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Original Article

Genetic polymorphisms of *UTS2* rs2890565 Ser89Asn in coronary heart disease and myocardial infarction in Chinese population

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Abstract: Objective: Atherosclerosis plays a key role in the inducibility and persistence of coronary heart disease. Clinical evidence, in vitro and in vivo studies have implicated Urotensin II (U-II/UTS2) in the development of atherosclerosis and coronary artery disease, contributing to the (patho) physiological regulation of cardiovascular homeostasis in humans. Increased U-II plasma levels have been reported in patients with atherosclerosis and coronary heart disease. Considering these, our objective was to evaluate possible role of the UTS2 gene polymorphisms (Thr21Met and Ser89Asn) in the genetic susceptibility to coronary heart disease and myocardial infarction in a Chinese population. Methods: A case-control study was designed to compare the distribution of alleles and genotypes between case group (subjects with myocardial infarction, n=409) and control group (subjects with coronary heart disease, n=830). The detection of UTS2 gene polymorphisms was achieved with PCR-RFLP technique. Results: We did not identify statistically significant differences between the myocardial infarction and coronary heart disease groups, neither with regard to the frequency of genotype/variant at the Ser89Asn locus nor at the Thr21Met locus. When stratified by sex, differences in genotype distribution of polymorphism Ser89Asn were only seen in female subjects in both additive tested inheritance model (OR=0.257, 95% CI: 0.074-0.896, P=0.033) and recessive tested inheritance model (OR=0.280, 95% CI: 0.082-0.955, P=0.042). For subjects with myocardial infarction, we identified statistically significant differences between the ST-segment elevation myocardial infarction and non ST-segment elevation myocardial infarction groups. Differences in genotype distribution of polymorphism Ser89Asn not Thr21Met were seen in both additive tested inheritance model (OR=0.202, 95% CI: 0.049-0.833, P=0.027) and recessive tested inheritance model (OR=0.208, 95% CI: 0.052-0.835, P=0.027). When stratified by sex, differences in genotype distribution of polymorphism Ser89Asn were only seen in male subjects in both additive tested inheritance model (OR=0.208, 95% CI: 0.049-0.890, P=0.034) and recessive tested inheritance model (OR=0.197, 95% CI: 0.047-0.824, P=0.026). Conclusions: Ser89Asn (S89N) polymorphisms of the UTS2 gene were significantly associated with coronary heart disease and myocardial infarction in Chinese population. Additionally, we demonstrated that Genotype Asn89Asn may imply a potential benefit role for myocardial infarction.

Keywords: Coronary heart disease (CAD), myocardial infarction, urotensin-II (U-II/UTS-II), single nucleotide polymorphisms (SNP)

Introduction

Urotensin II (U-II) is a cyclic peptide synthesized through proteolytic cleavage of a precursor molecule, prepro-U-II, and is a potent vasoconstrictor [1]. U-II has been identified in the heart [2], which is also known to display an abundant expression of U-II receptors.

It is now well established that U-II levels are significantly increased in several cardiovascular diseases such as coronary artery disease (CAD). Hassan et al. demonstrated up regulation of U-II in endothelial and sub endothelial cells of atherosclerotic human coronary arteries, and suggested a possible role for U-II in the pathogenesis of coronary atherosclerosis [3]. The above findings are in agreement with that of Maguire et al. showing the presence of U-II immunoreactivity in the plaque of atherosclerotic coronary arteries [4]. It is therefore reasonable to suggest that the increased expression

of U-II in coronary atherosclerosis may contribute to the pathophysiology of the coronary atherosclerosis disease through an endocrine or paracrine fashion.

Acute myocardial infarction is the leading cause of morbidity and mortality. The most common cause of MI is coronary atherosclerotic plaque rupture or erosion, resulting in exposure to thrombus formation [5]. As vasoconstriction is involved in unfavorable myocardial and vascular remodeling, changes of U-II secretion after myocardial infarction may deteriorate or counterbalance its clinical course. In patients with myocardial infarction both increased and decreased plasma U-II concentrations were demonstrated. Several authors have investigated plasma levels of U-II in patients with acute coronary syndrome (ACS). Joyal et al. found lower plasma U-II concentration in patients with ACS compared with patients with stable angina and healthy controls [6]. Khan et al. found raised plasma U-II concentration in patients with acute myocardial infarction as compared to healthy controls [7]. Magdalena et al. aimed to analyze whether plasma concentration reflects the severity of ACS and found that decreased plasma U-II concentration in patients with ACS could be associated with more severe injury of myocardium [8]. As the results of the performed studies are ambiguous, it may imply that U-II concentration is not a good marker of myocardial necrosis and the relationship between myocardial necrosis and plasma U-II concentration may be indirect. On the other hand, genetic factors are important determinants.

