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Altered Long Noncoding RNA Expression Precedes the Course of Parkinson's Disease—a Preliminary Report

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Abstract Parkinson's disease (PD) is a slowly progressing neurodegenerative disorder that affects approximately seven million patients worldwide. Despite intensive research, the molecular mechanisms initiating and promoting PD are still unknown. However, it is assumed that environmental factors trigger PD. Recent research demonstrated that long noncoding RNAs (lncRNA) interfere in transcriptional and translational processes modulating gene expression reflecting environmental influences. Nevertheless, there is no systematic analysis available that investigates the impact of lncRNAs on PD. In the current study, we performed a comprehensive analysis on expression levels of 90 well-annotated lncRNAs in 30 brain specimens deriving from 20 PD patients and 10 controls as a preliminary report on the significance of lncRNAs in PD. Expression profiling of lncRNAs revealed that five lncRNAs are significantly differentially expressed in PD. While H19 upstream conserved 1 and 2 is significantly downregulated in PD, lincRNA-p21, Malat1, SNHG1, and TncRNA are significantly upregulated. An analysis on expression levels and PD stages revealed that the identified dysregulated lncRNA are altered already in early disease stage and that they precede the course of PD. In summary, this is the first comprehensive analysis on lncRNAs in PD revealing significantly altered lncRNAs. Additionally, we found that lncRNA dysregulations precede the course of the disease. Thus, the five newly identified lncRNAs may serve as potential new biomarkers appropriate even in early PD. They may be used in monitoring disease progression and they may serve as potential new targets for novel therapeutic approaches.

Keywords Parkinson's disease · PD · Long noncoding RNA · lncRNA · Human brain tissue

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Introduction

Parkinson's disease (PD) is the most frequent neurodegenerative disorder altering the movement abilities [1]. This slowly progressing disease affects approximately seven million patients worldwide. In the population over 80 years, up to 4 % of persons get the disease [1]. It is estimated that there are 8 to 18 new PD cases per 100,000 persons per year [1].

Clinically, PD is characterized by progressive impairments of motoric abilities. Till date, the disease can only be treated inadequately: while the currently available drugs provide only a relief of the symptoms and a delay in progression, there are no causal therapies available. Despite intensive research, the molecular mechanisms initiating and promoting PD still remain unclear [2]. Recent research emphasizes that epigenomic factors modulating gene expression, transcription, and translation play a crucial role in the development of neurodegenerative diseases [2, 3]. Thus, it is assumed that the initiation is promoted by a combination of a genetic predisposition and environmental triggers [1–3].

Recently, it has been revealed that the vast majority of genetic information coded within the DNA is transcribed into RNA. This "dark matter" of noncoding RNA (ncRNA) is not only "transcriptional noise" but interferes in transcription, translation, and numerous other cellular mechanisms [4–7].

Consisting of RNA molecules of 200 bp to >10 kbp in length, the group of long noncoding RNA (lncRNA) is assumed to be responsible for numerous regulatory processes [8–10].

The best studied events that are linked to lncRNAs are imprinting and X-chromosome inactivation [11–13]. They are regulated by cis-acting master control regions. In case of X-chromosome inactivation experiments have shown that a single X-inactivation center (Xic) is sufficient for X-chromosome inactivation [13, 14]. With regard to functions and mechanisms of lncRNAs, Xic contains the best characterized lncRNA: X-inactive specific transcript (Xist) [13, 14]. This 17–20-kbp-long RNA marks one X-chromosome in the course of inactivation [12]. Only the X-chromosome that is inactivated expresses Xist subsequently leading to the inactivation of the whole X-chromosome and the change of chromatin and transcription by binding Polycomb repressive complex 2 (PRC2) being responsible for H3K27 trimethylation [13, 15–17]. However, Xist itself is controls by other lncRNAs, e.g., Tsix and Jpx, leading to a complex regulatory mechanism [14, 18, 19]. It is obvious that abnormalities in these regulations can lead to severe diseases. Besides the importance of lncRNAs in the field of tumorigenesis [20, 21], lncRNAs are closely linked to the development of cognitive dysfunctions, autism, schizophrenia, and Alzheimer's disease [22]. However, till date, there are no comprehensive studies available analyzing the significance of lncRNAs in Parkinson's disease.

In the current study, we addressed this question and performed expression profiling of 90 well-annotated lncRNAs in neurons separated from anterior cingulate gyrus of 30 human postmortem brain specimens. Tissue was derived from healthy donors as well as PD patients. Finding severe dysregulations of lncRNAs, the data presented in this study is a preliminary report on lncRNAs in PD that will boost research on lncRNAs in PD and will contribute to better understand the molecular mechanisms underlying PD as well as to develop new therapeutic approaches.

