C18ORF54 is differentially expressed in the brains of patients with Parkinson's Disease.

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Parkinson's Disease (PD) is characterized by loss of dopaminergic neurons in the substantia nigra of the basal ganglia¹. We mined published and public microarray datasets^{2,3,4} to identify genes whose expression was most different in the substantia nigra of patients with PD as compared to that of non-affected patients. We identified significant changes in expression of open reading frame 18 on chromosome 54 (C18ORF54) in the substantia nigra of patients with PD.

Keyword: Parkinson's Disease, motor disorders, substantia nigra, dopaminergic neurons, C18ORF54.

1 Parkinson's Disease is a neurodegenerative disorder with no cure. Patients with
2 Parkinson's Disease suffer from motor symptoms including rigidity, tremors, bradykinesia
3 (slowness of movement), and postural instability¹. With disease progression, non-motor
4 neuropsychiatric problems can emerge and worsen, including difficulties with cognition,
5 problems with executive functioning, and problems with mood and behavior¹. 40% of patients
6 with PD develop depression⁵ and approximately 50% of patients with PD will develop psychotic
7 symptoms - hallucinations⁶. Thus, motor and non-motor symptoms are a serious problem in
8 patient with PD. While treatment with dopamine precursor levodopa can provide significant
9 relief, such treatment is marked by "on" and "off" periods of effectiveness and the risk of
10 development of dyskinesias^{7,8}. Newer dopamine agonists such as pramipexole and ropinirole are
11 of limited efficacy⁹ and associated with the risk of impulse control disorders¹⁰. The development
12 of targeted treatments for patients with Parkinson's Disease, to halt disease progression in the
13 substantia nigra and control motor and neuropsychiatric symptoms in PD patients is required.

14 We mined published microarray datasets to identify the most striking changes in gene
15 expression in the substantia nigra of patients with Parkinson's Disease. We identified
16 C18ORF54 as among the most differentially expressed genes in the brains of patients with PD.
17 C18ORF54 may be of relevance to the biology of PD.

24 **Methods**

25 We used datasets GSE42966², GSE43490³, and GSE49036⁴ for this differential gene
26 expression analysis of the substantia nigra in Parkinson's Disease in conjunction with GEO2R.
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GSE42966 was generated with Agilent-014850 Whole Human Genome Microarray 4x44K (G4112F) technology with $n=6$ for substantia nigra from control subjects and $n=9$ for substantia nigra from patients with Parkinson's Disease. GSE43490 was generated with Agilent-014850 Whole Human Genome Microarray 4x44K (G4112F) technology with $n=5$ for substantia nigra from control subjects and $n=8$ for substantia nigra from patients with Parkinson's Disease. GSE49036 was generated with Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=8$ for substantia nigra from control subjects and $n=15$ for substantia nigra from patients with Parkinson's Disease. The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether C18ORF54 expression was significantly different when comparing substantia nigra from control subjects and from patients with Parkinson's Disease using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published and public microarray datasets^{2,3,4} to identify differentially expressed genes in patients with Parkinson's Disease.

C18ORF54 is differentially expressed in the Parkinson's Disease substantia nigra

1 We identified open reading frame 54 on chromosome 18 C18ORF54 as among the genes
2 whose expression changed most significantly genome-wide when comparing the transcriptional
3 profiles of the substantial nigra from control, non-affected subjects and from patients with
4 Parkinson's Disease (PD)². When sorting each of the transcripts identified by microarray based
5 on degree of change in expression between control subjects and patients with PD, C18ORF54
6 ranked 85 out of 45015 total transcripts (Table 1). Differential expression of C18ORF54 in the
7 substantial nigra of patients with PD was statistically significant (Table 1; $p=0.008465$).
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10 We mined a second microarray dataset³ to determine if differential expression of
11 C18ORF54 in patients with PD could be observed. C18ORF54 was again among the genes most
12 differentially expressed when comparing transcriptional profiles of the substantia nigra between
13 patients with PD and control subjects (Table 2). When sorting each of the transcripts identified
14 by microarray based on degree of change in expression between control subjects and patients
15 with PD, C18ORF54 ranked 146 out of 22627 total transcripts (Table 2). Differential expression
16 of C18ORF54 in the substantial nigra of patients with PD in this second dataset was statistically
17 significant (Table 2; $p=0.0006536$).
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21 We mined a third microarray dataset⁴ to determine if differential expression of
22 C18ORF54 in patients with PD could be further observed. C18ORF54 was again among the
23 genes most differentially expressed when comparing transcriptional profiles of the substantia
24 nigra between patients with PD and control subjects (Table 3). When sorting each of the
25 transcripts identified by microarray based on degree of change in expression between control
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1 subjects and patients with PD, C18ORF54 ranked 119 out of 54675 total transcripts (Table 2).
2 Differential expression of C18ORF54 in the substantia nigra of patients with PD in this second
3 dataset was statistically significant (Table 3; $p=0.00011668$).
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6 C18ORF54 is expressed at significantly higher levels in patients with Parkinson's Disease.

