Acta Neuropsychiatrica

cambridge.org/neu

Impact of ACE2 genetic variant on antidepressant efficacy of SSRIs

Negar Firouzabadi[1], Parisa Farshadfar[1], Maral Haghnegahdar[1], Ali Alavi-Shoushtari[2,3] and Vahid Ghanbarinejad[1]

[1]Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran; [2]Department of Psychiatry, UCLA-Kern, Bakersfield, CA, USA and [3]Department of Psychiatry, School of Medicine, Hafez Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

Original Article

Cite this article: Firouzabadi N, Farshadfar P, Haghnegahdar M, Alavi-Shoushtari A, and Ghanbarinejad V. (2022) Impact of ACE2 genetic variant on antidepressant efficacy of SSRIs. *Acta Neuropsychiatrica* **34**:30–36. doi: 10.1017/neu.2021.32

Received: 22 February 2021
Revised: 23 September 2021
Accepted: 23 September 2021
First published online: 4 October 2021

Key words:
major depressive disorder; genetic polymorphism; single nucleotide polymorphism; fluoxetine; sertraline

Author for correspondence:
Negar Firouzabadi,
Emails: Firouzabadi@sums.ac.ir, nfirouzabadi@yahoo.com

Abstract

Identification of a new axis of angiotensin-converting enzyme 2 (ACE2)/angiotensin (1–7)/Mas receptor, in the renin-angiotensin system (RAS), has opened a new insight regarding the role of RAS and angiotensin in higher brain functions. ACE2 catabolizes angiotensin II and produces angiotensin (1–7), an agonist of Mas receptor. Mice lacking the Mas receptor (angiotensin 1–7 receptor) exhibit anxiety-like behaviours. The present study was conducted to test the hypothesis of the involvement of ACE2 genetic variant (G8790A) on response to selective serotonin reuptake inhibitors (SSRIs). In a randomised control trial, 200 newly diagnosed Iranian patients with major depressive disorder completed 6 weeks of fluoxetine or sertraline treatment. Patients with a reduction of 50% or more in the Hamilton Rating Scale for Depression score were considered responsive to treatment. G8790A polymorphism was determined in extracted DNAs using restriction fragment length polymerase chain reaction method. Our results show that the A allele and AA and GA genotypes were significantly associated with better response to SSRIs ($p = 0.008$; OR = 3.4; 95% CI = 1.4–8.5 and $p = 0.027$; OR = 3.3, 95% CI = 1.2–9.2, respectively). Moreover, patients with GA and AA genotypes responded significantly better to sertraline ($p = 0.0002$; OR = 9.1; 95% CI = 2.4–33.7). The A allele was significantly associated with better response to sertraline ($p = 0.0001$; OR = 7.6; 95% CI = 2.5–23.3). In conclusion, our results confirm the role of G8790A in response to some SSRIs.

Significant outcomes

- Significant response to SSRIs in MDD patients with AA and GA genotypes ($p = 0.027$; OR = 3.3, 95% CI = 1.2–9.2).
- Significantly better response to sertraline in MDD patients with GA and AA genotypes ($p = 0.0002$; OR = 9.1; 95% CI = 2.4–33.7).

Significantly better response to sertraline in MDD patients with A allele ($p = 0.0001$; OR = 7.6; 95% CI = 2.5–23.3).

Limitations

- Small population of enrolled patients.
- Lack of genotype assessment in non-MDD patients and comparison with the MDD group.
- Limited available data due to lack of previous studies in other psychiatric disorders as well as other ethnicities.

Introduction

Depression is a common and deleterious psychiatric disorder and accounts for 4.4% of the world's total illnesses. More than 7.3% of people in the United States are affected by depression (Wakefield et al., 2007). Drastically, the census rises to 43% among Iranian elderly (Sarokhani et al., 2018). Despite a vague understanding of the exact biological mechanisms for depression, there are numerous and effective therapies used to improve patients (McKay et al., 2006). Many clinical pieces of evidence suggest that depression may be caused by dysregulation in neurotransmitters in different brain regions (Milak et al., 2005). Along with the conventional neurotransmitters, the renin-angiotensin system (RAS) has gained much attention in the pathophysiology and treatment of psychiatric and neuropsychiatric illnesses in recent years (Saab et al., 2007; Murck et al., 2012; Kalra et al., 2015; Firouzabadi et al., 2016; Vian et al., 2017; Chrissobolis et al., 2020; de Melo & Almeida-Santos, 2020).

Angiotensin-converting enzymes 1 and 2 (ACE1 and ACE2, respectively) are the two key enzymes of RAS found extensively in the central nervous system (CNS). ACE2 and its main product angiotensin (1–7) has been found in the brain regions responsible for the control of cardiovascular functions such as central regulation of blood pressure (Doobay et al., 2007). Additionally, ACE2 is highly expressed in the endothelial cells of the heart arteries and venules (Lovren et al., 2008). Hence, ACE2 has been considered as a therapeutic target for the management of hypertension and cardiac dysfunctions.

Angiotensin II, an important RAS neuropeptide, is the main product of ACE1 which facilitates the release of norepinephrine (Skidgel & Erdös, 2004). Angiotensins are involved in specialised brain functions such as memory, cognition and adaptive response to different stressful stimuli such as anxiety and depression (Voigt et al., 2005; Lazaroni et al., 2012). Recent research advocates the anxiolytic and antidepressive roles of brain ACE2/Ang (1–7)/Mas pathway (de Melo & Almeida-Santos, 2020). Studies show that mice lacking Mas receptor, the main functional receptor of angiotensin (1–7), show increased anxiety-like behaviour which is suggestive of the anxiolytic property of this peptide (Almeida-Santos et al., 2016). Chronic overexpression of angiotensin (1–7) also exhibits anxiolytic-like effects (Moura Santos et al., 2017).

