

Transcriptomic profiles in Parkinson's disease

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Impact statement

This work is relevant because a meta-analysis of transcriptomic studies of Parkinson's disease (PD) has not been attempted yet. Furthermore, two peripheral and easily obtainable tissues are being analyzed in this study, paving a way for future large-scale studies from these *in vivo* accessible tissues in PD. This work maps changes that are consistent between previous work and the current one and, particularly, maps the gene expression changes that might be of specific interest, because they are differentially changed even after applying robust and rigorous statistical methods. This aims to prompt readers to investigate into the qualities of specific gene expressions or pathways regarding Parkinson's disease.

Abstract

Transcriptomics in Parkinson's disease offers insights into the pathogenesis of Parkinson's disease but obtaining brain tissue has limitations. In order to bypass this issue, we profile and compare differentially expressed genes and enriched pathways (KEGG) in two peripheral tissues (blood and skin) of 12 Parkinson's disease patients and 12 healthy controls using RNA-sequencing technique and validation with RT-qPCR. Furthermore, we compare our results to previous Parkinson's disease *post mortem* brain tissue and blood results using the robust rank aggregation method. The results show no overlapping differentially expressed genes or enriched pathways in blood vs. skin in our sample sets (25 vs. 1068 differentially expressed genes with an $FDR \leq 0.05$; 1 vs. 9 pathways in blood and skin, respectively). A meta-analysis from previous transcriptomic sample sets using either microarrays or RNA-Seq yields a robust rank aggregation list of cortical gene expression changes with 43 differentially expressed genes; a list of *substantia nigra* changes with 2 differentially expressed genes and a list of blood changes with 1 differentially expressed gene being

statistically significant at $FDR \leq 0.05$. In cortex 1, KEGG pathway was enriched, four in *substantia nigra* and two in blood. None of the differentially expressed genes or pathways overlap between these tissues. When comparing our previously published skin transcription analysis, two differentially expressed genes between the cortex robust rank aggregation and skin overlap. In this study, for the first time a meta-analysis is applied on transcriptomic sample sets in Parkinson's disease. Simultaneously, it explores the notion that Parkinson's disease is not just a neuronal tissue disease by exploring peripheral tissues. The comparison of different Parkinson's disease tissues yields surprisingly few significant differentially expressed genes and pathways, suggesting that divergent gene expression profiles in distinct cell lineages, metabolic and possibly iatrogenic effects create too much transcriptomic noise for detecting significant signal. On the other hand, there are signs that point towards Parkinson's disease-specific changes in non-neuronal peripheral tissues in Parkinson's disease, indicating that Parkinson's disease might be a multisystem disorder.

Keywords: Biomarkers, brain, neurodegeneration, blood, transcriptomics, skin

Experimental Biology and Medicine 2021; 246: 584–595. DOI: 10.1177/1535370220967325

Introduction

Parkinson's disease (PD) has an appreciable rate of clinical misdiagnosis. The diagnosis made by experienced

clinicians is ~90% concordant with the following pathological diagnosis.^{1,2} Furthermore, PD is not a uniform disease where next to causal monogenetic mutations (<10% of all

PD patients), many low-risk loci account for much of the genetic risk in sporadic PD.³ Although the effect size of each locus is small, the combined effect increases the risk of PD substantially.⁴ These loci change gene expression levels rather than changing the coded protein qualities.⁵ Both monogenetic and sporadic changes in PD result in pathognomic loss of neurons in *substantia nigra* (SN) and in accumulation of misfolded α -synuclein in Lewy bodies. The incidence of PD increases with aging, which reflects the accumulation of changes caused by altered gene expression.⁶ Therefore, mapping differentially expressed genes (DEGs) in PD is useful in discovering these multiple changes and could offer diagnostic and prognostic markers and possible treatment options.

However, there are substantial obstacles. PD is canonically thought to be a disease affecting a specific population of neurons. Obtaining tissue for transcriptomic analyses from brain has some limitations, since it is performed *post mortem* on advanced PD patients. Another factor is that RNA-molecule is instable and the length of *post mortem* interval and RNA integrity must be considered. A possibility to bypass these obstacles is to sample peripheral tissues, especially the blood, for gene expression. There is evidence that blood gene expression profile does not differ significantly from the brain.^{7,8} Thus, analyzing blood for easily obtainable biomarkers in PD has become widespread.^{9–17}

Another issue with gene expression studies in PD is the lack of reproducibility of the findings. For example, from a set of 22 DEGs identified with microarray from PD whole blood by Scherzer *et al.*,¹⁸ only three were found overlapping from 1367 DEGs in a subsequent study by Kauczynska *et al.*⁹ Lack of reproducibility between datasets could come from different analytic procedures and some from the inherent genetic and transcriptional heterogeneity of PD. PD has been associated with aberrant splicing¹⁹ and using microarrays or polymerase chain reaction (PCR) limits mapping this heterogeneity. Unlike arrays, RNA sequencing (RNA-Seq) does not require probes but can detect alternatively spliced or novel transcripts.^{20,21} RNA-Seq has also been shown to be highly accurate for quantifying expression levels and has less background noise. This enables a smaller sample size, while still having more statistical power compared to microarrays.^{22,23}

In this study, we compare two peripheral tissues in PD—whole blood and skin. We identified the DEGs using RNA-Seq with subsequent validation with RT-qPCR. In addition to describing DEGs, our second goal is to describe enriched functional pathways in PD whole blood and skin. Thirdly, we aim to compare our results with previous datasets from central nervous system (CNS) and blood. The usage of different methods has limited comparing and integrating previous data and thus far enabled only descriptive reviews of PD transcriptomics.²⁴ Meta-analysis could possibly offer a more reproducible set of DEGs or functional pathways for PD, but the use of different methods, annotations, and the publication of incomplete DEG lists hampers integrating the results of previous work. In order to bypass this issue, we use the robust rank aggregation method²⁵ to analyze the aggregated results from our study and previous datasets. The aim here is to determine which DEGs and enriched

pathways are ranked the highest within a specific tissue and whether CNS tissue changes overlap with those from heterogenous peripheral tissue populations.

