Angiotensin Converting Enzyme Gene Insertion/Deletion Polymorphism in Migraine Patients

Migren Hastalarında Anjiyotensin Dönüştürücü Enzim Gen İnsersiyon/Delesyon Polimorfizmi

Belgin Alaşehirli[1], Mehmet Gür[2], Aylin Akçalı[3], Sırma Geyik[4], Binnur Bülbül[5], Derya Sayar[6], Mustafa Yılmaz[3], Abdurrahman Neyal[7], Münife Neyal[3]

[1]Gaziantep Üniversitesi Tıp Fakültesi, Farmakoloji Anabilim Dalı, Gaziantep, Türkiye
[2]İskenderun Devlet Hastanesi, İskenderun, Hatay, Türkiye
[3]Gaziantep Üniversitesi Tıp Fakültesi, Nöroloji Anabilim Dalı, Gaziantep, Türkiye
[4]Medical Park Hastanesi, Nöroloji Bölümü, Gaziantep, Türkiye
[5]Mega Park Hastanesi, Nöroloji Bölümü, Kahramanmaraş, Türkiye
[6]Gaziantep 25 Aralık Devlet Hastanesi, Nöroloji Bölümü, Gaziantep, Türkiye
[7]Gaziantep Cengiz Gökçek Devlet Hastanesi, Nöroloji Bölümü, Gaziantep, Türkiye

Turk Norol Derg 2009;15:161-165

ÖZET

Amaç: Anjiyotensin dönüştürücü enzim inhibitörü ilaçların migren atak sıklığı üzerine etkili oldukları gösterilmiştir. Bu çalışmada anjiyotensin dönüştürücü enzim geninin migren patofizyolojisi ile ilişkisi araştırılmak istenmiştir.

Hastalar ve Yöntem: Anjiyotensin dönüştürücü enzim geninin I/D polimorfizmlerinin migren atakları ile ilişkisini araştırmak üzere 102 migren hastası (35 auralı migren, 67 aurasız migren) ile yaş ve cinsiyet olarak uygun 75 kontrol üzerinde çalışılmıştır. Migren atak sıklığı ve başlangıç yaşları da anjiyotensin dönüştürücü enzim genotipleri ile beraber değerlendirilmiştir.

Bulgular: Auralı ve aurasız migren hastaları ve kontrol grubu anjiyotensin dönüştürücü enzim genotipleri yönünden birbirileri ile karşılaştırılabilir olarak gözlendi (sırasıyla; p= 0.88 ve p= 0.76, p= 0.624). Atak sıklığı ile anjiyotensin dönüştürücü enzim genotipleri arasında ilişki saptanmadı (p= 0.125) ancak anjiyotensin dönüştürücü enzim-II genotipine sahip olanlarda migren atakları daha genç yaşta başlıyordu (p= 0.021).

Yorum: Anjiyotensin dönüştürücü enzim gen polimorfizmi ile ilgili farklı popülasyonlarda ve genç migren hastalarında çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Anjiyotensin dönüştürücü enzim, polimorfizm, genetik, migren.

ABSTRACT

Angiotensin Converting Enzyme Gene Insertion/Deletion Polymorphism in Migraine Patients

Belgin Alaşehirli[1], Mehmet Gür[2], Aylin Akçalı[3], Sırma Geyik[4], Binnur Bülbül[5], Derya Sayar[6], Mustafa Yılmaz[3], Abdurrahman Neyal[7], Münife Neyal[3]

[1]Department of Pharmacology, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey
[2]Iskenderun State Hospital, Iskenderun, Hatay, Turkey
[3]Department of Neurology, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey
[4]Clinic of Neurology, Medical Park Hospital, Gaziantep, Turkey
[5]Clinic of Neurology, Mega Park Hospital, Kahramanmaras, Turkey
[6]Clinic of Neurology, Gaziantep 25 Aralik State Hospital, Gaziantep, Turkey
[7]Clinic of Neurology, Gaziantep Cengiz Gokcek State Hospital, Gaziantep, Turkey

Objective: The beneficial effects of angiotensin converting enzyme inhibitor drugs on migraine attack frequency have been shown. We aimed to study the relationship between the angiotensin converting enzyme gene and migraine pathophysiology.

