ID2 is differentially expressed in the brains of patients with Parkinson's Disease.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

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^{2,3} datasets to identify genes whose expression was most different in the brains of patients with PD as compared to that of non-affected patients. We identified significant changes in expression of the gene encoding the transcription factor ID2 in the substantia nigra of patients with PD.

Keyword: Parkinson's Disease, motor disorders, substantia nigra, dopaminergic neurons, ID2.

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 depression⁴ and approximately 50% of patients with PD will develop psychotic
7 symptoms - hallucinations⁵. 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^{6,7}. Newer dopamine agonists such as pramipexole and ropinirole are
11 of limited efficacy⁸ and associated with the risk of impulse control disorders⁹. 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 the
16 transcription factor ID2 as among the most differentially expressed genes in the brains of patients
17 with PD. ID2 may be of relevance to the biology of PD.

18 **Methods**

19 We used datasets GSE43490 and GSE24378 for this differential gene expression analysis
20 of the substantia nigra in Parkinson's Disease in conjunction with GEO2R. GSE43490 was
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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. GSE24378 was generated with Affymetrix Human X3P Array technology with $n=9$ dopaminergic neurons from control subjects and $n=8$ for dopaminergic neurons 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 ID2 expression was significantly different when comparing substantia nigra or dopaminergic neurons 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} to identify differentially expressed genes in patients with Parkinson's Disease.

ID2 is differentially expressed in the Parkinson's Disease substantia nigra

We identified ID2 as among the genes whose expression changed most significantly genome-wide when comparing the transcriptional profiles of the substantia nigra from control subjects with that of patients with Parkinson's Disease (PD)². When sorting each of

the transcripts identified by microarray based on degree of change in expression between control subjects and patients with PD, ID2 ranked 195 out of 22627 total transcripts (Table 1). Differential expression of ID2 in the substantia nigra of patients with PD was statistically significant (Table 1; $p=0.0008524$).

We mined a second microarray dataset³ to determine if differential expression of ID2 in patients with PD could be observed in an independent dataset. ID2 was again among the genes most differentially expressed when comparing transcriptional profiles of controls and patients with PD, here in dopaminergic neurons (Table 2). When sorting each of the transcripts identified by microarray based on degree of change in expression between control subjects and patients with PD, ID2 ranked 214 out of 61359 total transcripts (Table 2). Differential expression of ID2 in dopaminergic neurons from patients with PD was statistically significant (Table 2; $p=4.14E-03$).

ID2 is expressed at significantly higher levels in patients with Parkinson's Disease.

We obtained exact mRNA expression levels for the differentially expressed ID2 transcript, from control subjects and from patients with PD, to directly compare ID2 expression levels. We also performed a statistical test to determine if this difference in expression was statistically significant. ID2 was expressed at higher levels in the substantia nigra of patients with PD, and this difference was statistically significant (Figure 1; $p=0.0005$). We calculated a mean fold change of 1.2154 ± 0.0713 in ID2 expression when comparing substantia nigra from patients with PD to that of control subjects (Table 1).

1 In dopaminergic neurons, we observed the opposite, with increased expression of ID2 in
2 patients with Parkinson's Disease. This difference was not statistically significant (Figure 2;
3 $p=0.0683$). We calculated a mean fold change of 0.3421 ± 0.4949 in ID2 expression when
4 comparing dopaminergic neurons from patients with PD to that of control subjects (Table 2).
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6 Thus, we identified ID2 as a differentially expressed gene in the substantia nigra of
7 patients with Parkinson's Disease and in isolated dopaminergic neurons from patients with PD
8 and found that ID2 was expressed at significantly higher levels in the substantia nigra, but at
9 lower levels in isolated dopaminergic neurons.
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12 **Discussion**

13 The inhibitor of DNA binding ID2 is a basic helix-loop-helix transcription factor-type
14 protein that can impair transactivation by functioning similar to a decoy, heterodimerizing with
15 competent transcription factors but lacking a DNA-binding domain to execute transcriptional
16 activation¹⁰. One important binding partner of Id2 is the retinoblastoma tumor suppressor Rb¹¹.
17 Id2 is required for the development of Langerhans dendritic cells¹² and important for
18 development of natural kill cells; mice deficient in Id2 do not possess lymph nodes or Peyer's
19 patches but have a functional spleen¹³.
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22 ID2 has critical functions in the nervous system, required for the development of
23 oligodendrocytes¹⁴ and for specification of the neural crest¹⁵. Earliest expression of Id2 can be
24 detected in the ventricular zone of the neuroepithelium and later in Purkinje cells of the
25 cerebellum, layers 2, 3 and 5 of the cerebral cortex and in mitral cells of the olfactory bulb¹⁶.
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1 In developing neuroblasts Id2 can dimerize with the E protein E47 to block
2 transactivation of the cyclin-dependent kinase inhibitor p57^{Kip2} by E47. Thus, Id2 can alter cell
3 cycle kinetics in neuroblastoma and in neuroblasts by repressing expression of a CDK
4 inhibitor¹⁷.

