



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Review

Genetic diversity of CX3CR1 gene and coronary artery disease: New insights through a meta-analysis

Stavros Apostolakis^a, Virginia Amanatidou^a, Emmanouil G. Papadakis^b, Demetrios A. Spandidos^{a,*}^a Laboratory of Clinical Virology, Faculty of Medicine, University of Crete, Voutes, 71110 Heraklion, Greece^b Onassis Cardiac Surgery Centre, Athens, Greece

ARTICLE INFO

Article history:

Received 11 February 2009

Received in revised form 6 March 2009

Accepted 24 March 2009

Available online 5 April 2009

Keywords:

CX3CR1

Polymorphism

Coronary artery disease

ABSTRACT

A significant portion of current medical research is devoted to the pursuit of genetic markers that can be used to identify disease or predict susceptibility to disease. In such a quest many investigators hypothesized that genetic variations that alter signalling pathways involved in atherosclerosis affect susceptibility to coronary artery disease (CAD). Fractalkine (FKN) is a small cytokine involved in monocyte chemotaxis and activation. Two single nucleotide polymorphisms, V249I and T280M, have been identified in the receptor coding sequence of FKN. The polymorphisms alter ligand–receptor affinity and are believed to influence an individual's susceptibility to atherosclerosis. Several investigators have tested the latter hypothesis with inconsistent results. In order to clarify the effect of the two polymorphisms on susceptibility to CAD we performed a meta-analysis, using pooled data retrieved from seven case–control studies. In total, 2000 CAD patients and 2841 subjects without evidence of cardiovascular disease were included in the meta-analysis. The 280M allele was associated with a reduced risk for CAD in the heterozygous state. Consequently, this effect was attributed to the only 280M-containing haplotype: I₂₄₉M₂₈₀. The latter haplotype was found to be significantly more frequent in the control population's gene pool. Although we do not believe that the retrieved odds ratios render the T280M polymorphism a candidate genetic marker for clinical applications, we do believe that the above genotype–phenotype interaction is indicative of the strong associations between FKN-induced pathways and CAD.

© 2009 Elsevier Ireland Ltd. All rights reserved.

Contents

1. Introduction	9
1.1. Reasons for investigating CX3CR1	9
1.2. CX3CR1 and atherosclerosis	9
1.3. Functional consequences of CX3CR1 genotypes	9
2. Materials and methods	10
2.1. Literature search	10
2.2. Statistical analysis	10
3. Results	12
3.1. Hardy–Weinberg equilibrium and linkage disequilibrium analysis	12
3.2. Genotype–phenotype association	13
3.3. Haplotype–phenotype association	13
4. Discussion	13
4.1. T280M polymorphism and risk for coronary artery disease	13
4.2. Limitations of meta-analyses	13
4.3. Limitations of candidate gene association studies	13
4.4. A quest for genetic markers	14
5. Conclusion	14
References	14

* Corresponding author. Tel.: +30 2810 394631; fax: +30 2810 394759.

E-mail addresses: spandidos@spandidos.gr, stavrosapos@hotmail.com (D.A. Spandidos).

1. Introduction

1.1. Reasons for investigating CX3CR1

Chemokines are small chemotactic cytokines responsible for cell activation and trafficking in response to mainly inflammatory stimuli. They consist of an expanding family of approximately 50 ligands and 20 receptors which are classified into four sub-groups based on the number and structural arrangement of conserved cysteine residues within their amino-terminal polypeptide sequence (C, CC, CXC and CX3C) [1,2]. CXC chemokines have a single amino acid separating the two amino-terminal cysteine residues of the protein, while CC chemokines have no amino acid separating the amino-terminal cysteines [1–4]. Fractalkine (FKN) is the single member of the CX3C sub-family, with three amino acids separating the two amino-terminal cysteine residues [5]. Moreover, the recently discovered lymphotactin (XCL1) and single C motif chemokine 1 (SCM1- or XCL2) are currently the only known members of the C sub-family, and lack two of the four conserved cysteines in the mature protein [5]. Chemokines induce cell activation by binding to specific seven-transmembrane G-protein coupled cell-surface receptors on target cells. Six human CXC chemokine receptors, 10 human CC chemokine receptors, and a single receptor for each of the CX3C and C chemokine sub-families have been identified to date [5]. An unusual characteristic of most chemokine receptors is their high affinity for multiple ligands. Chemokines interacting with their receptors on the cell surface lead to the generation of an intracellular signal via the G-protein complex, resulting in cell chemotaxis towards the source of the chemokine [5].

Fractalkine or CXCL1, the only currently known member of the CX3C chemokine sub-family, exists as membrane-bound and in soluble form [6,7]. Membrane-bound FKN consists of an extracellular domain of 76 amino acids connected to an extended mucin-like stalk, followed by transmembrane and intracellular domains of 34 amino acids [6,7]. Soluble FKN is released, presumably by proteolysis, at the membrane-proximal region by a TNF- α -converting enzyme. Soluble FKN is an efficient chemoattractant for monocytes and natural killer cells. The FKN receptor (CX3CR1) is a seven-transmembrane domain G-protein coupled receptor, and FKN binds to it with high affinity, activating intracellular signalling and directly mediating monocyte adhesion [6,7].

