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Aberrant Expression of Long Non-Coding RNAs in Schizophrenia Patients

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Background: Dysfunction of long non-coding RNAs (lncRNAs) has been demonstrated to be involved in psychiatric diseases. However, the expression patterns and functions of the regulatory lncRNAs in schizophrenia (SZ) patients have rarely been systematically reported.

Material/Methods: The lncRNAs in peripheral blood mononuclear cells (PBMCs) were screened and compared between the SZ patients and demographically-matched healthy controls using microarray analysis, and then were validated by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) method. Three verified significantly dysregulated lncRNAs of PBMCs were selected and then measured in SZ patients before and after the antipsychotic treatment. SZ symptomatology improvement was measured by Positive And Negative Syndrome Scale (PANSS) scores.

Results: One hundred and twenty-five lncRNAs were significantly differentially expressed in SZ patients compared with healthy controls, of which 62 were up-regulated and 63 were down-regulated. Concurrent with the significant decrease of the PANSS scores of patients after the treatment, the PBMC levels of lncRNA NONHSAT089447 and NONHSAT041499 were strikingly decreased ($P<0.05$). Down-regulation of PBMC expression of NONHSAT041499 was significantly correlated to the improvement of positive and activity symptoms of patients ($r=-0.444$ and -0.423, respectively, $P<0.05$, accounting for 16.9% and 15.1%, respectively), and was also significantly associated with better outcomes (odds ratio 2.325 for positive symptom and 12.340 for activity symptom).

Conclusions: LncRNA NONHSAT089447 and NONHSAT041499 might be involved in the pathogenesis and development of SZ, and the PBMC level of NONHSAT041499 is significantly associated with the treatment outcomes of SZ.

MeSH Keywords: Drug Therapy • Microarray Analysis • RNA, Long Noncoding • Schizophrenia

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Background

Schizophrenia (SZ) is one of the most severely disabling mental disorders, which usually begins in early adulthood and features disordered symptoms such as hallucinations, delusions, disturbed communication, reduced motivation, and blunted affect. SZ has been estimated to have a median lifetime prevalence of 4.0 per 1000 persons worldwide [1], and is a global devastating health and socioeconomic burden. Current evidence demonstrates that SZ is attributable to the interactions between environmental and genetic factors [2]; however, the mechanisms of the pathogenesis of SZ are still unclear. We currently lack reliable and simple biomarkers for the diagnosis of SZ and prognosis of antipsychotic treatment, which hampers the early diagnosis and effective treatment of SZ patients [3,4].

LncRNAs are non-coding transcripts of longer than 200 nucleotides, and were previously often considered to be transcriptional 'noise' [5]. Recently, accumulating evidence has revealed that a number of lncRNAs play critical roles in the regulation of gene expression, and cell proliferation and differentiation, and participate in the pathogenesis and development of various diseases [6–9], especially neuropsychiatric disorders and neurodegenerative diseases such as Alzheimer's disease [10], major depressive disorder [11], Parkinson's disease [12], and autism spectrum disorders [13]. Barry et al. showed that dysregulation of lncRNA Gomafu led to defective alternative splicing patterns which link to SZ, implying the role of Gomafu in SZ [8]. Rao et al. reported that lncRNA MIAT was significantly associated with paranoid SZ among the Chinese Han population [14]. Recently, Ren et al. demonstrated that 2 lncRNA modules were significantly associated with early-onset SZ [15]. However, few studies have investigated lncRNA expression profiles in the peripheral blood cells of SZ patients, and the response of lncRNAs to the antipsychotic medications is unclear.

The present study systematically screened the differentially expressed lncRNAs in the peripheral blood mononuclear cells (PBMCs) from SZ patients compared with healthy controls, using microarray method. We then observed the changes in the PBMC levels of 3 verified significantly dysregulated lncRNAs in response to antipsychotic treatment in SZ patients, and analyzed the association of the variation of the lncRNA expression with the improvement of symptoms. This study provides new insights into the mechanisms underlying the pathogenesis of SZ, and suggests that lncRNAs might be considered as novel biomarkers for the diagnosis of SZ and prognosis of the related treatment, and potentially as therapeutic targets.

Material and Methods

Patients

A total of 106 SZ patients aged 20–50 years who met the diagnostic criteria based on the Diagnostic and Statistical Manual of Mental Disorders by American Psychiatric Association [16] were prospectively enrolled between December 2013 and May 2015 at the No. 102 Hospital of the People's Liberation Army (Changzhou, China). All patients were of Han ethnicity and did not take any antipsychotic medications for at least 3 months before the enrollment. Patients with history of severe medical diseases, structural brain disorders, cognitive disability, other psychiatric disorders, unstable psychiatric features, or movement disorders were excluded. In addition, patients who had post-traumatic amnesia for over 24 h or received blood transfusion therapy within 1 month or electroshock therapy within 6 months were also excluded from the study.

Forty-eight age-, gender-, and ethnicity-matched healthy control subjects without any family history of major psychiatric disorders (e.g., SZ, major depressive disorder and bipolar disorder) within the last 3 generations, and without any history of blood transfusion therapy or severe traumatic injury within 1 month, were recruited. All the control subjects were also of Han ethnicity.

The study was approved by the Institutional Review Board of No. 102 Hospital of the People's Liberation Army. Written informed consent was obtained from each subject.

