**ABSTRACT:** Background: The study of functional connectivity by means of magnetic resonance imaging (MRI) in asymptomatic LRRK2 mutation carriers could contribute to the characterization of the prediagnostic phase of LRRK2-associated Parkinson’s disease (PD). The objective of this study was to characterize MRI functional patterns during the resting state in asymptomatic LRRK2 mutation carriers.

Methods: We acquired structural and functional MRI data of 18 asymptomatic LRRK2 mutation carriers and 18 asymptomatic LRRK2 mutation noncarriers, all first-degree relatives of LRRK2-PD patients. Starting from resting-state data, we analyzed the functional connectivity of the striatocortical and the nigrocortical circuitry. Structural brain data were analyzed by voxel-based morphometry, cortical thickness, and volumetric measures.

Results: Asymptomatic LRRK2 mutation carriers had functional connectivity reductions between the caudal motor part of the left striatum and the ipsilateral precuneus and superior parietal lobe. Connectivity in these regions correlated with subcortical gray-matter volumes in mutation carriers. Asymptomatic carriers also showed increased connectivity between the right substantia nigra and bilateral occipital cortical regions (occipital pole and cuneus bilaterally and right lateral occipital cortex). No intergroup differences in structural MRI measures were found. In LRRK2 mutation carriers, age and functional connectivity correlated negatively with striatal volumes. Additional analyses including only subjects with the G2019S mutation revealed similar findings.

Conclusions: Asymptomatic LRRK2 mutation carriers showed functional connectivity changes in striatocortical and nigrocortical circuits compared with noncarriers. These findings support the concept that altered brain connectivity precedes the onset of classical motor features in a genetic form of PD. © 2016 International Parkinson and Movement Disorder Society

**Key Words:** LRRK2; Parkinson’s disease; functional MRI; connectivity
Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) are the most common known cause of inherited Parkinson’s disease (PD). Potential disease modification treatments for LRRK2-PD, such as LRRK2 kinase inhibitors, are currently under intense study. If effective, it is hoped that these treatments could also have a positive effect in sporadic PD cases because LRRK2-PD is clinically similar to sporadic PD and responds to the same therapies.

It is now widely accepted that a prolonged prodromal phase antedates the onset of motor manifestations in sporadic as well as in genetic forms of PD. Still, although clinical abnormalities have been reported in prediagnostic LRRK2-PD, most studies suggest that the LRRK2 prodromic phase could be clinically quite silent. Imaging studies are therefore particularly important in the identification of subjects in this phase of LRRK2-PD.

Magnetic resonance imaging (MRI) techniques have demonstrated structural and functional abnormalities in asymptomatic LRRK2 mutation carriers. In structural MRI analyses, gray-matter (GM) volume increases have been reported in the caudate nucleus and in the cuneus and decreases in the right prefrontal and orbitofrontal regions. Task-based functional MRI (fMRI) results in these subjects have shown changes in imagery-related activity in different cortical and subcortical brain areas. Only 1 study assessed resting-state functional connectivity in asymptomatic LRRK2 mutation carriers by means of fMRI. In this study, asymptomatic LRRK2 mutation carriers showed reduced interaction between the right inferior parietal cortex and the dorsoposterior putamen but also increased interaction of this cortical area with the ventroanterior putamen, suggesting a reorganization of corticostriatal circuits in these subjects.

The aim of the current work was to evaluate whole-brain resting-state functional connectivity and structural brain measures in a group of asymptomatic LRRK2 mutation carriers. We hypothesized that functional nigral and striatal connectivity might be altered in this group of subjects at high risk of developing PD, a reflection of underlying subclinical brain dysfunction. We also hypothesized that LRRK2 mutation carriers might show subtle signs of GM structural alteration.

Methods

Participants

Study participants were recruited consecutively between May 2012 and May 2013 among first-degree relatives of a previously identified cohort of LRRK2-PD patients at the Movement Disorders Unit, Hospital Clinic de Barcelona. Exclusion criteria were the presence of parkinsonism, other neurodegenerative disorders, and general exclusion criteria for MRI scanning (such as claustrophobia or pacemakers). Thirty-six first-degree relatives of LRRK2-PD patients, who came from 17 families, agreed to participate in the study. The presence of a LRRK2 gene mutation, assessed as previously described, was identified in 18 subjects. Among them, 13 (72.2%) were carriers of the G2019S mutation, 3 (16.7%) of the R1441G mutation, and 2 (11.1%) of the R1441C mutation. Participants were not aware of their genetic status at the time of the study.

