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Association Between *SRC-1* Gene Polymorphisms and Coronary Artery Aneurysms Formation in Taiwanese Children With Kawasaki Disease

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Background: Kawasaki disease (KD) patients who experience a cardiovascular complication known as a coronary artery aneurysm (CAA) are at high risk of developing ischemic heart disease, which may lead to sudden death. The etiology of CAA in KD patients is unclear, and this study aims to clarify the relationship between steroid receptor coactivator-1 (*SRC-1*) gene polymorphisms and CAA pathogenesis. *Methods:* We investigated four *SRC-1* gene polymorphisms (rs11894248, rs17791703, rs7572475, and rs9309308) and their correlation with KD with CAA susceptibility in 327 Taiwanese people (279 KD patients without CAA and 48 KD patients with CAA). *Results:* The results indicated a statistically significant difference in genotype and allele frequency distributions at the *SRC-1* four single nucleotide polymorphisms (SNPs) between KD patients with and without CAA ($P < 0.01$). Additionally, *Smad3* gene polymorphism (rs12901071) is well known to be associated with KD patients. In our results, *Smad3* SNP did not provide a statistically significant difference between KD patients with and without CAA. *Conclusion:* Our data show that *SRC-1* polymorphisms may be the underlying cause of CAA; therefore, the polymorphisms examined in this study warrant further investigation. J. Clin. Lab. Anal. 28:435–439, 2014.

Key words: Kawasaki disease (KD); coronary artery aneurysm (CAA); steroid receptor coactivator-1 (*SRC-1*)

INTRODUCTION

Kawasaki disease (KD) is an acute, self-limited, and systemic vasculitis that is one of the leading inducers of heart disease in children (1–3). KD patients with cardiac complications, particularly those of the coronary artery, are known to suffer frequently from this syndrome, which develops in 15–25% of children diagnosed with KD who remain untreated (4, 5). KD patients with cardiovascular complications are at high risk of developing ischemic heart disease that can lead to myocardial infarction and sudden death (5). Coronary artery aneurysm (CAA) develop in 25% of untreated patients and 3–5% of patients treated with intravenous immunoglobulin (IVIG) within the first 10 days of fever onset (6). The etiology of CAA in KD patients is unclear.

Transforming growth factor β (TGF-β) is a multifunctional peptide that regulates proliferation, differentiation, apoptosis, and migration in many cell types. TGF-β induces transformation of cells of different lineages to myofibroblasts that mediate damage to the arterial wall. TGF-β pathway genes, *TGFB2*, *TGFBR2*, and *Smad3* genetic variants and their haplotypes are associated with KD susceptibility, CAA formation, aortic root dilatation, and IVIG treatment response in different cohorts

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(7). *Smad3* is a key signaling molecule in the pathway and was associated with both KD susceptibility and formation of CAA (7). The steroid receptor coactivator-1 (*SRC-1*) is a Smad3/4 transcriptional partner facilitating the functional link between Smad3 and p300/CBP and enhances TGF-β medicated transcription (8). *SRC-1* is a transcriptional coactivator and the first identified member of the *p160 SRC* gene family that includes *SRC-2* and *SRC-3* (9–11). These transcriptional coactivators interact with nuclear receptors in a ligand-binding dependent manner and recruit general coactivators, such as cAMP-responsive element binding protein (CREB) binding protein (CBP) or p300, to the target gene promoter for activation of gene transcription (10, 11). SRC-1 is expressed in endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and neointima cells. SRC-1 expression in these cells facilitates estrogen/estrogen receptor (ER)-mediated vasoprotection through the inhibition of neointima after a vascular injury (12).

In the present study, we examined 327 patients with a past history of KD, 48 of whom were Taiwanese children diagnosed with CAA. In a complication study, we investigated whether the identified gene polymorphisms were associated with CAA.

MATERIALS AND METHODS

Patients and Sample Collection

We identified and enrolled 327 individuals into this study. All individuals attended the Department of Pediatrics, China Medical University Hospital in Taichung from 1998 to 2011 and fulfilled the diagnostic criteria for KD (13–17). 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 CAA was identified when either the right coronary artery or the left coronary artery showed a dilated diameter of 3 mm in children younger than 5 years or of 4 mm in children older than 5 years (18). The normal population was Han Chinese in Beijing (CHB) data from HapMap database (http://hapmap.ncbi.nlm.nih.gov/).