Materials and Methods

Sample Collection and Preparation of Tissue Specimen

In this study, we selected 30 well-characterized human postmortem brain tissue samples that were stored at −80 °C. All samples were provided by the Neurobiobank Munich (NBM) and were clinically as well as neuropathologically classified according to the NBM standard protocols. The extent of Parkinson's disease (PD)-associated changes was assessed according to the classification recommended by McKeith et al. [23]. Cases with hypoxia, inflammation, infection, infarction, and tumor were excluded. Written informed consent was obtained according to the guidelines of the local ethics committee. We included 10 control cases without PD and 20 cases with PD. Age distribution of controls ranged from 46 to 85 years with mean age of 66 years, and age distribution of PD cases ranged from 57 to 85 years with mean age of 74 years. Sex distribution showed 6 female and 4 male patients in the control cohort (female to male ratio of 1.5) and 11 female and 9 male patients in the PD cohort (female to male ratio of 1.2). Detailed information on patients can be found in Table 1. According to McKeith, PD can be assigned based on the pattern of Lewy-related pathology in brain stem, limbic, and neocortical regions [23]. Early PD cases show Lewy-related pathology only in brain stem regions. Subsequently, basal forebrain and limbic regions are affected (such as the cingulate gyrus). In late PD, Lewy-associated pathology can also be detected in neocortical regions. By selecting the cingulate gyrus as target region in this study, we were able to perform an analysis of lncRNA expression preceding the course of Parkinson's disease as the anterior cingulate gyrus is affected in limbic and neocortical type PD but not in brain stem type PD. Additionally, whereas the substantia nigra shows a loss of neurons and reactive gliosis in very early PD with a raddled state during advancing of PD, the anterior cingulate gyrus shows an active state during a long time period. Thus, the anterior cingulate gyrus represents the ideal anatomical region to be studied in order to reveal molecular mechanisms preceding and promoting PD. As the cortex consists of a crude mixture of different cell types [24] and as molecular alterations are cell type specific [22], we performed an enrichment of neurons using a NeuN selective antibody as described previously [25] to further increase the validity of our approach.

Extraction of Long Noncoding RNAs, Reverse Transcription Reaction, and Quantitative Polymerase Chain Reaction

We performed enrichment of lncRNAs by using the miRNeasy Micro Kit (Qiagen) and the RNeasy MinElute Kit (Qiagen) according to the manufacturer's protocols. Detecting the 260/280 nm absorbance ratio using a NanoDrop device (Thermo Fischer), we determined the quantity and quality of lncRNA. In all cases, the absorption ratio was between 1.90 and 2.20. To avoid amplification bias, no preamplification of lncRNA was performed and lncRNA was directly processed for subsequent analysis. Reverse transcription reaction (RT) was performed using the human LncProfiler qPCR Assay Kit (SBI) including polyadenylation reactions optimized for lncRNAs as described previously [26, 27]. We used equal amounts of 50 ng lncRNA with a concentration of 10 ng/μl. To boost cDNA yield, reverse transcription reactions were performed using Oligo dT primers and random primer. All procedures were performed in accordance with the manufacturer's protocols. As a first approach to assess the importance of lncRNAs in PD, we selected the top 90

