ITPKC and *SLC11A1* gene polymorphisms and gene-gene interactions in Korean patients with Kawasaki disease

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ABSTRACT

ITPKC and *SLC11A1* gene polymorphisms and gene-gene interactions in Korean patients with Kawasaki disease

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Kawasaki disease (KD) is an acute systemic vasculitis. Both the etiology of KD and the cause of Bacille Calmette-Guérin (BCG) injection site erythema characteristically observed in the disease are poorly understood. Many candidate KD-associated genes have been studied. The present study investigated the association between KD and single nucleotide polymorphisms (SNPs) in two candidate genes: inositol 1,4,5-triphosphate 3-kinase (*ITPKC*), one of the most well-studied KD-associated genes, and solute carrier 11a1 (*SLC11A1*), which is reportedly associated with the hypersensitive reaction to the BCG strain in Koreans. Potential associations between BCG injection site erythema and SNPs in the *ITPKC* and *SLC11A1* genes were also evaluated. Gene-gene interactions between *ITPKC* and *SLC11A1* in KD and BCG injection site erythema were also analyzed. Three tagging SNPs in

ITPKC and five tagging SNPs in *SLC11A1* were genotyped in 299 KD patients and 210 control children. Allele, genotype, and gene-gene interactions were analyzed between the KD and control groups and between subjects with/without BCG injection site erythema. SNP rs28493229 in *ITPKC* was associated with KD and coronary artery complications. SNP rs77624405 in *SLC11A1* was associated with KD. Comparisons of KD patients with and without BCG injection site erythema revealed that SNP rs17235409 in *SLC11A1* was associated with erythema, but no erythema-associated SNPs in *ITPKC* were identified. Interactions between *ITPKC* rs28493229_GG and *SLC11A1* rs17235409_GA and between *ITPKC* rs10420685_GG and *SLC11A*1 rs17235409_AA were significantly associated with BCG injection site erythema. In conclusion, this study identified several important polymorphisms in the *ITPKC* and *SLC11A1* genes in Koreans. The genetic variants identified in this study affected KD and erythema of BCG injection sites independently and through gene-gene interactions. In addition, the effects of the polymorphisms were age dependent.

Key words: Kawasaki disease, *ITPKC*, *SLC11A1*, Single-nucleotide polymorphism, Gene-gene interaction, coronary arterial lesions, BCG

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I. INTRODUCTION

Kawasaki disease (KD) is an acute systemic vasculitis that primarily affects children younger than 5 years of age. There are no specific laboratory tests for the diagnosis of KD; therefore, diagnosis is usually made according to clinical criteria.[1] Following Japan, Korea has the second highest prevalence of KD in the world, and the incidence of this disease is increasing steadily.[2] Although KD is a self-limiting disease, if not treated properly with intravenous immune globulin (IVIG), coronary artery lesions (CALs) can occur. In developed countries, KD is the most common cause of acquired heart disease.

KD exhibits an epidemic pattern. The peak incidence is at 9–11 months of age, coinciding with waning maternal immunity. Symptoms are similar to those of other infectious diseases, and the disease course is usually self-limited. For these reasons, KD was once thought to be an infectious disease. Several organisms were reported as causative agents, including viruses and species of

some bacteria, such as *Streptococcus* and *Staphylococcus*. However, no causative infectious organisms have been isolated from patients.[3]

Genetic predisposition to KD has also been suggested. Worldwide, the annual incidence of KD is highest (and increasing) in Japan, Korea, and Taiwan. This incidence is 10–20 times higher than that of Western countries. Interestingly, the same incidence level in people of Japanese ancestry living in Hawaii indicates that the predilection for oriental populations might not be due solely to geographic factors. KD also exhibits familial accumulation. The relative risk for siblings is about 10-fold greater, and a recent study revealed that there are more two-generation KD patients than would be expected. HLA types and allotypes of immunoglobulin in relation to KD have been studied, but the results differ by study and region.[4-6] Lack of agreement between studies may be due to differences in genetic background among the different races involved. Thus, KD can be thought of as a complex multifactorial disease that could develop in association with as yet unidentified infectious organisms in children with a predisposing genetic background.[7-9]

With the recent advent of genome-wide association studies, remarkable progress has been made in identifying candidate genes associated with KD.[10] One of the most interesting candidate genes is inositol-1,4,5-triphosphate 3-kinase (*ITPKC*), which was first reported in Japan.[11] Different single-nucleotide polymorphisms (SNPs) in the *ITPKC* gene have been reported in several countries.[12] [11,13-16] However, a previous study[17] found no

polymorphisms in *ITPKC* in Korean KD patients. Interestingly, a recent study reported that polymorphisms in *ITPKC* are associated with reactivation of Bacille Calmette-Guérin (BCG) injection scars.[18]

Erythema and induration of the BCG injection site is a supporting feature of the clinical picture of KD[19,20] and is present in ~30–50% of KD patients, especially those younger than 2 years of age.[21-23] Although erythematous changes in the BCG inoculation site in patients with human herpes virus type 6 infection have been reported,[24] this clinical feature is known as a specific finding for KD. Chun et al. used BCG in the development of an animal model of KD in programmed death-1 gene knockout mice.[25] These findings suggest a possible correlation between BCG and the pathogenesis of KD.

