OXFORD

International Journal of Neuropsychopharmacology (2020) 23(11): 721–730

doi:10.1093/ijnp/pyaa050
Advance Access Publication: 22 July 2020
Regular Research Article

REGULAR RESEARCH ARTICLE

A Study in First-Episode Psychosis Patients: Does Angiotensin I-Converting Enzyme Activity Associated With Genotype Predict Symptom Severity Reductions After Treatment With Atypical Antipsychotic Risperidone?

João V. Nani, Caroline Dal Mas, Camila M. Yonamine, Vanessa K. Ota, Cristiano Noto, Sintia I. Belangero, Jair J. Mari, Rodrigo Bressan, Quirino Cordeiro, Ary Gadelha, Mirian A. F. Hayashi

Department of Pharmacology (Drs Nani, Dal Mas, Yonamine, and Hayashi), Department of Psychiatry (Drs Nani, Noto, Mari, Bressan, and Gadelha), and Department of Genetics (Drs Ota and Belangero), Laboratory of Integrative Neuroscience (LiNC) (Dr Belangero), First-episode Psychosis Program (Drs Noto and Cordeiro), Universidade Federal de Sao Paulo (UNIFESP), São Paulo, Brazil; National Institute for Translational Medicine (INCT-TM, CNPq), Ribeirão Preto, Brazil (Drs Nani and Hayashi).

Correspondence: Prof. Mirian A. F. Hayashi, PhD, Departamento de Farmacologia, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP), Rua 3 de maio 100, Ed. INFAR, 3rd floor, CEP 04044-020, São Paulo, Brazil (mhayashi@unifesp.br or mirianhayashi@yahoo.com).

Abstract

Background: Our previous studies showed increased angiotensin I-converting enzyme (ACE) activity in chronic schizophrenia patients compared with healthy control (HC) volunteers, and the relevance of combining ACE genotype and activity for predicting schizophrenia was suggested.
Methods: ACE activity was measured in plasma of ACE insertion/deletion (I/D) genotyped HC volunteers (n = 53) and antipsychotic-naïve first-episode psychosis (FEP) patients (n = 45) assessed at baseline (FEB-B) and also after 2 months (FEP-2M) of treatment with the atypical antipsychotic risperidone.
Results: ACE activity measurements showed significant differences among HC, FEP-B, and FEP-2M groups ($F = 5.356$, $df = 2$, $P = .005$) as well as between HC and FEP-2M (post-hoc Tukey's multiple comparisons test, $P = .004$). No correlation was observed for ACE activity increases and symptom severity reductions in FEP as assessed by total Positive and Negative Syndrome Scale ($r = -0.131$, $P = .434$). FEP subgrouped by ACE I/D genotype showed significant ACE activity increases, mainly in the DD genotype subgroup. No correlation between ACE activity and age was observed in FEP or HC groups separately ($r = 0.210$, $P = .392$), but ACE activity level differences observed between these groups were influenced by age.
Conclusions: The importance of measuring the ACE activity in blood plasma, associated with ACE I/D genotyping to support the follow-up of FEP patients, did not show correlation with general symptom amelioration in the present study. However, new insights into the influence of age and I/D genotype for ACE activity changes in FEP individuals upon treatment was demonstrated.

Key Words: Angiotensin-converting enzyme (ACE), first-episode psychosis (FEP), risperidone, genotype, enzyme activity

Received: December 19, 2019; **Revised:** June 8, 2020; **Accepted:** July 14, 2020

Significance Statement

Our study suggest the power of measuring ACE activity in blood plasma, associated with ACE I/D genotyping to better understand the neuropathological mechanisms underlying the symptoms of SCZ and potentially support clinical decisions.

Introduction

Angiotensin I-converting enzyme (ACE) is mainly responsible for the conversion and generation of the hypertensive octapeptide angiotensin II, although this enzyme is also capable of catalyzing the degradation of neuropeptides as bradykinin and neurotensin (Masuyer et al., 2014; Dal Mas et al., 2018). Interestingly, bradykinin and neurotensin (Binder et al., 2001; van den Buuse et al., 2005; Martin et al., 2008; LaCrosse and Olive 2013; Boules et al., 2014; Feifel et al., 2016), as well as the ACE activity (Gadelha et al., 2015a, 2015b; Dal Mas et al., 2018), were independently associated with the pathophysiology of psychiatric disorders such as schizophrenia (SCZ) (Rodriguez et al., 2020).

Several associations for ACE suggested a possible convergence with SCZ pathophysiology (Zhang et al., 2014; Gadelha et al., 2015a, 2015b; Mazaheri and Saadat 2015) in which dopamine dysregulation (which is still the main target for the available treatment hitherto) seems to be the common final pathway to SCZ symptoms (Laruelle et al., 1996; Klein et al., 2018). In fact, the currently preconized SCZ treatment employs mainly first- (or typical) and second-generation (or atypical) antipsychotic drugs, which, in fact, are not able to improve the cognitive symptoms claimed to be one of the most responsible for the functional impairments reported in SCZ (O'Tuathaigh et al., 2017). Interestingly, convergent evidence from human and animal studies implicated ACE activity in cognitive performance in chronic SCZ (Gadelha et al., 2015a). Moreover, elevated ACE activity levels and also its main substrate, angiotensin II, were both independently associated with worse cognitive function (Yasar et al., 2018).

