Adaptive and pathological connectivity responses in Parkinson's disease brain networks

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Article

Keywords: assortativity, network connectivity, Parkinson's disease, treatment response, levodopa, subthalamic gene therapy, LRRK2, GBA

Posted Date: October 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-927446/v1>

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Version of Record: A version of this preprint was published at Cerebral Cortex on January 1st, 2022. See the published version at <https://doi.org/10.1093/cercor/bhac110>.

Abstract

Functional imaging has been used extensively to identify and validate disease-specific networks as biomarkers in neurodegenerative disorders. It is not known, however, if connectivity patterns in these networks differ with disease progression compared to the beneficial adaptations that may also occur over time. To distinguish the two responses, we focused on assortativity, the tendency for network connections to link nodes with similar properties. High assortativity is associated with unstable, inefficient flow through the network. Low assortativity, by contrast, involves more diverse connections that are also more robust and efficient. We found that in Parkinson's disease (PD), network assortativity increased with over time. Assortativity was high in clinically aggressive genetic variants, but low for genes associated with slow progression. Dopaminergic treatment increased assortativity despite improving motor symptoms, but subthalamic gene therapy, which remodels PD networks, reduced this measure compared to sham surgery. Assortativity may therefore be useful in evaluating disease-modifying therapies.

Introduction

Disease-related network patterns identified with functional brain imaging are increasingly being used to gain insight into neurodegenerative disorders and to track disease progression and treatment responses in patient populations^{1–5}. Given the brain's enormous capacity to compensate for dysfunction—network changes are apparent on imaging long before the onset of symptoms—it is difficult to determine whether the novel connections that arise reflect underlying pathology or an adaptive response to the disease. Such studies are particularly challenging in neurodegenerative disorders such as Parkinson's and Alzheimer's, where it is difficult to identify individuals decades before they develop symptoms unless they have a rare, inherited form of the disorder.

In the case of Parkinson's disease (PD), cross-comparisons have begun to provide insight into the connectivity patterns that are associated with more aggressive or more benign disease courses. The dominant disease network, termed the PD-related covariance pattern (PDRP), consists of nodes that are either more or less metabolically active than in healthy subjects, and these differences from baseline intensify so that pattern expression increases with disease progression^{3,6,7}. Although PDRP (which correlates primarily with motor dysfunction) and its cognition-related counterpart network, the PD-related cognitive pattern (PDCP)^{3,8}, were identified in patients with idiopathic PD, we recently found that patients with PD caused by the *LRRK2* G2019S mutation or *GBA1* variants express the same disease networks⁸. Although this makes sense (after all, the patients have clinical and pathological features of sporadic PD, regardless of genotype), we speculated that a deeper analysis of functional connectivity within the PDRP and PDCP spaces might provide clues as to why *LRRK2*-associated PD tends to progress more slowly, and *GBA1*-associated PD more rapidly, than sporadic disease. This turned out to be the case: both genotypes were associated with abnormal increases in connectivity compared to idiopathic PD, but the pattern was quite different. *LRRK2* mutation led to the formation of additional connections within the

network core, particularly between the cerebellum and putamen, which may represent protective adaptations⁸. *GBA1* variants, on the other hand, were associated with gain in connections outside the core, involving mainly cortico-cortical projections⁸. Of note, conventional network metrics such as degree centrality, clustering coefficient, and characteristic path length did not distinguish the slow and rapidly progressive PD genotypes.

This led us to ask whether other descriptors of functional connectivity within PD networks or subgraphs would be more informative. Specifically, we hypothesized that pathological connectivity patterns would be distinguished by their assortativity. Defined as the link-averaged correlation coefficient of degree centrality across pairs of connected nodes, assortativity can be thought of as an index of connectional diversity within a network⁹. When a network is dominated by links between nodes of different degree centrality (negative degree pair correlation), it is termed disassortative. Such networks are robust and transfer information efficiently along the graph. When connections predominate between nodes of similar degree centrality, the network is termed assortative (positive degree pair correlation). Assortative networks are more vulnerable to random attack than their disassortative counterparts¹⁰ and information transfer is comparatively less efficient^{11–14}. Given these properties, we hypothesized that pathological responses, such as those observed over time in patient populations, would be associated with increasing assortativity of disease networks. Adaptive responses, on the other hand, would be accompanied by lower assortativity or disassortativity in the disease network space. To test this hypothesis, we measured assortativity for the PDRP and PDCP as a function of disease progression, genotype, and response to two different treatments: intravenous levodopa infusion, which acutely improves motor symptoms, and subthalamic AAV2-GAD gene therapy, which remodels motor networks over time⁴.

Results

Network assortativity is higher in PD than in healthy controls

We measured PDRP assortativity, degree centrality, clustering coefficient, characteristic path length, and small-worldness in FDG PET scans previously taken from 96 PD patients and 22 healthy control (HC) subjects (**Table S1A**)¹⁵. We evaluated the same metrics in FDG PET scans from an independent group of 146 PD and 39 HC subjects (**Table S1B**) studied under similar conditions^{16,17}. Group-level analysis showed that PDRP assortativity was greater in PD than in healthy controls ($P_{\text{CORR}} < 0.001$, compared to HC in both populations) (**Fig. 1A; Fig. S1A, B**). Analysis of the other network metrics revealed only inconsistent differences between the patient and control samples (**Table 1**).

We evaluated the same network metrics in 20 patients and 20 healthy control subjects scanned with resting-state fMRI (rs-fMRI) using an individual case approach (see Methods)⁷ (**Table S1C**). Again, we found greater assortativity in the rs-fMRI-based PDRP space in the patient group compared to controls ($P_{\text{CORR}} < 0.005$; **Fig. 1B, left**). The findings were confirmed in a large rs-fMRI testing set composed of 102

PD and 18 matched HC subjects scanned as part of a multicenter imaging database (**Table S1C**). As with PET, PDRP assortativity was elevated in these patients ($P_{\text{CORR}} < 0.001$, compared to HC; **Fig. 1B, right**), while group differences were inconsistent for the other network metrics (**Table 1**). At the individual patient level, PDRP assortativity was stable, with excellent test-retest reliability in repeat rs-fMRI scans from the North Shore cohort (ICC=0.83 [0.58-0.93, 95% CI] $p < 0.0001$; see Methods).