Further evidence supporting the role of RAS in neuroregulation suggests that drugs which are clinically effective in treating depression antagonize angiotensin 1 receptors (AT1) (Gard et al., 1999). On the other hand, ACE inhibitors like captopril and angiotensin II antagonists (ARBs) such as losartan have anti-depressant effects in humans and animals (Braszko et al., 2003; Saavedra et al., 2011; Diniz et al., 2018; Luo et al., 2020). Previous studies showed that angiotensin II negatively regulates *ACE2* expression, but the expression can be restored by ARB administration. Since angiotensin II is in close interactions with other neurotransmitters, such as norepinephrine and serotonin, and the role of these neurotransmitters in depression are well documented, disruptions in RAS elements may also account for the pathophysiology of depression (Vian et al., 2017). Inhibition of the classical RAS pathway is involved in the upregulation of *ACE2*, and as a result, angiotensin-(1–7)/Mas pathway is activated resulting in increased angiotensin (1–7) levels. Increased levels of angiotensin-(1–7) and activation of Mas receptors exhibit neuroprotective and mood stabilising effects (Almeida-Santos et al., 2016; Kangussu et al., 2017).

As it is known, serotonin contributes to the pathophysiology of depression and decline in brain serotonin levels can cause relapse in recovered depressed patients (Cowen, 2008). ACE2 is proposed to indirectly modulate brain serotonin levels, a neurotransmitter involved in brain neurogenesis (Alenina & Klempin, 2015, Klempin et al., 2018). As reported, ACE2 has a significant role in absorption of tryptophan, the precursor of serotonin, in the gut. Decline in plasma levels of tryptophan in ACE2-defcient mice has shown to reduce serotonin levels in the brain, affecting neurogenesis (Klempin et al., 2013). It may be postulated that increased activity of ACE2 may result in higher serotonin levels in the brain.

Selective serotonin reuptake inhibitors (SSRIs) are one of the most prominent drugs used in the treatment of depression. Sertraline and fluoxetine are among the drugs of choice used in major depressive disorder (MDD) that selectively prevent serotonin reuptake from pre-synaptic neurones (Newhouse et al., 2000). Up to 40% of depressed patients fail to respond to first-line antidepressants (King et al., 2002; Arroll et al., 2005) and among those who do respond, only a very few will attain complete recovery (Joffe et al., 1996).

Among different contributors to the pathophysiology of depression and its treatment, genetics also plays a fundamental role (Levinson, 2006; Mullins & Lewis, 2017). Genetic variants of RAS, especially the ones located on the *ACE1* gene, are reported to be associated with depression (Baghai et al., 2006; Firouzabadi et al., 2012) as well as antidepressant response (Baghai et al., 2004; Bondy et al., 2005; Bahramali et al., 2016). *ACE2* is a polymorphic gene in humans with approximately 140 SNP loci determined in the human genome. Among the SNPs identified on the *ACE2* gene is the G8790A (rs2285666) polymorphism. This variant, which is located in an intronic position adjacent to an exon, might influence *ACE2* gene expression with alternative splicing mechanisms. Substitution of a G with an A at the 8790th base has been reported to result in mRNA montage changes (Yang et al., 2015). The mutant variant of this polymorphism is proposed to show higher serum ACE2 levels, which is suggestive of the higher production of angiotensin (1–7). Some studies on the association of this variant with blood pressure suggest a significant link between rs2285666 polymorphism and higher blood pressure (Yi et al., 2006; Zhong et al., 2006; Niu et al., 2007). On the other hand, a report by Vangjeli et al., is suggestive of the protective role of this variant against fatal cardiovascular events in female subjects (Vangjeli et al., 2011).

Regarding the positive effect of higher ACE2 activity as well as antidepressant properties of angiotensin (1–7), it could be postulated that the mutant allele of G8790A might influence antidepressant response. No study to date has investigated the role of G8790A (rs2285666) polymorphism in association with depression and treatment response in depressed patients.

Based on previous reports that are suggestive of the role of special genetic variants of RAS as an indicator of better response to antidepressants, we examined the relationship between G8790A polymorphism of the *ACE2* gene and response to sertraline versus fluoxetine in a group of MDD patients.

Material and methods

The pharmacogenetics project was conducted in 2019, and the study was approved by Ethics Committee of Shiraz University of Medical Sciences with the ethical code and grant number of 13096 and was in accordance with Declaration of Helsinki. This study was a part of a randomised clinical trial of MDD patients aged 17–62 years old who referred to Imam Reza Hospital, Shiraz, Iran. All participants were of Iranian descent (Persian ethnicity) and born from Iranian parents. All patients were diagnosed as new cases and did not have any history of previous mental and psychiatric illnesses. The diagnosis of MDD was made by a psychiatrist and based on the DSM-V criteria. Informed consent was signed by the patient or their guardian before entering the study.