RNA extraction

Whole blood (5 ml) from each subject was collected in EDTA-containing anticoagulant tubes and processed within 1 h. PBMCs were isolated from the blood samples through density gradient centrifugation, collected and stored at –80°C until use. Total RNA was isolated from PBMCs using Trizol regent (Invitrogen, Carlsbad, CA, USA) and RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturers' protocols, followed by Turbo DNase treatment (Life Technologies, Carlsbad, CA, USA), quantification by NanoDrop ND-2000 (Thermo Scientific, Delaware, ME, USA), RNA integrity detection by gel electrophoresis, and reverse transcription (Superscript III; Invitrogen).

LncRNA microarray

RNA samples from 3 SZ patients (patient #1: male, 23 years old; patient #2: male, 31 years old; patient #3: female, 28 years old) and 3 healthy controls (patient #1: male, 20 years old; patient #2: male, 33 years old; patient #3: female, 26 years old) were used for lncRNA microarray profiling. The RNA sample labeling, microarray hybridizing, and washing were conducted

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according to the manufacturer's standard protocols. Briefly, total RNA was transcribed to double-stranded cDNA, synthesized into cRNA, labeled with Cyanine-3-CTP, and then hybridized onto the Agilent Human lncRNA array v4.0 (4X180 K, Design ID: 062918, Agilent Technologies, Santa Clara, CA, USA). After washing, the arrays were then scanned by the Agilent Scanner G2505C (Agilent Technologies). Array images were analyzed using Feature Extraction software 10.7.1.1 (Agilent Technologies) to extract the raw data, which were further normalized with the quantile algorithm using Genespring software (Version 12.5; Agilent Technologies). The probes that were all flagged as "P" in at least 1 out of the 2 groups were chosen for further data analysis. LncRNAs data were shown as fold-changes relative to the controls. Differentially expressed lncRNAs were identified by fold-changes and P values through t-test. The threshold for up- and down-regulated expression was a fold-change ≥2.0 and a P value ≤0.05. Finally, hierarchical clustering was conducted to display the distinguishable lncRNA expression patterns among samples.

Real-time quantitative reverse-transcription PCR (qRT-PCR)

According to the microarray results, the 10 most dysregulated lncRNAs were chosen for further validation by qRT-PCR in 106 SZ patients versus 48 healthy controls. Blood samples were collected and PBMCs were isolated. Total RNAs were isolated from PBMCs using Trizol reagent (Invitrogen), and complementary DNA was synthesized using the Reverse Transcription TaqMan RNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA) according to the manufacturer's instructions. Real-time PCR was performed using a 7900HT Real-Time PCR System (Applied Biosystems). Data were analyzed using the SDS 2.3 software (Applied Biosystems) and DataAssist v3.0 software. The expression levels of lncRNAs were normalized to β-actin and were calculated using $2^{-\Delta\Delta Ct}$ method.

Medical intervention and symptom assessment

According to the microarray and qRT-PCR results, 3 verified significantly dysregulated lncRNAs were further measured the expression variation in the PBMCs of 30 SZ patients before and after the antipsychotic treatment by qRT-PCR. Among these patients, 5 were treated with risperidone (starting dosage 2 mg, average dosage 3.9 mg, range 2–6 mg), 7 with ziprasidone (starting dosage 40 mg, average dosage 125 mg, range 40–140 mg), 8 with quetiapine (starting dosage 100 mg, average dosage 520 mg, range 100–800 mg), and 10 with olanzapine (starting dosage 5 mg, average dosage 12 mg, range 5–20 mg).

Positive and Negative Syndrome Scale (PANSS) is commonly used to evaluate the severity of symptoms of patients with SZ [17]. PANSS contains 33 items, including 3 for aggressiveness, 7 for positive symptoms, 7 for negative symptoms, and 16 for general psychopathological symptoms [17]. In this study, symptoms of patients were assessed at baseline and at 6 weeks after the antipsychotic treatment by experienced psychiatrists using the PANSS. The symptom improvement was reflected by the variation of the symptomatology scores and total score before and after the treatment. The reduction rate of symptomatology scores was calculated as the variation of symptomatology score before and after the medication treatment relative to the pre-medication symptomatology score.

Statistical analysis

Data are expressed as the mean ± standard deviation or percentages where appropriate, and were compared between SZ patient and healthy control groups using the Statistical Package for Social Sciences for Windows 22.0 (SPSS Inc. Chicago, IL, USA). The chi-square test was used to compare the categorical demographic variables, and the *t* test was used to compare quantitative demographic variables. The Mann-Whitney U test was used to compare the PBMC levels of the top 10 differentially expressed lncRNAs by microarray between SZ and healthy controls subjects. The paired-sample *t* test was for the comparison of the expression levels of lncRNAs in SZ patients between before and after the treatment. Pearson correlation analysis was performed to evaluate the correlation of change of the lncRNA expression level with the improvement of symptomatology scores. Regression analysis was then carried out using the variation of lncRNA NONHSAT041499 expression as independent variable and improvement of PANSS positive and activity symptoms as dependent variables. Stepwise regression analysis was to determine the lncRNA NHSAT041499 accountability of symptomatological improvement in SZ patients. ΔR^2 was assessed to show the percentage of the variation of positive and activity subscales with the NHSAT041499 variation. Then, according to the reduction rate of symptomatology scores before and after the medication, SZ patients were divided into better (score reduction rate equal to or more than 50%) and worse (score reduction rate less than 50%) treatment outcome subgroups. Logistic regression analysis was then conducted to observe the association of NHSAT041499 with the treatment outcomes of patients, which was assessed by odds ratio (OR) and P values. P<0.05 (2-tailed) was considered statistically significant.

