LUCRĂRI ORIGINALE

Deletion allele of the ACE gene is not a risk factor for asthma predisposition

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ABSTRACT

Background: Angiotensin-converting enzyme (ACE) has an important role in inactivation of bradykinin and tachykinins which known as powerful bronchoconstrictors. It has been demonstrated that an insertion (I)/deletion (D) genetic variations within the ACE gene greatly influence the plasma level of ACE. Objective: The aim of the present study was to determine the frequencies of ACE D and I alleles and ACE DD, DI and II genotypes in asthmatic patients and controls with Iranian Azeri-Turkish origin and to compare the frequency of the ACE genotypes between asthmatic patients and controls. Methods: We genotyped 212 healthy controls including 73 males and 138 females, as well as 62 patients with asthma, including 28 males and 34 females by PCR. Results: Of the 212 healthy controls: 1) the prevalence of DD, DI, and II genotypes were 83(39.151), 92(43.396) and 37(17.453), respectively. 2) the frequency (%) was 257(60.9) for D allele and 165(39.1) for I allele. 3) D and I allele frequencies were 0.61 and 0.39 respectively. Of the 62 patients with asthma: 1) the prevalence of DD, DI, and II genotypes were 17(27.42), 31(50) and 14(22.58), respectively. 2) the frequency (%) was 65(52.42) for D allele and 59(47.58) for I allele. 3) D and I allele frequencies were 0.52 and 0.48 respectively. Statistical analysis showed that studied groups (female + male patients group and female + male controls group) were in Hardy–Weinberg equilibrium. Our findings imply that I/D ratio was 0.61/0.39 in all controls and 0.6/0.4 in male or female controls. Significant differences were not found in the ACE genotype or allele frequencies between studied groups regarding all cases versus all controls, female cases versus female controls, male cases versus male controls. Conclusion: We have concluded that deletion allele of the ACE gene is not a risk factor for asthma predisposition.

Keywords: *ACE, Asthma, Iranian Azeri-Turkish*

REZUMAT

Deleţia alelei genei ACE nu este un factor de risc pentru predispoziţia către astm

Context general. *Angiotensin-convertaza serică (ACE) are un rol important în inactivarea bradikininei şi tahikininelor, cunoscute ca bronhoconstrictoare puternice. A fost demonstart faptul că inserţia (I)/deleţia (D) în cadrul genei ACE influenţează major nivelul plasmatic al ACE.* ***Obiectiv.*** *Scopul prezentului studiu a fost să determine frecvenţa alelelor I şi D pentru ACE şi a genotipurilor DD, DI and II pentru ACE la pacienţi astmatici şi subiecţi control sănătoşi de etnie iraniană cu origine azero-turcă şi să compare frecvenţa genotipurilor ACE între pacienţii astmatici şi subiecţii control.* ***Metode.*** *Am genotipat prin PCR 212 subiecţi de control sănătoşi, incluzând 73 de bărbaţi şi 138 de femei şi 62 de pacienţi cu astm - 28 de bărbaţi şi 34 de femei. Rezultate. Dintre cei 212 subiecţi sănătoşi control: 1) prevalenţa genotipurilor DD, DI şi II a fost 83 (39.151), 92 (43.396) şi respectiv 37 (17.453); 2) frecvenţa (%) a fost 257 (60.9) pentru alela D şi 165 (39.1) pentru alela I; 3) frecvenţa alelelor D şi I a fost 0.61 şi respectiv 0.39. Dintre cei 62 de pacienţi cu astm: 1) prevalenţa genotipurilor DD, DI şi II a fost 17 (27.42), 31 (50) şi respectiv 14 (22.58); 2) frecvenţa (%) a fost 65 (52.42) pentru alela D şi 59 (47.58) pentru alela I; 3) frecvenţa alelelor D şi I a fost de 0.52 şi 0.48. Analiza statistică a arătat că grupurile de studiu (grupul pacienţilor femei + bărbaţi şi grupul subiecţilor control femei + bărbaţi) au fost în echilibru Hardy-Weinberg. Rezultatele noastre implică faptul că raportul I/D a fost de 0.61/0.39 în rândul tuturor subiecţilor control şi 0.6/0.4 pentru bărbaţii sau femeile control. Nu au fost găsite diferenţe semnificative privind genotipul ACE sau frecvenţa alelelor comparând toate cazurile versus toţi subiecţii control, pacientele de sex feminin versus subiecţii control de sex feminin, pacienţii de sex masculin versus subiecţii control de sex masculin.* ***Concluzii.*** *Am concluzionat că deleţia alelelor genei ACE nu reprezintă un factor de risc pentru predispoziţia către astm.*

