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# Association of *Angiotensin-converting Enzyme* Gene Polymorphism with Susceptibility to Asthma Disease in Western Iran

**Ali Moini[1], Mohammad Reza Khirehgesh[2], Amir Sarkhosh[3], Atena Godarzi[3], Asad Vaisi-Raygani[4], and Amir Kiani[5]**

*[1] Department of Internal Medicine, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*[2] Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*[3] Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*[4] Fertility and Infertility Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*[5] Regenerative Medicine Research Center (RMRC), Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran*

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## ABSTRACT

Asthma is a complex disease caused by a combination of multiple genetic and environmental factors. Angiotensin-converting enzyme (ACE) is involved in the pathogenesis of asthma by inactivating bradykinin, substance P, and neurokinin A. It has been shown that the level of ACE variation in serum is associated with an insertion-deletion (I/D) polymorphism. So, this study aimed to investigate the association of these polymorphisms with asthma in western Iran.

In this case-control study, 111 asthmatic patients as a case group and 80 healthy subjects as a control group were evaluated. The ACE gene polymorphism was determined by the PCR method. The relationship between genotypes done by the $\chi 2$ test and the relative risk of disease with genetic polymorphism (Odds Ratio) was performed using logistic regression.

The frequency of I/D genotypes (included in II, ID, and DD) between patient and control groups had no significant difference. In addition, none of the genotypes in the patient and control groups show any significant differences between men and women. However, the frequency of ID and DD genotypes was considerably different between the male patient groups (over and under 40 years old). Hence, these genotypes are suggested to be a risk factor for asthma.

The results of our study indicate that *ACE* gene polymorphism is not significantly associated with asthma in the west of Iran.

**Keywords:** Asthma; Genotype; Single nucleotide polymorphism

## INTRODUCTION

Asthma is a chronic respiratory disease identified by the increased response of the tracheobronchial tree to a variety of stimuli and is associated with symptoms such as shortness of breath, cough, chest tightness, and

**Corresponding Author:** Amir Kiani, PhD;
Regenerative Medicine Research Center (RMRC), Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran. Tel: (+98 918) 831 6929, Fax: (+98 83) 3427 6493, E-mail: amir1kiani@yahoo.com, akiani@kums.ac.ir

decreases in expiratory flow. The main features of asthma are airways inflammation, airways hyperresponsiveness, and mucous hypersecretion.[1-3] The disease is caused by gene-environment interactions and is prevalent in both children and adults. However, the nature of its genetic essence is not clearly defined.[4,5] Forecasts show that the number of people with asthma will reach more than 400 million by 2025.[6] The prevalence of asthma varies in different countries, and it is almost 8.9% in Iranian people.[7]

Among the genetic factors associated with asthma, more than 40 genes, the *angiotensin-converting enzyme (ACE)* gene plays an important role.[8-10] This enzyme, dipeptidyl peptidase, locates mainly in the capillary blood vessels of the lungs and plays a central role in the conversion of angiotensin I (Ang I) to angiotensin II (Ang II), ACE is also involved in inactivating some inflammatory peptides such as substance P and bradykinin.[11-13] Ang II is the main effector peptide on the renin-angiotensin system. This octapeptide affects the vasoconstriction and homeostasis of the cardiovascular system. Also, illustrate the proliferative, pro-inflammatory, and pro-fibrotic performance.[14,15]

One of the genetic risk factors for respiratory disorders is the Insertion/Deletion (I/D) polymorphisms in the *ACE* gene. This gene, 21-kilobases (kb), is located on chromosome 17q23 and contains 25 introns and 26 exons.[10,16] The common *ACE* polymorphism is an I/D of a 287 bp DNA fragment inside intron 16. Plasma ACE levels are highest in individuals with the homozygous D allele (DD), followed by heterozygotes (ID) and homozygous I allele (II).[17] The correlation of ACE I/D polymorphism with asthma susceptibility has been examined in several studies. This study aimed to determine the relationship between polymorphism and asthma risk among the population of western Iran.

