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The effect of *LRRK2* mutations on the cholinergic system in manifest and premanifest stages of Parkinson's disease: a cross-sectional PET study

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Summary

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See Online for appendix

Contributors

VS and AJS had the idea for and designed the study. DJW, JV, ES, SM, NV, JM, NN, JZ, CPZ, JOA, ZKW, MJA, and TJR contributed to data acquisition. S-YL, JFF, ES, SM, RM, AP-S, JEA, MC, PC, MJM, and MS contributed to data analysis. S-YL, VS, and AJS contributed to design and writing of the report. All authors read and approved the final version for publication.

Declaration of interests

S-YL reports a scholarship from Capital Medical University during the conduct of the study. CPZ reports grants from the National Institutes of Health (NIH; National Institute of Neurological Disorders and Stroke [NINDS]) and salary support from the Department of Veterans Affairs during the conduct of the study. The Mayo Clinic, JOA, and ZKW have a financial interest in technologies related to the *PARK8* locus in familial Parkinson's disease and the *LRRK2* mutation 6055G→A (Gly2019Ser) in autosomal-dominant parkinsonism, which have been licensed to Athena Diagnostics; to date, JOA and ZKW have received royalties of less than US\$1500 through the Mayo Clinic in accordance with its royalty-sharing policies. AJS reports grants from the Michael J Fox Foundation during the conduct of the study; personal fees from AbbVie, Kyowa, Roche/Genentech, and Pfizer outside the submitted work; and grants from NTCCell, Cundill Foundation, Pacific Parkinson's Research Institute, and Weston Brain Institute outside the submitted work. All other authors declare no competing interests.

Background—Markers of neuroinflammation are increased in some patients with *LRRK2* Parkinson's disease compared with individuals with idiopathic Parkinson's disease, suggesting possible differences in disease pathogenesis. Previous PET studies have suggested amplified dopamine turnover and preserved serotonergic innervation in *LRRK2* mutation carriers. We postulated that patients with *LRRK2* mutations might show abnormalities of central cholinergic activity, even before the diagnosis of Parkinson's disease.

Methods—Between June, 2009, and December, 2015, we recruited participants from four movement disorder clinics in Canada, Norway, and the USA. Patients with Parkinson's disease were diagnosed by movement disorder neurologists on the basis of the UK Parkinson's Disease Society Brain Bank criteria. *LRRK2* carrier status was confirmed by bidirectional Sanger sequencing. We used the PET tracer *N*-¹¹C-methyl-piperidin-4-yl propionate to scan for acetylcholinesterase activity. The primary outcome measure was rate of acetylcholinesterase hydrolysis, calculated using the striatal input method. We compared acetylcholinesterase hydrolysis rates between groups using ANCOVA, with adjustment for age based on the results of linear regression analysis.

Findings—We recruited 14 patients with *LRRK2* Parkinson's disease, 16 *LRRK2* mutation carriers without Parkinson's disease, eight patients with idiopathic Parkinson's disease, and 11 healthy controls. We noted significant between-group differences in rates of acetylcholinesterase hydrolysis in cortical regions (average cortex $p=0.009$, default mode network-related regions $p=0.006$, limbic network-related regions $p=0.020$) and the thalamus ($p=0.008$). *LRRK2* mutation carriers without Parkinson's disease had increased acetylcholinesterase hydrolysis rates compared with healthy controls in the cortex (average cortex, $p=0.046$). Patients with *LRRK2* Parkinson's disease had significantly higher acetylcholinesterase activity in some cortical regions (average cortex $p=0.043$, default mode network-related regions $p=0.021$) and the thalamus (thalamus $p=0.004$) compared with individuals with idiopathic disease. Acetylcholinesterase hydrolysis rates in healthy controls were correlated inversely with age.

Interpretation—*LRRK2* mutations are associated with significantly increased cholinergic activity in the brain in mutation carriers without Parkinson's disease compared with healthy controls and in *LRRK2* mutation carriers with Parkinson's disease compared with individuals with idiopathic disease. Changes in cholinergic activity might represent early and sustained attempts to compensate for *LRRK2*-related dysfunction, or alteration of acetylcholinesterase in non-neuronal cells.