Table 1 Overview of samples used in this study

Case	Diagnosis	Stage	Age (years)	Sex	PMI (h)	Region
Dnr_01	Control	No PD	61	Male	24	Anterior cingulate gyrus
Dnr_02	Control	No PD	63	Female	14	Anterior cingulate gyrus
Dnr_03	Control	No PD	66	Female	<38	Anterior cingulate gyrus
Dnr_04	Control	No PD	80	Female	<15	Anterior cingulate gyrus
Dnr_05	Control	No PD	46	Male	62.5	Anterior cingulate gyrus
Dnr_06	Control	No PD	60	Female	14	Anterior cingulate gyrus
Dnr_07	Control	No PD	60	Male	71	Anterior cingulate gyrus
Dnr_08	Control	No PD	75	Female	24	Anterior cingulate gyrus
Dnr_09	Control	No PD	85	Male	25	Anterior cingulate gyrus
Dnr_10	Control	No PD	59	Female	11.5	Anterior cingulate gyrus
Dnr_11	PD	Limbic	79	Male	23	Anterior cingulate gyrus
Dnr_12	PD	Limbic	77	Female	48	Anterior cingulate gyrus
Dnr_13	PD	Limbic	74	Male	<24	Anterior cingulate gyrus
Dnr_14	PD	Brain stem	76	Female	18	Anterior cingulate gyrus
Dnr_15	PD	Limbic	72	Female	11	Anterior cingulate gyrus
Dnr_16	PD	Limbic	69	Female	50	Anterior cingulate gyrus
Dnr_17	PD	Neocortical	79	Female	<37	Anterior cingulate gyrus
Dnr_18	PD	Neocortical	73	Male	24	Anterior cingulate gyrus
Dnr_19	PD	Limbic	72	Male	28	Anterior cingulate gyrus
Dnr_20	PD	Limbic	57	Male	<60	Anterior cingulate gyrus
Dnr_21	PD	Limbic	83	Male	19	Anterior cingulate gyrus
Dnr_22	PD	Neocortical	63	Female	22	Anterior cingulate gyrus
Dnr_23	PD	Neocortical	66	Female	12	Anterior cingulate gyrus
Dnr_24	PD	Neocortical	85	Female	57	Anterior cingulate gyrus
Dnr_25	PD	Neocortical	84	Female	18	Anterior cingulate gyrus
Dnr_26	PD	Brain stem	71	Male	26	Anterior cingulate gyrus
Dnr_27	PD	Neocortical	74	Female	14	Anterior cingulate gyrus
Dnr_28	PD	Neocortical	71	Male	20	Anterior cingulate gyrus
Dnr_29	PD	Brain stem	83	Female	48	Anterior cingulate gyrus
Dnr_30	PD	Brain stem	70	Male	60	Anterior cingulate gyrus

We used 10 control and 20 PD cases. PD stage was assessed according to the criteria suggested by McKeith

Dnr donor, *PMI* postmortem interval

disease-associated lncRNAs that are annotated in the lncRNA database (lncRNAdb) [28]. These notable lncRNAs have already been described as key factors in a vast majority of human diseases such as cancers (ANRIL, HOTAIR, HOTAIRM1, MALAT1, MEG3, Tsix, Xist) [20, 29], neurdegeneration (BASE1AS, BC200, NEAT1) [30, 31], and neurodevelopmental/psychiatric diseases (Gomafu, NRON, Sox2ot) [32–35]. Analysis was performed using the predesigned and validated qPCR primer library set of the human LncProfiler qPCR Assay Kit (SBI). Predesigned assays have been validated by the supplier across numerous cell types for high specificity and robustness. Quantitative PCR (qPCT) was performed on a LightCycler 480 II device (Roche) using the SensiFAST SYBR No-ROX Kit (Bioline) in combination with standard protocols. The relative amount of cDNA was calculated using the comparative C_T method ($\Delta\Delta C_T$) [36]. To enhance data quality, three valid normalizers were used according to the guidelines for real-time qPCR experiments [37].

Computational Data Analysis

Computational analysis of stably expressed lncRNAs was performed using NormFinder algorithm [38]. We determined the stability value (sv) for each lncRNA. High expression stability is reflected by a low sv, and low expression stability is reflected by a high sv. We assumed stable expression for stability values of ≤0.009. Only highly abundant lncRNAs with C_T (cycle threshold) values of≤32 were assumed as suitable references. Graphical data visualization by heat map generation was performed using the Gene-E software (http://www.broadinstitute.org/cancer/software/GENE-E/).

Statistical analysis was performed applying unpaired *t* test. We using Prism 6 software suite (GraphPad) as statistical environment. Statistical significance was assumed for p values <0.05.

Results

Identification of Stably Expressed lncRNAs

In this study, we performed an analysis on 90 well-annotated lncRNAs that are annotated in the lncRNAdb [28]. As we explored neuronal enriched fractions of 30 human brain tissue samples of the anterior cingulate gyrus, we initially performed an analysis on lncRNA expression stability in order to identify stably expressed lncRNAs that serve as appropriate normalisers in the subsequent analysis.

Applying NormFinder algorithm [38] on 2700 individual expression data, the sv of each lncRNA was determined. Only lncRNAs with stability values of ≤0.009 were assumed as stably expressed. Additionally, we assumed only highly abundant lncRNAs with mean cycle threshold (C_T) values of ≤32 as suitable references.

Applying these criteria, only 3 out of 90 explored lncRNAs fulfilled the requirements for suitable normalizers (Table 2 and Supplementary Table S1): Gas5-family (growth arrest specific 5) showed a sv of 0.007 and a mean C_T of 31.05; highly accelerated region 1B (HAR1B) showed a sv of 0.008 and a mean C_T of 31.23; and small nucleolar RNA host gene 4 (SNHG4) showed a sv of 0.009 and a mean C_T of 31.75 (Table 2 and Supplementary Fig. S1). Thus, these three lncRNAs were regarded as appropriate references and used for normalization strategy in the subsequent analysis.