In inbred mice, resistance and/or susceptibility to the growth of BCG is controlled by the Bcg locus, also known as the natural resistance–associated macrophage protein 1 (*NRAMP1*) gene. The *NRAMP1* gene was later renamed solute carrier 11a1 (*SLC11A1*), and the human homologue was subsequently isolated. Interactions of macrophages with bacterial lipopolysaccharide and/or natural killer cell– or T cell–derived interferon-γ are regulated by *SLC11A1*. Polymorphisms in *SCL11A1* are thought to induce a hypersensitivity reaction to the BCG strain. A relationship between *SCL11A1* polymorphisms and KD has been reported.[26]

This study examined polymorphisms in the *ITPCK* gene in Korean patients with KD. Polymorphisms in *SLC11A1* were also examined to determine a

possible association with KD. The relationship between KD susceptibility and polymorphisms in these genes was also examined. As other studies have investigated interactions between these two genes, especially with respect to synergistic effects,[27-32] gene-gene interactions and/or synergistic effects between *SCL11A1* and *ITPKC* polymorphisms in KD were also investigated.

II. MATERIALS AND METHODS

1. Subject groups

We evaluated 299 patients with typical KD who were admitted to Severance Children's Hospital between January 1, 2012, and October 31, 2015. The patients were diagnosed using criteria of the Japanese Kawasaki Disease Research Committee.[33] Incomplete or atypical KD cases were excluded. The control group included healthy children with no past history of KD who had visited the endocrinology clinic of our hospital for cosmetic reasons.

The KD patients were dichotomized based on age: below or over the age of 24 months, as erythema of BCG scar sites is predominantly observed in children younger than 24 months.

This study was approved by the Institutional Review Board Ethics Committee of Yonsei University College of Medicine (2008-0055-010).

2. Genomic DNA extraction and whole-gene sequencing

Genomic DNA was extracted using a QIAmp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). DNA was quantified using an Epoch microplate spectrophotometer (BioTek, Winooski, VT, USA).

In order to identify causative variants, we sequenced the *SLC11A1* and *ITPKC* genes in 24 KD patients less than 24 months old who appeared to have BCG injection site erythema. Genomic sequences for analysis were obtained from the GenBank (http://www.ncbi/nlm.nih.gov/) database. Polymerase chain reaction (PCR) primers for amplifying the exon and promoter regions (2 kb upstream from exon 1) of the genes were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/).[34] Each amplified PCR fragment was sequenced using BigDye Terminator chemistry on an ABI Prism 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. The results were then expanded to include all KD patients and controls. DNA polymorphisms were identified using the PolyPhred program (http://droog.gs.washington.edu/polyphred/).[35] Tagging SNPs were selected using Haploview (version 4.2). Among SNPs selected according to whole-gene sequencing of *SLC11A1* and *ITPKC* through linkage disequilibrium (LD) analysis, genotyping of tagging SNPs was carried out for all samples using the TaqMan® fluorogenic 5'-nuclease assay (Applied Biosystems). The polymorphism rs2290692 was added in the 3'-UTR of the *ITPKC* gene, as it was recently identified in Han Chinese and is known to be associated with susceptibility to KD.[15] Real-time PCR was performed using a QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems). After PCR and genotyping, the data were analyzed using 7500 SDS 2.3 software (Applied Biosystems).

3. Statistical analysis

Statistical analysis was performed using SAS software (version 9.0). To test for associations with KD, the v2 or Cochran-Armitage trend tests were used to compare allele and genotype frequencies between cases and controls. A p-value less than 0.05 was considered indicative of statistical significance.

Synergistic effects between *SLC11A1* and *ITPKC* genetic polymorphisms were analyzed using combination modes of multiple genetic loci. Searches for the best combination pattern of the genetic polymorphisms in surveyed genes were carried out based on the principle of maximization of both cross-validation consistency and test balance accuracy in order to evaluate interactions between the two genes of interest in relation to KD.

III. RESULTS

1. Clinical characteristics of the subjects

A total of 299 KD patients and 210 healthy control children were recruited (Table 1). Of the 299 KD patients, 77 (25.75%) had BCG injection site erythema. When the subjects were dichotomized by age above or below 24 months, the percentage of patients exhibiting erythema of the BCG site differed ($p<0.001$). A total of 62 of 114 (54.39%) younger patients had erythema of the BCG injection site. Among the older subjects, only 15 of 185 (8.11%) exhibited BCG injection site erythema.

