Is There a Relation Between Insertion/Deletion Polymorphism of the Angiotensin Converting Enzyme Gene in Patients of Migraine with Aura and Migraine without Aura in Region of Eastern Turkey?

Türkiye'nin Doğu Bölgesinde Auralı ve Aurasız Migren Hastalarında Anjiyotensin Konverting Enzim Geninin İnsersiyon/Delesyon Polimorfizmiyle İlişkisi Var mıdır?

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ABSTRACT Objective: Angiotensin-converting enzyme (ACE) is one of the key enzymes in the renin-angiotensin-aldosterone system which modulates vascular tension and blood pressure. Many authors have reported an association between ACE-D allele and coronary heart disease and other cardiovascular diseases. The mechanism underlying the positive associations between the ACE-D alleles and diseases are not yet clear. Alterations of the renin-angiotensin system have been implicated in the pathogenesis of various diseases. Several angiotensin I converting enzyme inhibitors and an angiotensin II receptor blocker were demonstrated to have a clinically important prophylactic effect in migraine. Previous reports showed an association between migraine without aura and ACE-D allele polymorphism. In this study it is aimed to evaluate whether the DD genotype could also be associated with the frequency and duration of migraine without aura (MoA) and migraine with aura (MwA). **Material and Methods:** One hundred one migraine patients (37 MoA, 64 MwA) and 101 healthy non-migrainous controls from the region of Eastern Turkey were included in this study. The genotype characteristics were determined by the PCR analysis using DNA extracted from peripheral blood. **Results:** There was no significant difference in allelic frequency (I and D) and genotype polymorphism (DD, DI and II) of the ACE gene in migraine patients and controls (genotype frequency: p= 0.08). ACE genotype distribution was similar in migraine patients and healthy control groups. Turkish patients MoA and MwA did not show any difference in incidence of the ACE-DD genotype. **Conclusion:** We conclude that the I/D polymorphism at the ACE locus does not play any role in the pathogenesis and progression of migraine.

Key Words: Migraine with aura; migraine without aura; headache; polymorphism, genetic

ÖZET Amaç: Anjiyotensing konverting enzim (ACE), vasküler tansiyon ve kan basıncını düzenleyen renin-anjiyotensin-aldosteron sisteminde anahtar enzimlerden biridir. Birçok araştırmacı, koroner kalp hastalıkları ve diğer kardiyovasküler hastalıklar ile (ACE)-D alleli arasında bir ilişkinin olduğunu bildirmişlerdir. ACE-D allelleri ve hastalıklar arasında pozitif ilişkinin temelini oluşturan mekanizmalar henüz açık değildir. Renin-anjiyotensin sistemindeki değişimler, çeşitli hastalıkların patogenezinde gösterilmiştir. Birkaç anjiyotensin I konverting enzim inhibitörü ve bir angitensin II reseptör bloklayıcısının migrende klinik olarak önemli bir profilaktik etkiye sahip olduğu gösterilmiştir. Önceki raporlarda, aurasız migren ve ACE–D allel polimorfizimi arasında bir ilişki olduğu gösterilmiştir. Bu çalışmada DD genotipinin, auralı (MoA) ve aurasız migren (MwA) süresi ve sıklığı ile ilişkisinin olup olmadığının değerlendirilmesi amaçlandı. **Gereç ve Yöntemler:** Türkiye'nin doğu bölgesinde 101 migren hastası (37 MoA ve 64'ü MwA) ve 101 migren olmayan sağlıklı kontroller bu çalışmaya dahil edildi. Genotip özellikleri, periferik kandan ekstrakte edilmiş DNA kullanılarak PCR analizi ile tespit edildi. **Bulgular:** Migren hastaları ve kontrollerde ACE geninin allelik insidasında (I ve D) ve genotip polimorfizminde (DD, ID, II) anlamlı farklılıklar yoktu (genotip sıklığı p= 0.08). ACE genotip dağılımı, migren hastalarda ve kontrol grubunda benzerdi. MoA yada MwA'lı Türk migren hastalarında ACE-DD genotip insidasında farklılık gösterilemedi. **Sonuç:** Biz ACE lokusundaki insersiyon/delesyon polimorfizimin migrenin progresyonunda ve patogenezinde herhangi bir rol oynamadığını düşünmekteyiz.

