



Matrix metalloproteinase gene polymorphisms in patients with coronary artery disease

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Abstract

Matrix metalloproteinases (MMPs) play an important role in the pathogenesis of atherosclerosis, the pathology underlying the majority of coronary artery disease (CAD). In this study we tested the hypothesis that polymorphic variation in the MMP genes influences the risk of developing atherosclerosis. We analyzed functional polymorphisms in the promoter of the MMP-1, MMP-3, MMP-9 and MMP-12 genes in 183 Brazilian Caucasian individuals submitted to coronary angiography, of which 67 (37%) had normal coronary arteries (control group) and 116 (63%) had CAD (CAD patient group). The -1607 1G/2G MMP-1, -1171 5A/6A MMP-3, -1562 C/T MMP-9, -82 A/G MMP-12 polymorphisms were analyzed by PCR followed by restriction digestion. No significant differences were observed in allele frequencies between the CAD patients and controls. Haplotype analysis showed no differences between the CAD patients and controls. There was a significant difference in the severity of CAD, as assessed by the number of diseased vessels, in MMP-1 1G/1G homozygous individuals and in those homozygous for the 6A allele of the MMP-3 polymorphism. However, multivariate analysis showed that diabetes mellitus was the only variable independently associated with CAD severity. Our findings indicated that MMP polymorphisms have no significant impact on the risk and severity of CAD.

Key words: atherosclerosis, coronary artery disease, gene polymorphisms, matrix metalloproteinases.

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Introduction

Atherosclerosis, the underlying pathology of coronary artery disease (CAD), is a common, multifactorial disorder with both genetic and environmental components. Its heritability is estimated to be 50-60%, but its genetic basis remains incompletely understood (Lusis, 2000). Matrix metalloproteinases (MMPs) form a family of zinc-dependent enzymes with proteolytic activity against connective tissue proteins such as collagen, proteoglycans and elastin (Visse and Nagase, 2003). Several lines of evidence have implicated MMPs in both atherogenesis and the precipitation of acute coronary syndromes by regulating connective

tissue remodeling, thus determining the volume expansion of the atherosclerotic plaque, its stability and the potential for smooth muscle cell proliferation (Newby, 2005). Previous studies have found that MMP-1 (interstitial collagenase), MMP-3 (stromelysin-1), MMP-9 (92-kD gelatinase or gelatinase B) and MMP-12 (macrophage metalloelastase) are expressed in atheroma at high levels compared with normal vessel walls (Jones *et al.*, 2003; Newby, 2005). These MMPs have the capacity to degrade virtually all components of the extracellular matrix in the arterial wall (collagens, elastin, proteoglycans, laminin, fibronectin, etc.). According to the important role of MMPs in the atherogenesis it has been shown that genetic variation affecting the expression of MMPs influences the susceptibility and progression of various diseases, including cardiovascular diseases (Ye, 2000, 2006). Expression of MMPs is regulated primarily at the transcriptional level where the

promoter of the genes responds to different regulators (Nagase and Woessner, 1999). Some functional polymorphisms have been described in the regulatory region of MMP genes (Ye, 2000). Therefore, the aim of the study reported in this paper was to ascertain if polymorphisms in the promoter of MMP-1, MMP-3, MMP-9 and MMP-12 genes are associated with the risk of developing CAD in a Brazilian population of European ancestry.

Subjects and methods

Study population

A total of 183 consecutive and unrelated Brazilian Caucasian individuals admitted for routine angiography, for investigation of chest pain and/or CAD suspected were recruited for the study from the Hemodynamics Service of the Hospital de Clínicas de Porto Alegre (Rio Grande do Sul, Brazil) as described previously (Simon *et al.*, 2003). Individuals with angiography-proven $\geq 50\%$ stenosis in a major coronary artery or one of their branches constituted the CAD group (116 individuals) and those without angiographically significant lesion constituted the control group (67 individuals). All individuals answered a standard questionnaire concerning their personal history and we recorded demographic and clinical data (Table 1). The studied population is descended from Europeans, mainly from Portugal, Spain, Italy and Germany (Salzano and Freire-Maia, 1970). The study was approved by the Institutional Ethics Committee of the Universidade Luterana do Brasil. All subjects gave written informed consent for a blood sample drawn for DNA extraction to be used in studies approved by the Hospital Ethics Committee. Individuals reporting regular smoking during the last five years were considered to be smokers. Hypertension was defined as present if the mean blood pressure was higher than 140/90 mmHg, or if the patient was already under treatment with antihypertensive drugs at study inclusion. Diabetes mellitus was diagnosed as present if the fasting blood glucose level was ≥ 126 mg dL⁻¹ or if the individuals were on treatment with insulin or other antidiabetic agents at the time of inclusion. Dyslipidemia