## MATERIALS AND METHODS

### Inclusion and Exclusion Criteria

All volunteer subjects must be able to provide informed, written consent for participation in this study. Inclusion criteria included age 20 to 70 years old, a history of at least two years of asthma with a forced expiratory volume in one second (FEV1) less than 70%, and the FEV1 to forced vital capacity (FVC) ratio be less than 70%. Exclusion criteria were cardiovascular disease, pneumonia, pneumothorax, or other systemic and pulmonary diseases other than bronchial asthma, severe respiratory failure with cyanosis or confusion, pregnant women, and smokers.

### Population and Procedures

In this case-control study, 111 clinically diagnosed cases with asthma disease, match based on age and gender, and 80 healthy volunteers were included. Patients enrolled in this study were diagnosed by a pulmonologist conforming to clinical pulmonary symptoms such as hearing the wheezing sound in the lungs. Healthy participants showed no symptoms of a respiratory disorder, allergy, asthma, or inflammatory diseases. Peripheral blood (1 mL) of each individual was collected in an ethylene diamine tetraacetic acid (EDTA) vial. Then genomic DNA was isolated by the phenol-chloroform method.[18] Polymerase chain reaction (PCR) recognition of the I/D polymorphism of the *ACE* gene was done by specific forward and reverse primers. The sequences of forwarding and reverse primers used were
5′CTGGAGACCACTCCCATCCTTTCT3′
and 5′GATGTGGCCATCACATTCGTCAGAT3′, respectively. The PCR amplification consisted of an initial denaturation for 5 min at 95ºC, followed by 35 cycles of 1 min denaturation at 95ºC, annealing for 1 min at 58ºC, and extension for 1 min at 72ºC. The final extension step was performed at 72ºC for 10 min. The PCR product was analyzed by electrophoresis on a 1.5% agarose gel for 40 min. The genotypes of the individuals were determined as follows: Individuals with genotype DD having a band of 190 bp, ID two band of 490 bp and 190 bp, and individuals with II genotype, have a band in region 490 bp (Figure 1 A). To prevent the loss of the heterozygous genotype (ID), in all cases the first PCR that showed the DD genotype is subjected to the second PCR with specific ACE-I/D primers. The sequences of forwarding and reverse primers used were
5′TGGGACCACAGCGCCCGCCACTAC3′
and 5′TCGCCAGCCCTCCCATGCCCATAA3, respectively. PCR cycling conditions were performed with an initial denaturation step of 5minat 95°C, followed by 35 cycles of denaturation at 95 C for 1min, annealing at 65.7°C for 1min, and extension at 72°C for 1min. Observation of the 335 bp PCR product confirms the presence of the I allele, although there is no band for the DD genotype (Figure 1 B). This study was approved by the Medical Ethics Committee of Kermanshah University of Medical Sciences (Code

IR.KUMS.MED.REC.1400.037), and an informed consent letter was signed by participants.

**Statistical Analysis**

Chi-square ($\chi 2$) test was used to compare the distribution of different qualitative data between the study groups. The relationship between genotypes and disease was examined as the Odds ratio (OR) with a 95% confidence interval (95% CI). Statistically, the value of $p<0.05$ is considered significant. Logistic regression analysis was performed to assess the association between gene polymorphism and asthma risk with SPSS 16 statistical package.

