 4). A 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 cytokines definitely involved in the pathogenesis of KD are potential markers of disease susceptibility: e.g., tumour necrosis factor-α, Interleukin-1, 10, and

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

Clinical parameters[b]	Patient with KD		
	AC ($n = 32$)	non AC ($n = 35$)	p value[c]
Age, year	1.52 ± 0.87	2.0 ± 2.0	0.21
WBC, $\times 10^3$/mm^3	14.9 ± 6.0	14.6 ± 5.7	0.85
Haemoglobin, g/dl	11.5 ± 1.0	11.16 ± 0.95	0.28
Platelet, $\times 10^3$/mm^3	360 ± 120	420 ± 120	0.17
ESR, mm/h	80 ± 33	78 ± 34	0.89
CRP, mg/dl	9.6 ± 7.4	9.2 ± 5.7	0.80
GOT, IU/l	130 ± 160	45 ± 31	0.02
GPT, IU/l	110 ± 140	42 ± 35	0.03
Fever duration (day) (before IVIG)	6.1 ± 1.6	5.6 ± 1.5	0.22
Fever duration (day) (after IVIG)	1.7 ± 2.3	1.4 ± 1.6	0.63
Total fever duration (day)	7.8 ± 2.2	6.8 ± 2.1	0.13

[a] *17β-HSD8* gene diplotypes contain haplotype AC.
[b] Data for each group are expressed as mean $\pm$ SD.
[c] Student t-test.

18[22–25].

This study focused on the variant of *17β-HSD8* 3′UTR genetic polymorphisms (rs421446 and rs213213) previously investigated for biosynthesis of oestrogens and androgens in mammals[16]. Our data linked the correlation between KD and rs421446 of *17β-HSD8* polymorphism, and AA homozygous genotype frequency in KD patients was significantly higher than in controls (Table 1). However, the call rate of rs421446 in KD patients was slightly low. TaqMan assay (Applied Biosystems) to validate 10% of all samples gave concordant results. The rs421446 locus is located in the promoter region (-176) of *17β-HSD8* gene within the sequences from -260 to -75 of *17β-HSD8* promoter region required for full transcriptional activity[26]. As the SNP rs421446 is located in an important region of *17β-HSD8* gene promoter, it may contribute to the different risk to develop KD via different transcriptional activity. In addition, our results also indicated haplotypes of *17β-HSD8* gene play a significant role in creating susceptibility to KD in the Taiwanese population. Table 2 shows the AC haplotype present in an estimated 35.6% of KD patients. AC haplotype seems to be a susceptibility factor for developing KD in our cohort. We also observed that individuals with AC haplotype seemed more "at-risk" for KD progression (OR $=$ 1.46, 95% CI $=$ 1.05–2.01; $p = 0.022$). Briefly, these haplotypes may be involved in a potential role of *17β-HSD8* gene in KD pathogenesis, although the precise mechanism remains to be determined.

Since inflammation is believed to play a role in pathogenesis of cardiovascular events, measuring inflammation markers may facilitate predicting the risk of these events[27]. For example, C-reactive protein (CRP) level evaluation may provide a useful method to assess risk of cardiovascular diseases in apparently healthy persons[28,29]. 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[3]. 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[30]. 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 GTP 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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