Anahtar Kelimeler: Auralı migren; aurasız migren; başağrısı; polimorfizm, genetik

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Migraine is the most frequent primary headache disorder. It is a neurovascular disorder in which the primary abnormality is thought to be a neuronal excitability underlined by a complex genetic susceptibility. Epidemiogenetic studies have shown that migraine without aura (MoA) and migraine with aura (MwA) are polygenic conditions.[1] The three known migraine genes have been identified by the study of the unique monogenic variety of migraine, i.e. familial hemiplegic migraine. These genes all encode ion transporters: the P/Q type calcium channel, a calcium/potassium ATPase and a sodium channel. According to the latter hypothesis about the mechanisms of migraine attacks, poorly known triggers initiate a cortical wave of depolarisation that is responsible for the transient aura symptoms. This cortical spreading depression induces several biochemical changes which, by diffusion through the extracellular space, stimulate the trigeminovascular fibers. These fibers release vasoactive neuropeptides that initiate the neurogenic inflammation. Trigeminovascular fibers transmit nociceptive information centrally via the brainstem. The trigeminovascular fibers also activate the parasympathetic system that is responsible for the persistence of vasodilation in meningeal vessels.[2]

The pathophysiology of migraine is not yet fully understood but may involve painful vasodilatation of cerebral blood vessels and/or the release of vasoactive neurotransmitters from the perivascular axons in the dura mater after activation of the trigeminovascular system. Paterna proposed the "trigemino-vascular theory" of migraine headache, which claims that neurogenic inflammations of meningeal blood vessels were evoked by excitation of trigeminovascular fibers.[3] Angiotensin I-converting enzyme (ACE) is one of the key enzymes in the renin-angiotensin-aldosterone system, which modulates vascular tension and blood pressure.[4] The renin-angiotensin system (RAS) is a circulatory cascade system primarily involved in the regulation of blood pressure and serum electrolytes. The key enzyme in this system is ACE which converts angiotensin I to the potent vasoconstrictor angiotensin II. The RAS has been said to be involved in the pathogenesis of several diseases including fibrosis in the heart, kidney, lung and liver during chronic inflammation through the regulation of cell growth, inflammation, oxidative stress and fibrosis. The ACE gene insertion/deletion (I/D) polymorphism was first identified in 1990. The ACE-D, a deletion polymorphism of a 190-bp fragment of intron 16 of the ACE gene allele, has been shown to result in higher levels of circulating enzyme in a dose dependent manner.[4] The role of the ACE gene I/D polymorphism as a risk factor has been investigated in several diseases.[5] Migraine is in part associated with the cerebral circulation. The mechanism underlying the positive associations between the ACE-D alleles and diseases are not yet clear. Previous reports showed an association between migraine without aura and ACE-D allele polymorphism.[6] We aimed to investigate the ACE genotype as a possible risk factor for MwA and MoA in a population of the Elazig Region of East Anatolia, Turkey.

MATERIAL AND METHODS

In our hospital-based case-control study, 101 migraine patients (90 women and 11 men) (64 MwA and 37 MoA) were recruited from Department of Neurology, School of Medicine, Fırat University, in Elazig region of Eastern Turkiye. The migraine patient group consisted of 101 Turkish migraine patients with their diagnose based on Headache Classification Subcommittee of the International Headache Society ICHD-2 criteria.[7] One hundred one normal healthy volunteers composed the control group (82 women and 19 men). Control subjects did not suffer from migraine or tension-type headache. All the subjects were Turkish. The mean ages and sex distribution did not differ significantly between the two groups. After the complete description of the study to the subjects, all subjects gave informed written consent which was in accordance with the Declaration of Helsinki and the study was approved by the local ethics committee.