Patients and Methods: In the present study, to assess whether the angiotensin converting enzyme insertion/deletion (I/D) gene polymorphisms have an effect on migraine attacks, we studied the angiotensin converting enzyme genotypes of 102 migraine patients (35 cases of migraine with aura and 67 of migraine without aura) and 75 age-and sex-matched normal volunteers. Frequency and age of onset of migraine attacks were also assessed according to angiotensin converting enzyme genotypes.

Results: Patients with migraine with and without aura were comparable with each other and the control group with respect to angiotensin converting enzyme genotypes (respectively; p= 0.88 and p= 0.76, p= 0.624). We could not determine a relationship between angiotensin converting enzyme genotypes and attack frequency (p= 0.125), but cases with angiotensin converting enzyme-II genotype showed a significantly younger age for onset of migraine attacks in comparison with the I/D genotype patients (p= 0.021).

Conclusion: We believe that further angiotensin converting enzyme gene studies are warranted in younger age groups of patients with migraine and also in different populations.

Key Words: Angiotensin-converting enzyme, polymorphism, genetic, migraine disorders.

INTRODUCTION

While the genetic tendency to migraine has been known for a long time, no clear genetic link has been shown except for a few forms. Angiotensin converting enzyme (ACE) is an enzyme of the peptidase M2 family, which is the key enzyme for two enzymatic cascades. Firstly, it converts angiotensin I to angiotensin II, which is a strong constrictor of vascular smooth muscle and an inducer of aldosterone. Secondly, it degrades bradykinin, which is an important factor for relaxation of vessels (1).

ACE is under strong genetic control (2). Various human ACE gene polymorphisms have already been suggested in the physiopathology of a variety of cardiovascular disorders, and also of carotid artery diseases and cerebral lacunar infarcts (3-7). A relationship between migraine without aura and ACE-D allele was also shown in previous studies (8,9). A number of studies revealed the beneficial effects of ACE inhibitor drugs on reducing the frequency of migraine attacks (10-12). These results encourage the idea that the ACE gene may have an important role in migraine physiopathology. However, the role of the ACE gene polymorphism in most of these conditions, as in migraine pathophysiology, is not fully understood yet.

The aims of the present study were 1. To assess the distribution of ACE gene alleles and genotypes in migraine patients and control cases in the Turkish population; and 2. To evaluate if the ACE gene insertion/deletion (I/D) polymorphism has an effect on frequency and age of onset of migraine attacks.

PATIENTS and METHODS

The study design was approved by the local ethics committee.

Patient Group

One hundred and two migraine patients (35 cases of migraine with aura and 67 of migraine without aura) who were identified according to the classification of the International Headache Society and consented to participate were enrolled into the present study (13).

All cases were between 15-40 years of age and none was using any medication that may have an effect on ACE. None of the cases had chronic systemic illnesses.

A previously prepared questionnaire including demographic parameters, present complaints, past medical history, age of onset of the attacks, frequency of attacks du-

ring the last three months, symptomatology and associated features of the attacks, and medication taken was completed for all patients. The age of onset of migraine attacks in each group according to allele distribution was used for the assessment of association of ACE polymorphisms and age of onset of migraine attacks.

Frequency of the migraine attacks in the last three months was obtained as an average number per month and the number of days that the patient could not continue her/his daily or professional life. Migraine patients were divided into two groups according to the attack frequency as: 1. Three or less times per month (less frequent group) and 2. More than three times per month (frequent group).

The associated features were defined as the symptoms present during the headache period. Aura symptoms were evaluated separately.

Control Group

Seventy-five age and sex-matched normal volunteers were enrolled into the study as a control group. The control group was gathered from hospital staff and medical students who did not have a history of migraine or chronic illnesses.

Data of the control group on demographic parameters, detailed past medical history and medications used were noted on the previously prepared questionnaire.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes as described by Miller et al. (14). The ACE I/D gene polymorphism was determined by polymerase chain reaction using primers flanking the polymorphic region of intron 16 (15). Electrophoresis of the amplified products in 2% agarose gel allowed detection of a 190 bp fragment (deletion, D allele) and of a 490 bp fragment (insertion, I allele) (15).

