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Polymorphism of stromal cell-derived factor-1 selectively upregulates gene expression and is associated with increased susceptibility to coronary artery disease

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ABSTRACT

Stromal cell-derived factor-1 (SDF-1) plays critical roles in vascular development and hematopoiesis. Here, we investigated the function of SDF-1 rs1801157G/A polymorphism in various immune cells and examined its association with susceptibility to coronary artery disease (CAD). Protein and mRNA levels of SDF-1 were tested in peripheral CD4+ T cell, CD8+ T cells, monocytes, and natural killer (NK) T cells from healthy donors with different genotypes of rs1801157G/A polymorphism. Prevalence of the polymorphism was compared between CAD patients and healthy controls. Data revealed that SDF-1 mRNA and protein were detectable in CD4+ T cells, CD8+ T cells, monocytes and NK T cells. Interestingly, both protein level and mRNA level of SDF-1 were significantly increased in the monocytes with rs1801157AA genotype, whereas the same phenomenon was not observed in the other three cell types. Blockage of CD14 completely inhibited the upregulation of SDF-1 in the monocytes with rs1801157AA genotype. Association analysis showed that frequencies of the rs1801157AA genotype and A allele were significantly higher in CAD cases than in controls (odds ratio [OR] = 2.28, 95% confidence interval [CI], 1.50–3.29, $p < 0.0001$, and OR = 1.46, 95% CI, 1.21–3.73, $p < 0.0001$, respectively). Also, prevalence of rs1801157AA genotype was further increased in cases with ST-elevation myocardial infarction (OR = 1.65, 95% CI, 1.04–2.56, $p = 0.028$). Our data suggest a novel pathway for regulating SDF-1 and a new risk factor for CAD.

1. Introduction

Coronary artery disease (CAD) is the leading cause of hospitalization and the predominant contributor to mortality in China. It is now widely spread accepted that atherosclerosis is not only merely a lipid disorder but also a chronic inflammatory disease [1]. The immune system plays critical roles in the initiation and propagation of atherosclerosis. Studies have shown that dysregulation of immune cells and chemokines results in the progression of atherosclerosis, plaque instability and the subsequent onset of acute coronary syndrome (ACS, including unstable angina pectoris and acute myocardial infarction) [2,3].

Stromal cell-derived factor-1 (SDF-1) is a small (8 kDa) homeostatic chemokine that was originally described as an efficacious lymphocyte chemo-at tractant and regulator of hematopoiesis, and was soon after also characterized as a modulator of multiple physiological processes [4–6]. SDF-1 is a pleiotropic chemokine that is widely expressed in different organs including the brain, lung, colon, heart, kidney, and liver where it acts as a chemoat tractant for immature and mature hematopoietic cells; it thus plays an important role in inflammation and immune surveillance of tissues [5,6]. Additionally, SDF-1 serves as an emergent salvage signal for initiating tissue regeneration and repair. Recent study has revealed high level of SDF-1 in atherosclerotic plaques [5,6]. However, the mechanism remains unclear.

SDF-1 is a potent chemo-at tractant for monocyte, whereas monocytes play important roles in atherosclerosis [7–10].

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Monocyte involvement in the development of atherosclerotic plaques was first reported by Kottke et al., with monocyte accumulation demonstrated in porcine atherosclerotic lesions [7]. Several related processes, which involve monocytes, have been identified in CAD. Monocytes promote destabilization of the fibrous cap leading to the plaque rupture. This is mainly orchestrated by matrix metalloproteinases (MMPs) [8,9]. Monocytes have a role in thrombus propagation contributing to the coagulation cascade during the acute event. Patients with ACS show features of procoagulant monocyte activation with exposure of tissue factor [10]. Monocyte adherence to extracellular matrix and extravasation to the injured tissue induces the expression of a multitude of cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 [11,12].

Single nucleotide polymorphisms (SNPs) may cause functional changes of genes and affect the development of diseases. The SDF-1 rs1801157G/A polymorphism has a G to A mutation at position 801 in 30-untranslated region in its b transcriptional splice variant. It has been shown to be associated with an increased risk of lymphoma, breast, and lung cancers [13–15]. However, functions of the SNP remain unclear. In this study, we investigated the effect of the SNP on different immune cells and its association with susceptibility to CAD.

