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Association of Urotensin II Gene Polymorphism with Intracranial Haemorrhagia and Hypertension

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Abstract

Purpose: To investigate the association of S89N polymorphism in exon 3 of the urotensin II (UTS2) gene with intracranial haemorrhagia (ICH) and hypertension (HT).

Material and Methods: One hundred and eighteen subjects, 24 with a diagnosis of intracranial haemorrhagia (ICH) associated with hypertension, 44 with only hypertension and 50 healthy subjects as a control group, were included between 2013 and 2014. All the subjects were tested for G to A transition in codon 266 in the *UTS2* gene by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The distributions of genotypes and allele frequencies were compared between the groups.

Results: *UTS2* gene S89N polymorphism for hypertension and healthy control: GA genotype and A allele were found to be related to HT (p=0.004, p=0.0002, respectively). Existance of A allele found to be a risk factor for HT.

***UTS2* gene S89N polimorphism for ICH and healthy control:** GA genotype and A allele were found to be related to ICH (p=0.0105, p=0.0069, respectively). Existing of A allele was found to be a risk factor for ICH. Subjects in the ICH group showed a significant deviation from Hardy-Weinberg equilibrium (HWE) (p=0.036). The Hardy-Weinberg equilibrium is a principle stating that the genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors. No significant differences were noted in distribution of genotypes and allele frequencies between the HT and ICH groups.

Conclusion: The results of this study suggest that UTS2 single gene (S89N) polymorphism is associated with HT and ICH. Existance of A allele seems like a risk factor for hypertension and ICH occurred in association with HT.

Keywords: Gene haemorragica, hypertension, intracerebral, intracranial, pollymorphism, stroke ,Urotensin 2

Introduction

Stroke is known to be one of the leading causes of death worldwide. Intracerebral haemorrhage (ICH) is a severe form of stroke subtype, more likely to result in death or major disability than cerebral infarction or subarachnoidal haemorrhage (5, 7). Risk factors for ICH include elevated arterial blood pressure (BP), diabetes mellitus, hyperlipidemia, smoking, heavy drinking, prior symptomatic stroke or transient ischemic attacks, ischemic heart diseases, and hepatic diseases. It has been reported that 45-65% of nonlobar ICHs would be prevented if the effects of hypertension were eliminated (13). The gene for urotensin II (UTS2 or UII) is located at chromosome 1p36-p32 and encodes a peptide that was originally isolated and characterized in

fish. In mammals, UTS2 was first reported to have a vasoconstrictor action in rat thoracic aorta denuded of endothelium, increasing smooth muscle cell proliferation and vascular tone in vitro; this indicates a possible pathogenic contribution of this peptide to essential hypertension(3, 4)

Urotensin 2 (UT2) is reported to be one of the most potent endogenous vasoconstrictors identified so far. Elevated plasma levels of UT2 and UTS2 receptor expression have been demonstrated in numerous diseased conditions, including hypertension, preeclampsia, diabetes, atherosclerosis, heart failure, pulmonary hypertension, renal failure, and the metabolic syndrome (8). Recently, we showed that maternal serum UTS2 levels were significantly higher in subjects with preeclampsia compared the control group (4). Another study demonstrated that UT2 increases cerebral blood flow when administered into the cerebral ventricle and exacerbates ischemic brain lesions in rats. UT2 treatment resulted in a significant increase of hemispheric infarction volume 24 h after the induction of ischemia relative control rats (3).

In this study, we aimed to investigate a possible contribution of SNPs in the *UTS2* gene to development of ICH causing from hypertension.

Material and Methods

This is a retroprospective study. The subjects were included from the database of following institutions: Sifa University Neurosurgery Department, İzmir-Buca Seyfi Demirsoy Hospital Neurosurgery Department, İzmir Tepecik Training and Research Center Hospital Norology Department. The genetic analyses were performed at Gaziantep University Medical Biology and Genetics Department. This study was performed between 2013-2014.