Network assortativity increases with disease progression

If high assortativity in disease networks such as PDRP indicates pathological patterns of connectivity, we would expect to see steady increases in this measure over time. We therefore measured PDRP assortativity in a group of 15 early-stage PD patients who underwent longitudinal metabolic imaging at baseline, 24 months, and 48 months^{18,19} (**Table S2A**). Assortativity (**Fig. 2A**) was elevated in the PDRP space at baseline and 24 months ($P_{\text{CORR}} < 0.001$ at both time points, compared to healthy control (HC) subjects), with further increases at 48 months ($P_{\text{CORR}} < 0.001$, compared to baseline and 24 months, as well as HC). We next performed a cross-sectional comparison of age- and gender-matched groups of PD patients with early (0-4 years), intermediate (5-9 years), and long (>10 years) duration of symptoms, along with healthy control subjects (n=20 in each group; **Table S2B**). PDRP assortativity (**Fig. 2B**) was elevated in all three PD groups ($P_{\text{CORR}} < 0.001$); values were higher ($P_{\text{CORR}} < 0.001$) with each successive increment in disease duration.

To visualize the changes in PDRP assortativity seen with advancing disease, we constructed two-dimensional (2D) matrix displays of degree–degree correlations in the PDRP space (**Fig. 2C**) for the early and late PD groups and the HC subjects. Each of the patient groups exhibited an assortative connectivity pattern; this relationship, demonstrated by an elliptical joint probability distribution (see Methods), was more pronounced in late than in early PD patients but was not present in healthy subjects.

Genotypic differences in assortativity parallel the rate of clinical progression

We next asked whether disease network assortativity differed for clinically matched PD patients carrying genetic variants for slower (*LRRK2*) or faster (*GBA1*) disease progression^{8,20,21}. As noted in the introduction, we recently found that although both genotypes express the same disease networks as sporadic PD, the three groups differ in the pattern of connectivity within the disease network spaces⁸. We therefore measured assortativity in the PDRP and PDCP networks in these patient groups, which were carefully matched so that the observed network effects were unlikely to have been caused by differences in age or motor symptoms (**Table S2C**).

Assortativity in the PDRP space (**Fig. 3A**) was elevated for PD-GBA ($P_{\text{CORR}} < 0.05$, compared to HC as well as PD-LRRK2) but did not differ from normal for PD-LRRK2 ($P_{\text{CORR}} = 1.00$). Given our recent findings that genotypic differences in PD network connectivity were more pronounced at the subnetwork level⁸, we partitioned the PDRP space into core and periphery zones (**Table S3A**) based on metabolic valence as described elsewhere²² (see Methods). Assortativity in the PD-LRRK2 PDRP core was lower

than in the other groups ($P_{\text{CORR}} < 0.01$, compared to PD-GBA and HC) (**Fig. 3B**), even though degree centrality was elevated⁸.

To examine connectivity patterns in the PDRP core in greater detail, we reconstructed corresponding subgraphs for the two PD genotypes. PD-LRRK2 showed a dense cluster of interconnected high-degree nodes (**Fig. 3C, left**) at the center of the core zone, involving the putamen, globus pallidus, thalamus, and supplementary motor area (SMA), as well as the amygdala, parahippocampal gyrus, and insula. These were connected to an outer rim of low-degree nodes in the pons, cerebellum, and hippocampus, which were also linked to each other. This configuration was therefore characterized by a neutral connectivity pattern, i.e., a combination of assortative and disassortative connections, for the subgraph as a whole. By contrast, for PD-GBA (**Fig. 3C, right**), a set of interconnected low-degree nodes in the globus pallidus, thalamus, and SMA formed the rim of the subgraph. Sparse connections linked these to a cluster of interconnected high-degree nodes in the interior of the core zone.

Analogous group differences were seen in the PDCP space (**Fig. 4A**): network assortativity was higher in PD-GBA than in the other groups ($P_{\text{CORR}} < 0.05$, compared to HC; $P_{\text{CORR}} < 0.001$, compared to PD-LRRK2), but PD-LRRK2 assortativity did not differ from HC ($P_{\text{CORR}} > 0.34$). This is consistent with the general lack of cognitive decline in PD-LRRK2. Genotypic differences were also more pronounced in the PDCP core zone (**Table S3C**) compared to the whole network. As in the PDRP core, the PDCP core was least assortative in PD-LRRK2 (**Fig. 4B**) ($P_{\text{CORR}} < 0.001$, compared to HC and PD-GBA) but most assortative in PD-GBA ($P_{\text{CORR}} < 0.001$, compared to HC and PD-LRRK2).

The genotypic differences in PDCP core assortativity are highlighted in the corresponding correlation matrix displays and subgraph reconstructions (**Fig. 4C, D**). For PD-LRRK2, the connectivity pattern in this subgraph was disassortative on average, with prominent connections linking high- and low-degree nodes in frontal and parietal association regions. For PD-GBA, the PDCP core was assortative. In contrast to the PD groups, healthy control subjects exhibited a balance of assortative and disassortative connections in the same subgraph.

Dopaminergic treatment increases PDRP assortativity

Given that PDRP assortativity rises with disease progression, we were particularly interested in whether treatment would affect this measure. On the one hand, dopaminergic therapy improves symptoms; on the other hand, the disease continues to progress, as indicated by rising expression of PDRP³.

We first examined changes in network assortativity in 14 PD subjects who underwent metabolic imaging in the unmedicated baseline state (*off*) state and during intravenous levodopa infusion (*on*), titrated to maximal clinical benefit without abnormal involuntary movements²³. The clinical and demographic features of these subjects are provided in **Table S2D**. In the PDRP space, assortativity (**Fig. 5A**) was elevated in the off-state ($P_{\text{CORR}} = 0.001$, compared to HC). Values increased further during

levodopa infusion ($P_{\text{CORR}} < 0.05$, *on* compared to *off*), reaching even more abnormal levels ($P_{\text{CORR}} < 0.001$, *on* compared to HC). Interestingly, this increase (worsening) in network assortativity was not accompanied by changes in degree centrality (**Table 2**) or nodal hierarchy (see Methods), given that the rank order of degree centrality in the network space was similar for the two treatment conditions ($r_s = 0.69$ [0.57 - 0.78, 95% confidence interval (CI)], $p < 0.001$; Spearman correlation).

