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Polymorphisms of Transmembrane Channel-like 1 Gene are Associated with Kawasaki Disease in Korean Population

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Accepted 19 November 2009

Abstract

Kawasaki disease (KD) is believed to be infectious but etiology and the mechanism of development remain elusive. The aim of this study was to investigate the association between transmembrane channel-like 1 (TMC1) gene and KD. One hundred nine KD patients and 424 normal controls were enrolled. Of all KD patients, 34 developed coronary artery lesions (CALs). Eleven single nucleotide polymorphisms (SNPs) within TMC1 gene were selected and SNP genotyping was performed by the direct sequencing. Genotype frequencies were analyzed with the SNPAnalyzer, Helixtree, and SNPStats programs. In the present study, six SNPs (rs7851577, rs10781105, rs2589615, rs1663743, rs1373628, and rs1373626) were significantly associated with the risk of KD. In further haplotype analysis, one haplotype (CGGACCCT) showed a significant association between KD and control groups. These results suggest that TMC1 gene may be a susceptibility gene for KD in Korean population.

Keywords: Kawasaki disease, Polymorphism, Transmembrane channel-like 1 gene, Haplotype

Kawasaki disease (KD) is an acute, self-limited vasculitis of infants and children characterized by the following: prolonged fever unresponsive to antibiotics; polymorphous skin rash; erythema of the oral mucosa, lips, and tongue; erythema of the palms and soles; bilateral conjunctival injection; and cervical lymphadenopathy[1]. KD is often complicated by coronary artery aneurysm[2]. It is the leading cause of acquired heart disease in children from East Asian countries[3] and may be a potential risk factor for adult ischemic heart disease and/or sudden death in early adulthood[4-7]. Despite a widely held belief that KD is an infectious disease, its etiology and the mechanism of the development of KD remain elusive.

Transmembrane channel-like 1 (TMC1) gene is considered a member of a family of 8 genes encoding transmembrane proteins (UniProt, http://beta.uniprot.org; SwissProt, http://www.expasy.org). It is located on chromosome 9q21.12 and contains 24 exons[8]. TMC1 protein consists of 760 amino acids and the molecular mass of TMC1 is 87,768 Da. Moreover, it is predicted to contain 6 transmembrane domains and to have cytoplasmic orientation of N and C termini. TMC1 is mainly expressed in the bone marrow, the brain, the kidney, the prostate, and the testis. The specific function of this gene is not fully defined. However, it is known to be required for normal function of cochlear hair cells[9,10]. Mutations in this gene have been associated with progressive postlingual hearing loss and profound prelingual deafness[11,12]. Recently, Tlili *et al.* reported that mutations of the TMC1 gene are responsible for autosomal recessive nonsyndromic hearing impairment in Tunisian families[13]. The aim of this study was to investigate the association between TMC1 gene and KD in 109 KD patients and 424 healthy control subjects.

Associations between TMC1 Polymorphisms and Kawasaki Disease

To evaluate whether TMC1 is associated with KD in Korean population, 11 SNPs were genotyped. Locations of 11 SNPs on the TMC1 gene region are shown in Figure 1. No deviation of Hardy-Weinberg equilibrium (HWE) in genotype distributions of each SNP was found in KD and control subjects (data not shown).

