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Platelet Endothelial Cell Adhesion Molecule-1 Gene Polymorphisms are Associated with Coronary Artery Lesions in the Chronic Stage of Kawasaki Disease

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Background: Kawasaki disease is the most common cause of pediatric acquired heart disease. The role of platelet endothelial cell adhesion molecule-1 in the inflammatory process has been documented. To date, no report has investigated the relationship between coronary artery lesions of Kawasaki disease and platelet endothelial cell adhesion molecule-1 polymorphisms.

Methods: A total of 114 Kawasaki disease children with coronary artery lesions and 185 Kawasaki disease children without coronary artery lesions were recruited in this study. The TaqMan assay was conducted to identify the genotype in this case-control study.

Results: In three single nucleotide polymorphisms (Leu125Val, Ser563Asn, and Arg670Gly) of platelet endothelial cell adhesion molecule-1, we found that the Leu-Ser-Arg haplotype was associated with a significantly increased risk for coronary artery lesions in the chronic stage (odds ratio 3.05, 95% confidence interval 1.06-8.80, p = 0.039), but not for coronary artery lesions in the acute stage. Analysis based on the diplotypes of platelet endothelial cell adhesion molecule-1 also showed that Kawasaki disease with one or two alleles of Leu-Ser-Arg had a significantly increased risk of chronic coronary artery lesions (odds ratio 3.38, 95% confidence interval 1.11-10.28, p = 0.032) and had increased platelet counts after Kawasaki disease was diagnosed, as compared to those with other diplotypes.

Conclusions: The haplotype of platelet endothelial cell adhesion molecule-1 Leu-Ser-Arg might be associated with the increased platelet counts and the following risk of chronic coronary artery lesions in a dominant manner in Kawasaki disease.

Key Words: Coronary artery lesions • Gene polymorphisms • Kawasaki disease • PECAM-1

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INTRODUCTION

Kawasaki disease (KD) is an acute self-limiting systemic vasculitis that usually affects infants and children under 5 years of age. The top incidences of KD in children under 5 years of age are estimated to be 239.6/100,000 in Japan,[1] 113.1/100,000 in Korea,[2] and 49/100,000 in Taiwan.[3] KD is manifested by fever more than 5 days and is accompanied by at least four out of five additional manifestations: skin rash, red eyes, red lips and mouth, swollen and red hands and feet, and swol-

len lymph nodes in the neck area.[4,5]

The inflammation of blood vessels in the coronary arteries and subsequent coronary artery aneurysms with thrombosis or rupture are the most important cardiac complications in KD. Patients who develop these cardiovascular complications are at risk of developing ischemic heart disease, and may experience myocardial infarction and sudden death.[1,2,4-6] Although treatment with intravenous immunoglobulin (IVIG) and aspirin is an effective therapy for KD, not all patients respond and the cause of refractory KD remains unclear.[7] Acute coronary artery lesions (CALs) develop in 20-48% children with KD and chronic CALs develop in approximately 20-30% of untreated children with KD. However, 2-6% of children with KD treated with IVIG still develop chronic CALs.[5] The cause of CALs in KD remains unknown,[8] but it is believed to be caused by infectious agents, immune dysregulation, and genetic susceptibility.[4,7]

Platelet endothelial cell adhesion molecule-1 (PECAM-1) is constitutively expressed on the surface of hematopoietic cells, including platelets, neutrophils, monocytes, and T and B lymphocytes.[9] It is a multifunctional vascular cell adhesion molecule involved in the diverse roles in vascular biology, such as the maintenance of a vascular permeability barrier, regulation of multiple stages of monocytes and neutrophils migration through vascular walls, and mechano-sensing of endothelial cell response to fluid shear stress.[10-14] There are three major single-nucleotide polymorphisms (SNPs) in PECAM-1 genes: Leu125Val (at codon 125 altering a leucine to a valine) is in the first Ig-like domain involved in the homophilic interaction in exon 3, Ser563Asn (at codon 563 altering an serine to a asparagine) is in the sixth Ig-like domain in exon 8, which may participate in the heterophilic interaction with integrins, and Arg670Gly (at codon 670 altering an arginine to a glycine) is in the cytoplasmic domain in exon 12, playing a key role in signal transduction.[9,10] Homophilic PECAM-1 interactions are involved in leukocyte transmigration, especially in drawing leukocytes to the luminal surface of endothelial cells and initiating diapedesis.[11] During the atherosclerotic process, PECAM-1 is a key participant in the adhesion cascade leading to the extravasation of leucocytes.[15] An in vivo study demonstrated that the intravenous injection of anti-PECAM-1 antibodies in mice prolongs the length of time to the first detectable intravascular adhesion of platelet aggregates on injured endothelium.[12] Therefore, PECAM-1 is suggested to influence the role of platelets in atherogenesis at sites of endothelial damage.[15] In addition, PECAM-1 polymorphism has been shown to be associated with the increased risk of coronary artery disease and myocardial infarction.[9,15-21] PECAM-1 SNPs of Ser563Asn and Arg670Gly are associated with myocardial infarction in the Japanese,[9] and PECAM-1 SNPs of Leu125Val are associated with myocardial infarction in patients with type 2 diabetes in the Slovene population (Caucasians).[21] The Leu125Val polymorphism of PECAM-1 is also associated with coronary artery disease in Chinese Singaporeans,[16] and Indian Singaporeans.[9]

Early recognition of chronic CALs formation is an important issue for intensive follow-up of acute CALs and aggressive treatment of KD. To our knowledge, there are no prior studies which have investigated the association of these three major PECAM-1 SNPs (Leu125Val, Ser563Asn, and Arg670Gly) with the risk of CALs in KD. The aim of this study was to evaluate the possible association of three PECAM-1 SNPs with the development of chronic CALs among Taiwanese KD children with acute CALs.