The purpose of the present study was to assess the impact of the UTS2 Ser89Asn (S89N) and Thr21Met (T21M) polymorphism on the incidence and types of myocardial infarction in a case-control study in Chinese population. To the best of our knowledge, no data are available on the effect of the UTS2 polymorphisms on myocardial infarction compared with coronary artery disease.

Materials and methods

The investigation was conducted according to principles outlined in the Declaration of Helsinki. For research involving human participants, informed consent has been obtained from the patients or the guardian of the patients. The research has been approved by the Ethics Committee of the Peking University First Hospital.

Subjects

Study subjects were enrolled consecutively from the Department of Cardiology, Peking University First Hospital between October 2013 and June 2015. The study included 409 patients admitted to our division of cardiology for acute myocardial infarction and 830 control subjects which were admitted in the same period diagnosed as coronary heart disease without history of myocardial infarction. The Study subjects all underwent coronary angiography. The diagnosis of MI was based on typical electrocardiographic changes as well as on increases in the serum activities of enzymes such as creatine kinase, aspartate aminotransferase, and lactate dehydrogenase and in the serum concentration of troponin T/I. The diagnosis was confirmed by the presence of a wall-motion abnormality on left ventriculography as well as by identification of the responsible stenosis in any of the major coronary arteries or in the left main trunk by coronary angiography.

All study individuals were genetically unrelated ethnic Han Chinese and all came from North China.

Blood samples, DNA isolation and polymerase chain reaction restriction fragment length polymorphism

Blood samples from cases and controls were collected and stored at -80°C, Genomic DNA was extracted from EDTA-anticoagulated peripheral venous blood from each individual using the QIAamp DNA blood kit (Qiagen). The typing was performed using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique by means of an Applied Biosystems® 2720 Thermal Cycler. PCR optimization for each primer set was validated by temperature gradient and primers are listed in **Table 1**. The thermo cycling procedure consists of initial denaturation step at 95°C for 4 min, denaturation at 95°C for 30 s, annealing temperatures (58°C for 30 s for Thr21Met) and (60°C for 30 s for Ser89Asn) with 35 cycles, extension at 72°C for 30 s), and final extension

Table 1. Urotensin II gene polymorphisms Thr21Met and Ser89Asn with location, primer sequences, sizes of the amplicons and annealing temperatures

Reference SNP Number	Aminoacid changes	Enzyme	Nucleotide composition (5'→3')	Expected size of PCRproduct (bp)	Annealing temperature (°C)
rs228648	Thr21Met	*TaiI*	GGAAACCAACGTATTTCATC	141	60
			GCAAAAGAGGCAACTTACAGC		
rs2890565	Ser89Asn	*RsaI*	GTGCCTGTCTGTCTGCATTCA	263	58
			GAGTCCTGTAAAACCAGTACAG		

Table 2. Clinical characteristics of patients with myocardial infarction compared with controls

Demographic characteristic	Cases	Controls	t	χ^2	P
Sex					
Male	314 (0.768)	534 (0.643)			
Female	95 (0.232)	296 (0.357)		19.616	<0.001
Mean age (years)	63.05±12.50	63.88±10.27	1.164		0.245
Hypertension					
Yes	275 (0.672)	568 (0.684)			
No	134 (0.328)	262 (0.316)		0.18	0.671
Diabetes					
Yes	170 (0.416)	348 (0.419)			
No	239 (0.584)	482 (0.581)		0.015	0.903
Hyperlipidaemia					
Yes	218 (0.533)	477 (0.575)			
No	191 (0.467)	353 (0.425)		1.934	0.164
Hyperhomocysteinemia					
Yes	56 (0.137)	119 (0.143)			
No	353 (0.863)	711 (0.857)		0.094	0.759
Smoker					
Yes	257 (0.628)	415 (0.500)			
No	152 (0.372)	415 (0.500)		18.188	<0.001

(72°C for 7 min). Thr21Met polymorphism was identified by TaiI restriction enzyme and restricted on this region. The wild-type DNA (21T) yielded two bands with 83 and 58 bp, while the mutated DNA (21M) showed three bands with 141, 83, and 58 bp in size. Ser89Asn polymorphism was identified by RsaI restriction enzyme and restricted on this region. The 89S generated three bands with 161, 84 and 18 bp, while 89N showed two bands with 245 and 18 bp. Digested PCR products were resolved by 3% agarose gel electrophoresis, stained by using ethidium bromide and photographed under UV illumination. Genotyping was conducted in a blinded fashion.