Cuvinte-cheie: *ACE, astm, iranieni azero-turci*

Introduction

Asthma is defined as a heterogeneous disease and several sets of genes and environmental factors play important roles in the asthma predisposition [1]. Results of recent studies on asthma in different ethnic groups indicated that genetic variations in 64 genes have been associated with asthma [1, 2]. It has been demonstrated that angiotensin-converting enzyme (ACE) plays critical roles in the asthma pathogenesis and neurogenic inflammation with different mechanisms such as inactivation of bradykinin and neurokinin A [3,4]. The ACE gene is located on the chromosome 17q23 and has 25 introns and 26 exons. Presence or absence of deletion of a 287-bp element within intron 16 leads to ACE genotypes [5-7]. ACE genotypes (insertion homozygote (II), deletion homozygote (DD), and heterozygotes (DI)) could influence the level of ACE in plasma. ACE DD genotype accounts for about double level of ACE production in plasma compare to those of the ACE II genotype. ACE DI genotype results in intermediate level of ACE production in plasma [7,8]. Presence of D allele within the ACE gene results in production of high plasma levels of ACE [9]. A large body of investigations demonstrated that ACE D allele/DD genotype

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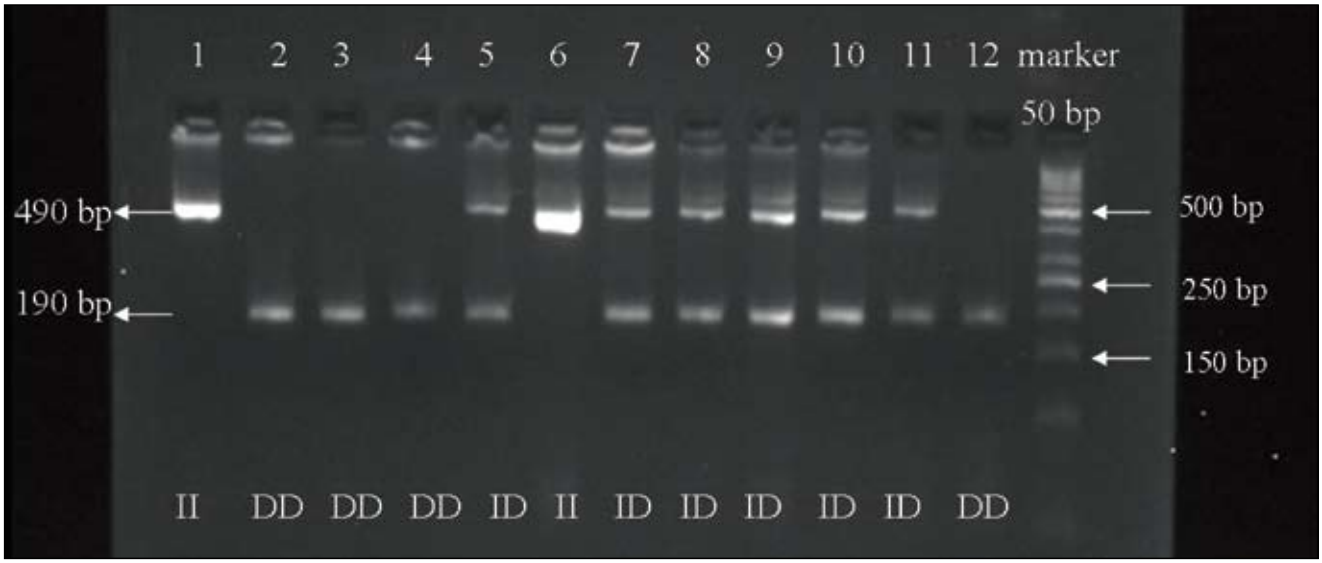
Figure 1. ACE genotyping of 12 samples (DD, ID, and, II) on 2% agarose gel