Genomic DNA Extraction and Genotyping

All blood samples were collected using venipuncture for genomic DNA isolation. Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Genomic DNA kit; Qiagen, Valencia, CA). We genotyped four SNPs in *SRC-1* gene (rs11894248, rs17791703, rs7572475, and rs9309308) and one SNP in *Smad3* gene (rs12901071). The primers and probes used to detect SNPs were from the ABI Assays-on-Demand kit (ABI: Applied Biosystems Inc., Foster City, CA). Reactions were performed according to the manufacturer's protocol. Briefly, PCR was performed in the presence of 2× TaqMan Universal PCR Master Mix, assay mix, and genomic DNA (15 ng). The probe for fluorescence signal detection was from the ABI Prism 7900 Real-Time PCR System. This study was approved by the Human Studies Committee of China Medical University Hospital, and informed consent was obtained from either the participants or their parents.

Statistical Analysis

The allelic and genotype frequency distributions for the polymorphism of KD were determined through χ^2 analysis using SPSS software (version 10.0, SPSS, Inc., Chicago, IL). A *P*-value of less than 0.05 was considered statistically significant. Allelic and genotype frequencies were expressed as percentages of the total number of alleles and genotypes. Odds ratios (OR) were calculated from allelic and genotype frequencies with a 95% confidence interval (95% CI). Adherence to the Hardy–Weinberg equilibrium constant was tested using a χ^2 test with one degree of freedom.

RESULTS

In this study, 327 KD patients, including 216 males and 111 females (Table 1), were analyzed. In total, 279 patients were without CAA (175 males and 104 females) and the mean age of the onset was 20.88 ± 18.60 months. Forty-eight patients suffered from CAA (41 males and 7 females) and the mean age of onset was 21.96 ± 21.60 months. Adjuvant therapy was administered according to individual considerations.

Table 2 plots *SRC-1* genotypic and allelic frequencies of rs11894248, rs17791703, rs7572475, and rs9309308, genotype distributions in Hardy–Weinberg equilibrium. We compared the genotype or allele frequencies between KD patients without CAA and with CAA and noted a statistically significant difference in genotype and allele frequencies for rs11894248, rs17791703, rs7572475, and rs9309308 SNPs in KD patients without CAA and with CAA ($P < 0.01$). In rs11894248, the frequency of the "TT" genotype was lower for KD patients with CAA (45.8%) as compared to the group consisting of KD patients without CAA (77.8%). The frequency of the "TC + CC" genotype was higher in KD patients with CAA (54.2%) than in KD

TABLE 1. Clinical Characteristics of KD Patients With CAA and Without CAA

	KD	Without CAA	With CAA
Number (%)	327	279 (85.32)	48 (14.68)
Age at onset (month)	21.00 ± 19.08	20.88 ± 18.60	21.96 ± 21.60
Male/female ratio	216/111	175/104	41/7

TABLE 2. Genotypic and Allelic Frequencies of SRC-1 Genetic Polymorphisms in KD Patients With and Without CAA

