

# Calcitonin Gene Related Peptide Gene Polymorphism in Migraine Patients

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**ABSTRACT:** *Objective:* Calcitonin gene related peptide (CGRP), which has a vasodilator effect, is held responsible for neurogenic inflammation and vasodilatation of the cranial vessels in migraine pathophysiology. In this study, we investigated the association between alpha CGRP gene polymorphism (CALCA T-692C) and migraine. *Material and Methods:* One hundred and thirty-four female migraineurs and 96 healthy female cases were enrolled in the study. The patient group was further subdivided into migraine with and without aura groups. The CALCA T-692C gene polymorphism was identified using polymerase chain reaction (PCR) technique and restriction fragment length polymorphism (RFLP). *Results:* The genotype and allele frequencies of CALCA T-692C gene polymorphism did not differ between the migraine and control groups. Between the migraine with and without aura subgroups, there was no difference. No association was seen between the CALCA T-692C gene polymorphisms and migraine attack severity and frequency. *Conclusion:* Our study did not show any association between CALCA T-692C gene polymorphism and migraine.

**RÉSUMÉ:** *Polymorphisme du gène du peptide relié au gène de la calcitonine chez les patients souffrant de migraine. Objectif :* Le peptide relié au gène de la calcitonine (PRGC), qui a un effet vasodilatateur, est tenu responsable de l'inflammation neurogène et de la vasodilatation des vaisseaux crâniens dans la pathophysiologie de la migraine. Nous avons étudié l'association entre le polymorphisme du gène du PRGC alpha (CALCA t-692C) et la migraine. *Méthode :* Cent trente-quatre femmes migraineuses et 96 femmes témoins ont été incluses dans l'étude. Le groupe de patientes a été subdivisé en deux groupes, selon que la migraine était accompagnée ou non d'une aura. Le polymorphisme CALCA T-692C a été identifié au moyen de la technique de réaction en chaîne de la polymérase (PCR) et polymorphisme de longueur des fragments de restriction. *Résultats :* Nous n'avons pas constaté de différence entre le génotype et les fréquences alléliques du polymorphisme du gène CALCA T-692C dans le groupe de femmes migraineuses et le groupe témoin ainsi qu'entre les groupes de femmes migraineuses avec et sans aura. Il n'existait pas d'association entre les polymorphismes du gène CALCA T-692C et la sévérité et la fréquence des crises de migraine. *Conclusion :* Notre étude n'a pas mis en évidence d'association entre le polymorphisme CALCA T-692C et la migraine.

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Migraine is a common disorder characterized by unilateral throbbing headache with autonomic symptoms such as nausea, vomiting and photophobia. Its pathogenesis is still obscure, but genetic tendency and environmental factors are known to be responsible for its etiology.<sup>1,2</sup> The trigeminovascular system is activated at the onset of migraine attack and the release of substance P, neurokinin A and CGRP at the sensorial nerve endings result in vasodilatation and neurogenic inflammation of cranial blood vessels. The induced pain signals are transmitted to the thalamus and perceived as headache by the cerebral cortex.<sup>1</sup>

It has been demonstrated that CGRP levels increase in the blood obtained from the jugular vein during migraine attack and normalize after the cessation of the headache.<sup>3</sup> Intravenous infusion of CGRP also causes a migraine-like headache.<sup>4</sup> Conveniently, the administration of CGRP antagonists provides

cessation of the attack.<sup>5</sup> The effect of nitric oxide which has an important role in migraine pathogenesis is also suggested to occur by means of CGRP release at the trigeminal nerve terminals.<sup>6</sup> Calcitonin gene related peptide is one of the members of calcitonin family (calcitonin, amilin, adrenomedullin, alpha and beta CGRP) and is mainly released in

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the central nervous and cardiovascular system. It causes vasodilatation in blood vessels, regulates vascular tonus and angiogenesis, modulates pain sensation in the nervous system,<sup>7</sup> potentiates the effect of substance P<sup>8</sup> and acts as a neurotropic factor.<sup>9</sup> Alpha and beta CGRP are also known as CALCA and CALCB. The human CALCA gene is located on chromosome 11p15.2-p15.1 and codes for both calcitonin and alpha-CGRP. CALCA T-692C is one of the identified single nucleotide polymorphisms loci of the CALCA gene (<http://www.ncbi.nlm.nih.gov/projects/SNP/>).

The molecules of CGRP have to be properly synthesized to exhibit biologic activity. As the result of genetic polymorphisms, the molecular structure, function and reaction can be altered. The association of various genetic polymorphisms with migraine has been well researched.<sup>10-11</sup> In this study, we investigated the frequency of alpha CGRP gene polymorphism (CALCA T-692C) in migraine patients and its relationship with migraine attack frequency and severity.

