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ACE genetic variability and response to fluoxetine: lack of association in depressed patients

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Abstract

Evidences suggest that besides the neurotransmitters contributing to the development of depression, renin-angiotensin system (RAS) may also have a substantial role. Certain polymorphisms of RAS are associated with over activity of RAS & depression. Considering that antidepressants reduce the actions of angiotensin II, the main product of RAS, this may come into mind that genetic polymorphisms of the mentioned system may affect the outcome of therapy in depressed patients. In the present study, 100 newly diagnosed depressed patients, according to DSM-IV criteria, were treated with 20 mg of fluoxetine for 8-12 weeks. Patients were categorized into responsive and non-responsive groups according to 50% reduction in symptoms. Genotype frequencies of angiotensin-converting enzyme (ACE) gene [ACE (I/D, A-240T and A2350G)] were then determined in DNAs extracted from venous blood of the patients using polymerase chain reaction–restriction fragment length polymorphism (PCR– RFLP) and PCR. Results indicate that polymorphisms studied and their haplotypes were not associated with better response to fluoxetine. However, a strong association between age and treatment in depressed Iranian patients was observed (P=0.001). In conclusion, unlike previous reports, this study does not support the hypothesis of special genotypes of RAS contributing to a better response to antidepressants in depressed patients.

Keywords: Major depressive disorder, Angiotensin-converting enzyme, Genetic polymorphism, Fluoxetine

1. Introduction

There is a considerable heterogeneity in the biological factors that may affect the risk of developing depression, among which genetic factors are of great importance (1). According to previous reports, genetic factors account for 40–70% of the risk for developing depression (2). Genetic and epigenetic factors may influence both gene expression and protein function via different molecular systems, which may predispose an individual to this illness (3).

Several lines of evidence suggest that along with the mono aminergic system being involved in the pathogenesis of depression, renin-angiotensin system (RAS) may also play a crucial role (4-6). Angiotensin-converting enzyme (ACE), an essential enzyme in this system, not only plays a key role in RAS resulting in production of angiotensin II (Ang II), but it is also believed to be responsible for degeneration of neuro-kinins. Neuro-kinins, a family of neurotransmitters in the central nervous system (CNS), are believed to conduct a

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major role in regulation of emotions (7, 8). Furthermore, Ang II is suggested to be a potent neuropeptide interacting with other neurotransmitters involved in the pathophysiology of depression (9, 10). On the other hand, the role of inflammation in depression is undeniable (11). The hypothesis that antidepressants may act by reducing the effect of Ang II, is obtained from the clinical findings that ACE inhibitors, which prevent the production of angiotensin II, elevate mood in depressed patients (12, 13). On the other hand, evidences indicate that clinically active antidepressants reduce the effect of Ang II (14). The relationship between overactivity of RAS and depression has been repeatedly reported (4, 6). Therefore, functional polymorphisms determining the level of Ang II, may also be one of the determinants of therapy outcome with antidepressants in depressed patients. As reported in a German population, carriers of D allele of ACE gene insertion/deletion (I/D) polymorphism respond better to treatment with antidepressants than those with the II genotype (15).

To our knowledge, the association between RAS polymorphisms and response to antidepressants has not been studied in an Iranian population to date. Therefore, the aim of this study was to investigate the relationship between different variants of ACE (I/D, A-240T and A2350G) and response to fluoxetine treatment in a group of depressed Iranian patients.

2. Methods

2.1. Study population

This work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and Uniform Requirements for manuscripts submitted to biomedical journals. The study was approved by the local committee for ethics of medical experiments on human subjects of Shiraz University of Medical Sciences. The written consent was obtained from the participants prior to the interview. All patients and controls were of Caucasian origin from the same geographical area.

In total, 100 newly diagnosed, unrelated patients (male: 31, female: 69, mean age±SD: 33.4±11.3) suffering from major depressive disorder (MDD) were enrolled in this study. The MDD sufferers were diagnosed according to DSM-IV criteria (16) and by an experienced psychiatrist. Exclusion criteria were as follows: a family history of schizophrenia; bipolar disorder in first-degree relatives; a personal history of schizophrenia, manic or hypomanic episode, mood incongruent psychotic symptoms, active substance dependence or primary organic disease, history of cardiovascular diseases, such as hypertension, coronary artery disease, myocardial infarction or heart failure; current treatment with an antipsychotic or a mood stabilizer; ACE inhibitors or angiotensin receptor blockers and the presence of serious medical con-

Table 1. Primers, PCR condition and locations of ACE I/D, A-240T and A2350G polymorphisms on DNA.

