Journal of the Neurological Sciences 342 (2014) 107–113

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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

Matrix metalloproteinase-1 and matrix metalloproteinase-12 gene polymorphisms and the risk of ischemic stroke in a Tunisian population

Khouloud Chehaibi [a,*], Mohamed Yahia Hrira [b], Samir Nouira [c], Faouzi Maatouk [d], Khaldoun Ben Hamda [d], Mohamed Naceur Slimane [a]

[a] Research Unit: UR 12ES09 Dyslipidemia and Atherogenesis, Faculty of Medicine, Monastir 5019, Tunisia
[b] Research Unit: UR 07/06, Faculty of Pharmacy, Monastir 5019, Tunisia
[c] Emergency Department, CHU Fattouma Bourguiba, Monastir 5000, Tunisia
[d] Department of Cardiovascular Diseases, Fattouma Bourguiba Hospital, Monastir 5000, Tunisia

ARTICLE INFO

Article history:
Received 19 January 2014
Received in revised form 15 April 2014
Accepted 23 April 2014
Available online 2 May 2014

Keywords:
Matrix metalloproteinases (MMPs)
Ischemic stroke
Type 2 diabetes mellitus
Polymorphism
Haplotype
Risk factors
Important joint effect

ABSTRACT

Matrix metalloproteinases (MMPs) play an important role in early atherosclerosis, extracellular matrix remodeling, plaque rupture and myocardial infarction. MMP gene polymorphisms contribute to the risk of developing cardiovascular diseases. In this study, we investigated, for the first time, the association between MMP-1-16071G/2G, MMP-12 -82A/G and MMP-12 1082A/G genotypes and haplotypes and the risk of ischemic stroke (IS) among patients with type 2 diabetes mellitus (T2DM). To examine whether these genetic polymorphisms are associated with susceptibility to IS, 196 patients with IS and 192 controls were examined by PCR-based RFLP. When the analyses were adjusted for multiple risk factors, no interaction between T2DM and MMP-1-1607 1G/2G polymorphism on the risk of ischemic stroke was found ($p = 0.074$). However, MMP-12 polymorphisms genotypes were associated with the higher risk of IS in diabetic patients compared with total patients. The -82G-1082G haplotype of MMP-12 polymorphisms was associated with higher risk of ischemic stroke in diabetic patients [AOR = 2.33; 95% CI (1.25–3.62), $P = 0.032$]. These findings showed that there was an important joint effect of the MMP-12 polymorphisms and T2DM on the risk of IS and therefore it can be considered as a potential marker of cerebrovascular disorders in diabetic patients.

1. Introduction

Stroke physiopathology is complex at the molecular level and involves a wide variety of proteins, including the matrix metalloproteinases (MMPs) [38]. Extracellular matrix (ECM) remodeling is an essential process in the pathogenesis of atherosclerosis and coronary heart diseases (CHD). Matrix metalloproteinases (MMPs) a family of peptidase enzymes are secreted by many types of cells as proenzymes. On activation by proteolytic cleavage, activated enzymes are capable of degrading many extracellular matrix components, resulting in weakening the fibrous cap and predisposing the atherosclerotic plaques to disruption and embolic events. Because MMPs appear to be involved in monocyte invasion and vascular smooth muscle cell migration, derangement of MMP regulation is considered to be a critical factor in the development of vascular lesions [46] and remodeling which is recognized as a determinant of major vascular pathologies including atherosclerosis and restenosis [29]. MMP expression can vary among individuals due to genetic diversity, which could influence the susceptibility of presenting with vascular disease [14]. Genetic polymorphisms located in the promoter region of the MMP genes could lead to increased gene expression and could be associated with predisposition to various diseases [55]. Overexpression of MMPs enzymes in advanced lesions may contribute to the thinning of the plaque cap and to the development of ischemic events resulting from plaque rupture [3]. MMP-1 and MMP-12 genes contain single nucleotide polymorphisms. Most of the investigated polymorphisms have previously been shown to alter the gene expression [26,47]. MMP-1 is the only MMP that can cleave native collagen types I and III, which are major structural components of the fibrous plaque cap. MMP-1 might play a significant role in fibrous plaque disruption by contributing to the degradation of interstitial collagens and thinning of the fibrous cap [39]. A single-guanine (1G)-to-(2G) polymorphism located at the MMP-1 promoter region (MMP-1-1607 1G/2G, rs1799750) that affects the transcription level of the gene has been identified. It has been demonstrated that the promoter comprising the 2G allele has significantly greater transcriptional activity compared with the 1G promoter, because the 2G allele creates an E26 (Ets) transcription factor binding site and increases transcription capacity [47]. MMP-12 displays a broad substrate specificity, including ECM proteins such as fibronectin,

* Corresponding author. Tel.: +216 73 462 200; fax: +216 73 460 737.
E-mail address: khouloudchehaibi5@gmail.com (K. Chehaibi).