Detection of Significantly Dysregulated lncRNAs in Parkinson's Disease

In order to compute the relative expression levels of the investigated lncRNAs, we applied the comparative C_T method [36]. According to the guidelines for real-time qPCR experiments that were promoted by Abdel Nour et al., we used all three stably expressed lncRNAs (Gas5 family, HAR1B, and SNHG4) as normalizers to enhance data quality [37] in calculating expression levels (Fig. 1).

Statistical testing on expression data revealed that five lncRNAs were significantly differentially expressed in PD compared with controls (Fig. 2 and Supplementary Table S2): while only one lncRNA was significantly downregulated in PD, four lncRNAs were significantly upregulated in PD. The two ~400-bp-long conserved elements H19 upstream conserved 1 and 2 (Huc 1 and 2) that can be found ~10 kb of the 5′ end of the H19 gene showed a significant twofold decrease in PD compared with controls ($p = 0.0142$, unpaired *t* test, Fig. 2a). The lincRNA-p21 that is a transcription target of p53 and HIF1α showed a significant twofold increase in PD compared with controls ($p = 0.0224$, unpaired *t* test, Fig. 2b). The lncRNA metastasis-associated lung adenocarcinoma transcript 1 (Malat1) showed a significant threefold increase in PD compared with controls ($p = 0.0475$, unpaired *t* test, Fig. 2c). Small nucleolar RNA host gene 1 (SNHG1) showed a significant twofold increase in PD compared with controls ($p = 0.0039$, unpaired *t* test, Fig. 2d). Tiny noncoding RNA (TncRNA) showed a significant twofold increase in PD compared with controls ($p = 0.0427$, unpaired *t* test, Fig. 2e).

Thus, the five identified differentially expressed lncRNAs H19 upstream conserved, lincRNA-p21, Malat1, SNHG1, and TncRNA may be crucial for the understanding of the molecular mechanisms occurring in the disease and may serve as new targets for an advanced molecular therapy.

Altered Expression of lncRNAs Precedes the Course of Parkinson's Disease

Parkinson's disease is a neurodegenerative disorder that shows a distinct spread throughout the brain during the course of the disease. In order to perform analysis on the dynamics of lncRNAs during progression of PD, all cases analyzed in this study were classified according to the guidelines suggested by McKeith et al., which were revealed by the dementia with Lewy bodies (DLB) consortium [23]. The assignment of disease stage is based upon the pattern of Lewy-related pathology in brain stem, limbic, and neocortical regions [23]. Early PD cases show Lewy-related pathology only in brain stem regions (e.g., in the locus caeruleus and the substantia nigra).

Table 2 Expression stability of identified references

lncRNA name	Stability value	Intragroup variation		Intergroup variation		CT value (mean)
		Control	PD	Control	PD	
GAS5-family	0.007	0.001	0.001	0.000	0.000	31.053
HAR1B	0.008	0.001	0.001	−0.005	0.005	31.226
SNHG4	0.009	0.001	0.001	0.002	−0.002	31.752

Performing NormFinder algorithm, we detected three highly abundant stably expressed lncRNAs that show stability values of ≤0.009 and C_T values of ≤32: GAS5-family, HAR1B, and SNHG4. Indicated are stability values as well as the intragroup and intergroup variations of all three lncRNAs

