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snoRNAs are deregulated in patients with Parkinson's Disease

Parkinson tanısı almış hastalarda snoRNA'ların deregülasyonu

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Abstract

Purpose: Small nucleolar RNAs are ranging from 65 to 300 nucleotides in length that mediate post-transcriptional RNA modifications. They don't have a 5′-Cap and a poly-A tail and are categorized as C/D box snoRNAs, H/ACA box snoRNAs, and small Cajal body-specific RNAs. snoRNAs have essential roles in important biological processes such as transcription, RNA splicing, cell cycle, and etc. In this study, we tried to reveal differential expressions of snoRNAs in PBMCs of patients with Parkinson's Disease by microarray analysis.

Materials and methods: Patients (n=3) who are considered to have a unilateral onset history and a good response to dopaminergic treatment in the first years were included in the study. 10 ml peripheral blood sample was taken for peripheral blood mononuclear cell isolation. Total RNA was extracted using GeneAll® Hybrid-R™ kit and microarray analysis was performed by using Affymetrix GeneChip Human ST 2.0 platform. Raw data were extracted using Affymetrix Command Console Software 1.1. KEGG pathway and GO terms analyses were performed and protein-protein interaction of host genes were determined by using STRING database.

Results: Data from patients revealed that there were 28 snoRNAs were downregulated and 3 snoRNAs were upregulated.

Conclusion: Here in this study, we evaluated the differential expressions of snoRNAs in patients with a definitive diagnosis of PD by microarray analysis and observed deregulated expressions of some snoRNAs. Differential expression of snoRNA may cause changes in the transcriptional activity of host genes and thus can serve as biomarkers for diseases.

Key words: Parkinson, snoRNA, microarray.

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Öz

Amaç: Küçük nükleolar RNA'lar 65 ila 300 nükleotit uzunluğundadır ve transkripsiyon sonrası RNA modifikasyonlarına aracılık ederler. 5'-uçları ve poli-A kuyrukları yoktur. C/D box snoRNA'lar, H/ACA box snoRNA'lar ve küçük kajal gövdesine özgü RNA'lar olarak sınıflandırılırlar. snoRNA'lar, transkripsiyon, RNA eklenmesi, hücre döngüsü vb. gibi önemli biyolojik süreçlerde önemli rollere sahiptir. Bu çalışmada, Parkinson tanısı alan hastaların PBMC'lerinde snoRNA'ların ifade değişimlerini mikroarray analizi ile ortaya koymaya çalıştık.

Gereç ve yöntem: Unilateral başlangıç öyküsü olan ve ilk yıllarda dopaminerjik tedaviye iyi yanıt verdiği düşünülen hastalar (n=3) çalışmaya alındı. Periferik kan mononükleer hücre izolasyonu için 10 ml periferik kan örneği alındı. Total RNA, GeneAll® Hybrid-R™ kiti kullanılarak izole edildi ve Affymetrix GeneChip Human ST 2.0 platformu kullanılarak mikrodizin analizi yapıldı. Ham datalar, Affymetrix Command Console Software 1.1 kullanılarak çıkarıldı. KEGG yolak ve Gen ontoloji analizleri yapıldı ve lokus genlerin protein-protein değişimi STRING veri tabanı kullanılarak gerçekleştirildi.

Bulgular: Elde edilen veriler, 28 snoRNA'nın upregüle olduğunu ve 3 snoRNA'nın downregüle olduğunu ortaya çıkardı

Sonuç: Bu çalışmada, mikrodizin analizi ile kesin Parkinson tanısı alan hastalarda snoRNA'ların ifade değişimlerini değerlendirdik ve bazı snoRNA'ların deregüle ekspresyonlarını gözlemledik. snoRNA'lardaki ifade değişimi, lokus genlerin transkripsiyonel aktivitesinde değişikliklere neden olabilir ve bu nedenle hastalıklar için biyobelirteçler olarak değerlendirilebilirler.

Anahtar kelimeler: Parkinson, snoRNA, mikrodizin.

