1 **Autophagin-3 (ATG4C) is differentially expressed in the brains of patients with**
2 **Parkinson's Disease.**

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7 Parkinson's Disease (PD) is characterized by loss of dopaminergic neurons in the substantia
8 nigra of the basal ganglia¹. We mined published microarray datasets^{2,3} to identify genes whose
9 expression was most different in the substantia nigra of patients with PD as compared to that of
10 non-affected patients. We identified significant changes in expression of the gene encoding
11 autophagin-3 (ATG4C) in the substantia nigra of patients with PD.
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27 Keyword: Parkinson's Disease, motor disorders, substantia nigra, dopaminergic neurons,
28 ATG4C.

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 ATG4C as
16 among the most differentially expressed genes in the brains of patients with PD. ATG4C may be
17 of relevance to the biology of PD.

18 **Methods**

19 We used datasets GSE49036² and GSE43490³ for this differential gene expression
20 analysis of the substantia nigra in Parkinson's Disease in conjunction with GEO2R. GSE49036
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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. 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. 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 ATG4C 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 microarray datasets^{2,3} to identify differentially expressed genes in patients with Parkinson's Disease.

ATG4C is differentially expressed in the Parkinson's Disease substantia nigra

We identified ATG4C as among the genes whose expression changed most significantly genome-wide when comparing the transcriptional profiles of the substantia nigra from control, non-affected subjects and from 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, ATG4C ranked 64 out of 54675 total transcripts (Table 1). Differential expression of ATG4C in the substantia nigra of patients with PD was statistically significant (Table 1; $p=0.00005685$).

We mined a second microarray dataset³ to determine if differential expression of ATG4C in patients with PD could be observed. ATG4C was again among the genes most differentially expressed when comparing global transcriptional profiles of the substantia nigra in patients with PD with that of control subjects (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, ATG4C ranked 177 out of 22627 total transcripts (Table 2). Differential expression of ATG4C in the substantia nigra of patients with PD in this second dataset was statistically significant (Table 2; $p=0.0007613$).

ATG4C is expressed at significantly higher levels in patients with Parkinson's Disease.

We obtained exact mRNA expression levels for the differentially expressed ATG4C transcript, from control subjects and from patients with PD, to directly compare ATG4C expression levels. We also performed a statistical test to determine if this difference in expression was statistically significant. ATG4C 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 1.1017 ± 0.0556 in ATG4C expression when comparing substantia nigra from patients with PD to that of control subjects (Table 1).

1 In the second microarray dataset queried, we again observed increased expression of
2 ATG4C in the substantia nigra of patients with PD; this difference was statistically significant
3 (Figure 2; $p=0.0039$). We calculated a mean fold change of 1.3194 ± 0.1154 in ATG4C
4 expression when comparing substantia nigra from patients with PD to that of control subjects
5 (Table 2).
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8 Thus, we identified ATG4C as a differentially expressed gene in the substantia nigra of
9 patients with Parkinson's Disease and found that ATG4C was expressed at significantly higher
10 levels in the PD substantia nigra.
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12 **Discussion**

