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Ndel1 oligopeptidase activity as a potential biomarker of early stages of schizophrenia

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ABSTRACT

Our previous studies showed reduced Ndel1 enzyme activity in patients with chronic schizophrenia (SCZ), and only a subtle *NDEL1* mRNA increases in antipsychotic-naïve first-episode psychosis (FEP) individuals compared to matched healthy controls (HC). Aiming to refine the evaluation of Ndel1 enzyme activity in early stages of psychosis, we compared 3 groups composed by (1) subjects at ultra-high-risk (UHR) for psychosis, (2) a cohort comprising antipsychotic-naïve FEP individuals (assessed in three moments, at baseline (FEP-0), and after 2 months (FEP-2 M) and one year (FEP-1Y) of treatment with risperidone), and (3) a HC group. There was no significant difference in Ndel1 enzyme activity between UHR and HC, but this activity was significantly lower in FEP compared to HC. Conversely, Ndel1 activity in HC groups was higher than in FEP even before (FEP-0) or after the treatment with risperidone (FEP-2 M and FEP-1Y), and with progressive decrease of Ndel1 activity and significant improvement of symptoms observed after this treatment. In addition, a positive correlation was observed for Ndel1 activity with clinical symptoms as assessed by PANSS, while a negative correlation was seen for GAF scores. Our results suggest that reductions in Ndel1 activity in FEP may be possibly related to responses to the illness, rather than to the pharmacological effects of antipsychotics, which might be acting essentially in the symptoms suppression. This hypothesis might be further evaluated in prospective long-term follow-up studies with a larger sample cohort.

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1. Introduction

In the last decade, scientific research in psychosis has experienced a major shift in its focus, and a better understanding of the trajectories from normal development to the first manifestations allowing the diagnosis of schizophrenia (SCZ) was found to offer new insight in the possibility of prevention. One of the most well established strategy to investigate early illness trajectories in psychosis is to evaluate putative prodromal stage also known as ultra-high risk (UHR) for psychosis, which may be composed by individuals who are genetically predisposed to psychosis due to a first degree relative affected by mental disorder or by any population presenting sub-threshold SCZ symptoms that may be detectable weeks, months or even years before the first episode of psychosis (FEP) (Yung and McGorry, 1996; Yung et al., 1998; Yung et al., 2005). The assessment of individuals in FEP, especially before the confounder effect of antipsychotic medications, is also crucial to evaluate the disorder progression and to identify reliable biomarkers of illness progression.

SCZ trajectory is shaped by a multifactorial combination of genetic and environmental factors continually influencing the neurodevelopment and neuroprogression (Davis et al., 2014; Stein and Broome, 2015). Several

Abbreviations: SCZ, schizophrenia; UHR, ultra-high-risk; FEP, first episode of psychosis; FEP-0, FEP patients at baseline; FEP-2 M, FEP patients after 2 months of treatment; FEP-1Y, FEP patients after 1 year of treatment; HC, healthy control; BD, Bipolar Disorder; APS, attenuated positive symptoms; Ndel1, Nuclear distribution element-like 1; *DISC1*, *Disrupted-in-Schizophrenia 1*; SD, standard deviation; AUF, arbitrary units of fluorescence; GEE, generalized estimating equation; PANSS, Positive and Negative Syndrome Scale; CGI, Clinical Global Impression; GAF, Global Assessment of Functioning; CDSS, Calgary Depression Scale for Schizophrenia; YMRS, Young Mania Rating Scale; MADRS, Montgomery-Åsberg Depression Rating Scale; BMI, body mass index; SPSS, Statistical Package for Social Sciences.

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risk genes potentially associated to SCZ have been identified, and *Disrupted-in-Schizophrenia 1* (*DISC1*) gene is among the most investigated genetic factor (Brandon and Sawa, 2011; Niwa et al., 2016; Tomoda et al., 2017; Dahoun et al., 2017). The major ligand of the *DISC1* protein product is the nuclear distribution element-like 1 (Ndel1), which possesses enzyme activity and cleaves neuropeptides as bradykinin and neurotensin that also have essential roles in neurogenesis and antipsychotic response, respectively (Hayashi et al., 2005; Camargo et al., 2007; Hayashi et al., 2015; Bradshaw and Hayashi, 2017). Ndel1 and DISC1 complex formation is critical for neuritogenesis, neurite outgrowth and neuronal migration (Ozeki et al., 2004; Duan et al., 2007; Hayashi et al., 2010; Bradshaw and Hayashi, 2017). Failures in Ndel1/DISC1 interactions can affect the formation of brain structures during embryogenesis (Kamiya et al., 2005; Kamiya et al., 2006; Bradshaw and Hayashi, 2017). Interestingly, the Ndel1-DISC1 complex formation also modulates Ndel1 enzyme activity (Hayashi et al., 2005), whose suppression was demonstrated to decrease neurite outgrowth *in vitro* (Hayashi et al., 2010; Bradshaw and Hayashi, 2017).