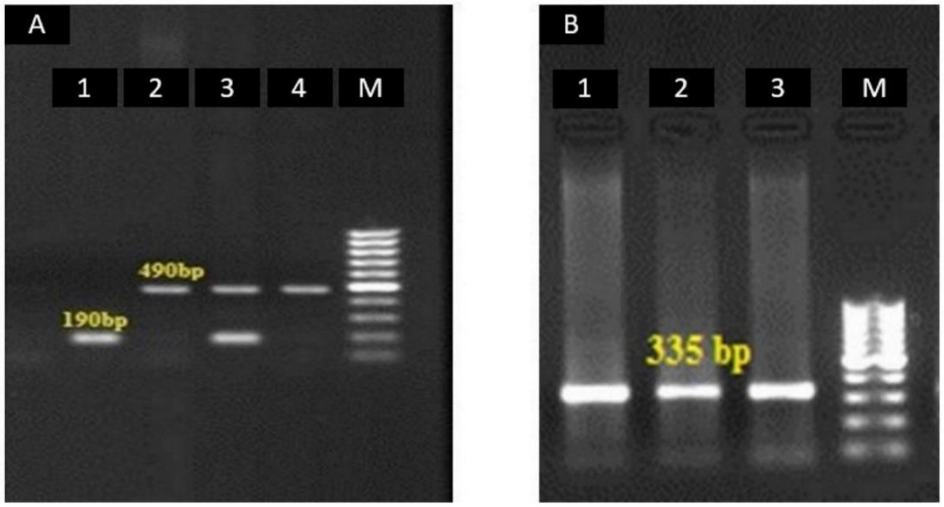


**Figure 1. (A) Electrophoresis result of first PCR productions. Lane1, DD genotype; Lane2 and 4, II genotype; Lane3, ID genotype; M, Molecular weight marker (100bp). (B) Electrophoresis result of second PCR productions with the use of insertion-specific primers. Lane 1-3, I allele (335 bp); M, Molecular weight marker (100bp)**

## RESULTS

**Demography Characteristics and Frequency Distribution of *ACE* Gene Polymorphism in Patient and Control Groups**

Demographic and clinical data in the asthmatic patients and control are shown in Table 1. Statistical analysis indicated that the frequency of I/D genotypes did not significantly show a difference between patient and control groups ($p$=0.275). The total frequency distribution of ACE-I/D alleles showed no significant association between patients and control groups ($p$=0.154). Although the D allele increased the risk of disease by 1.63 times, it was not significantly OR=1.63 ($p$=0.15) (Table 2). The frequency of DD as compared with the II genotype in the patient group (over 40 and under 40) did not demonstrate a significant difference ($p$=0.278). Although DD genotype increased the relative risk of an outbreak of the disease by 1.39 times, this difference was not significant OR=1.39 ($p$=0.28). Also, the frequency of ID as compared with the DD genotype did not show a significant difference ($p$=0.71).

The frequency of DD as compared with the II genotype between the patient and control groups did not demonstrate a significant difference ($p$=0.111). Also, there was no meaningful relationship between the increase of DD genotype and outbreak of asthma compared with II genotype; however, DD genotype increased by 1.50 times the risk of the outbreak of asthma, this difference was not significant OR=1.50 ($p$=0.11). The frequency of ID as compared with the DD genotype did not demonstrate a significant difference ($p$=0.386). Despite the increase of ID genotype made increased the risk of asthma by 1.3 times, it was not meaningful OR=1.32 ($p$=0.38).

The frequency of ID as compared with the II genotype in the patient group did not determine a significant difference ($p$=0.271). Although ID genotype increased the relative risk of an outbreak of the disease by 1.70 times, this difference was not significant OR=2.26 ($p$=0.27).

**Table 1. Demographic and clinical data in the asthmatic patients and control**

| Age | | Asthma patients (n=111) | |
|---|---|---|---|
| | | Male | Female |
| | <40 | 22 (56.4%) | 48 (66.7%) |
| | >40 | 17 (43.6%) | 24 (33.3%) |
| Sickness severity | Mild | 11 (33.3%) | 22 (66.7%) |
| | Moderate | 19 (33.3%) | 38 (66.7%) |
| Drug | Theophylline+ Salbutamol+Beclometasone | 27 (73%) | 57 (77%) |
| | Theophylline+Beclometasone | 1 (2.7%) | 0 (0%) |
| | Theophylline+Salbutamol | 9 (24.3%) | 17 (23%) |
| Sex (Male/Female) | | Asthma patients | Control |
| | | 37 (33.3%) / 74 (66.7%) | 47 (58.8%) / 33 (41.3%) |

