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Original Article

Association of natriuretic peptide polymorphisms with left ventricular dysfunction in southern Han Chinese coronary artery disease patients

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Abstract: Background: Left ventricular dysfunction (LVD) occurs with myocardial ischemia and coronary artery disease (CAD). The natriuretic peptide system has compensatory vasodilatory, natriuretic and paracrine effects on LVD and subsequent heart failure. The aim of this study was to investigate the relationship between natriuretic peptide polymorphisms and risk of LVD in CAD patients. Methods: We recruited 747 consecutive Southern Han Chinese patients with angiographically confirmed CAD, 201 had a reduced left ventricle ejection fraction (LVEF ≤45%, LVD group) and 546 had a preserved left ventricle ejection fraction (LVEF >45%). The reduced and preserved LVEF groups were matched by gender and age. Taqman assays were performed to identify five polymorphisms in the *NPPA-NPPB* locus (rs5065, rs5063, rs632793, rs198388 and rs198389). Results: Single-locus analyses found no significant difference in the allele and genotype frequencies of the reduced and preserved LVEF group, even after adjusting for confounding factors. Subgroup analyses performed by hyperlipidemia (HLP) demonstrated 3 polymorphisms, rs632793 (OR = 0.31, 95% CI 0.1-0.93, P = 0.04), rs198388 (OR = 0.26, 95% CI 0.09-0.79, P = 0.02) and rs198389 (OR = 0.26, 95% CI 0.09-0.80, P = 0.02) were associated with the reduced risk of LVD. No CAD-susceptible haplotypes were identified. Multifactor dimensionality reduction analysis did not detect any gene-to-gene interactions among the five loci. Three loci (rs5063, rs632793 and rs198388) formed the best model with the maximum testing accuracy (39.89%) and cross-validation consistency (10/10). Conclusion: Three *NPPA-NPPB* polymorphisms (rs632793, rs198388 and rs198389) were associated with reduced risk of LVD in CAD patients with HLP.

Keywords: Left ventricular dysfunction, coronary artery disease, natriuretic peptide, polymorphism, heart failure

Introduction

Left ventricular dysfunction (LVD) is an early stage of heart failure (HF) characterized by a reduced ejection fraction and a depressed level of left ventricular wall motility [1]. Coronary artery disease (CAD) and myocardial infarction (MI), the cardiac polygenic disorders [2-4], are contributing factors to the development of LVD. Other diseases, such as cardiomyopathy, hypertension (HTN) and valvular disease, may also lead to LVD. LVD with subsequent HF is characterized by a continuous interaction between the underlying myocardial dysfunction and a series of compensatory mechanisms. A host of hemodynamic and neurohormonal factors, such as the adrenergic and renin-angiotensin-aldosterone systems (RAAS) are triggered to modulate left ventricle remodeling of the vascular tree once LVD occurs. One of the key neuroendocrine axes is the natriuretic peptide system. This system consists of five peptides, atrial peptide (ANP), urodilatin (an isoform of ANP), B-type natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and dendroaspis natriuretic peptide (DNP) [5]. These peptides share a similar molecular structure and biological functions (natriuresis, diuresis and vasodilation) [6]. ANP and BNP are mainly synthesized and secreted by cardiomyocytes in response to increased cardiac wall tension and volume loading [7]. The secretion of natriuretic peptides from cardiac ventricles is increased with LVD. The extent of ANP and BNP release is in

line with the degree of cardiac dysfunction and left ventricular failure, making them useful markers in evaluating the severity of these phenomena [8]. Circulating natriuretic peptide levels are helpful to assess the prognosis in patients with CAD and MI, with or without LVD [9, 10]. The persistence of elevated levels of natriuretic peptides for several months after MI suggests a continued risk of pathologic remodeling and the development of LVD and HF [11].

The *NPPA* (natriuretic peptide precursor A) and *NPPB* (natriuretic peptide precursor B) genes lie in tandem 9.7kb apart on chromosome 1. Mice with homozygous mutations of the *NPPA* gene have right and left ventricular hypertrophy. This hypertrophy is increased disproportionately (relative to controls) in response to transverse aortic constriction, suggesting that ANP negatively regulates matrix remodeling in the myocardium [12]. In contrast, *NPPB* gene knockout mice exhibit multifocal fibrotic lesions in the cardiac ventricles. These increase in size and number in response to ventricular overload, indicating that BNP acts as an anti-fibrotic factor [13]. Several polymorphisms within the *NPPA-NPPB* locus have been reported to be correlated with inter-individual variation in circulating natriuretic peptide concentrations [14, 15], contributing to ambulatory cardiovascular disease states such as stroke [16], HF [17], CAD [18] and HTN [15, 19]. The nonsynonymous coding polymorphism rs5065 is significantly associated with increased circulating BNP and amino-terminal BNP (NT-proBNP) levels in severe HF patients [20]. The rs5065 and rs5063 polymorphisms correlate with increased left ventricular mass index and left ventricular septal thickness in HTN patients [21]. The minor *NPPA-NPPB* allele, rs632793, rs198388 and rs198389 are associated with increased circulating ANP and BNP concentrations and a reduced rate of cardiovascular readmission in CAD patients [15, 22]. Surprisingly, these polymorphisms have not been evaluated as markers for LVD. Therefore, we investigated the relationship between common *NPPA-NPPB* polymorphisms and the presence of LVD in CAD patients.

