LncRNA NONRATT021972 in Neuropathic Pain Patients with Type 2 Diabetes

Kai LI¹, Yuan JIAO¹ and Hongyu ZHANG²*

¹Department of Anesthesiology, China Japan Union Hospital of Jilin University, Changchun
²Department of Endocrinology, China Japan Union Hospital of Jilin University, Changchun
*Corresponding author

Keywords: LncRNA, Diabetic, Neuropathic pain.

Abstract. Objective to explore LncRNA NONRATT021972 in diabetic neuropathic pain and detailed mechanisms in clinical patient. Methods According to inclusion criteria, 154 Chinese patients with type 2 diabetes were enrolled as experimental group, and the same numbers of normal adults were paired as control group with the same baseline status. Patients without diabetes but neuropathy were also enrolled to explore exclusive role of LncRNA NONRATT021972 in neuropathy. Vein blood sampling was performed in both groups for further experiment. Quantitative Real-time PCR was performed to examine expression of LncRNA NONRATT021972 in blood. Neuropathic pain scores were calculated with data collected from Neuropathic Pain Questionnaire in Both groups. Result Compared with control group, patients with type 2 diabetes had a significantly higher concentration of LncRNA NONRATT021972 in blood (P<0.05) and more severe symptoms of neuropathic pain. Moreover, among patients with type 2 diabetes, the concentration of LncRNA NONRATT021972 was positively associated with neuropathic pain scores, suggesting pain-aggravation effect of LncRNA NONRATT021972 was concentration-dependent. However, no association was observed between neuropathy and LncRNA NONRATT021972 in patients without diabetes but neuropathy. Conclusion LncRNA NONRATT021972 was increased in type 2 diabetes, and was positively associated with neuropathic pain scoring in patients with type 2 diabetes.

Introduction

Long non-protein-coding RNA (LncRNAs) belongs to family of small RNAs, and is characterized with transcripts that are >200 nucleotides in molecular length. LncRNAs can be transcribed, ranging from 195 bp up to a couple of kilobases in length according to the sequence of LncRNA nearby protein-coding genes [1, 2]. Previous studies have indicated that LncRNA was involved in occurrence and progressing of diabetes, and produced a complex regulatory network through interactions with transcription factors in type 2 diabetes. On the other hand, LncRNAs were also involved in the pathological progression of nervous system, and had multiple effects on neuropathic pain [3].

Diabetes mellitus has become a global health issue in recent years, with a global incidence of 14.6 %. In clinical scenario, neuropathic pain is one of the most common chronic complications in patients with type 2 diabetes, and character with typical symptoms of pathological pain, including hyperalgesia, spontaneous pain and specific alldynia [4, 5]. Thus, it is urgent to develop novel therapy for neuropathic pain of type 2 diabetes due to the enormous number of diabetes patients and its adverse effects.

NONRATT021972 (NONRA) is an LncRNA which was proved with diabetes promoting effect, and its sequence was well determined (http://www.noncode.org/show_ma.php?id=NONRATT021972). Animal experiments have showed that expression of NONRATT021972 increased in diabetic mice, and participated in the transmission of nociceptive signaling, especially in neuropathic pain. What’s more, there are data suggesting NONRA silence was related to decreased inflammation, however, although researchers found NONRA could regulate P2X7 and
P2X3 receptors in dorsal root ganglia, no detailed mechanisms were explored in the field of inflammation. In the clinical application, it is unclear whether NONRA could be a predictor for type 2 diabetes, and there is also no report about how NONRA influenced neuropathic pain of type 2 diabetes in clinical scenario. Accordingly, we hypothesized that NONRA could be a potential biomarker for neuropathic pain of type 2 diabetes in clinical scenario.

Materials and Methods

Clinical Samples

154 patients with type 2 diabetes were enrolled as experimental group from local hospital. All patients signed informed consent and consent with following examinations. The inclusion criteria were as follows: male, aging (55-65 years), type 2 diabetes, without history of hypertension, no history of chronic kidney disease. The same number of healthy subjects was enrolled from the same hospital as control group, which are matched to experimental group with similar baseline states. To further investigate whether NONRA is exclusively a biomarker of pain, we enrolled patients without diabetes but neuropathy. The inclusion criteria were as follows: patients with confirmed neuropathic pain, especially patients with polyneuropathy (ie. distal symmetric sensory polyneuropathy patients, small fibers neuropathy patients or localized neuropathy), patients without diabetes. The diagnosis standard of neuropathy is accordance with statement of American Diabetes Association. Distal symmetric sensory polyneuropathy group (DPN group) had 76 samples, while small fibers neuropathy group (SFN group) 46 samples, localized neuropathy group (LN group) 65 samples. Blood samples harvested from both groups were collected and allowed to coagulate for 30 min at room temperature. All samples were centrifuged for 10 min (1300×g). The collected serum was centrifuged for another 10 min (3000×g) to remove any remaining cellular components. Supernatants were transferred into 500μL EP tube and stored immediately at −80 °C.

