KMT5B 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 microarray datasets^{2,3,4} 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 histone lysine N-methyltransferase KMT5B in the substantia nigra of patients with PD.

Keyword: Parkinson's Disease, motor disorders, substantia nigra, dopaminergic neurons, KMT5B.

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^{2,3,4} to identify the most striking changes in gene
15 expression in the substantia nigra of patients with Parkinson's Disease. We identified KMT5B as
16 among the most differentially expressed genes in the brains of patients with PD. KMT5B may
17 be of relevance to the biology of PD.

18 **Methods**

19 We used datasets GSE43490², GSE49036³ and GSE24378⁴ for this differential gene
20 expression analysis of the Parkinson's Disease substantia nigra in conjunction with GEO2R.

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. 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 KMT5B 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). GEO2R only provides exact mRNA expression values for the top 250 most differentially expressed genes.

Results

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

KMT5B is differentially expressed in the Parkinson's Disease substantia nigra

1 We identified the gene encoding the histone lysine N-methyltransferase KMT5B as
2 among the genes whose expression changed most significantly genome-wide when comparing
3 the transcriptional profiles of the substantia nigra from control, non-affected subjects and from
4 patients with Parkinson's Disease (PD)². When sorting each of the transcripts identified by
5 microarray based on degree of change in expression between control subjects and patients with
6 PD, KMT5B ranked 115 out of 22627 total transcripts (Table 1). Differential expression of
7 KMT5B in the substantia nigra of patients with PD was statistically significant (Table 1;
8 $p=0.0004756$).

12 We mined a second microarray dataset³ to determine if differential expression of KMT5B
13 in patients with PD could be observed in the substantia nigra. KMT5B was again among the
14 genes most differentially expressed when comparing global transcriptional profiles of the
15 substantia nigra in patients with PD with that of control subjects (Table 2). When sorting each of
16 the transcripts identified by microarray based on degree of change in expression between control
17 subjects and patients with PD, KMT5B ranked 714 out of 54675 total transcripts (Table 2).
18 Differential expression of KMT5B in the substantia nigra of patients with PD in this second
19 dataset was statistically significant (Table 2; $p=0.00099483$).

23 We mined a third microarray dataset to examine whether changes in KMT5B expression
24 could be found in isolated dopaminergic neurons from patients with PD. Differential expression
25 of KMT5B could also be observed in dopaminergic neurons from patients with PD (Table 3).
26 When sorting each of the transcripts identified by microarray based on degree of change in
27 expression between dopaminergic neurons from control subjects and from patients with PD,
28

1 KMT5B ranked 459 out of 61359 total transcripts (Table 3). Differential expression of
2 KMT5B in dopaminergic neurons from patients with PD was statistically significant (Table 3;
3 $p=8.79E-03$).
4

6 KMT5B is expressed at significantly higher levels in patients with Parkinson's Disease.

7 We obtained exact mRNA expression levels for the differentially expressed KMT5B
8 transcript, from control subjects and from patients with PD, to directly compare KMT5B
9 expression levels. We also performed a statistical test to determine if this difference in
10 expression was statistically significant. KMT5B was expressed at higher levels in the substantia
11 nigra of patients with PD, and this difference was statistically significant (Figure 1; $p=0.0061$).
12 We calculated a mean fold change of 1.4664 ± 0.2284 in KMT5B expression when comparing
13 substantia nigra from patients with PD to that of control subjects (Table 1).
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17 Thus, we identified KMT5B as a differentially expressed gene in the substantia nigra of
18 patients with Parkinson's Disease and found that KMT5B was expressed at significantly higher
19 levels in the substantia nigra of patients with PD.
20

