

Relationship between major depressive disorder and ACE gene I/D polymorphism in a Turkish population

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Received: 11/9/2015 – Accepted: 4/7/2016

DOI: 10.1590/0101-60830000000077

Abstract

Background: Major depressive disorder (MDD) is a complex disease and a significant health problem that is prevalent across the world. Angiotensin-converting enzyme (ACE) has an important role in renin-angiotensin system (RAS) and converts inactive angiotensin I to a potent vasopressor and aldosterone-stimulating peptide angiotensin II. Levels of ACE in plasma vary according to the insertion/deletion (I/D) polymorphism of ACE gene. **Objective:** The aim of the current study was to examine the influence ACE gene I/D variations on the risk of MDD. **Methods:** In the present case-control study, we analyzed ACE I/D polymorphism in 346 MDD patients and 210 healthy subjects using polymerase chain reaction technique. **Results:** Comparing the two groups, no significant difference was observed with regard to either genotype distributions or allele frequencies of the I/D polymorphism of ACE gene. **Discussion:** Our findings suggest that the ACE I/D polymorphism is not associated with MDD in Turkish case-control study. Further studies are still needed.

Inanir S et al. / Arch Clin Psychiatry. 2016;43(2):27-30

Keywords: Major depressive disorder, angiotensin-converting enzyme, polymorphism.

Introduction

Major depressive disorder (MDD) is a common psychiatric disease, often associated with morbidity and mortality. Although many factors were considered as the cause of this disease, its etiology has not been ascertained yet. Studies with families, twins and fosterlings reveal that genetic factors play an important role in its etiology¹. The prevalence of disease varies according to countries and age groups². Studies suggest that 10% to 15% of the general population will be exposed to clinical depression during their life¹. Although the etiopathogenesis of the disease is not fully understood yet, it has been estimated that a genetic susceptibility could be effective in the onset of MDD³. Also, MDD is because of a complex genetic heterogeneity. Recent studies have shown that different genetic variants may contribute to the development of the MDD such as ACE gene polymorphism⁴⁻⁷.

ACE gene, localized on chromosome 17q23, undergoes a polymorphism deriving from the presence (insertion, I) or absence (deletion, D) within intron 16, of a 287 base pair ALU repeat sequence⁸. Although each allele are codominant effect to ACE levels, the homozygosity for I and D alleles have the lowest and highest levels of the enzyme, respectively. Additionally, the heterozygosity have no effect to serum ACE levels^{5,9}. ACE is also member of the renin-angiotensin system and acts a part in the conversion of angiotensin I to angiotensin II⁵. Angiotensin II is a peptide hormone which acts as a stimulator of proinflammatory cytokines and interferes with hypothalamic-pituitary-adrenal (HPA) axis activation in response to stress^{5,10}. It is mentioned from the effect of the brain renin-angiotensin system in regulation of mood¹¹. It has been stated that ACE I/D polymorphism may be associated with suicide and MDD⁵. ACE is responsible for degeneration of neurokinins that play an important role in regulation of emotions. Angiotensin II is both a potent neuropeptide and have essential roles in cognitive processes¹². In some studies, it has been shown that ACE may contribute to the pathophysiology of psychiatric diseases by interacting with central

dopamine⁷. Therefore, it is important the investigation of ACE gene polymorphisms which are affecting an active to mood and personal stress. This case control study was designed to determine whether the ACE I/D gene polymorphism is related with susceptibility to MDD in Turkish population or not.

Material and methods

Subjects

In our study, 346 patients diagnosed with MDD (74 male and 272 female) and 210 healthy volunteers (75 male and 134 females as control group) who were recruited to Tokat Gaziosmanpasa University, Department of Psychiatry were included to the study. According to Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria, the patient group comprises individuals diagnosed with major depression. Both the study group and control group were recruited from the Turkish population. Subjects included to the study were greater than 18 years old. Informed written consent was obtained from all patients and subjects before enrollment to the study, according to the ethical guidelines of the 2008 Declaration of Helsinki and the investigation was approved by the ethical, investigation and biosecurity committee of Gaziosmanpasa University Faculty of Medicine.

