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Research Article

Detection and Prediction of Neurodegenerative Disorders by Long Noncoding RNA Signature Analysis among Bangladeshi Population

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Long noncoding RNAs (lncRNAs) have appeared lately as a new class of genes that may control and mediate cellular processes. It plays a critical role in many diseases, including neurodegenerative disorders. HOTAIR and MKRN2-42:1 are two lncRNAs that have been seen upregulated in various diseases including in the peripheral blood of the patients suffering from Parkinson's disease (PD) in different countries. It has also been seen that Meg3v1 and NEAT1 lncRNAs are expressed highly in patients suffering from motor neuron disease (MND) from various countries, though the association between the reason for the onset of this disease and the expression of these specific lncRNAs is not clear. The primary aim of this study was to observe expression variations for specific lncRNAs in the peripheral blood of PD and MND patients compared with healthy individuals and to look for lncRNAs that might be related to the pathogenesis of PD and MND. The aim of this study was also to investigate the association of lncRNA sequence as biomarkers with the risk of having neurodegenerative disorders (PD and MND) in the population of Bangladesh. The purpose of this research also included the possibility of prediction of these neurodegenerative disorders by analyzing and comparing the data which was acquainted from the study where 20 PD patients and 20 healthy subjects and 25 MND patients and 25 healthy subjects of Bangladesh were examined. Their peripheral blood was taken, and plasma was separated. Later, RT-PCR was performed to observe the changes in the expression of specific lncRNAs. Results showed that expression of HOTAIR and MKRN2-42:1 was positively correlated ($p < 0.05$) with the symptoms (5) of Parkinson's suspects in comparison to the healthy subjects. Similarly, Meg3v1 and NEAT1 lncRNAs were expressed significantly ($p < 0.05$) in MND patients rather than that in healthy controls. Data were analyzed using GraphPad Prism 7.0, where $p < 0.05$ was considered statistically significant. Successful prediction of the possibility of neurodegenerative disorder by analyzing data regarding the expression of lncRNAs as biomarkers may help in advance clinicians to stratify high-risk patients and prepare for appropriate medical intervention.

1. Introduction

Noncoding RNAs take part as regulators of gene expression and have a crucial role in mediating different signaling pathways. Recently, ncRNAs are thought as one of the causes of the pathogenesis of multiple diseases. Noncoding RNAs are considered as novel transcripts which own no probable protein-coding components. However, rising and collecting biochemical and genetic shreds of proof have regularly found out their vital regulatory roles in growth of disease and ailment contexts [1, 2]. Theoretically, regulatory ncRNAs are divided into two groups considering their lengths. RNAs that are shorter than 200 nucleotides (nt) in length are called small RNA, for instance, microRNA

(miRNA, 22-25 nt), Piwi-interacting RNA (piRNA, 21-35 nt), small nucleolar RNA (snoRNA, 60-170 nt), and transfer RNA (tRNA, 70-100 nt). ncRNAs longer than 200 nt are defined as long noncoding RNAs (lncRNAs) that include approximately 10~30% of transcripts in not only human (GENCODE 32) but also mouse (GENCODE M23) genomes, showing that they may take part broadly in the mammal physiology which is mostly undiscovered till now. Classification of lncRNAs can be done according to their genomic location. They can be transcribed from introns (intronic lncRNA), coding exons, $3'$ or $5'$ untranslated regions ($3'$ or $5'$ UTRs), and in an antisense direction overlapping with their transcripts (natural antisense transcript (NAT)) [3, 4]. In regulatory regions, upstream promoters (promoter upstream transcript (PROMPT)), enhancers (eRNA), intergenic regions (lincRNA), and telomeres can also generate lncRNAs [5]. It has been proven in many previous studies that lncRNAs possess crucial roles in regulating cellular processes [6]. It has been shown in different studies that in the nucleus, lncRNAs take part approximately in all types of gene regulation, from keeping nuclear structure to transcription, etc. [7]. The expression ranges and forms of a wide variety of lncRNAs are susceptible to change before or during Parkinson's disease (PD) and motor neuron (MN) disease [8]. Parkinson's disease and motor neuron disease are developing as a threat these days worldwide, including in Bangladesh. Therefore, it has turned out to be pressing to find out and prove prognostic biomarkers that can acknowledge subjects at the largest risk for future cognitive degeneration as well as speed up the testing of preventive plans. Recent research of combinatorial biomarkers shows that they may have a greater potential to seize the heterogeneity as well as multifactorial complexity of numerous neurodegenerative disorders, than a conventional, and signify numerous potential risk factors for Parkinson's disease (PD) and motor neuron (MN) disease with numerous cross-sectional research additionally correlating information with imaging and fluid biomarkers [9]. lncRNAs can be treated as biomarkers for neurodegenerative disorder [10]. This study may help in the detection and prediction of the possibility to have such neurodegenerative disorders in the future by analyzing these biomarkers. Here, we are going to find 2 (HOTAIR, MKRN2-42:1) specific lncRNAs which are expressed in the patients suffering from Parkinson's disease and 3 ($Meg3^{v1}$, NEAT1) specific lncRNAs which are expressed in the patients suffering from motor neuron disease.

