TARC/CCL17 gene polymorphisms and expression associated with susceptibility and coronary artery aneurysm formation in Kawasaki disease

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BACKGROUND: Kawasaki disease (KD) is a systemic vasculitis of unknown etiology. Thymus and activation-regulated chemokine/chemokine ligand 17 (*TARC/CCL17*) is one of the Th2 chemokines and has been suggested as a candidate gene for conferring susceptibility to Th2 associated with allergy diseases. This study examined the correlation between gene polymorphisms and plasma levels of TARC/CCL17 in patients with KD and the outcomes of KD.

METHODS: A total of 381 KD patients and 564 controls were subjected to determination of five tagging single-nucleotide polymorphisms of *TARC/CCL17*. In addition, plasma TARC/CCL17 levels were measured by enzyme-linked immunosorbent assay.

RESULTS: Polymorphisms of *TARC/CCL17* were significantly different between normal children and patients with KD. A allele of rs4784805 has better intravenous immunoglobulin (IVIG) treatment response to KD. Furthermore, plasma TARC/CCL17 levels were higher in KD patients than that in controls before IVIG treatment. After IVIG treatment, plasma TARC/CCL17 levels decreased significantly.

CONCLUSION: This study provides the first evidence supporting the association between *TARC/CCL17* polymorphisms, susceptibility of KD, and IVIG responses in KD patients.

Kawasaki disease (KD), also known as mucocutaneous lymph node syndrome, is an acute febrile systemic vasculitis that was first described by Kawasaki *et al*. in 1974 in English (1), but its etiology remains unknown till now. It occurs most commonly in children younger than 5 y of age, particularly in children younger than 2 y of age, and the clinical presentation of KD is prolonged fever, conjunctivitis, diffuse mucosal inflammation, polymorphous skin rashes, indurative edema of the hands and feet associated with peeling of finger tips, and nonsuppurative lymphadenopathy (2). In developed countries, KD has been the leading cause of acquired heart diseases in children (2,3). The most serious complication of KD is the occurrence of coronary artery lesions (CALs), including myocardial infarction, coronary artery fistula formation (4), coronary artery dilation, or coronary artery aneurysm (CAA) (5). In addition, KD is the major cause of acquired CAA in childhood (6). The prevalence of KD in children is the highest in Japan, followed by Korea and Taiwan, and the lowest in Europe (5). Therefore, it is possible that a genetic background plays an important role in the pathogenesis of KD.

Thymus and activation-regulated chemokine/chemokine ligand 17 *(TARC/CCL17)* is a member of the CC chemokine group (7). It is a ligand of the CC chemokine receptor (8), which is selectively expressed on Th2 cells (9) and serves for the recruitment and migration of cells bearing this receptor (7–9). *TARC/CCL17* has been suggested as a candidate gene for conferring susceptibility to Th2 associated with allergy diseases and is highly implicated in the pathogenesis of atopic dermatitis (10,11) and bronchial asthma (12). There are several lines of evidence pointing out an abnormal Th1/Th2 balance in KD patients (13). Recently, it has been shown that the incidence of atopic dermatitis and bronchial asthma among children with KD was nine times and three times greater than that of controls (14,15), respectively. In addition, a single-nucleotide polymorphism (SNP) in a C-to-T substitution at position -431 (431C/T) in the 5′-flanking region was demonstrated that significantly increased the levels of serum TARC/CCL17 concentrations (16). Therefore, this study examined the correlation between *TARC/CCL17* gene polymorphisms and plasma levels of TARC/CCL17 in patients with KD and the outcomes of KD.

RESULTS

Association of *TARC/CCL17* Polymorphism With Susceptibility to KD

Table 1 shows the characteristics of the subjects. A total of 381 KD patients and 564 controls were enrolled in this study. In the selected population, 66.8% of cases and 55.5% of controls were male. The mean age of patients and controls was 1.7 ± 1.6 y (SD) and 5.4 ± 3.7 y, respectively. Children were predominant