Subthalamic gene therapy reduces PDRP assortativity

We previously found that AAV2-GAD gene therapy induces therapeutic remodeling of brain networks⁴, which gives rise to what we called the AAV2-GAD-related metabolic pattern (GADRP). An important test of our hypothesis was therefore to determine whether this disease-modifying treatment also diminishes assortativity. We analyzed metabolic imaging data from PD patients scanned at baseline before randomization to either gene therapy or sham surgery as part of a double-blind randomized clinical trial, and again 12 months after surgery²⁴ (for demographic information see **Table S2E**).

For each group and time point, we measured assortativity for both PDRP and GADRP (**Table S3D**)⁴. PDRP assortativity (**Fig. 5B**) was elevated at baseline in the gene therapy group ($P_{\text{CORR}} < 0.001$, compared to HC) but fell to normal levels 12 months after treatment ($P_{\text{CORR}} < 0.001$, compared to baseline). Nodal hierarchy in the PDRP space was not appreciably altered by the gene therapy ($r_s = 0.63$ [0.49 - 0.74, 95% CI], $p < 0.05$; Spearman correlation).

In the Sham group (**Fig. 5C**), PDRP assortativity increased somewhat at 12 months ($P_{\text{CORR}} < 0.05$, compared to baseline) but did not differ from HC at either time point ($P_{\text{CORR}} > 0.50$). At baseline, the rank order of nodal degree in the PDRP space was similar for the sham surgery and gene therapy groups ($r_s = 0.57$ [0.42 - 0.69, 95% CI], $p < 0.001$; Spearman correlation), and nodal hierarchy for this network remained the same 12 months after sham surgery ($r_s = 0.84$ [0.77 - 0.89, 95% CI], $p < 0.001$; Spearman correlation).

The independent GADRP network (**Table S3D**)⁴ showed lower assortativity (**Fig. 6A, top**) 12 months after gene therapy compared to baseline and HC ($P_{\text{CORR}} < 0.05$). GADRP assortativity was higher 12 months after sham surgery compared to baseline ($P_{\text{CORR}} < 0.01$; **Fig. 6B, top**), which reached abnormally elevated levels at endpoint compared to HC ($P_{\text{CORR}} < 0.001$). The divergence of the GADRP assortativity responses in the gene therapy and Sham groups can be seen in the graphical displays of the treatment-induced network. In the gene therapy group, GADRP exhibited an assortative baseline configuration (**Fig. 6A, middle**), with a cluster of high degree nodes, representing the caudate nuclei, thalamus, and frontal regions, which linked to pairs of interconnected lower degree nodes in the putamen and supramarginal gyrus bilaterally. Twelve months after treatment (**Fig. 6A, bottom**), degree centrality increased in most GADRP nodes, with a concurrent decline in network assortativity. In the sham surgery group, the baseline configuration (**Fig. 6B, middle**) was also assortative, with nodal degree in similar rank order to that observed at baseline in the gene therapy group ($r_s = 0.53$ [0.37 - 0.66, 95% CI], $p < 0.05$; Spearman

correlation). The network configuration seen after sham surgery (**Fig. 6B, bottom**), however, was strikingly different, with an overall reduction in degree and an increase in network assortativity. Despite these changes, GADRP nodal hierarchy was not appreciably altered after sham surgery ($r_s=0.54$ [0.38 - 0.67, 95% CI], $p<0.05$; Spearman correlation). This contrasted with the substantial network remodeling following gene therapy, given the revision of nodal hierarchy that took place over the same time period ($r_s=0.13$ [-0.7 - 0.32, 95% CI], $p=0.67$).

Increase in module-to-module bridging connections in the PDRP core

In previous work, we reported an increase in GADRP connections following STN AAV2-GAD gene therapy⁴. We now observed parallel connectivity changes in the PDRP space of these subjects, which were similar to those seen in PD-LRRK2⁸. Compared to baseline, eight new PDRP connections developed in the 12 months following gene therapy (**Table S4**). It is worth noting that three of these overlapped with the novel connections observed in PD-LRRK2⁸. In both groups (**Fig. 7A**), these connections linked basal ganglia and limbic nodes, which are located in separate modules within the PDRP core²².

As previously reported in PD-LRRK2⁸, such connections reduce the modularity of the PDRP core ($P_{\text{CORR}}<0.001$, relative to HC; **Fig. 7B**), and indeed comparable modularity reductions were also seen after gene therapy ($P_{\text{CORR}}<0.001$, compared to baseline and HC; **Fig. 7B**). Thus, low PDRP assortativity, as occurs intrinsically in PD-LRRK2 and following STN AAV2-GAD in sporadic PD, was accompanied by reductions in subgraph modularity linked to increases in bridging connections between core modules.

Discussion

Elevated assortativity has been observed in neurodegenerative disorders including Alzheimer's disease²⁵ and frontotemporal dementia²⁶. Here, we quantify this graph metric in specific, previously validated disease networks identified in the resting state with either FDG PET or rs-fMRI^{3,8,27}. Furthermore, to date, assortativity has not been used to discern the effects of specific interventions on connectivity patterns in these networks. In this study, we found that PDRP assortativity is elevated in the course of PD, indicating over-reliance on connections between nodes of similar degree centrality, which is associated with unstable network configurations and inefficient information^{10,11,14}. Disease network assortativity was diminished by subthalamic AAV2-GAD, but not by levodopa, suggesting a shift toward a more stable connectivity pattern after the former intervention. In functional brain networks, assortativity is only minimally correlated with other graph metrics²⁸—indeed, we observed no consistent changes in degree centrality or characteristic path length—which underscores the unique information this metric provides about the architecture and dynamic properties of the underlying circuitry¹².

