

# Angiotensin-Converting Enzyme Insertion/Deletion Gene Polymorphism in General Population of West Azarbaijan, Iran

Isa Abdi-rad,<sup>1</sup> Morteza Bagheri<sup>2</sup>

<sup>1</sup>Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran

<sup>2</sup>Department of Genetics, Urmia University of Medical Sciences, Urmia, Iran

**Keywords.** angiotensin-converting enzyme, genetic polymorphism, gene frequency, alleles, Iran

**Introduction.** Angiotensin-converting enzyme (*ACE*) gene polymorphisms have been studied in different ethnic populations. We assessed the *ACE* gene insertion/deletion alleles frequencies and genotype distributions in a group of individuals from among the general population of west Azarbaijan, Iran, in order to determine the prevalence of deletion/deletion (D/D), deletion/insertion (D/I) and insertion/insertion (I/I) genotypes.

**Materials and Methods.** In a cross-sectional study, 167 healthy individuals were enrolled in a randomized fashion. They were selected from the general population of west Azerbaijan, Iran. Polymerase chain reaction was carried out to determine the *ACE* D/D, D/I and I/I genotypes.

**Results.** The *ACE* alleles were D and I in 59.7% and 40.3% of the women, 59.3% and 40.7% of the men and 59.6% and 40.4% of all of the participants, respectively. The D/D, D/I, and I/I genotypes were observed in 22 (40.7%), 20 (37%), and 12 (22.2%) men, 41 (36.3%), 53 (46.9%), and 19 (16.8%) women, and 63 (37.7%), 73 (43.7%), and 31 (18.6%) participants, respectively. Statistical analysis showed that differences in *ACE* D and I alleles and genotype frequencies were not significant between the men and the women.

**Conclusions.** In this study on the general population of west Azarbaijan, the frequencies of the *ACE* D and I alleles were 59.6% and 40.4%. The *ACE* D/D, D/I and I/I genotypes were seen in 37.7%, 43.7%, and 18.6% of the studied population, respectively.

IJKD 2011;5:86-92  
www.ijkd.org

## INTRODUCTION

Identification of specific individual genetic backgrounds has a critical role in understanding of the genetic variations among a population and in determining the susceptibility to different human diseases. These variations include several types of polymorphisms, the most frequently seen of which being single nucleotide polymorphisms (SNPs). They are located in the regulatory elements of several important gene sequences. Each gene variation has a specific impact on the natural selection and predisposition to different diseases. Most of the human diseases are complex and

multifactorial. It has been demonstrated that several genes' variations have been associated with the human diseases.<sup>1-3</sup>

Recently, a large body of studies has been focused on angiotensin-converting enzyme (*ACE*) gene variations in human diseases.<sup>4</sup> Angiotensin-converting enzyme, as a circulating and membrane-bound enzyme, has an important role in the conversion of angiotensin I to angiotensin II and degradation of bradykinin, which mediates a wide range of cellular functions in different tissues.<sup>5-7</sup> It has been shown that plasma level of *ACE* varies significantly in different populations, but it is

similar between the members of a family.<sup>6-8</sup> It has been demonstrated that the production level of ACE is under the control of some gene variations.<sup>9,10</sup> The *ACE* gene is mapped on chromosome 17q23. It contains 25 introns and 26 exons and shows a polymorphism which is characterized by the presence or absence of deletion of a 287-bp element within intron 16.<sup>11-13</sup> Presence or absence of a 287-bp element in the *ACE* gene leads to the insertion/deletion (I/D) polymorphisms, and the D/D, I/D, and I/I genotypes.<sup>10,14,15</sup>

The D allele of the *ACE* gene results in high plasma levels of ACE.<sup>16</sup> It has been reported that the D allele or the D/D genotype of the *ACE* gene predisposes individuals to several human diseases, including Alzheimer disease, diabetes mellitus, polycystic kidney disease, hypertension, coronary artery disease, and pregnancy loss.<sup>17-25</sup> Other studies reported no association between the *ACE* gene polymorphisms and various disease conditions.<sup>26-28</sup> On the other hand, the D/I and I/I genotypes result in intermediate and low plasma levels of ACE, respectively.<sup>10</sup> Frequencies of the D and I alleles of the *ACE* gene and its genotype distribution in different populations have been reported in several studies. To our knowledge, the allele's frequencies and genotypes distribution of the *ACE* gene has not been investigated in the general population of west Azarbaijan of Iran. We aimed to determine the allele's frequencies and genotypes distribution of the *ACE* gene in the general population of west Azarbaijan, Iran.

## MATERIALS AND METHODS

### Studied Population

This study was approved by the ethical committee of Urmia University of Medical Sciences. In a cross-sectional study, 167 healthy individuals among volunteers were enrolled by the randomization method. All participants were from West Azarbaijan, Iran. They were selected by genetic counseling sessions, which were taken place in the genetic department of Urmia University. Randomization was performed by a simple consecutive method after excluding individuals with any accompanying disorders in physical examinations, and also, by considering familial history and laboratory tests.