Serum Isolation of NONRA and RT-PCR

Total RNA was isolated from the serum using the RNA pure Circulating Reagent (CWBIIO, Beijing, cat. no. CW2281) with routine protocols. Briefly, serum sample (300 μL) was added to three times volumes of RNA pure Circulating Reagent and mixed thoroughly via vortex. Storage samples to maintain reaction at room temperature for five minutes. Further, 1/5 volume of chloroform was added, mixed vigorously for 30s, and incubated for 5 min at room temperature. Centrifugate mixture for 20 min (12,000×g, 4 °C). The supernatant was transferred to a new sterile EP tube, and the same volume of isopropanol was added and mixed thoroughly for 30 min at room temperature. Centrifugate mixture for 20 min (12,000×g, 4°C). The precipitate was transferred and rinsed twice with 1 mL of 75 % ethanol (dilute with DD H2O). Isolated RNA was eluted by RNase-free water (20 μL) with routine protocols. Total RNA (1000 ng) was prepared as standard template for reverse transcription with the Revert Aid First Strand cDNA Synthesis Kit (Thermos, USA). PCR amplification of NONRA and GAPDH (control) was performed according to reported method. The primers were as follows: NONRA, 5-TGTTTCGTCAACTGTCAGCT-3, antisense 5-GGGATGTTCAAAGCTTCA-3; The estimated length of the PCR product was 250 bp and 199 bp for NONRA and GAPDH, respectively. The thermal cycling protocols were as follows: 95 °C for 30 s; 40 cycles of amplification at 95 °C for 5 s; 60°C for 30 s. The results of PCR were analyzed by the software with PCR instrument (ABI7500).

Scoring with Neuropathic Pain Questionnaire

All subjects in two groups received and completed Neuropathic Pain Questionnaire (NPQ). Data collect from questionnaire were assessed by three independent expertized individuals for neuropathic pain scores. NPQ scoring was performed according to guideline recommendation.

Statistical Analysis

SPSS 16.0 software was used for data processing. Measurement data are normal distribution to
mean ± SD. T test was performed for data with normal distribution and equal variance. Logistic
Linear regression was performed to determine association among variate.

Result

NONRA Increased in Type 2 Diabetes

Compared with control group, serum concentration of NONRA indeed increased in patients with
type 2 diabetes (P<0.05), suggesting increase of NONRA was associated with increased blood
glucose or occurrence of type 2 diabetes and NONRA might be a novel biomarker of type 2
diabetes (Table 1). Moreover, NPQ showed that incidence of neuropathic pain significantly
increased in diabetes group (Table 2).

Nonra Was Associated with Neuropathic Pain in Type 2 Diabetes

Further intra-group analysis showed that serum levels of LncRNA NONRATT021972 varied
relatively greatly among patients with type 2 diabetes (Table 1), and similar trend was observed in
neuropathic pain scoring, verified by NPQ. Logistic Linear regression showed that neuropathic pain
scoring was positively related to the level of NONRA, suggesting pain-aggravation effect of
NONRA was concentration-dependent (Figure 1). Moreover, symptom types of neuropathic pain
increased in patients with high level of NONRA.

NONRA Was Not Associated with Neuropathic Pain in Patients without Type 2 Diabetes

Further analysis showed that, compared with controls, the level of NONRA did not significantly
change in DPN group, and similar phenomenon were observed in SLN versus control and LN
versus control (Table 3).

Table 1. Analysis of blood glucose and NONRA relative expression.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>blood glucose</th>
<th>NONRA relative expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>5.12±1.023</td>
<td>1.00±0.107</td>
</tr>
<tr>
<td>D2M group</td>
<td>13.27±2.972*</td>
<td>2.98±0.693*</td>
</tr>
</tbody>
</table>

Compared with Control group*P<0.05

Table 2. Numbers of patients with different NPQ score in two groups.

<table>
<thead>
<tr>
<th>Categories Based on Score</th>
<th>Control group (n=154)</th>
<th>D2M group (n=154)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤5</td>
<td>112</td>
<td>38</td>
</tr>
<tr>
<td>6-8</td>
<td>19</td>
<td>44</td>
</tr>
<tr>
<td>9-11</td>
<td>15</td>
<td>37</td>
</tr>
<tr>
<td>≥12</td>
<td>8</td>
<td>35</td>
</tr>
</tbody>
</table>

Figure 1. Association between NPQ score and NONRA relative expression.
Discussion
Although LncRNAs accounts for a quite high proportion of RNA profile in mammalian, current studies only revealed a small number of LncRNAs which have been functionally proved, and functions of most LncRNAs are unclear. Recent evidence from animal experiments indicated that the abnormal expression of LncRNAs participates in the occurrence and progression of type 2 diabetes, in which LncRNA ONRAT021972 was a biomarker with promising clinical potential [6].

Our studies firstly shown that increased serum level of NONRA was positively associated with grade neuropathic pain in patients with type 2 diabetes, suggesting NONRA was biomarker or predictive factor for neuropathic pain of type 2 diabetes. Further clinical trials explored that there was no association between neuropathy and NONRA in patients without diabetes, suggesting NONRA was a biomarker for type 2 diabetes and not exclusively a marker for neuropathy.

Previous reports indicated that NONRA was involved in type 2 diabetes and mostly induced hypersensitivity of nervous system, which finally resulted in neuropathic pain [7]. Moreover, animal studies have showed that NONRA aggravated diabetic neuropathic pain via P2X3 or other P2 receptors in dorsal root ganglia [8]. However, as described above, such studies were mainly focused on animal studies or underlying mechanisms, while no concrete data were offered in clinical scenario. This report made the first excellent answer in clinical research.

In summary, NONRA was positively associated with neuropathic pain scoring in patients with type 2 diabetes. Inhibition of NONRA could be a target for alleviate neuropathic pain.

Acknowledgement
This research was financially supported by Jilin province Financial Foundation SCZSY201719, 3D515B173430.

References