2. Methods and Materials

2.1. Blood Sample Collection and Whole RNA Extraction. To validate the expression of specific lncRNAs in the plasma of blood samples from Parkinson's and motor neuron disease patients of the Bangladeshi population, blood samples were taken from a total of 552 Bangladeshi individuals where a total number of 138 Parkinson's disease patients, 138 motor neuron disease patients, and 276 healthy people, all having a Bengali ethnic background, were enrolled in this study. This study was performed after receiving clearance from the Ethical Review Committee of Dhaka Medical College Hospital. The identity of the patients and the information acquired after analysis were disclosed. Data were used for research purposes only [11]. All the subjects fulfilled a questionnaire providing data on age, sex, symptoms, duration of disease, blood pressure, etc. 5 mL of venous blood was drawn from each individual maintaining all aseptic safety measures with the assistance of a trained person and was instantly shifted to an Ethylenediaminetetraacetate (EDTA) containing vacuum tube [12]. Then, that tube was then centrifuged for 10 minutes at 4,000 rpm. Plasma was then transferred with a micropipette taking care not to take any red cells. Appropriate aliquots of plasma were stored in centrifuge tubes. All samples (plasma and whole blood) were stored at -80°C. RNA extraction from the plasma was performed by using a TRIzol kit according to the manufacturer's instruction. RNA extraction and purification combined phase separation with the speed of microspin technology.

2.2. Preparation of cDNA. After extracting RNA, cDNA was made from whole RNA. Total extracted RNAs were used to construct the GoScript™ Reverse Transcription System (origin: Promega, USA) cDNA according to the manufacturer's instructions. Then, PCR was performed to acquire an enhanced cDNA. At last, products were purified (AMPure XP system) and assessed (qTOWER).

2.3. Performing RT-PCR. The Analytik Jena $qTOWER^3$ G Real-Time PCR Thermal Cycler machine was used to perform RT-PCR. Expression of lncRNAs was demonstrated by using a quantitative real-time PCR (qRT-PCR) with qTOWER. Briefly, reactions were performed in a mixture (20 μL) containing 1 μL cDNA template, 10 μL 2x SYBR-Green PCR Mix, 8 μL H_2O, and 0.5 μL each of sense and antisense primers. A total of 4 lncRNAs was taken to be differentially expressed, using qRT-PCR. The sequences of the primers were designed for use in RT-PCR. 45 healthy samples were used as the control. After qRT-PCR amplification, a melting curve analysis was performed to confirm reaction specificity, and the fold change (FC) of each lncRNA was calculated via the ΔCt method. 2–5 μL of the diluted cDNA product is recommended per 50 μL PCR reaction. The list of temperatures and cycles for RT-PCR for 4 lncRNAs will be found in supplemental table S1, S2, S3, and S4. The list of forward and reverse primers which have been used for 4 specific lncRNAs and internal control will be found in supplemental table S5.

The relative mRNA expression levels were analyzed and expressed relative to threshold cycle values by delta-delta Ct ($2^{-\Delta\Delta Ct}$) method. GAPDH was used as an internal control. Here, $\Delta Ct = Ct(\text{target gene}) - Ct(\text{reference gene})$, where reference gene is GAPDH. $\Delta\Delta Ct = \Delta Ct\,(\text{target sample}) - \Delta Ct(\text{reference sample})$, where reference sample is the control sample.

3. Result

3.1. Sample Size. According to a formula of scientific research sample size,

$$n = \frac{Z^2 pq}{d^2}, \tag{1}$$

where n is the desired sample size; Z is the standard normal distribution, usually set at 1.96 at 5% level which corresponds to 95% confidence level; p is the proportion of patients which is 10%, so $p = 0.1$; and d is the acceptable error. It is usually set at 5% (0.05). $q = (1 - p) = 1 - 0.1 = 0.9$. n is the $(1.96)^2(0.1)\ (0.9)/(0.05)^2$. n is 138.

We conducted this experiment in two parts:

(1) Estimation of Ct values from RT-PCR

(2) Analysis of specific lncRNAs' frequency

3.2. Status of the Expression Level of Different lncRNAs in Different Study Groups. Mean $2^{-\Delta Ct}$ values of expression of HOTAIR, MKRN2-42:1, Meg3^{v1}, and NEAT1 were analyzed between cases and controls of our study groups where GAPDH was used as an internal control. Here, $\Delta Ct = Ct\,(\text{target gene}) - Ct(\text{reference gene})$, where reference gene is GAPDH. Mean ± SEM$2^{-\Delta Ct}$ values of expression of HOTAIR and MKRN2-42:1 lncRNAs in the Parkinson's patient group and control subject are shown in Figure 1(a). The mean ± SD of $2^{-\Delta Ct}$ values of expression of HOTAIR lncRNAs was 869.66 ± 3070.28 ($n = 138$) and 0.00096 ± 0.00056 ($n = 138$) in the Parkinson's patient group and control group, respectively. The mean $2^{-\Delta Ct}$ values of expression of HOTAIR lncRNAs of the Parkinson's patient group were significantly higher ($p < 0.0001$) compared to the control group.