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Introduction

Parkinson's disease is a heterogeneous neurological disorder with variable signs and pathological features. The characteristics of genetic parkinsonism have long been a focus of interest.¹ Autosomal-dominant inherited Parkinson's disease caused by *LRRK2* mutations is one of the commonest forms of genetic parkinsonism. The prevalence of *LRRK2* Gly2019Ser mutations is roughly 4% in familial Parkinson's disease and 1% in sporadic Parkinson's disease worldwide,² and in specific populations (eg, Ashkenazi Jews) the frequency can be as high as 28%.^{2,3} The phenotype of *LRRK2* Parkinson's disease is

generally indistinguishable from that of idiopathic Parkinson's disease in routine clinical practice, but slower progression of motor dysfunction has been reported.² The non-motor features of *LRRK2* Parkinson's disease include better olfactory performance, less cognitive decline, and lower prevalence of rapid-eye-movement (REM) sleep behaviour disorder compared with idiopathic Parkinson's disease.^{2,4-7} These differences are even more striking in the prodromal stage.

Lewy body pathology is the most common finding in the brains of people with *LRRK2* Parkinson's disease. However, an absence of Lewy pathology was reported in 35% of individuals carrying the *LRRK2* Gly2019Ser mutation and in more than 50% of people with other *LRRK2* mutations.⁸ Pleomorphic pathology can be detected within families with the same mutation,⁹ and the frequency of non-motor manifestations differs between *LRRK2* carriers with or without Lewy bodies.⁸ Previous studies suggest a role for *LRRK2* in the regulation of inflammation, and increased amounts of neuroinflammation markers in serum have been noted in people with the *LRRK2* Gly2019Ser mutation with or without Parkinson's disease, compared with healthy controls and patients with idiopathic Parkinson's disease.^{10,11} Taken together, these observations imply possible differences in disease pathogenesis from idiopathic Parkinson's disease in people with *LRRK2* mutations.

The general pattern of presynaptic dopaminergic degeneration in *LRRK2* Parkinson's disease is similar to that reported in idiopathic disease, showing a rostrocaudal gradient and asymmetric severity.^{7,12,13} Compensatory mechanisms are incompletely understood. In *LRRK2* Parkinson's disease, very early decreases in dopamine transporter binding are noted, as well as increased dopamine turnover.¹²⁻¹⁵ People carrying *LRRK2* mutations but without Parkinson's disease have been shown to have amplified serotonin transporter binding in multiple regions of the brain,¹⁶ as assessed by PET with the radiotracer ¹¹C-3-amino-4-(2-dimethylaminomethylphenylsulfaryl)-benzonitrile (¹¹C-DASB).

Besides monoamine neurotransmitters, brain cholinergic function is reduced in idiopathic Parkinson's disease, which has been linked to the presence of REM sleep behaviour disorder, hyposmia, cognitive decline, and freezing of gait.¹⁷⁻¹⁹ However, acetylcholinesterase activity has not been investigated to date in *LRRK2* mutation carriers with or without Parkinson's disease. Therefore, we aimed to compare the activity of acetylcholinesterase in people with *LRRK2* mutations with and without Parkinson's disease with that in patients with idiopathic Parkinson's disease and healthy controls, using PET.

Methods

Participants

We did a cross-sectional PET study at the University of British Columbia, Vancouver, BC, Canada. We included individuals carrying an *LRRK2* mutation with and without Parkinson's disease, patients with idiopathic Parkinson's disease, and healthy controls. The age range for recruitment was 19–85 years, and we attempted to match groups by age. The exception was healthy female controls, who we restricted to age older than 30 years. *LRRK2* mutation carriers were from nine well-documented families¹⁶ with a history of autosomal-dominant Parkinson's disease, and they were referred to our study from four movement disorder

clinics in Canada, Norway, and the USA. We recruited patients with idiopathic Parkinson's disease from Vancouver, and we advertised in a local newspaper and by poster in Vancouver for healthy controls matched by age using stringent criteria. We excluded patients with dementia, neuropsychiatric disease, or who were receiving cholinergic drugs. The study was approved by the Clinical Research Ethics Board of the University of British Columbia. All participants provided written informed consent.