2. Materials and methods

2.1. Patients and controls

The study group included 592 CAD patients and 625 healthy controls recruited from Changzheng Hospital and Guangzhou Liuhuaqiao Hospital. The diagnosis of CAD was confirmed by coronary angiography performed with the Judkins technique using a quantitative coronary angiographic system, and it was defined by angiography with at least one main coronary vessel >50% luminal narrowing. All the CAD patients were newly diagnosed and previously untreated. Cases with valvular heart disease, thromboembolism, collagen disease, disseminated intravascular coagulation, advanced liver disease, renal failure, malignant disease, septicemia or on steroid therapy were excluded from the study. In the same period, 625 subjects who underwent regular physical examinations at the same hospital were recruited as controls. They were diagnosed free of CAD by their medical history of CAD or angiography, free of clear ischaemic changes by electrocardiography and without chest pain symptoms. Individuals with congestive heart failure, peripheral vascular disease, rheumatic heart disease, pulmonary heart disease, tumor, chronic kidney or hepatic disease were excluded from the study. All individuals enrolled were from the Han population in China. Social demographic information, family history of CAD, past history and lifestyle factors were obtained through questionnaire interview. Written informed consent was obtained from each participant. The study was approved by the Review Boards of Changzheng Hospital and Guangzhou Liuhuaqiao Hospital. Each study participant provided a peripheral blood sample.

2.2. DNA extraction and genotyping

Genomic DNA was extracted from 5 ml frozen whole blood using the DNA Extraction Kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's protocol. The SDF-1 rs1801157G/A polymorphism was identified by the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay. The primers were 5′-CAGTCAACCTGGGCAAAGCC-3′, and 5′-AGCTTTGGTCCTGAGAGTCC-3′. PCR was performed in a total reaction volume of 20 μl containing 2 μl of 10× PCR buffer (Qiagen Inc., Hilden, Germany), 1.5 mM $MgCl_2$, 0.5 μM of each primer (shown in Table 1), 0.2 mM dNTP, 1.2 U Taq polymerase (Qiagen Inc., Hilden, Germany) and 200 ng of genomic DNA. After an initial denaturation at 95 °C for 5 min, the DNA was amplified for 35 cycles at 94 °C for 30 s, 55–60 °C for 30 s, and 72 °C for 30 s, with a final elongation at 72 °C for 5 min on the Gene-Amp PCR System 9700 (PE Applied Biosystems, Foster City, CA, USA). PCR products containing the three polymorphic sites were then digested with the restriction enzymes MspI by using the conditions recommended in the manufacturer's instructions. The digested PCR products were fractionated on 2% agarose Tris–borate–EDTA gel (Agarose 1000; Gibco BRL, Rockville, MD, USA) and stained with ethidium bromide (product size after digestion shown in Table 1). To confirm the genotyping results, more than 15% of PCR-amplified DNA samples were examined by DNA sequencing. Results between PCR and DNA sequencing analysis were 100% concordant.

2.3. Peripheral CD4+ T cells, CD8+ T cells, monocytes and natural killer (NK) T cells

Mononuclear cells were isolated from peripheral blood of 60 healthy individuals with after Ficoll-Hypaque centrifugation, in which 20 subjects were rs1801157GG genotype, 20 subjects were rs1801157GA genotype, and 20 subjects were rs1801157AA genotype. Purified peripheral blood mononuclear cells (PBMC) were then washed and resuspended in phosphate-buffered saline containing 2% fetal bovine serum. Extraction of CD4+ T cells, CD8+ T cells, monocytes and NKT cells were conducted according to previously described methods.

2.4. Quantitative real-time PCR of SDF-1 mRNA

Total cellular RNA was extracted from peripheral white blood cells with TRIzol LS reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Purified total RNA was measured and assessed for purity by determining absorbance at 260 and 280 nm and then was stored at −80 °C until testing. Reverse transcriptase reaction was conducted. Real-time PCR using SYBR green fluorescence was performed with 20 ng of cDNA in a total

Table 1
General characteristics of the CAD patients and control group.