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14 We mined published microarray data^{2,3} to discover genes associated with Parkinson's
15 Disease in the tissues most classically associated with the disease, the substantia nigra of the
16 basal ganglia. We identified the gene encoding autophagin-3, ATG3, as among the most
17 differentially expressed genes in the substantia nigra of patients with Parkinson's Disease.
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20 ATG4 members belong to the C54 peptidase family of cysteine proteases important for
21 cleavage of ATG8 and subsequent targeting to phosphatidylethanolamine (PE) in autophagosome
22 membranes during mammalian autophagy^{10,11}. ATG4 cleavage of ATG8 at a carboxy-terminal
23 arginine residue exposes a conserved glycine which is conjugated to PE in a process involving
24 the E1-like activating enzyme ATG7 and the E2-like conjugating enzyme ATG3¹⁰. ATG4
25 members in humans include ATG4A, ATG4B, ATG4C and ATG4D; ATG4B is thought to be the
26 major processor of ATG8, with weak cleavage capacity of ATG4C against ATG8 homologues
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1 LC3-B and GATE-16 observable *in vitro*¹¹. Recombinant caspase-8, caspase-3 and calpain can
2 each cleave ATG4C *in vitro*, and cleavage of ATG4C can be observed in HeLa cells during
3 apoptosis induced by either staurosporine or the tumor necrosis factor-related apoptosis-inducing
4 ligand (TRAIL)¹². The amino acid sequences immediately following the amino-terminal DEVD
5 caspase cleavage consensus site of both ATG4C and ATG4D represent a latent mitochondrial
6 targeting signal, and caspase-cleaved ATG4D localizes to the mitochondrial matrix¹³ ; this is the
7 same caspase cleavage site where ATG4C is cleaved during apoptosis¹². Other cellular contexts
8 in which involvement of ATG4C has been observed is in mouse thymocytes, where treatment
9 with CPS, an agonist for the non-selective cation channel TRPV1 resulted in induction of
10 autophagy, increased expression of ATG4C mRNA and oxidation of ATG4C dependent on
11 reactive oxygen species (ROS)¹⁴. ATG4C has been reported as a target of microRNA miR-376b;
12 expression of miR-376b results in impairment of autophagy induced by either rapamycin or by
13 starvation¹⁵.

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19 Roles for ATG4C in cancer have been described including in the MCF-7 breast cancer
20 cell line where depletion of ATG4C resulted in impairments in mammosphere formation *in vitro*,
21 mirroring the effect of depletion of the ATM kinase, suggesting interaction of the DNA repair
22 and autophagy pathways¹⁶; ATM expression correlated with ATG4C expression in every human
23 breast cancer subtype examined. In glioma, expression of ATG4C associated with overall
24 survival of the patient and median relapse-free survival in patients with low ATG4C was
25 significantly higher than that of patients with high ATG4C expression¹⁷. In epithelial ovarian
26 cancer, overall survival of patients with low ATG4C tumor expression was significantly longer
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1 than that of patients with high tumor expression of ATG4C, and the expression of ATG4C
2 correlated with that of caveolin-1¹⁸. Other human diseases in which ATG4C has been described
3 as important include Kashin-Beck Disease, a form of osteoarthritis characterized by multi-joint
4 deformities and retardation of skeletal growth¹⁹; SNPs in ATG4C significantly associate with risk
5 of developing KBD and chondrocytes from patients with KBD express significantly lower levels
6 of ATG4C than in controls¹⁹. Thus, expression of ATG4C is implicated in a number of human
7 diseases.
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11 While we could not find any literature specifically implicating ATG4C in the biology of
12 Parkinson's Disease, autophagosomal clearance of the mitochondria, mitophagy, appears to be a
13 relevant cellular process to the pathogenesis of Parkinson's Disease - mutations in the mitophagy
14 gene Parkin cause hereditary recessive early-onset Parkinson's Disease²⁰⁻²². If validated in
15 separate transcriptome analyses of substantia nigra from patients with Parkinson's Disease, the
16 effect of ATG4C depletion and over-expression *in vivo* in the substantia nigra can be evaluated
17 through conditional deletion or expression in wild-type mice or in mouse models of Parkinson's
18 Disease²³⁻²⁶. ATG4C may be relevant to the genesis, maintenance or progression of Parkinson's
19 Disease.
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References