Previous studies of the group showed subtle *NDEL1* mRNA increases in FEP individuals compared to healthy controls (HC) (Ota et al., 2015), while reduced Ndel1 enzyme activity was demonstrated by us in chronic SCZ patients with no significant association to any specific clinical aspects or to the employed antipsychotics (Gadelha et al., 2013). Considering the low Ndel1 activity in chronic SCZ, we hypothesized a possible progressive decrease of Ndel1 activity among HC, UHR and FEP subjects, with a relative improvement in FEP after risperidone treatment.

Therefore, the objective of this study was to evaluate and compare the Ndel1 oligopeptidase activity levels in three different groups, namely HC, UHR for psychosis, and antipsychotic-naïve FEP individuals, who were also assessed along the treatment with risperidone. In addition, we investigated the Ndel1 activity levels as a possible biomarker of early stages of SCZ and the association of Ndel1 activity levels with symptoms severity before and after the treatment with risperidone.

2. Methods

2.1. Subjects

This study was approved by the Research Ethics Committee of UNIFESP [CEP No. 1427/16]. A written informed consent was obtained from all participants or their representatives, prior their inclusion. Clinical and laboratory investigations were strictly conducted according to the principles expressed in the Declaration of Helsinki.

Exclusion criteria were the same for all groups, as listed: (i) presence of neurological and general medical comorbidities, (ii) history of brain traumatic injury, (iii) presence of intellectual disability, or (iv) pregnancy or post-partum period.

2.1.1. Ultra-high-risk (UHR) subjects

The UHR group involved male and female help-seeking subjects, with ages between 14 and 26 years old, who were regularly attending a high-risk research clinic, in the PRISMA Early Intervention Program. The classification of UHR was confirmed by Comprehensive Assessment of At-Risk Mental States (CAARMS), and individuals were classified as: (i) attenuated positive symptoms (APS), (ii) brief limited intermittent psychotic syndrome (BLIPS), or (iii) 1st degree relative with psychotic disorder + functional decline in the last year (HDec) (Yung and McGorry, 1996; McGlashan and Johannessen, 1996; Klosterkötter et al., 2001; Yung et al., 2008). The development of psychosis or mania in UHR subjects of the present study was monitored for up to 2 years. However, as UHR and first-episode psychosis (FEP) subjects were recruited at different clinical facilities, these individuals were not included in the FEP group of the present study.

2.1.2. First-episode of psychosis (FEP) subjects

Antipsychotic-naïve FEP subjects, aged between 15 and 42 years, were recruited from an outpatient early psychosis clinic, in the Centre for Integrated Mental Health of Santa Casa de São Paulo (CAISM). The inclusion and exclusion criteria for FEP subjects were described in details in the flowchart (Fig. 1).

For the follow-up study, the FEP subjects were assessed, for clinical evaluation and sample collection, two months (FEP-2 M, mean = 2.57 (SD = 1.27) months) and one-year (FEP-1Y, mean = 14.2 (SD = 3.19) months) after the treatment with risperidone (1–8 mg).

2.1.3. Healthy control (HC) group

HC group was composed by mentally health volunteers recruited in a center for job seeking assistance. Only individuals without any history of current or previous psychiatric diagnoses, and with negative family history for severe psychiatric illness were included. Although the HCs were matched to the UHR and FEP groups by sex, in this study, it was not possible to match all groups for age, mainly due to the relative low average age of UHR group and the consequent expected difficulties in recruiting young (mainly under age) HC volunteers. Nevertheless, we controlled for potential differences in age as covariates in all statistical analyzes.

2.2. Blood samples collection

Blood samples were collected from all subjects (i.e. HC, UHR and FEP) into heparin vacuum tubes (BD Vacutainer, BD, NJ, USA). The blood was collected from FEP subjects, at baseline (FEP-0) and also at 2 months (FEP-2 M) and one year (FEP-1Y) of treatment with risperidone (Fig. 1). The plasma was collected, prepared and stored essentially as previously described (Gadelha et al., 2013).