**Table 2. Distribution of ACE-I/D polymorphisms and allelic frequencies in asthmatic patients and control groups**

| ACE-I/D polymorphisms | Control (n= %) | Patient (n= %) | OR (95%CI, *p*) |
|---|---|---|---|
| II | 7 (8.8%) | 17 (15.3%) | 1.44 (0.910-2.305, 0.118) |
| ID | 45 (56.2%) | 64 (57.7%) | |
| DD | 28 (35%) | 30 (27%) | |
| | *p*=0.275 | | |
| II | 7 (8.8%) | 17 (15.3%) | |
| ID+DD | 73 (91.2%) | 94 (84.7%) | 1.88 (0.743–4.789, 0.182) |
| | *p*=0.177 | | |
| Alleles | | | |
| I | 59 (36.9%) | 98 (44.1%) | |
| D | 101 (63.1%) | 124 (55.9%) | 1.63 (0.94-1.43, 1, 0.15) |
| | *p*=0.154 | | |

CI, confidence interval; OR, odds ratio

**Frequency Distribution of *ACE* Gene Genotypes in the Patient Group Based on Age of Onset**

As shown in Table 3, the frequency of DD as compared with the II genotype between the over and under 40 years old male patients demonstrated a significant difference (*p*=0.049). DD genotype increased the relative risk of an outbreak of the disease by 3.26 times, more inclined toward the significant difference OR=3.26 (*p*=0.07). Also, the frequency of ID as compared with the DD genotype displayed a significant difference (*p*=0.033). ID genotype significantly increased the relative risk of asthma outbreak by 8.72 times, OR=8.72 (*p*=0.05). The frequency of ID as compared with the DD genotype between the over and under 40 years old female patients displayed a significant difference (*p*=0.038). The frequency of ID as compared with the II genotype did not determine a significant difference (*p*=0.075). ID genotype made increased the relative risk of the outbreak of asthma by 3.55 times, more inclined toward the significant difference OR=3.55 (*p*=0.085). The frequency distribution ratio in the patient group

showed no significant relationship between the age of disease outbreak and the frequency of alleles I and D. Measures of disease frequency in over and under 40 years old male patients, showed no significant difference of outbreak of disease between I and D alleles; however, it tended to be meaningful ($p$=0.077). Genotype distribution of ACE-I/D polymorphisms within the asthmatic patients is shown in Figure 2.

**Table 3. Frequency distribution of ACE-I / D polymorphisms in asthmatic patients based on age (over and under 40) and gender, and the relative risk of the outbreak of the diseases.**

| | Patients | | | |
|---|---|---|---|---|
| **ACE-I/D** | **Male** | | **Female** | |
| **polymorphisms** | **<40** | **>40** | **<40** | **>40** |
| DD vs. II | 8 (72.7%) | 1 (20%) | 11 (68.8%) | 10 (66.7%) |
| | 3 (27.3%) | 4 (80%) | 5 (31.3%) | 5 (33.3%) |
| | ($p$=0.049) OR (95%CI, pvalue): 3.26 (0.90-11.75, $p$=0.07) | | ($p$=0.90) OR (95%CI, pvalue): 1.10 (0.244 - 4.963, $p$=0.90) | |
| ID vs. DD | 11 (57.9%) | 12 (92.3%) | 32 (74.4%) | 9 (47.4%) |
| | 8 (42.1%) | 1 (7.7%) | 11 (25.6%) | 10 (52.6%) |
| | ($p$=0.033) OR=8.72 (0.93-81.75, $p$=0.05) | | ($p$=0.038) OR=0.30 (0.10-0.95, $p$=0.04) | |
| ID vs. II | 11 (78.6%) | 12 (75%) | 32 (86.5%) | 9 (64.3%) |
| | 3 (21.4%) | 4 (25%) | 5 (13.5%) | 5 (35.7%) |
| | ($p$=0.818) OR=1.22 (0.222-6.730, $p$=0.81) | | ($p$=0.075) OR=3.55 (0.84-15.05, $p$=0.085) | |

CI, confidence interval; OR, odds ratio.