Three unique structural and functional characteristics of the FKN/CX3CR1 pathway make it an attractive field of investigation in atherogenesis. Firstly, FKN displays properties of both chemokines and adhesion molecules [5–7]. It also possesses chemoattractant activities for the most important inflammatory cells implicated in atherogenesis [5–7]. Finally, unlike other chemokines, FKN interacts exclusively with a single receptor. Thus, cells expressing CX3CR1 are undoubtedly targets of FKN-induced signalling [5,7].

1.2. CX3CR1 and atherosclerosis

Several *in vitro* and *in vivo* studies in the recent literature have assessed the potential role of CX3CR1/FKN-induced pathways in atherosclerosis. Notably, Wong et al. reported the high expression of CX3CR1 in the foam cells and coronary artery smooth muscle cells of human atherosclerotic arteries, but not in normal human arterial tissue [8]. Moreover, Lesnik et al. and Combadière et al. independently reported that the deletion of CX3CR1 in apoE $^{-/-}$ mice decreases susceptibility to atherosclerosis, suggesting that, in mice, CX3CR1/FKN interaction promotes atherogenesis [9,10]. Most recently, Landsman et al. reported that the enforced survival of monocytes and plaque-resident phagocytes, including foam cells, restored atherogenesis in CX3CR1-deficient mice. The authors concluded that FKN–CX3CR1 interactions confer an essential survival

signal whose absence leads to the increased death of monocytes and/or foam cells [11].

1.3. Functional consequences of CX3CR1 genotypes

Since they were first described by Faure et al. two single nucleotide polymorphisms (SNPs) in the CX3CR1 coding sequence (V249I and T280M) have been noted in the scientific literature [12]. The SNPs had two appealing characteristics: they were frequently found among Caucasians; thus, a phenotype–genotype interaction attributed to these SNPs would have a clinical significance. Secondly, they resulted in a dysfunctional receptor [12–15].

Both the V249I and T280M SNPs of the CX3CR1 gene have been associated with a decreased risk of atherosclerosis. In retrospective studies, these polymorphisms have consistently been associated with reduced prevalence of atherosclerotic disease endpoints, including cerebrovascular disease, coronary endothelial dysfunction, the incidence of acute coronary syndromes and the angiographic severity of coronary artery stenosis [13,14,16–24]. Many investigators have explored the mechanisms underlying this phenotype–genotype interaction. First, Faure et al. compared FKN binding to primary peripheral blood mononuclear cells (PBMCs) from HIV-infected patients homozygous for CX3CR1-V₂₄₉T₂₈₀ versus CX3CR1-I₂₄₉M₂₈₀. Scatchard regression analysis of the binding data revealed a significantly reduced binding affinity of ¹²⁵I-labeled FKN to cells from CX3CR1-I₂₄₉M₂₈₀ homozygotes versus wild-type CX3CR1-V₂₄₉T₂₈₀ cells, providing an indication that the specific genotype affects the integrity of the receptor. Furthermore, the total number of binding sites per cell was dramatically reduced in CX3CR1-I₂₄₉M₂₈₀ homozygotes versus wild-type controls. The authors concluded that the T280M polymorphism directly affects ligand recognition [12]. Similarly, Moatti et al., using ¹²⁵I-FKN, reported that FKN binding-site density was approximately 40% lower on PBMCs from individuals carrying the VI genotype (either VITT or VITM) than on PBMCs from individuals bearing the reference genotype VVTT [13]. McDermott et al. further assessed the putative mechanism underlying the atheroprotective effect of the 280M allele and concluded that FKN-dependent cell-to-cell adhesion under conditions of physiologic shear is severely reduced in cells expressing CX3CR1-M280. This was associated with a marked reduction in the kinetics of FKN binding as well as with the reduced FKN-induced chemotaxis of primary leukocytes from donors homozygous for CX3CR1-M280 [14]. In contrast to these findings, in a study by Daoudi et al., PBMCs from individuals carrying the CX3CR1-I₂₄₉M₂₈₀ haplotype were reported to adhere more potently to membrane-bound FKN than PBMCs from homozygous CX3CR1-V₂₄₉T₂₈₀ donors. Similar excess adhesion was observed in CX3CR1-I₂₄₉M₂₈₀-transfected human embryonic kidney (HEK) cell lines tested using two different methods: the parallel plate laminar flow chamber and the dual pipette aspiration technique [15].

Nevertheless, the exact functional effect of the CX3CR1-M280 allele is unclear. Regardless of whether it functions as a gain- or loss-of-function variant, there is convincing data that it functions abnormally. Moreover, the 280M allele has consistently been reported to interfere with individual susceptibility to atherosclerosis.

However, despite the initial impressively unvarying results that revealed an atheroprotective effect of the 280M variant, more recent sufficiently powered studies using similar case–control designs have resulted in different phenotype–genotype interactions. Furthermore, even those studies that demonstrated an atheroprotective effect of the 280M allele were not sufficiently powered to identify whether this effect was attributed to the homozygous or heterozygous state. Finally, several investigators did not report estimated haplotype frequencies and a haplotype–genotype interaction may have been overlooked.

Table 1
Characteristics of the included studies.

