RESEARCH ARTICLE

Plasma CXCL1 levels and TRAF3IP2 variants in patients with myocardial infarction

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Background: IL-17A plays an important role in inflammatory responses in myocardial infarction (MI). IL-17A signals through its receptor, for which, Act1 (TRAF3IP2) functions as a key upstream adaptor in the pathway.

Aim: To compare frequencies of functional polymorphisms of TRAF3IP2 (rs13210247, rs33980500) between patients with MI and healthy controls.

Methods: The selected SNPs were studied in 201 Iranian MI patients and 201 healthy blood donors from Fars Province by PCR-RFLP in association with clinicopathologic criteria of patients. CXCL1 plasma levels in 126 MI patients and 50 normal subjects were measured by ELISA.

Results: A significant increase in the mutant (T) allele of TRAF3IP2 rs33980500 in patients with diastolic dysfunction of the heart ($P = .01$) was observed. The highest correlation, however, was observed between the TRAF3IP2 rs33980500 TT genotype and T allele with left main coronary artery stenosis ($P = .01$, $P < .001$; OR = 31.03). T allele of TRAF3IP2 rs33980500 was also associated with female gender, family history of cardiovascular disease, and mechanical complications of heart ($P = .04$, $P = .02$, and $P = .01$, respectively). Moreover, TRAF3IP2 rs13210247 (G) correlated with mechanical complications of the heart ($P = .01$). A significant increase in the plasma levels of CXCL1 chemokine in patients ($P = .0006$) associated with TT genotype of TRAF3IP2 (rs33980500) was observed ($P = .04$).

Conclusion: The gene variants of Act1 adaptor are associated with correlates of poor outcome in patients with MI and plasma CXCL1 levels.

KEYWORDS

CXCL1, IL-17A, myocardial infarction, TRAF3IP2

1 | INTRODUCTION

Myocardial infarction (MI) is one of the chief causes of death worldwide. Inflammatory responses have been confirmed to be the hallmarks of MI.¹ Indeed, MI leads to an intricate inflammatory reaction

accompanied by cytokine secretion and recruitment of inflammatory cells into the infarcted myocardial region.^{2,3} Among cytokines, IL-17A triggers the production and activation of chemokines, inflammatory cytokines, matrix metalloproteinases (MMPs), and caspases 3 and 9 that cause apoptosis in endothelial cells and cardiomyocytes.⁴ Increased frequency of Th17 cells and excessive production of IL-17, IL-6, IL-23 are demonstrated in patients with acute myocardial infarction (AMI).^{5,6} In addition, percutaneous coronary intervention (PCI), which is a common therapeutic intervention to attenuate

Abbreviations: Act1, actin-related protein 1; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; HDL, high-density lipoprotein; IL-17A, interleukin 17A; LDL, low-density lipoprotein; MI, Myocardial infarction; MMP, matrix metalloproteinases; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; TG, triglyceride; Th, T-helper; TNF, tumor necrosis factor.

the damage of ischemic myocardium can cause ischemic-reperfusion injury in heart by elevated expression of IL-17 and IL-17RA receptor subunits.⁷⁻⁹ IL-17A receptor consists of IL-17RA and IL-17RC chains where binding of IL-17A induces the onset of downstream signaling pathways through recruitment of Act1 adaptor molecule. Act1 eventually activates several transcription factors and transcription of chemokines, cytokines, and MMPs. Enhanced IL-17A signaling via the activation of a number of mitogen-activated protein kinases (MAPK) causes elevated expression of downstream proinflammatory targets. In addition, IL-17A signaling may cause Act1 interaction with TNF receptor-associated factor 2 (TRAF2) and TRAF5 and lead to increased stability of CXCL1 mRNA. Thus, the inflammatory and pro-atherogenic function of IL-17A can be aggravated.^{10,11} Act1-deficient mice models have revealed that Act1-dependent IL-17 signaling pathway is pivotal for pathogenesis of EAE,¹² collagen-induced arthritis (CIA), and colitis.^{13,14} Previous reports reveal that the rs33980500 and rs13210247 SNPs in TRAF3IP2 gene are markedly associated with inflammatory diseases.^{15,16} According to the role of IL-17A signals in augmentation of inflammatory conditions and its link to the pathogenesis of MI, we hypothesized that polymorphisms in Act1, the key adaptor molecule in IL-17A signaling pathway, are correlated with myocardial infarction or its outcome in patients. Selection of polymorphisms was based on a thorough search on all validated SNPs within TRAF3IP2 gene in NCBI databases. Based on previous studies, the rs33980500 SNP affects downstream signaling by altering the expression of protein (Act1), and along with rs13210247 is associated with several inflammatory diseases. Therefore, we studied both SNPs in the TRAF3IP2 adaptor (Act1) gene in patients who were hospitalized because of myocardial infarction. The SNP frequencies were also defined in a group of healthy blood donors in the same geographic region. Since previous studies have shown that certain genotypes of rs33980500 from TRAF3IP2 reinforce the stability of CXCL1 mRNA, we also evaluated the relationship of CXCL1 plasma levels with TRAF3IP2 genotypes in patients.

