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Investigating the link between MCP-1 A-2518G, RANTES G-403A, CX3CR1 V249I and MTHFR C677T gene polymorphisms and the risk of acute myocardial infarction among Egyptians

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ABSTRACT

Background: Acute myocardial infarction (AMI) is one of the leading causes of death among Egyptians. Monocyte chemoattractant protein-1 (MCP-1), regulation on activation normal T cell expressed and secreted (RANTES) and fractalkine (FKN) are chemokines that act as components of inflammatory response while methylenetetrahydrofolate reductase (MTHFR) is important enzyme in folate metabolism essential for homocysteine metabolism. Hyperhomocysteinemia has been linked to AMI. MCP-1 A-2518G, RANTES G-403A, CX3CR1 V249I and MTHFR C677T are important polymorphisms identified in MCP-1, RANTES, CX3CR1 and MTHFR genes respectively. There are conflicting data in the literature about their association with AMI. Therefore, the aim of the current study was to investigate the contribution of these gene variants to risk of AMI among Egyptians.
Subjects and methods: The study comprised 200 subjects; 100 AMI patients and 100 age-matched healthy controls. The MCP-1, RANTES, CX3CR1 and MTHFR genotypes were determined by restriction fragment length polymorphism (PCR-RFLP).
Results: Genotypes distributions for RANTES, fractalkine and MTHFR genes were significantly different between AMI patients and controls ($p = 0.0221$, 0.0498 and 0.0083) while those results in MCP-1 were not significantly different. A significant risk for AMI with concurrent presence of RANTES (AG/AA), fractalkine (VV) and MTHFR (CT/TT) genotypes was observed.
Conclusions: 1 - Each of MTHFR 677T, RANTES-403A and CX3CR1 249V alleles is considered an independent risk factor for AMI. 2 - Concurrent presence of high risk genotypes of RANTES (AG/AA), fractalkine (VV) and MTHFR (CT/TT) increases risk of AMI more than their individual risks. 3 - MCP-1 polymorphism is not associated with AMI among Egyptians.

1. Introduction

AMI is a significant cause of mortality and morbidity in the world. The AMI is a consequence of a cascade of thrombotic events following atherosclerotic plaque rupture causes occlusion of the coronary artery, interrupting blood supply and oxygenation to myocardium (Boateng and Sanborn, 2013).

These events are mediated by the interaction between leucocytes and endothelium. In response to chemoattractant signals (chemokines), monocytes and T-cells adhere to inflamed endothelium where they recognise oxidised low-density lipoproteins (ox-LDL) and acquire an activated phenotype in the vessel wall (Rahman et al., 2015).

Monocyte chemoattractant protein-1 (MCP-1 or CCL2 in the newest nomenclature) is a chemokine secreted by a variety of cells as a response to several proinflammatory stimuli (Marsillach et al., 2005; Kiyici et al., 2006). MCP-1 triggers activation, chemotaxis and transendothelial migration of monocytes or macrophages to inflammatory lesions by interacting with the membrane receptor CCR2 in monocytes (O'Hayre et al., 2008).

While RANTES (regulation on activation normal T cell expressed and secreted); also known as CCL5; is another chemokine which belongs to the CC chemokine family (Appay and Rowland-Jones, 2001). RANTES is expressed in macrophages, endothelial cells, lymphocytes, vascular smooth muscle cells and atherosclerotic plaques suggesting its contribution in the development of atherosclerosis (Tavakkoly-Bazzaz et al., 2011).

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Fractalkine (FKN) is a unique proinflammatory member of the CX3C chemokine family that exists in both soluble and membrane-bound forms. It is expressed by vascular endothelial cells of the brain, heart and peripheral blood vessels. CX3CR1; the specific receptor of FKN; is expressed on the cell membrane of monocytes, NK cells and T cells. The interaction between FKN and CX3CR1 results in inflammation (Bazan et al., 1997).

In coronary artery disease (CAD) platelets aggregation and clot formation were also linked to elevated homocysteine levels (Deeparani et al., 2009). Elevated plasma homocysteine levels may arise from genetic factors autosomal recessive severe deficiency of methylenetetrahydrofolate reductase (MTHFR) (Goyette et al., 1995) and nutritional factors as deficiency of folate, vitamin B_6 or vitamin B_{12} (Kang et al., 1991). MTHFR is an important enzyme for the folate metabolism. Specifically, it converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate in a multistep process, in which MTHFR converts the amino acid homocysteine to another amino acid, methionine which can be used by the body as a building block for proteins and other important compounds (Goyette et al., 1995).

It has been shown that many patients with history of myocardial infarction do not have any conventional risk factors, suggesting the contribution of an uncharacterized genetic component 3a. Previous studies from our lab demonstrated the link between AMI in Egyptians and several variants in genes relevant to cardiovascular homeostasis (Gad et al., 2012; Hashad et al., 2014; Abdel Rahman et al., 2015).