Polymorphisms	Primer sequence (5′-3′)	Location	Restriction enzyme digestion	Allele	DNA fragment size (bp)	References
ACE I/D	F-CTG GAG ACC ACT CCC ATC CTT TCT	Intron 16	none	I	490	19
				D	290	
	R- GAT GTG GCC ATC ACA TTC GTC AGA T					
A-240T	F- TCG GGC TGG GAA GAT CGA GC	5′UTR	XbaI at 37 0C/24 h	A	137	21
				T	114+23	
	R- GAG AAA GGG CCT CCT CTC TCT					
A2350G	F-CTG ACG AAT GTG ATG GCC GC	Intron 17	BstUI at 60 0C/24 h	A	122	22
				G	100+22	
	R-TTG ATG AGT TCC ACG TAT TTC G					

PCR: Polymerase Chain Reaction; ACE: Angiotensin-converting enzyme; UTR: untranslated region.

dition interfering with treatment. At the time of diagnosis (week 0) and after 8-12 weeks of treatment with 20 mg fluoxetine (FLUOXETINE-ABIDI ®) the severity of depression was measured using Hamilton Rating Scale for Depression (HAM-D) (17) and by a specialized psychiatrist. The patients were then categorized in to two groups of responsive and non-responsive. The level of assessment was calculated based on 50% reduction in psychiatric symptoms after the course of treatment.

2.2. DNA extraction and genotype determination

Genomic DNAs were extracted from whole blood leukocytes using a salting out method (18). The extracted DNAs were solved in sterile distilled water and stored at 4 °C for further PCR analysis. PCR amplification/detection of ACE I/D was carried out using standard protocol (19). In order to avoid mistyping of ID as DD genotype, all DD genotypes were reconfirmed by another typing system(20). PCR amplification of A-240T and A2350G was performed using primers mentioned in Table 1(19, 21, 22). In each reaction, 100–200 ng of genomic DNA was amplified in 15 µl of 1× PCR master mix (67 mM Tris base, pH8.8, 16.6 mM(NH4)2SO4, 2 mM MgCl2, 0.1 % Tween-20, 200 lMdNTPs, 5 % glycerol, 100 lg/ml cresol red) containing 0.2–2.0 lM of each primer and 0.5 U of Taq DNA polymerase (Cinnagen Inc., Tehran, Iran). All genes were amplified under the same procedure which is of great advantage to reduce workload (23). The program under which the amplification took place was a modified form of the previous studies. After initial denaturation at 96 ºC for 2 min, PCR was performed for 5 cycles, each one comprised of denaturation at 96 ºC for 40 s, annealing at 60 ºC for 50 s and extension at 72 ºC for 30 s followed by 25 cycles of denaturation at 96 ºC for 40 s, annealing at55 ºC for 50 s and the extension at 72 ºC for 30 s. An Eppendorf gradient Mastercycler (Hamburg, Germany) PCR machine was used as the thermal cycler. PCR

Table 2. Genotype and allele frequencies in responsive and non-responsive patients.

Variables	Responsive patients (N=69)	Non-responsive patients (N=31)	*Pc*
ACE I/D			0.32
II	30(43.5%)	12(38.7%)	
ID	26(37.7%)	16(51.6%)	
DD	13(18.8%)	3 (9.7%)	
Alleles			0.76
I	86(62.3%)	40(64.5%)	
D	52(37.7%)	22(35.5%)	
ACE A-240T			0.99
AA	28(40.6%)	13(41.9%)	
AT	34(49.3%)	15(48.4%)	
TT	7(10.1%)	3(9.7%)	
Alleles			0.90
A	90(65.2%)	41(66.1%)	
T	48(34.8%)	21(33.9%)	
ACE A2350G			0.36
AA	25(36.2%)	8(25.8%)	
AG	34(49.3%)	20(64.5%)	
GG	10(14.5%)	3(9.7%)	
Alleles			0.70
A	84(60.9 %)	36(58.1%)	
G	54(39.1%)	26(41.9%)	

ACE: Angiotensin-converting enzyme; Pc: P value for Chi-square test.

products (7 µl) were digested with the specified enzymes mentioned in Table 1. Digested fragments were separated by electrophoresis on 3 % agarose (Invitrogen® UltraPure) gel after an overnight incubation (Table 1). They were then stained by ethidium bromide and visualized in a UV transilluminator. It is to mention that all of the samples were genotyped at least twice and reconfirmed.

3. Statistics

Hardy–Weinberg equilibrium (HWE) for the distributions of genotypes was estimated by Arlequin 3.1 software package. A one-way analysis of variance (ANOVA procedure) was performed to detect significant differences in HAM-D mean scores between the genotypes after -8-12 weeks of treatment. Adjusted associations were investigated by logistic regression models. Odds ratio (OR) and 95% confidence intervals (CI) were obtained. *P* value <0.05 was considered as statistically significant. SPSS 15 for Windows (SPSS inc. Chicago, IL, USA) was applied for statistical analysis.