Genomic DNA was extracted from venous blood using the standard phenolchloroform method.[3] The DNA samples were then stored at 4°C until

used as a template DNA in polymerase chain reaction (PCR). PCR was performed on the genomic DNA samples with primers as previously reported.[8] The ACE genotype was determined by PCR amplification of a genomic DNA fragment on intron 16 of the ACE gene as previously described by Rigat et al and Saiki et al.[8,9] The oligonucleotide primers were sense (forward): 5'CTGGAGACCACTCCCATCCTTTCT3' and antisense (reverse): 5'GATGTGGCCATCACATTCGTCAGAT3'. Amplification was performed with 0.5 μmol of each primer. The PCR product was a 190 bp fragment in the absence, and a 490 bp fragment in the presence of the insertion. The samples were amplified using a thermal cycler (Eppendorf) and the products separated on 1.5% agarose gel. PCR reactions were carried out in a total volume of 50 mL, using approximately 100 ng DNA, 2.5 mmol/L MgCl2, 200 mol/L dNTPs, 12.5 ng of each primer, and 0.5 units of Taq DNA polymerase (Promega, Madison, WI, USA) in the PCR buffer provided by the manufacturer (10 mmol/L Tris-HCl, pH 9.0, and 50 mmol/L KCl). PCR involved a denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min for 30 cycles. Amplification was confirmed by electrophoresing 5 mL of the PCR product on a 1.5% agarose gel containing ethidium bromide and subsequent visualization under ultraviolet illumination.

Patients and control individuals were genotyped for the ACE gene promoter polymorphism. The ACE gene consists of either an insertion (I) allele or a deletion (D) allele forming three possible genotypes: II, ID or DD. Amplified ACE gene fragments without insertion (D allele) and with insertion (I allele) of approximate 190 and approximate 490 bp, respectively.

STATISTICAL ANALYSES

The differences in the frequency of ACE alleles and genotypes between groups were evaluated by the gene-counting method and comparison of groups by the χ^2 test. The level of significance was set at $p<0.05$. The odds ratios associated with each genotype of ACE and their 95% confidence intervals were determined by using unconditional logistic regression. Data are presented as mean ± SD. All calculations were performed with the SPSS 15.0 statistical package (SPSS Inc, Chicago, IL, USA).

RESULTS

In this study, migraine group consisted of 90 women and 11 men. Mean age in the patient group was 33.40 ± 9.34 (min: 14, max: 53). In the control group with 82 women and 19 men, mean age was 34.13 ± 8.45 (min: 15, max: 56). There were no significant differences between the cases and controls for the mean age or gender distribution, and this suggested that the matching based on these two variables was adequate. Genotypes for over 90% of the total genomic DNA samples included in the case and control groups were obtained for the ACE I/D polymorphism. The allele frequency and genotype distribution of I/D polymorphism in patients and the controls are presented in Table 1. The distributions of the ACE I/D promoter for migraine patients and controls did not deviate significantly from Hardy-Weinberg equilibrium.

We did not observe any significant difference in the allele frequency and genotype distribution of the I/D polymorphism at ACE locus between patients and controls (allele: $X^2= 0.01$, df= 1, p= 0.91, genotype: $X^2= 5.05$, df= 2, p= 0.08). There was no existence of a difference among the frequency and duration of headache in each subgroup of mi-

TABLE 1: Distribution of allele and genotype frequencies of the ACE I/D polymorphism in migraine patients and control groups.

Group	Genotype distributions (%)			Allel frequency	
	D/D	I/D	I/I	I	D
Migraine (n-101)*	38 (37.6)	57(56.43)	1(0.99))	0.34	0.66
Controls (n-101)	32(31.68)	68(67.32)	6 (5.94)	0.35	0.65

*All migraineurs (MwA and MoA) are combined into one group. No significant differences were detected (Genotype: $X^2= 5.05$, df= 2, p= 0.08; alleles: $X^2= 0.01$, df=1, p= 0.91).

graine patients stratified by ACE genotype. For the DD genotype, there was no difference between female and male in migraine patients. In 37.8% of females and 36.4% of males were detected DD genotype.