Materials and methods

Study subjects

The study was conducted in concordance with the Declaration of Helsinki and approved by the local Ethics Committee. All subjects gave their written consent to participate in the study. The RNA-Seq from whole blood included 12 patients with clinically diagnosed idiopathic PD and 12 matched healthy controls (HC). Patient characteristics chosen for skin RNA-Seq have been published previously.²⁶ The inclusion of patients was based on: (1) a diagnosis of idiopathic PD according to the QSBB criteria²⁷; (2) on standard medical treatment for PD; and (3) no other severe diagnoses based on medical interview. The demographic data, history of disease, and clinical data were documented, and a summary of participant characteristics and comparison with the previously published skin dataset is presented in Table 1. Clinical disease markers were assessed using validated instruments including the Movement Disorders Unified Parkinson's Disease Rating Scale (MDS-UPDRS),²⁸ the Hoehn and Yahr Scale (HY),²⁹ the Schwab and England Activities of Daily Living Scale (SE-ADL),³⁰ and the Mini Mental State Examination (MMSE).³¹

PD patients selected for the whole blood RNA-Seq had a mean age of 72.2 (± 10.0) years with a mean disease duration of 6.9 (± 6.5) years and mean HY stage of 2.7 (± 1). The results were validated with qRT-PCR with further 59 PD and 33 HC blood samples (including the 12 + 12 from RNA-Seq), and a breakdown of clinical characteristics can be found in Supplementary Table 1.

Sampling and RNA sequencing

At the time of the medical interview, venous blood of all study subjects was collected into Tempus Blood RNA Tubes (Thermo Fisher Scientific Inc., CA, USA) for the whole blood RNA-Seq. The tubes were immediately frozen and stored in liquid nitrogen at -80°C . The RNA was extracted applying Tempus Spin RNA Isolation Kit (Thermo Fisher Scientific Inc., CA, USA) combined with DNase treatment (RNase-Free DNase Set, Qiagen, Hilde, Germany), according to the manufacturers' protocols. The globin mRNA was removed from the extracted total RNA using GLOBINclear Kit, human (Thermo Fisher Scientific Inc, CA, USA). The lowest acceptable concentration of globin clear RNA was 36.5 ng/ μL for PD patients and 39.5 ng/ μL for the control group; 50 ng of each RNA sample was amplified with Ovation RNA-Seq System V2 Kit (NuGen Technologies Inc., CA, USA) and the output double stranded DNA was used to prepare SOLiD 5500 W System DNA fragment libraries according to the manufacturers' protocols (Thermo Fisher Scientific Inc, CA, USA). Barcoding adapters were applied, and the 12 libraries were pooled prior to sequencing. Fragment (single-end) sequencing chemistry was applied with SOLiD 5500 W XL platform resulting in

Table 1. Clinical characteristics of blood and skin RNA-Seq patients.

Subj. no	Gender (% of males)	Age ^a	Disease onset age ^a	Duration of disease ^a	1st degree relatives with PD (%)	HY ^a	SE-ADL ^a	MMSE ^a	MDS-UPDRS ^a
Blood RNA-Seq patients									
1	M	73	65	9	No	3	80	30	77
2	F	82	74	9	No	4	60	26	79
3	M	67	50	17	No	4	70	28	103
4	M	69	68	1.2	No	3	80	30	56
5	F	85	70	15	No	2.5	60	23	44
6	M	85	67	18	No	4	40	27	159
7	F	68	65	3	Yes	1.5	100	30	26
8	F	71	66	5	No	3	70	24	73
9	F	48	47	1.1	No	1.5	90	30	33
10	F	69	67	2	No	2.5	80	29	72
11	M	76	75	1.7	No	2	90	25	36
12	M	73	72	1	No	1	95	29	22
Total	50%	72.2 (±10.0)	65.5 (±8.6)	6.9 (±6.5)	8%	2.7 (±1)	76.3 (±17.2)	27.6 (±2.5)	65 (±38.7)
Skin RNA-Seq patients									
1	M	60	46	14	No	2.5	80	28	83
2	F	82	74	9	No	4	60	26	79
3	F	77	68	9	No	3	70	30	68
4	M	63	58	4	No	2.5	90	30	40
5	F	76	73	3	No	3	80	27	77
6	M	81	80	1	No	2.5	90	30	43
7	M	67	64	3	No	2	90	29	65
8	F	68	65	3	No	1.5	100	30	26
9	M	75	64	11	No	4	70	30	105
10	F	69	67	2	No	2.5	80	29	72
11	F	80	76	4	No	2	95	25	28
12	F	65	55	10	No	4	60	30	114
Total	42%	71.9 (±7.5)	65.8 (±9.6)	6.1 (±4.3)	0%	3.1 (±0.9)	80.4 (±13.2)	28.7 (±1.8)	66.7 (±28.1)
P-value	0.70	0.95	0.93	0.71	0.33	0.75	0.51	0.24	0.91

