Laboratory Research

Genotype frequency of gelatinase B C-1562 T polymorphism in coronary heart disease and myocardial infarction

Hong JIANG,[1,2] Dieter Niederacher,[3] Ming DU,[3] Roger Marx,[1] Thomas Scheffold,[1] Rolf Michael Klein[1]

[1] *Heart Center Wuppertal, University of Witten-Herdecke, Wuppertal, Germany*
[2] *Department of Cardiology, Zhongshan Hospital, Shanghai Medical College, Fudan University, Shanghai, China*
[3] *Molecular Genetics Laboratory of the Gynecology Division, Henrich-Heine University Duesseldorf, Germany*

Background One of the characteristics of atherosclerosis is a change in the content of extracellular matrix in the arterial wall. Gelatinase B, a member of the family of matrix metalloproteinase, can regulate extracellular matrix metabolism and play a role in the pathogenesis of atherosclerosis, coronary heart disease (CHD) and myocardial infarction (MI). Gelatinase B is polymorphic due to a C to T change at the position -1562 bp in the promoter region. Its relationship with gene product concentration in serum and its role in mediating the risk of CHD and MI in Germans is still unknown. **Methods** We enrolled 102 controls and 322 patients with angiographically documented CHD, including a sub-group of 173 patients with acute or chronic MI and 80 patients with acute coronary syndrome (ACS). All patients and controls were Germans and genotyped by polymerase chain reaction and digestion with SphI. **Results** We found that several classical risk factors for CHD and MI, including hypercholesterolemia and cigarette smoking, were significantly increased in CHD and MI patients compared with controls. Serum levels of gelatinase B and tissue inhibitor of metalloproteinase-1 were increased in the peripheral blood of patients with acute coronary syndrome. No significant differences in genotype or allelic frequencies between CHD, MI and control subjects of either men or women were found. Our search for a possible association of the polymorphisms with CHD and MI by logistic regression analysis was also negative. The serum concentrations of gelatinase B showed no differences between genotypes. **Conclusions** Our data showed that gelatinase B might provide an index of plaque activity in ACS, but gelatinase B protein was not affected by genotypes. Also, the T variant of gelatinase B was not associated with CHD or MI in Germans. (***J Geriatr Cardiol* 2004;1(2):114-118.**)

Key Words gelatinase B gene; polymorphism; coronary heart disease; myocardial infarction; gelatinase B protein

Introduction

Disturbed extracellular matrix (ECM) metabolism occurs throughout all stages of human atherosclerosis.[1] Matrix metalloproteinases (MMPs) are released by macrophages and a variety of other cells; they are capable of degrading all of the components of ECM, and therefore play a role in remodelling it. Gelatinase B (MMP-9, or 92-kD gelatinase, or type 4 collagenase) is the largest downstream member of the MMP family proteolytic cascade. Brown et al[2] reported that gelatinase B is present in coronary atherectomy samples of patients with unstable angina, and may contribute to the weakening of the cap and subsequent plaque rupture.[3] Its inhibitor is a tissue inhibitor of metalloproteinases-1 (TIMP-1). An imbalance between MMPs and their inhibitors may contribute to the disease conditions. Elevated plasma concentrations of gelatinase B and TIMP-1 have also been noted in the coronary circulation and in peripheral blood of patients with acute coronary syndrome (ACS), including unstable angina and acute myocardial infarction.[4,5] Therefore, gelatinase B plays a role in the pathogenesis of coronary heart disease (CHD) and myocardial infarction (MI). Gelatinase B single base polymorphism, ie, C to T transition, which results in an substitution of cytosine (C) to thymidine (T) in the gelatinase B gene at position -1562 bp upstream from the start of transcription, has been described. Zhang et al[6] reported that the frequencies of TT homozygotes and CT heterozygotes of gelatinase B in French men with three-vessel CHD were significantly higher than in one- and two-vessel CHD. This substitution

Corresponding author: Hong JIANG, Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai, China
Tel: 86-21-64434817; E-mail: jiangh01@yahoo.com

leads to a higher transcriptional activity of the gene in vitro. Our aim was therefore to determine whether C/T polymorphism of the gelatinase B gene influences the risk of CHD and MI in Germans.