The insertion/deletion (I/D) polymorphism in the 16th intron of the *ACE* gene (locus rs4646994) is the most studied genetic basis known to influence the ACE expression in humans, in which individuals bearing the D allele express more ACE than the I allele carriers (Rigat et al., 1990). In contrast to its well-known association with several pathologies or processes, there is still a lack of knowledge in the roles of this polymorphism in the psychiatry field. In spite of some paradoxical works proposing the opposite (Segman et al., 2002; Song and Lee 2015; Hui et al., 2014), we have demonstrated that ACE I/D polymorphism was associated with SCZ only if this ACE I/D polymorphism was concomitantly considered with the ACE activity levels (Gadelha et al., 2015b), possibly elucidating the discrepancies reported by others (Crescenti et al., 2009; Hui et al., 2015; Mazaheri and Saadat, 2015). More precisely, we have proposed that the differences between the ACE activity of each SCZ patient and the average mean value expected for each respective genotype subgroup of control patients was a better predictor of SCZ than the ACE dichotomized (high/low) values or ACE I/D genotype alone (Gadelha et al., 2015b). However, the interpretation of these several previous findings, including ours, is limited by the fact they all were conducted in chronic SCZ patients under treatment with antipsychotics, making it more difficult to separate the original characteristics from the disease from those determined by the treatment with antipsychotics.

The investigation of antipsychotic-naïve first-episode psychosis (FEP) patients is particularly helpful for understanding the biology at the onset of psychotic disorders. At this phase, before the intervention with antipsychotic medications, the potential to contribute to the evaluation of the disease before the influence of medication is recognized, and it may also allow the assessment of the pharmacotherapy effects in the follow-up studies (Anderson et al., 2014; Ito et al., 2015; Elowe and Conus 2017; Gay et al., 2017; Whale et al., 2017).

Therefore, the objective of the present study was to measure and compare the ACE activity levels in plasma of FEP patients before (FEP-B) and 2 months after the treatment with risperidone (FEB-2M), which were also compared with a healthy control (HC) group matched by sex and age. We aimed to correlate the ACE activity levels with the clinical aspects, including the response to the treatment with the atypical antipsychotic risperidone. Eventual differences due to the influence of ACE I/D polymorphism genotypes or age on ACE activity measures in FEP compared with HC individuals or due to the treatment with risperidone are also discussed herein.

Methods

Participants

This study was approved by the Research Ethics Committee of UNIFESP (CEP No. 1427/16), and written informed consent was obtained from all recruited participants. Clinical and laboratory investigations were strictly conducted according to the principles expressed in the Declaration of Helsinki. Antipsychotic-naïve FEP patients aged between 15 and 46 years old were recruited from an outpatient early psychosis clinic: the Centre for Integrated Mental Health of Santa Casa de São Paulo. The HC group was composed by mentally healthy volunteers recruited in a center for job-seeking assistance, and only individuals without any history of current or previous psychiatric diagnosis and with negative family history for severe psychiatric illness were included. The main inclusion and exclusion criteria are summarized in Figure 1.

The structured clinical interview for DSM-IV was applied by trained psychiatrists to confirm the diagnosis. The clinical evaluation also included the Positive and Negative Syndrome Scale (PANSS) (Higuchi et al., 2014), the Calgary Depression Scale for Schizophrenia (Bressan et al., 1998), the Global Assessment of Functioning and Global Clinical Impression (Lima et al., 2007), Young Mania Rating Scale. All available information, including medical records, was used for the diagnosis.

The blood of HC and FEP patients (before the introduction of antipsychotic therapy and after 2 months of treatment with risperidone, namely FEP-B and FEP-2M, respectively) was collected into heparin vacuum tubes (BD Vacutainer), and the plasma was processed essentially as previously described (Gadelha et al., 2013). In summary, the blood samples were immediately processed or kept at 4°C up to 12 hours, and the plasma was carefully recovered after centrifugation at 2000 × *g* for 10–15 minutes at room temperature. Plasma aliquots were stored in sterile plastic microtubes (Axygen Inc.) at –20°C