Since these patients were newly diagnosed, they did not have a history of antidepressant medication. Patients with family history of any other psychiatric disorders such as schizophrenia; bipolar disorder, mania and psychosis in the first-degree relatives; active substance dependence or primary organic disease; current treatment with antipsychotics, mood stabilisers, ACE inhibitors or ARBs and cardiovascular diseases such as coronary artery disease, myocardial infarction or heart failure were excluded from our study. Notably, only patients diagnosed with MDD with no other medical comorbidities other than anxiety or sleep disorder were included in our study. The rate of anxiety in enrolled patients was 43%. The only medication other than an SSRI (sertraline or

https://doi.org/10.1017/neu.2021.32 Published online by Cambridge University Press

fluoxetine) that was received by the patients was a benzodiazepine. Patients were randomly categorised into two groups receiving either fluoxetine (FLUOXETINE-ABIDI®) or sertraline (SERTRALINEABIDI®).

A fixed dose of 20 mg of fluoxetine and 50 mg of sertraline per day for 6 weeks were used. A benzodiazepine was prescribed for patients experiencing sleep disorders. After completing the 6-week treatment period, patients were evaluated again by the Hamilton questionnaire. Patients were classified into responsive and non-responsive groups based on the score obtained from the 21-item Hamilton Rating Scale for Depression (HAMD-21) questionnaire. A minimum of 50% reduction in the initial score (before taking the medication) was considered as responsive to treatment. Five millilitre of whole blood was obtained from enrolled patients for further DNA extraction and genotyping.

Allocation of patients was based on the simple randomisation method in which two sets of envelopes were provided containing the name of the prescribed drugs inside. The psychiatrist sequentially opened the shuffled envelopes to assign the treatment group for each enrolled individual. The prescribing psychiatrist and the patients were not blinded to the treatment, but the analyst was blinded.

DNA extraction

The DNA obtained from each patient was extracted from whole blood samples using the salting-out method (Miller et al., 1988). Yields of DNAs were evaluated using NanoDrop®.

Genotyping

G8790A polymorphism was genotyped using restriction fragment length polymorphism (PCR-RFLP) assay (Zhong et al., 2006). Oligonucleotide primers were synthesised based on GenBank for the PCR. The applied primer sequences included

Forward primer: 5′-TTCTCCCTGCTCCTATACTACCG-3′ and Reverse primer:

5′-TTCATTCATGTCCTTGCCCTTA-3′.

PCR procedure was conducted using 12.5 µl Master Mix (1X), 2.5 µl primer and 2 µl DNA at a primer annealing temperature of 60 °C. For RFLP, 5 µl of amplicons were digested with 0.8 µl of AluI restriction endonuclease over 16 h at 37°C. Then, the loading dye was added to the digested product and run on a 1% agarose gel for 30 min at a 100 (v). After electrophoresis, stained products were visualised using a UV trans-illuminator. All samples were genotyped at least twice for reconfirmation.

After digestion with AluI restriction enzyme, G allele band was observed at 817 bp, A allele was observed at 589 and 228 bp and GA heterozygote bands were observed at 817, 589, and 228 bp (Fig. 1).

Statistical analysis

Data were analysed using SPSS® 16.0 for Windows (SPSS Inc., Chicago, IL, USA) software. Power analysis was performed using the statistical program G*Power (http://www.gpower.hhu.de/; Faul et al., 2007).

The frequency of alleles and genotypes is presented as percentages. For calculating the normality of the data, Kolmogorov–Smirnov test was used. Continuous variables were compared using a parametric test (Student's *t*-test). Quantitative variables are demonstrated as mean ± SD (mean ± SD). Chi-square test (χ^2) was used for comparing categorical parameters between groups. Logistic regression analysis was performed to assess the influence of sex on the role of different variants of ACE2 on response to treatment. To assess the association of genotypes and allele frequencies with response to treatment, odds ratio (OR) and the corresponding 95% confidence interval (CI) are reported. $p < 0.05$ was considered statistically significant.

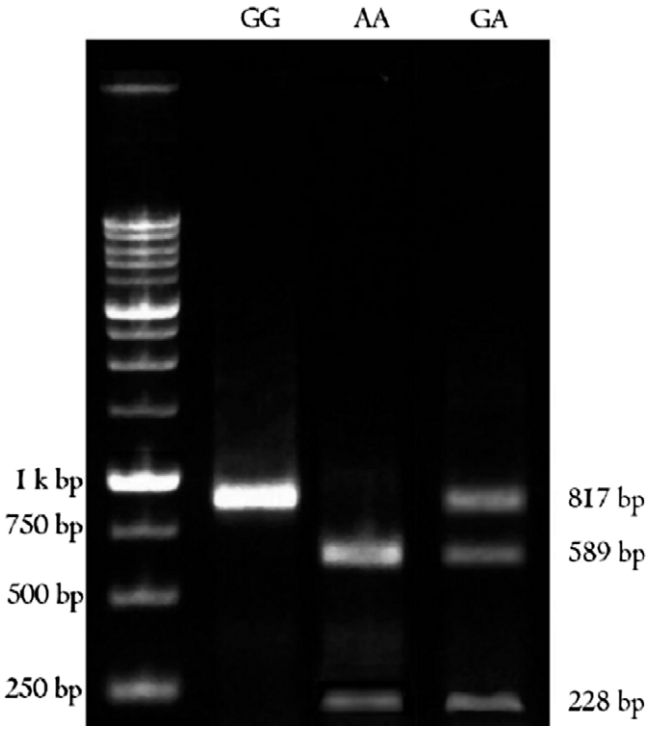

Fig. 1. Agarose gel electrophoresis of the PCR-RFLP products using AluI restriction enzyme. The GG genotype appeared as single-band at 817 bp. The AA genotype appeared as two-bands at 589 and 228 bp and GA genotype appeared as three-bands at 817, 589, and 228 bp.