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in the study population. There were 9.7% of KD with CAL formation and 4.2% with aneurysm formation. Among these KD patients, 12.9% were resistant to initial intravenous immunoglobulin (IVIG) treatment. The distribution of SNP genotypes was in accordance with the Hardy–Weinberg equilibrium for both cases and controls (Table 2). The distribution of *TARC/CCL17* genotypes between the KD patients and healthy children differed significantly (Table 2). In the comparison of the alleles distribution between the KD patients and the normal control, the *TARC/CCL17* SNP rs223895, rs223899, and rs223828 showed significant associations with KD under three genetic models (genotype model: $P = 0.0262$, $P = 0.0095$, and $P = 0.0028$; dominant model: $P = 0.0149$, $P = 0.0031$, and $P = 0.0007$; allelic model: $P = 0.0065$, $P = 0.0033$, and $P = 0.0013$, respectively). Furthermore, the T-allele frequency of rs223895, A-allele frequency of rs223899, and T-allele frequency of rs223828 were all significantly lower in patients with KD than that in the normal control (Table 2; $P = 0.0062$, $P = 0.0025$, and $P = 0.0011$, respectively).

Table 1. Basal characteristics of patients with KD and normal controls

Characteristic	Patients with KD ($n = 381$)	Normal controls ($n = 564$)
Male gender, *n* (%)	247 (66.8)	313 (55.5)
Mean (SD), age (y)	1.7 ± 1.6	5.4 ± 3.7
Age range (y)	0–11	0–23
CAL formation	37 (9.7%)	
Aneurysm formation	16 (4.2%)	
IVIG resistance	49 (12.9%)	

CAL, coronary artery lesion; IVIG, intravenous immunoglobulin; KD, Kawasaki disease.

Association Between *TARC/CCL17* Genetic Polymorphism and CAL, Aneurysm Formation, or IVIG Responsiveness

In the comparison of the distribution of alleles and the risk of CAL formation, as shown in Table 3, there were no differences between genotypes of *TARC/CCL17* and CAL formation. Further analysis found that KD patients with rs16956811 GG genotype ($P = 0.0451$) and rs223828 CC genotype ($P = 0.0389$) had higher risk of aneurysm formation; however, the significance disappeared after Bonferroni correction (Table 4). Moreover, genetic polymorphisms of rs223895, rs4784805, and rs223828 were also associated with aneurysm formation; however, also in this case, the significance disappeared after Bonferroni correction (see **Supplementary Table S1** online). In addition, pharmacogenomics approaches indicated that patients with KD have A allele of rs4784805 ($P = 0.0037$ and $P = 0.0046$, respectively) with better IVIG treatment response (Table 5).

Plasma TARC/CCL17 Levels in the KD Patients and Controls

We measured the plasma TARC/CCL17 protein expressions in the KD patients ($n = 92$) and age-matched febrile controls ($n = 99$) using enzyme-linked immunosorbent assay kits. As shown in Figure 1, we found higher TARC/CCL17 levels in the KD patients than that in the controls (398.01 ± 35.18 and 127.33 ± 14.18 pg/ml, respectively; $P < 0.001$). The TARC/CCL17 levels were greatly decreased after IVIG treatment, and the lowest levels were noted at the subacute stage at least 3 wk after IVIG treatment in KD patients (303.94 ± 29.51 and 160.14 ± 14.64 pg/ml, respectively; both $P < 0.001$). In addition, we found that the plasma TARC/CCL17 levels of pre-IVIG, post-IVIG, and subacute stages did not differ significantly in

Table 2. Genotype and allele frequencies of the *CCL17* gene in controls and patients with KD

		Case (%)	Control (%)	Allele	Case (%)	Control (%)	Genotype	Dominant	Recessive	Allelic
	Genotype	($n = 381$)	($n = 564$)		($n = 381$)	($n = 564$)	*P* value	*P* value	*P* value	*P* value
rs223895	TT	50 (13.9)	98 (18.8)	T	263 (36.5)	448 (43.0)	**0.0262***	**0.0149***	0.0548	**0.0065****
	CT	163 (45.3)	252 (48.3)	C	457 (63.5)	594 (57.0)				
	CC	147 (40.8)	171 (32.8)							
rs4784805	AA	2 (0.6)	3 (0.6)	A	42 (5.9)	61 (5.8)	0.9857	0.8688	0.9983	0.8759
	AC	38 (10.7)	55 (10.4)	C	666 (94.1)	999 (94.2)				
	CC	314 (88.7)	472 (89.0)							
rs16956811	GG	1 (0.3)	0 (0.0)	G	11 (1.5)	12 (1.2)	0.4799	0.6709	0.2306	0.5064
	GT	9 (2.5)	12 (2.3)	T	705 (98.5)	1016 (98.8)				
	TT	348 (97.2)	502 (97.7)							
rs223899	AA	32 (8.9)	63 (12.4)	A	216 (30.1)	374 (36.9)	**0.0095****	**0.0031****	0.1032	**0.0033****
	AG	152 (42.3)	248 (48.9)	G	502 (69.9)	640 (63.1)				
	GG	175 (48.8)	196 (38.7)							
rs223828	TT	26 (7.5)	53 (10.2)	T	183 (26.2)	348 (33.5)	**0.0029****	**0.0007****	0.1680	**0.0013****
	TC	131 (37.5)	242 (46.5)	C	515 (73.8)	692 (66.5)				
	CC	192 (55.0)	225 (43.3)							