MATERIALS AND METHODS

Subjects

A total of 299 unrelated children who met the established criteria of KD (Japanese Kawasaki Disease Research Committee, 1984) were enrolled in the Department of Pediatrics, Kaohsiung Veterans General Hospital in Taiwan. Medical records of all children who received a diagnosis of KD between 1990 and 2010 were reviewed for age, sex, CALs complications, and laboratory data, including baseline white blood cell with differential counts (band, neutrophils, monocytes, eosinophil and lymphocytes), and platelet counts, including platelet 1 counts and platelet 10 counts. Platelet 1 counts mean that we collected the platelet counts within one day of the patients' being diagnosed with KD (n = 299), and platelet 10 counts mean that we collected the highest platelet counts within 10 days of the patients' being diagnosed with KD.

Most of the patients were treated with high-dose

IVIG (2 g/kg) within 10 days of fever onset as a single infusion for 10-12 h without concomitant aspirin therapy at Kaohsiung Veterans General Hospital.[4] Fever was defined as an oral or rectal temperature ≥ 38 °C, and antipyretic medication such as acetaminophen was used to control fever. After the fever subsided, low dose aspirin (3-5 mg · kg^{-1} · day^{-1}) was prescribed until all signs of inflammation were resolved and CALs regressed as detected by 2-dimensional echocardiography.[4,15] Patients who had defervescence within 48 hrs after the completion of IVIG treatment were defined as IVIG-responsive, and those whose fever persisted or recrudesced for at least 48 hrs but not longer than 7 days after completion of IVIG treatment were defined as IVIG resistant. Some KD patients of this study did not receive IVIG treatment because the illness had been more than 10 days after the onset of KD and the patients had no evidence of systemic inflammation after 10 days of illness. All patients had 2-dimensional echocardiography performed at the time of diagnosis and again at weeks 2, 4, and 8 after treatment, and annually as follow-up. Acute and chronic CALs were defined as CALs within 2 weeks of illness and beyond 1 year, respectively. CALs were defined as follows: (1) internal lumen diameter ≥ 3 mm in children < 5 years old; (2) internal lumen diameter ≥ 4 mm in children ≥ 5 years old; (3) internal lumen diameter 1.5-fold greater than that of an adjacent segment; or (4) clearly irregular coronary artery lumen. The intra- and inter-rater reliabilities for 2-dimensional echocardiography were qualified, which were described in our previous paper.[16] Blood samples were collected for PECAM-1 gene polymorphism analysis after obtaining informed consent from the parents of all subjects. The protocol was approved by the Institutional Review Board of Kaohsiung Veterans General Hospital.

Polymorphism genotyping

Genomic DNA for genotyping was extracted and purified from the whole-blood samples of all subjects using PUREGENE DNA purification kits according to the manufacturer's instructions (Gentra, 2008). The detection of genotypes of PECAM-1 SNPs in 125 C/G, 563 G/A, and 670 A/G was performed by the Taqman real-time polymerase chain reaction (PCR) method, and then analyzed with ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) in a 96-well format. PCR reactions were carried out in reaction mixes containing 10 ng DNA, 5 μl 2 × TaqMan Universal PCR Master Mix (Applied Biosystems), 0.5 μl 20 × Primer/Probe mixture, and ddH2O to a final volume of 10 μl. The PCR program was as follows: 95 °C for 10 min followed by 45 cycles of 15 s at 92 °C, and 1 min at 60 °C. A single no-template control in each 96-well format was used for quality control. The allelic-specific fluorescence data from each plate was analyzed using the SDS v1.3.1 software (Applied Biosystems, 2005) to automatically determine the genotype of each sample. Subsequently, a senior researcher independently reviewed all absolute quantification curves for fluorescence data in the Taqman assays. Finally, 20% of the samples were randomly selected and run in duplicate to ensure accuracy of genotyping.

Statistical analysis

For each tested SNP, departure from the Hardy-Weinberg equilibrium in the control children was evaluated using the chi-square test with one degree of freedom. Simple logistic regression was used to evaluate the relationships of allelic types, genotypes, haplotypes, and diplotypes of three SNPs in PECAM-1 with the risk of CALs, including acute CALs and chronic CALs. In addition, the impact of diplotype (with vs. without one or two alleles of Leu-Ser-Arg) on the risk of acute CALs or chronic CALs was further analyzed in stratification analysis based on the demographic, clinical, and laboratory variables, which had statistical significance in the initial simple logistic regression models (such as Platelet 10 counts being included, but Platelet 1 counts being excluded). The "p for interaction" obtained from other sets of models with the laboratory variable (such as platelet 1 levels: ≤ 400 vs. > 400), the diplotype of allele (with vs. without one or two alleles of Leu-Ser-Arg), and their interaction terms (platelet 1 levels * diplotypes of Leu-Ser-Arg allele). The odds ratio (OR) and 95% confidence intervals (CI) were all estimated by simple logistic regression. $p < 0.05$ was considered statistically significant, and the False Discovery Rate corrected p-value was applied for multiple testing of the three SNPs.[17] The statistical software packages SPSS (Version 12.0) and SAS/Genetics (Version 9.1.3) were used to perform all statistical analysis.

