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Original Article

MADD-FOLH1 polymorphisms and serum homocysteine level in patients with coronary heart disease

Dong-Feng Wu[1,2], Rui-Xing Yin[2], Jian Wu[1,2], Xiao-Li Cao[3], Wu-Xian Chen[2]

[1]Department of The Cadre Integrated Ward, People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, Guangxi, People's Republic of China; [2]Department of Cardiology, Institute of Cardiovascular Diseases, The First Affiliated Hospital, Guangxi Medical University, Nanning, Guangxi, People's Republic of China; [3]Department of Neurology, The First Affiliated Hospital, Guangxi Medical University, Nanning, Guangxi, People's Republic of China

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Abstract: Background: The MAP-kinase activating death domain-folate hydrolase 1 gene region (MADD-FOLH1) involved in folate metabolism. The present study was determined to detect the association of SNPs in MADD-FOLH1 and serum homocysteine level in coronary heart disease (CHD) patients. Methods: Genotypes of 6 MADD-FOLH1 SNPs were determined by the Snapshot technology platform in 494 unrelated CHD patients, including 84 stable angina (SA) patients, 236 unstable angina (UA) patients and 174 acute myocardial infarction (AMI) patients. Results: Serum homocysteine level in the group AMI and UA were higher than that in the group SA ($P<0.05$). Serum homocysteine level in CHD patients was different among different rs7395662 and rs3736101 SNPs genotypes ($P<0.01$), the subjects with rs7395662 GG genotype had higher serum homocysteine level than that with rs7395662 AG or AA genotype ($P<0.05$), and this association was mainly present in the group UA but not in the group SA and AMI. The subjects with rs3736101 GA/AA genotypes had higher serum homocysteine level than that with rs3736101 GG genotype ($P<0.05$), and this association was mainly present in the group AMI but not in the group SA and UA. However, there were no significant differences in genotypic frequency among groups of SA, UA and AMI ($P>0.05$). Conclusions: Serum homocysteine was related to the clinical manifestation of CHD. The rs7395662 and rs3736101 SNP were associated with serum homocysteine level in CHD, but not associated with the clinical manifestation of CHD.

Keywords: MAP-kinase activating death domain-folate hydrolase 1 gene, single nucleotide polymorphism, coronary heart disease, homocysteine

Introduction

Coronary heart disease (CHD) remains a major cause of worldwide morbidity and mortality, despite therapeutic advances that control many risk factors such as low-density lipoprotein cholesterol (LDL-C) to levels lower than previously possible [1]. Therefore, many research programs have been tried to identify new risk factors to prevent CHD [2], with special attention to homocysteine, genetic and prospective studies showed that serum homocysteine is significantly associated with ischemic stroke (IS) and CHD risk [3-5]. Meta-analysis shows that an increase of 5 μmol/L in plasma homocysteine level enhances the risk of cardiac vascular disease by 1.6- to 1.8-fold, which is similar to the risk seen with an increase of 20 mg/dL (0.52 mmol/L) in cholesterol concentration [6]. The level of serum homocysteine is affected by lifestyle, such as the supplement of the vitamin B12 and folic acid decrease serum homocysteine level [7]. In addition, the serum homocysteine is also partly genetic determined, the methylene tetrahydrofolate reductase (MTHFR) 677C>T polymorphism (rs1801133) is among the strongest genetic predictors of serum homocysteine [8]. However, other genes involved in the folic acid pathway also influence the serum homocysteine level.

The folic acid pathway is essential for hundreds of intracellular transmethylation reactions including DNA methylation and nucleic acid synthesis, processes that are closely related to homocysteine metabolism [9]. Folic acid defi-

ciency and abnormal metabolism of folic acid can lead to high serum homocysteine level [10]. In a previous genome wide association study (GWAS), a novel single nucleotide polymorphism (SNP) of rs7395662 in or near MADD-FOLH1 have been found association with serum lipid levels [11, 12] and the risk of CHD [13]. As a member of the MADD-FOLH1 gene cluster mentioned in the GWAS, the FOLH1 gene encodes a type II transmembrane glycoprotein belonging to the M28 peptidase family. The protein acts as a glutamate carboxypeptidase on different alternative substrates, including the nutrient folate and the neuropeptide N-acetyl-l-aspartyl-l-glutamate [14]. A mutation in this gene may be associated with impaired intestinal absorption of dietary folates, resulting in low blood folate levels and consequent hyperhomocysteinemia [15, 16]. However, the SNPs in these region were not reported to influence serum homocysteine levels in general population or CHD patients, Therefore, the purpose of the present study was to detect the association of 6 SNPs in or near FOLH1 with serum homocysteine concentrations in the CHD patients.

