Association of IL-1*Ra* Gene Polymorphism, but no Association of IL-1 β and IL-4 Gene Polymorphisms, With Kawasaki Disease

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The purpose of this study was to evaluate whether IL-1 β (IL-1 β promoter and IL-1 β exon 5), IL-1 receptor antagonist (IL-1 *Ra*), and IL-4 (IL-4 promoter and IL-4 intron 3) gene polymorphisms act as markers of susceptibility to Kawasaki disease (KD), or of the severity of the disease. The study included 107 KD patients and 103 normal controls. Polymorphisms for cytokine genes were detected by polymerase chain reaction (PCR). Genotypes and allelic frequencies for cytokine gene polymorphisms in both groups were compared. No significant

difference was observed in the genotypes and allelic frequencies of cytokines between patients with coronary aneurysm and without. In addition, there was no significant association in the genotype and allelic frequencies of IL-1 β , IL-4, and IL-6 in patients with KD. The genotype I/II for IL-1*Ra* and the frequency of allele II for IL-1*Ra* are associated with a higher susceptibility to KD, and thus may be useful markers for predicting the development of KD. *J. Clin. Lab. Anal.* 19:99–102, 2005.

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Key words: IL-1 *Ra* gene; IL-1 β gene; IL-4 gene; Polymorphism; Kawasaki disease

INTRODUCTION

Kawasaki disease (KD) is an acute systemic vasculitis that predominantly affects infants and young children. KD is the leading cause of acquired heart disease in the pediatric age group in Taiwan. Although the epidemiologic features of this disease suggest that it is related to an infectious agent, the cause remains unknown. There have been many reports on the abnormal immune response in the acute phase of this disease. Increased serum cytokines during KD, such as interleukin-1 (IL-1), IL-2, IL-4, IL-6, IL-10, and tumor necrosis factor- α have been reported (1–4). The results suggest that cytokines play an important role in the onset of this disease. The cytokine network is complex because cytokines have both diverse and overlapping functions, including effects that are promoted or inhibited by other cytokines.

The stimulus for the increased serum levels of cytokines in KD remains unknown. Polymorphisms in genes encoding these crucial immunomodulatory molecules may result in an altered level of expression, and hence must be considered important candidate genes for KD susceptibility and severity. In this study we investigated a total of five polymorphisms: two in the IL-1 β gene, one in the IL-1 *Ra* gene, and two in the IL-4 gene. We compared genotypes and allele frequencies in a normal healthy population and 107 patients with KD.

MATERIALS AND METHODS

We enrolled patients with KD from the Department of Pediatrics at the China Medical University Hospital from July 1997 to December 2002.

The study group included 107 patients (65 males and 42 females, 0.2–3.2 years old, mean age = 1.7 years), all of whom met the appropriate diagnostic criteria for KD. All of the patients received treatment with intravenous gamma globulin (2 gm/Kg/day for 1 day, plus oral aspirin at 80 mg/Kg/day). Participant blood samples were obtained on the day of admission. Two-dimensional echocardiography was used to detect the presence of any coronary-artery lesions. Coronary arteries with diameters of ≥ 4 mm were classified as abnormal. A total of 29 patients developed a coronary-artery aneurysm, while 78 patients revealed no evidence of coronary-artery aneurysm. We also studied 103 healthy children (79 males and 24 females, 0.4–3.0 years old, mean age = 1.7 years) who served as the control group. Control samples were tested in parallel with patient samples. In this study the genes including interleukin-I β

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TABLE 1. Main characteristics of cytokine gene polymorphisms and techniques used for screening

