



The association between CGRP gene -624 T→C polymorphism and headache severity in Turkish migraine patients

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Abstract

Aim: This study aims to explore -624 T→C polymorphism on CGRP gene in migraine patients, and the relation between this polymorphism and migraine attacks.

Materials and Methods: The study registered a total of 101 migraine patients, 17 with aura and 84 without aura and 80 healthy volunteers. CGRP -624 T→C polymorphism in migraine patients and the control group was evaluated using PCR-RFLP method.

Results: Although no significant difference could be found between genotype and allele frequencies of CGRP -624T→C polymorphism in the patient and control groups ($p>0.05$). In addition, a statistically significant positive correlation was established between the severity of headache and C allele of this gene in migraine patients ($p<0.05$).

Conclusion: The fact that C allele of CGRP gene is at a higher rate in those with severe migraine attacks suggests that this allele can render neuroinflammation more marked by increasing the amount of CGRP. However, how CGRP -624T→C polymorphism influences the expression level of the gene is not known for sure yet.



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Introduction

According to international headache criteria, migraine is a severe and recurrent headache attacks that comes in the form of attacks, can last up to 4-72 hours, is mostly unilateral, throbbing or compressive, includes nausea and vomiting, light and smell sensitivity, and negatively affects the individual's quality of daily life [1]. The situation in our country is similar to the global frequency and is reported as 16% in general, 25% for women and 9% for men [2].

Migraine is a neurovascular syndrome characterized by vasodilatation of meningeal blood vessels [3]. This vasodilatation is accompanied by activation of the perivascular trigeminal and sensorial nerves [4]. In the neurovascular migraine model, intra-cranial blood vessels release neuropeptides like substance P, calcitonin gene-related peptide (CGRP), neurokinin A, when the trigeminal ganglia nerves are activated [1, 5]. Human CALCA/CGRP locus (MIM# 114130) is localized on chromosome 11p15.2-p15 [3]. The gene codes both calcitonin and the calcitonin gene-related peptide alpha (α -CGRP) through alternative splicing in the neural tissues. α -CGRP, which

is a small neuropeptide composed of 37 amino acids, is produced by peripheral and central nervous system under normal conditions. α -CGRP, which is considered a central neuropeptide, is also secreted from ventral tegmental area (VTA), amygdale, and nerve fibers projected between ventral striatum areas. It has been shown in all cranial nuclei, except for the dorsal motor nucleus of the vagus nerve. α -CGRP has a strong vasodilator effect not only on the peripheral organs, but also the brain [6]. It is considered to play fundamental roles in the pathology of vascular headaches. CGRP levels have been shown to increase in the serum collected from the external jugular vein during attacks in migraine and cluster headaches [5]. Interestingly, it is known that plasma level of CGRP in the external jugular veins increases throughout the headache phase of migraine, and these increased levels are restored to normal with the recovery of headache. Besides, it is known that triptans used in attack treatment reduce CGRP levels, which are found higher in the serum during the recovery phases of the pain [3].

The most common polymorphisms of the CALCA gene include T692C, -1786T > C, -624 (T/C) and -1752C > G polymorphisms. CALCA -624 (T/C) gene polymorphism is a single nucleotide polymorphism at -624 in the promoter region of the gene. It is characterized by the T/C

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base transition at the position. The genotypes observed in the CALCA -624 (T/C) gene polymorphism are TT, CT and CC. The gene polymorphisms have been associated with various diseases such as ovarian cancer, Parkinson's disease, migraine, schizophrenia, essential hypertension and bone mineral density [7-11].

CALCA locus that produces many different products with many clinically important functions has made this gene an interesting topic of study to explore relations between different diseases and polymorphisms on the gene. To our knowledge, there is no study examining the relations between CGRP gene polymorphisms and migraine in literature. In the current investigation we aimed to explore the distribution of -624T→C promoter polymorphism on CALCA gene in the healthy Turkish population and migraine patients, and whether it is related with migraine attacks.