Procedures

We confirmed *LRRK2* carrier status by bidirectional Sanger sequencing. Patients with Parkinson's disease were diagnosed by movement disorder specialists on the basis of the UK Parkinson's Disease Society Brain Bank criteria.²⁰ We did neurological tests in patients with Parkinson's disease in the OFF-state (ie, off medication for at least 12 h) and in participants with *LRRK2* mutations without Parkinson's disease, and these tests included Hoehn and Yahr stage, Unified Parkinson's Disease Rating Scale (UPDRS) or Movement Disorder Society (MDS)-UPDRS III, Montreal Cognitive Assessment (MoCA), and the Beck Depression Inventory (BDI).

We did PET scans in three-dimensional (3D) mode on a GE Advance Nx1 tomograph (General Electric Medical Systems, Milwaukee, WI, USA) with a spatial resolution of approximately 6 mm³. We obtained 16 frames over 80 min after intravenous administration of a bolus of mean 331.8 MBq (SD 28.1) of the radiotracer *N*-¹¹C-methylpiperidin-4-yl propionate (¹¹C-PMP)—three frames for 40 s, three for 60 s, two for 150 s, two for 300 s, and six for 600 s. We spatially coregistered and realigned PET frames for every participant with rigid-body transformation to reduce motion artifacts, then we normalised the frames to a common ¹¹C-PMP template image derived from a group of healthy controls. A standardised set of regions of interest was placed automatically on the head of the caudate, putamen, orbitofrontal cortex, medial prefrontal cortex, temporal pole, medial temporal lobe, middle temporal gyrus, lateral temporal gyrus, anterior cingulate cortex, posterior cingulate cortex, precuneus, angular gyrus, superior parietal gyrus, supramarginal gyrus, occipital cortex, and thalamus bilaterally, using the software package MEDx version 3.43 (Sensor Systems, Sterling, VA, USA). We estimated rates of acetylcholinesterase hydrolysis using reference tissue-based linear least squares analysis in MATLAB version R2016A (Mathworks, Natick, MA, USA), with striatum (average activity of caudate and putamen) as the reference input tissue.²¹

Statistical analysis

We compared age and MoCA scores using ANOVA and post-hoc analyses. We assessed sex as a binomial variable with Fisher's exact test. We ascertained differences in disease duration between patients with idiopathic Parkinson's disease and those with *LRRK2* Parkinson's disease by the independent two-tailed *t* test. We compared Hoehn and Yahr stage as a categorical variable using the Mann-Whitney test. For MDS-UPDRS III and the BDI, the homogeneity of variances is not normal, thus we compared these assessments using the Kruskal-Wallis or Mann-Whitney test.

To balance the number of regions investigated and the power of statistical comparisons with a relatively small number of participants, we investigated differences in rate of acetylcholinesterase hydrolysis among widely identified functional networks essential to cognitive processes. We calculated the rate of acetylcholinesterase hydrolysis in cortex by averaging the rate of acetylcholinesterase hydrolysis over all cortical regions of interest. We defined the rate of acetylcholinesterase hydrolysis in default mode network regions as the average of the hydrolysis rate estimated for the precuneus, angular gyrus, supramarginal gyrus, medial prefrontal cortex, posterior cingulate gyrus, medial temporal lobe, and lateral temporal gyrus.²² We defined the rate of acetylcholinesterase hydrolysis in limbic network regions as the average of temporal pole, orbitofrontal cortex, anterior cingulate gyrus, posterior cingulate gyrus, and medial temporal lobe.²³ We compared rates of acetylcholinesterase hydrolysis between groups using ANOVA and ANCOVA to adjust for the effect of age. We also did ANCOVA to adjust for both age and sex, to further assess the effect of *LRRK2* mutations on acetylcholinesterase hydrolysis rates. We did pairwise post-hoc analyses using Fisher's least significant difference analysis. We did linear regression analyses of acetylcholinesterase hydrolysis rates on age in healthy controls and in the full participant dataset.