Standard Protocol Approvals and Patient Consent

The study was approved by the Ethics Committee of Hospital Clinic de Barcelona (May 2012; code 7723). Written informed consent was obtained from all subjects prior to the study.

Clinical Assessment

All study participants were clinically evaluated at a single center by the same researcher team (D.V., E.T., and C.P.). Motor signs were assessed through the Movement Disorders Society Unified Parkinson’s Disease Rating Scale (MDS-UPDRS-III). None of the participants showed signs of parkinsonism, as determined by neurological examination at the time of the study. Smell was evaluated with the 40-item University of Pennsylvania Smell Identification Test (UPSIT; Sensionics, Spanish version). The Hospital Anxiety and Depression scale (HADS) and the Beck Depression Inventory-II were used to quantify depression and anxiety symptoms. Dysautonomic features were assessed with the SCOPA-AUT scale. Global cognitive function was tested by the Mini-Mental State Examination (MMSE).

Statistical Analyses of Demographic and Clinical Data

Normality of distribution of quantitative variables was assessed with the Kolmogorov-Smirnov test. Normally distributed variables were analyzed using independent-sample Student t tests and Pearson’s product-moment correlations; otherwise, Mann-Whitney U tests and Spearman’s rank correlations were used. Qualitative variables were analyzed using Pearson’s chi-square test. Nonimaging statistical analyses were performed with SPSS version 18.0 (SPSS Inc.) with a 2-sided type I error threshold of 5%.

MRI Acquisition, Processing, and Analyses

Images for all subjects were obtained with a 3T MRI scanner (MAGNETOM Trio, Siemens, Germany), using an 8-channel head coil. The scanning...
protocol included a resting-state, 10-minute-long functional gradient-echo echo-planar imaging sequence (300 T2*-weighted volumes, TR = 2 seconds, TE = 19 milliseconds, flip angle = 90°, slice thickness = 3 mm, FOV = 240 mm, 128 × 128 matrix), in which subjects were instructed to keep their eyes closed, not to think of anything in particular, and not to fall asleep; with a high-resolution 3-D structural T1-weighted MPRAGE sequence acquired sagittally (TR = 2.3 seconds, TE = 2.98 milliseconds, 240 slices, FOV = 256 mm; 1 mm isotropic voxel) and a T2-weighted axial FLAIR sequence (TR = 9 seconds, TE = 96 milliseconds).

**Cortical Thickness**

The processing pipeline for cortical thickness analyses with FreeSurfer (version 5.3; available at: http://surfer.nmr.harvard.edu) is provided as supplemental data. Comparisons between groups were performed with a vertex-by-vertex general linear model using Qdec. An initial vertex-wise threshold was set at \( P < 0.05 \) to find clusters. To avoid false-positives, Monte Carlo simulation with 10,000 repeats was performed. Results are reported at a cluster-wise probability significance level set at \( P < 0.05 \).

**Subcortical Volumetry**

Subcortical volumes, including putamen, caudate, and estimated total intracranial volume (eTIV), were obtained automatically via whole-brain segmentation with FreeSurfer.

**Voxel-Based Morphometry**

Structural data were analyzed with FSL-VBM, a voxel-based morphometry (VBM) style analysis carried out with FSL (release 5.0.4; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL); see Supplementary Materials. A voxel-wise general linear model was applied, and statistical significance was established through permutation testing (5000 permutations), randomizing the study groups and generating a sampling distribution of GM volume differences against which the actual group differences were compared. Statistical significance was set at \( P < 0.05 \), corrected for multiple comparisons using family-wise error (FWE) control.

**Processing of fMRI**

The preprocessing of resting-state images was performed with FSL and AFNI (http://afni.nimh.nih.gov/afni) tools. Briefly, it included removal of the first 5 volumes to allow for T1 equilibration, grand mean scaling, linear trend removal, and temporal filtering (0.01-0.1 Hz). To control for the effect of head movement and other nonneural sources of signal variation, motion correction and regression of nuisance signals (6 motion parameters, cerebrospinal fluid, and white matter) were performed. To remove the effects of timepoints corrupted by motion, a scrubbing procedure was applied, using a root mean square interframe intensity difference threshold equal to the 75th percentile + 1.5 times the interquartile range. In addition, head motion was calculated as the average Euclidean displacement between consecutive timepoints for rotatory and translatory motion.