ICH was diagnosed with brain computed tomography (CT) read by a radiologist. CT examinations were repeated routinely within a few days of admission. An additional CT scan was also performed when a clinical deterioration was noticed. The subjects with the following conditions were excluded: 1) patients with ICH due to aneurysmal rupture, arteriovenous malformations, moyamoya disease, and infective endocarditis, as well as those receiving anticoagulants or antiplatelet agents, 2) patients undergoing a second CT >120 hours after the onset of ICH due to facts that hematoma margin became illy defined, 3) patients who died or who underwent neurosurgery before a second CT examination. We classified the infarctions according to the system of the National Institute of Neurological Disorders and Stroke. All subjects were evaluated by a neurologist before the first CT for the following data: level of consciousness, aphasia, neglect or other discrete deficits in higher cortical function, abnormalities in visual fields, eye position, pupillary function, gaze, motor and sensory function, and plantar responses. A GCS score was calculated from the written description of the examination. Motor weakness was graded from 0 (complete plegia) to 5 (full strength). Clinical outcome was assessed by the modified Rankin Scale (grades 0 to 5) at 30 days or at discharge, if sooner. Death represented a Rankin score of 6.

Hypertension was defined to be present if patients fulfilled at least one of the following criteria: 1) a history of antihypertensive medication, 2) a history of hypertension diagnosed by a referring physician or 3) systolic blood pressure greater than 160 mmHg or diastolic blood pressure greater than 95 mmHg on two or more discrete occasions, after 1 month has lapsed from the onset of the ICH.

The primary aim of the study was to identify whether or not there is relation between ICH occurred related with HT and *UTS2* S89N gene polymorphsm.

The study protocol was approved by Instutional Ethic Committee of the Gaziantep University Hospital.

Genetical Analyses

All subjects were analyzed for S89N polymorphism in the *UTS2* gene. Genomic DNA was extracted from peripheral blood samples by using Ultra Clean Blood Spin DNA isolation kit according to the kit's protocol (MO BIO Laboratories, Inc., 2746 Loker Ave West, Carlsbad, CA 92010). Polymerase chain reaction-restriction

fragment length polymorphysm (PCR-RFLP) was used to examine S89N polymorphism (14). The nucleotide transition from G to A in codon 266 (amino acid transition from Ser to Asp at amino acid position 89) eliminates an Afa I site. The PCR fragments amplified with forward primer (F-5'-gtgcctgtctgtctgcattca-3') and reverse primer (R-5'-gagtcctgtaaaaccagctacag-3') were digested with AFA I and analyzed by 4% agarose gel electrophoresis. The 89S is expected to show three bands with 161, 84 and 18 bp, while 89N shows two bands with 245 and 18bp.

Statistical Method

SPSS 9.0 for Windows was used for statistical analysis. Results were reported as mean ± standart deviation. Statistical evaluation of demographics was performed using Student's *t-test.* A χ^2 test was used for statistical analysis of allele frequencies and the distrubition of genotypes in the case and control groups. Hardy-Weinberg equilibrium (HWE) was calculated using the De-finetti program (http://ihg.gsf.de/cgi-bin/hw/hwa2.pl). $p<0.05$ was considered to be statistically significant.

Results

A total of 118 patients were included: 44 patients with ICH, 24 patients with HT, and 50 subjects for the healthy control The remaining patients with ICH (n=44, 19 women and 25 men; mean age, 64.8±12.5 years) served as subjects for the present study. Demographics including age, and body mass index were similar between the groups ($p>0.05$). Demographics are shown in Table 1. Table 1 shows comparison of the groups according to the age and body mass index. Data is shown as mean (SD) (Table 1).

Table1. *shows comparison of the groups according to the age and body mass index. Data is shown as mean (SD).*

	ICH	HT	Control	P value
Age (Years)	59.2±	58.5±	58.2±	0.06
Body Mass Index	32±1.3	31±0.3	30±0.4	0.13

The mean systolic arterial pressures of the subjects at the admission in ICH group, HT group, and Control were 180±11mmHg, 160 ±10 mmHg and 120±8 mmHg respectively ($p<0.05$). The mean diastolic arterial pressures of the subjects in ICH group, HT group, and Control were 120±11mmHg, 119 ±10 mmHg and 85±3 mmHg respectively ($p<0.05$). No statistically significant differences in systolic and diastolic blood pressures were observed between the ICH and HT group. ICH were occurred at lobar (41%), thalamic (27%) ,putaminal (25%), pontine (5%), serebellar (2%) locations (Table 2).

Table2. *Shows location of Intraserebral Hemorrhage.*

	ICH occurred in association with HT (n=44)
Lobar	18 (%41)
Thalamic	12 (%27)
Putaminal	11 (%25)
Pontine	2 (%5)
Cerebellar	1 (%2)
Total	44

Severe headache was the most common symptomps reported (61.3%) in the patients with ICH occurred related with HT. Table 3 shows clinical features at onset of initial neurological findings and clinical outcomeof the ICH (Table 3). Table 4 shows CT Findings in the ICH group. (Table 4).

