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Original Article

Association between Insertion/Deletion Polymorphism in Angiotension Converting Enzyme and Susceptibility to Schizophrenia

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Abstract

Background: The activity of angiotension converting enzyme (ACE; OMIM: 106180) in different brain regions of patients with schizophrenia changed, suggesting a possible involvement of ACE in psychiatric disorders. Genetic polymorphism of insertion/deletion (I/D; dbSNP rs4646994) in the gene encoding *ACE* has been well defined.

Methods: The present case-control study was performed on 363 (268 males, 95 females) in-patients with schizophrenia diagnosis, and 363 (268 males, 95 females) healthy blood donor controls. The genotypes of I/D *ACE* polymorphism were determined using PCR method. PCR products were separated and sized by electrophoresis on a 2% agarose gel. The insertion allele (I) was detected as a 478 bp band, and the deletion allele (D) was visualized as a 191 bp band. The association between genotypes of the I/D polymorphism and the schizophrenia risk was examined by use of odds ratios (OR) and 95% of confidence intervals (CIs).

Results: Among females, the II genotype significantly decreased the risk of schizophrenia compared with the DD genotype (OR=0.18, 95%CI: 0.04-0.72, *P*=0.015). There was significant linear trend for the number of the I allele and schizophrenia risk among females (Chi^2=5.19, *P*=0.023). There was no significant association between I/D polymorphism and susceptibility to schizophrenia among male subjects. There was significant interaction between gender and the II genotype (*P*=0.031).

Conclusion: The II genotype of the I/D polymorphism has a protective effect for schizophrenia among females.

Keywords: Angiotensin converting enzyme, Iranian population, Polymorphism, Risk, Schizophrenia

Introduction

Angiotensin converting enzyme (EC 3.4.15.1; ACE; OMIM: 106180), is a circulating and membrane bound enzyme in the renin-angiotensin system and can modulate dopamine turnover in the midbrain (1, 2). The ACE has an important role in the conversion of angiotensin I to angiotensin II and degradation of bradykinin, a potent vasodilator, which mediates a wide range of cellular functions in different tissues (1). The ACE is widely distributed on the surface of endothelial and epithelial cells. The insertion/deletion (I/D) polymorphism of the *ACE* gene (dbSNP rs4646994) is defined as either the presence (insertion, I) or absence (deletion, D), of a 287 base pair insert in intron 16 of the gene (3, 4). The D allele has been associated with a higher ACE activity in the serum and tissue than the I allele (5). The D allele of the *ACE* I/D polymorphism lead to higher expression of the ACE mRNA (6).

ACE I/D polymorphism is associated with development of several multifactorial diseases such as, diabetes mellitus (7), hypertension (8), coronary artery disease (9, 10), diabetic nephropathy (7, 11), pre-eclampsia (12), and cerebral infraction (13).

In addition to the roles of ACE in cardiovascular and renal homeostasis, there is convincing evidence that angiotensin II and its metabolites play an important role in the central nervous system; it has been implicated in dementia, depression, anxiety and epilepsy (14-16). The activity of ACE in different brain regions of patients with schizophrenia changed, suggesting a possible involvement of ACE in psychiatric disorders (17-21).

Several studies investigating the association between *ACE* I/D genetic polymorphism and risk of schizophrenia have provided inconsistent results (22-27). Therefore, the association between this polymorphism and susceptibility to schizophrenia is still an open question.

In order to clarify the effect of *ACE* I/D genotype on the risk of developing schizophrenia, the present case-control study was carried out.

Materials and Methods

Subjects

This was a population-based case-control study. A detailed description of the study subjects has been reported in our previous report (28). Briefly, 363 (268 males, 95 females) schizophrenia in-patients participated in the study. The patients were chronic cases. Each face-to-face interview was conducted by three psychiatrics. Inclusion criteria for patients were being aged between 16-65 years and having chronic schizophrenia. The patients were diagnosed as chronic schizophrenia according to clinical interview using SCID-I (clinician version) to confirm and document DSM-IV diagnosis. These patients had no other psychiatric disorder; including schizoaffective disorder, major depressive, episode with psychotic features, substance misuse, bipolar disorder, or mental retardation. A total of 363 (268 males, 95 females) healthy blood donors (with no history of psychiatric disorders, cancers, psychotic disorder including schizophrenia, bipolar disorder, major depressive) frequency matched with the patients according to age and gender was also studied, as a control group. Considering the high heterogeneity of the Iranian population (29, 30), the participants of the both cases and control groups were selected from Persian Muslims (Caucasians) living in Fars province (southern Iran). Because it has been reported that polymorphisms of *ACE* are associated with several types of cancers (14, 31), the subjects of the both groups had negative history of cancers.

Written informed consent was obtained from each participant. This study was approved by the Shiraz University Ethics Committee.