4. Results

Role of sex and age was assessed on the level of response between non-responsive and responsive group. There was no statistically significant difference between these two groups with respect to sex. However, there was a significant difference between the two groups with regard to age (P=0.001)

Table 2 shows genotype and allele frequencies of studied patients. Regarding different polymorphisms, no significant difference in HAM-D scores was observed between the two studied groups.

Haplotype analyses in Table 3 shows the existence of seven common haplotypes in ACE gene and found no significant difference in HAM-D scores between the two groups for either haplotype distributions.

5. Discussion

To the best of our knowledge, this is the first report determining three genetic polymorphisms of ACE with respect to response to fluoxetine in a sample of depressed Iranian patients. As stated, MDD is the most common psychiatric disorder which affects almost 20% of the individuals experiencing a depressive episode during their lifetime (24). Despite lack of survey in the middle east, some analysis report that 26.9% of individual suffer from MDD in Iran (25). Taking into account that depression is a multifactorial illness, in which multiple genetic and environmental factors may be crucial, the role of genetics is not negligible (26, 27).

RAS is believed to be one of the systems contributing to both pathogenesis and treatment of MDD (4). Recently, it has been implicated that RAS has certain special roles in regulation of cerebral blood flow and cerebro protection, stress, depression and memory consolidation (28). The fact that antidepressant properties for both ACE inhibitors and losartan have been reported, strengthens the presumption of RAS involvement in drug response to antidepressants (29).

Although a study showing a strong association between ACE A2350G and depression was previously reported in a sample of Iranian patients (6), no study has been made to date to investigate a relationship between RAS polymorphisms and antidepressant treatment in an Iranian population. Genetic polymorphisms of RAS elements reported to be associated with altered ACE

Table 3. Haplotype frequencies (ACE I/D, A-240T, A2350G) in three investigated samples.

Haplotype (ACE I/D, A-240 T, A2350G)	Responsive patients (2n=138)	Non-responsive patients (2n=62)	OR; (95% CI)
D T G	34 (24.63%)	16 (25.80%)	0.48; 95% CI:0.24 - 0.94
I A A	45 (32.60%)	19 (30.64%)	0.53; 95% CI:0.28 - 0.99
D A G	18 (13.04%)	8 (12.90%)	0.55; 95% CI:0.22 - 1.33
D A A	24 (17.39%)	12 (19.35%)	0.47; 95% CI:0.22 - 0.99
I A G	3 (2.17%)	2 (3.22%)	0.38; 95% CI:0.06 - 2.34
D T A	10 (7.24%)	4 (6.45%)	0.63; 95% CI:0.19 - 2.1
I T A	4 (2.89%)	1 (1.61%)	1.04; 95% CI:0.11 - 0.94

Odds ratio; CI: Confidence interval.

activity may also influence responsiveness to antidepressant treatment in diverse manners in different ethnic groups. Since along with the D allele of ACE I/D, the G allele of ACE A2350G and T allele of ACE -240T have been reported to be associated with higher serum ACE levels (6, 30), it was hypothesized that depressed patients carrying the mentioned variants might respond differently to fluoxetine treatment. In association studies, several reports have been suggesting a relationship between some genetic polymorphisms of RAS and depression (15, 29). Moreover, a couple of studies have shown associations between ACE I/D polymorphism and a better pharmacological treatment in depressed patients. However, in the sample of depressed Iranian patients observed in this study, no association was observed between the suspected polymorphisms and response to treatment. Taking into account that studied polymorphisms are located on introns, makes these variants unlikely to be functional to influence the expression of ACE mRNA directly but linkage disequilibrium with a putative silencer DNA fragment may exist (31). As our study covers known polymorphisms in a region between intron 16 and 3′ UTR of ACE gene, existence of other functional polymorphisms in 5′ region of this gene cannot be excluded (32).

Subgroup analysis in depressed Iranian patients, showed no significant difference in response to treatment in females with regard to studied polymorphisms. Conversely, in the German population an association between D allele of ACE I/D and good response to antidepressant treatment was observed in female patients but not males (15).

Haplotype analysis in the study group showed no significant association between the studied haplotypes and level of response to treatment, which may be due to small sample size of newly diagnosed depressed patients.

However, our finding in this study was the strong association between age and response to fluoxetine. A report by Joyce *et al*., also shows that young patients receiving fluoxetine respond better to treatment comparing with patients of older age (33). This may be due to alteration in brain serotonin receptors believed to reduce with age. This finding may be of importance in understanding differential antidepressant response in various groups of patients (34).

6. Conclusion

In summary, neither ACE I/D nor any individual single nucleotide polymorphisms of the ACE gene studied were in association with better response to fluoxetine in depressed Iranian patients. However; it is suggested that in a larger sample size, more precise results may be achieved.

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Conflict of Interest

None declared.

8. References

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