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Original Article

Relationship between polymorphism Ser89Asn of UTS2 gene and atherosclerotic cerebral infarction in Chinese Hunan Han population

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Abstract: As an endogenous vasoactive peptide, Urotensin-II (UTS2) is involved in the development of atherosclerosis. But there are no related reports focusing on UTS2 polymorphism and stroke risk so far. 308 patients with atherosclerotic cerebral infarction and 351 healthy controls were enrolled in this study. UTS2 gene Ser89Asn polymorphism was genotyped by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Genotypic and allelic frequencies of UTS2 Ser89Asn revealed no significant difference between ACI group and control group. For all subjects, Blood pressure, Fasting blood sugar (FBS) and blood lipids were not significantly different among the three genotypes of Ser89Asn. For Chinese Hunan Han population, UTS2 Ser89Asn may be not associated with ACI risk and also had nothing to do with levels of blood pressure, FBS or blood lipids.

Keywords: Urotensin-II (UTS2), polymorphism, atherosclerotic cerebral infarction (ACI), restriction fragment length polymorphism (RFLP)

Introduction

Stroke is the leading cause of death in China, as a serious threat to human health and a heavy burden to the community and the family [1]. But effective treatment for it is still lacking. Atherosclerotic cerebral infarction (ACI) is the most common subtype of stroke in China. ACI is generally regarded as a complex disease resulted from interactions between genetic factors and multiple environmental factors [2, 3]. Susceptibility genes probably play an important role in the development of stroke. Hypertension, hyperlipidemia, diabetes, heart disease, atherosclerosis, abnormal coagulation and metabolic syndrome are recognized risk factors for ACI. Those risk factors are also found to be related to genetic backgrounds [4, 5]. Therefore, it is especially vital to figure out susceptible genes of ACI.

Urotensin-II (UTS2) is one kind of vasoactive peptides, whose encoding gene is located on 1p36. UTS2 express mainly in the cardiovascular system, central nervous system and endocrine tissues [6, 7]. At present, studies have confirmed UTS2 is an endogenous mitogen involved in the process of atherosclerosis and may be caused by the following mechanisms: promoting the proliferation of endothelial cells, smooth muscle cell proliferation and migration, inhibition of apoptosis [8]; unregulated expression of collagen-1; reducing expression of matrix metalloproteinase-I [9]; activation of NADPH oxidase and plasminogen activator inhibitor-I [10]; accelerating macrophage-derived foam cell formation [11, 12]. Upregulation of Urotensin II and its receptors had been found in parts of human aortic atherosclerotic lesions [13]. The researchers had found that polymorphism Ser89Asn of UTS2 was associated with type 2 diabetes [14, 15] and essential hypertension [16]. A study found UTS2 can promote the development of atherosclerotic in cholesterol-fed rabbits [17]. Further, animal studies indicated that plaque development can be attenuated by UTS2 receptor antagonism [18, 19]. But there are no related reports focusing on UTS2 and ACI risk so far. So in this study, we

Table 1. Clinical data of ACI group and control group

Clinical data	ACI subgroup (n=308)	Control group (n=351)	*P*
Mean Age (years)	64.3±10.2	64.1±9.4	0.7935
Male/Femal	194/114	219/132	0.8750
BMI (kg/m^2)	23.71±2.32	23.42±2.51	0.1258
Smoking history (Yes/No)	201/107	232/119	0.8213
Drinking history (Yes/No)	232/76	202/149	0.2060
SBP (mmHg)	141.42±19.36	128.67±10.75	0.0000*
DBP (mmHg)	84.81±11.57	83.27±13.11	0.1126
FBS (mmol/l)	6.19±2.24	5.41±1.43	0.0000*
TC (mmol/l)	4.54±1.14	4.27±0.93	0.0009*
TG (mmol/l)	2.01±1.13	1.89±1.06	0.1602
HDL (mmol/l)	1.26±0.33	1.36±0.28	0.0000*
LDL (mmol/l)	2.69±0.84	2.56±0.63	0.0239*

*$P < 0.05$.

tried to find out whether Ser89Asn polymorphism of UTS2 gene is associated with ACI risk.