2 | METHODS AND MATERIALS

2.1 | Study population

All confirmed MI cases ($n = 201$) referring to the affiliated hospitals of Shiraz University of Medical Sciences in a 1-year period were included in this study. MI diagnosis was approved by collaborating cardiologist on the basis of typical ECG changes and increased cardiac markers. The MI diagnosis was confirmed by coronary angiography. The patients' clinical and pathological criteria are shown in Table 1. Control individuals ($n = 201$) were selected from among healthy blood donors of the same age range and gender (mean age $\pm$ SD = 57.80 $\pm$ 11.8 years, 175 Male/26 Female) who referred to Fars Blood Transfusion Center. First, they were examined by a physician and were evaluated for underlying diseases, including: cardiovascular diseases, hyperthyroidism, hypertension, pulmonary diseases, stroke, diabetes, cancer, and autoimmune diseases.

TABLE 1 Demographical Criteria of patients

Characteristic	
No. of subjects	201
Age (means $\pm$ SD)	59.30 $\pm$ 12.4
Sex (male/female, no %)	175 (87.1)/26 (12.91)
BMI (112)	
<18.4	1 (1.8)
18.5-24.9	52 (46.4)
25-30	45 (40.2)
>30	13 (11.6)
HLP (163)	
Yes	42 (25.8)
No	121 (74.2)
DM (162)	
Yes	40 (24.7)
No	122 (75.3)
Family history (163)	
Yes	48 (29.4)
No	115 (70.6)
HTN (163)	
Yes	113 (69.3)
No	50 (30.7)
Smoking (163)	
Yes	78 (47.9)

Second, they were asked and examined for recent infectious diseases including common cold, influenza, lung infection, urinary tract infection, brucellosis, tuberculosis, and typhoid and excluded if positive. Third, the recipients of recent surgery and dentistry and those who were on any medications such as antibiotics, aspirin, propranolol, or had recently received any vaccine or blood products were excluded from study. Travel to endemic malaria areas in the country, tattooing, acupuncture, high or low blood pressure and anemia, favism, and risky behaviors were also considered as exclusion criteria. Blood samples from these individuals were further tested for infectious diseases such as hepatitis C, hepatitis B, AIDS, and syphilis. All of these 201 peoples were negative for those criteria.

The lipid panel (HDL, LDL, TG, and cholesterol) and blood glucose levels were not studied due to the fact that the control individuals were not fasting. Having said so, we only included individuals who had their lipid profile and blood sugar levels checked up to 3 months before sampling and turned normal.

Body mass index (BMI) was calculated as weight in kilograms divided by height in square meters. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg or the use of oral antihypertensive therapy. Type 2 diabetes mellitus (T2DM) was diagnosed according to World Health Organization criteria.¹⁷ Hyperlipidemia was defined as elevated levels of any or all lipids and/or lipoproteins in the blood.