Results

Table 1 shows the demographic data of MDD patients treated with sertraline and fluoxetine. Among 100 MDD patients treated with sertraline, 25 were men and 75 were women. The mean age and BMI of the sertraline group were 35.4 ± 12.9 years and 24.6 ± 4.0 kg/m^2 , respectively. Regarding 100 MDD patients treated with fluoxetine, 33 were men and 67 were women. In the fluoxetine group, the mean age and BMI were 33.5 ± 10.7 years and 24.7 ± 3.8 kg/m^2 , respectively. There was no significant difference in age, gender, and BMI between responders to sertraline and fluoxetine ($p > 0.05$). HAM-D scores were calculated at the beginning of the study, week 0, and after 6 weeks. Patients whose HAM-D scores were dropped to more than 50% after the 6-week treatment period were considered as responsive to treatment.

Genotype and allele distribution of *ACE2* gene polymorphism are presented in Table 2. As shown, patients with GA and AA genotype responded significantly better to SSRIs (fluoxetine and sertraline) compared to GG genotype ($p = 0.027$; OR = 3.3; 95% CI = 1.2–9.2). The A allele was associated with better response to SSRIs than the G allele ($p = 0.008$; OR = 3.4; 95% CI = 1.4–8.5). As shown in Tables 3 and 4, subgroup analyses indicated that patients with GA and AA genotypes responded significantly better to sertraline ($p = 0.0002$; OR = 9.1; 95% CI = 2.4–33.7). Additionally, the A allele was significantly associated with better response to sertraline ($p = 0.0001$, OR = 7.6, 95% CI = 2.5–23.3).

https://doi.org/10.1017/neu.2021.32 Published online by Cambridge University Press

Table 1. Demographic characteristics of patients receiving SSRIs (sertraline and fluoxetine)

	Variable	Responsive (N = 46)	Non-responsive (N = 54)	*p*-value
Sertraline (N = 100)	Sex (male/female) N%	(13/33) 28.2% vs. 71.8%	(12/42) 22.2% vs. 77.8%	0.881
	Age (years)	36.7 ± 13.7	33.9 ± 11.8	0.275
	BMI (kg/m^2)	24.5 ± 3.7	24.7 ± 4.4	0.808
	HAM-D 1 (week 0)	26.5 ± 10.8	27.3 ± 9.5	0.694
	HAM-D 2 (week 6)	11.5 ± 6.9	22.6 ± 10.3	0.0001
	Variable	Responsive (N = 70)	Non-responsive (N = 30)	
Fluoxetine (N = 100)	Sex (male/female) N%	(23/47) 32.9% –67.1%	(10/20) 33.3%–66.7%	0.963
	Age (years)	32.4 ± 10.3	33.0 ± 11.7	0.798
	BMI (kg/m^2)	24.7 ± 3.9	24.7 ± 3.8	0.926
	HAM-D 1 (week 0)	24.8 ± 6.5	25.4 ± 7.1	0.682
	HAM-D 2 (week 6)	7.7 ± 5.4	23.1 ± 6.9	0.0001

BMI, body mass index; kg, kilogram; m^2, meter square; HAM-D, Hamilton Depression Rating Scale.

Table 2. Genotype and allele frequencies of G8790A variant in patients treated with SSRIs

Variants in total population		Responsive (N = 116)	Non-responsive (N = 84)	*p*-value	OR; 95% CI
Genotypes	GG	96	79	0.027	3.3; 1.2–9.2
	GA	14	4		
	AA	6	1		
Alleles	G	206	162	0.008	3.4; 1.4–8.5
	A	26	6		
	A	5	1		

CI, confidence interval; OR, odds ratio.

However, different genotypes of G8790A were not associated with response in patients treated with fluoxetine ($p = 0.05$).

Discussion

Like most complex disorders, depression is a polygenic illness arising from the combined effect of many genetic variants (Wray et al., 2014). Pharmacotherapy of mood disorders such as depression has been significantly improved over the past decades by introducing SSRIs. As with all antidepressants, however, 30%–40% of depressed patients do not respond satisfactorily to SSRIs. Since MDD has a strong genetic base, pharmacogenetics may offer great promise for individualizing antidepressant therapy (Tsai & Hong, 2003).

Results of our study indicated a significant association between A allele of G8790A on *ACE2* gene and better response to SSRIs. Subgroup analyses revealed a significant association between A allele and response to sertraline in MDD patients. Additionally, carriers of AA and GA variants responded significantly better to sertraline.

To date, no studies regarding the association between ACE2 activity and depression have been conducted in the human population. It has been reported that in a neurological disease-like multiple sclerosis, the level of ACE1 activity is significantly elevated compared to the control group, while the level of ACE2 activity is significantly decreased (Kawajiri et al., 2009).

Regarding G8790A polymorphism and response to antidepressants in MDD, no reports have been released as yet. However, a report by Han et al. investigated the effect of ACE2 variants with comorbidity of depression and coronary heart disease and showed a significant association between rs2285666 variants and depression in depressed CHD patients. Moreover in the same report, association of rs2285666 variants with lower serum levels of Ang (1–7) was observed that confirmed the role of rs2285666 on Ang (1–7) levels and susceptibility to depression (Han et al., 2020). Regarding neurological complications, results of a study by Wu et al. (2017) reported that G8790A polymorphism in the *ACE2* gene in type 2 diabetes mellitus is significantly associated with cerebral stroke. Additionally, a study by Strafella et al. (2020) suggested a link between ACE2 variants and neurological complications observed in COVID-19.

