Interleukin-18 Gene 105A/C Genetic Polymorphism is Associated With the Susceptibility of Kawasaki Disease

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Interleukin-18 (IL-18)-656T/G, -607A/C, and -137C/G promoter polymorphisms had been reported associated with Kawasaki disease (KD). An IL-18 genetic A/C polymorphism at coding position 105 (rs549908) has been linked with asthma, rheumatoid, and systemic lupus erythematosus. We tested a hypothesis that the IL-18 105A/C genetic polymorphism confers KD susceptibility. Study participants were Taiwanese KD patients and a healthy control group. Our data indicated that the frequency of C allele was significantly higher in the patient group (13.9%) than in the control group (2.7%; $P < 0.0001$, odds ratio [OR] = 5.93; 95% confidence interval [CI] = 2.57–13.73). Therefore, persons with

the C allele may have higher risk of developing KD. In addition, compared with the haplotype frequencies between case and control groups, the KD patients with TACC haplotype appeared to be a significant “at-risk” haplotype compared with other haplotypes (OR: 4.62, 95% CI: 1.71–12.43; $P = 0.001$). KD patient with the TAGA haplotype appeared to be a significant “protective” haplotype compared with other haplotypes (OR: 0.51, 95% CI: 0.29–0.89; $P = 0.017$). Our results suggest that 105A/C polymorphism and the haplotypes in IL-18 gene are associated with the risk of KD in Taiwanese population. Clin. Lab. Anal. 23:71–76, 2009. © 2009 Wiley-Liss, Inc.

Key words: Kawasaki disease (KD); interleukin 18 (IL-18); polymorphism

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INTRODUCTION

Kawasaki disease (KD) is an acute febrile vasculitic syndrome of early childhood which presents 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 1967 (2). Cardiac sequelae, such as coronary artery lesions (CAL), are one of the most important aspects of this disease (3–6). The cause of the disease is a syndrome of unknown etiology but is generally believed to be an infectious agent (7).

The immune response is characterized by the accumulation and expansion of T-helper 1 (Th1) lymphocytes (8) and increased amounts of several proinflammatory cytokines, such as interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α) (9–11). Interleukin-18 (IL-18), a proinflammatory member of the IL-1 cytokine superfamily, is recognized as an important regulator of innate and acquired immune responses. It plays multiple roles in chronic inflammation, autoimmune diseases, and various cancers and infectious diseases (12–15). The most prominent biologic property of IL-18 is its ability to induce the production of IFN- γ in the presence of IL-12. Consequently, IL-18 was originally designated “IFN-inducing factor” (16). Furthermore, IL-18 also stimulates the expression of TNF- α , enhances the differentiation of T cells into the Th1 (proinflammatory) phenotype, and impairs the synthesis of the anti-inflammatory cytokine IL-10 (17–19).

Single nucleotide polymorphisms (SNPs) in the IL18 gene have been reported to be associated with different chronic inflammatory diseases, such as Crohn's disease and diabetes mellitus (20–24). Recently, significant association between polymorphism of IL-18 promoter region and the development of KD has also been reported (25).

In this study, we hypothesized that IL-18 105A/C genetic variants in the coding region confer KD susceptibility, we examined and compared IL-18 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 IL-18 and KD severity.

MATERIALS AND METHODS

Study Population

We enrolled patients with KD from the Department of Pediatrics at the China Medical University Hospital. The study group included 132 patients, all of whom met the criteria proposed by the Japanese Kawasaki Disease Research Committee (Research Committee on KD) (Table 1). All patients were treated with intravenous immunoglobulin (IVIG; 2 g/kg infused over 8–12 hr) and oral aspirin (80–100 mg/kg/day). Echocardiographs were obtained by the pediatric cardiologist before or within 2 weeks of IVIG administration. CAL were diagnosed from the echocardiograms using the criteria proposed by the Japanese Kawasaki Disease Research Committee (Research Committee on KD): coronary arteries were classified as abnormal if the internal lumen diameter was >3 mm in children younger than 5 years or >4 mm in children 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 136 unrelated healthy children (49 boys, 87 girls, 0.4–7.0-years old, mean age 3.2 years) who served as the control group. All blood samples were drawn before IVIG therapy in the KD patient group. Control samples were tested in parallel with patient samples. The ethics committee of the China Medical University Hospital Institutional Review Board approved the study, and written informed consent was obtained from parents of all subjects.