In the current study 4 single nucleotide polymorphisms (SNPs), MCP-1 A-2518G, RANTES G-403A, CX3CR1 V249I and MTHFR C677T, were investigated for their association with the incidence of AMI in Egyptians.

MCP-1 A-2518G is a SNP in the regulatory region of the MCP-1 promoter while RANTES G-403A is a SNP that has been identified in the promoter of RANTES gene. Both MCP-1 and RANTES genes are localized on chromosome 17. MCP-1 A-2518G is suggested to increase the level of MCP-1 expression and circulating levels in response to inflammatory stimuli (Rovin et al., 1999; McDermott et al., 2005) while RANTES G-403A might have a functional effect increasing the RANTES expression (Liu et al., 1999; Nickel et al., 2000).

V249I SNP is one of 2 common polymorphisms identified in the CX3CR1 gene. Some studies reported the association of the CX3CR1 I249 allele in homo- and heterozygote conditions with a marked reduction in risk of acute coronary events (Fong et al., 1998; Moatti et al., 2001; Singh et al., 2012; Pucci et al., 2013).

MTHFR C677T gene polymorphism was identified in the MTHFR gene and the homozygosity for this SNP was associated with decreased specific enzyme activity, increased thermolability, and elevated homocysteine levels (Frosst et al., 1995), mainly in subjects with low levels of plasma folate (Jacques et al., 1996). Several studies have tried to explore the association between MTHFR C677T gene polymorphism and the incidence of CAD but the results were inconsistent.

To our knowledge no association studies were performed to investigate the association of any of these SNPs and the incidence of CAD in the Egyptian population.

2. Materials and methods

2.1. Study population

Random unrelated 100 healthy controls were recruited for the study from the volunteers attending the blood bank at 57357 Hospital in Cairo, Egypt. Out of the controls, 40 were females, aged between 41 and 55 years, and 60 were males, 42 to 55 years of age. On the other hand, random unrelated 100 AMI patients, divided into 45 females (age range 45 and 55 years) and 55 males (age range 44 and 55 years) were recruited from the intensive care unit of the National Heart Institute, Imbaba, Giza.

Patients were included if they had a diagnosis of an acute single or multi-vessel CAD verified by clinical presentation, ECG changes, and/or cardiac markers elevation. Written informed consent was obtained from each participant in the study that abided by the Helsinki declaration. Information on personal and family medical history and health-relevant behaviours, including exercise and diet was obtained by a routine questionnaire filled in by blood donors at the time of venesection. Exclusion criteria for both patients and controls included any concomitant acute or chronic severe diseases such as renal failure, hepatic insufficiency, cardiovascular disease, other than MI, and diabetes mellitus.

2.2. Specimen collection

Fasting blood samples (4 ml) were collected in EDTA-coated vacuum tubes stored at 4 °C for DNA extraction.

2.3. Purification of DNA from human blood by spin protocol

DNA purification was done using QIAamp DNA Blood Mini Kits (Qiagen, Germany). DNA eluted in buffer AE was ready for direct addition to PCR reaction. The purified DNA was free of protein, nucleases, and other contaminants or inhibitors (Greenspoon et al., 1998; Fahle and Fischer, 2000).

2.4. Determination of the MCP-1 A-2518G polymorphism

The determination of the MCP-1 A-2518G polymorphism was carried out by PCR-RFLP. The PCR was performed under the following cycling condition: 95 °C for 5 min, followed by 95 °C for 20 s, 65 °C for 20 s, and 72 °C for 45 s for 15 cycles, followed by another 20 cycles of 95 °C for 20 s, 55 °C for 20 s, and 72 °C for 45 s, with a final extension step at 72 °C for 7 min.

Forward 5-CCGAGATGTTCCCAGCACAG-3primer and a reverse 5-CTGCTTTGCTTGTGCCTCTT-3primer were used for the PCR reactions.

The resulting PCR product size was 930 bp. The nucleotide substitution corresponding to position −2518 (A/G) creates a *Pvu*II (New England Biolabs) restriction site. Digestion resulted in 708- and 222-bp fragments for the G allele (Fig. 1) (Ramasawmy et al., 2006).

2.5. Determination of the RANTES G-403Apolymorphism

Determination of the RANTES G-403Apolymorphism was carried out by PCR followed by restriction digestion (PCR-RFLP)·The PCR reaction was performed in a final volume of 25 μl containing 1 μl genomic DNA, 3.5 pmoles of each primer, 2 mM of $MgCl_2$, 1× buffer, 0.2 mM