## MATERIAL AND METHODS

One hundred and thirty-four female migraine patients and 96 healthy female control cases were enrolled in the study. All the migraineurs were examined by the neurologists and the diagnosis of migraine was established in accordance with the criteria of international classification of headache disorders II.<sup>12</sup> Hemiplegic migraine was excluded. The control groups consisted of the healthy volunteers, health care personal and postpartum females who were hospitalized in the obstetric clinic. The frequency of migraine attacks was recorded as the number of attacks in a month. The severity of the headache was determined with the visual analog scale. The history of hypertension, diabetes mellitus, smoking habit and the existence of cardiovascular disease in the family of the migraineurs and controls were recorded.

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cgcatctgtacctfgcaactcaccctcaggcccagcaggtcatcgcc
ccctcctcacacatgtaatggatctgaagagtacccgggacagtcgg
ggagatggagattcggaaagtatccatggagatcttacagaatcccctgt
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aaccttaaaatctcacttgggcaggggtctgggatcagagttggaagag
tccctacaatcctggacccttccgcaaatcgtgaaaccaggggtggag
tggggcgaggggtcaaaaccaggccggactgagaggtgaaattcacca
tgacgtcaaactgccctcaaatcccgctcacttta

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**Figure 1:** The sequencing of the region which contains CALCA T-692C gene polymorphism. Italic and bold letters were used for the primer sequences. The underlined and bold letters represent the restriction site for PshA I (GACNN↓NNGTC).

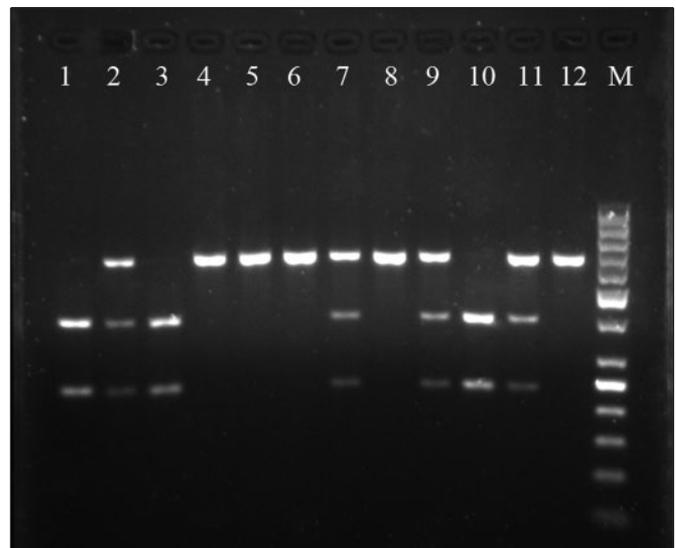
The study was approved by the local ethics committee and informed consent was obtained from the study cases.

## DNA Isolation

DNA was isolated from peripheral blood, collected into tubes containing ethylenediamine-tetraacetic acid (EDTA) by high pure PCR template preparation kit (Roche USA). The CALCA T-692C gene polymorphism (rs 3781719) were identified using polymerase chain reaction (PCR) technique and restriction fragment length polymorphism (RFLP) assay.<sup>13</sup>

A total of 25 µL PCR mixture containing 1X Taq Buffer with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.5 mM MgCl<sub>2</sub>, deoxynucleotide triphosphates (0.2 mM of each), 0.5 nmol of sense and anti-sense primers (sense: 5'-cgcatctgtacctfgcaact-3', anti-sense: 5'-taaagtgagcgggaattga-3'), 1.25 Unit of Taq DNA polymerase and 200 ng of DNA. All reagents for PCR amplification and gel electrophoresis were purchased from Roche (ELİPS, Istanbul, Turkey). All other chemicals were from Sigma and Merck (BO&GA, Istanbul, Turkey). DNA amplifications were performed with a Techne (TechGene) DNA Thermal Cycler by an initial melting step of 94° for five minutes, 38 cycles of 94° C for 50 seconds, 57° for 50 seconds and 72° C for one minute, and a final extension step of 72°C for ten minutes.

Polymerase chain reaction primers were generated to amplify the 636 base pair (bp) fragment encompassing the CALCA T-692C region. Figure 1 shows the sequencing of the region which contains CALCA T-692C gene polymorphism. The PCR products were electrophorized on 2% agarose gels, stained with ethidium bromide, and checked under UV light transillumination.



**Figure 2:** EtBr stained gel of PshA I digested PCR products of CALCA T-692C shows the TT genotype (636 bp; lane 4, 5, 6, 8, and 12), the TC genotype (636 bp, 401 bp, and 235 bp; lane 2, 7, 9, and 11), the CC genotype (401 bp, and 235bp; lane 1, 3, and 10), lane M is a size marker (50 bp DNA Ladder).