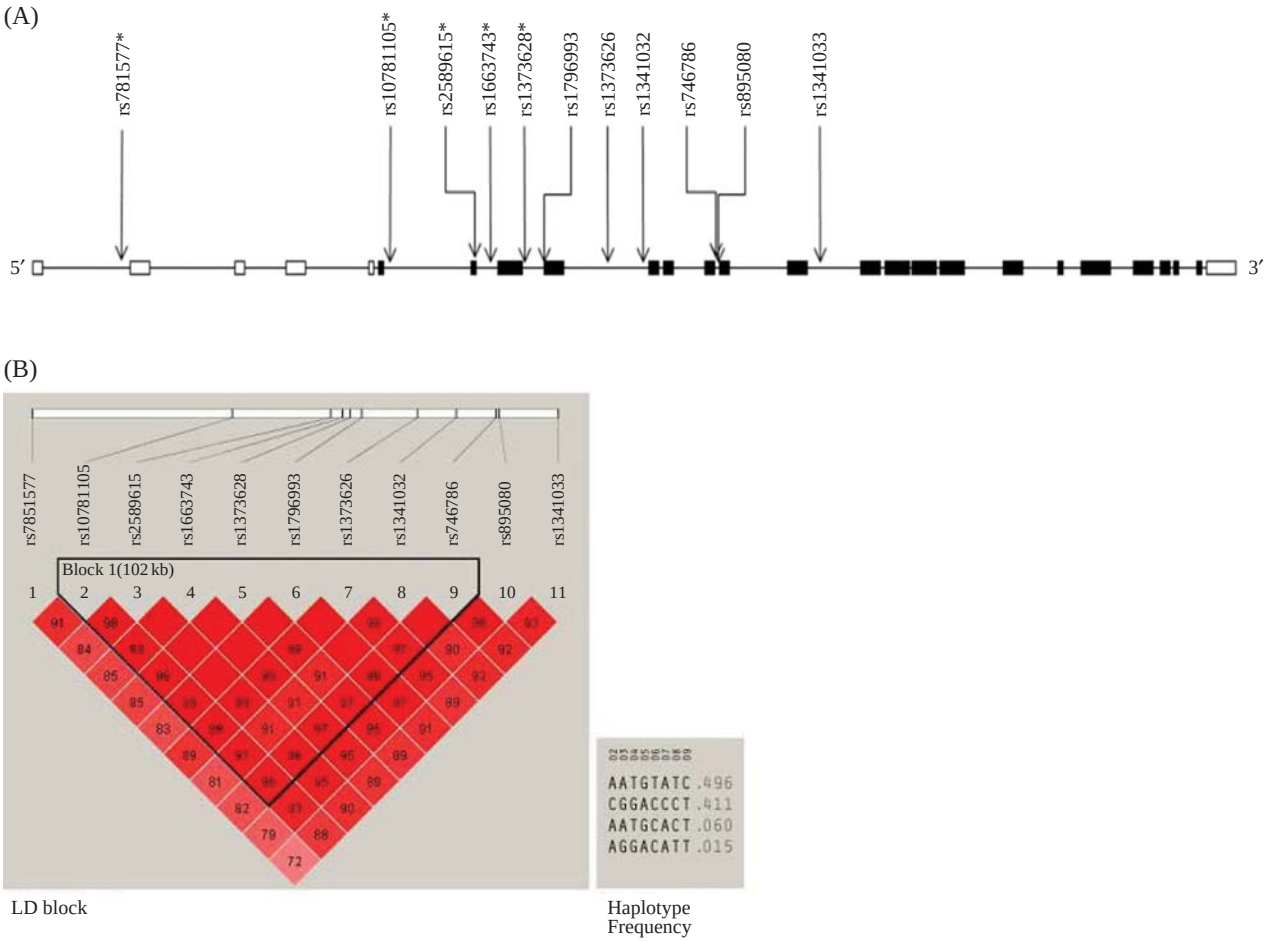

Figure 1. Gene map and linkage disequilibrium (LD) in transmembrane channel-like 1 (TMC1) gene. A, Gene map of single nucleotide polymorphisms (SNPs) in TMC1. Exons are marked with box. The coding region is black-boxed and untranslated regions are white-boxed. Asterisk (*) indicates a significant SNP. Arrow indicates the location of each SNP. B, LD coefficient (|D′|) and LD blocks among SNPs of TMC1. A block consists of rs10781105, rs2589615, rs1663743, rs1373628, rs1796993, rs1373626, rs1341032, and rs746786.

Table 1. Primer of each single nucleotide polymorphism in TMC1 gene.

SNP	Size (bp)	Sense (5′-3′)	Anti-sense (5′-3′)
rs7851577	295	CCCTCAAACCCAGAATTTTGAGT	TTCCAAAGTTTACGCACAGGACT
rs10781105	336	GCTATGAAGGGACAGAACAAGCC	GGATGGAGCTGGAAGACATTATCC
rs2589615	386	CTGTTTGCTGAACTTGATTCCTC	GGCATTGTATAAGATGCTGTAGC
rs1663743	503	TATCTGCAGCATACCTTTCTGTG	GTGCTATTGTACTCCAGCTTGGG
rs1373628	433	CACATGGCCCCATTTCCTAGAAG	TTTCCTCCTCCTCTTCCCCAGCT
rs1796993	398	CAGTGTATGTGTGTGTGTGTGTG	CCTGCATCAGTGTTCAGATTTG
rs1373626	396	GGTGAGCTCCACAACCTGAATTC	TACCTGGCATGTCTATAGGGAAG
rs1341032	357	GGTGCAGCTTGTGAGTTTTTGAG	GAACAAGGTGGGATCATAGATGTG
rs746786	427	AGGCCAAAAAGCTAGACTGAGGT	CACATCAGCATTATGTGACTCCA
rs895080	474	TCAAGGTCTCAGGCTTGCCCAGG	CGTTACTTAGTGTCAGATGCAGG
rs1341033	375	AAATCATGTGCTATACGTAACCC	AGTCAAATGACCTTGTGCAGTTC

