Biochemical and Biophysical Research Communications 435 (2013) 319–322

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Association between MIF gene polymorphisms and carotid artery atherosclerosis

Min-Yu Lan, Yung-Yee Chang, Wei-Hsi Chen, Yu-Lung Tseng, Hung-Sheng Lin, Shung-Lon Lai, Jia-Shou Liu *

Department of Neurology, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, 123, Ta-Pi Road, Niaosong, Kaohsiung 833, Taiwan

ARTICLE INFO

Article history:
Received 17 January 2013
Available online 26 March 2013

Keywords:
Macrophage migration inhibitory factor
Atherosclerosis
Polymorphism
Carotid artery
Cerebral infarction
Sonography

ABSTRACT

Atherosclerosis is a chronic inflammatory disorder. Macrophage migration inhibitory factor (MIF) is a potent cytokine that plays an important role in the regulation of immune responses. Polymorphisms including five- to eight-repeat CATT variants ($(CATT)_{5-8}$) and G-173C in the promoter region of the MIF gene are associated with altered levels of MIF gene transcription. The purpose of the study is to investigate the relationship between promoter polymorphisms of the MIF gene and the severity of carotid artery atherosclerosis (CAA). The severity of CAA was assessed in 593 individuals with a history of ischemic stroke by using sonographic examination, and the MIF promoter polymorphisms of these individuals were genotyped. The carriage of $(CATT)_7$ (compared to genotypes composed of $(CATT)_5$, $(CATT)_6$, or both), carriage of C allele (compared to GG), and carriage of the haplotype $(CATT)_7$-C (compared to genotypes composed of $(CATT)_5$-G, $(CATT)_6$-G, or both) were significantly associated with an increase in the severity of CAA. We conclude that polymorphisms in the MIF gene promoter are associated with CAA severity in ischemic stroke patients. These genetic variants may serve as markers for individual susceptibility to CAA.

1. Introduction

Atherosclerosis is a chronic inflammatory disorder [1]. Endothelial damage caused by vascular risk factors triggers a series of cellular responses which, coupled with the actions of chemical mediators such as cytokines, can lead to the development of atherosclerotic lesions. Epidemiological studies have found a link between inflammation, or immune response, and atherosclerosis. Chronic bacterial infection, particularly with *Chlamydia pneumoniae* or *Helicobacter pylori*, has been found to be significantly associated with the development of atherosclerosis [2], unstable angina [3], myocardial infarction [4] and stroke [5,6]. Systemic autoimmune diseases are associated with a significant increase in the risk of cardiovascular disease that cannot be explained solely by traditional risk factors [7,8]. Therefore, investigating the factors governing the responsiveness of the immune system could be a new strategy for identifying innate risk factors for atherosclerosis.

Macrophage migration inhibitory factor (MIF) is a cytokine that was originally isolated from T lymphocytes and identified as an inhibitor of the random migration of macrophages [9]. The secretion of MIF from inflammatory cells can be induced by exposure to oxidized low-density lipoprotein [10] or other cytokines, such as tumor necrosis factor-α and interleukin-γ [11]. MIF has been shown to play a pivotal role in the regulation of both the innate and adaptive immune responses [12]. In addition to macrophages and lymphocytes [13], MIF is also constitutively expressed by smooth muscle cells [14] and endothelial cells [15] in normal blood vessels.

The MIF gene, located at chromosome 22q 11.2, is a small gene consisting of three exons that are 205, 173, and 183 base pairs in length (UCSC Genome Browser, http://genome.ucsc.edu/cgi-bin/hgGateway). The MIF promoter contains a CATT short tandem repeat (STR) at position −794. This repeat is polymorphic and can contain five- to eight-repeat variants ($(CATT)_{5-8}$). A higher repeat number is associated with an increase in basal and stimulus-induced MIF gene expression [16]. In addition, the 5′-flanking region has a single nucleotide polymorphism (SNP), G-173C (rs755622). The functional significance of this polymorphism has been confirmed using transient transfections in the T lymphoblast cell line, in which the −173C transfected cells had a significant increase in MIF expression [17].