http://dx.doi.org/10.1016/j.jns.2014.04.036
0022-510X/

laminin, vitronectin, type IV collagen, and heparan sulfate [5,15]. MMP-12 not only digests elastin, but also degrades the basement membrane, which enables macrophages to penetrate injured tissues during inflammation. The A allele of the MMP-12 -82A/G polymorphism (rs2276109) shows a higher affinity for the transcription factor activator protein-1 (AP-1) and higher gene expression in reporter gene assays [26]. The MMP-12 1082A/G (357Asn/Ser, rs652438) polymorphism is located in the coding region of the hemopexin domain that is responsible for MMP-12 activity, while the function of this polymorphism remains unknown [25]. Studies have found that polymorphisms in MMP genes that modulate their transcription or activity are associated with the outcome of the coronary artery disease (CAD). Studies assessing the effects of polymorphisms in the MMP-1 on cardiovascular events have found inconsistent associations [16,43]. The MMP-1-16071G/2G variant has been associated with the risk of coronary heart disease and with several cancers also [22,27,40,41,56,57]. The 2G allele has been linked to increased incidence or progression of several diseases, including periodontitis [1,7,21,31,45], cardiovascular disorders [43,57], coronary heart disease in diabetes mellitus patients [10], arteriosclerosis [42], degenerative disc disease [50], endobronchial tuberculosis [29], arthritis [37] and lung conditions [24,36]. -1607 2G allele is also the independent significant risk factor for carotid plaque presence [9]. In addition, a common functional MMP-12 (-82A/G) promoter polymorphism that increases expression of MMP-12 is associated with increased coronary artery stenosis in diabetic patients [26] and it has been associated with coronary atherosclerosis [54,55]. MMP-12 -82A/G was also associated with coronary artery aneurysm formation in patients with Kawasaki disease with the G allele conferring increased risk [48]. The MMP-12 rs2276109 gene polymorphism may contribute to susceptibility to systemic sclerosis (SSc), and in particular to diffuse cutaneous SSc (dcSSc) and pulmonary fibrosis in an Italian population [34]. The functional significance of the second single-nucleotide polymorphism MMP-12 1082 A/G (rs652438), is unclear, but has been correlated with breast cancer outcome [49]. MMP-12 expression levels were found to be upregulated in recurrent versus non-recurrent stage IB lung cancer [6] and correlated with local recurrence and metastasis [20]. Haplotype analysis was performed to investigate the combined effect of some linked MMP polymorphisms and some diseases. A study showed that haplotypes of MMP-1 and MMP-12 were associated with the decline of lung function [27]. Some MMP polymorphisms were shown to be associated with both atherosclerosis and chronic obstructive pulmonary disease [17,52]. In addition, a trend of the GG-A haplotype of MMP-1 -16071G/2G and MMP-12 1082A/G towards the prediction of future clinical events was found in patients with CAD (coronary artery disease) [23]. We hypothesized that the joint effects or haplotypes of MMP polymorphisms are stronger than the individual effect of each polymorphism and because MMP-1 and MMP-12, genes are clustered on chromosome 11q22.3, we therefore addressed, for the first time, whether there were any associations between MMP-1-16071G/2G, MMP-12 -82A/G and MMP-12 1082A/G genotypes and haplotypes and the risk of ischemic stroke in a Tunisian population among patients with and without diabetes.

2. Material and methods

2.1. Subjects

Blood was sampled from 196 patients (102 males, 94 females; ages ranging from 40 to 85 years, with an average age of 63.52 ± 11.45 years) who presented to the emergency department at Fattoyuma Bourguiba University Hospital in Monastir, Tunisia with ischemic stroke (117 diabetic and 79 nondiabetic) from March 2011 to March 2012. In addition to age, the other inclusion criterion was clinical diagnosis of ischemic stroke, causing a measurable neurological deficit (defined as impairment of language, motor function, cognition, gaze, or vision, or as neglect). Ischemic stroke was defined as the rapid development of focal or global disturbance of cerebral function, with symptoms lasting 24 h or longer, or leading to death, with no apparent cause other than vascular origin after exclusion of hemorrhage by computed tomographic scan or magnetic resonance imaging of the brain. Diabetic subjects were defined by a fasting plasma glucose ≥7.0 mmol/l, or by the use of anti-diabetic drugs [12]. Patients were considered diabetic if diabetes was previously known. Exclusion criteria for cases included clinical presentation suggestive of subarachnoid hemorrhage, even if the initially computed tomography scan is normal. In addition, none of the subjects had any of the following conditions: transient ischemic attack, cerebral infarction due to cardiogenic events, cerebral hemorrhage, cerebral venous thrombosis, systematic inflammatory and autoimmune diseases, brain tumors, valvular heart disease, cancers, rheumatoid arthritis, heart failure, acute coronary syndrome, peripheral vascular disease, arteriovenous malformation, or aneurysm, presumed pericarditis or presence of either ventricular thrombus or aneurysm related to recent acute myocardial infarction and no anti inflammatory or oral anticoagulants drugs has been taken by any patient. Concomitant liver and kidney diseases and thyroid disease were not also found in these patients. After at least brain computed tomography or magnetic resonance imaging scan was performed to rule out hemorrhagic stroke, patient recruitment from 3 to 6 h was enrolled since onset of stroke. Medical personnel of the emergency department provided ischemic stroke care within the first hours to time of initial diagnosis, treatment, and initial hospitalization. 192 healthy subjects consisting of blood donors were recruited as controls (110 males, 82 females; ages ranging from 40 to 85 years, with an average age of 61.69 ± 7.0 years). Matching is required for case control study for the elimination of bias in comparison between cases and controls. It assures that no large imbalance between cases and controls occurs. Controls were matched with cases for age and sex. Before sampling, it was verified by direct interview by two experienced neurologists if they had neurological disorders. All participants completed a structured questionnaire as performed previously by [37] in order to verify the stroke free-status. In addition, physical examination and complete clinical history, including stroke risk factors, were taken for all participants. Controls had no history of stroke who were symptomatically normal (had no history of arterial or venous thrombosis); all free of any history of obesity, hypertension, dyslipidemia, diabetes mellitus, or CAD were included in the study. Cerebrovascular diseases, brain aneurysms, Alzheimer's disease, dementia or Parkinson's disease, kidney/liver diseases, hematological diseases, tumors, peripheral vascular diseases, and autoimmune diseases were excluded from these controls. Hypertension was defined as systolic pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg in at least two separate measurements, or in the case of a hypertension history [53]. Obesity was defined as BMI ≥30 kg/m^2. Hypercholesterolemia was considered present if total cholesterol serum levels were ≥5 mmol/l or if the subject was undergoing a treatment with cholesterol lowering drugs. As ethnic differences may influence conclusions reached in SNP-association studies, all participants were of Tunisian origin and were consented to participate in the study. This study was approved by our hospital ethical committee.

2.2. Genotyping

For each polymorphism, a DNA sequence containing the polymorphic site was amplified by PCR using primers described in supplemental Table 1. The primers, probes and reaction conditions are available upon request. Genotyping was performed by laboratory personnel blinded to case–control status. As a quality control measure, and to assess reliability of genotyping, we performed double-sampling restriction fragment length polymorphism-PCR in more than 10% of the samples and the results were consistent. Negative control reactions without DNA were included in each well in all the genotyping steps (PCR amplification and enzymatic digestions). No PCR product was detected from any of the negative control reactions. The amplicon was digested with an appropriate restriction enzyme that cleaves only 1 of the 2 alleles. The methods

Table 1
Sequences of PCR primers.