We judged p values less than 0.05 significant. We corrected multiple comparisons by controlling the false discovery rate within each region or network using the Benjamini-Hochberg procedure. We did statistical analyses using SPSS for Windows, version 19.0 (SPSS, Chicago, IL, USA) and MATLAB.

Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between June, 2009, and December, 2015, 30 people carrying *LRRK2* mutations were identified, 14 with Parkinson's disease and 16 without the disease. Of these 30 people, 17 had the Gly2019Ser mutation (eight with Parkinson's disease), seven had the Arg1441Cys mutation (four with Parkinson's disease), five had the Asn1437His mutation (two with Parkinson's disease), and one person without Parkinson's disease had the Arg1441His mutation. Between April, 2014, and April, 2016, eight patients with idiopathic Parkinson's disease were identified, and 11 healthy controls were recruited by advertising.

No differences in demographics were noted between patients with *LRRK2* Parkinson's disease and those with idiopathic disease, whereas the people carrying an *LRRK2* mutation without Parkinson's disease were significantly younger than the other study groups (table 1). The severity of motor deficit indicated by Hoehn and Yahr stage, disease duration, and MDS-UPDRS III did not differ between patients with *LRRK2* Parkinson's disease and idiopathic disease. Only one participant had a MoCA score lower than 24, and this person did not have Parkinson's disease but was a *LRRK2* mutation carrier (score of 22). No between-group differences were noted in MoCA scores (table 1); however, BDI scores

varied significantly between groups ($p=0.006$), and post-hoc analyses showed higher scores in patients with *LRRK2* Parkinson's disease than in those with idiopathic disease ($p=0.006$).

Significant negative correlations between age and mean rate of acetylcholinesterase hydrolysis were noted in cortical regions of all participants (table 2; appendix) and in healthy controls only (table 2; figure 1). ANCOVAs with age as a covariate were done to control for this potentially confounding effect. Rates of acetylcholinesterase hydrolysis differed significantly between patients with *LRRK2* Parkinson's disease, those with idiopathic Parkinson's disease, people carrying an *LRRK2* mutation without Parkinson's disease, and healthy controls in the average cortex ($p=0.009$), default mode network-related regions ($p=0.006$), limbic network-related regions ($p=0.020$), and the thalamus ($p=0.008$; table 3, figure 2). In the premanifest stage, *LRRK2* mutation carriers without Parkinson's disease showed increased rates of acetylcholinesterase hydrolysis in average cortex compared with healthy controls ($p=0.046$; table 3, figure 2). In patients with *LRRK2* Parkinson's disease, rates of acetylcholinesterase hydrolysis in the average cortex, default mode network-related regions, and limbic network-related regions were raised marginally compared with healthy controls but were not significant after correction for multiple comparisons (table 3). Significantly higher rates of acetylcholinesterase hydrolysis were detected in patients with *LRRK2* Parkinson's disease compared with individuals with idiopathic Parkinson's disease in average cortex ($p=0.043$), default mode network-related regions ($p=0.021$), and the thalamus ($p=0.004$; table 3). Acetylcholinesterase activity noted in one patient with *LRRK2* Parkinson's disease and a BDI score of 21 was comparable with that of other individuals in this study group.

We also did ANCOVA with both age and sex as covariates, although the effect of sex was not significant in healthy controls ($p>0.05$ for all regions). However, inclusion of an additional covariate resulted in loss of power such that only the comparison between patients with *LRRK2* Parkinson's disease and those with idiopathic Parkinson's disease in the thalamus was significant after correction for multiple comparisons ($p=0.008$; appendix).

Discussion

Our findings show that acetylcholinesterase activity is highest in *LRRK2* mutation carriers with and without Parkinson's disease compared with healthy controls, and activity of this enzyme is lowest in patients with idiopathic Parkinson's disease. Activity of acetylcholinesterase differed significantly between patients with *LRRK2* Parkinson's disease and those with idiopathic disease.

