Brief Communication *Highlight article*

Longitudinal dysregulation of long non-coding RNAs in Parkinson's disease

Amica C Müller-Nedebock[1,2], Katelyn Cuttler[1], Abigail L Pfaff[3,4] and Sulev Kõks[3,4]

[1]Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town 7602, South Africa; [2]South African Medical Research Council/Stellenbosch University Genomics of Brain Disorders Research Unit, Stellenbosch University, Cape Town 7602, South Africa; [3]Perron Institute for Neurological and Translational Science, Nedlands 6009, Australia; [4]Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Murdoch 6150, Australia
Corresponding author: Sulev Kõks. Email: Sulev.Koks@murdoch.edu.au

Impact Statement

In this study, we have analyzed genome-wide expression profile of long non-coding RNAs (lncRNAs) in 835 Parkinson's patients and 725 healthy controls and compared these changes over the five years of disease progression. We identified a specific set of lncRNAs that were distinctively dysregulated in Parkinson's patients, indicating their potential role in the progression of the disease. This is the first study where lncRNA were analyzed longitudinally in Parkinson's patients and shows their potential as progression biomarkers.

Abstract

Long non-coding RNAs (lncRNAs) have been suggested as potential biomarkers for Parkinson's disease (PD). This study aimed to identify blood-based lncRNA transcripts that are dysregulated in PD over time and could serve as peripheral biomarkers. Using RNA-sequencing data from the Parkinson's Progression Markers Initiative, differential expression between case and control groups at five different time points was detected, and pathway analysis was conducted. Seven transcripts, not previously linked to PD, were consistently dysregulated across all time points, while PD-linked lncRNAs were dysregulated at some but not all time points. Pathway analysis highlighted pathways, known to be affected in PD. This suggested that dysregulated lncRNA transcripts could play a role in PD pathogenesis by affecting well-known PD pathways and highlighted their potential as longitudinal biomarkers for PD. Further studies are needed to validate these findings and explore the potential use of identified lncRNAs as diagnostic and therapeutic targets.

Keywords: PD, lncRNA, PPMI, RNA-Seq, long non-coding RNA

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Introduction

Despite decades of research, the debilitating neurodegenerative movement disorder, Parkinson's disease (PD), remains difficult to diagnose and treat due to its clinical heterogeneity and complex nature/etiology. Consequently, the identification of biomarkers that could improve disease detection, progression, and treatment is crucial and is an active field of research[1] of the key ongoing studies to identify such markers, is the Parkinson's Progression Markers Initiative (PPMI)[2]—an international, observational study of people with PD or at risk for PD and healthy volunteers. This study was designed to obtain comprehensive longitudinal data from its participants, with the ultimate goal of identifying biomarkers to accelerate PD therapeutics. Given that several long non-coding RNAs (lncRNAs) have been reported to be dysregulated in Parkinson's patients, as well as PD cell and mice models,[3] lncRNAs have been proposed as potential PD biomarkers[4] but have not yet been investigated in the PPMI cohort.

Briefly, lncRNAs are a class of RNA transcripts longer than 200 bp in length, with key biological roles including the regulation of transcription, mRNA processing, and protein activity as well as the organization of multiprotein complex assembly.[5] Indeed a growing body of evidence has implicated lncRNAs in complex human diseases and supports the notion that lncRNAs could also represent novel targets for PD diagnosis, progression, and/or treatment.[6,7] Consequently, this study aimed to build on this evidence by using longitudinal PPMI RNA-Sequencing data to identify potential blood-based lncRNA markers/transcripts that are dysregulated in PD over time and identify possible pathways influenced by them.

Table 1. Number of individuals included in the study by their disease status.

Visit	Cases	Controls
BL	835	725
V02 (6 months)	475	388
V04 (year 1)	529	356
V06 (year 2)	531	319
V08 (year 3)	366	193

BL: baseline.