^aAverage (± standard deviation).

reads with length of 75 bp. Methods of skin RNA-Seq preparation are described in detail in our previous paper.²⁶

RNA sequencing data analysis

LifeScope software was used for aligning RNA-Seq reads to the hg19 reference genome. The alignment was performed with recommended default parameters. Gene-level read counts were obtained from LifeScope alignment summary statistics and DeSeq2 package in R³² was used to test the differential expression of genes. Detected differential expression of genes was considered statistically significant at a false discovery rate (FDR) ≤ 0.05. Following, a pathway enrichment analysis was performed using the hypergeometric test implemented by ClusterProfiler in R³³ and KEGG pathway annotations and pathway results were considered significant at *P*-adjusted ≤ 0.05. A reanalysis from the previously published skin data²⁶ was done with the ClusterProfiler package for R (Input DEGs with an FDR of ≤ 0.05, cut-off for pathways at *P*-adjusted ≤ 0.05).

Validation with RT-qPCR

In a larger group of 59 PD patients and 33 controls (including the RNA-Seq samples), validation top DEGs of whole blood RNA sequencing data with RT-qPCR was conducted. Total-RNA was converted to cDNA using random primers and High Capacity cDNA Reverse Transcription Kit with an

RNase Inhibitor (Applied Biosystems). TaqMan Gene Expression Assay with VIC-label was used as the housekeeping gene (Actin) and FAM as the gene of interest. The samples were prepared using the TaqMan[®] Gene Expression Master Mix (Applied Biosystems). The genes that statistically had the largest change in gene expression were OGT; UBE2J1, KIR2DL3, LCE5A, ENOSF1, FAM219B, CTSL, IL8R1, MIAT, SBSN, ETV1, SPRRE2, CTSD, KRTDAP, and S100A2. Following, duplex quantitative real-time PCR (qRT-PCR) analysis was performed in three replicates to minimize technical errors. The data were analyzed using the 2^{-ΔCT} method.

Meta-analysis of previously published gene expression datasets from blood, cortex, and SN

For the comparison of our work with previously published transcription studies' results, a search was conducted on PubMed. Minimal criteria for including these gene expression results in our analysis were: (1) the list of DEGs is openly accessible (2) the list is original data, (3) list did not contain selectively presented gene expressions, lists with statistical significance cut-offs were allowed, (4) idiopathic PD vs. control comparison, (5) there are enough lists per tissue type for further analysis. The search yielded three lists from cortex,^{34–36} five from SN,^{37–41} and five from blood.^{13,16,42–44} Details of the input lists can be found in Supplementary Table 2. The meta-analysis of the gene

expression datasets published by the authors of this paper and other previously published PD gene expression studies was conducted using the Robust rank aggregation (RRA) method by Kolde *et al.*²⁵ This method summarizes the ranked DEGs lists reported by the authors of these studies by assigning significance scores for the genes that appear more frequently in the top of the ranked lists of differentially expressed genes. This method allows for incomplete ranked gene lists to be included as well, therefore we also included gene rankings where only DEGs with significant *P*-values were published or a fold change threshold was applied. We conducted the meta-analysis separately in each type of tissue (three in total: cortex, SN, and blood). All gene identifiers were converted to Entrez IDs prior to analysis with RRA. The *N* parameter was calculated as $N(i) + N(\text{avg. } u)$, where $N(i)$ denotes the number of genes present on all the different microarray chips used by the studies included in our meta-analysis and $N(\text{avg. } u)$ denotes the average number of genes per microarray that were not included in the intersected $N(i)$. The *N* was calculated separately for each set of ranked gene lists in different tissues. DEGs were considered statistically significant at $\text{FDR} \leq 0.05$. For pathway analysis that was conducted with ClusterProfiler KEGG package for R DEGs with a score/*P*-value ≤ 0.05 were included and the results were considered statistically significant at $\text{FDR} \leq 0.05$.

Results

Differential expression of genes and pathways in PD blood

The RNA-Seq yielded 21.4 ± 3.3 million reads (mean \pm SD) per sample, out of which $73.6 \pm 0.8\%$ aligned to the genome at least once. No cut-offs to reads were applied; >20 million reads per sample in a 12-sample set should provide optimal statistical power for this analysis. Sequencing deeper than 10M reads and having a sample size >12 does not significantly improve statistical power and precision for detecting DEGs.^{45–47}

Raw data were subjected to differential expression testing with DeSeq2. Differential expression testing of whole blood RNA-Seq data resulted in 25 DEGs between control and PD samples (Table 2). Most of DEGs in blood are upregulated, but notably UBE2J1 (also known as HIP2) is downregulated, with a foldchange of 0.63. Significantly upregulated genes include A2M, LINC00612, FAM219B, KIR2DL3, OGT, ENOSF1, MIAT, IL18R1 and the only other downregulated gene CUTALP. Pathway analysis from PD blood shows one enriched pathway—cholesterol metabolism.