Given the role of MIF as a crucial mediator of host immune responses, genetic factors governing its expression could contribute to individual susceptibility to inflammatory disease and atherosclerosis. The purpose of this study was to investigate the relationship between polymorphisms in the MIF gene and the severity of carotid artery atherosclerosis (CAA) in a cohort of Taiwanese patients with ischemic stroke.

* Corresponding author. Address: Department of Neurology, Chang Gung Memorial Hospital – Kaohsiung Medical Center, 123, Ta-Pi Road, Niaosong, Kaohsiung 833, Taiwan. Fax: +886 7 7317123x3390.
E-mail address: josefliu@ms15.hinet.net (J.-S. Liu).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.02.129

2. Material and methods

The study protocol was approved by the Institutional Review Board of Chang Gung Memorial Hospital (No. 97-0443B). Informed consent was obtained from all participants.

2.1. Study population

This study included 593 consecutive patients admitted to the Department of Neurology at the Chang Gung Memorial Hospital, Kaohsiung, following ischemic stroke (acute neurological symptoms with corresponding acute ischemic lesions on diffusion-weighted brain MR images) or a transient ischemic attack. Demographic and clinical data about vascular risk factors including age, gender, status of cigarette smoking, hypertension, diabetes mellitus, and cardiac disease (coronary artery disease, arrhythmia, valvular heart disease, or congestive heart failure) were obtained. Patients' body weight and height were measured upon admission, and the body mass index was calculated. The levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides in the blood were measured within 24 h of admission, with the subjects in a fasting state. The blood level of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation [18].

2.2. Sonographic study of the carotid arteries

A real-time B-mode sonographic examination was performed to assess plaques in the carotid arteries. A plaque was defined as an area of focal wall thickening $\geqslant$ 0.13 cm. The number of plaques and extent of lumen diameter reduction in the segments of the common carotid artery, the carotid bulb and the internal carotid artery were recorded. The severity of the plaques in each segment was evaluated using grading criteria proposed by Sutton-Tyrrell et al. [19]: grade 0, no plaque; grade 1, one small plaque (thickness <30% lumen diameter); grade 2, one medium plaque (30–50% vessel diameter) or multiple small plaques; grade 3, one large plaque (>50% vessel diameter) or multiple plaques with at least one medium plaque; and grade 4, complete occlusion. The severity of atherosclerotic plaques in the carotid arteries in an individual was represented by a plaque score that was the sum of grades in the six (three on each side) arterial segments.

2.3. Genetic study

Genomic DNA was extracted from peripheral blood leukocytes using commercial kits (QIAamp DNA Blood Mini Kit, QIAGEN). Genotyping for the CATT STR was carried out as described previously [17]. SNP G-173C was genotyped with the AB 7500 Real-Time PCR System using the TaqMan® assay following the manufacturer's conditions (Applied Biosystems, Foster City, CA, USA). Samples with ambiguous genotypes that were not separated by discrete clusters were re-genotyped.

2.4. Statistical analysis

Subjects in the study population were classified into three groups: mild (plaque score = 0 or 1; N = 167, 28.2%), moderate (plaque score = 2–4; N = 212, 35.8%), or severe CAA (plaque score = 5 or higher; N = 214, 36.0%).

Genotype distribution was tested for the Hardy–Weinberg equilibrium. The PHASE program was implemented to reconstruct the haplotypes of the two polymorphisms [20]. Linkage disequilibrium between the two polymorphisms was determined using EH Plus software [21]. Univariate analysis was performed using the chi-square test to compare allele, genotype and haplotype distributions in subjects with mild, moderate or severe CAA. Genotype (or haplotype)-related risks were tested assuming a dominant relationship between the risk allele (or haplotype) and CAA severity (i.e., "carriage of the risk-conferring allele (or haplotype)" *vs.* "homozygous for the reference allele (or haplotype)"). Multinomial logistic regression, adjusted for age, gender, diabetes, hypertension, cardiac disease, cigarette smoking, body mass index, and HDL-C and LDL-C levels, was used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) of the likelihood of either moderate or severe CAA relative to mild CAA for the risk-conferring genetic variant. A p-value < 0.05 was considered to be statistically-significant. All data were analyzed with SPSS version 12.0 software (Chicago, IL, USA).