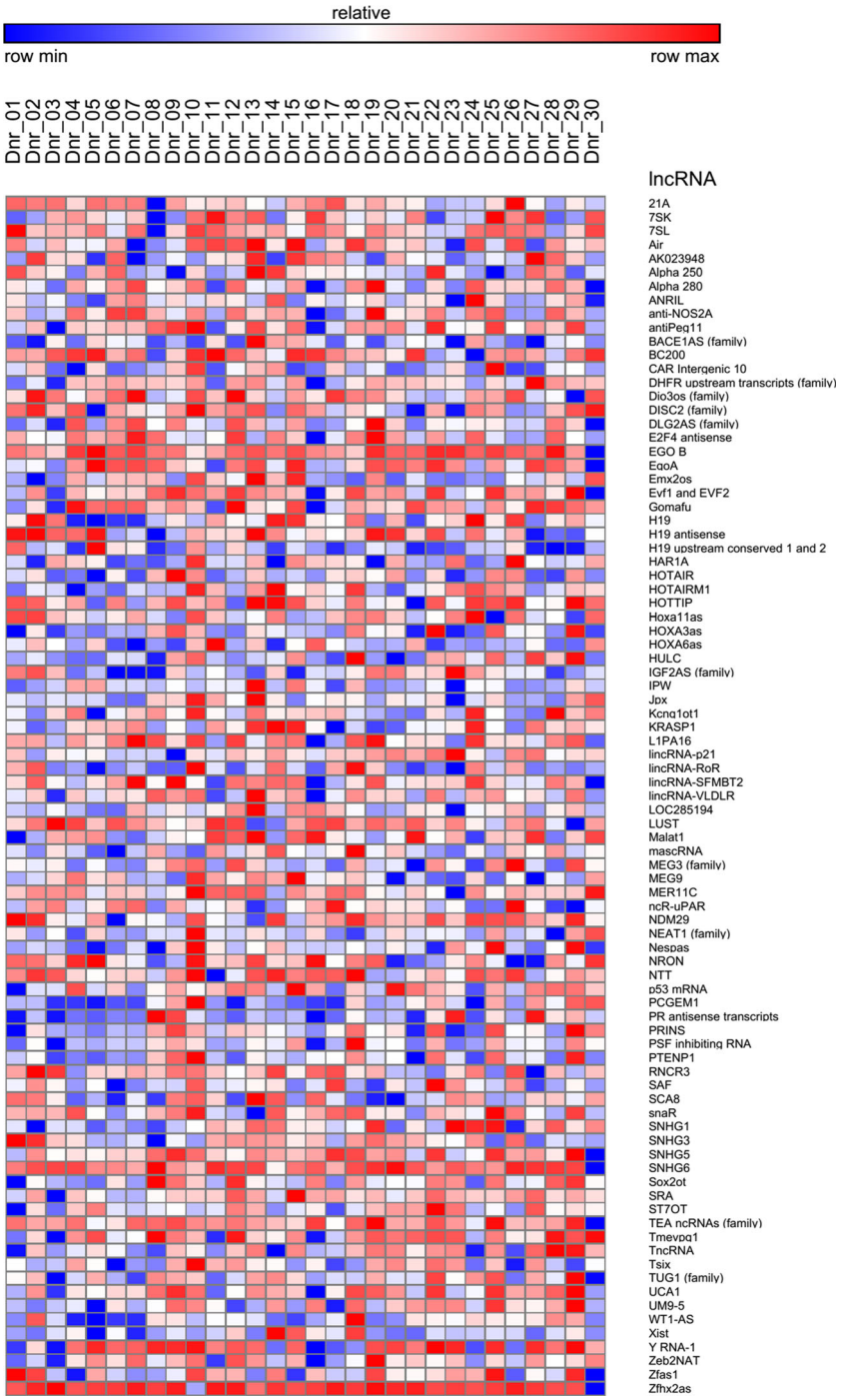

Fig. 1 Heatmap showing expression levels of lncRNAs analyzed in this study. Data were normalized according to the comparative C_T method using the three stably expressed references GAS5-family, HAR1B, and SNHG4. Low relative expression levels correspond with *blue marks*; high relative expression levels correspond with *red marks*. Donors 1 to 10 are controls; donors 11 to 30 are PD patients. *Dnr* donor (color figure online)

Subsequently, basal forebrain and limbic regions are affected (e.g., amygdala and the cingulate gyrus). In late PD, Lewy-associated pathology can also be detected in neocortical regions (e.g., frontal and occipital cortex). By selecting the cingulate gyrus as target region in this study, we were able to perform an analysis of lncRNA expression preceding the course of Parkinson's disease.

Neuropathological staging of the 20 PD cases included in this study showed that 4 cases corresponded with brain stem type, 8 corresponded with limbic type, and 8 corresponded with neocortical type (Table 1). Performing stage-dependent analysis on expression profiles of the five lncRNAs that we identified as differentially expressed in PD (H19 upstream conserved, lincRNA-p21, Malat1, SNHG1, and TncRNA) revealed that alterations can already be detected in early, brain stem type PD (Fig. 3 and Supplementary Table S3). The significantly downregulated lncRNA H19 upstream conserved 1 and 2 was already downregulated in brain

