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ORIGINAL ARTICLE

Genetic Polymorphism of SMAD5 is Associated With Kawasaki Disease

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Abstract Mothers against decapentaplegic homolog (SMAD) proteins are intracellular mediators of members of the transforming growth factor-β (TGF-β) superfamily, which are activated by bone morphogenetic proteins (BMPs). On activation, SMAD5 forms heterometric SMAD complexes, which are translated to the nucleus where they regulate gene transcription. TGF-β induces T cell activation and cardiovascular disease, two important features of Kawasaki disease (KD), whereas BMP is associated with coronary artery disease. In this study, we hypothesized that single nucleotide polymorphisms (SNPs) of SMAD5 may be associated with KD and coronary arterial lesions (CALs). Genotyping for 15 SNPs of the SMAD5 gene (rs3764941, rs10085013, rs6596284, rs7356756, rs13179769, rs13166063, rs1109158, rs4585442, rs4146185, rs12719481, rs6865297, rs3206634, rs6871224, rs1057898, and rs7031) was performed by direct sequencing of 105 KD patients and 303 healthy adult controls. We also compared the allele frequencies between a CAL group ($n = 31$) and a normal coronary group ($n = 74$). Results showed that among the 15 SNPs, rs3206634 was significantly associated with KD in a recessive model (odds ratio = 2.31, $p = 0.019$), whereas there was no association between any of the 15 SNPs and CALs. These findings may be used as a risk factors development of KD or for future generations of therapeutic treatments for KD.

Keywords Mothers against decapentaplegic homolog proteins · SMAD · Transforming growth factor-beta type II receptor · TGF-β · Kawasaki disease · Coronary artery lesion · Polymorphism

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Introduction

Kawasaki disease (KD) is a common cause of acquired heart disease in children of developed nations and may be associated with serious cardiovascular sequelae in adulthood [33]. The cardiovascular complications of KD are coronary artery lesions (CALs), such as myocardial infarction, coronary artery dilatation, coronary fistula, or coronary artery aneurysm (CAA).

It is known that transforming growth factor-β (TGF-β) plays a crucial role in the pathogenesis of multiple cardiovascular diseases. For example, it has been shown that TGF-β can induce neoangiogenesis, cardiomyocyte hypertrophy, calcification, and fibrosis [11, 27]. In addition, alteration of TGF-β signaling has been implicated in the pathophysiology of several vascular disorders, including Marfan [24] and Loeys-Dietz syndromes [18] due to their association with CAA and aortic root dilatation, which result from apoptosis caused by the dysregulation of TGF-β activation and signaling. Evidence of this type of cardiovascular remodeling, as well as the role of TGF-β in T-cell activation, suggests the possibility that the TGF-β gene may be related to the pathogenesis of KD and CALs.

Our previous study showed that a TGFBR2 gene polymorphism (rs6550004) was associated with the development of KD [9]. It is possible that gene polymorphisms

may act as important candidate genes for the identification of KD susceptibility due to their role in the alteration of the transcription and translation of modulatory molecules that they may encode. Mothers against decapentaplegic homolog (SMAD) proteins are intracellular mediators of members of the TGF-β superfamily [12]. One such protein, SMAD5, is activated by bone morphogenetic proteins in the TGF-β pathway and is involved in vasculogenesis and angiogenesis [4]. As such, under the premise that the TGF-β pathway influences KD susceptibility and severity, we hypothesized that single nucleotide polymorphisms (SNPs) of SMAD5 may be associated with KD and CALs.

Materials and Methods

Subjects

Study subjects included 105 patients diagnosed with KD and 303 controls (Table 1). KD patients enrolled in this study were patients from the Department of Pediatrics at Kyung Hee University Medical Center from 2003 to 2005. Diagnosis of KD was made according to the guidelines of the Japanese Kawasaki Disease Research Committee [21]. Clinical characteristics of the KD group are listed in Table 2. Mean age of the patients was 32 months (34 girls and 71 boys), and 26.6 % of them had a CAL, which was defined as a diameter of ≥3 mm of either the left or right coronary artery in patients <5 years of age, a diameter of or ≥4 mm in patients >5 years of age, or a diameter that was >1.5 times that of an adjacent vessel [3]. The control group was composed of healthy adults without any history of KD (Table 1). Mean age of the control group was 41 years (median 39.4).