1. Dauer, W. and Przedborski, S., 2003. Parkinson's disease: mechanisms and models. *Neuron*, 39(6), pp.889-909.
2. Dijkstra, A.A., Ingrassia, A., de Menezes, R.X., van Kesteren, R.E., Rozemuller, A.J., Heutink, P. and van de Berg, W.D., 2015. Evidence for immune response, axonal dysfunction and reduced endocytosis in the substantia nigra in early stage Parkinson's disease. *PloS one*, 10(6), p.e0128651.
3. Corradini, B.R., Iamashita, P., Tampellini, E., Farfel, J.M., Grinberg, L.T. and Moreira-Filho, C.A., 2014. Complex network-driven view of genomic mechanisms underlying Parkinson's disease: analyses in dorsal motor vagal nucleus, locus coeruleus, and substantia nigra. *BioMed research international*, 2014.
4. Cummings, J.L., 1992. Depression and Parkinson's disease: a review. *The American journal of psychiatry*.
5. Fénelon, G. and Alves, G., 2010. Epidemiology of psychosis in Parkinson's disease. *Journal of the neurological sciences*, 289(1-2), pp.12-17.
6. Parkinson Study Group, 2004. Levodopa and the progression of Parkinson's disease. *New England Journal of Medicine*, 351(24), pp.2498-2508.
7. Lees, A.J., 1989. The on-off phenomenon. *Journal of Neurology, Neurosurgery & Psychiatry*, 52(Suppl), pp.29-37.
8. Stowe, R., Ives, N., Clarke, C.E., Ferreira, J., Hawker, R.J., Shah, L., Wheatley, K. and Gray, R., 2008. Dopamine agonist therapy in early Parkinson's disease. *Cochrane Database of Systematic Reviews*, (2).
9. Weintraub, D., Siderowf, A.D., Potenza, M.N., Goveas, J., Morales, K.H., Duda, J.E., Moberg, P.J. and Stern, M.B., 2006. Association of dopamine agonist use with impulse control disorders in Parkinson disease. *Archives of neurology*, 63(7), pp.969-973.
10. Ichimura, Y., Kirisako, T., Takao, T., Satomi, Y., Shimonishi, Y., Ishihara, N., Mizushima, N., Tanida, I., Kominami, E., Ohsumi, M. and Noda, T., 2000. A ubiquitin-like system mediates protein lipidation. *Nature*, 408(6811), pp.488-492.
11. Li, M., Hou, Y., Wang, J., Chen, X., Shao, Z.M. and Yin, X.M., 2011. Kinetics comparisons of mammalian Atg4 homologues indicate selective preferences toward diverse Atg8 substrates. *Journal of Biological Chemistry*, 286(9), pp.7327-7338.

12. Norman, J.M., Cohen, G.M. and Bampton, E.T., 2010. The in vitro cleavage of the hAtg proteins by cell death proteases. *Autophagy*, 6(8), pp.1042-1056.
13. Betin, V.M., MacVicar, T.D., Parsons, S.F., Anstee, D.J. and Lane, J.D., 2012. A cryptic mitochondrial targeting motif in Atg4D links caspase cleavage with mitochondrial import and oxidative stress. *Autophagy*, 8(4), pp.664-676.
14. Farfariello, V., Amantini, C. and Santoni, G., 2012. Transient receptor potential vanilloid 1 activation induces autophagy in thymocytes through ROS-regulated AMPK and Atg4C pathways. *Journal of leukocyte biology*, 92(3), pp.421-431.
15. Korkmaz, G., Le Sage, C., Tekirdag, K.A., Agami, R. and Gozuacik, D., 2012. miR-376b controls starvation and mTOR inhibition-related autophagy by targeting ATG4C and BECN1. *Autophagy*, 8(2), pp.165-176.
16. Antonelli, M., Strappazzon, F., Arisi, I., Brandi, R., D'Onofrio, M., Sambucci, M., Manic, G., Vitale, I., Barilà, D. and Stagni, V., 2017. ATM kinase sustains breast cancer stem-like cells by promoting ATG4C expression and autophagy. *Oncotarget*, 8(13), p.21692.
17. Wen, Z.P., Zeng, W.J., Chen, Y.H., Li, H., Wang, J.Y., Cheng, Q., Yu, J., Zhou, H.H., Liu, Z.Z., Xiao, J. and Chen, X.P., 2019. Knockdown ATG4C inhibits gliomas progression and promotes temozolomide chemosensitivity by suppressing autophagic flux. *Journal of Experimental & Clinical Cancer Research*, 38(1), p.298.
18. Zeng, Y., Chen, M., Ganesh, S., Hu, S. and Chen, H., 2020. Clinicopathological and prognostic significance of caveolin-1 and ATG4C expression in the epithelial ovarian cancer. *PloS one*, 15(5), p.e0232235.
19. Wu, C., Wen, Y., Guo, X., Yang, T., Shen, H., Chen, X., Tian, Q., Tan, L., Deng, H.W. and Zhang, F., 2017. Genetic association, mRNA and protein expression analysis identify ATG4C as a susceptibility gene for Kashin–Beck disease. *Osteoarthritis and Cartilage*, 25(2), pp. 281-286.
20. Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. and Shimizu, N., 1998. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *nature*, 392(6676), pp.605-608.
21. Lücking, C.B., Dürr, A., Bonifati, V., Vaughan, J., De Michele, G., Gasser, T., Harhangi, B.S., Meo, G., Denèfle, P., Wood, N.W. and Agid, Y., 2000. Association between early-onset Parkinson's disease and mutations in the parkin gene. *New England Journal of Medicine*, 342(21), pp.1560-1567.