Gene	Polymorphism	Forward primer (5′–3′)	Reverse primer (5′–3′)	Restriction enzyme
MMP-1	(G-1607GG)	5′-TCGTGAGAATGTCTTCCCATT-3′	5′-TCTTGGATTGATTTGAGATAAGTGAAATC-3′	XmnI
MMP-12	(A-82G)	5′-GAGATAGTCAAGGGATGATATCAGC-3′	5′-AAGAGCTCCAGAAGCAGTGG-3′	PvuII
MMP-12	(A1082G)	5′-GGGATAATTTGGCTCTGGTCTTCAA-3′	5′-CCATGGGAACCATAGAAAAGA-3′	MfeI

used to type MMP-1-16071G/2G, MMP-12 -82A/G and MMP-12 1082A/G polymorphisms have been described previously [11,25]. Genotypes of the 1G/2G polymorphism in the MMP-1 promoter were determined by PCR-based XmnI restriction fragment length polymorphism [47]. A polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) introducing a PvuII and MfeI restriction site was performed, respectively for MMP-12 -82A/G and MMP-12 1082A/G polymorphisms. The digested products were electrophoresed on a 3% agarose gel containing ethidium bromide and were visualized under ultraviolet light.

3. Statistical analysis

Statistical analysis was performed on SPSS v.17.0 software. Data were expressed as mean ± SD (continuous variables) or as percentages of total (categorical variables). Differences between the diabetic ischemic stroke patients, nondiabetic ischemic stroke patients and healthy controls were analyzed using Student's unpaired *t* test for parametric variables. Hardy–Weinberg equilibrium (HWE) was applied to test the observed and expected genotype frequencies for cases and controls (χ^2 test). Each of the SNP in these genes was analyzed for Hardy–Weinberg equilibrium (HWE). All statistical tests were two-tailed and adjusted *p*-values for genotype and allele frequencies were obtained adjusting for age, sex, obesity, smoking, hyperlipidemia and hypertension. *P*-values were considered to be statistically significant when <0.05. First, we investigated the associations between individual MMP polymorphism and the risk of ischemic stroke in separate logistic regression model adjusting for covariates. Second, we investigated the associations between MMP haplotypes and the risk of ischemic stroke using PHASE 2.0 software [51] and this association was also adjusted for age, sex, obesity, smoking, hyperlipidemia and hypertension. Haplotypes that had a frequency below 0.02% were combined into a "rare haplotypes" category. The odds ratios (OR) and 95% confidence intervals (CI) for the risk of ischemic stroke were calculated. The linkage disequilibrium (LD) statistics were calculated among SNPs using SNPSTAT computer software.

4. Results

4.1. Clinical and biological characteristics of the study population

The study population characteristics are summarized in Table 2. There were no significant demographic differences (age and gender) and BMI between patients with or without diabetes and control groups. The results presented were obtained from the analysis of the whole population (men and women). All comparisons were re-done after dichotomization of men, and no differences in the results were revealed (data not shown). Diabetic ischemic patient group compared with controls, showed a significantly higher prevalence of conventional risk factors for stroke, including high levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), triglycerides (TG), smoking, glucose, and lower level of HDL-C ($p < 0.05$). On the other hand, LDL-C levels and total cholesterol (TC) were not significantly different ($p > 0.05$). SBP ($p < 0.001$), DBP ($p < 0.001$), glucose ($p < 0.001$), and TG level ($p = 0.004$) were found to be higher in nondiabetic ischemic stroke patient group than in the controls, while HDL-C level ($p = 0.55$), LDL-C level ($p = 0.94$), and TC ($p = 0.54$) were not significantly different ($p > 0.05$). There were no differences in the mean age, BMI and sex between the diabetic ischemic stroke patient group and the nondiabetic ischemic stroke patient group. TC, LDL-C and HDL-C were not significantly different ($p > 0.05$) in the nondiabetic ischemic stroke patient group when compared with the diabetic ischemic patient group. While, TG was found to be higher in the nondiabetic ischemic stroke patient group than in the diabetic ischemic patient group ($p = 0.035$). However, glucose was found to be higher in the diabetic ischemic stroke patient group than in the nondiabetic ischemic patient one ($p < 0.001$).

4.2. Distribution of MMP polymorphisms among cases and controls

The genotypes 2G/2G and 1G/2G of MMP-1 or A/G and G/G of both MMP-12 polymorphisms were combined because of the small number of patients in each category. The χ^2 test was used to test Hardy–Weinberg equilibrium. Genotype distributions of MMP-1-1607

Table 2
Clinical and biological characteristics of the study population.

Characteristics	Controls ($n = 192$)	Cases		*p*-values		
		DM(+) I stroke ($n = 117$)	DM(−) I stroke ($n = 79$)	P1	P2	P3
Age (years) mean ± SD	61.69 ± 7.0	64.23 ± 12.31	62.46 ± 10.02	0.20	0.47	0.22
Gender (male/female) [*n*%]	110/82 (57.29%/42.70%)	63/54 (53.84%/46.15%)	39/40 (49.36%/50.63%)	0.55	0.28	0.31
BMI (kg/m^2) mean ± SD	26.29 ± 4.28	25.74 ± 4.05	26.19 ± 3.34	0.29	0.86	0.84
Current smokers	(22) 11.45%	33(28.20%)	(15)18.98%	0.031	0.55	0.72
Systolic blood pressure (mm Hg)	123.20 ± 12.78	153.19 ± 29.29	162.37 ± 34.98	<0.001	<0.001	<0.001
Diastolic blood pressure (mm Hg)	77.88 ± 7.06	89.08 ± 15.5	84.74 ± 16.28	<0.001	<0.001	<0.001
Systemic hypertension	0(0%)	73(62.39%)	62(78.48%)	<0.001	<0.001	<0.001
Total cholesterol (mmol/l)	4.46 ± 0.79	4.61 ± 1.15	4.53 ± 1.16	0.17	0.54	0.88
Triglycerides (mmol/l)	1.15 ± 0.51	1.32 ± 0.61	1.37 ± 0.68	0.009	0.004	0.035
HDL cholesterol (mmol/l)	1.14 ± 0.34	1.05 ± 0.33	1.11 ± 0.43	0.02	0.55	0.94
LDL cholesterol (mmol/l)	2.78 ± 0.75	2.95 ± 1.19	2.78 ± 1.20	0.12	0.94	0.56
Glucose (mmol/l)	4.91 ± 0.78	9.07 ± 3.89	6.01 ± 1.73	<0.001	<0.001	<0.001

DM(+) I stroke, ischemic stroke patients with diabetes; DM(−) I stroke, ischemic stroke patients without diabetes; p1, control versus DM(+) I stroke; p2, control versus DM(−) I stroke; p3, DM(+) I stroke versus DM (−) I stroke. BMI, body mass index; HDL-C and LDL-C = high- and low-density lipoprotein cholesterol, respectively. Data are reported as means ± SD or as number with percent in parentheses.

