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Major Histocompatibility Complex Class I Chain-Related Gene Polymorphisms: Associated with Susceptibility to Kawasaki Disease and Coronary Artery Aneurysms

Yao-Yuan Hsieh,[1,2] Chi-Chen Chang,[2] Chin-Mu Hsu,[1,3] Shih-Yin Chen,[4] Wen-Hsin Lin,[5] and Fuu-Jen Tsai[3,4,6]

Background: Kawasaki disease (KD) involves a complex interaction of immunoinflammatory process, cytokine activation, and genetic factors. We aimed to investigate whether genetic variations in a major histocompatibility complex (MHC) class could be used as markers of susceptibility in KD and coronary artery aneurysm lesions (CALs). *Methods:* Individuals were divided into following groups: (1) normal controls; (2) KD with CAL; (3) KD without CAL. Polymorphisms for MHC class I chain-related genes A (MICA) (rs2301747, rs2256184, rs2848716), MICB (rs2855804, rs3132464, rs2516400), BAT3 (rs750332), MSH5 (rs1150793), and chromosome 6 open reading frame 27 (C6orf27, rs707928) were genotyped with polymerase chain reaction and the TaqMan® allelic discrimination assay. Genotypes, alleles, and haplotype in each group were compared. *Results:* Genotype and allele frequency of MICB*rs2516400 polymorphisms in each group were significantly different. MICB (rs2516400)*C-related genotypes/alleles are correlated with development of KD and CAL. Proportions of rs2516400*TT/TC/CC were (1) 1/39/60%, (2) 0/0/100%, and (3) 0/0/100%. Other single-nucleotide polymorphisms were not associated with KD susceptibilities. Haplotypes (rs2301747-rs2256184-rs2848716-rs2855804-rs3132464-rs2516400-rs750332-rs1150793-rs707928) G-G-G-C-T-C-T-A-A, C-A-G-T-T-C-T-A-A, and G-G-G-C-C-C-T-A-A were associated with higher susceptibilities for KD. The G-G-G-T-T-T-T-G-G and C-G-G-T-T-T-T-A-A haplotypes were associated with lower susceptibilities. *Conclusion:* MICB*rs2516400 polymorphisms and some MHC class I-related haplotypes are associated with KD susceptibility. MICB and MHC class I genetic variations might contribute to the pathogenesis of KD and CAL.

Introduction

Kawasaki disease (KD) is a complex vasculitis disease, which is associated with immunologic and genetic changes. KD is characterized by persistent fever, nonpurulent conjunctivitis, oropharyngeal inflammation, induration and erythema of hands and feet, rash, and cervical lymphadenopathy. Despite intensive research, the etiology of KD remains unclear. Current theories suggest that KD is an immunologically mediated vasculitis (Kamizono *et al.*, 1999). The features of KD are immune activation and cytokine-mediated generalized vasculitis. The injured vascular tissues show subendothelial edema, vascular damage, gap formation, and fenestration of endothelial cells, which contribute to the pathogenesis of this disorder (Leung *et al.*, 1989).

Vascular endothelial cells express major histocompatibility complex (MHC) molecules on their surface for presenting antigenic peptides to T cells, which initiate acquired immune responses (Danese *et al.*, 2007). Human lymphocyte antigen (HLA) from the MHC has been reported to be associated with immune-mediated vascular diseases (Pay *et al.*, 2007). HLA has regulatory functions in both the innate and adaptive immune responses. Recently, with the clinical application of molecular genetic technology, some HLA polymorphisms have been identified to be associated with KD. As the vascular endothelium is a functional barrier between the vessel wall and the bloodstream, endothelial cell damage or vascular injury of some disorders might lead to the expression and release of HLA molecules (Coupel *et al.*, 2007).

MHC class I chain-related genes (MICs) are located within MHC class I region of chromosome 6. MICs, MICA and MICB, are located centromeric to human leukocyte antigen B (HLA-B) on chromosome 6 (Fig. 1). Recent work supports the findings that MICA is associated with several autoimmune

[1]School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan.
[2]Division of Infertility Clinic, Hsieh Yao-Yuan Womens' Hospital, Taichung, Taiwan.
[3]Department of Medical Genetics, China Medical University Hospital, Taichung, Taiwan.
[4]Graduate Institute of Chinese Medical Science, China Medical University Hospital and [5]School of Pharmacy Undergraduate Program, Department of Medicine, China Medical University, Taichung, Taiwan.
[6]Department of Health and Nutrition Biotechnology, Asia University, Taichung, Taiwan.