Informed consent was obtained from the parent or legal guardian of each patient according to Declaration of Helsinki guidelines. The study and study protocol were approved by the Ethics Review Committee of Medical Research Institute and Institutional Review Boards of the Medical Research Institute at Kyung Hee University Medical Center.

Table 1 Demographic data of KD and control groups

Demographic data	KD (n = 105)		Control (n = 303)	
Sex (%)				
Male	71	67.7	132	43.6
Female	34	34.3	171	56.4
Mean ± SD age (mo)	31.6 ± 24.2		41.0 ± 13.3	

KD Kawasaki disease

SNP Selection and Genotyping

The SMAD5 gene is located on chromosome 5q31, and SNP locations of the gene range from nucleotide positions 135466657–135518898 according to the National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/snp). A total of 15 SNPs of the SMAD5 gene (rs6871224, rs6871224, rs7356756, rs13166063, rs7031, rs6865297, rs12719481, rs1057898, rs13179769, rs1109158, rs4146185, rs6596284, rs4585442, rs3764941, and rs10085013) were selected based on heterozygosity (>0.1), and genotyping was performed using an Illumina Sentrix Array Matrix chip.

Patient genomic DNAs was extracted from the leukocytes from whole blood samples using a Qiagen DNA Extraction kit (Qiagen, Tokyo, Japan). SNP genotyping was performed using the Golden-Gate SNP Genotyping Assay and an Illumina BeadStation 500 GX (Illumina, San Diego, CA) according to the manufacturer's instructions. Briefly, DNA samples were reacted with biotin, and oligonucleotides of the assay (oligos) were subsequently hybridized to the biotin-activated DNA. After polymerase chain reaction, products with biotin-activated fluorescence were decoded by the Illumina BeadArray Reader (Illumina, San Diego, CA). SNPs with low Illumina Array quality design scores were excluded from analysis.

Statistical Analysis

SNPStats (http://bioinfo.iconcologia.net/SNPstats) and Helix Tree programs (Golden Helix, Bozeman, MT, USA) were used for statistical analysis. Fisher's exact test values were calculated as needed. Multiple logistic regression models for each SNP were performed to calculate the odds ratio (OR), 95 % confidence interval (CI), and corresponding p values. Statistical significance was regarded as $p < 0.05$.

Results

The genetic association between 15 SNPs of the SMAD5 gene and susceptibility to KD was investigated. The

Table 2 Clinical characteristics of the KD group

Characteristics (n = 105)	
Coronary artery (%)	
Normal	73 (69.5)
CAL	32 (30.5)
No. of IVIG treatments (%)	
More than twice	9 (8.6)

KD Kawasaki disease, *CAL* coronary artery lesion, *IVIG* intravenous gamma globulin

Table 3 Genotype frequencies of polymorphisms of the SMAD5 gene in KD patients and control subjects