3. Results

The demographic and clinical characteristics of the study population are shown in Table 1. The allele, genotype and reconstructed haplotype distributions of MIF polymorphisms are presented in Table 2. The most frequent CATT STR allele was $(CATT)_6$ (47%), whereas $(CATT)_8$ was rare and only detected in three cases (0.3%). The frequencies of the G and C allele of the G-173C SNP were 81.5% and 18.5%, respectively. Genotype frequencies of the two polymorphisms were in good agreement with the Hardy–Weinberg equilibrium at both loci ($p > 0.05$, chi-square test). The two polymorphisms were in significant linkage disequilibrium, with the −173C allele strongly associated with $(CATT)_7$ ($p < 0.00001$). The three most common haplotypes, $(CATT)_5$-G,

Table 1
Demographic and clinical characteristics in the study population.

	All Subjects	Carotid Artery Atherosclerosis			p[a]
		Mild	Moderate	Severe	
Case number	593	167	212	214	
Age (years)	65.3 ± 11.1	59.3 ± 11.5	66.1 ± 10.0	69.0 ± 9.9	<0.001
Male gender (%)	62.7	63.5	60.4	64.5	0.662
Diabetes mellitus (%)	41.8	31.1	48.1	43.9	0.003
Hypertension (%)	71.8	63.5	72.2	78.0	0.007
Cardiac disease (%)	23.4	19.2	19.8	30.4	0.011
Cigarette smoker (%)	31.0	34.7	27.4	31.8	0.292
Body mass index (kg/m^2)	24.8 ± 3.4	25.3 ± 3.5	24.6 ± 3.4	24.5 ± 3.2	0.037
HDL-cholesterol (mg/dl)	40.2 ± 10.8	41.0 ± 11.3	40.6 ± 11.3	39.2 ± 9.9	0.227
LDL-cholesterol (mg/dl)	129 ± 36	123 ± 33	128 ± 36	133 ± 38	0.018

Continuous variable expressed as the mean ± standard deviation.
[a] Comparisons among the three groups of different severity of carotid artery atherosclerosis.

Table 2
Distributions of alleles, genotypes and haplotypes of the polymorphisms with respect to the severity of carotid artery atherosclerosis (n = 593).

	All Subjects	Carotid artery atherosclerosis			p[a]
		Mild	Moderate	Severe	
(CATT)n					
5	436	122 (28.0)[b]	161 (36.9)	153 (35.1)	0.017
6	558	177 (31.7)	191 (34.2)	190 (34.1)	0.003[c]
7	189	35 (18.5)	70 (37.0)	84 (44.5)	
8	3	0 (0)	2 (66.7)	1 (33.3)	
5/5	83	28 (33.7)	30 (36.2)	25 (30.1)	0.032
5/6	202	58 (28.7)	76 (37.6)	68 (33.7)	0.001[d]
6/6	134	49 (36.6)	42 (31.3)	43 (32.1)	
5/7	67	8 (12.0)	25 (37.3)	34 (50.7)	
6/7	86	21 (24.4)	29 (33.7)	36 (41.9)	
7/7	18	3 (16.7)	8 (44.4)	7 (38.9)	
5/8	1	0 (0)	0 (0)	1 (100)	
6/8	2	0 (0)	2 (100)	0 (0)	
G-173C					
G	966	287 (29.7)	343 (35.5)	336 (34.8)	0.031
C	220	47 (21.4)	81 (36.8)	92 (41.8)	
GG	395	124 (31.4)	140 (35.4)	131 (33.2)	0.013
GC	176	39 (22.2)	63 (35.8)	74 (42.0)	0.027[e]
CC	22	4 (18.2)	9 (40.9)	9 (40.9)	
Haplotype, $(CATT)_n$-G/C					
$(CATT)_5$-G	436	122 (28.0)	161 (36.9)	153 (35.1)	0.031
$(CATT)_6$-G	529	165 (31.2)	182 (34.4)	182 (34.4)	0.003[f]
$(CATT)_7$-C	189	35 (18.5)	70 (37.0)	84 (44.5)	
$(CATT)_6$-C	29	12 (41.4)	9 (31.0)	8 (27.6)	
$(CATT)_8$-C	3	0 (0)	2 (66.7)	1 (33.3)	