Results

Microarray analysis

Microarray analysis showed there were 125 lncRNAs significantly differentially expressed in SZ patients compared with healthy controls (fold change ≥2, *P*<0.05), among which 62 were up-regulated and 63 were down-regulated (Supplementary Table 1).

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Table 1. Top 20 aberrantly expressed lncRNAs in peripheral blood mononuclear cells from Schizophrenia patients versus healthy controls by microarray analysis.

lncRNA	Fold-change	P-value	Style	Chromosome start end
ENST00000394742	5.6865215	0.001366189	Down	Chr12 13100085 13106891
TCONS_l2_00025502	5.130027	0.016324848	Down	Chr6 143360562 143363461
NONHSAT041499	5.1150675	0.004036811	Up	Chr15 32806172 32819079
NONHSAT098126	4.610542	0.047743667	Up	Chr4 121606074 121631566
NONHSAT003974	4.4428	0.006958588	Up	Chr1 75428997 75430802
ENST00000519337	4.2703557	0.020613242	Up	Chr8 103866683 103868303
ENST00000563823	3.775364	0.012858684	Down	Chr1 672557231 72661585
NONHSAT021545	3.6979194	0.023714427	Up	Chr11 59570202 59573350
ENST00000496491	3.6021621	0.026623152	Up	Chr3 149095565 149104370
ENST00000521622	3.5583007	0.00341083	Down	Chr8 106810555 107072719
NONHSAT 089447	3.514152	0.038919978	Up	Chr3 46598887 46601178
TCONS_l2_00021339	3.4050841	0.010671916	Down	Chr4 147030377 147043080
TCONS_l2_00024969	3.370955	0.017096879	Down	Chr6 147182804 147240209
NONHSAT104778	3.2426178	0.03928101	Up	Chr5 157174512 157174715
ENST00000581634	3.0800865	0.024413016	Up	Chr18 76265165 76266410
TCONS_l2_00000563	2.9737065	0.030129751	Down	Chr1 143398221 143401768
NONHSAT074892	2.9536893	0.03315027	Down	Chr2 149666582 149685052
NONHSAT005508	2.947432	0.030145906	Up	Chr1 118628601 118629785
TCONS_l2_00012438	2.896232	0.027099133	Down	Chr19 28409545 28447647
NONHSAT016970	2.8774061	0.04668065	Up	Chr10 129849591 129850501

The top 20 differentially expressed lncRNAs are shown in Table 1. In hierarchical clustering analysis, the normalized expression of the 125 significantly differentially expressed lncRNAs was recorded to generate a heat map, from which a general difference of the lncRNA expression in blood samples from SZ patients versus healthy control subjects were clearly displayed (Figure 1).

Clinical characteristics of the patients

As shown in Table 2, the mean age of patients and healthy controls was 30.49±12.86 and 29.61±12.32 years, respectively. There was no significant difference in age, gender, residential location, sibling status, education, marital status, or family history of mental disorders between SZ patients and healthy controls (P>0.05, Table 2).

qRT-PCR validation

To validate the results of the microarray assay, 10 of the top 20 significantly differentially expressed lncRNAs (including 5 up-regulated lncRNAs: NONHSAT098126, NONHSAT089447, NONHSAT021545, NONHSAT041499, and NONHSAT104778, and 5 down-regulated lncRNAs: ENST00000394742, TCONS_l2_00025502, ENST00000563823, ENST00000521622, and TCONS_l2_00021339) were chosen for further validation in larger blood samples from 106 patients versus 48 healthy controls using qRT-PCR method. Results showed that the expression of lncRNAs NONHSAT089447, NONHSAT021545, NONHSAT041499, NONHSAT098126, and NONHSAT104778 was consistent with the microarray results, of which the first 3 lncRNAs exhibited significant difference of expression between patients and healthy controls (P<0.05) (Figure 2). These first 3 up-regulated lncRNAs were then chosen for further study.