We re-genotyped approximately 20% of the samples to verify the initial results. The check confirmed the previous genotyping results by 100%. Ten percent of individuals from each study group were randomly chosen, directly sequenced and the results were 100% concordant.

All of the UTS2 gene polymorphisms (Thr21Met and Ser89Asn) were in HWE (P>0.05) in population controls.

Statistical analysis

Statistical analysis was carried out using the SPSS 19.0. Independent t-test was used to examine the statistical significance of the difference of age and sex in different groups. Differences of genotype distribution and allele frequency were tested by chi-square analysis. Fisher's exact test was used when the expected number of samples in a group was less than

Table 3. Allele frequency and genotype distribution of urotensin II gene polymorphisms Thr21Met and Ser89Asn in cases and controls

SNP	Cases	Controls	OR (95% CI)	P	[a]OR (95% CI)	P
rs2890565						
Additive						
GG	215 (0.526)	432 (0.520)				
AG	167 (0.408)	339 (0.408)	0.990 (0.773-1.267)	0.935	0.973 (0.757-1.251)	0.832
AA	27 (0.066)	59 (0.071)	0.920 (0.567-1.492)	0.734	0.961 (0.588-1.571)	0.873
Dominant						
GG	215 (0.526)	432 (0.520)				
AG+AA	194 (0.474)	398 (0.480)	0.979 (0.773-1.241)	0.863	0.971 (0.764-1.236)	0.813
Recessive						
GG+AG	382 (0.934)	771 (0.929)				
AA	27 (0.066)	59 (0.071)	0.924 (0.576-1.480)		0.972 (0.602-1.570)	0.908
Alleles						
G	597 (0.730)	1203 (0.725)				
A	221 (0.270)	457 (0.275)	0.974 (0.807-1.176)	0.788		
rs228648						
Additive						
GG	190 (0.466)	366 (0.443)				
AG	172 (0.422)	379 (0.458)	0.874 (0.680-1.124)	1.294	0.870 (0.675-1.123)	0.285
AA	46 (0.113)	82 (0.099)	1.081 (0.723-1.614)	0.705	1.140 (0.758-1.714)	0.53
Dominant						
GG	190 (0.466)	366 (0.443)				
AG+AA	218 (0.534)	461 (0.557)	0.911 (0.718-1.156)	0.442	0.961 (0.685-1.349)	0.819
Recessive						
GG+AG	362 (0.887)	745 (0.901)				
AA	46 (0.113)	82 (0.099)	1.154 (0.788-1.692)	0.461	1.220 (0.827-1.800)	0.316
Alleles						
G	552 (0.676)	1111 (0.672)				
A	264 (0.324)	543 (0.328)	0.979 (0.818-1.170)	0.812		

[a]Adjusted for clinical characteristics. SNP, single nucleotide polymorphism.

five. Two sided testing was used to evaluate statistical significance. Multivariate logistic regression analysis was used when age and sex were adjusted. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated to identify the relationship between SNPs and case groups. Linkage disequilibrium was analyzed with SHEsis software (http://analysis.bio-x.cn/myAnalysis.php).

Results

Cases and controls

There were no statistically significant differences between the control and case groups for age. Male were more common in case group (76.8% in case group vs. 64.3% in control group; P<0.001) (**Table 2**).

Analysis of HWE and the associations between SNPs of the UTS2 gene with myocardial infarction

The genotypes of control were in Hardy-Weinberg equilibrium (HWE) (P>0.05).

The genotype and allele frequencies of UTS2 polymorphisms in case and control groups were summarized in **Table 3**. The frequencies of genotype and allele were adjusted for age, sex and concomitant diseases.