The mean ± SD of $2^{-\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs was 2323.04 ± 5884.49 ($n = 138$) and 0.00091 ± 0.00058 ($n = 138$) in the Parkinson's patient group and control group, respectively. The mean $2^{-\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs of the Parkinson's patient group were significantly higher ($p < 0.0001$) compared to the control group.

Mean ± SEM$2^{-\Delta Ct}$ values of expression of Meg3^{v1} and NEAT1 lncRNAs in the motor neuron disease patient group and control subject are shown in Figure 1(b). The mean ± SD of $2^{-\Delta Ct}$ values of expression of Meg3^{v1} lncRNAs was 13.51 ± 32.56 ($n = 138$) and 0.0014 ± 0.0015 ($n = 138$) in the motor neuron disease patient group and control group, respectively. The mean $2^{-\Delta Ct}$ values of expression of Meg3^{v1} lncRNAs of the motor neuron disease patient group were significantly higher ($p < 0.0001$) compared to the control group.

The mean ± SD of $2^{-\Delta Ct}$ values of expression of NEAT1 lncRNAs was 8.075 ± 14.07 ($n = 138$) and 0.0017 ± 0.0024 ($n = 138$) in the motor neuron disease patient group and control group, respectively. The mean $2^{-\Delta Ct}$ values of expression of NEAT1 lncRNAs of the motor neuron disease patient group were significantly higher ($p < 0.0001$) compared to the control group.

3.3. Correlation and Regression Analyses between Different Parameters of Study Subjects. The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of lncRNAs and symptoms was calculated. The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of HOTAIR lncRNAs and "tremors and trembling of hands, arms, jaw, face" symptom was $r = 0.6342$ in the Parkinson's patient group. Though this indicates that there was a positive correlation between these two variables in Parkinson's patients, the correlation was significant where $p = 0.0027 < 0.05$. Here, R-squared value = 0.4022 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of HOTAIR lncRNAs and "tremors and trembling of hands, arms, jaw, face" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 2).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of HOTAIR lncRNAs and "stiffness of the arms, legs and trunk" symptom was $r = 0.5497$ in the Parkinson's patient group. Though this indicates that there was a positive correlation between these two variables in Parkinson's patients, the correlation was significant where $p = 0.0120 < 0.05$. Here, R-squared value = 0.3021 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of HOTAIR lncRNAs and "stiffness of the arms, legs and trunk" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 2).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of HOTAIR lncRNAs and "face slowness of movement" symptom was $r = 0.4440$ in the Parkinson's patient group. Though this indicates that there was a positive correlation between these two variables in Parkinson's patients, the correlation was significant where $p = 0.0499 < 0.05$. Here, R-squared value = 0.1971 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of HOTAIR lncRNAs and "face slowness of movement" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 2).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of HOTAIR lncRNAs and "poor balance and coordination" symptom was $r = 0.4440$ in the Parkinson's patient group. Though this indicates that there was a positive correlation between these two variables in Parkinson's patients, the correlation was significant where $p = 0.0499 < 0.05$. Here, R-squared value = 0.1971 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of HOTAIR lncRNAs and "poor balance and coordination" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 2).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of HOTAIR lncRNAs and "speech difficulty" symptom was $r = 0.4963$ in the Parkinson's patient group. Though this indicates that there was a positive correlation between these two variables in Parkinson's patients, the correlation was significant where $p = 0.0260 < 0.05$. Here, R-

