Lack of association between urotensin-II (*UTS2*) gene polymorphisms (Thr21Met and Ser89Asn) and migraine

Betül Ozan[1], Seniz Demiryürek[1]*, Muhammad Safdar[2], Yusuf Inanc[3], Abdullah Tuncay Demiryürek[4]

[1]Department of Physiology, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey, [2]Department of Medical Biology, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey, [3]Department of Neurology, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey, [4]Department of Medical Pharmacology, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey

ABSTRACT

Migraine is a common neurovascular brain disorder with heterogeneous clinical presentation, including recurrent headache attacks. The pathophysiology of migraine is complex, and a number of genomic regions have been associated with the development of migraine. In this study, we analyzed the allele and genotype frequencies of the urotensin-II gene (*UTS2*) polymorphisms, Thr21Met and Ser89Asn, among Turkish patients with migraine. A total of 146 patients with migraine (14 with aura [MA group] and 132 without aura [MO group]) were genotyped for Thr21Met and Ser89Asn polymorphisms and compared with 154 age- and sex-matched healthy controls. The *UTS2* gene polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). No significant differences were observed in allele and genotype frequencies for Thr21Met and Ser89Asn polymorphisms between the patients with migraine and control group. Similarly, we did not observe significant differences in allele and genotype frequencies between MA and MO and control group. Moreover, the haplotype analysis showed no association between *UTS2* gene haplotypes (MN, MS, TN, and TS) and migraine. In summary, Thr21Met and Ser89Asn polymorphisms of the *UTS2* gene are not risk factors for migraine in our sample of Turkish migraine patients.

KEY WORDS: Aura; migraine; urotensin-II; *UTS2* gene; polymorphism; Thr21Met; Ser89Asn

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INTRODUCTION

Migraine is a multifactorial genetic disease characterized by recurrent headache attacks. The clinical presentation depends on the patient's genetic background as well as environmental factors [1-3]. A number of clinical subtypes of migraine have been described, two of which are migraine without aura (MO) and migraine with aura (MA) [4]. The annual prevalence of migraine in the Unites States (US) is 12%, and the prevalence is threefold higher in women compared to men [5]. In the eastern region of Turkey, the prevalence was reported as 10.3% in men and 23.1% in women [6]. The etiology of migraine is not well understood. Population-based studies showed that the familial risk of migraine has been increased [2,3,7]. A recent genome-wide association (GWA) study demonstrated a weak association between migraine and the following three single nucleotide polymorphisms (SNPs): rs2651899 of the PR domain containing 16 gene (*PRDM16*), rs10166942 of the transient receptor potential cation channel subfamily M member 8 (*TRPM8*), and rs11172113 of the low density lipoprotein receptor-related protein 1 (*LRP1*) [8]. Although a genetic component of migraine has been recognized, the type and number of genes involved are largely unknown.

Human urotensin-II (U-II), an 11-amino acid cyclic peptide, is a potent vasoconstrictor. U-II and its receptor, UT, are widely expressed throughout the central nervous, cardiovascular, renal, pulmonary, and metabolic systems. Elevated plasma levels and increased expression of U-II are found in many pathological conditions, including renal failure, hypertension, atherosclerosis, cirrhosis, congestive heart failure, diabetes, and metabolic syndrome [9]. For example, increased expression of U-II was observed in vascular smooth muscle cells due to hypoxia [10]. U-II may alter brain regions by directly affecting the vascular structures during the initiation and duration of migraine attacks.

The U-II gene (*UTS2*) is located on the human chromosome region 1p36 and contains 5 exons [11]. Recent GWA studies identified the 1p36 region as one of the susceptibility

*Corresponding author: Seniz Demiryürek, Department of Physiology, Faculty of Medicine, University of Gaziantep, University Road, Gaziantep 27310, Turkey. Tel.: 90-342-3606060 Ext.74204. Fax: 90-342-3601617. E-mail: sdemiryurek@gantep.edu.tr

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loci for migraine [8,12]. Moreover, a study on Turkish patients showed that *UTS2* Thr21Met polymorphism was associated with the risk of migraine, but not *UTS2* Ser89Asn polymorphism [13]. The aim of this study was to further investigate the association between the two *UTS2* polymorphisms and migraine in a Turkish population.