SNP	Genotype	Control *n* (%)	KD *n* (%)	Model	OR (95 % CI)	*p*
rs6871224	A/A	95 (35.7)	38 (36.2)	Log-additive	1.17 (0.83–1.64)	0.37
	A/G	127 (47.7)	55 (52.4)	Dominant	1.06 (0.66–1.72)	0.8
	G/G	44 (16.5)	12 (11.4)	Recessive	1.62 (0.81–3.25)	0.16
rs3206634	T/T	51 (34.5)	38 (36.2)	Log-additive	1.35 (0.94–1.95)	0.1
	T/C	64 (43.2)	55 (52.4)	Dominant	1.16 (0.68–1.98)	0.59
	C/C	33 (22.3)	12 (11.4)	Recessive	2.31 (1.12–4.76)	**0.019**
rs7356756	T/T	107 (35.8)	38 (36.5)	Log-additive	1.14 (0.82–1.60)	0.44
	T/C	143 (47.8)	53 (51)	Dominant	1.08 (0.67–1.74)	0.74
	C/C	49 (16.4)	13 (12.5)	Recessive	1.42 (0.73–2.77)	0.3
rs13166063	T/T	107 (35.7)	38 (36.5)	Log-additive	1.17 (0.83–1.64)	0.37
	T/C	144 (48)	54 (51.9)	Dominant	1.09 (0.68–1.75)	0.72
	C/C	49 (16.3)	12 (11.5)	Recessive	1.54 (0.77–3.05)	0.21
rs7031	G/G	105 (37.1)	38 (36.2)	Log-additive	1.14 (0.82–1.60)	0.43
	T/G	130 (45.9)	55 (52.4)	Dominant	1.01 (0.63–1.63)	0.96
	T/T	48 (17)	12 (11.4)	Recessive	1.64 (0.82–3.27)	0.15
rs6865297	A/A	95 (35.7)	38 (36.5)	Log-additive	1.18 (0.83–1.66)	0.35
	A/G	127 (47.7)	54 (51.9)	Dominant	1.08 (0.67–1.76)	0.74
	G/G	44 (16.5)	12 (11.5)	Recessive	1.60 (0.80–3.22)	0.17
rs12719481	A/A	107 (36)	38 (36.5)	Log-additive	1.16 (0.83–1.63)	0.38
	A/G	141 (47.5)	54 (51.9)	Dominant	1.54 (0.73–3.24)	0.76
	G/G	49 (16.5)	12 (11.5)	Recessive	1.08 (0.67–1.74)	0.2
rs1057898	T/T	107 (35.8)	38 (36.9)	Log-additive	1.21 (0.86–1.70)	0.27
	T/C	143 (47.8)	54 (52.4)	Dominant	1.12 (0.69–1.80)	0.65
	C/C	49 (16.4)	11 (10.7)	Recessive	1.70 (0.84–3.46)	0.13
rs13179769	A/C	107 (35.3)	38 (36.9)	Log-additive	1.18 (0.84–1.65)	0.34
	A/A	147 (48.5)	53 (51.5)	Dominant	1.12 (0.70–1.80)	0.65
	C/C	49 (16.2)	12 (11.7)	Recessive	1.51 (0.76–3.00)	0.23
rs1109158	A/A	107 (36)	38 (36.5)	Log-additive	1.16 (0.83–1.63)	0.38
	T/A	141 (47.5)	54 (51.9)	Dominant	1.08 (0.67–1.73)	0.77
	T/T	49 (16.5)	12 (11.5)	Recessive	1.55 (0.78–3.09)	0.2
rs4146185	A/A	105 (36.8)	38 (36.2)	Log-additive	1.15 (0.82–1.61)	0.42
	T/A	132 (46.3)	55 (52.4)	Dominant	1.02 (0.64–1.65)	0.92
	T/T	48 (16.8)	12 (11.4)	Recessive	1.63 (0.82–3.24)	0.15
rs6596284	T/T	105 (36.1)	38 (36.5)	Log-additive	1.14 (0.81–1.59)	0.44
	T/C	138 (47.4)	53 (51)	Dominant	1.07 (0.66–1.72)	0.78
	C/C	48 (16.5)	13 (12.5)	Recessive	1.44 (0.74–2.83)	0.27
rs4585442	A/A	107 (36.1)	38 (37.2)	log-additive	1.21 (0.86–1.70)	0.28
	A/G	140 (47.3)	53 (52)	dominant	1.11 (0.69–1.80)	0.66
	G/G	49 (16.6)	11 (10.8)	recessive	1.70 (0.84–3.46)	0.13
rs3764941	A/A	107 (35.7)	38 (36.5)	log-additive	1.17 (0.83–1.64)	0.37
	A/C	144 (48)	54 (51.9)	Dominant	1.09 (0.68–1.75)	0.73
	C/C	49 (16.3)	12 (11.5)	Recessive	1.54 (0.77–3.07)	0.2
rs10085013	T/T	107 (35.7)	38 (36.5)	Log-additive	1.17 (0.83–1.64)	0.37
	T/A	144 (48)	54 (51.9)	Dominant	1.09 (0.68–1.75)	0.73
	A/A	49 (16.3)	12 (11.5)	Recessive	1.54 (0.77–3.07)	0.2

Bold value is statistically significant

KD Kawasaki disease, *SNP* single nucleotide polymorphism, *OR* odds ratio, *CI* confidence interval

Table 4 Genotype frequencies of polymorphisms of the SMAD5 gene in a normal coronary-artery group and a CAL group with KD