Assortativity increases with disease progression

Network assortativity increases with disease progression, and is also influenced by intrinsic factors such as genotype. Disease progression is relatively fast in PD patients with *GBA1* variants²¹, and

indeed assortativity in the PDRP/PDCP core zones was greater than in clinically matched carriers of the more benign *LRRK2*-G2019S mutation²⁰. In the latter genotype, assortativity was reduced in the core subgraphs of both networks, suggesting more efficient information transfer^{12,14}. The current findings extend our previous observation that the gain in functional connections in PD-*LRRK2* occurred primarily in the core zone of the two PD networks⁸. The relatively benign clinical course seen with this mutation can be attributed to the enhanced integration of signal across these subgraphs, as implied by the observed reduction in core assortativity, as well as the relatively low modularity and increased synchronization reported previously in these patients⁸. In aggregate, the altered pattern of functional connectivity seen in PD-*LRRK2* may represent a previously unrecognized adaptation to the disease process. The pattern in PD-GBA was quite different, however, in that connectional gain was not centralized to the PDRP/PDCP core zones as it was in PD-*LRRK2*⁸. The genotypic difference in core assortativity was particularly striking, however, with significant increases in PD-GBA compared to PD-*LRRK2* in both networks. These changes suggest that signal is more segregated in the core zones of PD-GBA patients, consistent with a pathological response rather than a beneficial adaptation.

We also note that while cognitive functioning was grossly preserved in both patient groups⁸, genotypic differences in core assortativity were greater for the PDCP compared to PDRP. The propensity for neocortical Lewy body formation is also greater in PD-GBA patients^{29,30}. Thus, the increases in PDCP core assortativity seen in these patients suggest that pathological connectivity changes develop in this subgraph before clinically identifiable cognitive decline. In this regard, the disassortativity observed in the PDCP core zone in PD-*LRRK2* patients may be compatible with an adaptive response that slows the transition to dementia in patients with this mutation. Prospective imaging studies will be needed to determine whether increased baseline assortativity in the PDCP core predicts future cognitive deterioration in individual PD patients.

Changes in assortativity distinguish treatment responses

Mean PDRP expression increases with disease progression, paralleling the changes in network assortativity seen over time in the same groups of patients. While these measurements relate to a fixed PDRP, it is also possible to identify similar yet topographically more extensive disease patterns at each successive time point^{6,31}. In this context, the magnitude of the corresponding eigenvalue would rise, leading potentially to parallel increases in network assortativity^{12,32}. It is therefore not surprising that for PDRP, assortativity and mean pattern expression both increase with advancing disease.

That said, the two network measures exhibit divergent responses to treatment. Levodopa administration consistently improves motor symptoms in PD patients by replenishing nigrostriatal dopamine. Although levodopa lowers PDRP expression levels^{7,33}, network assortativity increased in these individuals during treatment. It is conceivable that transient increases in PDRP assortativity occur with each levodopa dose. Under such circumstances, repeated daily administration of the drug may lead over time to development of a maladaptive connectivity pattern in the network space³⁴. It would be interesting

to see whether changes in PDRP assortativity appear with chronic treatment and, if so, how they relate to the appearance of LID.

A much different set of network changes follow subthalamic AAV2-GAD gene therapy: PDRP expression levels increase over time, consistent with disease progression⁴, yet PDRP assortativity substantially declined in the treatment group. Treatment reduced assortativity in the GADRP space to subnormal levels and extensively reorganized network structure, as indicated by treatment-mediated changes in nodal hierarchy for this network but not for PDRP.

Thus, assortativity provides complementary information not obtained by routine network expression measurements. In this context, it is worth noting that treatment-mediated modulation in PDRP assortativity (**Table 2**) was not associated with parallel changes in degree centrality or the other connectivity metrics. The response of PDRP small-worldness to treatment is interesting in this regard. Assortativity in this network increased with levodopa and sham surgery, and declined with gene therapy. PDRP small-worldness, by contrast, declined toward normal after either levodopa or gene therapy. Given that significant clinical improvement occurred with both treatments, it is possible that normalization of PDRP small-worldness is a feature of the symptomatic benefit seen with both interventions^{4,22}. The reductions in PDRP assortativity, on the other hand, may reflect longer-term adaptive responses that occur with gene therapy but not with acute levodopa treatment.

The present study suggests that while symptomatic treatment suppresses PDRP expression, the efficacy of potential disease-modifying treatments can be assessed by analyzing the adaptive connectivity patterns within the network space. Ideally, to customize treatment, disease networks should be assessed at the individual patient level. In this study, treatment effects were evaluated at the group level using FDG PET. Nonetheless, the rs-fMRI findings show that single-subject measurements are reliable and potentially suitable for the evaluation of individual patients. Such studies will be an important next step in the design of customized interventions for PD and other neurological disorders.

Methods

Study samples

Network metrics were computed for previously validated motor and cognition-related PD topographies described elsewhere^{1,3}. Group differences in the measures, as well as the effects of disease progression, genotype, and treatment were explored in previously published datasets referenced below (**Table S1A-S2E**). PD patients in each sample were diagnosed according to UK Parkinson Disease Society Brain Bank criteria³⁵. The subjects were evaluated at the time of imaging according to the Unified Parkinson's Disease Rating Scale (UPDRS)³⁶ at least 12 hours after the last medication dose. Ethical permission for these studies was obtained from the Institutional Review Boards of the participating institutions. Written consent was obtained from each subject following detailed explanation of the procedures.

Study cohorts

1. Comparison of network metrics in PD and healthy control subjects

(a) [^{18}F]-fluorodeoxyglucose (FDG PET). We studied 96 PD patients and 22 age- and gender-matched healthy control (HC) subjects (HC1, $n=22$) who were scanned with FDG PET at North Shore University Hospital as part of long-term diagnostic project¹⁵ (**Table S1A**). For validation, we studied an independent cohort of 146 PD and 39 HC subjects who were scanned with FDG PET as part of two subsequently published ascertainment studies^{16,17} (**Table S1B**).