2.3. Ndel1 enzyme activity measurements

Ndel1 enzyme activity was measured essentially as previously described (Gadelha et al., 2013). Hydrolysis of substrate at 37 °C was monitored by measuring the fluorescence at λ_{Ex} = 420 nm and λ_{Em} = 320 nm, in a F-7000 spectrofluorimeter (Hitachi Ltd., Ibaraki, Japan). The Ndel1 activity (expressed here in nM/min) was calculated employing the following formula: nM/min = AUF/s × 60 s × 1000.

2.4. Data analysis

Parametric (Student's *t*-test) and non-parametric (Spearman's Rho) tests were used accordingly to variables distribution. Chi-square was adopted for categorical variables, such as sex and ethnic background. Numerical variables as age, years of education and scales of symptoms mean values were analyzed using ANOVA one-way test and post-hoc Bonferroni. Ndel1 enzyme activity was analyzed using ANCOVA and the age and sex were considered as covariates comparing the differences among the HC, UHR, FEP-0, FEP-2 M and FEP-1Y groups. We considered as criteria for exclusion of outliers, only values >5 times the standard deviation (SD). All distribution was checked using Kolmogorov-Smirnoff test.

In order to compare the FEP groups before and after the treatment, we verified the correlation of Ndel1 enzyme activity with clinical aspects, age, and year of studies, using Spearman's Rho correlation. Generalized estimating equation (GEE) models were used to assess within- and between-groups effects for longitudinal data analysis of Ndel1 enzyme activity. Due to the non-normal distribution of Ndel1 values, with a positively skewed distribution, we used gamma models, with log link specification, and an autoregressive covariance structure (AR-1), which best fit the data. The independent variables were group (i.e. HC, UHR and FEP) and time (which applied only for FEP). Age and gender were included as covariates. Due to the non-linearity of the models, the estimated β coefficients were transformed into rate ratio (RR)

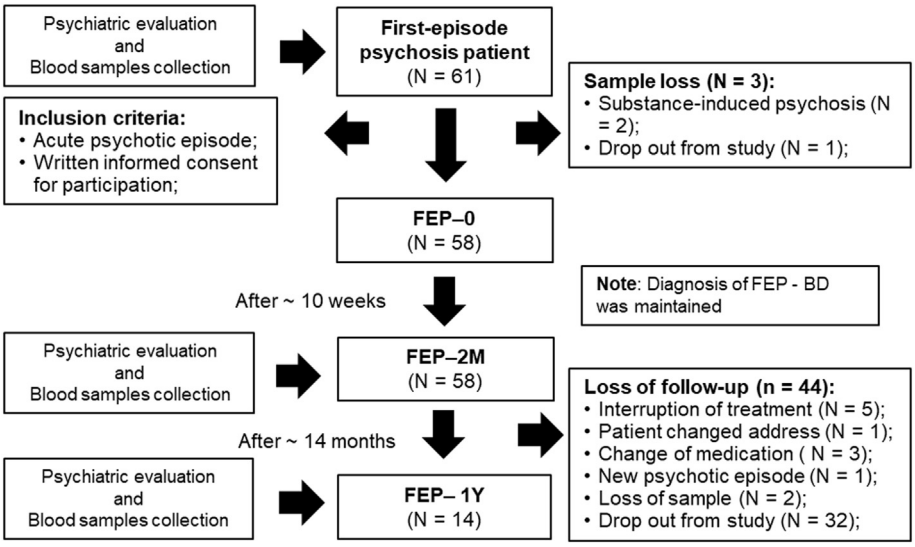

Fig. 1. Flow chart of the study with the inclusion and exclusion criteria of samples and the reason for loss of patients in FEP during the follow-up assessment.

estimates. Differences were considered significant for values of $p \leq 0.05$. Data analyses were performed using the SPSS Version 14.0.

3. Results

3.1. Demographic characteristics of the groups

The UHR group was composed by 15 individuals, from which 12 were classified as attenuated positive symptoms (APS), one presented brief and intermittent psychotic symptoms (BLIPS), and one patient was reported to have genetic risk plus functional decline in the last year (HDec). At the blood collection time, 6 UHR subjects were under the use of anti-depressive or antipsychotic medication, while other 9 individuals were not medicated. In the following 2-years after the first assessment, only 3 individuals converted to FEP, and they were identified as UHR-FEP and UHR-BD in Fig. 2. None of them was included into the FEP group.