**Definition of Regions of Interest and Extraction of Temporal Courses**

Structures used as regions of interest (ROIs) for seed-based functional connectivity analyses included the substantia nigra and striatum. The striatum was divided into (left and right) caudal motor, rostral motor, executive, and limbic regions using the Oxford-GSK-Imanova Striatal Connectivity Atlas (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases/striatumconn). Supplementary data include an analysis of thalamic functional connectivity.

To define the substantia nigra, motion-corrected individual resting-state data sets were temporally averaged and normalized to a Montreal Neurological Institute (MNI) template at a 2-mm resolution. These normalized images were averaged across subjects, and left and right nigral masks were manually drawn by a trained radiologist (H.B.) based on the mean image. Supplementary Figure 1 displays the ROI segmentation scheme used.

To obtain each seed region’s resting-state time series, its respective masks were linearly registered to each subject’s T1-weighted image and subsequently linearly registered to native functional space using FSL FLIRT. To obtain region-specific temporal courses, we initially thresholded each probabilistic mask at 30% and subsequently extracted a weighted mean of the time series of all voxels inside them in native space, without smoothing.

**Functional Connectivity Analysis**

Each seed region’s time course was correlated with the time series of every brain voxel using smoothed (6-mm FWHM Gaussian kernel) functional images normalized to MNI space after resampling to \( 4 \times 4 \times 4 \) mm\(^3\) voxel size, producing a Pearson’s \( r \) coefficient correlation map. These were then converted to \( Z \) maps using Fisher’s \( r \)-to-\( Z \) transformation. A Voxelwise general linear model was applied using nonparametric testing including all brain voxels. Statistical significance was established through permutation testing (5000 permutations). Significance level was set at \( P < 0.05 \) using threshold-free cluster enhancement, corrected for multiple comparisons by controlling the FWE across space.

To assess the relationship between functional connectivity alterations and both clinical and volumetric data, mean connectivity values were obtained by averaging individual \( Z \) values in all voxels inside the clusters of significant intergroup differences if results contained more than 10 voxels. Pearson correlation
TABLE 1. Demographic and clinical data.

| Age, y | 43.33 ± 12.07 | 44.72 ± 12.48 | 0.339/0.736 |
| Sex, % male | 9 (50%) | 7 (38.9%) | 0.458/0.502 |
| Mutation, n (%) | 02195G | 13 (72.2%) | NA |
| R1441C | 2 (11.1%) |
| R1441G | 3 (16.7%) |
| Years of education | 13.44 ± 5.08 | 15.28 ± 4.66 | 1.128/0.267 |
| MDS-UPDRS I score | 2.89 ± 4.52 | 2.39 ± 3.70 | 157.5°/0.881 |
| MDS-UPDRS II score | 0.22 ± 0.43 | 0.28 ± 0.58 | 160.0°/0.930 |
| MDS-UPDRS III score | 0.39 ± 0.98 | 0.06 ± 0.24 | 134.5°/0.147 |
| UPSIT score | 32.72 ± 3.74 | 33.0 ± 3.45 | 0.223/0.818 |
| SCOPA-AUT score | 8.44 ± 6.68 | 7.56 ± 4.88 | 0.456/0.651 |
| HADS total score | 8.0 ± 5.69 | 7.83 ± 6.05 | 0.085/0.933 |
| HADS Anxiety score | 5.17 ± 3.26 | 5.39 ± 3.24 | 0.205/0.839 |
| HADS Depression score | 2.83 ± 2.68 | 2.44 ± 3.37 | 0.383/0.704 |
| BDI | 5.12 ± 6.19 | 4.06 ± 4.87 | 0.566/0.575 |
| MMSE score | 29.17 ± 0.92 | 29.17 ± 0.99 | 159.5°/0.932 |

Results are described by mean ± SD. Test statistics (Test stat) refer to Student t, Mann-Whitney’s U (t), or Pearson’s chi-square (χ²).