TABLE 2 Primer sequences, restriction endonucleases, PCR product lengths, and restriction patterns

SNPs	Primer sequences	Restriction enzyme	Product length (bp)	Length of final fragments (bp)
TRAF3IP2 rs33980500	Forward -5'-GGGCTCCAACCACAGACT-3'	Tru1I (MseI)	441	CC: 441
	Reverse -5'-GTGAGGACTCCAAGAATTATC-3'			CT: 441, 345, 96 TT: 345, 96
TRAF3IP2 rs13210247	Forward -5'-GGGGACAACCTAAGGCAAGGA-3'	MvaI (BstNI)	220	AA: 220
	Reverse -5'-GACGCTTCAAACCTATTCCTG-3'			AG: 220, 166, 54 GG: 166, 54

2.2 | Blood samples and DNA extraction

Venous blood of 6 ml was collected from all subjects in tubes containing ethylene diamine tetra acetic acid (EDTA) anticoagulant in the first 12 hours after admission. Plasma was separated for ELISA test within an hour after blood collection and DNA extraction was further performed by salting out method. DNA concentration and protein contamination were determined by means of spectrophotometer in 260 and 280 wavelength. The concentration of DNA samples was standardized to 0.3 µg/µL.

2.3 | Genotyping

We studied two SNPs in TRAF3IP2 gene: one coding polymorphism rs33980500 and an intronic one called rs13210247. PCR and RFLP methods were established and used for genotyping of noted SNPs. PCR procedure was performed in a 15 µL total reaction volume containing 200 µM of each dNTPs, 300 ng genomic DNA, 2 mM of MgCl₂, 10X PCR Buffer, 1U Taq DNA polymerase, and 1.0 µM of each primer. Then, the related restriction enzyme was added to PCR products and incubated at 37°C overnight in a dry block. The primers sequences, required restriction enzyme, recognition site of restriction enzyme, and length of cleaved products are shown in Table 2. The enzymatically cleaved products were electrophoresed on a 3.5 agarose gel containing 2.5 µL safe stain and visualized by UV light at 254 nm (Figures 1 and 2).

2.4 | Cytokine measurement

CXCL1 plasma levels in 126 MI patients and 50 normal subjects were measured by the Human CXCL1 (MGSA alpha, Thermo scientific, USA) ELISA assay. Due to financial inadequacies, chemokine measurement was not performed in all patients and healthy individuals. Selected patients and healthy controls representing different genotypes were included in this assay.

3 | RESULTS

3.1 | Genotype distributions and allelic prevalence

The genotype distributions and allelic prevalence of studied SNPs are shown in Table 3. Two studied SNPs in patient and control groups followed Hardy-Weinberg equilibrium. As indicated in Table 3, TRAF3IP2

FIGURE 1 PCR-RFLP products corresponding to the TRAF3IP2 rs33980500 polymorphism **FIGURE 2** PCR-RFLP products corresponding to the TRAF3IP2 rs13210247 polymorphism

(rs33980500) and TRAF3IP2 (rs13210247) SNPs showed no significant difference in allelic and genotype distributions between patients and controls (Table 3). Moreover, logistic binary regression after correction for sex and age confirmed the results of chi-square showing that TRAF3IP2 rs13210247 and rs33980500 alleles ($P = .35$ and $P = .31$, respectively) and genotypes ($P > .05$ for all genotypes) do not increase the susceptibility to myocardial infarction. The analysis based on dominant, recessive, and additive models showed no increase in the

TABLE 3 The distribution of studied SNPs genotypes and allelic frequencies in the MI patients and the controls

Genotypes and alleles	MI patients N (%)	Controls N (%)	P value	OR (95%CI)	Relative risk
TRAF3IP2 rs33980500 Genotypes					
CC	155 (79.9)	163 (81.1)	.49	CC: 0.927 (0.563-1.524)	0.962
CT	34 (17.5)	36 (17.9)		CT: 0.974 (0.581-1.633)	0.987
TT	5 (2.6)	2 (1.0)		TT: 2.633 (0.505-13.732)	1.466
Alleles					
C	344 (88.6)	362 (90.00)	.56	0.864 (0.549-1.359)	0.930
T	44 (11.4)	40 (10.00)			
TRAF3IP2 rs13210247 Genotypes					
AA	173 (86)	169 (84.9)	.95	AA: 1.097 (0.628-1.914)	1.048
AG	26 (13)	28 (14.1)		AG: 0.907 (0.511-1.611)	0.952
GG	2 (1)	2 (1)		GG: 0.990 (0.138-7.097)	0.995
Alleles					
A	372 (92.5)	366 (92)	.79	1.084 (0.645-1.829)	1.042
G	30 (7.5)	32 (8)			

susceptibility to myocardial infarction by the mutant genotypes (data not shown).