TMC1, transmembrane channel-like 1; SNP, single nucleotide polymorphism; bp, base pair

In Table 2, logistic analysis of codominant, dominant, and recessive models showed that there were significant differences between patients with KD and control subjects. Of the eleven SNPs, six SNPs were stati-

Table 2. Genotype frequencies of TMC1 gene polymorphism in patients with Kawasaki disease and control subjects.

SNP Location	Genotype	KD n=109 (%)	Control n=424 (%)	Codominant OR (95% CI)	*P*	Dominant OR (95% CI)	*P*	Recessive OR (95% CI)	*P*
rs7851577 (intron 1)	G/G	23 (21.1)	138 (32.5)	0.73 (0.55-0.99)	0.040	0.55 (0.34-0.92)	0.017	0.78 (0.48-1.29)	0.340
	A/G	59 (54.1)	199 (46.9)						
	A/A	27 (24.8)	87 (20.5)						
rs10781105 (intron 5)	A/A	28 (25.7)	153 (36.1)	0.72 (0.54-0.98)	0.035	0.61 (0.38-0.98)	0.037	0.70 (0.42-1.18)	0.190
	A/C	57 (52.3)	201 (47.4)						
	C/C	24 (22.0)	70 (16.5)						
rs2589615 (exon 6)	A/A	26 (23.9)	144 (34.0)	0.75 (0.56-1.02)	0.064	0.61 (0.38-0.99)	0.039	0.79 (0.47-1.32)	0.370
	A/G	59 (54.1)	203 (47.9)						
	G/G	24 (22.0)	77 (18.2)						
rs1663743 (intron 6)	T/T	26 (23.9)	144 (34.0)	0.75 (0.56-1.02)	0.064	0.61 (0.38-0.99)	0.039	0.79 (0.47-1.32)	0.370
	T/G	59 (54.1)	203 (47.9)						
	G/G	24 (22.0)	77 (18.2)						
rs1373628 (intron 7)	G/G	26 (23.9)	144 (34.0)	0.75 (0.56-1.02)	0.064	0.61 (0.38-0.99)	0.039	0.79 (0.47-1.32)	0.370
	A/G	59 (54.1)	203 (47.9)						
	A/A	24 (22.0)	77 (18.2)						
rs1796993 (exon 8)	C/C	27 (24.8)	108 (25.5)	1.13 (0.84-1.53)	0.410	0.96 (0.59-1.57)	0.880	1.49 (0.88-2.51)	0.130
	T/C	61 (56.0)	205 (48.4)						
Glu81Lys	T/T	21 (19.3)	111 (26.2)						
rs1373626 (intron 8)	A/A	28 (25.7)	148 (34.9)	0.74 (0.55-1.00)	0.049	0.64 (0.40-1.04)	0.064	0.70 (0.42-1.18)	0.190
	A/C	57 (52.3)	206 (48.6)						
	C/C	24 (22.0)	70 (16.5)						
rs1341032 (intron 8)	T/T	23 (21.1)	119 (28.1)	0.83 (0.62-1.12)	0.230	0.69 (0.41-1.14)	0.140	0.89 (0.54-1.45)	0.640
	T/C	59 (54.1)	209 (49.3)						
	C/C	27 (24.8)	96 (22.6)						
rs746786 (intron 11)	C/C	21 (19.3)	112 (26.4)	0.87 (0.65-1.17)	0.360	0.66 (0.39-1.12)	0.120	1.01 (0.62-1.65)	0.960
	T/C	61 (56.0)	206 (48.6)						
	T/T	27 (24.8)	106 (25.0)						
rs895080 (intron 11)	T/T	27 (24.8)	144 (34.0)	0.79 (0.59-1.06)	0.120	0.64 (0.40-1.03)	0.062	0.85 (0.51-1.42)	0.540
	T/C	58 (53.2)	198 (46.7)						
	C/C	24 (22.0)	82 (19.3)						
rs1341033 (intron 13)	T/T	35 (32.1)	114 (26.9)	1.19 (0.89-1.58)	0.240	1.29 (0.82-2.03)	0.280	1.24 (0.75-2.03)	0.400
	T/C	49 (45.0)	196 (46.2)						
	C/C	25 (22.9)	114 (26.9)						

