in vivo *28*: 885-890 (2014)

The Relationship Between *ACE* Polymorphism and Panic Disorder

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Abstract. *Background: The angiotensin converting enzyme (ACE) gene, which has been found to have an insertion and deletion polymorphism (I/D), is of increasing interest in etiology and treatment of various psychiatric disorders such as panic disorder. The present study aimed to investigate the relationship between ACE polymorphism and panic disorder. Materials and Methods: In this study, 43 patients diagnosed with panic disorder at the Erenköy Mental and Neurological Diseases Training and Research Hospital, Istanbul and 41 healthy controls were enrolled. The ACE gene insertion/deletion polymorphism of exon 16 was evaluated using the polymerase chain reaction method. Results: There was a significant association between I/D genotype and panic disorder (p=0.003). However, the frequency of the I allele was found to be significantly higher in patients compared to controls (p=0.002). In addition, we recognized a significant association between I/D polymorphism and respiratory-type panic disorder in patients. Carriers of the D allele also had an increased risk of respiratory type panic disorder patients (p=0.034). Moreover, the result of Spearman correlation analysis showed an association with ACE D allele and severity of panic disorder (p<0.001). Conclusion: We suggest that the I/D polymorphism of the ACE gene is associated with panic disorder and particularly respiratory-type panic disorder in patients. The I/D polymorphism of the ACE gene seems to influence therapeutic outcome in patients suffering from panic disorder. Our results indicate that ACE D allele is associated with the severity of panic disorder.*

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Key Words: ACE (angiotensin converting enzyme), polymorphism, panic disorder.

The angiotensin converting enzyme (*ACE*) gene is located on chromosome 17q23 and has 26 exons. The gene has been described with an insertion and deletion polymorphism (I/D) of a 287 bp Alu repeat within intron 16 (1), resulting in three possible genotypes homozygous DD, and II, and heterozygous ID (2). *ACE* I/D polymorphism was found to be associated with serum and tissue ACE enzyme levels (3). Presence of the D allele was associated with increased ACE enzyme activity (4).

Panic disorder (PD) is a severe anxiety disorder characterized by sudden attacks of immediate physical discomfort and fear of dying or losing control (5). The most important feature of PD is the existence of distress that peaks and disappears very quickly (6). Repeated panic attacks are a main characteristic of PD with feelings of extreme fear accompanied by marked neurodegenerative symptoms (7). PD occurs more frequently in women than in men (8), and has been linked to respiratory abnormalities such as chronic hyperventilation associated with hypocapnia related to hypersensitivity of the respiratory control system (9).

Disordered breathing plays a very important role in the pathophysiology of PD. Studies indicate that patients with PD present respiratory disorders more frequently than controls (10). Evidence of greater variability and irregularity in the respiratory patterns of patients with PD was found but could not explain the reason for respiratory abnormalities in PD (11).

The aim of this study was to investigate the relationship between *ACE* polymorphism and PD. Another goal of the study was to recognize any associations between clinical parameters and *ACE* polymorphism in patients with PD.

Materials and Methods

Participants. The study group consisted of 43 patients with PD and 41 healthy controls. The participants, who attended to Polyclinic of Psychiatry of Erenköy Mental and Neurological Disease Training and Research Hospital, Istanbul, Turkey were diagnosed with PD with or without agoraphobia according to the Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV) diagnostic criteria (12). The healthy control (HC) group was balanced according to age, sex, and education and consisted of outpatients that did not have any psychiatric comorbid diagnosis or substance abuse; non-medical volunteers with no history of disease were selected.

Patients with PD were asked to complete the Body Sensations Questionnaire (BSQ) for identification of their RPD subtype (13). The BSQ scale contains 17 items concerning the degree to which patients fear somatic symptoms commonly associated with PAs (*e.g.*, heart palpitations, dizziness, *etc.*). Items are rated on a five-point scale ranging from 1=not frightened or worried by this sensation to 5=extremely frightened by this sensation. The total score therefore ranges from 17 to 85. Patients scoring five or more items as serious or very serious among the seven items that describe respiratory symptoms (2, 3, 4, 5, 6, 7, and 17) were characterized as having RPD. Otherwise, patients were characterized as having NRPD. Unfortunately, there is no standard procedure for this characterization.

Patients with PD were assessed for the Panic Disorder Severity Scale, which contains items that evaluate the severity of seven dimensions of PD and associated symptoms: 1) frequency of panic attacks; 2) distress during panic attacks; 3) anticipatory anxiety (worry about future panic attacks); 4) agoraphobic fear and avoidance; 5) interoceptive fear and avoidance (*i.e.*, apprehension and avoidance of bodily sensations) 6) interference in or impairment of work functioning; and 7) impairment of, or interference in social functioning (14, 15). The scale was administered by a clinician using a scripted interview.

DNA isolation. Blood samples from all participants were collected in tubes containing ethylene diamine tetra acetic acid. Genomic DNA was extracted from peripheral whole blood using iPrep PureLink gDNA Blood Kit with the iPrep Purification Instrument (In vitrogen, Life Technologies, California, USA).