Besides the periphery, all RAS elements are distributed in different brain regions. Angiotensins, the main RAS components, are involved in high brain functions including memory, cognition, anxiety and depression (Ciobica et al., 2009). Injection of angiotensin II induces anxiety-like behaviour in animal models (Georgiev et al., 1990). Angiotensin (1–7), the product of ACE2, results from angiotensin II degradation. Mice lacking Mas, a functional receptor of angiotensin (1–7), exhibit elevated anxiety-like behaviour suggestive of anxiolytic-like effects of angiotensin (1–7) (Santos et al., 2003; Almeida-Santos et al., 2016). An increase in antidepressants' effects through a decline in levels of angiotensin II was reported in several studies (Gard et al., 1994, 1999). Unlike angiotensin II, an increase in angiotensin (1–7) levels has been proposed to show antidepressant effects (Kangussu et al., 2013). Psychiatric disorders such as depression are associated with overactivity of ACE1 and the resultant, higher levels of angiotensin II (Baghai et al., 2006; Firouzabadi et al., 2012). Angiotensin II activates AT1 receptors expressed at BBB endothelial cells. Activation of AT1 receptors ends in the phosphorylation of a tight junction protein, called occludin. Hence, an increase in angiotensin II degradation and angiotensin (1–7) production through a rise in ACE2 activity in A allele carriers (Wu et al., 2017) decreases the expression of occludin protein

https://doi.org/10.1017/neu.2021.32 Published online by Cambridge University Press

Table 3. Genotype and allele frequencies in patients treated with sertraline

G8790A variants in total population		Responsive to sertraline (N = 46)	Non-responsive (N = 54)	*p*-value	OR; 95% CI
Genotypes	GG	30	51	0.0002	9.1; 2.4–33.7
	GA	11	2		
	AA	5	1		
Alleles	G	71	104	0.001	7.6; 2.5–23.3
	A	21	4		

Table 4. Genotype and allele frequencies in patients treated with fluoxetine

G8790A variants in total population		Responsive to fluoxetine (N = 70)	Non-responsive (N = 30)	*p*-value	OR; 95% CI
Genotypes	GG	66	28	1	0.8; 0.1–4.9
	GA	3	2		
	AA	1	0		
Alleles	G	135	58	1	1.1; 0.2–5.7
	A	5	2		

leading to an increase in drug passage across BBB, increased CNS permeability of drugs and thereby increasing their effectiveness.

It is worth pointing out that since G8790A polymorphism is located on an intron of *ACE2* gene, adjacent to an exon, it may influence *ACE2* gene expression with alternative splicing mechanisms (Yang et al., 2015). Additionally, G8790Avariant is also in strong linkage disequilibrium with other functional SNPs in *ACE2* gene that may contribute to physiologic actions of G8790A (Kramkowski et al., 2006).

As a limitation of our study, we should point to rather the small sample size of enrolled patients and lack of comparison between MDD and non-MDD patients. In addition, since no previous studies was performed in other psychiatric disorders as well as other ethnicities, available data are limited. Therefore, no comparison can be made for discussing our findings. However, the results of our study may provide preliminary insights for further investigations. The results of our study and other pharmacogenetics studies may assist in the smarter selection of SSRIs for the management of MDD. Personalized medicine provides a better chance of treating patients as well as achieving remission.

At last, it is worth noting that depression is a heterogeneous illness both aetiologically and phenotypically, and two patients diagnosed with MDD may have few symptoms in common (Østergaard et al., 2011). GWAS studies have provided interesting clues for various diseases. However, they are mostly underpowered for complex traits such as MDD (Ripke et al., 2013). Moderate heritability, heterogeneity in genetics and also non-genetic factors and high prevalence of MDD make it a challenging disorder for GWAS. However, for common multifactorial illnesses, GWAS is considered the most promising method (Levinson et al., 2014). Another important fact is that besides the CONVERGE study, which was conducted in depressed Han Chinese women, MDD GWAS has been limited to the European ethnicity (Ormel et al., 2019). Therefore, no data regarding other ethnicities such as Iranian are at reach at present.

Our study investigated a rare susceptibility allele that are usually excluded in GWAS (Vadgama et al., 2021). The studied variant (rs2285666) affects ACE2 activity which is proposed to be altered in MDD and thus be associated with depression. However, this association should be studied in the future. It is noteworthy that other ACE variants such as ACE I/D, which have been vastly studied in MDD association studies, have not been reported in GWAS as well.

Conclusion

Here we showed that GA and AA carriers of G8790A polymorphism of *ACE2*gene and the A was associated with better response to SSRIs. Patients with GA and AA genotypes responded significantly better to sertraline. In conclusion, our results confirm the role of G8790A in response to some SSRIs.

Conflict of interest. The authors of the manuscript have no conflicts of interest to declare.

Funding. This study was supported by Shiraz University of Medical Sciences, Grant No. 13096.

Declarations. The protocol of this study was reviewed and approved by the Ethics committee of Shiraz University of Medical Sciences with the ethical code of 13096. Each patient signed an informed consent form approved by the local institutional Review Board.

All methods were carried out in accordance with relevant guidelines and regulations.

Data availability statement. The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Contributions of authors statement. N.F.: design of the work, analysis and interpretation of data for the work, writing the manuscript, revising the manuscript critically for important intellectual content, draughting the work and final approval of the manuscript.

M.H.: writing the draft of the manuscript, revising the manuscript critically for important intellectual content and final approval of the manuscript.

P.F.: conducting the project, analysis and interpretation of data for the work, draughting the manuscript and final approval of the manuscript.

V.G.: conducting the project, final approval of the manuscript.