Characteristics	CAD (n = 592)	Control (n = 625)	p Value
Age (years)	56.6 ± 9.2	55.7 ± 10.1	>0.05
Gender (M/F)	355/237	348/273	>0.05
BMI (kg/m^2)	26.3 ± 5.8	23.4 ± 4.6	<0.05
Smoking, n (%)	251 (42.4)	89 (14.2)	<0.05
Hypertension, n (%)	277 (46.8)	95 (15.2)	<0.05
Diabetes, n (%)	199 (33.6)	64 (10.2)	<0.05
TG (mmol/L)	2.3 ± 0.8	1.1 ± 0.4	<0.05
TC (mmol/L)	5.9 ± 1.5	4.1 ± 0.6	<0.05
HDL-C (mmol/L)	0.8 ± 0.3	1.3 ± 0.4	<0.05
LDL-C (mmol/L)	3.8 ± 1.0	3.0 ± 0.4	<0.05
Hemoglobin (g/L)	138 ± 15.8	131 ± 16.1	>0.05
White blood cells (10^9/L)	6.5 ± 1.9	6.4 ± 1.7	>0.05
Red blood cells (10^{12}/L)	4.1 ± 0.7	4.2 ± 0.9	>0.05
Platelets (10^9/L)	248 ± 32	269 ± 33	>0.05
Coronary angiography, n (%)			
1- Vessel	146 (24.6)	–	
2- Vessel	188 (31.8)	–	
3- Vessel	258 (43.6)	–	
STEMI, n (%)			
Yes	263 (44.4)		
No	329 (55.6)		

Data are mean ± SD.
CAD, coronary artery disease; BMI, body mass index; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; STEMI, ST-elevation myocardial infarction.

volume of 20 μL. Quantitative real-time PCR reaction was carried out using Platinum SYBR Green qPCR SuperMix UDG (Invitrogen) using 0.25 nm of each sense and antisense primer. The amount of SDF-1 cDNA was estimated by quantitative polymerase chain reaction (qPCR) amplified using the sense primer 5′-TTACCCGCSDF-1AGACAAG T-3′ and the antisense primer 5′-AGGCAATCACSDF-1ACCCAGT-3′, human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was amplified using the sense primer 5′-GAAGGT-GAAGGTCGGA-3′ and the antisense primer 5′-GGGTCATTGATGG-CAAC-3′. The PCR reaction was performed for 40 cycles as follows: 95 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s in a Chromo Real Time PCR Detection (Bio-Rad, Hercules, USA). In quantitative RT-PCR analysis the expression level of SDF-1 mRNA was calculated according to the Pfaffl method, in which Ct values for SDF-1 were the mean fold change + SEM for three independent determinations corrected by GAPDH Ct values from control samples, considering efficiency values.

2.5. SDF-1 excretion from different immune cells

Purified CD4+ T cells, CD8+ T cells, monocytes or NK T cells were resuspended in RPMI1640 medium at the concentration of 3×10^6 cells/ml and cultivated in 37 °C, 5% CO_2 incubator for 24 h. Supernatants were than harvested and tested for SDF-1 by ELISA (R&D systems, UK). Assays were performed according to the manufacturer's guidelines. The sensitivity of kits was 2 pg/ml, and inter- and intra-assay assessments of reliability of the kit were conducted. Data were only used when the inter- and intra-assays produced scores of CV < 14% and CV < 0.03%, respectively.

2.6. CD14 blocking experiments

Purified monocytes were resuspended in PBS at the concentration of 1×10^6 cells/ml. Anti-CD14 antibody (Invitrogen) or Anti-CD16 antibody (eBioscience) as a control was added and cultivated for 1 h. Cells were then centrifuged, resuspended in RPMI1640 at the concentration of 3×10^6 cells/ml, and cultivated in 37 °C, 5% CO_2 incubator for 24 h. Supernatants were than harvested and tested for SDF-1 by ELISA (R&D systems, UK).

2.7. Statistical analysis

The SPSS statistical software package ver.19.0 (SPSS Inc., Chicago, USA) was used for statistical analysis. Demographic data between the study groups were compared by the chi-square test and by the student *t*-test. MRNA level and SDF-1 excretion data were compared by the student *t*-test. The polymorphism was tested for deviation from Hardy–Weinberg equilibrium by comparing the observed and expected genotype frequencies using the chi-square test. For SNP analysis, genotype and allele frequencies were compared between groups using the chi-square test. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated using unconditional logistic regression. *p* Values less than 0.05 were considered significant.