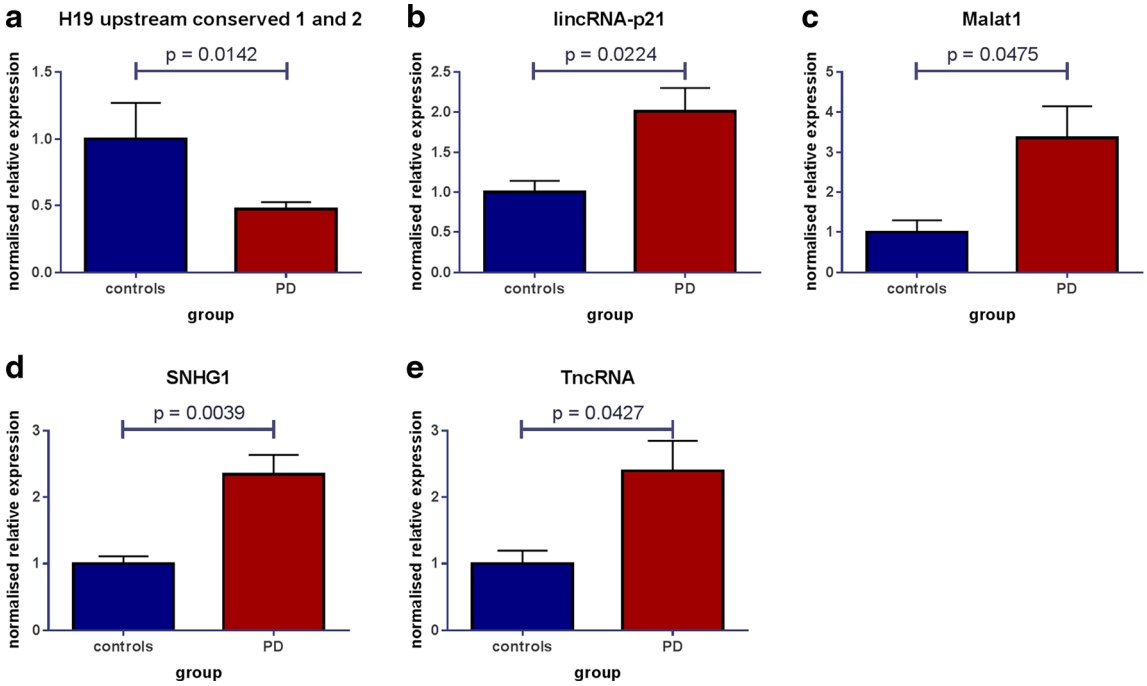

Fig. 2 Normalized relative expression levels of differentially expressed lncRNAs. Indicated are the normalized relative expression levels of the five lncRNAs that were significantly differentially expressed in PD compared with controls. H19 upstream conserved 1 and 2 shows a significantly decreased expression in PD compared with controls (**a**). The lncRNAs lincRNA-p21 (**b**), Malat1 (**c**), SNHG1 (**d**), and TncRNA (**e**) show significantly increased expression levels in PD compared with controls. Indicated are mean and SEM (color figure online)

stem type PD and then remained at decreased levels in the progression of PD with a significantly decreased expression in neocortical PD compared with limbic PD ($p = 0.0309$, unpaired t test, Fig. 3a). In case of the significantly upregulated lincRNA-p21, we found that this lncRNA was already upregulated in early PD and remained at high levels during disease progression with a significantly increased expression in brain stem type PD ($p = 0.0050$, unpaired t test), limbic type PD ($p = 0.0199$, unpaired t test), and neocortical type PD compared with controls ($p = 0.0399$, unpaired t test, Fig. 3b). The significantly upregulated lncRNA Malat1 showed already increased expression in brain stem type PD, highest expression levels were reached in limbic PD cases with a significantly increased expression in limbic type PD compared with controls ($p = 0.0027$, unpaired t test), in neocortical type PD expression deceases compared with limbic type PD ($p = 0.0291$, unpaired t test, Fig. 3c). The significantly overexpressed SNHG1 lncRNA showed increased levels already in brain stem type PD cases, during the course of the disease, expression further increased with a significantly increased expression in brain stem type PD ($p = 0.0262$, unpaired t test), limbic type PD ($p = 0.0072$, unpaired t test), and neocortical type PD compared with controls ($p = 0.0035$, unpaired t test, Fig. 3d). The significantly overexpressed TncRNA showed already overexpression in early brain stem type PD cases, and the overexpression remained stable during the course of the disease a significantly increased expression in limbic type PD ($p = 0.0247$, unpaired t test) and neocortical type PD compared with controls ($p = 0.0301$, unpaired t test, Fig. 3e).

Summing up these findings, we revealed that the expression of differentially expressed lncRNAs was already altered in early PD, e.g. expression of TncRNA was already significantly increased in limbic type PD and remained at high levels during progression of PD, and expression of lincRNA-p21 and SNHG1 was already significantly increased in early brain stem type PD and then remained at overexpressed levels during the course of PD. As morphological changes in the investigated target brain region (anterior cingulate gyrus) can be detected earliest in limbic stage, altered expression of these identified lncRNAs precedes the course of Parkinson's disease.