### Molecular Procedures

After obtaining written consent from all

the participants of this study, 3 mL to 5 mL of peripheral blood was drawn and poured in ethylenediaminetetraacetic acid (EDTA)-containing tubes and stored. The DNA was isolated by using a manual method, same as described previously by Miller and colleagues with some modifications.<sup>29</sup> The obtained buffy coat from the stored peripheral blood samples were resuspended in 15-mL polypropylene tubes with 4 mL of lysis buffer (10 mM Tris-HCl, 400 mM sodium chloride, and 2mM Na<sub>2</sub>EDTA, pH 8.2). The samples were digested for 24 to 48 hours at 37°C with 250  $\mu$ L of 10% sodium dodecyl sulfate and 100  $\mu$ L of proteinase K enzyme. Then, 1800  $\mu$ L of saturated sodium chloride (6 M) was added to the samples and was mixed slowly for 10 seconds. This step was followed by centrifugation at 3000 rpm for 30 minutes. The pellet was precipitated at the bottom of the centrifugation tubes. The supernatant was transferred into a new 15-mL polypropylene tube. Approximately, 2 volumes of cold absolute ethanol (96% to 100%) were added to the supernatant solution.

To obtain DNA precipitation, the tubes should be mixed by inversion several times. The DNA particles were transferred to a clean 1.5-mL microcentrifuge tube. The DNA pellet was removed from the solution by centrifuging at 14 000 rpm for 2 minutes. The 1.5-mL microcentrifuge tube containing DNA pellet was kept at 56°C for 20 minutes to remain dry. The DNA pellets were dissolved in a 50- $\mu$ L to 100- $\mu$ L Tris-EDTA buffer (10 mM Tris-HCl, 0.2 mM Na<sub>2</sub>EDTA, pH 7.5). The DNA purity was examined via evaluating the A<sub>260</sub>/A<sub>280</sub> ratios of 1.8 to 2.0. Polymerase chain reaction (PCR) was carried out to determine the *ACE* D/D, I/D, and I/I genotypes. Amplification of the PCR products, which is a 490-bp fragment for the I allele and a 190-bp fragment for the D allele, was carried out using the following:

Forward primers:

5'-CTGGAGACCACTCCCATCCTTCT-3'

Reverse Primer:

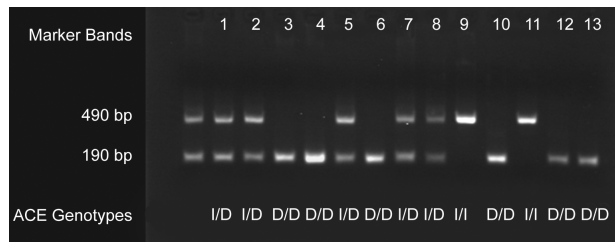
5'-GATGTGGCCATCACATTCGTCAGAT-3'

The PCR profile consisted of 35 cycles (denaturized at 94°C in 1 minute, annealing at 60°C in 1 minute, and extension at 72°C for 1 minute) was used.<sup>12</sup> Two-percent agar gel with ethidium bromide was used for electrophoresis of the amplified fragments. Presence or absent from 490-bp and 190-bp fragments were monitored by

an ultraviolet transilluminator. Presence of both 490-bp and 190-bp amplified fragments indicates the heterozygous I/D genotype. Only the presence of a 490-bp amplified fragment and absence of a 190-bp amplified fragment indicates the homozygote I/I genotype, and the presence of a 190-bp amplified fragment without a 490-bp shows the homozygote D/D genotype.

**RESULTS**

The ACE D/I genotyping by electrophoresis in 13 samples are shown in Figure 1. The frequency of the genotypes in the studied population (54 women and 113 men) is demonstrated in Table 1. The frequency of the genotypes in the women, men, and all participants were fit to the Hardy-Weinberg equilibrium regarding the ACE D/I allele and genotype frequencies. The ACE alleles were D and I in 59.7% and 40.3% of the women, 59.3% and 40.7% of the men and 59.6% and 40.4% of all of the participants, respectively. The D/D, D/I, and I/I genotypes were observed in 22 (40.7%), 20 (37%), and 12 (22.2%) men, 41 (36.3%), 53 (46.9%), and 19 (16.8%) women, and 63 (37.7%), 73 (43.7%), and 31 (18.6%) participants, respectively. Statistical analysis showed that differences in ACE D and I alleles and genotypes frequencies were not significant between the men and the women (Table 1).



**Figure 1.** Angiotensin-converting enzyme genotyping (D/D, I/D and, I/I) by electrophoresis on 2% agar gel in 13 samples. ACE indicates angiotensin-converting enzyme; I, insertion; and D, deletion.