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- 2 22. Geisler, S., Holmström, K.M., Skujat, D., Fiesel, F.C., Rothfuss, O.C., Kahle, P.J. and
- 3 Springer, W., 2010. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/
- 4 SQSTM1. *Nature cell biology*, 12(2), pp.119-131.
- 5 23. Fredriksson, A. and Archer, T., 1994. MPTP-induced behavioural and biochemical deficits: a
- 6 parametric analysis. *Journal of neural transmission-Parkinson's disease and dementia*
- 7 *section*, 7(2), pp.123-132.
- 8 24. Yavich, L., Oksman, M., Tanila, H., Kerokoski, P., Hiltunen, M., van Groen, T., Puoliväli, J.,
- 9 Männistö, P.T., García-Horsman, A., MacDonald, E. and Beyreuther, K., 2005. Locomotor
- 10 activity and evoked dopamine release are reduced in mice overexpressing A30P-mutated
- 11 human α -synuclein. *Neurobiology of disease*, 20(2), pp.303-313.
- 12 25. Li, Y., Liu, W., Oo, T.F., Wang, L., Tang, Y., Jackson-Lewis, V., Zhou, C., Geghman, K.,
- 13 Bogdanov, M., Przedborski, S. and Beal, M.F., 2009. Mutant LRRK2 R1441G BAC
- 14 transgenic mice recapitulate cardinal features of Parkinson's disease. *Nature neuroscience*,
- 15 12(7), pp.826-828.
- 16 26. Brooks, S.P. and Dunnett, S.B., 2009. Tests to assess motor phenotype in mice: a user's guide.
- 17 *Nature Reviews Neuroscience*, 10(7), pp.519-529.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
64	228190_at	0.00005685	4.8512669	1.85782	1.1017 ± 0.0556	ATG4C	autophagy related 4C cysteine peptidase

Table 1: ATG4C 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 ATG4C 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
177	A_24_P182539	0.0007613	3.887375	-0.42084	1.3194 ± 0.1154	ATG4C	autophagy related 4C cysteine peptidase

Table 2: ATG4C 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 ATG4C in PD substantia nigra when compared to control substantia nigra, the gene and gene name are listed in this chart.

Figure 1: ATG4C is expressed at significantly higher levels in the substantia nigra of patients with Parkinson's Disease.

The mRNA expression of ATG4C 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 ATG4C expression between the normal substantia nigra and substantia nigra from patients with PD, a *p*-value, listed above.

Figure 2: ATG4C is expressed at significantly higher levels in the substantia nigra of patients with Parkinson's Disease.

The mRNA expression of ATG4C 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 ATG4C expression between control substantia nigra and substantia nigra from patients with PD, a *p*-value, listed above.