Total number of each SNP is different, because genotypes of some SNPs are unreadable

TMC1, transmembrane channel-like 1; SNP, single nucleotide polymorphism; KD, Kawasaki disease; OR (95% CI), odds ratio (95% confidence interval); SNP, single nucleotide polymorphism

stically associated with the risk of KD. The frequencies of GG, GA, and AA genotypes for the rs7851577 were 32.5%, 46.9%, and 20.5% in the control subjects and 21.1%, 54.1%, and 24.8% in patients with KD, respectively. The SNP rs7851577 was significantly associated with KD in the codominant (OR=0.73, 95% CI=0.55-0.99, *P*=0.040) and dominant (OR=0.55, 95% CI=0.34-0.92, *P*=0.017) models, respectively. The frequencies of AA, AC, and CC genotypes for the rs10781105 were 36.1%, 47.4%, and 16.5% in the control subjects, 25.7%, 52.3%, and 22.0% in patients with KD, respectively. The SNP rs10781105 showed a statistically significant association with KD in the codominant model (OR=0.72, 95% CI=0.54-0.98, *P*=0.035). The frequencies of AA AG, and GG genotypes for the rs2589615 were 34.0%, 47.9%, and 18.2% in the control subjects, 23.9%, 54.1%, and 22.0% in patients with KD, respectively. The SNP rs2589615 showed a significant association with KD in the dominant model (OR=0.61, 95% CI=0.38-0.99, *P*=0.039). The frequencies of TT, TG, and GG genotypes for the rs1663743 were 34.0%, 47.9%, and 18.2% in the control subjects, 23.9%, 54.1%, and 22.0% in patients with KD, respectively. The SNP rs1663743 was significantly associated with KD in the dominant model (OR=0.61, 95% CI=0.38-0.99,

Table 3. Genotype frequencies TMC1 gene polymorphism in patients Kawasaki disease with and without coronary artery lesions.

SNP Location	Genotype	Without CALs n (%)	With CALs n (%)	Codominant OR (95 CI)	*P*	Dominant OR (95 CI)	*P*	Recessive OR (95 CI)	*P*
rs7851577 (intron 1)	A/A	17 (24.3)	9 (26.5)	1.13 (0.62-2.07)	0.690	0.89 (0.35-2.28)	0.810	1.58 (0.60-4.17)	0.360
	A/G	40 (57.1)	16 (47.1)						
	G/G	13 (18.6)	9 (26.5)						
rs10781105 (intron 5)	A/A	17 (24.3)	10 (29.4)	0.71 (0.39-1.30)	0.260	0.77 (0.31-1.93)	0.580	0.50 (0.17-1.48)	0.190
	A/C	35 (50.0)	19 (55.9)						
	C/C	18 (25.7)	5 (14.7)						
rs2589615 (exon 6)	A/A	15 (21.4)	10 (29.4)	0.66 (0.36-1.22)	0.180	0.65 (0.26-1.66)	0.380	0.50 (0.17-1.48)	0.190
	A/G	37 (52.9)	19 (55.9)						
	G/G	18 (25.7)	5 (14.7)						
rs1663743 (intron 6)	T/T	15 (21.4)	10 (29.4)	0.66 (0.36-1.22)	0.180	0.65 (0.26-1.66)	0.380	0.50 (0.17-1.48)	0.190
	T/G	37 (52.9)	19 (55.9)						
	G/G	18 (25.7)	5 (14.7)						
rs1373628 (intron 7)	G/G	15 (21.4)	10 (29.4)	0.66 (0.36-1.22)	0.180	0.65 (0.26-1.66)	0.380	0.50 (0.17-1.48)	0.190
	A/G	37 (52.9)	19 (55.9)						
	A/A	18 (25.7)	5 (14.7)						
rs1796993 (exon 8)	C/C	18 (25.7)	8 (23.5)	1.00 (0.54-1.85)	0.990	1.12 (0.43-2.93)	0.810	0.86 (0.30-2.47)	0.770
	T/C	38 (54.3)	20 (58.8)						
Glu81Lys	T/T	14 (20.0)	6 (17.6)						
rs1373626 (intron 8)	A/A	17 (24.3)	10 (29.4)	0.71 (0.39-1.30)	0.260	0.77 (0.31-1.93)	0.580	0.50 (0.17-1.48)	0.190
	A/C	35 (50.0)	19 (55.9)						
	C/C	18 (25.7)	5 (14.7)						
rs1341032 (intron 8)	C/C	18 (25.7)	8 (23.5)	0.94 (0.51-1.71)	0.830	1.12 (0.43-2.93)	0.810	0.72 (0.25-2.05)	0.540
	T/C	36 (51.4)	20 (58.8)						
	T/T	16 (22.9)	6 (17.6)						
rs746786 (intron 11)	T/T	18 (25.7)	8 (23.5)	1.00 (0.54-1.85)	0.990	1.12 (0.43-2.93)	0.810	0.86 (0.30-2.47)	0.770
	T/C	38 (54.3)	20 (58.8)						
	C/C	14 (20.0)	6 (17.6)						
rs895080 (intron 11)	T/T	14 (20.0)	11 (32.4)	0.66 (0.36-1.22)	0.180	0.52 (0.21-1.32)	0.170	0.67 (0.24-1.88)	0.440
	T/C	39 (55.7)	17 (50.0)						
	C/C	17 (24.3)	6 (17.6)						
rs1341033 (intron 13)	T/T	23 (32.9)	11 (32.4)	1.08 (0.61-1.90)	0.790	1.02 (0.43-2.45)	0.960	1.23 (0.46-3.30)	0.680
	T/C	33 (47.1)	15 (44.1)						
	C/C	14 (20.0)	8 (23.5)						