(b) rs-fMRI. To further substantiate the finding in single case measurements of PDRP assortativity, we studied a cohort of 20 PD and 20 age- and gender-matched HC subjects (HC2, $n=20$) at North Shore, who were scanned with resting-state fMRI (rs-fMRI)⁷ (**Table S1C**). To validate the findings, we additionally analyzed baseline rs-fMRI scans from 102 PD and 18 HC who were studied as part of Parkinson's Progression Markers Initiative (PPMI; <https://www.ppmi-info.org/>)³⁷ (**Table S1C**).

2. Change in network metrics with disease progression

(a) Longitudinal progression. We studied a longitudinal cohort of early PD patients ($n=15$) who were scanned with FDG PET at baseline, 24 months and 48 months, and a matched group of healthy control subjects (HC3; $n=15$) (see **Table S2A**). Limited metabolic data from these subjects have appeared previously¹⁸.

(b) Comparison of groups with increasing symptom duration. We compared three age- and gender-matched groups of PD patients with motor symptoms of short (0-4 years; $n=20$), intermediate (5-9 years; $n=20$), and long (≥ 10 years; $n=20$) duration, and age- and gender-matched healthy control subjects (HC4; $n=20$) (see **Table S2B**).

3. Effects of PD genotype on network metrics

We studied patients with PD associated with either the *LRRK2*-G2019S mutation (PD-LRRK2; $n=14$) or *GBA1* variants (PD-GBA; $n=12$), and matched healthy control subjects (HC5; $n=14$) (**Table S2C**). Clinical details and limited network data from these patients have appeared previously⁸.

4. Effects of levodopa infusion on network metrics

We studied PD subjects ($n=14$) who underwent metabolic imaging in the unmedicated state (*off*) and during intravenous levodopa infusion (*on*), and a matched group of healthy control subjects (HC6; $n=14$) (**Table S2D**). In each patient, the levodopa dose was titrated to achieve maximal clinical benefit, without eliciting involuntary movements. Experimental details and limited metabolic data from these patients have appeared previously²³.

5. Effects of subthalamic gene therapy and sham surgery on network metrics

We analyzed baseline and 12-month FDG PET data from PD patients who were randomized to either subthalamic AAV2-GAD (n=16) or sham surgery (n=21) as part of a double blind surgical trial, and a matched group of healthy control subjects (HC7; n=22)²⁴ (**Table S2E**). The results of graph analysis of the gene therapy-induced network topography have appeared previously⁴.

Imaging

FDG PET imaging

Study participants at North Shore underwent metabolic imaging with FDG PET on a GE Advance tomograph (General Electric, Milwaukee, WI) at The Feinstein Institutes for Medical Research (Manhasset, NY) as described in detail elsewhere^{6,38}. Study participants from other centers underwent metabolic imaging on their respective FDG PET platforms^{16,17,24}. In all centers, subjects fasted overnight prior to PET imaging; anti-parkinsonian medications were withheld for at least 12 hours before the scanning session. Scans from each subject were realigned and spatially normalized to a standard Montreal Neurological Institute (MNI)-based PET template and smoothed with an isotropic Gaussian kernel (10 mm) in all directions to improve the signal-to-noise ratio. Image processing was performed using Statistical Parametric Mapping (SPM5) software (Wellcome Trust Centre for Neuroimaging, Institute of Neurology).

MR imaging

All participants received MR scans including structural and functional imaging. During the resting-state sequence they were instructed to rest quietly with eyes open and not to sleep.

Study participants at North Shore were scanned on a General Electric 3.0 Tesla Signa HDxt scanner. For each subject, a 3D T1 image was acquired using a SPGR protocol with repetition time between 2 RF pulses (TR) = 7.6 ms, echo time (TE) = 2.98 ms, TI = 650ms, field of view (FOV) = 240 mm, flip angle (FA) = 8°, and voxel size = 1×1×1 mm³. For rs-fMRI, 240 volumes × 40 slices were acquired for 8 minutes with TR = 2000 ms, TE = 28 ms, FOV = 240 mm, FA = 77° and voxel size = 3 mm. Further details on scanning parameter can be found elsewhere⁷.

PPMI study participants were scanned on a 3T Siemens Trio Tim scanner. For each subject, a 3D T1 image was acquired using a MPRAGE protocol with repetition time (TR) = 2300 ms, echo time (TE) = 2.98 ms, field of view (FOV) = 256 mm, flip angle (FA) = 9°, and voxel size = 1×1×1 mm³. For rs-fMRI, 210 volumes × 40 slices were acquired for 8 minutes and 24 seconds with TR = 2400 ms, TE = 25 ms, FOV = 224 mm, FA = 80° and voxel size = 3.3 mm. Further details on scanning parameter can be found on the website (<http://www.ppmi-info.org/wp-content/uploads/2017/06/PPMI-MRI-Operations-Manual-V7.pdf>). Image preprocessing was performed using the FMRIB Software Library (FSL; www.fmrib.ox.ac.uk/fsl) for rs-fMRI scans from both cohorts. It included motion correction, brain extraction, spatial smoothing (kernel = 8 mm; FWHM) and temporal high-pass filtering (cutoff frequency = 1/100 Hz). Rs-fMRI volumes were

registered to the individual patient's structural T1 image and then to the standard MNI 152 template. Further details of image preprocessing can be found elsewhere⁷.

Network analysis

1. PD-related networks

We used previously validated PD-related metabolic patterns associated with motor and cognitive dysfunction, known respectively as PDRP and PDCP^{3,33}.

These patterns were identified using spatial covariance analysis of FDG PET data from PD patients and HC subjects as described previously^{6,31,39}. Based on prior graphical analysis, we used nodal valence (region weight sign) to partition both networks into core and periphery subgraphs²². For the PDRP (38 nodes according to the AAL atlas⁴⁰ (see below; **Table S3A**), the 20 nodes with positive metabolic valence (i.e., those with region weights ≥ 1.0) were found to form a discrete topological core zone^{8,22}, whereas the remaining 18 nodes had negative valence (i.e., region weights ≤ -1.0) and comprised the network periphery⁸. For PDCP (35 nodes; **Table S3C**), the core was composed of the 16 nodes with negative valence⁸, which corresponded to the cortical core of the default mode network (DMN)^{3,31,41}. The assignment of each PDRP and PDCP node to the core or periphery of the respective network is given in **Tables S3A** and **S3C**. For both networks, assignments based on metabolic valence agreed well with the results of modularity maximization and information theoretic community detection algorithms⁸.