6 Increased expression of Id2 during brain development in mice results in microcephaly
7 and these mice are seizure-prone. In neural stem cells from Id2 transgenic mice, increased Id2
8 expression results in apoptosis in the early neuroepithelium¹⁸. While activation of Id2 in
9 intermediate progenitors has no effect, activation of Id2 in maturing neuroepithelium features
10 activation of p53 target gene expression¹⁸. Together the data suggested Id2 is a negative
11 regulator of neural stem cell renewal¹⁸.

14 Id2 is required for specification of dopaminergic neurons during adult olfactory
15 neurogenesis¹⁹. The olfactory bulbs of Id2-deficient mice have significantly decreased numbers
16 of dopaminergic periglomerular neurons; neuroblasts prematurely differentiate into the astroglial
17 lineage and the migratory stream is disorganized¹⁹.

20 The number of primary and secondary neurospheres is significantly increased upon
21 ectopic expression of Id2, indicating that Id2 supports the proliferation of neural stem cells from
22 the cortex²⁰. Addition of Id2 to NeuroD::E47 complexes *in vitro* abrogates E-box binding by
23 NeuroD::E47, indicating that inhibition of gene expression through Id2-bHLH dimerization
24 might contribute to Id2 restriction of neuronal differentiation²⁰.

27 The amino-terminus of Id2 possesses three phosphorylation sites; neural progenitor cells
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1 expressing mutant Id2 unable to be phosphorylated display significantly higher proliferation
2 rates, supporting a role for Id2 in promoting proliferation of NPCs, and of Id2 phosphorylation as
3 a mechanism to control this pro-proliferative capacity²¹. Protein phosphatase PP2A may be
4 responsible for dephosphorylation of Id2.
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6 In normal NPCs, ID2 is a direct transcriptional target of p53; depletion of ID2 can block
7 increased proliferation and self-renewal found in p53-deficient NPCs (22). Thus, ID2 functions
8 antagonistically with p53 in controlling proliferation of NPCs²². Together, the data reveal a
9 critical role for Id2 in influencing lineage commitment in the brain through control of
10 proliferation in neural progenitors; p53 appears to play a key role in restricting Id2 function, and
11 phosphorylation of Id2 may be another mechanism to regulate Id2 function.
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15 It is unclear why dopaminergic neurons from patients with PD express lower amounts of
16 ID2 while the bulk substantia nigra of patients with PD expresses significantly lower amounts of
17 ID2. In both cases, however, ID2 is among the genes whose expression changes most
18 significantly system-wide when compared to control subjects. If validated in separate datasets
19 generated with tissues from patients with Parkinson's Disease, the effect of ID2 depletion and
20 over-expression *in vivo* in the substantia nigra can be evaluated through conditional deletion or
21 expression in mice. ID2 may be relevant to the genesis, maintenance or progression of
22 Parkinson's Disease.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
195	A_32_P69368	0.0008524	3.841401	-0.52132	1.2154 ± 0.0713	ID2	inhibitor of DNA binding 2, HLH protein

Table 1: ID2 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 ID2 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
214	g464183_3p_at	4.14E-03	-3.19632873	-4.46	0.3421 ± 0.4949	ID2	inhibitor of DNA binding 2, HLH protein

Table 2: ID2 is differentially expressed in dopaminergic neurons from patients with Parkinson's Disease.

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 ID2 in PD dopaminergic neurons when compared to dopaminergic neurons from control subjects, the gene and gene name are listed in this chart.

Figure 1: ID2 is expressed at significantly higher levels in the substantia nigra of patients with Parkinson's Disease.

The mRNA expression of ID2 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 ID2 expression between the normal substantia nigra and substantia nigra from patients with PD, a *p*-value, listed above.

Figure 2: Dopaminergic neurons from patients with Parkinson's Disease express lower levels of ID2.

The mRNA expression of ID2 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 ID2 expression between dopaminergic neurons from control subjects and dopaminergic neurons from patients with PD, a *p*-value, listed above.