dsSNP ID		KD without CAA $N = 279$ (%)	KD with CAA $N = 48$ (%)	Normal[a] N (%)	P-value[b]	P-value[c]	OR (95% CI)[d]	OR (95% CI)[e]
rs11894248	Genotype				<0.001	0.761		
	TT	217 (77.8)	22 (45.8)	102 (74.5)			Ref	Ref
	TC + CC	62 (23.3)	26 (54.2)	35 (25.5)			4.14 (2.19–7.80)	4.07 (2.14–7.76)
	Allele type				<0.001	0.774		
	T	493 (88.4)	68 (70.8)	237 (86.5)			Ref	Ref
	C	65 (11.6)	28 (29.2)	37 (13.5)			3.12 (1.88–5.20)	3.07 (1.82–5.17)
rs17791703	Genotype				<0.001	0.761		
	AA	217 (77.8)	22 (45.8)	102 (74.5)			Ref	Ref
	AG + GG	62 (22.2)	26 (54.2)	35 (25.5)			4.14 (2.19–7.80)	4.07 (2.14–7.76)
	Allele type				<0.001	0.774		
	A	493 (88.4)	68 (70.8)	237 (86.5)			Ref	Ref
	G	65 (11.6)	28 (29.2)	37 (13.5)			3.12 (1.88–5.20)	3.07 (1.82–5.17)
rs7572475	Genotype				<0.001	0.729		
	AA	217 (77.8)	22 (45.8)	103 (74.6)			Ref	Ref
	AT + TT	62 (22.2)	26 (54.2)	35 (25.4)			4.14 (2.19–7.80)	4.07 (2.14–7.76)
	Allele type				<0.001	0.708		
	A	493 (88.4)	69 (71.9)	238 (86.9)			Ref	Ref
	T	65 (11.6)	27 (28.1)	36 (13.1)			2.97 (1.77–4.97)	2.92 (1.73–4.94)
rs9309308	Genotype				<0.001	0.548		
	TT	215 (77.1)	22 (45.8)	103 (75.2)			Ref	Ref
	TC + CC	64 (22.9)	26 (54.2)	34 (24.8)			3.97 (2.11–7.47)	3.86 (2.03–7.35)
	Allele type				<0.001	0.540		
	C	68 (12.2)	28 (29.2)	238 (86.9)			Ref	Ref
	T	490 (87.8)	68 (70.8)	36 (13.1)			2.97 (1.79–4.93)	2.87 (1.71–4.82)

KD, Kawasaki disease; OR, odds ratio; CI, confidence interval from unconditional logistic regression analysis.
[a]Normal population from Han Chinese in Beijing (CHB; data from HapMap database, http://hapmap.ncbi.nlm.nih.gov/).
[b]P-value from chi-square test; compared KD patients with CAA and without CAA.
[c]P value from chi-square test; compared KD patients with normal population from Han Chinese in Beijing.
[d]Unadjusted odd ratio.
[e]Odds ratios were estimated adjusting for gender.

patients without CAA (23.3%). In comparison with the "TT" genotype, the OR of "TC + TT" was 4.14 (95% CI, 2.19–7.80, $P < 0.001$). The allelic frequency of "C" was higher in KD patients with CAA (29.2%) than in KD patients without CAA (11.6%). In comparison to the "T" allele, the OR for the "C" allele was 3.12 (95% CI, 1.88–5.20, $P < 0.001$), the data show statistically significant differences in this comparison. In rs17791703, rs7572475, and rs9309308 SNPs, genotype and allele frequencies also showed statistically significant differences with CAA. Our data indicated that these four *SRC-1* SNPs may lead to a high risk of development of CAA in KD patients. We also compare KD patients with normal population from HapMap database in *SRC-1* SNPs; there were no statistically significant differences in these comparisons. The result of gender comparison also showed no statistically significant differences in this comparison.

Table 3 shows the *Smad3* SNP rs12901071 and indicates that the frequency of the "TT" genotype was higher in KD patients with CAA (77.1%) than in KD patients without CAA (66.3%). The frequency of the "TC + CC" genotype was lower in KD patients with CAA (22.9%) than in KD patients without CAA (33.7%). As compared to the "TT" genotype, the OR of "TC + CC" was 0.59 (95% CI, 0.29–1.20, $P = 0.140$). The allelic frequency of "C" was lower in KD patients with CAA (11.5%) than in the KD patients without CAA (18.6%). The OR for the "T" allele was 0.57 (95% CI, 0.29–1.10, $P = 0.109$). The data show nonstatistically significant differences in this comparison. We also compare KD patients with normal population from HapMap database in *Smad3* SNPs; there were well-known statistically significant differences in these comparisons. The result of gender comparison also showed no statistically significant differences in this comparison.