Table 1. Gene Positions and Polymerase Chain Reaction Primer and Probe Sequences for Nine Single-Nucleotide Polymorphisms Genotyped in the SDF-1 Gene

Gene name	SNP database ID	Nucleotide change	Reporter 1 dye	Allele	Reporter 1 quencher	Reporter 2 dye	Allele	Reporter 2 quencher	Context sequence	PCR conditions (annealing temperature)
MICA	rs2301747	C/G	VIC	C	NFQ	FAM	G	NFQ	AGGGCGGGGCTCCTGTGC[C/G]CTGTCGGTGGCGC	62°C
	rs2256184	A/G	VIC	A	NFQ	FAM	G	NFQ	TCACGTGCTGAATGCT[A/G]AGGGCCTGGAT	60°C
	rs2848716	C/G	VIC	C	NFQ	FAM	G	NFQ	TCACCATTTCCTA[C/G]AAGCCCCTCAAC	60°C
MICB	rs2855804	C/T	VIC	C	NFQ	FAM	T	NFQ	CCTCCAGACCCC[C/T]AAGGAGAGCCCTGAT	60°C
	rs3132464	C/T	VIC	C	NFQ	FAM	T	NFQ	TTCTCTCAGAGAAAGGG[C/T]GAATCT GATTTGGGGCA	62°C
	rs2516400	C/T	VIC	C	NFQ	FAM	T	NFQ	AGTGGATTTTGCC[C/T]AAGTAAAAACTCGGA	60°C
BAT3	rs750332	C/T	VIC	C	NFQ	FAM	T	NFQ	ACGCTGGCCAAGTCCC[C/T]ATGATCCCAGC AAGCACA	62°C
MSH5	rs1150793	A/G	VIC	A	NFQ	FAM	G	NFQ	TCCCGCTGCAATGTGT[A/G]TCTTGTAGGAG TTAAGGGA	62°C
C6orf27	rs707928	A/G	VIC	A	NFQ	FAM	G	NFQ	AGCCTGAATGTTTGAAACT[A/G] GGGCTGTGCTGGAAAA	62°C

The genotyping method was designed by TaqMan® Genotyping Assays (Applied Biosystems).
BAT, human lymphocyte antigen-B associated transcript; SNP, single-nucleotide polymorphism; PCR, polymerase chain reaction.

diseases. MIC proteins are considered to be markers of "stress" in the epithelia and act as ligands for cells expressing a common activating natural killer (NK)-cell receptor. MICA molecules appear to be highly flexible and polymorphic (Stephens 2001). The functional relevance and implications of MIC polymorphism have to be yet fully discerned.

A large number of genes have been previously identified between the class I and class II gene families within the HLA class I region. Genetic studies suggest HLA class I genes might be associated with KD development (Huang *et al.*, 2000). Several studies have indicated that genes in the HLA region contribute to KD susceptibility. These genes include HLA-B–associated transcript (BAT). BAT3, a member of the BAT family, is originally identified as one of the genes located within MHC. BAT appears to regulate the production of inflammatory cytokines associated with neurological disorders (Gnjec *et al.*, 2008). MSH5, another single-nucleotide polymorphism (SNP) within the HLA region, is involved in DNA mismatch repair (MMR) and meiotic recombination (Wang *et al.*, 2008). Despite some reports in the literature about the effects of these genetic variations upon human disorders, few investigators have demonstrated the association of KD with MIC, BAT, MSH, or the chromosome 6 open reading frame 27 (C6orf27).

The aim of this study was to assess whether these markers within the MHC class I region are associated with KD. A total of 6 genetic variations and related haplotypes within MHC class I region were evaluated, including MICA (rs2301747, rs2256184, rs2848716), MICB (rs2855804, rs3132464, rs2516400), BAT3 (rs750332), MSH5 (rs1150793) and C6orf27 (rs707928). We tried to search for association of these MHC genes with susceptibility to KD and the occurrence of coronary artery aneurysm lesions (CALs) in Taiwanese children. To the best of our knowledge, this is the first survey of this sort.

Materials and Methods

Taiwanese children with and without the histories of KD were recruited and divided into following groups: (1) normal controls ($n = 668$); (2) KD with CAL ($n = 29$); (3) KD without CAL ($n = 63$). All children with KD were evaluated at China Medical University Hospital and met the criteria of KD. Every patient underwent regular echocardiography examinations, beginning during the acute stage of KD, at 2 and 6 months after disease onset, and once a year thereafter. A CAL was identified when either the right or left coronary artery showed a dilated diameter of 3 mm in children younger than 5 years or 4 mm in children older than 5 years (Akagi *et al.*, 1992). The control group consisted of healthy children randomly selected from the Han Chinese Cell and Genome Bank (Hung *et al.*, 2005). Control subjects were matched for sex and age with the study patients. The estimated prevalence of KD is fewer than 1 in 1000 children; therefore, it should be assumed that there were no KD cases in the control group. This series was approved by the ethical committee and institutional review board of China Medical University Hospital. Informed consents were signed by all individuals who donated their blood.