Table 3
Lipid and lipoprotein concentrations and BMI according to the -82A/G polymorphism genotype in the MMP-12 gene.

Parameter	DM(+) I stroke ($n = 117$)		DM(−) I stroke ($n = 79$)		Controls ($n = 192$)		*P* values		
	AA	AG/GG	AA	AG/GG	AA	AG/GG	P1	P2	P3
TC (mM)	4.50 ± 1.12	4.86 ± 1.19	4.38 ± 1.14	4.98 ± 1.12	4.43 ± 0.79	4.80 ± 0.84	0.10	0.40	0.53
HDL-C (mM)	1 ± 0.28	1.17 ± 0.42	1.13 ± 0.45	1.06 ± 0.39	1.15 ± 0.33	1.03 ± 0.39	0.17	0.1	0.15
LDL-C (mM)	2.89 ± 1.20	3.09 ± 1.16	2.59 ± 1.10	3.40 ± 1.33	2.75 ± 0.74	3.23 ± 0.86	0.09	0.07	0.43
TG (mM)	1.32 ± 0.57	1.31 ± 0.70	1.41 ± 0.75	1.22 ± 0.40	1.14 ± 0.51	1.17 ± 0.52	0.81	0.63	0.77
BMI (kg/m^2)	25.53 ± 2.89	26.31 ± 6.15	26.17 ± 3.30	26.26 ± 3.56	26.08 ± 4.04	26.27 ± 6.28	0.41	0.77	0.12

p1, control versus DM(+) I stroke; p2, control versus DM(−) I stroke; p3, DM(+) I stroke versus DM(−) I stroke.

1G/2G, MMP-12 -82A/G and 1082A/G polymorphisms were consistent with Hardy–Weinberg equilibrium ($\chi^2 = 1.92$, $p = 0.31$; $\chi^2 = 3.21$, $P = 0.13$ and $\chi^2 = 1.70$, $p = 0.34$, respectively). To investigate whether these polymorphisms had any effect on lipid and metabolic parameters, we compared the characteristics among the genotype groups in controls and in patients with or without diabetes. Association was tested for each polymorphism separately. Results of analyses for single polymorphisms are shown in Tables 3, 4 and 5. Plasma concentrations of total cholesterol, TG, LDL-C, HDL-C and BMI did not differ significantly between subjects carrying the 1G/1G genotype and those carrying the 2G2G/1G2G genotypes of the MMP-1 polymorphism in both ischemic stroke patients (with or without diabetes) and control groups (p 0.05). The same results were obtained for the other MMP-12 polymorphisms. The effects of MMP polymorphisms (MMP-1-16071G/2G, MMP-12 -82A/G and MMP-12 1082A/G) on serum lipid levels in ischemic stroke patients with and without diabetes mellitus in different sex groups were investigated and revealed no differences in the results (data not shown).

4.3. Association between individual MMP polymorphisms and ischemic stroke risk

The genotype and allele frequencies in patients and controls are presented in Table 6. On the basis of logistic regression analysis with adjustment for sex, age, smoking, obesity, dyslipidemia and hypertension, there was a difference in the genotype distributions of the MMP-1 polymorphism in total ischemic stroke patients (diabetics and nondiabetics) and controls. Indeed, the 2G2G/1G2G genotype was more frequent in total cases (diabetics and nondiabetics) compared with controls. However, differences did not reach statistical significance [AOR = 1.14; 95% CI (0.76–1.52); $p = 0.080$]. On the other hand, no interaction between diabetes and MMP-1-1607 1G/2G polymorphism on the risk of ischemic stroke was found [AOR = 1.10; 95% CI (0.39–1.49); $p = 0.074$]. We also found no association between nondiabetic subjects and MMP-1 polymorphism [AOR = 1.06; 95% CI (0.75–1.35); $p = 0.119$]. As MMP-1 polymorphism, the association between individual MMP-12 polymorphisms and ischemic stroke risk was also evaluated using regression model and adjusted for sex, age, smoking, obesity, dyslipidemia and hypertension. No interaction between nondiabetic and MMP-12 -82A/G polymorphism on the risk of ischemic stroke was found [AOR = 1.23; 95% CI (0.99–1.46); $p = 0.858$]. Whereas, we found an association between diabetic subjects and MMP-12 polymorphism [AOR = 1.83; 95% CI (1.10–2.67); $p = 0.033$]. Risk associated with ischemic stroke in total patients was also significant in MMP-12 -82A/G present genotypes with [AOR = 1.79; 95% CI (1.29–2.69); $p = 0.001$]. For MMP-12 1082A/G, the genotypes were associated with ischemic stroke risk in diabetic patients [AOR = 1.73; 95% CI (1.12–2.63); $p = 0.013$] and in total patients [AOR = 1.68; 95% CI (1.22–2.56); $p = 0.001$] but not in nondiabetic subjects [AOR = 1.25; 95% CI (0.87–1.58); $p = 0.354$].

4.4. Haplotype analysis

Haplotype analysis was only performed to investigate the effect of two linked MMP-12 polymorphisms combined and ischemic stroke risk in both ischemic stroke (diabetic and nondiabetic) and control groups. Indeed, there are total of four common haplotypes (>2%) among both cases and controls with their adjusted *p*-values using the most common haplotype as reference (Table 7). The difference in frequency distribution of all common haplotypes was examined between patients and controls and found a significant haplotype effect of -82G-1082G [AOR = 2.28; 95% CI (1.29–3.55), $P = 0.029$] on ischemic stroke risk in total patients and in diabetic patients [AOR = 2.33; 95% CI (1.25–3.62), $P = 0.032$] but not in nondiabetic patients [AOR = 1.45; 95% CI (0.97–2.25), $P = 0.065$]. The significant results are a consequence of the association of MMP-12 1082A/G and MMP-12 -82A/G polymorphisms with the risk of ischemic stroke. The low LD ($r^2 = 0.082$) in controls between these two polymorphisms indicates the separate involvement of both polymorphisms in this association.

