Association between peripheral lncRNA expression and coping style in schizophrenia patients

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Abstract

In order to explore the relationship between peripheral lncRNA expression and coping style in schizophrenia patients, this study screened the peripheral blood mononuclear cells in 5 patients and 5 controls, and 10 differentially expressed lncRNAs were selected and validated in 96 patients and 50 controls by qPCR. Compared to control group, three lncRNAs (NONHSAT089447, NONHSAT021545, NONHSAT041499) were up-regulated in schizophrenia group. Among them, NONHSAT089447, NONHSAT021545 were negatively associated with positive coping style, and other four lncRNAs (ENST00000394742, NONHSAT089447, NONHSAT021545, NONHSAT041499) were positively related to negative coping style. Positive coping style scores in higher-expression of PR4 and PR6 subgroup were significantly lower than those in lower-expression subgroup. While on the other hand, negative coping style scores in higher-expression of NONHSAT089447, NONHSAT021545, NONHSAT041499 subgroup were significantly higher than those in lower-expression subgroup. In conclusion, three lncRNAs (NONHSAT089447, NONHSAT021545, NONHSAT041499) were over-expressed in schizophrenia patients, probably playing a role in the epigenetic process of choosing coping style.

Keywords: Schizophrenia; Coping style; lncRNA; Epigenetics

1. Introduction

Recent researches [1, 2] show that schizophrenia (SZ) has been one of the most disabling mental disorders with onset usually in early adulthood, with a lifetime prevalence of 1%, imposing great social burden and paralyzing individuals' life. Kafali et al. and Wen et al. [3, 4] argued that it usually takes two years or more to progress from prodromal symptoms to typical symptoms, during which there is usually an ultra-high risk (UHR). Some researches emphasised [5, 6] that in spite of the generally accepted fact that an early diagnosis and intervention would yield a better clinical prognosis in schizophrenia patients, treatment or intervention of any kind for SZ patients in UHR would be ethically controversial. Therefore, a specific clinical biomarker would be an urgent need for psychiatric researchers. A great many studies [7-9] have demonstrated that genetic contribution has been playing a leading role in the development process of SZ, one study has specifically pointed out that SZ has a hereditability as high as 70% ~ 85%.

Noncoding RNAs (ncRNAs) can be divided into small ncRNA and long ncRNA according to their length. In recent years, noncoding RNAs (ncRNAs) has become the focus of study around the globe, among which microRNA has been fully tapped in a great variety of diseases. Unfortunately, inconsistent results have made it so difficult to yield significant conclusions. Therefore, more valid biomarkers for psychiatric diseases warrant notice. lncRNAs are noncoding
transcripts longer than 200 nucleotides, that though being identified later, are increasingly appreciated as important regulators of gene expression. More and more studies [10-13] have verified that lncRNA might play extensive and significant roles in gene expression regulation of a great many biological processes, such as individuals’ growth and development, cell apoptosis, proliferation and differentiation. Wang et al. [14] state that lncRNA could regulate the stability of target mRNA, and also regulate gene transcription by binding to promoter, meanwhile, lncRNA might also serve its biological function by interacting with miRNA. Studies have indicated [15-18] that the differential lncRNA expression might be involved into the pathogenesis of cardiovascular diseases, regenerative diseases, Type 2 diabetes and many other diseases, including tumors. But currently, there have been few studies on lncRNA in psychiatric diseases so far, and no studies on relationship of lncRNA expression and coping style. Given the fact that lncRNA extensively exists in brain tissue, and plays significant roles in brain development and senior cognitive function, its role of regulating pathogenesis in psychiatric diseases holds tremendous potential. This study explored the differential expression of lncRNA for the aim of confirming its association with individuals’ coping style.

2. Material and methods

2.1. Participants

2.1.1. Study group

A total of 96 SZ patients, 51 male and 45 female, who met the diagnostic criteria as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition for SZ were enrolled from No.904 Hospital of the PLA from August 2018 to June 2020. All patients, aged from 15 to 80, were drug naive from any antipsychotic treatment, or in the absence of antipsychotic medication within at least 3 months before enrollment. The exclusion criteria were as follow: a personal history of severe medical diseases, other psychiatric disorders, structural brain disorders, cognitive disability, unstable psychiatric features and movement disorders. Also, patients who had brain injury causing traumatic amnesia longer than 24 hours, and who received blood transfusion within 1 month or electroconvulsive therapy within 6 months, were excluded from the study.