ACE; angiotensin-converting enzyme, I: insertion (490 bp fragment) and D: deletion (190 bp fragment)

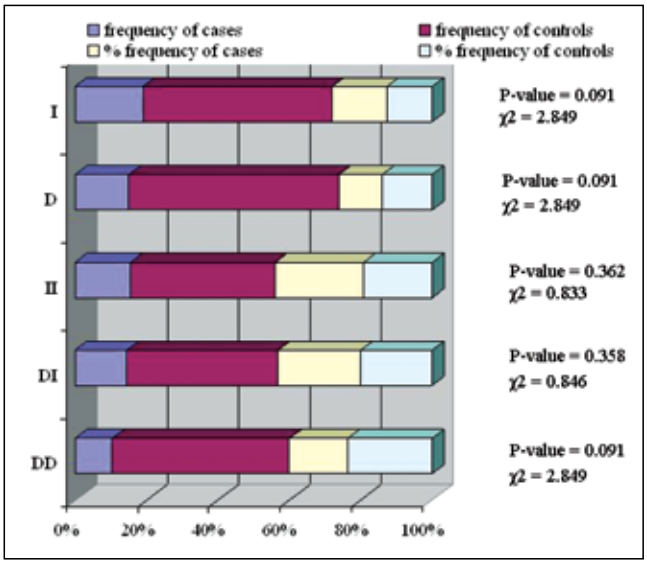
Table I. The frequency of ACE DD, DI, II genotypes, and D, I alleles among asthmatics and controls (male+female)

	Patients F(F%)	Controls F(F%)	OR (95% C.I.)	$\chi 2$	P-Value
DD	17(27.419)	83(39.151)	0.587(0.315-1.094)	2.849	0.091
DI	31(50)	92(43.396)	1.304(0.74-2.3)	0.846	0.358
II	14(22.581)	37(17.453)	1.38(0.69-2.759)	0.833	0.362
D	65(52.419)	257(60.9)	0.707(0.473-1.058)	2.849	0.091
I	59(47.581)	165(39.1)	1.414(0.945-2.116)	2.849	0.091

F: Frequency; Hardy–Weinberg equilibrium analysis: Female+Male patients group: $\chi 2 = 0.0003 < 3.84$, P-value = 0.999> 0.05, n=62, p=0.52, q= 0.48; Female+Male controls group: $\chi 2 = 1.8802 < 3.84$, P-value = 0.390> 0.05, n=212, p=0.61, q=0.39

predisposes individuals to human disorders [10-19], but others are inconsistence with theses findings [20-23]. The aim of the present study was to determine the frequencies of ACE D and I alleles and ACE DD, DI, and II genotypes in asthmatic patients and controls with Iranian Azeri-Turkish origin and to compare the frequency of the ACE genotypes between asthmatic patients and controls.

Figure 2. The frequency of ACE DD, DI, II genotypes, and D, I alleles among asthmatics and healthy controls (male+female)