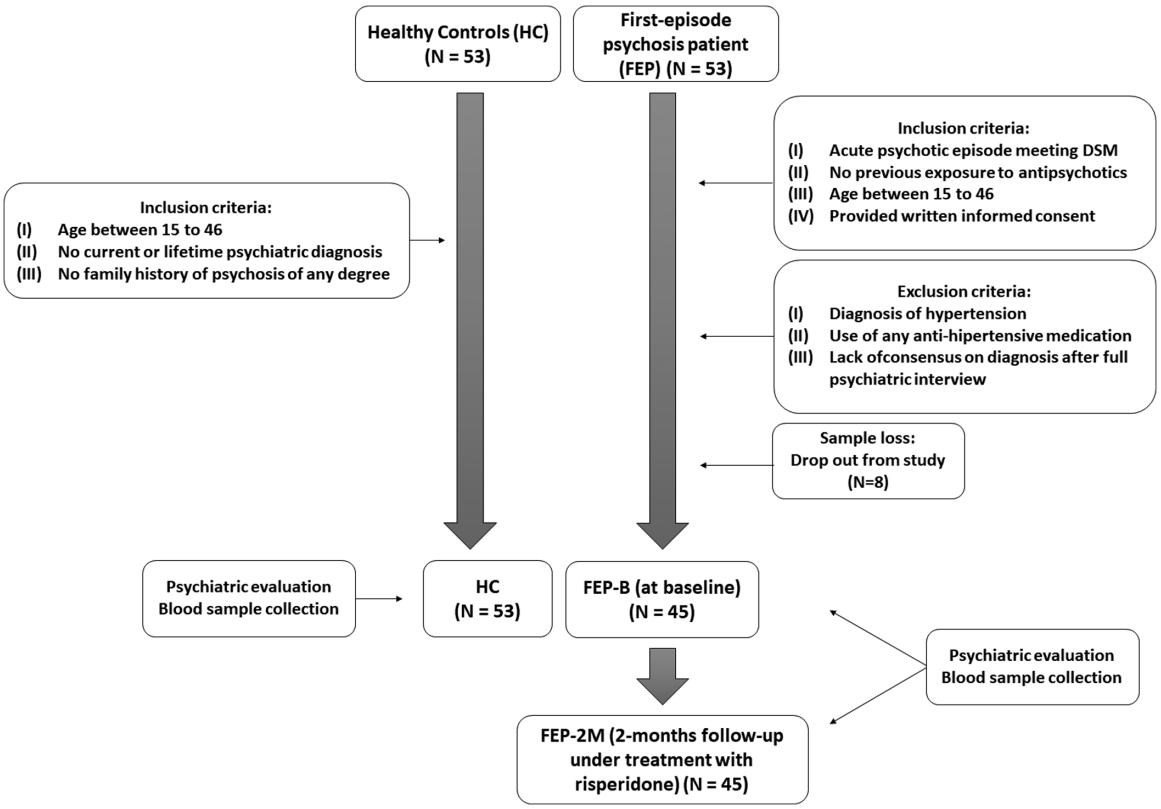

Figure 1. Flow chart of the study with the inclusion and exclusion criteria of samples and the follow-up assessment.

until use, when they were thawed in a wet ice bath before use. Previous work has assured the total ACE activity did not change for storage at –20°C and periods up to 6 months (Carmona et al., 2006; Gadelha et al., 2015b).

ACE Activity Measurements

The ACE activity was measured essentially as previously described (Gadelha et al., 2015b). The increase of fluorescence due to the substrate hydrolysis was measured at 37°C using a Hitachi F-7000 spectrofluorimeter (Hitachi Ltd., Ibaraki, Japan) at λ_{Ex}=420 nm and λ_{Em}=320 nm. The reaction was prepared on 96-wells black plate by adding 5 μL of the plasma in 200 μL of buffer (100 mM NaCl, 50 mM Tris-HCl, pH 7.4) and subsequent addition of 5 μM Abz-FRKP-EDDnp substrate in the absence or presence of 0.5 μM of ACE specific inhibitor lisinopril (Sigma-Aldrich, St Louis, MO). ACE activity was determined by the difference in the rate of hydrolysis, and the specific activity of ACE was confirmed by the addition of inhibitor lisinopril. ACE activity was expressed in nM/min. The ACE activity of antipsychotic-naïve FEP-B (at baseline) was compared with both risperidone-treated FEP-2M (2 months follow-up) and HC groups matched by age and sex.

DNA Extraction and Genotyping

DNA was isolated from fresh whole-blood samples by using a Gentra Puregene kit (Qiagen, Germantown, MD) strictly following the manufacturer's instructions. ACE I/D genotyping was performed using polymerase chain amplification technique followed by restriction fragment length polymorphism methodology as previously described (Gadelha et al., 2015b).

Statistical Analysis

The variable distribution was verified by the Gaussian distribution using the Kolmogorov-Smirnoff tests for the total sample and in each comparison group. Chi-square was adopted for categorical variables such as sex, ethnic background, and genotype frequency. To measure the mean differences in ACE activity between FEP patients and HC groups, we used the ANOVA 1-way test with the Tukey post-hoc comparison test. The possible associations of ACE activity and variables, such as the improvement of symptoms according to PANSS total scale, that present nonparametric distributions were investigated using the nonparametric correlation test (Spearman's Rho). Possible associations between ACE activity and polymorphism interactions were investigated by logistic regression models. Statistical significance was defined as $P \leq .05$. Data analyses were performed by using the SPSS Statistics software version 22.0 (IBM Corporation, Endicott, NY).

Results

Sociodemographic and Clinical Characteristics

No significant differences were observed for age and sex between the groups composed by (1) FEP (n=45) participants and (2) HC (n=53) volunteers, as shown in the sociodemographic characteristics table (Table 1). As one would expect for the incidence of a first psychosis episode, the members of the FEP group originally showed relatively low mean average age value (26 years old), varying between from 15 to 46 years old, without significant differences in the distribution between the groups (χ^2=2.790, P<.413, df=2) (Table 1). However, the HC group was

Table 1. Sociodemographic Characteristics of the Groups

		HC (N = 53)		FEP (N = 45)		Statistics		
		n	%	n	%	Test value	*P* value	df
Gender	Male	27	51	28	62	0.322	.289	1
	Female	26	49	17	38			
Educational level (years of education)	≤10	23	43	37	81	0.361	< .001[a]	1
	>10	30	57	8	19			
Ethnic background	Caucasian	35	66	23	51	3.152	.603	1
	Non-Caucasian	18	34	22	49			
Age, M (SD)	Years	27 (7)		26 (8)		0.557	.457	1
	Min	16		15				
	Max	44		46				

Abbreviations: df, degrees of freedom; FEP, first-episode psychosis; HC, healthy control.
[a]Statistical significance was defined as $P \leq .01$.

Table 2. Clinical Characteristics in FEP Group

		FEP (n = 45)		Statistics	
		FEP-B	FEP-2M	Test value	P value
Panns	Total	93.54 (22.43)	67.30 (22.97)	45.88	<.001[a]
	Positive	26.59 (7.18)	14.08 (6.36)	86.53	<.001[a]
	Negative	21.03 (7.18)	19.68 (8.21)	1.55	.221
	Depression	25.68 (12.85)	16.67 (7.74)	147.65	<.001[a]
	Disorganization	26.94 (7.93)	19.79 (6.95)	349.91	<.001[a]
	Excitement	29.54 (13.61)	14.32 (6.92)	35.60	<.001[a]
CDSS		4.08 (4.71)	3.15 (4.76)	0.74	.398
GAF		29.83 (13.01)	56.93 (18.75)	48.01	<.001[a]
CGI		5.10 (0.91)	3.42 (1.39)	38.05	<.001[a]
YMRS		11.00 (10.27)	1.50 (1.20)	86.53	<.001[a]

