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Association of Promoter Genetic Variants in *Interleukin-10* and Kawasaki Disease With Coronary Artery Aneurysms

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Background: Kawasaki disease (KD) is an acute, self-limited vasculitis in infants and young children. Interleukin-10 (IL-10) is a potent cytokine that exerts pleiotropic effects on immunoregulation and inflammation. Elevated IL-10 serum levels have been reported in the KD patients. *Methods:* In this study, we investigated whether *IL-10* genetic polymorphisms contribute to coronary artery aneurysm (CAA) development among KD patients in Taiwan. A total of 58 KD patients with CAA and 277 unrelated healthy children matched for sex and age were enrolled for this study. *Results:* Higher G allele frequencies of *IL-10* at -1082 position were observed in KD patients with CAA compared to the controls ($P = 0.016$, OR: 2.86, 95% CI, 1.17–6.98). In addition, higher *IL-10* GCC haplotype frequencies were also observed in KD patients with CAA ($P = 0.016$, OR: 2.85, 95% CI, 1.17–6.98). *Conclusion:* Our data support the possibility that *IL-10* gene polymorphisms may be related with CAA development of KD in Taiwanese population. J. Clin. Lab. Anal. 28:461–464, 2014.

Key words: Kawasaki disease; coronary artery aneurysms; interleukin-10; genetic polymorphism

INTRODUCTION

Kawasaki disease (KD), an acute, self-limited, and systemic form of vasculitis, is a leading cause of acquired heart disease in infants and young children (1). KD patients are at increased risk of developing ischemic heart disease, which can lead to myocardial infarction and sudden death (2). There is speculation that KD is triggered by a combination of infectious agents, host immune dysregulation, and genetic susceptibility (3).

Interleukin-10 (IL-10) is a potent cytokine, mainly produced by monocytes, macrophages, T- and B-lymphocytes, and exerts pleiotropic effects on immunoregulation and inflammation (4, 5). Several studies indicate associations between *IL-10* genetic polymorphisms and immunological disease, including diabetes, rheumatoid arthritis, Sjögren's syndrome, and systemic

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lupus erythematosus (6–10). In addition, the serum levels of IL-10 are elevated in the acute phase of KD patients, suggesting that IL-10 has been reported to be involved in the pathogenesis of KD (11, 12). Studies about *IL-10* genetic polymorphisms and KD in Asian populations have also been reported (13, 14). The major positive findings are located at the *IL-10* (-819 C/T, rs1800871) and *IL-10* (-592 C/A, rs1800872). According to Weng et al., the homozygous variant genotype CC of *IL-10*–819 and *IL-10*–592 was associated with 80% and 79% reduction in risk of CAA, respectively (13). According to Jin et al., Korean KD children with one or two copies of the *IL-10* (-627C) allele are at significantly higher risk of early CAA (14).

The purpose was to investigate potential associations between the *IL-10* genetic polymorphisms and CAA development in Taiwanese KD patients. We focused on three genetic polymorphisms: -1082A/G, -819T/C, and -592A/C and assessed their possible associations with KD CAA development.

PATIENTS AND METHODS

Patients

The KD patients were enrolled from the Department of Pediatrics at China Medical University Hospital in Taichung, Taiwan (15). All patients were diagnosed according to clinical criteria summarized by the American Heart Association (16). A CAA diagnosis was made when either the right or left coronary artery showed a dilated diameter $\geqq$3 mm in children younger than 5 years of age or $\geqq$4 mm in older children (16).

For the control group, we randomly selected 277 healthy children matched for sex and age from the Han Chinese Cell and Genome Bank, which consists of 3,312 unrelated Han Chinese who were recruited based on their geographic distribution across Taiwan (17). Estimated KD prevalence was less than 1/1,000, therefore, it was assumed that the control group had zero KD cases. However, sampling for rare disease would have limitation. There would be some problems, such as sampling bias and genetic power estimations (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html).

Our research proposal was approved by the Human Studies Committee of China Medical University Hospital. Informed consent was obtained from the participants, their parents, or both parties.

Genotyping of IL-10 Genetic Polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes (DNA Isolation kit for Mammalian Blood, Roche Applied Science, Taiwan). The three *IL-10* genetic polymorphisms were analyzed with TaqMan(R) SNP Genotyping Assays and an ABI PRISM 7700 Sequence Detection System (Applied Biosystems).

TABLE 1. Characteristics of the Taiwanese Kawasaki Disease With CAAs Patients

Characteristics	
Number of patients	58
Age (years) at time of diagnosis	
Median	1.25
Range	0.17–7.92
Sex, number of patients	
Male	41
Female	17

Statistical Analysis

Genotype and allelic frequency distributions for the targeted polymorphisms in both KD patients and controls were analyzed using a chi-squared test. Statistical significance was determined at $P < 0.017$ (0.05/3). Allelic frequencies are expressed as percentages of total alleles. Odds ratios (ORs) were calculated for genotype and allelic frequencies (95% confidence interval [CI]). Haplotypes were inferred from unphased genotype data using the Bayesian statistical method in the Phase 2.1 software program (18). All statistical tests were performed using SPSS 12.0 for Windows XP (SPSS, Inc., Chicago, IL).