A.A.-S.: data gathering from patients, revising the manuscript critically for important intellectual content, draughting the work and final approval of the manuscript.

References

Alenina N and Klempin F (2015) The role of serotonin in adult hippocampal neurogenesis. *Behavioural Brain Research* **277**(5), 49–57.

Almeida-Santos AF, Kangussu LM, Moreira FA, Santos RA, Aguiar DC and Campagnole-Santos MJ (2016) Anxiolytic-and antidepressant-like effects of

angiotensin-(1-7) in hypertensive transgenic (mRen2) 27 rats. *Clinical Science* **130**(14), 1247–1255.

Arroll B, Macgillivray S, Ogston S, Reid I, Sullivan F, Williams B and Crombie I (2005) Efficacy and tolerability of tricyclic antidepressants and SSRIs compared with placebo for treatment of depression in primary care: a meta-analysis. *The Annals of Family Medicine* **3**(5), 449–456.

Baghai T, Binder E, Schule C, Salyakina D, Eser D, Lucae S, Zwanzger P, Haberger C, Zill P, Ising M (2006) Polymorphisms in the angiotensin-converting enzyme gene are associated with unipolar depression, ACE activity and hypercortisolism. *Molecular Psychiatry* **11**, 1003.

Baghai TC, Schule C, Zill P, Deiml T, Eser D, Zwanzger P, Ella R, Rupprecht R and Bondy B (2004) The angiotensin I converting enzyme insertion/deletion polymorphism influences therapeutic outcome in major depressed women, but not in men. *Neuroscience Letters* **363**(1), 38–42.

Bahramali E, Firouzabadi N, Yavarian I, Shayesteh MRH, Erfani N, Shoushtari AA and Asadpour R (2016) Influence of ACE gene on differential response to sertraline versus fluoxetine in patients with major depression: a randomized controlled trial. *European Journal of Clinical Pharmacology* **72**(9), 1059–1064.

Bondy B, Baghai TC, Zill P, Schule C, Eser D, Deiml T, Zwanzger P, Ella R and Rupprecht R (2005) Genetic variants in the angiotensin I-converting-enzyme (ACE) and angiotensin II receptor (AT1) gene and clinical outcome in depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **29**(6), 1094–1099.

Braszko JJ, Karwowska-Polecka W, Halicka D and Gard PR (2003) Captopril and enalapril improve cognition and depressed mood in hypertensive patients. *Journal of Basic and Clinical Physiology and Pharmacology* **14**(4), 323–344.

Chrissobolis S, Luu A, Waldschmidt R, Yoakum M and D'souza MS (2020) Targeting the renin angiotensin system for the treatment of anxiety and depression. *Pharmacology Biochemistry and Behavior* **199**(10), 173063.

Ciobica A, Bild W, Hritcu L and Haulica I (2009) Brain renin-angiotensin system in cognitive function: pre-clinical findings and implications for prevention and treatment of dementia. *Acta Neurologica Belgica* **109**, 171.

Cowen PJ (2008) Serotonin and depression: pathophysiological mechanism or marketing myth? *Trends in Pharmacological Sciences* **29**(9), 433–436.

de Melo LA and Almeida-Santos AF (2020) Neuropsychiatric properties of the ACE2/Ang-(1-7)/Mas pathway: a brief review. *Protein and Peptide Letters* **27**(6), 476–483.

Diniz CR, Casarotto PC, Fred SM, Biojone C, Castrén E and Joca SR (2018) Antidepressant-like effect of losartan involves TRKB transactivation from angiotensin receptor type 2 (AGTR2) and recruitment of FYN. *Neuropharmacology* **135**(10), 163–171.

Doobay MF, Talman LS, Obr TD, Tian X, Davisson RL and Lazartigues E (2007) Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin-angiotensin system. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **292**(1), R373–R381.

Faul F, Erdfelder E, Lang A-G and Buchner A (2007) G* Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods* **39**(2), 175–191.

Firouzabadi N, Ghazanfari N, Alavi Shoushtari A, Erfani N, Fathi F, Bazrafkan M and Bahramali E (2016) Genetic variants of angiotensin-converting enzyme are linked to autism: a case-control study. *PLOS ONE* **11**(4), e0153667.

Firouzabadi N, Shafiei M, Bahramali E, Ebrahimi SA, Bakhshandeh H and Tajik N (2012) Association of angiotensin-converting enzyme (ACE) gene polymorphism with elevated serum ACE activity and major depression in an Iranian population. *Psychiatry Research* **200**(2-3), 336–342.

Gard PR, Mandy A and Sutcliffe MA (1999) Evidence of a possible role of altered angiotensin function in the treatment, but not etiology, of depression. *Biological Psychiatry* **45**(8), 1030–1034.

Gard PR, Mandy A, Whiting JM, Nickels DP and Meakin AJ (1994) Reduction of responses to angiotensin II by antidepressant drugs. *European Journal of Pharmacology* **264**(3), 295–300.

Georgiev V, Stancheva S, Kambourova T and Getova D (1990) Effect of angiotensin II on the Vogel conflict paradigm and on the content of dopamine and noradrenaline in rat brain. *Acta Physiologica et Pharmacologica Bulgarica* **16**, 32–37.

Han W, Wei Z, Dang R, Guo Y, Zhang H, Geng C, Wang C, Feng Q and Jiang P (2020) Angiotensin-II and angiotensin-(1-7) imbalance affects comorbidity of depression and coronary heart disease. *Peptides* **131**(6), 170353.