Materials and Methods

Study population

The study was initiated after approval by Firat University Human Experiments Ethics Committee. It was approved on 09.03.2006 with number 2006/4/7. The study enrolled 101 migraine patients whose migraine headache was diagnosed according to International Classification of Headache Disorders III (ICHD3) criteria [12]. In the patient group, 17 participants had migraine with aura and 84 participants had migraine without aura. Headache attacks were evaluated as mild, moderate and severe. Headache attacks of the patients were grouped according to their points on the visual analog scale (VAS) as mild, 1-3; moderate, 4-6; and severe, 7-9. When the patients were grouped according to the severity of headaches, the decisions were based on the mean VAS values of each headache attack they had in the last 3 months. The patients had not received any migraine preventive treatment in the last three months, not have any genetical, neurological, psychiatric or systemic disease, other than migraine. Patients with diabetes, hypertension, people who have previously had a stroke or transient ischemic attack, myocardial infarction, intellectual disability, hemiplegic migraine, presence dementia, those who were pregnant, those using non-steroidal anti-inflammatory drugs and anticoagulants before the test, and individuals who were physically unable to donate enough blood were excluded in the study.

The control group consisted of 80 healthy volunteers, including subjects matched for ethnic background and age. Each potential healthy control individual underwent a neurological examination to exclude the presence of migraine or any other primary headache disorder as well as other chronic pain syndromes. Other exclusion criteria for the control group are the same as for the patient group. After approval of the local ethics committee was obtained for the study protocol, patients were informed about the study and consent forms for participation were taken from the patients.

Genotyping of the 624T→C promoter polymorphism on CGRP gene

DNA from 2 ml of venous blood from subjects according to the kit procedure (Cat No: A1120, Promega, USA)

and stored at +40C. New primers were designed from the gene arrangement in order to determine 624T→C promoter polymorphism on CGRP gene (rs8924099) (GenBank Ace. No: XI5943). The PCR reaction mixture consisted of 50 ng of genomic DNA, 20 pmol/μl each primer, 1.25 U Taq polymerase, 2 mM dNTP, 2 mM MgCl₂ and 1XPCR buffer. PCR conditions were completed at 35 cycles, initial denaturation at 94°C for 10 minutes, denaturation at 94°C for 1 minute, primer annealing at 56°C for 1 minute, elongation at 72°C for 1 minute, final elongation at 72°C for 5 min. PCR products were then treated with 1 unit of PshAI restriction enzyme at 37°C for 16 hour according to the manufacturer's recommendations. The 260bp/base pair PCR products were subjected to 2% agarose gel electrophoresis containing 4 μl ethidium bromide, visualized with CCD and re-evaluated with gel analysis software. The wild-type allele provides a cleavage site for the PshAI restriction enzyme, resulting in 154 and 106 bp products after digestion of the 260b PCR product.

Statistical analysis

Data were analyzed using SPSS ver.10 program (SPSS, Chicago, IL). Results were expressed as mean±standard deviation or percentage. The suitability of the values obtained from the groups to normal distributions was analyzed with the one-sample Kolmogorov-Smirnov test. Student-t test was used to compare the clinical characteristics of the groups. Allele frequency was calculated from the genotypes of both groups and analyzed with the Hardy Weinberg Equivalence test. Genotype and allele differences in the two groups were compared with the Chi-square test and odds ratio and 95% confidence interval values were calculated. The relationship between genotype and allele groups and clinical features was tested with the Pearson correlation test. P values below 0.05 were considered significant.

Results

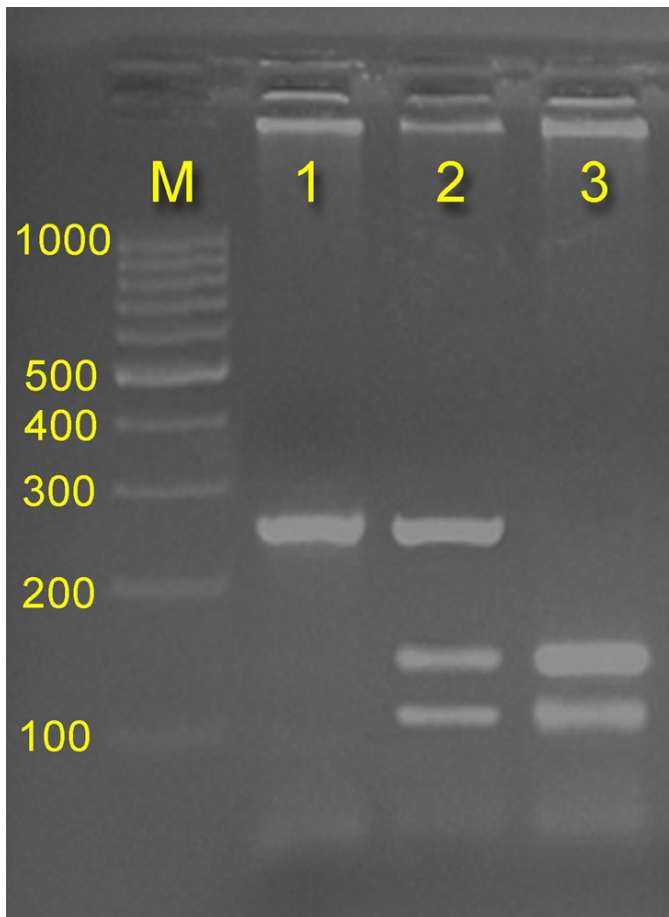
Of the patients included in the study, 90 were females and 11 were males. Mean age in the patient group was 33.40±9.34(min: 14, max: 53) in the healthy control group, there were 72 females and 8 males. Mean age was 34.13±8.45(min: 15, max: 56). The RFLP method is quite suitable for determining the -624T→C polymor-