Discussion

Parkinson's disease is the most frequent neurodegenerative disorder that shows altered movement abilities [1]. During the progression of PD, there is a spread of pathological synuclein deposits beginning in the brain stem and successively affecting limbic and neocortical regions in a distinct pattern [23]. However, the molecular mechanisms underlying and initiating PD still remain unknown. It is assumed that the

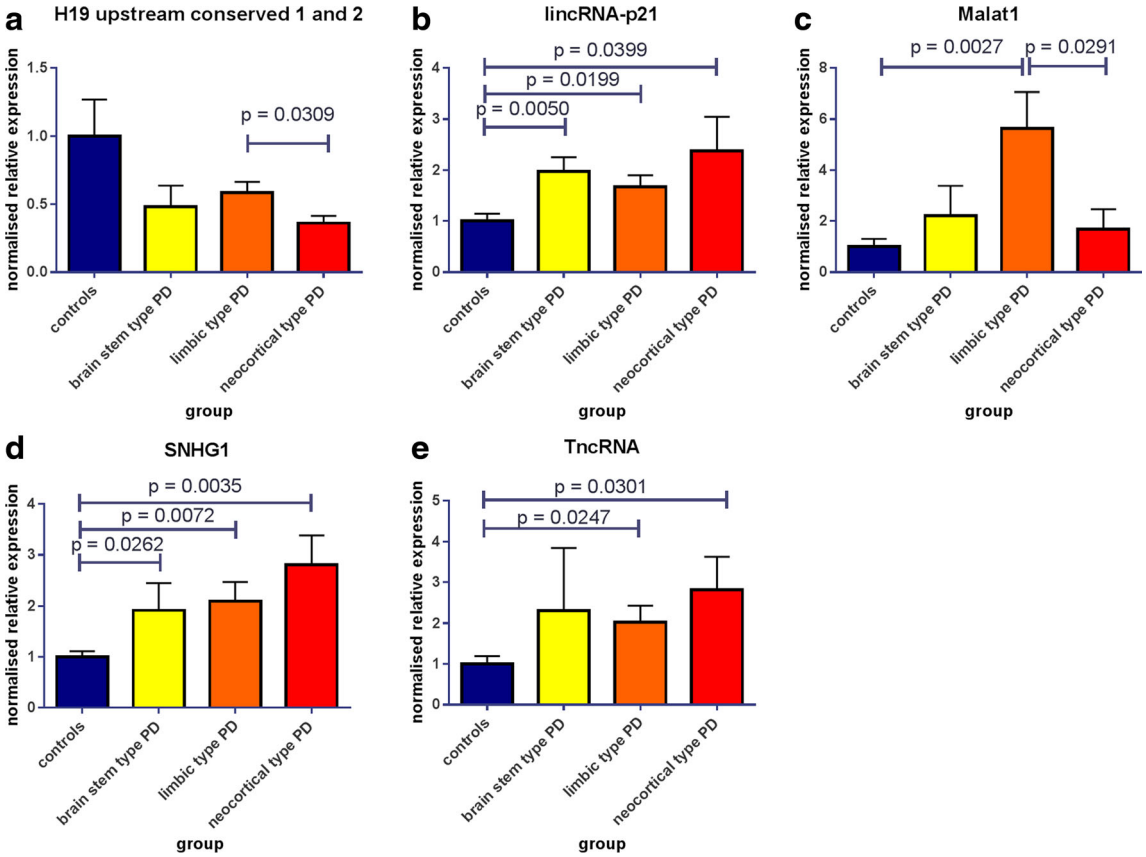

Fig. 3 PD stage-dependent normalized relative expression levels of differentially expressed lncRNAs. Indicated are the PD stage-dependent normalized relative expression levels of the five lncRNAs that were significantly differentially expressed in PD compared with controls. PD stages were assigned to brain stem type, limbic type, and neocortical type according to the classification of McKeith et al. [23]. In early PD, pathological changes can be detected in brain stem regions only. During the course of the disease, basal forebrain and limbic regions, such as the cingulate gyrus, are affected. In late PD, there are also pathological changes in neocortical regions. The lncRNAs H19 upstream conserved 1 and 2 (**a**), lincRNA-p21 (**b**), Malat1 (**c**), SNHG1 (**d**), and TncRNA (**e**) show altered expression in the anterior cingulate gyrus during the course of PD. Indicated are mean and SEM

initiation of neurodegenerative diseases is promoted by a combination of genetic predisposition and environmental influences [2, 3]. In this context, especially mechanisms modulating transcription and translation are supposed to be crucial in disease initiation and progression. The recent discovery of long noncoding RNAs opened up a new field of transcriptional and translational control [22].