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Introduction

As noncoding RNA, small nucleolar RNAs (snoRNAs) are ranging from 65 to 300 nucleotides in length that mediate post-transcriptional RNA modifications. SnoRNAs do not have a 5′Cap and a poly-A tail, and are classified as C/D box snoRNAs (SNORDs), small Cajal body-specific RNAs (scaRNAs) and H/ACA box snoRNAs (SNORAs). Both types of snoRNAs bind to specific protein partners to form complexes called snoRNPs (small nucleolar ribonucleoprotein complexes). snoRNAs are mainly encoded by intronic regions of both protein-coding and non-coding genes [1-3]. Common tasks of snoRNAs include 2'-O-methylation and pseudouridylation of rRNAs. C/D box snoRNAs guide the dioxymethylation of nucleotides, while H/ACA box snoRNAs are responsible for pseudouridylation [4]. snoRNAs play essential roles in biological processes such as transcription, RNA splicing, cell cycle regulation, chromosome segregation, and genomic imprinting [5-7]. Studies have shown that snoRNAs can also regulate cell physiology by performing miRNA-like functions within cells [8].

Parkinson's disease (PD) is a disorder with abnormalities of movement and is clinically diagnosed by bradykinesia, postural instability, tremor, and rigidity of the arms and legs [9]. Clinical diagnosis of PD can show heterogeneity, thus some other conditions such as parkinsonism secondary to vascular disease, essential tremor and progressive supranuclear palsy can mimic PD [10]. Ribosomal RNA (rRNA) is transcribed in the nucleolus and dysregulation of the nucleolus can cause cellular stress which is associated with neurodegenerative diseases including PD [11]. Recent studies have reported several snoRNAs in different organisms.

In this study, we aimed to identify deregulated expressions of snoRNAs in PBMCs of patients with Parkinson's Disease by microarray analysis.

Materials and methods

Patient selection criteria

Ethical approval and patient consent were taken for the study. Patients (n=3) diagnosed with "Parkinson's Disease" were included in our study, considering that they had a unilateral onset history and had a good response to dopaminergic treatment in the first years. The control group consisted of individuals (n=2) over the age of 50, who were determined by excluding secondary parkinsonism cases due to other causes and parkinsonism related to other degenerative diseases.

Sample collection and PMBC preparation

A 10 ml peripheral blood sample was taken using K2EDTA anticoagulant tubes and an "Informed Voluntary Consent Form" was taken from the patients meeting the inclusion criteria. Peripheral blood mononuclear cells (PBMC) are prepared by using Ficoll-Histopaque density gradient centrifugation within 3 hours and directly prepared for RNA isolation.

RNA Isolation

The total RNA was isolated from PBMCs of samples with GeneAll® Hybrid-R™ kit according to manufacturer's instructions. The quantity and integrity of RNA samples were measured by NanoDrop ND-1000 (Thermo Scientific) and Agilent 2100 bioanalyzer using the RNA 6000 Nano Chip. Samples with a purity of 1.8 to 2.0 were accepted for microarray analysis.

RNA labeling, microarray hybridization, and scanning

Microarray analysis was performed by using Affymetrix GeneChip Human ST 2.0 platform. 1µg of total RNA was used as input and then converted into double-stranded cDNA. The cDNA was then fragmented and end-labeled by a TdT (terminal deoxynucleotidyl transferase) reaction. Fragmented and end-labeled cDNAs were hybridized for 16 hours at 45 °C and 60 rpm with the GeneChip Human Gene 2.0 ST oligonucleotide arrays including 53,617 probes. Chips were then stained and washed in the GeneChip Fluidics Station 450 (Affymetrix) and scanned with an Affymetrix Model GCS3000 scanner.

Raw data preparation

Raw data were extracted using Affymetrix Command Console Software 1.1. The raw CEL file which contain intensity data was used for further analysis. Data normalization was performed with Affymetrix Power Tools, R 3.3.3. Hierarchical cluster analysis was performed using complete linkage and Euclidean distance as a measure of similarity.

Microarray data analysis

Data showing expression differences between patients and controls were filtered. Fold changes limits were accepted as "1,5". Values <1,5 indicate downregulation while >1,5 indicate upregulation.

Statistical analysis

GraphPad Prism software (version 7.0; GraphPad) was used for statistical data analysis. Comparisons among groups were performed using Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's test. $p<0.05$ value was accepted as statistically significant.

Gene ontology and KEGG pathway analysis

KEGG pathway and GO terms analyses were performed to analyze the biological process, molecular functions, and biochemical pathways of snoRNAs determined by microarray analysis.

