Original Article

Association of ACE Gene I/D polymorphism with migraine in Kashmiri population

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Abstract

Introduction: Migraine is a complex, recurrent headache disorder that is one of the most common complaints in neurology practice. The role of various genes in its pathogenesis is being studied. We did this study to see whether an association exists between ACE gene I/D polymorphism and migraine in our region. **Materials and Methods:** The study included 100 patients diagnosed with migraine and 121 healthy controls. The study subject were age and gender matched. The analysis was based on Polymerase Chain Reaction (PCR) and included following steps: DNA extraction from blood, PCR and Restriction Fragment Length Polymorphism (RFLP). **Results:** Out of 100 cases, 69 were females and 31 were males. Fifty-seven were having migraine without aura and 43 had migraine with aura. 45 of the cases had II polymorphism, 40 had ID polymorphism and 15 had DD polymorphism in ACE gene. **Conclusion:** We were not able to find a statistically significant association between ACE gene I/D polymorphism with migraine. The reason for difference in results between our study and other studies could be because of different ethnicity in study populations. So a continuous research is needed in this regard in order to find the genes and different polymorphism that increase the susceptibility of Kashmiri population to migraine.

Key Words

ACE gene, gene polymorphism, migraine

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Introduction

Migraine headache is a complex, recurrent headache disorder that is one of the most common complaints in neurology practice.[1] There are two main types of migraine and they are migraine without aura and migraine with aura. Migraines without aura is the most common type, accounting for more than 80% of all migraines. Based on different studies, the overall prevalence of migraine among men and women in the US is estimated at 12%.[2-4] In Asia the sex-specific migraine prevalence has been reported as 11.3-14.4% in women and 3.6-6.7% in men.[5]

Much research has been done to unravel the pathogenesis of migraine and the role of genetics has always been emphasized.

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The genetic basis of migraine is likely to be complex and in some individuals an additive effect of more than one genetic source may come into play. Individuals prone to migraine have a genetic threshold that renders them susceptible to an acute migraine attack depending upon the balance between excitation and inhibition at various levels of the nervous system.

Many people have shown interest in ACE gene I/D polymorphism in order to determine its role in health and disease states including migraine. In humans, the gene encoding ACE is located on the long arm of chromosome 17 (17q23). The gene is 21 kilo bases (kb) long and comprises 26 exons and 25 introns. More than 160 *ACE* gene polymorphisms have been listed till date and the most extensively studied polymorphism involves the presence (insertion, I) or absence (deletion, D) of a 287-bp sequence of DNA in intron 16 of the gene giving rise to three possible genotypes: II, ID or DD. It has been found that the mean ACE activity levels is different depending upon the type of I/D genotype. It has been found that there is relative excesses of the II genotype and commensurate deficiencies of the DD genotype in athletes like swimmers, runners and mountain climbers. ACE gene I/D polymorphism has been associated with a number of diseases like hypertension, diabetic nephropathy and Alzheimer's disease. Previous studies have demonstrated

different results regarding whether an association between the *ACE* polymorphisms and migraine exists. As a result, the importance of the ACE ID polymorphism is not clearly understood. So we undertook this case-control study to see whether an association exists between ACE gene polymorphism and migraine in patients attending Outpatient services at Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Soura a tertiary-care hospital in Jammu and Kashmir, a state in North India.

Materials and Methods

The study entitled "Association of ACE gene I/D polymorphism with migraine" was conducted in the Sher-I-Kashmir Institute of Medical sciences, Soura, Srinagar, in the Department of Neurology and Department of Immunology and Molecular Medicine over a period of 2 yrs from January 2013. Clearance from local SKIMS ethical committee was taken prior to start of study. The study included 100 patients diagnosed with migraine and 121 healthy controls. The study subject were age and gender matched. Migraine was diagnosed according to the criteria from the International Headache Society.[6] A detailed medical history was obtained from each patient, and all study participants underwent a physical examination. The migraine patients who had any cardiovascular or cerebrovascular disease or hypertension, and smoked were excluded from this study. All patients with migraine were unrelated. Control subjects, who had never experienced a migraine headache, and had no family history of migraine were selected from the outpatient-neurology clinic. The controls had no cardiovascular or cerebrovascular disease or hypertension and none smoked.