[a] Comparisons among the three groups of different severity of carotid artery atherosclerosis.
[b] Number (% in this allele or genotype groups).
[c] $(CATT)_7$ compared with $(CATT)_{5-6}$ (i.e., $(CATT)_5$ and $(CATT)_6$).
[d] Carriage of $(CATT)_7$ compared with $(CATT)_{5-6}$ homozygote (i.e., $(CATT)_5/(CATT)_5$, $(CATT)_5/(CATT)_6$, and $(CATT)_6/(CATT)_6$).
[e] Carriage of C compared with GG.
[f] Carriage of $(CATT)_7$-C compared with $(CATT)_{5-6}$-G homozygote (i.e., $(CATT)_5$-G/$(CATT)_5$-G, $(CATT)_5$-G/$(CATT)_6$-G, and $(CATT)_6$-G/$(CATT)_6$-G); $(CATT)_6$-C or $(CATT)_8$-C were not included in the analysis.

$(CATT)_6$-G, and $(CATT)_7$-C, accounted for 97.3% of the estimated haplotypes. The three haplotypes $(CATT)_5$-C, $(CATT)_7$-G and $(CATT)_8$-G were absent in the study population.

Significant differences in allele and genotype frequencies for the CATT STR and G-173C were observed in the three groups of patients with different CAA severities (Table 2). For further analyses related to the CATT STR, a composite allele of the 5 or 6-repeat variant, $(CATT)_{5-6}$, was used because of similar distributions of the two alleles with regard to the severity of CAA (p = 0.423), and $(CATT)_8$ was not included because of its low frequency in the study population. When compared with the $(CATT)_{5-6}$ homozygote (i.e., $(CATT)_5/(CATT)_5$, $(CATT)_5/(CATT)_6$, and $(CATT)_6/(CATT)_6$), the carriage of 7-repeat variant [i.e., $(CATT)_5/(CATT)_7$, $(CATT)_6/(CATT)_7$, and $(CATT)_7/(CATT)_7$] was associated with an increase in the severity of CAA (p = 0.001). For G-173C, there was a positive association between the carriage of the C allele (combined GC and CC genotypes compared with GG) and an increase in the severity of CAA (p = 0.027). Multinomial logistic regression revealed that, compared to the $(CATT)_{5-6}$ homozygote, the carriage of $(CATT)_7$ was significantly associated with moderate (adjusted OR 1.94, 95% CI 1.15–3.27, p = 0.012) and severe CAA (adjusted OR 2.75, 95% CI 1.62–4.69, p < 0.001) (Table 3). The carriage of the C allele of the G-173C SNP was significantly associated with severe CAA when compared with the GG homozygote (adjusted OR 2.03, 95% CI 1.24–3.33, p = 0.005).

In order to evaluate the risk of haplotype-related CAA, the cases were classified into two groups according to the carriage of the haplotype $(CATT)_7$-C (N = 561, accounting for 94.6% cases of the study population; those carrying the haplotypes $(CATT)_6$-C or $(CATT)_8$-C were not included for analysis due to their low frequencies). The carriage of $(CATT)_7$-C (i.e., $(CATT)_5$-G/$(CATT)_7$-C, $(CATT)_6$-G/$(CATT)_7$-C, and $(CATT)_7$-C/$(CATT)_7$-C), when compared with the $(CATT)_{5-6}$-G homozygote (i.e., $(CATT)_5$-G/$(CATT)_5$-G, $(CATT)_5$-G/$(CATT)_6$-G, and $(CATT)_6$-G/$(CATT)_6$-G), was associated with an increased severity of CAA (p = 0.003) (Table 2). Multinomial logistic regression revealed that the carriage of $(CATT)_7$-C independently increased the probability of either moderate (adjusted OR 1.90, 95% CI 1.12–3.25, p = 0.018) or severe CAA (adjusted OR 2.55, 95% CI 1.48–4.41, p = 0.001) (Table 3).