From a total of 61 FEP subjects selected for this study, 3 were excluded or abandoned the study at baseline (FEP-0), and a total of 58 FEP subjects completing 2-months follow-up were considered for the present analysis. From those, only 14 FEP subjects completed the one-year follow-up. The reasons for this high rate of losses (N = 44) are presented in the flowchart, and the most frequent reason was not related to clinical reasons (see Fig. 1). In this FEP sample, six patients fulfilling these criteria at baseline (FEP-0) received the diagnosis of bipolar disorder (BD) in the first follow-up assessment (FEP-2 M), and they were maintained in the study (identified in Fig. 2 as FEP-BD).

The average age of each group composed by 15 UHR, 58 FEP and 102 HC individuals was 17.9 (SD = 3.4), 25.9 (SD = 7.3) and 29.1 (SD = 6.5) years, respectively ($F = 20.8$, $df = 2$, $p = 0.001$) (Table 1). On the other hand, we did not observe significant difference for sex ($Chi^2 = 2.74$, $df = 2$, $p = 0.256$) or ethnic background ($Chi^2 = 0.05$, $df = 2$, $p = 0.972$) among these groups.

3.2. Ndel1 enzyme activity in plasma of UHR, FEP and HC individuals

The mean value for Ndel1 enzyme activity, adjusted by age and sex, was significantly different among the UHR, FEP-0, FEP-2 M, FEP-1Y and HC groups ($F_1 = 14.09$, $df = 6$, $p = 0.001$; $\eta^2 = 0.273$). The Ndel1 enzyme activity of FEP-0 group was significantly lower compared to HC (post-hoc Bonferroni, $p = 0.001$), but not compared to UHR (post-hoc Bonferroni, $p = 0.239$). However, no significant difference was observed between HC and UHR groups (post-hoc Bonferroni, $p = 0.999$). Significant lower Ndel1 enzyme activity was observed in FEP-2 M compared to HC and UHR (post-hoc Bonferroni, $p = 0.001$ and $p = 0.023$, respectively), and in FEP-1Y compared to HC and UHR (post-hoc Bonferroni, $p = 0.001$ and $p = 0.015$, respectively) groups (Fig. 2). In addition, the Ndel1 activity was not significantly different between the non-medicated ($N = 9$) and medicated ($N = 6$) UHR subgroups, composed by subjects using anti-depressive or antipsychotic medication ($t = 0.171$, $df = 1$, $p = 0.686$).

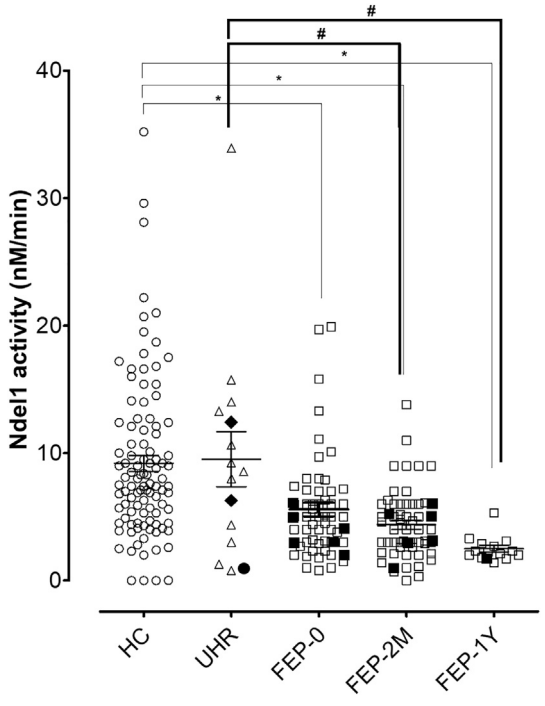

Fig. 2. Scatter plot for Ndel1 enzyme activity measurements. Ndel1 enzyme activity were measured in plasma of healthy controls (HC, ○) and compared to ultra-high-risk (UHR, △), and first episode of psychosis (FEP, □) at baseline (FEP-0), and 2 months, (FEP-2 M) and one year (FEP-1Y) after treatment with risperidone. UHR subjects with diagnosis for FEP (UHR-FEP, ♦) or bipolar disorder (UHR–BD, ●) and FEP subjects with diagnosis for bipolar disorder (FEP-BD, ■), are identified. Differences were considered significant for $p \leq 0.05$, after ANOVA analysis adjusted for age and sex, and post-hoc Bonferroni, for comparisons to *HC, #UHR, and §FEP-0.

Table 1
Sociodemographic characteristics.