Materials and methods

Study population

A total of 494 unrelated CHD patients were continuously recruited from hospitalized patients in the First Affiliated Hospital, Guangxi Medical University. The selected CHD patients were subject to significant coronary stenosis (≥50%) in at least either one of the three main coronary arteries or their major branches (branch diameter ≥2 mm). The enrolled patients are divided into 84 stable angina patients (SA), 236 unstable angina patients (UA) and 174 acute myocardial infarction patients (AMI), the modality of the first manifestation of CHD based on the symptoms, electrocardiogram and biochemical examination. The diagnostic criteria of AMI were as follows: chest pain lasting >30 min before enrollment and myocardial infarction confirmed by significant rise of creatin kinase MB and troponin I levels; The diagnostic criteria of UA were as follows: chest pain with an accelerating pattern or prolonged duration (>20 min) or recurrent episodes at rest or with minimal effort with documented transient ST-segment elevation or ST-segment depression of 0.1 mV in at least two contiguous electrocardiograph leads; The diagnostic criteria of SA were as follows: chest discomfort, including spreading to the left shoulder and arm, which could be relieved with nitroglycerin or rest. These patients had a down sloping or horizontal ST-segment depression >1 mm in an exercise test [17]. All enrolled patients were Han Chinese from Guangxi, the People's Republic of China. A standard questionnaire was used to ascertain the general information and medical history for all participants. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Informed consent was obtained from all subjects after they received a full explanation of the study.

Biochemical measurements

A 5 ml of venous blood samples were obtained from all enrolled subjects after at least 12 hours of fasting. The levels of serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and LDL-C in samples were determined by enzymatic methods with commercially available kits. Serum apolipoprotein (Apo) A1 and ApoB levels were detected by the immunoturbidimetric immunoassay. Serum homocysteine was measured by using enzymatic cycling method.

SNP selection and genotyping

The SNPs were selected on the basis of the following criterion: (1) SNPs information was obtained from NCBI dbSNP Build 132 (http://www.Ncbi.nlm.nih.gov/SNP/); (2) SNPs were restricted to minor allele frequency (MAF) >5%; (3) SNPs were reported to associated with CHD risk; and (4) SNPs located on exon that might be associated with the gene function.

Genomic DNA was extracted from leucocytes of venous blood using the phenol-chloroform method, Genotyping of the SNPs were performed by the Snapshot technology platform [18, 19]. The sense and antisense primers were: rs7120118F: 5'-TGCTCCCCTCTTCCAAACCACT-3', rs7120118R: 5'-TCCTTCTCCCCAAGACCTCACTC-3'; rs326214F: 5'-TGGCTCATGACAGGTGGTGCTA-3', rs326214R: 5'-TAGCAGCGGGATGACAGGAAAC-3'; rs326217F: 5'-CCCAGGGACGTTCCTTGTGTAA-3', rs326217R: 5'-CCTGGTTGCAACATCCACAGAAT-3'; rs7395662F: 5'-CTGTGGCTCCCACATCACTGG-3', rs7395662R: 5'-AAATGATTTTCCCTGCATGCTAGTT-3';

Table 1. General characteristics and serum homocysteine level among different subtypes of CHD

Parameter	SA (stable angina)	UA (unstable angina)	AMI (acute myocardial infarction)	F	*P*
Number	84	236	174	–	–
Age (years)	62.10±8.70	63.63±9.91	61.01±10.66	4.300	0.014
Systolic blood pressure (mmHg)	136.14±17.93	140.97±27.59	128.82±23.68[a,b]	11.968	0.000
Diastolic blood pressure (mmHg)	78.48±11.20	82.98±16.05	77.71±11.69[b]	8.130	0.000
Pulse pressure (mmHg)	57.67±11.86	57.50±19.08	51.11±18.57[a,b]	7.290	0.001
Cigarette smoking [*n* (%)]	48 (57.1)	106 (44.9)	88 (50.6)	3.977	0.137
Alcohol consumption [*n* (%)]	16 (19.0)	54 (22.9)	46 (26.4)	1.812	0.404
Total cholesterol (mmol/L)	4.34±0.89	4.55±1.25	4.51±1.11	1.032	0.357
Triglyceride (mmol/L)	1.46±0.65	1.73±1.11	1.56±0.95	2.759	0.064
HDL-C (mmol/L)	1.07±0.21	1.20±0.39[c,d]	1.05±0.31	10.941	0.000
LDL-C (mmol/L)	2.81±0.75	2.69±1.13[c,d]	2.74±0.97	0.455	0.635
Apolipoprotein (Apo) AI (g/L)	0.93±0.16	1.05±0.45	0.95±0.24	6.417	0.002
ApoB (g/L)	0.90±0.22	0.91±0.33	0.94±0.25	0.924	0.398
Homocysteine (mmol/l)	14.39±3.59	16.30±5.33[e]	16.13±6.07[e]	4.115	0.017