RESULTS

Demographic, clinical data, and laboratory findings in KD patients with/without CALs in the acute and chronic stages

The demographic data and times of IVIG treatment between KD patients with and without CALs in the acute and chronic stages (n = 299) are summarized in Table 1.

Of the KD patients, there were 114 (38.1%) patients with acute CALs and 185 (61.9%) patients without acute CALs. An increased risk of acute CALs was found for males. There were no significant differences for age, KD diagnosis year, and ethnicity between those complicated with and without acute CALs. Of the KD patients with acute CALs, there were 33 (28.9%) patients with chronic CALs formation and 81 (71.1%) patients without chronic CALs formation. There were no significant differences for age, sex, and ethnicity between those complicated with and without chronic CALs. However, chronic CALs complication decreased when we compared the KD diagnosis year "≥ 2004" with "< 2004". In addition, the patients who ever underwent ≥ 2 rounds of IVIG treatment had a more increased risk for acute and chronic CALs than the patients who even just underwent IVIG treatment once. For the follow-up of those 33 patients with chronic CALs, we found that 5 patients had giant aneurysm, and 1 patient with giant aneurysm developed ischemic symptoms. No patient with chronic CAL had ever undergone interventional procedures such as percutaneous coronary intervention or coronary artery bypass grafting in our hospital.

In laboratory findings on KD patients (Table 2), there were significantly increased platelet 10 counts (OR 2.63, 95% CI 1.21-5.71, p = 0.015) in those with acute CALs as compared to those without acute CALs. There were no significant differences for platelet 1 counts, white blood

Table 1. Comparisons of demographic and clinical data in KD patients between different status of acute CALs and chronic CALs

Factor/category	KD (n = 299)				KD with acute CALs (n=114)			
	Without acute CALs (n = 185)	With acute CALs (n=114)	OR* (95% CI)	p value	Without chronic CALs (n = 81)	With chronic CALs (n = 33)	OR* (95% CI)	p value
	Number (%)	Number (%)			Number (%)	Number (%)		
Age (Months) (Mean ± SD)	27.92 ± 24.79	25.35 ± 22.80	-	0.369	24.22 ± 21.41	28.13 ± 26.07	-	0.409
≤ 12 months	55 (29.7)	41 (36.0)	1.00		28 (34.6)	13 (39.4)	1.00	
12-60 months	112 (60.5)	59 (51.8)	0.72 (0.43-1.21)	0.220	46 (56.8)	13 (39.4)	0.59 (0.24-1.45)	0.248
> 60 months	18 (9.7)	14 (12.3)	1.07 (0.48-2.40)	0.871	7 (8.6)	7 (21.2)	2.08 (0.60-7.17)	0.248
Sex								
Female	82 (44.3)	33 (28.9)	1.00		24 (29.6)	9 (27.3)	1.00	
Male	103 (55.7)	81 (71.1)	1.95 (1.19-3.22)	0.008	57 (70.4)	24 (72.7)	1.12 (0.46-2.77)	0.801
KD diagnosis year								
< 2004	113 (61.1)	69 (60.5)	1.00		43 (53.1)	26 (78.8)	1.00	
≥ 2004	72 (38.9)	45 (39.5)	1.02 (0.64-1.65)	0.924	38 (46.9)	7 (21.2)	0.31 (0.12-0.78)	0.013
Times of IVIG treatment								
1	139 (75.1)	76 (66.7)	1.00		60 (74.1)	16 (48.5)	1.00	
≥ 2	6 (3.2)	22 (19.3)	6.71 (2.61-17.25)	< 0.001	12 (14.8)	10 (30.3)	3.13 (1.15-8.53)	0.026
None	40 (21.6)	16 (14.0)	0.73 (0.38-1.39)	0.341	9 (11.1)	7 (21.2)	2.92 (0.94-9.04)	0.064
Ethnicity								
Fukienese	123 (73.7)	82 (80.4)	1.00		62 (86.1)	20 (66.7)	1.00	
Hakka	15 (9.0)	10 (9.8)	1.00 (0.43-2.33)	1.000	5 (6.9)	5 (16.7)	3.10 (0.81-11.82)	0.097
Mainlander	29 (17.4)	10 (9.8)	0.52 (0.24-1.12)	0.094	5 (6.9)	5 (16.7)	3.10 (0.81-11.82)	0.097
Missing value	18	12			9	3		

* OR, odds ratio; p value is estimated by logistic regression or t-test; CALs, coronary artery lesions; IVIG, intravenous immunoglobulin; KD, Kawasaki disease.

Table 2. Odds ratios for KD with acute CALs and chronic CALs according to various laboratory findings