3. Results

3.1. Expression of SDF-1 in different immune cells

Although studies have shown that SDF-1 is expressed from different tissues, whether immune cells can excrete the chemokine remains unclear. We collected PBMCs from 90 healthy donors, in which 20 subjects with rs1801157GG genotype, 20 subjects with rs1801157GA genotype, and 20 subjects with rs1801157AA genotype. CD4+ T cells, CD8+ T cells, monocytes and NK T cells were isolated. We first tested mRNA levels of SDF-1 in these cells. Data revealed that all the 4 cell types had detectable SDF-1 mRNA (data not shown). We further tested whether these cells could excrete SDF-1. After 24 h cultivation, SDF-1 were detected in the supernatant of all the four purified cell types, in which monocytes revealed the highest expression (data not shown). These data suggest that peripheral CD4+ T cells, CD8+ T cells, monocytes and NK T cells can express SDF-1.

3.2. rs1801157 SNP increases SDF-1 expression in monocytes

We compared mRNA level of SDF-1 in CD4+ T cells, CD8+ T cells, monocytes and NK T cells from the subjects with different rs1801157 genotypes (Fig. 1). In the CD4+ T cells (Fig. 1A), CD8+ T cells (Fig. 1B), and NK T cells (Fig. 1D), we did not observe any significant changes of SDF-1 mRNA level among subjects with different genotypes. However, subjects with rs1801157AA genotype revealed significantly higher level of SDF-1 mRNA than those with rs1801157GG genotype ($p < 0.001$) or rs1801157GA genotype ($p < 0.001$) in monocytes (Fig. 1C). Also, we compared SDF-1 excretion of CD4+ T cells, CD8+ T cells, monocytes and NK T cells from the subjects with different rs1801157 genotypes (Fig. 2). Similarly, we did not observe any significant changes of SDF-1 protein level in CD4+ T cells (Fig. 2A), CD8+ T cells (Fig. 2B), and NK T cells (Fig. 2D) from subjects with different genotypes, whereas subjects with rs1801157AA genotype revealed significantly higher level of SDF-1 protein (540 pg/ml) than those with rs1801157GG genotype (189 pg/ml, $p < 0.01$) or rs1801157GA genotype (256 pg/ml, $p < 0.01$) in monocytes (Fig. 2C). These data suggest that rs1801157 SNP selectively increase SDF-1 expression in monocytes.

3.3. Blockage of CD14 inhibits the function of rs1801157 in monocytes

Since rs1801157 SNP selectively increase SDF-1 expression in monocytes, whereas monocytes specifically express CD14, we hypothesized whether the CD14 signaling pathway could be involved in regulating the function of rs1801157. Anti-CD14 antibody was used to block CD14 and anti-CD16 antibody was used as a control. After 24 h of blocking CD14, cases with different rs1801157 genotypes demonstrated similar level of SDF-1 in the supernatant of monocytes (Fig. 3A), whereas after blocking CD16, subjects with rs1801157AA still showed higher SDF-1 level in the supernatant of monocytes compared to those with rs1801157GG genotype or rs1801157GA genotype (Fig. 3B). These data indicate that the CD14 signaling pathway plays critical roles in modulating the function of rs1801157.

3.4. rs1801157 SNP is associated with increased risk of CAD

We analyzed the association between the rs1801157 SNP and CAD. A total of 592 CAD cases and 625 healthy controls were recruited. The clinical characteristics of all the subjects are shown in Table 1. The genotype and allele frequencies of the SDF-1 rs1801157G/A SNP in CAD cases and controls are summarized in Table 2. The genotype distributions of this polymorphism among the controls were in agreement with the Hardy–Weinberg equilibrium ($p > 0.05$). The genotype frequencies of rs1801157G/A polymorphism were 49.3% for GG, 33.6% for GA, and 17.1% for AA among the cases, and 58.2% for GG, 33.1% for GA, and 8.7% for AA among the controls (Table 2). Prevalence of the rs1801157AA genotype was significantly higher in patients than in controls (OR = 2.28, 95%CI: 1.50–3.29, $p < 0.0001$; data were adjusted for age and sex). Also, frequency of the rs1801157A allele was significantly increased in cases compared to healthy donors (OR = 1.46, 95%CI: 1.21–1.73, $p < 0.0001$; data were adjusted for age and