was defined by levels of triglycerides ≥ 150 mg dL⁻¹, HDL cholesterol < 40 mg dL⁻¹ and LDL cholesterol > 100 mg L⁻¹, or if the individual was receiving lipid-lowering treatment at inclusion. Overweight or obesity was defined as a body-mass index (BMI) ≥ 25 kg m⁻².

DNA analysis

High-molecular weight DNA was extracted from whole blood using a non-enzymatic technique for DNA analysis (Lahiri and Nurnberger, 1991) and amplified using the polymerase chain reaction (PCR). The -1607 1G/2G MMP-1 polymorphism was analyzed as described by Dunleavy *et al.* (2000a), this polymorphism being characterized by the presence of 1 guanine base (1G) or 2 guanine bases (2G). Carriers of the 2G allele seem to have higher MMP-1 activity than carriers of the 1G allele (Rutter *et al.*, 1998) as well as a reduced risk of coronary artery disease (Ye *et al.*, 2003). The -1171 5A/6A MMP-3 polymorphism was analyzed as described by Dunleavy *et al.* (2000b), this polymorphism being characterized by the presence of 5 adenine bases (5A) or 6 adenine bases (6A). Carriers of the 6A allele have lower MMP-3 expression than carriers of the 5A allele (Ye *et al.*, 1996) and a higher coronary atheroma growth (Ye *et al.*, 1995; Humphries *et al.*, 1998; de Maat *et al.*, 1999). The -1562 C/T MMP-9 polymorphism was analyzed as described by Morgan *et al.* (2003), this polymorphism being caused by a single base change C→T. Carriers of the T allele have higher MMP-9 transcriptional activity than carriers of the C allele (Pollanen *et al.*, 2001) and a higher severity to coronary atherosclerosis (Zhang *et al.*, 1999; Pollanen *et al.*, 2001; Morgan *et al.*, 2003). The -82 A/G MMP-12 polymorphism was analyzed as described by Zhang *et al.* (2001), this polymorphism being caused by a single base change A→G. Carriers of the A allele have higher MMP-12 transcriptional activity than carriers of the G allele and higher coronary stenosis (Jormsjo *et al.*, 2000).

Statistical analysis

Allele frequencies were determined by direct count of the alleles. Departures from Hardy-Weinberg equilibrium and differences between groups were evaluated by the chi-square test. The quantitative variables were compared between groups using the Student *t*-test or Mann-Whitney non-parametric test when indicated. Haplotype frequencies were determined using the SNPAnalyzer computer software which employs an expectation-maximization algorithm (Yoo *et al.*, 2005). Comparisons between genotype groups were performed by using unpaired *t*-tests or chi-square tests, as appropriate. Logistic regression analysis was performed to eliminate confounding factors, and the dependent variable was the number of diseased vessels (single-vessel lesion and two or three-vessel lesion). The independent variables tested were age, body mass index, dyslipidemia, diabetes mellitus, gender, hypertension,

Table 1 - Clinical and demographic characteristics of coronary artery disease (CAD) patients and controls. Data reported as percentages, except for age (years).

	CAD patients (n = 116)	Controls (n = 67)	p value
Age (years)	62.5 ± 10.7	56.4 ± 11.7	0.001
Male gender	75.0	44.8	< 0.001
Diabetes mellitus	16.4	13.4	0.59
Dyslipidemia	44.0	26.9	0.02
Hypertension	78.4	73.1	0.41
Overweight or obese	72.4	70.1	0.74
Smoker (current or former)	31.9	28.3	0.62

MMP genotypes and smoking status. A significance level of $p < 0.05$ was considered to be significant.