Table 1. Characteristics of the study subjects

	KD patients			Controls (210)	*p*-value
	All (299)	< 24 month (114)	≥ 24month (185)		
Age (month)	41.81 ± 39.49	11.81 ± 5.88	60.33 ± 40.05	115.97 ± 35.24	
Gender (M:F)	194:105	81:33	113:72	130:80	
BCG injection site erythema	77	62	15	NA	<0.001†
WBC	12,900.73 ± 5,315.07	13,594.09 ± 5,195.91	12,432.82 ± 5,359.14	6,910.71 ± 2,01.68	<.0001 *
Platelet	399.97 ± 182.73	425.72 ± 181.55	382.60 ± 182.02	304.70 ± 71.17	<.0001 *
ESR	63.51 ± 32.49	59.93 ± 33.15	65.98 ± 31.90	11.00 ± 9.81	<.0001 *
CRP	57.75 ± 53.02	51.09 ± 47.92	62.33 ± 55.95	9.152 ±14.61	0.0686 *
LDH	334.75 ± 145.66	334.06 ± 115.38	335.21 ± 163.21	241.21 ± 41.67	<.0001 *
Coronary complication	55	13	42	NA	0.008†
Recur of KD	48	18	30	NA	0.296†

* *p*-value for comparison between KD patients and controls; † *p*-value for comparison between < 24-month-old KD patients (younger age group) and ≥ 24-month-old KD patients (older age group)

KD patients exhibited elevations in white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, and lactate dehydrogenase (LDH) level compared with control subjects.

CALs were observed in 55 of 299 patients (18.39%). CALs occurred more frequently (p=0.008) in the older age group (42 of 185 patients, 22.70%) compared with the younger age group (13 of 114 patients, 11.40%). Forty-eight patients (16.05%) had recurrence of clinical symptoms. However, there was no difference in recurrence of clinical symptoms between the older age group (30 of 185 patients, 16.22%) and the younger age group (18 of 114 patients, 15.79%).

2. Polymorphisms of *ITPKC* and *SLC11A1* gene and association with clinical or laboratory findings

According to the results of preliminary exon sequencing for 24 patients, 7 SNPs in *ITPKC* and 10 SNPs in *SLC11A1* were selected. No novel SNPs were identified. Based on the results of LD analyses, three tagging SNPs in *ITPKC* and five tagging SNPs in *SLC11A1* were selected. The frequencies for the entire study group and the control group are listed in Table 2.

Comparisons of the genotype and allele frequencies in the patient and control groups revealed a significant difference only for rs28493229 in the intron of *ITPKC* (p=0.0446 and p=0.0219, respectively). Over-representation of the C allele in rs28493229 of *ITPKC* was observed (Table 3). Similar results were observed in the younger patients after dichotomization based on age (p=0.0392 and p=0.0307, respectively).

Although no significant difference between the patient and control groups was observed for rs77624405 in exon 12 of *SLC11A1*, when the patients were dichotomized, a significant difference was found in the younger patients (p=0.0083 and p=0.0092, respectively). Overrepresentation of the A allele in rs77624405 of *SLC11A1* gene was observed. (Table 4)

The relationship between the identified SNPs and laboratory data was also assessed. The laboratory data differed between the KD patients and normal controls, and the alleles of the related SNPs were heterogeneous.

Table 2. Frequency of SNPs in the *ITPKC* and *SLC11A1* genes for the patient and control groups