2.1.2. Control group

50 healthy controls, 27 male and 23 female, without any family history of major psychiatric disorders (SZ, bipolar disorder, and major depressive disorder) within the last three generations were recruited. Similarly, all healthy controls were without any history of blood transfusion or severe traumatic event within 1 month. Patients and healthy controls were matched in gender, age and ethnicity. The study was approved by the local Institutional Review Board. Written informed consent was obtained from all participants.

2.2. Measuring instrument

Trait Coping Style Questionnaire has 20 items, including two factors, namely positive coping and negative coping, each of which has 10 items. This questionnaire adopts 5-point Likert scale, with definitely no scoring 1 and definitely yes scoring 5. As Wang reported [19] this questionnaire has verified reliability and validity, meeting psychometric criteria. The psychological assessing work is done by three psychiatric doctors, who were collectively trained for consistency.

2.3. Blood collection and RNA extraction

Whole blood (5 ml) was collected in EDTA anticoagulant tube from each subject and processed within 1hour. PBMCs were isolated from the blood through density gradient centrifugation and stored at -80°C until use. Total RNAs were isolated from the PBMCs using Trizol(Invitrogen, Carlsbad, CA, USA)and the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol, quantified by NanoDrop (Thermo Scientific, Delaware, ME, USA), DNase treated (TurboDNase, Life Technologies) and reverse transcribed (Superscrip III; Invitrogen). The integration of RNA was confirmed by gel electrophoresis.

2.4. lncRNA microarray expression profiling

RNA samples from 5 SZ patients (male, 23 years; male, 31 years; male, 33 years; female, 28 years; female, 42 years) and 5 controls (male, 20 years; male, 33 years; male, 34 years; female, 26 years; female, 43 years) were used for lncRNA microarray profiling. lncRNA expression was measured by Human IncRNA 3.0 array (Arraystar, Santa Clara, CA, USA).
The sample labeling, microarray hybridization and washing were performed based on the manufacturer’s standard protocols. Afterwards, the labeled RNAs were hybridized onto the microarray. Having washed and stained the slides, the arrays were scanned by the Agilent Scanner G2505C (Agilent). The scanned images were analyzed using Feature Extraction software (version10.7.1.1, Agilent Technologies) and Gene spring software (version 12.5; Agilent Technologies). 5 up-regulated lncRNAs (NONHSAT021545, NONHSAT041499, NONHSAT089447, NONHSAT098126, NONHSAT104778) and 5 down-regulated lncRNAs (ENST00000394742, ENST00000521622, ENST00000563823, TCONS_l2_00021339 and TCONS_l2_00025502) were chosen for further validation in larger sample using the qRT-PCR method.

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

According to microarray results, the top lncRNAs with the highest expression changes were chosen for further validation with qRT-PCR. Blood samples from 96 SZ patients and 50 controls were used to validate the candidate lncRNAs. Total RNAs were isolated from the PBMCs using Trizol reagent (Invitrogen®, USA) for quantitative detection of lncRNA. Complementary DNA was synthesized using the Reverse Transcription TaqMan RNA Reverse Transcription Kit (Applied Biosystems, inc., USA) according to the manufacturer’s instructions. Real-time PCR was performed using Applied Biosystems 7900HT Real-Time PCR System (Applied Biosystems, Inc., USA). Data were collected using the SDS 2.3 software (Applied Biosystems, Inc.) and DataAssist v3.0 software. After normalized to β-Actin, the expression levels of lncRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.6. Statistical analysis

Statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) for Windows 17.0. Mann–Whitney U test was performed to test the differences of 10 lncRNAs between SZ and healthy controls subjects. lncRNAs data were presented as fold change relative to the control group (control=1). Pearson correlation test was carried out for testing the correlation of lncRNAs expression level change with coping style scores. The difference in coping style between higher lncRNA expression subgroup and lower lncRNA expression subgroup were also measured by independent sample t test. All statistical tests were two-tailed and P-values of <0.05 was considered to indicate significant differences.