Methods

Study population

The study population consisted of three groups: control group, CHD group, and MI group (a sub-group of the CHD group) (Table 1). The CHD group consisted of 322 patients (235 males, 87 females; median age 62.6 ± 10.8) with angiographically documented CHD. Percutaneous transluminal coronary angioplasty showed that there was at least one stenosing major coronary vessel and the stenosis was over 50%.

The MI group consisted of 173 patients (135 males, 38 females; median age 61.8 ± 11.5) with acute or chronic MI. Among them, 80 were ACS patients. The diagnosis of MI was based on typical electrocardiographic changes and increases in serum enzyme activities, including creatinine kinase, aspartate aminotransferase, and lactate dehydrogenase.

The control group comprised 102 chest pain patients (43 males, 59 females; median age 59.6 ± 15.1) with normal left ventricular function and without documented evidence of CHD. There were no significant differences in ages among the CHD, MI and control groups. All patients and control subjects in the study were Germans and signed a written informed consent form.

Determination of biochemical parameters

Total cholesterol and triglyceride were measured in fasting serum specimens by standard techniques at all visits.

Isolation of DNA

EDTA blood samples (5 ml) were obtained from peripheral venous blood of all subjects after cardiac catheterization and all blood samples were stored at 4℃ until DNA extraction was performed using an extraction kit (QIA amp DNA blood midi kit) from QIAGEN.

Polymerase chain reaction and genotyping

Gelatinase polymorphism is due to a C to T substitution at position -1562. This polymorphism creates a new SphI restriction site and consequently a new restriction-fragment length polymorphism (RFLP). To detect this RFLP, polymerase chain reaction (PCR) containing the genomic sites was used to amplify a 435 base pair fragment of the promoter region. The PCR was performed using 25 ng template DNA in a final reaction volume of 25 μl containing 1 × PCR reaction buffer, 0.2 mmol/L of each deoxynucleotide, and 2.0 U of Taq DNA polymerase (Pharmacia) with 15 pmol of each upstream and downstream primer (upstream primers: 5'- GCC TGG CAC ATA GTA GGC CC -3'; downstream primers: 5'-TCT CTC AGC CAG CCG GCA TC-3') as previously published.[6] Thermal cycling was carried out with an initial 95℃ denaturation step for 5 min followed by 33 cycles of denaturation at 95℃ for 30s, annealing at 62℃ for 30 s, extension at 72℃ for 40s and a final extension for 7 min at 72℃. To determine the genotype of the gelatinase, after checking that the amplification was successful by running 5 μl of the PCR product on a 2% agarose gel, 4 μl was digested with 4 units of restrictive enzyme of SphI (Roche diagnostics, Mannheim, Germany) for 3 hr at 37℃. Recognition sequence for SphI is GCATGC. The T allele was cleaved at the -1562 polymorphic site, generating two fragments of 247 and 188 base pairs, but the C allele was not cut. The fragments obtained were separated on 2% agarose gels, followed by ethidium bromide staining and ultraviolet visualization (Fig. 1).

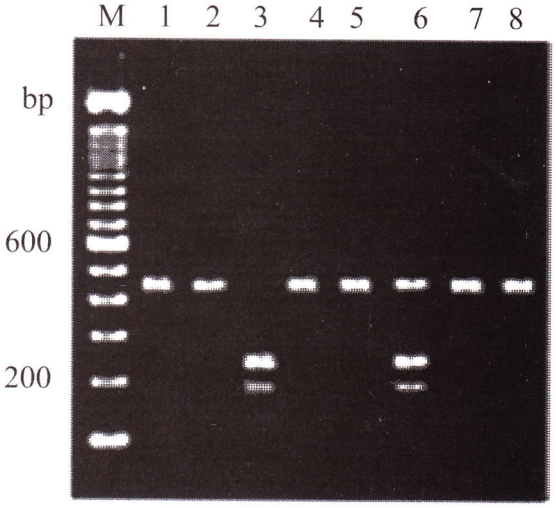

Fig. 1. Agarose gels. Electrophoresis image showing PCR products. M: 100 bp DNA marker (lane 1); sample, 1,2,4, 5,7,8, homozygous for C allele (Wild-type CC); sample 6, heterozygous (TC); sample 3, homozygous for T allele (TT)