Materials and methods

Ethics statement

The ethics committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine approved this study. All the authors followed the guidelines of the World's Association Declaration of Helsinki. Written informed consent for the study was obtained from each patient.

Study cohort

A total of 747 consecutive patients undergoing coronary angiography for the diagnosis and intervention of CAD were admitted to Ruijin Hospital from January 2008 to December 2012. Coronary angiography was performed via the femoral or radial artery approach, according to the clinical standards of the American College of Cardiology/American Heart Association guidelines for coronary angiography [23]. Standard Judkins techniques were used for angiography [24]. Physicians performing the studies were blinded to the study protocol. CAD was defined as ≥50% luminal obstruction at least one or more major coronary epicardial coronary vessel. The patients were grouped according to the number of significantly stenotic vessels, single-, double- or triple-vessel disease. Any patient with cardiomyopathy, congenital heart disease, pulmonary heart disease, valvular heart disease, stroke, infection, tumor or immune system disorders was excluded. Left ventricular (LV) function was calculated by the echocardiography staff just before angiography. Imaging (e.g., M-mode, 2D) and color-flow Doppler echocardiography were performed. LV ejection fraction (EF), volumes and internal dimensions were measured according to the American Society of Echocardiography recommendations [25]. LVD was defined as (modified Simpson's rule) LVEF≤ 45% [26]. All patients were interviewed to ascertain their sociodemographic, economic and health status characteristics as well as lifestyle (e.g., habits, tobacco usage). Ultrasound staff was blinded to polymorphism analysis results. Subjects were residents of Shanghai, a population that is primarily of Southern Han Chinese ethnicity.

Data collection

Complete clinical history and information on conventional cardiovascular risk factors were obtained by reviewing the patients' medical records. HTN was defined as a systolic blood pressure >140 mmHg, a diastolic blood pressure >90 mmHg or the use of anti-hypertensive

Table 1. The baseline characteristics of study population

Characteristics	Reduced LVEF (n = 201)	Preserved LVEF (n = 546)	*P* value[b]
Age, year[a]	64.26±11.76	64.29±8.98	0.97
Male sex, n (%)	157 (78.1)	417 (76.4)	0.62
Risk factors			
Hypertension, n(%)	135 (67.2)	418 (76.6)	0.01
Diabetes, n (%)	58 (28.9)	160 (29.3)	0.91
Hyperlipidaemia, n (%)	37 (18.4)	145 (26.6)	0.02
Stroke history, n (%)	21 (10.4)	67 (12.3)	0.49
Smoking habit (%)	89 (44.3)	209 (38.4)	0.15
Angiographic profile			
Single vessel disease (SVD), n (%)	46 (22.9)	184 (33.7)	0.01
Double vessel disease (DVD), n (%)	68 (33.8)	186 (34.1)	
Triple vessel disease (TVD), n (%)	87 (43.3)	176 (32.2)	

[a]Data are expressed as mean ± SD. [b]The unpaired t-test is used for age and the x^2 test is used for other categorical characteristics.

drugs. Smoking habit was classified as smokers (ex-smoker and current smokers) or non-smokers. Diabetes mellitus (DM) was defined as a fasting serum glucose level above 7.0 mmol/L, a two-hour postprandial glucose greater than 11.1 mmol/L, or the use of diabetic medications. The diagnosis and management of Hyperlipidemia (HLP) was performed according to the National Cholesterol Education Program Adult Treatment Panel III Guidelines (NCEP ATIII) [27]. Stroke was defined and classified based according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [28].

DNA isolation and genotyping

Blood was drawn using EDTA-collection tubes. Genomic DNA was extracted from peripheral blood leukocytes according to standard phenol-chloroform methods, and stored at -20°C until batch genotyping. Taqman assay was used to genotype SNPs (Single nucleotide polymorphisms). SNP Taqman probes and primers were designed using the Applied Biosystems Assay-by-Design Service for SNP genotyping. The sample DNA was amplified by PCR following the recommendations of the manufacturer. Thermal cycling was done on a Gene Amp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). Allele detection and genotype determination were performed using the fluorescence mode of an ABI PRISM 7900HT Sequence Detector (Applied Biosystems, Foster City, CA, USA). Genotyping was performed without knowledge of the case-control status of the subjects. Ten percent of samples from patients and controls were sequenced to estimate the quality of genotyping. No discrepancy was found. Five SNPs located in the *NPPA-NPPB* locus (rs5065, rs5063, rs632793, rs198388 and rs198389) were investigated.

Statistical analysis

Continuous variables were reported as the mean ± standard deviation (SD) or median with 5th and 95th percentiles. Categorical measures were reported as percentages. The chi-square test or Fisher's exact test were used to examine the goodness of fit between the observed allelic frequencies and the allelic frequencies expected by Hardy Weinberg Equilibrium (HWE). HWE was determined using the web program- http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl. Differences in the allelic and genotypic frequencies of cases and controls were evaluated using the chi-square test. An unpaired t-test was used to test group differences of continuous variables. Genotype frequency of the subjects specified by different genetic models (allelic, additive, dominant, recessive and homozygote comparison) was analyzed by multivariate logistic regression adjusted for confounding factors. A two-tailed $P<0.05$ was regarded as statistically significant. Data management and statistical analyses were performed using SPSS software version 20.0 (SPSS, Chicago, IL, USA).