Differential expression of genes and pathways in PD skin and comparison with blood

Skin RNA-Seq yielded 1068 DEGs (data available upon request). A clear pattern of global downregulation appears, with 874 of total DEGs being downregulated.²⁶ Nine enriched pathways from PD skin were detected (Table 3). If blood and skin gene expressions are compared using the standard cut-off values for significance, no overlap in DEGs or pathways is to be found (Figure 1.)

Table 2. Differentially expressed genes from PD blood.

Gene	<i>P</i> -adjusted
UBE2J1	0.002456533032969
OGT	0.004525065433154
C15orf17	0.005583830092225
LUC7L3	0.005583830092225
C12orf33	0.008624302294215
STAT4	0.008624302294215
PTPN4	0.008624302294215
A2M	0.00966346623602
ENOSF1	0.011203502577878
MIAT	0.012084815414542
CD36	0.016921618832212
IL18R1	0.018943723956247
PYHIN1	0.018943723956247
PPT1	0.018943723956247
LOC253039	0.018943723956247
SYTL2	0.023012888462431
EPM2AIP1	0.02323506201148
PCGF3	0.025439081994619
PKM2	0.025439081994619
ZNF767	0.032474874139179
RAB32	0.037059204955581
PRPF4B	0.037487943308261
STAR9D9	0.038488470301337
AGPAT4	0.048712754929204
GMPPA	0.048712754929204

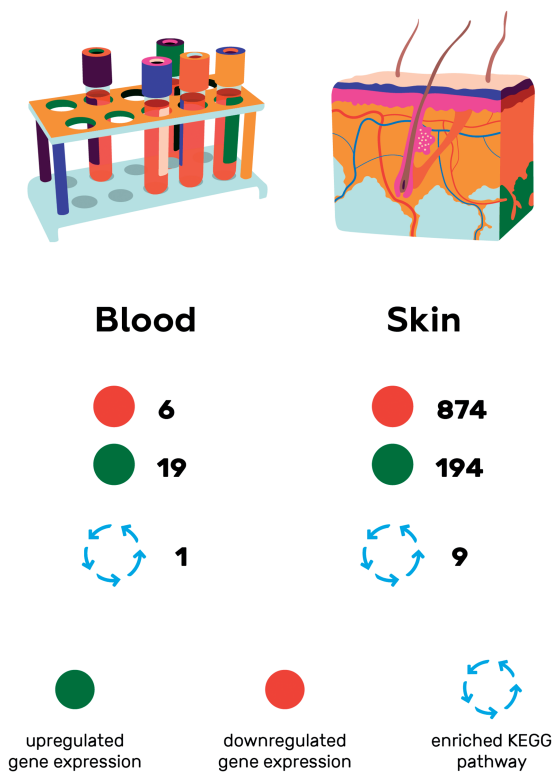
Results of the meta-analysis

When five previously published sets of DEGs from PD blood^{13,16,42–44} and the current work were integrated into an RRA list, only one gene remained significant: IL18R1. It is an interleukin receptor linked with proinflammatory responses that belongs to the immunoglobulin superfamily. Pathway enrichment analysis of the top 163 DEGs from blood RRA list found two enriched KEGG pathways: hematopoietic cell lineage ($\text{FDR} = 0.001$) and prion diseases ($\text{FDR} = 0.037$).

Thereafter, we decided to investigate how these findings from peripheral tissues in PD correlate to transcriptomic profiles from CNS. In order to do that, aggregated ranked gene list was created based on the results of three previous studies on the genes expression in cortex^{34–36} and five studies on SN^{37–41} (Figure 2). In case of differentially expressed genes in the cortex of PD patients, RRA resulted in 43 significant genes (data available upon request) despite differences in the location and exact cell composition of cortical samples. Topmost significant DEG is PENK, which is elevated in the brain during oxidative stress.⁴⁸ Furthermore, heat shock proteins HSPA1B, HSPA6, and SERPINH1 and growth factors BDNF, VGF, and CSF3 were elevated. KEGG pathway analysis based on 475 DEGs yielded one enriched pathway: legionellosis. In the case of differentially expressed genes in the SN of PD patients, RRA yielded two significant genes: LMO3 and RIMS3. LMO3 is an oncogene predominantly expressed in the brain, while RIMS3 is involved in vesicle trafficking. Pathway analysis from the 390 DEGs yielded four KEGG pathways to be enriched: calcium signaling pathway, synaptic vesicle cycle, proteoglycans in cancer and dopaminergic synapse.

Table 3. Enriched KEGG pathways from PD skin.

ID	Description	GeneRatio	BgRatio	P value	P. adjust	q value	Count
hsa05016	Huntington disease	44/434	193/7528	9.3451827163023e-16	2.68206743957876e-13	2.59697709168822e-13	44
hsa04932	Non-alcoholic fatty liver disease (NAFLD)	36/434	149/7528	6.6323638782285e-14	9.5174421652579e-12	9.21549507290697e-12	36
hsa05012	Parkinson disease	33/434	142/7528	2.46947683273819e-12	2.36246616998621e-10	2.28751538190485e-10	33
hsa00190	Oxidative phosphorylation	31/434	133/7528	1.07724856969573e-11	7.72925848756688e-10	7.48404269472824e-10	31
hsa03010	Ribosome	31/434	153/7528	4.8176225916021e-10	2.38471885093517e-08	2.3090620819618e-08	31
hsa05010	Alzheimer disease	33/434	171/7528	4.98547494969025e-10	2.38471885093517e-08	2.3090620819618e-08	33
hsa04714	Thermogenesis	36/434	231/7528	3.27933904006457e-08	1.34452900642647e-06	1.30187294222112e-06	36
hsa04723	Retrograde endocannabinoid signaling	24/434	148/7528	3.35738799540263e-06	0.000120446294335069	0.000116625056682407	24
hsa04260	Cardiac muscle contraction	13/434	78/7528	0.000458898234034117	0.0146337547964213	0.0141694893315798	13

**Figure 1.** A schematic comparison of the original RNA-Seq data from Parkinson's disease blood and skin samples visualizing the differences in number of DEGs and KEGG pathways and showing no overlap.