Abbreviations: CDSS, Calgary Depression Scale for Schizophrenia; CGI, Clinical Global Impression; FEP-B, FEP patients at baseline before treatment; FEP, first-episode psychosis; FEP-2M, FEP patients after 2 months of treatment with antipsychotics; GAF, Global Assessment of Functioning; PANSS, Positive and Negative Syndrome Scale; YMRS, Young Mania Rating Scale.
[a]Statistical significance was defined as $P \leq .01$.

composed of better educated individuals relative to the FEP group ($t = 0.361$, $df = 1$, $P < .001$), which was determined by the difficulties/limitation we have faced to recruit mentally healthy young volunteers in the general population paired for age, as we mainly recruit them in a center for job-seeking assistance. Therefore, as an alternative, we had to include several students from our university (medical school) in the healthy volunteers group studied here.

All FEP participants had blood collected at baseline (FEP-B group), and soon after they were primarily medicated with risperidone (1–4 mg). Blood from all these FEP participants was collected again after 2 months of treatment (FEP-2M). At this point, 28 participants (about 60%) maintained exclusively the use of this atypical antipsychotic risperidone, while 17 FEP participants (about 40%) had the atypical antipsychotic treatment changed to olanzapine. More importantly, despite this change in medication, which was motivated by several reasons (including due to nonresponse and/or adverse side effects), a significant improvement of the general symptoms assessment (PANSS, Calgary Depression Scale for Schizophrenia, Global Assessment of Functioning, Global Clinical Impression, and YMRS) scores of all FEP patients was observed after the 2 months of treatment with risperidone (Table 2). In fact, a significant improvement of positive, depression, disorganization, and excitement dimensions in PANSS was observed, but with no changes in negative symptoms (Table 2). As the medication change mostly occurred at the 2-month clinical follow-up and no blood samples were further collected after this medication change, there was no reason to find any correlation between this change in medication and ACE activity, as in fact, it was not observed.

ACE Activity in Plasma of FEP and HC Individuals

The mean value of ACE activity significantly differed among the FEP-B, FEP-2M, and HC groups ($F = 5.356$, $df = 2$, $P = .005$). FEP at baseline before the treatment with antipsychotics (FEP-B) showed only a numerically and nonsignificant trend toward increased levels of ACE activity compared with HC (post-hoc Tukey's multiple comparisons test, $P = .160$). However, this ACE activity was significantly higher after the 2-month treatment with risperidone, as observed for the comparisons between HC and the FEP-2M (post-hoc Tukey's multiple comparisons test, $P = .004$) but not for comparisons between the FEP-B at baseline and FEP-2M (post-hoc Tukey's multiple comparisons test, $P = .350$) (Figure 2).

Moreover, no significant correlation between ACE activity level increases and symptom improvement, as evidenced by the total PANSS score decreases, was noticed for FEP individuals after the 2-month treatment with risperidone ($r = -0.131$, $P = .434$) (Figure 3).

ACE Activity in FEP and HC Groups Separated by Genotype

Polymorphism analysis of our present cohort showed that the distribution of ACE Insertion/Deletion (I/D) genotype between the FEP (DD 22%, DI 53%, II 25%) and HC (DD 36%, DI 43%, II 21%) groups was not statistically different (chi-squared = 2.17, $df = 2$, $P = .337$). As expected, a higher prevalence of DI genotype was observed in both HC (n = 23, 43%) and FEP (n = 24, 53%) groups, but with no statistical difference in ACE activity levels between the HC and FEP with DI genotype subgroups either before or after the treatment for 2 months with the atypical antipsychotic risperidone (Figure 4).

In addition, as one would expect, the HC individuals with II genotype showed the lowest levels of ACE activity ($m = 6.15 \pm 3.12$ nM/min), which was also significantly lower compared with DD or DI subgroups of HCs ($t = 3.783$, $df = 51$, $P = .030$). On the other hand, the ACE activity in FEP individuals with II genotype was not significantly lower than DD or DI

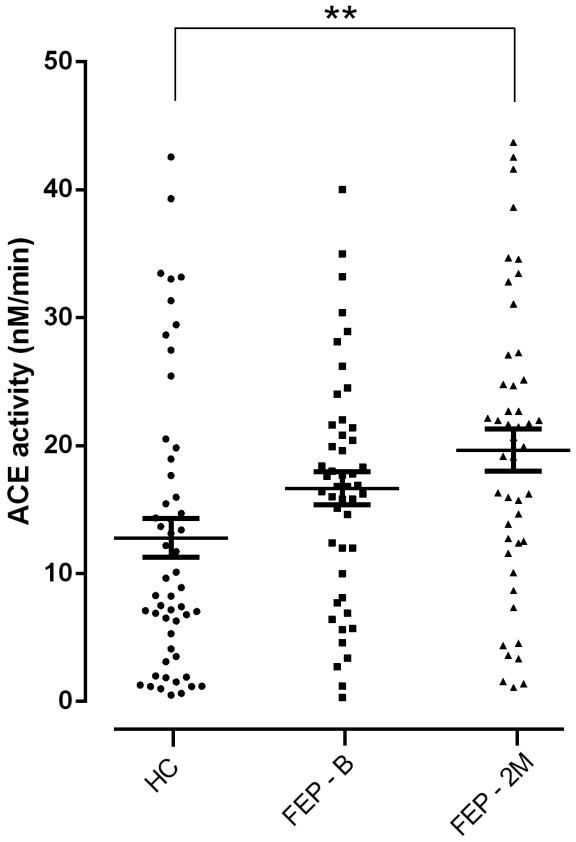

Figure 2. Scatter plot for angiotensin I-converting enzyme (ACE) activity measurements. ACE activity was measured in healthy controls (HC) and first-episode psychosis (FEP) participants before (FEP-B, antipsychotic-naïve individuals) and after treatment for 2 months with risperidone (FEP-2M). Despite the statistical difference between HC and FEP after treatment, no significant differences in the mean value for ACE activity were observed between the FEP-B and FEB-2M groups. One-way ANOVA, Tukey post-hoc comparison test, $^{*}P \leq .05$ and $^{**}P \leq .01$.