For the rs-fMRI datasets, we used the fPDRP, a PD-related motor pattern topographically similar to the PET-based PDRP⁷. The fPDRP was identified using independent component analysis of rs-fMRI data from patients and control subjects in conjunction with bootstrap resampling⁷. It is composed of 39 nodes according to the AAL atlas⁴⁰ (**Table S3B**).

2. AAV2-GAD-related pattern (GADRP)

We utilized the previously reported AAV2-GAD-related metabolic pattern (GADRP) identified in trial participants scanned with FDG PET at baseline and after subthalamic gene therapy^{4,24}. This network was extracted from the metabolic data using ordinal trends canonical variates analysis (OrT/CVA), a form of supervised principal component analysis⁴². The details of this analysis and its results are provided elsewhere⁴. The GADRP was composed of 14 nodes according to the AAL atlas (**Table S3D**). Because of its relatively small size, this network could not be reliably partitioned; graphical analysis was therefore performed on the network as a whole. Preliminary graph theory analysis of the GADRP has appeared previously⁴. In the current study, we extended the analysis to the PDRP and additionally measured graphical metrics for this space in the various groups and time points.

Graph theory and network metrics

Defining nodes and edges

As noted above, for each network, nodes were defined based on the AAL atlas⁴⁰, in which we parcellated the 3D image of the whole brain, normalized to MNI space. This produced 95 standardized anatomical regions-of-interest (ROIs) as described previously^{4,8,22}. For the significant clusters identified by voxel-wise network analysis, we identified corresponding AAL nodes (see **Table S3A-D** for listings). For each node, we computed normalized metabolic activity for FDG PET scans and mean time series for rs-fMRI scans.

Different network spaces were used in the various analyses. Most of the studies were analyzed in PDRP space for FDG PET⁶ and fPDRP space for rs-fMRI data⁷. As mentioned above, comparison of PD genotypes (PD-LRRK2 vs. PD-GBA) relied on prespecified subnetworks of the PDRP and PDCP (i.e., network core and periphery) defined in an earlier study⁸. Lastly, in addition to the PDRP, we used the treatment-induced AAV2-GAD gene therapy network (GADRP) reported previously⁴ to assess the changes in graph metrics that occurred with this intervention and with sham surgery in this network space and in the PDRP. The details of each of these networks are provided in Supplementary **Tables S3A-S3D**.

In this study, we used AAL ROI data from FDG PET (globally normalized regional metabolic activity) and rs-fMRI (filtered regional mean time series) to construct matrices of pairwise correlations at the group and subject levels. For group-level analysis of the FDG PET scans, we used bootstrap methods (in-house Matlab script; MATLAB R2020a) to generate 100 samples for each group. For each iteration, we computed pairwise nodal correlation coefficients (Pearson correlations). The median values of the iterates (100 bootstrap correlation estimates) were used to create an adjacency matrix for the network/subgraph in each group. For subject-level analysis of the rs-fMRI scans, regional mean time series data were filtered using a continuous Gabor wavelet transform in the 0.01-0.12 Hz frequency range (*cwt* function in MATLAB R2020a). We then computed pairwise nodal correlation coefficients to create an adjacency matrix for the network in each subject/group. These calculations were performed using the Wavelet Toolbox and Statistics and Machine Learning Toolbox in MATLAB R2020a.

Network metrics

To assess group differences in connectivity parameters within the relevant network spaces, we used undirected graphs for simplicity in hypothesis testing and computational ease. The following metrics were computed on weighted graphical links:

1. *Assortativity coefficient*: the correlation coefficient between the degrees of all nodes on two opposite ends of a link^{11–13}. The details of calculating the assortativity coefficient are described elsewhere¹³. For a given network, we consider assortativity in a group of subjects to be increased if the assortativity coefficient, which is described as assortative for positive values, neutral for ≈ 0 , or disassortative for negative values, is significantly elevated compared to values for the same network

computed in a different group. Analogously, assortativity is considered to be reduced when the coefficient computed for the same network is lower than in a comparison group.

2. *Degree centrality*: the number of connections (edges) within the network or subgraph, divided by the total number of nodes in the same space.
3. *Clustering coefficient*: a measure of the likelihood that the nearest neighbors of a node will also be connected.
4. *Characteristic path length*: the shortest path length between two nodes averaged over all pairs of nodes in a given network. High characteristic path length implies less efficient information transfer through the network^{43,44}.
5. *Small-worldness*: the ratio of clustering coefficient to characteristic path length, normalized to corresponding parameters from an equivalent random graph⁴¹. This measure quantifies the ratio of segregation to integration of information sources in the network space.

These parameters were computed using the Brain Connectivity Toolbox⁴⁴ and an in-house Matlab script (MATLAB R2020a). We present assortativity and the other network metrics over a range of connectivity thresholds as described previously^{4,8}. In the current study, thresholds ranged from $r=0.3$ to 0.6 , at 0.05 increments, corresponding to graph densities between 25% and 60% ^{4,8}. At lower thresholds ($r<0.3$, graph density $>60\%$), however, group differences may be difficult to discern because of the inclusion of random, non-specific links. At higher thresholds ($r>0.6$, graph density $<25\%$), graphs may disconnect and distort group comparisons. By plotting the results over a range of thresholds, we demonstrated that group differences in a given metric were robust beyond two or three adjacent levels.

Stability of network metrics

To evaluate test-retest reliability for PDRP network metrics, the 8-min rs-fMRI acquisitions in the North Shore PD cohort ($n=20$) were divided into two 4-min scans, which were randomly assigned to the test or retest conditions as described elsewhere⁷. Test-retest reliability was evaluated by computing the intraclass correlation coefficient (ICC) and 95% confidence interval (CI) across subjects and correlation thresholds.