Blood samples were taken from both the groups and a written pre informed consent was obtained from all the cases and controls. Five milliliters of peripheral blood was obtained from each subject in ethylenediaminetetraacetic acid (EDTA) containing vials (200 µl of 0.5 M, pH = 8.0) and stored at −20 °C till use.

Deoxyribonucleic acid (DNA) extraction was performed according to the manufacturer's protocol for Qiagen DNA extraction kits (Qiagen, Hilden, NRW, Germany). DNA content was quantified by spectrophotometric absorption (Nanodrop Spectrophotometer, BioLab, Scoresby, VIC, Australia).

Polymerase chain reaction (PCR) was performed using an iCycler Thermal Cycler (Bio-Rad, Hercules, CA, USA).

Molecular detection of the ACE I/D polymorphism was performed by PCR amplification of intron 16. The template DNA (0.5-1.0 ug) was used in a PCR under stringent conditions to avoid the possibility of false positives for ACE genotyping. The reactions was be carried out with 10 pmol of each primer: Forward primer, 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', and reverse primer, 5'-GAT GTG GCC ATC TTC GTC AGA T-3', in a final volume of 25 µL containing 1.5 mM MgCl2, 25 mM KCl, 5 mM Tris-HCl (pH 8.4), 0.25 mM each of deoxyribonucleotide triphosphate (dNTP, Biotools, Spain) and 1 unit Taq polymerase (Biotools, Spain). Amplification was carried out in a DNA thermal cycler (Pamcycler thermocycler) for 30 cycles with denaturation extension at 72 °C for 2 min. The PCR products were separated on a 2% agorose gel and DNA were visualized by ethidium bromide staining. The PCR product is a 490-bp fragment in the presence of the insertion (I) allele. Thus, each DNA sample revealed one of three possible patterns after electrophoresis: A 490-bp band (genotype I/I), a 190-bp band (genotype D/D), or both 490-bp and 190-bp bands (genotype I/D).

Restriction fragments were visualized after ethidium bromide staining of the agarose gel with the use of an ultraviolet transilluminator.

Results

Out of 100 cases taken in this study, 57 were having migraine without aura and 43 had migraine with aura. Demographic features of the studied cases like age distribution, residence, marital status are given in Table 1 below.

The main objective of this study was to know the association between ACE gene I/D polymorphism and migraine in the kashmiri populations. The results of this study are presented in Tables 2-6.

Table 1: Depicting the demographic features of studied cases

Demographic feature	Number of patients
Sex distribution	
Males	31
Females	69
Age distribution	
18-30	45
31-40	37
41-50	14
>51	04
Residence	
Rural	38
Urban	62
Marital status	
Married	68
Unmarried	32

Table 2: Below shows the number of patients of migraine with and without aura in different age groups

Age (in years)	Total number of migraine cases	Males (*n* = 31)		Females (*n* = 69)	
18-30	45	M*	07	M	15
		MwA**	09	MwA	14
			16		29
31-40	37	M	08	M	16
		MwA	03	MwA	10
			11		26
41-50	14	M	02	M	07
		MwA	01	MwA	04
			03		11
>51	04	M	01	M	01
		MwA	00	MwA	02
			01		03

M = Migraine without aura, MwA = Migraine with aura

On comparing cases of migraine with aura and migraine without aura, we were not able to find a statistically significant relation between the two with regards to ACE gene I/D polymorphism.

Direct visualization of ACE I/D PCR products, electrophoresed on 2% agarose gel, by ethidium bromide staining [Figure 1]. *A 490 base pair ACE I allele and 190 base pair ACE D allele is seen. Results from 8 patients are shown.*
II homozygote genotypes: Lane 6 and 8.

Table 3: Assocition of ace i/d polymorphism with migraine patients

SNP	Migraine	Controls	*P* value
DD*	15	11	>0.5
ID**	40	45	>0.5
II***	45	65	>0.5
Total	100	121	>0.5

*ACE gene DD polymorphism, **ACE gene ID polymorphism, ***ACE gene II polymorphism

Table 4: Association of ace i/d polymorphism with migraine without aura

SNP	Migraine without aura	Controls	*P* value
DD	9	11	>0.5
ID	23	45	>0.5
II	25	65	>0.5
Total	57	121	>0.5