MATERIALS AND METHODS

Study population and collection of blood samples

The study group included 146 patients with migraine, and 154 unrelated healthy volunteers with no history of migraine. All patients were registered at the outpatient clinic of the Neurology Department, Faculty of Medicine, University of Gaziantep. This case-control study was approved by the local Ethics Committee, and it was conducted in accordance with the guidelines in the Declaration of Helsinki. Informed consent was obtained from all subjects prior to the study.

The patients were diagnosed by an interview as having either MA or MO. The questionnaires were prepared according to the International Headache Society (IHS) criteria for diagnosing migraine. Demographic details and clinical data such as migraine type and frequency, past medical history, hypertension history, concomitant drug history, and relevant family history were collected. The records of the cranial magnetic resonance imaging (MRI), computed brain tomography, and biochemical laboratory tests were evaluated for all patients. The control group was also subjected to the questionnaire by a neurologist specialized in migraines. The healthy control subjects were matched for age and gender and had no clinical evidence or family history of migraine or other neurological diseases. In addition, they were without a history of coronary artery disease, diabetes mellitus, hypertension, inflammatory and autoimmune diseases, or genetic disorders. The participants were all of Caucasian origin, and from the same geographical area (southeastern Turkey).

Venous blood samples were obtained for the molecular analysis of *UTS2* gene polymorphisms. Genomic DNA was extracted from whole blood using a salting-out method as described previously [14] and stored at -20°C.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

Two primer sets were used, covering two polymorphic regions (Thr21Met and Ser89Asn) of the *UTS2* gene [sequence in the National Center for Biotechnology Information (NCBI) database; NT_021937.19 contig]. The primers were designed with Primer Premier v.5.0 software (Premier Biosoft International, USA). The nucleotide composition of the primer sequences, amplicon size, and annealing temperatures are provided in Table 1. The PCR protocol included initial denaturation at 95°C for 4 minutes, 30 cycles of denaturation (95°C for 30 seconds) for Thr21Met (T21M, rs228648, G62A) and 32 cycles of denaturation for Ser89Asn (S89N, rs2890565, C266T), annealing for 30 seconds at temperatures given in Table 2, elongation at 72°C for 30 seconds, and final elongation at 72°C for 4 minutes. The PCR was conducted on GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA).

UTS2 Thr21Met polymorphism is characterized by G to A transition which leads to amino acid substitution of threonine to methionine at amino acid position 21. We used *NlaIII* restriction enzyme to identify the A/G mutation in the *UTS2* gene. The wild-type DNA (21T) produced two bands, 109 and 32 bp long respectively, while the mutated DNA (21M) produced three bands, 57, 52, and 32 bp in size, respectively.

A C/T mutation is present in *UTS2* Ser89Asn polymorphism. This mutation results in amino acid substitution of serine to asparagine at amino acid position 89. We identified the C/T mutation by *RsaI* restriction enzyme. The wild-type DNA (89S) produced three bands, 161, 84 and 18 bp long respectively, while the mutated DNA (89N) yielded two bands (245 and 18 bp in size). Digested PCR products were resolved by size using 3% agarose gel electrophoresis and stained by ethidium bromide.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) or percentage. GraphPad Instat version 3.05 (GraphPad Software Inc., San Diego, CA, USA) was used for the statistical analysis. Deviation from Hardy–Weinberg equilibrium (HWE) was determined by comparing the observed and

TABLE 1. Analyzed *UTS2* gene polymorphisms, their gene locations, primer sequences, sizes of the amplicons, annealing temperatures and related amino acid changes