Materials and methods

Study participants

Blood-derived RNA-Sequencing data were available for PPMI study participants at five different time points: at the time of diagnosis, that is, baseline (BL), six months (V02), one year (V04), two years (V06), and three (V08) years from enrolment in the study. The PPMI data set used in this study included controls without PD, and cases with confirmed PD, prodromal PD and scan without evidence of dopaminergic deficit (SWEDD) PD. The RNA-sequencing of the PPMI data set has been outlined elsewhere,[8,9] and a summary of the individuals included in this study at each time point is given in Table 1. Consent from all the study participants was previously obtained by the PPMI and conformed with the ethical standards of the World Medical Association Declaration of Helsinki.[2]

Data analysis

Differential expression analysis. Fastq files from whole transcriptome sequencing were used to call the expression of transcripts with Salmon.[10] The human genome version used was GRCh38 and lncRNAs were annotated by using GENCODE version 38, containing 55,446 annotated transcripts of lncRNAs.[11] Differential transcript expression between the case and control groups was detected using "DESeq2," separately for every visit.[12]

Pathway analysis of longitudinal changes. To identify possible pathways synergistically regulated by differentially expressed lncRNAs across all five time points, the LncPath function of the R lncPath package (LncRNAs2Pathways, https://cran.r-project.org/web/packages/LncPath/) was used.[13] This mapped the lncRNAs of interest into a lncRNA-mRNA interaction network and returned the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways significantly influenced by the lncRNAs (Benjamini-Hochberg (BH)'s False Discovery Rate (*FDR*) < 0.01). Importantly, unlike many other tools, LncRNAs2Pathways can prioritize pathways influenced by only a few lncRNAs or even a single lncRNA.

Statistical analysis

Formal statistical analysis of differential expression of the lncRNA transcripts was performed by using the "DESeq2" package for R, and only the BH FDR-corrected lncRNA transcripts below 0.05 were reported. All analyses were performed using R and a bar plot was generated using the R package "ggplot2."[14]

Table 2. Total lncRNA transcripts differentially expressed between cases and controls at each of the five different time points (FDR < 0.05).

	Time point				
	BL	V02	V04	V06	V08
Down-regulated	1,046	152	93	148	4
Up-regulated	1,743	2,068	142	10,045	49
Total	2,789	2,220	235	10,193	53

BL: baseline.

Data sharing

Data used in the preparation of this article can be obtained from the PPMI database upon application (www.ppmi-info.org/data).

Results

LncRNAs are dysregulated in PD

Numerous lncRNA transcripts were significantly dysregulated in cases compared to controls at each of the five timepoints (Table 2), ranging from 2,789 transcripts at baseline to 53 transcripts at three years follow-up. The fluctuating number of study participants at each time point (Table 1) likely influenced the power of our data set to detect differentially expressed transcripts and could account for the varying numbers of transcripts identified across the different time points.

Interestingly, seven transcripts from six unique genes were dysregulated across all five time points that are six months, one year, two years and three years after the diagnosis (Table 3).

LncRNAs previously linked to PD. Several lncRNA's have previously been reported to be dysregulated in PD patients, as well as PD cell and mice models.[3] Therefore, we examined whether these previously reported lncRNAs were differentially expressed in our data set. As seen in Table 4, multiple PD-linked lncRNA transcripts were indeed differentially expressed in cases compared to controls at four of the five time points. No such transcripts were detected at time point five (V08) or three years after the diagnosis.

Pathway analysis of longitudinal changes

As mentioned a total of seven lncRNA transcripts were differentially expressed across all five time points (Table 3). We performed functional pathway analysis to identify KEGG pathways influenced by these seven lncRNAs and to identify common themes of these transcripts. This analysis yielded 13 statistically significant pathways (*BH FDR* < 0.05; Figure 1).

Discussion

Using longitudinal RNA-Sequencing data of PD cases and controls, we aimed to identify blood-based lncRNA transcripts that were dysregulated in cases over time. Furthermore, we aimed to identify possible pathways influenced by these transcripts.

Table 3. Long non-coding RNA transcripts dysregulated across all five time points (FDR < 0.05).

Transcript	Gene symbol	Transcript length	Ensemble transcript ID	Ensemble gene ID
AC106801.1–001	AC106801.1	1,845	ENST00000518054	ENSG00000253519
LINC02863–201	LINC02863	405	ENST00000422204	ENSG00000238160
LINC02863–202	LINC02863	767	ENST00000454380	ENSG00000238160
NFE4–201	NFE4	1,072	ENST00000420058	ENSG00000230257
RP11-22E12.2-007	RP11–22E12.2	3,353	ENST00000684049	ENSG00000288700
RP11-875O11.5-001	RP11–875O11.5	1,242	ENST00000397703	ENSG00000284948
SCAT8–201	SCAT8	1,454	ENST00000429530	ENSG00000236345

ID: identifier.