Study	Location	Race	Cases (n)	Controls (n)	Age (cases)	Age (controls)	Type of cases ^a	Type of controls ^a
Moatti et al. [13]	Paris, France	Caucasian	151	249	47.3 ± 7.1	48.1 ± 7.1	ACS	Randomly selected
McDermott et al. [24]	Maryland, USA	Caucasian	197	142	62 ± 1	50 ± 1	Positive CA (≥50%) CVD	Negative CA (<50%) No signs of CVD
McDermott et al. [14]	Massachusetts, USA	Caucasian	204	1655	65.9 ± 8.4	58.8 ± 9.8		
Niessner et al. [18,23]	Vienna, Austria	Caucasian	720	432	63.4 ± 11.9	57.1 ± 15.8	Positive CA (≥60%)	Negative CA (<60%) or negative Stress ECG
Rios et al. [19]	Porto Alegre, Brazil	Caucasian	219	149	61.2 ± 10.7	58.5 ± 11.4	Positive CA (≥75%)	Negative CA (<75%)
Apostolakis et al. [5]	Crete, Greece	Caucasian	210	165	63.7 ± 0.7	63.2 ± 0.9	Positive CA (≥70%)	Negative CA (<70%)
Nassar et al. [16]	Nova Scotia, Canada	Caucasian	149	149	45.4 ± 4	67 ± 6.4	Positive CA (≥50%) (age <50 years)	No history of CAD
Nassar et al. [16]	Nova Scotia, Canada	Caucasian	150	149	73.7 ± 5.4	67 ± 6.4	Positive CA (≥50%) (age >65 years)	No history of CAD
Total			2000	2941				

ACS: acute coronary syndrome; CA: coronary angiography; CVD: cardiovascular disease; CAD: coronary artery disease.

^a Percentages indicate degree of lumen's diameter reduction.

Consequently, we hypothesized that a meta-analysis provides a better insight into the relations among CX3CR1 genotypes and the risk of CAD.

2. Materials and methods

2.1. Literature search

We identified all population-based case-control studies published before December 2008 on the CX3CR1 T280M and V249I polymorphisms and their association with the risk for CAD. The literature was scanned by a formal search of the MEDLINE electronic database for the terms CX3CR1 or FKN in combination with *polymorphism*, *mutation* or *genetics*. Any references that contained data evaluating CAD risk with polymorphisms in CX3CR1 gene were retrieved, including review articles. Bibliographies of pertinent articles and reviews were searched for additional references. Relevant textbooks and foreign language articles were also reviewed.

Two investigators (SA and DAS) independently applied inclusion criteria for articles. All disagreements were resolved through discussion. The following inclusion criteria were used: (1) the incidence of greater than 100 case subjects, (2) identification of CAD either through coronary angiography or through clinical presentation, (3) cohort or case-control design with controls, (4) sufficient data provided to determine the odds ratio (OR) or relative risk (RR) and confidence intervals (CIs) by comparing CAD patients to non-CAD controls and (5) sufficient data reported to determine haplotype frequencies and perform a linkage disequilibrium analysis.

From each study we abstracted the mean age of participants, geographical location, race of participants, numbers of cases and controls, definition of coronary artery disease, definition of CAD-free controls, frequency of each genotype, genotyping methods and laboratory procedures.

2.2. Statistical analysis

Genotype distributions for each polymorphism were first compared to values predicted by the Hardy-Weinberg equilibrium (HWE) through χ^2 analysis. Linkage disequilibrium was measured using the classic statistic, disequilibrium coefficient. Linkage disequilibrium analysis was performed by *CubeX* analysis software for each study separately and for the whole sample [25].

Fixed-effect pooled odds ratios were calculated. Heterogeneity among studies was assessed using the Breslow-Day χ^2 and the I^2 statistics. I^2 describes the percentage of variation in point estimates that may be attributable to true differences across studies rather than random errors. In the presence of substantial heterogeneity ($I^2 > 60\%$), a DerSimonian-Laird random effects model was used as the pooling method.

Studies that reported a comparison of two case samples, such as different age groups, were included in the meta-analysis as independent studies.

Four out of six studies that genotyped their population for V249I and T280M polymorphisms, did not report estimated haplotype frequencies. Therefore, we performed a haplotype analysis based on the genotype data. In order to avoid discrepancies in estimated haplotype frequencies resulting from different methods of calculation, we re-calculated the haplotype frequencies for the six included studies. Haplotype analysis was performed by *CubeX* analysis software [25].

In all cases, *p*-values less than 0.05 were considered to be statistically significant. The analyses were performed using *SPSS v15* (SPSS Inc., Chicago, IL, USA) and the *Review Manager 5* (RevMan) v5.0. (The Cochrane Collaboration, 2008).

Table 2
Estimated haplotype frequencies and linkage disequilibrium analysis of the included studies.