The wild-type genotype GG at the Thr21Met locus was observed in 190 (46.6%) of the myocardial infarction patients, whereas the frequencies of variant genotypes GA and AA were 172 (42.2%) and 46 (11.3%) respectively. In the control group, the frequencies of genotypes

Table 4. Genotype distribution of Ser89Asn in the urotensin II gene in cases and controls stratified by sex

rs2890565	Cases	Controls	OR (95% CI)	P	[a]OR (95% CI)	P
Male						
Additive						
GG	162 (0.516)	284 (0.532)				
AG	128 (0.408)	221 (0.414)	1.015 (0.759-1.358)	0.918	1.011 (0.755-1.355)	0.94
AA	24 (0.076)	29 (0.054)	1.451 (0.817-2.576)	0.202	1.453 (0.817-2.586)	0.204
Dominant						
GG	162 (0.516)	284 (0.532)				
AG+AA	152 (0.484)	250 (0.468)	1.066 (0.806-1.409)	0.654	1.063 (0.803-1.407)	0.67
Recessive						
GG+AG	290 (0.924)	505 (0.946)				
AA	24 (0.076)	29 (0.054)	1.441 (0.823-2.522)	0.199	1.446 (0.824-2.537)	0.198
Alleles						
G	452 (0.720)	789 (0.739)				
A	176 (0.280)	279 (0.261)	1.101 (0.883-1.374)	0.393		
Female						
Additive						
GG	53 (0.558)	148 (0.50)				
AG	39 (0.411)	118 (0.399)	0.923 (0.572-1.490)	0.743	0.829 (0.507-1.357)	0.455
AA	3 (0.032)	30 (0.101)	0.279 (0.082-0.953)	0.031	0.257 (0.074-0.896)	0.033
Dominant						
GG	53 (0.558)	148 (0.50)				
AG+AA	42 (0.442)	148 (0.50)	0.792 (0.498-1.261)	0.326	0.716 (0.444-1.155)	0.171
Recessive						
GG+AG	92 (0.968)	266 (0.899)				
AA	3 (0.032)	30 (0.101)	0.289 (0.086-0.970)	0.033	0.280 (0.082-0.955)	0.042
Alleles						
G	145 (0.763)	414 (0.699)				
A	45 (0.237)	178 (0.301)	0.722 (0.495-1.053)	0.09		

[a]Adjusted for clinical characteristics.

were 366 (44.3%) for GG, 379 (45.8%) for GA and 82 (9.9%) for AA. However, there were no statistical differences in genotype or allele frequency at the Thr21Met locus between case and control group.

The wild-type genotype GG at the Ser89Asn locus was observed in 215 (52.6%) of the myocardial infarction patients, whereas the frequencies of variant genotypes GA and AA were 167 (40.8%) and 27 (6.6%) respectively. In the control group, the frequencies of genotypes were 432 (52%) for GG, 339 (40.8%) for GA and 59 (7.1%) for AA. Concerning the UTS2 Ser89Asn gene polymorphisms, there were no statistical differences in genotype or allele frequency between case and control group.

We did not identify statistically significant differences between the myocardial infarction and coronary heart disease groups, neither with regard to the frequency of genotype variant at the Ser89Asn locus nor at the Thr21Met locus.

Association of UTS2 gene polymorphisms with myocardial infarction stratified by sex

UTS2 Ser89Asn genotype distribution was analyzed with stratification by sex. When case group was compared with control group, the significant association regarding to the frequency of variant genotypes at the Ser89Asn locus was only seen in female subjects in both additive tested inheritance model (OR= 0.257, 95% CI: 0.074-0.896, P=0.033) and recessive tested inheritance model (OR= 0.280, 95% CI: 0.082-0.955, P=0.042) (**Table 4**).

Table 5. Allele frequency and genotype distribution of urotensin II gene polymorphisms Thr21Met and Ser89Asn with types of myocardial infarction