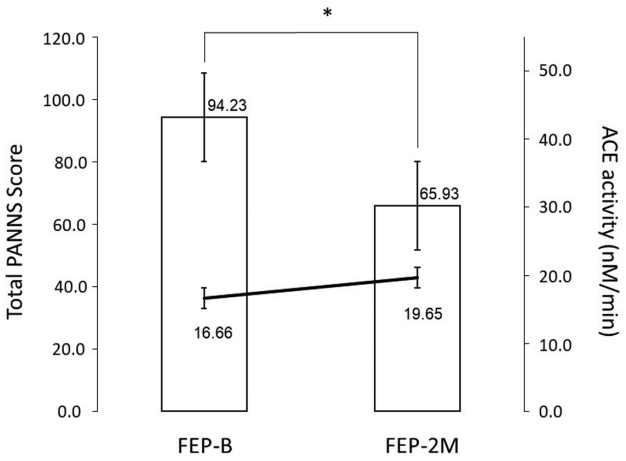

Figure 3. Angiotensin I-converting enzyme (ACE) activity levels and clinical symptoms improvement of first-episode psychosis (FEP) group participants. The statistically significant reduction of symptoms after 2 months of treatment with antipsychotics (FEP-2M) compared with baseline antipsychotic-naïve individuals (FEP-B), as evaluated by total Positive and Negative Syndrome Scale (PANSS) score, is indicated by the bar, while the levels of ACE activity are depicted by the continuous bold line. Paired Student's *t* test analysis, with $^{*}P \leq .05$ for comparisons with FEP-B.

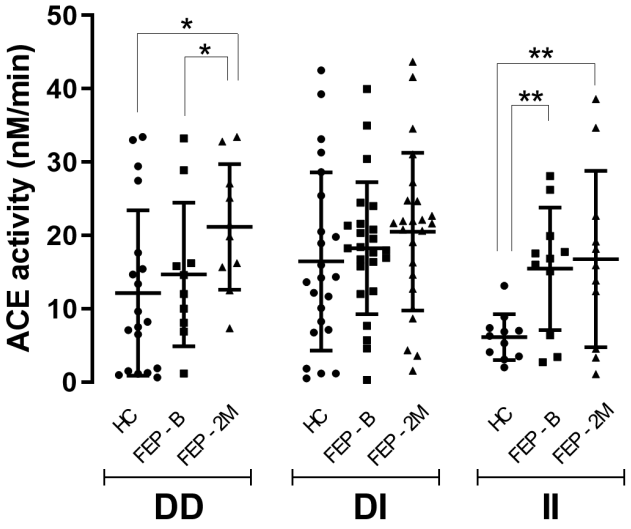

Figure 4. Mean values for angiotensin I-converting enzyme (ACE) activity for each genotype subgroup from the healthy controls (HC), first-episode psychosis (FEP) participants before (FEP-B, antipsychotic-naïve individuals) and after treatment for 2 months with risperidone (FEP-2M) groups. A prevalence of the DI genotype was observed in both groups (HCs and FEP), and a higher ACE activity level in FEP after the treatment (FEP-2M) was noticed only for the DD genotype subgroup. The II genotype subgroup for HCs presented the lowest ACE enzyme activity levels and it is statistically lower compared with the DI genotype; this lower activity in II genotype could not be observed in the FEP group, either at baseline (FEP-B) or after the treatment for 2 months (FEP-2M). One-way ANOVA, Tukey post-hoc comparison test, $^{*}P \leq .05$ and $^{**}P \leq .01$.

subgroups of FEP ($t=0.909$, $df=84$, $P=.479$), either before (FEP-B, $m=15.47 \pm 8.34$ nM/min) or after the 2-month treatment with risperidone (FEP-2M, $m=16.78 \pm 12.02$ nM/min) (Figure 4).

Interestingly, when ACE activity was analyzed separately for each ACE I/D genotype, a significant increase was observed only for DD genotype subgroup of FEP individuals ($t=2.406$, $df=9$, $P=.043$) (Figure 4), suggesting a possible strong influence of the I allele or the heterozygosity for this response, which may possibly explain the lack of significative differences in ACE activity of the entire FEP group after the treatment with risperidone (Figure 2). However, considering the trend for a higher ACE activity throughout treatment with risperidone, it would be possible to expect that these differences would become statistically significant most probably only after longer periods of follow-up than those employed in the present analysis.

In addition, the DD genotype subgroup showed differences in ACE activity only between HC and FEP-2M ($t=2.206$, $df=27$, $P=.036$) but not between HC and FEP-B at baseline ($t=0.381$, $df=27$, $P=.705$), reinforcing the effect of the genotype in the degree of ACE activity changes after the treatment with risperidone. On the other hand, ACE activity of the DI genotype subgroup did not show differences for comparisons between HC and FEP either before (FEP-B) or after the treatment with risperidone for 2 months (FEP-2M) (Figure 4). Interestingly, II genotype FEP individuals also showed higher ACE activity observed either before (FEP-B) ($t=2.839$, $df=20$, $P=.010$) or after the treatment for 2 months (FEP-2M) ($t=3.468$, $df=20$, $P=.002$) compared with HC volunteers (Figure 4), and this could potentially explain why the ACE activity could not be significantly increased by the treatment with risperidone.