Table3. *shows clinical features at onset of initial neurological findings and clinical outcome of the ICH.*

Clinical features at onset	Patient number (n=44)
Severe headache	27 (61.3%)
Seizure or Convulsion	7
Vomiting	10
Vertigo or dizziness	5
Initial neurological findings	
Glasgow coma scale	12.5±2.3
Manuel muscle strength test	
Upper limb	3.5±1.2
Lower limb	3.6±1.5
Clinical Outcome	
Modified Rankin Scale	2.4±2.0

Table4. *CT Findings in the ICH group.*

Hematoma volume	29.3(cm^3)
Ventricular enlargement	5
Mass effect	30
Ventricular extension	5
Subarachnoid extension	4

UTS2 gene S89N polymorphism: GA genotype and A allele were found to be related with hypertension (p=0.004, p=0.0002). Existance of A allele seems to be a risk factor for HT. The observed genotype counts did not deviate significantly from those expected according to the Hardy-Weinberg Equilibrium (p>0.05). Table 5 shows the frequencies of genotype and allele distributions of *UTS2* gene polymorphism in HT and healthy controls (Table 5).

Table5. *Shows the frequencies of genotype and allele distributions of UTS2 gene polymorphism in HT and healthy controls.*

	Hypertension n (%)	**Controls n (%)**	**Odds Ratio (% 95 C.I)**	***P* value**
Genotype				
GG	10 (42)	42 (84)	0.14 (0.04-0.41)	0.0004*
GA	12 (50)	8 (16)	5.25 (1.75-15.79)	0.0043*
AA	2 (8)	0 (0)	11.22 (0.52-243.57)	0.1022*
GA+AA	14 (58)	8 (16)	7.35 (2.42-22.28)	0.0004*
Total	**24**	**50**		
Allele				
G	32 (67)	92 (92)	0.17 (0.07-0.44)	0.0002
A	16 (33)	8 (8)	5.75 (2,25-14.71)	0.0002
HWE *P*	0.5402	0.5386		
*:Fischer exact test				

UTS2 gene S89N polimorphism: GA genotype and A allele related with ICH (p=0.0105, p=0.0069). Existing of A allele is a risk factor for ICH. Group of ICH shows a significantly deviation from HWE (p=0.036). Table 6 shows the frequencies of genotype and allele distributions of *UTS2* gene polymorphism in intracerebral hemorrhage (ICH) and healthy controls(Table 6).

Table6. *The frequencies of genotype and allele distributions of UTS2 gene polymorphism in intracerebral hemorrhage (ICH) and healthy controls*

	ICH n (%)	Controls n (%)	Odds Ratio (% 95 C.I)	*P* value
Genotype				
GG	25 (57)	42 (84)	0.25 (0.09-0.65)	0.0057*
GA	18 (41)	8 (16)	3.63 (1.38-9.55)	0.0105*
AA	1 (2)	0 (0)	3.48 (0.13-87.77)	0.4681*
GA+AA	19 (43)	8 (16)	2.69 (1.31-5.54)	0.0057*
Total	**44**	**50**		
Allele				
G	68 (77)	92 (92)	0.29 (0.12-0.71)	0.0069*
A	20 (23)	8 (8)	3.38 (1.40-8.13)	0.0069*
HWE *P*	0.0360	0.5386		
*:Fischer exact test				

No significant differences were noted in distribution of genotypes and allele frequencies are between the HT and ICH groups. Table 7 shows the frequencies of genotype and allele distrubition of UT-II gene polymorphysm in HT and ICH (Table 7).

Table7. *The frequencies of genotype and allele distrubition of UT-II gene polymorphysm in HT and ICH.*

	n (%)	ICH n (%)	Odds Ratio (% 95 C.I)	*P* value
Genotype				
GG	10 (42)	25 (57)	0.54 (0.19-1.48)	0.3112*
GA	12 (50)	18 (41)	1.22 (0.71-2.08)	0.6101*
AA	2 (8)	1 (2)	3.90 (0.33-45.54)	0.2827*
Total	**24**	**44**		
Allele				
G	32 (67)	68 (77)	0.58 (0.26-1.28)	0.2558
A	16 (33)	20 (23)	1.7 (0.77-3.71)	0.2558
*:Fischer exact test				

Discussion

The results of this study sugget that GA genotype and A allele in regards to UT2 (S89N) polymorphism might be related with HT and ICH. If GA genotype and A allele exist in HT group, they have high risk for ICH.