All individuals accepted the peripheral blood sampling for genotype analyses. Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Roche Genomic DNA kit). SNP discovery and genotyping for MICA (rs2301747, rs2256184, rs2848716), MICB (rs2855804, rs3132464, rs2516400), BAT3 (rs750332), MSH5 (rs1150793),

and C6orf27 (rs707928) were obtained from dbSNP (http://ncbi.nih.gov/SNP/) and Applied Biosystems (www.appliedbiosystems.com) (Table 1). The genetic variations were detected by TaqMan(R) Genotyping Assays (Applied Biosystems). SNPs were detected by polymerase chain reaction (PCR) system with TaqMan allelic discrimination assay (Applied Biosystems) (Table 1) (Ricci *et al.*, 2009). Briefly, the PCR was carried out in a total volume of 12.5 μL using the following amplification protocol: denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, and annealing and extension at 60°C–62°C for 1 min. Further, genotyping of each sample was automatically attributed by measuring the allele-specific fluorescence on the ABI Prism 7700 Sequence Detection System, using the SDS 1.9.1 software for allelic discrimination (Applied Biosystems). Genotype frequencies of each SNP were determined by direct counting. The resulting spectra were processed with SpectroTyper (Sequenom) software.

The genotype frequency and allelic frequency distributions of these polymorphisms in both KD patients and controls were analyzed by the χ^2 method. Haplotypes were estimated in compound heterozygotes using the PHASE program. Haplotype analysis was performed using Haploview. The SAS system with χ^2 test was utilized for statistical analyses. Allelic frequencies were expressed as a percentage of the total number of alleles. Odds ratios were calculated from genotype frequencies and allelic frequencies with 95% confidence interval. A p-value of <0.05 was considered statistically significant.

Results

Genotype distribution and allele frequency of MICB* rs2516400 polymorphisms in each group were significantly different (Table 2). Proportions of MICB rs2516400*T homozygote/heterozygote/C homozygote in (1) controls, (2) KD with CAL, and (3) KD without CAL were (1) 1/39/60%, (2) 0/0/100%, and (3) 0/0/100%, respectively (Table 2). MICB (rs2516400)*C-related genotypes and alleles are correlated with higher susceptibilities for KD and CAL. In contrast, other SNPs (MICA*rs2301747, MICA*rs2256184, MICA*rs2848716, MICB*rs2855804, MICB*rs3132464, BAT3*rs750332, MSH5*rs1150793, C6orf27*rs707928) are not associated with KD or CAL susceptibilities (Table 2).

Haplotypes (MICA*rs2301747-MICA*rs2256184-MICA*rs2848716-MICB*rs2855804-MICB*rs3132464-MICB*rs2516400-BAT3*rs750332-MSH5*rs1150793-C6orf27*rs707928) G-G-G-C-T-C-T-A-A, C-A-G-T-T-C-T-A-A, and G-G-G-C-C-C-T-A-A haplotypes are associated with higher susceptibilities for KD (Table 3). The G-G-G-T-T-T-G-G and C-G-G-T-T-T-A-A haplotypes are associated with lower susceptibilities for KD. Further, the C-G-G-C-T-C-T-A-A haplotype is associated with higher susceptibility for KD and CAL. The C-A-G-C-T-C-T-A-A is associated with higher susceptibility for CAL in KD individuals (Table 3). Pairwise linkage disequilibriums between these SNPs associated with KD are presented in Figure 2.

Discussion

KD, an acute, self-limited, and systemic vasculitis, is one of the leading causes of acquired heart disease in children (Burns and Glodé, 2004). Although KD is a mysterious disease of unknown etiology and pathogenesis, it is believed to be caused by infectious agents, host immune dysregulation, and genetic susceptibility (Tse *et al.*, 2002). The development of KD involves a complex interaction between immunoinflammatory process, cytokines activation, and genetic factors. KD is a multisystemic disorder with a possible underlying pathology of immune-mediated vasculitis (Burns and Glodé, 2004). Recent studies suggest a potential role of bacterial toxins in the immunopathogenesis of KD (Lin *et al.*, 1992). During the acute stage of KD, activation of vascular endothelial cells and increased serum levels of proinflammatory cytokines are involved in the occurrence of inflamed and injured vessels (Lin *et al.*, 1992). The vascular inflammation might cause the development of CAL and cardiac complications. Patients with these cardiovascular complications are at increased risk of ischemic heart disease, which may lead to myocardial infarction and sudden death (Kato *et al.*, 1996). Although the administration of immunoglobulin significantly reduces the development of CALs, 2%–15% of KD patients suffer from this complication (Muta *et al.*, 2004).

Recent studies have shown that the expression of soluble HLA might have important implications in the pathogenesis of immune-mediated vascular diseases (Coupel *et al.*, 2007). The HLA-B35, B75, and Cw09 are associated with KD susceptibilities (Oh *et al.*, 2008). HLA-B*5801 allele is a genetic marker for severe cutaneous adverse reactions caused by allopurinol (Hung *et al.*, 2005). The HLA-DR gene variations were not associated with susceptibility for KD and CALs in Taiwanese population (Huang *et al.*, 2007). HLA-G plays a crucial role for the susceptibility to KD and CAL (Kim *et al.*, 2008). In our previous survey, we observed an association between HLA-E gene polymorphism and KD (Lin *et al.*, 2009).