The study is more than sufficiently powered with an n=726 to detect a small-medium effect in allelic frequency between the two groups. Using the GPOWER (www.psycho.uni-duesseldorf.de/aap/projects/gpower) software (version 2.0), to detect a real difference in allelic frequency with a power of 0.95, α=0.01, *df*=1, Lambda=17.84, and an effect size of 0.2; a minimum sample of 446 would be necessary.

DNA extraction and genotyping analysis

Blood samples were obtained from patient and control groups. Immediately after collection, whole blood was stored at -20 ºC until use. Genomic DNA for PCR was isolated from whole blood using the thawed blood samples, as described previously (32). Genotypic analysis for the polymorphism of *ACE* was determined by PCR assay, using specific primers of *ACE* (Forward: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'; Reverse: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3') as described previously (23). PCR products were separated and sized by electrophoresis on a 2% agarose gel and visualized under UV light after ethidium bromide staining. The insertion allele (I) was detected as a 478 bp band, and the deletion allele (D) was visualized as a 191 bp band. To test for contamination, negative controls (tubes containing the PCR mixture, without the DNA template) were incubated in every run. Any sample with ambiguous result due to low yield was retested and a random selection of 15% of all samples was repeated. No discrepancies were discovered upon replicate testing.

Statistical analysis

A Chi-square test was performed for the I/D *ACE* polymorphism to determine if the sample groups demonstrated Hardy-Weinberg equilibrium. The difference in genotypic frequencies be-

tween gender groups was determined using the Chi-square test of goodness of fit. The association between the *ACE* I/D polymorphism and the development of schizophrenia was examined by use of the odds ratios (OR) and 95% of confidence intervals (CIs). Statistical analyses were performed using the SPSS version 11.5 statistical software package (SPSS Inc, Chicago, IL, USA). A probability of $P<0.05$ was considered statistically significant. All *P* values were two-tailed.

Results

Table 1 shows the prevalence of genotypes of the I/D *ACE* polymorphism in cases and controls. The prevalence of the genotypes among controls (For males: $Chi^2=1.58$, df=1, $P=0.208$; For females $Chi^2=2.35$, df=1, $P=0.125$) were consistent with those expected from the Hardy-Weinberg equilibrium. Considering that there was no significant difference between gender groups for the genotypes, the gender groups were pooled.

Among females, the II genotype significantly decreased the risk of schizophrenia compared with the DD genotype (OR=0.18, 95% CI: 0.04-0.72, $P=0.015$). There was significant linear trend for the number of the I allele and schizophrenia risk among females ($Chi^2=5.19$, $P=0.023$). There was no significant association between genotypes (ID and II) and risk of schizophrenia among male subjects. There was significant interaction between gender and the II genotype ($P=0.031$).

Table 1: Genotypic frequency of I/D *ACE* in schizophrenia patients and controls

Polymorphism/Gender	Controls	Cases	OR	95% CI	*P*-Value
Males					
DD	105	100	1.0	-	-
ID	133	141	1.11	0.77-1.59	0.562
II	30	27	0.94	0.52-1.70	0.850
Females					
DD	30	40	1.0	-	-
ID	53	52	0.73	0.40-1.35	0.323
II	12	3	0.18	0.04-0.72	0.015
Total					
DD	135	140	1.0	-	-
ID	186	193	1.0	0.73-1.36	0.997
II	42	30	0.68	0.40-1.16	0.689

Discussion

The present study showed that although there was no significant association between the genotypes of I/D *ACE* polymorphism and susceptibility to schizophrenia among male subjects; among females, the II genotype significantly decreased the risk of schizophrenia compared with the DD genotype (Table 1). Therefore, the II genotype of the polymorphism of I/D of *ACE* has a protective effect for schizophrenia among

The I allele is associated with lower level of ACE activity (5) and lower expression of the ACE mRNA (6). On the other hand, it has been reported that in brain regions of schizophrenia patients the activity of ACE was changed (17-21). Taken together, we hypothesized that the II genotype compared with the DD genotype increased (or alternatively the II genotype decreased) the risk of schizophrenia. We found that there is significant association between the *ACE* genotypes and risk of schizophrenia among female subjects (Table 1). This finding is consistent with the previous reports from Turkey (20) and India (21).

Our finding is not consistent with other reports from Japan, Taiwan, Spain, and Jews (24-27). Different associations between the *ACE* polymorphism and risk of schizophrenia reported by investigators, at least in part, might be interpreted by the effect of interaction between gender and

the I/D polymorphism of *ACE*. We know that sex ratio of participants is not equal between the above-mentioned studies.
A meta-analysis of *ACE* I/D polymorphism associated with gastric cancer determined that the effects of the different genotypes differed between Asians and Caucasians (29). Considering that the prevalence of the D allele of *ACE* I/D polymorphism varies between populations (12, 13, 22-27), and the fact that ethnicity may influence the associations in multifactorial disease (32-35), replication of this study (with larger sample size) in other countries is recommended.

Conclusion

Our present case-control study indicating that the II genotype of the polymorphism of I/D of *ACE* has a protective effect for schizophrenia among females.

Ethical Considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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