Genotype determination

This study was conducted in Gaziosmanpasa University, Department of Medical Biology and Genetics laboratories. Blood samples were collected from patients and controls. Genomic DNA was isolated from whole venous blood samples using a commercial DNA isolation kit (Sigma-Aldrich, Germany). In addition, after isolated, the DNA should be stored at -20C. ACE gene I/D polymorphism genotypes

were determined by polymerase chain reaction (PCR) using the primers and conditions described earlier^{8,11}. Reactions were performed with 10 pmol of each primer: sense oligo, 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', and antisense oligo, 5'-GAT GTG GCC ATC ACA TTC GTC AAG T-3'. Amplification was performed in a thermal cycler for 30 cycles with denaturation at 94°C for 40 s, annealing at 56°C for 40 s and extension at 72°C for 40 s, followed by a final extension at 72°C for 10 min. PCR products were analyzed on 2% agarose gels after staining with ethidium bromide and were visualized using a UV transilluminator. The polymorphisms detected by PCR were evident as an approximately 490-bp fragment in the presence of the insertion (I) allele and as an approximately 190-bp fragment in the absence of insertion (D) allele. In heterozygous samples, two bands (490 and 190 bp) were detected. In order to confirm the accuracy and reproducibility of this method, each PCR reaction included internal controls for each genotype. Second PCR was performed to confirm samples whose results were not clear.

Statistical analysis

All statistical analyses of data were performed using the computer software SPSS version 15.0 for Windows and OpenEpi Info software package program. Genotype distribution of the ACE gene and allele frequency between MDD patients and controls were compared by Chi-Square test. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated. Hardy-Weinberg equation was applied to the genotypes of the patients and controls. *P* values 0.05 or less were considered statistically significant.

Results

Demographic variables and baseline characteristics of patients were presented in Table 1. The mean age \pm standard deviation (SD) was 38.12 ± 12.74 years in patients and 36.47 ± 9.33 years in control group. There were 74 (21.4%) males, 272 (78.6%) females and 75 (35.7%) males, 134 (63.8%) females in patient and control groups, respectively. The observed and expected frequencies of the polymorphism in both patient and control group were in Hardy-Weinberg equilibrium. It is determined that there was no statistical significant difference in the allele and genotype frequencies of ACE I/D polymorphism in patients and control groups ($p > 0.05$, OR 1.18, 95% CI 0.91-1.51) (Table 2). Agarose gel electrophoresis patterns of ACE gene I/D polymorphisms were represented in Figure 1.

Table 1. Clinical and demographic characteristics of the patients with MDD and healthy controls

Characteristics	Control group, n (%)	Study group, n (%)
Gender, male/female	75/134 (35.7/63.8)	74/272 (21.4/78.6)
Age, mean \pm SD, years	36.47 \pm 9.33	38.12 \pm 12.74
Weight, mean \pm SD, years		70.97 \pm 10.54
BMI, mean \pm SD, years		26.51 \pm 3.78
Age of onset		33.81 \pm 12.25
Disease duration		4.75 \pm 5.12
Civil status		
Single/Married		73/165 (30.7/69.3)
Background of psychological disorders		
No/Yes		148/90 (62.2/37.8)
Family history		
No/Yes		150/88 (63.0/37.0)
Depression score		19.22 \pm 11.22
Anxiety score		22.07 \pm 14.48
Pain score		19.62 \pm 11.30

BMI: body mass index.

Table 2. Genotype and allele frequencies of ACE gene polymorphisms in MDD patient and control groups

Gene	MDD patients n = 346	Healthy controls n = 210	p	OR (CI 95%)
ACE (I/D) Genotypes				
I/I	44 (12.7%)	34 (16.19%)	0.40	
I/D	163 (47.1%)	101 (48.09%)		
D/D	139 (40.1%)	75 (35.71%)		
II: ID+DD	44:302	34:176	> 0.05	0.75 (0.46-1.23)
II-ID:DD	207:139	135:75	> 0.05	0.77 (0.53-1.10)
Alleles				
I	251 (36.2%)	169 (40.23%)	0.18	1.18 (0.91-1.51)
D	441 (63.7%)	251 (59.76%)		

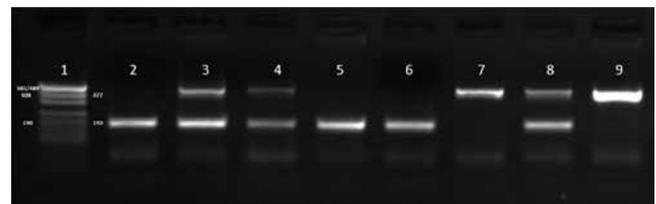


Figure 1. Agarose gel electrophoresis patterns for I/D genotypes and genetic variations of ACE gene.