MICs belong to a multicopy gene family located within the HLA class I region of the short arm of human chromosome 6. One member of MHC genes is the MICA gene, which is characterized by its high degree of polymorphisms. There were over 50 MICA alleles described (Rueda *et al.*, 2002). MIC encodes for proteins that have a completely different organization, expression, and products from classical HLA class I gene products. The predicted amino acid sequence of MICA chain suggests that it folds similarly to typical class I chains and might have the capacity to bind peptides or other short ligands. MICA is predicted to have a specialized function in antigen presentation or T-cell recognition (Mizuki *et al.*, 1997). MICA was found to be associated with Addison's disease (Gambelunghe *et al.*, 1999), Behcet disease (Mizuki *et al.*, 1997), etc.

Some MICA polymorphisms have been shown to influence various chronic inflammatory conditions (Folwaczny *et al.*, 2011). Some MIC gene polymorphisms and expressions are associated with autoimmune diseases (Li *et al.*, 2010). Some MIC molecules are receptors on NK cells, T4 cells, and T8 cells that mediate host antitumor immune response (Kopp *et al.*, 2009). Recently, Folwaczny *et al.* (2011) demonstrated the association between the MIC SNPs and the susceptibility to chronic periodontitis. Some MIC genetic variation was also associated with ulcerative colitis (Li *et al.*, 2010). The functional role of MIC genes in the pathogenesis of rheumatoid arthritis has been demonstrated (López-Arbesu *et al.*, 2007).

The gene coding for BAT lies adjacent to TNF in the central MHC. BAT polymorphisms are also associated with asthma susceptibilities (Migita *et al.*, 2005), rheumatoid arthritis (Martinez *et al.*, 2004), Chagas cardiomyopathy (Ramasawmy

Table 2. Genotype and Allele Distributions of MICA, MICB, BAT3, and MSH5 Polymorphisms in the Taiwanese Kawasaki Disease Patients and Controls

Gene name (SNP database ID)	Genotype/ allele	Ctrl	KD total	KD CAL(+)	KD CAL(−)	Ctrl vs. KD total			KD CAL (+) vs. Ctrl			KD CAL (−) vs. Ctrl			KD CAL (+) vs. CAL (−)		
						p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI
MICA		(*n* = 668)	(*n* = 92)	(*n* = 29)	(*n* = 63)												
rs2301747	GG	22 (0.03)	3 (0.03)	0 (0)	3 (0.05)	0.81	1.04	0.3–3.57	0.60	0.00		0.67	1.56	0.45–5.43	0.48	0.00	
	GC	189 (0.28)	29 (0.32)	9 (0.31)	20 (0.32)		1.17	0.73–1.88		1.09	0.49–2.43		1.21	0.69–2.12		0.90	0.35–2.33
	CC	457 (0.68)	60 (0.65)	20 (0.69)	40 (0.63)		1.00			1.00			1.00			1.00	
	Allele G	233 (0.17)	35 (0.19)	9 (0.16)	26 (0.21)	0.60	1.11	0.75–1.65	0.71	0.87	0.42–1.79	0.37	1.23	0.78–1.94	0.41	0.71	0.31–1.62
	Allele C	1103 (0.83)	149 (0.81)	49 (0.84)	100 (0.79)		1.00			1.00			1.00			1.00	
MICA		(*n* = 660)	(*n* = 93)	(*n* = 30)	(*n* = 63)												
rs2256184	GG	115 (0.17)	15 (0.16)	7 (0.23)	8 (0.13)	0.80	0.84	0.44–1.6	0.65	1.62	0.59–4.45	0.37	0.59	0.26–1.34	0.28	2.72	0.77–9.62
	AG	306 (0.46)	41 (0.44)	14 (0.47)	27 (0.43)		0.87	0.54–1.39		1.21	0.52–2.85		0.75	0.43–1.31		1.61	0.6–4.34
	AA	239 (0.36)	37 (0.4)	9 (0.3)	28 (0.44)		1.00			1.00			1.00			1.00	
	Allele G	536 (0.41)	71 (0.38)	28 (0.47)	43 (0.34)	0.53	0.90	0.66–1.24	0.35	1.28	0.76–2.15	0.16	0.76	0.52–1.11	0.10	1.69	0.9–3.16
	Allele A	784 (0.59)	115 (0.62)	32 (0.53)	83 (0.66)		1.00			1.00			1.00			1.00	
MICA		(*n* = 666)	(*n* = 93)	(*n* = 30)	(*n* = 63)												
rs2848716	CC	66 (0.1)	12 (0.13)	4 (0.13)	8 (0.13)	0.27	1.63	0.79–3.32	0.81	1.33	0.42–4.22	0.14	1.82	0.77–4.35	0.41	0.73	0.18–2.94
	GC	314 (0.47)	49 (0.53)	13 (0.43)	36 (0.57)		1.39	0.87–2.24		0.91	0.42–2		1.73	0.97–3.08		0.53	0.2–1.36
	GG	286 (0.43)	32 (0.34)	13 (0.43)	19 (0.3)		1.00			1.00			1.00			1.00	
	Allele C	446 (0.33)	73 (0.39)	21 (0.35)	52 (0.41)	0.12	1.28	0.94–1.76	0.81	1.07	0.62–1.84	0.08	1.40	0.96–2.03	0.41	0.77	0.4–1.45
	Allele G	886 (0.67)	113 (0.61)	39 (0.65)	74 (0.59)		1.00			1.00			1.00			1.00	
MICB		(*n* = 662)	(*n* = 93)	(*n* = 30)	(*n* = 63)												
rs2855804	TT	5 (0.01)	0 (0)	0 (0)	0 (0)	0.69	0.00		0.39	0.00		0.43	0.00				
	CT	297 (0.45)	43 (0.46)	10 (0.33)	33 (0.52)		1.04	0.67–1.61		0.61	0.28–1.31		1.33	0.79–2.24		0.45	0.18–1.12
	CC	360 (0.54)	50 (0.54)	20 (0.67)	30 (0.48)		1.00			1.00			1.00			1.00	
	Allele T	307 (0.23)	43 (0.23)	10 (0.17)	33 (0.26)	0.98	1.00	0.69–1.43	0.24	0.66	0.33–1.32	0.45	1.18	0.77–1.78	0.15	0.56	0.26–1.24
	Allele C	1017 (0.77)	143 (0.77)	50 (0.83)	93 (0.74)		1.00			1.00			1.00			1.00	
MICB		(*n* = 656)	(*n* = 92)	(*n* = 30)	(*n* = 62)												
rs3132464	CC	1 (0)	0 (0)	0 (0)	0 (0)	0.89	0.00		0.53	0.00		0.89	0.00				
	TC	85 (0.13)	13 (0.14)	6 (0.2)	7 (0.11)		1.10	0.59–2.07		1.68	0.67–4.22		0.85	0.38–1.94		1.96	0.6–6.46
	TT	570 (0.87)	79 (0.86)	24 (0.8)	55 (0.89)		1.00			1.00			1.00			1.00	
	allele C	87 (0.07)	13 (0.07)	6 (0.1)	7 (0.06)	0.83	1.07	0.58–1.96	0.31	1.56	0.65–3.74	0.67	0.84	0.38–1.36	0.28	1.86	0.6–5.79
	allele T	1225 (0.93)	171 (0.93)	54 (0.9)	117 (0.94)		1.00			1.00			1.00			1.00	