7 We obtained exact mRNA expression levels for the differentially expressed C18ORF54
8 transcript, from control subjects and from patients with PD, to directly compare C18ORF54
9 expression levels. We also performed a statistical test to determine if this difference in
10 expression was statistically significant. C18ORF54 was expressed at higher levels in the
11 substantia nigra of patients with PD, and this difference was statistically significant (Figure 1;
12 $p=0.0056$). We calculated a mean fold change of 1.8993 ± 0.6725 in C18ORF54 expression
13 when comparing substantia nigra from patients with PD to that of control subjects (Table 1).
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17 In the second microarray dataset queried, we again observed increased expression of
18 C18ORF54 in the substantia nigra of patients with PD; this difference was statistically
19 significant (Figure 2; $p=0.0268$). We calculated a mean fold change of 1.5061 ± 0.1060 in
20 C18ORF54 expression when comparing substantia nigra from patients with PD to that of control
21 subjects (Table 2).
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24 In the third microarray dataset queried, we again observed increased expression of
25 C18ORF54 in the substantia nigra of patients with PD; this difference was statistically
26 significant (Figure 3; $p=0.0001$). We calculated a mean fold change of 1.1175 ± 0.0670 in
27 C18ORF54 expression when comparing substantia nigra from patients with PD to that of control
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1 subjects (Table 3).

2 Thus, we identified C18ORF54 as a differentially expressed gene in the substantia nigra
3
4 of patients with Parkinson's Disease and found that C18ORF54 was expressed at significantly
5 higher levels in the substantia nigra of patients with PD.
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7 **Discussion**

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9 We mined public and published microarray data to discover genes associated with
10 Parkinson's Disease in the tissues most classically associated with the disease, the substantia
11 nigra of the basal ganglia. We identified open reading frame 18 on chromosome 54 (C18ORF54)
12 as among the most differentially expressed genes in the substantia nigra of patients with
13 Parkinson's Disease.
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15 A comprehensive description of C18ORF54 is lacking, but several reports document
16 differential or perturbed expression of C18ORF54 in specific contexts. C18ORF54 is one of 701
17 genes associated with progression through the cell cycle from the G1/S boundary until M phase.
18 C18ORF54 co-localized with γ -tubulin and could be found at centrosomes¹¹. The mouse
19 homolog of C18ORF54, LAS2, was identified as a Pas1 modifying gene in a study identifying
20 single nucleotide polymorphisms associated with development of chemically-induced lung
21 tumors among 21 unique inbred strains; Pas1 had previously been identified as a lung adenoma
22 susceptibility loci using inbred mice¹². 40% of human lung adenocarcinomas surveyed
23 possessed deletion of LAS2, indicating that loss of LAS2 was a recurrent event in
24 human lung tumorigenesis¹². LAS2 control of cancer cell growth was assayed by expression of
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1 allelic variants of LAS2, LAS2-S and LAS2-R in the LM2 cell line derived from papillary
2 tumors induced by urethane in A/J mice¹². LAS2-R represents a Par2-resistant LAS2 allele;
3 Par2 had been associated with inhibitory properties in mice expressing the Pas1 lung cancer
4 susceptibility allele¹³. In nude mice xenografted with LM2 cells expressing LAS2-R, tumor size
5 was significantly decreased as compared to LM2-LAS2-S or LM2 control xenografts,
6 demonstrating LAS2 control of cell proliferation¹³. Depletion of LAS2 in LM2 cells over-
7 expression LAS2-R resulted in significant decreases in proliferation and colony formation *in*
8 *vitro*¹². Together, the data suggested that a LAS2 variant could confer significant risk of
9 development of lung adenocarcinoma through mechanisms involving control of cell
10 proliferation¹². C18ORF54 has been identified as one of 268 genes repressed by the p53-LIN37/
11 DREAM pathway¹³. In a study of multiple myelomas, C18ORF54 was found to be a p53-
12 associated genes in multiple myelomas cell lines and primary tumors; among other p53-
13 associated genes, C18ORF54 was classified by investigators as “unknown” with regard to
14 function¹⁴. A separate study of anti-myeloma properties of thalidomide found C18ORF54 was
15 down-regulated in the human multiple myeloma cell line OPM2, both after treatment with
16 lenalidomide and after depletion of cereblon, a known teratogenic target of thalidomide¹⁵. Thus,
17 C18ORF54 appears to influence cell cycle and cell proliferative capacity, is described as a
18 repressed transcriptional target of the p53 pathway, may be associated with the centrosome, and
19 its expression is perturbed in human cancers.