Table 5: Association of ace i/d polymorphism with migraine with aura

SNP	Migraine with aura	Controls	*P* value
DD	6	11	>0.5
ID	17	45	>0.5
II	20	65	>0.5
Total	43	121	>0.5

Table 6: Assocition of ace i/d polymorphism in migraine without aura in relation to migraine cases

SNP	Migraine without aura	Migraine with aura	*P* value
DD	9	6	>0.5
ID	23	17	>0.5
II	25	20	>0.5
Total	57	43	>0.5

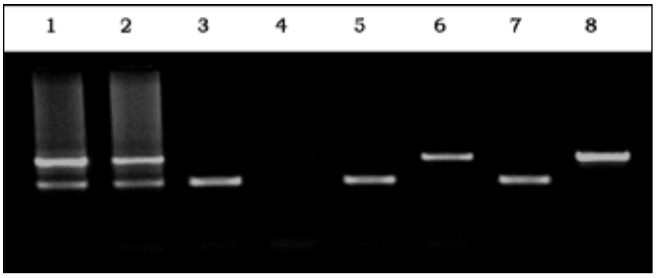

Figure 1: Representative gel picture is shown in figures 1

DD homozygote: Lanes 3, 5 and 7
ID heterozygotes: Lane: 1 and 2.
Control (D. H_2O): 4

As is evident from the results, no association could be found between ACE I/D polymorphism and migraine in the studied group.

Discussion

As of now, the pathogenesis of migraine is not known completely. It was previously considered to be a vascular phenomenon that resulted from intracranial vasoconstriction followed by rebound vasodilation. Currently, however, the neurovascular theory describes migraine as primarily a neurogenic process with secondary changes in cerebral perfusion associated with a sterile neurogenic inflammation. Some authors have proposed a dopaminergic basis for migraine.[7] Another theory proposes that deficiency of magnesium in the brain triggers a chain of events, starting with platelet aggregation and glutamate release and finally resulting in the release of 5-hydroxytryptamine, which is a vasoconstrictor. In clinical studies, oral magnesium has shown benefit for preventive treatment and intravenous magnesium may be effective for acute treatment, particularly in certain subsets of migraine patients.[8] The serotonin receptor 5-hydroxytryptamine (5-HT) is believed to be the most important receptor in the headache pathway. Immunohistochemical studies have detected 5-hydroxytryptamine-1D (5-HT1D) receptors in trigeminal sensory neurons, including peripheral projections to the dura and within the trigeminal nucleus caudalis (TNC) and solitary tract, while 5-HT1B receptors are present on smooth muscle cells in meningeal vessels; however, both can be found in both tissues to some extent and even in coronary vessels. The importance of seratonin in migraine is also determined from the fact that 5HT1B/1D receptor agonists that is triptans are very effective in abolishing an episode of migraine headache.

Migraine has a strong genetic component. Approximately 70% of migraine patients have a first-degree relative with a history of migraine. The risk of migraine is increased four-fold in relatives of people who have migraine with aura.[9] Large national registry-based twin studies have confirmed a consistently higher concordance of migraine in monozygotic twins versus dizygotic twins. In one such study, using a polygenic multifactorial model, researchers estimated that inheritance accounts for 40-50% of an individual's susceptibility to migraine.[10]

Angiotensin I-converting enzyme (ACE) is a part of the kinin–kallikrein cascade. It metabolizes bradykinin, a strong vasodilator, forming the inactive metabolite bradykinin. In addition to a prominent role in blood pressure regulation through the kinin–kallikrein cascade, ACE is the main active product of the renin–angiotensin system (RAS), a dipeptidyl carboxypeptidase that converts the inactive decapeptide, angiotensin I to angiotensin II, an active octapeptide and potent vasoconstrictor (angiotensin II). RAS maintains the long-term regulation of blood pressure and blood volume in the body.[11] As the actual pathogenesis of migraine is still not

known so it is difficult to summarise the role of ACE gene in migraine but its importance is established by the facts that ACE gene polymorphism affects the vascular reactivity through difference in serum ACE level and that ACE inhibitors have been successfully used for migraine prophylaxis. It was because of these effects of ACE that studies were done to look for an association between ACE gene polymorphism and migraine.