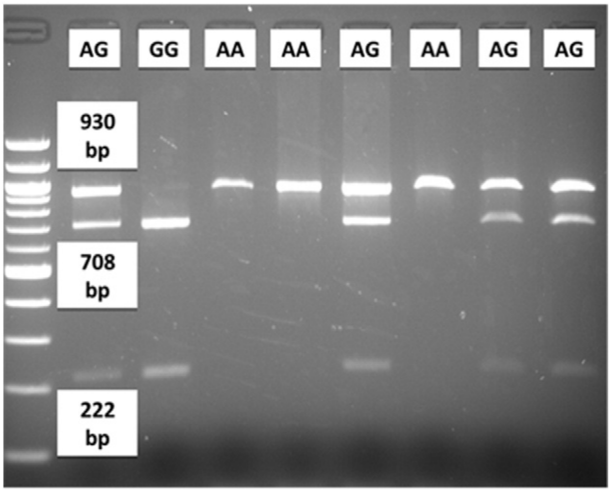

Fig. 1. Representative of 2% agarose gel electrophoresis by *PvuII* RFLP of MCP-1 gene.

dNTPs, and 1 U *Taq* polymerase. PCR cycles were as follows: initial denaturation at 95 °C for 2 min followed by 35 cycles each of denaturation at 95 °C for 40 s, annealing at 50 °C for 40 s and extension at 72 °C for 40 s. A final extension step was carried out at 72 °C for 5 min.

The primers used were forward: 5′-GCC TCA ATT TAC AGT GTG-3′ and reverse 5′-TGC TTA TTC ATT ACA GAT GTT-3′. The resulting PCR product size was 135 bp.

Enzyme digestion (RFLP) of PCR product was carried out using 4 U of *MaeIII* enzyme for the reactions were incubated at 55 °C overnight. Digestion with *MaeIII* yields 112 and 23 bp fragments when G allele is present, while the mutant A allele was detected as a band of 135 bp. The digested products were subjected to electrophoresis on 3% agarose gel stained with ethidium bromide (Fig. 2) (Nahas et al., 2012).

2.6. Determination of the CX3CR1 V249I polymorphism

The determination of the CX3CR1 V249I polymorphism was also carried out by PCR-RFLP. The PCR reaction was performed in a final volume of 25 μl containing 5 μl genomic DNA, 10.5 pmoles of each primer, 4 mM of $MgCl_2$, 1 × buffer, 0.4 mM dNTPs, and 1 U *Taq* polymerase. PCR cycles were as follows: initial denaturation at 94 °C for 3 min followed by 35 cycles each of denaturation at 94 °C for 30 s, annealing at 52 °C for 40 s and extension at 72 °C for 55 s. A final extension step was carried out at 72 °C for 10 min.

The primers used were forward: 5′-CCGAGGTCCTTCAGGAAATCT-3′ and reverse 5′-TCAGCATCAGGTTCAGGAACTC-3″. The resulting PCR product size was 588 bp.

Enzyme digestion (RFLP) of PCR product was carried out using 4 U of *AclI* enzyme for the reactions were incubated at 37 °C for 3 h. Digestion with *AclI* yields 383 and 205 bp fragments when V allele is present, while the mutant I allele was detected as a band of 588 bp. The digested products were subjected to electrophoresis on 2% agarose gel stained with ethidium bromide (Fig. 3) (Kimouli et al., 2009).

2.7. Determination of the MTHFR C677T polymorphism

Determination of the MTHFR C677T polymorphism was carried out by PCR followed by restriction digestion (PCR-RFLP)·The PCR program consisted of initial denaturation at 94 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 63 °C for 1 min and extension at72°C for 1 min. A final extension at72°C for 5 min was done once.

The primers used were forward: 5′-TGAAGGAGAAGGTGTCTG CGGGA-3′ and reverse 5′-AGGACGGTGCGTGAGAGTG-3′.

The resulting PCR product size was 198 bp. The nucleotide substitution corresponding to position 677 (C/T) creates a *HinfI* (New England Biolabs) restriction site. Digestion resulted in 175- and 23-bp fragments for the T allele (Fig. 4) (Deeparani et al., 2009).

2.8. Statistical analysis

Statistical analysis was performed using the statistical programs SPSS and GraphPad Prism. Data are represented as mean ± SEM. To compare differences between groups odds ratio (Fisher's exact test), nonparametric student *t*-test (Mann-Whitney) and nonparametric one-way ANOVA (Kruskal-Wallis) were used.

In all statistical tests two-tailed *p* value ≤0.05 was considered statistically significant.

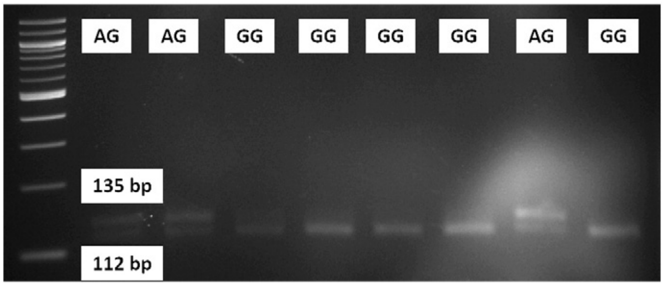

Fig. 2. Representative of 3% agarose gel electrophoresis by *MaeIII* RFLP of RANTES gene.