Material and methods

The ethical committee of Urmia University of Medical Sciences approved the present study. Totally, 212 healthy controls, including 73 males and 138 females, as well as 62 patients with asthma, including 28 males and 34 females from Azerbaijan province of Iran (North-West of Iran), participated in the present study. The cases and controls, all with Azeri origin, had good fitness regarding demographic characters. Asthmatic cases were diagnosed and sequentially selected among patients referred to Imam Hospital affiliated to Urmia University of Medical Sciences. The inclusion criteria include: 1) age > 15 years old, 2) history of asthma, 3) non-smoker, 4) spirometry test findings of FEV1/FVC<0.7, and FEV1<70% predicted and 15% increase in FEV1 after bronchodilator inhalation, and 5) asthma must be clinically confirmed by pulmonologist. Controls randomly selected in department of genetics of Urmia University of Medical Sciences. Individuals with any accompanied disorders such as genetic and congenital diseases or history of atopy were excluded from the study. Informed written consent has been taken from every participant. 5 ml peripheral blood was used for DNA extraction via salting out method described by Miller et al [24]. A single polymerase chain reaction (PCR) performed for detection of ACE DD, ID, and II, genotypes in every sample. PCR carried out using forward primer: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and reverse Primer: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3' and PCR program containing of 35 cycles (denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72oC for 1 min) was used 6. Electrophoresis on

Table II. The frequency of ACE DD, DI, II genotypes, and D, I alleles among asthmatic females and healthy females

	Patients F(F%)	Controls F(F%)	OR (95% C.I.)	χ2	P-Value
DD	9(26.471)	52(37.681)	0.595(0.258-1.374)	1.498	0.221
DI	17(50)	64(46.377)	1.156(0.546-2.45)	0.144	0.705
II	8(23.529)	22(15.942)	1.622(0.65-4.047)	1.091	0.296
D	35(51.471)	168(60.87)	0.682(0.4-1.162)	1.993	0.158
I	33(48.529)	108(39.13)	1.467(0.86-2.5)	1.993	0.158

F: Frequency; Hardy–Weinberg equilibrium analysis: Female patients group: χ2 = 2.549E-05< 3.84, P-value = 0.999> 0.05, n=34, p=0.51, q=0.49; Female controls group: χ2 = 0.0965< 3.84, P-value = 0.952> 0.05, n=138, p=0.6, q=0.4

Figure 3. The frequency of ACE DD, DI, II genotypes, and D, I alleles among asthmatic females and healthy females

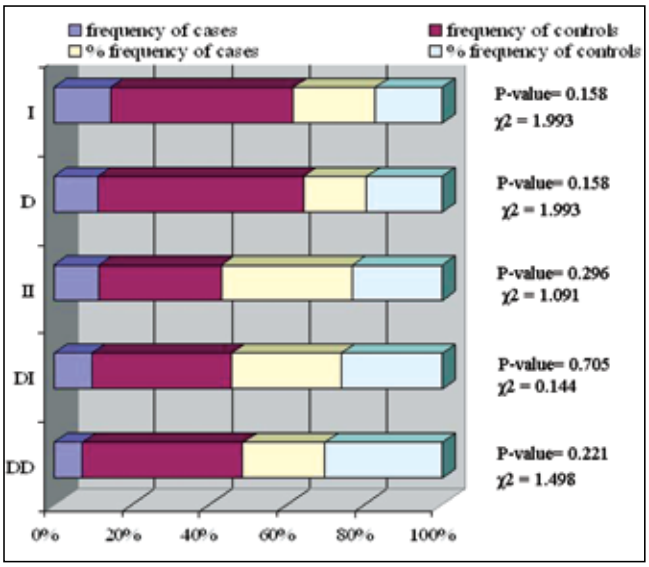
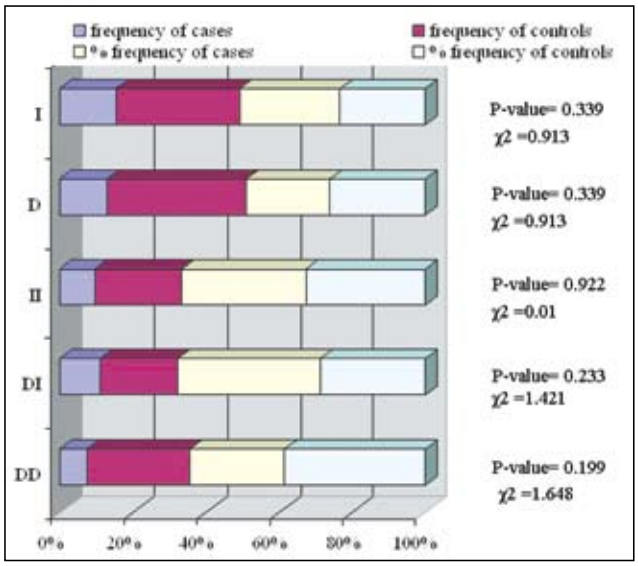