Factor/category	KD (n = 299)				KD with acute CALs (n = 114)			
	Without acute CALs (n = 185)	With acute CALs (n = 114)	OR* (95% CI)	p value	Without chronic CALs (n = 81)	With chronic CALs (n = 33)	OR* (95% CI)	p value
	Number (%)	Number (%)			Number (%)	Number (%)		
Plt1 count (× 1000/Cumm)								
< 150	2 (1.1)	2 (1.8)	1.00		0 (0)	0 (0)	1.00	
150-400	117 (63.2)	71 (62.3)			7 (8.6)	2 (6.1)		
> 400	66 (35.7)	41 (36.0)	1.01 (0.62-1.65)	0.960	74 (91.4)	31 (93.9)	1.47 (0.29-7.46)	0.645
Plt10 count (× 1000/Cumm)								
< 150	0 (0)	0 (0)	1.00		1 (1.2)	1 (3.0)	1.00	
150-400	34 (18.4)	9 (7.9)			50 (61.7)	21 (63.6)		
> 400	151 (81.6)	105 (92.1)	2.63 (1.21-5.71)	0.015	30 (37.0)	11 (33.3)	0.85 (0.36-1.99)	0.709
WBC count (× 10^3/mm^3)								
< 4	3 (1.6)	0 (0)	1.00		0 (0)	0 (0)	1.00	
4-9.9	36 (19.5)	18 (15.8)			13 (16.0)	5 (15.2)		
> 9.9	146 (78.9)	96 (84.2)	1.43 (0.77-2.64)	0.259	68 (84.0)	28 (84.8)	1.07 (0.35-3.29)	0.905
PMN (%)								
< 41	28 (15.1)	13 (11.4)	1.00		7 (8.6)	6 (18.2)	1.00	
41-73	141 (76.2)	83 (72.8)	1.27 (0.62-2.58)	0.513	59 (72.8)	24 (72.7)	0.48 (0.14-1.56)	0.219
> 73	16 (8.6)	18 (15.8)	2.42 (0.95-6.21)	0.065	15 (18.5)	3 (9.1)	0.23 (0.05-1.22)	0.084
Lym (%)								
< 20	32 (17.3)	29 (25.4)	1.00		21 (25.9)	8 (24.2)	1.00	
20-45	128 (69.2)	74 (64.9)	0.64 (0.36-1.14)	0.128	54 (66.7)	20 (60.6)	0.97 (0.37-2.55)	0.954
> 45	25 (13.5)	11 (9.6)	0.49 (0.20-1.16)	0.103	6 (7.4)	5 (15.2)	2.19 (0.52-9.23)	0.286
Band (%)								
0-5	145 (78.4)	85 (74.6)	1.00		70 (86.4)	15 (45.5)	1.00	
> 5	40 (21.6)	29 (25.4)	1.24 (0.72-2.14)	0.447	11 (13.6)	18 (54.5)	7.64 (3.00-19.45)	< 0.001
Monocyte (%)								
< 3	21 (11.4)	6 (5.3)	1.00		3 (3.7)	3 (9.1)	1.00	
3-7	121 (65.4)	85 (74.6)			57 (70.4)	28 (84.8)		
> 7	43 (23.2)	23 (20.2)	0.84 (0.47-1.48)	0.535	21 (25.9)	2 (6.1)	0.18 (0.04-0.84)	0.029
Eosinophil (%)								
< 1	14 (7.6)	15 (13.2)	1.00		15 (18.5)	0 (0)	1.00	
1-5	149 (80.5)	89 (78.1)	0.56 (0.26-1.21)	0.139	58 (71.6)	31 (93.9)		
> 5	22 (11.9)	10 (8.8)	0.42 (0.15-1.21)	0.107	8 (9.9)	2 (6.1)	0.59 (0.12-2.93)	0.518

* OR, odds ratio; p value is estimated by simple logistic regression.

CALs, coronary artery lesions; KD, Kawasaki disease; Lym, lymphocytes; Plt1 count, the platelet counts collected within one day of the patient' being diagnosed with KD; Plt10 count, the highest platelet counts collected within 10 days of the patient' being diagnosed with KD; PMN, neutrophils; WBC, white blood cell.

cell and with differential counts between those complicated with and without acute CALs. Of the KD patients with acute CALs, there were increased risks with chronic CALs in band neutrophils > 5% (OR 7.64, 95% CI 3.00-19.45, p < 0.001), but reduced risk in monocyte > 7% (OR 0.18, 95% CI 0.04-0.84, p = 0.029). There were no significant differences for platelet 1 counts, platelet 10 counts, white blood cell, neutrophils, lymphocytes, and

eosinophil between those complicated with and without chronic CALs.

Because a limited number of KD children exists with the variant genotype, haplotype, or diplotype of PECAM-1, multiple logistic regression model cannot be used to control the impact of confounders on CALs. Therefore, sex, KD diagnosis year, times of IVIG treatment, platelet 10 count, white blood cell, neutrophils, lymphocytes, band, monocyte, and eosinophil were further controlled in the stratification analysis to evaluate the genetic impact of PECAM-1 on risk of acute CALs or chronic CALs.

The genotypes, allelic types, haplotypes and diplotypes in KD patients with/without CALs in the acute and chronic stages

The genotypic and allelic frequencies of the PECAM-1 SNPs in the KD patients with and without CALs and complicated with or without chronic CALs progressing from the acute CLAs are shown in Table 3. There were

Table 3. Odds ratios for KD with acute CALs and chronic CALs by various genotypes and allelic types of PECAM-1