SNP	STEMI	Non-STEMI	OR (95% CI)	P	[a]OR (95% CI)	P
rs2890565						
Additive						
GG	59 (0.541)	53 (0.491)				
AG	47 (0.431)	46 (0.426)	0.918 (0.529-1.591)	0.76	0.940 (0.515-1.715)	0.839
AA	3 (0.028)	9 (0.083)	0.299 (0.077-1.165)	0.068	0.202 (0.049-0.833)	0.027
Dominant						
GG	59 (0.541)	53 (0.491)				
AG+AA	50 (0.459)	55 (0.509)	0.817 (0.479-1.392)	0.456	0.782 (0.439-1.390)	0.402
Recessive						
GG+AG	106 (0.972)	99 (0.917)				
AA	3 (0.028)	9 (0.083)	0.311 (0.082-1.183)	0.072	0.208 (0.052-0.835)	0.027
Alleles						
G	165 (0.757)	152 (0.704)				
A	53 (0.243)	64 (0.296)	0.763 (0.498-1.167)	0.212		
rs228648						
Additive						
GG	47 (0.431)	44 (0.415)				
AG	48 (0.440)	44 (0.415)	1.021 (0.572-1.824)	0.943	0.974 (0.519-1.828)	0.936
AA	14 (0.128)	18 (0.170)	0.728 (0.324-1.638)	0.442	1.014 (0.417-2.466)	0.975
Dominant						
GG	47 (0.431)	44 (0.415)				
AG+AA	62 (0.569)	62 (0.585)	0.936 (0.545-1.608)	0.811	0.985 (0.549-1.767)	0.958
Recessive						
GG+AG	95 (0.872)	88 (0.830)				
AA	14 (0.128)	18 (0.170)	0.720 (0.338-1.535)	0.394	1.027 (0.445-2.371)	0.95
Alleles						
G	142 (0.651)	132 (0.623)				
A	76 (0.349)	80 (0.377)	0.883 (0.596-1.309)	0.536		

[a]Adjusted for clinical characteristics. SNP, single nucleotide polymorphism.

However, there was no statistical difference in allele frequency at the Ser89Asn locus between case and control groups among female subjects. We did not find statistical differences in genotype or allele frequencies at the Ser89Asn locus between case and control groups among male subjects.

UTS2 Thr21Met genotype distribution was analyzed with stratification by sex, we did not find any statistical difference (data not shown).

Association of UTS2 gene polymorphisms with types of myocardial infarction

For subjects with myocardial infarction, we identified statistically significant differences between the ST-segment elevation myocardial infarction and non ST-segment elevation myocardial infarction groups. Differences in genotype distribution of polymorphism Ser89Asn not Thr21Met were seen in both additive tested inheritance model (OR=0.202, 95% CI: 0.049-0.833, P=0.027) and recessive tested inheritance model (OR=0.208, 95% CI: 0.052-0.835, P=0.027) (**Table 5**). When stratified by sex, differences in genotype distribution of polymorphism Ser89Asn were only seen in male subjects in both additive tested inheritance model (OR=0.208, 95% CI: 0.049-0.890, P=0.034) and recessive tested inheritance model (OR=0.197, 95% CI: 0.047-0.824, P=0.026) (**Table 6**).

Linkage disequilibrium was analyzed with SHEsis software

We used SHEsis software to analyze linkage disequilibrium between Thr21Met locus and

Table 6. Association of UTS2 gene polymorphisms Ser89Asn with types of myocardial infarction stratified by sex

rs2890565	STEMI	Non-STEMI	OR (95% CI)	P	[a]OR (95% CI)	P
Male						
Additive						
GG	50 (0.532)	37 (0.493)				
AG	41 (0.436)	29 (0.387)	1.046 (0.553-1.980)	0.89	1.141 (0.572-2.275)	0.708
AA	3 (0.032)	9 (0.120)	0.247 (0.062-0.975)	0.034	0.208 (0.049-0.890)	0.034
Dominant						
GG	50 (0.532)	37 (0.493)				
AG+AA	44 (0.468)	38 (0.507)	0.857 (0.467-1.573)	0.618	0.879 (0.460-1.680)	0.696
Recessive						
GG+AG	91 (0.968)	66 (0.880)				
AA	3 (0.032)	9 (0.120)	0.242 (0.063-0.927)	0.027	0.197 (0.047-0.824)	0.026
Alleles						
G	141 (0.750)	103 (0.687)				
A	47 (0.250)	47 (0.313)	0.730 (0.453-1.178)	0.197		
Female						
Additive						
GG	9 (0.60)	16 (0.485)				
AG	6 (0.40)	17 (0.515)	0.627 (0.182-2.164)	0.459	0.579 (0.132-2.541)	0.469
AA	0 (0.00)	0 (0.000)				
Dominant						
GG	-	-	-	-	-	-
AG+AA	-	-	-	-	-	-
Recessive						
GG+AG	-	-	-	-	-	-
AA	-	-	-	-	-	-
Alleles						
G	24 (0.80)	49 (0.742)				
A	6 (0.20)	17 (0.258)	0.721 (0.252-2.061)	0.54		

[a]Adjusted for clinical characteristics.