		HC	UHR	FEP	Statistics			
		N = 102	N = 15	N = 58		Test value	df	*p*-value
Sex, N(%)	Male	64 (63)	11 (73)	42 (72)	All Groups[1]	2.74	2	0.256
					HC × UHR[1]	0.63	1	0.428
	Female	38 (37)	4 (27)	14 (28)	HC × FEP[1]	2.45	1	0.116
					UHR × FEP[1]	0.01	1	0.895
Educational Level, mean (SD)	≤10[a]	10 (13)	8 (57)	26 (44)	All groups[1]	13.9	2	0.001**
					HC × UHR[1]	14.5	1	0.001**
	≥10[a]	67 (87)	6 (43)	28 (56)	HC × FEP[1]	19.6	1	0.001**
					UHR × FEP[1]	0.35	1	0.359
Age, mean (SD)	Years	29.1 (6.5)	17.9 (3.4)	25.9 (7.3)	All Groups[2]	20.08	2	0.001**
					HC × UHR[3]	6.7	2	0.001**
					HC × FEP[3]	3.6	2	0.013*
					UHR × FEP[3]	4.3	2	0.001**
Ethnic background, N (%)	Caucasian	41 (63)	8 (61)	28 (60)	All groups[1]	0.05	2	0.972
					HC × UHR[1]	0.01	1	0.910
	Non-caucasian	24 (37)	5 (39)	18 (40)	HC × FEP[1]	0.05	1	0.813
					UHR × FEP[1]	0.001	1	0.965

SD = standard deviation.
[a] Years of education.
* $p \le 0.05$.
** $p \le 0.001$.
[1] Chi-square test.
[2] ANOVA one-way test.
[3] post-hoc Bonferroni.

Although we did not observe significant differences between the UHR and FEP-0 groups after analysis of multiple comparisons considering all groups, namely HC, UHR and FEP (including FEP-0, FEP-2 M and FEP-1Y) (Fig. 2), the mean values for Ndel1 enzyme activity of these two groups were numerically different (UHR, mean = 9.5 (SD = 8.35) nM/min, and FEP-0, mean = 5.57 (SD = 4.06) nM/min). Therefore, we performed a comparative analysis only for FEP-0 and UHR, which confirmed that this difference was statistically different between these groups after ANCOVA analysis ($F_1 = 3.19$; df = 3; $p = 0.029$, $\eta^2 = 0.127$).

FEP group was assessed at three different moments, namely FEP-0 (baseline), FEP-2 M and FEP-1Y under treatment with risperidone, showing a statistically significant reduction in Ndel1 enzyme activity only between FEP-0 and FEP-1Y (post-hoc Bonferroni, $p \le 0.05$) (Fig. 3), and ANCOVA analysis for these three groups suggested a significant progressive decrease in the Ndel1 activity values along the treatment ($F_1 = 3.44$, df = 4, $p = 0.011$; $\eta^2 = 0.108$). Interestingly, 6 patients from FEP-0 group, who had the diagnosis for BD (FEP-BD) confirmed (as identified in Fig. 2), did not show a significant change in the Ndel1 activity mean values along the treatment with risperidone (t-Student test, $t = 0.250$, df = 5, $p = 0.812$, for FEP-2 M compared to FEP-0) (Supplemental Fig. 1). As only one single FEP-BD patient could be assessed after 1 year, it was not considered for this statistical analysis, and the small representativeness of this subgroup is accepted here as an important limitation.

In the generalized estimating equation (GEE) model, no influence of sex ($Chi^2 = 1.44$, $p = 0.230$) or age ($Chi^2 = 1.40$, $p = 0.237$) on Ndel1 activity was noticed, but there were effects of group ($Chi^2 = 29.66$, $p \le 0.001$) and time ($t = 62.83$, $p \le 0.001$) on this activity. As for the groups, Ndel1 activity, relative to HC, was significantly lower in the FEP group at baseline (FEP-0, RR = 0.514, $p \le 0.001$); whereas it was numerically, but not statistically, higher in the UHR group (RR = 0.806, $p = 0.344$). In the FEP group, effects of time were significant at 2 months (RR = 0.786, $p = 0.046$) and 1 year (RR = 0.428, $p \le 0.001$) under treatment with risperidone.