DISCUSSION

KD is a systemic vasculitis involving small and medium size blood vessels all over the body, virtually involving the coronaries. Arterial remodeling or revascularization may occur in KD with coronary arteritis. The advancing of stenosis in KD results from active remodeling with proliferation and neoangiogenesis. The growth factors are observably expressed at the aneurysms (19). Although the majority of patients with KD recover without long-term

TABLE 3. Genotypic and Allelic Frequencies of *Smad3* Genetic Polymorphism in KD Patients With and Without CAA

dsSNP ID		KD without CAA $N = 279$ (%)	KD with CAA $N = 48$ (%)	Normal N (%)	P-value[a]	P-value[b]	OR (95% CI)[d]	OR (95% CI)[e]
rs12901071	Genotype				0.140	0.044		
	TT	185 (66.3)	37 (77.1)	79 (58.1)			Ref	Ref
	TC + CC	94 (33.7)	11 (22.9)	57 (41.9)			0.59 (0.29–1.20)	0.55 (0.26–1.13)
	Allele type				0.109	0.087		
	C	104 (18.6)	11 (11.5)	211 (77.6)			Ref	Ref
	T	454 (81.4)	85 (88.5)	61 (22.4)			0.57 (0.29–1.10)	0.54 (0.28–1.05)

KD, Kawasaki disease; OR, odds ratio; CI, confidence interval from unconditional logistic regression analysis.
[a]Normal population from Han Chinese in Beijing (CHB; data from HapMap database, http://hapmap.ncbi.nlm.nih.gov/).
[b]*P*-value from chi-square test; compared KD patients with CAA and without CAA.
[c]*P*-value from chi-square test; compared KD patients with normal population from Han Chinese in Beijing.
[d]Unadjusted odd ratio.
[e]Odds ratios were estimated adjusting for gender.

consequences, the disorder is associated with vasculitis affecting the coronary arteries and occasionally other muscular arteries, resulting in CAA in more than 20% of untreated patients (5, 20). Two to three percent of untreated patients die of coronary artery thrombosis, myocardial infarction, or, rarely, aneurysm rupture. Patients with a large (8 mm or more) CAA are at a long-term risk of developing aneurysm thrombosis or coronary artery stenosis and myocardial infarction, even years after the acute illness (5). In view of the frequency and severity of coronary artery complications, there has been intense interest in treatments to reduce the risk of CAA (21).

TGF-β may contribute to aneurysm formation by promoting the generation of myofibroblasts growth factor that may also be involved in the induction of regulatory T-cells in KD (22). *TGFB2*, *TGFBR2*, and *Smad3* were found to have a significant association with the susceptibility of KD (7). Genetic polymorphisms in TGF-β signaling pathway are associated with KD susceptibility, but not coronary artery lesions formation or IVIG treatment response in the Taiwanese population (23). We showed *SRC-1* gene polymorphism was strongly associated with CAA complication of KD, but not *Smad3*. *SRC-1* interacts with the transcriptional coactivators p300/CBP, but not with *Smad3*, since SMAD proteins are differentially expressed in target tissues for TGF-β, the tissue-specific amounts of endogenous SMAD proteins may contribute to the cooperative actions (8, 24). SMAD proteins are differentially expressed in target tissue for TGF-β. The tissue-specific amounts of endogenous SMAD proteins may contribute to the cooperative actions (24).

SRC-1 serves as an in vivo coactivator for ER in the blood vessel wall to comediate the effect of estrogen on vasoprotection during vascular wall remodeling after an injury. Conversely, SRC-1 downregulation or loss-of-function mutation will reduce or impair the ER function in the vascular wall and thereby enhance the neointima formation in response to a vascular injury (12). Mutant form of SRC-1, lacking the CBP/p300 binding site, failed to upregulate Smad3/4-dependent transcription, while full-length SRC-1 potentiated p300–Smad3 interactions and also triggered the TGF-β signaling pathway (8). In early stages of cancer, TGF-β functions as a tumor suppressor because it inhibits proliferation, induces apoptosis, and mediates differentiation. Conversely, in later stages of cancer, TGF-β promotes tumor progression through increasing tumor cell invasion and metastasis. Thus, TGF-β can have opposing roles, likely dependent, in part, on whether the cancer is early or late stage (25).

In conclusion, we have shown that *SRC-1* gene polymorphisms susceptible to the development of KD complication of CAA are associated with genetic predisposition in Taiwanese children of Han Chinese ethnic background. Our research also shows that genetic polymorphism in the TGF-β signaling pathway, particularly the *SRC-1* gene, is associated with susceptibility to CAA as a complication from KD.

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