RESULTS

The characteristics of the 58 KD patients with CAA were shown in Table 1. The median age at time of diagnosis was 1.25 (0.17–7.92) years old. The sex distribution of the KD patients was with a male/female ratio of 2.4:1. Significant differences were observed between KD patients with CAA and controls in allele frequency (Table 2; $P = 0.016$, G allele: OR = 2.86, 95% CI, 1.17–6.98) and genotypic frequency ($P = 0.015$, AG genotype: OR = 3.01, 95% CI, 1.2–7.54) for the *IL-10*/-1082 genetic polymorphism. No significant differences were also found between KD patients with CAA and controls for any allele or genotype frequency. Haplotype frequencies were estimated for the three genetic polymorphisms. As shown in Table 2, ATA, ACC, and GCC haplotypes were identified. A significantly higher frequency of the GCC haplotype was observed in KD patients with CAA compared to the controls ($P = 0.016$, OR: 2.85, 95% CI, 1.17–6.98).

DISCUSSION

Studies on *IL-10* genetic polymorphisms at positions -1082(G/A), -819(C/T), and -592(C/A) generating the ATA, ACC, and GCC haplotypes have revealed an

TABLE 2. Allele, Genotype, and Haplotype Frequency Distributions of *IL-10* Genetic Polymorphisms in the Taiwanese KD Patients With CAAs and Controls

Gene name (nucleotide change; SNP database ID)	Genotype	Ctrl	KD With CAAs	*P*	OR	95% CI
IL-10 -1082 (A/G)						
(rs1800896)	GG	0 (0.0%)	0 (0.0%)	***0.015****		
	AG	14 (5.1%)	8 (13.8%)		3.01	1.2–7.54
	AA	263 (94.9%)	50 (0.862)		1.00	
	G allele	14 (2.5%)	8 (6.9%)	***0.016****	2.86	1.17–6.98
	A allele	540 (97.5%)	108 (93.1%)		1.00	
IL-10 -819 (C/T)						
(rs1800871)	TT	115 (41.4%)	28 (48.3%)	0.112	0.68	0.28–1.61
	CT	138 (49.6%)	21 (36.2%)		0.42	0.17–1.03
	CC	25 (9.0%)	9 (15.5%)		1.00	
	T allele	368 (66.2%)	77 (66.4%)	0.968	1.01	0.66–1.54
	C allele	188 (33.8%)	39 (33.6%)		1.00	
IL-10 -592 (C/A)						
(rs1800872)	CC	23 (8.3%)	9 (16.0%)	0.110	1.68	0.70–4.02
	AC	134 (48.4%)	21 (36.2%)		0.67	0.36–1.24
	AA	120 (43.3%)	28 (48.3%)		1.00	
	C allele	180 (32.5%)	39 (33.6%)	0.814	1.05	0.69–1.61
	A allele	374 (67.5%)	77 (66.4%)		1.00	
Haplotype (-1082/-819/-592)						
	ATA	186 (67.3%)	39 (66.4%)	0.843		
	ACC	84 (30.2%)	15 (26.7%)	0.463		
	GCC	7 (2.5%)	4 (6.9%)	***0.016****		

CI, confidence interval; Ctrl, control subject; OR, odds ratio.
Numbers in parentheses indicate allele or genotype frequency percentages.
Numbers in bold italics indicate statistically significant differences.
Allele and genotype frequency distributions in the polymorphism at the *IL-10* (-1082) position (rsl 800896) in the KD patients and controls were analyzed using the chi-square test method (2 × 2 tables).
The *P* values were adjusted by using Bonferroni's correction. Statistical significance was considered as *P*-value < 0.017 (0.05/3).

association with IL-10 protein production (19). The GCC haplotype has been shown to be associated with high IL-10 production, while the ATA haplotype showed lower transcriptional activity, producing low levels of IL-10 protein (19). The -1082 (G/A) polymorphism lies in a putative E-twenty-six (ETS)-like transcription factor binding site (20), while the -819 (C/T) polymorphism may affect an estrogen receptor element (21). Similarly, -592 (C/A) polymorphism has been shown to be in a region of negatively regulatory function (20, 21), making those important loci for study in relation to disorders affected by IL-10 protein production. In addition, the serum levels of IL-10 are elevated in the KD patients (12). The use of IL-10 therapy has shown promise in certain clinical trials aimed at cardiovascular diseases, such as Crohn's disease, endotoxemia, and shock, suggesting that IL-10 may also play a regulating role in the microvasculature. KD, a systemic vasculitis, has recently associated with *IL-10* (13, 14). The major positive findings are located at the *IL-10* (-819 C/T, rs1800871) and *IL-10* (-592 C/A, rs1800872), which are negative in this study. Sampling bias may be one of the possible reasons to lead to different results. However, Jin et al. reported that Korean KD children with one or two copies of the *IL-10* (-627C) allele showed significantly higher frequencies of early CAA (14). In our results, higher *IL-10* GCC haplotype frequencies were also observed in KD patients with CAA, suggesting that the *IL-10* GCC genetic haplotype may be a risk factor for CAA development in Taiwanese KD children.

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