Material and methods

Subjects

ACI group: Cerebral infarction patients hospitalized in Department of Neurology, Xiangya Hospital from December 2012 to December 2013 were included in our study. All the cases were examined by cranial CT and/or MRI scanning and conformed with diagnostic criteria passed by the Fourth National Cerebrovascular Diseases Conference in 1995. Exclusion criteria: arteritis; trauma; blood diseases; tumors; vascular malformations or aneurysm; serious liver or kidney disease; autoimmune diseases; pregnancy; receiving lipid-lowering therapy in half a year. After screening, final samples consisted of 308 ACI patients (194 males and 114 females, average age 64.3±10.2 years old).

Control group: 351 healthy volunteers (219 males and 132 females, average age 64.1±9.4 years) were recruited from Health Management Center of Xiangya Hospital. Clinical and imaging examinations were conducted to exclude stroke. Liver and kidney diseases, autoimmune diseases, pregnancy or receiving lipid-lowering therapy in half a year was ruled out.

All subjects were non-consanguineous Chinese Hunan Han population. This study was approved by the Ethics Committee of Xiangya Hospital, Central South University (Changsha, Hunan province, China). All cases and controls gave written informed consent.

Blood biochemistry tests and DNA extraction

10 ml peripheral venous blood was collected from subjects with fasting 12 hours later. 5 ml was tested for determination of blood lipid and blood sugar; Another 5 ml (EDTA-anticoagulant) was used to extract DNA by conventional phenol/chloroform method.

Polymorphisms analysis

Primers were designed according to the reference [15] and synthesized by Sangon Biotech Co. Ltd (Shanghai, China). The sequences of corresponding primer: 5'-gagtcctgtaaaaccagtacag-3' (upstream); 5'-gtgcctgtctgtctgcattca-3' (downstream). PCR amplification parameters: 95°C predegeneration for 5 min, 95°C degeneration for 45 s, then 60°C denaturation for 45 s, finally 72°C extension for 45 s; totally 34 cycles, finally 72°C full extension for 10 mins. PCR products were digested at 37°C for 3 hours by restriction enzyme RsaI (Promega, Madison, USA).

The digested products were electrophoresed on a 2% agarose gel containing ethidium bromide (0.5×TBE, 120 volts). Enzyme-digested products of homozygous (CC) showed three bands with 161 bp, 84 bp and 18 bp respectively. Homozygous TT enzyme-digested products were presented with 245 bp and 18 bp whereas heterozygous (CT) enzyme-digested products exhibited four bands of 245 bp, 161 bp, 84 bp and 18 bp separately. To be clear, the length of 18 bp band is too small to be found in agarose gel electrophoresis.

Statistical analysis

Direct counting method was used to calculate the frequencies of genotypes and alleles. The chi-square test was employed for count data. Comparison of measurement data between the two groups was exercised by *t* test. Comparison between three groups was conducted by analy-

Table 2. Genotypes and alleles of polymorphism Ser89Asn of UTS2

Group	n	Genotypes, n (%)			*P1*	Alleles, %		*P2*
		CC	CT	TT		C	T	
ACI	308	144 (46.8)	121 (39.3)	43 (13.9)	0.7916	66.4	33.6	0.8449
Control	351	169 (48.2)	137 (39.0)	45 (12.8)		67.7	32.3	

All $P > 0.05$.

Table 3. Association between SER89ASN genotypes and traditional stroke risk factors

Stroke Risk Factors	ACI group			*P*1	Control Group			*P*2
	CC (144)	CT (121)	TT (43)		CC (169)	CT (137)	TT (45)	
TC (mmol/l)	4.42±1.04	4.61±1.27	4.74±1.10	0.1912	4.19±0.85	4.32±1.04	4.44±0.83	0.2064
LDL (mmol/l)	2.64±0.79	2.69±1.01	2.83±0.86	0.4719	2.58±0.69	2.57±0.76	2.48±0.81	0.7124
TG (mmol/l)	1.90±1.10	2.13±1.62	2.01±1.13	0.3766	1.91±0.86	1.84±0.89	1.94±0.93	0.7163
HDL (mmol/l)	1.29±0.32	1.25±0.29	1.22±0.41	0.3767	1.33±0.32	1.39±0.30	1.36±0.27	0.2351
SBP (mmHg)	142.17±25.29	143.41±19.10	139.49±16.34	0.6003	129.61±14.32	127.11±12.93	129.29±11.65	0.2550
DBP (mmHg)	85.59±14.32	83.51±12.23	86.16±11.09	0.3423	84.52±15.91	82.57±12.65	85.08±13.35	0.4122
FBS (mmol/l)	6.10±2.58	6.25±1.72	6.33±2.01	0.7770	5.52±1.61	5.27±0.98	5.43±1.46	0.3359