Study	Polymorphism	Hardy-Weinberg equilibrium		Estimated haplotype frequencies				Estimated haplotype frequencies (controls)				D^a	r^{2a}
		Cases	Controls	V ₂₄₉ T ₂₈₀	V ₂₄₉ M ₂₈₀	I ₂₄₉ T ₂₈₀	I ₂₄₉ M ₂₈₀	V ₂₄₉ T ₂₈₀	V ₂₄₉ M ₂₈₀	I ₂₄₉ T ₂₈₀	I ₂₄₉ M ₂₈₀		
Moatti et al. [13]	V249I T280M	0.18 1.55	0.15 0.1	0.798	0.0	0.0993	0.1026	0.7149	0.0	0.1345	0.1506	1.0	0.4445
McDermott et al. [24]	V249I T280M	0.45 4.4	0.03 0.61	0.7817	0.0	0.0838	0.1345	0.7042	0.0	0.0986	0.1972	1.0	0.5848
McDermott et al. [14]	V249I T280M	1.85 0.01	6.17 0.46	0.7304	0.0	0.1324	0.1373	0.7018	0.0	0.1157	0.1825	1.0	0.5253
Niessner et al. [18,23]	V249I T280M	0.41 0.01	0.21 2.17	0.7332	0.0016	0.1113	0.154	0.7564	0.0	0.0998	0.1439	1.0	0.5217
Rios et al. [19]	V249I T280M	7.75	9.47										
Apostolakis et al. [5]	V249I T280M	0.34 0.06	3.64 1.45	0.7214	0.0	0.15	0.1286	0.7	0.0	0.1212	0.1788	1.0	0.508
Nassar et al. [16] <50	V249I T280M	0.01 1.28	0.08 0.07	0.7067	0.0171	0.144	0.1321	0.6544	0.0	0.1376	0.2081	1.0	0.4974
Nassar et al. [16] >65	V249I T280M	2.11 0.51	0.08 0.07	0.625	0.0203	0.1622	0.1925	0.6544	0.0	0.1376	0.2081	1.0	0.4974
Total	V249I T280M	0.45 0.86	5.68 70.2	0.7295	0.0035	0.1199	0.1471	0.7089	0.0	0.1155	0.1756	1.0	0.5186

^a D^a and r^2 statistics refer to control groups.

Table 3
Genotype distribution in cases and controls.

Study	Cases (n)											Controls (n)															
	VVTT	VVTM	IITT	IIMM	IITM	VITT	VITM	TT	TM	MM	VV	VI	II	VVTT	VVTM	IITT	IIMM	IITM	VITT	VITM	TT	TM	MM	VV	VI	II	
Moatti et al. [13]	97	0	2	3	2	24	23	123	25	3	97	47	7	126	0	2	5	12	51	53	179	65	5	126	104	19	
McDermott et al. [24]	122	0	1	7	3	28	36	151	39	7	122	64	11	70	0	1	7	4	22	38	93	42	7	70	60	12	
McDermott et al. [24]	105	0	3	4	4	44	44	152	48	4	105	88	11	794	0	23	51	52	285	450	1102	502	51	794	735	126	
Niessner et al. [18,23]	390	2	9	17	28	114	160	513	190	17	392	274	54	245	0	1	12	11	73	89	319	100	13	245	163	24	
Rios et al. [19]	-	-	-	-	-	-	-	-	-	-	80	120	19	-	-	-	-	-	-	-	-	-	-	-	56	84	9
Apostolakis et al. [5]	111	0	6	3	9	42	39	159	48	3	111	81	18	86	0	1	3	16	22	37	109	53	3	86	59	20	
Nassar et al. [16] <50	69	4	3	3	7	25	38	97	49	3	73	63	13	63	0	6	7	4	25	44	94	48	7	63	69	17	
Nassar et al. [16] >65	53	5	2	7	6	37	38	92	49	9	58	77	15	-	-	-	-	-	-	-	-	-	-	-	-	-	
Total n	947	11	26	44	59	314	378	1287	448	46	1038	814	148	1384	0	34	85	99	478	711	1896	810	86	1440	1274	227	

Values refer to number of patients.

Table 4
The independent effect of each genotype on susceptibility to coronary artery disease.

Genotype	Cases (n)	Controls (n)	Baseline risk	Odds ratios	95% confidence intervals	p
VVTT	947	1447		1		
VVTM	11	0	VVTT	7.74	1.43–41.8	0.02
IITT	26	40	VVTT	1.15	0.65–2.04	0.64
IIMM	44	92	VVTT	0.73	0.48–1.11	0.14
IITM	59	103	VVTT	0.81	0.56–1.18	0.27
VITT	314	503	VVTT	1.03	0.86–1.24	0.76
VITM	378	755	VVTT	0.83	0.71–0.99	0.03
VV	1038	1440		1		
VI	814	1274	VV	0.91	0.79–1.04	0.16
II	148	227	VV	0.86	0.67–1.1	0.22
VI or II	962	1587	VV	0.9	0.79–1.02	0.1
TT	1287	1990		1		
TM	448	858	TT	0.82	0.7–0.96	0.02
MM	46	93	TT	0.71	0.48–1.06	0.1
MM or TM	494	950	TT	0.83	0.72–0.97	0.02

Values refer to number of patients. Overall fixed-effect odds ratios are presented.

3. Results

An initial search with the terms *CX3CR1* and *polymorphism* retrieved 69 studies published since December 2008, of which 13 were related to atherosclerosis. Six studies were excluded as irrelevant, and as exclusively directed to peripheral arterial or internal carotid internal disease. The cohort of McDermott et al. included patients identified as suffering from cardiovascular disease in general. However, the majority of the included subjects (73%) reported symptoms or signs of CAD. Therefore, their data were included in the meta-analysis [14]. In their study, Nassar et al. reported independent odds ratios for two age groups. Therefore their data were included separately [16].

Seven case-control studies [13,14,16,17,19,23,24] were finally included in the meta-analysis (Table 1). In total, 2000 CAD patients were genotyped for the V249I polymorphism and 1781 CAD

patients for the two variations. In seven cohorts, 2841 controls (without evidence of coronary heart disease) were screened for the V249I polymorphism and 2792 controls for both genetic variations.