Our results accord with the clinical characteristics of *LRRK2* Parkinson's disease compared with those of idiopathic Parkinson's disease. Findings of a PET study using ^{11}C -PMP as a radiotracer showed that the clinical markers with the highest accuracy in identifying cholinergic disruption were presence of REM sleep behaviour disorder and fall history.²⁴ In our study, increased rates of acetylcholinesterase hydrolysis were noted in *LRRK2* mutation carriers without Parkinson's disease compared with healthy controls and in patients with *LRRK2* Parkinson's disease compared with people with idiopathic disease, indicating preserved and possibly upregulated cholinergic projections from the pedunculopontine

nucleus in individuals with *LRRK2* mutations. Our results accord with evidence of a lower prevalence of REM sleep behaviour disorder in this genetic subtype in people both with and without Parkinson's disease.^{4,6} Only one of eight patients with idiopathic Parkinson's disease had possible signs of mild REM sleep behaviour disorder in our study, with a score of seven on the screening questionnaire. The relative absence of REM sleep behaviour disorder in our patients with idiopathic Parkinson's disease, together with the fairly preserved cognition of the few study participants (n=49 with average score on MoCA >28), could account for our failure to find a difference in rates of acetylcholinesterase hydrolysis between people with idiopathic Parkinson's disease and healthy controls. Unfortunately, REM sleep behaviour disorder questionnaire scores are available for only a few *LRRK2* mutation carriers, so between-group analyses were not done.

The finding of higher rates of acetylcholinesterase hydrolysis in the thalamus in individuals with *LRRK2* mutations also accords with evidence of a longer mean time-to-first-fall in patients with *LRRK2* mutations and Parkinson's disease compared with individuals with idiopathic Parkinson's disease.² However, other evidence from quantitative motor evaluation studies has indicated a high prevalence of postural-instability-gait-difficulty subtype and gait abnormality in people with *LRRK2* Parkinson's disease.^{4,6,25} Unlike idiopathic Parkinson's disease, the increased proportion of postural-instability-gait-difficulty subtype was neither associated with raised UPDRS scores nor with cognitive decline in individuals with the *LRRK2* Gly2019Ser mutation. Thus, the underlying mechanisms for gait and balance abnormality in *LRRK2* Parkinson's disease remain unclear.^{4,6}

Hyposmia and cognitive decline are other clinical markers predicting cholinergic denervation in Parkinson's disease.^{18,19} In our study, increased acetylcholinesterase activity was noted in some cortical regions in patients with *LRRK2* Parkinson's disease compared with individuals with idiopathic Parkinson's disease, which accords with better olfactory performance in *LRRK2* mutation carriers.^{2,4-7} People with an *LRRK2* mutation showed more Stroop task-related functional MRI activity in multiple brain regions than did their relatives who were not *LRRK2* mutation carriers,²⁶ a finding that might be compatible with alterations in cholinergic-innervated default mode network areas, as we noted in our study.

The basis for greater central cholinergic activity in people with *LRRK2* Parkinson's disease than in individuals with idiopathic Parkinson's disease is uncertain. One possibility is that a lifelong mutation in *LRRK2* is associated with better compensatory capacity compared with idiopathic Parkinson's disease, which has disease onset later in life. In previous work, we showed normal uptake of ¹⁸F-6-fluoro-L-dopa in *LRRK2* mutation carriers without Parkinson's disease, despite significant decreases in ¹¹C-(6) α -dihydrotetrabenazine and ¹¹C-d-threomethylphenidate binding, implying preserved striatal L-dopa decarboxylase activity in *LRRK2* mutation carriers, even when the integrity of dopaminergic terminals is already disrupted.¹²⁻¹⁵ This finding is supported further by evidence of preserved serotonin transporter binding in a patient with *LRRK2* Parkinson's disease and normal ¹⁸F-6-fluoro-L-dopa uptake but typical parkinsonian signs, who was initially considered to have a scan without evidence of dopamine deficiency but was ultimately diagnosed with *LRRK2* Parkinson's disease,¹⁴ and by increased serotonin transporter binding in *LRRK2* mutation carriers without Parkinson's disease.¹⁶ In our study, augmented activity of

acetylcholinesterase in patients with *LRRK2* Parkinson's disease compared with people with idiopathic Parkinson's disease suggests a possible compensatory mechanism that happens in parallel with the progressive dopaminergic deficit, which might contribute to the somewhat more benign clinical phenotype of *LRRK2* Parkinson's disease.