TABLE 1. Genotypic and Allelic Frequencies of the IL-18 105 A/C Genetic Polymorphism in Patients With KD and Controls

IL-18 105 A/C	Patients with KD <i>N</i> = 132	Control <i>N</i> = 136	OR (95% CI)	<i>P</i> value
Genotype	<i>n</i> = 115 (%)	<i>n</i> = 132 (%)		
AA	84 (73.0)	125 (94.7)	Ref	<0.0001 ^a
AC	30 (26.1)	7 (5.3)	—	
CC	1 (0.9)	0 (0)	—	
AC+CC	31 (27.0)	7 (5.3)	6.59 (2.77–15.66)	
Allelic frequency				
A	198 (86.1)	257 (97.3)	Ref	<0.0001
C	32 (13.9)	7 (2.7)	5.93 (2.57–13.73)	

OR, odds ratio; CI, confidence interval.

^aCompared with IL-18 105A/C AC+CC genotype.

Genomic DNA Extraction and Genotyping of IL-18 Promoter and 105 A/C Genetic Polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Genomic DNA kit; Qiagen, Valencia, CA). Genotypes of the IL-18 promoter SNP at positions -656T/G, -607A/C, -137C/G were identified by the probe hybridization method employing the LightCycler 480 instrument (Roche Diagnostics, Indianapolis, IN) with corresponding primers and probes as described in Hsueh et al. Briefly, polymerase chain reaction (PCR) was performed in the presence of 0.5 μ M sense primer, 1 μ M antisense primer, 2 mM MgCl₂, 2 ml of 10 $\times$ Lightcycler DNA master hybridization probes (Roche), and 0.2 μ M of each fluorescence probe (25). After initial denaturation for 10 min at 95°C, 55 cycles were run, each consisting of denaturation (95°C for 0 sec), annealing (57°C for 10 sec), and extension (72°C for 20 sec). The melting curve analysis was performed as follows: one cycle, 95°C for 0 sec, 45°C for 60 sec, followed by the gradual increase of 0.1°C/sec to the target temperature of 80°C. In addition, the IL-18 105 A/C genetic variant (rs549908) was genotyped by PCR and restriction fragment length polymorphism. We used two primers (5'-TGT-TTA-TTG-TAG-AAA-ACC-TGG-AAT-T-3'; 5'-CCT-CTA-CAG-TCA-GAA-TCA-GT-3') for amplifying 148 bp PCR product. Two fragments of 123 and 25 bp were present during digestion with Tag I restriction enzyme. A detailed description of PCR procedures is presented in (26).

Statistical Analysis

The Hardy–Weinberg equilibrium was tested for each marker using χ^2 test. Chi-square test was used to determine statistically significant differences in allele/genotype frequencies between case and control groups. Allelic frequencies were expressed as percentage of the total number of alleles. The haplotype combination at IL-18: -656T/G, -607A/C, -137C/G, and 105A/C were estimated using Haploview version 4.1 based on an accelerated EM algorithm (27). The differences in the

distribution of the haplotype frequencies between the two groups were assessed with a χ^2 test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were obtained using logistic regressions to determine associations between IL-18 alleles/genotypes/haplotypes and KD susceptibility. All data were analyzed with SPSS Version 13.0 software (SPSS Inc., Chicago, IL). A P value < 0.05 was considered statistically significant.