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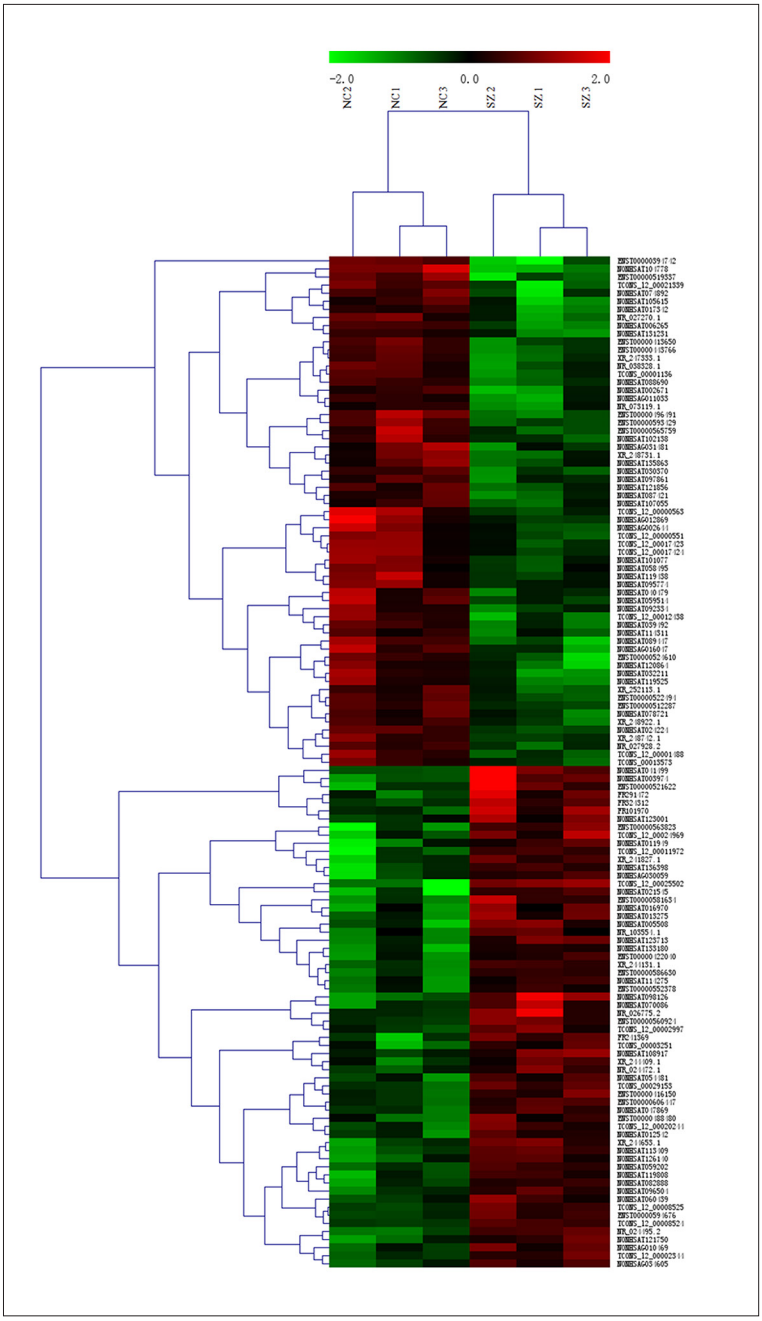

Figure 1. Hierarchical clustering analysis of differentially expressed lncRNAs in peripheral blood mononuclear cells from schizophrenia patients versus normal controls. Rows represent differentially expressed lncRNAs and columns represent blood samples. Color scale depicts the relative lncRNA expression; red shows up-regulation and green shows down-regulation. The 2.0, 0, and -2.0 indicate fold-changes in the corresponding spectrum. NC represents blood samples from normal controls and SZ represents samples from SZ patients. The differentially expressed lncRNAs were self-segregated into NC and SZ clusters.

LncRNA expression, and symptomatology scores and total score before and after the treatment in SZ patients

As shown in Table 3, ΔCT values of lncRNAs NONHSAT089447 and NONHSAT041499 were significantly increased in patients after the treatment (P<0.001), indicating the significant down-regulation of these lncRNA expression by the treatment. Consistently, the symptomatology scores and total score were significantly decreased after the treatment (P<0.001, Table 3).

Down-regulation of lncRNA NONHSAT041499 expression was correlated with symptom improvement in SZ patients

Pearson correlation analysis revealed that the down-regulation of the lncRNA NONHSAT041499 expression was significantly correlated with the improvement of positive and activity symptoms after the treatment (r=–0.444 and –0.423, respectively, P<0.05, Table 4).

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Table 2. Demographic characteristics of patients with schizophrenia and healthy controls.

	Patients (n=106)	Controls (n=48)	t	*P*-value
Age (years)	30.49±12.86	29.61±12.32	–0.379	0.705
Gender (n/%)			1.523	0.683
Female	52 (49.1%)	25 (52.1%)		
Male	54 (50.9%)	23 (47.9%)		
Inhabitants (n/%)			1.958	0.892
Urban	47 (44.3%)	22 (45.8%)		
Rural	59 (55.7%)	26 (54.2%)		
Sibling status (n/%)			2.123	0.096
Only- child	62 (58.5%)	26 (54.2%)		
Non-only child	44 (41.5%)	22 (45.8%)		
Education (n/%)			2.325	0.075
Junior high school or below	59 (55.7%)	21 (43.8%)		
Senior high school or above	47 (44.3%)	27 (56.2%)		
Marital status (n/%)			1.538	0.356
Married	50 (47.2%)	28 (58.3%)		
Unmarried	56 (52.8%)	20 (41.7%)		
Family history of mental disorders (n/%)			1.621	0.125
With	19 (17.9%)	7 (14.6%)		
Without	87 (82.1%)	41 (85.4%)		

LncRNA NONHSAT041499 down-regulation was significantly associated with improvement of treatment outcomes of SZ patients

Step-wise regression analysis revealed that the down-regulation of lncRNA NONHSAT041499 expression as independent variable accounted for 16.9% (ΔR^2=0.169) and 15.1% (ΔR^2=0.151) of the improvement of positive and activity symptoms, respectively (Table 5).