Linkage disequilibrium (LD) and haplotype blocks within 5 SNPs of the *NPPA-NPPB* locus were identified using the online computer platform SHEsis- http://analysis.bio-x.cn/myAnalysis.php [29]. LD coefficients were calculated using the formula $D'=D/D_{max}$ or D/D_{min}. Haplotypes with a frequency higher than 1% were examined and significance was estimated by reference to the most frequent haplotype. Haplotype analysis was performed using the Haplo.stats program developed by R language version 3.0.2 (http://www.r-project.org). The

Table 2. Genotype distributions and allele frequencies of the 5 examined polymorphisms between the reduced and preserved LVEF groups in CAD patients and the risk prediction under various genetic models

SNP ID (rs number)	Genotype and allele	Cases	Control	P_{X2}[a]	Genetic models	Unadjusted			Adjusted[b]		
						OR	95% CI	*P* value	OR	95% CI	*P* value
rs5065	GG	0	0	0.75	Allelic comparison	0.81	0.22-2.97	0.75	0.84	0.23-3.11	0.80
	AG	3	10		Dominant model	0.81	0.22-2.98	0.75	0.84	0.23-3.12	0.80
	AA	198	535		Recessive model	–[c]	–	–	–	–	–
	G (%)	0.7	0.9	0.75	Homozygote comparison	–	–	–	–	–	–
	A (%)	99.3	99.1		Additive model	0.81	0.22-2.98	0.75	0.84	0.23-3.12	0.80
rs5063	TT	4	7	0.78	Allelic comparison	1.09	0.74-1.61	0.65	1.06	0.72-1.58	0.76
	CT	32	86		Dominant model	1.06	0.69-1.62	0.79	1.04	0.67-1.60	0.87
	CC	165	452		Recessive model	1.56	0.45-5.39	0.48	1.43	0.40-5.13	0.58
	T (%)	10.0	9.2	0.65	Homozygote comparison	1.57	0.45-5.42	0.48	1.46	0.41-5.20	0.56
	C (%)	90.0	90.8		Additive model	1.09	0.75-1.58	0.66	1.06	0.72-1.55	0.77
rs632793	GG	6	9	0.50	Allelic comparison	1.07	0.76-1.50	0.69	1.06	0.75-1.50	0.75
	AG	42	120		Dominant model	1.01	0.69-1.48	0.95	1.02	0.69-1.50	0.93
	AA	153	416		Recessive model	1.83	0.64-5.22	0.26	1.56	0.53-4.56	0.42
	G (%)	13.4	12.7	0.69	Homozygote comparison	1.81	0.64-5.18	0.27	1.54	0.52-4.52	0.43
	A (%)	86.6	87.3		Additive model	1.07	0.77-1.49	0.70	1.06	0.75-1.48	0.75
rs198388	TT	5	14	0.50	Allelic comparison	0.84	0.61-1.17	0.30	0.81	0.58-1.13	0.22
	CT	45	145		Dominant model	0.8	0.56-1.16	0.25	0.78	0.53-1.14	0.20
	CC	151	386		Recessive model	0.97	0.34-2.72	0.95	0.85	0.30-2.44	0.76
	T (%)	13.7	15.9	0.30	Homozygote comparison	0.91	0.32-2.58	0.86	0.82	0.28-2.35	0.71
	C (%)	86.3	84.1		Additive model	0.84	0.61-1.17	0.30	0.81	0.59-1.13	0.22
rs198389	GG	6	10	0.43	Allelic comparison	0.96	0.68-1.33	0.79	0.95	0.68-1.34	0.77
	AG	42	132		Dominant model	0.89	0.61-1.29	0.54	0.89	0.61-1.31	0.56
	AA	153	402		Recessive model	1.64	0.59-4.58	0.34	1.49	0.52-4.27	0.45
	G (%)	13.4	14.0	0.79	Homozygote comparison	1.58	0.56-4.41	0.39	1.44	0.51-4.13	0.49
	A (%)	86.6	86.0		Additive model	0.96	0.69-1.33	0.79	0.95	0.68-1.33	0.77

[a]*P* values were calculated by x^2 test for differences in genotypes and alleles between the two groups. [b]ORs adjusted for age, gender, HTN, DM, HLP, Stroke history, smoking habit and angiographic profile. [c]data not available.

main functions in Haplo.stats were implemented: Haplo.em was used to calculate maximum likelihood estimates of haplotype probability using a "progressive insertion" algorithm that progressively inserted batches of loci into haplotypes of growing lengths. Haplo.cc and Haplo.glm were used to calculate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for each haplotype, respectively. These two approaches computed the regression of a trait on haplotypes and other covariates based on a generalized linear model [30, 31]. Haplo.score was used to calculate score statistics and test the difference in haplotype frequencies between cases and controls on the basis of simulated *P* (P_{sim}) values that were obtained from 1000 replicates [32].