		Genotypes			*P*	Alleles		*P*
ITPKC		CC	CT	TT		C	T	
rs2561531	Controls	130(61.90)	70(33.33)	10(4.76)	0.9568	330(78.57)	90(21.43)	0.8556
	KD patients	184(61.54)	99(33.11)	16(5.35)		467(78.09)	131(21.91)	
		GG	GC	CC		G	C	
rs28493229	Controls	178(84.76)	32(15.24)	0(0.00)	0.0446	388(92.38)	32(7.62)	0.0219
	KD patients	231(77.26)	64(21.40)	4(1.34)		526(87.96)	72(12.04)	
		AA	AG	GG		A	G	
rs10420685	Controls	114(54.29)	76(36.19)	20(9.52)	0.8360	304(72.38)	116(27.62)	0.7774
	KD patients	156(52.17)	116(38.80)	27(9.03)		428(71.57)	170(28.43)	
		GG	GC	CC		G	C	
rs2290692	Controls	65(30.95)	97(46.19)	48(22.86)	0.9331	227(54.05)	193(45.95)	0.9075
	KD patients	89(29.77)	143(47.83)	67(22.41)		321(53.68)	277(46.32)	
SLC11A1		CC	CT	TT		C	T	
rs7573065	Controls	194(92.38)	15(7.14)	1(0.48)	0.4048	403(95.95)	17(4.05)	0.8146
	KD patients	273(91.30)	26(8.70)	0(0.00)		572(95.65)	26(4.35)	
		GG	GA	AA		G	A	
rs2276631	Controls	143(68.10)	59(28.10)	8(3.81)	0.9088	345(82.14)	75(17.86)	0.6878
	KD patients	209(69.90)	79(26.42)	11(3.68)		497(83.11)	101(16.89)	
		CC	CT	TT		C	T	
r17221959	Controls	186(88.57)	24(11.43)	0(0.00)	0.202	396(94.29)	24(5.71)	0.2195
	KD patients	253(84.62)	46(15.38)	0(0.00)		552(92.31)	46(7.69)	
		GG	GA	AA		G	A	
rs77624405	Controls	204(97.14)	6(2.86)	0(0.00)	0.0723	414(98.57)	6(1.43)	0.076
	KD patients	280(93.65)	19(6.35)	0.(0.00)		579(96.82)	19(3.18)	
		GG	GA	AA		G	A	
rs17235409	Controls	165(78.57)	42(20.00)	3(1.43)	0.8859	372(88.57)	48(11.43)	0.7799
	KD patients	239(79.93)	55(18.39)	5(1.67)		533(89.13)	65(10.87)	

Table 3. Frequencies of *ITPKC* rs28493229

		Genotypes				Alleles		
		GG	GC	CC	*P*	G	C	*P*
All	Controls	178 (84.76)	32 (15.24)	0 (0.00)	0.0446	388 (92.38)	32 (7.62)	0.0219
	KD patients	231 (77.26)	64 (21.40)	4 (1.34)		526 (87.96)	72 (12.04)	
< 24 month	Controls	178 (84.76)	32 (15.24)	0 (0.00)	0.0392	388 (92.38)	32 (7.62)	0.0307
	KD patients	86 (75.44)	27 (23.68)	1 (0.88)		199 (87.28)	29 (12.71)	
≥ 24month	Controls	178 (84.76)	32 (15.24)	0 (0.00)	0.0646	388 (92.38)	32 (7.62)	0.0523
	KD patients	145 (78.38)	37 (20.00)	3 (1.62)		327 (88.38)	43 (11.62)	

Table 4. Frequencies of *SLC11A1* rs77624405

		Genotypes			Alleles		
		GG	GA	*P*	G	A	*P*
All	Controls	204 (97.14)	6 (2.86)	0.0723	414 (98.57)	6 (1.43)	0.076
	KD patients	280 (93.65)	19 (6.35)		579 (96.82)	19 (3.18)	
< 24 month	Controls	204 (97.14)	6 (2.86)	0.0083	414 (98.57)	6 (1.43)	0.0092
	KD patients	103 (90.35)	11 (9.65)		217 (95.18)	11 (4.82)	
≥ 24month	Controls	204 (97.14)	6 (2.86)	0.4252	414 (98.57)	6 (1.43)	0.4294
	KD patients	177 (95.68)	8 (4.32)		362 (97.84)	8 (2.16)	

The rs2290692 polymorphism in *ITPKC* was associated with elevations in WBC and platelet counts and LDH levels in the KD group, as compared with the control group. When the KD group was dichotomized based on age above and below 24 months, a different polymorphism pattern was observed. In the younger patients, elevations in WBC count and LDH level were associated with different SNPs compared with the non-dichotomized KD group. The

rs28493229 polymorphism was associated with elevated WBC count, and the rs10420685 polymorphism was associated with elevated LDH level in the younger patients. Elevated platelet level in the younger patients was associated with the same rs28493229 SNP, similar to the non-dichotomized KD group. Elevated ESR was associated with polymorphism rs2290692 in the younger patients but not in the non-dichotomized KD group. In the older patients, polymorphism rs2290692 was associated with elevated WBC count, similar to the non-dichotomized KD group. Polymorphism rs28493229 was associated with elevated platelet count in the older patients, a pattern that differed compared with that of the non-dichotomized KD group and younger patients (Table 5).

Table 5. Association between laboratory data and polymorphisms in the *ITPKC* and *SLC11A1* genes

ITPKC gene	SNP	Values	p Value
KD Vs Control	rs2290692_GC	Elevation of WBC	0.0209
	rs2290692_GC	Elevation of platelet	0.0241
	rs2290692_CC	Elevation of LDH	0.0396
Younger group Vs Control	rs28493229_CC	Elevation of WBC	0.0004
	rs2290692_GC	Elevation of platelet	0.0461
	rs2290692_GC	Elevation of ESR	0.0248
	rs10420685_AG	Elevation of LDH	0.0139
Older group Vs Control	rs2290692_GC	Elevation of WBC	0.0328
	rs28493229_CC	Elevation of platelet	0.0078
SLC11A1 gene	SNP	Values	p Value
Younger age group Vs Control	rs2276631_GA	Elevation of AST	0.0218
Older age group Vs Control	rs17235409_GA	Elevation of LDH	0.0498