HT is one of the most important risk factor for spontaneous ICH, particularly in those who are not compliant with antihypertensive medication, are 55 years of age or younger, or are smokers. Improved control of hypertension appears to reduce the incidence of ICH (9, 10, 11, 12). In the Hypertension Detection and Follow-up Program, persons with HT (defined as a diastolic blood pressure of at least 95 mmHg) who were 30 to 69 years of age and who received standardized antihypertensive theraphy had a risk for stroke (including intracerebral hemorrhage) of 1.9 per 100 persons, as compared with a risk of 2.9 per 100 persons in those who received

routine community care (11). Mortality rate at 1 month for ICH, varies between 28 and 52%, with most deaths occuring within the first few days after the onset of symptoms (6). Poor outcome is reported to be associated with the following conditions: large size haemorrhage, reduced level of consciousness on admission, low GCS score, initial elevated BP, intraventricular extension and older age.

UTS2 is reported to be one of the most potent endogenous vasocontsrictors identified so far. It has been suggested to have profound and potentially lethal pressor and vasoconstrictor effects in non-human primates in vivo. The distrubition of UTS2 receptors has been mapped using immunochemistry, confirming target binding sites in cardiovascular tissues including coronary arteries, internal mamary arteries and ventricular cardiomyocytes. Therefore it is considered likely that UTS2 functions as an endocrine hormone with cardiovascular actions. UTS2 concentrations are found to be increased in cardiac failure, renal failure, and diabetes as well as in hypertensive states (1,2).

In one study, maternal serum UTS2 levels has been found to be significantly higher in a preeclampsia group compared to those of healthy subject. Additionally, UTS2 levels and mean arterial pressure were positively correlated in both groups, while UTS2 levels were found to be highest in a severe preeclampsia group (4). It is nortworthy to remember that pregnancy-associated ICH is an infrequent but severe complication: the estimated mortality of pregnancy-associated ICH is 9-38%, which contributes to more than 12% of all maternal deaths in most countries. Pregnancy-associated ICH is usually caused by preeclampsia, eclampsia or cerebrovascular malformation, and several studies have even suggested that pregnancy associated ICH may be a main cause of death in pre-eclampsia patients. Hypertension, which is associated with both ischemic and haemorrhagic stroke, is a primary feature of preeclampsia (1).

It has been reported that UTS2 receptors are expressed in central nervous system (3). It has been previously shown that intravenous injection of UTS2 had minimal effects on cerebral blood flow (CBF) (3). However, its administration (10 nmol) directly into the lateral cerebral ventricle induced gradual and long lasting increase in CBF (+61% at 1 h post-injection, $p<0.05$). It has also been reported that these UTS2 mediated CBF increases were not related to the transient systemic pressor actions of the peptide and were reduced by nitric oxide synthase inhibition (61 vs 17%, $p<0.05$). Intracerebroventricular administration of UTS2 following the induction of cerebral ischemia, failed to alter residual CBF in the affected cerebral hemisphere. Nonetheless, following reperfusion (90 min after ischemia), UTS2 treated animals displayed a remarkable hyperperfusion compared to vehicle-treated rats (+168%, $p<0.05$). The volume of infarction was significantly increased in UTS2 treated rats (+40%, $p<0.05$). The authors suggested that the results of their study provide the first evidence of increased cerebral blood flow when UTS2 administered into the cerebral ventricle and exacerbates brain damage following an ischemic insult..

Intravenous UTS2 induces a sustained hyperemia through a pathway involving the production of NO, and secondly, UTS2 exacerbation ischemic brain lesion. Therefore, we aimed to show whether or not there is a relation between *UTS2* gene polymorphysm and intracerebral hemorrhage occured due to systemic hypertension.

In the present study, none of the subjects in control group had AA allele whereas one patient had AA allele, 18 patients had GA allele in ICH group. The results suggest that if a person with systemic HT have GA genotype or A allele for *UTS2* (S89N) gene polymorhysm might be in a high risk for ICH. Therefore, these patients should be treated appropriately for systemic HT, and should be monitored for their blood pressure regularly.

Conclusion

The results of this study showed that A allele for *UTS2* gene polymorphysm (S89N) is a risk factor for ICH occured related with HT. GA genotype and A allele were related both with HT and ICH.

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