Gene-to-gene interactions in the occurrence of LVD were evaluated using the open-source multifactor dimensionality reduction (MDR) software version 3.0 (www.epistasis.org) [33, 34]. MDR collapsed high-dimensional multilocus-genotype variables into a single dimensional multilocus-genotype variable by sorting the genotypes into two levels, high- or low- risk. All possible combinations of these 5 polymorphisms were tested. A probabilistic naïve Bayes classifier with 10-fold cross-validation was used to estimate the prediction accuracy and the empirical *P*-value. The data are divided into 10 divisions equally. 9/10 of the data is regarded as training set and then the remaining 1/10 is tested. A single best model with maximal testing accuracy and cross-validation consistency was determined by measuring the number of times of 10 divisions of the data. The *P*-value <0.05 was considered statistically significant using a 1000-fold permutation test.

Table 3. Stratified analyses of the 5 *NPPA-NPPB* polymorphisms with the risk of LVD in CAD patients with HLP under the additive genetic model

Study ID (rs number)	Group	Unadjusted			Adjusted[a]		
		OR	95% CI	*P* value	OR	95% CI	*P* value
rs5065	HLP	–[b]	–	–	–	–	–
	Non-HLP	0.91	0.24-3.49	0.89	0.97	0.25-3.77	0.96
rs5063	HLP	0.69	0.28-1.71	0.42	0.69	0.26-1.81	0.45
	Non-HLP	1.24	0.82-1.88	0.31	1.19	0.78-1.82	0.41
rs632793	HLP	0.35	0.12-1.00	0.05	0.31	0.10-0.93	0.04
	Non-HLP	1.31	0.91-1.89	0.15	1.30	0.90-1.89	0.16
rs198388	HLP	0.30	0.10-0.86	0.03	0.26	0.09-0.79	0.02
	Non-HLP	0.99	0.69-1.40	0.93	0.97	0.68-1.39	0.86
rs198389	HLP	0.31	0.11-0.88	0.03	0.26	0.09-0.80	0.02
	Non-HLP	1.17	0.82-1.68	0.39	1.17	0.81-1.69	0.40

[a]ORs adjusted for age, gender, HTN, DM, Stroke history, smoking habit and angiographic profile. [b]data not available.

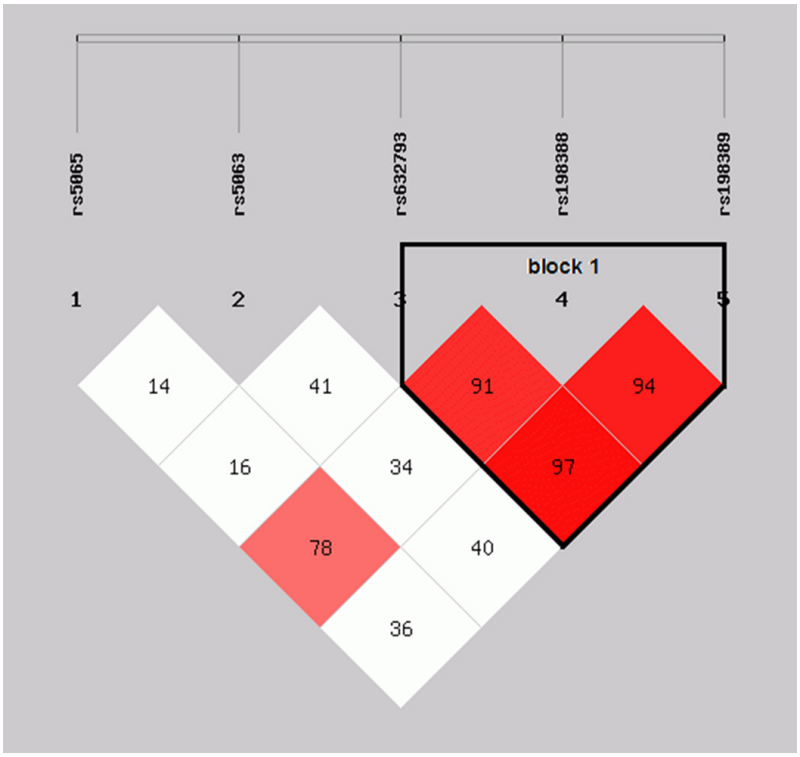

Figure 1. Block structure of linkage disequilibrium (LD) for five genotyped single nucleotide polymorphisms (SNPs) in the *NPPA-NPPB* locus. Stronger correlations between these SNPs are noted by red color in the intersecting squares linking each pair of SNPs.

Results

Patient characteristics

Clinical characteristics of CAD patients are shown in **Table 1**. Of the total 747 CAD patients, 201 had reduced (≤45%) LVEF and 546 had preserved LVEF (>45%). The reduced LVEF group was matched with the preserved LVEF group for age (P = 0.97) and gender (P = 0.62). There was no significant difference in the distribution of other confounding factors including DM (P = 0.91), smoking habit (P = 0.15) and stroke history (P = 0.49). The reduced LVEF group had a significantly lower proportion of HTN (P = 0.01) and HLP (P = 0.02) than the preserved LVEF group. The angiographic profile of the reduced LVEF group was significantly different from that of the preserved LVEF group (P = 0.01) (**Table 1**).