Exploring this new field of lncRNA expression, the identification of suitable references that serve as normalizers is essential [37, 39, 40]. As there were no studies available analyzing neurons of the human anterior cingulate cortex, we explored 90 lncRNAs according to their expression stability using the NormFinder algorithm [38]. We identified three lncRNAs that are highly abundant and stably expressed in controls and PD samples and thus are appropriate as normalizers: GAS5-family, HAR1B, and SNHG4. Applying the comparative C_T method and computational analysis, we found that five lncRNAs are significantly differentially expressed in PD. H19 upstream conserved 1 and 2 is significantly downregulated, and lincRNA-p21, Malat1, SNHG1, and TncRNA are significantly upregulated in PD. H19 upstream conserved 1 and 2 (Huc 1 and 2) are ~400-bp-long conserved elements that are located ~10 kb at the 5′ end of the H19 gene. It is proposed that these genomic regions interact with imprinting control regions and epigenetically regulated silencers and that the transcription products are overexpressed and stabilized in various human tissues [41, 42]. The identified lincRNA-p21 represents a transcription target of p53 and HIF1α, and recent research indicated that lincRNA-p21 regulates mRNA translation, gene expression, protein stability, as well as p53-dependent apoptosis and cell cycle arrest [43]. Hall et al. hypothesized that lincRNA-p21 represents a functional key role in cell cycle arrest and in apoptosis [43]. Furthermore, they found that loss of lincRNA-p21 resulted in the evasion of cellular apoptosis and cell cycle arrest [43]. The lncRNA Malat1 is reported to be highly abundant in neurons [31]. Experimental knockdown of Malat1 resulted in a decreased synaptic density while the overexpression showed a cell-autonomous increase in synaptogenesis [44]. In case of the lncRNA SNHG1, it was shown that SNHG1 promotes

cellular proliferation and affects p53 stability as well as downstream p53 regulated pathways [45, 46]. TncRNA is hypothesized being a new target of TP53 that may play a role in mediating DNA damage response, but there is also evidence for associations with Malat1 lncRNA [47, 48]. Summing these findings up, the lncRNAs that we identified to be overexpressed in PD are reported being highly abundant in neurons and being closely linked with synaptogenesis (Malat1) as well as being linked with cellular proliferation, cell cycle control and apoptosis (lincRNA-p21, SNHG1, and TncRNA). Interestingly, analysis on lncRNA expression levels and disease stage revealed that altered expression of lncRNAs can already be detected in early PD, e.g. overexpression of TncRNA was already in limbic type PD statistically significant, and overexpression of lincRNA-p21 and SNHG1 was already in very early brain stem type statistically significant and remained at high levels during the course of the disease. As we analyzed the anterior cingulate gyrus, a brain region where morphological changes can be detected earliest in limbic stage PD, altered expression of these lncRNAs precedes the course of PD. Thus, these lncRNAs may serve as early biomarkers in PD.

In summary, we were able to identify five lncRNAs (H19 upstream conserved, lincRNA-p21, Malat1, SNHG1, and TncRNA) in this preliminary report that are differentially expressed in neurons of the anterior cingulate gyrus in PD brains. While H19 upstream conserved is significantly downregulated, lincRNA-p21, Malat1, SNHG1, and TncRNA are significantly upregulated in PD. It is worthwhile to mention that these lncRNAs have already been connected with synaptogenesis, cellular proliferation, and apoptosis. This is in good perception of the current notion of PD [2, 3, 31]. Interestingly, the expression of these lncRNAs precede the course of the disease: already in early brain stem type PD, altered lncRNA expression can be detected with lincRNA-p21 and SNHG1 showing significantly increased expression levels in brain stem type PD and remaining at high levels during the course of PD. It is noteworthy that the analyzed target region, the anterior cingulate gyrus, does not show morphological alterations in this early brain stem type PD according to the classification recommended by McKeith et al. [23]. Commonly, the cingulate gyrus is only affected in advancing PD, i.e., in limbic and neocortical type PD. Thus, it can be assumed that these newly identified differentially expressed lncRNAs may be appropriate as novel biomarkers. They may even serve as potential early indicators for PD. Thus, the data presented in this study will help to better understand the molecular mechanisms occurring during initiation and progression of PD. They will boost further research in the field of lncRNAs and neurodegeneration and will help to establish novel biomarkers and to develop new molecular approaches for molecularly targeted therapies.

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Compliance with Ethical Standards

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Conflict of Interest The authors declare that they have no conflict of interest.

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