Statistical Evaluation

Statistical analysis was made for comparisons of:

1. The ACE I/D gene polymorphism of patient and control groups,

2. The ACE I/D gene polymorphism of migraine patients with and without aura,

3. The ACE I/D gene polymorphism and age of migraine onset,

4. The ACE I/D gene polymorphism and attack frequency.

Statistical evaluation was made using chi-square test, Student's-t and unidirectional variant analysis (ANOVA), and $p< 0.05$ was accepted as statistically significant. The demographic characteristics of the patients and volunteers were compared by chi-square test.

RESULTS

Eighty-four females and 18 males were included in the patient group, while 54 female and 21 male cases were included in the control group. The ages of the patients and control cases were between 15-40 years (means 30.9 and 29.2 years, respectively) ($p= 0.10$ and $p= 0.10$, respectively).

Out of 102 patients, 67 cases had migraine without aura and 35 patients had migraine with aura. The genotype distribution and the allele frequencies of the ACE gene polymorphism for migraine patients and the control group are shown in Table 1.

Migraine and control groups did not show a significant difference in distribution of D and I alleles ($p= 0.62$). There were no differences in allele distribution when migraine patients with and without aura were compared with each other and with the control group ($p= 0.92$, $p= 0.88$ and $p= 0.76$, respectively).

There was no significant difference between patient and control groups with respect to ACE genotypes ($p= 0.917$). Patients with migraine with and without aura were comparable according to ACE genotypes ($p= 0.118$). Similarly, when we compared migraine cases with aura and without aura separately with the control group, ACE genotypes of both groups were compatible with the control group ($p= 0.772$ and $p= 0.857$, respectively).

Table 1. D and I alleles and ACE genotypes in migraine (with aura and without aura) and control groups

	Allele frequency		ACE genotypes		
	D (%)	I (%)	DD (%)	ID (%)	II (%)
Migraine group (n= 102)	119 (58.3)	85 (41.7)	38 (37.3)	43 (42.2)	21 (20.6)
With aura (n= 35)	41 (58.6)	29 (41.4)	12 (34.3)	17 (48.6)	6 (17.1)
Without aura (n= 67)	78 (58.2)	56 (41.8)	26 (38.8)	26 (38.8)	15 (22.4)
Control group (n= 75)	91 (60.7)	59 (39.3)	30 (40.0)	31 (41.3)	14 (18.7)

ACE: Angiotensin converting enzyme.

Table 2. ACE alleles and ACE genotypes in lower and higher frequency attack groups

		Allele frequency		ACE genotypes		
	Migraine	**D (%)**	**I (%)**	**DD (%)**	**ID (%)**	**II (%)**
Attack frequency ≤ 3 (n= 53)	With aura (n= 19)	18 (47.3)	20 (52.7)	5 (26.3)	8 (42.1)	6 (31.6)
	Without aura (n= 34)	37 (54.4)	31 (45.6)	12 (35.3)	13 (38.2)	9 (26.5)
Attack frequency > 3 (n= 49)	With aura (n= 16)	23 (71.8)	9 (28.2)	7 (43.8)	9 (56.3)	0 (0)
	Without aura (n= 33)	41 (62.1)	25 (37.9)	14 (42.4)	13 (39.4)	6 (18.2)

ACE: Angiotensin converting enzyme.

Of 102 migraine patients, 53 (19 with aura, 34 without aura) cases reported three or less attacks, while 49 (16 with aura, 33 without aura) patients reported more than three attacks per month in the last three months. Sixty-four (D) and 34 (I) alleles were found in the cases that experienced more than three attacks per month, while 55 (D) and 51 (I) alleles were found in the cases that reported three or less attacks per month. Distribution of alleles and genotypic characteristics according to attack frequency per month are shown in Table 2. We could not determine a statistically significant relationship between ACE genotypes and attack frequency (p= 0.125).

In evaluation of the relationship between ACE genotypes and age of onset of migraine attacks, the average age of onset was 20.8 ± 6.5 years in DD, 24.1 ± 6.5 years in ID and 19.5 ± 5.6 years in II genotype cases. Cases with II genotype showed a significantly earlier age of onset of migraine attacks in comparison with ID genotype patients (p= 0.021).