Results

Clinical and demographic baseline characteristics of the population study are summarized in Table 1. No significant differences were observed between patients and controls for some CAD risk factors (diabetes mellitus, hypertension, overweight/obesity and smoking), although patients were significantly older and there were more males and dyslipidemics in the patient group than in the control group.

The allele and genotype frequencies of the MMP polymorphisms in CAD patients and controls are presented in Table 2. The observed genotype frequencies were in Hardy-Weinberg equilibrium in the total sample and in all subgroups. No significant differences were observed in genotype frequencies between the 116 CAD patients and 67 control individuals for any of the polymorphisms analyzed.

Since the MMP-1, MMP-3 and MMP-12 genes are in the same chromosome cluster (11q22.3), haplotype analysis was undertaken and the estimated haplotype frequencies are presented in Table 3. No significant differences were observed between CAD patients and controls in regard to haplotype frequencies. The haplotype 1G 6A A (MMP-1, MMP-3 and MMP-12 genes, respectively), that carry the risk alleles, showed similar frequencies between patients and controls.

To assess the influence of the MMP genotypes on the severity of coronary atherosclerosis we divided the CAD patient group ($n = 116$) into two sub-groups, one sub-group containing patients with a single-vessel lesion ($n = 40$) and the other sub-group containing patients with two ($n = 40$) or three ($n = 36$) major epicardial coronary arteries with a $> 50\%$ reduction in luminal diameter. The proportion of patients with two or more diseased vessels was significant higher in individuals with the MMP-1 1G/1G genotype (86%) than those with the 1G/2G and 2G/2G genotypes (61%) ($p < 0.05$). We also observed a significant difference in the severity of CAD in individuals with MMP-3 6A/6A when compared with those with the 5A/6A and 5A/5A genotypes ($p < 0.05$). No significant association was observed between the MMP-9 and MMP-12 genotypes and CAD severity. Logistic regression analysis was performed to assess the independent influence of MMP-1 and MMP-3 genotypes on the severity of CAD but after adjusting for confounding variables we found that diabetes mellitus was the only variable independently associated with CAD severity ($p < 0.05$).

Discussion

A major contribution of a single gene is very unlikely for a multifactorial disease such as CAD, where a large number of disease mechanisms results in one clinical out-

Table 2 - Allele and genotype frequencies of matrix metalloproteinase (MMP) gene polymorphisms in coronary artery disease (CAD) patients and control individuals.

Allele and genotype	Frequency (% in parentheses)		p value
	Patients (n = 116)	Controls (n = 67)	
MMP-1 (-1607 1G/2G)			
Allele			
1G	115 (49.6)	62 (46.3)	0.54
2G	117 (50.4)	72 (53.7)	
Genotype			
1G/1G	29 (25.0)	15 (22.3)	0.82
1G/2G	57 (49.1)	32 (47.8)	
2G/2G	30 (25.9)	20 (29.9)	
MMP-3 (-1171 5A/6A)			
Allele			
5A	102 (44.0)	62 (46.0)	0.67
6A	130 (56.0)	72 (54.0)	
Genotype			
6A/6A	37 (32.0)	17 (25.0)	0.52
5A/6A	56 (48.0)	38 (57.0)	
5A/5A	23 (20.0)	12 (18.0)	
MMP-9 (-1562 C/T)			
Allele			
C	206 (88.8)	113 (84.3)	0.21
T	26 (11.2)	21 (15.7)	
Genotype			
CC	92 (79.3)	46 (68.6)	0.10
CT	22 (19.0)	21 (31.4)	
TT	2 (1.7)	-	
MMP-12 (-82 A/G)			
Allele			
A	197 (84.9)	118 (88.0)	0.40
G	35 (15.1)	16 (12.0)	
Genotype			
AA	85 (73.3)	52 (77.6)	0.67
AG	27 (23.3)	14 (20.9)	
GG	4 (3.4)	1 (1.5)	

Table 3 - Haplotype frequencies of the matrix metalloproteinase (MMP) gene polymorphisms (MMP-1, MMP-3 and MMP-12) in coronary artery disease (CAD) patients and control individuals.