BDI, Beck Depression Inventory-II; HADS, Hospital Anxiety and Depression Scale; MMSE, Mini-Mental State Examination; SCOPA-AUT, Scales for Outcomes in Parkinson’s disease — Autonomic; UPDRS, Unified Parkinson’s Disease Rating Scale; UPSIT, University of Pennsylvania Smell Identification Test; NA, not applicable.

was then used to correlate these values with age, MDS-UPDRS-III, and UPSIT scores. In addition, partial correlations were used to correlate age and connectivity values with striatal volumes obtained with FreeSurfer, taking eTIV into account.

Image analysis was performed by independent investigators (B.S. and H.B.) blinded to the clinical and genetic status of study subjects.

Results

Demographic and Clinical Data

Mean age was similar between mutation carriers and noncarriers, with similar ranges (27 to 66 and 23 to 67 years, respectively; Table 1). All participants were right-handed. No significant group differences were found in years of education or MDS-UPDRS (I, II or III) and UPSIT scores. Likewise, no significant differences in scores were found for other nonmotor features.

Functional Connectivity

Asymptomatic LRRK2 mutation carriers showed differences in functional connectivity compared with asymptomatic noncarriers. Specifically, we found LRRK2 mutation carriers to have reduced connectivity between the caudal motor subdivision of the left striatum (including the caudal portions of the putamen and caudate nucleus) and the ipsilateral precuneus and superior parietal lobule (P < 0.05, FWE-corrected; see Table 2 and Fig. 1). LRRK2 mutation carriers also displayed reduced functional connectivity between the executive subdivision of the left striatum and the anterior-medial part of the superior frontal gyrus (MNI coordinates 6, 54, 24; cluster size 448 mm³; P = 0.036, FWE-corrected).

Compared with noncarriers, LRRK2 mutation carriers showed increased functional connectivity between the right substantia nigra and the occipital pole and cuneus bilaterally, and the right lateral occipital cortex (Table 2 and Fig. 1).

LRRK2 mutation carriers also displayed reduced functional connectivity between the occipital region of the left thalamus and the occipital cortex bilaterally, including the occipital pole, calcarine cortex, lateral occipital cortex and lingual gyrus, and the left superior parietal lobule (see Supplementary Material). Functional connectivity in mutation carriers was also reduced between the temporal part of the left thalamus and bilateral dorsomedial portions of the precentral gyrus, postcentral gyrus, and supplementary motor area (see Supplementary Fig. 3).

No other significant functional connectivity changes were found when other seed regions were tested. No significant differences in head motion were observed between LRRK2 mutation carriers and noncarriers.

TABLE 2. Differential connectivity in asymptomatic LRRK2 mutation carriers and noncarriers

| Topography (maximum) | Volume (mm³) | Voxels | MNI coordinates | P |
|----------------------|--------------|--------|-----------------|---|
| Right substantia nigra seed | 9344 | 146 | 14, -94, 20 | 0.003 |
| Right occipital pole | 768 | 12 | 26, -94, -12 | 0.008 |
| Right lateral occipital cortex | 640 | 10 | 50, -82, 8 | 0.037 |
| Left substantia nigra seed | 576 | 9 | -50, -78, -16 | 0.038 |
| Superior parietal lobule | 2368 | 37 | -30, -70, 52 | 0.019 |
| Precuneus | 1728 | 27 | -6, -58, 60 | 0.013 |

Description of clusters of significant between-group functional connectivity differences (P < 0.05 FWE-corrected). Asymptomatic LRRK2 mutation carriers showed reduced left striatocortical and increased right nigrocortical connectivity compared with noncarriers. MNI coordinates, x, y, z MNI coordinates of the maximum.
Comparisons Between G2019S Mutation Carriers and LRRK2 Mutation Noncarriers

Structural and functional intergroup analyses excluding the 5 subjects with LRRK2 mutations other than G2019S were performed. Thirteen G2019S carriers were contrasted with 18 noncarriers. Functional analyses showed that G2019S mutation carriers had reduced connectivity between the caudal motor subdivision of the left striatum and both the left precuneus and the superior parietal lobule (Fig. 2).

G2019S mutation carriers also displayed increased connectivity of the right substantia nigra with the occipital pole and calcarine cortex bilaterally and with the right lateral occipital cortex (Fig. 2).