Determination of serum gelatinase B and TIMP-1 levels

Gelatinase B/TIMP-1 concentrations were quantified in 10 μl serum using the BAYER IMMUNO 1© immunoassay system. Concentrations were reported in ng/ml. The detectable range was 0-2 000 ng/ml.

Statistical analysis

Statistical analysis was performed with the SPSS (version 10.0) package. Data were expressed as mean ± SD. Continuous variable means were tested by analysis of variance (ANOVA). Frequencies were compared using

the *Chi*-square test and Fisher's exact test. The gelatinase genotype was calculated according to a dominant (CC vs TT + TC) or additive (CC vs TC and CC vs TT) genetic model. We performed multivariable logistic regression analysis for the effect of the gelatinase B polymorphism and other coronary risk factors on MI and CHD. MI and CHD were dependent variables, while the independent variables included gelatinase genotype [0 for CC, 1 for TC and TT combined (dominant effect of the T allele)], smoking status (0 = nonsmoker, 1 = smoker), metabolic variables (0 = for absence of hypercholesterolemia, 1 = for presence) and gender (0 = women, 1 = men). We used forward LR stepwise selection in this analysis. The odds ratio and 95% CI were also calculated. $P < 0.05$ was taken as the limit of significance.

Results

Comparison of CHD, MI and control groups for coronary risk factors

As expected, several classical risk factors for CHD and MI, including hypercholesterolemia and cigarette smoking, were significantly increased in CHD and MI patients compared with the controls (Table 1).

Coronary risk factors for CHD and MI by gelatinase genotype

Distributions of coronary risk factors in subjects grouped by their genotype are shown for both groups (CHD, MI) in Table 2. There was no association between any of the risk factors and the T allele.

Allele and genotype frequencies of C/T gelatinase B polymorphism in patients and controls

The distribution of C/T genotypes and allele frequencies at the position -1562 of the gelatinase gene is shown in Table 3. The gelatinase T allele frequencies were 0.17, 0.19 and 0.12 for men in CHD, MI and controls respectively and 0.10, 0.09 and 0.15 for women. No difference in either genotype distribution or allele frequencies was found between CHD and MI patients and controls for either men or women.

Multivariate logistic modeling for CHD and MI

Our search for a possible association between the gelatinase polymorphisms and CHD and MI by Logistic regression analysis was negative (Table 4).

Effects of gelatinase B polymorphisms on its serum concentration

The serum concentration of gelatinase B in 49 controls and 80 ACS patients was 80.31 ± 15.81 ng/ml and 245.5 ± 225.0 ng/ml ($P < 0.01$), and of TIMP-1 was 319.16 ± 90.07 ng/ml and 448.29 ± 148.97 ng/ml ($P < 0.01$). Serum levels of gelatinase B and TIMP-1 were increased in the peripheral blood in patients with ACS.

To determine whether the concentration was affected by genotypes, we found that the serum concentration of gelatinase B differed among the 3 genotypes (wild type CC 235.9 ± 220.93 ng/ml, heterozygote TC and mutation homozygote TT 269.22 ± 238.22 ng/ml).

Discussion

Our aim of the present study was to determine the possible role of gelatinase B gene polymorphisms in predicting CHD and MI. However, we found no association between C-1562T polymorphism of the human gelatinase B gene and CHD or MI. Also, genotype has no effect on the serum level of gelatinase B protein.