**DISCUSSION**

Frequencies of the D allele and D/D genotype of the ACE gene in different populations are shown in Table 2 and Figure 2. Our results are along with some published data for ACE D allele frequency from Italian, German, American, British, Turkish, Greek, Australian, African-American, French, Colombian, white European, Spanish, and Lebanese populations (Table 2).<sup>5,30-54</sup> Findings of this study

**Table 2.** Frequencies of Angiotensin-Converting Enzyme D Allele in Different Populations

Population	Allele D (%)
Thai <sup>30,31</sup>	3
Javanese-Indonesian <sup>31,32</sup>	24
Singaporean <sup>31,33</sup>	31
Japanese <sup>31,34</sup>	33
Malaysian pooled <sup>31</sup>	35
Taiwanese <sup>31,35</sup>	36
Chinese <sup>34,36</sup>	37
Korean <sup>5,37</sup>	41
Indian <sup>31</sup>	42
Asian Indians <sup>31,38</sup>	45
Italian <sup>5,25</sup>	47
Germany <sup>5,39</sup>	49
American <sup>31,40</sup>	52
British <sup>5,41</sup>	53
Turkisey <sup>42,43</sup>	53.4
Greek <sup>5,44</sup>	54
Australian <sup>31,45</sup>	54
African-American <sup>5,46</sup>	55
FranceFrench <sup>46,47</sup>	56
African- American <sup>31,48</sup>	59
Colombian <sup>5,49</sup>	59
White European <sup>5,50</sup>	59
Iranian*	60
Spanish <sup>5,51</sup>	62
Lebanese <sup>5</sup>	62
Arab <sup>31,52</sup>	73
Emirati <sup>5,53</sup>	74
Kuwaiti <sup>5,54</sup>	75

\*Present study

**Table 1.** Angiotensin-Converting Enzyme Genotypes and Alleles Among a Population of West Azabaijanes\*

ACE	All (n = 167)	Men (n = 54)	Women (n = 113)	OR (95% CI)	P
Genotype					
D/D	63 (37.7)	22 (40.7)	41 (36.3)	1.21 (0.62 to 2.35)	.58
I/D	73 (43.7)	20 (37.0)	53 (46.9)	0.67 (0.34 to 1.29)	.23
I/I	31 (18.6)	12 (22.2)	19 (16.8)	1.41 (0.63 to 3.18)	.40
Allele					
D	199 (59.6)	64 (59.3)	135 (59.7)	0.98 (0.62 to 1.56)	.93
I	135 (40.4)	44 (40.7)	91 (40.3)	1.02 (0.64 to 1.63)	.93

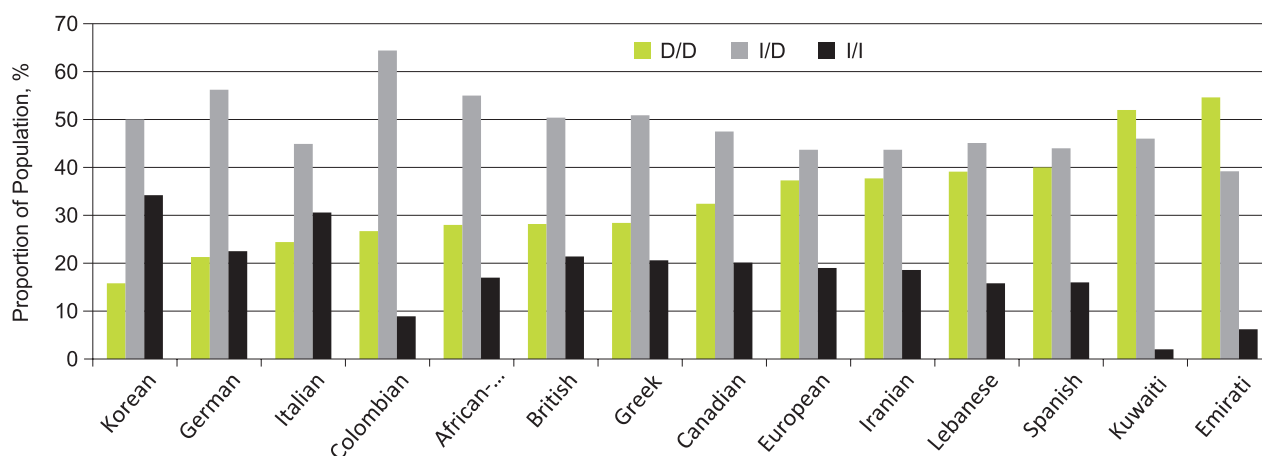
\*Values in parentheses are percents. ACE indicates angiotensin-converting enzyme; I, insertion; D, deletion; OR, odds ratio; and CI, confidence interval.

are inconsistent with some others, including the Thai, Javanese-Indonesian, Singaporean, Japanese, Malaysian, Taiwanese, Chinese, Korean, Indian, Asian Indians, Arab, Emirati, and Kuwaiti studies (Table 2).