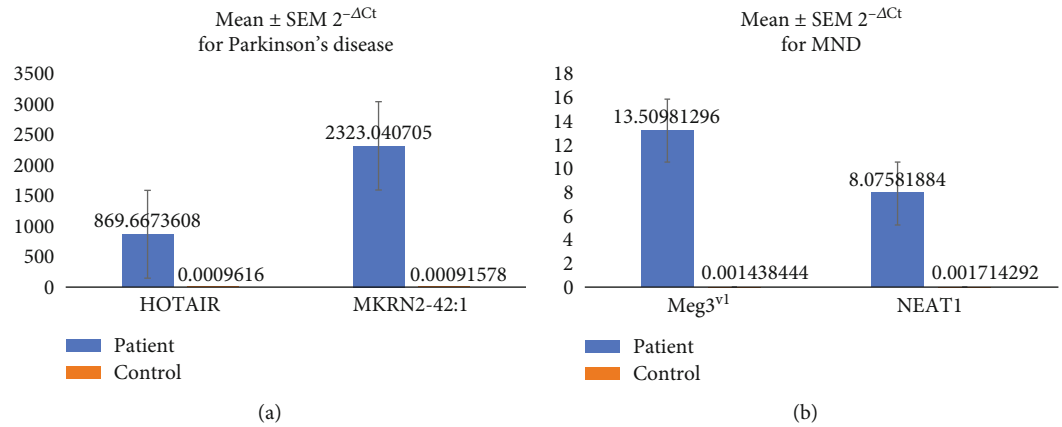

Figure 1: Mean $2^{-\Delta Ct}$ values of expression of HOTAIR and MKRN2-42:1 in PD patients and control group. Data are presented as mean ± SEM$2^{-\Delta Ct}$. Student's t-test was performed to analyze data. (a) Mean ± SEM$2^{-\Delta Ct}$ values of expression of HOTAIR and MKRN2-42:1 in different study subjects. $p\,(0.0001) < 0.05$ was considered as the level of significance. Mean ± SEM$2^{-\Delta Ct}$ values of expression of Meg3^{v1} and NEAT1 in the MND patient's and control group. Data are presented as mean ± SEM$2^{-\Delta Ct}$. Student's t-test was performed to analyze data. (b) Mean $2^{-\Delta Ct}$ values of expression of Meg3^{v1} and NEAT1 in different study subjects. $p\,(0.0001) < 0.05$ was considered the level of significance.

squared value = 0.2463 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of HOTAIR lncRNAs and "speech difficulty" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 2).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs and "tremors and trembling of hands, arms, jaw, face" symptom was $r = 0.6464$ in the Parkinson's patient group. Though this indicates that there was a positive correlation between these two variables in Parkinson's patients, the correlation was significant where $p = 0.0021 < 0.05$. Here, R-squared value = 0.4179 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs and "tremors and trembling of hands, arms, jaw, face" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 3).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs and "stiffness of the arms, legs and trunk" symptom was $r = 0.5013$ in the Parkinson's patient group. Though this indicates that there was a positive correlation between these two variables in Parkinson's patients, the correlation was significant where $p = 0.0243 < 0.05$. Here, R-squared value = 0.2513 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs and "stiffness of the arms, legs and trunk" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 3).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs and "face slowness of movement" symptom was $r = 0.4980$ in the Parkinson's patient group. Though this indicates that there was a positive correlation between these two variables in Parkinson's patients, the correlation was significant where $p = 0.0255 < 0.05$. Here, R-squared value = 0.2480 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs and "face slowness of movement" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 3).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs and "poor balance and coordination" symptom was $r = 0.4446$ in the Parkinson's patient group. Though this indicates that there was a positive correlation between these two variables in Parkinson's patients, the correlation was significant where $p = 0.0495 < 0.05$. Here, R-squared value = 0.1976 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs and "poor balance and coordination" symptom was significant. Regression analysis between these two variables were performed. F-test was used to analyze significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 3).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs and "speech difficulty" symptom was $r = 0.4980$ in the Parkinson's patient group. Though this indicates that there was a positive correlation between these two variables in Parkinson's patients, the correlation was significant where $p = 0.0255 < 0.05$. Here, R-squared value = 0.2480 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs

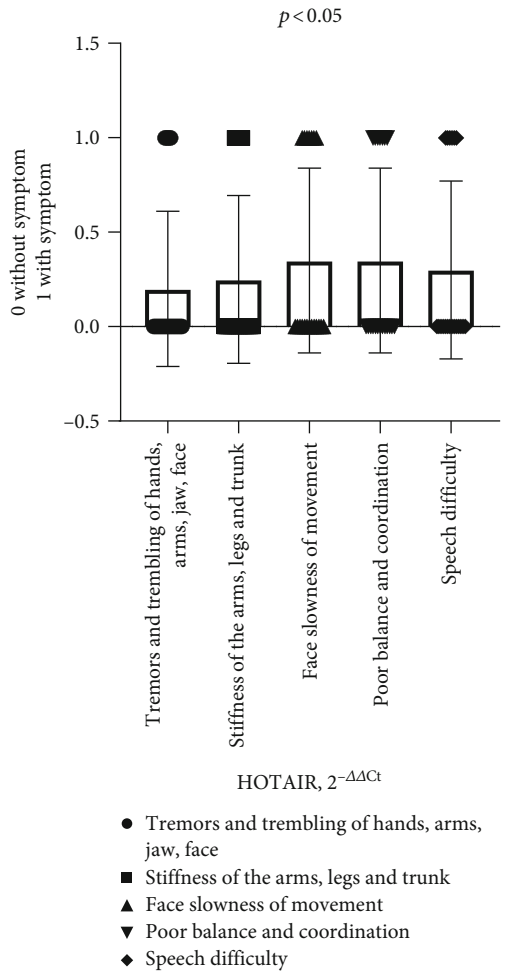

Figure 2: Correlation and regression analyses of $2^{-\Delta\Delta Ct}$ values of expression of HOTAIR lncRNAs with "tremors and trembling of hands, arms, jaw, face"; "stiffness of the arms, legs and trunk"; "face slowness of movement"; "poor balance and coordination"; and "speech difficulty" symptoms ($r = 0.6342$, $r = 0.5497$, $r = 0.4440$, $r = 0.4440$, and $r = 0.4963$, respectively). Linear regression was performed to analyze the data.