To further validate the association of NONHSAT041499 with the antipsychotic treatment outcomes, SZ patients were divided into better and worse treatment outcome subgroups according to the symptomatology score reduction rate. Taking the down-regulation of NONHSAT041499 expression as an independent variable, and the improvement of positive and activity symptoms as dependent variables, logistic regression analysis was carried out. The results showed that for positive symptom, the OR determined by NONHSAT041499 down-regulation (calculated as the increase of CT value, ΔCT) of better treatment outcome subgroup against worse treatment outcome subgroup was 2.325, and for activity symptom the OR was 12.340 (Table 6)

Discussion

Current treatment for SZ mainly comprises dopamine receptors system [18,19], 5-HT receptors [20], and GABA system [21] drugs, but the pharmacological mechanisms remain elusive. This makes it difficult for more effective treatment and prognosis of the outcomes. LncRNAs play important roles in various pathologic processes, including neuropsychiatric disorders and neurodegenerative diseases [11,13]. To date, only a few studies reported that lncRNAs were significantly associated with SZ [14,15]. There have been few reports on lncRNA expression profiling in SZ patients. Only a recent study demonstrated a microarray profiling of lncRNAs of SZ patients, with the focus on the analysis of co-expression network of lncRNAs and mRNAs and their correlation [15]. The association between these lncRNAs and the treatment outcomes of SZ patients is still unclear.

This study systematically screened the differentially expressed lncRNAs in SZ patients in comparison to healthy controls and demonstrated that lncRNAs NONHSAT089447, NONHSAT021545, and NONHSAT041499 were significantly up-regulated

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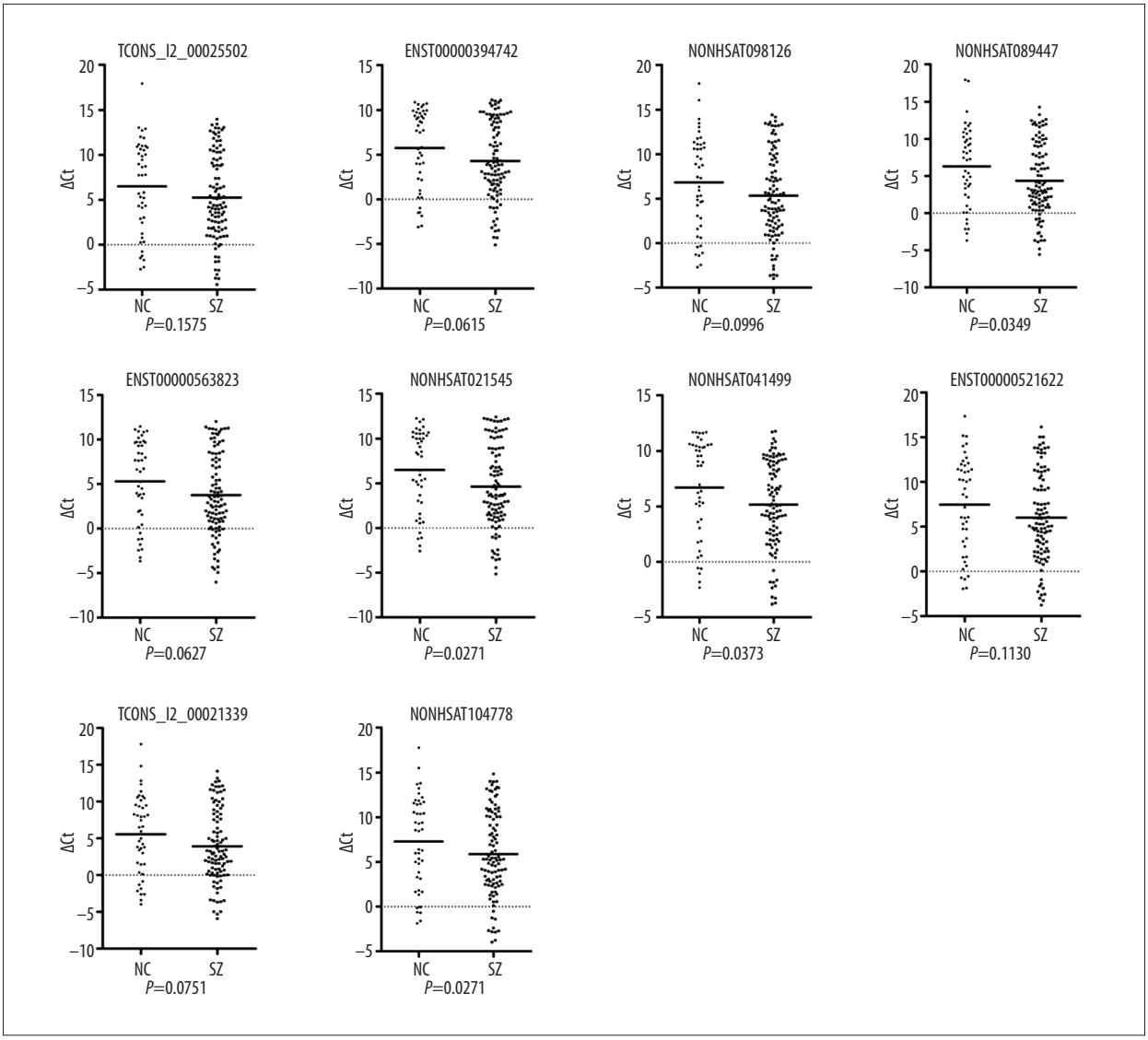

Figure 2. Validation of the expression of lncRNAs by qRT-PCR analysis in the peripheral blood mononuclear cells from schizophrenia patients (n=106) and normal controls (n=48). The line represents the median value, and the plots were constructed by using GraphPad Prism 5 software. Statistical difference was analyzed using the Mann-Whitney U test.