The analysis of TRAF3IP2 (rs33980500) and TRAF3IP2 (rs13210247) haplotypes was performed by LD2SNPing v2.0 and statistical EPI-INFO 7 softwares. There was no significant difference between healthy individuals and patients with MI in respect to TRAF3IP2 (rs33980500) and TRAF3IP2 (rs13210247) haplotypes (Table 4, $P = .05$).

3.2 | Associations of TRAF3IP2 (rs33980500) and TRAF3IP2 (rs13210247) genes variants with clinicopathologic characteristics of patients

T allele and TT genotype in TRAF3IP2 (rs33980500) polymorphism were associated with female gender, diastolic dysfunction, mechanical complications of heart, left main artery stenosis, anterolateral hypokinesis, and left ventricular ejection fraction; however, CT genotype was associated with positive family history of the diseases. Binary regression analysis confirmed the association of T allele with gender ($P = .043$, OR = 2.24), family history of cardiovascular disease ($P = .024$, OR = 2.30), diastolic dysfunction ($P = .01$, OR = 3.76), mechanical complications of the heart ($P = .01$, OR = 2.7), and left main artery stenosis ($P < .0001$, OR = 31.033) (Table 5).

Also binary regression analysis confirmed the association of the G allele of TRAF3IP2 rs13210247 with the mechanical complications of the heart ($P = .01$, OR = 3.29), but in spite of the primary analysis, correlation with left main artery stenosis ($P = .06$, OR = 2.826) and diastolic dysfunction did not reach the significant level ($P = .06$, OR = 4.27) (Table 6).

3.3 | Comparison of CXCL1 plasma levels between patients and controls

In this study, plasma levels of CXCL1 in 126 patients and 50 controls were measured. Due to the lack of normal distribution in data, approved by

TABLE 4 The TRAF3IP2 (rs13210247, rs33980500) haplotype distribution in the patients with myocardial infarction and control

Group	Haplotype TRAF3IP2 (rs13210247, rs33980500) Number (%)			
	CA	TA	CG	TG
Patients	169 (87.1)	11 (5.4)	3 (1.5)	11 (5.9)
Control	173 (87.2)	10 (4.8)	6 (3)	10 (5)
P value	.95	.77	.33	.77

Kolmogorov-Smirnov and Shapiro-Wilk statistical tests, we used the non-parametric Mann-Whitney test for comparison between groups. Statistical analysis showed a significant difference in the plasma levels of CXCL1 chemokine between MI patients and healthy controls ($P = .0006$, Figure 3).

3.4 | Associations between TRAF3IP2 (rs33980500) and (rs13210247) variants and CXCL1 chemokine levels

We investigated the association between plasma levels of CXCL1 chemokine with studied polymorphisms in patients and controls. Due to the low number of patients in each cluster of genotypes and high variance in the plasma levels of CXCL1, we used Kruskal-Wallis test for data analysis. We found a significant correlation between plasma contents of CXCL1 and TT genotype of TRAF3IP2 (rs33980500) polymorphism in patients ($P = .04$, Figure 4A).

We also studied the association between plasma levels of CXCL1 and TRAF3IP2 (rs13210247) polymorphism in the healthy controls and MI patients. There again we observed non-significantly higher plasma levels of CXCL1 chemokine and the two patients with GG genotype ($P = .056$, Figure 4B). However, the low number of patients in this group decreased the power of this result.

TABLE 5 Associations between clinicopathologic manifestations and TRAF3IP2 (rs33980500) in patients with MI