Polymorphism of rs17235409 in *SLC111A1* gene was associated with elevated LDH in the older age group, and polymorphism of rs2276631 in *SLC11A1 gene* was associated with increased aspartate aminotransferase (AST) level in the younger age group. Besides these two polymorphisms of *SLC11A1* gene, there was no related SNP sites found in *SLC11A1* gene with inflammatory markers. (Table 5)

3. Interactions between the *ITPKC* and *SLC11A1* genes in KD patients

When comparing the KD patients and controls, no significant interactions between the *ITPKC* and *SLC11A1* genes were observed. However, after the KD patients were dichotomized based on age above and below 24 months, interactions between *ITPKC* rs2561531CC and *SLC11A1* rs17221959 CT and between *ITPKC* rs2561531 CC and *SLC11A1* rs77624405 GA appeared to exert a protective effect against KD symptoms in the younger patients (Table 6).

Table 6. Gene-gene interactions between the *ITPKC* and *SLC11A1* genes in KD patients

All		OR (95% CI)	*P*
ITPKC rs2561531_CC	SLC11A1 rs17221959_CT	0.841 (0.387-1.828)	0.6614
ITPKC rs2561531_CC	SLC11A1 rs77624405_GA	0.249 (0.047-1.328)	0.1036
< 24 month		OR (95% CI)	*P*
ITPKC rs2561531_CC	SLC11A1 rs17221959_CT	0.379 (0.154-0.931)	0.0343
ITPKC rs2561531_CC	SLC11A1 rs77624405_GA	0.128 (0.025-0.667)	0.0147

4. Associations of polymorphisms in the *ITPKC* and *SLC11A1* genes with BCG injection site erythema

No SNPs exhibiting a relationship with BCG injection site erythema were found in *ITPKC* in the KD group. Polymorphism rs17235409 in *SLC11A1* exhibited a relationship with BCG injection site erythema only in the younger-age KD patients (Table 7).

Table 7. Association of the rs17235409 polymorphism in *SLC11A1* with BCG injection site erythema

	Genotypes	BCG (+)	Control	*P*
All	GG	62 (80.52)	155 (78.68)	0.895
	GA	14 (18.18)	38 (19.29)	
	AA	1 (1.30)	4 (2.03)	
	G	138 (89.61)	348 (88.32)	0.6694
	A	16 (10.39)	46 (11.68)	
< 24 month	GG	53 (85.48)	32 (66.67)	0.0369
	GA	8 (12.90)	15 (31.25)	
	AA	1 (1.61)	1 (2.08)	
	G	114 (91.94)	79 (82.29)	0.0306
	A	10 (8.06)	17 (17.71)	

5. Association of *ITPKC* and *SLC11A1* gene interactions with BCG injection site erythema in KD patients

As shown in Table 8, statistically significant associations were found for interactions between rs28493229 of *ITPKC* and rs17235409 of *SLC11A1* (OR=5.1; p=0.0166) and between rs10420685 of *ITPKC* and rs7235409 of *SLC11A1* (OR=12.51; p=0.0082) in the older-age KD patients.

Table 8. Gene-gene interactions of *ITPKC* and *SLC11A1* genes with BCG injection site erythema

All		**OR (95% CI)**	***P***
ITPKC rs28493229_GG	SLC11A1 rs17235409_GA	0.031 (0.093-1.031)	0.0562
ITPKC rs10420685_GG	SLC11A1 rs17235409_AA	<0.001 (<0.001 - >999.999)	0.9854
≥ 24month		**OR (95% CI)**	***P***
ITPKC rs28493229_GG	SLC11A1 rs17235409_GA	5.105 (1.345-19.372)	0.0166
ITPKC rs10420685_GG	SLC11A1 rs17235409_AA	12.51 (1.927-82.01)	0.0082

6. Associations of polymorphisms in the *ITPKC* and *SLC11A1* genes with CALs and clinical symptom recurrence

As shown in Table 9, polymorphism rs28493229 in *ITPKC* was associated with CALs. However, no *ITPKC* or *SLC11A1* SNPs were found to be associated with the recurrence of clinical symptoms (data not shown).