Reference SNP number	Location	Amino acid change	Nucleotide composition (5'→3')	Expected size of PCR product	Annealing temperature (°C)
rs228648	Exon 1	Thr21Met	GGAAACCAACGTATTTCATC GCAAAAGAGGCAACTTACAGC	141 bp	55°C
rs2890565	Exon 4	Ser89Asn	GTGCCTGTCTGTCTGCATTCA GAGTCCTGTAAAACCAGTACAG	263 bp	57.7°C

SNP: single nucleotide polymorphism; bp: base pair

TABLE 2. Demographic and clinical characteristics of participants

	Patients (n=146)	Controls (n=154)	*p*
Mean age (years)*	33.8±9.9	33.4±8.8	0.7114
Sex			
Female n (%)	112 (76.7)	117 (76.0)	
Male n (%)	34 (23.3)	37 (24.0)	0.9884
Alcohol intake n (%)	2 (1.4)	4 (2.6)	0.6850
Cigarette smoking n (%)	31 (21.2)	33 (21.4)	0.9670
First degree family history of migraine n (%)			
Positive	31 (21.2)		
Negative	115 (78.8)		
Migraine type n (%)			
MA	14 (9.6)		
MO	132 (90.4)		
Migraine attack frequency n (%)			
1-3/week	80 (54.8)		
1-3/month	55 (37.7)		
<1-3/month	11 (7.5)		
Hypertension history n (%)			
Positive	13 (8.9)		
Negative	133 (91.1)		
Phonophobia n (%)	145 (99.3)		
Photophobia n (%)	146 (100.0)		
Nausea n (%)	146 (100.0)		
Vomiting n (%)	143 (97.9)		

*Data are presented as mean±standard deviation (SD). MA: Migraine with aura; MO: Migraine without aura.

expected genotype frequencies using the Chi-square test. Differences in genotype and allele frequencies were calculated by the Chi-square or Fisher's exact test. Differences in the mean values of the two groups were determined using the unpaired Student's *t*-test. The risk was calculated as odds ratio (OR) with 95% confidence (CI) interval. Haplotype analysis was performed using SHEsis online software (http://analysis.bio-x.cn/myAnalysis.php). The level of statistical significance was set at $p < 0.05$.

RESULTS

Among 146 migraine patients, 14 were diagnosed with MA and 132 with MO. The demographic characteristics of the study population are shown in Table 2. There were no significant differences between the patient and control group in the mean age, sex distribution, alcohol consumption, and cigarette smoking. Both groups were in HWE ($p > 0.05$).

The distribution of SS and SN genotypes of *UTS2* Ser89Asn polymorphism was 90.4% and 9.6%, respectively, in the patients with migraine compared to 92.2% and 7.8%, respectively, in controls (Table 3). Interestingly, we did not detect the NN genotype neither in the control nor in patient group. No significant differences were observed in allele and genotype frequencies for Ser89Asn polymorphism between the two groups (Table 3). The frequency of TT, TM, and MM genotypes of *UTS2* Thr21Met polymorphism was 7.6%, 45.5%, and 46.9%, respectively, in the patient group, and 7%, 53.1%, and 39.9%, respectively, in the control group. The two groups did not differ significantly in the genotype and allele frequencies of Thr21Met polymorphism (Table 4). Similarly, we did not observe significant differences in allele and genotype frequencies between MA and MO and control group (Table 3 and 4). The haplotype analysis of Ser89Asn and Thr21Met polymorphisms showed no association between MN, MS, TN, and TS haplotypes and migraine (Table 5). Ser89Asn and Thr431Asn polymorphisms were not associated with the family history of migraine, migraine attack frequency, or hypertension history (Table 6 and 7).

DISCUSSION

In the present study, we showed no significant association between migraine and *UTS2* Thr21Met and Ser89Asn polymorphisms. Similarly, no significant relationship was observed between the *UTS2* gene haplotypes and migraine in our sample of Turkish patients.