Gelatinase B single base polymorphism, that is C to T transition in the gelatinase B gene at position 1562 bp upstream from the start site of transcription, has been described. A study with French people[6] showed that the frequencies of TT homozygotes and CT heterozygotes for gelatinase B in three-vessel CHD were significantly higher than in one- and two-vessel disease, but there was no difference between one - vessel and two - vessel disease and

Table 1. Baseline characteristics of the study population

Variable	Control group (n = 102)	CHD group (n = 322)	MI group (n = 173)
Age (yr)	59.6 ± 15.1	62.6 ± 10.8	61.8 ± 11.5
Gender (female/male)	59/43	87/235	38/135
BMI (kg/m^2)	27.0 ± 5.0	27.2 ± 3.9	27.2 ± 4.2
Habitual smoking(%)	27	54*	59*
Hypertension(%)	73	70	66
Diabetes mellitus(%)	17	24	21
Hypercholesterolemia(%)	59	81*	81*

The values are presented as mean ± SD; BMI = body mass index; CHD = coronary heart disease; MI = myocardial infarction; * $P < 0.001$ compared with control group

Table 2. Prevalence of coronary risk factors in cases (CHD, MI) grouped by genotype

	CHD		MI	
	CT + TT (n = 89)	C (n = 233)	CT + TT (n = 52)	C (n = 121)
Gender(female/male)	16/73	71/162	6/46	32/89
Age(yr)	60.7 ± 9.9	63.4 ± 11.1	60,9 ± 10.1	62.3 ± 12.1
BMI(kg/m^2)	26.5 ± 3.6	27.5 ± 4.0	26.4 ± 3.4	27.7 ± 4.4
Habitual smoking(%)	60	51	67	56
Hypertension(%)	73	70	72	64
Diabetes mellitus(%)	19	25	16	22
Hypercholesterolemia(%)	86	80	87	79

BMI = body mass index; No differences in classical risk factors between CC, CT and TT genotypes in CHD and MI patients

Table 3. Allele and genotype frequencies of C/T gelatinase B polymorphisms in patients and controls

Variables	Men (n = 413)			Women (n = 184)		
	Controls (n = 43)	CHD (n = 235)	MI (n = 135)	Controls (n = 59)	CHD (n = 87)	MI (n = 38)
Genotypes n (%)						
TT	0	7(3)	5(3.7)	1(1.7)	2(2.3)	1(2.6)
TC	10(23.3)	66(28.1)	41(30.4)	16(27.1)	14(16.1)	5(13.2)
CC	33(76.7)	162(68.9)	89(65.9)	42(71.2)	71(81.6)	32(84.2)
Dominant effects						
P(CC/TT + TC)		0.30	0.18		0.14	0.14
Additive effects						
P(TC & CC)		0.45	0.30		0.11	0.11
P(TT & CC)		0.23	0.18		0.89	0.85
Allele frequency (percentage)						
T	0.12	0.17	0.19	0.15	0.10	0.09
C	0.88	0.83	0.81	0.85	0.90	0.91

There were no differences in frequency between cases and controls for any of the allele or genotype distributions

Table 4. Results of Logistic regression analysis for CHD and MI

Variable	Beta	SE	*P* value	OR(95%CI)
Predictive model for CAD				
Gender	1.313	0.301	0.000	3.72(2.06-6.70)
Smoker	0.959	0.314	0.002	2.61(1.41-4.83)
Hyperchol.	1.453	0.309	0.000	4.28(2.33-7.84)
C-1562T variant			ns	
Predictive model for MI				
Gender	1.533	0.349	0.000	4.63(2.34-9.18)
Smoker	1.099	0.347	0.002	3.00(1.52-5.93)
Hyperchol.	1.512	0.361	0.000	4.54(2.24-9.21)
C-1562T variant			ns	