3.1. Hardy-Weinberg equilibrium and linkage disequilibrium analysis

Genotype frequencies were in agreement with those predicted by the HWE in cases and controls in the majority of the included sub-populations (Table 2). Significant disassociations were identified in the sub-groups of three studies (Table 2). In the whole sample the estimated genotype frequencies of control subjects deviated significantly from those predicted by the HWE for the V249I and T280M polymorphisms ($\chi^2 = 5.68$ and 70.2 respectively, $df = 1$).

In accordance with the previously reported data, linkage disequilibrium (LD) analysis indicated a strong association between

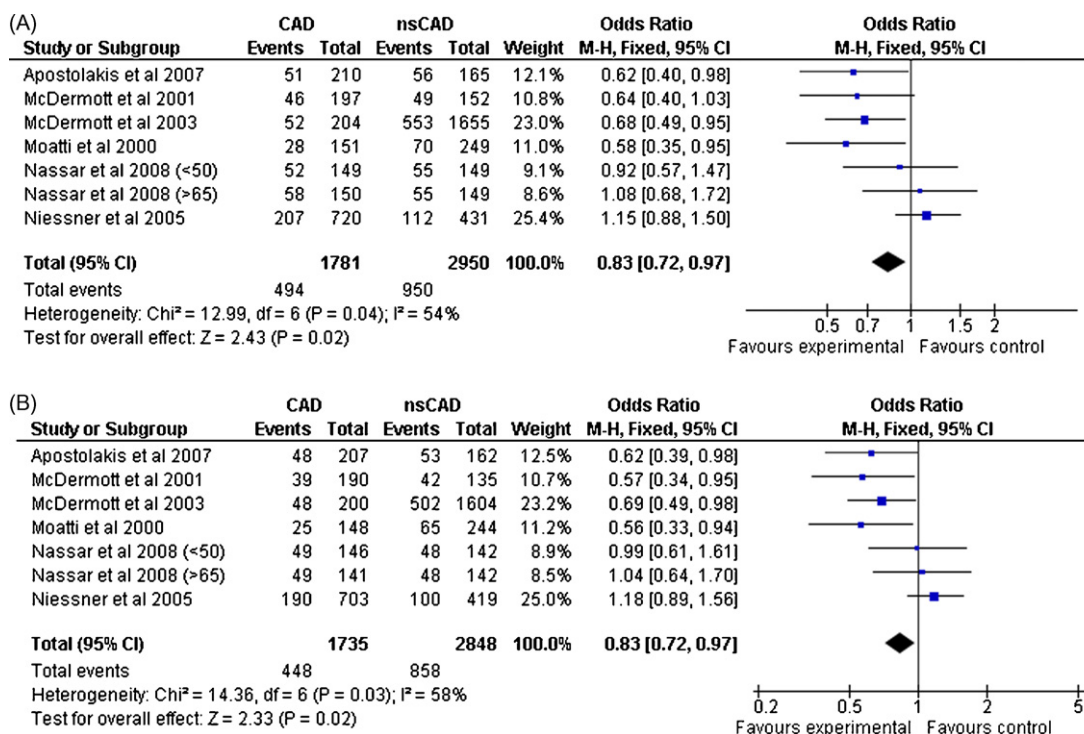


Fig. 1. Odds ratios (ORs) for coronary artery disease (CAD) comparing the TM or MM carriers versus TT carriers (A), and TM carriers versus TT carriers (B). In both cases the TT genotype was considered to be baseline risk. Values refer to the number of patients. Squares indicate the OR in each study. Horizontal lines represent the 95% confidence interval. The pooled ORs are indicated by black diamonds.

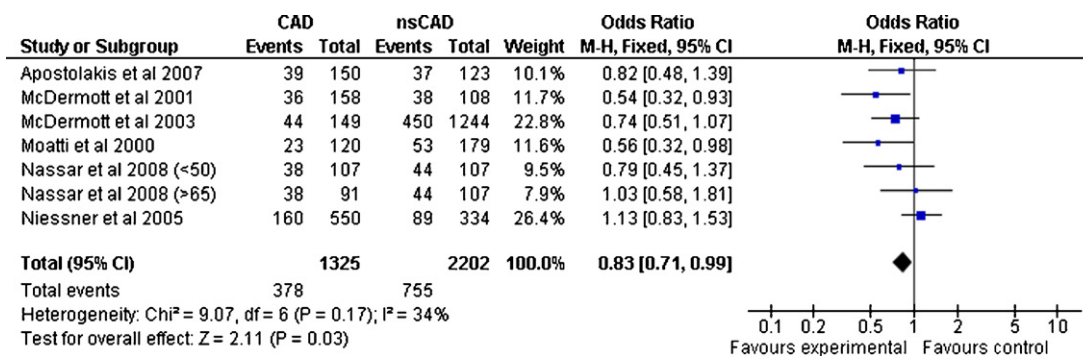


Fig. 2. Odds ratios (ORs) for coronary artery disease (CAD) comparing the VITM versus VVTM carriers. VVTM genotype was considered to be baseline risk. Values refer to the number of patients. Squares indicate the OR in each study. Horizontal lines represent the 95% confidence interval. The pooled ORs are indicated by black diamonds.

the two loci. Four out of eight studies conclusively found complete LD, and reported three haplotypes and six combined genotypes of a potential of four haplotypes and nine genotypes (Table 4). Three studies identified the extremely rare VVTM genotype. Thus, an association analysis of the whole sample (cases and controls) indicated strong (but not complete) LD between the two loci ($D' = 0.99$).