Ser89Asn locus based on R2 value. The R2 value was 0.12, which indicated that Thr21Met locus and Ser89Asn locus were not in linkage disequilibrium.

Discussion

The present study did not identify statistically significant differences between the case and control groups, neither with regard to the frequency of genotype variant at the Ser89Asn locus nor at the Thr21Met locus. When stratified by sex, differences in genotype distribution of polymorphism Ser89Asn were only seen in female subjects. For subjects with myocardial infarction, we identified statistically significant differences between the ST-segment elevation myocardial infarction and non ST-segment elevation myocardial infarction groups. Differences in genotype distribution of polymorphism Ser89Asn not Thr21Met were seen in both additive tested inheritance model and recessive tested inheritance model. When stratified by sex, differences in genotype distribution of polymorphism Ser89Asn were only seen in male subjects. Our study suggests that Ser89Asn (S89N) polymorphisms of the UTS2 gene were significantly associated with coronary heart disease and myocardial infarction in Chinese population. Additionally, we demonstrated that Genotype Asn89Asn may imply a potential benefit role for myocardial infarction.

The gene for urotensin II (*UTS2*) is located at chromosome 1p36-p32 and encodes a somatostatin-like peptide identified as a potent vasoconstrictor. The vasoconstrictive effect of U-II is mediated via GPR14, also known as UT,

which is the specific receptor of U-II. U-II and its receptor UT are widely distributed in vascular and cardiac tissues [2] and they play an important role in cardiovascular diseases such as hypertension [9, 10], coronary artery disease [3], and heart failure [11]. In addition to clinical evidence, in vitro and in vivo studies have further implicated U-II in the development of atherosclerosis and coronary artery disease, contributing to the (patho) physiological regulation of cardiovascular homeostasis in humans. Migration and proliferation of vascular smooth muscle cells are important features of coronary atherosclerosis. U-II has been shown to induce proliferation of arterial smooth muscle cells through the activation of RhoA/Rho-kinase signal transduction pathway, which is one of the hallmark pathological features of atherosclerosis [12]. In addition to its mitogenic effects, U-II has been shown to stimulate hyperlipidemia by enhancing depot lipase activity in Coho salmon [13]. There is also emerging evidence for the U-II's ability to regulate pancreatic function by inhibiting insulin release and thus predisposing to diabetes [14], a potential risk factor to atherosclerosis. Moreover, U-II was also shown to act synergistically with mildly oxidized LDL, a factor known to be associated with atherosclerosis, in inducing DNA synthesis in vascular smooth muscle cells via the cSrc/PKC/MAPK pathway [15]. Another characteristic of coronary atherosclerosis is the deposition of extracellular matrix proteins, and in particular collagen type I in the sub endothelial layer [16]. Other data have demonstrated that U-II increases precollege production by cardiac fibroblasts [17] and endothelial cells [18]. In the previous study, we examined the direct effects of U-II on collagen synthesis in cultured rat aortic smooth muscle cells (VSMCs) [19]. We demonstrated that U-II stimulates collagen secretion and increases the mRNA expression of collagen I in a concentration-dependent manner. TGF-β1 is a potent profibrotic factor that is implicated in pathological fibrosis and that potentially acts in cardiovascular fibrosis. We explored the role of TGF-β1 in the effects of U-II on collagen synthesis in VSMCs. We found that U-II promotes collagen I production in VSMCs through mechanisms that include TGF-β1 expression and secretion [20]. In another study we provided evidence for the first time that U-II could induce VEGF expression in adventitial fibroblasts by concentration-dependent manner and that VEGF was involved in UII-induced collagen synthesis [21]. Ames et al. demonstrated increased U-II and UT in atheroma of coronary arteries [1]. Maguire et al. have demonstrated the increased expression of U-II in atherosclerotic human coronary artery. They found that, in atherosclerotic coronary artery, U-II immunoreactivity localized to regions of macrophage infiltration [4]. Hassan et al. investigated the expression of U-II in coronary arteries of normal control subjects and patients with coronary atherosclerosis and demonstrated up regulation of U-II in endothelial, myointimal and medial smooth cells of atherosclerotic human coronary arteries. In general, U-II mRNA expression shown by in situ hybridization matched that of U-II immunoreactivity shown by immunohistochemistry. The expression of endothelial U-II appears to be associated with the presence of inflammation and lipid deposition. Greater expression of U-II was noted in endothelial cells of lesions with sub endothelial inflammation or fibro fatty lesion compared with that of endothelial cells underlined by dense fibrosis or minimal intimal thickening [3].