Visualization

To visualize assortativity effects along a graph, we generated 2D displays of the joint probability distribution of remaining degree (i.e., the total degree centrality for each node, minus the connected edge) for random pairs of nodes in the network space^{11,13}. This was done using the bivariate Gaussian copula with the correlation matrix $S=[1 \quad ; \quad 1]$. Poisson marginal distributions ($=1000$ random numbers, mean= λ) were computed for each variable, where ρ is the degree-degree correlation coefficient and λ is the average degree of the network^{11,13}. These two parameters were estimated from the empiric data from each group and network. The simulation steps were performed using an in-house Matlab script (Statistics and Machine Learning Toolbox, MATLAB R2020a).

To visualize differences in connectivity patterns across groups or conditions, graphs were displayed at the minimum threshold (Level 1, $r=0.30$) for a given network. For assortative configurations (mean $p>0$), an exemplar with assortativity coefficient between the mean value and the mean $+0.5$ sd was selected from the bootstrap samples described above. For disassortative configurations (mean $p<0$), an exemplar was selected with assortativity coefficient between the mean value and the mean -0.5 sd. Graphical configurations were constructed using an in-house Matlab script (mathematics/graph and network algorithms toolbox, MATLAB R2020a).

Lastly, to assess differences in nodal organization between groups and/or conditions, we ordered the nodes hierarchically according to degree centrality. For each configuration, we computed the Spearman rank-order correlation coefficient for the median graph at minimum threshold ($r=0.30$). Significant correlations ($p\leq 0.05$) reflected similar nodal hierarchy across groups or conditions. Uncorrelated rank order implied the presence of structural differences in the two graphs.

Statistical analysis

Network expression values were compared across groups using student's t -test or one-way analysis of variance (ANOVA) with Tukey-Kramer HSD post-hoc tests for multiple comparison correction. For the graph analysis, the bootstrapped data or subject data were used to assess group differences in the network parameters for the relevant subgraphs. Group differences in each of the parameters were evaluated using the general linear model with post-hoc Bonferroni tests across graph thresholds. These analyses were performed using IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, N.Y., USA) or GraphPad Software (Version 7.0, La Jolla California, USA). Results were considered significant for $p<0.05$, corrected.

Data availability

Deidentified data will be made available on reasonable request from interested investigators for the purpose of replicating results.

Declarations

Acknowledgments

The authors thank Drs. Susan Bressman, Rachel Saunders-Pullman, Matthew During, and Michael G. Kaplitt for subject referral and valuable discussions. Special thanks to Drs. Madhavi Tripathi and Maja Trost for sharing FDG PET data from their subjects, to the Parkinson's Disease Progression Markers Initiative (PPMI) of The Michael J. Fox Foundation for Parkinson's Research for access to the rs-fMRI database, and to V.L. Brandt and Mr. Peter Brown for helpful comments and editorial suggestions.

Funding: Aspects of this work were supported by the National Institute of Neurological Disorders and Stroke (P50 NS 071675 [Morris K. Udall Center of Excellence for Parkinson's Disease Research at The Feinstein Institute for Medical Research] to D.E.), The Michael J. Fox Foundation for Parkinson's Research

(Grant #14878 to A.V.), and Neurologix, Inc. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the National Institute of Neurological Disorders and Stroke. K.A.S. is the recipient of a Research Fellowship for Clinical Neurologists (PF-FBS-1929) from the Parkinson's Foundation and the Leopoldina Fellowship Program of the German National Academy of Sciences Leopoldina (LDS 2016-08).

Author contributions

A.V., K.A.S., and D.E. contributed to the conception and design of the study; M.N. and V.D. acquired the data; A.V., K.A.S., C.C.T., Y.Y.C. and D.E. performed voxel-based network analysis; N.N., A.V., K.A.S., P.G.S., and D.E. performed graph theory analysis and quantitative network measurements; A.V., K.A.S., N.N., A.R., and D.E. prepared the figures; and A.V., K.A.S. and D.E. drafted the article. All authors revised the article for intellectual content.

Competing interests

D.E. has received consultant fees from MeiraGTx and has received research support from the NIH (NINDS, NIDCD, NIAID) and The Michael J. Fox Foundation for Parkinson's Research. All other authors declare no relevant competing interests.

Materials and correspondence

Deidentified data will be made available on reasonable request to the corresponding author (D.E.) for the purpose of replicating results.

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Tables

Table 1: Group differences in PDRP network metrics

Scanning Modalities	Cohort (PD/HC) ¹	Assortativity coefficient	Degree centrality	Clustering coefficient	Char. path length	Small-worldness
FDG PET	North Shore (96/22)	+++	+++	0	+++	0
	Multisite (146/39)	+++	+++	–	+++	—
Resting-state fMRI	North Shore (20/20)	++	0	0	0	0
	Multisite (102/18)	+++	—	++	–	+

¹ See Table S1A-D.

PD = Parkinson’s disease; HC = healthy control

Increase: +, ++, +++: p<0.05, p<0.01, p<0.001 (PD > HC) for comparison of PD with HC (see text).

Decrease: -, --, ---: p<0.05, p<0.01, p<0.001 (PD < HC).

No difference: 0 (PD = HC)

Table 2: Treatment-mediated changes in PDRP network metrics

Intervention ¹ (number of subjects)	Assortativity coefficient	Degree centrality	Clustering coefficient	Characteristic path length	Small-worldness
Levodopa infusion (14) (<i>on</i> vs. <i>off</i> medication)	↑	0	0	↑	↓
STN AAV2-GAD (16) (12 months vs. baseline)	↓↓↓	↑↑↑	↓↓↓	↑↑↑	↓↓↓
Sham surgery (21) (12 months vs. baseline)	↑	0	↑↑↑	0	0

¹ See Table S2D-E.

Increase: ↑, ↑↑, ↑↑↑: $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. baseline (see text), for comparison of treatment with baseline.

Decrease: ↓, ↓↓, ↓↓↓: $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. baseline (see text), for comparison of treatment with baseline.