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoAI, apolipoprotein AI; ApoB, apolipoprotein B. [a]P<0.05, in comparison with group SA; [b]P<0.05, in comparison with group UA; [c]P<0.05 in comparison with group SA; [d]P<0.05 in comparison with group AMI; [e]P<0.05 in comparison with group SA.

rs3736101 and rs1051006F: 5'-CGGCCTTT-AGGAACCTGCTGAC-3', rs3736101 and rs105 1006R: 5'-TTGGCTGAATCGGGGAGTGTAA-3'.

Statistical analyses

The statistical analyses were carried out using the statistical software package SPSS 13.0 (SPSS Inc., Chicago, Illinois). Quantitative variables were expressed as mean ± standard deviation. Qualitative variables were expressed as percentages. Allele frequency was determined by direct counting. The difference in genotype distribution and sex ratio between the groups was used by chi-square analysis. The general characteristics between groups were tested by analysis of variance. P<0.05 was considered statistically significant.

Results

The general characteristics and serum homocysteine level in CHD patients

As shown in **Table 1**, the age in group UA was older than that in group SA and group AMI (P<0.05). The blood pressure was different among different CHD clinic manifestations, the systolic blood pressure and pulse pressure were lower in group AMI than that in the group SA and group UA (P<0.05), the diastolic blood pressure were higher in the group UA than that in the group SA and group AMI (P<0.05). The group UA had the highest serum HDL-C and ApoAI levels than in the group SA and group AMI (P<0.05). The serum homocysteine level in the group AMI and group UA were significant higher than that in the group SA (P<0.05).

MADD-FOLH1 polymorphisms and serum homocysteine level

As shown in **Table 2**, the level of serum homocysteine in the CHD patients were significant different among the different genotypes in the rs7395662 and rs3736101 SNPs (P<0.01 for both), the subjects with rs7395662 GG genotype had higher serum homocysteine levels than the subjects with rs7395662 AG or AA genotype (P<0.01), and this association was mainly present in the group UA but not in the groups of SA and AMI. The subjects with rs3736101 GA/AA genotypes had higher serum homocysteine levels than the subjects with rs3736101 GG genotype (P<0.01), and this association was mainly present in the group AMI but not in the group SA and UA. There was no significant difference in serum homocysteine levels between different genotypes in the SNPs of rs326214, rs326217, rs1051006 and rs7120118 (P>0.05).

The genotypic frequencies distribution and clinical manifestations of CHD

The genotypic frequencies and the genotype distributions of MADD-FOLH1 SNPs are shown in **Table 3**. There were no significant differences in the genotypic frenquencies among different clinical manifestation of CHD (P>0.05).

Table 2. Association of MADD-FOLH1 SNPs and serum homocysteine levels in CHD patients