Haplotype			Patients*	Control individuals**
MMP-1	MMP-3	MMP-12		
1G	5A	A	72 (31.0)	36 (26.9)
2G	6A	A	56 (24.1)	34 (25.4)
1G	6A	A	42 (18.1)	24 (17.9)
2G	6A	G	30 (12.9)	12 (8.9)
2G	5A	A	28 (12.1)	24 (17.9)
1G	6A	G	2 (0.9)	2 (1.5)
2G	5A	G	2 (0.9)	2(1.5)

*N = 116 (% in parentheses). **N = 67 (% in parentheses).

come. Several gene polymorphisms of the matrix extracellular system have currently been identified as risk factors for atherosclerosis and/or acute coronary syndromes. In our study, we assessed the potential association between CAD and MMP gene functional polymorphisms on the basis of the relevance of such genes to the pathophysiology of atherosclerosis. Matrix metalloproteinases can be categorized into the several groups, including collagenases (MMP-1), stromelysins (MMP-3), gelatinases (MMP-9), and metalloelastase (MMP-12), with the MMP-1, -3, -9 and -12 genes all having polymorphisms in their promoter regions associated with different transcriptional activity *in vitro*. Furthermore, three genes (MMP-1, -3 and -12) are located on the same chromosome, allowing haplotype analysis and consequently the association of risk alleles. In our sample of Caucasian Brazilian patients submitted to coronary angiography, no significant differences were observed in genotype frequencies between CAD patients and control subjects for the MMP polymorphisms analyzed. In addition, our results did not show significant association between haplotypes carrying risk-alleles and CAD.

Several studies show that MMPs are associated with CAD, but studies with MMP polymorphisms are relatively few and have produced controversial results. The MMP-3 polymorphism is the MMP genetic variant most studied in relation to atherosclerosis. The MMP-3 gene promoter 5A/6A polymorphism has been analyzed in a number of genetic epidemiological studies and there is consistent evidence for the association of this polymorphism with CAD (Ye *et al.*, 1995; Humphries *et al.*, 1998; de Maat *et al.*, 1999; Schwarz *et al.*, 2002; Beyzade *et al.*, 2003; Hirashiki *et al.*, 2003). However, in our study, we found no association between MMP-3 polymorphism and CAD, although we did observe a significant difference in the severity of CAD in patients with the MMP-3 6A/6A genotype compared with patients with the 5A/6A and 5A/5A genotypes. However, this difference did not remain after multivariate analysis.

Only two studies have been carried out concerning atherosclerosis and the MMP-1 polymorphism, with the results of these studies being controversial. Ghilardi *et al.* (2002) found that homozygosity for the 2G allele in association with MMP-3 6A homozygosity predicts an increase in the risk of internal carotid artery stenosis. On the other hand, Ye *et al.* (2003) reported a reduction in the risk of CAD in 2G homozygous individuals. In our study, we found that the number of patients with two or more diseased vessels was significant higher in patients with the MMP-1 1G/1G genotype compared to those with the 1G/2G or 2G/2G genotypes, but this difference did not remain after multivariate analysis.

In respect to MMP-12 polymorphism only one study related to CAD has been published, in which Jormsjo *et al.* (2000) reported that the MMP-12 polymorphism did not influence coronary artery luminal dimensions in patients who underwent percutaneous transluminal coronary angio-

graphy with stent implantation. However, when only diabetic patients were analyzed the G allele was associated with a greater luminal diameter compared with the A allele. In our study, no association was found when the entire patient group or diabetic subset were examined.

Our results were not consistent with the findings from some previous studies of CAD and MMP-9 polymorphism, which found an association between the -1562T allele and coronary atherosclerosis (Zhang *et al.*, 1999; Pollanen *et al.*, 2001; Morgan *et al.*, 2003). Nevertheless, other studies have also been published which found no association between the MMP-9 polymorphism and the risk of CAD (Blankenberg *et al.*, 2003; Haberbosch and Gardemann, 2005). However, Haberbosch and Gardemann (2005) showed that patients with the TT genotype had a higher degree of CAD than the other genotypes in subgroups of individuals with high apolipoprotein B levels, high lipoprotein A and/or fibrinogen levels. This suggests that the influence of the MMP-9 genotypes on CAD can be modulate by complex interactions. In this context, contradictory results on the association between the -1562 C/T MMP-9 polymorphism and MMP-9 levels have been reported in the literature. Blankenberg *et al.* (2003) found that the T allele of this polymorphism was associated with increased MMP-9 plasma levels in patients with CAD, in a fairly codominant fashion. On the other hand, Demacq *et al.* (2006) found no association between plasma MMP-9 activity and the -1562 C/T genotypes in healthy subjects, suggesting that the polymorphism would contribute to an increased cardiovascular risk under conditions of induced MMP-9 expression.