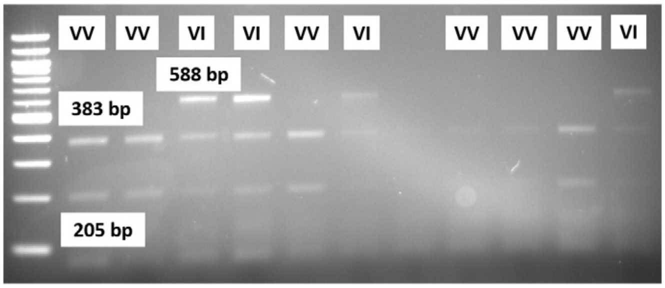

Fig. 3. Representative of 2% agarose gel electrophoresis by *AclI* RFLP of fractalkine gene.

3. Results

3.1. Lipid profile

Lipid profile of AMI patients and controls is summarized in Table 1. The mean serum total cholesterol concentration in AMI patients is not significantly different from controls ($p = 0.5796$), whereas mean serum triglycerides showed a significant increase reaching to 1.42 fold of the control group ($p < 0.0001$).

3.2. Genotyping of the MCP-1A-2518G polymorphism

No significant difference in the MCP-1 genotype distribution was observed between AMI patients (AA 56%, AG 36% and GG 8%) and controls (AA 45%, AG 48%, and GG 7%) (Mann-Whitney test, $p = 0.1857$). The corresponding allele frequencies for AMI patients were A 74% and G 26% and for controls A 69% and G 31% (Mann-Whitney test, $p = 0.2689$) (Table 2). No deviation from HWE was observed in the SNP's genotype distribution (HWE *p*-value = 0.87).

3.3. Genotyping of RANTES G-403Apolymorphism

The genotype distribution for RANTES gene was significantly different between AMI patients (GG 55%, AG 43% and AA 2%) and controls (GG 72%, AG 24%, AA 4%) (Mann-Whitney test, $p = 0.0221$). The corresponding allele frequencies for patients were G 76.5% and A 23.5% and for controls C 84% and T 16% with no significant difference. The odds ratio between homozygous GG genotype and AA+ AG genotypes was 2.104 ($p = 0.0185$) (Table 2). No deviation from Hardy-Weinberg

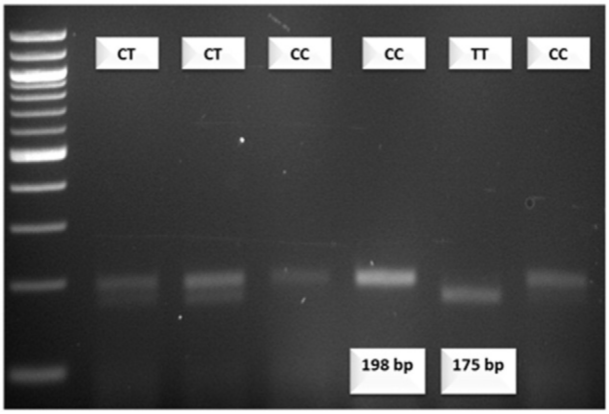

Fig. 4. Representative of 3% agarose gel electrophoresis by *Hinf I* RFLP of MTHFR gene.

Table 1
Lipid profile of the study groups.

	AMI	Controls
Serum total cholesterol (mg/dl)	207.4 ± 5.7	219.7 ± 9.1
Serum total triglycerides (mg/dl)	157.2 ± 7.0[***]	110.8 ± 7.6

[***] Significant difference from the control group at $p < 0.0001$.

equilibrium (HWE) was observed in the SNP's genotype distribution (HWE p-value = 0.42).

3.4. Genotyping of the CX3CR1 V249I polymorphism

The CX3CR1 genotype distribution was significantly different between AMI patients (VV 70%, VI 29% and II 1%) and controls (VV 57%, VI 40% and II 3%) (Mann-Whitney test, $p = 0.0498$). The corresponding allele frequencies for AMI patients were V 84.5% and I 15.5% and for controls V 77% and I 23% (Table 2) however no significant difference was observed in alleles frequencies between AMI patients and controls. No deviation from HWE was observed in the SNP's genotype distribution (HWE p-value = 0.12).

3.5. Genotyping of MTHFR C677T polymorphism

The genotype distribution for MTHFR gene was significantly different between AMI patients (CC 55%, CT 34% and TT 11%) and controls (CC 73%, CT 25%, TT 2%) (Mann-Whitney test, $p = 0.0083$). The corresponding allele frequencies for patients were C 72% and T 28% and for controls C 85.5% and T 14.5% (Mann-Whitney test, $p = 0.001$)

Table 2
Alleles and genetic models for MCP-1 A-2518G, RANTES G-403A, CX3CR1 V249I and MTHFR C677TSNPs in AMI patients and controls