3. Results

3.1. Comparison of lncRNA expression between SZ patients and controls

As shown in the Table S2, altogether three lncRNAs (PR4, PR6 and PR7) in SZ patients were significantly up-regulated compared to healthy controls ($z=-2.460 \sim -2.038, P<0.05$). (Table 1)

### Table 1 Comparisons of lncRNA expression between case group and their controls ($\bar{x} \pm S$)

<table>
<thead>
<tr>
<th>lncRNAs</th>
<th>NC (n=50)</th>
<th>SZ (n=96)</th>
<th>$z$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR1</td>
<td>5.713±4.417</td>
<td>4.162±4.075</td>
<td>-2.141</td>
<td>0.0605</td>
</tr>
<tr>
<td>PR2</td>
<td>6.485±5.101</td>
<td>5.263±4.173</td>
<td>-1.587</td>
<td>0.1563</td>
</tr>
<tr>
<td>PR3</td>
<td>6.914±5.213</td>
<td>5.401±4.611</td>
<td>-1.701</td>
<td>0.0987</td>
</tr>
<tr>
<td>PR4</td>
<td>6.314±5.176</td>
<td>4.261±4.669</td>
<td>-2.289</td>
<td>0.0341</td>
</tr>
<tr>
<td>PR5</td>
<td>5.322±4.546</td>
<td>3.819±4.622</td>
<td>-1.902</td>
<td>0.0621</td>
</tr>
<tr>
<td>PR6</td>
<td>6.511±4.463</td>
<td>4.544±4.312</td>
<td>-2.381</td>
<td>0.0269</td>
</tr>
<tr>
<td>PR7</td>
<td>6.801±4.446</td>
<td>5.131±3.935</td>
<td>-2.231</td>
<td>0.0380</td>
</tr>
<tr>
<td>PR8</td>
<td>7.165±5.132</td>
<td>6.317±4.168</td>
<td>-1.698</td>
<td>0.1128</td>
</tr>
<tr>
<td>PR9</td>
<td>5.241±5.121</td>
<td>3.989±4.123</td>
<td>-1.856</td>
<td>0.0749</td>
</tr>
<tr>
<td>PR10</td>
<td>7.105±5.124</td>
<td>5.987±4.735</td>
<td>-1.701</td>
<td>0.1069</td>
</tr>
</tbody>
</table>

Note: PR1=ENST00000394742, PR2=TCONS_l2_00025502, PR3=NONHSAT098126, PR4=NONHSAT089447, PR5=ENST00000563823, PR6=NONHSAT021545, PR7=NONHSAT041499, PR8=ENST00000521622, PR9=TCONS_l2_00021339, PR10=NONHSAT104778
### 3.2. Association between IncRNA expression and coping style scores

Pearson correlation analysis results showed that the expression levels of PR4 and PR6 were negatively related to PC (P<0.05), and the expression levels of PR1, PR4, PR6 and PR7 were positively related to NC (P<0.05 ~ P<0.01). (See Table 2)

**Table 2** Correlation analysis of IncRNAs’ expression level and coping style in case group (r)

<table>
<thead>
<tr>
<th>Coping style</th>
<th>PR1</th>
<th>PR2</th>
<th>PR3</th>
<th>PR4</th>
<th>PR5</th>
<th>PR6</th>
<th>PR7</th>
<th>PR8</th>
<th>PR9</th>
<th>PR10</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>0.155</td>
<td>-0.216</td>
<td>-0.140</td>
<td>-0.390*</td>
<td>0.102</td>
<td>-0.337*</td>
<td>-0.273</td>
<td>-0.154</td>
<td>-0.142</td>
<td>-0.216</td>
</tr>
<tr>
<td>NC</td>
<td>0.336*</td>
<td>0.275</td>
<td>-0.206</td>
<td>0.374*</td>
<td>-0.294</td>
<td>0.475**</td>
<td>0.402*</td>
<td>-0.244</td>
<td>-0.220</td>
<td>0.261</td>
</tr>
</tbody>
</table>

*Note: *P<0.05, **P<0.01

### 3.3. Comparison of coping style between higher and lower IncRNA expression subgroups

Those whose expression levels of PR4, PR6 and PR7 that were less than QL(P25) were classified into lower expression subgroup, and Those whose expression levels of PR4, PR6 and PR7 that were more than QL(P75) were classified into higher expression subgroup. Comparison of PC and NC between these two subgroups were made, and the results indicated that the PC of PR4 and PR6 higher expression subgroups were significantly lower than those of PR4 and PR6 lower expression subgroups (P<0.05 ~ P<0.01), and the NC of PR4, PR6 and PR7 higher expression subgroups were significantly higher than those of PR4, PR6 and PR7 lower expression subgroups (P<0.05 ~ P<0.01). (See Table 3)