SNPs/ Genotype	Homocysteine (umol/L)							
	n	CHD	n	SA	n	UA	n	AMI
rs7395662								
AA	184	16.34±6.27	32	13.81±3.60	88	17.51±6.49	64	16.00±6.65
AG	238	15.14±4.26	44	14.62±3.85	120	14.97±4.02	74	15.73±4.82
GG	50	17.50±6.77[a,b]	8	15.50±1.28	16	19.28±5.00[a,b]	26	17.02±8.42
F	–	5.319		0.885		8.592		0.410
P	–	0.005		0.417		0.000		0.664
rs326214								
GG	262	15.88±5.71	44	14.48±3.21	126	16.55±5.69	92	15.62±6.53
GA	192	15.91±5.24	40	14.30±4.02	90	15.95±5.03	62	16.89±5.99
AA	20	14.92±3.71			10	15.20±3.66	10	14.64±3.94
F	–	0.304		0.051		0.521		1.041
P	–	0.738		0.821		0.595		0.355
rs326217								
TT	272	15.90±5.63	44	14.48±3.21	128	16.64±5.63	100	15.57±6.33
TC	184	15.89±5.31	40	14.30±4.02	88	15.81±5.10	56	17.16±6.14
CC	18	14.74±3.88			10	15.20±3.66	8	14.16±4.31
F	–	0.390		0.051		0.827		1.586
P	–	0.677		0.821		0.439		0.208
rs1051006								
AA	188	15.77±5.51	40	14.50±3.37	86	17.25±5.59	62	14.55±6.04
AG	232	15.93±5.76	38	13.80±3.92	108	15.53±5.46	88	17.29±6.42
GG	52	16.00±3.56	8	16.55±2.41	30	16.39±3.69	14	14.84±3.76
F		0.058		1.996		2.498		3.985
P		0.943		0.143		0.080		0.020
rs3736101								
GG	422	15.63±5.45	76	14.17±3.63	202	16.36±5.50	144	15.37±6.01
GA/AA	50	17.96±4.99	8	16.55±2.41	22	15.78±3.78	20	20.92±5.54
F		8.340		3.272		0.230		15.282
P		0.004		0.074		0.632		0.000
rs7120118								
CC	272	16.01±5.62	44	14.48±3.21	128	16.64±5.59	100	15.88±6.36
CT	186	15.66±5.31	40	14.30±4.02	90	15.77±5.12	56	16.44±6.25
TT	14	16.09±3.63			6	17.13±2.74	8	15.31±4.18
F		0.244		0.051		0.766		0.203
P		0.784		0.821		0.466		0.816

[a]P<0.05, in comparison with AA genotype; [a]P<0.05, in comparison with AG genotype.

Discussion

It is well determined that the increased of serum homocysteine level is related to increased of the CHD risk [2], however, the association of the serum homocysteine level and clinical manifestation of the CHD was little explored. In the present study, we showed that the serum homocysteine level was different in different CHD manifestations, the groups of AMI and UA had significant higher serum homocysteine level than that in the group SA. It is well known that the acute coronary syndromes (ACS) including AMI and UA, was triggered by coronary plaque rupture and erosion, which is always marked by an increase of serum levels pro-inflammatory cytokines [20]. While recently reports have also established that homocyste-

Table 3. MADD-FOLH1 genotype frequencies and distribution among different subtypes of CHD

Genotype	SA [n (%)]	UA [n (%)]	AMI [n (%)]		
	N=84	N=236	N=164	x^2	P
rs7395662					
AA	32 (38.1)	88 (39.3)	64 (39.0)		
AG	44 (52.4)	120 (53.6)	74 (45.1)	8.333	0.080
GG	8 (9.5)	16 (7.1)	26 (15.9)		
rs326214					
GG	44 (52.4)	126 (55.8)	92 (56.1)		
GA/AA	40 (40/0)	100 (90/10)	72 (62/10)	0.350	0.839
rs326217					
TT	44 (52.4)	128 (56.6)	100 (61.0)		
TC/CC	40 (40/0)	98 (88/10)	64 (56/8)	1.776	0.411
rs1051006					
AA	40 (47.6)	86 (38.5)	62 (37.8)		
AG	36 (42.9)	108 (48.2)	88 (53.7)	5.193	0.268
GG	8 (9.5)	30 (13.4)	14 (8.5)		
rs3736101					
GG	76 (90.5)	202 (90.2)	144 (87.8)		
GA/AA	8 (9.5)	22 (9.8)	20 (12.2)	0.687	0.709
rs7120118					
CC	44 (52.4)	128 (57.1)	100 (61.0)		
CT/TT	40 (40/0)	96 (90/6)	64 (56/8)	1.721	0.423

ine influences endothelial function leading to a prothrombotic environment, platelet activation and endothelial leukocyte interactions though enhances inflammatory responses [21]. Therefore, the relation of serum homocysteine level and the stability of CHD may attribute to the inflammatory responses cause by homocysteine, however, the mechanism needs further research.