SNP	Allele/genotype(s)	AMI n (%)	Controls n (%)	OR (95% confidence interval)	p-Value
MCP-1	Genotypes distribution				
A-2518G	AA	56 (56%)	45 (45%)		0.1857
	AG	36 (36%)	48 (48%)		
	GG	8 (8%)	7 (7%)		
	Alleles frequency				
	A	148 (74%)	138 (69%)	1.3	0.3188
	G	52 (26%)	62 (31%)	(0.8–2.0)	
	Dominant model				
	AA	56 (56%)	45 (45%)	1.6	0.1571
	AG/GG	44 (44%)	55 (55%)	(0.9–2.7)	
	Recessive model				
	GG	8 (8%)	7 (7%)	1.2	1.000
	AA/AG	92 (92%)	93 (93%)	(0.4–3.3)	
RANTES	Genotypes distribution				
G-403A	GG	55 (55%)	72 (72%)		0.0221[*]
	AG	43 (43%)	24 (24%)		
	AA	2 (2%)	4 (4%)		
	Alleles frequency				
	A	47 (23.5%)	32 (16%)	1.6	0.0783
	G	153 (76.5%)	168 (84%)	(1.0–2.7)	
	Dominant model				
	AG/AA	45 (45%)	28 (28%)	2.1	0.0185[*]
	GG	55 (55%)	72 (72%)	(1.2–3.8)	
	Recessive model				
	AA	2 (2%)	4 (4%)	0.5	0.6827
	GG/AG	98 (98%)	96 (96%)	(0.1–2.7)	
CX3CR1	Genotypes distribution				
V249I	VV	70 (70%)	57 (57%)		0.0498[*]
	VI	29 (29%)	40 (40%)		
	II	1 (1%)	3 (3%)		
	Alleles frequency				
	I	31 (15.5%)	46 (23%)	0.6	0.0754
	V	169 (84.5%)	154 (77%)	(0.4–1.0)	
	Dominant model				
	VV	70 (70%)	57 (57%)	1.8	0.0776
	VI/II	30 (30%)	43 (43%)	(1.0–3.2)	
	Recessive model				
	II	1 (1%)	3 (3%)	0.3	0.6212
	VV/VI	99 (99%)	97 (97%)	(0.1–2.7)	
MTHFR C677T	Genotypes distribution				
	CC	55 (55%)	73 (73%)		0.0083[**]
	CT	34 (34%)	25 (25%)		
	TT	11 (11%)	2 (2%)		
	Alleles frequency				
	T	144 (72%)	171 (85.5%)	2.3	0.0014[**]
	C	56 (28%)	29 (14.5%)	(1.4–3.8)	
	Dominant model				
	CT/TT	55 (55%)	73 (73%)	2.2	0.012[*]
	CC	45 (45%)	27 (27%)	(1.2–4.0)	
	Recessive model				
	TT	11 (11%)	2 (2%)	6.1	0.0184[*]
	CC/CT	89 (89%)	98 (98%)	(1.3–28.0)	

OR: odds ratio.

[*] means p value <0.05.

[**] means p value <0.01.

(Table 2). The odds ratio between homozygous TT genotype and CC + CT genotypes was 6.1 ($p = 0.0184$) while the odds ratio between the T allele and the C allele was 2.3 ($p = 0.0014$). No deviation from Hardy-Weinberg equilibrium (HWE) was observed in the SNP's genotype distribution (HWE p-value = 0.09).

3.6. Linkage disequilibrium between MCP-1 and RANTES SNPs

Linkage disequilibrium was examined between the investigated MCP-1 and RANTES SNPs as both of them are located on the same chromosome but the results showed no linkage disequilibrium ($D' = -0.037$ and $r^2 = 0.0001$).

3.7. The combined effect of MCP-1, RANTES, CX3CR1 and MTHFR SNPs on the risk for myocardial infarction

To evaluate the interaction between the different genotypes of these SNPs in the incidence of AMI, the combined effect of MCP-1, RANTES, CX3CR1 and MTHFR SNPs was examined by calculating adjusted ORs for all of the combinations of two, three and four of the investigated genotypes (Tables 3–5). The reference group consisted of individuals with the putatively most advantageous combinations of the genotypes

Table 3
Combined effects of two genotypes (MCP-1, RANTES, CX3CR1 and MTHFR) and risk of AMI.

MCP-1	RANTES	AMI	Controls	Odds ratio (confidence interval)	*p*-Value
AA	GG	30	31		
AA	AA/AG	26	14	1.9 (0.8–4.4)	0.1527
AG/GG	GG	25	40	0.6 (0.3–1.3)	0.2813
AG/GG	AA/AG	19	15	1.3 (0.6–3.0)	0.6688

MCP-1	CX3CR1	AMI	Controls	Odds ratio (confidence interval)	*p*-Value
AA	VI/II	17	17		
AA	VV	39	28	1.4 (0.6–3.2)	0.5261
AG/GG	VV	30	29	1.0 (0.4–2.4)	1
AG/GG	VI/II	14	26	0.5 (0.2–1.4)	0.24