Table 4. Number of differentially expressed transcripts from lncRNA genes previously reported as dysregulated in Parkinson's disease.

	LncRNA gene	Time point	BL	V02	V04	V06
Down-regulated						
	GAS5		2			
	LINC-PINT		1			2
	MALAT1		4		1	
	NEAT1			1	2	1
	SNHG1		7			1
	XIST		1			
	Total		15	1	3	4
Up-regulated						
	H19		1			1
	HOTAIR					2
	HOXA-AS2		1			2
	LINC-PINT		3			1
	MALAT1		2		2	1
	MAPT-AS1			1		4
	MEG3		1	2		4
	NEAT1		3	1		
	PANTR1			1		2
	PART1					3
	RMST					2
	SNCA-AS1					1
	XIST					1
	Total		11	5	2	24

BL: baseline; LINC-PINT: long intergenic non-protein coding RNA, P53 induced transcript; XIST: X inactive specific transcript; HOTAIR: HOX transcript antisense RNA; RMST: Rhabdomyosarcoma 2 associated transcript.

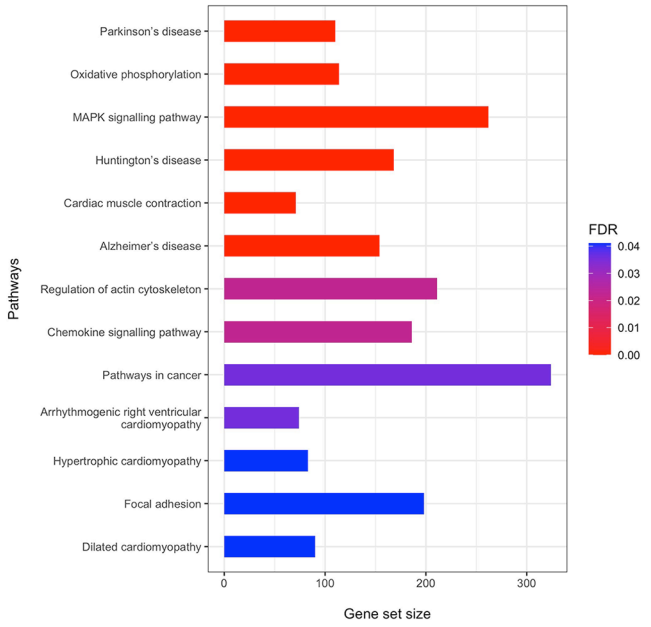

Figure 1. Bar plot of all statistically significant (*FDR* < 0.05) enriched KEGG pathways identified using LncRNAs2 Pathways.

Interestingly, seven lncRNA transcripts from six unique genes, not previously linked to PD, were found to be longitudinally dysregulated across all five time points, highlighting their possible involvement in disease development and progression. This possible involvement was also supported by the findings from the pathway analysis that identified pathways which could largely be interpreted based on our current understanding of PD. For instance, it is well established that "oxidative phosphorylation" is involved in PD onset and progression and that the dysregulation of the same molecular signaling pathways, such as "MAPK signaling," are implicated in the onset and the progression of cancers and neurodegenerative diseases, including PD, Alzheimer's disease, and Huntington's disease, albeit with different outcomes.[15]

Moreover, pathways related to the "regulation of the actin cytoskeleton" and "focal adhesion" were also dysregulated in this study. It has been shown that these can be altered in neurodegenerative disorders. MAPT, an important protein in multiple neurodegenerative disorders, is known to be involved in the stabilization of the axonal cytoskeleton, while *LRRK2* may also have a role in regulating the cytoskeleton.[16] The *LRRK2* G2019S mutation, in particular, has also been shown to attenuate microglial motility by inhibiting focal adhesion kinase.[17]

Finally, and perhaps most intriguingly, four pathways related to the heart, including three cardiomyopathy pathways and one cardiac muscle contraction pathway, were also enriched in our data set. This finding supports the increasing evidence that cardiovascular abnormalities are likely important non-motor symptoms in the early stages of PD, which worsen over time with disease progression.[18,19] The management of cardiovascular symptoms should therefore be considered in the general management of PD cases to circumvent sudden cardiac death.[19]

Overall, the pathway analysis results supported the notion that the seven longitudinally dysregulated transcripts could play a role in PD pathogenesis and progression by affecting/regulating already-known disease pathways. However, as a limitation of our descriptive study, we acknowledge that we have not experimentally shown the impact that these lncRNAs have on PD.