Single-locus analysis

Ninety-nine percent of the samples were successfully identified. The genotype and allele frequencies of these 5 polymorphisms, as well as their risk prediction under different genetic models, are listed in **Table 2**. No deviation from HWE was observed in both the reduced LVEF group and the preserved LVEF group (P>0.05). There was no difference in the allele or genotype frequencies of the two groups. The risk estimates for LVD with these 5 polymorphisms did not change after adjusting for age, gender, HTN, DM, HLP, stroke history, smoking habit and angiographic profile.

Stratification analyses were performed to investigate the interactive effect of *NPPA-NPPB* polymorphisms and confounding factors on LVD. When data were classified by HLP (**Table 3**) and adjusted for other confounding factors, three polymorphisms of the *NPPA-NPPB* locus

Table 4. Haplotype frequencies of the 5 *NPPA-NPPB* polymorphisms and their risk prediction of LVD in CAD patients

Haplotype	Cases (%)	Controls (%)	P_{sim}	Unadjusted			Adjusted[a]		
				OR	95% CI	*P* value	OR	95% CI	*P* value
Total[b]									
A-C-G-T-G	7.09	7.76	0.95	1.02	0.66-1.58	0.96	1.05	0.67-1.63	0.84
A-T-A-C-A	5.44	4.44	0.47	1.19	0.70-2.02	0.47	1.24	0.72-2.12	0.44
A-T-G-T-G	4.03	3.93	0.90	1.01	0.54-1.87	0.90	0.92	0.49-1.74	0.80
A-C-A-C-A	79.13	78.86	0.86		Reference			Reference	
Block1[c]									
A-T-A	1.26	2.40	0.17	0.51	0.19-1.34	0.16	0.41	0.15-1.11	0.08
G-T-G	11.93	12.03	0.99	0.97	0.68-1.38	0.96	0.97	0.68-1.38	0.86
A-C-A	84.81	83.44	0.51		Reference			Reference	

P_{sim}: simulated *P* value. [a]ORs adjusted for age, gender, HTN, DM, HLP, Stroke history, smoking habit and angiographic profile. [b]Alleles in total haplotype were arrayed in order of rs5065, rs5063, rs632793, rs198388 and rs198389. [c]Alleles in block 1 haplotype were arrayed in order of rs632793, rs198388 and rs198389.

Table 5. Summary of the multifactor dimensionality reduction analysis

Best combination of each model	Testing accuracy	Cross-validation consistency	*P* value[a]
rs198388	0.3963	9	0.8834
rs632793, rs198388	0.3788	5	0.9281
rs5063, rs632793, rs198388	0.3989	10	0.7708

[a]*P* value based on 1000 permutations.

were significantly associated with LVD (rs632793: *P* = 0.04, OR=0.31, 95% CI 0.10-0.93; rs198388: *P* = 0.02, OR = 0.26, 95% CI 0.09-0.79; rs198389: *P* = 0.02, OR=0.26, 95% CI 0.09-0.80). No such difference was found in the non-HLP population.

Haplotype analysis

Considering these 5 polymorphisms were located on the same chromosome, we performed a linkage analysis (**Figure 1**). Strong linkage patterns were observed between rs632793, rs198388 and rs198389 (D'≥0.91), suggesting that these 3 loci in the *NPPA-NPPB* locus were in the same block (block 1, **Figure 1**). Haplotype analyses were performed to investigate combinational effects of these 5 polymorphisms on LVD risk (**Table 4**). Four haplotypes (alleles in order of rs5065, rs5063, rs632793, rs198388 and rs198389) had frequencies ≥1% and were included in the haplotype analyses. The A-C-A-C-A haplotype was the most frequent (79.13%in the reduced LVEF group and 78.86% in the preserved LVEF group). Using the A-C-A-C-A haplotype as a reference, no significant difference was found between the other three allele combinations and the reference haplotype in prediction of LVD. Similarly, no difference was found for all the haplotypes in block1 (alleles in order of rs632793, rs198388 and rs198389), even after adjusting for confounding factors.

Gene-to-gene Interactions

An exhaustive MDR analysis that estimated possible interactions of different gene polymorphisms with LVD was performed (**Table 5**). Each best model was accompanied with its testing accuracy, cross-validation consistency and significant level determined by permutation testing. The single-locus model including rs198388 generated a testing accuracy of 39.63% and a cross-validation consistency of 9/10. Three polymorphisms, rs5063, rs632793 and rs198388 constituted the best overall MDR model with the highest testing accuracy of 39.89% and a maximal cross-validation consistency of 10. None of these models was significant in predicting LVD risk in CAD patients (*P*>0.05).