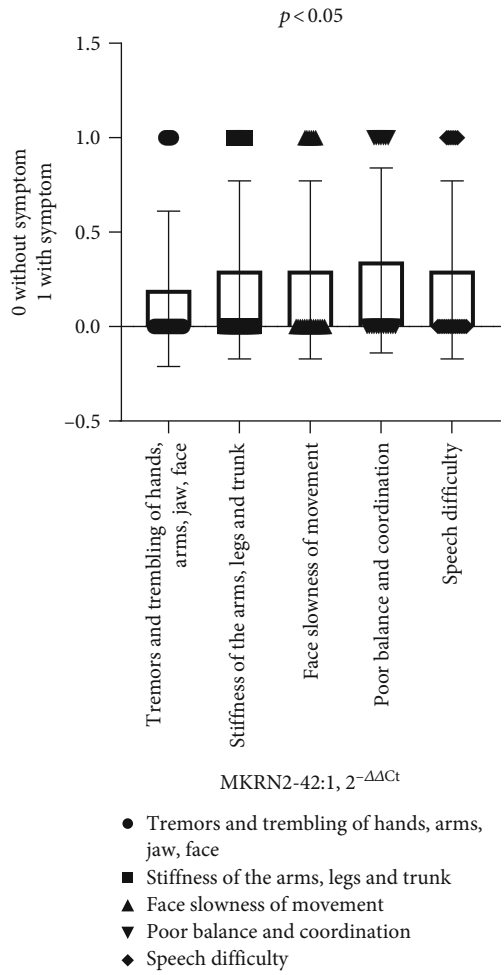

Figure 3: Correlation and regression analyses of $2^{-\Delta\Delta Ct}$ values of expression of MKRN2-42:1 lncRNAs with "tremors and trembling of hands, arms, jaw, face"; stiffness of the arms, legs and trunk"; "face slowness of movement"; "poor balance and coordination"; and "speech difficulty" symptoms ($r = 0.6464$, $r = 0.5013$, $r = 0.4980$, $r = 0.4446$, and $r = 0.4980$, respectively). Linear regression was performed to analyze the data.

and "speech difficulty" symptom was significant. Regression analysis between these two variables was performed. *F*-test was used to analyze significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 3).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "muscle aches, cramps, twitching" symptom was $r = 0.6438$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p = 0.0005$ (<0.05). Here, *R*-squared value = 0.4145 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "muscle aches, cramps, twitching" symptom was significant. Regression analysis between these two variables was performed. *F*-test was used to analyze significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 4).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "clumsiness and stumbling" symptom was $r = 0.7842$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p < 0.0001$ (<0.05). Here, *R*-squared value = 0.6149 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "clumsiness and stumbling" symptom was significant. Regression analysis between these two variables was performed. *F*-test was used to analyze the significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 4).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "slurred speech, swallowing or chewing difficulty" symptom was $r = 0.9404$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these