5. Discussion

This study is the first one conducted on the Tunisian population that investigates the association between MMP-1-16071G/2G, MMP-12 -82A/G and MMP-12 1082A/G genotypes and haplotypes and the risk of ischemic stroke among patients with diabetes. In our study, MMP-1 and MMP-12 gene variants had no effects on BMI and lipid/lipoprotein profiles in both ischemic stroke patients (with or without diabetes). In addition, the lack of association between the MMP-1 gene variant and the risk of ischemic stroke in patients with and without diabetes was in accordance with a study in CAD [24] and in cervical artery dissection patients [4]. However, in other studies, 2G allele of MMP-1 was associated with the risk of coronary heart disease (CHD) [57], CHD in diabetic patients [10] and with stable plaques [13]. This controversy was explained by the fact that MMP-1 expression in the arterial wall is increased in individuals carrying the 2G allele, which

Table 4
Lipid and lipoprotein concentrations and BMI according to the 1082A/G polymorphism genotype in the MMP-12 gene.

Parameter	DM(+) I stroke ($n = 117$)		DM(−) I stroke ($n = 79$)		Controls ($n = 192$)		*P* values		
	AA	AG/GG	AA	AG/GG	AA	AG/GG	P1	P2	P3
TC (mM)	4.71 ± 1.11	4.51 ± 1.18	4.38 ± 1.12	4.76 ± 1.19	4.48 ± 0.79	4.39 ± 0.80	0.53	0.44	0.40
HDL-C (mM)	1.10 ± 0.36	1.01 ± 0.30	1.10 ± 0.37	1.13 ± 0.52	1.13 ± 0.32	1.17 ± 0.40	0.31	0.29	0.44
LDL-C (mM)	2.97 ± 1.17	2.93 ± 1.21	2.61 ± 1.14	3 ± 1.26	2.80 ± 0.75	2.71 ± 0.75	0.22	0.11	0.60
TG (mM)	1.39 ± 0.64	1.24 ± 0.58	1.29 ± 0.64	1.47 ± 0.73	1.16 ± 0.53	1.09 ± 0.47	0.50	0.08	0.07
BMI (kg/m^2)	25.62 ± 2.97	25.87 ± 4.90	26.35 ± 3.54	25.96 ± 3.09	25.97 ± 3.76	26.37 ± 5.51	0.40	0.76	0.43

p1, control versus DM(+) I stroke; p2, control versus DM(−) I stroke; p3, DM(+) I stroke versus DM(−) I stroke.

Table 5
Lipid and lipoprotein concentrations and BMI according to the -16071G/2G polymorphism genotype in the MMP-1 gene.

Parameter	DM(+) I stroke ($n = 117$)		DM(−) I stroke ($n = 79$)		Controls ($n = 192$)		*P* values		
	1G/1G + 2G/2G	1G/2G	1G/1G + 2G/2G	1G/2G	1G/1G + 2G/2G	1G/2G	P1	P2	P3
TC (mM)	4.52 ± 1.15	4.71 ± 1.14	4.50 ± 1.15	4.58 ± 1.19	4.52 ± 0.76	4.35 ± 0.84	0.39	0.40	0.37
HDL-C (mM)	0.97 ± 0.24	1.15 ± 0.4	1.05 ± 0.34	1.20 ± 0.5	1.26 ± 0.30	0.94 ± 0.30	0.56	0.17	0.44
LDL-C (mM)	3.02 ± 1.26	2.87 ± 1.11	2080 ± 1.20	2.72 ± 1.22	2.75 ± 0.73	2.83 ± 0.78	0.38	0.58	0.34
TG (mM)	1.16 ± 0.48	1.48 ± 0.7	1.36 ± 0.66	1.37 ± 0.72	1.11 ± 0.54	1.20 ± 0.46	0.44	0.44	0.41
BMI (kg/m^2)	25 ± 2.7	26.73 ± 5.21	26.28 ± 3.5	26.06 ± 3.15	25.35 ± 2.98	26.95 ± 5.56	0.15	0.15	0.26

p1, control versus DM (+) I stroke; p2, control versus DM (−) I stroke; p3, DM (+) I stroke versus DM (−) I stroke.

would retard collagen accumulation and thereafter would retard plaque progression [57], or it would, in the opposite, participate in fibrous plaque disruption or erosion leading to clinical manifestations of cardiovascular diseases. Concerning the MMP-12 polymorphisms, the effect of MMP-12 -82A/G on type 2 diabetes has been investigated. In the current study, G allele carriers were associated with the risk of ischemic stroke and the relative risk was more important in diabetic patients which suggested that MMP-12 -82A/G may be a risk factor for ischemic stroke in diabetic patients. Our study was in accordance with a study of Shimizu et al. [48] in coronary artery aneurysm (CAA) patients and in contradiction with another studies in coronary aneurysms [30], intracranial aneurysms [58] and in vulnerable plaques in Chinese Han population [33]. However, in a previous study of Jormsjö et al. [26], the A allele showed a higher promoter activity of MMP-12 and was associated with increased coronary artery stenosis in diabetic patients. The effect of 1082A/G polymorphism of the MMP-12 on type 2 diabetes has not been investigated. In the current study, we showed, for the first time, an association between the MMP-12 1082A/G polymorphism and the risk of ischemic stroke which was also more important in diabetic patients. This data suggested that MMP-12 1082 A/G polymorphism may be also a risk factor for ischemic stroke in diabetic patients. Similar to our results, MMP-12 1082A/G polymorphism has been also correlated with the outcome in breast cancer [49], in lung cancer among men (Li [32]) and in early-stage non small cell lung cancer (NSCLC) [19]. Whereas, it was not associated with the incidence of restenosis or clinical events in a Tunisian population with CAD [23]. In addition, our study showed that diabetes seemed to increase significantly the risk of ischemic stroke which was in accordance with many previous studies [2,8,44]. The effect of MMP-12 polymorphisms on stroke may be mediated through a mechanism other than lipid metabolism. It could be that these MMP-12 variants expressed variable effects based on a variable interaction with other genetic and environmental factors or possibly through mechanisms involving inflammation. Indeed, in addition to degrading ECM proteins, MMP-12 may promote macrophage recruitment to the vessel wall by activating TNF-α or by modulating levels of proinflammatory cytokines such as monocyte chemotactic protein-1 (MCP-1) [18]. Cytokines are able to induce these enzymes in cervical blood vessel walls. In our study, we only evaluated the association of MMP-12 polymorphisms with the risk of ischemic stroke in haplotype combinations. The results of haplotype analysis were consistent with the genotype analysis. Our finding showed that the -82G-1082G haplotype of MMP-12 was associated with higher risk of ischemic stroke in diabetic patients. Our results were not in accordance with a study of Haq et al. [17] in patients with chronic obstructive pulmonary disease (COPD). In this study, haplotype analysis was only performed for MMP-12 gene variants. It is possible that functional polymorphisms of other MMP genes such as

Table 6
Distribution of genotypes and alleles of 3 single nucleotide polymorphisms in MMP genes.