**Table 1: The characteristics of the migraine and control cases**

	Migraine (n=134)	Control(n=96)	P
Age	37,21±10,01	35,01±7,47	0,069
Hypertension (%)	16,3	17,2	0,51
Diabetes mellitus (%)	1,5	4,5	0,206
Smoking (%)	47,1	17,7	0,003
Family history of vascular disease (%)	58,9	26,4	0,008

**Table 2: The genotype frequencies of calcitonin gene related peptide gene**

	TT	TC	CC	P
Migraine group (%)	42,5	42,5	14,9	0,44
Control group (%)	43,7	46,8	9	
Migraine with aura subgroup (%)	36,5	46,2	17,3	0,52
Migraine without aura subgroup (%)	46,4	40,2	13,4	

**Table 3: The allele frequencies of calcitonin gene related peptide gene**

	T	C	P
Migraine group (%)	63,8	36,2	0,258
Control group (%)	67,2	32,8	
Migraine with aura subgroup (%)	59,6	40,4	0,241
Migraine without aura subgroup (%)	66,5	33,5	

Three  $\mu$ l of PCR product were digested with 0.5  $\mu$ l of the Fast Digest restriction enzyme PshA I (BoxI); an enzyme that cuts DNA at specific recognition nucleotide sequences (5'...GACNN↓NNGTC...3'), in 1X Fast Digest green buffer for 1 hour at 37°C. The wild allele (thymine) produced a single 636 bp fragment, a mutant allele (cytosine) produced two fragments of 235 bp and 401 bp. The restriction digest products were visualized under UV light transillumination after electrophoresis on a 2% agarose gel and ethidium bromide staining (Figure 2).

### Statistical analysis

The data were presented as mean±standard deviation or percentage frequency. Allele frequencies were calculated from the genotypes of all subjects. Hardy–Weinberg equilibrium was assessed by  $\chi^2$  analysis. Allele and genotype frequencies were compared by the standard contingency table analysis using chi-square and Fisher's exact test probabilities. The association of genotype and allele groups with the clinical characteristics was tested with Pearson correlation test.  $p < 0.05$  was considered statistically significant. Statistical analyses were performed with the SPSS 19.0 software.

### RESULTS

The means of ages did not differ between the migraine and control groups (37.2±10.01 and 35.01±7.47, respectively.) A close rate of hypertension and diabetes mellitus were observed in both groups, but smoking and family history of vascular disease were significantly more frequent in the migraine group ( $p < 0.01$ ) (Table 1).

No difference was found between the genotypes and allele frequencies of the migraine and control groups. The percentage of the migraine with aura was 38.8% (52/134) of all migraineurs. The genotype and allele frequencies in the migraine with and without aura subgroups also showed no difference. No association was found between the genotype and allele frequencies and the severity and frequency of migraine attacks (Table 2 and 3).

### DISCUSSION

Calcitonin gene related peptide is accepted to effect the development of migraine headache.<sup>13</sup> It can be hypothesized that the CALCA gene polymorphisms can modulate the CALCA level or function and, subsequently, migraine development. In the present study, it was found that the genotypic and allelic

distributions of CALCA T-692C gene polymorphism are not different in the female migraine patients and are not associated with migraine attack severity and frequency. Additionally, no difference was observed among migraine with and without aura subgroups. As far we could find in the PubMed search, there are a few published reports investigating the association between the CALCA gene polymorphism and migraine.<sup>14-15</sup> However, different types of CALCA gene polymorphisms were investigated in our study and each of these studies. Menon et al<sup>15</sup> did not find any difference in CALCA 16 bp deletion among Australian migraine with and without aura patients and control cases. Lemos et al<sup>16</sup> investigated the frequencies of CALCA rs1553005 gene polymorphisms in a Portuguese population and could not show a significant difference between migraineurs with and without aura and controls. In the same study brain natriuretic factor gene polymorphisms were additionally investigated, and the coexistence of AT genotype with CALCA GC-genotype was found to increase the risk of migraine to 1.8 fold. In our study, the distribution of a different type of CALCA polymorphisms (CALCA T-692C) was for the first time evaluated in migraine patients, and no difference was found from the controls. Apart from the migraine headache, CALCA gene polymorphisms have recently been studied in Parkinson's disease, schizophrenia, essential hypertension.<sup>16-17</sup> While no association was seen with Parkinson's disease and schizophrenia<sup>17</sup>, C allele was found to increase the risk of essential hypertension.<sup>16</sup>

The small number of study cases and the absence of male cases are the limitations of the study. Although, to date, no association between CALCA gene polymorphisms and migraine could be shown, more studies with larger case numbers and with different CALCA gene polymorphisms are needed. Gene polymorphisms can also show distinct distributions in different ethnic and racial populations.

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