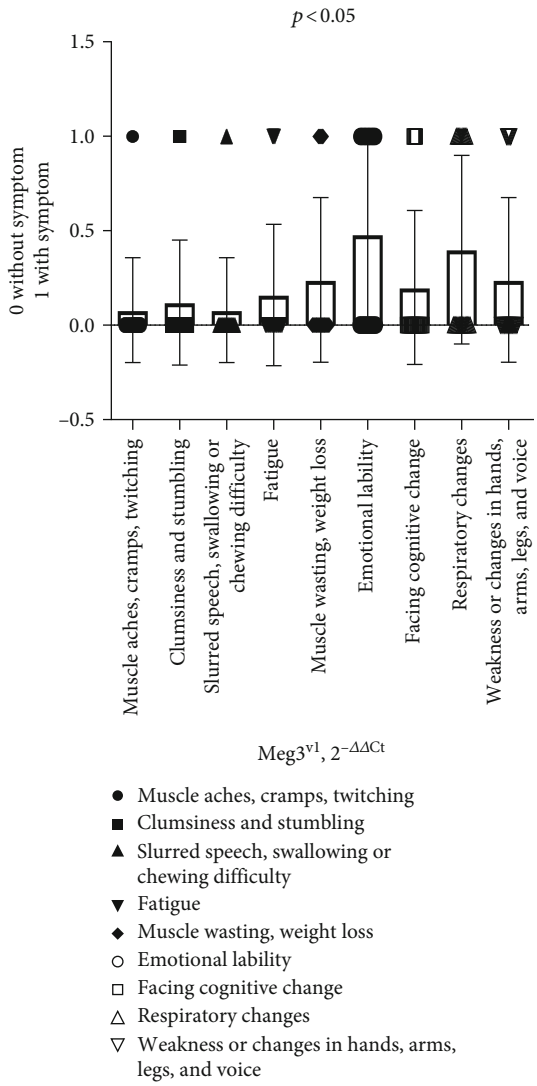

Figure 4: Correlation and regression analyses of $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs with "muscle aches, cramps, twitching"; "clumsiness and stumbling"; "slurred speech, swallowing or chewing difficulty"; "fatigue"; "muscle wasting, weight loss"; "emotional lability"; "facing cognitive change"; "respiratory changes"; and "weakness or changes in hands, arms, legs, and voice" symptoms ($r = 0.6438$, $r = 0.7842$, $r = 0.9404$, $r = 0.6679$, $r = 0.5259$, $r = 0.3087$, $r = 0.5867$, $r = 0.3508$, and $r = 0.5233$, respectively). Linear regression was performed to analyze the data.

two variables in motor neuron disease patients, the correlation was significant where $p < 0.0001$ (<0.05). Here, R-squared value = 0.8844 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "slurred speech, swallowing or chewing difficulty" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 4).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "fatigue" symptom was $r = 0.6679$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p = 0.0003$ (<0.05). Here, R-squared value = 0.4460 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "fatigue" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 4).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "muscle wasting, weight loss" symptom was $r = 0.5259$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p = 0.0069$ (<0.05). Here, R-squared value = 0.2766 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "muscle wasting, weight loss" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 4).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "emotional lability" symptom was $r = 0.3087$ in the motor neuron disease patient group. Though this indicates that there was a low degree of positive correlation between these two variables in motor neuron disease patients, the correlation was not significant where $p = 0.1333$ (>0.05). Here, R-squared value = 0.09528 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "emotional lability" symptom was low significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. No significant association between these two variables was found during analysis ($p > 0.05$) (Figure 4).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "facing cognitive change" symptom was $r = 0.5867$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p = 0.0021$ (<0.05). Here, R-squared value = 0.3442 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "facing cognitive change" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 4).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ and "respiratory changes" was $r = 0.3508$ in the motor neuron disease patient group. This indicates that there was a low degree of positive correlation between these two variables in motor neuron disease patients. But the correlation was not significant because $p = 0.0856$ ($p > 0.05$). Regression analysis between these two

variables was performed. F-test was used to analyze the significance between the two variables. No significant association between these two variables was found during analysis ($p > 0.05$) (Figure 4).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "weakness or changes in hands, arms, legs, and voice" symptom was $r = 0.5233$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p = 0.0073$ (<0.05). Here, R-squared value = 0.2738 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of $Meg3^{v1}$ lncRNAs and "weakness or changes in hands, arms, legs, and voice" was significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 4).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "muscle aches, cramps, twitching" symptom was $r = 0.6480$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p = 0.0005$ (<0.05). Here, R-squared value = 0.4199 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "muscle aches, cramps, twitching" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 5).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "clumsiness and stumbling" symptom was $r = 0.5535$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p = 0.0041$ (<0.05). Here, R-squared value = 0.3064 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "clumsiness and stumbling" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 5).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "slurred speech, swallowing or chewing difficulty" symptom was $r = 0.7095$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p < 0.0001$ (<0.05). Here, R-squared value = 0.5033 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "slurred speech, swallowing or chewing difficulty" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze the

