Association between matrix metallopeptidase 1 and type 2 diabetes mellitus coexisting with coronary heart disease in a Han Chinese population

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ABSTRACT. Matrix metallopeptidase 1 (MMP-1) has been reported to be involved in the coexistence of type 2 diabetes mellitus (T2DM) and coronary heart disease (CHD). We sought to examine the association between the *MMP-1* gene polymorphism and coexistence of T2DM and CHD in a Han Chinese population. We extracted genomic DNA from the peripheral blood of 794 subjects, including 378 patients with coexisting T2DM and CHD and 416 healthy controls. We selected several single nucleotide polymorphisms of the *MMP-1* gene and genotyped them using the MassARRAY system, before analyzing the data with Haploview 4.0 and SPSS 20.0. A statistical difference was found in the distribution of rs1799750 genotypes between the patient and control groups (P = 0.041). The frequency of the 2G/2G genotype was 44.25 and 37.0% among patients and control subjects, respectively. Moreover, the frequency of the 2G allele was 65.9% among patients

and 59.6% in the control group, and this difference was found to be significant (P = 0.010). Elevated body mass index was also associated with the 2G/2G genotype. Thus, *MMP-1* rs1799750 may be involved in the development of coexisting T2DM and CHD in the Han Chinese population.

Key words: Type 2 diabetes mellitus; Coronary heart disease; Matrix metallopeptidase 1; Polymorphism

INTRODUCTION

Type 2 diabetes mellitus (T2DM) frequently coexists with coronary heart disease (CHD) (El-Atat et al., 2004). Increased release of free fatty acids induces the generation of reactive oxygen species (ROS) and elevates oxidative stress in T2DM patients, leading to endothelial dysfunction and promoting the occurrence of CHD (Meerarani et al., 2006). Therefore, T2DM and CHD may interact in patients to intensify the severity of both diseases. However, the exact mechanisms underlying this interaction remain unclear.

Matrix metalloproteinases (MMPs) are endopeptidases that depend upon calcium (Ca^{2+}) and contain zinc (Zn^{2+}). They belong to a large, highly structurally homologous group of proteases known as the metzincin superfamily (Nagase and Woessner, 1999). These zinc-peptidase enzymes use hydrolysis to degrade various extracellular matrix proteins, and are known to be involved in tissue reconstruction and repair, cell migration, angiogenesis, chronic inflammatory reactions, wound healing, and tumor invasion and metastasis (Vu and Werb, 2000; Visse and Nagase, 2003). Matrix metallopeptidase 1 (*MMP-1*) forms part of an MMP gene cluster on chromosome 11q22.3. It encodes a secreted enzyme that breaks down interstitial collagen types I, II, and III. MMP-1 can thin and disrupt the fibrous cap of atherosclerotic plaques (Nikkari et al., 1995), and thus may be associated with coronary atherosclerosis, a condition that can easily lead to CHD.

The 1G/2G polymorphism of *MMP-1* has been reported to be associated with CHD in Caucasian populations (Ye et al., 2003). In addition, high glucose concentrations in endothelial cells and macrophages have been shown to increase MMP-1 expression and activity (Death et al., 2003). Heightened glucose concentration also disrupts certain intracellular signal transduction pathways, such as those involving ROS that activate *MMP-1* expression (King and Wakasaki, 1999; Li and Karin, 1999). Therefore, we speculated that *MMP-1* might be associated with coexisting T2DM and CHD.

We chose six single nucleotide polymorphisms (SNPs) of the *MMP-1* gene to study: rs1799750, rs498186, rs996999, rs2071232, rs1938901, and rs2239008. rs1799750 and rs498186 are located in the *MMP-1* promoter, whereas rs2239008 lies within the 3'-untranslated region. The remainder are located in introns or the untranslated region.

MATERIAL AND METHODS

Subjects

A total of 378 patients were recruited from our hospital. T2DM diagnoses were made at least six months before enrolment in this study. Patients were defined as diabetic according

to: 1) a report of having been informed of having diabetes, and/or 2) use of oral hypoglycemic drugs or insulin, or 3) a fasting plasma glucose level ≥126 mg/dL, or 4) a 2-h oral glucose tolerance test result ≥200 mg/dL. CHD was diagnosed if patients had a history of myocardial infarction or showed abnormal electrocardiographic results. The control group consisted of 416 unrelated healthy subjects having undergone health examinations at our hospital. The study was approved by the local ethics committee, and written informed consent was obtained from all subjects prior to participation.