PECAM-1 SNPs	Genotype	KD (n = 299)		KD with acute CALs (n = 114)		With acute CALs vs. without acute CALs		With chronic CALs vs. without chronic CALs	
		Without acute CALs (n = 185)	With acute CALs (n = 114)	Without chronic CALs (n = 81)	With chronic CALs (n = 33)				
		N (%)	N (%)	N (%)	N (%)	OR* (95% CI)	p value	OR* (95%CI)	p value
Leu125Val	Val/Val	30 (16.2)	13 (11.4)	8 (9.9)	5 (15.2)	1.00		1.00	
(C>G)	Leu/Val	83 (44.9)	49 (43.0)	37 (45.7)	12 (36.4)	1.36 (0.65-2.86)	0.413	0.52 (0.14-1.89)	0.320
	Leu/Leu	72 (38.9)	52 (45.6)	36 (44.4)	16 (48.5)	1.67 (0.79-3.50)	0.177	0.71 (0.20-2.52)	0.597
	Val/Val	30 (16.2)	13 (11.4)	8 (9.9)	5 (15.2)	1.00		1.00	
	Leu/Val+Leu/Leu	155 (83.8)	101 (88.6)	73 (90.1)	28 (84.9)	1.50 (0.75-3.02)	0.252	0.61 (0.19-2.04)	0.425
	Val allele	143 (38.6)	75 (32.9)	53 (32.7)	22 (33.3)	1.00		1.00	
	Leu allele	227 (61.4)	153 (67.1)	109 (67.3)	44 (66.7)	1.29 (0.91-1.82)	0.156	0.97 (0.53-1.79)	0.928
Ser563Asn	Asn/Asn	59 (31.9)	40 (35.1)	29 (35.8)	11 (33.3)	1.00		1.00	
(G>A)	Ser/Asn	90 (48.6)	58 (50.9)	45 (55.6)	13 (39.4)	0.95 (0.57-1.60)	0.848	0.76 (0.30-1.93)	0.566
	Ser/Ser	36 (19.5)	16 (14.0)	7 (8.6)	9 (27.3)	0.66 (0.32-1.34)	0.246	3.39 (1.01-11.34)	0.047
	Asn/Asn+Ser/Asn	149 (80.5)	98 (86.0)	74 (91.4)	24 (72.7)	1.00		1.00	
	Ser/Ser	36 (19.5)	16 (14.0)	7 (8.6)	9 (27.3)	0.68 (0.36-1.28)	0.231	3.96 (1.33-11.79)	0.013
	Asn allele	208 (56.2)	138 (60.5)	103 (63.6)	35 (53.0)	1.00		1.00	
	Ser allele	162 (43.8)	90 (39.5)	59 (36.4)	31 (47.0)	0.84 (0.60-1,17)	0.300	1.55 (0.87-2.76)	0.141
Arg670Gly	Gly/Gly	60 (32.4)	41 (36.0)	30 (37.0)	11 (33.3)	1.00		1.00	
(A>G)	Arg/Gly	89 (48.1)	58 (50.9)	44 (54.3)	14 (42.4)	0.95 (0.57-1.60)	0.857	0.87 (0.35-2.17)	0.762
	Arg/Arg	36 (19.5)	15 (13.2)	7 (8.6)	8 (24.2)	0.61 (0.30-1.26)	0.179	3.12 (0.91-10.64)	0.069
	Gly/Gly+Arg/Gly	149 (80.5)	99 (86.9)	74 (91.4)	25 (75.8)	1.00		1.00	
	Arg/Arg	36 (19.5)	15 (13.2)	7 (8.6)	8 (24.2)	0.63 (0.33-1.21)	0.162	3.38 (1.11-10.28)	0.032
	Gly allele	209 (56.5)	140 (61.4)	104 (64.2)	36 (54.5)	1.00		1.00	
	Arg allele	161 (43.5)	88 (38.6)	58 (35.8)	30 (45.5)	0.82 (0.58-1.14)	0.236	1.49 (0.84-2.67)	0.176

* OR, odds ratio; p-value is estimated by simple logistic regression; CALs, coronary artery lesions; KD, Kawasaki disease.

no significant differences in the genotypes among the PECAM-1 SNPs of Leu125Val, Ser563Asn, and Arg670Gly between the KD patients with and without acute CALs. Of the KD patients with acute CALs, the Ser/Ser genotype was higher than Asn/Asn and/or Ser/Asn genotypes (OR 3.39, 95% CI 1.01-11.34, p = 0.047; OR 3.96, 95% CI 1.33-11.79, p = 0.013) in the PECAM-1 SNP of Ser563Asn in chronic CALs. The Arg/Arg genotype was higher than the Gly/Gly+Arg/Gly genotype in chronic CALs (OR 3.38, 95% CI 1.11-10.28, p = 0.032).

Linkage disequilibrium was observed between three SNPs of PECAM-1, such as Leu125Val and Ser563Asn (D' = 0.941, r^2 = 0.711), Leu125Val and Arg670Gly (D' = 0.932, r^2 = 0.705), as well as Ser563Asn and Arg670Gly (D' = 1, r^2 = 0.989). Ser563Asn and Arg670Gly were in almost complete linkage disequilibrium.

In Table 4, there were no significant differences in haplotype and diplotype of Leu-Ser-Arg between the KD patients with and without acute CALs. However, of the KD patients with acute CALs, we found the Leu-Ser-Arg haplotype (125-563-670) was associated with a significantly increased risk for chronic CALs compared to non-Leu-Ser-Arg haplotypes (OR 3.05, 95% CI 1.06-8.80, p = 0.039). In analyses of the diplotypes of PECAM-1 polymorphisms, those with one or two alleles of Leu-Ser-Arg were shown to have a significantly increased risk of chronic CALs as compared to without the allele of Leu-Ser-Arg among the KD patients with acute CALs (OR 3.38, 95% CI 1.11-10.28, p = 0.032).

Comparisons of laboratory data by PECAM-1 diplotype

We further stratified KD patients and KD with acute CALs patients respectively, into two groups based on laboratory data to assess the impact of PECAM-1 diplotype (with Leu-Ser-Arg alleles vs. without any Leu-Ser-Arg alleles) on risk of acute CALs and chronic CALs, respectively (Table 5). For KD with acute CALs patients, diplotype with Leu-Ser-Arg alleles was significantly associated with the increased risk of chronic CALs in female patients (OR 46.00, 95% CI 4.03-525.13, p = 0.002), higher platelet 10 counts (> 400,000/cumm; OR 3.33, 95% CI 1.09-10.20, p = 0.035), and lower monocytes% (≤ 7%; OR 4.08, 95% CI 1.09-15.26, p = 0.036). However, no interactions were found between PECAM-1 diplotype and any laboratory data on the risk of acute CALs or chronic CALs except sex in chronic CALs.