A multiple linear regression analysis was also performed to evaluate if it was possible to predict the different groups (HC, FEP-B, and FEP-2M) considering the ACE activity and genotype

Table 3. Frequency of Genotypes, ACE Activity (nM/min) and PANSS in FEP Before (FEP-B) and After (FEP-2M) Treatment for 2-Months with the Atypical Antipsychotic Risperidone

FEP participants (n = 45)					
	FEP-B	FEP-2M	Statistics		
Genotype	DD		t	*P* value	df
Frequency (%)	10 (22.2)		2.311	<.046[a]	9
ACE activity, mean (SD)	14.7 (11.3)	21.2 (8.6)			
PANSS total, mean (SD)	92.0 (14.2)	69.8 (20.1)	3.083	<.013[a]	
Correlation	Spearman's		Rho = 0.176	.627	
Genotype	DI		t	*P* value	df
Frequency (%)	24 (53.3)		0.716	.480	23
ACE activity, mean (SD)	18.3 (9.2)	20.5 (10.8)			
PANSS total, mean (SD)	92.5 (15.2)	66.5 (18.1)	6.804	<.001[b]	
Correlation	Spearman's		Rho = −0.349	.094	
Genotype	II		t	*P* value	df
Frequency (%)	11 (24.5)		0.362	.724	10
ACE activity, mean (SD)	15.5 (8.4)	16.8 (12.1)			
PANSS total, mean (SD)	97.8 (25.4)	61.5 (22.0)	4.976	<.001[b]	
Correlation	Spearman's		Rho = 0.348	.295	

Abbreviations: ACE, angiotensin I-converting enzyme; DD, deletion/deletion; df, degrees of freedom; FEP-B, FEP patients at baseline before treatment; FEP, first-episode psychosis; FEP-2M, FEP patients after 2 months of treatment with antipsychotics; ID, deletion/insertion; II, insertion/insertion; PANSS, Positive and Negative Syndrome Scale.

*a*Statistical significance was defined as $P \leq .05$

*b*Statistical significance was defined as $P \leq .01$

as prediction variables. Although the genotype could not predict these groups (|t| = 1.507; *P* = .134), the ACE activity alone did (|t| = 3.467; *P* < .0007). However, even considering the genotypes, we could not find any correlation between ACE activity and symptom improvement as evidenced by the total PANSS (Table 3).

ACE Activity in FEP Stratified by Age

Significant differences in ACE activity were not observed between HC and FEP-B subgrouped by age (t = 1.405, df = 5, *P* = .229), and there was only a significant difference between the FEP and HCs for the oldest subgroup (35–45 years) (post-hoc Tukey's multiple comparisons test, *P* = .009) (Figure 5A). On the other hand, significant differences in ACE activity were observed between the HC and FEP-2M (t = 3.259, df = 5, *P* = .009) groups and also between the subgroup 15 to 25 years (post-hoc Tukey's multiple comparisons test, *P* = .04) and subgroup 35 to 45 years (post-hoc Tukey's multiple comparisons test, *P* = .02) (Figure 5B). Interestingly, FEP-0 and FEP-2M showed a significant difference between the subgroups (t = 2.580, df = 5, *P* = .032), but also a significant difference in the increase of ACE activity in the subgroup at ages younger than 25 years old was observed after treatment with antipsychotics regardless of the genotype (post-hoc Tukey's multiple comparisons test, *P* = .02) (Figure 5C).

Discussion

In the present work, we observed that the ACE activity measured in antipsychotic-naïve FEP-B did not significantly differ from the HC group (Figure 2), but this activity significantly increased in the FEP-2M group (Figure 2; supplemental Figure 1), with an interesting influence of the age on this effect (Figure 5). In spite of the lack of statistically significant differences noticed in total FEP-B compared with the HC group, significantly higher ACE activity was observed between the FEP-B and HC groups (at ages older than the 35 years subgroup) and between the antipsychotic-treated FEP-2M and HC groups (at ages younger than 25 years and older than 35 years subgroups) (Figure 5A,B). On the other hand, when we compared the FEP-B and FEP-2M groups, the subgroups at ages younger than 25 years old showed significant increases of ACE activity after the treatment (Figure 5C), and this could be signaling an important influence of age and the possible cognitive reservoir or recovery ability (which could be decreased with ageing) of these individuals to the deleterious effects eventually imposed by the long-lasting treatment or by disease progression (Bartrés-Faz et al., 2000; Basso et al., 2005; Yasar et al., 2018).

Moreover, if subgrouped by ACE I/D genotype, we noticed a significant higher ACE activity in the II genotype subgroup of FEP compared with HCs, even before (at baseline) or after the treatment, while the other genotype subgroups did not show significant differences, possibly suggesting the influence of the genotype for the ACE activity levels regardless of the treatment. On the other hand, the effect of the treatment in FEP individuals was statistically significant only for the DD genotype FEP subgroup, whereas the other I allele carriers, namely DI or II genotype subgroups, showed no significant change in ACE activity even after the treatment for 2 months with the antipsychotic risperidone (Figure 4; supplemental Figure 1), suggesting a higher response for the DD genotype FEP subgroup to the treatment with this antipsychotic, regardless of the symptom amelioration. Although in chronic SCZ, the ACE activity was significantly lower in II genotype FEP patients compared with the D allele carrier subgroups of HC individuals (Gadelha et al., 2015b), the II genotype FEP subgroup did not show any difference in average ACE activity compared with the D allele carrier subgroups either before or after the treatment with risperidone (Figure 4; supplemental Figure 2), as the effect of the treatment was in fact more prominent in the DD genotype FEP subgroup relative to other I allele carrier subgroups (Figure 4). Taken together, these data suggest that the differences in ACE activity between the SCZ/FEP and HC existed even before the intervention with antipsychotics,