MMP genotypes	DM(+) I stroke ($n = 117$)	DM(−) I stroke ($n = 79$)	controls ($n = 192$)	Total patient ($n = 196$)	*P** values			
Major Minor	N (%)	N (%)	N (%)	N (%)	P1	P2	P3	P4
Alleles					*AOR (95% CI)			
MMP1 G-1607GG					$P = 0.074$	$P = 0.119$	$P = 0.56$	$P = 0.080$
1G/1G	61(52.1%)	45(57%)	121(63%)	106(54.1%)				
1G/2G + 2G/2G	56(47.9%)	34(43%)	71(37%)	90(45.9%)	*1.10 (0.39–1.49)	*1.06 (0.75–1.35)	*1.09 (0.66–1.42)	*1.14 (0.76–1.52)
Allele 1G	0.69	0.65	0.71	0.67				
Allele 2G	0.31	0.35	0.29	0.33				
MMP12 A-82G					$P = 0.033$	$P = 0.858$	$P = 0.41$	$P = 0.001$
A/A	82(70.08%)	59(74.68%)	180(93.75%)	141(71.93%)				
G/G + A/G	35(29.91%)	20(25.31%)	12(6.25%)	55(28.06%)	*1.83 (1.10–2.67)	*1.23 (0.99–1.46)	*1.03 (0.98–1.56)	*1.79 (1.29–2.69)
Allele A	0.78	0.81	0.95	0.79				
Allele G	0.22	0.19	0.05	0.21				
MMP12 A1082G					$P = 0.013$	$P = 0.354$	$P = 0.19$	$P = 0.001$
A/A	58(49.57%)	47(59.49%)	145(75.52%)	105(53.57%)				
G/G + A/G	59(50.42%)	32(40.50%)	47(24.47%)	91(46.42%)	*1.73 (1.12–2.63)	* 1.25 (0.87– 1.58)	*1.01 (0.76– 1.47)	*1.68 (1.22–2.56)
Allele A	0.65	0.71	0.79	0.68				
Allele G	0.35	0.29	0.21	0.32				

DM(+) I stroke, ischemic stroke patients with diabetes; DM(−) I stroke, ischemic stroke patients without diabetes; p1, control versus DM(+) I stroke; p2, control versus DM(−) I stroke; p3. DM (+) I stroke versus DM (−) I stroke; p4, controls versus total patient.
*P**-values adjusted by logistic regression for sex, age, smoking, obesity, dyslipidemia and hypertension.
* Adjusted odds ratios (AOR) are relative to genotype, CI: confidence interval.

Table 7
Estimation of the main haplotype frequencies.

Haplotypes		DM(+) I stroke ($n = 117$)			DM(−) I stroke ($n = 79$)			Total patients ($n = 196$)			Controls ($n = 192$)
MMP12 (-82A/G)	MMP12 (1082A/G)	Frequency	AOR (95%CI)	*P** value	Frequency	AOR (95%CI)	*P** value	Frequency	AOR (95%CI)	*P** value	Frequency
A	A	0.614	1[a]		0.662	1[a]		0.620	1[a]		0.751
A	G	0.274	1.33 (0.77–2.22)	0.081	0.248	1.26 (0.65–2.15)	0.095	0.257	1.49 (0.89–2.42)	0.089	0.196
G	A	0.045	1.45 (0.87– 2.32)	0.322	0.036	1.19 (0.75 – 2.21)	0.371	0.050	1.52 (0.98–2.52)	0.291	0.030
G	G	0.066	2.33 (1.25 – 3.62)	0.032	0.053	1.45 (0.97–2.25)	0.065	0.072	2.28 (1.29–3.55)	0.029	0.022

*P** adjusted for sex, age, smoking, obesity, dyslipidemia and hypertension.
AOR: adjusted odds ratios.
1[a] Haplotype used as reference.

MMP-3 may affect the association between these two polymorphisms of MMP-12 and the risk of ischemic stroke. More studies are needed to confirm these findings.

5.1. Conclusion

Even the little sample size of our population, our study concluded that only the MMP-12 polymorphisms are associated with higher risk of ischemic stroke in diabetic patients in singly and in haplotype combination. Our results suggest that polymorphisms of the MMP-12 gene may be linked with T2DM coexisting with ischemic stroke, and therefore it can be considered as a potential marker of cerebrovascular disorders in diabetic patients. These findings need to be investigated in larger populations to better clarify this association and further investigations are needed to explain the potential role of MMP-12 in diabetes and its complications.

Conflict of interest

The authors declare there is no conflict of interest.

Acknowledgments

We would like to thank Dr. Jesús M. Martín-Campos, Hospital de la Santa Creu i Sant Pau, Servei de Bioquímica, Barcelona, Spain for help in the statistical analysis. A part of this work was supported by a grant from “Ministère de l'Enseignement Supérieur, de la Recherche Scientifique et de la Technologie-Tunisie (grant no. 800780).”

