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Polymorphisms of MMP-2 gene are associated with systolic heart failure prognosis

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ABSTRACT

Background: MMP-2 is a proteolytic enzyme involved in myocardial remodeling that occurs in congestive heart failure (HF). We hypothesized MMP-2 genetic variations could influence the prognosis of systolic HF.
Methods: To test our hypothesis, we performed a follow-up study of 605 patients with systolic HF. Three single nucleotide polymorphisms (SNPs) of MMP-2 (rs243864, rs243866, rs17859821) were analyzed by restriction fragment length polymorphism (RFLP) methods.
Results: Totally 526 patients (86.9%) were followed up. At follow up (median 24 months), 116 patients (22.1%) died, 102 patients (19.4%) were readmitted because of HF. One, two, three and four year survival rate was 86.9%, 81%, 77.9% and 77.9%. MMP-2 rs17859821 A allele carriers had lower all cause death rate, cardiac death rate and MACE rate than did GG genotype carriers (OR = 0.655, 0.580, 0.705; $P = 0.030$, 0.008, 0.011). After adjustment for age, bundle branch block, LVEF and NYHA grade by using cox regression analysis, MMP-2 A allele carriers had lower cardiac death rate and MACE rate than did GG genotype carriers(OR = 0.643 and 0.746; $P<0.05$). However, the genotypes had no association with plasma levels of proMMP-2. Haplotype analysis had confirmed the above results. MMP-2 rs243866, rs243864 had no association with systolic HF prognosis.
Conclusion: The findings of the present study suggest that MMP-2 rs17859821 A allele was associated with better prognosis of systolic HF in the northern Han Chinese population.

1. Introduction

Heart failure (HF) is a major public health problem, associated with high mortality and poor outcome. However, susceptibility to and outcomes of HF are highly variable [1]. Some descriptions of the twin [2–4] or familial correlation of HF together with advances in genetic research have clued to the role of genetics in HF [5]. Though the HF phenotype is quite complex which has contributed to the difficulties in discovering the role of common genetic variation, it's still reasonable to speculate that genetic underpinnings for HF that arises from systolic dysfunction may vary from those predisposing to HF due to diastolic dysfunction [6].

Although the inciting stimuli can be diverse, an important structural milestone in patients with progressive heart failure is myocardial remodeling [7]. It is now well recognized that changes in the myocardial extracellular matrix (ECM) contribute to the progressive remodeling process. Among the pathways that contribute to ECM remodeling, the activation of a family of proteolytic enzymes known as the metalloproteinases (MMPs) appears to be particularly important. This proteolytic system degrades a wide spectrum of ECM proteins and is constitutively expressed in a large number of cell and tissue types, including myocardium [8].

Previous studies have found that MMP-2 level is elevated during the progression of LV dilation and dysfunction [9–12]. Researches have also validated that elevated MMP-2 activity contributes to myocardial stunning in ischemic reperfusion injury and direct impairment of myocardial contraction [13–16].

Importantly, the polymorphisms identified thus far often affect the promoter region of the MMP gene, thereby influencing critical steps in the binding of transcription factors or the overall efficiency of transcription. MMP gene polymorphisms have now been identified for several of the MMP subtypes. The polymorphisms were already shown to be associated with certain diseases like stroke [17], diabetic

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Table 1
Baseline characteristics of the follow-up population and lost follow-up patients.

	Follow-up ($n=526$)	Lost follow-up ($n=79$)	P value
Male %	437/83.1	62/78.5	NS
Age SD	58.36 ± 12.78	55.03 ± 15.01	0.035
BMI	24.42 ± 3.72	24.25 ± 3.78	NS
Ischaemic %	335/63.7	52/65.8	NS
NYHA class %			
II	176/34	29/37.2	
III	167/32.2	28/35.9	NS
IV	175/33.8	21/26.9	
LVEDD mm	65.17 ± 9.11	64.64 ± 8.88	NS
LVEF %	35.87 ± 8.04	36.67 ± 9.35	NS
Hypertension %	235/44.7	38/48.1	NS
Diabetes %	118/22.4	16/20.3	NS
Smoke %			
Non-smoker	181/34.4	26/32.9	
Smoke now	142/27	16/20.3	NS
ceased	203/38.6	37/46.8	
Uric	396.88 ± 149.89	370.14 ± 137.80	NS
FPG	5.71 ± 2.07	5.67 ± 2.33	NS
NT-proBNP	2127.95 ± 1719.23	2418.21 ± 1938.59	NS
CRP	12.49 ± 30.68	6.98 ± 10.18	0.003
Hs-CRP	4.28 ± 4.38	3.54 ± 4.01	NS

Values are means ± SD or percent of patients. BMI, body mass index; NYHA, New York Heart Association functional classification; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; Uric, uric acid; FPG, fast plasma glucose; CRP, C reactive protein; Hs-CRP, hypersensitive C reactive protein.

retinopathy [18], cancer [19,20], aneurysms [21] and heart failure [22,23]. Among them, Vasku et al. [22] had found that T allele frequency of the -790T/G MMP-2 polymorphism was significantly higher in patients with chronic heart failure than in subjects without clinical signs of cardiovascular disease as well as of the C allele frequency of the -735C/T MMP-2 gene polymorphism. However, the association between polymorphisms of MMP-2 with the prognosis of systolic heart failure in Chinese population hasn't been determined yet.