Comparing the DEGs from three different tissues (blood, cortex, and SN) yielded no overlap (Table 4). Also, reanalysis of enriched pathways from these tissues using KEGG database yielded no overlapping pathways (Table 5). When comparing all RRA lists with the currently only skin DEGs list, only two genes overlap between cortex and skin: G0S2 and C6. No statistically significant pathway was found comparing four different tissues in PD.

Discussion

RNA-seq results from blood and skin yielded very different number of significantly changed DEGs (25 vs. 1068), but no

overlap. In the blood, a gene was found which has previously shown to have altered expression in PD venous blood—UBE2J1^{9,18,49} The protein encoded by this gene is a ubiquitin-conjugating enzyme located in the membrane of the endoplasmic reticulum. UBE2J1 is involved in ubiquitin-proteasome system, which targets proteins for degradation.⁵⁰ In PD, the ubiquitin-proteasome system is impaired and contributes to aberrant protein degradation. UBE2J1 is also linked with HD, where it ubiquitinylates aberrant protein huntingtin.⁵¹ Another gene, A2M encodes for a protease inhibitor and cytokine transporter that is linked with AD where it contributes to the degradation of A-beta.⁵² The only statistically significant pathway in the blood was cholesterol metabolism. Interestingly, epidemiological studies demonstrate an association between higher levels of cholesterol and lower risk of PD.⁵³ A study showing higher plasma levels of (S)24-OH-cholesterol, a metabolite possibly reflecting brain cholesterol metabolism, was linked to lower odds of having PD.⁵⁴ In the PD brain, aberrant alpha-synuclein impacts cholesterol metabolism which neurons depend on in synapse formation. Altered levels of cholesterol in neuronal cultures lead to impaired formation of synapses and less neurotransmitter release.⁵⁵ DEGs in the skin reflect the PD-related dermal changes.²⁶ A reanalysis of this data yielded three KEGG pathways that were associated with neurodegeneration. Huntington disease (HD) pathway contains genes that are involved in vesicular transport, Ca²⁺ signaling and (through disrupted Ca²⁺ signaling), mitochondrial dysfunction. Notably, a study⁵⁶ compared post mortem brain tissues between PD and HD and found an overlap of 25% of DEGs. The second neurodegenerative pathway enriched in PD skin was Alzheimer's disease (AD) pathway which contains genes that increase in the production of amyloidogenic A-beta peptides or affect posttranslational processing of proteins. Lastly, KEGG Parkinson's disease pathway itself, which characterizes the intracellular accumulation of alpha-synuclein and impaired mitochondrial function, is enriched in PD skin. The fact that major neurodegenerative pathways are mapped in a peripheral tissue in PD suggests two things. Firstly, that there are global pan-neurodegenerative mechanisms involved in PD. Secondly, these pan-neurodegenerative mechanisms are detectable