Table 1. α-CGRP genotype and allele frequencies in the control and migraine patients.

α-CGRP	Patients (n=101)	Controls (n=80)	Odd ratio	P value
Genotypes				
TT	75 (74.2%)	61 (76.2%)		
TC	25 (24.7%)	19 (23.7%)	1.07 (0.54-2.12)	0.65
CC	1 (0.99%)	0 (0%)		
Alleles				
T	0.87	0.88		
C	0.13	0.12	0.57 (0.33-0.98)	0.055

Table 2. -624T→C promoter polymorphism and severity of pain in migraine group.

Severity of pain	Genotypes			Alleles	
	TT	TC	CC	T	C
Mild	4 (5.3 %)	0 (0 %)	0 (0 %)	8	0
Moderate	34 (45.3 %)	8 (32 %)	0 (0 %)	76	8
Severe	37 (49.3 %)	17 (68 %)	1 (100 %)	92	19

**Figure 1.** Genotyping of CGRP- α gene -624 T→C (rs8924099) polymorphism by PshAI RFLP. 260 bp PCR products belonging to the promoter of CGRP- α gene were obtained by agarose gel (2.5%) electrophoresis with ethidium bromide staining. M= 100-bp DNA ladder marker (Fermentas, USA), Lane-1 Polymorphic CC genotype was undigested (260 bp fragments), Lane-2: T/C genotype was digested into two fragments (260+154 + 106 bp fragments), Lane-3: TT wild genotype was digested into two fragments (154 + 106 bp fragments).

phism in the CGRP gene, and genotyping was successfully performed in patient and control groups (Figure 1).

The comparison of 101 Turkish migraine patients, and 80 healthy voluntary individuals, who constituted the control group, in terms of CGRP -624T→C polymorphism genotype frequencies did not show a significant difference ($X^2 = 0.834$, $df=2$, $P = 0.659$). No significant difference

was found when comparing the patient and control groups in terms of allele frequencies ($X^2 = 0.092$, $df = 1$, $p = 0.87$). Allele and genotype frequencies of the patient and control groups are presented in Table 1.

A positive correlation was found between the severity of pain and -624C allele ($p=0.046$). Severity of pain, genotype and allele frequencies distribution is presented Table 2.

But, when the patient group was divided into aura and non-aura, no relationship was found between pain intensity and polymorphism. However, a nearly significant value was detected in the aura group. With the post-hoc analyzes made using the G power 3.1 program, 95% power was obtained in the current sampling. The effect size (W) of the study is 0.338.

Discussion

This study evaluated -624T→C polymorphism on CGRP gene in Turkish migraine patients and the control group. Although there was not any statistically significant difference between the genotypes and allele frequencies of -624T→C polymorphism in the patient and control group. When pain intensity was classified according to the patient's statement and the VAS pain scale in patients with migraine attacks, a statistically significant positive correlation was shown between this pain scale and the C allele. In addition, a statistically significant positive correlation was established between severity of headache and C allele in the patient group.