Clinical manifestations	TRAF3IP2 (rs33980500)			OR (95% CI)			P value Chi ²	P value Regression	TRAF3IP2 (rs33980500)			OR (95%CI)	P value Chi ²	P value Regression
Gender (194)	CC	CT	TT	TT: 1 (Reference)			.1	.12	C	T		2.235 (1.026-4.868)	.04	.04
Male (169)	139 (82.2)	26 (15.4)	4 (2.4)	CC VS TT: 0.46 (0.048-4.375)					304 (89.9)	34 (10.1)				
Female (25)	16 (64)	8 (32)	1 (4)	CT VS TT: 1.231 (0.12-12.652)					40 (80)	10 (20)				
Family history (157)	CC	CT	TT	TT: 1 (Reference)			.01	.02	C	T		2.302 (1.118-4.740)	.02	.02
Negative (110)	95 (86.4)	12 (10.9)	3 (2.7)	CC VS TT: 1.011 (0.101-10.063)					202 (91.8)	18 (8.2)				
Positive (47)	32 (68.1)	14 (29.8)	1 (2.1)	CT VS TT: 3.5 (0.320-38.282)					78 (83)	16 (17)				
Diastolic function (135)	CC	CT	TT	TT: 1 (Reference)			.05	.11	C	T		3.763 (1.272-11.136)	.01	.017
Normal (46)	42 (91.3)	4 (8.7)	0 (0)	CC VS TT: 0.224 (0.011-4.437)					88 (95.7)	4 (4.3)				
Diastolic dysfunction(89): [grade I (74) + grade II (14) + grade III (1)]	66 (74.1)	20 (22.5)	3 (3.4)	CT VS TT: 0.651 (0.28-14.945)					152 (85.4)	26 (14.6)				
Mechanical complications (144)	CC	CT	TT	TT: 1 (Reference)			.03	.05	C	T		2.7 (1.255-5.807)	.01	.01
None (87)	76 (87.4)	10 (11.5)	1 (1.1)	CC VS TT: 0.263 (0.023-2.992)					162 (93.1)	12 (6.9)				
Ventricular septal rupture (VSR) (6) + ischemic mitral regurgitation (IMR) (51)	40 (70.2)	15 (26.3)	2 (3.5)	CT VS TT: 0.750 (0.06-9.418)					95 (83.3)	19 (16.7)				
Anterolateral hypokinesia (130)	CC	CT	TT	TT: 1 (Reference)			.05	.07	C	T		3.857 (0.938-15.865)	.05	.06
Negative (125)	102 (81.6)	21 (16.8)	2 (1.6)	CC VS TT: 0.122 (0.005-3.258)					225 (90)	25 (10)				
Positive (5)	2 (40)	3 (60)	0 (0)	CT VS TT: 0.814 (0.032-20.777)					7 (70)	3 (30)				
Left main coronary artery (162)	CC	CT	TT	TT: 1 (Reference)			.01	.05	C	T		31.033 (6.165-156.214)	.001	.001
Normal (148)	120 (81.1)	26 (17.6)	2 (1.3)	CC VS TT: 0.075 (0.009-0.597)					266 (89.9)	30 (10.1)				
With Stenosis (14)	9 (64.3)	3 (21.4)	2 (14.3)	CT VS TT: 0.115(0.012- 1.146)					2 (75)	7 (25)				
Diastolic dysfunction (135)	CC	CT	TT	TT: 1 (Reference)			.05	.15	C	T		3.763 (1.272-11.136)	.01	.02
No (46)	42 (91.3)	4 (8.7)	0 (0)	CC VS TT: 0.224 (0.11-4.437)					88 (95.7)	4 (4.3)				
Yes (89)	66 (74.1)	20 (22.5)	3 (3.4)	CT VS TT: 0.651 (0.028-14.945)					152 (85.4)	26 (14.6)				

(Continues)

TABLE 5 (Continued)

Clinical manifestations	TRAF3IP2 (rs33980500)			OR (95% CI)		P value Chi ²	P value Regression	TRAF3IP2 (rs33980500)		OR (95%CI)		P value Chi ²	P value Regression
	CC	CT	TT	TT: 1 (Reference)				C	T	1.874 (0.768-4.570)		.16	.17
Systolic function (122)							.34	178 (89.9)	20 (10.1)				
Normal (99)	80 (80.8)	18 (18.2)	1 (1)	CC VS TT: 0.200 (0.01-3.366)				38 (82.6)	8 (14.4)				
Systolic dysfunction (23): mild (13)+ moderate (5) + severe (2) + very severe (3)	16 (69.6)	6 (26.1)	1 (4.3)	CT VS TT: 0.333 (0.018-6.191)									
Ejection fraction (144)							.05	84 (84)	16 (16)	0.488 (0.233-1.024)		.07	.06
<35 (50)	37 (74)	10 (20)	3 (6)	CC VS TT: 14.653 (0.738-290.990)				172 (91.5)	16 (8.5)				
>35 (94)	78 (83)	16 (17)	0 (0)	CT VS TT: 11.000 (0.514-235.200)									