All *P* values were cases compared with controls; OR = odds ratio; CI = confidence interval; ns = no significance

the controls. No difference was found between the frequencies of the two alleles in either the French control population[6] (C allele 0.87, T allele 0.13) or in our own control population (C allele 0.88, 0.85, T allele 0.12, 0.15 for men and women respectively, $P = 0.80$ vs 0.59) in a *Chi* square test. However, we found no association between C-1562T polymorphism of the human gelatinase B gene and CHD or MI in Germans. Multiple variable logistic regression analysis also indicated that the T-allele of gelatinase B was not an independent risk factor for CHD and MI. There was a significant sex difference between our patients and controls. However, our analysis was performed separately for male and female patients. We found no significant difference in either genotype or allele frequencies between CHD or MI subjects and controls for either men or women. Although several classical risk factors for CHD and MI, including habitual smoking and hypercholesterolemia, were significantly increased compared with controls (Table 1), there was no difference between genotypes for any of the risk factors (Table 2) in CHD and MI patients. The alleles in our study were in Hardy-Weinberg equilibrium.

Imbalance between MMPs and their inhibitors can serve as an important mechanism for atherosclerosis. Our data showed that gelatinase B might provide an index of plaque activity in ACS, but our study failed to confirm differences in the gelatinase B concentrations between CC, CT and TT genotypes. In our study, sequence variation in this region of gelatinase B did not affect the expression of either gelatinase B or the balanced control of matrix remodeling. Our study failed to confirm an association of T allele with several risk factors for CAD and MI. An in vitro study showed[6] that C to T substitution resulted in a loss of binding of a nuclear protein to the promoter region of gelatinase B. It therefore led to a higher transcriptional activity of this gene, and might lead to different gene expression among genotypes. Since the CA repeat sequence, which is situated about 100 bp upstream of the first exon in the promoter of gelatinase B, had also been shown to influence transcriptional activity,[7,8] other polymorphisms may also be involved in the regulation of gelatinase B gene expression in vivo.[9]

In conclusion, our case control study suggests that the T variant of gelatinase B at position -1562 is not associated with CHD or MI in Germans. Our study shows that CHD is not the result of a single gene or any single environmental factor, but is caused by multifactorial interactions of genetic and environmental components. Further studies are required to determine which combinations of factors influence the risk of CHD.

References

1. Kuzuya M, Iguchi A. Role of matrix metalloproteinases in vascular remodeling. *J Atheroscler Thromb* 2003;10(5):275-82.
2. Brown DL, Hibbs MS, Kearney M, et al. Identification of 92-kD gelatinase in human coronary atherosclerotic lesions. Association of active enzyme synthesis with unstable angina. *Circulation* 1995; 91:2125-31.
3. Ferroni P, Basili S, Martini F, et al. Serum metalloproteinase 9 levels in patients with coronary artery disease: a novel marker of inflammation. *J Investig Med* 2003;51(5):295-300.
4. Inokubo Y, Hanada H, Ishizaka H, et al. Plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 are increased in the coronary circulation in patients with acute coronary syndrome. *Am Heart J* 2001; 141: 211-7.
5. Kai H, Ikeda H, Yasukawa H, et al. Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes. *J Am Coll Cardiol* 1998;32:368-72.
6. Zhang B, Ye S, Herrmann SM, et al. Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation* 1999; 99: 1788-94.
7. Shimajiri S, Arima N, Tanimoto A, et al. Shortened microsatellite d(CA)21 sequence down-regulates promoter activity of matrix metalloproteinase 9 gene. *FEBS Lett* 1999;455:70-4.
8. Sato H, Kita M, Seiki M. v-Src activates the expression of 92-kDa type IV collagenase gene through the AP-1 site and the GT box homologous to retinoblastoma control elements. A mechanism regulating gene expression independent of that by inflammatory cytokines. *J Biol Chem* 1993;268(31):23460-8.
9. Zhang B, Henney A, Eriksson P, et al. Genetic variation at the matrix metalloproteinase-9 locus on chromosome 20q12.2-13.1. *Hum Genet* 1999;105(5):418-23.