MCP-1	MTHFR	AMI	Controls	Odds ratio (confidence interval)	*p*-Value
AA	CC	28	30		
AA	CT/TT	28	15	2.0 (0.9–4.5)	0.1082
AG/GG	CC	27	43	0.7 (0.3–1.4)	0.2872
AG/GG	CT/TT	17	12	1.5 (0.6–3.7)	0.4952

RANTES	CX3CR1	AMI	Controls	Odds ratio (confidence interval)	*p*-Value
GG	VI/II	17	35		
GG	VV	38	36	2.2 (1.0–4.5)	0.0455*
AG/AA	VV	31	21	3.0 (1.4–6.8)	0.0102*
AG/AA	VI/II	14	8	3.6 (1.3–10.2)	0.0201*

RANTES	MTHFR	AMI	Controls	Odds ratio (confidence interval)	*p*-Value
GG	CC	28	55		
GG	CT/TT	27	16	3.3 (1.5–7.1)	0.0024**
AG/AA	CC	27	18	2.9 (1.4–6.2)	0.0052**
AG/AA	CT/TT	18	11	3.2 (1.3–7.7)	0.0092**

CX3CR1	MTHFR	AMI	Controls	Odds ratio (confidence interval)	*p*-Value
VI/II	CC	19	32		
VV	CT/TT	33	16	3.5 (1.5–7.9)	0.003**
VV	CC	36	41	1.5 (0.7–3.0)	0.3623
VI/II	CT/TT	12	11	1.8 (0.7–5.0)	0.3094

* means p value <0.05.
** means p value <0.01.

Table 4
Combined effects of three genotypes (MCP-1, RANTES, CX3CR1 and MTHFR) and risk of AMI.

MCP-1	RANTES	CX3CR1	AMI	Controls	Odds ratio (confidence interval)	*p*-Value
AA	GG	VI/II	9	14		
AA	GG	VV	21	17	1.9 (0.7–5.5)	0.293
AG/GG	GG	VV	17	19	1.4 (0.5–4.0)	0.5988
AG/GG	GG	VI/II	8	21	0.6 (0.2–1.9)	0.5525
AA	AA/AG	VV	18	11	2.5 (0.8–7.8)	0.162
AA	AA/AG	VI/II	8	3	4.1 (0.9–19.9)	0.1411
AG/GG	AA/AG	VV	13	10	2.0 (0.6–6.6)	0.3762
AG/GG	AA/AG	VI/II	6	5	1.9 (0.4–8.0)	0.4748

MCP-1	RANTES	MTHFR	AMI	Controls	Odds ratio (confidence interval)	*p*-Value
AA	GG	CC	15	22		
AA	GG	CT/TT	15	9	2.4 (0.9–7.0)	0.1196
AG/GG	GG	CC	13	33	0.6 (0.2–1.5)	0.2541
AG/GG	GG	CT/TT	12	7	2.5 (0.8–7.9)	0.1588
AA	AA/AG	CC	13	8	2.4 (0.8–7.2)	0.1722
AA	AA/AG	CT/TT	13	6	3.2 (1.0–10.2)	0.0891
AG/GG	AA/AG	CC	14	10	2.0 (0.7–5.8)	0.1992
AG/GG	AA/AG	CT/TT	5	5	1.5 (0.4–6.0)	0.723

MCP-1	CX3CR1	MTHFR	AMI	Controls	Odds ratio (confidence interval)	*p*-Value
AA	VI/II	CC	10	12		
AA	VV	CT/TT	21	10	2.5 (0.8–7.8)	0.1576
AG/GG	VV	CC	18	23	0.9 (0.3–2.7)	1
AG/GG	VV	CT/TT	12	6	2.4 (0.7–8.7)	0.2157
AA	VV	CC	18	18	1.2 (0.4–3.5)	0.7913
AA	VI/II	CT/TT	7	5	1.7 (0.4–7.0)	0.7207
AG/GG	VI/II	CC	9	20	0.5 (0.2–1.7)	0.3835
AG/GG	VI/II	CT/TT	5	6	1.0 (0.2–4.3)	1

RANTES	CX3CR1	MTHFR	AMI	Controls	Odds ratio (confidence interval)	*p*-Value
GG	VI/II	CC	11	26		
GG	VV	CT/TT	21	7	7.1 (2.3–21.5)	0.0004***
AA/AG	VV	CC	19	12	3.7 (1.4–10.3)	0.014*
AA/AG	VV	CT/TT	12	9	3.2 (1.0–9.6)	0.0533
GG	VV	CC	17	29	1.4 (0.5–3.5)	0.6409
GG	VI/II	CT/TT	6	9	1.6 (0.5–5.5)	0.5249
AA/AG	VI/II	CC	8	6	3.2 (0.9–11.3)	0.1056
AA/AG	VI/II	CT/TT	6	2	7.1 (1.2–40.1)	0.0388*

* means p value <0.05.
** means p value <0.01.
*** means p value <0.001.

Table 5
Combined effects of four genotypes (MCP-1, RANTES, CX3CR1 and MTHFR) and risk of AMI.