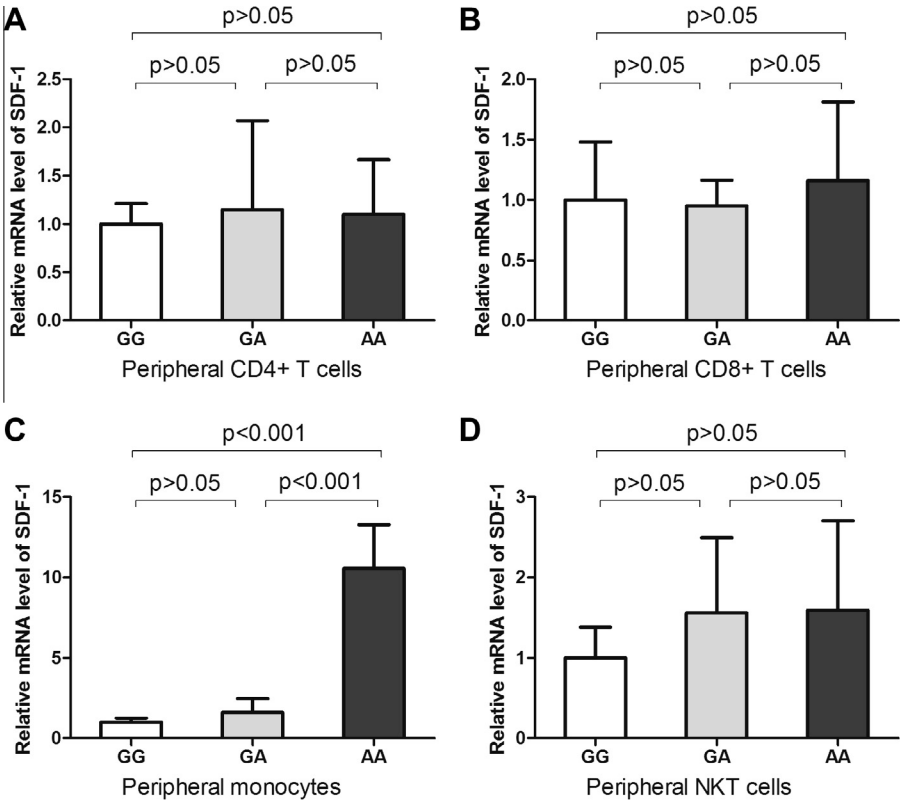

Fig. 1. Relative mRNA level of SDF-1 in peripheral CD4+ T cells (A), CD8+ T cells (B), monocytes (C), and natural killer (NK) T cells (D) with different rs1801157 genotypes. The expression level of SDF-1 mRNA was calculated according to the Pfaffl method, in which Ct values for SDF-1 were the mean fold change+ SEM for three independent determinations corrected by GAPDH Ct values. Values of GG genotype were normalised to 1.00.

