KMT2A/MLL1 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 KMT2A (also known as MLL1) in the substantia nigra of patients with PD.

Keyword: Parkinson's Disease, motor disorders, substantia nigra, dopaminergic neurons, KMT2A.

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 KMT2A,
16 also known as MLL1 as among the most differentially expressed genes in the brains of patients
17 with PD. KMT2A may be of relevance to the biology of PD.

24 **Methods**

25 We used datasets GSE43490², GSE8397³ and GSE24378⁴ for this differential gene
26 expression analysis of the Parkinson's Disease substantia nigra in conjunction with GEO2R.
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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. GSE8397 was generated with Affymetrix Human Genome U133A Array technology with $n=11$ for substantia nigra from control subjects and $n=26$ 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 KMT2A 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.

KMT2A is differentially expressed in the Parkinson's Disease substantia nigra

1 We identified the histone lysine N-methyltransferase KMT2A as among the genes whose
2 expression changed most significantly genome-wide when comparing the transcriptional profiles
3 of the substantia nigra from control, non-affected subjects and from patients with Parkinson's
4 Disease (PD)². When sorting each of the transcripts identified by microarray based on degree of
5 change in expression between control subjects and patients with PD, KMT2A ranked 43 out of
6 22627 total transcripts (Table 1). Differential expression of KMT2A in the substantia nigra of
7 patients with PD was statistically significant (Table 1; $p=0.000142$).
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10 We mined a second microarray dataset³ to determine if differential expression of KMT2A
11 in patients with PD could be observed in the substantia nigra. KMT2A was again among the
12 genes most differentially expressed when comparing global transcriptional profiles of the
13 substantia nigra in patients with PD with that of control subjects (Table 2). When sorting each of
14 the transcripts identified by microarray based on degree of change in expression between control
15 subjects and patients with PD, KMT2A ranked 4278 out of 22283 total transcripts (Table 2);
16 thus, the change in KMT2A expression between controls and patients with PD, albeit less
17 significant than in the first dataset, was greater than 80.8% of all transcripts measured.
18 Differential expression of KMT2A in the substantia nigra of patients with PD in this second
19 dataset was statistically significant (Table 2; $p=1.92E-02$).
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24 We mined a third microarray dataset to examine whether changes in KMT2A expression
25 could be found in isolated dopaminergic neurons from patients with PD. Differential expression
26 of KMT2A could also be observed in dopaminergic neurons from patients with PD (Table 3).
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When sorting each of the transcripts identified by microarray based on degree of change in expression between dopaminergic neurons from control subjects and from patients with PD, KMT2A ranked 413 out of 61359 total transcripts (Table 3). Differential expression of KMT2A in dopaminergic neurons from patients with PD was statistically significant (Table 3; $p=7.67E-03$).

KMT2A is expressed at significantly higher levels in patients with Parkinson's Disease.

We obtained exact mRNA expression levels for the differentially expressed KMT2A transcript, from control subjects and from patients with PD, to directly compare KMT2A expression levels. We also performed a statistical test to determine if this difference in expression was statistically significant. KMT2A was expressed at higher levels in the substantia nigra of patients with PD, and this difference was statistically significant (Figure 1; $p=0.0001$). We calculated a mean fold change of 1.5261 ± 0.1297 in KMT2A expression when comparing substantia nigra from patients with PD to that of control subjects (Table 1).

Thus, we identified KMT2A as a differentially expressed gene in the substantia nigra of patients with Parkinson's Disease and found that KMT2A was expressed at significantly higher levels in the substantia nigra of patients with PD.

Discussion

We mined published microarray data^{2,3,4} to discover genes associated with Parkinson's Disease in the tissues most classically associated with the disease, the substantia nigra of the

1 basal ganglia. We identified KMT2A as among the most differentially expressed genes in the
2 substantia nigra of patients with Parkinson's Disease.

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4 Histone lysine N-methyltransferase KMT2A/MLL1 is one member of the MLL/
5 COMPASS family of SET domain-containing enzymes that catalyze di- and tri-methylation of
6 lysine 4 on Histone H3 (H3K4me2 and H3K4me3) in complexes containing RbBP5, WDR5,
7 Ash2L and Dpy30¹¹⁻¹³. H3K4me3 marks actively transcribed promoters and is enriched at
8 transcription start sites¹⁴⁻¹⁶. KMT2A can interact with histone acetyltransferase MOF1, and
9 purified KMT2A/MOF1 complexes can both methylate H3K4 and acetylate lysine 16 on Histone
10 H4¹⁷. Structural features of KMT2A include a plant homeodomain (PHD) finger motif, a Win
11 motif for WDR5 interaction, a transactivation domain, a bromodomain and three AT-hooks at its
12 amino-terminus that can bind DNA¹².