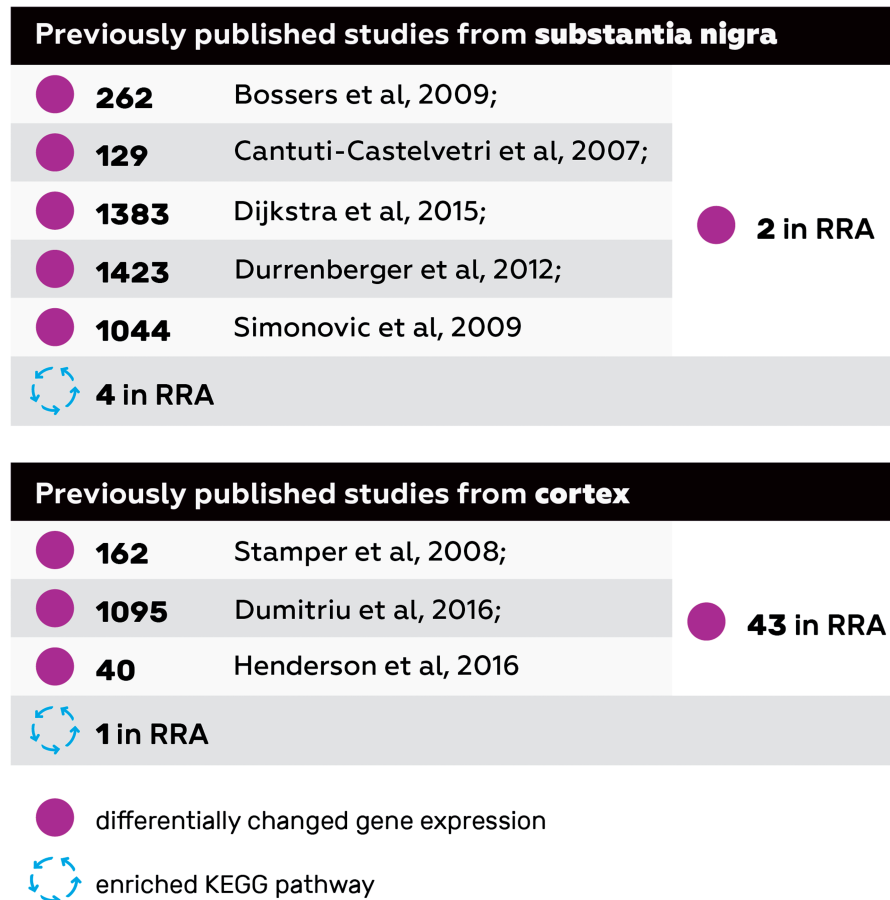


Figure 2. A schematic overview of previously published cortex and *substantia nigra* RNA-Seq and microarray studies showing the number DEGs in each single study, overlapping DEGs and KEGG pathways in the RRA analysis.

from a peripheral tissue. A group of enriched pathways emerge from skin that are linked to metabolic changes in PD, for example oxidative phosphorylation pathway, which is another key player in neurodegenerative diseases.⁵⁷ Non-alcoholic fatty liver disease (NAFLD) is a pathway of lipid accumulation which is affected by insulin resistance and leads to elevated oxidative stress though mitochondrial beta-oxidation of fatty acids in the liver. NAFLD has already been linked to AD,⁵⁸ the implication in PD needs to be investigated. Another pathway related to lipid metabolism affected in PD skin is thermogenesis pathway in which chemical energy is converted into heat in brown adipose tissue that is controlled by sympathetic nervous system. This has also been shown to be implicated in murine models of HD,⁵⁶ a key regulator in this pathway is peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC1 α) was upregulated in PD skin samples.²⁶ Its activation was demonstrated to improve the phenotype in mouse models of PD.⁵⁹ Retrograde endocannabinoid signaling pathway affects synaptic plasticity and neurotransmission in the brain. It is suggested dyskinesias in PD might be caused through disrupted endocannabinoid signaling among other effectors.^{60,61} Initially, it was thought that endocannabinoids are exclusive to the CNS, but further research has described their involvement in peripheral tissues. In the skin, changes in endocannabinoid system are

linked to melanoma,⁶² which is interesting considering the epidemiological bidirectional link between PD and melanoma.⁶³

When comparing our sample sets from skin and blood it is clear that skin yields more significant changes. Low DEG count from blood might be due to higher number of different cell lineages, metabolic, and possibly iatrogenic effects that create too much transcriptomic noise for detecting significant signal. The lack of overlap between results of skin and blood lead us to investigate comparability with previous studies. As the output of individual experiments can be noisy, data integration could increase the signal and lessen false positive findings. Therefore, we performed meta-analysis using the RRA method²⁵ of the lists of DEGs from previously published open-access studies and the present study in hopes of finding robust PD-specific changes (Figure 3). Another significant reason that lead us to perform this meta-analysis was to allow for a comparison between tissues of different origin (especially the CNS and the periphery).

In a descriptive review of transcriptomic studies of PD, whole blood shows significant changes in pathways involved in inflammation, mitochondrial function, immune function, protein chaperones, RNA processing, and programmed cell death.²⁴ In our analysis using the RRA method, the only significant DEG in blood was

Table 4. Significantly changed gene expressions from blood, cortex, *substantia nigra* after RRA analysis.