Furthermore, increased activity of acetylcholinesterase is present in *LRRK2* mutation carriers without Parkinson's disease compared with healthy controls, indicating a gene mutation-related mechanism that is apparent before manifestation of clinical signs of Parkinson's disease. This upregulation, as noted, could be a form of prodromal compensation similar to upregulation in the serotonin system in *LRRK2* mutation carriers without Parkinson's disease.¹⁶ However, cholinergic activity in non-neuronal cells—eg, glia or inflammatory cells—should also be considered. In human beings, acetylcholinesterase is expressed not only in neurons but also in astrocytes, oligodendrocytes, microglia, and endothelial cells in the CNS,^{27,28} and it is widely expressed in white blood cells—including T and B lymphocytes, monocytes, and macrophages.²⁹ Although the catalytic activity of acetylcholinesterase in non-neuronal cells is much lower than that in neurons, amounts of protein are equivalent under normal conditions;²⁶ under oxidative stress, transcription and release of acetylcholinesterase in astrocytes rises further, which cannot be attributed to passive leakage from dying cells.²⁸ Therefore, the increase in rates of acetylcholinesterase hydrolysis detected in our study might be affected by both upregulation of cholinergic neurons and activation of glia. Besides astrocytes, acetylcholine has long been recognised as an important mediator of neuroinflammation through the so-called cholinergic anti-inflammation pathway in microglia,³⁰ even though direct evidence is scant for increased acetylcholinesterase transcription in microglia related to neurodegeneration. High uptake of ¹¹C-donepezil has been noted in peripheral inflammatory or infective disease in both mice and human beings, and uptake is augmented 1 day after an increase of ¹⁸F-fluorodeoxyglucose signal in the same inflammatory sites. This occurrence could be attributed to proliferation of peripheral immune cells and upregulation of associated cholinergic signalling mechanisms.²⁹ Of note, *LRRK2* can also directly modulate inflammatory pathways downstream to toll-like receptors.^{10,11} In people with the *LRRK2* Gly2019Ser mutation (both with and without Parkinson's disease), amounts of neuroinflammation markers in serum were increased significantly compared with healthy controls or people with idiopathic Parkinson's disease,¹⁰ in accordance with the association of *LRRK2* mutations with hyperinflammation from cell culture and animal models.¹¹ In our study, upregulation of acetylcholinesterase activity was already significant in the prodromal stage and persisted after disease became manifest, thus potentially compatible with initiation of inflammatory activity early in the course of neurodegeneration and persistence in manifest disease.

Although α -synuclein-positive Lewy bodies or Lewy neurites are seen in most patients with *LRRK2* Parkinson's disease, Lewy pathology was absent in the brains of 21–35% of people with the *LRRK2* Gly2019Ser mutation and 57–70% of individuals with other *LRRK2* mutations.^{8,9} In our study, 13 (43%) of 30 *LRRK2* mutation carriers had mutations other than Gly2019Ser (ie, Arg1441Cys [n=7], Asn1437His [n=5], and Arg1441His [n=1]), so it is possible that some participants might not have Lewy pathology in the brain. The potential pathological differences between *LRRK2* Parkinson's disease and idiopathic Parkinson's

disease might be associated with differential involvement of cholinergic and serotonergic systems. Since both α -synuclein and *LRRK2* mutations have an important role in the neuroinflammation process, the relation between microglial activation, α -synuclein, and *LRRK2* mutations in the pathogenesis of Parkinson's disease is an area for further study.¹¹

Our study had some limitations. First, no clinical measures were made of REM sleep behaviour disorder and hyposmia, so objective correlations between severity of these signs and rates of acetylcholinesterase hydrolysis could not be investigated. Second, some participants did not have scores for MoCA and BDI (mainly because of language barriers). However, the aim of assessing these scales was to exclude obvious clinical differences that might account for differences in PET. Third, we used the Benjamini-Hochberg procedure to correct for multiple comparisons, which controlled the false discovery rate rather than family-wise error rate and is less stringent than the Bonferroni procedure. However, in view of the exploratory nature of our study and challenges in recruiting people with *LRRK2* mutations, a larger sample size would have been less feasible, and less stringent corrections have been applied accordingly.