SNP	Genotype	Normal CA *n* (%)	CAL *n* (%)	Model	OR (95 % CI)	*p*
rs6871224	A/A	31 (41.9)	7 (22.6)	Log-additive	1.68 (0.87–3.24)	0.12
	A/G	35 (47.3)	20 (64.5)	Dominant	2.47 (0.95–6.46)	0.054
	G/G	8 (10.8)	4 (12.9)	Recessive	1.22 (0.34–4.40)	0.76
rs3206634	T/T	31 (41.9)	7 (22.6)	Log-additive	1.68 (0.87–3.24)	0.12
	T/C	35 (47.3)	20 (64.5)	Dominant	2.47 (0.95–6.46)	0.054
	C/C	8 (10.8)	4 (12.9)	Recessive	1.22 (0.34–4.40)	0.76
rs7356756	T/T	31 (42.5)	7 (22.6)	Log-additive	1.60 (0.84–3.05)	0.15
	T/C	33 (45.2)	20 (64.5)	Dominant	2.53 (0.97–6.62)	0.049
	C/C	9 (12.3)	4 (12.9)	Recessive	1.05 (0.30–3.72)	0.94
rs13166063	T/T	31 (42.5)	7 (22.6)	Log-additive	1.69 (0.87–3.26)	0.12
	T/C	34 (46.6)	20 (64.5)	Dominant	2.53 (0.97–6.62)	0.049
	C/C	8 (11)	4 (12.9)	Recessive	1.20 (0.33–4.34)	0.78
rs7031	G/G	31 (41.9)	7 (22.6)	Log-additive	1.68 (0.87–3.24)	0.12
	T/G	35 (47.3)	20 (64.5)	Dominant	2.47 (0.95–6.46)	0.054
	T/T	35 (47.3)	4 (12.9)	Recessive	1.22 (0.34–4.40)	0.76
rs6865297	A/A	31 (42.5)	7 (22.6)	Log-additive	1.69 (0.87–3.26)	0.12
	A/G	34 (46.6)	20 (64.5)	Dominant	2.53 (0.97–6.62)	0.049
	G/G	8 (11)	4 (12.9)	Recessive	1.20 (0.33–4.34)	0.78
rs12719481	A/A	31 (42.5)	7 (22.6)	Log-additive	1.69 (0.87–3.26)	0.12
	A/G	34 (46.6)	20 (64.5)	Dominant	2.53 (0.97–6.62)	0.049
	G/G	8 (11)	4 (12.9)	Recessive	1.20 (0.33–4.34)	0.78
rs1057898	T/T	31 (42.5)	7 (23.3)	Log-additive	1.56 (0.80–3.05)	0.19
	T/C	34 (46.6)	20 (66.7)	Dominant	2.43 (0.92–6.37)	0.062
	C/C	8 (11)	3 (10)	Recessive	0.90 (0.22–3.66)	0.89
rs13179769	A/C	31 (42.5)	7 (23.3)	Log-additive	1.66 (0.86–3.23)	0.13
	A/A	34 (46.6)	19 (63.3)	Dominant	2.43 (0.92–6.37)	0.062
	C/C	8 (11)	4 (13.3)	Recessive	1.25 (0.35–4.51)	0.74
rs1109158	A/A	31 (42.5)	7 (22.6)	Log-additive	1.69 (0.87–3.26)	0.12
	T/A	34 (46.6)	20 (64.5)	Dominant	2.53 (0.97–6.62)	0.049
	T/T	8 (11)	4 (12.9)	Recessive	1.20 (0.33–4.34)	0.78
rs4146185	A/A	31 (41.9)	7 (22.6)	Log-additive	1.68 (0.87–3.24)	0.12
	T/A	35 (47.3)	20 (64.5)	Dominant	2.47 (0.95–6.46)	0.054
	T/T	8 (10.8)	4 (12.9)	Recessive	1.22 (0.34–4.40)	0.76
rs6596284	T/T	31 (42.5)	7 (22.6)	Log-additive	1.60 (0.84–3.05)	0.15
	T/C	33 (45.2)	20 (64.5)	Dominant	2.53 (0.97–6.62)	0.049
	C/C	9 (12.3)	4 (12.9)	Recessive	1.05 (0.30–3.72)	0.94
rs4585442	A/A	31 (43.1)	7 (23.3)	Log-additive	1.57 (0.81–3.07)	0.18
	A/G	33 (45.8)	20 (66.7)	Dominant	2.48 (0.95–6.53)	0.055
	G/G	8 (11.1)	3 (10)	Recessive	0.89 (0.22–3.61)	0.87
rs3764941	A/A	31 (42.5)	7 (22.6)	Log-additive	1.69 (0.87–3.26)	0.12
	A/C	34 (46.6)	20 (64.5)	Dominant	2.53 (0.97–6.62)	0.049
	C/C	8 (11)	4 (12.9)	Recessive	1.20 (0.33–4.34)	0.78
rs10085013	T/T	31 (42.5)	7 (22.6)	Log-additive	1.69 (0.87–3.26)	0.12
	T/A	34 (46.6)	20 (64.5)	Dominant	2.53 (0.97–6.62)	0.049
	A/A	8 (11)	4 (12.9)	Recessive	1.20 (0.33–4.34)	0.78