ACE I/D polymorphism. The template DNA was used in a polymerase chain reaction (PCR) under stringent conditions to avoid the possibility of false positives for ACE genotyping. The reactions were carried out with 10 pmol of each primer: forward primer, 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', and reverse primer, 5'-GAT GTG GCC ATC ACT TTC GTC AGA T-3', in a final volume of 50 μl containing 0.5 μl DFS-Taq DNA Polymerase (Bioron, Ludwighafen, Germany), 1 μl 100 mM dNTP mix (PCR grade, Invitrogen, Life Technologies, California, USA) and 1.5 μl $MgCl_2$. Amplification was carried out in a DNA thermal cycler (Verite Thermal Cycler, Applied Biosystems) for 30 cycles. The PCR products were separated on a 2% agorose gel and DNA was visualized by ethidium bromide staining. The PCR product is a 490-bp fragment in the presence of the insertion (I) allele. Thus, each DNA sample revealed one of three possible patterns after electrophoresis: a 490 bp band (genotype II), a 190-bp band (genotype DD), or both 490 bp and 190 bp bands (genotype ID) (Figure 1).

Statistical analysis. Statistical analyses were performed using the *SPSS software package*, version 21.0 (IBM, London, United Kingdom). Clinical laboratory data are expressed as means±SD. Mean values were compared between patients with PD and controls by the unpaired Student's *t*-test. Differences in the distribution of *ACE* genotypes or alleles between cases and controls were tested using chi-square (χ^2) and Fischer's exact test. *ACE* I/D allelic frequencies were estimated by gene counting methods. Values of $p<0.05$ were considered statistically significant.

Results

The PD group consisted of 28 female and 15 male participants (total 43; mean age 34.36±9.94) and the HC group consisted of 17 female and 24 male participants (total 41; mean age 65.39±11.41). There were significantly more females than males in the PD group ($p=0.025$).

The frequencies of *ACE* gene polymorphism II, ID and DD among the patients in the PD group and in the control group are shown in Table I. We observed that there was an association between ID genotype and PD ($p=0.003$). The frequency of the I allele was found to be significantly higher in patients compared to controls ($\chi^2=9.665$; OR=1.510, 95% CI=1.142-1.997, $p=0.002$).

The distribution of *ACE* II, ID and DD genotypes by respiratory type subgroup are shown in Table II. Because the DD genotype was rare in this study group, we combined the ID genotype and with the DD genotype, assuming a dominant genetic model. Carriers of the D allele also had an increased risk of respiratory type subgroup ($\chi^2=5.372$; OR=1.853, 95% CI=0.878-3.910, $p=0.034$), (Table II). In addition, the statistical analyses showed that values for the respiratory type group differed significantly from non-respiratory type patients in the panic disorder group with a greater frequency of I and D alleles in the former Table II. There were no significant differences between alleles and respiratory type subgroups.

The Spearman correlation analysis showed that the *ACE* D allele was correlated with severity of PD in Table III ($p<0.001$).

Discussion

ACE is an important enzyme in the renin angiotensin cascade converting angiotensin I to the physiologically-active octapeptide, angiotensin. This system controls the fluid-electrolyte balance and systemic blood pressure (16). Besides their effects on the cardiovascular, renal, endocrine and

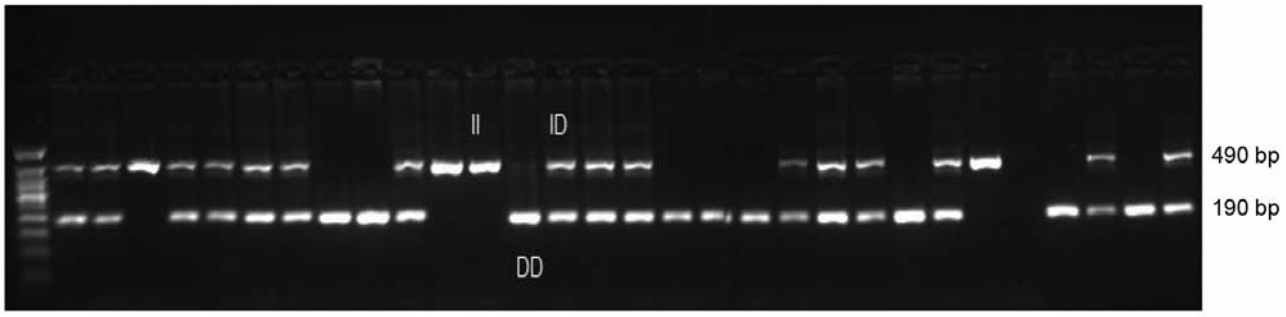

Figure 1. *Individuals homozygous for the D allele (DD genotype) were identified by the presence of a single 190 bp product. Those homozygous for the I allele (II genotype) were identified by the presence of a single 490 bp product. Heterozygous individuals (ID genotype) were identified by the presence of both 190 bp and 490 bp products.*

Table I. *The distribution of the ACE genotypes and allelic frequency in the study groups.*

ACE	Panic disorder (n)	%	Control (n)	%	Total	*p*-Value
Genotype						
II	12	27.9	10	24.4	22	*0.003
ID	26	60.5	13	31.7	39	
DD	5	11.6	18	43.9	23	
Allele						
I allele	50	58.13	33	41.24	83	*0.002
D allele	36	41.87	49	59.76	85	0.912

n: Number of individuals; values are reported as number of patients (percentage of the total group). *p-Values less than 0.05 denoted statistical significance.