All $P > 0.05$.

sis of variance method. SPSS18.0 statistical software package for windows (SPSS Inc., Chicago, IL, USA) was used for statistical processing. $P < 0.05$ was considered statistically significant (Two-tailed).

Results

Clinical characteristics of subjects

The main clinical data of the ACI group and control group as shown in **Table 1**. In both groups, no significant difference was found in mean age, sex, body mass index (BMI), smoking history, drinking history, diastolic blood pressure (DBP) and Triglyceride (TG) level ($P > 0.05$). TC level, systolic blood pressure (SBP) and fasting blood sugar (FBS) in ACI group were significantly higher than the control group ($P < 0.05$); high density lipoprotein (HDL) levels of ACI subgroup were lower than the control group ($P < 0.05$).

Distribution of genotypes and alleles

Genotype frequencies of Ser89Asn were CC 46.8% (144/308), CT 39.3% (121/308) and TT 13.9% (43/308) in an ACI group while CC 48.2% (169/351), 39.0% (137/351) and 12.8 (45/351) in the control group. Allele frequencies of Ser89Asn were C 66.4% and T 33.6% in ACI group and C 67.7% and T 32.3% in the control group. As shown in **Table 2**, genotypic and allelic frequencies between two groups were not significantly different ($P > 0.05$).

Association studies between Ser89Asn genotypes and traditional stroke risk factors

In order to ascertain the relationship between Ser89Asn polymorphism and levels of blood pressure, FBS and blood lipids, we compared the blood pressure and blood lipids among three distinct genotypes between ACI group and control group. We discovered that no matter in which groups, Ser89Asn genotype did not affect levels of blood pressure, FBS, TC, TG, LDL or HDL (**Table 3**).

Discussion

UTS2 is a cyclic peptide composed of only 11 amino acid residues and exerts a series of physiological effects in endocrine, cardiovascular, renal, and immune functions. UTS2 and its receptor are widely expressed throughout the cardiovascular, central nervous and metabolic systems [20]. The up-regulated expression of UTS2 and its receptor have been indicated in several diseases, including metabolic syndrome, atherosclerosis, diabetes, hypertension [21, 22]. Numerous studies have proved that UTS2 can bind to and activate its receptor to stimulate proliferation of endothelial, smooth muscle cell and chemotaxis of monocytes [23, 24]. So UTS2 and its receptor are thought to play an important role in atherosclerosis development.

In this study, we conducted a case-control study to test the association of UTS2 Ser89Asn polymorphism with ACI risk. But there was no significant difference found in genotypic or allelic frequencies of Ser89Asn between ACI group and control group. While published literatures reported that polymorphism Ser89Asn of UTS2 was related to hypertension or type 2 diabetes [14-16]. And the plasma level of UTS2 was found to be up-regulated in hypertensive patients [16, 25]. Also UTS2 had been proved to influence blood pressure in pathological conditions *in vivo* [26-28], however the underlying mechanisms are still unclear. So our study further analyzed the relationship between three genotypes of polymorphisms Ser89Asn and traditional stroke risk factors. But our results found that Ser89Asn genotype did not affect blood pressure, FBS levels, both in stroke group and the control group. Then we explored the relevance between Ser89Asn polymorphism and lipid metabolism. We found that the blood lipid levels of stroke groups or controls were not significantly different regardless of different genotypes.

In summary, our study indicated that UTS2 Ser89Asn may be not associated with ACI risk for the Chinese Hunan Han population and also had nothing to do with levels of blood pressure, FBS or blood lipids. Larger samples and ethnically diverse populations are necessary to confirm this finding.

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Disclosure of conflict of interest

None.

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