Discussion

In this study we assessed the association of ACE I/D polymorphism in patients with MDD. When we consider prevalence and etiologies of MDD, we think that the genetic background of MDD should be investigated. In this study, it was not found any association between patient and control groups in Turkish MDD population in terms of ACE I/D polymorphism. ACE, membrane-bound endopeptidase, is expressed in many tissues including brain¹³. It is reported that ACE in neuroepithelial cells degrades neurotransmitters such as substance P, which has a role in depression¹⁴ and that angiotensin II interacts with dopamine in some specific parts of brain¹⁵. Angiotensin II, acting as a stimulator of proinflammatory cytokines, makes changes on the hypothalamic-pituitary-adrenal (HPA) axis activation that develops in response to stress. ACE gene variations play a significant role in the HPA axis hyperactivity in depression. It has shown that variations in the ACE gene have an important impact on the HPA axis hyperactivity found in depression¹⁶. Captopril, an ACE inhibitor, is proved to have an antidepressant effect¹⁷. The fact that ACE gene variations affect serum enzyme level causes functional changes. ACE I/D polymorphism has been the subject of association studies related to various physiopathological conditions including mood disorder. It was reported that the depressive symptoms in schizophrenics was clearly associated with ACE I allele in the Chinese population⁷ and that D allele had a protective effect for schizophrenia in the Spanish population¹⁸. The researchers were also interested in whether ACE gene polymorphism affected the responses to therapeutic agents. It was reported that high activity of ACE genotype was associated with unfavorable response to conventional therapy in schizophrenic patients¹⁹. Kucukali *et al.* indicated that there was an association between D/D genotype and bipolar disorder in patients order and their first-degree relatives²⁰. On the other hand, data collected previously suggested that ACE I/D polymorphism could possibly be a biological marker of depression²¹⁻²⁴. On the basis of these findings, the studies related to the genetic background of the MDD patients will make an important contribution to the clarification of the relationship between ACE gene and MDD.

A meta-analysis evaluating the data of several studies indicated that there was no association between schizophrenia and ACE I/D

polymorphism²⁵, and the same result was supported in a study with the Croatian population²⁶, in consistent with our study results. While it was reported that bipolar disorder was not associated with I/D polymorphism²⁷, another study suggested that it was associated with I/D genotype in the Asian race²⁸. ACE gene polymorphism was reported not to be related to panic disorder^{29,30}, although the frequency of I allele is higher in men than women³¹. Another study stated that there was an association between ACE gene polymorphism and MDD only in female patients³², as consistent to our results. Arinami *et al.* found that D/D genotype increased sensitivity to affective disorder³³. However, mood disorders that started in childhood were reported not to be associated with ACE polymorphism³⁴. Studies conducted to examine the association between MDD and ACE did not show any link with ACE I/D polymorphism^{14,35-39}. The same result was supported in Chinese population where it was reported that ACE gene polymorphism did not affect response to the treatment¹³. Also, Zill *et al.* suggest that aberrations in ACE promoter DNA methylation may be an underlying cause of MD⁴⁰. Depression were also significantly associated with an increase in cortisol secretion and, it is stated that ACE gene rs4295, rs4311, rs4343, rs4291, rs4333 and rs4351 variations influence cortisol secretion⁴¹. ACE gene A2350G polymorphism was closely associated with MDD among Iranian population¹². Stewart *et al.* reported that there was no difference between MDD and control groups with regard to allele and genotype⁴². Considering this informations, we think that ACE I/D polymorphism was not related to the development of MDD in Turkish patients. Because the number of studies performed on the association of the ACE gene with Turkish MDD is limited, we think that the present study provides an important contribution to the literature.

Conclusions

In the present study, we not found a significant association between the ACE gene I/D polymorphism and MDD in Turkish population. The results cannot explain the role of ACE I/D polymorphism in the development of MDD. This polymorphism is not a susceptibility factor to MDD in the Turkish population. Although the present study does not provide any difference between the groups, we believe that there is a need for more comprehensive studies in different populations.

Conflict of interest

The authors declare no conflict of interest.

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