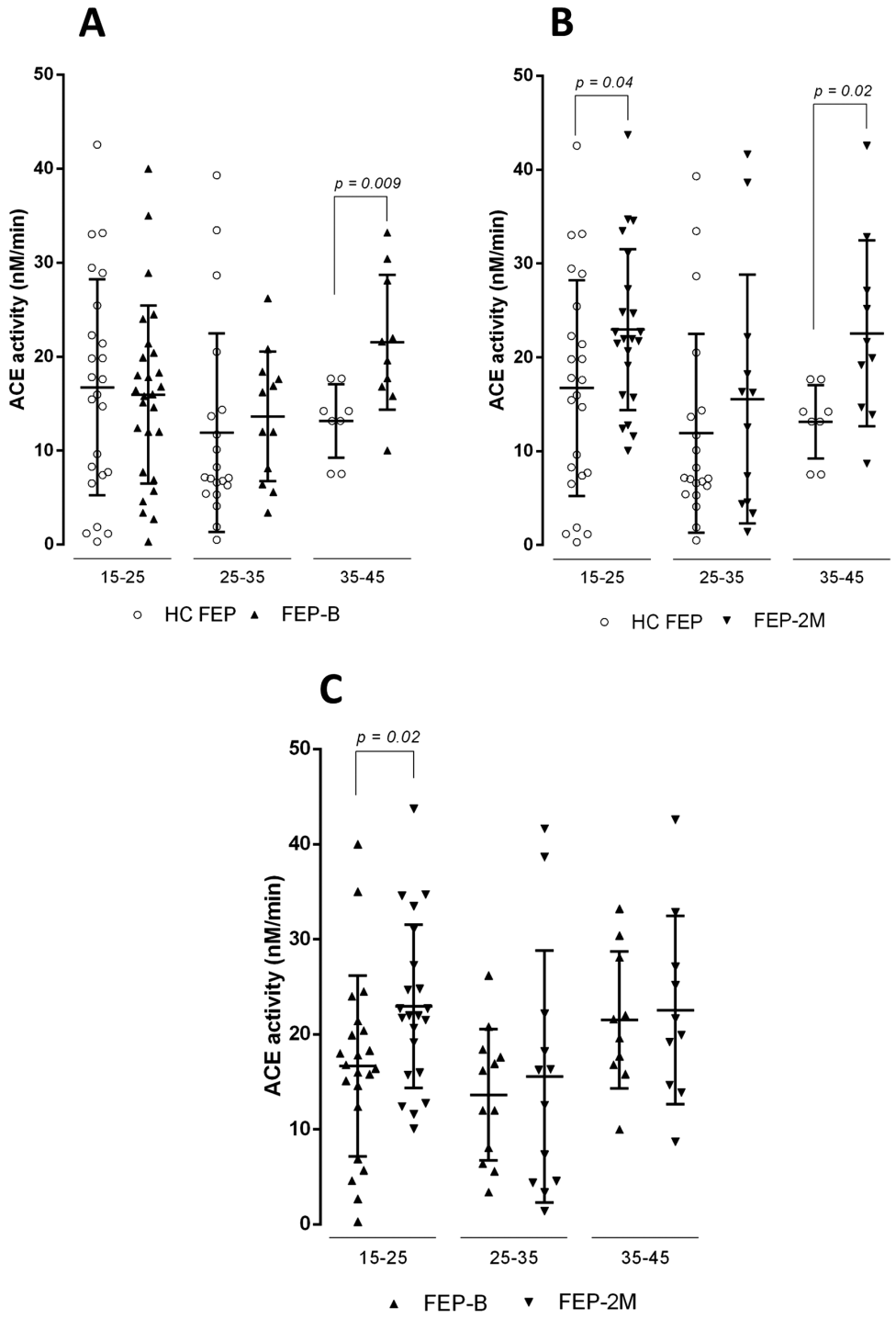

Figure 5. Scatter plot for ACE activity measurements of first-episode psychosis (FEP) patients stratified by age. ACE activity was measured in healthy controls (HC), FEP before (FEP-B, antipsychotic-naïve individuals) and after treatment for 2 months (FEP-2M). The samples were separated based on their age into 3 subgroups of similar size. The significant differences between the FEP and HC groups are indicated by the asterisks (*). *$P < .05$ for 1-way ANOVA.

which is also clearly influenced by the treatment, with more or less intensity depending on the ACE I/D genotype or age and also on the original level of ACE activity at baseline. In other words, it seems that only initially lower levels of ACE activity are susceptible to increases determined by the treatment. In fact, the effect of treatment on ACE activity was not the same for all ACE I/D genotype or age subgroups, and treatment with risperidone significantly changed the ACE activity only for the DD genotype FEP subgroup ($t = 2.311$, $df = 9$, $P = .046$), while other I allele carrier genotypes showed only a trend for increases in ACE activity (Figure 4). Hence, we could demonstrate here that increases of ACE activity due to the treatment with antipsychotics also varies with the ACE I/D genotype in FEP, which is in line with our previous study in chronic treated SCZ (Gadelha et al., 2015b). However, no significant correlation could be observed between the increases of ACE activity and

the symptom improvement determined by the treatment with risperidone (r = –0.131, P = .434), as denoted by the significative decreases of PANSS scores (t = 45.88, df = 1, P < .001) (Figure 3). We believe this possibly could be due to the modest increase of ACE activity observed in the present studied conditions, as determined by the limited sample size number or short period of follow-up (up to 2 months).