3.2. Genotype–phenotype association

The effect of each genotype was independently assessed (Tables 3 and 4). No association was established between the V249I polymorphism and CAD. The 280M allele (TM or MM) was significantly more common in controls (OR = 0.83; 95% CI: 0.72–0.97; $p = 0.02$) in the homozygous or heterozygous state. When the 280M allele homozygotes and heterozygotes were independently assessed, only the 280TM genotype produced statistically significant results (OR = 0.82; 95% CI = 0.7–0.96; $p = 0.02$) (Fig. 1). In accordance with the latter observation the VITM combined genotype was significantly more common in the control subjects (OR = 0.83; 95% CI: 0.71–0.99; $p = 0.03$) (Fig. 2).

Finally, the rare VVTM genotype was identified only in case subjects and this observation proved statistically significant (OR = 7.74; 95% CI: 1.43–41.8; $p = 0.02$). However, the rarity of the VVTM genotype makes any conclusion rather unsafe.

No other genotype grouping gave statistically significant results.

3.3. Haplotype–phenotype association

Haplotype frequencies of each study and the total sample are reported in Table 2. The strong linkage disequilibrium between the V249I and T280M polymorphisms resulted in three predominant haplotypes and the extremely rare V₂₄₉M₂₈₀. The latter haplotype accounts for less than 0.5% of the entire population's gene pool and was not considered to be of potential clinical significance. We assessed the effect of the I₂₄₉T₂₈₀ and I₂₄₉M₂₈₀ haplotypes on the risk for CAD independently taking the V₂₄₉T₂₈₀ haplotype as a baseline risk. A significant predominance of the I₂₄₉M₂₈₀ haplotype was observed in the control population compared to case subjects (OR = 0.81; 95% CI: 0.71–0.92; $p = 0.001$). No association was observed between the I₂₄₉T₂₈₀ haplotype and susceptibility to CAD (Fig. 3).

4. Discussion

4.1. T280M polymorphism and risk for coronary artery disease

The present meta-analysis confirms that the genetic diversity of the CX3CR1 gene influences an individual's susceptibility to CAD. A protective effect was attributed to the 280M allele occurring in

the heterozygous state. However, the strongest statistical significance was obtained when the haplotype frequencies of the two polymorphisms were evaluated in the studied sub-populations. The I₂₄₉M₂₈₀ haplotype was significantly more frequent in the gene pool of the control subjects. In both a fixed and random effect model the observation retained its statistical significance.

There is strong evidence from *in vitro* studies and studies in animal models suggesting that a dysfunctional CX3CR1 receptor reduces the progression of atherosclerosis probably by inhibiting the FKN-induced inflammatory pathways [8–11,26,27]. The 280M allele has been related to markedly reduced and delayed binding of FKN to the CX3CR1 receptor and decreased FKN-induced chemotaxis [13–15]. These previously reported data strongly justify an atheroprotective effect of the 280M allele and consistently justify the atheroprotective effect of the only 280M-containing haplotype: I₂₄₉M₂₈₀. However, meta-analyses have limitations, which may lead to misleading conclusions.

4.2. Limitations of meta-analyses

Heterogeneity of the included studies is the most important drawback when analysing genotyping data. Variation in clinical definitions, genotyping and statistical methods and most important ethnic and race polymorphy can influence the outcome of a meta-analysis. In the present study, race bias was minimised since all the included studies were conducted in Caucasians. Furthermore, in order to avoid clinical heterogeneity, we included in the meta-analysis, studies that assessed the effect of the two variations in CAD, and excluded other forms of cardiovascular disease (peripheral arterial or occlusive carotid artery disease). Moreover, all investigators used strict clinical or angiographic criteria for CAD. Finally, we excluded studies conducted in over-selected populations, such as CAD patients developing restenosis after percutaneous coronary intervention. Since the V249I and T280M polymorphisms are in strong linkage disequilibrium a genotype–phenotype effect could not have been attributed to a single allele *per se*. Thus, we constructed haplotypes and assessed the effect of each haplotype on susceptibility to CAD independently. Using the above methodology, we clarified that the I₂₄₉M₂₈₀ haplotype exhibits a favourable effect.

4.3. Limitations of candidate gene association studies

The present meta-analysis is based on candidate gene association (CGA) studies. Compared to genome-wide association studies, CGA studies are less expensive, less technically challenging, and can establish more powerful phenotype–genotype interactions if applied in large-scale case–control cohorts. However, using this method, only the genes suspected of affecting a disease are tested. As a result, many genotype–phenotype interactions not attributed