(*continued*)

Table 2. (Continued)

Gene name (SNP database ID)	Genotype/ allele	Ctrl	KD total	KD CAL(+)	KD CAL(−)	Ctrl vs. KD total			KD CAL (+) vs. Ctrl			KD CAL (−) vs. Ctrl			KD CAL (+) vs. CAL (−)		
						p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI
MICB		(*n* = 624)	(*n* = 85)	(*n* = 29)	(*n* = 56)												
rs2516400	TT	7 (0.01)	0 (0)	0 (0)	0 (0)	***< 0.005***	0.00		< 0.005	0.00		< 0.005	0.00				
	CT	245 (0.39)	0 (0)	0 (0)	0 (0)		0.00			0.00			0.00				
	CC	372 (0.6)	85 (1)	29 (1)	56 (1)		1.00			1.00			1.00			1.00	
	allele T	259 (0.21)	0 (0)	0 (0)	0 (0)	***< 0.005***	0.00		< 0.005	0.00		< 0.005	0.00				
	allele C	989 (0.79)	170 (1)	58 (1)	112 (1)		1.00			1.00			1.00			1.00	
BAT3		(*n* = 678)	(*n* = 93)	(*n* = 30)	(*n* = 63)												
rs750332	CC	21 (0.03)	1 (0.01)	0 (0)	1 (0.02)	0.53	0.35	0.05–2.61	0.41	0.00		0.51	0.54	0.07–4.13	0.34	0.00	
	CT	156 (0.23)	23 (0.25)	5 (0.17)	18 (0.29)		1.07	0.65–1.77		0.64	0.24–1.71		1.31	0.74–2.34		0.49	0.16–1.48
	TT	501 (0.74)	69 (0.74)	25 (0.83)	44 (0.7)		1.00			1.00			1.00			1.00	
	allele C	198 (0.15)	25 (0.13)	5 (0.08)	20 (0.16)	0.67	0.91	0.58–1.42	0.18	0.53	0.21–1.34	0.70	1.10	0.67–1.82	0.16	0.48	0.17–1.35
	allele T	1158 (0.85)	161 (0.87)	55 (0.92)	106 (0.84)		1.00			1.00			1.00			1.00	
MSH5		(*n* = 678)	(*n* = 91)	(*n* = 30)	(*n* = 61)												
rs1150793	GG	8 (0.01)	0 (0)	0 (0)	0 (0)	0.37	0.00		0.73	0.00		0.22	0.00				
	AG	113 (0.17)	19 (0.21)	4 (0.13)	15 (0.25)		1.30	0.75–2.24		0.76	0.26–2.22		1.61	0.87–2.98		0.47	0.14–1.57
	AA	557 (0.82)	72 (0.79)	26 (0.87)	46 (0.75)		1.00			1.00			1.00			1.00	
	allele G	129 (0.1)	19 (0.1)	4 (0.07)	15 (0.12)	0.69	1.11	0.67–1.84	0.46	0.68	0.24–1.9	0.32	1.33	0.75–2.36	0.24	0.51	0.16–1.61
	allele A	1227 (0.9)	163 (0.9)	56 (0.93)	107 (0.88)		1.00			1.00			1.00			1.00	
C6orf27		(*n* = 658)	(*n* = 93)	(*n* = 30)	(*n* = 63)												
rs707928	GG	98 (0.15)	8 (0.09)	3 (0.1)	5 (0.08)	0.15	0.61	0.28–1.36	0.71	0.59	0.17–2.1	0.10	0.63	0.23–1.70	0.46	0.94	0.19–4.58
	GA	290 (0.44)	49 (0.53)	13 (0.43)	36 (0.57)		1.27	0.8–2.01		0.86	0.4–1.87		1.52	0.87–2.66		0.57	0.23–1.43
	AA	270 (0.41)	36 (0.39)	14 (0.47)	22 (0.35)		1.00			1.00			1.00			1.00	
	allele G	486 (0.37)	65 (0.35)	19 (0.32)	46 (0.37)	0.60	0.92	0.67–1.27	0.41	0.79	0.45–1.38	0.93	0.98	0.67–1.44	0.52	0.81	0.42–1.55
	allele A	830 (0.63)	121 (0.65)	41 (0.68)	80 (0.63)		1.00			1.00			1.00			1.00	