Table II. *The distrubution of ACE genotypes by respiratory disorder type*

ACE	Respiratory type (n)	%	Non-Respiratory type (n)	%	p-Value
Genotype					
II	6	17.6	5	55.6	*0.034
ID+DD	28	82.4	4	44.4	
Allele					
I allele	40	32.26	14	53.84	0.052
D allele	84	67.74	12	46.16	0.277

n: Number of individuals; values are reported as number of patients (percentage of the total group). *p-Values less than 0.05 denoted statistical significance. Differences in the distribution of genotypes and alleles between groups were tested Fischer's exact test.

Table III. *The association of the ACE genotype and allele frequency with panic disorder severity*

	ACE Genotype	ACE I Allele	ACE D Allele
Panic disorder severity	–0.277	–0.177	0.253

Spearman correlation analysis showing that there is an association with ACE D allele and Panic disorder severity (p<0.001).

peripheral autocrine nervous systems, angiotensins also have central effects within the brain (17). Additionally, a neurotransmitter called substance P is also known to be degraded by *ACE* (18).

In addition to its vascular and cardiovascular effects there are many studies about ACE and its cognitive, behavioural and neurophysiological effects (19).

Studies of the renin angiotensin system have suggested the association between *ACE* deletion allele and age-related cognitive decline, depressive illness (20) the *ACE* insertion allele has been found to be related to Alzheimer's disease, the frequency of the I allele was being higher in this patient group (21). The renin-angiotensin system is thought to play an important role in regulating mood by dopamine release control (22). It was suggested that co-localization of angiotensin in the brain areas which contain dopamine synthesizing neurones is affected by *ACE* (23). There is an increasing interest in the I/D polymorphism of *ACE* regarding its possible role in the etiology and treatment of neuropsychiatric disorders such as depression. Depressive patients with *ACE* DD and ID genotypes were found to respond better to antidepressant treatment thanthose with the II genotype (24). The *ACE* gene is a candidate gene for psychiatric disorders, because of the effects on the neurotransmitters such as dopamine. It was also

hypothesized that the D allele is involved in the development of schizophrenia (25).

An association between *ACE* I/D polymorphism and PD is interesting in the light of previous findings regarding aberrations in respiratory control in patients with PD. Our finding that ID genotype and D allele variants of *ACE* are more common in patients with PD is in line with previous reports (1, 26).

The *ACE* D allele is associated with greather activity of *ACE* in serum (22), and was found to be more prevalent in a population of depressed Japanase patients (27). These data support our findings that the frequencies of *ACE* gene ID is higher in this Turkish population ($p<0.05$).

ACE is also expressed in the central nervous system, where its primary function comprises degradation of neuropeptides including substance P. Substance P, is a putative neuropeptide neurotransmitter that is highly expressed in brain regions involved in response to stress (29, 30). There might be an association between D allele prevalence and neuropeptide switch.

It was assumed that angiotensin are candidate genes in PD (1, 7, 30). Researchers suggest that angiotensin II may influence individual variation of ventilation, as well as anxiety (31). We analyzed the D allele prevalence in patients with PD by respiratory disorder type. The group with respiratory disorder PD differed significantly from non-respiratory type ($p<0.032$). We suggest that *ACE* I/D polymorphism and in particular the D allele, may be associated with respiratory symptom in patients with PD.

Several mechanisms might explain the influence of the *ACE* gene polymorphisms on drug response. Baghai *et al*. reported that the D allele of the *ACE* gene is associated with enhanced response to antidepressant drugs (29). Efficacy is probably not a result of competitive displacement of angiotensin II from its receptor, but may be due to the action of *ACE* on the post receptor signal transduction cascade (13). Angiotensin I receptor antagonists such as losartan might have antidepressant-like effects in the treatment of hypertension (30). Based on that, Khoury *et al*. determined a significant association between the presence of *ACE* inhibitor/angiotensin receptor blocker medication and decreased post traumatic stress disorder symptoms in a cross-sectional study on 505 individuals ($p<0.014$) (31). As a result of high prevalence of *ACE* I/D polymorphism in patients with PD in this Turkish population, *ACE* inhibitors or ARB could be used to enhance the treatment.

In conclusion, the results of our investigations suggest that the I/D polymorphism of the *ACE* gene is associated with PD and respiratory type PD in particular. Our results indicate that the *ACE* D allele is involved in respiratory type PD and to severity of PD. Due to the small number of participants and both positive and negative findings this study should be regarded as preliminary.

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Received February 3, 2014
Revised June 5, 2014
Accepted June 9, 2014