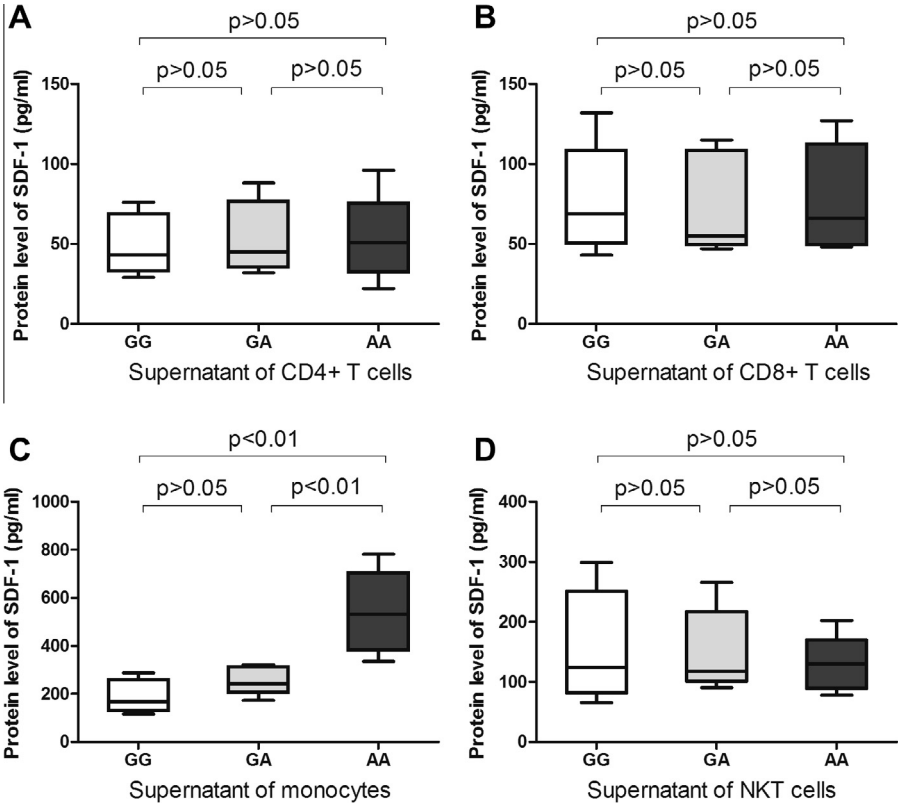

Fig. 2. Protein level of SDF-1 in the supernatant of purified peripheral CD4+ T cells (A), CD8+ T cells (B), monocytes (C), and natural killer (NK) T cells (D) with different rs1801157 genotypes.

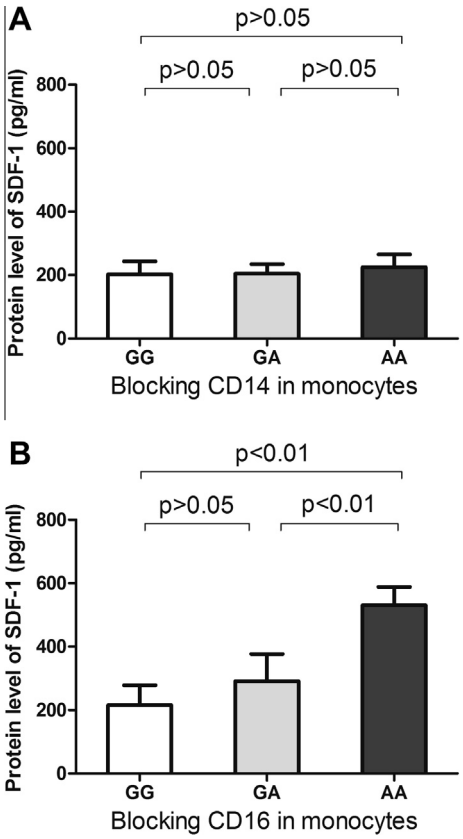

Fig. 3. After blocking with anti-CD14 antibody (A) or anti-CD16 antibody (B), protein level of SDF-1 in the supernatant of purified monocytes with different rs1801157 genotypes.

Table 2
SDF-1 rs1801157 SNP between CAD cases and controls.

Polymorphisms	Cases (N = 592) (%)	Controls (N = 625) (%)	OR (95% CI)*	p Value
SDF-1 (rs1801157)				
Genotype				
GG	292 (49.3)	364 (58.2)	1.00	
GA	199 (33.6)	207 (33.1)	1.12 (0.84–1.55)	0.153
AA	101 (17.1)	54 (8.7)	2.28 (1.50–3.29)	<0.0001[a]
Allele				
G	783 (66.1)	935 (74.8)	1.00	
A	401 (33.9)	315 (25.2)	1.46 (1.21–1.73)	<0.0001[a]

[a] p Value < 0.05.
* Data were adjusted for age and sex.

sex). These data suggest that SDF-1 rs1801157G/A polymorphism is associated with an increased susceptibility to CAD in the Chinese population.

3.5. rs1801157 SNP is associated with ST-elevation myocardial infarction (STEMI)

We further evaluated the association of the SDF-1 polymorphism with clinical-pathological factors in CAD patients. The stratification analysis included coronary vessel status and STEMI status (Table 3). Data revealed that prevalence of rs1801157AA genotype was significantly increased in patients with STEMI than those without STEMI (OR = 1.65, 95%CI, 1.04–2.56, p = 0.028), whereas the polymorphism did not show any significant differences in CAD cases with different vessels affected (p > 0.05) (Table 3). Since STEMI is treated as an emergency with either urgent coronary angiography and percutaneous coronary intervention or with thrombolysis, our results indicate that SDF-1 rs1801157 SNP can be used as marker for the severity of CAD.

4. Discussion

In the current study, we identified that SDF-1 could be excreted by CD4+ T cells, CD8+ T cells, monocytes and NK T cells. More importantly, we observed rs1801157AA genotype could increase SDF-1 expression in monocytes but not in CD4+ T cells, CD8+ T cells, and NK T cells. Blocking CD14 could inhibit the function of rs1801157 in monocytes. In addition, the polymorphism was associated with increased susceptibility to CAD, and further correlated with CAD cases with STEMI.