KD, Kawasaki disease.

*Significant ($0.01 \le P < 0.05$) values are in bold. **Significant ($P < 0.01$) values are in bold.

Table 3. Genotype and allele frequencies of *CCL17* gene in patients having KD with or without CAL formation

		CAL (%)	Without (%)	Allele	CAL (%)	Without (%)	Genotype	Dominant	Recessive	Allelic
	Genotype	($n = 37$)	($n = 336$)		($n = 37$)	($n = 336$)	*P* value	*P* value	*P* value	*P* value
rs223895	TT	7 (20.0)	41 (12.9)	T	29 (41.4)	226 (35.5)	0.5017	0.5935	0.2945	0.3299
	CT	15 (42.9)	144 (45.3)	C	41 (58.6)	410 (64.5)				
	CC	13 (37.1)	133 (41.8)							
rs4784805	AA	1 (2.9)	1 (0.3)	A	6 (8.6)	35 (5.6)	0.2146	0.5707	0.1918	0.2895
	AC	4 (11.4)	33 (10.6)	C	64 (91.4)	589 (94.4)				
	CC	30 (85.7)	278 (89.1)							
rs16956811	GG	1 (2.9)	0 (0.0)	G	3 (4.3)	8 (1.3)	0.1060	0.2622	0.0997	0.0874
	GT	1 (2.9)	8 (2.5)	T	67 (95.7)	624 (98.7)				
	TT	33 (94.2)	308 (97.5)							
rs223899	AA	4 (11.8)	26 (8.2)	A	21 (30.8)	188 (29.6)	0.7339	0.9167	0.5131	0.8205
	AG	13 (38.2)	136 (42.8)	G	47 (69.2)	448 (70.4)				
	GG	17 (50.0)	156 (49.0)							
rs223828	TT	2 (6.1)	23 (7.5)	T	14 (21.2)	164 (26.6)	0.5859	0.3013	1.0000	0.3414
	TC	10 (30.3)	118 (38.3)	C	52 (78.8)	452 (73.4)				
	CC	21 (63.6)	167 (54.2)							

CAL, coronary artery lesion; KD, Kawasaki disease.

Table 4. Genotype and allele frequencies of the *CCL17* gene in patients with KD with aneurysm or without aneurysm

	Genotype	Aneurysm ($n = 16$) (%)	Without aneurysm ($n = 362$) (%)	Allele	Aneurysm ($n = 16$) (%)	Without aneurysm ($n = 362$) (%)	Genotype (*P* value)	Dominant (*P* value)	Recessive (*P* value)	Allelic (*P* value)
rs223895	TT	2 (13.3)	48 (14.0)	T	8 (26.7)	252 (36.8)	0.2860	0.1302	1.0000	0.2569
	CT	4 (26.7)	156 (45.6)	C	22 (73.3)	432 (63.2)				
	CC	9 (60.0)	138 (40.4)							
rs4784805	AA	0 (0.0)	2 (0.6)	A	0 (0.0)	41 (6.1)	0.4478	0.2339	1.0000	0.2489
	AC	0 (0.0)	37 (11.0)	C	32 (100.0)	629 (93.9)				
	CC	16 (100.0)	296 (88.4)							
rs16956811	GG	1 (6.2)	0 (0.0)	G	2 (6.3)	9 (1.3)	0.0502	0.3733	**0.0451***	0.0840
	GT	0 (0.0)	9 (2.7)	T	30 (93.7)	669 (98.7)				
	TT	15 (93.8)	330 (97.3)							
rs223899	AA	2 (12.5)	30 (8.8)	A	8 (25.0)	206 (30.3)	0.3618	0.2646	0.6453	0.5233
	AG	4 (25.0)	146 (43.0)	G	24 (75.0)	474 (69.7)				
	GG	10 (62.5)	164 (48.2)							
rs223828	TT	1 (6.3)	25 (7.6)	T	4 (12.5)	177 (26.8)	0.0704	**0.0389***	1.0000	0.0973
	TC	2 (12.5)	127 (38.5)	C	28 (87.5)	483 (73.2)				
	CC	13 (81.2)	178 (53.9)							

KD, Kawasaki disease.