	IL-1 β promoter	IL-1 β exon 5	IL-1 <i>Ra</i>	IL-4 intron 3	IL-4 promoter
Type of polymorphism	Single base C/T	Single base	86-bp VNTR	70bp VNTR	Single base C/T
Site of polymorphism	Position-511	Exon-5	Intron 2	Intron 2	Position-500
PCR primers	5'-TGGCATTGATCT- GGTTCATC-3'	5'-GTTGTCATCAGA- CTTGACC-3'	5'-CTCAGCAAC- ACTCTAT-3'	5'-AGGCTGAAA- GGGGGAAAGC-3'	5'-ACTAGGCCTCA- CCTGATACG-3'
Upstream	5'-GTTTAGGAAT- CTTCCCACTT-3	5'-TTCAGTTCATATG- GACCAGA-3'	5'-TCCTGGTC- TGCAGGTAA-3'	5'-CTGTTACCTCA- ACTGCTCC-3'	5'-GTTGTAATGCA- GTCCTCTG-3'
Downstream					
PCR conditions					
Denaturation	95', 0.5 minutes	95', 0.5 minutes	95', 0.5 minutes	95', 0.5 minutes	95', 0.5 minutes
Annealing	55', 0.5 minutes	55', 0.5 minutes	58', 0.5 minutes	60', 0.5 minutes	57', 0.5 minutes
Extension	72', 0.5 minutes	72', 0.5 minutes	72', 0.5 minutes	72', 0.5 minutes	72', 0.5 minutes
No. of cycles	30	30	35	30	32
Digestion	Yes (<i>Ava</i> I) IL-1 β -511*	Yes (<i>Taq</i> I)	No I:410	No	Yes (<i>Bsm</i> FI)
Allele size, bp	C:190 + 114 IL-1 β -511* T:304	E1:135 + 114 E2:249	II:240 III:500 IV:325 V:595	RP1:183 RP2:253	IL-4-590* C:192 + 60 IL-4-590* T:252

IL-1 β , interleukin-1 β ; IL-1*Ra*, interleukin-1 receptor antagonist; C/T, C-to-T substitution; VNTR, variable number of tandem repeats; G/A, G-to-A substitution; PCR, polymerase chain reaction; SSOP, sequence-specific oligonucleotide probe.

(IL-1 β) promoter, IL-1 β exon 5, IL-1 receptor antagonist, IL-4 intron3, and IL-4 promoter in all of the subjects were analyzed.

This study was carried out with the approval of the Human Studies Committee of China Medical University Hospital. Information was obtained from the patients' families. The genomic DNA was prepared from peripheral blood. Purified genomic DNA was amplified by PCR amplification. The PCR products were analyzed by electrophoresis on agarous gel, and each allele was recognized according to its size. The details for each polymorphism analysis are given in Table 1.

A Chi-squared test was used to compare the genotype and allelic frequency distribution of the cytokine gene for the patients with KD and the control subjects. A Chi-squared test was also used to compare patients with and without a coronary-artery aneurysm. A value of $P < 0.05$ was considered to represent a significant difference between the tested populations.

RESULTS

Five polymorphisms in three genes were analyzed in both the patients and the control subjects. We found that the KD patients had a significantly higher frequency of the genotype I/II and allele II in the IL-1 *Ra* gene compared to controls ($P = 0.05$). The polymorphisms in the other genes studied did not reveal any association with KD when compared with the control

TABLE 2. Distribution of interleukin genotypes for patients suffering Kawasaki disease and also for healthy controls subjects

Gene polymorphism	Genotype	Kawasaki patients (<i>n</i> = 107) <i>n</i> (%)	Control (<i>n</i> = 103) <i>n</i> (%)	<i>P</i> value
IL-1 β promoter	CC	41 (38.3)	27 (26.2)	0.17
	CT	45 (42.1)	52 (50.5)	
	TT	21 (19.6)	24 (23.3)	
IL-1 β exon 5	E1/E1	105 (98.1)	98 (95.1)	0.27
	E1/E2	2 (1.9)	5 (4.9)	
	E2/E2	0 (0)	0 (0)	
IL-1 <i>Ra</i>	I/I	89 (83.3)	95 (92.2)	0.05
	I/II	16 (14.9)	6 (5.8)	
	II/II	1 (0.9)	1 (1.0)	
	I/IV	0 (0)	0 (0)	
IL-4 promoter	IV/IV	0 (0)	1 (1)	0.64
	CC	6 (5.6)	6 (5.8)	
	CT	42 (39.3)	34 (33.0)	
IL-4 intron 3	TT	59 (55.1)	63 (61.2)	0.66
	RP1/RP1	62 (57.9)	66 (64.1)	
	RP1/RP2	38 (35.6)	33 (32.0)	
	RP2/RP2	6 (5.6)	4 (3.9)	
	RP0/RP1	1 (0.9)	0 (0)	

group (Tables 2 and 3). We did not find any difference in genotypes and allelic frequencies between KD patients with and without coronary aneurysm for the IL-1 β promoter polymorphism ($P = 0.75$; $P = 0.45$, respec-