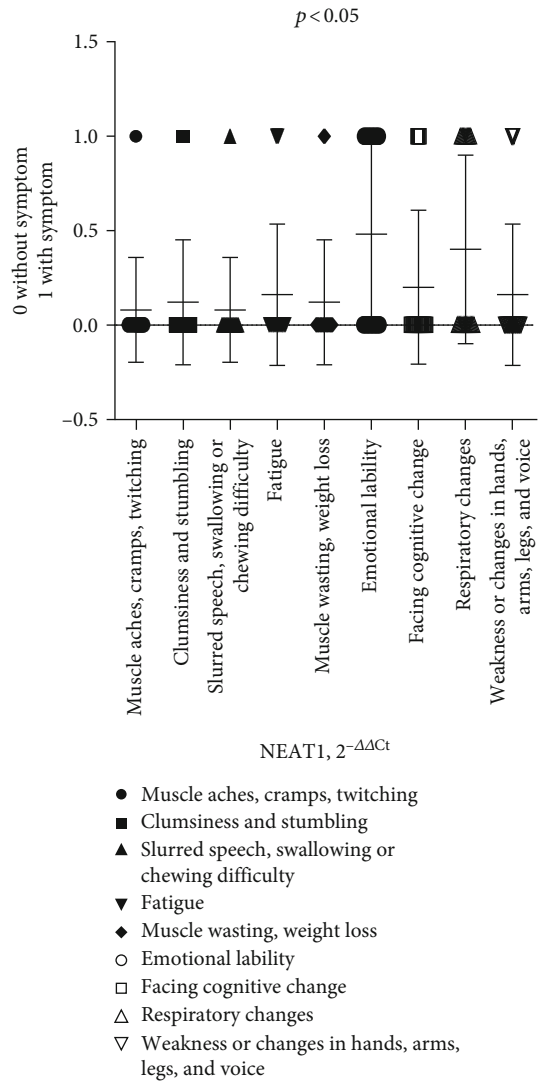

Figure 5: Correlation and regression analyses of $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs with "muscle aches, cramps, twitching"; "clumsiness and stumbling"; "slurred speech, swallowing or chewing difficulty"; "fatigue"; "muscle wasting, weight loss"; "emotional lability"; "facing cognitive change"; "respiratory changes"; and "weakness or changes in hands, arms, legs, and voice" symptoms ($r = 0.6480$, $r = 0.5535$, $r = 0.7095$, $r = 0.4565$, $r = 0.7482$, $r = 0.2296$, $r = 0.5865$, $r = 0.1983$, and $r = 0.6743$, respectively). Linear regression was performed to analyze the data.

significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 5).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 and "fatigue" was $r = 0.4565$ in the motor neuron disease patient group. This indicates that there was a positive correlation between these two variables in motor neuron disease patients; the correlation was significant where $p = 0.0218$ (<0.05). Here, R-squared value = 0.2084 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "fatigue" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze the

significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 5).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "muscle wasting, weight loss" symptom was $r = 0.7482$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p < 0.0001$ (<0.05). Here, R-squared value = 0.5598 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "muscle wasting, weight loss" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 5).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "emotional lability" symptom was $r = 0.2296$ in the motor neuron disease patient group. Though this indicates that there was a low degree of positive correlation between these two variables in motor neuron disease patients, the correlation was not significant where $p = 0.2697$ (>0.05). Here, R-squared value = 0.05270 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "emotional lability" symptom was low significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. No significant association between these two variables was found during analysis ($p > 0.05$) (Figure 5).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "facing cognitive change" symptom was $r = 0.5865$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p = 0.0021$ (<0.05). Here, R-squared value = 0.3440 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "facing cognitive change" symptom was significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 5).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 and "respiratory changes" was $r = 0.1983$ in the motor neuron disease patient group. This indicates that there was a low degree of positive correlation between these two variables in motor neuron disease patients. But the correlation was not significant because $p = 0.3421$ ($p > 0.05$). Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. No significant association between these two variables was found during analysis ($p > 0.05$) (Figure 5).

The correlation coefficient between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "weakness or changes in hands, arms, legs, and voice" symptom was $r = 0.6743$ in the motor neuron disease patient group. Though this indicates that there was a positive correlation between these two variables in motor neuron disease patients, the correlation was significant where $p = 0.0002$ (<0.05). Here, R-squared value = 0.4547 which shows that the correlation between $2^{-\Delta\Delta Ct}$ values of expression of NEAT1 lncRNAs and "weakness or changes in hands, arms, legs, and voice" was significant. Regression analysis between these two variables was performed. F-test was used to analyze the significance between the two variables. Significant association between these two variables was found during analysis since $p < 0.0001$ (Figure 5).

4. Conclusion

Over the past decade, many proofs represent that long noncoding RNAs (lncRNAs) are expressed in a wide range and play crucial roles in gene regulation [13]. Recent research has started to clarify how the biosynthesis of lncRNAs varies from that of mRNAs and is connected with their definite subcellular localizations and activities. lncRNAs can regulate chromatin function, mediate the assembly as well as actions of membraneless nuclear bodies, change the constancy and translation of cytoplasmic mRNAs, and impede signaling pathways which are controlled by lncRNAs' localization and their specified interactions with DNA and RNA as well as proteins [7]. Many of these roles eventually influence gene expression in various physiopathological as well as biological forms, which include neuronal disorders, immune responses, and cancer [14]. Tissue-specific and condition-specific expression patterns recommend that lncRNAs are prospective biomarkers and can be used as a rationale to target them clinically [15]. The major aim of our study was to find out the association of 5 specific lncRNAs with specific disease conditions which can lead to future hope for molecular treatment of these diseases, for example, CRISPR cas-9 and siRNA treatment. This study only shows the association, but it directs the probability of future study whether the expression of these lncRNAs is the reason for the occurrence of these diseases. Here, we tried to find out the association of expression of HOTAIR and MKRN2-42:1 lncRNAs with the symptoms of Parkinson's disease patients and find out the association of expression of MALAT-1, Meg3^{v1}, and NEAT1 lncRNAs with the symptoms of motor neuron disease patients in Bangladesh. In our case-control study, both patients and controls belonged to the same ethnic background and all shared a common geographic origin. We have estimated the Ct values of expression of HOTAIR, MKRN2-42:1, MALAT-1, Meg3^{v1}, and NEAT1 in the Bangladeshi population through RT-PCR. We have also studied all the symptoms and blood pressure levels to establish lncRNAs as potential biomarkers of Parkinson's disease patients and motor neuron disease patients in Bangladesh.