Figure 4. The frequency of ACE DD, DI, II genotypes, and D, I alleles among asthmatic males and healthy males



2% agarose gel containing ethidium bromide was done for separation of PCR products. Presence/absent of 490/190 bp fragments were detected by UV transilluminator. Presence of two fragments of 490/190 bp indicates the heterozygous I/D genotype. Presence of a fragment of 490 bp along with absence of a fragment of 190 bp shows homozygote II genotype. Also, presence of a fragment of 190 bp along with absence a fragment of 490 bp indicates the homozygote DD genotype.

Results

The results of our study are summarized in Tables I-III. We studied two groups, including female+male controls group (212 healthy controls including 73 Males and 138 females) and female+male cases group (62 patients with asthma including 28 Males and 34 females). Of the 212 healthy controls: 1) the prevalence of DD, DI, and II genotypes were 83(39.151), 92(43.396) and 37(17.453) respectively. 2) the frequency (%) was 257(60.9) for D allele and 165(39.1) for I allele. 3) D and I allele frequencies were 0.61 and 0.39 respectively. Of the 62 patients with asthma: 1) the prevalence of DD, DI and II genotypes were 17(27.419), 31(50) and 14(22.581), respectively. 2) the frequency (%) was 65(52.42) for D allele and 59(47.58) for I allele. 3) D and I allele frequencies were 0.52 and 0.48 respectively. The distribution of ACE genotypes/alleles and also the allele frequencies in males and females in both the cases and controls groups are shown in the tables 1-3. Statistical analysis showed that the studied groups (female+male patients group: χ2 = 0.0003< 3.84, P-value = 0.999> 0.05 and female+male controls group: χ2 = 1.8802<

Table III. The frequency of ACE DD, DI, II genotypes, and D, I alleles among asthmatic males and healthy males

	Patients F(F%)	Controls F(F%)	OR (95% C.I.)	χ2	P-Value
DD	8(28.571)	31(42.466)	0.542(0.211-1.39)	1.648	0.199
DI	14(50)	27(36.986)	1.704(0.707-4.108)	1.421	0.233
II	6(21.429)	15(20.548)	1.055(0.363-3.063)	0.01	0.922
D	30(53.571)	89(60.959)	0.739(0.397-1.376)	0.913	0.339
I	26(46.429)	57(39.041)	1.353(0.727-2.52)	0.913	0.339

F: Frequency; Hardy–Weinberg equilibrium analysis: Male patients group: χ2 = 0.0007< 3.84, P-value = 0.999> 0.05, n=28, p=0.54, q=0.46; Male controls group: χ2 = 3.6284< 3.84, P-value = 0.162> 0.05, n=73, p=0.6, q=0.4

3.84, P-value = 0.390> 0.05) were in Hardy–Weinberg equilibrium. Also, males (male patients group: $\chi 2$ = 0.0007< 3.84, P-value = 0.999> 0.05, male controls group: $\chi 2$ = 3.6284< 3.84, P-value = 0.162> 0.05) and females (female patients group: $\chi 2$ = 2.549E-05< 3.84, P-value = 0.999> 0.05, female controls group: $\chi 2$ = 0.0965< 3.84, P-value = 0.952> 0.05) showed an excellent fitness to Hardy–Weinberg equilibrium. Our findings implied that the I/D ratio was 0.61/0.39 in all controls and 0.6/0.4 in male or female controls. Significant differences were not found in the ACE genotype or allele frequencies between studied groups regarding all cases versus all controls, female cases versus female controls, male cases versus male controls. OR (95% C.I.), $\chi 2$ and P-Value were shown amongst the mentioned groups in Tables I-III. We didn't find any association between asthma and the ACE gene polymorphism in the present study. Figure 1 shows the status of PCR products in 12 samples. The frequency of ACE DD, DI, II genotypes and D, I alleles among cases and healthy controls are presented in the figures 2-4 regarding males+females, females and males groups.