DISCUSSION

This study demonstrated that the Leu-Ser-Arg haplotype is associated with an increased risk of developing CALs in the chronic stage of KD patients, and also

Table 4. Odds ratios for KD with acute CALs and chronic CALs according to various diplotypes and haplotypes of PECAM-1

PECAM-1 polymorphisms	KD (n = 299)		KD with acute CALs (n = 114)					
	Without acute CALs (n = 185)	With acute CALs (n = 114)	Without chronic CALs (n = 81)	With chronic CALs (n = 33)	With acute CALs vs. without acute CALs		With chronic CALs vs. without chronic CALs	
	N (%)	N (%)	N (%)	N (%)	OR* (95% CI)	p value	OR* (95% CI)	p value
Haplotype (125-563-670)								
Leu-Asn-Gly	203 (54.9)	137 (60.1)	102 (63.0)	35 (53.0)				
Val-Ser-Gly	1 (0.3)	1 (0.4)	1 (0.6)	0 (0)				
Val-Asn-Gly	5 (1.4)	1 (0.4)	1 (0.6)	0 (0)	1.00		1.00	
Val-Ser-Arg	137 (37.0)	73 (32.0)	51 (31.5)	22 (33.3)				
Leu-Ser-Gly	0 (0)	1 (0.4)	0 (0)	1 (1.5)				
Leu-Ser-Arg	24 (6.5)	15 (6.6)	7 (4.3)	8 (12.1)	1.02 (0.52-1.98)	0.965	3.05 (1.06-8.80)	0.039
Diplotype (125-563-670/125-563-670)								
Without the allele of Leu-Ser-Arg	162 (87.6)	99 (86.8)	74 (91.4)	25 (75.8)	1.00		1.00	
With one or two alleles of Leu-Ser-Arg	23 (12.4)	15 (13.2)	7 (8.6)	8 (24.2)	1.07 (0.53-2.14)	0.855	3.38 (1.11-10.28)	0.032

* OR, odds ratio; p-value is estimated by logistic regression; CALs, coronary artery lesions; KD, Kawasaki disease.

Table 5. Odds ratios for KD with acute CALs or chronic CALs according to the Leu-Ser-Arg allele of PECAM-1 diplotypes

Lab data	With one or two alleles of Leu-Ser-Arg	KD (n=299)		KD with acute CALs (n=114)		With acute CALs vs. without acute CALs		p for interaction	With chronic CALs vs. without chronic CALs		p for interaction
		Without acute CALs (n = 185)	With acute CALs (n = 114)	Without chronic CALs (n = 81)	With chronic CALs (n = 33)						
		N (%)	N (%)	N (%)	N (%)	OR* (95% CI)	p value		OR* (95% CI)	p value	
Sex											
Female	No	74 (90.2)	26 (78.8)	23 (95.8)	3 (33.3)	1.00			1.00		
	Yes	8 (9.8)	7 (21.2)	1 (4.2)	6 (66.7)	2.49 (0.82-7.55)	0.107		46.00 (4.03-525.13)	0.002	
Male	No	88 (85.4)	73 (90.1)	51 (89.5)	22 (91.7)	1.00		0.065	1.00		0.007
	Yes	15 (14.6)	8 (9.9)	6 (10.5)	2 (8.3)	0.64 (0.26-1.60)	0.343		0.77 (0.15-4.13)	0.763	
KD diagnosis year											
< 2004	No	100 (88.5)	58 (84.1)	39 (90.7)	19 (73.1)	1.00			1.00		
	Yes	13 (11.5)	11 (15.9)	4 (9.3)	7 (26.9)	1.46 (0.61-3.47)	0.392		3.59 (0.94-13.79)	0.062	
≥ 2004	No	62 (86.1)	41 (91.1)	35 (92.1)	6 (85.7)	1.00		0.250	1.00		0.664
	Yes	10 (13.9)	4 (8.9)	3 (7.9)	1 (14.3)	0.61 (0.18-2.06)	0.421		1.94 (0.17-21.94)	0.591	
Times of IVIG treatment											
1	No	124 (89.2)	66 (86.8)	53 (88.3)	13 (81.2)	1.00			1.00		
	Yes	15 (10.8)	10 (13.2)	7 (11.7)	3 (18.8)	1.25 (0.53-2.94)	0.605		1.75 (0.40-7.69)	0.461	
≥ 2	No	6 (100.0)	19 (86.4)	12 (100.0)	7 (70.0)	-			-		
	Yes	0 (0.0)	3 (13.6)	0 (0.0)	3 (30.0)	-	1.000#	0.496	-	0.078#	-
None	No	32 (80.0)	14 (87.5)	9 (100.0)	5 (71.4)	1.00			-		
	Yes	8 (20.0)	2 (12.5)	0 (0.0)	2 (28.6)	0.57 (0.11-3.04)	0.512		-	0.175#	
Plt 10 count (× 1000/Cumm)											
≤ 400	No	31 (91.2)	9 (100.0)	7 (100.0)	2 (100.0)	-			-		
	Yes	3 (8.8)	0 (0)	0 (0)	0 (0)	-	1.000#	-	-	-	-
> 400	No	131 (70.8)	90 (78.9)	67 (82.7)	23 (69.7)	1.00		-	1.00		-
	Yes	20 (10.8)	15 (13.2)	7 (8.6)	8 (24.2)	1.09 (0.53-2.25)	0.812		3.33 (1.09-10.20)	0.035	
Band (%)											
≤ 5	No	127 (87.6)	77 (90.6)	64 (91.4)	13 (86.7)	1.00			1.00		
	Yes	18 (12.4)	8 (9.4)	6 (8.6)	2 (13.3)	0.73 (0.30-1.77)	0.489		1.64 (0.30-9.05)	0.570	
> 5	No	35 (87.5)	22 (75.9)	10 (90.9)	12 (66.7)	1.00		0.158	1.00		0.443
	Yes	5 (12.5)	7 (24.1)	1 (9.1)	6 (33.3)	2.23 (0.63-7.90)	0.215		5.00 (0.51-48.75)	0.166	
Monocyte (%)											
≤ 7	No	121 (85.2)	80 (87.9)	56 (93.3)	24 (77.4)	1.00			1.00		
	Yes	21 (14.8)	11 (12.1)	4 (6.7)	7 (22.6)	0.79 (0.36-1.73)	0.560		4.08 (1.09-15.26)	0.036	
> 7	No	41 (95.3)	19 (82.6)	18 (85.7)	1 (50.0)	1.00		0.088	1.00		0.819
	Yes	2 (4.7)	4 (17.4)	3 (14.3)	1 (50.0)	4.32 (0.73-25.65)	0.108		6.00 (0.29-124.10)	0.246	