On the basis of the conflicting results of these association studies, it is possible to assume that the MMP promoter polymorphisms can have some effect on CAD, but this influence may be slight and perhaps restricted to some specific environment-genotype effect. Some studies have presented a smoking-genotype interaction in the risk of CAD in regard to the MMP-3 polymorphism (Humphries *et al.*, 2002; Liu *et al.*, 2003), while the MMP-12 polymorphism has been shown to be associated with CAD in diabetic patients (Jormsjo *et al.*, 2000) and the MMP-9 polymorphism was reported to be associated with the extent of CAD in individuals with high apolipoprotein B levels, high lipoprotein A plasma concentrations and high fibrinogen levels, or with combinations of increased levels of these coronary risk factors (Haberbosch and Gardemann, 2005).

It is important to mention that the statistical power to detect differences in the present study may be limited due to the small sample size of the control group. The sample size of CAD patient ($n = 116$) and control group ($n = 67$) were large enough to detect differences in odds ratio higher than 3.0 with 90% power at a $p < 0.05$, but the study was under-powered for detecting small increases in the risk of presenting CAD.

In conclusion, our study found no evidence for MMP gene variations as being independent risk factor for coro-

nary artery disease. Our results indicated that MMP gene polymorphisms probably have no significant impact on the risk and severity of coronary heart disease in the Brazilian Caucasian sample studied.