TABLE 3. Distribution of interleukin allelic frequencies for patients suffering Kawasaki disease and also for healthy controls subjects

Gene polymorphism	Allele frequency	Kawasaki patients (n = 107) n (%)	Control (n = 103) n (%)	P value
IL-1 β promoter	C	127 (59.3)	106 (51.5)	0.10
	T	87 (40.7)	100 (48.5)	
IL-1 β exon 5	E1	212 (99.1)	201 (97.6)	0.28
	E2	2 (0.9)	5 (2.4)	
IL-1 <i>Ra</i>	I	195 (91.1)	196 (95.1)	0.05
	II	18 (8.4)	8 (3.9)	
	III	1 (0.5)	0 (0)	
	IV	0 (0)	2 (1.0)	
IL-4 promoter	C	54 (25.2)	46 (22.3)	0.49
	T	160 (74.8)	160 (77.7)	
IL-4 intron 3	RP1	163 (76.2)	165 (80.1)	0.38
	RP2	50 (23.4)	41 (19.9)	
	RP0	1 (0.4)	0 (0)	

tively), IL-1 β exon5 polymorphism ($P=0.47$; $P=0.47$, respectively), IL-1*Ra* polymorphism ($P=0.14$; $P=0.39$, respectively), IL-4 promoter polymorphism ($P=0.51$; $P=0.20$, respectively), or IL-4 intron3 polymorphism ($P=0.30$; $P=0.30$, respectively).

DISCUSSION

Cytokines with polymorphic gene sequences are potential markers of disease susceptibility because their gene products are involved in KD pathogenesis (2,3). This study provides evidence for a genetic association between KD and the IL-1 *Ra* gene. The IL-1 *Ra* is structurally related to IL-1 α and IL-1 β , and competes with these molecules for occupation of IL-1 cell surface receptors. IL-1 *Ra* plays a role as an important regulator of inflammation, and is currently being evaluated in clinical trials (5). Five alleles of the IL-1 *Ra* gene have been reported, corresponding to two to six copies of an 86-basepair (bp) sequence repeat located in intron 2 of chromosome 2 (6). Because three potential protein-binding sites are located in this 86-bp sequence, the number of repeats may influence gene transcription and protein production. In a previous study the presence of the IL-1 *Ra* allele II was associated with enhanced IL-1 β production in vitro (7). The type II IL-1 *Ra* allele has also been found in association with a variety of autoimmune diseases, including alopecia areata, lichen sclerosus, systemic lupus erythematosus, ulcerative colitis, and late-onset psoriasis (8–12).

IL-1 receptor antagonist levels are elevated in the blood of patients with a variety of infectious and immune conditions. The term IL-1 receptor antagonist

refers to two different proteins encoded by the same gene but generated by alternative splicing of two different first exons. The secreted isoform is produced by hepatocytes, and is regulated by proinflammatory cytokines as an acute-phase protein. The KD is an acute inflammatory disease of vessels. The IL-1 *Ra* genes code several proteins that may be key components in the pathogenesis of KD. In this study we observed that the IL-1 *Ra* genotype I/II and II allele are associated with higher susceptibility to KD. These observations suggest that genes that help regulate the level of IL-1 production may be useful in predicting the occurrence of KD. The IL-1 *Ra* genotype I/II and II allele may be involved in the formation of KD through a complex pathway. Such as change the pathway of signal transduction between cells and then change the function of transcription. Endogenous production of IL-1 *Ra* is an important antiinflammatory mechanism in human disease. IL-1 *Ra* binds to the IL-1 receptor with an avidity equal to that of IL-1, and thus fails to stimulate the cells. In our KD patients we observed an imbalance in this system because the relative levels of IL-*Ra* production were not adequate to effectively block the proinflammatory effects of IL-1.

The IL-1 β promoter and IL-1 β exon 5 gene polymorphisms are not useful for predicting a susceptibility to KD. A previous report (13) showed that IL-1 genes may play a role in the severity of the disease rather than in susceptibility to the disease itself. However, in this study we found no difference in the association of IL-1 gene polymorphisms between patients with and without coronary aneurysm.