Total number of each SNP is different, because genotypes of some SNPs are unreadable

TMC1, transmembrane channel-like 1; CALs, coronary artery lesions; SNP, single nucleotide polymorphism; OR (95% CI), odds ratio (95% confidence interval); SNP, single nucleotide polymorphism

P=0.039). The frequencies of GG, GA, and AA genotypes for the rs1373628 were 34.0%, 47.9%, and 18.2% in the control subjects, 23.9%, 54.1%, and 22.0% in patients with KD, respectively. The SNP rs1373628 was also associated with KD in the dominant model (OR=0.61, 95% CI=0.38-0.99, *P*=0.039). The frequencies of AA, AC, and CC genotypes for the rs1373626 were 34.9%, 48.6%, and 16.5% in the control subjects, 25.7%, 52.3%, and 22.0% in patients with KD, respectively. The SNP rs1373626 showed a significant association with KD in the codominant model (OR=0.74, 95% CI=0.55-1.00, *P*=0.049). The missense SNP rs1796993 (Glu81Lys) was not statistically associated with KD. The other 4 SNPs (rs1341033, rs895080, rs746786, and rs1341032) were not associated with KD (Table 2).

We also assessed the association of the TMC1 polymorphisms with the risk of coronary artery lesions (CALs) in KD patients. Unfortunately, there was no association between each SNP of TMC1 and the risk of CALs in KD patients (Table 3).

Table 4. Haplotype analysis of TMC1 gene polymorphism in patients with Kawasaki disease and control subjects.

Haplotype	Kawasaki		Control		Kawasaki, Control Frequencies	Chi Square	*P*
	+	–	+	–			
AATGTATC	102.0	116.0	424.0	420.0	0.468, 0.502	0.824	0.364
CGGACCCT	104.0	114.0	334.0	510.0	0.477, 0.396	4.729	0.030
AATGCACT	8.0	210.0	58.0	786.0	0.037, 0.069	3.048	0.081
AGGACATT	2.0	216.0	13.8	830.2	0.009, 0.016	0.608	0.436

Table 5. Genotype frequencies of TMC1 SNPs in each population.