The baseline characteristics were analyzed between the control group and PD patient group and MND patient group. Age; sex; symptom duration; "tremors and trembling of hands, arms, jaw, face"; "stiffness of the arms, legs and trunk"; "face slowness of movement"; "poor balance and

coordination"; "speech difficulty"; and systolic blood pressure (SBP) and diastolic blood pressure (DBP) of PD patients and control group and age, sex, symptom duration, "muscle aches, cramps, twitching"; "clumsiness and stumbling"; "slurred speech, swallowing or chewing difficulty"; "fatigue"; "muscle wasting, weight loss"; "emotional lability"; "facing cognitive change"; "respiratory changes"; and "weakness or changes in hands, arms, legs, and voice" of MND patients and control group were the main baseline characteristics of our study.

In our study, we found out that there was a negative correlation between all the symptoms and Ct values of expression of all the lncRNAs mentioned previously though p values were significant ($p < 0.05$). For HOTAIR, all the symptoms ("tremors and trembling of hands, arms, jaw, face"; "stiffness of the arms, legs and trunk"; "face slowness of movement"; "poor balance and coordination"; "speech difficulty"; and systolic blood pressure (SBP) and diastolic blood pressure (DBP) of PD patients) and Ct values of expression of HOTAIR lncRNA showed negative correlation though p values were significant which indicate that patients with these symptoms (who are known as Parkinson's disease patient) show a high level of expression of HOTAIR lncRNAs in their peripheral blood in comparison with the control group. We also found out that all the symptoms ("tremors and trembling of hands, arms, jaw, face"; "stiffness of the arms, legs and trunk"; "face slowness of movement"; "poor balance and coordination"; "speech difficulty"; and systolic blood pressure (SBP) and diastolic blood pressure (DBP) of PD patients) and Ct values of expression of MKRN2-42:1 lncRNA showed negative correlation though p values were significant which indicate that patients with these symptoms (who are known as Parkinson's disease patient) show high level of expression of MKRN2-42:1 lncRNAs in their peripheral blood in comparison with the control group.

In the case of MND, all the symptoms of MND showed a negative correlation with the expression of lncRNAs ($Meg3^{v1}$, NEAT1) though p values were significant for most of the cases except some symptoms. For $Meg3^{v1}$, all the symptoms ("muscle aches, cramps, twitching"; "clumsiness and stumbling"; "slurred speech, swallowing or chewing difficulty"; "fatigue"; "muscle wasting, weight loss"; "emotional lability"; "facing cognitive change"; and "weakness or changes in hands, arms, legs, and voice" of MND patients) (except "respiratory changes" for which $p > 0.05$) and Ct values of expression of $Meg3^{v1}$ lncRNA showed negative correlation though p values were significant which indicate that patients with these symptoms (who are known as motor neuron disease patient) show a high level of expression of $Meg3^{v1}$ lncRNAs in their peripheral blood in comparison with the control group.

For NEAT1, all the symptoms ("muscle aches, cramps, twitching"; "clumsiness and stumbling"; "slurred speech, swallowing or chewing difficulty"; "fatigue"; "muscle wasting, weight loss"; "emotional lability"; "facing cognitive change"; and "weakness or changes in hands, arms, legs, and voice" of MND patients) (except "respiratory changes" for which $p > 0.05$) and Ct values of expression of NEAT1 lncRNA showed negative correlation though p values were significant which indicate that patients with these symptoms (who are known as motor neuron disease patient) show a high level of expression of NEAT1 lncRNAs in their peripheral blood in comparison with the control group.

From the above discussion, it can be concluded that lncRNAs can be considered as biomarkers for neurodegenerative disorder patients though, here, the sample volume was not large. To confirm this hypothesis, further research is needed to be done. Successful prediction of the possibility of neurodegenerative disorder by analyzing data regarding the expression of lncRNAs as biomarkers may help advance clinicians to stratify high-risk patients and prepare for appropriate medical intervention.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Supplementary Materials

The list of temperatures and cycles for RT-PCR for 4 lncRNAs will be found in supplemental table S1, S2, S3, and S4. Here, Table S1: Parkinson's disease: temperatures and cycles for RT-PCR for HOTAIR. Table S2: Parkinson's disease: temperatures and cycles for RT-PCR for MKRN2-42:1. Table S3: motor neuron disease: temperatures and cycles for RT-PCR for Meg3v1. Table S4: motor neuron disease: temperatures and cycles for RT-PCR for NEAT1. The list of forward and reverse primers which have been used for 4 specific lncRNAs and internal control will be found in supplemental table S5. *(Supplementary Materials)*

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