Correlation Between Functional Connectivity, Clinical and Structural Data

In the carrier group, age significantly correlated with mean connectivity Z scores in the clusters of

(rotation (degrees): carriers, 0.03 ± 0.01; noncarriers, 0.03 ± 0.02; P = 0.662; translation (mm): carriers, 0.07 ± 0.03; noncarriers, 0.06 ± 0.05; P = 0.445).
significant intergroup differences in left caudal motor striatal connectivity \((r = -0.475, \ P = 0.046)\). This effect was not observed in the noncarrier group \((r = -0.356, \ P = 0.147)\).

Techniques such as the Bonferroni method for FWE control involving highly correlated measures (eg, basal ganglia volumes) can be excessively conservative.\(^{26}\) We accordingly assessed a possible correlation between connectivity and a single measure, the mean striatal volume, obtained by averaging putaminal and caudate volumes. We observed a significant correlation between Z scores in the clusters of intergroup left caudal motor striatal connectivity differences and this compound striatal volume measure in the carrier group, but not in the noncarrier group (see Table 3 and Fig. 1).

Post hoc correlation analyses between connectivity and volumes of individual striatal nuclei also revealed significant effects in LRRK2 mutation carriers. In the carrier group, Z scores in the clusters of intergroup left caudal motor striatal connectivity differences correlated positively with bilateral caudate and putamen volumes. No significant correlations were observed in the noncarrier group (see Table 3 and Fig. 1).

Also, no significant correlations were observed between Z scores in the clusters of right substantia nigra connectivity and subcortical volumes. Mean connectivity (in the regions in which significant intergroup differences were found) and MDS-UPDRS-III or UPSIT scores showed no correlations, either in the whole sample or in the noncarrier or carrier subgroups (all \(P > 0.2\), uncorrected).

**Structural MRI Findings**

GM volumes (VBM analysis and volumetric measures), cortical thickness measures, and eTIV did not differ significantly between groups (Supplementary Table 2). Age correlated significantly and negatively with striatal volumes in the carrier group (especially the putamina), but not in noncarriers (see Table 3).

**Discussion**

In the present study, asymptomatic LRRK2 mutation carriers showed resting-state functional connectivity reductions between the left striatum and both the ipsilateral precuneus and superior parietal lobule, and connectivity increments between the right substantia nigra and bilateral occipital cortical regions including the occipital poles and cunei, and the ipsilateral lateral occipital cortex. These connectivity reductions correlated with striatal volumes. Analyses including only subjects with the G2019S mutation revealed similar findings.

In manifest idiopathic PD, reduced corticostriatal functional connectivity has been described in previous resting-state fMRI studies in which decreased connectivity between the thalamus and sensorimotor cortices\(^{27}\) and between the striatum and thalamus, midbrain,
pons, and cerebellum was observed. Even in untreated patients in early stages of idiopathic PD, decreased functional connectivity of the caudate with frontal and insular cortices and in mesolimbic-striatal and corticostriatal loops has been observed.

Information concerning functional connectivity alterations in asymptomatic LRRK2 carriers is very limited. In healthy G2019S LRRK2 mutation carriers, a resting-state study reported the presence of striatocortical connectivity reorganization. As in the current study, connectivity changes between the posterior striatum and the parietal cortex consisted of positive correlations in mutation noncarriers and negative ones in carriers. In the present study, the striatal connections showing differences between groups were mostly reductions in the functional connectivity in mutation carriers. However, for the nigral connectivity differences, an increase in functional connectivity was observed, occurring between the right substantia nigra and the occipital cortex. Interestingly, increased resting-state functional connectivity between the right substantia nigra and the occipital cortex has been described in patients with idiopathic REM sleep behavior (IRBD) disorder, a condition considered to represent prodromal PD. As most IRBD patients are eventually diagnosed with a synucleinopathy, especially PD, our findings may represent connectivity changes in prediagnostic PD. In the present study, the striatal connections showing differences between groups are mostly negative for the mutation carriers and positive for the noncarriers. For the nigral connectivity differences, the opposite pattern was observed. Although the biological significance of negative correlations is controversial, different studies revealed that the brain activity is organized into anticorrelated functional networks. The nigral and striatal connectivity alterations observed in our study might reflect a reorganization of these network dynamics, as previously described in patients with idiopathic PD.