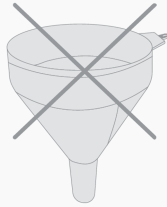
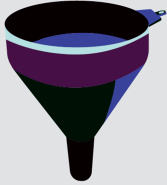
Tissue	Gene ID	Score	FDR	SYMBOL
Blood	8809	1.28802794373959e-05	0.050954385454338	IL18R1
<i>Substantia nigra</i>	55885	3.93808672219532e-06	0.00859806402721747	LMO3
<i>Substantia nigra</i>	9783	5.21727186117565e-06	0.00859806402721747	RIMS3
Cortex	5179	2.27636804825777e-05	0.026952197691372	PENK
Cortex	4548	4.8484098221707e-05	0.0274016611662194	MTR
Cortex	56261	6.94298847117046e-05	0.0274016611662194	GPCPD1
Cortex	1440	0.00016460604283402	0.0324822591192467	CSF3
Cortex	29102	0.00016460604283402	0.0324822591192467	DROSHA
Cortex	3310	0.00016460604283402	0.0324822591192467	HSPA6
Cortex	3627	0.000329206064413617	0.0389772851187413	CXCL10
Cortex	50486	0.000329206064413617	0.0389772851187413	G0S2
Cortex	8289	0.000329206064413617	0.0389772851187413	ARID1A
Cortex	627	0.000392790400009593	0.0389772851187413	BDNF
Cortex	203523	0.00043728447392925	0.0389772851187413	ZNF449
Cortex	54431	0.000441864529348627	0.0389772851187413	DNAJC10
Cortex	23332	0.000493800064848919	0.0389772851187413	CLASP1
Cortex	3303	0.000493800064848919	0.0389772851187413	HSPA1A
Cortex	57822	0.000493800064848919	0.0389772851187413	GRHL3
Cortex	3315	0.000658388044250058	0.041505605825661	HSPB1
Cortex	729	0.000658388044250058	0.041505605825661	C6
Cortex	7874	0.000658388044250058	0.041505605825661	USP7
Cortex	57542	0.000683883429288061	0.041505605825661	KLHL42
Cortex	151613	0.000701108206514544	0.041505605825661	TTC14
Cortex	486	0.000822970002727162	0.0423650644882157	FXYD2
Cortex	6401	0.000822970002727162	0.0423650644882157	SELE
Cortex	85369	0.000822970002727162	0.0423650644882157	STRIP1
Cortex	23704	0.000987545940390364	0.0449713228239304	KCNE4
Cortex	283742	0.000987545940390364	0.0449713228239304	FAM98B
Cortex	7425	0.000987545940390364	0.0449713228239304	VGf
Cortex	3304	0.00115211585734979	0.0470381094862812	HSPA1B
Cortex	5304	0.00115211585734979	0.0470381094862812	PIP
Cortex	5469	0.00115211585734979	0.0470381094862812	MED1
Cortex	58517	0.00123118103210076	0.0472408735878559	RBM25
Cortex	4009	0.00131667975371558	0.0472408735878559	LMX1A
Cortex	6415	0.00131667975371558	0.0472408735878559	SELENOW
Cortex	6539	0.00131667975371558	0.0472408735878559	SLC6A12
Cortex	126308	0.00144191115876224	0.0473996041471313	MOB3A
Cortex	4922	0.00148123762959785	0.0473996041471313	NTS
Cortex	871	0.00148123762959785	0.0473996041471313	SERPINH1
Cortex	96610	0.00148123762959785	0.0473996041471313	BMS1P20
Cortex	2168	0.00164578948510674	0.0487153687591595	FABP1
Cortex	3337	0.00164578948510674	0.0487153687591595	DNAJB1
Cortex	91748	0.00164578948510674	0.0487153687591595	ELMSAN1
Cortex	51116	0.00181033532035238	0.0498473725417957	MRPS2
Cortex	54541	0.00181033532035238	0.0498473725417957	DDIT4
Cortex	56116	0.00181033532035238	0.0498473725417957	PCDHB@

IL18R1. It is an interleukin receptor linked with proinflammatory responses of the immunoglobulin superfamily. PD is characterized by a dysregulated inflammatory environment. In a PD model of MPTP-treated mice, GM-CSF administration induced IL18R1, which was argued to be due to GM-CSF induced neuroprotective mechanisms.⁶⁴ IL18R1 has also been associated with neuroinflammation in multiple sclerosis.⁶⁵ Altered levels of IL18R1 found in PD blood underline the relevance of neuroinflammatory processes in neurodegeneration. In the enrichment analysis of PD blood, RRA lists two pathways which were significant: hematopoietic cell lineage and prion diseases pathway. Hematopoietic cell lineage pathway is a part of the immune system, and changes in this lineage have previously been published in PD.^{44,66} Prion diseases pathway is

possibly linked to PD prion-like propagating qualities.⁶⁷ In order to compare the transcriptomic profiles in PD of our current peripheral tissues and the aggregated results from peripheral blood with previous transcriptomic results from CNS, aggregated lists of these sample sets needed to be made. The two specific localizations in the CNS that had enough transcriptomic sample sets for an RRA analysis—SN and cortex showed no overlap in DEGs or pathways. There were multiple DEGs in cortex RRA list (Table 4) with only two (G0S2 and C6) showing an overlap with the skin RNA-Seq results. G0/G1 switch gene 2 (G0S2) is a major regulator of lipid metabolism in adipocytes.⁶⁸ Complement component 6 (C6) is a protein in the lytic membrane attack complex (MAC) macromolecule C5b-9. The MAC inserts itself into foreign cells, causing lysis. It has been shown

Table 5. Enriched KEGG pathways from blood, cortex, *substantia nigra* after RRA analysis.

Tissue	ID	Description	GeneRatio	BgRatio	P value	P. adjust	q value	geneID	Count
Blood	hsa04640	Hematopoietic cell lineage	14/181	97/7914	7.7189077408129e-06	0.00129677650045657	0.00124315040457302	933/2208/2322/7850/ 948/930/3552/2323	8
Blood	hsa05020	Prion diseases	8/181	35/7914	0.000481311510902407	0.0404301669158022	0.0387582427200359	712/714/3552/713	4
<i>Substantia nigra</i>	hsa04020	Calcium signaling pathway	14/181	193/7914	0.00012816581859865	0.0305034648264787	0.0273870117637115	4923/5027/2066/6543/ 8913/5159/489/3708/ 185/80228/2065/ 3710/444/23236	14
<i>Substantia nigra</i>	hsa04721	Synaptic vesicle cycle	8/181	78/7914	0.000383587904674655	0.0456469606562839	0.04098333392889236	6857/4905/5864/535/ 23025/440279/22999/ 6571	8
<i>Substantia nigra</i>	hsa05205	Proteoglycans in cancer	13/181	204/7914	0.000779054186583262	0.0479601005770201	0.043060152220854	286/2066/7480/5962/ 3708/1499/10451/ 6198/6383/2065/ 3710/7473/2549	13
<i>Substantia nigra</i>	hsa04728	Dopaminergic synapse	10/181	131/7914	0.000806052110538154	0.0479601005770201	0.043060152220854	10681/2932/1813/6323/ 3708/6571/5528/ 2893/3710/23236	10
Cortex	hsa05134	Legionellosis	10/207	56/7914	1.50285900432062e-06	0.00039074334112336	0.000367013988423561	3310/3303/3304/4791/ 5970/7100/840/4792/ 10767/3305	10