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References

- Beyzade S, Zhang S, Wong YK, Day IN, Eriksson P and Ye S (2003) Influences of matrix metalloproteinase-3 gene variation on extent of coronary atherosclerosis and risk of myocardial infarction. *J Am Coll Cardiol* 41:2130-2137.
- Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G, Meyer J, Cambien F, Tiret L and AtheroGene Investigators (2003) Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. *Circulation* 107:1579-1585.
- de Maat MP, Jukema JW, Ye S, Zwinderman AH, Moghaddam PH, Beekman M, Kastelein JJ, van Boven AJ, Bruschke AV, Humphries SE, *et al.* (1999) Effect of the stromelysin-1 promoter on efficacy of pravastatin in coronary atherosclerosis and restenosis. *Am J Cardiol* 83:852-856.
- Demacq C, de Souza AP, Machado AA, Gerlach RF and Tanus-Santos JE. (2006) Genetic polymorphism of matrix metalloproteinase (MMP)-9 does not affect plasma MMP-9 activity in healthy subjects. *Clin Chim Acta* 365:183-187.
- Dunleavey L, Beyzade S and Ye S (2000a) Rapid genotype analysis of the matrix metalloproteinase-1 gene 1G/2G polymorphism that is associated with risk of cancer. *Matrix Biol* 19:175-177.
- Dunleavey L, Beyzade S and Ye S (2000b) Rapid genotype analysis of the stromelysin gene 5A/6A polymorphism. *Atherosclerosis* 151:587-589.
- Ghilardi G, Biondi ML, DeMonti M, Turri O, Guagnellini E and Scorza R (2002) Matrix metalloproteinase-1 and matrix metalloproteinase-3 gene promoter polymorphisms are associated with carotid artery stenosis. *Stroke* 33:2408-2412.
- Haberbosch W and Gardemann A (2005) Gelatinase B C(-1562)T polymorphism in relation to ischaemic heart disease. *Scand J Clin Lab Invest* 65:513-522.
- Hirashiki A, Yamada Y, Murase Y, Suzuki Y, Kataoka H, Morimoto Y, Tajika T, Murohara T and Yokota M (2003) Association of gene polymorphisms with coronary artery disease in low- or high-risk subjects defined by conventional risk factors. *J Am Coll Cardiol* 42:1429-1437.
- Humphries SE, Luong LA, Talmud PJ, Frick MH, Kesaniemi YA, Pasternack A, Taskinen MR, and Syvanne M (1998) The 5A/6A polymorphism in the promoter of the stromelysin-1 (MMP-3) gene predicts progression of angiographically determined coronary artery disease in men in the LOCAT gemfibrozil study. *Atherosclerosis* 139:49-56.
- Humphries SE, Martin S, Cooper J and Miller G (2002) Interaction between smoking and the stromelysin-1 (MMP3) gene 5A/6A promoter polymorphism and risk of coronary heart disease in healthy men. *Ann Hum Genet* 66:343-352.
- Jones CB, Sane DC and Herrington DM (2003) Matrix metalloproteinases: A review of their structure and role in acute coronary syndrome. *Cardiovasc Res* 59:812-823.
- Jormsjo S, Ye S, Moritz J, Walter DH, Dimmeler S, Zeiher AM, Henney A, Hamsten A and Eriksson P (2000) 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* 86:998-1003.
- Lahiri DK and Nurnberger JI Jr (1991) A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 19:5444.
- Liu PY, Chen JH, Li YH, Wu HL and Shi GY (2003) Synergistic effect of stromelysin-1 (matrix metallo-proteinase-3) promoter 5A/6A polymorphism with smoking on the onset of young acute myocardial infarction. *Thromb Haemost* 90:132-139.
- Lusis AJ (2000) Atherosclerosis. *Nature* 407:233-241.
- Morgan AR, Zhang B, Tapper W, Collins A and Ye S (2003) Haplotype analysis of the MMP-9 gene in relation to coronary artery disease. *J Mol Med* 81:321-326.
- Nagase H and Woessner Jr JF (1999) Matrix metalloproteinases. *J Biol Chem* 274:21491-21494.
- Newby AC (2005) Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. *Physiol Rev* 85:1-31.
- Pollanen PJ, Karhunen PJ, Mikkelsen J, Laippala P, Perola M, Penttila A, Mattila KM, Koivula T and Lehtimaki T (2001) Coronary artery complicated lesion area is related to functional polymorphism of matrix metalloproteinase 9 gene: An autopsy study. *Arterioscler Thromb Vasc Biol* 21:1446-1450.
- Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ and Brinckerhoff CE (1998) A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res* 58:5321-5325.
- Salzano FM and Freire-Maia N (eds) (1970) Problems in Human Biology. A Study of Brazilian Populations. Wayne State University Press, Detroit, 200 pp.
- Schwarz A, Haberbosch W, Tillmanns H and Gardemann A (2002) The stromelysin-1 5A/6A promoter polymorphism is a disease marker for the extent of coronary heart disease. *Dis Markers* 18:121-128.
- Simon D, Paludo CA, Ghisleni GC, Manfroi WC and Roisenberg I (2003) Association studies between -1185A/G von Willebrand factor gene polymorphism and coronary artery disease. *Braz J Med Biol Res* 36:709-714.
- Visse R and Nagase H (2003) Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. *Circ Res* 92:827-839.
- Ye S (2000) Polymorphism in matrix metalloproteinase gene promoters: Implication in regulation of gene expression and susceptibility of various diseases. *Matrix Biol* 19:623-629.
- Ye S (2006) Influence of matrix metalloproteinase genotype on cardiovascular disease susceptibility and outcome. *Cardiovasc Res* 69:636-645.

- Ye S, Watts GF, Mandalia S, Humphries SE and Henney AM (1995) Preliminary report: Genetic variation in the human stromelysin promoter is associated with progression of coronary atherosclerosis. *Br Heart J* 73:209-215.
- Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE and Henney AM (1996) Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. *J Biol Chem* 271:13055-13060.
- Ye S, Gale CR and Martyn CN (2003) Variation in the matrix metalloproteinase-1 gene and risk of coronary heart disease. *Eur Heart J* 24:1668-1671.
- Yoo J, Seo B and Kim Y (2005) SNPAnalyzer: A web-based integrated workbench for single-nucleotide polymorphism analysis. *Nucleic Acids Res* 33:W483-488.
- Zhang B, Ye S, Herrmann SM, Eriksson P, de Maat M, Evans A, Arveiler D, Luc G, Cambien F, Hamsten A *et al.* (1999) Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation* 99:1788-1794.
- Zhang B, Dhillon S, Geary I, Howell WM, Iannotti F, Day IN and Ye S (2001) Polymorphisms in matrix metalloproteinase-1, -3, -9, and -12 genes in relation to subarachnoid hemorrhage. *Stroke* 32:2198-2202.

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