It was well explored that the serum homocysteine was influenced by many factors, including lifestyles and genetic factor, the methylene tetrahydrofolate reductase (MTHFR) 677C>T polymorphism is among the strongest genetic predictors of homocysteine [8], However, other genes involve in the folic acid pathway also influences the serum homocysteine level. Of these genes, the FOLH1 involved in folate uptake and retention, which hydrolyses dietary folate, has received the most attention with regard to its potential to modulate plasma folate status [15]. Studies reported that the 1561C→T SNP in FOLH1 was associated with elevated plasma folate concentrations [16], and interacted significantly with smoking in determining plasma homocysteine [22]. A novel SNP of rs7395662 assigned as MADD-FOLH1 locus of chromosome 11p, have initially found associated with serum lipid levels and the risk of CHD in a previous GWAS [11]. However, SNPs in this gene cluster were not reported to influence serum homocysteine level in CHD patients. In the present study, we selected the rs7395662 SNP and the other five SNPs that might related to the MADD-FOLH1 gene function, and to determine the associations of these SNPs and serum homocysteine level. The results shows that the level of serum homocysteine in the overall CHD patients were significant influenced by rs7395662 and rs3736101 SNPs, the subjects with rs7395662 GG genotype had higher serum homocysteine level than the subjects with rs7395662 AG or AA genotype, and this association was mainly present in the group UA but not in the group SA and group AMI. The subjects with rs3736101 GA/AA genotypes had higher serum homocysteine levels than the subjects with rs3736101 GG genotype, and this association was mainly present in the group AMI but not in the group SA and UA. Which suggest that the rs3736101 and rs7395662 SNPs influence the serum homocysteine levels in CHD patients. The mechanism of the rs7395662 and rs3736101 SNP influence the serum homocysteine was not involved in present study, the rs7395662 SNP is located in a flanking locus between MADD and FOLH1, which represents a gene desert close to the centromere with no known gene on the 500-kb flanking region [11], So whether SNP rs7395662 is a functional loci or just a tagging loci needs to be determined by further functional studies. The rs3736101 SNP, a "G" to "A" substitution at cDNA position 2509 base position on exon 13 of the MADD gene, result in an amino acid "arginine" to "glutamine" substitution, that might influences the MADD gene function, MADD encodes mitogen-activated protein kinase activating death domain, which interacted with tumor necrosis factor alpha receptor 1 to activate mitogen-activated pro-

tein kinase and propagate the apoptotic signal [23]. Genetic study and functional study showed the MADD involved in glucose metabolism [24]. However, the MADD gene have not been implicated in folate metabolism, these data may provide a new idea for the association between blood glucose metabolism and homocysteine.

As mentioned above, the serum homocysteine level was related with the stability of CHD, and serum homocysteine level was influenced by genetic factors, therefore, we performed a chi-square test to detect whether the MADD-FOLH1 SNPs were associated with the stability of CHD, in the present study, we did not found the SNP was associated with the stability of CHD. It is well known that the stability of CHD was influenced by multiple channels, such as systemic and coronary inflammatory, activation of innate and adaptive immunity, activation of adaptive Immunity, environmental, physical, emotional stressors and so one [25], furthermore, the two SNPs did not cover the whole gene cluster and could not overall explain the associations of this gene with the diseases. Also, there is potential limitation in this study, the sample size especially the numbers of the group SA was relatively small. Therefore, further studies with larger sample sizes are needed to confirm our results.

Conclusions

The present study shows the serum homocysteine level in the group AMI and group UA were significant higher than that in the group SA. The subjects with rs7395662 GG genotype had higher serum homocysteine level than the subjects with rs7395662 AG or AA genotype in the group UA. The subjects with rs3736101 GA/AA genotypes had higher serum homocysteine level than the subjects with rs3736101 GG genotype in the group AMI. The genotypic frequencies were not significant different among groups of SA, UA and AMI, suggesting the serum homocysteine level was related to the clinical manifestation of CHD, the rs7395662 and rs3736101 SNP were associated with serum homocysteine level in CHD, but not associated the clinical manifestation of CHD.

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Disclosure of conflict of interests

None.

Authors' contribution

DFW participated in the design, performed the statistical analyses, and drafted the manuscript. RXY conceived the study, participated in the design, and helped to draft and edit the manuscript. JW, XLC, WXC collected the data and the samples. All authors read and approved the final manuscript.

Address correspondence to: Rui-Xing Yin, Department of Cardiology, Institute of Cardiovascular Diseases, The First Affiliated Hospital, Guangxi Medical University, 22 Shuangyong Road, Nanning 530021, Guangxi, People's Republic of China. E-mail: yinruixing@163.com

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