4. Discussion

In this candidate gene association study, we used the presence of atherosclerotic plaques as the phenotype of CAA. Although intima-media thickness is more frequently used as a surrogate marker for atherosclerosis, it is a heterogeneous entity and in part reflects non-atherosclerotic reactions associated with the local modification of flow and mural tension [22]. Moreover, plaques in the carotid arteries are generally considered to be a marker better than intima-media thickness in predicting future cardiovascular syndromes [23,24]. In addition, the high prevalence of carotid plaques in our study population, coupled with the grading of CAA severity using a scoring system, also helped to increase the statistical power of this study.

In the present study, we demonstrated that the genetic variants of MIF, including the 7-repeat variant of the CATT STR, the C allele of the G-173C SNP, and the haplotype composed of the two alleles, are associated with an increase in the severity of carotid plaques in patients with ischemic stroke. MIF has been shown to participate in critical steps of the development of atherosclerotic lesions, including promoting leukocyte adhesion and infiltration [25], uptake of oxidized low-density lipoprotein [26], cytokine secretion [11], the expression of matrix metalloproteinases [27], T cell activation, [12] and the migration of vascular smooth muscle cells [28]. The expression of MIF in the lesions is up-regulated along with the progression of atherosclerosis [10]. Blockage of MIF activity leads to the regression of atherosclerotic plaques and the

Table 3
Association analyses between genotypes and haplotypes of the polymorphisms and severity of carotid artery atherosclerosis.

Carotid atherosclerosis	Moderate *vs.* mild			Severe *vs.* mild		
(N = 590)[a]	$(CATT)_7$ Positive	$(CATT)_{5-6}$ Homozygote	p	$(CATT)_7$ Positive	$(CATT)_{5-6}$ Homozygote	p
Adjusted OR[b] (95% CI)	1.94 (1.15–3.27)	1	0.012	2.75 (1.62–4.69)	1	<0.001
(N = 593)	CC + GC	GG	p	CC + GC	GG	p
Adjusted OR (95% CI)	1.60 (1.00–2.60)	1	0.050	2.03 (1.24–3.33)	1	0.005
(N = 561)[c]	$(CATT)_7$-C Positive	$(CATT)_{5-6}$-G Homozygote	p	$(CATT)_7$-C Positive	$(CATT)_{5-6}$-G Homozygote	p
Adjusted OR (95% CI)	1.90 (1.12–3.25)	1	0.018	2.55 (1.48–4.41)	1	0.001

Abbreviations: OR, odds ratio; CI, confidence interval.
[a] Cases carrying $(CATT)_8$ not included.
[b] Adjusted for age, sex, diabetes mellitus, hypertension, cardiac disease, cigarette smoking, body mass index, and high-density lipoprotein cholesterol and low-density lipoprotein cholesterol levels.
[c] Cases carrying $(CATT)_6$-C and $(CATT)_8$-C not included.

attenuation of the inflammatory response in the lesion [29]. In addition, the MIF gene polymorphisms tested in the current study have been recognized to be functional in transcriptional activities [16,17]. The basal serum levels of MIF are higher in carriers of the C allele of the G-173C polymorphism, 7-repeat allele of the CATT STR and a haplotype composed of these two alleles [17,30]. Taken together, our findings indicate that an increase in MIF expression governed by the genetic variants in the MIF gene promoter may stimulate the development of CAA.

Data on genetic variants of the MIF gene in relation to cardiovascular disorders are still limited. Previous studies have shown that −173C is associated with an increased risk for coronary heart disease in a Chinese population [31] and in females in a German population [32], but not for myocardial infarction in Russian and Czech populations [33]. A study on proinflammatory gene profiles in stroke patients showed that G-173C was not associated with a history of ischemic stroke [34]. Apart from the diversity in ethnic groups and study designs, one important reason for the discrepancy of these study results could be a difference in atherosclerosis phenotypes in each study. To our knowledge, the present study is the first to investigate the association between genetic variants of MIF and CAA. As an "intermediate phenotype" for cardiovascular disorders, carotid plaques could be more powerful than disease outcome (e.g., stroke) for detecting the association with genetic factors by avoiding the incomplete penetrance that may occur in case-control studies.