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There is limited information describing functions of C18ORF54 in the brain or
associated with disorders of the brain. Investigators searching for genetic contributions to

1 neuroticism based on association of genotype with subject survey responses identifying with
2 concepts characteristic of neuroticism (e.g., “worry/vulnerability” or “anxiety/tension”)
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4 described C18ORF54 as a genetic risk factor for neuroticism, significantly associating with the
5 “general neuroticism factor”; this study utilized genotyping data at 805,246 markers in 488,377
6 subjects. C18ORF54 was unique among genes identified as risk factors for the development of
7 the “general neuroticism factor” (along with STARD6) in that it displayed evidence of
8 interaction at the chromatin level with multiple, independent risk factors for neuroticism across
9 cell type¹⁶. In a separate study, C18ORF54 was found to be significantly differentially
10 expressed and up-regulated following cannabidiol treatment of mesenchymal stem cells from
11 human gingiva (hGMSC); cannabidiol treatment of hGMSC activated expression of genes
12 associated with differentiation of neural precursor cells¹⁷. We could identify one report
13 describing expression of C18ORF54 in dopaminergic neurons. Investigators studying
14 acrylamide-associated neurotoxicity treated rat N27 dopaminergic cells with acrylamide and
15 performed mass spectrometry to search for ACR-adducted proteins¹⁸. A peptide representing the
16 rat homolog of C18ORF54 with the sequence KcGDKIPELLILK was among 103 unique peptides
17 identified by shotgun proteomics as featuring a cysteine residue with an acrylamide adduct¹⁸.
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23 If validated in separate datasets generated with tissues from patients with Parkinson’s
24 Disease, the effect of C18ORF54 depletion and over-expression *in vivo* in the substantia nigra
25 can be evaluated through conditional deletion or expression in mice. C18ORF54 may be
26 relevant to the genesis, maintenance or progression of Parkinson’s Disease.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
85	34534	0.008465	2.968417	-4.48	1.8993 ± 0.6725	C18orf54	chromosome 18 open reading frame 54

Table 1: C18ORF54 is differentially expressed in the Parkinson's Disease substantia nigra.

The rank of differential expression, the probe/transcript ID, the *p*-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of C18ORF54 in PD substantia nigra when compared to control substantia nigra, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
146	A_32_P162374	0.0006536	3.949345	-0.28518	1.5061 ± 0.1060	C18orf54	chromosome 18 open reading frame 54

Table 2: C18ORF54 is differentially expressed in the Parkinson's Disease substantia nigra.

The rank of differential expression, the probe/transcript ID, the *p*-value with respect to global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, the fold change of C18ORF54 in PD substantia nigra when compared to control substantia nigra, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
119	229442_at	0.00011668	4.5719392	1.22255	1.1175 ± 0.0670	C18orf54	chromosome 18 open reading frame 54

Table 3: C18ORF54 is differentially expressed in the Parkinson's Disease substantia nigra.

The rank of differential expression, the probe/transcript ID, the *p*-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of C18ORF54 in PD substantia nigra when compared to control substantia nigra, the gene and gene name are listed in this chart.

Figure 1: C18ORF54 is expressed at significantly higher levels in the substantia nigra of patients with Parkinson's Disease.

The mRNA expression of C18ORF54 in normal substantia nigra from control subjects (left) and in the substantia nigra of patients with PD (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in C18ORF54 expression between the normal substantia nigra and substantia nigra from patients with PD, a *p*-value, listed above.

Figure 2: C18ORF54 is expressed at significantly higher levels in the substantia nigra of patients with Parkinson's Disease.

The mRNA expression of C18ORF54 in normal substantia nigra from control subjects (left) and in the substantia nigra of patients with PD (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in C18ORF54 expression between control substantia nigra and substantia nigra from patients with PD, a *p*-value, listed above.

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