Joffe R, Sokolov S and Streiner D (1996) Antidepressant treatment of depression: a metaanalysis. *The Canadian Journal of Psychiatry* **41**(10), 613–616.

Kalra J, Prakash A, Kumar P and Majeed ABA (2015) Cerebroprotective effects of RAS inhibitors: beyond their cardio-renal actions. *Journal of the Renin-Angiotensin-Aldosterone System* **16**(3), 459–468.

Kangussu LM, Almeida-Santos AF, Bader M, Alenina N, Fontes MAP, Santos RA, Aguiar DC and Campagnole-Santos MJ (2013) Angiotensin-(1-7) attenuates the anxiety and depression-like behaviors in transgenic rats with low brain angiotensinogen. *Behavioural Brain Research* **257**(1 Pt 2), 25–30.

Kangussu LM, Almeida-Santos AF, Moreira FA, Fontes MA, Santos RA, Aguiar DC and Campagnole-Santos MJ (2017) Reduced anxiety-like behavior in transgenic rats with chronically overproduction of angiotensin-(1-7): role of the Mas receptor. *Behavioural Brain Research* **331**(5), 193–198.

Kawajiri M, Mogi M, Higaki N, Matsuoka T, Ohyagi Y, Tsukuda K, Kohara K, Horiuchi M, Miki T, Kira JI (2009) Angiotensin-converting enzyme (ACE) and ACE2 levels in the cerebrospinal fluid of patients with multiple sclerosis. *Multiple Sclerosis* **15**(2), 262–265.

King M, Davidson O, Taylor F, Haines A, Sharp D and Turner R (2002) Effectiveness of teaching general practitioners skills in brief cognitive behaviour therapy to treat patients with depression: randomised controlled trial. *British Medical Journal* **324**(7343), 947–947.

Klempin F, Beis D, Mosienko V, Kempermann G, Bader M and Alenina N (2013) Serotonin is required for exercise-induced adult hippocampal neurogenesis. *Journal of Neuroscience* **33**(19), 8270–8275.

Klempin F, Mosienko V, Matthes S, Villela DC, Todiras M, Penninger JM, Bader M, Santos RA and Alenina N (2018) Depletion of angiotensin-converting enzyme 2 reduces brain serotonin and impairs the running-induced neurogenic response. *Cellular and Molecular Life Sciences* **75**(19), 3625–3634.

Kramkowski K, Mogielnicki A and Buczko W (2006) The physiologic significance of the alternative. *Journal of Physiology and Pharmacology* **57**, 529–539.

Lazaroni TL, Raslan ACS, Fontes WR, de Oliveira ML, Bader M, Alenina N, Moraes MF, dos Santos RA and Pereira GS (2012) Angiotensin-(1-7)/Mas axis integrity is required for the expression of object recognition memory. *Neurobiology of Learning and Memory* **97**(1), 113–123.

Levinson DF (2006) The genetics of depression: a review. *Biological Psychiatry* **60**(2), 84–92.

Levinson DF, Mostafavi S, Milaneschi Y, Rivera M, Ripke S, Wray NR and Sullivan PF (2014) Genetic studies of major depressive disorder: why are there no GWAS findings, and what can we do about it? *Biological Psychiatry* **76**(7), 510–512.

Lovren F, Pan Y, Quan A, Teoh H, Wang G, Shukla PC, Levitt KS, Oudit GY, Al-Omran M, Stewart DJ (2008) Angiotensin converting enzyme-2 confers endothelial protection and attenuates atherosclerosis. *American Journal of Physiology-Heart and Circulatory Physiology* **295**(4), H1377–H1384.

Luo H, Wu P-F, Cao Y, Jin M, Shen T-T, Wang J, Huang J-G, Han Q-Q, He J-G, Deng S-L (2020) Angiotensin-converting enzyme inhibitor rapidly ameliorates depressive-type behaviors via bradykinin-dependent activation of mTORC1. *Biological Psychiatry* **88**(5), 415–428.

Mckay KM, Imel ZE and Wampold BE (2006) Psychiatrist effects in the psychopharmacological treatment of depression. *Journal of Affective Disorders* **92**(2-3), 287–290.

Milak MS, Parsey RV, Keilp J, Oquendo MA, Malone KM and Mann JJ (2005) Neuroanatomic correlates of psychopathologic components of major depressive disorder. *Archives of General Psychiatry* **62**(4), 397–408.

Miller S, Dykes D and Polesky H (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16**(3), 1215.

Moura Santos D, Ribeiro Marins F, Limborço-Filho M, de Oliveira ML, Hamamoto D, Xavier CH, Moreira FA, Santos RAS, Campagnole-

https://doi.org/10.1017/neu.2021.32 Published online by Cambridge University Press

Santos MJ and Peliky Fontes MA (2017) Chronic overexpression of angiotensin-(1-7) in rats reduces cardiac reactivity to acute stress and dampens anxious behavior. *Stress-the International Journal on the Biology of Stress* **20**(2), 189–196.

Mullins N and Lewis CM (2017) Genetics of depression: progress at last. *Current Psychiatry Reports* **19**(8), 43.

Murck H, Schüssler P and Steiger A (2012) Renin-angiotensin-aldosterone system: the forgotten stress hormone system: relationship to depression and sleep. *Pharmacopsychiatry* **45**(3), 83–95.

Newhouse PA, Krishnan K, Doraiswamy PM, Richter EM, Batzar ED and Clary CM (2000) A double-blind comparison of sertraline and fluoxetine in depressed elderly outpatients. *The Journal of Clinical Psychiatry* **61**(8), 559–568.