Table 9. Relationship between *ITPKC* polymorphism rs28493229 and CALs

		Genotypes				Alleles		
		GG	GC	CC	*P*	G	C	*P*
All	No CAL patients	181 (78.35)	49 (21.21)	1 (0.43)	0.043	411 (88.96)	51 (11.04)	0.087
	CAL patients	40 (72.73)	12 (21.82)	3 (5.45)		92 (83.64)	18 (16.36)	

IV. DISCUSSION

The KD-associated gene *ITPKC* was first identified and studied in Japan.[11]

Data have demonstrated relatively higher frequencies of *ITPKC* in Japanese and European populations compared with Taiwanese populations.[12] Consistent with geographic differences in SNPs, one study conducted in Korea did not find similar polymorphisms in *ITPKC*.[36] In the present study, KD was carefully classified and an appropriate control group was selected to ensure the validity of the results. Patients with atypical or incomplete KD were thus excluded, as were those treated with IVIG before the fourth day of fever. In the present study, a significant increase in the frequency of allele C in rs28493229 was found in KD patients overall. The same increase was observed in the younger group of KD patients. Contrary to previous reports from Korea, this result demonstrated that polymorphisms in *ITPKC* are indeed associated with KD in Korea. However, the frequency was low (0.12) compared with that reported for other countries, including Japan (0.16).[12]

The rs28493229 polymorphism in *ITPKC* was also significantly associated with CALs. In our study, only genotype showed a statistically significant difference but not in alleles. Onouchi reported a significant relationship between the C allele and CALs.[12] In the present study, however, the frequency of the C allele was higher in CALs, but no statistically significant difference was found. By contrast, the same SNP in Taiwanese children was not significantly associated with CALs. These results provide some explanation for the variety of country-specific SNPs.[18,37]

A number of inflammatory markers, such as ESR, CRP, WBCs, platelets,

and LDH, are typically elevated in the peripheral blood of KD patients during the acute phase of the disease. The patients in the present study also exhibited increased WBC and platelet counts, as well as elevated LDH and CRP levels and an increase in the ESR. A previous study reported no significant correlations between SNPs in *ITPKC* and various inflammatory markers.[18] In the present study, however, several polymorphisms in *ITPKC* and *SLC11A1* were associated with elevations in WBC and platelet counts and LDH level in the KD group relative to the controls, as shown in Table 5. The association with elevated inflammatory markers was more pronounced for polymorphisms in *SLC11A1* compared with those in *ITPKC*. Furthermore, this association differed in older versus younger patients.

The results of this study suggest that KD is not a single disease entity. Differences in age of onset, clinical picture, pattern of laboratory data, and response to IVIG treatment in conjunction with age-related differences in SNPs suggest that KD would be more appropriately designated Kawasaki 'syndrome,' as it more closely resembles a group of heterogeneous entities that present a similar clinical picture.

A typical laboratory finding in KD is elevated platelet counts. This is very important, because low-dose aspirin treatment is used to prevent thrombosis associated with elevations in platelet count. The exact mechanism underlying the development of thrombocytosis in KD is unknown. It can be speculated that the increase in serum IL-6 level during the acute phase of the disease triggers

megakaryocyte maturation in the bone marrow, resulting in an increase in the number of platelets in the peripheral blood.[38] According to our data, SNPs in *ITPKC* (but not *SLC11A1*) are associated with thrombocytosis. These results indicate that *ITPKC* is more closely associated with inflammation in KD than *SLC11A1*.

Another interesting finding of the present study is that polymorphism rs28493229 in *ITPKC* has a protective effect against KD symptoms (OR 0.563; 95% CI 0.343–0.923; p=0.0229). We expected that the most-studied *ITPKC* SNP, rs28493229, would instead have a triggering effect on KD.[39] Some studies have shown no association between KD and polymorphism rs28493229 in *ITPKC*.[13] The opposite effect was observed in the present study, however. Along with the above-mentioned increases in the production of many inflammatory cytokines during the acute phase of KD, there is a simultaneous increase in the production of the anti-inflammatory cytokine IL-10.[10,39,40] A number of systems within the human body contribute to the maintenance of homeostasis. When inflammation arises, anti-inflammatory processes work simultaneously to restore homeostasis. Considered within this context, the rs28493229 polymorphism in *ITPKC* might play a role in suppressing the initiation of KD as a means of maintaining homeostasis.

In the process of choosing tagging SNPs, we added rs2290692 in the 3'-UTR of *ITPKC*. Han Chinese KD patients have higher frequencies of the C allele ($p<0.001$) compared with disease-free subjects.[15] However, no statistically

significant difference in C-allele frequency between KD patients and controls was observed in the present study. This result further demonstrates the geographic differences in the genetic background of KD.