STRING database analysis

Protein-Protein interaction network analysis was performed using the STRING database (https://string-db.org/).

Results

In order to identify differentially expressed snoRNAs, PBMCs of patients were used for microarray analysis. All microarray data were filtered from among 5,638 Refseq non-coding transcripts. Data from patients revealed that there were 28 snoRNAs were downregulated of which 5 of them were members of the H/ACA box and 15 of them were C/D boxes. Three snoRNAs were upregulated and 1 of them was a member of the H/ACA box whereas 2 of them were C/D boxes. HeatMap analysis of host genes were shown in Figure 1. snoRNA orthological gene database (http://snoopy.med.miyazaki-u.ac.jp) was used for the identification of their localizations (protein-coding genes, introns, ORFs and etc) and target RNAs of snoRNAs. The corresponding data were shown in Table 1.

We used ShinyGo v0.741 Gene Ontology Enrichment Analysis algorithm (http://bioinformatics.sdstate.edu/go74/). Gene ontology analysis was used for revealing biological, molecular, and functional processes in host genes of differentially expressed snoRNAs. In analysis, patients and control groups were compared and all processes were determined by taking the $p<0.05$ value into consideration (Figure 2A-C). GO categories of the corresponding host genes of snoRNAs are given in Table 2. KEGG pathway analysis was performed to determine the relationship in host genes of differentially expressed snoRNAs. According to KEGG analysis, we observed that the major pathway with high significance is related to the ribosome (Figure 3). Protein-protein interactions (PPI) were performed by STRING database. Protein interactions of host genes were shown in Figure 4.

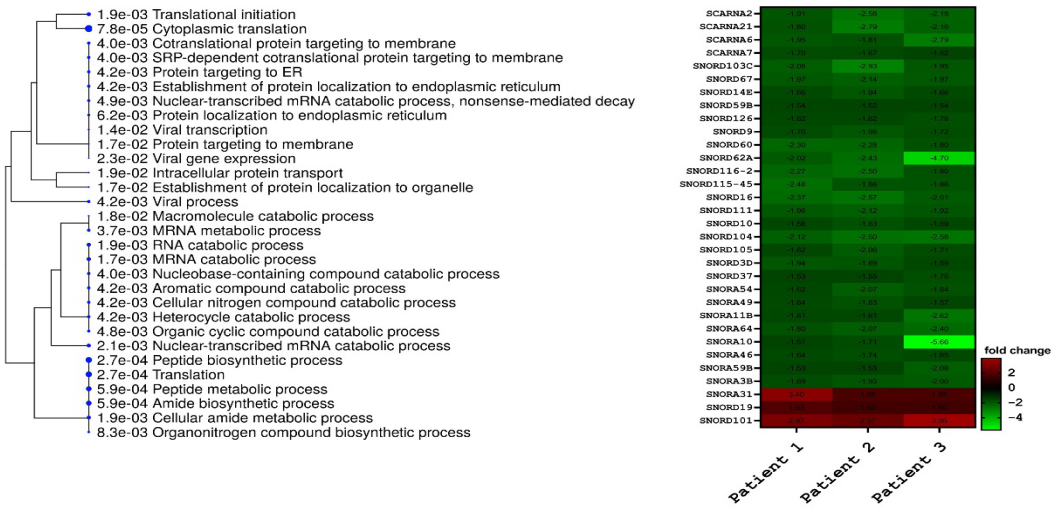

Figure 1. HeatMap analysis of the host genes of snoRNAs with deregulated expressions