In summary, these observations demonstrate abundant expression of the vasoactive peptide U-II in coronary atherosclerosis. The expression of endothelial U-II appears to be associated with the presence of inflammation and lipid deposition suggesting that increased expression of U-II may contribute to arterial remodeling associated with coronary atherosclerosis, by increasing cellular proliferation and extracellular matrix deposition. These findings demonstrate up regulation of U-II in endothelial and sub endothelial cells of atherosclerotic human coronary arteries, and suggest a possible role for U-II in the pathogenesis of coronary atherosclerosis.

The effects of U-II on cardiovascular function in pathological conditions are still controversial and remain to be explained. It has been reported that U-II can protect the heart against ischemia-reperfusion (I/R) through enhancing coronary flow and reducing cardiac contractility [22]. A recent study found that U-II receptor antagonist SB-710411 protects rat heart against ischemia-reperfusion injury via RhoA/ROCK pathway [23]. Plasma U-II has been observed to be raised in patients with acute myocardial infarction and a lower U-II response

is associated with more severe injury of myocardium [7], indicating a possible cardio protective role for this peptide. Reactive oxygen species (ROS) and antioxidant pathway are involved in the protective effect of U-II [24]. To explore the potential molecular mechanisms, Gong et al. treated cultured cardiomyocytes with H_2O_2 to induce oxidative stress and found that U-II pretreatment significantly reduced the number of apoptotic cardiomyocytes induced by H_2O_2 by partly abolishing the increase of pro-apoptotic protein Bax and the decrease of anti-apoptotic protein Bcl-2 in cardiomyocytes induced by H_2O_2. U-II rapidly promoted the phosphorylation of ERK and upregulated CSE level and H_2S production, which in turn activated ERK signaling to protect cardiomyocytes from apoptosis under oxidative stress. These results suggest that increased plasma U-II level may protect cardiomyocytes at the early-phase of acute myocardial infarction in patients.

Given that the effects of single polymorphisms on the development of MI are likely to be small, the association between a given polymorphism and the prevalence of MI might be influenced by the absence or presence of conventional coronary risk factors. Furthermore, conventional risk factors, such as hypertension, hypercholesterolemia, and diabetes mellitus, may themselves have genetic components and these components may interact with gene polymorphisms associated with MI. Many studies have focused on U-II polymorphisms as risk factors for these health outcomes, it has been suggested that the SNPs in UTS2 gene (Thr21Met and/or Ser89Asn) may affect susceptibility to hypertension, diabetes, pre-eclampsia, systemic sclerosis and Behcet's disease. In this study, we aimed to investigate the significance of UTS2 gene polymorphism in pathogenesis of myocardial infarction in Chinese population. Our study found that Ser89Asn (S89N) polymorphisms of the UTS2 gene were significantly associated with myocardial infarction in Chinese population. Additionally, we demonstrated that Genotype Asn89Asn may imply a potential benefit role for myocardial infarction. Several studies on the SNPs in UTS2 gene have suggested a role of U-II in the development of diabetes. It was reported that there were statistical differences in the allele frequency and genotype distribution of Thr21Met and Ser89Asn polymorphisms in UTS2 gene between case (subjects of Type 2 diabetes) and control groups suggesting Thr21Met may be associated with type 2 diabetes in Han people of China [25-28]. Ser89Asn, but not Thr21Met, was reported to be significantly associated with susceptibility of developing type 2 diabetes, increased plasma insulin level, and higher homeostasis model assessment of insulin resistance index (HOMA-IR) in Japanese subjects [29-31]. Ong et al. [32] also showed that plasma U-II level was related to Ser89Asn, but not to Thr21Met, in Hong Kong Chinese (29 hypertensive and 54 normotensive unrelated subjects). Ser89Asn was also significantly associated with higher fasting plasma insulin levels HOMA-IR and HOMA-β% in that study. However, neither Thr21Met nor Ser89Asn was associated with hypertension in this study. Yi et al. assessed the significance of polymorphism of the gene for UTS2 as risk factors for essential hypertension in two populations (Han and Dongxiang Populations) from north-western China and found that in the Han population there were significant differences in Ser89Asn genotype and allele frequencies between patients and controls [33]. Recently, Liu et al. explored the potential association between the UTS2 gene polymorphisms Ser89Asn and essential hypertension (EH) risk in a larger population of Northern Han Chinese. Significant associations were found in genotype distribution and allele frequencies between hypertensive and normotensive groups. However, when the data were subjected to logistic regression analysis after adjusting for confounding risk variables, no significant associations were found [34]. Yoshiji et al. [35] aimed to assess the genetic risk for myocardial infarction (MI) in individuals with or without conventional coronary risk factors. To a certain extent, our results on Ser89Asn are inconsistent with that of Yoshiji et al. We did not identify statistically significant differences between the myocardial infarction and coronary heart disease groups, neither with regard to the frequency of genotype variant at the Ser89Asn locus nor at the Ser89Asn locus. Yoshiji et al. have shown that the G→A (Ser89Asn) polymorphism of UTS2 was associated with the prevalence of MI only in hypertensive individuals but not other subjects (with or without hypercholesterolemia/diabetes mellitus). The possible explanations may be that the control subjects in our study are all diagnosed with coronary heart disease with or without conventional coronary