DISCUSSION

The need for new prophylactic drugs with fewer adverse effects remains a subject of research for pharmaceutical companies. The observation of improvement in migraine attacks in a patient with hypertension encouraged the idea that lisinopril, an ACE inhibitor drug, may have a role in migraine treatment. The first study recommended that lisinopril, even in low doses, may be effective in migraine prophylaxis. It is well tolerated, and the efficacy of ACE inhibitors may be more relevant in patients with ACE D/D gene polymorphism (11,12). Various studies concerning ACE and migraine revealed similar data on the role of ACE inhibitors in migraine attack prophylaxis. In addition, blocking the conversion of angiotensin I to angiotensin II may inhibit free radical activity, increase prostacyclin synthesis and block degradation of bradykinin enkephalin and substance P, which may additionally serve to alter the sympathetic activity (16,17).

In the present study, distribution of ACE allele frequency and genotype distribution in migraine cases and normal controls were comparable. Migraine cases with or without aura also showed compatible genotype distribution when compared with each other and with the control cases.

Previously, a higher incidence of the ACE gene DD genotype was found in migraine patients without aura and a determining role of the ACE gene DD genotype in the frequency of migraine attacks was suggested (8,18). Inconsistently, another study pointed out a slight protective effect of ACE-DD against migraine in male patients (19). These trials suggested the involvement of the renin-angiotensin-aldosterone system in some part of the migraine pathogenesis. Unfortunately, we were unable to determine a relationship between the ACE I/D gene polymorphism and attack frequency in migraine cases with or without aura, similar to Schürks et al. (20). There are very complex relationships between circulating peptides that play a role in pain and ACE. The identification of these mechanisms will permit a better understanding of the role of the ACE gene.

The present study was cross-sectional. Therefore, in most of the cases, treatment strategies had been planned prior to the patient's enrollment into the study, and none of the cases had a headache diary for the last three months. Since evaluation of serum ACE activity was not a goal of this study, we did not find it relevant to change or stop the antimigraine drugs that the patients were accustomed to taking, though in the study design, we excluded the cases using any drugs that could affect ACE.

Collection of data about the frequency of the attacks in the present study was anamnestic, based primarily on the patient's memory, and thus may cause a bias in the results. However, three months is a relatively short period to affirm and all cases were able to state the number of attacks confidently. To stress the consistency, questions about the migraine frequency were asked in two different ways in two different parts of the questionnaire. The three-month period may seem to be a short time period, but since migraine attack frequency showed an irregular distribution over time, we did not feel that selection of a different time period would add much to our data about the frequency of migraine attacks per month.

Another point that should be considered in the present study is whether the migraine cases that were enrolled into the study from the outpatient clinics represented those in whom attacks were already more frequent, thus requiring neurology consultation. By coincidence, in the present study, the number of the cases with more than three attacks per month was very similar with the number of cases with less than three attacks per month. Therefore, we do not think that enrollment of the migraine cases from outpatient clinics created a bias in the present study.

In our study, age of onset of migraine was found to be younger in cases with ACE-II genotype than in cases with ACE-ID, but we do not have a satisfactory explanation for the role of ACE-II genotype in the age of onset of migraine attacks. However, since data about this topic is very limited, we believe that it warrants testing in larger groups of migraine patients and also in different populations.

ACKNOWLEDGEMENT

We thank Dr. Hasan Herken for his kind help with the statistical analysis of our data.