*Significant ($0.01 \leq P < 0.05$) values are in bold.

the occurrence of CAL, CAA, and the responsiveness in KD patients (data not shown; all $P > 0.1$).

Plasma TARC/CCL17 Levels in the Different Genotypes of KD Patients and Controls

We explored the functional effect of the genetic polymorphisms of TARC gene expression. As shown in **Table 6**, TARC/CCL17 levels were higher in the patients with KD than that in the controls ($P < 0.001$). However, no significant correlation between genetic polymorphisms and protein expression was noticed.

DISCUSSION

The immunopathogenesis of KD remains unknown. Several studies have pointed out an imbalanced immunity between Th1 and Th2 reaction in patients with KD. Sekiya *et al.* (16) first demonstrated a functional SNP in the 5′-flanking region (-431 C/T) of the *TARC/CCL17* gene; however, there was no significant

Table 5. Genotype and allele frequencies of the *CCL17* gene in patients with KD responding or not responding to IVIG treatment

	Genotype	Resistant ($n = 49$) (%)	Responsive ($n = 332$) (%)	Allele	Resistant ($n = 49$) (%)	Responsive ($n = 332$) (%)	Genotype (P value)	Dominant (P value)	Recessive (P value)	Allelic (P value)
rs223895	CC	4 (8.7)	46 (14.9)	C	30 (32.6)	229 (37.2)	0.5261	0.7097	0.3637	0.3963
	CT	22 (47.8)	137 (44.5)	T	62 (67.4)	387 (62.8)				
	TT	20 (43.5)	125 (40.6)							
rs4784805	AA	0 (0.0)	2 (0.7)	A	0 (0.0)	41 (6.8)	**0.0200***	**0.0046****	1.0000	**0.0037****
	AC	0 (0.0)	37 (12.2)	C	92 (100.0)	563 (93.2)				
	CC	46 (100.0)	263 (87.1)							
rs16956811	GG	1 (2.2)	0 (0.0)	G	4 (4.3)	7 (1.1)	0.0597	0.1296	0.1307	**0.0436***
	GT	2 (4.3)	7 (2.3)	T	88 (95.7)	605 (98.9)				
	TT	43 (93.5)	299 (97.7)							
rs223899	AA	2 (4.5)	30 (9.7)	A	24 (26.7)	189 (30.7)	0.5127	0.7317	0.4020	0.4382
	AG	20 (44.4)	129 (41.9)	G	66 (73.3)	427 (69.3)				
	GG	23 (51.1)	149 (48.4)							
rs223828	TT	2 (4.8)	24 (8.0)	T	17 (20.2)	163 (27.1)	0.4161	0.2015	0.7545	0.1820
	TC	13 (30.9)	115 (38.2)	C	67 (79.8)	439 (72.9)				
	CC	27 (64.3)	162 (53.8)							

IVIG, intravenous immunoglobulin.

*Significant ($0.01 \le P < 0.05$) values are in bold. **Significant ($P < 0.01$) values are in bold.