Table 1. snoRNAs detected in the PBMCs of the patients

snoRNAs	BOX	Target RNA	Organization	Locus	Expression
SCARNA2	C/D	U2 snRNA	Monocistron	mono:SCARNA2 (mgU2-25/61)	Downregulated
SCARNA21	Unknown	Unknown	Unknown	Unknown	Downregulated
SCARNA6	C/D	U5 snRNA	Intronic	ATG16	Downregulated
SCARNA7	C/D	U1 snRNA	Intronic	KPNA4	Downregulated
SNORD103C	C/D	18S rRNA	Intronic	PUM1	Downregulated
SNORD67	C/D	U6 snRNA	Intronic	CKAP5	Downregulated
SNORD14E	Unknown	Unknown	Unknown	Unknown	Downregulated
SNORD126	Unknown	Unknown	Unknown	Unknown	Downregulated
SNORD9	C/D	U6 snRNA	Intronic	CHD8	Downregulated
SNORD60	C/D	28S rRNA	Intronic	AA974833	Downregulated
SNORD62A	C/D	18S rRNA	Intronic	PRRC2B	Downregulated
SNORD116-2	C/D	Unknown	Unknown	hmm16326423	Downregulated
SNORD115-45	Unknown	Unknown	Unknown	Unknown	Downregulated
SNORD16	C/D	18S rRNA	Intronic	RPL4	Downregulated
SNORD111	C/D	28S rRNA	Intronic	SF3B3	Downregulated
SNORD10	Unknown	U6 snRNA, 28S rRNA	Intronic	EIF4A1	Downregulated
SNORD104	C/D	28S rRNA	Polycistron	Poly:10:AC025362.12	Downregulated
SNORD105	C/D	18S rRNA	Intronic	P2RY11	Downregulated
SNORD3D	Unknown	Unknown	Unknown	Unknown	Downregulated
SNORD37	C/D	28S rRNA	Intronic	EEF2	Downregulated
SNORA54	H/ACA	28S rRNA	Intronic	NAP21L4	Downregulated
SNORA49	H/ACA	Unknown	Intronic	EP400	Downregulated
SNORA11B	H/ACA	Unknown	Intronic	C14orf159	Downregulated
SNORA64	H/ACA	28S rRNA	Intronic	MYRIP, RPS2	Downregulated
SNORA10	H/ACA	18S rRNA, 28S rRNA	Intronic	RPS2	Downregulated
SNORA46	H/ACA	18S rRNA	Intronic	AHSA1, CNOT1	Downregulated
SNORA59B	Unknown	Unknown	Unknown	Unknown	Downregulated
SNORA3B	Unknown	Unknown	Unknown	Unknown	Downregulated
SNORA31	H/ACA	18S rRNA, 28S rRNA	Intronic	TPT1	Upregulated
SNORD19	C/D	18S rRNA	Intronic	GNL13	Upregulated
SNORD101	C/D	Unknown	Intronic	RPS12	Upregulated

Figure 2. Gene ontology analysis A) Molecular Function B) Biological Process C) Cellular component

Table 2. High Level "GO Terms" Category of the Host Genes of snoRNAs

GO Category	Host Genes
Cellular localization	RPS12 RPS2 EEF2 MYRIP RPL4 CKAP5 KPNA4
Catabolic process	RPS12 CNOT1 PUM1 RPS2 RPL4 SF3B3
Macromolecule localization	RPS12 RPS2 MYRIP RPL4 CKAP5 KPNA4
Response to stress	CNOT1 TPT1 PUM1 EEF2 P2RY11
Response to external stimulus	CHD8 TPT1 PUM1 EEF2 P2RY11
Regulation of signaling	CHD8 CNOT1 TPT1 PUM1 MYRIP
Regulation of response to stimulus	CHD8 CNOT1 TPT1 PUM1 P2RY11
Cellular component biogenesis	CNOT1 RPS2 RPL4 CKAP5
Regulation of biological quality	CNOT1 TPT1 PUM1 MYRIP
Immune system process	PUM1 EEF2 P2RY11
Immune response	PUM1 EEF2 P2RY11
Response to endogenous stimulus	CNOT1 EEF2 P2RY11
Biological process involved in interspecies interaction between organisms	TPT1 PUM1 KPNA4
Regulation of molecular function	AHSA1 RPS2 P2RY11
Behavior	CHD8 PUM1
Regulation of immune system process	PUM1 P2RY11
System process	CHD8 EEF2
Response to biotic stimulus	TPT1 PUM1
Cell cycle process	CNOT1 CKAP5
Regulation of cellular component biogenesis	CNOT1 CKAP5
Regulation of developmental process	CNOT1 TPT1
Response to other organism	TPT1 PUM1
Maintenance of cell number	CNOT1 TPT1