21 **Discussion**

22 We mined published microarray data^{2,3,4} to discover genes associated with Parkinson's
23 Disease in tissues classically associated with the disease, the substantia nigra of the
24 basal ganglia. We identified KMT5B as among the most differentially expressed genes in the
25 substantia nigra of patients with Parkinson's Disease.
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1 KMT5B, or SUV420H1 functions as a histone lysine methyltransferase at lysine 20 of
2 histone H4 (H4K20)¹¹. Mono-methylation of H4K20 by Pr-Set7/SET8¹² is required for
3 subsequent di- and tri-methylation of H4K20 to produce the H4K20me2 and H4K20me3 marks.
4 While H4K20me1 is associated with facultative heterochromatin, H4K20me3 is a feature of
5 constitutive heterochromatin¹³; methylation of H4K20 is generally thought to result in
6 transcriptional repression of the target gene. Mice deficient for both KMT5B and KMT5C
7 (SUV420H2) manifest perinatal lethality and lack virtually all H4K20me2 and H4K20me3¹⁴. B-
8 cells from KMT5B -/- KMT5C -/- mice are deficient in immunoglobulin gene class switching
9 due to defects in recombination¹⁴. In human fibroblasts, the H4K20me2 and H4K20me3 marks
10 accumulate during quiescence and is associated with chromatin compaction; levels of
11 H4K20me1 increase during S phase and during quiescence these marks become H4K20me2 and
12 H4K20me3¹⁵. Depletion of KMT5C in human fibroblasts results in significant increase in
13 percentage of cells in S phase, and over-expression of KMT5B results in cell cycle arrest in G2¹⁵.
14 In neural stem progenitor cells isolated from the subventricular zone of the adult baboon brain,
15 10,000 loci were marked by H4K20me3, translating to enrichment of H4K20me3 at 763 unique
16 genes¹⁶. KMT5B is implicated in diseases of the brain. In patient-derived tumor cells from
17 patients with diffuse intrinsic pontine glioma, deletion of KMT5B resulted in increases in
18 migration and invasion¹⁷. A sequencing study of candidate genes in neurodevelopmental
19 disorders revealed association of predicted gene-disruptive mutations in KMT5B with autism¹⁸.
20 A separate exome sequencing study of 4351 patients with neurodevelopmental disorders
21 uncovered mutations in KMT5B predicted to be pathogenic¹⁹.

1 In mice, deficiency of neuron-specific H4K20 demethylase *LSD1* results in deficits in
2 spatial learning and memory accompanied by gain of H4K20 methylation and increases in
3 transcriptional elongation and initiation²⁰, and in zebrafish, depletion of the H4K20 demethylase
4 PHF8 results in apoptosis in the developing brain and neural tube, suggesting association
5 between H4K20 methylation and neuronal cell survival²¹. Thus, KMT5B catalyzes generation of
6 H4K20me2 and H4K20me3 mark; this mark is generally associated with transcriptional
7 repression and can be found enriched at genomic sites in stem cells from the adult primate brain,
8 and KMT5B is implicated in multiple human diseases of the brain. Because of the involvement
9 of the H4K20me2 mark in the double-stranded DNA break (DSB) response²², models of KMT5B
10 loss-of-function have been proposed suggesting impaired differentiation or apoptosis of neural
11 stem cells following DSB events during proliferation, concomitant with improper activation of
12 regulators of neuronal differentiation²³.

17 It remains unclear what relation KMT5B over-expression in the substantia nigra of
18 patients with Parkinson's Disease has with cellular pathology found in patients with Parkinson's
19 Disease. It would be of interest to assess global H4K20me2 and H4K20me3 in isolated
20 dopaminergic neurons from patients with Parkinson's Disease and any subsequent changes in
21 expression of genes marked by methylation at H4K20. If validated in separate datasets
22 generated with tissues from patients with Parkinson's Disease, the effect of KMT5B depletion
23 and over-expression *in vivo* in the substantia nigra can be evaluated through conditional deletion
24 or expression, in wild-type mice and in mouse models of Parkinson's Disease²⁴⁻²⁷. KMT5B may
25 be 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
115	A_23_P326739	0.0004756	4.078204	-0.00245	1.4664 ± 0.2284	KMT5B	lysine methyltransferase 5B

Table 1: KMT5B 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 KMT5B 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	Gene	Gene name
714	218242_s_at	0.00099483	3.7345037	-0.67479	KMT5B	lysine methyltransferase 5B

Table 2: KMT5B 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 KMT5B 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	Gene	Gene name
459	g12803398_3p_at	8.79E-03	-2.87286762	-4.49	KMT5B	lysine methyltransferase 5B

Table 3: KMT5B 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 KMT5B in dopaminergic neurons from patients with PD when compared to dopaminergic neurons from control subjects, the gene and gene name are listed in this chart.

Figure 1: KMT5B is expressed at significantly higher levels in the substantia nigra of patients with Parkinson's Disease.

The mRNA expression of KMT5B 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 KMT5B expression between the normal substantia nigra and substantia nigra from patients with PD, a *p*-value, listed above.