TABLE 6 Associations between clinicopathologic manifestations and TRAF3IP2 (rs13210247) in patients with MI

Clinical manifestations (N)	TRAF3IP2 (rs13210247)			P value Chi ²		OR(95%CI)		TRAF3IP2 (rs13210247)		P value Chi ²		OR(95%CI)	
	AA	AG	GG					A	G				
Mechanical complications (150)						GG: 1 (Reference) AA VS GG: 0.175 (0.007-4.391)				.01	.01	3.291 (1.272-8.516)	
None (93)	86 (92.5)	7 (7.5)	0 (0)			GT VS GG: 0.511 (0.18-14.282)		179 (96.2)	7 (3.8)				
Ventricular septal rupture (VSR)(6) + ischemic mitral regurgitation (IMR) (51)	45 (78.9)	11 (19.3)	1 (1.8)					101 (88.6)	13 (11.4)				
Diastolic dysfunction (141)						GG: 1 (Reference) AA VS GG: 0.582 (0.023-14.595) GT VS GG: 1.933 (0.060-62.171)		A	G	.04	.06	4.279 (0.963-19.017)	
No (47)	45 (95.7)	2 (4.3)	0 (0)					92 (97.9)	2 (2.1)				
Yes (94)	79 (84)	14 (14.9)	1 (1.1)					172 (91.5)	16 (8.5)				
Left main coronary artery (168)						GG: 1 (Reference) AA VS GG: 0.075 (0.004-1.297) GT VS GG: 0.150 (0.007-3.092)		A	G	.05	.055	2.826 (0.979-8.156)	
Normal (154)	133 (86.4)	20 (13)	1 (0.6)					286 (92.8)	22 (7.2)				
With stenosis (14)	10 (71.4)	3 (21.4)	1 (7.2)					23 (82.1)	5 (17.9)				

4 | DISCUSSION

In our study, the highest correlation was observed between the risk allele of TRAF3IP2 rs33980500 with left main coronary artery stenosis. Few studies have investigated the inflammatory processes in left main coronary artery stenosis among which a recent one showed a positive correlation between serum Chemerin, as an inflammatory marker, and the degree of stenosis.¹⁸

This mutant/risk allele is reported to be associated with increased inflammation.¹⁹ Inflammation reduces nitric oxide, cyclic guanosine, and activity of protein kinase G which lead to increase in myocardial

hypertrophy and increased resting due to hypo phosphorylation of the cytoskeletal protein, titin, that can lead to a decrease in cardiac output.²⁰ Moreover, high serum levels of CRP along with leukocyte count are reported to predict poor outcome in patients with unprotected left main coronary artery stenosis.^{21,22}

We also found significantly increased mutant/risk alleles of TRAF3IP2 (rs33980500, rs13210247) in MI patients with diastolic dysfunction compared with other patients. Myocardial infarction leads to loss of contractility and impairment of heart ventricular dilation resulting in reduction of systolic and diastolic function. Physiological phase diastolic function including active relaxation and passive filling are both affected by myocardial ischemia and myocardial infarction. Active relaxation after a heart attack is postponed, while changes in left ventricular stiffness depend on the rate of myocardial infarction and remodeling after myocardial infarction. Interstitial edema and fibrosis increase the stiffness of the ventricle wall. MI patients with diastolic dysfunction have poorer prognosis compared with MI patient without diastolic dysfunction.²³⁻²⁵ Therefore, the increased frequencies of TRAF3IP2 inflammatory variants in patients may be considered as a correlate of poor prognosis. The increase in the same mutant/risk alleles of TRAF3IP2 in patients with left main coronary artery stenosis is in line with this suggestion. Many studies show the role of inflammation in atherosclerotic plaque formation, atherosclerosis progression, and narrowing of the arteries.^{26,27}

Interestingly, we observed an increase in the frequencies of mutant/risk alleles of TRAF3IP2 (rs33980500, rs13210247) in patients with ventricular septal rupture (VSR). Mechanical complications of myocardial infarction include ventricular septal rupture, free wall rupture, and ischemic mitral regurgitation. Mechanical complications

FIGURE 3 Comparison of CXCL1 chemokine plasma levels between MI patients and healthy controls