* OR, odds ratio; p-value is estimated by logistic regression; # p-value is estimated by Fisher's exact test; CALs, coronary artery lesions; KD, Kawasaki disease; Plt10 count, the highest platelet counts collected within 10 days of the patients' being diagnosed with KD.

exerts the dominant manner according to the diplotypes analysis; however, the Leu-Ser-Arg haplotype is not found in KD patients with acute CALs. To the best of our knowledge, this is the first paper to investigate the increased risk of chronic CALs development among PECAM-1 SNPs.

The mechanism of IVIG treatment in KD is unknown and a second dose of IVIG has been recommended for KD patients with persistent or recrudescent fever.[4,5] In our studies, receiving ≥ 2 courses of IVIG treatment was strongly associated with developing acute and chronic CALs. In additional IVIG infusion, this probably reflects ongoing active vasculitis and prolonged inflammation, leading to significant coronary artery abnormalities.[18]

Substantially elevated platelet counts develop after the acute phase of KD, and the vascular endothelial inflammation with platelet adhesion and leukocyte activation will play the primary role in aneurysm formation.[19,20] The mortality in KD was due to coronary vasculitis, which occurs concomitantly with a marked elevation of the platelet count and a hypercoagulable state.[5,19] Our results were consistent with this observation. In our study, for KD patients with acute CALs, there was a significant increase in platelet 10 counts. The possible mechanisms for CALs formation, especially in the acute CALs, may be that the CD40 ligand on the activated platelets induces endothelial cells in the blood vessels to secrete chemokines and to express adhesion molecules, causing endothelial cell damage by recruiting inflammatory cells.[20]

In genetic susceptibility, the Onouchi[7] summarized some candidate genes previously tested for association with the risk of CALs in KD, including 5,10-methylenetetrahydrofolate reductase, c-reactive protein, interleukin 10, Fc fragment of gamma immunoglobulins, low affinity IIa, receptor, angiotensin II receptor, vascular endothelial growth factor receptor 2, CD14 antigen, vascular endothelial growth factor A, tumor necrosis factor-α, mannose-binding lectin, matrix metallopeptidase 13, angiotensin I converting enzyme, tissue inhibitor of metalloproteinase 2, and CD40 ligand. However, there was little focus placed on chronic CALs formation.

Platelet endothelial cell adhesion molecule-1 (PECAM-1) is a transmembrane glycoprotein with 6 immunoglobulin-like extracellular domains (encoded by exons 3-8), a short transmembrane domain (encoded by exon 9), and a short cytoplasmic tail (encoded by exons 10-16) and multifunctional vascular cell adhesion molecule involved in the diverse roles in vascular biology, such as the maintenance of a vascular permeability barrier, regulation of multiple stages of monocytes and neutrophils migration through venular walls, and mechanosensing of endothelial cell response to fluid shear stress, angiogenesis, platelet function, and thrombosis.[9-14]

The role of PECAM-1 in the inflammatory process has been previously documented, and polymorphisms in PECAM-1 might affect its function. The transendothelial migration means that leukocytes, in ameboid fashion, enter sites of inflammation by squeezing through the borders between the endothelial cells that line postcapillary venules. PECAM-1is an integral membrane protein with a key role in transendothelial migration.[23] PECAM-1 homophilic interactions on the cell surface can transduce “outside-in” signals and activate MAPK/ERKs and small GTPases, impacting both cadherin-mediated cell-cell and integrin-mediated cell-matrix interactions and activating specific-intracellular-signaling pathways, which mediate rapid and reversible opening of the vascular barrier, and leukocyte adhesion and migration through the endothelium,[24] thereby facilitating leukocyte extravasation. PECAM-1 may also serve as a costimulatory agonistic receptor capable of modulating integrin function in human platelets during adhesion and aggregation at the sites of minor endothelial damage.[20,25] The junctional molecule PECAM-1 in the endothelial cell junction is a powerful regulator of endothelial adhesiveness.[26]