In the National Center for Biotechnology Information (NCBI) records, more than 160 *ACE* gene polymorphisms are listed, most of which are single nucleotide polymorphisms (SNPs). Only 34 of those polymorphisms are located in coding regions with 18 of them being missense mutations. It was Rigat and Co-workers[12] who first described a polymorphism involving the presence (insertion, I) or absence (deletion, D) of a 287-bp sequence of DNA in intron 16 of the gene (NCBI ref.SNP ID: rs1799752). They found that the mean ACE activity levels in DD carriers were approximately twice that found in II genotype individuals. Intermediate levels were shown in subjects with the ID genotype which pointed towards codominancy. The I/D polymorphism accounted for approximately half (47%) of the observed variance in ACE levels in this study group. Later studies showed that the involvement of the I/D polymorphism is also detected in tissue ACE levels.[13,14] Associations between the I/D polymorphism and disease are expected as evidence favors the hypothesis that functional polymorphisms, linked with the I/D polymorphism, are involved in pathophysiological conditions through the renin-angiotensin and kinin-kallikrein systems. A lot number of studies have been published investigating the association between the I/D polymorphism and numerous clinical outcomes. These investigations included not only the presence or incidence of disease, but also covered other aspects, such as symptoms and manifestations, efficacy of drugs and therapies, interaction with other genetic or environmental factors, recovery rates, disease progression, and survival. Many studies have been conducted in the last decade to study the association of migraine with ACE gene I/D polymorphism. The result of these studies have shown a lot of variation and disparity. About four studies[15-17] have found ACE gene DD polymorphism to be associated with Migraine with Aura whereas one study showed it be associated with Migraine without Aura.[18]

Till date only one study has been done from India by Joshi *et al*.,[16] who studied the role of the ACE ID and MTHFR C677T polymorphisms in genetic susceptibility of migraine in a north Indian population. They found that ACE DD genotype showed significant association in migraine patients with aura (MA) but a marginal significance in female MA patients in comparison with healthy controls. They also found synergistic role of ACE (DD)*MTHFR (CT) interaction, showing a positive association in total migraine with aura patients as well as female migraine patients with aura when compared with healthy controls.

However some published studies failed to show any association between ACE gene I/D polymorphism and Migraine.[19-22] In fact, one of the studies findings indicate that ACE-DD may have a slight protective effect against migraine in male patients.[19] A Systematic Review and Meta-Analysis by Markus Schürks *et al*., in 2010 concluded that the *ACE* II genotype is protective against both migraine with and without aura among non-Caucasian populations. There was no association among Caucasians.[23]

One study done in Kashmiri population did not show an association between migraine and single nucleotide polymorphisms of CACNA1A gene.[24] This study included a total of 25 patients of migraine, which were compared with age and sex matched 25 healthy controls. 20 of the patients (80%) were females and 5 (20%) were males. The polymorphic analysis of CACNA1A gene revealed the presence of only the wild form of the gene for the codon E993V in both case and control groups.

We conducted our study in Jammu and Kashmir, a Northern state of India. The frequency of consangious marriage is quite high in this population. Our study compared 100 migraine patients with age and sex-matched healthy 121 controls. In the previous study by Joshi *et al*.,[16] a total of 150 migraine patients, 220 non-migraine headache patients (Disease controls) and 150 age-sex matched normotensive healthy controls were enrolled. In our study out of 100 patients, 31 were Males and 69 were Females. 57 had Migraine without aura and 43 had Migraine with aura. Among the 100 cases in our study, 15 had DD, 40 had ID and 45 had II genotype while out of 121 controls, 11 had DD, 45 had ID and 65 had II genotype. In contrary to the previous study done in northern India,[16] we were not able to find a statistically significant association between ACE gene I/D polymorphism with migraine as compared to controls. The reason for difference in results between our study and the studies showing an association between ACE gene I/D polymorphism and migraine including the one done in India could be because of different ethnicity in study populations as several authors have suggested that the frequency I/D genotype vary according to ethnicity.[17] The Kashmiri population is slightly different from the rest of country in being ethnically related to Aryans. This does not mean that ACE gene does not play any role in migraine but maybe has a little role only or some other SNP is involved. So a continuous research is needed in this regard in order to find the genes and different polymorphism that increase the susceptibility of Kashmiri population to migraine.

Bullet points.

ACE gene I/D polymorphism is not associated with migraine.

Further research is needed to determine the genetic influence on migraine.

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