In addition to the aforementioned transcripts, multiple transcripts from lncRNAs previously linked to PD were

differentially expressed in cases compared to controls. Some of these had fluctuating levels over different time and none were detected at time point five (three years from diagnosis), also likely due to the varying levels of participants at each time point.

Notably, many of the identified lncRNA's have roles in neuroinflammation.[3] For instance, the lncRNA GAS5 is known to be involved in inflammatory responses and has been found to be upregulated in a PD mouse model.[20] Interestingly, GAS5 can positively regulate NLRP3 expression by competitively sponging miR-223-3p. Inhibiting the activation of NLRP3 has been shown to reduce the occurrence of neuroinflammation. Therefore, GAS5 could accelerate PD progression by targeting this axis.[20] Similarly, SNHG1 has been elevated in the brains of PD patients and *in vitro*. SNHG1 also functions as a ceRNA for miR-7 which can activate the aforementioned NLRP3 inflammasome pathway.[21] Furthermore, MALAT1 is highly elevated in PD model mice and elevates NRF2 expression, which also activates the inflammasome and the production of reactive oxygen species.[21] Overall, these results support the role of neuroinflammation in Parkinson's pathogenesis and the notion that lncRNAs play a role in its regulation.

Interestingly, two of the dysregulated lncRNAs, HOTAIR and XIST, may play a role in regulating the PD-gene, *LRRK2.* HOTAIR (upregulated at two years after enrolment) has been shown to promote PD pathogenesis *in vitro* and *in vivo* by upregulating *LRRK2*.[22]

An XIST transcript was initially downregulated in PD patients at baseline and upregulated two years later. In previous studies, XIST lncRNA has been shown to be upregulated in PD and modulates Sp1/LRRK2 to accelerate disease progression. Indeed, both *in vitro* and *in vivo* studies have shown that knocking down XIST or overexpression of its partner miRNA, miR-199a-3p, can rescue neurodegeneration and alleviate PD-associated symptoms.[23] This finding highlights XIST as a potential therapeutic target to perhaps slow PD progression.

LncRNAs represent an interesting new class of potential therapeutics. As a direct regulators of the gene expression, they can be used as therapeutics and transferred by using RNA transfer technologies in combination with the nanoparticles. At the same time, lncRNAs are very good targets for the antisense oligonucleotides and can be blocked by using antisense oligonucleotides. The fact that lncRNAs regulate multiple genes that can be pathogenetic or protective for the PD, makes them very attractive and versatile target for future drug development.

Conclusions

Unlike many genomic studies, we examined lncRNAs instead of the "functional genome" to gain insight into which lncRNAs may be involved in PD progression. The study identified longitudinally dysregulated transcripts not previously implicated in PD, which could represent novel peripheral biomarkers for PD detection and progression. However, we acknowledge that lncRNAs demonstrate tissue and even cell-type-specific expression and that the differential expression of lncRNAs observed in the blood of our cases may therefore not be truly representative of the changes present in the substantia nigra and other affected brain regions.[24,25] Given that PD is a systemic disease, the identified dysregulated lncRNAs in the blood of PD patients could nonetheless reflect the changes caused by the molecular pathological processes of the disease and may be relevant to improve our understanding of PD progression. Therefore, further investigation of these lncRNAs and their associated pathways is warranted.

AUTHORS' CONTRIBUTIONS

ACM-N contributed to conception, data analysis, execution of the research project, writing and reviewing the manuscript. KC contributed to writing and reviewing the manuscript. ALP contributed to writing and reviewing the manuscript. SK contributed to conception, data analysis, organization, and execution of the research project, writing and reviewing the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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DATA AVAILABILITY

Raw data are available from the PPMI website (www.ppmi-info.org/data (accessed on 19 January 2021)).

ORCID ID

Sulev Kõks https://orcid.org/0000-0001-6087-6643

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