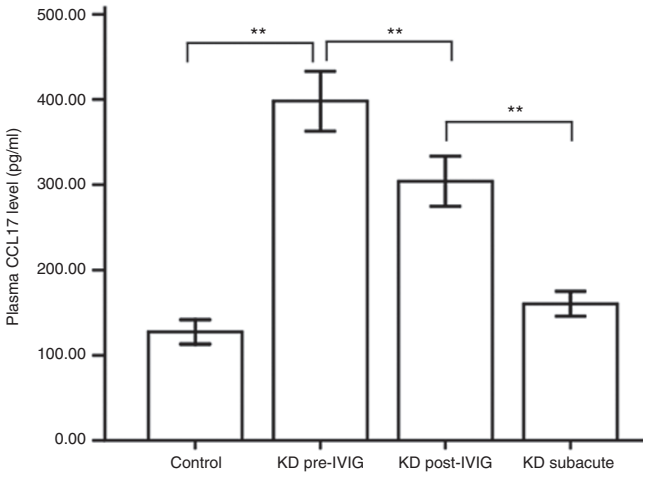

Figure 1. Comparison of plasma TARC/CCL17 levels by enzyme-linked immunosorbent assay (ELISA) between controls ($n = 99$) and patients with Kawasaki disease (KD) ($n = 92$). There were higher TARC/CCL17 levels in the KD patients than in the controls before intravenous immunoglobulin (IVIG) treatment. Furthermore, the TARC/CCL17 levels were greatly decreased after IVIG treatment and the lowest levels at subacute stage at least 3 wk after IVIG treatment in KD patients. **$P < 0.01$. Data are presented as mean ± SE.

association of the SNP with susceptibility to bronchial asthma and atopic dermatitis (10,16). In this study, we report the correlation between *TARC/CCL17* genetic polymorphisms and KD. Furthermore, we also found that plasma TARC/CCL17 protein levels changed dynamically over the course of the illness. Plasma TARC/CCL17 levels were higher in KD patients than that in controls ($P < 0.001$) before IVIG treatment. After IVIG treatment, plasma TARC/CCL17 levels decreased significantly ($P < 0.001$). However, the change of TARC/CCL17 levels was not associated with IVIG treatment response and the occurrence of CAL and CAA. The plasma TARC/CCL17 levels were markedly increased in patients with KD in the acute stage and greatly decreased after IVIG treatment.

TARC/CCL17, a ligand for CC chemokine receptor 4 and CC chemokine receptor 8, serves for the recruitment and migration of lymphocytes of the Th2 phenotype (8,9) and direct T-cell and macrophage recruitment into areas of allergic inflammation. In human and animal studies, it is demonstrated that TARC/CCL17 mRNA and protein are potently induced by the Th2 cytokine, interleukin-4 (IL-4), through induction of promoter binding of signal transducer and activator of transcription 6 (STAT6) (17,18). IL-4/signal transducer and activator of transcription 6 signaling during T-cell development contributes to polarized patterns of cytokine expression manifested by differentiated Th cells (19). IL-4, mainly produced by Th2 T cells, mast cells, basophils, and eosinophils, appears to be a crucial factor in allergic responses (20). However, prolonged IL-4 stimulation might result in the downregulation of TARC/CCL17 expression through classical negative-feedback loops including the activation of suppressors of cytokine signaling families (21). Hijnen *et al.* (22) demonstrated that TARC/CCL17 levels in patients with atopic dermatitis were significantly higher than those in healthy controls. Furthermore, they can be objective parameters for disease severity–specific atopic dermatitis and treatment monitoring. In our KD patients, we found that significantly high levels of TARC/CCL17 have also been observed than those in the controls, which was compatible with our previous findings of increased IL-4/IL-5 expression in patients with KD (13). Moreover, our previous study showed that IL-4, IL-5, and eotaxin increased significantly after IVIG treatment (13). Not

Table 6. Differences in TARC levels among patients with KD and control subjects stratified by CCL17 genotype

		Patients with KD		Control subjects	
SNP	Genotype	*n*	Mean ± SD	*n*	Mean ± SD
rs223895	TT	9	303.27 ± 233.91[a]	14	91.98 ± 69.23
	CT	39	465.39 ± 387.14	50	121.94 ± 108.90
	CC	30	296.22 ± 244.63	27	108.91 ± 108.85
	P value		0.0802		0.6138
rs4784805	AA	0	–	0	–
	AC	7	317.44 ± 184.21	13	113.40 ± 82.09
	CC	71	386.70 ± 341.92	80	117.24 ± 108.74
	P value		0.6002		0.9036
rs16956811	GG	0	–	0	–
	GT	1	506.37	1	151.03
	TT	77	386.51 ± 332.97	93	111.83 ± 104.53
	P value		0.7216		0.7100
rs223899	AA	7	327.44 ± 236.43	8	95.73 ± 70.67
	AG	37	458.14 ± 384.14	47	120.20 ± 113.62
	GG	34	300.06 ± 236.13	34	110.07 ± 99.02
	P value		0.1059		0.7982
rs223828	TT	3	196.16 ± 27.23	5	49.74 ± 19.49
	TC	34	485.80 ± 394.44	47	111.26 ± 91.24
	CC	38	310.38 ± 262.43	42	120.47 ± 119.59
	P value		0.0506		0.3538

KD, Kawasaki disease; SNP, single-nucleotide polymorphism.