SNP single nucleotide polymorphism, *CA* coronary artery, *CAL* coronary artery lesion, *OR* odds ratio, *CI* confidence interval

genotype frequencies of the 15 SNPs in the KD and the control groups are listed in Table 3. One SNP, rs3206634 (T>C), was found to be associated with KD in a recessive model (OR = 2.31, 95 % CI = 1.12–4.76, $p = 0.019$). In KD patients, genotype frequencies of the SNPs were compared between those with normal coronary arteries and those with a CAL (Table 4). Interestingly, no significant association between the selected SNPs and CAL presence in KD patients was observed. It was found that all 15 SNPs under study were in Hardy–Weinberg equilibrium ($p < 0.05$, data not shown).

Discussion

KD is an acute vasculitic syndrome occurring worldwide that mainly affects children <5 of age [7, 15, 36]. Although the etiology of KD remains unknown, an infectious trigger affecting genetically susceptible hosts is suspected. Genetic and immunologic studies suggest that T-cell activation and regulation play important roles in the pathogenesis of KD [6]. The reason for the development of CALs in patients with KD also remains yet to be determined.

The annual incidence of KD in Asian countries is >tenfold higher than in other countries [22]. The incidence rate of KD in Japan, Korea, and Taiwan alone was 222.9, 113.1, and 69.0/100,000 children, respectively [15, 20, 25]. Epidemiologic findings of KD suggest that genetic predisposition might play a role in the etiology of the disease [28]. Indeed, the higher incidence of KD in Asian descendants compared with other ethnic populations suggests that a genetic predisposition might play an important role in susceptibility to the disease [13]. In addition, there is evidence that the incidence of KD is greater among siblings than in the general population [36].

Shimizu et al. [28] reported that genetic polymorphisms of TGFB2, TGFBR2, and SMAD3 may play an important role in the susceptibility to KD and the development of CALs. Recently, numerous candidate genes have been proposed as markers of susceptibility and as indicators for the formation of CALs in several populations. Genes with candidate SNPs for the development of KD include the following: the genes for C–C chemokine receptor 5 [8], angiotensin-II type-1 receptor [37], vascular endothelial growth factor receptor 2 [16, 38], interleukin-4 [10], tumor necrosis factor-α [2], macrophage migration inhibitory factor [30], CD40 ligand [14], inositol 1,4,5-trisphosphate 3 kinase [23], and E-selectin [29]. Such SNPs may strongly influence the biological activity of the corresponding translated proteins, thus leading to potential clinical implications.

A genome wide scan recently showed a genetic association of KD with SMAD [28]. The polymorphism of SMAD located on chromosome 5q31 resulted in different transcriptional levels of mature mRNA caused by interfering RNA splicing efficiency. The SMAD family comprises transcription factors that function as signal transducers of TGF-β superfamily members. Studies investigating the expression, activation, or involvement of both SMAD and TGF-β family members in cardiovascular diseases are constantly being performed [12].