References

[1] Astolfi CM, Shinohara AL, da Silva RA, Santos MC, Line SR, de Souza AP. Genetic polymorphisms in the MMP-1 and MMP-3 gene may contribute to chronic periodontitis in a Brazilian population. J Clin Periodontol 2006;33:699–703.
[2] Banerjee C, Moon YP, Paik MC. Duration of diabetes and risk of ischemic stroke. The Northern Manhattan Study. Stroke 2012;43:1212–7.
[3] Brinckerhoff CE, Matrisian LM. Matrix metalloproteinases: a tail of a frog that became a prince. Nat Rev Mol Cell Biol 2002;3:207–14.
[4] Buss A, Pech K, Roelver S, Bloemeke B, Klotzsch C, Breuer S. Functional polymorphisms in matrix metalloproteinases -1, -3, -9 and -12 in relation to cervical artery dissection. BMC Neurol 2009:9–40.
[5] Chandler S, Cossins J, Lury J, Wells G. Macrophage metalloelastase degrades matrix and myelin proteins and processes a tumour necrosis factor-alpha fusion protein. Biochem Biophys Res Commun 1996;228:421–9.
[6] Cho NH, Hong KP, Hong SH, Kang S, Chung KY, Cho SH. MMP expression profiling in recurred stage IB lung cancer. Oncogene 2004;23:845–51.
[7] De Souza AP, Trevilatto PC, Scarel-Caminaga RM, Brito RB, Line SR. MMP-1 promoter polymorphism: association with chronic periodontitis severity in a Brazilian population. J Clin Periodontol 2003;30:154–8.
[8] Djelilovic-Vranic J, Alajbegovic A, Zelija-Asimi V, Niksic M, Tiric-Campara M, Salcic S, et al. Predilection role diabetes mellitus and dyslipidemia in the onset of ischemic stroke. Med Arh 2013;67(2):120–3.
[9] Djurić T, Stojković L, Zivković M, Končar I, Stanković A, Djordjević A, et al. Matrix metalloproteinase-1 promoter genotypes and haplotypes are associated with carotid plaque presence. Clin Biochem 2012;45:1353–6.
[10] Drzewoski J, Sliwińska A, Przybyłowska K, Sliwiński T, Kasznicki J, Zurawska-Klis M, et al. Gene polymorphisms and antigen levels of matrix metalloproteinase-1 in type 2 diabetes mellitus coexisting with coronary heart disease. Kardiol Pol 2008;66:1042–8.
[11] Dunleavey L, Beyzade S, Ye S. Rapid genotype analysis of matrix metalloproteinase-1 gene 1G/2G polymorphism that is associated with risk of cancer. Matrix Biol 2000;19:175–7.
[12] Expert Committee on the Diagnosis, Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2003;26:S5–S20.
[13] Fiotti N, Moretti ME, Bussani R, Altamura N, Zamolo F, Gerloni R, et al. Features of vulnerable plaques and clinical outcome of UA/NSTEMI: relationship with matrix metalloproteinase functional polymorphisms. Atherosclerosis 2011;215:153–9.
[14] Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002;90:251–62.
[15] Gronski Jr TJ, Martin RL, Kobayashi DK, Walsh BC, Holman MC, Huber M, et al. Hydrolysis of a broad spectrum of extracellular matrix proteins by human macrophage elastase. J Biol Chem 1997;272:12189–94.
[16] Han Y, Wu Z, Zhang X, Yan C, Xi S, Yang Y, et al. Impact of matrix metalloproteinase-1 gene variations on risk of acute coronary syndrome. Coron Artery Dis 2008;19:227–30.
[17] Haq I, Chappell S, Johnson SR, Lotya J, Daly L, Morgan K, et al. Association of MMP-12 polymorphisms with severe and very severe COPD: a case control study of MMPs-1, 9 and 12 in a European population. BMC Med Genet 2010;11:7. http://dx.doi.org/10.1186/1471-2350-11-7.
[18] Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. Science 1997;277:2002–4.
[19] Heist RS, Marshall AL, Liu G, Zhou W, Su L, Neuberg D, et al. Matrix metalloproteinase polymorphisms and survival in stage I non-small cell lung cancer. Clin Cancer Res 2006;12(18):5448–53.
[20] Hofmann HS, Hansen G, Richter G, Taege C, Simm A, Silber RE, et al. Matrix metalloproteinase-12 expression correlates with local recurrence and metastatic disease in non-small cell lung cancer patients. Clin Cancer Res 2005;11:1086–92.
[21] Hou T, Gao L, Zheng J, Liu Z, Wu C, Liu Q, et al. Matrix metalloproteinase-1 gene polymorphisms and periodontitis susceptibility: a meta-analysis based on 11 case–control studies. Gene 2013;521:111–5.
[22] Hughes S, Agbaje O, Bowen RL, Holliday DL, Shaw JA, Duffy S, et al. Matrix metalloproteinase single nucleotide polymorphisms and haplotypes predict breast cancer progression. Clin Cancer Res 2007;13:6673–80.
[23] Jguirim-Souissi I, Jelassi A, Slimani A, Addad F, Hassine M, Hamda KB, et al. Matrix metalloproteinase-1 and matrix metalloproteinase-12 gene polymorphisms and the outcome of coronary artery disease. Coron Artery Dis 2011;22:388–93.
[24] Jinju Li, Ghio Andrew J, Cho Seung-Hyun, Brinckerhoff Constance E, Sidney A, Liedtke W, Simon. Diesel exhaust particles activate the matrix-metalloproteinase-1 gene in human bronchial epithelia in a β-arrestin-dependent manner via activation of RAS. Environ Health Perspect 2009;117:400–9.
[25] Joos L, He JQ, Shepherdson MB, Connett JE, Anthonisen NR, Paré PD, et al. The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. Hum Mol Genet 2002;11:569–76.
[26] Jormsjö S, Ye S, Moritz J, Walter DH, Dimmeler S, Zeiher AM, et al. Allele specific regulation of matrix metalloproteinase-12 gene activity is associated with coronary artery luminal dimensions in diabetic patients with manifest coronary artery disease. Circ Res 2000;86:998–1003.
[27] Kanamori Y, Matsushima M, Minaguchi T, Kobayashi K, Sagae S, Kudo R, et al. Correlation between expression of the matrix metalloproteinase-1 gene in ovarian cancers and an insertion/deletion polymorphism in its promoter region. Cancer Res 1999;59:4225–7.
[28] Kuo HP, Wang YM, Wang CH, He CC, Lin SM, Lin HC, et al. Matrix metalloproteinase-1 polymorphism in Taiwanese patients with endobronchial tuberculosis. Tuberculosis 2008;88:262–7.
[29] Kuzuya M, Iguchi A. Role of matrix metalloproteinases in vascular remodeling. J Atheroscler Thromb 2003;10:275–82.
[30] Lamblin N, Bauters C, Hermant X, Lablanche JM, Helbecque N, Amouyel P. Polymorphisms in the promoter regions of MMP-2, MMP-3, MMP-9 and MMP-12 genes as determinants of aneurysmal coronary artery disease. J Am Coll Cardiol 2002;40:43–8.