**Table 3** Comparisons of coping style between higher IncRNA-expression subgroup and lower IncRNA-expression in (X ±S)

<table>
<thead>
<tr>
<th>Coping style</th>
<th>PR4</th>
<th>PR5</th>
<th>PR6</th>
<th>PR7</th>
</tr>
</thead>
</table>

*Note: *P<0.05, **P<0.01

### 3.4. Regression analysis of IncRNA upon coping style

Taking PR1 ~ PR10 as independent variables, and PC and NC as dependent variables, regression function was established. The results demonstrated that PR6 and PR7 were entered into the NC regression function (P<0.05 ~ P<0.01), accounting for 11.3% of the NC, and PR4 and PR6 were entered into the PC regression function (P<0.01), accounting for 8.7% of the PC. (See Table 4)

**Table 4** Stepwise analysis for effects of IncRNA on schizophrenia patients’ coping style

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>B</th>
<th>SE</th>
<th>ΔR²</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>PR6</td>
<td>0.784</td>
<td>0.185</td>
<td>0.076</td>
<td>4.251</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>PR7</td>
<td>0.533</td>
<td>0.134</td>
<td>0.037</td>
<td>2.751</td>
<td>0.009</td>
</tr>
<tr>
<td>PC</td>
<td>PR4</td>
<td>0.563</td>
<td>0.156</td>
<td>0.062</td>
<td>3.612</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>PR6</td>
<td>0.394</td>
<td>0.097</td>
<td>0.025</td>
<td>2.163</td>
<td>0.007</td>
</tr>
</tbody>
</table>
4. Discussion

Currently, as reviewed by Insel [20] due to unclear etiology, the diagnosis of schizophrenia has always been limited to symptomatology, making early diagnosis and intervention impossible. Potential biomarkers, such as lncRNA, would probably shed light on this situation.

This study used microarray analysis to screen out 125 lncRNAs that were differentially expressed between SZ group and control group. Ten of them were selected for PCR verification, and 3 of them were significantly up-regulated in SZ patients, indicating that lncRNA might have been involved into the pathogenesis of SZ. A couple of studies [21-23] have proved that the temporal and spatial specificity of lncRNA have been precisely epigenetically regulated, exerting important impact upon central nervous system, thus abnormal expression of lncRNA probably resulting in psychiatric disorders. Another studies [24-26] showed that lncRNA might be involved into Alzheimer’s disease, Gomafu/MIAT/Rncr2 is proposed to control retinal cell specification, stem cell differentiation and alternative splicing of schizophrenia related genes. These lncRNA happen to be located at SZ related chromosome region as Gianfrancesco et al. [27] examined in their study, indicating that abnormality of lncRNA might lead to the onset of schizophrenia. The three lncRNAs that were up-regulated in this study might be worth of exploring for a better understanding of pathogenesis of SZ.

The dominating theory about the etiology of schizophrenia has been that both genetic and environmental factors contribute to its onset. And on the other hand, coping, defined as both cognitive and behavioral efforts against internal requests or external stress, has been identified as a mediating factor between environmental stress and psychiatric diseases [28], and one study [29] has been confirmed that improper coping style manifests itself in SZ patients. Our study results demonstrated a significant association between the expression levels of three lncRNAs, namely PR4, PR6 and PR7, and coping styles, namely positive coping style and negative coping style, and the expression levels of PR4 and PR6 accounted for 8.7% of positive coping style, and PR6 and PR7 accounted for 11.3% of negative coping style, suggesting that lncRNAs might play a role in SZ patients’ choosing coping style. This conclusion accords well with Spadaro’s study [30], indicating that lncRNA might be involved into individuals’ behavioral readjustment. Consistently, Hasan’s study [31] proved that a positive coping style would improve prognosis in SZ patients.

5. Conclusion

In conclusion, PR4, PR6 and PR7 that were significantly expressed in SZ patients may have the potential to become one of the clinical joint markers for SZ diagnosis. They probably play important roles in the pathogenesis of SZ, which warrants further evidences.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All authors declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position on presented in, or the review of, the manuscript entitled.

Statement of informed consent

The present research was approved by the local ethics committee, and written informed consent was obtained from all individual participants included in the study.
References