3.3. Clinical symptoms severity

As expected, the treatment of FEP subjects with risperidone determined a significant decay in almost all symptomatic dimensions and clinically measured functional variables, as evaluated by total Positive and Negative Syndrome Scale (PANSS) ($F = 25.6$, df = 2, $p = 0.001$) (Fig. 3), Young Mania Rating Scale (YMRS) ($F = 15.3$, df = 2, $p \le 0.001$), and Global Assessment of Functioning (GAF) ($F = 38.3$, df = 2, $p \le 0.001$) (Table 2). However, although the significant decrease in the PANSS positive symptoms ($F = 58.1$, df = 2, $p \le 0.001$), no significant changes were observed for the Clinical Global Impression (CGI) ($F = 1.5$, df = 2, $p = 0.220$), Calgary Depression Scale for Schizophrenia (CDSS) ($F = 2.1$, df = 2, $p = 0.125$) and PANSS negative symptoms ($F = 0.904$, df = 2, $p = 0.408$) (Table 2).

In addition to the significant decrease of total PANSS scores along the one year follow-up ($F = 25.6$, df = 2, $p = 0.001$), a significant reduction in total PANSS scores at FEP-2 M (post-hoc Bonferroni, $p = 0.001$) and

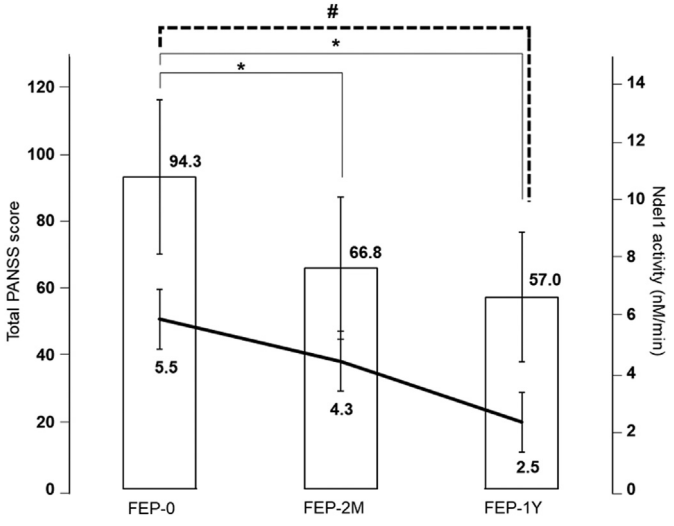

Fig. 3. Clinical symptoms improvement as evaluated by total PANSS and Ndel1 enzyme activity. The statistically significant reduction of total PANSS score in first episode of psychosis (FEP) subjects after 2 months (FEP-2 M) or one year (FEP-1Y) treatment with risperidone is indicated by the thin line bars, while the significant difference in Ndel1 activity decrease is shown by the dashed line bar. Differences were considered significant for $p \le 0.05$, after ANOVA one-way and post-hoc Bonferroni analysis for comparisons of total PANSS * and Ndel1 enzyme activity # (compared to FEP-0 at baseline).

Table 2
Clinical characteristics of FEP.

Clinical parameter, Mean, (SD)	FEP subjects			Statistics[a]		
	FEP-0 (N = 58)	FEP-2 M[b] (N = 58)	FEP-1Y[b] (N = 14)	Test value	df	*p*-value
CDSS	5.2 (5.7)	3.0 (4.8)	3.2 (5.2)	2.1	2	0.125
GAF	33.8 (13.8)	58.6 (16.8)	62.5 (15.0)	38.3	2	0.001**
CGI	5.3 (3.5)	4.1 (6.7)	2.8 (1.3)	1.5	2	0.220
YMRS	14.6 (13.2)	1.1 (1.3)	1.2 (1.4)	15.3	2	0.001**
PANSS Positive	34.8 (7.8)	19.0 (8.9)	15.0 (6.3)	58.3	2	0.001**
PANSS Negative	26.6 (10.5)	25.2 (10.0)	22.4 (7.1)	0.9	2	0.408
Total PANSS	94.3 (25.1)	66.8 (20.7)	57.0 (15.2)	25.6	2	0.001**

SD = standard deviation.
[a] ANOVA one-way test.
[b] after treatment with risperidone.
** $p \leq 0.001$.

FEP-1Y (post-hoc Bonferroni, $p = 0.001$) was also observed. However, the total PANSS values were not statistically different between FEP-2 M and FEP-1Y groups (post-hoc Bonferroni $p = 0.278$) (Fig. 3).

3.4. Correlation of Ndel1 enzyme activity with the symptoms severity

We observed a significant positive correlation between the Ndel1 enzyme activity and total PANSS scores (Spearman's rho = 0.270, $p = 0.005$), whereas the Ndel1 activity were negatively correlated with GAF (Spearman's rho = −0.303, $p = 0.002$) in the FEP follow-up arm of the study. No correlation between Ndel1 activity measures and CDSS was observed. In addition, a positive correlation of Ndel1 activity values at baseline (in FEP-0) with the decreases of PANSS score values ($r = 0.489$; $p = 0.001$) was also noticed.