in SZ patients. Down-regulation of NONHSAT089447 and NONHSAT041499 was concurrent with the improvement of symptoms of patients after the anti-psychotropic medication. These results suggest that these lncRNAs might be involved in the pathogenesis and development of SZ and could be considered as novel potential treatment targets. Reportedly, lncRNAs are transcribed in complex patterns (e.g., intergenic, overlapping, and antisense patterns) relative to the adjacent protein-coding genes [9], and participate in the regulation of the target gene expression by inducing chromatin remodeling and targeting transcription factors [7,22], suggesting the complexity of the regulatory pathways of lncRNAs. An integrated co-expression network analysis revealed significant correlation between lncRNAs and mRNAs, and that the lncRNAs, together with mRNAs, constructed co-expressed modules, some of which were associated with early-onset SZ [15]. Barry et al. showed that lncRNA Gomafu directly binds to the splicing factors, such as serine/arginine-rich splicing factor 1, to regulate the alternative splicing patterns whose defection is linked to SZ [8]. Ishizuka et al. reported that lncRNA Gomafu indirectly modulated RNA-binding protein Celf3 and other splicing factors to regulate the functions of the SZ-related genes, thus playing roles in SZ [23]. How these lncRNAs modulate these SZ-related genes to regulate SZ warrants further investigation.

Traditionally, diagnosis of SZ is based on the clinical symptoms [16,17]. Functional neuroimaging techniques have been developed to detect the neurotransmitters (e.g., dopamine and

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Table 3. LncRNA expression, symptomatology scores and total score before and after the antipsychotic treatment in schizophrenia patients.

Item	Baseline (n=30)	After treatment (n=30)	t	P
NONHSAT089447 (ΔCT)	3.23±4.26	5.13±3.51	–4.577	<0.001
NONHSAT021545 (ΔCT)	3.67±4.12	4.04±4.55	–0.858	0.398
NONHSAT041499 (ΔCT)	4.56±3.92	6.77±3.13	–5.056	<0.001
Positive subscale	19.94±5.98	9.32±4.78	8.993	<0.001
Negative subscale	21.29±8.24	10.58±4.56	8.778	<0.001
General psychopathology subscale	40.90±10.85	19.71±5.05	11.29	<0.001
Total score	81.84±18.13	39.61±7.64	13.80	<0.001
Lack of response	10.10±4.57	6.00±2.42	5.507	<0.001
Disturbance of thought	12.42±3.97	5.64±2.59	9.455	<0.001
Activity	5.84±2.19	3.29±2.12	7.289	<0.001
Paranoid	7.16±2.78	3.58±1.03	7.271	<0.001
Depression	10.68±3.30	5.74±2.62	8.093	<0.001
Aggressiveness	13.03±3.73	7.07±2.02	9.276	<0.001

Table 4. Correlation between the down-regulation of lncRNA expression and improvement of symptoms in schizophrenia patients.

Item	NONHSAT089447 (r value)	NONHSAT041499 (r value)
Positive subscale	–0.122	–0.444*
Negative subscale	–0.160	–0.065
General psychopathology subscale	–0.093	–0.107
Total score	–0.146	–0.216
Lack of response	–0.143	–0.064
Disturbance of thought	0.139	0.116
Activity	–0.193	–0.423*
Paranoid	–0.172	–0.016
Depression	–0.035	0.149
Aggressiveness	–0.323	–0.110

* $P<0.05$ represented significant difference for the correlation between the lncRNA with the symptoms.

Table 5. LncRNA NONHSAT041499 down-regulation accountability of symptomatology improvement of schizophrenia patients by step-wise regression analysis.

Dependent variables	Regression model	Partial regression coefficient	Standard error	Standard coefficient	*t*	ΔR^2	*P* value
Δ Positive symptom	Constant	–7.965	1.464		–5.440		0.000
	Δ NONHSAT041499	–1.144	0.429	–0.444	–2.666	0.169	0.012
Δ Activity symptom	Constant	–1.800	0.438		–4.106		0.000
	Δ NONHSAT041499	–0.323	0.128	–0.423	–2.515	0.151	0.018

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Table 6. Association between lncRNA NONHSAT041499 down-regulation and treatment outcomes of schizophrenia patients by logistic regression analysis.

Dependent variables	Regression model	*B*	Standard error	Wals	Odds ratio	Determination coefficient	*P* value
Δ Positive symptom	Constant	–1.912	0.800	5.717	0.148		0.017
	Δ NONHSAT041499	0.844	0.319	7.018	2.325	0.366	0.008
Δ Activity symptom	Constant	–4.347	1.831	5.637	0.013		0.013
	Δ NONHSAT041499	2.513	1.010	6.186	12.340	0.607	0.018

glutamate) implicated in SZ, and SZ-associated regional brain activity [24]. However, at present it is difficult to diagnose SZ because neither single clinical symptoms nor neurotransmitters are unique for SZ. Symptoms or SZ-related brain activity are usually manifested or detected when SZ is developed to a certain stage, which means such symptoms or brain activity-based diagnosis might lead to the delay of the SZ treatment. Accurate and early diagnosis of SZ is very important. This study demonstrates that lncRNAs NONHSAT089447, NONHSAT021545, and NONHSAT041499 were significantly up-regulated in SZ patients and that NONHSAT089447 and NONHSAT021545 down-regulation was significantly correlated with improvement of symptoms by the anti-psychotropic treatment, suggesting these lncRNAs could be used as new non-invasive biomarkers for the diagnosis of SZ. Particularly, based on the findings that lncRNAs play roles in SZ via upstream regulating the SZ-related genes [8,15,23], it is anticipated that lncRNAs could be even considered as early diagnostic biomarkers for SZ, which will be beneficial for the early treatment of SZ. Our next study, with larger patient numbers, will be carried out to validate the diagnostic value of NONHSAT041499 in SZ.