Discussion

ACE as membrane-bound ectoenzyme is presented on epithelial cells such as microvilli, brush border of placenta, kidney, intestine, choroid plexus, neuroepithelial cells such as subfornical organ, pallidonigral dendrites, median eminence, male genital tract such as testes, prostate, epididymides, endothelial cells of vascular system [25-28], smooth muscle cells and adventitial layers of blood vessels [29, 30]. Soluble ACE is widely distributed in body fluids such as seminal plasma, blood, urine, lung edema, amniotic fluid, cerebrospinal fluid, lymph, and on the luminal surface of endothelial cells of pulmonary system [28, 32-36]. ACE as a peptidyldipeptide hydrolase has an important role in the rennin-angiotemnsin system. ACE converts angiotensin I into the potent vasopressor angiotensin II [26]. Angiotensin II is known as vasoconstrictor and aldosterone activator which leads to increased pressure of blood [26]. Consequently, inhibitors of ACE are helpful in management of human disease such as hypertension, heart failure, and coronary disease [37-41]. Additionally, ACE inactivates the vasodilator bradikinin as well as tachykinins [42]. In human airways, the neuropeptides such as substance P and neurokinin A are defined as tachykinins and localized in sensory airway nerves [42, 43]. Chronic airway inflammation results in asthma via airway hyper-responsiveness/remodeling [44]. The pathophysiology of asthma is triggered by different inflammatory process. Bradikinin and tachykinins participate in the pathogenesis of asthma and neurogenic inflammation [43, 44].

Bradykinin causes vascular permeability and mucosal edema, as well as induces the production of mucus by neuropeptides [3]. Rigat et al (1990) reported that there are two alleles of insertion (I) and deletion (D) and three genotypes of I/I, I/D and D/D, regarding the presence or absence of a 287 bp element in intron [16] of ACE gene [7]. Findings of Tiret et al (1992) implied that presence of the ACE D allele leads to increased serum level of ACE and tissue specific activity of ACE enzyme [9]. The present investigation evaluated the association between the ACE gene insertin/delletion mutations and the susceptibility to the asthma. To the best of our knowledge, the current study is the first in its own type for determination of the ACE insertion/deletion mutations in asthmatic Iranian Azeri-Turkish patients which conducted from 2008 through 2010. At first, we determined the frequencies of ACE D and I alleles and ACE DD, DI, and II genotypes in asthmatic patients and controls and then the frequency of the ACE genotypes between asthmatic patients and controls were compared. In our study, the I/D ratio was 0.39/0.61 in controls which means that the D allele is dominant. This finding is similar to that of the studies in Caucasian such as Rigat et al (0.411/0.59) [7], Lindpaintner et al (0.4449/0.5551) [45] and Benessiano et al (0.43/0.57) [48]; but, inconsistence with the findings from Japan where showed that the I allele is dominant, Furuya et al (0.66/0.34) [46], Tomita et al (0.654/0.346) [47], and Tomita 0.6131/0.387 [54]. We didn't find any association between asthma and the ACE deletion in the present study. The results of some studies suggested that ACE DD genotype played a significant role in the susceptibility to asthma [48-53]. Some others are consistence with our results [54, 55]. Our study has a limitation due to the sample size, and it would be necessary to replicate with a large sample size of asthmatic patients. Other studies concerning gene-gene and gene-environmental interactions could help to explain different aspects of asthma predisposition and would be helpful to find possible association between asthma and candidate genes and gene variations. We have concluded that deletion allele of the ACE gene is not a risk factor for the asthma predisposition.

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