For last, no significant correlation was observed between Ndel1 enzyme activity and the several clinical parameters assessed in the UHR group, including GAF (Spearman's rho = −0.228; $p = 0.433$), Montgomery-Åsberg Depression Rating Scale (MADRS) (Spearman's rho = 0.073; $p = 0.804$), or YMRS (Spearman's rho = 0.295; $p = 0.353$) (Supplemental Table 1).

4. Discussion

Aiming to evaluate the Ndel1 activity in each stages of illness, Ndel1 enzyme activity was measured in UHR for psychosis and in antipsychotic-naïve FEP individuals. Despite the absence of significant differences between the UHR and HC groups, lower Ndel1 enzyme activity in FEP individuals compared to HC was shown for the first time at baseline (FEP-0) and also after treatment. In fact, Ndel1 activity decreased after 2 months (FEP-2 M) and one year of treatment (FEP-1Y) in parallel with the administration of the atypical antipsychotic risperidone is in line with our previous findings, which showed a lower Ndel1 enzyme activity in chronic SCZ patients compared to HC (Gadelha et al., 2013) (Supplemental Fig. 2). In our current set of analyses, a significant positive correlation between the Ndel1 enzyme activity and total PANSS and negative correlation with GAF scores were observed in FEP group, suggesting that the reduction of Ndel1 activity in FEP-2 M patients is compatible with the improvement of the symptoms evaluated by PANSS or GAF scales. In addition, Ndel1 activity in FEP-0 may predict the response to the treatment, as it shows positive correlation with the decreases of PANSS score values after 2 months of treatment, namely in FEP-2 M ($r = 0.489$; $p = 0.001$). However, the treatment with risperidone was not able to restore or approximate the Ndel1 enzyme activity to the levels measured at pre-morbid stage (FEP-0) or to the levels in HC.

In our view, these results are compatible with the hypothesis that although conventional medications are efficient in reduce symptoms severity, they have limited action in modify the illness trajectory. Indeed, the integration of our data to recent theoretical models of longitudinal evolution of psychosis might suggest some possibilities. Although we did not observe a significant correlation between Ndel1 enzyme activity and specific symptom or dysfunction, it is not possible to exclude that Ndel1 activity measures could reflect specific functionality or cognitive deficit that we were not able to capture here, as determined by us for other oligopeptidase (Gadelha et al., 2015a, 2015b). Another possibility is that there is a phase of physiological decrease of Ndel1 activity around the period covering the FEP follow-up study, which could suggest a possible compensatory response to the subtle increase of *NDEL1* expression, as significant decrease of expression after treatment with risperidone was also reported (Ota et al., 2015).

First episode psychosis has been postulated as being associated with intense brain auto-toxicity (Pedrini et al., 2012). In this context, auto-toxicity could be the resultant from oxidative imbalances (Gubert et al., 2013), inflammatory response due to microglial activation (Réus et al., 2015), glutamate toxicity, brain-derived neurotrophic factor (BDNF) deprivation and intense dopamine release (Jauhar et al., 2018). This combination of variables could be beneficial in inhibiting new synapses formation, dendritic arborization and connectivity in hyperactive brain areas directly involved in psychotic pathophysiology. After the resolution of one or more episodes, it becomes necessary to restore the connectivity lost in acute episodes, and the possible roles of Ndel1 in neuritogenesis becomes associated to treatment responsiveness in chronic SCZ (Gadelha et al., 2013), which presents higher Ndel1 activity compared to FEP-2M, as demonstrated here (Supplemental Fig. 2). Interestingly, morphometry studies based on structural magnetic resonance imaging (MRI) analysis have suggested more widespread structural brain abnormalities in chronic SCZ compared to FEP patients, suggesting a possible potential impact of antipsychotics on brain structural modification evidenced by the reduction of brain volumes, which was speculated to be due to the loss of grey matter (Olabi et al., 2011; Torres et al., 2016). We hypothesize here that Ndel1 enzyme activity could be more likely signaling possible damages due to the psychosis event, which could also be potentially influenced by the neurotoxic effects of therapy with antipsychotics, that may ultimately undo the initial treatment gains as suggested by others (Olabi et al., 2011; Torres et al., 2016). We could also simply consider that Ndel1 activity decrease was due to the declining clinical course of psychotic disorder which may not be completely hampered by the treatment with antipsychotics, as they may have their action limited to the control of symptoms and not necessarily to preempt the disease progression and the overall decline of the patients (Schnack et al., 2016; Dragioti et al., 2017; Kotov et al., 2017). A more detailed trajectory of Ndel1 activity should be further explored in specific longitudinal studies in which extension in its early trajectory would allow predicting unfavorable outcomes in the follow-up.