In line with our findings in prediagnostic PD, changes in functional connectivity have been found in the prediagnostic phase of other neurodegenerative disorders. In Alzheimer’s, decreased functional connectivity occurs mainly between the precuneus and limbic regions. In preclinical Huntington’s disease, weakened and strengthened connectivity has been observed in the frontostriatal network, thalamus, anterior insula, and memory centers. Although the meaning of these functional changes is unclear, our results suggest that the neurodegenerative process has already started in some of these mutation carriers, leading to brain connectivity changes.

No significant GM atrophy was detected in our asymptomatic mutation carriers, either with VBM or a more sensitive technique such as cortical thickness analysis. Correlation analyses, nonetheless, showed that mutation carriers with more marked striatal connectivity reductions tended to have lower striatal volumes, suggesting that LRRK2 mutation carriers with the lowest levels of striatal connectivity already display subtle signs of neurodegeneration. As LRRK2 mutation penetrance is age dependent, this hypothesis is in line with the correlation observed between age and both striatal connectivity and striatal volumes in the carrier group. It cannot be ruled out, however, that the observed connectivity reductions (and associated variation in subcortical volumes) can be at least partially explained by the effect of LRRK2 mutations. Previous studies showed that the LRRK2 gene is involved in neuronal morphogenesis as well as striatal synaptic transmission and synaptogenesis during development. The follow-up of these subjects is critical to determining if the observed connectivity reductions or smaller subcortical volumes can predict the occurrence of PD.

**TABLE 3. Correlations between subcortical volumes and both age and connectivity measures**

| Left caudal motor striatal connectivity (rho/P) | Age (rho/P) |
|---------------------------------------------|-------------|
| Mean striatal volume | 0.63/0.007 | 0.05/0.842 | −0.67/0.003 | −0.23/0.382 |
| L caudate | 0.65/0.004 | 0.07/0.801 | −0.54/0.024 | −0.14/0.592 |
| L putamen | 0.53/0.029 | 0.05/0.861 | −0.65/0.005 | −0.21/0.425 |
| R caudate | 0.61/0.010 | −0.01/0.997 | −0.52/0.031 | −0.20/0.442 |
| R putamen | 0.57/0.018 | 0.07/0.805 | −0.68/0.003 | −0.24/0.354 |

Left caudal motor striatal connectivity, mean Z scores in the clusters of significant intergroup differences in connectivity with the caudal motor subdivision of the left striatum; mean striatal volume, average of bilateral caudate and putaminal volumes; L, left; R, right; rho, partial correlation coefficient (controlling for intracranial volume).
different underlying neuropathological substrates, represent limitations when interpreting our results. Similarly, our study did not include either healthy controls unrelated to LRRK2-PD patients or a group of symptomatic LRRK2-PD patients for comparison purposes. The inclusion of functional and structural analyses can be considered strengths of this study. Structural connectivity techniques could help to clarify in future studies the mechanisms underlying the findings observed.