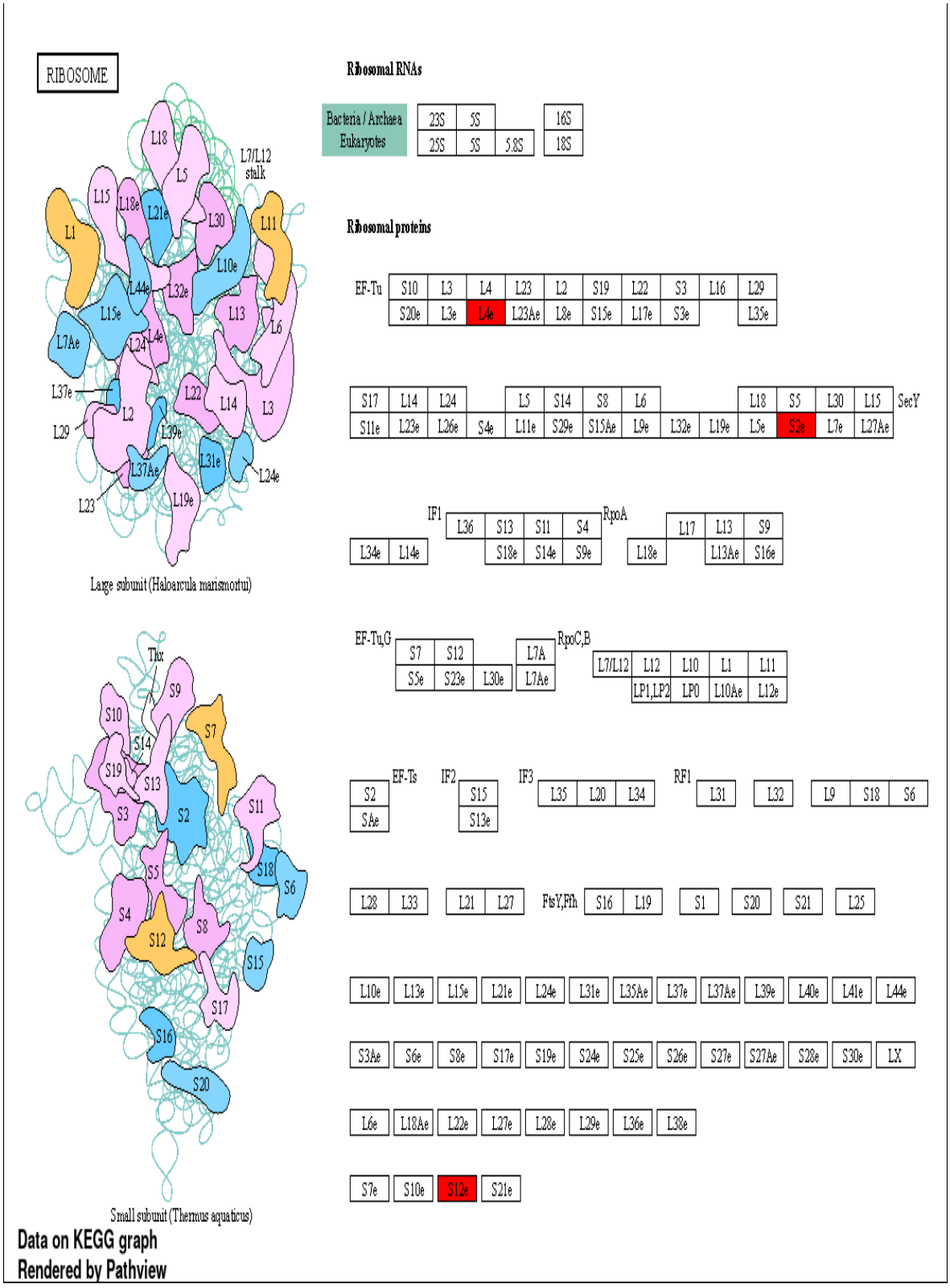

Figure 3. KEGG pathway analysis

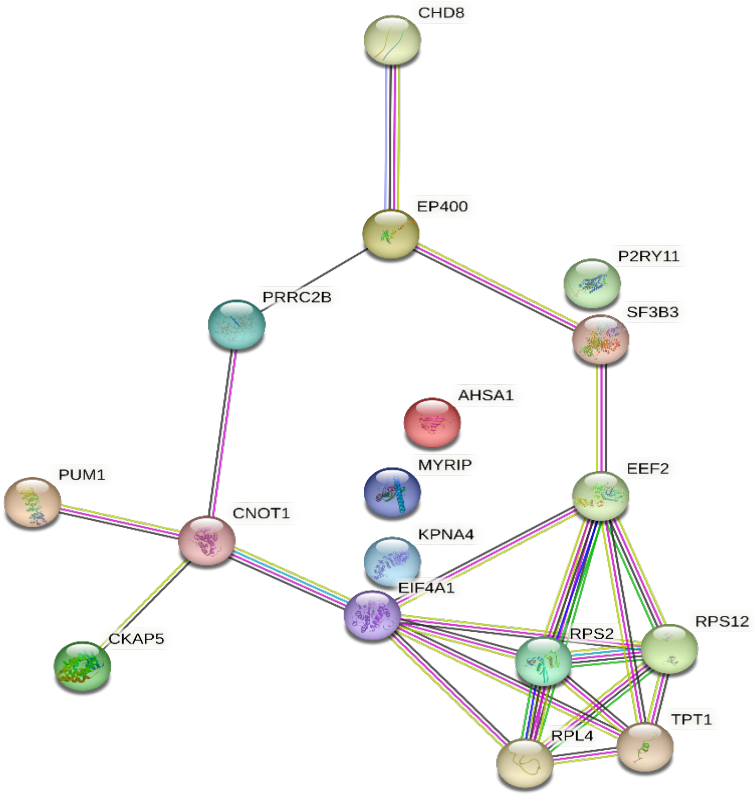

Figure 4. Protein-Protein interactions using STRING Database

Discussion

In our study, we revealed the deregulated expressions of snoRNAs in the PBMCs isolated from patients with PD. GO terms and pathway analysis have shown that snoRNAs are involved in several biological processes, mainly in cytoplasmic translation. With the advance of the in-silico algorithms, it is possible to determine the host genes and target RNAs of snoRNAs as shown in Table 1.

snoRNAs, which are involved in many physiological and pathological processes, also have tumor suppressor and oncogenic functions in various cancer types. In a study, it was reported that snoRNAs are overexpressed in human breast and prostate cancers, and this overexpression supports tumorigenicity both in vitro and in vivo [12]. In a study that investigated the expression of ncRNAs in central nervous system diseases, two snoRNAs whose expression was deregulated prior to amyloid plaque formation were identified in a mouse Alzheimer's model [13]. The role of snoRNAs in Parkinson's disease is unknown, but one study identified four snoRNAs (SNORA52, SNORD15A, SNORD134, and SNORD57) that were significantly upregulated in a model of MPP+-induced Parkinson's disease [14]. Cavaillé et al. reported four snoRNAs (MBII-13, MBII-52, MBII-85, and MBI-36) expressed in the central nervous system of mice [15]. The presence of snoRNAs can also be detected in human cerebrospinal fluid samples [13]. Altered snoRNA expression may result from disease processes and changes in the transcriptional activity of host genes and thus serve as biomarkers for diseases.

Peripheral blood mononuclear cells (PBMCs) are one of the novel sources in many disorders [16] and could mimic the conditions of some tissues [17]. Because PBMCs are subsidiary in the functionalities of the immune system they can be a source of biomarkers [18].

In conclusion, there is a fact that the heterogeneity of PD causes diagnostic difficulties. The limitation of the study is a very limited number of cases with motor movement deficits can be diagnosed as PD. Therefore, here in this study, we evaluated the differential expressions of snoRNAs in patients with a

definitive diagnosis of PD by microarray analysis and observed deregulated expressions of some snoRNAs. We believe that this study can contribute to the literature. More studies on PD patients can help to determine new biomarkers for the disease.

Conflict of interest: No conflict of interest was declared by the authors.

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Authors' contributions to the article

P.E.T. and A.G.T. constructed the main idea and hypothesis of the study. P.E.T., A.G.T. and F.G.S. developed the theory and arranged/edited the material and method section. L.S.B. has chosen the patients included in the study. P.E.T., A.G.T. have done the evaluation of the data in the Results section. Discussion section of the article was written by P.E.T., A.G.T.; P.E.T., A.G.T., F.G.S. and L.S.B. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.