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17 KMT2A is critical for memory consolidation in the brain^{18,19}. Mice with conditional
18 deletion of KMT2A in excitatory forebrain neurons of the hippocampus manifest significant
19 defects in associative memory, measured by percent freezing in a fear-conditioning test, and in
20 spatial memory measured by escape latency in a water-maze test¹⁸. KMT2A-deficient mice,
21 unlike KMT2B-deficient mice, are not impaired in object recognition memory¹⁸. H3K4me3
22 marks are present in significantly lower quantities on the chromatin of hippocampal neurons
23 from KMT2A-deficient mice, and these cells express 4,964 genes at decreased levels, supporting
24 activation of gene expression in the brain by KMT2A deposition of H3K4me3 associated with
25 consolidation of associative and spatial memory¹⁸. Conditional deletion of KMT2A in the adult
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1 prefrontal cortex using Camk-Cre results in increased anxiety and impairments in cognition in
2 the absence of locomotor defects¹⁹. Medial prefrontal layer V pyramidal neurons from KMT2A-
3 deficient mice display significant defects in working memory-associated synaptic facilitation and
4 temporal summation, indicating KMT2A is important for synaptic plasticity in the prefrontal
5 cortex¹⁹. Globally, only 31 genes displayed significant decreases in H3K4me3 in cortical
6 neurons from KMT2A-deficient mice, including the cortical and striatal transcription factor
7 Meis2¹⁹. Neural stem cells (NSC) from the subventricular zone (SVZ) of KMT2A-deficient
8 mice fail to differentiate into neurons while glial differentiation remains intact; expression of a
9 central regulator of the neurogenic program, Dlx2, is lost in KMT2A-deficient SVZ cells and
10 ectopic expression of Dlx2 can restore neuronal differentiation capacity to these cells²⁰. In
11 KMT2A-deficient SVZ cells, chromatin at Dlx2 is marked by both H3K4me3 and H3K27me3
12 and these cells fail to express Dlx2, while Dlx2 is marked by high-levels of H3K4me3 in wild-
13 type SVZ cells, suggesting that resolution of bivalent chromatin in neural precursors by MLL2 is
14 important for neurogenesis by supporting activation of a neurogenic gene expression program²⁰.

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20 Importantly, mice deficient for KMT2A in the postnatal forebrain fail to properly respond
21 to stimulant drugs and dopaminergic agonists; mice with conditional deletion of KMT2A in the
22 central striatum manifest increased anxiety behaviors; genes regulated by KMT2A-mediated
23 transcriptional repression included serotonin receptor 5HTR2A²¹. Moreover, timing-dependent
24 long-term potentiation in medium spiny neurons from the striatum of KMT2A-deficient mice is
25 largely defective²¹. Thus, KMT2A has established roles in memory formation, catalyzes
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1 methylation of lysine 4 on Histone H3, and is important for expression of genes associated with
2 the differentiation and function of neurons in the mammalian brain. If validated in separate
3 datasets generated with tissues from patients with Parkinson's Disease, the effect of KMT2A
4 depletion and over-expression *in vivo* in the substantia nigra can be evaluated in wild-type mice
5 or in mouse models of Parkinson's Disease²²⁻²⁵. KMT2A may be relevant to the genesis,
6 maintenance or progression of Parkinson's Disease.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
43	A_24_P127812	0.000142	4.608046	1.07283	1.5261 ± 0.1297	KMT2A	lysine methyltransferase 2A

Table 1: KMT2A 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 KMT2A 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
4278	212080_at	1.92E-02	2.4496449	-3.531672	KMT2A	lysine methyltransferase 2A

Table 2: KMT2A 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 KMT2A 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
413	Hs2.406233.1.S1_3p_s_at	7.67E-03	2.93228244	-4.48	KMT2A	lysine methyltransferase 2A

Table 3: KMT2A 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 KMT2A 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: KMT2A is expressed at significantly higher levels in the substantia nigra of patients with Parkinson's Disease.

The mRNA expression of KMT2A 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 KMT2A expression between the normal substantia nigra and substantia nigra from patients with PD, a *p*-value, listed above.