Our results indicate that the common genetic variation in the *UTS2* gene unlikely plays a role in the development of migraine. However, these results are not in agreement with the findings reported by Geyik et al. [13] who found that Thr21Met polymorphism was associated with the risk for migraine. Several factors could have contributed to the disparate results. First, although their study was conducted at the same university as ours, Geyik et al. [13] did not specifically indicate the ethnic background of their study population. In our study, all participants were Caucasians and of Turkish origin. Another difference is that Geyik et al. [13] only included

TABLE 3. Distribution of *UTS2* Ser89Asn (S89N, rs2890565) polymorphism between groups

Genotypes/Alleles	Controls (n=154) n (%)	Patients (n=146) n (%)	*p*	OR (95% CI)
SS	142 (92.2)	132 (90.4)		
SN	12 (7.8)	14 (9.6)	0.6826	1.255 (0.560-2.812)
NN	0 (0.0)	0 (0.0)		
S	296 (96.1)	278 (95.2)		
N	12 (3.9)	14 (4.8)	0.6895	1.242 (0.565-2.733)
MO		(n=132)		
SS	142 (92.2)	119 (90.2)		
SN	12 (7.8)	13 (9.8)	0.6753	1.293 (0.568-2.940)
NN	0 (0.0)	0 (0.0)		
S	296 (96.1)	251 (95.1)		
N	12 (3.9)	13 (4.9)	0.6824	1.278 (0.573-2.851)
MA		(n=14)		
SS	142 (92.2)	13 (92.9)		
SN	12 (7.8)	1 (7.1)	1.0000	0.910 (0.110-7.569)
NN	0 (0.0)	0 (0.0)		
S	296 (96.1)	27 (96.4)		
N	12 (3.9)	1 (3.6)	1.0000	0.914 (0.114-7.300)

OR: Odds ratio; CI: Confidence interval; MA: Migraine with aura; MO: Migraine without aura.

TABLE 4. Distribution of *UTS2* Thr21Met (T21M, rs228648) polymorphism between groups

Genotypes/Alleles	Controls (n=143) n (%)	Patients (n=145) n (%)	*p*	OR (95% CI)
TT	10 (7.0)	11 (7.6)		
TM	76 (53.1)	66 (45.5)	0.6462	0.790 (0.315-1.977)
MM	57 (39.9)	68 (46.9)	1.0000	1.085 (0.430-2.738)
T	96 (33.6)	88 (30.3)		
M	190 (66.4)	202 (69.7)	0.4595	1.160 (0.817-1.647)
MO		(n=131)		
TT	10 (7.0)	9 (6.8)		
TM	76 (53.1)	61 (46.6)	0.8115	0.892 (0.341-2.333)
MM	57 (39.9)	61 (46.6)	0.8072	1.189 (0.451-3.138)
T	96 (33.6)	79 (30.2)		
M	190 (66.4)	183 (69.8)	0.4446	1.170 (0.816-1.678)
MA		(n=14)		
TT	10 (7.0)	2 (14.3)		
TM	76 (53.1)	5 (35.7)	0.2219	0.329 (0.056-1.927)
MM	57 (39.9)	7 (50.0)	0.6272	0.614 (0.111-3.393)
T	96 (33.6)	9 (32.1)		
M	190 (66.4)	19 (67.9)	1.0000	1.067 (0.465-2.447)

OR: Odds ratio; CI: Confidence interval; MA: Migraine with aura; MO: Migraine without aura.

patients with MA, while in our study, both MA and MO patients were investigated.

The effect of Thr21Met and Ser89Asn polymorphisms on the structure, function, and plasma levels of U-II is still unclear, and requires further studies. Previously, our molecular modeling analysis revealed that the two polymorphic regions of U-II protein interact with neighboring residues. Specifically, changes in 7 and 25 amino acid residues in U-II were associated withThr21Met and Ser89Asn polymorphisms, respectively [15].