FIGURE 4 The functional relevance of the studied TRAF3IP2 SNPs on CXCL1 chemokine production. (A) The plasma levels of CXCL1 (pg/mL) compared between patients and controls based on the TRAF3IP2 rs33980500 genotype. (B) The plasma levels of CXCL1 (pg/mL) compared between patients and controls based on the TRAF3IP2 rs13210247 genotype

after a heart attack occur due to the lack of proper blood flow which leads to myocardial necrosis, neutrophil infiltration, activation of MMPs, and degradation of collagen by serine proteases which lead to slip myocytes, thin walls, increased wall stress, ventricular dilatation, and eventually ventricular septal rupture. The incidence of these three mechanical effects is very low, but is associated with very poor prognosis.²⁸ Previous studies have shown that western diet increases the expression of TRAF3IP2 in mice²⁹ and male transgenic mice with cardiomyocyte-specific TRAF3IP2 overexpression develop spontaneous myocardial hypertrophy, fibrosis, and dysfunction where a gender-dependent effect of TRAF3IP2 is suggested.³⁰ In addition, dipeptidyl peptidase-4 inhibition results in simultaneous reduction of TRAF3IP2, proinflammatory cytokine, and growth factor expression, as well as collagen induction in primary cardiac fibroblasts.²⁹ This phenomenon which reverses diastolic dysfunction through targeting TRAF3IP2 expression and its downstream inflammatory signaling has cardioprotective effects.²⁹

We observed an increase in the mutant/risk allele of TRAF3IP2 (rs33980500) in MI patients with anterolateral hypokinesia. Hypokinesia is defined as less movement in parts of heart walls within each heartbeat. An inadequate blood supply leads to wall motion abnormalities, a condition such as myocardial infarction. Hypokinesia can reduce ejection fraction, cardiac output, and even cause organ failure. Patients with wall motion abnormality have lower cardiac output and higher CRP than individuals without the disorder.³¹ In our study, there was a trend of increase in T allele and TT genotype in patients with lower EFs (Table 5). This observation is noteworthy because rs33980500 results in the substitution of an asparagine for an aspartic acid at position 10 of ACT1, which due to alternative splicing of its mRNA two ACT1 isoforms with different functionality are produced in fibroblasts but not in T cells.³² Therefore, fibroblasts remain responsive to IL-17A and its signaling. Once again, these results underline the significance of TRAF3IP2 gene variants and its protein regulation in different diseases.

Our finding showed an increase in the mutant/risk allele of TRAF3IP2 (rs33980500) polymorphism in female patients. It is known that the clinical signs, treatment, diagnosis, and prognosis of cardiovascular disease in men and women are different.^{33,34} Previous studies have shown more deaths among women than men after MI.³⁵ Our results once again highlight the gender differences in genetics of myocardial infarction.

In this study, also we compared the plasma levels of CXCL1 chemokine between patients with MI and healthy subjects, and investigated the correlation between different genotypes of TRAF3IP2 polymorphisms and the plasma levels of CXCL1 in MI patients and healthy controls. CXCL1 is one of the target genes of IL-17A signaling pathway, levels of which may be affected by functional polymorphisms in TRAF3IP2 adaptor in this pathway. Elevated level of CXCL1 in patients with myocardial infarction is already reported.^{7,36,37} However, the interesting finding was the significant correlation between TT genotype of TRAF3IP2 (rs33980500) and plasma levels of CXCL1 in MI patients. TT genotype reinforces the IL-17A signaling pathway in a direction, which leads to the stability

of CXCL1 mRNA and by this mechanism may have led to increased levels of CXCL1 in the blood.

Our study had limitations in terms of cost and logistics for expanding sample size (which affected the power of study, especially for rs13210247 alleles) as well as testing lipid profile and blood sugar, blood pressure, and BMI of control individuals at the time of sampling.

5 | CONCLUSION

Our study showed no significant difference in the genotype distributions and allelic prevalence between cases and controls, however, we observed associations between mutant/risk alleles of studied polymorphisms of TRAF3IP2 and correlates of poor prognosis of MI. TRAF3IP2 is the coding gene of Act1 adaptor molecule in the IL-17A signaling pathway which is increasingly known to be a main player in the pathogenic inflammation of various diseases. Our results re-emphasize the role of inflammatory genetic factors in the coronary artery diseases and may provide information to identify molecules that can be targeted in therapeutic approaches.

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