Discussion

We evaluated the relationship of 5 common *NPPA-NPPB* locus polymorphisms with LVD in a large Chinese population. To the best of our knowledge, this is the first such study performed in CAD patients. The principal finding of

the study indicated none of the *NPPA-NPPB* polymorphisms was associated with LVD in overall analysis. Haplotype analysis confirmed the lack of association of these 5 examined polymorphisms with LVD. A subgroup analysis of HLP patients identified a significant association between 3 polymorphisms (rs632793, rs198388 and rs198389) and a reduced risk of LVD after adjusting for environmental covariates. Our results confirmed a previous study of 1,164 Europeans undergoing primary coronary artery bypass graft (CABG) surgery with cardiopulmonary bypass (CPB) [35]. No significant associations were reported between the 2 *NPPA-NPPB* polymorphisms (rs5065 and rs5063) and ventricular dysfunction after CABG with CPB. Three polymorphisms (rs632793, rs198388 and rs198389) were protective of the ventricular dysfunction after CABG with CPB. These 3 polymorphisms are located in the *NPPA-NPPB* promoter region that has a tandem array of possible cis regulatory elements. These are known to be gene regulators and targets for gene up regulation via different signaling pathways [36]. Various physiologic stimuli including mechanical stretch, ischemic injury and hypoxia, as well as inflammatory mediators activate regulation of the *NPPA-NPPB* promoter, resulting in increased secretion of natriuretic peptide [37]. These SNPs are arranged in tandem on chromosome 1 and may coordinately regulate gene expression, through shared enhancer elements [15]. Further work will be required to determine the mechanisms by which these polymorphisms alter transcript stability or biological activity of the peptides.

A relationship was identified between the *NPPA-NPPB* polymorphisms in CAD patients with HLP and LVD. Natriuretic peptides are believed to be important in lipid metabolism, probably promoting adipose tissue lipolysis through increased cyclic guanosine monophosphate (cGMP) production [38]. The migration of human mesangial cells, may play a role in the pathogenesis of atherosclerosis. Oxidized LDL and lysophosphatidylcholine stimulate the migration of human mesangial cells. This migration is inhibited by ANP and BNP, possibly via a cGMP-dependent process [39]. BNP broadly inhibits Ang II-stimulated steroidogenesis by a number of mechanisms including the modulation of cholesterol biosynthesis, inhibition of Ang II- induced expression of scavenger receptor class B type I (SR-BI) , LDL receptors (LDLR), uptake of cholesterol from HDL and LDL into adrenocortical cells, inhibition of cholesterol transfer through the mitochondrial inner membrane, and reduction of steroid synthesis in primary human adrenocortical cells [40].

MDR is a novel method of analyzing genotype-genotype and genotype-phenotype associations. The advantage of MDR is that high-order gene-gene interactions can be detected in a relatively small sample population without the influence of dimensionality and genetic models. We did not detect any gene-gene interactions affecting LVD risk in CAD patients examined with MDR. This may be explained by the possibilities that the effect of the *NPPA-NPPB* promoter on LVD is predominant, and the interactive effects among genes may be overwhelmed by this main effect. Racial genetic diversity of the 5 examined polymorphisms could also explain this finding. There is a relatively lower genotype frequency of these 5 *NPPA-NPPB* polymorphisms in Chinese than in Caucasians [14, 15, 22]. The *NPPA-NPPB* polymorphisms probably had more complex genetic effects on the Chinese population than the Caucasian population. Additional studies of the pleiotropic effect of *NPPA-NPPB* polymorphisms are warranted.

This study had several limitations. First, the study was case-control in design, which precludes comments on causality. Second, only 5 *NPPA-NPPB* loci were evaluated. We could not exclude the possibility that other common SNPs or rare variations would affect disease progression. Third, we only evaluated genes related to the natriuretic peptide systems. LVD is a complex process that involves interactions of the adrenergic nervous system, RAAS and natriuretic peptides [41-43]. The interaction of natriuretic peptide genes with other neurohumoral factors, such as angiotensin and adrenergic genes, merits further study.

In conclusion, rs632798, rs198388 and rs198389 polymorphisms of the *NPPA-NPPB* locus might be additive genetic factors influencing disease progression of LVD in CAD patients with HLP. Rs5065 and rs5063 polymorphisms were not independent determinants of LVD in CAD patients. Our study reinforces the belief that natriuretic peptides may be protective of LVD, especially those with atherogenic dyslipidemia.

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Disclosure of conflict of interest

None.

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References

[1] Armstrong PW. Left ventricular dysfunction: causes, natural history, and hopes for reversal. Heart 2000; 84 Suppl 1: i15-17.

[2] Wu Z, Lou Y, Jin W, Liu Y, Lu L and Lu G. The C161T polymorphism in the peroxisome proliferator-activated receptor gamma gene (PPAR-gamma) is associated with risk of coronary artery disease: a meta-analysis. Mol Biol Rep 2013; 40: 3101-3112.

[3] Wu Z, Lou Y, Lu L, Liu Y, Chen Q, Chen X and Jin W. Heterogeneous effect of two selectin gene polymorphisms on coronary artery disease risk: a meta-analysis. PLoS One 2014; 9: e88152.

[4] Wu Z, Lou Y, Jin W, Liu Y, Lu L, Chen Q and Zhang R. The Connexin37 gene C1019T polymorphism and risk of coronary artery disease: a meta-analysis. Arch Me Res 2014; 45: 21-30.

[5] Cea LB. Natriuretic peptide family: new aspects. Curr Med Chem Cardiovas Hematol Agents 2005; 3: 87-98.

[6] Rubattu S, Sciarretta S, Valenti V, Stanzione R and Volpe M. Natriuretic peptides: an update on bioactivity, potential therapeutic use, and implication in cardiovascular diseases. Am J Hypertens 2008; 21: 733-741.

[7] Lang RE, Tholken H, Ganten D, Luft FC, Ruskoaho H and Unger T. Atrial natriuretic factor--a circulating hormone stimulated by volume loading. Nature 1985; 314: 264-266.