Active TGF-β peptides bind to TGFBR2, which recruits and activates TGFBR1 or activin A receptor type II-like 1 (ACVRL1). The activated type-I receptor phosphorylates receptor-specific SMAD molecules: TGFBR1 phosphorylates SMAD2 and 3, and ACVRL1 phosphorylates SMAD1, 5, and 8 through BMP signaling. The activated SMADs form a larger complex with SMAD4 and enter the nucleus to regulate gene transcription [28]. Recently, cross-use of ligand and receptors between TGF-β and the BMP subfamilies was reported. As with the TGF-β peptides, ligands of the BMP subfamily can signal through type I and II receptors, from which transduction occurs by way of SMADs 1, 5, and 8 [19]. In addition, Bharathy et al. [5] reported that mutation of TGFBR2 can inhibit SMAD 2,3 activation and increase SMAD 1,5 activation. This type of mutation can thus cause alteration of TGF-β signaling and may be associated with the pathogenesis of KD. Genetic variation in the TGF-β pathway may influence KD susceptibility, disease outcome, and response to therapy [17, 28].

Alterations in TGF-β signaling can generate genetic conditions that predispose affected patients to thoracic aortic aneurysm and dissections. TGF-β signaling through the TGFBR2 receptor in endothelial cells plays an important role in cardiac development. It promotes myocardial fibrosis and remodeling in coronary artery disease [26].

SMAD complexes have also been suggested to be associated with cardiovascular disease by several studies. For example, it has been shown that mutation of SMAD3 can cause aneurysm–osteoarthritis syndrome [35]. SMAD3 has also been observed to be related to KD susceptibility and CAA formation [28]. SMAD4 plays a role in the SMAD cascade for TGF-β and BMP signaling, and the mutation of SMAD4 is involved in aortopathy, mitral valve dysfunction, and juvenile polyposis syndrome [1].

There has been no previous report regarding the relationship between SMAD5 gene polymorphism and KD. In fact, only a few reports are available investigating the function of SMAD5. It is known that SMAD5 is involved in vasculogenesis and angiogenesis [4]. In a mouse model, it was shown that SMAD5 gene is required for the survival of embryonic stem cell differentiated myocytes in vitro and mouse cardiomyocytes in vivo [32]. In another SMAD5-null phenotype animal study, SMAD5-mediated signaling was observed to be essential in blood vessel development [34].

Our previous study showed that a TGFBR2 gene polymorphism, rs6550004, was associated with the development of KD. Under the assumption that TGF-β signaling pathways play a critical role in the cardiovascular system, as well as in KD, we selected SMAD5 as the TGF-β-related effector gene. Frequencies of 15 SNPs in SMAD5 were compared between a KD-diagnosed group and control group. The SNPs selected were usually located on the 3′-untranslated region (UTR) or intron. Six of the SNPs (rs1057898, rs12719481, rs3206634, rs6865297, rs6871224, and rs7031) were variants of the 3′-UTR, whereas the remaining nine SNPs (rs10085013, rs1109158, rs13166063, rs13179769, rs3764941, rs4146185, rs4585442, rs6596284, and rs7356756) were variants of intron [31]. One SNP, rs3206634 (T>C), was found to be significantly associated with KD in a recessive model (OR = 2.31, $p = 0.019$), suggesting that the minor allele C may increase the risk of development of KD approximately 2.3-fold compared with the control group in the Korean population. A comparison of SNP frequencies between a group with normal coronary arteries and a group with CALs was also performed. No difference in SNP frequencies was observed between the groups, and thus it was concluded that none of the 15 SNPs are associated with the development of CALs in KD patients. Due to the fact that the correlations of SMAD1–SMAD4 and KD were not analyzed in this study, future studies involving other SMAD complexes in KD patients may greater elucidate the relationship of TGF-β effectors and KD.

One major limitation of this study was relatively small population size. Recently a genome-wide association was used to identify novel susceptibility loci, including SMAD, as important factors for KD. Although the SMAD gene has several known functions, its mechanism and role in the susceptibility to specific diseases are still unclear. Therefore, future analysis of a larger KD patient group is needed to verify the results of this study.

In conclusion, we found that the SNP, rs3206634, of the SMAD5 gene may be associated with susceptibility to KD, although this SNP is not associated with the formation of CALs. After verification by larger-scale studies, the association between rs3206634 and KD may be used as a candidate gene of KD or as a basis for the development of therapeutic treatments.