The association of ACE activity with the genotype was observed in populations with other mental disorders, such as depression (Firouzabadi et al., 2012). Despite the significant increases in ACE activity after the treatment with risperidone in FEP individuals with DD genotype, this treatment determined only a nonsignificant trend for higher ACE activity in D allele carrier FEPs as well as for the II genotype FEP subgroup, which showed significantly higher mean ACE activity value relative to the II genotype subgroup of FEP at baseline (i.e., even before treatment) (Figure 4; Table 3). In the present study, significant differences in ACE activity according to genotypes was observed only for the II genotype HC subgroup, in which a significant lower mean value for ACE activity was noticed (Tables 2 and 3; Figure 4; supplemental Figures 1 and 2). These differences between FEP and HC groups may be signaling for a possible association with worse cognitive functioning in FEP individuals compared with HCs, with a remarkable effect of the treatment in the DD genotype FEP subgroup, as we have also previously observed in chronic SCZ patients (Gadelha et al., 2015a). However, we recognize that an important limitation of this study is the relatively short-term follow-up and also the present unfeasibility to perform the cognitive measurements.

The atypical antipsychotic risperidone is often indicated for the treatment of SCZ symptoms due to its general good tolerability and efficacy in addition to the fact it is one of the cheapest antipsychotic currently available for clinical use (Gilbody et al., 2016). Herein, in the 2-month follow-up, risperidone needed to be replaced by olanzapine for the treatment of approximately 40% of the FEP patients due to diverse reasons (including nonresponse or unacceptable adverse effects), although no significant differences in ACE activity increases or total PANSS scores decreases could be observed compared with the other 60% of FEP patients continuing the treatment with risperidone. In addition, as discussed above, no significant correlations between symptoms and ACE activity could be observed, even when considering the genotypes subgroups separately, although this separation by genotype showed the important influence of D allele homozygosity for the more effective increases in ACE activity observed after the treatment with antipsychotics.

Our current data are still not sufficient to determine whether improvement in symptoms after the treatment with antipsychotics contributed to the observed increases in the ACE activity. However, considering our previous work (Gadelha et al., 2015a), we believe it is possible to hypothesize that the increased ACE activity 2 months after the FEP may be possibly signaling for a worse outcome and continuous progression of the disease, regardless of the improvement of symptoms as denoted by the significant decreases in PANSS scores after the treatment with risperidone (as shown here), which more likely cannot preclude the cognitive functioning decline or eventual impairments in cognition due to increased ACE activity as also reported by others (Poddar et al., 2020; Schaefer et al., 2020).

Interestingly, correlation analyses performed by others also showed that memory and working memory were more significantly associated with SCZ onset age, negative symptoms, and side effects in women, while processing speed correlated with antipsychotic dosage in men and side effects in women (Li et al., 2018), and this should be considered in our future work. In the case of cognition and functional outcomes, there is a debate regarding the maintenance and dose reduction/discontinuation of antipsychotics (Hori et al., 2018; Omachi and Sumiyoshi 2018; Fu et al., 2019).

Although further investigations on a large population are still necessary to test whether the D allele of the ACE gene polymorphism is susceptible to memory deterioration, it was possible to demonstrate here the importance of ACE I/D genotyping assessment to monitor the possible increased risk of cognitive worsening in SCZ patients under antipsychotic medication who may also require neurocognitive rehabilitation or specific add-on pharmacotherapy, to improve working memory, or to avoid impairments in cognition.

Herein, we recognize as an important limitation the sample size, the short-term follow-up analysis, and the inability to perform cognitive assessments. Therefore, further studies with a larger FEP cohort and increased follow-up period (at least 5 years or more), including cognitive measurements, is now envisioned to confirm the present hypothesis. Although technically challenging in our clinical practice for FEP individuals, the cognitive performance measurements by administration of a comprehensive neurocognitive battery would greatly add important information to the present report.

In addition, although the HC and FEP groups were matched for age and sex, there was a significantly higher number of years of education in the control HC group. Despite this, it is important to mention that we could not observe any correlation between the education level and ACE activity (rho = –275, P = .340). We also could not preclude other confounding factors, such as comorbidities or diet, among other individual peculiarities that could influence our present results, such as inflammation, which unfortunately could not be evaluated and controlled in the present study.

Taken together, our present data suggest the power of measuring the ACE activity in blood plasma, associated with ACE I/D genotyping, to support the follow-up of FEP or chronic SCZ individuals. These phenomena might be important for understanding the neuropathological mechanisms underlying the symptoms of SCZ and for supporting clinical decisions in FEP and chronic SCZ.

Supplementary Materials

Supplementary data are available at International Journal of Neuropsychopharmacology (IJNPPY) online.

Acknowledgments

The authors are grateful for the access to the spectrofluorimeter F7000 (Hitachi, Japan) used at the Department of Biophysics, UNIFESP/EPM. We also thank the executive secretary, Rosemary Alves de Oliveira, for great administrative support and Marcela Nering for technical assistance.

João V. Nani is a recipient of a fellowship from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, no. 2019/09207-3). Caroline Dal Mas received a fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Camila M. Yonamine was the recipient of a fellowship from FAPESP (no. 2012/08941-6). Mirian A. F. Hayashi was also supported by FAPESP (proc. nos. 2013/13392–4 and 2017/02413-1) and CNPq (proc. nos. 477760/2010–4, 557753/2010–4, 508113/2010–5, 311815/2012-0, 475739/2013-2).

Dr M. A. F. Hayashi is also the recipient of a fellowship from CNPq (311815/2012-0 and 309337/2016-0). Rodrigo Bressan and Sintia Belangero were supported by FAPESP (proc. no. 2017/25016–8). In addition, this study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES) - Finance Code 001.

Statement of Interest

The authors declare no conflicts of interest.

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