Numbers in parentheses indicate percentage of the genotype or allele frequency. Numbers in bold italics indicate significant differences.
CAL, coronary artery aneurysm lesion; CI, confidence interval; Ctrl, control subject; OR, odds ratio; KD, Kawasaki disease; MICA, major histocompatibility complex class I chain-related genes.

Table 3. Haplotype Distributions of MICA*rs2301747-MICA*rs2256184-MICA*rs2848716-MICB*rs2855804-MICB*rs3132464-MICB*rs2516400-BAT3*rs750332-MSH5*rs1150793-C6orf27*rs707928 Genes in the Taiwanese Kawasaki Disease Patients and Controls

	Estimated haplotype frequency				*Estimated haplotype frequency*				p-*Value*			
Haplotype	*Ctrl & KD total*	*Ctrl & KD (+)*	*Ctrl & KD (−)*	*KD(+) & KD (−)*	*Ctrl (n=679)*	*KD total (n=93)*	*KD CAL(+) (n=30)*	*KD CAL (−) (n=63)*	*Ctrl vs. KD total*	*KD CAL (+) vs. Ctrl*	*KD CAL (−) vs. Ctrl*	*KD CAL (+) vs. CAL (−)*
C-A-C-C-T-C-T-A-A	21%	21%	21%	24%	22%	20%	21%	20%	0.6655	0.9441	0.7727	0.2783
C-A-G-C-T-C-T-A-A	14%	14%	15%	12%	15%	12%	8%	14%	0.2549	0.1181	0.8479	***0.0495***
C-G-G-C-T-C-T-A-A	9%	9%	9%	11%	9%	12%	17%	10%	0.1527	***0.0345***	0.8041	0.907
G-G-G-T-T-T-G-G	5%	6%	6%	—	6%	1%	2%	1%	***0.0047***	0.1423	***0.0159***	—
C-G-G-C-T-C-T-A-G	4%	5%	4%	—	5%	2%	2%	1%	0.0749	0.401	0.1015	—
C-A-C-C-T-C-C-A-G	4%	4%	4%	2%	4%	3%	3%	4%	0.5347	0.5244	0.9826	0.9115
C-A-C-C-T-C-T-A-G	4%	4%	4%	5%	4%	6%	6%	6%	0.0979	0.2368	0.1624	0.1964
C-A-G-T-T-T-T-A-A	3%	3%	3%	—	3%	1%	1%	1%	0.0577	0.3696	0.1032	—
G-G-G-C-T-C-T-A-A	3%	3%	3%	—	3%	6%	0%	5%	***0.0122***	0.2441	0.0946	—
C-G-G-T-T-T-T-A-A	2%	3%	3%	—	3%	0%	7%	0%	***0.0322***	0.0596	0.0646	—
G-G-G-C-T-C-C-A-G	2%	2%	3%	1%	2%	3%	0%	4%	0.8994	0.2486	0.2558	0.3216
C-A-G-C-T-C-T-G-A	2%	2%	2%	—	2%	1%	1%	1%	0.2212	0.5233	0.3051	—
C-A-C-T-T-T-T-A-G	2%	2%	2%	—	2%	0%	0%	0%	0.0595	0.2764	0.1205	—
C-G-G-T-T-T-C-A-G	2%	2%	2%	—	2%	0%	1%	0%	0.163	0.7794	0.1376	—
G-G-G-C-T-C-T-A-G	2%	2%	1%	—	2%	2%	3%	1%	0.8015	0.3038	0.5639	—
C-A-G-T-T-C-T-A-A	2%	1%	2%	5%	1%	4%	2%	5%	***0.0013***	0.7122	***2.00E-04***	0.146
G-G-G-T-T-T-C-A-G	1%	1%	1%	—	1%	0%	0%	1%	0.2581	0.3471	0.4779	—
C-G-G-C-C-C-T-A-A	1%	1%	1%	—	1%	1%	1%	1%	0.5432	0.7837	0.5473	—
C-G-G-C-T-C-C-A-G	1%	1%	1%	—	1%	1%	2%	1%	0.8341	0.7847	0.6405	—
C-A-G-C-T-C-C-A-G	1%	1%	1%	—	1%	1%	0%	0%	0.7759	0.4034	0.235	—
G-G-G-C-C-C-T-A-A	1%	—	—	—	1%	3%	—	—	***0.0315***	—	—	—
C-A-G-C-T-C-C-A-G	—	1%	—	2%	—	—	2%	—	—	0.3829	—	0.9326