Tissues	Blood	Cortex	Skin	Substantia Nigra
RNA-Seq	12/12 20/20	13/11 29/44	12/12	
RNA microarray	51/39 40/20 50/22 30/29	15/14		10/9 7/8 9/8 12/7 8/8
RRA procedure				
Number of DEGs	1 IL 18R1	43 ^A	1068 ^B	2 LMO3, RIMS3
KEGG pathways	2 hematopoietic cell lineage, prion diseases	1 legionellosis	9 ^{**}	4 ^{***}
Overlap		2 DEGs (cortex, skin) - GOS2, C6		

* Huntington disease, nonalcoholic fatty acid liver disease, Parkinson disease, Oxydative phosphorylation, Ribosome, Alzheimer disease, Thermogenesis, Retrograde endocannabinoid signaling, cardiac muscle contraction

** calcium signaling, synaptic vesicle cycle, proteoglycans in cancer, dopaminergic synapse

A list of DEGs can be found in Supplementary Table 5

B list of DEGs can be found in Supplementary Table 4

Figure 3. A schematic overview of the studies involved in RRA analysis (51/39 is the number of Parkinson's disease patients and healthy controls in the original study respectively), the output DEGs and KEGG pathways, and the overlap between the investigated studies.

immunohistochemically in the brains of AD and PD⁶⁹ where its abundant staining is one of the signs of active neuroinflammation in neurodegeneration. The only significantly enriched pathway in the cortex of PD was legionellosis pathway; how this relates to PD remains to be known. A component of this pathway TLR/MYD88 signaling has previously been associated with neurodegenerative diseases.⁷⁰ In the RRA of SN, four enriched pathways were significant: calcium signaling, synaptic vesicle cycle, proteoglycans in cancer, and dopaminergic synapse pathway. Genes that give rise to PD have a known causal role in Ca²⁺ homeostasis which is regulated in the calcium signaling pathway.⁷¹ Synaptic vesicle cycle pathway has already been associated with PD previously.⁷² Proteoglycans in cancer pathway might be altered in PD due to

neuroinflammation, modulating the fibrotic process in the extracellular matrix as previously reported in neurodegeneration.^{73,74} Enriched dopaminergic synapse pathway in an RRA list from SN describes the pathognomonic changes of PD.

Due to the novelty of using RRA in analyzing transcriptome data, the optimal number of studies that need to be integrated for the results to be relevant still needs to be determined. If feasible, integrating studies with small numbers could be an alternative to increasing sample size per study in obtaining more reproducible and robust results. Another aspect that should be considered in future transcriptomic studies of PD that might yield more reproducible results, is dividing PD patients into endophenotypes. Also, in the current work and, indeed, in many of the

previous studies, the effects of dopaminergic medication and natural disease course remain undistinguishable, as most of the samples are from patients already receiving dopaminergic therapy. However, so far it is not been clearly shown that therapy naïve patients have very different transcriptomic profiles.⁹

Taken together, the differential expression of very few genes remained significant in direct comparison between PD blood and skin and in ranked aggregation lists and there was virtually no overlap between tissues. There are multiple pathways that lead to the pathognomonic sign of dopaminergic neuron death in PD, for an example alpha-syn aggregation and mitochondrial stress. There is a possibility that changes in adjacent DEGs in a pathway that converge into having the same metabolic effect is why transcriptomic studies so far have failed to yield robust reproducible results. Reproducing the exact DEGs even in the same tissue type has proven to be difficult and thus the comparison between tissues of different origin is hampered, especially when taking the current meta-analysis into regard. However, there are signs that point towards changes in non-neuronal tissues in PD, indicating that PD might be a multisystem disorder.

Key points:

- RNA-Seq analysis from PD whole blood and skin yielded very different results, blood being a lot more heterogeneous. There were no exact overlapping DEGs or enriched KEGG pathways.
- Integrating previous studies from blood, cortex, and SN into robust ranked aggregation lists yields virtually no significantly changed DEGs and no overlap between tissues. Notable exceptions are IL18R1 from blood, LMO3 and RIMS3 from SN, PENK from cortex and G0S2, and C6 from cortex and skin.
- Since the DEGs show great interstudy variability, there are also only a few significantly enriched pathways and no direct overlap between tissues in PD.
- Peripheral tissues should be used in transcriptomic studies of PD, because of their accessibility *in vivo* and moderate comparability to PD specific changes in CNS.
- Of the two peripheral tissues we investigated, skin shows more PD specific changes. The epidemiological link between PD and melanoma indicates that there might be some pathomechanistic similarities in these diseases. This warrants more transcriptomic studies from PD skin.

AUTHORS' CONTRIBUTIONS

LK drafted the manuscript and was involved in analyzing and validation processes. SK analyzed the raw data and revised the manuscript. PT and AP revised the manuscript. LKE gathered the clinical data. FL contributed in the RRA and pathway analysis. ER contributed in the transcriptomic analysis. KMK ran the validating tests.

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.





ETHICAL APPROVAL

Ethical approval was received from Research Ethics Committee of the University of Tartu.

FUNDING

This work was supported by Estonian Research Council Personal research funding [PUT1239].

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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(Received June 17, 2020, Accepted September 17, 2020)