SNP	Genotype	Korean		European	Chinese	Japanese	Sub-Saharan
		Kawasaki	Control				
rs7851577	GG	0.211	0.325	0.293	0.178	0.205	0.712
	GA	0.541	0.469	0.414	0.467	0.500	0.237
	AA	0.248	0.205	0.293	0.356	0.295	0.051
rs10781105	AA	0.257	0.361	0.190	0.222	0.250	0.915
	AC	0.523	0.474	0.552	0.511	0.500	0.085
	CC	0.220	0.165	0.259	0.267	0.250	0.000
rs2589615	AA	0.239	0.340	0.119	0.200	0.200	0.517
	AG	0.541	0.479	0.542	0.511	0.533	0.433
	GG	0.220	0.182	0.339	0.289	0.267	0.050
rs1663743	TT	0.239	0.340	0.103	0.200	0.205	0.517
	TG	0.541	0.479	0.552	0.511	0.523	0.431
	GG	0.220	0.182	0.345	0.289	0.237	0.052
rs1373628	AA	0.220	0.182	0.333	0.289	0.273	0.050
	AG	0.541	0.479	0.550	0.511	0.523	0.433
	GG	0.239	0.340	0.117	0.200	0.205	0.517
rs1796993	CC	0.248	0.255	0.583	0.341	0.341	0.617
	CT	0.560	0.484	0.400	0.545	0.500	0.317
	TT	0.193	0.262	0.017	0.114	0.159	0.067
rs1373626	AA	0.257	0.349	0.203	0.222	0.250	0.617
	AC	0.523	0.486	0.542	0.511	0.500	0.333
	CC	0.220	0.165	0.254	0.267	0.250	0.050
rs1341032	CC	0.211	0.281	0.400	0.341	0.295	0.017
	CT	0.541	0.493	0.483	0.523	0.523	0.250
	TT	0.248	0.226	0.117	0.136	0.182	0.733
rs746786	CC	0.193	0.264	0.017	0.114	0.159	0.100
	CT	0.560	0.486	0.400	0.523	0.500	0.483
	TT	0.248	0.250	0.583	0.364	0.341	0.417
rs895080	TT	0.248	0.340	0.150	0.244	0.227	0.183
	TC	0.532	0.467	0.533	0.444	0.455	0.617
	CC	0.220	0.193	0.317	0.311	0.318	0.200
rs1341033	TT	0.321	0.269	0.483	0.333	0.386	0.167
	TC	0.450	0.462	0.450	0.511	0.432	0.600
	CC	0.229	0.269	0.067	0.156	0.182	0.233

From SNP database (http://www.ncbi.nlm.nih.gov/sites/entrez)

Associations between TMC1 Haplotypes and Kawasaki Disease

One linkage disequilibrium (LD) block including eight SNPs (rs10781105, rs2589615, rs1663743, rs1373628, rs1796993, rs1373626, rs1341032, and rs746786) was constructed by the Gabriel method (Figure 1). Four haplotypes in the block had frequencies greater than 0.1 (Figure 1). In the analysis of haplotype association, only one haplotype (CGGACCCT) showed a significant association between KD and control groups (case/control frequency=0.477/0.396, chi square=4.726, P=0.030) (Table 4). Taken together, the results suggest that TMC1 gene may be affect the development of KD.

Next, we investigated ethnic differences of each SNP using website (http://www.ncbi.nlm.nih.gov/SNP) (Table 5). Genotype frequencies of most SNP in Korean population were similar to them in Asian

population.

Discussion

KD is an acute febrile vasculitis occurring predominantly in infants and young children. Although its etiology still remains elusive, epidemiological findings suggest that genetic factors play a role in the pathogenesis of KD. Recently, several genetic polymorphisms were reported to be associated with increased susceptibility to KD. Cheung *et al.* reported that C-reactive protein (CRP) +1444 C/T and tumor necrosis factor-α (TNF-α) -308 G/A polymorphisms were associated with predisposition to KD[14]. Ikeda *et al.* reported that allele and genotype frequencies of matrix metalloproteinase 13 (MMP13) -77A/G polymorphism showed significant differences between KD patients with CALs and without CALs[15]. Onouchi *et al.* identified a functional SNP (itpkc_3) in the inositol 1,4,5-trisphosphate 3-kinase C (ITPKC) gene on chromosome 19q13.2 that was significantly associated with KD susceptibility and also with an increased risk of CALs in both Japanese and US children[16]. Hsueh *et al.* reported the association of vascular endothelial growth factor C-634 g polymorphism in Taiwanese children with KD[17]. Jin *et al.* showed that the IL-10 (-627 A/C) promoter polymorphism may be associated with coronary aneurysms and low serum albumin in Korean children with KD[18]. Nishimura *et al.* reported that a polymorphism in the promoter of the CD14 gene (CD14/-159) is associated with the development of CAL in patients with KD[19]. These results suggest that several other genes significantly contribute to the KD susceptibility.