Numbers in bold italics indicate significant differences.
Haplotypes frequency of >1% are listed.

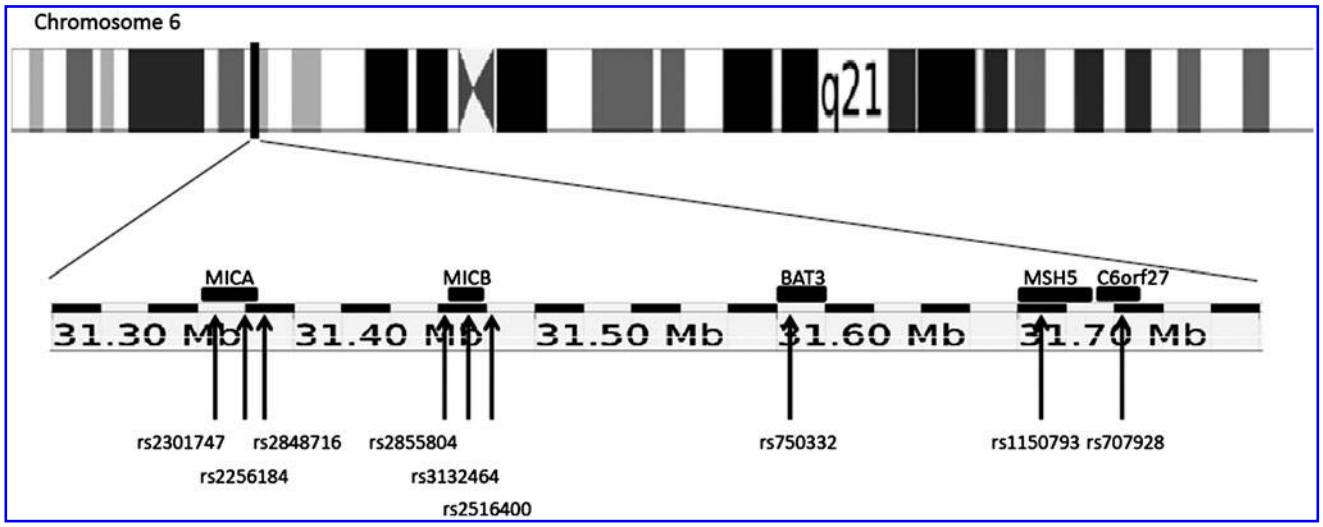

FIG. 1. Map of major histocompatibility complex class I chain-related genes (MICA) (rs2301747, rs2256184, rs2848716), MICB (rs2855804, rs3132464, rs2516400), HLA-B associated transcript 3 (BAT3) (rs750332), MSH5 (rs1150793), and chromosome 6 open reading frame 27 (C6orf27, rs707928) lying within the major histocompatibility complex region on chromosome 6p21.3.

et al., 2006), Alzheimer's disease pathology (Gnjec *et al.*, 2008), malaria (Diakite *et al.*, 2009), etc. BAT3, a nuclear protein, encodes a large proline-rich protein with unknown function. BAT3 is capable of modulating transforming growth factor (TGF) signaling and acts as a positive regulator of TGF of collagen expression (Kwak *et al.*, 2008). The intracellular protein of BAT3 is involved in DNA damage-induced apoptosis (Simhadri *et al.*, 2008). BAT3 could directly bind to NKp30 and engaged NKp30 on NK cells. BAT3 also triggered NKp30-mediated cytotoxicity, which was essential for tumor rejection (Pogge von Strandmann *et al.*, 2007). BAT3 is an essential regulator of p53-mediated responses to genotoxic stress (Sasaki *et al.*, 2007). BAT3 also controls DNA damage-induced acetylation of p53 (Sasaki *et al.*, 2007).