MCP-1	RANTES	CX3CR1	MTHFR	AMI	Controls	Odds ratio (confidence interval)	*p*-Value
AA	GG	VI/II	CC	6	10		
AG/GG	GG	VV	CC	8	17	0.8 (0.2–2.9)	0.7468
AA	AA/AG	VV	CC	9	6	2.5 (0.6–10.6)	0.289
AG/GG	AA/AG	VV	CC	10	6	2.8 (0.7–11.6)	0.289
AA	GG	VV	CC	9	12	1.3 (0.3–4.7)	1
AG/GG	GG	VI/II	CC	5	16	0.5 (0.1–2.2)	0.4753
AA	AA/AG	VI/II	CC	4	2	3.3 (0.5–24.0)	0.3476
AG/GG	AA/AG	VI/II	CC	4	4	1.7 (0.3–9.3)	0.6734
AA	GG	VV	CT/TT	12	5	4.0 (0.9–17.1)	0.0844
AG/GG	GG	VV	CT/TT	9	2	7.5 (1.2–47.0)	0.0473*
AA	AA/AG	VV	CT/TT	9	5	3.0 (0.7–13.3)	0.2723
AG/GG	AA/AG	VV	CT/TT	3	4	1.3 (0.2–7.6)	1
AA	GG	VI/II	CT/TT	3	4	1.3 (0.2–7.6)	1
AG/GG	GG	VI/II	CT/TT	3	5	1.0 (0.2–5.8)	1
AA	AA/AG	VI/II	CT/TT	4	1	6.7 (0.6–74.6)	0.1486
AG/GG	AA/AG	VI/II	CT/TT	2	1	3.3 (0.2–45.1)	0.5459

* means p value <0.05.

(low-risk genotypes) which are the wild types AA for MCP-1, GG for RANTES and CC for MTHFR. The only exception was Fractalkine where VI/II genotypes (heterozygotes + homozygous mutants) were used as references due to the reported protective effect of the SNP.

When combinations of two at-risk genotypes were examined (Table 3), the concurrent presence of RANTES (AG/AA) and Fractalkine (VV) high-risk genotypes and RANTES (AG/AA) and MTHFR (CT/TT) high-risk genotypes posed a higher risk of AMI (OR, 3.0; 95% CI, 1.4 to 6.8) and (OR, 3.2; 95% CI, 1.3 to 7.7) respectively. Also the concurrent presence of the high risk genotypes of Fractalkine (VV) and MTHFR (CT + TT) a >3-fold risk to AMI (OR, 3.5; 95% CI, 1.5 to 7.9). In contrast, no statistically significant effects between MCP-1 genotypes and any of the high risk genotypes in the other SNPs.

The same results were confirmed by studying the combined effects of 3 SNPs together (Table 4).

In comparing RANTES, Fractalkine and MTHFR the concurrent presence of the high risk genotypes in any 2 SNPs showed a significant increase in the risk of AMI, however the concurrent presence of the high risk genotypes of the 3 showed a >3-folds risk for the incidence but the results didn't reach the significance level ($p = 0.0533$). Also when MCP-1 was included as one of the SNPs in the studying the combined effects of three SNPs, no significant effects were observed.

The evaluation of the combined effects of all the four genotypes (Table 5)revealed that the concurrent presence of MCP-1 AG/GG, RANTES GG, fractalkine VV and MTHFR CT/TT showed >7 folds risk to AMI and (OR, 7.5; 95% CI, 1.2 to 47.0) confirming the interaction between fractalkine and MTHFR.

4. Discussion

Induction of chemokines is an important component of the inflammatory response associated with several diseases. Analysis of patient biopsies and animal models by *in situ* hybridization or immunostaining has shown the expression of MCP-1 mRNA and protein in ischemic myocardium, which correlates with the accumulation of leukocytes (Kumar et al., 1997).

The current study results showed no significant difference in the genotype distribution or the allele frequencies of MCP-1 A-2518G polymorphism between AMI patients and control subjects suggesting that the MCP-1 polymorphism doesn't contribute to the incidence of AMI among Egyptians. Results are consistent with other studies performed on German (Simeoni et al., 2004), Czech (Cermakova et al., 2005), Turkish (Cam et al., 2008) and Chinese Han (Zhang et al., 2009), and in contrast with those in Hungarian (Szalai et al., 2001) and Tunisian (Jemaa et al., 2008) populations.

Another study in the Egyptian population that involved a relatively small number of subjects (30 AMI patients and 25 controls) showed a significant association between the MCP-1-2518G polymorphism and acute MI in the Egyptian population (El Mahgoub et al., 2011).

The conflicting results of the studies might be explained by the ethnic differences and sample size. The variations in other risk factors for CAD may also play a role. Jemaa et al. proposed that the variations in risk factors for CAD including age, smoking, hypertension, diabetes mellitus and dyslipidemia might play the main role in the MCP-1 A-2518G association with MI in the Tunisian population (Jemaa et al., 2008).