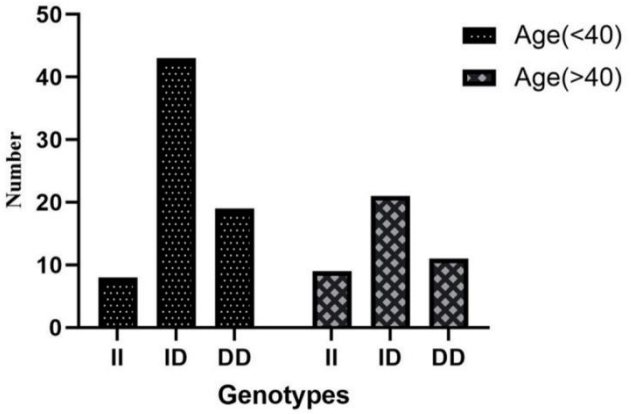


**Figure 2. Genotype distribution of ACE-I/D polymorphisms within the asthmatic patients**


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## DISCUSSION

Since the *ACE* gene polymorphism is associated with ACE plasma levels, it appears that the DD and II genotypes are associated with the highest and lowest plasma ACE levels, respectively.[19,20] To date, several studies have shown conflicting results regarding the association between the ACE polymorphism and asthma risk. This study aimed to investigate this relationship in the western Iranian population. In this study, we measured the frequency of *ACE* gene polymorphisms among 111 Iranian asthmatics and 80 healthy volunteers. Although the frequency of DD versus II genotype and ID versus DD genotype between the over 40 and under 40 years old male patients demonstrated a significant difference ($p<0.05$); however, we found no significant differences between ACE I/D polymorphisms among other patients and control groups. The following are the results of several studies. In 2012, a meta-analysis by Ding et al, showed that an increase in the frequency DD as compared with the II genotype was associated with an increased risk of asthma.[21] In 2012, the results of a meta-analysis study conducted by Zhang et al, showed that the risk of asthma outbreak in individuals with DD genotype increases compared to genotypes II and ID. Also, it was shown the increased risk of asthma outbreaks is significantly associated with the DD genotype in the Asian (China) population.[22] In 1999, another study was done by Chagani et al, showed I/D polymorphism in the *ACE* gene is not related to asthma onset.[23] In 2012, in a study accomplished by El-Shafei et al, on the frequency of genotypes, there was no significant difference in asthma onset in patients and control groups.[24] In 2009,a study in Turkish asthmatic patients conducted by Eryüksel et al, it was showed that the frequency of DD genotype in the patient group was significantly higher than the control group, but the ID genotype did not show a significant association. Overall, the results showed that DD genotype functions as a risk factor for asthma but has no role in exacerbating the disease.[25] Another study by Tomita et a. showed that there was no significant difference in serum ACE levels and genotype distribution in asthmatic patients.[26] In 2004, the results of a study by Orhan et al showed that the DD genotype in the *ACE* gene in asthma patients was higher than the control group and the frequency of D and I alleles was not significantly different between the control and patient groups.[27] In 1999, a study of the Japanese population by H Nakahama did not show a significant difference between *ACE* gene alleles in patient and control groups, and the results indicated that I/D polymorphisms in the *ACE* gene could not be considered as a risk factor for bronchial asthma.[28] Also, in 2013, Cortez et al, Presented a similar result.[29] In 2000, Gao et al, conducted a study on the association of I/D polymorphisms in the *ACE* gene with the risk of asthma in families. The results showed that the frequency of DD genotype is higher in patients with asthma and can be considered as an effective factor in obstructive pulmonary disease and the onset of asthma.[30] In 2008, the result of a study by Sekerli et al, showed that the frequency distribution of *ACE I/D* gene polymorphism is significantly different in the Greek and other European populations. This study shows that the frequency of genotypes can vary significantly between different populations.[31]

In conclusion, we failed to show an association between I/D polymorphisms in the *ACE* gene with susceptibility to asthma disease. In compaction with most previous studies, the main limitation of this study was the smaller number of participants in both patient and control groups. Also, our results are interpretable based on ethnic and genetic traits, and since different races affect ACE I/D polymorphisms and the outbreak of asthma, it is necessary to conduct this study in other racial groups with more samples.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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