[a]Data represent means ± SD.

surprisingly, the TARC/CCL17 levels were greatly decreased after IVIG treatment in KD patients, which raises the possibility of the activation of negative-feedback loops (23,24). Of note, there were still higher TARC/CCL17 levels at a subacute stage than those in the controls ($P < 0.001$). However, we found that the plasma TARC/CCL17 levels of pre-IVIG, post-IVIG, and subacute stages did not differ significantly in the occurrence of CAL, CAA, and the responsiveness in the KD patients. However, high levels of TARC/CCL17 mRNA expression are seen in some, but not all, human arteries with advanced atherosclerotic lesions which could play a role in mononuclear cell recruitment into atherosclerotic lesions and influence the subsequent inflammatory response (25).

In consonance with the findings of Sekiya *et al.* (16) and Tsunemi *et al.* (22) in allergic disease, we also found increased plasma TARC/CCL17 levels in the KD patients than those in the controls, but the differences were not significant. Indeed, we found that the TARC/CCL17 levels were not associated with the genotypes of *TARC/CCL17* in the patients with KD and in the controls, which reflects that the upregulation of TARC/CCL17 levels might be due to the disease itself and not because of the genotype difference.

Genetics might play an important role in the pathogenesis of KD (26). Genome-wide association study for KD was firstly performed by Burgner *et al.* (27). A total of 40 SNPs and 6 haplotypes were confirmed in an independent cohort of KD families. In Asian population, Onouchi *et al.* (28) identified the susceptibility loci on chromosome 19q13.2 and further confirmed the functional SNP of inositol 1, 4, 5-trisphosphate 3-kinase C in the disease activity of KD. Recently, it has been demonstrated that rs28493229 is associated with susceptibility to KD and CAL formation (28,29). We have also demonstrated that the A allele of rs72689236 located in the 5′-untranslated region of caspase 3 is very likely to be a risk allele in the development of aneurysm in patients with KD (30) and the association of *CTLA-4* (+49) A/G polymorphism with the CAL formation of KD, particularly in female patients (31). In this study, we revealed that *TARC/CCL17* gene polymorphisms have an association with the susceptibility of KD and were significantly associated with disease outcomes of KD patients in a Taiwanese population. Indeed, to the best of our knowledge, the allelic distribution of *TARC/CCL17* between ethnic groups is still unknown. Future studies of *TARC/CCL17* genotypes in patients with KD of other ancestries could prove to be quite interesting.

This study has potential limitations that should be reviewed. First, there were still higher TARC/CCL17 levels at the subacute stage at least 3 wk after IVIG treatment in KD patients as compared with the controls, and this should be ascertained through longitudinal studies to elucidate the time-dependent changes in TARC/CCL17 levels, especially during the convalescent stage of KD. Second, we cannot exclude the possibility that the higher TARC/CCL17 levels in the KD patients as compared with the controls were due to a longer duration of fever in the KD patients than that in the controls. Third, to identify low-frequency genetic polymorphisms in *TARC/CCL17*, application of direct sequencing is needed.

Conclusion

This study provides the first evidence supporting the association of *TARC/CCL17* polymorphisms with the susceptibility to KD and clinical outcome as well as significantly increased *TARC/CCL17* levels in KD patients. The role of elevated TARC levels in KD patients remains unknown. Further studies are warranted to investigate the pathophysiological basis for these findings with regard to KD.

METHODS

Patients Studied

A total of 381 patients with KD and 564 controls were enrolled in this study. The prevalence of KD is <1 in 1,000 children in a Taiwanese population. Therefore, we assumed that there was no KD case in the control group. Blood samples from the febrile age-matched control patients, who were admitted for upper and/or lower respiratory tract infections (including acute bronchiolitis, acute pharyngitis, acute bronchitis, croup, and acute tonsillitis), were used for cytokine comparison. All KD patients were initially treated with a single dose of IVIG (2 g/kg) during a 12-h period. This study was approved by the Institutional Review Board of the Chang Gung Memorial Hospital, and informed consent was obtained from either the parents or guardians of the children. Blood samples were collected within 24 h before IVIG treatment (pre-IVIG stage), within 3 d after IVIG treatment (post-IVIG stage), and at least 3 wk after IVIG treatment (subacute stage). Patients whose symptoms did not fit the KD criteria or those who had suffered from an acute fever for <5 d were excluded. All