RESULTS

Genotypic and allelic frequencies of IL-18 105A/C are shown in Table 1. Genotype distributions were in Hardy–Weinberg equilibrium. The A allele frequencies in KD patients and controls are 86.1% (198/230) and 97.3% (257/264), respectively. The C allele frequencies in KD patients and controls are 13.9% (32/230) and 2.7% (7/264), respectively. When we compared the frequencies between case and control groups, the results showed that the frequency of C allele was significantly higher in the patient group than in the control group (OR = 5.93; 95% CI = 2.57–13.73; P < 0.0001). Therefore, persons with the C allele may have higher risk of developing KD. A statistically significant difference in genotype frequency distribution was also found in KD patients and control individuals (P < 0.0001) (Table 1).

Haplotype frequencies were estimated using the three promoter polymorphisms (-656T/C, -607A/C, and -137C/G) and one coding region polymorphisms (105A/C) with haplotype frequencies > 5% (Table 2). Five major haplotypes of the IL-18 were present in the study population. The TACA and GCCA were the common haplotypes both in KD patients (32.7 and 30.3%, respectively) and health controls (39.8 and 38.4%, respectively) groups. When we compared the overall distribution of haplotype frequencies between KD patients and health controls, a significant difference was observed (P = 0.001, by χ^2 test from a 5 $\times$ 2 contingency table). In addition, compared with the haplotype frequencies between case and control groups, the KD patients with TACC and GCGA haplotypes appeared to be a significant “at-risk” haplotype

TABLE 2. Distribution of IL-18 Haplotype Frequencies in Patients With KD and Controls

Haplotype ^a	Patients with KD (%) ^b	Controls (%) ^c	OR (95% CI)	P value
TACA	32.7	39.8	0.73 (0.52–1.05)	0.086
GCCA	30.3	38.4	0.70 (0.49–1.01)	0.053
TAGA	7.6	13.9	0.51 (0.29–0.89)	0.017
TACC	8.0	1.8	4.62 (1.71–12.43)	0.001
GCGA	6.1	2.2	2.86 (1.10–7.43)	0.025

OR, odds ratio; CI, confidence interval.

^aOrder of single nucleotide polymorphisms comprising the IL-18 haplotypes: -656T/G, -607A/C, -137C/G, and 105A/C.

^{b,c}Percentages may not sum to 100% because of the presence of rare haplotypes (< 5%) not presented here.

TABLE 3. Genotypic of the IL-18 105 A/C Genetic Polymorphism in KD Patients With/Without Coronary Artery Lesions (CAL)

	IL-18 105A/C genotype			Total <i>n</i> = 113 (%) ^a
	AA <i>n</i> (%)	AC <i>n</i> (%)	CC <i>n</i> (%)	
KD patients				
With CAL	24 (70.6)	10 (29.4)	0 (0)	34/113 (30.1)
Without CAL	58 (73.4)	20 (25.3)	1 (1.3)	79/113 (69.9)

P value = 0.738.

^aTotal number *n* = 113 (not *n* = 115) owing to missing two cases of CAL data.

compared with other haplotypes (TACC: OR: 4.62, 95% CI: 1.71–12.43; *P* = 0.001; GCGA: OR: 2.86, 95% CI: 1.10–7.43; *P* = 0.025). KD patients with the TAGA haplotype appeared to be a significant “protective” haplotype compared with other haplotypes (OR: 0.51, 95% CI: 0.29–0.89; *P* = 0.017).

In addition, we try to analyze whether certain IL-18 105A/C genotypes are associated with development of CAL in the KD patients. The genotypic of IL-18 105A/C in KD patients with/without CAL was shown in Table 3. We observed 30.1% of KD patients with CAL development who were diagnosed by the criteria as described in the methodology above in the Taiwanese population. The AA genotype frequency in KD patients with/without CAL is 70.6% (24/34) and 73.4% (58/79), respectively, and the IL-18 105A/C genotypes possess C allele (AC+CC) in KD patients with/without CAL are 29.4% (10/34) and 26.6% (21/79), respectively.