Many sensorial fibers in the cranial structure originate trigeminal ganglion. Cerebral circulation is innervated by the CGRP-containing fibers in the trigeminal ganglia. CGRP is commonly co-localized with neuronal nitric oxide (NOS), amylin, pituitary adenylate cyclase activating peptide (PACAP), neurokinin A, and substance P. Immunohistochemical studies indicate that CGRP is a major component of the sensory nerve system. Although its role in the sensory neurons is not known for sure, CGRP is known to have a part as a vasodilator and anti-vasoconstrictor in the pathogenesis of headaches in migraine in situ hybridization studies have demonstrated that 40% of all neurons contain CGRP immunoreactivity and mRNA [13, 14].

CGRP in the trigeminal ganglia brings about significant modifications in the cerebral blood flow of vascular structures in the area innervated by trigeminal nerve. Through CGRP-1 receptor it has been argued that the trigemino-vascular system is charged with restoring balance in emergencies like intense vasoconstriction [15]. CGRP receptor mRNA has been shown in human trigeminal ganglion and human cranial arteries [13, 14]. Functional CGRP receptors operate through the cooperation of at least 3 different protein complexes, which are calcitonin-receptor-like receptor (CRLR), receptor-activity-modifying protein 1 (RAMP 1), and the cytoplasmic receptor component protein (RCP) [16]. It has been noted that there is a certain relation between migraine and CGRP, which is a biological marker of trigeminovascular activation. Ashina et al. found that CGRP increased in cubital venous blood in migraine patients outside of attacks [17]. It has been reported in a study that there is a marked positive correlation between plasma CGRP concentrations measured in

consecutive serum samples collected from the antecubital vein, and scores of headache severity in migraine attacks induced by 0.5 mg sublingual nitroglycerin administration. The same study had demonstrated a significant correlation between recovery of headache and a decreased in plasma CGRP concentration with the sumatriptan treatment administered for headache [18]. These data point to an obvious relation between migraine headache and CGRP secretion [19]. Besides, various studies have reported a significant positive correlation between plasma CGRP levels measured during migraine attacks and the severity of headache [3,18,19]. It has been stated that CGRP can contribute to the increase in local blood flow in migraine attacks by increasing vasodilatation [20]. But Tchivileva et al. could not detect a significant difference in terms of CGRP levels in external jugular or cubital fossa venous blood during or outside migraine attacks and stated that CGRP may not be a biomarker for migraine [21]. Furthermore, it is known that CGRP receptor antagonist agents are known to be successful in the treatment of acute migraine [22]. Nerves containing CGRP are associated with cranial blood vessels. Recently, Lassen et al. found that CGRP caused dilation in cerebral arteries. However, they stated that this caused a very small dilation and could not be the sole cause of migration induced by CGRP [23].

Besides, this allele was found to be higher in the migraine group with severe headache, in comparison to the two groups with mild and moderate headache. But, in the analysis performed using the TFSEARCH program, no protein factor binding to the -624 C polymorphism region was detected. We think that this polymorphism may co-segregate with some other CGRP gene polymorphisms and affect the severity of migraine pain. CGRP -624 C allele in the promoter area of the gene may enhance the serum levels of CGRP; and magnify its effect on the trigemino-vascular system. These enhanced serum levels may render headaches more severe in migraine patients. Or else, this allele may positively modify the effect of CGRP on its receptors in the trigeminovascular system by increasing the receptor sensitivity in a distinct manner, relative to other alleles, and thus headache may occur more severely.

Conclusion

In conclusion, over-expression or co-segregation with other gene polymorphism, effected gene expression or regulation, of the C allele of the gene in migraine patients may cause CGRP to be secreted at higher-than-normal levels and/or modification of its effect at the receptor level. However, how -624T→C polymorphism effects gene has not been yet. Studies that will reveal whether there is a relation between increased CGRP levels and -624C allele in patients with severe headache may shed light on this topic. The studies about subject are important to disclose the significance of CGRP polymorphisms in migraine. Additionally, due to the population-based design of the study, it may not be possible to generalize these results to the general population. Genetic polymorphisms often differ between ethnic groups. Additionally, the sample size of migraine cases was not large enough to detect a small effect from very low penetrance genes or SNPs. It is possible that this gene polymorphism is just one of many factors

contributing to pain severity in migraine. A large prospective study is needed to verify our findings.

Conflict of interest

The authors declare no declaration of interest. Only the authors are responsible for the content and writing of the article.

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Ethical approval

The study was initiated after approval by Firat University Human Experiments Ethics Committee. It was approved on 09.03.2006 with number 2006/4/7.

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