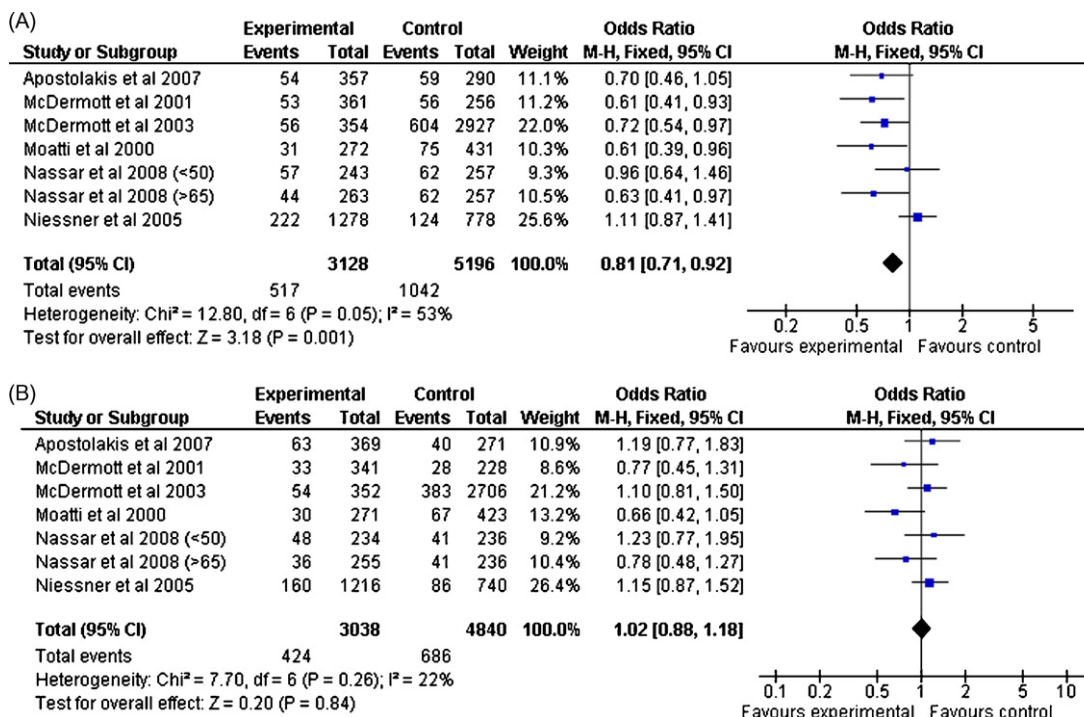


Fig. 3. Odds ratios (ORs) for coronary artery disease (CAD) comparing frequencies of the I²⁴⁹M²⁸⁰ haplotype (A) and the I²⁴⁹T²⁸⁰ in CAD patients and controls (fixed model). The V²⁴⁹T²⁸⁰ haplotype was considered to be baseline risk. Values refer to the number of alleles. Squares indicate the OR in each study. Horizontal lines represent the 95% confidence interval. The pooled ORs are indicated by black diamonds.

to the genes directly associated with atherosclerosis may be overlooked. Moreover, a significant association found in one study must be confirmed by additional studies to exclude false positive results, which are not uncommon in CGA studies. Additionally, the candidate gene approach does not provide information regarding the effect of polymorphisms on the functions of the tested genes. Genome-wide association studies, which provide an unbiased approach, would certainly be more effective in detecting unexpected genotype–phenotype interactions, especially in a complex and multifactorial disease such as atherosclerosis.

There is, however, a large body of data available in the scientific literature derived from very well-designed CGA studies reporting phenotype–genotype interactions. We believe that careful interpretation of this data would confer a better understanding of the mechanisms of the disease and, in some instances, aid in the development of diagnostic or prognostic tools. Linkage analysis, CGA analysis, genome-wide association studies and gene expression analysis have certain advantages and limitations. A combination of all methods would increase the possibility of identifying the specific genotypes underlying the so-called genetic predisposition to atherosclerotic disease.

4.4. A quest for genetic markers

It remains to be determined, however, whether such genetic information is useful in diagnostic and disease management practices. A significant portion of current medical research is devoted to the pursuit of genetic markers that can be used to identify disease or susceptibility to disease. However, all genetic alterations are not potential genetic markers. Ideally, a genetic marker should confer a high level of disease probability, and as such would be a useful diagnostic tool or predictor of prognosis. However, markers whose effects are not as strong may provide important information regarding disease pathophysiology, or help to identify new targets for therapeutic intervention. A marker may have functional consequences, such as altering the expression or function of a gene that

directly leads to disease. Nevertheless, positive association studies rarely prove the causality link between the mutation and the effects on disease.

In the present meta-analysis we do not intend to clarify causality between certain genotypes and susceptibility to disease. Moreover, we do not believe that the retrieved odds ratios from the present meta-analysis render the T280M polymorphism a candidate genetic marker for clinical applications. We believe, however, that the above genotype–phenotype interaction is indicative of the strong associations between FKN-induced pathways and CAD.

5. Conclusion

In conclusion, in a meta-analysis including seven populations studied worldwide, the I₂₄₉M₂₈₀ haplotype of the CX3CR1 gene was found to be significantly more common in control subjects compared to CAD patients. The latter observation confirms the atheroprotective effect of the above haplotype and underlines the role of the FKN-mediated pathway in atherosclerosis. We believe that in multifactorial conditions such as CAD, genotype–phenotype associations have minor clinical consequences in prognostic or diagnostic algorithms. They are, however, indicative of strong associations between biochemical pathways, compromised of a certain genetic variation, and disease.

References

- [1] Luster AD. Chemokines—chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436–45.
- [2] Curnock AP, Logan MK, Ward SG. Chemokine signalling: pivoting around multiple phosphoinositide 3-kinases. *Immunology* 2002;105:125–36.
- [3] Reape TJ. Chemokines and atherosclerosis. *Atherosclerosis* 1999;147:213–25.
- [4] Zernecke A, Shagdarsuren E, Weber C. Chemokines in atherosclerosis: an update. *Arterioscler Thromb Vasc Biol* 2008;28:1897–908.
- [5] Apostolakis S, Papadakis GE, Krambovitis E, Spandidos DA. Chemokines in vascular pathology. *Int J Mol Med* 2006;17:691–701.
- [6] Imai T, Hieshima K, Haskell C, et al. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. *Cell* 1997;91:521–30.