DISCUSSION

Currently, KD is thought to be an infectious disease with immunologic expressions that are caused by the genetic susceptible individuals (7). Polymorphic gene sequences of cytokines known to be involved in pathogenesis of KD are potential markers of disease susceptibility. Previous studies have examined the relationship between cytokine gene polymorphisms and the incidence of KD, including TNF- α , IL-1 RA, IL-10, and vascular endothelial growth factor (28–31).

In this study, we focused on the variant of IL-18 that is located at position 105 (initiating from ATG), which had previously been investigated for systemic lupus erythematosus, asthmatics, and rheumatoid arthritis patients, and therefore represents an A/C polymorphism (26,32,33). According to our data, we found a statistically significant association between KD and the IL-18 105A/C polymorphism in the coding region, and the C allele frequency in the polymorphism was significantly higher in KD than in control participants (Table 1). Our

results also indicated that the haplotypes of IL-18 promoter and coding region polymorphism play a significant role in creating susceptibility to KD in Taiwanese population (Table 2). As shown in Table 2, the TACC haplotype was estimated to be present in about 8.0% of patients with KD. The TACC haplotype seemed to be a susceptibility factor for developing KD in our Taiwanese cohort. We also observed that the KD patient with the TAGA haplotype appeared to be a significant “protective” haplotype compared with other haplotypes (OR: 0.51, 95% CI: 0.29–0.89; *P* = 0.017). Briefly, these haplotypes may involve in a potential role of IL-18 in KD pathogenesis, although the precise mechanism remains to be determined.

Previous study indicated that CAL will be present in about 25% of KD patients without therapy, and death may result from CA aneurysm rupture or thrombosis, myocardial infarction, or myocarditis (5). In this study, we observed 30.1% of KD patients with CAL and we analyzed the relationship between IL-18 105A/C polymorphism and CAL development in the KD patients. Our data showed that compared with the KD patients without CAL, the KD patients IL-18 105A/C genotype with C allele in KD patients seem to have higher frequency to develop CAL (29.4 vs. 26.6%), although the difference was not statistically significant (Table 3). In terms of haplotypes, we found none of the haplotypes had significantly different frequencies between KD patients with and without CAL.

Several functional studies have shown that the level of IL-18 production is related to the IL-18 promoter gene (34–37), and two SNP at positions -607 and -137 of the IL-18 promoter 1 region have been found to be associated with transcription activity of the IL-18 gene promoter (34). It has been shown that a change from C to A at position -607 disrupts a potential cAMP responsive element binding protein site, resulting in low IL-18 production (38). Similarly, a change at position -137 from G to C has been reported to affect the H4TF-1 nuclear factor-binding site (34,39). These studies showed low promoter activity for A and C alleles at position -607 and -137, respectively, and higher promoter activity for C and G alleles in those positions. Although in our study the variant of the IL-18 105A/C polymorphism does not produce any amino acid changes, but the polymorphism (with an A allele) at a significantly higher level appears in asthmatics and rheumatoid arthritis patients (32,33). Data from one functional study suggested that IL-18 production is higher in individuals with the 105 A/A genotype (37). Results from a linkage disequilibrium analysis indicated an association between the 105A allele and IL-18 up-regulation via the -137G allele (32,34,37). In our study, we observed the polymorphism of A allele frequency was significantly lower in

KD patients. The statistic power with α at 0.05 was more than 89% in this study (89.7%) According to the previous studies, the instability of IL-18 may contribute to KD susceptibility and pathogenesis.

In conclusion, our findings strongly suggest an association between the 105 coding region position of an IL-18 genetic variant and KD disease susceptibility, and the results also indicated that 105A/C polymorphism and the haplotypes of interleukin 18 gene (-656T/G, -607A/C, and -137C/G) are associated with susceptibility to KD in the Taiwanese population. According to our observations, those polymorphisms contribute to the genetic background of KD pathogenesis.

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