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**References**

1. West AB. Ten years and counting: moving leucine-rich repeat kinase 2 inhibitors to the clinic. Mov Disord 2015;30(2):180-189.
2. Tolosa E, Gaig C, Santamaria J, Compta Y. Diagnosis and the premotor phase of Parkinson disease. Neurology 2009;72(7 Suppl):S12-S20.
3. Healy DG, Falchi M, O’Sullivan SS, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson’s disease: a case-control study. Lancet Neurol 2008;7(7):583-590.
4. Gaig C, Vilas D, Infante J, et al. Nonmotor symptoms in LRRK2 G2019S associated Parkinson’s disease. PLoS One 2014;9(10):e108982.
5. Marras C, Schule B, Munhoz RP, et al. Phenotype in parkinsonian and nonparkinsonian LRRK2 G2019S mutation carriers. Neurology 2011;77(4):323-333.
6. van der Vet JP, van Nuenen BF, Bloem BR, Klein C, Siebner HR. Imaging the impact of genes on Parkinson’s disease. Neuroscience 2009;164(1):191-204.
7. Adams JR, van Netten H, Schulzer M, et al. PET in LRRK2 mutations: comparison to sporadic Parkinson’s disease and evidence for presymptomatic compensation. Brain 2005;128(Pt12):2777-2785.
8. Sierra M, Sanchez-Juan P, Martinez-Rodriguez MI, et al. Olfaction and imaging biomarkers in premotor LRRK2 G2019S-associated Parkinson disease. Neurology 2013;80(7):621-626.
9. Reetz K, Tadic V, Kasten M, et al. Structural imaging in the presymptomatic stage of genetically determined parkinsonism. Neurobiol Dis 2010;39(3):402-408.
10. Brockmann K, Groger A, Di Santo A, et al. Clinical and brain imaging characteristics in leucine-rich repeat kinase 2-associated PD and asymptomatic mutation carriers. Mov Disord 2011;26(13):2335-2342.
11. Thaler A, Mirelman A, Helmich RC, et al. Neural correlates of executive functions in healthy G2019S LRRK2 mutation carriers. Cortex 2013;49(9):2501-2511.
12. van Nuenen BF, Helmich RC, Ferraye M, et al. Cerebral pathological and compensatory mechanisms in the premotor phase of leucine-rich repeat kinase 2 parkinsonism. Brain 2012;135(Pt12):3687-3698.
13. Helmich RC, Thaler A, van Nuenen BF, et al. Reorganization of corticostriatal circuits in healthy G2019S LRRK2 carriers. Neurology 2015;84(4):399-406.
14. Gaig C, Esquerra M, Martí MJ, Munoz E, Valldedeoriola F, Tolosa E. LRRK2 mutations in Spanish patients with Parkinson disease: frequency, clinical features, and incomplete penetrance. Arch Neurol 2006;63(3):377-382.
15. Goetz CG, Tilley BC, Shaftman SR, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson’s Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. Mov Disord 2008;23(15):2129-170.
16. Doty RL, Bromley SM, Stern MB. Olfactory testing as an aid in the diagnosis of Parkinson’s disease: development of optimal discrimination criteria. Neurodegeneration 1995;4(1):93-97.
17. Zigmund AS, Snait P. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;67(6):361-370.
18. Beck AT, Steer RA, Brown GK. Manual for the Beck Depression Inventory. San Antonio, TX: Psychological Corporation; 1996.
19. Visser M, Marinus J, Stiggelbout AM, Van Hilten JJ. Assessment of autonomic dysfunction in Parkinson’s disease: the SCOPA-AUT. Mov Disord 2004;19(11):1306-1312.
20. Folstein MF, Folstein SE, McHugh PR. “Mini-mental state.” A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3):189-198.
21. Fischl B, Salat DH, Busa E, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuroen 2002;33(3):341-355.
22. Douaud G, Smith S, Jenkinson M, et al. Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. Brain 2007;130(Pt9):2375-2386.
23. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage 2012;59(3):2142-154.
24. Liu Y, Liang M, Zhou Y, et al. Disrupted small-world networks in schizophrenia. Brain 2008;131(Pt4):945-961.
25. Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3):189-198.
34. Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci U S A 2005;102(27):9673-9678.

35. Baggio HC, Segura B, Sala-Llonch R, et al. Cognitive impairment and resting-state network connectivity in Parkinson’s disease. Hum Brain Mapp 2015;36(1):199-212.

36. Sheline YI, Raichle ME, Snyder AZ, et al. Amyloid plaques disrupt resting state default mode network connectivity in cognitively normal elderly. Biol Psychiatry 2010;67(6):584-587.

37. Harrington DL, Rubinov M, Durgerian S, et al. PREDICT-HD investigators of the Huntington Study Group, Rao SM. Network topology and functional connectivity disturbances precede the onset of Huntington’s disease. Brain 2015;138(Pt8):2332-2346.

38. Pereira JB, Ibarretxe-Bilbao N, Marti MJ, Compta Y, Junqué C, Bargallo N, Tolosa E. Assessment of cortical degeneration in patients with Parkinson’s disease by voxel-based morphometry, cortical folding, and cortical thickness. Hum Brain Mapp 2012; 33(11):2321-2334.

39. Thaler A, Artzi M, Mirelman A, et al. A voxel-based morphometry and diffusion tensor imaging analysis of asymptomatic Parkinson’s disease-related G2019S LRRK2 mutation carriers. Mov Disord 2014;29(6):823-8277.

40. Parisiadou L, Yu J, Sgobio C, et al. LRRK2 regulates synaptogenesis and dopamine receptor activation through modulation of PKA activity. Nat Neurosci 2014;17(3):367–376.

Supporting Data

Additional supporting information may be found in the online version of this article at the publisher’s web-site.