Figure 2 shows the distribution of the ACE genotypes (D/D, D/I, and I/I) in the present study compared with the other studies.<sup>5,25,37,39,41,44,46,49-51,53-55</sup> The ACE genotypes frequencies in our study are consistent with some published data from African-American, British, Greek, Canadian, White European, Lebanese, and Spanish populations.<sup>5,41,44,46,50,51,55</sup> The frequency of ACE D/D genotype in our population is higher than that in the Korean, German, and Italian populations,<sup>5,25,37,39</sup> while it is lower than that in the Kuwaiti and Emirati populations.<sup>52,54</sup> In comparison with our study, the distribution of heterozygote ACE genotype (D/I) was similar in all groups, except for the Colombian people (64.4% versus 43.71%). Our results are similar to the

findings of two other studies on Iranian populations by Keikhaee and colleagues<sup>56</sup> and Mohammadi and coworkers<sup>57</sup> regarding the frequencies of the ACE I/D, and D/D genotypes, but not the I/I genotype (Table 3).

Different distributions of the ACE genotypes (D/D, D/I, and I/I) and alleles (D and I) in various populations are known to result in several human diseases. The most frequent variations in the DNA sequences can be used as a rich source of molecular marker with a wide range of biomedical applications such as association studies, linkage disequilibrium analysis, genotype-phenotype correlation, development of improved diagnostics and therapeutics, and studies on various drug response characteristics of individuals. Studies on the general populations can be used as a part of anthropometric and basic studies about gene-related diseases. In this regard, determining the distribution pattern of biomarkers not only in a



**Figure 2.** Distribution of different Angiotensin-converting enzyme genotypes (D/D, D/I, and I/I) in the present study and the other populations.<sup>5,25,37,39,41,44,46,49-51,53-55</sup> | indicates insertion and D, deletion.

**Table 3.** Comparison of Angiotensin-Converting Enzyme Genotypes and Alleles Frequencies Between Present Study and Other Iranian Studies\*

ACE	Keikhaee et al <sup>56</sup>	Mohammadi et al <sup>57</sup>	Present Study	Comparison With Keikhaee et al		Comparison With Mohammadi et al	
				OR (95% CI)	P	OR (95% CI)	P
<b>Genotype</b>							
D/D	41 (32.8)	31 (35.2)	63 (37.7)	0.80 (0.49 to 1.31)	.39	0.89 (0.52 to 1.53)	.69
D/I	60 (48.0)	24 (27.3)	73 (43.7)	1.18 (0.74 to 1.89)	.47	0.48 (0.27 to 0.84)	.01
I/I	24 (19.2)	33 (37.9)	31 (18.6)	1.04 (0.57 to 1.88)	.89	2.68 (1.49 to 4.80)	< .001
<b>Allele</b>							
D	142 (56.8)	86 (48.9)	199 (59.6)	0.89 (0.64 to 1.24)	.50	0.64 (0.44 to 0.93)	.02
I	108 (43.2)	90 (51.1)	135 (40.4)	1.12 (0.80 to 1.56)	.50	1.54 (1.06 to 2.22)	.02

\*Values in parentheses are percents. ACE indicates angiotensin-converting enzyme; I, insertion; D, deletion; OR, odds ratio; and CI, confidence interval.

person, but also in the different populations, is a useful method for diagnosis and prevention of diseases.

### CONCLUSIONS

In this study, 199 (59.6%) and 135 (40.4%) of the *ACE* gene alleles were D and I, respectively. The *ACE* D/D, D/I, and I/I genotypes were present in 63 (37.7%), 73 (43.7%), and 31 (18.6%) of the sample population from west Azarbaijan, respectively. To the best of our knowledge, this is the first study addresses the distribution of the *ACE* genotypes (D/D, D/I, and I/I) and alleles (D and I) in the general population of west Azarbaijan, Iran. For better understanding of the correlation between these gene polymorphisms, allele frequency, and diseases conditions, large cohort studies in different areas need to be conducted.

### ACKNOWLEDGMENTS

We would like to thank individuals and families who participated in our study. This study was supported by the research deputy of Urmia University of Medical Sciences.

### CONFLICT OF INTEREST

None declared.

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Correspondence to:  
Isa Abdi Rad MD, PhD  
Cellular and Molecular Research Center, Urmia University of  
Medical Sciences, Urmia, Iran  
Tel: +98 441 2240 166  
Fax: +98 441 2770 047  
E-mail: isaabdirad@umsu.ac.ir

Received April 2010  
Revised July 2010  
Accepted October 2010