Migraine involves different changes in brain function and connectivity, as well as in the vascular system. For example, migraineurs have an approximately two-fold increased risk for ischemic stroke compared to non-migraineurs [16]; also, altered vascular reactivity can be found in young migraineurs [17]. Moreover, it has been demonstrated that patients with chronic migraine have endothelial dysfunction and an increase in the arterial stiffness [18]. Nitric oxide (NO) is believed to contribute to the pathogenesis of migraine. NO acts as a mediator of neurogenic dilatation of cerebral arteries [19], and it has been recognized as an essential molecule associated with pain during migraine attacks [20]. Migraine attacks are associated with intra and extracranial arterial dilatation, and this effect may be related to NO [19,20]. At low concentrations, U-II induces vasodilation in an endothelial-dependent manner. Effects of U-II on the endothelium can occur through the release of NO, prostaglandins, and endothelium-derived hyperpolarizing factor [21]. Bicak et al. [22] reported low levels of U-II in the plasma

TABLE 5. Distribution of haplotype frequencies of *UTS2*Thr21Met and Ser89Asn polymorphisms in migraine patients and controls.

Thr21Met	Ser89Asn	Controls n (%)	Patients n (%)	*p*	OR (95% CI)
M	N	8 (2.8)	9 (3.2)	0.7599	1.160 (0.446-3.017)
M	S	182 (63.6)	193 (66.4)	0.4555	1.141 (0.806-1.616)
T	N	4 (1.4)	5 (1.6)	-	-
T	S	92 (32.2)	83 (28.8)	0.3794	0.852 (0.597-1.217)

OR: Odds ratio; CI: Confidence interval; Frequencies<3% in both control and case group were dropped from the analysis.

TABLE 6. Association between Ser89Asn (S89N, rs2890565) polymorphism and clinical variables

Variable	n (%)			*p*
	SS	SN	NN	
Family history of migraine				
Positive	28 (90.3)	3 (9.7)	0 (0.0)	1.0000
Negative	104 (90.4)	11 (9.6)	0 (0.0)	
Migraine attack frequency				
1-3 per week	72 (90.0)	8 (10.0)	0 (0.0)	0.9829
1-3 per month	50 (90.9)	5 (9.1)	0 (0.0)	
<1-3 per month	10 (90.9)	1 (9.1)	0 (0.0)	
Hypertension history				
Positive	11 (84.6)	2 (15.4)	0 (0.0)	0.3610
Negative	121 (91.0)	12 (9.0)	0 (0.0)	

TABLE 7. Association between Thr21Met (T21M, rs228648) polymorphism and clinical variables

Variable	n (%)			*p*
	TT	TM	MM	
Family history of migraine				
Positive	3 (9.7)	15 (48.4)	13 (41.9)	0.7766
Negative	8 (7.0)	51 (44.7)	55 (48.2)	
Migraine attack frequency				
1-3 per week	7 (8.8)	34 (42.5)	39 (47.7)	0.9097
1-3 per month	3 (5.6)	27 (50.0)	24 (44.4)	
<1-3 per month	1 (9.0)	5 (45.5)	5 (45.5)	
Hypertension history				
Positive	1 (7.7)	5 (38.5)	7 (53.8)	0.8592
Negative	10 (7.6)	61 (46.2)	61 (46.2)	

of migraine patients compared to healthy controls. However, it was also shown that plasma levels of U-II were significantly higher in MO patients [13]. Chuquet et al. [23] demonstrated that U-II administration into the lateral cerebral ventricle induced gradual and long-lasting elevation of cerebral blood flow in rats. In the same study, U-II exacerbated brain damage following an ischemic insult [23]. Finally, U-II induced norepinephrine, dopamine, and serotonin release from noradrenergic neurons [24]. Collectively, the above discussed findings indicate that U-II may play a role in the pathogenesis of migraine.

In conclusion, our study suggests no significant associations between *UTS2* Thr21Met and Ser89Asn polymorphisms and migraine. Thus, the *UTS2* may not be a susceptible gene for migraine in our sample of Turkish patients with migraine. However, these results should be cautiously interpreted due to the small sample size of our cohort. Further studies with larger cohorts are required to investigate the exact role of U-II in migraine.

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DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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