In conclusion, *LRRK2* gene mutation is associated with significantly increased brain cholinergic activity in individuals both with and without Parkinson's disease. Our results might account for some clinical characteristics of *LRRK2* Parkinson's disease, including less prevalent REM sleep behaviour disorder and cognitive impairment and better olfactory performance, and increased acetylcholinesterase activity could represent early and sustained attempts to compensate for *LRRK2*-related dysfunction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Research in context

Evidence before this study

We searched PubMed between Jan 1, 1970, and Dec 15, 2017, with the terms “Parkinson’s disease”, “positron emission tomography”, AND “acetylcholinesterase”, “AChE”, OR “acetylcholine”. We found 22 original PET studies reporting acetylcholinesterase data in the CNS of patients with idiopathic Parkinson’s disease; none of the studies had focused on populations at high risk of Parkinson’s disease (ie, carriers of a known pathogenic mutation). The major aim of previous studies was to assess the underlying disruption of cholinergic function in non-motor symptoms of Parkinson’s disease. Nine studies showed severe cholinergic denervation in parkinsonian dementia and interactive effects of cholinergic and dopaminergic deficits on cognitive impairment in Parkinson’s disease. Six studies showed the effect of cholinergic degeneration on gait, balance, and subsequent falls. Two studies showed the association between the presence of rapid eye movement (REM) sleep behaviour disorder and cortical and thalamic cholinergic denervation. Other studies showed cholinergic dysfunction in hyposmia, depression, and fatigue. Three studies further differentiated the cholinergic difference between parkinsonian syndromes, others showed evidence for sex effects and heterogeneity of cholinergic degeneration in Parkinson’s disease.

Added value of this study

To our knowledge, our study is the first to measure central acetylcholinesterase activity in populations at high risk of Parkinson’s disease. We report the effect of pathogenic *LRRK2* mutations on central cholinergic activity in people with and without Parkinson’s disease, and provide evidence of age-dependent changes in cholinergic activities in Parkinson’s disease patients and healthy controls.

Implications of all the available evidence

The finding that *LRRK2* mutation carriers have increased cholinergic activity in the brain might account for some clinical characteristics of this population, including lower prevalence of REM sleep behaviour disorder, olfactory dysfunction, and cognitive impairment. The amplification of acetylcholinesterase activity in *LRRK2* mutation carriers without Parkinson’s disease could reflect a compensation for early dopaminergic loss, since the two neurotransmitter systems interact in the manifestation of Parkinson’s disease. Available evidence so far indicates that *LRRK2* mutation carriers might differ from people with sporadic Parkinson’s disease in clinical features and in non-dopaminergic mechanisms.

Figure 1. Scatterplots showing rate of acetylcholinesterase hydrolysis versus age in healthy controls

(A) Rate of acetylcholinesterase hydrolysis versus age in the cortex, default mode network-related regions, and limbic network-related regions. Lines show the linear regression. (B) Rate of acetylcholinesterase hydrolysis versus age in the thalamus.

Figure 2. Rates of acetylcholinesterase hydrolysis in (A) cortex, (B) thalamus, (C) default mode network-related regions, and (D) limbic network-related regions

Horizontal line denotes the median, the box shows the IQR, and datapoints and errors bars depict 75th percentile + 1.5 × IQR and 25th percentile - 1.5 × IQR.