Niu W, Qi Y, Hou S, Zhou W and Qiu C (2007) Correlation of angiotensin-converting enzyme 2 gene polymorphisms with stage 2 hypertension in Han Chinese. *Translational Research* **150**(6), 374–380.

Ormel J, Hartman CA and Snieder H (2019) The genetics of depression: successful genome-wide association studies introduce new challenges. *Translational Psychiatry* **9**(1), 1–10.

Østergaard SD, Jensen S and Bech P (2011) The heterogeneity of the depressive syndrome: when numbers get serious. *Acta Psychiatrica Scandinavica* **124**(6), 495–496.

Ripke S, Wray NR, Lewis CM, Hamilton SP, Weissman MM, Breen G, Byrne EM, Blackwood DH, Boomsma DI, Cichon S (2013) A mega-analysis of genome-wide association studies for major depressive disorder. *Molecular Psychiatry* **18**(4), 497–511.

Saab YB, Gard PR, Yeoman MS, Mfarrej B, El-Moalem H and Ingram MJ (2007) Renin-angiotensin-system gene polymorphisms and depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **31**(5), 1113–1118.

Saavedra JM, Sánchez-Lemus E and Benicky J (2011) Blockade of brain angiotensin II AT1 receptors ameliorates stress, anxiety, brain inflammation and ischemia: therapeutic implications. *Psychoneuroendocrinology* **36**(1), 1–18.

Santos RA, Silva E, A.C. S, Maric C, Silva DM, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SVB, Lopes MT and Bader M (2003) Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proceedings of The National Academy of Sciences of The United States of America* **100**(14), 8258–8263.

Sarokhani D, Parvareh M, Dehkordi AH, Sayehmiri K and Moghimbeigi A (2018) Prevalence of depression among Iranian elderly: systematic review and meta-analysis. *Iranian Journal of Psychiatry* **13**, 55.

Skidgel RA and Erdös EG (2004) Angiotensin converting enzyme (ACE) and neprilysin hydrolyze neuropeptides: a brief history, the beginning and follow-ups to early studies. *Peptides* **25**(3), 521–525.

Strafella C, Caputo V, Termine A, Barati S, Gambardella S, Borgiani P, Caltagirone C, Novelli G, Giardina E, Cascella R (2020) Analysis of ACE2 genetic variability among populations highlights a possible link with COVID-19-related neurological complications. *Genes* **11**(7), 741.

Tsai S and Hong C (2003) Pharmacogenetics of selective serotonin reuptake inhibitor response in major depression. *Current Pharmacogenomics* **1**(1), 1–7.

Vadgama N, Kreymerman A, Campbell J, Shamardina O, Brugger C, Lee RT, Penkett CJ, Gifford CA, Mercola M, Nasir J (2021) SARS-CoV-2 susceptibility and ACE2 gene variations within diverse ethnic backgrounds. medRxiv.

Vangjeli C, Dicker P, Tregouet D-A, Shields DC, Evans A, Stanton AV and (for the MORGAM project*) (2011) A polymorphism in ACE2 is associated with a lower risk for fatal cardiovascular events in females: the MORGAM project. *Journal of the Renin-Angiotensin-Aldosterone System* **12**(4), 504–509.

Vian J, Pereira C, Chavarria V, Köhler C, Stubbs B, Quevedo J, Kim S-W, Carvalho AF, Berk M, Fernandes BS (2017) The renin-angiotensin system: a possible new target for depression. *BMC Medicine* **15**(1), 144.

Voigt J-P, Hörtnagl H, Rex A, Van Hove L, Bader M and Fink H (2005) Brain angiotensin and anxiety-related behavior: the transgenic rat TGR (ASrAOGEN) 680. *Brain Research* **1046**(1-2), 145–156.

Wakefield JC, Schmitz MF, First MB and Horwitz AV (2007) Extending the bereavement exclusion for major depression to other losses: evidence from the National Comorbidity Survey. *Archives of General Psychiatry* **64**(4), 433–440.

Wray NR, Lee SH, Mehta D, Vinkhuyzen AA, Dudbridge F and Middeldorp CM (2014) Research review: polygenic methods and their application to psychiatric traits. *Journal of Child Psychology and Psychiatry* **55**(10), 1068–1087.

Wu YH, Li JY, Wang C, Zhang LM and Qiao H (2017) The ACE 2 G8790A polymorphism: involvement in type 2 diabetes mellitus combined with cerebral stroke. *Journal of Clinical Laboratory Analysis* **31**, e22033.

Yang M, Zhao J, Xing L and Shi L (2015) The association between angiotensin-converting enzyme 2 polymorphisms and essential hypertension risk: a meta-analysis involving 14,122 patients. *Journal of the Renin-Angiotensin-Aldosterone System* **16**(4), 1240–1244.

Yi L, Gu Y, Wang X, An L, Xie X, Shao W, Ma L, Fang J, An Y, Wang F (2006) Association of ACE, ACE2 and UTS2 polymorphisms with essential hypertension in Han and Dongxiang populations from north-western China. *Journal of International Medical Research* **34**(3), 272–283.

Zhong J, Yan Z, Liu D, Ni Y, Zhao Z, Zhu S, Tepel M and Zhu Z (2006) Association of angiotensin-converting enzyme 2 gene A/G polymorphism and elevated blood pressure in Chinese patients with metabolic syndrome. *Journal of Laboratory and Clinical Medicine* **147**(2), 91–95.

https://doi.org/10.1017/neu.2021.32 Published online by Cambridge University Press