A secondary finding of this study is the fact that FEP patients, later diagnosed as having BD (FEP-BD), did not show the same trend of Ndel1 enzyme activity decrease after two months under treatment with

risperidone, in spite of the potentially important reduction of Ndel1 enzyme activity observed for one single FEP-BD individual assessed after one year of treatment (Supplemental Fig. 1). Interestingly, euthymic BD type 1 patients were also shown to have lower Ndel1 enzyme activity compared to HC group (Dal Mas et al., 2019) which may also suggest the specificity of Ndel1 activity as a biomarker of illness trajectory. However, the small sample size of FEP-BD in the present study does not allow us to categorically confirm this hypothesis, and this aspect need to be further explored in additional prospective studies.

It is important to recognize that this study has limitations as the sample size, the cross-sectional design, mainly for UHR, and the high rate of drop-outs mainly in FEP group, which in fact, were more due to patient's abandonment from continuing the treatment or participating in the study, or just because the patient could not be contacted to schedule the clinical consultation. In addition, although the HC and FEP groups were matched for sex, we noticed a clear and significant trend of more years of education in the HC group, and the mean age of this group was also significantly higher compared to other groups (Table 1). However, no significant correlation between the Ndel1 activity and years of education or age was observed. In addition, we showed that there is no influence of age or group (namely HC, UHR and FEP before or after treatment) on the distribution of measured Ndel1 activity values (Supplemental Fig. 3).

We also cannot exclude possible confounding factors influencing the present analysis, as the smoking status and diet, among others, could not be evaluated here. It is also worth to remark that Ndel1 enzyme activity measurement was conducted using plasma, and the true correspondence to brain activity values and function is uncertain, as well as the extension and ultimate mechanisms that determine its presence in blood still remain elusive. On the other hand, our recent works, using an animal model for SCZ, suggested a good correspondence between the measurements in blood and in several brain regions, and also with correspondent Ndel1 enzyme activity changes in blood and brain of animal treated with typical or atypical antipsychotics, for 30 days (Nani et al., 2019a), as also similarly observed for another oligopeptidase also involved in SCZ (Gadelha et al., 2015a, 2015b; Nani et al., 2019b).

Taken together, we show here that even under maintenance of the treatment with antipsychotics, the Ndel1 activity does not return to the levels determined at pre-morbid stage UHR or to those found in HC. On the other hand, the chronic SCZ subjects, mainly those non-resistant to treatment (NTR), show Ndel1 activity levels lower than in HC and UHR, but which is closer to those in drug-naïve FEP-0. Therefore, we suggest that, the Ndel1 activity measures could be a potential biomarker of early stage FEP and chronic SCZ, with some possible effect of the treatment in this activity regardless of the employed antipsychotic. Therefore, considering the accumulated evidences on the functions of Ndel1 activity in SCZ, further studies with longitudinal design and multimodal approach for a larger sample cohort should help elucidating Ndel1 potential role in predicting early illness trajectories in psychosis.

Author contribution

Caroline Dal Mas – performed experiments, data analyses and participation in writing.

João V. Nani – data analysis and participation in writing.

Cristiano Noto – recruitment and clinical evaluation of FEP patients.

Camila M. Yonamine – processing of samples and performed experiments.

Graccielle Rodrigues da Cunha – recruitment and clinical evaluation of UHR patients and databank management.

Rodrigo B. Mansur – data analysis.

Vanessa K. Ota – processing of blood samples and final revision of the text.

Sintia Iole Belangero – revision of the text.

Quirino Cordeiro – recruitment of patients and revision of the text.

Flávio Kapczinski – scientific discussions, data analysis and revision of the text.

Elisa Brietzke – scientific discussions, data analysis and revision of the text.

Rodrigo A. Bressan – revision of the text.

Ary Gadelha – recruitment of FEP and HC patients, data analysis and revision of the text.

Mirian A. F. Hayashi – conception, planning and execution of the work, organization of experiments, data analysis, redaction and final revision of the manuscript.

All authors contributed to this work and they have approved the final version of this manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2019.02.021.

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