At present, although drugs (mainly dopamine receptor system) have been employed, SZ is difficult to treat. For more effective treatment, it is therefore necessary to predict the treatment outcomes. In this study, through Pearson correlation, and step-wise and logistic regression analysis, we revealed that NONHSAT041499 down-regulation was significantly correlated to the improvement of positive and activity symptoms of patients after the medication treatment, and was also significantly associated with better outcomes. This result implies NONHSAT041499 might be considered as a potent prognosis factor for the treatment outcome. To the best of our knowledge, similar studies have not been reported yet. Recently, we reported that down-regulation of plasma miRNA-181b predicted symptom improvement of SZ patients after antipsychotic treatment [25]. These results suggest potential non-invasive molecular markers for the prognosis of SZ patients. Further studies with larger sample sizes are needed to confirm the potential of NONHSAT041499 as a prognostic factor for the treatment of SZ.

LncRNAs closely interact with the regulated mRNAs. LncRNA NONHSAT089447, NONHSAT021545, and NONHSAT041499 were found to be co-expressed with many mRNAs that regulate various biological processes, including neuron apoptosis, learning, memory, behavior, sensory perception of sound, synapse organization and activity, layer formation in the cerebral cortex, stress-activated protein kinase signaling pathway, Ras protein signal transduction, and small GTPase-mediated signal transduction (unpublished data). The detailed bioinformatics study of the above-mentioned lncRNAs is under investigation, and molecular mechanisms whereby these lncRNAs participate in the pathogenesis and development of SZ need to be extensively explored.

A limitation of this study is that the sample size is relatively small for the regression analysis. Small patient numbers in the control group relative to the SZ group might decrease the statistical power for the comparison of the lncRNA expression levels between SZ and control groups. Further studies with more patients and more variables are needed to validate the present results.

Conclusions

We systematically screened the differentially expressed lncRNAs in the PBMCs of SZ patients, and demonstrated that down-regulation of NONHSAT041499 was significantly associated with the symptom improvement and better treatment outcomes of SZ patients. This study will be beneficial for the investigation of the mechanisms underlying the pathogenesis and development of SZ, and suggests the potential usefulness of lncRNA NONHSAT041499 as a novel biomarker for the diagnosis of SZ and prognosis of the treatment, as well as being a potential treatment target.

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Supplementary Table 1. Differentially expressed lncRNAs in peripheral blood mononuclear cells from Schizophrenia patients versus healthy controls by microarray.

lncRNA	Fold-change	P-value	Style
TCONS_l2_00008525	2.0184042	0.007859847	Up
FR324312	2.5046852	0.019613983	Up
FR241369	2.795938	0.026778212	Up
NR_024495.2	2.6708856	0.001104883	Up
NONHSAG012869	2.7815604	0.048556764	Down
NONHSAG030059	2.4375992	0.04930478	Up
NONHSAT119438	2.386957	0.042039905	Down
XR_248742.1	2.0546277	0.013295304	Down
TCONS_l2_00017424	2.2616987	0.039616782	Down
NONHSAT123713	2.7456982	0.015772445	Up
TCONS_l2_00017423	2.3137672	0.04239666	Down
NONHSAT078721	2.090962	0.03983514	Down
NONHSAT104778	3.2426178	0.03928101	Up
NONHSAT016970	2.8774061	0.04668065	Up
NONHSAT006265	2.74296	0.003577456	Down
NONHSAT059514	2.416889	0.034944758	Down
NONHSAT041499	5.1150675	0.004036811	Up
NONHSAT092334	2.22519	0.045760788	Down
TCONS_00029153	2.2907834	0.005757978	Up
NONHSAT 089447	3.514152	0.038919978	Up
ENST00000519337	4.2703557	0.020613242	Up
NONHSAG016047	2.8309715	0.03872216	Up
FR101970	2.8698637	0.029207746	Up
ENST00000563823	3.775364	0.012858684	Down
NONHSAT121750	2.3670413	0.028498909	Up
NONHSAT135863	2.3467486	0.031746913	Down
NONHSAT021545	3.6979194	0.023714427	Up
NONHSAT011949	2.8012006	0.047748405	Up
NR_038328.1	2.4504633	0.025065137	Down
NONHSAT059202	2.1078913	0.003934796	Up
TCONS_00013573	2.016616	0.021455377	Down
XR_241827.1	2.519167	0.043524686	Up
NONHSAT123001	2.2459533	0.04912253	Up
NONHSAG034605	2.0021873	0.013193308	Up
ENST00000581634	3.0800865	0.024413016	Up
ENST00000522494	2.1406422	0.009605092	Up
NONHSAT120864	2.6860943	0.047448784	Down
NONHSAT108917	2.201703	0.025858361	Up
NONHSAG010469	2.170598	0.02606947	Up
NONHSAT040479	2.4519546	0.049309734	Down
ENST00000488480	2.2488601	0.04426648	Up