[31] Li G, Yue Y, Tian Y, Li JL, Wang M, Liang H, et al. Association of matrix metalloproteinase (MMP)-1, 3, 9, interleukin (IL)-2, 8 and cyclooxygenase (COX)-2 gene polymorphisms with chronic periodontitis in a Chinese population. Cytokine 2012;60:552–60.
[32] Su Li, Zhou Wei, Asomaning Kofi, Lin Xihong, Wain John C, Lynch Thomas J, et al. Genotypes and haplotypes of matrix metalloproteinase 1, 3 and 12 genes and the risk of lung cancer. Carcinogenesis 2006;27(5):1024–9.
[33] Li W, Jin X, Zhou Y, Zhu M, Lin X, Hu X, et al. Lack of independent relationship between the MMP-12 gene polymorphism and carotid plaque susceptibility in the Chinese Han population. Vasc Med 2012;17:310–6.
[34] Manetti M, Ibba-Manneschi L, Fatini C, Guiducci S, Cuomo G, Bonino C, et al. Association of a functional polymorphism in the matrix metalloproteinase-12 promoter region with systemic sclerosis in an Italian population. J Rheumatol 2010;37:1852–7.
[35] Massarotti M, Marchesoni A, Biondi ML, Marasini B. Polymorphism in the matrix metalloproteinase-1 promoter gene and severity of rheumatoid arthritis. J Rheumatol 2002;29:2241–2.
[36] Mercer BA, Wallace AM, Brinckerhoff CE, D'Armiento JM. Identification of a cigarette smoke responsive region in the distal MMP-1 promoter. Am J Respir Cell Mol Biol 2009;40:4–12.
[37] Meschia JF, Brott TG, Chukwudelunzu FE, Hardy J, Brown Jr RD, Meissner I, et al. Verifying the stroke-free phenotype by structured telephone interview. Stroke 2000;31(5):1076–80.
[38] Newby AC. Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. Physiol Rev 2005;85:1–31.
[39] Nikkari ST, O'Brien KD, Ferguson M, Hatsukami T, Welgus HG, Alpers CE, et al. Interstitial collagenase (MMP-1) expression in human carotid atherosclerosis. Circulation 1995;92:1393–8.
[40] Nishioka Y, Kobayashi K, Sagae S, Ishioka S, Nishikawa A, Matsushima M, et al. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter in endometrial carcinomas. Jpn J Cancer Res 2000;91:612–5.
[41] Nishioka Y, Sagae S, Nishikawa A, Ishioka Si, Kudo R. A relationship between matrix metalloproteinase-1 (MMP-1) promoter polymorphism and cervical cancer progression. Cancer Lett 2003;200:49–55.
[42] Orbe J, Fernandez L, Rodríguez JA, Rábago G, Belzunce M, Monasterio A, et al. Different expression of MMPs/TIMP-1 in human atherosclerotic lesions. Relation to plaque features and vascular bed. Atherosclerosis 2003;170:269–76.
[43] Pearce E, Tregouet DA, Samnegård A, Morgan AR, Cox C, Hamsten A, et al. Haplotype effect of the matrix metalloproteinase-1 gene on risk of myocardial infarction. Circ Res 2005;97:1070–6.
[44] Peters SA, Huxley RR, Woodward M. Diabetes as a risk factor for stroke in women compared with men: a systematic review and meta-analysis of 64 cohorts, including 775 385 individuals and 12 539 strokes. Lancet 2014;S0140-6736(14):60040–4. http://dx.doi.org/10.1016/S0140-6736(14)60040-4.
[45] Pirhan D, Atilla G, Emingil G, Sorsa T, Tervahartiala T, Berdeli A. Effect of MMP-1 promoter polymorphisms on GCF MMP-1 levels and outcome of periodontal therapy in patients with severe chronic periodontitis. J Clin Periodontol 2008;35:862–70.
[46] Rouis M. Matrix metalloproteinases : a potential therapeutic target in atherosclerosis. Curr Drug Targets Cardiovasc Haematol Disord 2005;5:541–8.
[47] Rutter JL, Mitchell TI, Butticè G, Meyers J, Gusella JF, Ozelius LJ, et al. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res 1998;58:5321–5.
[48] Shimizu C, Matsubara T, Onouchi Y, Jain S, Sun S, Nievergelt CM, et al. Matrix metalloproteinase haplotypes associated with coronary artery aneurysm formation in patients with Kawasaki disease. J Hum Genet 2010;55:779–84.
[49] Shin A, Cai Q, Shu XO, Gao YT, Zheng W. Genetic polymorphisms in the matrix metalloproteinase 12 gene (MMP-12) and breast cancer risk and survival: the Shanghai Breast Cancer Study. Breast Cancer Res 2005;7:R506–12.
[50] Song YQ, Ho DW, Karppinen J, Kao PY, Fan BJ, Luk KD, et al. Association between promoter -1607 polymorphism of MMP-1 and lumbar disc disease in Southern Chinese. BMC Med Genet 2008;9:38.
[51] Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89.
[52] Wallace AM, Sandford AJ. Genetic polymorphisms of matrix metalloproteinases: functional importance in the development of chronic obstructive pulmonary disease? Am J Pharmocogenomics 2002;2:167–75.
[53] Wang JG, Staessen JA, Franklin SS, Fagard R, Gueyffier F. Systolic and diastolic blood pressure lowering as determinants of cardiovascular outcome. Hypertension 2005;45:907–13.
[54] Ye S. Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. Matrix Biol 2000;19:623–9.
[55] Ye S. Influence of matrix metalloproteinase genotype on cardiovascular disease susceptibility and outcome. Cardiovasc Res 2006;69:636–45.
[56] Ye S, Dhillon S, Turner SJ, Bateman AC, Theaker JM, Pickering RM, et al. Invasiveness of cutaneous malignant melanoma is influenced by matrix metalloproteinase 1 gene polymorphism. Cancer Res 2001;61:1296–8.
[57] Ye S, Gale CR, Martyn CN. Variation in the matrix metalloproteinase-1 gene and risk of coronary heart disease. Eur Heart J 2003;24:1668–71.
[58] Zhang B, Dhillon S, Geary I, Howell WM, Iannotti F, Day IN, et al. Polymorphisms in matrix metalloproteinase-1, -3, -9, and -12 genes in relation to subarachnoid hemorrhage. Stroke 2001;32:2198–202.