REFERENCES

1. *Crisan D, Carr J. Angiotensin I-converting enzyme, genetic and disease association review. J Mol Diagnostic 2000;3:105-12.*
2. *Cambien FF, Alhenc-Gelas B, Herbeth JL, Andre R, Rakotovao MF, Gonzales J, et al. Familial resemblance of plasma angiotensin-converting enzyme level: The Nancy study. Am J Hum Genet 1988;43:774-80.*
3. *Evans AE, Poirier O, Kee F, Lecerf L, McCrum E, Falconer T, et al. Polymorphisms of the angiotensin-converting-enzyme gene in subjects who die from coronary heart disease. Q J Med 1994;87:211-4.*
4. *Mattace-Raso FU, van der Cammen TJ, Sayed-Tabatabaei FA, Van Popele NM, Asmar R, Schalekamp MA, et al. Angiotensin converting enzyme gene polymorphism and common carotid stiffness. The Rotterdam study. Atherosclerosis 2004;174:121-6.*
5. *Mattu RK, Needham EW, Galton DJ, Frangos E, Clark AJ, Caulfield M. A DNA variant at the angiotensin-converting enzyme gene locus associates with coronary artery disease in the Caerphilly Heart study. Circulation 1995;15:270-4.*
6. *Nakai K, Fusazaki T, Zhang T, Shiroto T, Osawa M, Kamata J, et al. Polymorphism of the apolipoprotein E and angiotensin I converting enzyme genes in Japanese patients with myocardial infarction. Coron Artery Dis 1998;9:329-34.*
7. *Zhang JH, Kohara K, Yamamoto Y, Nakura J, Tabaro Y, Fujisawa M, et al. Genetic predisposition to neurological symptoms in lacunar infarction. Cerebrovasc Dis 2004;17:273-9.*
8. *Paterna S, Di Pasquale P, D'Angelo A, Seidita G, Tuttolomondo A, Cardinale A, et al. Angiotensin-converting enzyme gene deletion polymorphism determines an increase in frequency of migraine attacks in patients suffering from migraine without aura. Eur Neurol 2000;43:133-6.*
9. *Joshi G, Pradhan S, Mittal B. Role of the ACE ID and MTHFR C677T polymorphisms in genetic susceptibility of migraine in a north Indian population. J Neurol Sci 2009;277:133-7.*
10. *Rahimtoola H, Buurma H, Tijssen CC, Leufkens HG, Egberts AC. et al. Reduction in the therapeutic intensity of abortive migraine drug use during ACE inhibition therapy-a pilot study. Pharmacoepidemiol Drug Saf 2004;13:41-7.*
11. *Schrader H, Stovner LJ, Helde G, Sand T, Bovim G. Prophylactic treatment of migraine with angiotensin converting enzyme inhibitor (lisinopril): Randomised, placebo controlled, crossover study. BMJ 2001;322:19.*
12. *Schuh-Hofer S, Flach U, Meisel A, Israel H, Reuter U, Arnold G. Efficacy of lisinopril in migraine prophylaxis-an open label study. Eur J Neurol 2007;14:701-3.*
13. *Headache Classification Committee of the International Headache Society. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. Cephalalgia 1988;8:13-39.*
14. *Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.*
15. *Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism in the human angiotensin converting enzyme gene. Nucleic Acids Res 1992;20:1433.*
16. *Goa KL, Balfour JA, Zuanetti G, Lisinopril. A review of its pharmacology and clinical efficacy in the early management of acute myocardial infarction. Drugs 1996;52:564-88.*
17. *Skidgel RA, Erdos EG. The broad substrate specificity of human angiotensin converting enzyme. Clin Exp Hypertens A 1987;9:243-59.*
18. *Kara I, Ozkok E, Aydin M, Orhan N, Cetinkaya Y, Gencer M, et al. Combined effects of ACE and MMP-3 polymorphisms on migraine development. Cephalalgia 2007;27:235-43.*
19. *Lin JJ, Wang PJ, Chen CH, Yueh KC, Lin SZ, Harn HJ. Homozygous deletion genotype of angiotensin converting enzyme confers protection against migraine in man. Acta Neurol Taiwan 2005;14:120-5.*
20. *Schürks M, Zee RY, Buring JE, Kurth T. ACE D/I polymorphism, migraine, and cardiovascular disease in women. Neurology 2009;72:650-6.*

Yazışma Adresi/Address for Correspondence

Prof. Dr. Münife Neyal

Gaziantep Üniversitesi Tıp Fakültesi
Nöroloji Anabilim Dalı
Gaziantep/Türkiye

E-posta: neyal@gantep.edu.tr

geliş tarihi/received 13/08/2009
kabul ediliş tarihi/accepted for publication 06/10/2009