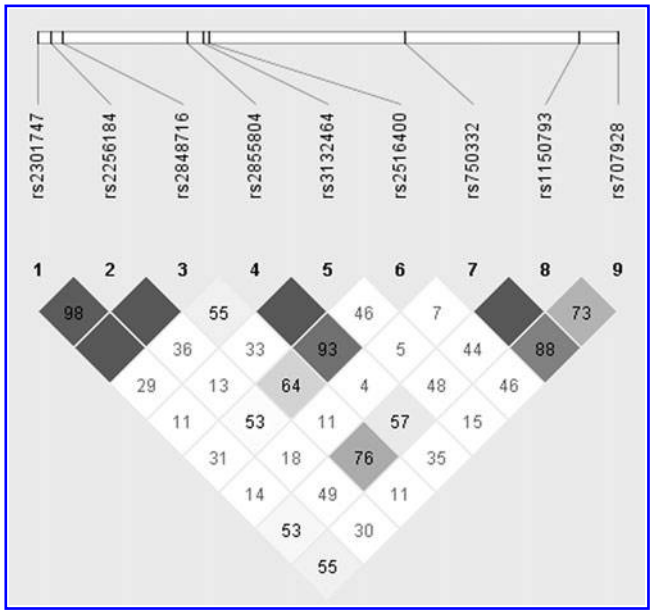

FIG. 2. Haplotype blocks of MICA (rs2301747, rs2256184, rs2848716), MICB (rs2855804, rs3132464, rs2516400), BAT3 (rs750332), MSH5 (rs1150793), and C6orf27 (rs707928) for the 668 control subjects and 92 Kawasaki disease patients constructed according to the confidence interval approach using Haploview software. Dark gray indicates linkage disequilibrium; white and light gray indicate evidence of recombination.

MSH5 plays functional roles in cellular processes, such as DNA damage response and meiotic homologous recombination (Yi *et al.*, 2005). The compromised MSH5 molecules play a crucial role in mismatch repair proteins (Xu *et al.*, 2010). MSH5 is associated with numerous disorders, including diabetics (Valdes *et al.*, 2009), lung cancer (Wang *et al.*, 2008), spermatogenesis (Xu *et al.*, 2010), etc. Severe cutaneous adverse reactions caused by allopurinol were associated with MSH5 (Hung *et al.*, 2005). MSH5*rs707915 is associated with diabetics (Valdes *et al.*, 2009). MSH5 C85T polymorphisms may be genetic determinants for human spermatogenesis impairment (Xu *et al.*, 2010).

Genetic studies of these multifactorial diseases such as KD are difficulty to approach because of the uncertainty of a polygenic trait. The SNPs are the most abundant types of DNA sequence variation in the human genome (Kwok and Gu 1999). The SNP markers provide a new way for the identification of complex gene-associated diseases. In this study, the genotype distributions, allelic frequencies, and haplotypes for MICB*rs2516400 polymorphisms in KD and non-KD patients were statistically different. MICB*rs2516400*C-related genotypes and alleles are correlated with the developments of KD and CAL. In contrast, other MHC genotypes/allele (MICA*rs2301747, MICA*rs2256184, MICA*rs2848716, MICB*rs 2855804, MICB*rs3132464, BAT3*rs750332, MSH5*rs1150793, C6orf27*rs707928) are not associated with KD susceptibilities. Some MHC haplotypes (G-G-G-C-T-C-T-A-A, C-A-G-T-T-C-T-A-A, and G-G-G-C-C-C-T-A-A) were associated with higher susceptibilities of KD with CAL. These findings suggested that MICB polymorphisms might be useful genetic markers in the prediction of the susceptibility to KD. It also suggested that some MHC genetic variations might be involved in disease

susceptibility and development. These SNPs might influence the production of soluble MICB by vascular endothelial cells, which further compromise the progression of KD.

In conclusion, MICB*rs2516400 polymorphisms and related haplotypes are associated with KD and CAL susceptibility. The related genetic variations might contribute to the pathogenesis of KD and CAL. MHC haplotypes (G-G-G-C-T-C-T-A-A, C-A-G-T-T-C-T-A-A, G-G-G-C-C-C-T-A-A) might be associated with higher susceptibilities for KD. These data suggest that some MICB molecules might be involved in the pathogenesis of KD. Some genotype/allele frequencies and haplotypes of MHC polymorphism might be useful markers for the prediction of KD susceptibility. This could provide the database for the further survey of the MHC gene polymorphisms. However, the real roles of MHC polymorphisms upon the KD remain to be clarified. Further, possible effects of other immune gene polymorphisms upon KD development merits further surveys.

Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Fuu-Jen Tsai, M.D., Ph.D.
Department of Medical Genetics
China Medical University Hospital
No.2 Yuh-Der Road
Taichung 402
Taiwan

E-mail: d0704@www.cmuh.org.tw