IL-4 is a cytokine with antiinflammatory properties, and its production is increased in KD patients. The IL-4 -590 T allele and IL-4 intron 3 RP gene polymorphisms have been related to different levels of cytokine production (14). In a study by Hunt et al. (15), the frequency of the T allele of the C/T polymorphism of the IL-4 gene was 14% in the control subjects. In the present study we found that the frequency of the T allele was 77.7 % in controls, in contrast to the findings by Hunt et al. The difference in results may reflect several factors. There may be genuine geographical differences in the data sets. Our data show that the C-T promoter and intron 3 RP1/RP2 polymorphism of the IL-4 gene do not confer protection against the development of KD and coronary aneurysm.

In conclusion, we found that the I/II genotype and II allele of IL-1 *Ra* are associated with KD. In a previous study, we also observed an association between KD and the polymorphic angiotensin-1-converting enzyme I/D gene sequence. These results illustrate that a different gene regulates KD pathogenesis. In addition, there appears to be no significant association between IL-1

gene and IL-4 gene polymorphisms and the potential for the formation of a coronary artery aneurysm in KD patients.

REFERENCES

1. Lin CY, Lin CC, Hwang B, Chiang BN. The changes of interleukin-2, tumor necrotic factor and gamma-interferon production among patients with Kawasaki disease. *Eur J Pediatr* 1991;150:179–182.
2. Hirao J, Hibi S, Andoh T, Ichimura T. High levels of circulating interleukin-4 and interleukin-10 in Kawasaki disease. *Int Arch Allergy Immunol* 1997;112:150–156.
3. Lin CY, Lin CC, Hwang B, Chiang BN. Serial changes of serum interleukin-6, interleukin-8, and tumor necrosis factor alpha among patients with Kawasaki disease. *J Pediatr* 1993;121:924–926.
4. Maury CPJ, Salo E, Pelkonen P. Elevated circulating tumor necrosis factor-alpha in patients with Kawasaki disease. *J Lab Clin Med* 1989;113:651–654.
5. Tsai FJ, Hsieh YY, Chang CC, et al. Polymorphisms for interleukin 1 β exon 5 and interleukin 1 receptor antagonist in Taiwanese children with febrile convulsions. *Arch Pediatr Adolesc Med* 2002;156:545–548.
6. Tarlow JK, Blakemore AIF, Lennard A, et al. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86 base pair tandem repeat. *Hum Genet* 1993;91:403–404.
7. Santtila S, Savinainen K, Hurme M. Presence of the IL-1 *Ra* allele 2 is associated with enhanced IL-1 beta production in vitro. *Scand J Immunol* 1998;47:195–198.
8. Tarlow JK, Clay FE, Cork MJ, et al. Severity of alopecia areata is associated with a polymorphism in the interleukin-1 receptor antagonist gene. *J Invest Dermatol* 1994;103:387–390.
9. Clay FE, Cork MJ, Tarlow JK, et al. Interleukin 1 receptor antagonist gene polymorphism association with lichen sclerosis. *Hum Genet* 1994;94:407–410.
10. Blakemore AIF, Tarlow JK, Cork MJ, et al. Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. *Arthritis Rheum* 1994;37:1380–1385.
11. Mansfield JC, Holden H, Tarlow JK, et al. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology* 1994;106:637–642.
12. Tarlow JK, Cork MJ, Clay FE, et al. Association between interleukin-1 receptor antagonist (IL-1ra) gene polymorphism and early and late-onset psoriasis. *Br J Dermatol* 1997;136:147–148.
13. Demeter J, Messer G, Ramisch S, et al. Polymorphism within the second intron of the IL-1 receptor antagonist gene in patients with hematopoietic malignancies. *Cytokines Mol Ther* 1996;2:239–242.
14. Cantagrel A, Navaux F, Nourhashemi F, et al. Interleukin-1 β receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms relationship to occurrence and severity of rheumatoid arthritis. *Arthritis Rheum* 1999;42:1093–1100.
15. Hunt PJ, Marshall SE, Weetman AP, et al. Cytokine gene polymorphisms in autoimmune thyroid disease. *J Clin Endocrinol Metab* 2000;85:1984–1988.