the KD patients underwent two-dimensional pulse Doppler and color flow imaging at least three times within 8 wk from the onset of the illness. If patients had abnormal coronary arteries, echocardiographic follow-up was scheduled every 3–6 mo within the first year, and then once annually until the affected coronary arteries resumed being normal as our previous reports (32,33). Echocardiography was performed with a SONOS 5500 or 7500 cardiac scanner (Philips, Andover, MA). Five- to eight-MHz sector phased array transducers were used for this study. Two-dimensional echocardiography was performed to visualize the diameter of the right and left coronary arteries on the parasternal short-axis view of the aorta (4). According to the Japanese Ministry of Health guidelines, a CAL was defined by the internal diameter of the coronary artery being >3 mm (4 mm, if the subject was older than 5 y of age) or the internal diameter of a segment being at least 1.5 times that of an adjacent segment, as observed in the echocardiogram. KD patients with coronary artery ectasia or dilation that was disappearing within the initial 8 wk after the onset of illness were defined as transient ectasia and not judged as CAL (30,34). In addition, coronary arteries were classified on the basis of presence or absence of aneurysms according to the criteria from the Japanese Circulation Society Joint Working Group. A CAA (including medium and giant aneurysms) was defined by the internal diameter of the coronary artery being at least 4 mm or, in children older than 5 y of age, the internal diameter of a segment being at least 1.5 times that of an adjacent segment as observed from echocardiography (30,34). IVIG responsiveness was defined as defervescence 48 h after the completion of IVIG treatment and no fever (defined as a temperature >38 °C) recurrence for at least 7 d after IVIG, with marked improvement or normalization of inflammatory signs (33,35). Blood samples were immediately placed in tubes containing heparin, and the remaining aliquots of plasma were stored at −80 °C until assay.

DNA Extraction

Blood cells were subjected to DNA extraction by treating them first with cell lysis solution (Cat. No.:158908; Qiagen Sciences, Germantown, MD) and RNase A (Cat. No.: R6513, Sigma-Aldrich, St. Louis, MO) for digestion ribonuclease for 15 min at 37 °C, and then protein precipitation solution (Cat. No.:158912, Qiagen Sciences) for digestion of nuclear protein. Total DNA was harvested by using Puregen manufacturer's followed by isopropanol precipitation.

Genotyping

Five tagging SNPs of *TARC/CCL17* (rs223895, rs4784805, rs16956811, rs223899, and rs223828) with a minimum allele frequency of >1% in the Han Chinese in Beijing population were selected from the HapMap database (http://hapmap.ncbi.nlm.nih.gov/). All polymorphisms of TARC/CCL17 located in the intron. Genomic DNA was extracted from whole-blood samples by using the standard method as described in our previous study (29). Genotyping was carried out using the TaqMan Allelic Discrimination Assay (Applied Biosystems, Foster City, CA). Briefly, the PCR was performed by using a 96-well microplate with the ABI 7500 Realtime PCR system (Applied Biosystems). The thermal cycle conditions were as follows: denaturing at 95 °C for 10 min, followed by 40 cycles of denaturing at 92 °C for 15 s, and annealing and extension at 60 °C for 1 min. After the PCR, fluorescence was measured and analyzed using the System SDS software version 1.3.1 (Applied Biosystems). The average genotyping successful rate in our laboratory is ~95.7%, so some individuals are without genotype data.

Measurement of Cytokines by Enzyme-Linked Immunoassay

We used enzyme-linked immunoassays to measure TARC/CCL17 (human TARC/CCL17; R&D Systems, Minneapolis, MN) according to the manufacturers' instructions.

Statistical Analysis

All data are presented as mean ± SE. Quantitative data were analyzed by using the Student's *t*-test or one-way ANOVA when appropriate. The least significant difference test was used for *post hoc* testing where appropriate. Changes in the data before and after IVIG treatment as well as subacute stage were tested by the paired sample *t*-test. The genotypes and allele frequencies associated with the KD patients and disease outcomes were tested by χ^2 test or Fisher's exact test when appreciate. Two-sided *P* values <0.05 were considered statistically significant. All statistical tests were performed using SPSS version 13.0 for Windows XP (SPSS, Chicago, IL).

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr

STATEMENT OF FINANCIAL SUPPORT

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