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References

1. Andrabi S, Bekheirnia MR, Robbins-Furman P, Lewis RA, Prior TW, Potocki L (2011) SMAD4 mutation segregating in a family with juvenile polyposis, aortopathy, and mitral valve dysfunction. Am J Med Genet A 155A:1165–1169
2. Arj-Ong S, Thakkinstian A, McEvoy M, Attia J (2010) A systematic review and meta-analysis of tumor necrosis factor α-308 polymorphism and Kawasaki disease. Pediatr Int 52:527–532
3. Arjunan K, Daniels SR, Meyer RA, Schwartz DC, Barron H, Kaplan S (1986) Coronary artery caliber in normal children and patients with Kawasaki disease but without aneurysms: an echocardiographic and angiographic study. J Am Coll Cardiol 8:1119–1124
4. Attisano L, Lee-Hoeflich ST (2001) The SMADS. Genome Biol 2:REVIEWS3010
5. Bharathy S, Xie W, Yingling JM, Reiss M (2008) Cancer-associated transforming growth factor beta type II receptor gene mutant causes activation of bone morphogenic protein-SMADs and invasive phenotype. Cancer Res 68:1656–1666
6. Burgner D, Davila S, Breunis WB, Ng SB, Li Y, Bonnard C et al (2009) A genome-wide association study identifies novel and functionally related susceptibility loci for Kawasaki disease. PLoS Genet. doi:10.1371/journal.pgen.1000319
7. Burns JC, Kushner HI, Bastian JF, Shike H, Shimizu C, Matsubara T et al (2000) Kawasaki disease: a brief history. Pediatrics 06:E27
8. Burns JC, Shimizu C, Gonzalez E, Kulkarni H, Patel S, Shike H et al (2005) Genetic variations in the receptor-ligand pair CCR5 and CCL3L1 are important determinants of susceptibility to Kawasaki disease. J Infect Dis 192:344–349
9. Choi YM, Shim KS, Yoon KL, Han MY, Cha SH, Kim SK et al (2012) Transforming growth factor beta receptor II polymorphisms are associated with Kawasaki disease. Korean J Pediatr 55:18–23
10. Chu MP, Wang D, Zhang YY, Li BQ, Zhou AH, Chen XW et al (2012) Pachyman treatment improves CD4+CD25+Treg counts and serum interleukin 4 and interferon γ levels in a mouse model of Kawasaki disease. Mol Med Rep 5(5):1237–1240
11. Clark-Greuel JN, Connolly JM, Sorichillo E, Narula NR, Rapoport HS, Mohler ER 3rd et al (2007) Transforming growth factor-beta1 mechanisms in aortic valve calcification: increased alkaline phosphatase and related events. Ann Thorac Surg 83:946–953
12. Euler-Taimor G, Heger J (2006) The complex pattern of SMAD signaling in the cardiovascular system. Cardiovasc Res 69:15–25
13. Holman RC, Christensen KY, Belay ED, Steiner CA, Effler PV, Miyamura J et al (2010) Racial/ethnic differences in the incidence of Kawasaki syndrome among children in Hawaii. Hawaii Med J 69:194–197
14. Huang FY, Chang TY, Chen MR, Chiu NC, Chi H, Lee HC et al (2008) Genetic polymorphisms in the CD40 ligand gene and Kawasaki disease. J Clin Immunol 28:405–410
15. Huang WC, Huang LM, Chang IS, Chang LY, Chiang BL, Chen PJ et al (2009) Epidemiologic features of Kawasaki disease in Taiwan, 2003–2006. Pediatrics 123:e401–e405
16. Kariyazono H, Ohno T, Khjoee V, Ihara K, Kusuhara K, Kinukawa N et al (2004) Association of vascular endothelial growth factor (VEGF) and VEGF receptor gene polymorphism with coronary artery lesions of Kawasaki disease. Pediatr Res 56:953–959
17. Kuo HC, Onouchi Y, Hsu YW, Chen WC, Huang JD, Huang YH et al (2011) Polymorphisms of transforming growth factor-β signaling pathway and Kawasaki disease in the Taiwanese population. J Hum Genet 56:840–845
18. Loeys BL, Schwarze U, Holm T, Callewaert BL, Thomas GH, Pannu H et al (2006) Aneurysm syndromes caused by mutations in the TGF-beta receptor. N Engl J Med 355:788–798
19. Miyazono K, Kamiya Y, Morikawa M (2010) Bone morphogenetic protein receptors and signal transduction. J Biochem 147:35–51
20. Nakamura Y, Yashiro M, Uehara R, Sadakane A, Tsuboi S, Aoyama Y et al (2012) Epidemiologic features of Kawasaki