In the present study, we investigated whether TMC1 gene polymorphisms are related to the development of KD in Korean children. This study showed that TMC1 is associated with KD in Korean children. Of the 11 SNPs selected in TMC1, six SNPs (rs7851577, rs10781105, rs2589615, rs1663743, rs1373628, and rs1373626) were significantly associated with KD. Moreover, one haplotype (CGGACCCT) showed a significant association with KD. These results suggest that TMC1 may be also related to the development of KD.

In conclusion, the results in the present study revealed that SNPs and haplotype in the TMC1 gene were significantly associated with KD. To our knowledge, this is the first report to assess on the genetic study between TMC1 gene polymorphisms and KD in Korean population. Since no other association studies concerning the TMC1 gene for KD have yet been reported, more studies as to confirm the possible role of TMC1 in the development of KD will be needed.

Materials & Methods

Patients

All patients with KD were recruited at the Department of Pediatrics at the Kyung Hee University Medical Center, Seoul, Korea. The KD group included 109 patients (76 males and 33 females; mean age ± S.D., 2.6 ± 2.1 years), all of whom met the appropriate diagnostic criteria for KD[20]. KD was diagnosed by its clinical features, which is fever persisting for at least 5 days and accompanied by at least 4 of the 5 classic clinical criteria: 1) changes in extremities; 2) polymorphous exanthem; 3) bilateral bulbar conjunctival injection; 4) changes in lips and oral cavity; 5) cervical lymphadenopathy (> 1.5 cm in diameter). Standard therapy consisted of a single intravenous immunoglobulin (IVIG) infusion (2 g/kg). A second IVIG infusion was administered when clinical symptoms (i.e., fever) persisted for 36 hours. Two-dimensional echocardiography was used to detect the presence of CALs, defined as coronary arteries with diameter larger than 3 mm (4 mm in patients older than 5 years). Of all patients studied, 34 developed CALs, while the remaining patients revealed no evidence of CALs. Four hundred twenty four normal controls (185 males and 239 females; mean age ± S.D., 41.7 ± 12.1 years) were included. In the control group, subjects with hypertension, hyperlipidemia, stroke, or other cardiac diseases were excluded. This study was approved by the Institutional Review Board of Kyung Hee University Medical Center, Seoul, Korea. Written informed consent was obtained from all subjects. Blood samples were obtained from all participants with informed written consent. Genomic DNA was extracted from blood samples using QIAamp® DNA mini kit (QIAGEN, Valencia, CA).

SNP Selection and Genotyping

In the TMC1 gene region, 11 SNPs [rs7851577 (intron 1), rs10781105 (intron 5), rs2589615 (exon 6, Asp15Asp), rs1663743 (intron 6), rs1373628 (intron 7), rs1796993 (exon 8, Glu81Lys), rs1373626 (intron 8), rs1341032 (intron 8), rs746786 (intron 11), rs895080 (intron 11), and rs1341033 (intron 13)] were selected using human SNP websites (http://www.hapmap.org/; genome build 34). The SNPs with unknown heterozygosity and minor allele frequency (below 5%) were excluded. SNP genotyping was performed using direct sequencing. Genomic DNA was amplified using the each primer (Table 1). The samples were sequenced using an ABI Prism 377 automatic sequencer (PE Applied

Biosystems, Foster City, CA). Sequence data were analyzed using the SeqManII software (DNASTAR Inc., Madison, WI).

Statistical Analysis

Statistical analyses were performed using the statistical Package for the Social Sciences software SAS (release 8.02; SAS Institute Inc, Cary, NC). The chi-square (χ^2) test was used to value Hardy-Weinberg equilibrium (HWE). SNP analyses were using the SNPAnalyzer, Helixtree, and SNPStats programs (http://bioinfo.iconcologia.net/index.php). Genotyping of each SNP was analyzed by logistic regression models using three models (codominant, dominant, and recessive models). Odds ratios (ORs), 95% confidence intervals (CIs), and corresponding *P* values, controlling gender as co-variables were calculated. A LD block of polymorphisms was tested using Haploview version 3.32. The significant level was set at 0.05.

Acknowledgements

This study was supported by the Governance Program of Kyung Hee University.

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