Among those polymorphisms, MMP-2 rs243866 (-1575G/A) is located immediately 5' to a half-palindromic potential estrogen receptor binding site. In estrogen receptor-positive MCF-7 cells the -1575G allele functions as an enhancer, whereas the -1575A allele reduces transcription activity [24]. According to Transfac Matrix Table (MatInspector V2.2), three transcription factors could be found in the T allele sequence (GKLF — gut-enriched Krueppel-like factor, S8 and Evi1 — ectopic viral integration site 1 encoded factor) but not in the G variant of -790 MMP-2 gene polymorphism (rs243864) [18]. Another polymorphism rs17859821 (-1059G/A) which doesn't involve in any known binding site of transcription factors was used as a reference SNP.

In the present study, we hypothesized that polymorphisms in the promoter region of MMP-2 gene were associated with systolic heart failure prognosis through their impact on the plasma levels of proMMP-2 in a northern Han Chinese population.

2. Materials and methods

2.1. Subjects and strategy

We recruited 605 unrelated consecutive patients who were referred to our centre between December 2003 and December 2006 with left ventricular ejection fraction (LVEF) ≤ 45% as assessed by echocardiography caused by myocardial infarction in the past ($n=387$) or idiopathic dilated cardiomyopathy ($n=218$). Patients with acute myocardial infarction, congenital heart disease and valvular disease were excluded. At recruitment, peripheral blood was drawn to determine MMP-2 genotypes. Interview, letter or telephone then followed up these patients after their admission to ascertain their vital status. The Institutional Review Board of Fuwai hospital approved the protocol, and informed consent was obtained from each participant.

2.2. Primary and secondary end point

The primary end point was the occurrence of an adverse cardiovascular event at follow up. An adverse cardiovascular event was defined as all cause death, cardiac death (pump failure, sudden death or myocardial infarction death). The secondary end point was hospitalization for cardiac reasons (new onset or worsening of heart failure symptoms), reinfarction and revascularization.

2.3. SNP selection

The MMP-2 gene is located on chromosome 16 at q13-21 and spans 27,049 bp with thirteen exons. There were 283 entries of SNPs for the MMP-2 gene in the public NCBI Single Nucleotide Polymorphism database (dbSNP, build126; http://www.ncbi.nlm.nih.gov/SNP/). Three MMP-2 SNPs were selected according to the following selection criteria: frequency of the minor allele ≥5% and previous evidence that sequence variants in the promoters influence expression [24,25].

2.4. Laboratory methods

2.4.1. Genotyping

Blood for genotyping was taken into EDTA-containing receptacles; DNA was isolated according to a standard phenol-chloroform method and stored at −20°C until required for batch genotyping. All SNPs were genotyped according to standard polymerase chain reaction and restriction fragment length polymorphism methods. Ninety-six randomly selected individuals were genotyped again for quality control with complete concordance.

2.4.2. Determination of MMP-2 levels

Plasma levels of the precursor of MMP-2 were measured using a commercially available ELISA kit (GE Healthcare UK Limited, UK), i.e., free proMMP-2 and that complexed with TIMP-2, but not the active form of MMP-2. It does not cross-react with MMP-1, -3, -7, -8, -9 and MT1-MMP.

2.5. Statistical analysis

We used single locus analyses to detect associations between the 3 tested SNPs and systolic heart failure prognosis. Hardy–Weinberg equilibrium (HWE) of the SNPs was evaluated by Fisher's exact test using the program HWE. The pattern of pairwise linkage disequilibrium (LD) between the SNPs was measured by D′ and r^2 calculated by the program Haploview. The distribution of alleles and genotypes of the SNPs were compared by the χ^2 test. In these analyses, homozygotes for the rare allele were pooled with heterozygotes. We

Table 2
All cause, cardiac, heart failure death and MACE rates in systolic heart failure patients with different genotypes of the three SNPs during follow-up.

	Number	All cause (n%)	P value/Pd[a]	Cardiac death (n%)	P value/Pd[a]	Heart failure (n%)	P value/Pd[a]	MACE (n%)	P value/Pd[a]
rs243864	526	116/22.1		110/20.9		88/16.7		231/43.9	
GG	6	2/33.3		2/33.3		1/16.7		2/33.3	
GT	116	24/20.7	0.763/0.822	21/18.1	0.558/0.523	16/13.8	0.619/0.345	46/39.7	0.483/0.246
TT	404	90/22.3		87/21.5		71/17.6		183/45.3	
rs17859821	517	116/22.4		110/21.3		88/17		227/43.9	
AA	37	7/18.9		5/13.5		5/13.5		14/37.8	
GA	193	33/17.1	0.047/0.014	30/15.5	0.010/0.003	25/13	0.098/0.031	72/37.3	0.028/0.008
GG	287	76/26.5		75/26.1		58/20.2		141/49.1	
rs243866	521	116/22.3		110/21.1		88/16.9		227/43.6	
GG	428	91/21.3		87/20.3		71/16.6		187/43.7	
GA	90	24/26.7	0.494/0.238	22/24.4	0.613/0.346	17/18.9	0.5/0.693	39/43.3	0.934/0.904
AA	3	1/33.3		1/33.3		0/0		1/33.3	

MACE: cardiac death, readmission for heart failure, reinfarction and revascularization.
[a]Dominant models based on minor allele of each locus.