- [7] Bazan JF, Bacon KB, Hardiman G, et al. A new class of membranebound chemokine with a CX3C motif. *Nature* 1997;385:640–4.
- [8] Wong BWC, Wong D, McManus BM. Characterization of fractalkine (CX3CL1) and CX3CR1 in human coronary arteries with native atherosclerosis, diabetes mellitus, and transplant vascular disease. *Cardiovasc Pathol* 2002;11:332–8.
- [9] Lesnik P, Haskell CA, Charo IF. Decreased atherosclerosis in CX3CR1^{-/-} mice reveals a role for fractalkine in atherogenesis. *J Clin Invest* 2003;111:333–40.
- [10] Combadière C, Potteaux S, Gao JL, et al. Decreased atherosclerotic lesion formation in CX3CR1/apolipoprotein E double knockout mice. *Circulation* 2003;107:1009–16.
- [11] Landsman L, Bar-On L, Zerneck A, et al. CX3CR1 is required for monocyte homeostasis and atherogenesis by promoting cell survival. *Blood* 2009;22:963–72.
- [12] Faure S, Meyer L, Costagliola D, et al. Rapid progression to AIDS in HIV+ individuals with a structural variant of the chemokine receptor CX3CR1. *Science* 2000;287:2274–7.
- [13] Moatti D, Faure S, Fumeron F, et al. Polymorphism in the fractalkine receptor CX3CR1 as a genetic risk factor for coronary artery disease. *Blood* 2001;97:1925–8.
- [14] McDermott DH, Fong AM, Yang Q, et al. Chemokine receptor mutant CX3CR1-M280 has impaired adhesive function and correlates with protection from cardiovascular disease in humans. *J Clin Invest* 2003;111:1241–50.
- [15] Daoudi M, Lavergne E, Garin A, et al. Enhanced adhesive capacities of the naturally occurring Ile249–Met280 variant of the chemokine receptor CX3CR1. *J Biol Chem* 2004;279:19649–57.
- [16] Nassar BA, Nanji AA, Ransom TP, et al. Role of the fractalkine receptor CX3CR1 polymorphisms V249I and T280M as risk factors for early-onset coronary artery disease in patients with no classic risk factors. *Scand J Clin Lab Invest* 2008;68:286–91.
- [17] Apostolakis S, Baritaki S, Kochiadakis GE, Igomienidis NE, Panutsopoulos D, Spandidos DA. Effects of polymorphisms in chemokine ligands and receptors on susceptibility to coronary artery disease. *Thromb Res* 2007;119:63–71.
- [18] Niessner A, Marculescu R, Kvakan H, et al. Fractalkine receptor polymorphisms V249I and T280M as genetic risk factors for restenosis. *Thromb Haemost* 2005;94:1251–6.
- [19] Rios DL, Callegari-Jacques SM, Hutz MH. Endothelial nitric oxide synthase and fractalkine chemokine receptor polymorphisms on angiographically assessed coronary atherosclerosis. *Clin Chim Acta* 2005;362:138–46.
- [20] Hattori H, Ito D, Tanahashi N, et al. T280M and V249I polymorphisms of fractalkine receptor CX3CR1 and ischemic cerebrovascular disease. *Neurosci Lett* 2005;374:132–5.
- [21] Ghilardi G, Biondi ML, Turri O, Guagnellini E, Scorza R. Internal carotid artery occlusive disease and polymorphisms of fractalkine receptor CX3CR1: a genetic risk factor. *Stroke* 2004;35:1276–9.
- [22] Gugl A, Renner W, Seinst G, et al. Two polymorphisms in the fractalkine receptor CX3CR1 are not associated with peripheral arterial disease. *Atherosclerosis* 2003;166:339–43.
- [23] Niessner A, Marculescu R, Haschemi A, et al. Opposite effects of CX3CR1 receptor polymorphisms V249I and T280M on the development of acute coronary syndrome: a possible implication of fractalkine in inflammatory activation. *Thromb Haemost* 2005;93:949–54.
- [24] McDermott DH, Halcox JP, Schenke WH, et al. Association between polymorphism in the chemokine receptor CX3CR1 and coronary vascular endothelial dysfunction and atherosclerosis. *Circ Res* 2001;89:401–7.
- [25] Gaunt TR, Rodríguez S, Day IN. Cubic exact solutions for the estimation of pairwise haplotype frequencies: implications for linkage disequilibrium analyses and a web tool 'CubeX'. *BMC Bioinformatics* 2007;8:428.
- [26] Lucas AD, Bursill C, Guzik TJ, Sadowski J, Channon KM, Greaves DR. Smooth muscle cells in human atherosclerotic plaques express the fractalkine receptor CX3CR1 and undergo chemotaxis to the CX3C chemokine fractalkine (CX3CL1). *Circulation* 2003;108:2498–504.
- [27] Apostolakis S, Krambovitis E, Vlata Z, Kochiadakis GE, Baritaki S, Spandidos DA. CX3CR1 receptor is up-regulated in monocytes of coronary artery diseased patients: impact of pre-inflammatory stimuli and rennin-angiotensin system modulators. *Thromb Res* 2007;121:387–95.