[8] Mohammed AA and Januzzi JL Jr. Natriuretic peptides in the diagnosis and management of acute heart failure. Heart Fail Clin 2009; 5: 489-500.

[9] Epshteyn V, Morrison K, Krishnaswamy P, Kazanegra R, Clopton P, Mudaliar S, Edelman S, Henry R and Maisel A. Utility of B-type natriuretic peptide (BNP) as a screen for left ventricular dysfunction in patients with diabetes. Diabetes Care 2003; 26: 2081-2087.

[10] Morrow DA, de Lemos JA, Blazing MA, Sabatine MS, Murphy SA, Jarolim P, White HD, Fox KA, Califf RM and Braunwald E. Prognostic value of serial B-type natriuretic peptide testing during follow-up of patients with unstable coronary artery disease. Jama 2005; 294: 2866-2871.

[11] Clerico A and Emdin M. Diagnostic accuracy and prognostic relevance of the measurement of cardiac natriuretic peptides: a review. Clin Chem 2004; 50: 33-50.

[12] Wang D, Oparil S, Feng JA, Li P, Perry G, Chen LB, Dai M, John SW and Chen YF. Effects of pressure overload on extracellular matrix expression in the heart of the atrial natriuretic peptide-null mouse. Hypertension 2003; 42: 88-95.

[13] Tamura N, Ogawa Y, Chusho H, Nakamura K, Nakao K, Suda M, Kasahara M, Hashimoto R, Katsuura G, Mukoyama M, Itoh H, Saito Y, Tanaka I, Otani H and Katsuki M. Cardiac fibrosis in mice lacking brain natriuretic peptide. Proc Natl Acad Sci U S A 2000; 97: 4239-4244.

[14] Del Greco MF, Pattaro C, Luchner A, Pichler I, Winkler T, Hicks AA, Fuchsberger C, Franke A, Melville SA, Peters A, Wichmann HE, Schreiber S, Heid IM, Krawczak M, Minelli C, Wiedermann CJ and Pramstaller PP. Genome-wide association analysis and fine mapping of NT-proBNP level provide novel insight into the role of the MTHFR-CLCN6-NPPA-NPPB gene cluster. Hum Mol Genet 2011; 20: 1660-1671.

[15] Newton-Cheh C, Larson MG, Vasan RS, Levy D, Bloch KD, Surti A, Guiducci C, Kathiresan S, Benjamin EJ, Struck J, Morgenthaler NG, Bergmann A, Blankenberg S, Kee F, Nilsson P, Yin X, Peltonen L, Vartiainen E, Salomaa V, Hirschhorn JN, Melander O and Wang TJ. Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. Nat Genet 2009; 41: 348-353.

[16] Rubattu S, Stanzione R, Di Angelantonio E, Zanda B, Evangelista A, Tarasi D, Gigante B, Pirisi A, Brunetti E and Volpe M. Atrial natriuretic peptide gene polymorphisms and risk of ischemic stroke in humans. Stroke 2004; 35: 814-818.

[17] Liguori A, Di Gregorio F, Napoli C, D'Armiento FP, Posca T, Di Benedetto A, Di Ieso N, Di Paolo E and Ferrara A. Atrial natriuretic factor and sympathetic activation in human heart failure. Riv Eur Sci Med e Farmacol 1994; 16: 61-67.

[18] Larifla L, Maimaitiming S, Velayoudom-Cephise FL, Ferdinand S, Blanchet-Deverly A, Benab-

dallah S, Donnet JP, Atallah A, Roussel R and Foucan L. Association of 2238T >C polymorphism of the atrial natriuretic peptide gene with coronary artery disease in Afro-Caribbeans with type 2 diabetes. Am J Hypertens 2012; 25: 524-527.

[19] Lynch AI, Boerwinkle E, Davis BR, Ford CE, Eckfeldt JH, Leiendecker-Foster C and Arnett DK. Pharmacogenetic association of the NPPA T2238C genetic variant with cardiovascular disease outcomes in patients with hypertension. Jama 2008; 299: 296-307.

[20] Vassalle C, Andreassi MG, Prontera C, Fontana M, Zyw L, Passino C and Emdin M. Influence of ScaI and natriuretic peptide (NP) clearance receptor polymorphisms of the NP System on NP concentration in chronic heart failure. Clin Chem 2007; 53: 1886-1890.

[21] Rubattu S, Bigatti G, Evangelista A, Lanzani C, Stanzione R, Zagato L, Manunta P, Marchitti S, Venturelli V, Bianchi G, Volpe M and Stella P. Association of atrial natriuretic peptide and type a natriuretic peptide receptor gene polymorphisms with left ventricular mass in human essential hypertension. J Am Coll Cardiol 2006; 48: 499-505.

[22] Ellis KL, Newton-Cheh C, Wang TJ, Frampton CM, Doughty RN, Whalley GA, Ellis CJ, Skelton L, Davis N, Yandle TG, Troughton RW, Richards AM and Cameron VA. Association of genetic variation in the natriuretic peptide system with cardiovascular outcomes. J Mol Cell Cardiol 2011; 50: 695-701.