The *SLC11A1* gene was originally named *NRAMP1*. *SLC11A1* encodes an iron-transporting protein that plays an important role in controlling susceptibility to *Mycobacterium tuberculosis* infection via regulation of IL-1 and TNF production as well as the activation of macrophages. With respect to BCG, it identifies host genes and proteins that play a key role in the response to mycobacterial infections.[41] As BCG injection site erythema is most common in younger KD patients, we searched for polymorphisms in this gene and found a total of 15 SNPs. A study by Ouchi et al.[26] showed that one variant of the *SLC11A1* gene is associated with KD in Japan. However, they could not find any polymorphisms in the *SLC11A1* gene, perhaps due to the age of the KD patients enrolled in the study. In contrast to Ouchi et al., who did not consider patient age in their analysis, in the present study, KD patients were dichotomized based on age less than versus over 24 months. In comparing the KD group as a whole with controls, no polymorphisms in *SLC11A1* were found, in agreement with the study of Ouchi et al. When KD patients under 24 months of age were considered, however, a statistically significant polymorphism, rs77624405, was identified, suggesting that this polymorphism in *SLC11A1* is associated with KD in patients under 24 months of age. This finding correlates well with the observed frequency of BCG injection site erythema in patients

under 24 months of age. Compared with control subjects, KD patients under 24 months of age tend to have a higher frequency of the A allele in rs2276631, whereas KD patients over 24 months of age tend to have a higher frequency of the A allele in rs17235409.

Gene-gene interactions have been linked to a number of important diseases, such as diabetes and essential hypertension.[42,43] In KD, interactions between *ITPKC* and the caspase-3 gene have been studied with respect to an association between IVIG unresponsiveness and development of coronary artery complications.[44,45] The potential role of interactions between *ITPKC* and *SLC11A1* were therefore examined in the present study. No meaningful interactions between these two genes were observed when the overall KD and control groups were analyzed. However, interactions between rs2561531 in *ITPKC* and rs17221959 in *SLC11A1* and between rs2561531 in *ITPKC* and rs77624405 in *SLC11A1* were found when the KD patients were considered based on age above and below 24 months, and these interactions were found to have a protective effect. The rs2561531 polymorphism in *ITPKC* is particularly interesting. This SNP was not identified in analyses of *ITPKC* in the KD patients. In analyses of potential associations between *ITPKC* and *SLC11A1*, however, a role for rs2561531 in *ITPKC* was revealed. Roles for rs17221959 and rs77624405 in *SLC11A1* were also identified.

Although BCG injection site erythema is not included in the diagnostic criteria for KD, it is an important clue, as there is no other disease associated

with erythematous changes at the BCG injection site. In a previous report, Lin et al. concluded that there is an association between the C allele of *ITPKC* SNP rs28493229 and BCG scars, although the frequency is low (8.04%).[18] In the present study, the C allele of *ITPKC* SNP rs28493229 was not significantly associated with BCG injection site erythema, however.

In younger KD patients, the *SLC11A1* SNP rs17235409 was associated with BCG injection site erythema. Although one Japanese study reported no polymorphisms in *SLC11A1*,[26] the results may have been affected by differences in nationality, year of introduction of BCG vaccination, or BCG coverage rate. A comparison of subjects of all ages with and without BCG injection site erythema indicated that *SLC11A1* SNP rs2276631 was associated with a decreased risk of KD (OR 0.161; 95% CI 0.038–0.689; p=0.0138). In subjects less than 24 months of age, however, the *SLC11A1* SNP rs17235409 was associated with increased risk of KD (OR 2.483; 95% CI 1.063–5.804; p=0.0357).

The *SLC11A1* gene is known to be associated with autoimmune and infectious diseases. The SNPs analyzed in this study are associated more with infectious than autoimmune disease. The SNPs rs7573065, rs2276631, rs17221959, and rs17235409 in *SLC11A1* are associated with tuberculosis and infection with *Mycobacterium* spp., with one meta-analysis reporting estimated ORs >1.[46] As BCG immunization is given to children at less than 1 month of age, the association of these SNPs with KD might be more significant in

younger children.

Analyses of interactions between *ITPKC* and *SLC11A1* showed a synergistic effect for BCG injection site erythema in patients over 24 months of age. Interactions between rs28493229 in *ITPKC* and rs17235409 in *SLC11A1* and between rs10420685 in *ITPKC* and rs7235409 in *SLC11A1* trigger BCG injection site erythema.

These results indicated that reactivation of BCG scars is associated with polymorphisms in either *ITPKC* or *SLC11A1* in patients under 24 months of age. In patients older than 24 months, however, reactivation of BCG scars is associated with synergism between the polymorphisms of these two genes rather than either of the genes alone.

During the acute phase of KD, striking immunological derangements occur, including changes in immune cells and marked inflammatory cytokine cascade stimulation.[3] In light of these changes, Kim suggested that acquired immune responses play an important role in the development of KD.[3] Recently, Ikeda et al. suggested that innate immunity, rather than acquired immunity, plays an important role in the development of vasculitis in KD.[47] Kusuda et al. reported that KD-specific molecules in the serum possess structures common to microbe-associated molecular pattern (MAMP) molecules of bacteria such as *Bacillus*, *Yersinia*, and *Staphylococcus* and that levels of these molecules decline after IVIG treatment.[48] Hara et al. suggested that increased levels of MAMP and damage-associated molecular pattern (DAMP) molecules in the

serum of acute-phase KD patients activate the immune system and vascular cells through innate immune pattern recognition receptors, leading to the release of various cytokines.[49] Heat shock protein, which is contained in considerable amounts in the BCG vaccine, is a well-known DAMP. It can thus be speculated that BCG-associated DAMPs play an important role in the development of KD.