Table 1

Demographic and clinical features

	<i>LRRK2</i> Parkinson's disease (n=14)	Idiopathic Parkinson's disease (n=8)	<i>LRRK2</i> mutation carrier without Parkinson's disease (n=16)	Healthy control (n=11)	Global p value [*]	p value for <i>LRRK2</i> Parkinson's disease vs idiopathic Parkinson's disease [*]
Age (years)	67.5 (51.8–77.3)	63.5 (58.0–71.8)	54.5 (32.5–57.0)	64.0 (51.0–69.0)	0.001	0.686
Sex ratio (men:women)	6:8	6:2	8:8	7:4	0.472	0.204
Hoehn and Yahr score	2.4 (0.6)	2.0 (0.0)	NA	NA	NA	0.059
Disease duration (months)	66.1 (57.9)	88.5 (36.8)	NA	NA	NA	0.342
MDS-UPDRS III score	31.3 (13.0)	25.2 (12.7)	5.5 (4.9)	NA	<0.0001	0.365
MoCA score [‡]	26.2 (1.9)	28.3 (0.8)	27.2 (2.3)	NA	0.081	0.084
BDI score [‡]	8.7 (6.1)	2.2 (1.4)	4.8 (2.9)	NA	0.006	0.006

Data are mean (SD) or median (IQR), unless otherwise indicated.

BDI=Beck Depression Inventory. MDS-UPDRS III=Movement Disorder Society Unified Parkinson's Disease Rating Scale part III. MoCA=Montreal Cognitive Assessment. NA=not applicable.

^{*} p values for age and MoCA calculated by ANOVA followed by post-hoc analyses. p values for sex ratio calculated by Fisher's exact test. p values for duration calculated by independent two-tailed t test. p values for Hoehn and Yahr score, MDS-UPDRS III, and BDI calculated by Kruskal-Wallis test or Mann-Whitney test. All p values were false discovery rate-corrected for multiple comparisons.

[‡] Data from 11 of 14 patients with *LRRK2* Parkinson's disease, nine of 16 *LRRK2* mutation carriers without Parkinson's disease, and seven of eight patients with idiopathic Parkinson's disease.

[‡] Data from five of 14 patients with *LRRK2* Parkinson's disease, six of 16 *LRRK2* mutation carriers without Parkinson's disease, and eight of eight patients with idiopathic Parkinson's disease.

Table 2

Linear regression between rate of acetylcholinesterase hydrolysis and age, by network or region of interest

	Slope ($\times 10^{-2}$)	R	p value
All participants			
Cortex average	-0.008	-0.337	0.018
Default mode network	-0.011	-0.443	0.002
Limbic network	-0.011	-0.471	0.002
Thalamus	-0.024	-0.356	0.016
Healthy controls			
Cortex average	-0.017	-0.722	0.024
Default mode network	-0.018	-0.743	0.024
Limbic network	-0.017	-0.681	0.028
Thalamus	-0.025	-0.507	0.111

All p values were false discovery rate-corrected.

Table 3

Rates of acetylcholinesterase hydrolysis, adjusted for age

Acetylcholinesterase hydrolysis rates (k3; min ⁻¹ × 10 ⁻²)				p value for between-group differences	p value for <i>LRRK2</i> mutation carrier without Parkinson's disease vs healthy control	p value for <i>LRRK2</i> Parkinson's disease vs healthy control	p value for idiopathic Parkinson's disease vs healthy control	p value for <i>LRRK2</i> Parkinson's disease vs idiopathic Parkinson's disease
<i>LRRK2</i> Parkinson's disease	Idiopathic Parkinson's disease	<i>LRRK2</i> mutation carrier without Parkinson's disease	Healthy control					
Cortex average	2.68 (0.39)	2.36 (0.22)	2.82 (0.24)	2.48 (0.27)	0.009	0.063	0.467	0.043
Default mode network	2.64 (0.36)	2.31 (0.22)	2.82 (0.22)	2.46 (0.29)	0.006	0.072	0.303	0.021
Limbic network	2.68 (0.34)	2.38 (0.23)	2.84 (0.25)	2.50 (0.29)	0.020	0.070	0.514	0.051
Thalamus	6.70 (1.17)	5.50 (0.88)	6.78 (0.57)	6.28 (0.59)	0.008	0.104	0.104	0.004

Data are mean (SD). Pairwise post-hoc analyses were done using Fisher's least significant difference analysis; multiple comparisons were corrected by controlling false discovery rate. All p values were false discovery rate-corrected for multiple comparisons.