Some limitations in this study should be noted. First, the study was conducted on stroke patients. Our findings still need to be confirmed in the general population and in other atherosclerosis-prone conditions. Second, this is a cross-sectional study. The causal role of the MIF genepolymorphisms for CAA would be better clarified by a longitudinal investigation. For the same reason, the state of glycemic and blood pressure control, which may strongly affect the development of carotid atherosclerosis, was not adjusted in the statistical analysis. Third, plasma levels of MIF were not measured in the study subjects. These data would be informative for the physiological relevance of the tested polymorphisms.

In conclusion, we demonstrated that polymorphisms in the MIF gene promoter are associated with CAA severity in ischemic stroke patients. These genetic variants may serve as markers for an individual's susceptibility to CAA. Given the biological plausibility that inflammation is relevant to atherosclerosis, our findings suggest that use of polymorphisms of pro-inflammatory genes as potential biomarkers for the risk of atherosclerosis might merit further investigation.

Acknowledgments

This research was supported by a grant from the Chang Gung Research Project to M.-Y. Lan (CMRPG870431)

References

[1] P. Libby, Inflammation in atherosclerosis, Nature 420 (2002) 868–874.
[2] S.C. Johnston, H. Zhang, L.M. Messina, et al., Chlamydia pneumoniae burden in carotid arteries is associated with upregulation of plaque interleukin-6 and elevated C-reactive protein in serum, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 2648–2653.
[3] R. Chousset, G. Montalescot, J. Collet, et al., Effect of prior exposure to *Chlamydia pneumoniae*, *Helicobacter pylori*, or cytomegalovirus on the degree of inflammation and one-year prognosis of patients with unstable angina pectoris or non-Q-wave acute myocardial infarction, Am. J. Cardiol. 86 (2000) 379–384.
[4] R. Sessa, M. Di Pietro, G. Schiavoni, et al., Prevalence of *Chlamydia pneumoniae* in peripheral blood mononuclear cells in Italian patients with acute ischaemic heart disease, Atherosclerosis 159 (2001) 521–525.
[5] M.H. Park, J.Y. Min, S.B. Koh, et al., *Helicobacter pylori* infection and the CD14 C(−260)T gene polymorphism in ischemic stroke, Thromb. Res. 118 (2006) 671–677.
[6] A.K. Njamnshi, K.N. Blackett, J.N. Mbuagbaw, et al., Chronic *Chlamydia pneumoniae* infection and stroke in Cameroon: a case-control study, Stroke 37 (2006) 796–799.
[7] M.B. Urowitz, A.A. Bookman, B.E. Koehler, et al., The bimodal mortality pattern of systemic lupus erythematosus, Am. J. Med. 60 (1976) 221–225.
[8] K.J. Warrington, P.D. Kent, R.L. Frye, et al., Rheumatoid arthritis is an independent risk factor for multi-vessel coronary artery disease: a case control study, Arthritis Res. Ther. 7 (2005). R984-981.
[9] J.R. David, Delayed hypersensitivity *in vitro*: its mediation by cell-free substances formed by lymphoid cell–antigen interaction, Proc. Natl. Acad. Sci. USA 56 (1966) 72–77.
[10] A. Burger-Kentischer, H. Goebel, R. Seiler, et al., Expression of macrophage migration inhibitory factor in different stages of human atherosclerosis, Circulation 105 (2002) 1561–1566.
[11] T. Calandra, J. Bernhagen, R.A. Mitchell, et al., The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor, J. Exp. Med. 179 (1994) 1895–1902.
[12] M. Bacher, C.N. Metz, T. Calandra, et al., An essential regulatory role for macrophage migration inhibitory factor in T-cell activation, Proc. Natl. Acad. Sci. USA 93 (1996) 7849–7854.
[13] J. Bernhagen, T. Calandra, R. Bucala, Regulation of the immune response by macrophage migration inhibitory factor: biological and structural features, J. Mol. Med. 76 (1998) 151–161.