risk factors, while the control subjects in Yoshiji et al. study only comprised individuals who visited the outpatient clinics of participating hospitals for an annual health checkup. They had no history of CHD, peripheral arterial occlusive disease, or other atherosclerotic diseases. In another study by Yoshiji et al. [36], they revealed that after multivariable logistic regression analysis with adjustment for age, sex, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia, the G→A (Ser89Asn) polymorphism of UTS2 (all genetic models) were significantly associated with MI in individuals with metabolic syndrome. The control subjects comprised individuals who visited the outpatient clinics of participating hospitals for an annual health checkup or who were community-dwelling individuals recruited to the prospective cohort study. They had no history of coronary heart disease, which is different from our control subjects who were diagnosed with coronary heart disease. In our study, when stratified by sex, differences in genotype distribution of polymorphism Ser89Asn was only seen in female subjects in both additive tested inheritance model (OR=0.257, 95% CI: 0.074-0.896, P=0.033) and recessive tested inheritance model (OR=0.280, 95% CI: 0.082-0.955, P=0.042). For subjects with myocardial infarction, we identified statistically significant differences between the ST-segment elevation myocardial infarction and non ST-segment elevation myocardial infarction groups. Differences in genotype distribution of polymorphism Ser89Asn not Thr21Met were seen in both additive tested inheritance model (OR=0.202, 95% CI: 0.049-0.833, P=0.027) and recessive tested inheritance model (OR=0.208, 95% CI: 0.052-0.835, P=0.027). When stratified by sex, differences in genotype distribution of polymorphism Ser89Asn was only seen in male subjects in both additive tested inheritance model (OR=0.208, 95% CI: 0.049-0.890, P=0.034) and recessive tested inheritance model (OR=0.197, 95% CI: 0.047-0.824, P=0.026). Ser89Asn (S89N) polymorphisms of the UTS2 gene were significantly associated with coronary heart disease and myocardial infarction in Chinese population. Additionally, we demonstrated that Genotype Asn89Asn may imply a potential benefit role for myocardial infarction.

In conclusion, our results suggest that subjects with Ser89Asn in the UTS2 gene may modify individual susceptibility to myocardial infarction in the Chinese population. It is hoped that the results presented in this report will lead to additional basic and clinical studies and further studies with large-scale are required to verify these findings in different ethnic groups.

Limitations

There are some limitations of this study. One of them is that the number of study subjects was relatively small and from single ethnic group. Therefore, large population studies are necessary to confirm the role of the UTS2 gene as a risk factor for myocardial infarction. Another limitation of this study is that although we applied a rigorous epidemiological design in selecting study subjects and adjusted for confounding factors in further statistical analysis to minimize the potential biases, inherent selection bias cannot be completely excluded.

Conclusion

In this case-control study, we have shown for the first time that Ser89Asn polymorphisms of the UTS2 gene were significantly associated with myocardial infarction in Chinese population. Therefore, blockade of the Urotensin-II system may become a promising therapeutic strategy for myocardial infarctions with specific UTS2 polymorphism. Additionally, we demonstrated that Genotype Asn89Asn polymorphisms may imply a potential benefit role for myocardial infarction. This knowledge and proposed mechanism may open up new possibilities for the discovery of the way of pathogenesis and treatment of myocardial infarction.

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Disclosure of conflict of interest

None.

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