[23] Scanlon PJ, Faxon DP, Audet AM, Carabello B, Dehmer GJ, Eagle KA, Legako RD, Leon DF, Murray JA, Nissen SE, Pepine CJ, Watson RM, Ritchie JL, Gibbons RJ, Cheitlin MD, Gardner TJ, Garson A Jr, Russell RO Jr, Ryan TJ and Smith SC Jr. ACC/AHA guidelines for coronary angiography: executive summary and recommendations. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Coronary Angiography) developed in collaboration with the Society for Cardiac Angiography and Interventions. Circulation 1999; 99: 2345-2357.

[24] Kushner FG, Hand M, Smith SC Jr, King SB 3rd, Anderson JL, Antman EM, Bailey SR, Bates ER, Blankenship JC, Casey DE Jr, Green LA, Hochman JS, Jacobs AK, Krumholz HM, Morrison DA, Ornato JP, Pearle DL, Peterson ED, Sloan MA, Whitlow PL and Williams DO; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. 2009 Focused Updates: ACC/AHA Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction (updating the 2004 Guideline and 2007 Focused Update) and ACC/AHA/SCAI Guidelines on Percutaneous Coronary Intervention (updating the 2005 Guideline and 2007 Focused Update): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation 2009; 120: 2271-2306.

[25] Gottdiener JS, Bednarz J, Devereux R, Gardin J, Klein A, Manning WJ, Morehead A, Kitzman D, Oh J, Quinones M, Schiller NB, Stein JH and Weissman NJ; American Society of Echocardiography. American Society of Echocardiography recommendations for use of echocardiography in clinical trials. J Am Soc Echocardiogr 2004; 17: 1086-1119.

[26] Nadir MA, Dow E, Davidson J, Kennedy N, Lang CC and Struthers AD. Myocardial ischaemia is associated with an elevated brain natriuretic pepide level even in the presence of left ventricular systolic dysfunction. Eur J Heart Fail 2014; 16: 56-67.

[27] Grundy SM, Cleeman JI, Merz CN, Brewer HB, Jr, Clark LT, Hunninghake DB, Pasternak RC, Smith SC Jr and Stone NJ. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation 2004; 110: 227-239.

[28] Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL and Marsh EE 3rd. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993; 24: 35-41.

[29] Shi YY and He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005; 15: 97-98.

[30] Lake SL, Lyon H, Tantisira K, Silverman EK, Weiss ST, Laird NM and Schaid DJ. Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. Hum Hered 2003; 55: 56-65.

[31] Stram DO, Leigh Pearce C, Bretsky P, Freedman M, Hirschhorn JN, Altshuler D, Kolonel LN, Henderson BE and Thomas DC. Modeling and E-M estimation of haplotype-specific relative risks from genotype data for a case-control study of unrelated individuals. Hum Hered 2003; 55: 179-190.

[32] Schaid DJ, Rowland CM, Tines DE, Jacobson RM and Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002; 70: 425-434.

[33] Pattin KA, White BC, Barney N, Gui J, Nelson HH, Kelsey KT, Andrew AS, Karagas MR and Moore JH. A computationally efficient hypothe-

sis testing method for epistasis analysis using multifactor dimensionality reduction. Genetic epidemiology 2009; 33: 87-94.
[34] Hahn LW, Ritchie MD and Moore JH. Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. Bioinformatics 2003; 19: 376-382.
[35] Fox AA, Collard CD, Shernan SK, Seidman CE, Seidman JG, Liu KY, Muehlschlegel JD, Perry TE, Aranki SF, Lange C, Herman DS, Meitinger T, Lichtner P and Body SC. Natriuretic peptide system gene variants are associated with ventricular dysfunction after coronary artery bypass grafting. Anesthesiology 2009; 110: 738-747.
[36] Martinez-Rumayor A, Richards AM, Burnett JC and Januzzi JL Jr. Biology of the natriuretic peptides. Am J Cardiol 2008; 101: 3-8.
[37] Onuoha GN, Nicholls DP, Patterson A and Beringer T. Neuropeptide secretion in exercise. Neuropeptides 1998; 32: 319-325.
[38] Dessi-Fulgheri P, Sarzani R and Rappelli A. Role of the natriuretic peptide system in lipogenesis/lipolysis. Nutr, Metab Cardiovasc Dis 2003; 13: 244-249.
[39] Kohno M, Yasunari K, Maeda K, Kano H, Minami M, Hanehira T and Yoshikawa J. Effects of cardiac natriuretic peptides on oxidized low-density lipoprotein- and lysophosphatidylcholine-induced human mesangial cell migration. Hypertension 2000; 35: 971-977.
[40] Liang F, Kapoun AM, Lam A, Damm DL, Quan D, O'Connell M and Protter AA. B-Type natriuretic peptide inhibited angiotensin II-stimulated cholesterol biosynthesis, cholesterol transfer, and steroidogenesis in primary human adrenocortical cells. Endocrinology 2007; 148: 3722-3729.
[41] Weber KT. Aldosterone in congestive heart failure. N Engl J Med 2001; 345: 1689-1697.
[42] Sisson DD. Neuroendocrine evaluation of cardiac disease. Vet Clin North Am Small Anim 2004; 34: 1105-1126.
[43] Schrier RW and Abraham WT. Hormones and hemodynamics in heart failure. N Engl J Med 1999; 341: 577-585.