The PECAM-1 SNPs have been reported in connection with coronary artery disease in different ethnic groups. However, the findings relating to the genotypes and alleles of PECAM-1 SNPs (Leu125Val, Ser563Asn, and Arg670Gly) remain controversial.[9,15-21] In one cross-sectional study of 142 myocardial infarction patients with Type 2 diabetic patients in the Slovene population (Caucasians) and 310 control subjects, the frequency of PECAM-1 Leu125Val Leu/Leu genotype was reported to be an independent risk factor.[21] Another study of Sasaoka et al. focusing on the Japanese population, with 136 myocardial infarction patients and 235 controls, found an increased frequency of 125Leu, 563Ser, and 670Arg alleles in myocardial infarction patients, and an increased frequency of Ser563Asn Ser/Ser and Arg670Gly

Arg/Arg genotypes in myocardial infarction patients.[9] Wei et al. reported that the genotypic distribution of Val/Val homozygous of Leu125Val was associated with higher occurrence of coronary artery disease compared to Leu/Leu and Leu/Val genotypes (OR 2.86, 95%CI 1.42-5.76, p = 0.005) in Chinese Singaporeans.[16] In this study, we showed that there were increased risks for chronic CALs formation in the genotypes of Ser563Asn Ser/Ser and Arg670Gly Arg/Arg (Table 3).

We found that all three PECAM-1 SNPs were in linkage disequilibrium with each other, and that Ser563Asn and Arg670Gly were in almost complete linkage disequilibrium. The Leu-Ser-Arg is inherited in a dominant manner and is a good marker for predicting chronic CALs formation in KD patients. In our results (Table 4), it is an attractive hypothesis that the haplotype (Leu-Ser-Arg) and the diplotype (Leu-Ser-Arg/ Leu-Ser-Arg) may be associated with the risk of chronic CALs. The possible mechanisms may be that 1) the allele of Leu-Ser-Arg is associated with a greater elevation of the platelet 1 and platelet 10 count than non-Leu-Ser-Arg, and may cause prolonged endothelial cell damage in coronary artery, and 2) PECAM-1cytoplasmic domain contains highly conserved-signaling motifs known as the intrinsic immunoreceptor-tyrosine inhibitory motifs and phosphorylated motifs can cause beta-catenin binding to vascular endothelial cadherin. But the allele of Leu-Ser-Arg in PECAM-1 interactions reduces the PECAM-1 phosphorylation state, less phosphoration in motifs, less beta-catenin bind to vascular endothelial cadherin, then leading to weakened junctional stability that is conducive to leukocyte transmigration,[21] so chronic CALs are produced.

CALs found in the chronic stage may lead to myocardial and vascular complications with long-term cardiac consequences.[4,5,7,22-26] Studies that have evaluated the association of PECAM-1 polymorphisms with risk of myocardial infarction show an increased risk for the haplotype (Leu-Ser-Arg).[9,27] In KD patients with coronary artery aneurysms, thrombosis was found to be due to sluggish blood flow within a dilated vascular space resulting from stenotic lesion developing in the proximal or distal end of aneurysms. In its clinical manifestation, myocardial infarction caused by thrombotic occlusion in aneurysmal and/or stenotic coronary arteries is the principal cause of death from KD.[5] Early recognition of CALs formation is an important in treatment of KD to reduce mortality and morbidity. In this study, we know higher platelet 10 count is associated acute CALs formation and the allele of Leu-Ser-Arg in PECAM-1 is associated chronic CALs formation. In clinical circumstances, we advise more aggressive treatment and frequent echocardiography to be considered in KD patients with these two high risk factors for CALs formation. And in our study, it is interesting to find that girls with the Leu-Ser-Arg allele of PECAM-1 diplotype are at a higher risk to develop chronic CALs (Table 5), and we still need a large independent cohort to validate the data.

There were limitations to this study. First, we did not study the expression of vascular endothelial cadherin and other adhesion molecules polymorphisms including intercellular adhesion molecule-1, vascular cell adhesion molecule 1, and E-selectin. Second, there is a "selection effect" of chronic CALs for KD cases that were diagnosed before 2004 (chronic CALs rate; < year 2004 vs. ≥ year 2004: 14.3% vs. 6.0%, X^2 = 5.00, p = 0.025; data not shown), but no "selection effect" of acute CALs (acute CALs rate; < year 2004 vs. ≥ year 2004: 37.9% vs. 38.2%, X^2 = 0.009, p = 0.924; data not shown) is found between two different periods of recruitment of KD cases in this study. Before 2004, we retrospectively collected blood samples and chart data of KD cases by use of the retrospective method but a prospective method has been used since 2004. However, this study is a case-control study, not a cross-sectional or cohort study. The focus of case-control study is to identify the genetic risk factor of coronary aneurysm in KD patients, not to estimate the prevalence rate of coronary aneurysm. The possible confounding of "different methods of KD case recruitment" on chronic CALs was adjusted by stratification analysis (Table 5). Because of the limited number of KD children with the variant genotype, haplotype, and diplotype of PECAM-1, multiple logistic regression model cannot be used to control the confounding effect of "KD diagnosis year" on chronic CALs. Third, the prevalence rate of chronic CALs in KD patients was overestimated in this study, and it is a common weakness of case-control studies.

CONCLUSIONS

In KD patients, the higher platelet 10 count has a

role in the pathogenesis of acute CALs. The haplotype of Leu-Ser-Arg can be used to predict the risk for chronic CALs formation in KD patients. These findings on PECAM-1 SNPs may have a favorable impact on the further treatment of KD.

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CONFLICT OF INTERESTS

All the authors declare no conflict of interest.

DISCLOSURES

None.

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