disease in Japan: results of the 2009–2010 nationwide survey. J Epidemiol 22:216–221
21. Newburger JW, Burns JC (1999) Kawasaki disease. Vasc Med 4:187–202
22. Onouchi Y (2012) Genetics of Kawasaki disease: what we know and don't know. Circ J 76:1581–1586
23. Onouchi Y, Gunji T, Burns JC, Shimizu C, Newburger JW, Yashiro M et al (2008) ITPKC functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. Nat Genet 40:35–42
24. Pannu H, Fadulu VT, Chang J, Lafont A, Hasham SN, Sparks E et al (2005) Mutations in transforming growth factor-β receptor type II cause familial thoracic aortic aneurysms and dissections. Circulation 112:513–520
25. Park YW, Han JW, Hong YM, Ma JS, Cha SH, Kwon TC et al (2011) Epidemiological features of Kawasaki disease in Korea, 2006–2008. Pediatr Int 53:36–39
26. Robinson PN, Arteaga-Solis E, Baldock C, Collod-Béroud G, Booms P, De Paepe A et al (2006) The molecular genetics of Marfan syndrome and related disorders. J Med Genet 43:769–787
27. Ruiz-Ortega M, Rodríguez-Vita J, Sanchez-Lopez E, Carvajal G, Egido J (2007) TGF-beta signaling in vascular fibrosis. Cardiovasc Res 74:196–206
28. Shimizu C, Jain S, Davila S, Hibberd ML, Lin KO, Molkara D et al (2011) Transforming growth factor-beta signaling pathway in patients with Kawasaki disease. Circ Cardiovasc Genet 4:16–25
29. Shirakawa T, Ikeda K, Nishimura S, Kuniba H, Nakashima K, Motomura H et al (2012) Lack of an association between E-selectin gene polymorphisms and risk of Kawasaki disease. Pediatr Int 54:455–460
30. Simonini G, Corinaldesi E, Massai C, Falcini F, Fanti F, De Martino M et al (2009) Macrophage migration inhibitory factor-173 polymorphism and risk of coronary alterations in children with Kawasaki disease. Clin Exp Rheumatol 27:1026–1030
31. SMAD5 (http://www.ncbi.nlm.nih.gov/gene/4090)
32. Sun Y, Zhou J, Liao X, Lü Y, Deng C, Huang P et al (2005) Disruption of SMAD5 gene induces mitochondria-dependent apoptosis in cardiomyocytes. Exp Cell Res 306:85–93
33. Tsuda E, Matsuo M, Naito H, Noguchi T, Nonogi H, Echigo S (2007) Clinical features in adults with coronary arterial lesions caused by presumed Kawasaki disease. Cardiol Young 17:84–89
34. Umans L, Cox L, Tjwa M, Bito V, Vermeire L, Laperre K et al (2007) Inactivation of SMAD5 in endothelial cells and smooth muscle cells demonstrates that SMAD5 is required for cardiac homeostasis. Am J Pathol 170:1460–1472
35. Van de Laar I, Oldenburg RA, Pals G, Roos-Hesselink JW, de Graaf BM, Verhagen JM et al (2011) Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. Nat Genet 43:121–126
36. Wang CL, Wu YT, Liu CA, Kuo HC, Yang KD (2005) Kawasaki disease: infection, immunity and genetics. Pediatr Infect Dis J 24:998–1004
37. Wu SF, Chang JS, Peng CT, Shi YR, Tsai FJ (2004) Polymorphism of angiotensin-1 converting enzyme gene and Kawasaki disease. Pediatr Cardiol 25:529–533
38. Yasukawa K, Terai M, Shulman ST, Toyozaki T, Yajima S, Kohno Y et al (2002) Systemic production of vascular endothelial growth factor and fms-like tyrosine kinase-1 receptor in acute Kawasaki disease. Circulation 105:766–769