SDF-1 or CXCL12 is a 68-amino-acid CXC chemokine with important roles in essential biological processes such as vascular and neuronal development and hematopoiesis. The response to SDF-1 occurs at a very early stage of embryonic development and appears to be widely operative whenever cell migration is required [4]. Indeed, mice lacking SDF-1 die prenatally and exhibit defects in vascularization, neuronal development, and hematopoiesis [16–18]. Besides these physiological functions, SDF-1 seems to be involved in pathological processes such as neoplasia, tumor progression, and chronic inflammation [8–14]. In immune system, the principal role of this chemokine is to regulate the trafficking and localization of myeloid, lymphoid, and progenitor cells between central and peripheral compartments [19,20]. SDF-1 is constitutively expressed in a broad range of tissues [21,22], and the major sources of SDF-1 expression are bone marrow stromal elements and endothelial cells [23,24]. Our study identified that CD4+ T cells, CD8+ T cells, monocytes and NK T cells could also express SDF-1, in which monocytes showed the highest excretion of SDF-1, indicating a boarder function of the chemokine in human beings.

The SDF-1 rs1801157G/A polymorphism has a G to A mutation at position 801 in 30-untranslated region in its b transcriptional splice variant. The SNP has been shown to be associated with an increased risk of lymphoma, breast, and lung cancers [13–15]. Researchers hypothesized that the SNP may increase SDF-1 expression. Our study for the first time showed that the SNP may upregulate SDF-1 excretion in monoctyes (Figs. 1 and 2), and blocking CD14 could interfere the function of rs1801157G/A polymorphism in monocytes (Fig. 3). This is important because it suggest a novel pathway of regulating SDF-1. Since SDF-1 is involved in various diseases including cancers, our knowledge may provide a new understanding of SDF-1 with diseases.

Monocytes play critical roles in atherosclerosis [8–12]. During the acute inflammatory phase that follows destabilization, monocytes regulate rupture of the atherosclerotic plaque, and acute thrombus formation in ACS; and during healing, monocytes reside in the myocardial tissue in the hypoxic phase during an acute coronary event and might promote myofibroblast accumulation, angiogenesis, and myocardial healing and remodeling, thus showing a protagonist or antagonist influence in post-ACS recovery. Our data suggest rs1801157G/A polymorphism may increase SDF-1 level in monocytes [8–12]. Since SDF-1 can modulate functions of monocytes, it is possible that the polymorphism can affect atherosclerosis through monocytes. Therefore, it is reasonable that the polymorphism is associated with increased risk of CAD (Table 2). Our study was performed in the Chinese population. It would be interesting to conduct similar research in different populations for comparison.

In conclusion, our study identified that rs1801157G/A polymorphism specifically upregulated SDF-1 expression through CD14

Table 3
Stratification analysis of rs1801157 polymorphism according to clinical-pathological characteristics in CAD patients.

rs1801157	Frequencies		OR (95% CI)*	*p* Value
Characteristics	*n* (%)	*n* (%)		
Vessel affected	Multiple (*n* = 446)	Single (*n* = 146)		
GG	215 (48.2)	77 (52.7)	1.00	
GA	150 (33.6)	49 (33.6)	1.07 (0.68–1.62)	0.664
AA	81 (18.2)	20 (13.7)	1.39 (0.73–2.39)	0.187
G	580 (65.0)	203 (69.5)	1.00	
A	312 (35.0)	89 (30.5)	1.15 (0.86–1.57)	0.159
STEMI	Yes (n = 263)	No (n = 329)		
GG	125 (47.5)	167 (50.8)	1.00	
GA	82 (31.2)	117 (35.6)	1.31 (0.70–2.44)	0.402
AA	56 (21.3)	45 (13.6)	1.65 (1.04–2.56)	0.028[a]
G	332 (63.1)	451 (68.5)	1.00	
A	194 (36.9)	207 (31.5)	1.26 (1.00–1.57)	0.050

OR, odds ratio; CI, confidence interval; CAD, coronary artery disease; STEMI, ST-elevation myocardial infarction.
[a] $p < 0.05$.
* Data were adjusted for age and sex.

signaling pathway, and was associated with increased susceptibility to CAD. This research sheds light on understanding the mechanism of SDF-1 and provides therapeutic strategies in cardiovascular diseases.

Author disclosure statement

No competing financial interests exist.

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