Genotyping

Peripheral blood was drawn from a vein into a sterile tube containing ethylenediamine tetraacetic acid. Plasma samples were stored at -20°C. Genomic DNA was extracted from frozen peripheral blood samples using a QIAamp Blood Mini Kit (QIAGEN Inc., Valencia, CA, USA) following the manufacturer protocol. Genotyping of all SNPs was performed using the MassARRAY platform (Sequenom, San Diego, CA, USA). Primer extension and polymerase chain reaction were performed using iPLEX enzyme (Sequenom) and HotStarTaq DNA polymerase (QIAGEN, Hilden, Germany), following manufacturer protocols. The resulting spectra were processed with MassARRAY RT software (version 3.0.0.4), and genotype data were analyzed using the MassARRAY Typer program (version 3.4; Sequenom).

Statistical analysis

All data were analyzed using SPSS 20.0 (IBM Corp., Armonk, NY, USA). Allele and genotype frequencies for each polymorphism and Hardy-Weinberg equilibrium were evaluated by chi-square tests. The association between CHD and each polymorphism was analyzed using chi-square tests or Fisher's exact tests. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the strength of the association between allele frequencies and CHD. Pairwise linkage disequilibrium (LD) statistics (D' and r^2) and haplotype frequencies were computed using Haploview 4.0 (Broad Institute, Cambridge, MA, USA) to construct haplotype blocks.

RESULTS

All observed genotype distributions in both patient and control groups were in line with Hardy-Weinberg equilibrium (P > 0.05). Strong LD was detected in relation to two pairs of SNPs (rs2239008-rs1938901 and rs996999-rs498186), which formed two haplotype blocks (D' > 0.9, Figure 1). Genotype distributions, allelic frequencies, and haplotypes in the patient and control groups are shown in Tables 1-3.

A statistical difference between the two groups was found in the distribution of *MMP-1* gene rs1799750 genotypes (P = 0.041). The frequency of the 2G/2G genotype was 44.2% in patients, and 37.0% among the controls (Table 2). Moreover, the distribution of rs1799750 alleles significantly differed between the patient and control groups (P = 0.010). In the former, the frequency of the 2G allele was 65.9%, while in the latter it was 59.6% (Table 1). Furthermore, we performed an association analysis to determine whether the haplotypes identified were associated with T2DM-CHD risk. However, these haplotypes did not differ between patients and controls (Table 3). Finally, we analyzed the association between rs1799750 genotype and

certain clinical characteristics among members of the patient group. The 2G/2G genotype was found to be associated with higher body mass index (BMI; Table 4).
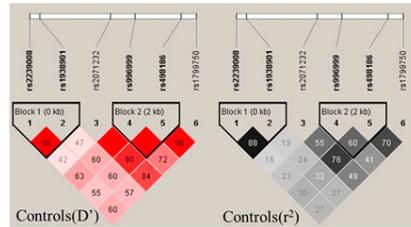

Figure 1. Linkage disequilibrium (LD) plot of *MMP-1* gene single nucleotide polymorphisms (SNPs) in the control group. Numbers in squares show pairwise r^2 values. Black squares indicate $r^2 = 1$ (i.e., perfect LD between a pair of SNPs). Empty squares indicate D' = 1 (i.e., complete LD between a pair of SNPs).

Table 1. *MMP-1* gene polymorphism allele frequencies.

SNP	Allele	Cases (378)		Controls (416)		P	OR (95%CI)
		N	%	N	%		
rs1799750	2G	498	65.9	496	59.6	0.010	1.308 (1.066-1.604)
	1G	258	34.1	336	40.4		
rs498186	A	388	51.5	440	52.9	0.570	0.944 (0.775-1.150)
	C	366	48.5	392	47.1		
rs996999	C	498	65.9	542	65.1	0.760	1.033 (0.839-1.271)
	T	258	34.1	290	34.9		
rs2071232	C	382	50.5	424	51.0	0.863	0.983 (0.807-1.197)
	T	374	49.5	408	49.0		
rs1938901	C	408	54.0	456	54.8	0.737	0.967 (0.793-1.178)
	T	348	46.0	376	45.2		
rs2239008	A	406	53.7	436	52.4	0.604	1.054 (0.865-1.283)
	G	350	46.3	396	47.6		

SNP = single nucleotide polymorphism, OR = odds ratio, CI = confidence interval.

Table 2. *MMP-1* gene polymorphism genotype frequencies.