[14] J.H. Pan, G.K. Sukhova, J.T. Yang, et al., Macrophage migration inhibitory factor deficiency impairs atherosclerosis in low-density lipoprotein receptor-deficient mice, Circulation 109 (2004) 3149–3153.
[15] J. Nishihira, Y. Koyama, Y. Mizue, Identification of macrophage migration inhibitory factor (MIF) in human vascular endothelial cells and its induction by lipopolysaccharide, Cytokine 10 (1998) 199–205.
[16] J.A. Baugh, S. Chitnis, S.C. Donnelly, et al., A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis, Genes Immun. 3 (2002) 170–176.
[17] R. Donn, Z. Alourfi, F. De Benedetti, et al., Mutation screening of the macrophage migration inhibitory factor gene: positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis, Arthritis Rheum. 50 (2002) 2402–2409.
[18] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (1972) 499–502.
[19] K. Sutton-Tyrrell, S.K. Wolfson Jr., T. Thompson, et al., Measurement variability in duplex scan assessment of carotid atherosclerosis, Stroke 23 (1992) 215–220.
[20] M. Stephens, N.J. Smith, P. Donnelly, A new statistical method for haplotype reconstruction from population data, Am. J. Hum. Genet. 68 (2001) 978–989.
[21] D. Curtis, P.C. Sham, Model-free linkage analysis using likelihoods, Am. J. Hum. Genet. 57 (1995) 703–716.
[22] P.J. Touboul, M.G. Hennerici, S. Meairs, et al., Mannheim intima-media thickness consensus, Cerebrovasc. Dis. 18 (2004) 346–349.
[23] A.V. Finn, F.D. Kolodgie, R. Virmani, Correlation between carotid intimal/medial thickness and atherosclerosis: a point of view from pathology, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 177–181.
[24] Y. Inaba, J.A. Chen, S.R. Bergmann, Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis, Atherosclerosis 220 (2012) 128–133.
[25] J.L. Gregory, M.T. Leech, J.R. David, et al., Reduced leukocyte-endothelial cell interactions in the inflamed microcirculation of macrophage migration inhibitory factor-deficient mice, Arthritis Rheum. 50 (2004) 3023–3034.
[26] T. Atsumi, J. Nishihira, Z. Makita, et al., Enhancement of oxidised low-density lipoprotein uptake by macrophages in response to macrophage migration inhibitory factor, Cytokine 12 (2000) 1553–1556.
[27] S. Onodera, K. Kaneda, Y. Mizue, et al., Macrophage migration inhibitory factor up-regulates expression of matrix metalloproteinases in synovial fibroblasts of rheumatoid arthritis, J. Biol. Chem. 275 (2000) 444–450.
[28] B.H. Schrans-Stassen, H. Lue, D.G. Sonnemans, et al., Stimulation of vascular smooth muscle cell migration by macrophage migration inhibitory factor, Antioxid. Redox. Signal 7 (2005) 1211–1216.
[29] J. Bernhagen, R. Krohn, H. Lue, et al., MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment, Nat. Med. 13 (2007) 587–596.
[30] T.R. Radstake, F.C. Sweep, P. Welsing, et al., Correlation of rheumatoid arthritis severity with the genetic functional variants and circulating levels of macrophage migration inhibitory factor, Arthritis Rheum. 52 (2005) 3020–3029.
[31] Z.X. Shan, Y.H. Fu, X.Y. Yu, et al., Association of polymorphism of macrophage migration inhibitory factor gene with coronary heart disease in Chinese population, Zhonghua Yi Xue Yi Chuan Xue Za Zhi 23 (2006) 548–550.
[32] C. Herder, T. Illig, J. Baumert, et al., Macrophage migration inhibitory factor (MIF) and risk for coronary heart disease: results from the MONICA/KORA Augsburg case-cohort study, 1984–2002, Atherosclerosis 200 (2008) 380–388.
[33] I.P. Tereshchenko, J. Petrkova, F. Mrazek, et al., The macrophage migration inhibitory factor (MIF) gene polymorphism in Czech and Russian patients with myocardial infarction, Clin. Chim. Acta 402 (2009) 199–202.
[34] A. Flex, E. Gaetani, P. Papaleo, et al., Proinflammatory genetic profiles in subjects with history of ischemic stroke, Stroke 35 (2004) 2270–2275.