Many researchers have concluded that the innate immune system is present at birth and does not change throughout life.[50] However, the results of the present study indicate that KD is associated with a difference in genetic background with respect to various inflammatory markers and reactivation of BCG scars in patients younger and older than 24 months. Interestingly, increasing evidence indicates that there are age-associated differences in maturation and function in the innate immune system.[51,52] In light of these factors, the results of the present study that show age-associated differences in BCG injection site erythema and genetic background in patients with KD are reasonable.

It is unclear whether BCG itself induces KD, whether the BCG injection site is reactivated as a result of specific processes that occur during initiation of KD, or whether reactivation of the BCG injection site is evidence of suppression of the inflammation process in KD. Nevertheless, it is clear that the BCG injection site exhibits inflammatory changes that manifest as erythema. Further study is needed to clarify the relationship between BCG injection site erythema and KD, as demonstrated by Chun et al. [25]

This study has several limitations. First, the study was not conducted at the functional gene level. Second, the power of the study was low. Third, the control group was not age matched. However, it was concluded that age matching would not significantly impact the results, as future development of KD in young, healthy children cannot be ruled out. Therefore, older subjects without a history of KD were considered a better choice for the control group.

V. CONCLUSION

In conclusion, this study identified polymorphisms in the *ITPKC* and *SLC11A1* genes in Korean KD patients. These polymorphisms are associated with increased production of inflammatory markers in the peripheral blood of patients with KD. The SNPs identified in this study are also associated with erythema of the BCG injection site in KD patients, but their effects differ depending on patient age.

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ABSTRACT (IN KOREAN)

한국의 가와사끼병 어린이들에서 *ITPKC* 유전자와 *SLC11A1* 유전자의 다형성과 두 유전자간의 상호작용

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가와사끼병은 급성 전신성 혈관염이고 아직 그 원인에 대해 명확히 알려진 바가 없다. 또한 가와사끼병에서만 볼 수 있는 특징적인 현상인 비씨지 예방접종 부위 홍반의 원인에 대해서도 완전히 이해되지 않았다. 최근 가와사끼병의 유전체 연구가 활성화되며 많은 후보 유전자들에 대한 활발한 연구가 진행되고 있다. 본 연구는 가와사끼병과 관련되어 가장 활발한 연구가 진행되고 있는 *ITPKC* 유전자와, 비씨지 균주의 과민반응에 관여하는 것으로 알려진 *SLC11A1* 유전자의 SNP가 가와사끼병에 걸린 한국 어린이들에서 어떠한 관계가 있는지 연구를 진행하였다. 비씨지 주사 부위 홍반과 두 유전자의 SNP와의 연관성도 분석하였다. 또한 두 유전자간의 상호작용에 대해서도 함께 살펴 보았다. 299명의 가와사끼병 환아와 210명의 대조군의 *ITPKC* 유전자의 세 가지 tagging SNP와 *SLC11A1* 유전자의 다섯 tagging SNP에 대한 유전자형을 분석하였다. Allele, genotype, 유전자 간 상호작용도 조사하였다. *ITPKC* 유전자의 rs28493229는 가와사끼병 및 관상 동맥 합병증과 연관이 있었다. *SLC11A1* 유전자의 SNP rs77624405는 가와사끼병 발병에 관여하였다. 비씨지 접종 부위의 홍반이 있는 환아와 없는 환아를 비교한 결과 *SLC11A1* 유전자의 rs17235409가 연관성을 보였으나 *ITPKC* 유전자에는 관련된 SNP가 없었다.

ITPKC 유전자의 rs28493229_GG와 *SLC11A1* 유전자의 rs17235409_GA, *ITPKC* 유전자의 rs10420685_GG와 *SLC11A1* 유전자의 rs17235409_AA가 비씨지 주사 부위 홍반에 영향을 주는 것으로 분석되었다. 결론적으로, 본 연구는 한국인에서의 가와사끼병에서 *ITPKC* 유전자와 *SLC11A1* 유전자의 다형성을 발견했다. 이 연구에서 genetic variants는 가와사끼병과 연관이 있으며, 비씨지 주사 부위 홍반과도 연관되어 있으며, 연령군에 따라서도 다른 역할을 하는 것으로 조사되었다.

핵심되는 말: 가와사끼병, *ITPKC*, *SLC11A1*, Single-nucleotide polymorphism, Gene-gene interaction, 관상동맥 합병증

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