The G-403A polymorphism in the RANTES gene has a key role in the expression of RANTES, which has been detected in a range of cells in atherosclerotic plaque (Simeoni et al., 2004).

The current study showed a significant difference in the genotype distribution between the AMI patients and the control subjects and a 2 folds higher risk of AMI in RANTES heterozygous and homozygous mutant genotypes (AG/AA) compared to the wild genotype GG.

These findings are consistent with the LURIC (Ludwigshafen risk and cardiovascular health) study results in Caucasians born in Germany (Simeoni et al., 2004) and other studies done on Han Chinese (Morawietz et al., 2001) and Spanish (Donato et al., 2009) populations. Another study reported that patients carrying the RANTES −403A allele had a significantly higher all cause mortality risk mainly due to cardiac events (Boger et al., 2005).

However our results are contradicting with other studies and meta analysis that reported no association (Donato et al., 2005; Liu et al., 2012; Cui et al., 2013). The discrepancy in the results might be explained by the involvement of RANTES as a chemokine and an inflammatory mediator in several inflammatory conditions and immune diseases that might interfere with the results as RANTES polymorphism was linked also with hepatitis B virus infection (Al-Qahtani et al., 2012) and papillary thyroid cancer (Kwon et al., 2013).

According to the current study results despite of the modest significant difference between AMI and controls in fractalkine receptor genotypes distribution yet the individuals carrying homozygous VV genotypes didn't show a significantly higher risk to AMI compared to those carrying the CX3CR1 249Iallele (VI/II). These results are consistent with another study done in Greece (Apostolakis et al., 2007). Another study suggested lack of association between CX3CR1 V249I polymorphism and peripheral artery diseases (Gugl et al., 2003). However these findings are contradicting with several studies that suggest that the CX3CR1 249I SNP possesses a protective effect against atherosclerosis and CAD (McDermott et al., 2001; Moatti et al., 2001; Apostolakis et al., 2009).

The current study results revealed that the MTHFR 677T allele is considered a risk factor for the development of AMI in Egyptians with the homozygous TT genotype having the highest risk.

These results are in harmony with several previous studies (Mager et al., 2002; Almawi et al., 2004) which stated that the homozygous TT genotype is significantly higher among CAD patients. A plausible mechanism of the involvement of MTHFR C677T polymorphism in AMI is the association of the MTHFR 677T with hyperhomocysteinemia (Frosst et al., 1995; Brilakis et al., 2003; Liang et al., 2014) as the MTHFR 677T allele leads to a thermolabile variant of the MTHFR enzyme with reduced activity (Kang et al., 1988; Kang et al., 1993). There is an increasing evidence that homocysteine may affect the coagulation system and the resistance of the endothelium to thrombosis (Malinow and Stampfer, 1994) and that it might interfere with the nitric oxide vasodilator and antithrombotic actions (Stamler and Slivka, 1996). Homocysteine also induces apoptotic DNA damage mediated by the increased intracellular generation of H2O2 (Huang et al., 2001). Plasma homocysteine level was reported to be a strong predictor of mortality in CAD (Nygard et al., 1997; Anderson et al., 2000).

Despite of the accumulating evidence of the impact on plasma homocysteine levels, there are conflicting reports regarding the risk of vascular disease in those with the MTHFR 677T allele. Some studies demonstrated lack of association between the increased frequency of TT homozygotes and the incidence of cardiovascular disease (Brugada and Marian, 1997; van Bockxmeer et al., 1997; Wilcken et al., 1997). The US physicians prospective study which demonstrated raised plasma homocysteine as a risk factor for the development of CAD (Stampfer et al., 1992), failed to show the thermolabile variant of MTHFR as an independent risk factor for MI (Ma et al., 1996).

In the current study the combined effects of the studied SNPs was also investigated as a hypothesis was made that the genetic effect of combinations of functionally relevant SNPs may additively or synergistically contribute to increased AMI risk. The results of the combined effect of the current study showed that the RANTES-403A, CX3CR1 249V and MTHFR 677T had synergistic effects where an individual carrying a combination of any 2 alleles of them will have a higher risk to AMI than individuals carrying one high risk allele. Although the current study results didn't reveal the CX3CR1 249V as an individual risk factor yet the combined effect results showed that this allele increases the risk of AMI in individuals carrying another high risk allele of either RANTES or MTHFR. The lack of significantly higher risk for AMI in individuals carrying the 3 high risk alleles might be due to the limited number of study subjects who were carrying this combination of alleles.

In conclusion the current study results showed that each of RANTES-403AG/AA, CX3CR1 V249I and MTHFR 677 CT/TT genotypes might be considered as individual risk factors for AMI. In addition, having the genotypes RANTES-GG, CX3CR1 VV and MTHFR CT/TT remarkably increased the risk to AMI. MCP-1A-2518G didn't associate individually or in combination with the other studied SNPs in the risk of AMI.

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