DISCUSSION

We did not observe any significant difference in the allele frequency and the genotype distribution of the I/D polymorphism at ACE locus between the patients and the controls. We have no data to clarify this point, however several authors were pointed out significant ethnic differences in the frequency of the I/D polymorphism as well as the associated ACE activity. The alternation of ACE activity due to the I/D polymorphism would result in changed levels of the neurotransmitters and vulnerability to cranial vascular activity. These states appear to be analogous to those found during migraine headache or aura. In addition, several ACE inhibitors and an angiotensin II receptor blocker were demonstrated to have a clinically important prophylactic effect in migraine. First, Bender reported that he successfully used an ACE inhibitor for prophylaxis of migraine in a small group. Then, one of the ACE inhibitor, lisinopril, was demonstrated to have a clinically important prophylactic effect in migraine in a randomized, placebo controlled, crossover study.[10] Moreover, the angiotensin II receptor blocker, candesartan, also provided effective migraine prophylaxis in a randomized controlled trial.[11] These trials suggested that the rennin angiotensin aldosterone system must be concerned at least in part in the pathogenesis of migraine.

There are contradictory results concerning the association between ACE I/D polymorphism and migraine. In particular, the absence of associations between ACE polymorphism and migraine reported in our studies are consistent with previous Turkish studies.[12]

However, other studies have reported a significant association between ACE I/D polymorphism and migraine.[3,13,14] This lack of replicability may be related to population stratification, distinctive environmental and genetic factors in different populations and differences in disease definition criteria between studies. The apparently conflicting results also suggest that the impact of ACE gene polymorphism in migraine patients is likely to be influenced by the ethnic origin. The frequency of ACE gene polymorphisms (I or D) differs among various ethnic populations as shown in Table 2.[15-18] If a *D/D* genotype was a significant and independent risk factor for the progression or development of migraine, then migraine patients of an ethnicity with a high prevalence of the D/D genotype would be expected to have a worse prognosis compared to those of an ethnicity with a lower prevalence.

Cakmak et al. in an earlier study reported the frequency of DD allele was increased in male patients with migraine. However, no significant association was found between genotype and allele frequency.[12] In our study, DD genotype frequency was found almost identical in female and male patients with migraine (37.8% vs 36.4%). Our results are in agreement with the Çakmak's study in migraine patients, which showed that the insertion/deletion polymorphism in the ACE gene does not make a significant contribution to the pathogenesis and progression of the disease.

We conclude that the D allele and the D/D genotype of ACE gene may not be a genetic risk factor for migraine patients. In this study, levels of ACE circulating in controls and subjects with headache were not examined. Since our data were only designed to estimate the frequency of ACE genotype, we have no definite information on the etiology of difference between MwA and MoA. There seems to be a possible relationship between ACE

TABLE 2: Frequency of the angiotensin converting enzyme (ACE) polymorphism in the normal population in different countries.

	ACE polymorphism (%)		
	I/I	I/D	D/D
Switzerland[16]	25.0	46.0	29.0
South-Korea[14]	44.3	40.9	14.8
Israel[15]	10.2	50.9	38.9
Turkey[11]	13.7	38.3	48.0
Kuwait[17]	2.0	46.0	52.0

activity and pathogenesis of migraine in other studies.[3,13,14] However, we did not find a significant relation between ACE gene I/D polymorphism and migraine in our study.

We conclude that the insertion/deletion polymorphism at the ACE locus does not appear to play a role in the etiology and progression of migraine. The results of our study suggested that the ACE-DD gene polymorphism could not have an important role in determining migraine attacks and the frequency of these attacks. Further evaluation in a larger study population is required to examine the relationship between ACE genotype and headache, especially on the biomolecular level.

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