lncRNA	Fold-change	P-value	Style
NONHSAT054481	2.1365309	0.033978045	Up
NONHSAT131231	2.2493892	0.022191135	Down
NONHSAT013275	2.7648053	0.026773857	Up
ENST00000560924	2.2441285	0.008775448	Up
TCONS_00003251	2.3368568	0.048376054	Up
NONHSAT133180	2.255505	0.039863095	Up
ENST00000422040	2.246702	0.034511745	Up
NONHSAT113409	2.6231997	0.006450792	Up
ENST00000552378	2.0455449	0.039099984	Up
NONHSAT098126	4.610542	0.047743667	Up
NONHSAT105615	2.6867616	0.037940864	Down
NONHSAT119525	2.5023258	0.041274253	Down
ENST00000565759	2.545508	0.038889498	Down
TCONS_00001136	2.401271	0.017750448	Down
NONHSAT003974	4.4428	0.006958588	Up
NONHSAT101077	2.382952	0.025674935	Down
TCONS_l2_00002344	2.021424	0.013821459	Up
XR_244131.1	2.320134	0.005847468	Up
NR_027928.2	2.035199	0.010459018	Down
NR_026775.2	2.709865	0.037649963	Up
TCONS_l2_00024969	3.370955	0.017096879	Down
TCONS_l2_00021339	3.4050841	0.010671916	Down
XR_252113.1	2.1277385	0.019082548	Down
TCONS_l2_00001488	2.42787	0.016129335	Down
ENST00000593429	2.664398	0.005580793	Down
NONHSAT032211	2.8629696	0.03303966	Down
NONHSAT039492	2.419247	0.012648112	Down
NONHSAT121856	2.2077594	0.013875067	Down
NONHSAT126140	2.4100482	0.026816849	Up
ENST00000413650	2.50962	0.010103071	Down
NONHSAG002644	2.5878246	0.046584073	Down
ENST00000512287	2.095495	0.00460248	Down
NONHSAT058495	2.0634727	0.04672545	Down
ENST00000416150	2.0931425	0.029960617	Up
XR_247333.1	2.482382	0.01222255	Down
NR_027270.1	2.7393317	0.023086803	Down
NONHSAT012542	2.0278077	0.03747045	Up
ENST00000594676	2.0075424	0.007863165	Up
FR291472	2.8738096	0.045134224	Up
NR_103554.1	2.3072157	0.048855953	Up
NONHSAT102138	2.3331935	0.035092298	Down

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Supplementary Table 1 conntinued. Differentially expressed lncRNAs in peripheral blood mononuclear cells from Schizophrenia patients versus healthy controls by microarray.

lncRNA	Fold-change	P-value	Style
NONHSAT095774	2.0473397	0.03872717	Down
XR_244653.1	2.6912992	0.01806327	Up
ENST00000521622	3.5583007	0.00341083	Down
TCONS_l2_00002997	2.1203494	0.024146989	Up
NONHSAT114311	2.045813	0.034777325	Down
ENST00000586630	2.2811098	0.009588628	Up
NONHSAT024224	2.280496	0.001247224	Down
NONHSAT060439	2.0503778	0.03933156	Up
ENST00000443766	2.5501273	0.006421687	Down
NONHSAT017342	2.3088417	0.027970523	Down
NONHSAG031481	2.6192472	0.04883049	Down
NONHSAT097861	2.0948513	0.03657174	Down
ENST00000524610	2.7294452	0.03419223	down
NONHSAT047869	2.046003	0.014862789	Up
ENST00000394742	5.6865215	0.001366189	Down
TCONS_l2_00008524	2.0070338	2.13E-04	Up
NONHSAT136398	2.4874713	0.046239804	Up
NR_024472.1	2.0432649	0.027397072	Up
NONHSAG011033	2.3757823	0.0297065	Down
ENST00000496491	3.6021621	0.026623152	Up
NONHSAT114275	2.109495	0.037710313	Up
ENST00000606447	2.0563893	0.011615175	Up

lncRNA	Fold-change	P-value	Style
XR_244409.1	2.0452442	0.049372125	Up
TCONS_l2_00000563	2.9737065	0.030129751	Down
NONHSAT082888	2.0611625	0.02947335	Up
NONHSAT005508	2.947432	0.030145906	Up
TCONS_l2_00000551	2.3637156	0.031189756	Down
NONHSAT070086	2.7337165	0.039751925	Up
XR_248731.1	2.5972993	0.022589074	Down
NONHSAT119808	2.2518048	0.03818275	Up
TCONS_l2_00012438	2.896232	0.027099133	Down
NONHSAT074892	2.9536893	0.03315027	Down
TCONS_l2_00020244	2.2335203	0.027745022	Up
NONHSAT107055	2.0738862	0.022926092	Down
NONHSAT030370	2.3975239	0.006883643	Down
NONHSAT087421	2.249703	0.021947894	Down
NONHSAT096504	2.0448937	0.02514679	Up
NONHSAT002671	2.6095362	0.02860338	Down
TCONS_l2_00011972	2.8288274	0.034055557	Up
NONHSAT088690	2.270206	0.006502805	Down
TCONS_l2_00025502	5.130027	0.016324848	Down
NR_073119.1	2.1553414	0.03583079	Down
XR_248922.1	2.0368042	0.019383209	Down

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