SNP	Genotype	Cases (378)		Controls (416)		P[a]	P[b]
		N	%	N	%		
rs1799750	2G/2G	167	44.2	154	37.0	0.210	0.041
	2G/1G	164	43.4	188	45.2		
	1G/1G	47	12.4	74	17.8		
rs498186	AA	94	24.9	120	28.8	0.472	0.334
	AC	200	53.1	200	48.1		
	CC	83	22.0	96	23.1		
rs996999	CC	164	43.4	178	42.8	0.753	0.932
	CT	170	45.0	186	44.7		
	TT	44	11.6	52	12.5		
rs2071232	CC	101	26.7	118	28.4	0.051	0.782
	CT	180	47.6	188	45.2		
	TT	97	25.7	110	26.4		
rs1938901	CC	117	31.0	118	28.4	0.168	0.132
	CT	174	46.0	220	52.9		
	TT	87	23.0	78	18.8		
rs2239008	AA	115	30.4	106	25.5	0.105	0.115
	AG	176	46.6	224	53.8		
	GG	87	23.0	86	20.7		

P[a] represents P values from tests of Hardy-Weinberg equilibrium in the healthy control group. P[b] signifies P-values from tests of genotype frequency differences between the case and control groups. SNP = single nucleotide polymorphism.

Table 3. *MMP-1* gene polymorphism haplotype frequencies.

Haplotype			Gene counting (frequency %)		P
Name			Cases	Controls	
Block 1 rs2239008-rs1938901					
HAP1	A	C	201	217	0.776
HAP2	G	T	172	187	0.876
Block 2 rs996999-rs498186					
HAP1	C	A	193	220	0.607
HAP2	T	C	127	145	0.709
HAP3	C	C	56	51	0.643

Table 4. Clinicodemographic characteristics of *MMP-1* rs1799750 genotype.

Variable	1G/1G	1G/2G	2G/2G	P
BMI (kg/m^2)				0.008
≤24	56	60	6	
>24	111	104	41	
Smoking				0.345
Yes	84	79	18	
No	83	85	29	
Alcoholism				0.061
Yes	98	75	24	
No	69	89	23	
Disease duration (years)				0.056
<5	76	54	18	
5-10	59	61	21	
>10	32	49	8	
Age (years)				0.294
≤40	37	29	6	
≥41	130	135	41	

BMI = body mass index.

DISCUSSION

Significant evidence exists showing that high blood glucose is a primary risk factor for cardiovascular disease (King and Wakasaki, 1999; Li and Karin, 1999). T2DM patients suffering from CHD are at increased risk of mortality compared to those without CHD (Kannel and McGee, 1979; Fuller et al., 1983).

Levels of MMPs are elevated in cultivated diabetic fibroblasts compared with those from healthy controls. This is significant because these cells play an important role in wound healing (Lobmann et al., 2006). In endothelial cells and macrophages from diabetes patients, high glucose levels increase the expression of MMPs and promote CHD (Death et al., 2003). In addition, diabetes mellitus enhances vascular MMP activity and increases the likelihood of developing CHD, as it is mediated by a ROS-sensitive pathway (Uemura et al., 2001; Ye et al., 2003). The above-cited evidence shows that T2DM-CHD coexistence may be associated with MMP activity.

In this study, we found that the *MMP-1* gene rs1799750 polymorphism was associated with coexisting T2DM and CHD. The body of research relating to this polymorphism has been growing in recent years. rs1799750 is located in the *MMP-1* promoter region, and its 2G allele is known to increase transcription of this gene (Rutter et al., 1998; Affara et al., 2011). This *MMP-1* 1G/2G polymorphism may influence the prevalence of CHD (Ye et al., 2003; Horne et al., 2007). Moreover, it has been associated with coexisting T2DM and CHD in Caucasian populations, and the 2G allele is present at a higher frequency in these patients than in healthy

control subjects (Drzewoski et al., 2008). Such findings are consistent with our results, which support the association between rs1799750 and coexisting T2DM and CHD.

The *MMP-1* promoter is known to contain essential response elements to the transcription factors activator protein-1 (AP-1) and E twenty-six (Ets), which can be active in the presence of high glucose levels. Insertion of an extra guanine at position -1607 in this promoter creates an additional Ets binding site (5'-GGAT-3') adjacent to the AP-1 site at -1602. Compared with the 1G allele, the 2G allele significantly increases *MMP-1* transcriptional activity, which in turn promotes coexisting T2DM and CHD. We also found that the 2G/2G genotype is associated with higher BMI.

The other polymorphisms under investigation, rs498186, rs996999, rs2071232, rs1938901, and rs2239008, showed no statistically significant differences in distribution between the study groups. Neither of the *MMP-1* haplotype blocks identified, rs2239008-rs1938901 and rs996999-rs498186, were associated with T2DM-CHD risk.

In conclusion, the rs1799750 polymorphism in the *MMP-1* promoter region may be associated with coexisting T2DM and CHD.

Conflicts of interest

The authors declare no conflict of interest.

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