No change: 0

Figures

Figure 1

Figure 1

PDRP assortativity is increased in PD patients. (A) Assortativity measurements in the PDRP space are elevated in FDG PET scans from 96 clinically ascertained PD patients compared to 22 healthy control (HC) subjects scanned at the North Shore site¹⁵ (Table S1A). PDRP assortativity was likewise increased in FDG PET scans from a combined validation cohort comprising 146 PD and 39 HC subjects from two other sites (Table S1B; Fig. S1). (B) Resting-state fMRI scans from 20 PD patients and 20 HC subjects

studied at North Shore (Table S1C) also revealed significant assortativity elevations in this patient sample. Similar increases in network assortativity were seen in rs-fMRI scans from 102 PD and 18 HC subjects studied at other sites as part of the Parkinson’s Disease Progression Markers Initiative (PPMI) multisite database (Table S1C; see text). Consistent group differences were not seen for the other PDRP network metrics (Table 1). [Levels 1 to 7 correspond to cutoff graph thresholds of $r=0.3$ to 0.6 in increments of 0.05 . $**p<0.01$ $***p<0.001$ compared to HC, corrected for multiple comparisons (see Methods).]

Figure 2

Figure 2

PDRP assortativity increases with disease progression. (A) Longitudinal PD cohort: Assortativity in the PDRP space rose over time in PD patients (Table S2A). (B) Cross-sectional PD cohort: PDRP assortativity was elevated in the three PD groups compared to HC (Table S2B). Values were higher with each successive 4-year increment in disease duration. [Levels 1 to 7 correspond to cutoff graph thresholds of $r=0.3$ to 0.6 in increments of 0.05 . *** $p<0.001$ compared to HC; ††† $p<0.001$ compared to PD-TP1 (A) or PD-Early (B). (C) Two-dimensional (2D) displays of the joint probability distribution of degree–degree correlations in the PDRP space for the groups in (B). Whereas the healthy control (HC) group showed a relatively symmetrical distribution of probability (p) values around the mean (center), the PD-Early and PD-Late groups exhibited an assortative connectivity patterns: the density of high p values along the main diagonal (dashed lines) represents a tendency for PDRP connections to link nodes with similar degree centrality. [The joint probability distribution for degree–degree correlations in the PDRP space was generated at threshold level 4 (vertical arrow in (B)). The color scale represents the computed probability that a randomly selected link connects nodes with degrees d_i and d_j (see Methods). Because mean degree centrality differed for the three groups, the centers of the joint distributions also varied. For comparable visualization, the x and y axes were shifted so that the mean value for each group was at or near the center of the corresponding display. N =number of nodes; D =mean degree centrality; ρ =assortativity coefficient.]

Figure 3

Figure 3

Influence of genotype on PDRP assortativity. (A) PD-GBA patients showed higher assortativity than PD-LRRK2 or healthy control (HC) subjects in the PDRP space as a whole. (B) In the PDRP core zone (see text), PD-LRRK2 patients showed lower assortativity than HC or PD-GBA subjects. [Levels 1 to 7 correspond to cutoff graph thresholds of $r=0.3$ to 0.6 in increments of 0.05 , corrected for multiple comparisons (see Methods). ** $p<0.01$, *** $p<0.001$ for PD-LRRK2 or PD-GBA relative to HC. † $p<0.05$,

†† $p < 0.01$ for PD-LRRK2 relative to PD-GBA.] (C) Connectivity patterns in the PDRP core zone for the genotypic patient groups in (B). PD-LRRK2 (left) exhibited a neutral pattern, with a combination of assortative and disassortative connections linking subgraph nodes. By contrast, in PD-GBA (right), the connectivity pattern for the subgraph was assortative at the same threshold ($|r| \geq 0.3$). [Core nodes are represented by discs, with radius proportional to the corresponding degree centrality. Yellow=high degree (top quartile); blue=low degree (bottom quartile); gray=intermediate degree (middle quartile). Connections were thresholded at $|r| \geq 0.3$ and displayed as gray lines. N=number of nodes; D=degree centrality; ρ =assortativity.]

Figure 4

Figure 4

Influence of genotype on PDCP assortativity. (A) PDCP assortativity was elevated in PD-GBA patients compared to PD-LRRK2 and the control group (HC). (B) In the PDCP core zone, PD-GBA again showed the highest assortativity, and LRRK2 the lowest. * $p < 0.05$, *** $p < 0.001$ for PD-LRRK2 or PD-GBA relative to HC. ††† $p < 0.001$ for PD-LRRK2 relative to PD-GBA. (C) Two-dimensional (2D) displays of the joint degree–degree probability distribution in the PDCP core for the three groups. PD-LRRK2 exhibited disassortativity,

as evident by the density of high p values along the secondary diagonal (dotted line). PD-GBA, by contrast, showed high p values along the main diagonal (dashed line). The joint probability distribution was spherical for HC subjects in the same subgraph. The joint degree probability distribution was generated at threshold level 3 (vertical arrow in (B)) for each group. [The color scale represents the probability that a randomly selected link connects nodes with degrees d_i and d_j . Because mean degree centrality differed for the three groups, the centers of the joint distributions also varied. For comparable visualization, the x and y axes were shifted so that the mean value for each group was at or near the center of the corresponding display.] (D) Connectivity patterns in the PDCP core zone for the groups in (C). In PD-LRRK2, the subgraph exhibited a disassortative configuration, whereas the pattern was assortative in PD-GBA and neutral in HC (see text). [Core nodes are represented by discs, with radius proportional to the corresponding degree centrality. Yellow= high degree (top quartile); blue= low degree (bottom quartile); gray=intermediate degree (middle quartile). Connections were thresholded at $|r| \geq 0.3$ and displayed as gray lines. N =number of nodes; D =degree centrality; ρ =assortativity.]

Figure 5

Figure 5

PDRP network assortativity: Changes with treatment. (A) Levodopa infusion: In the PDRP space, assortativity was elevated in the unmedicated (off) state compared to the healthy control (HC) group (Table S2D), but rose even higher during intravenous levodopa infusion (on). [***p<0.001, off or on compared to HC; †p<0.05, on compared to off.] (B) STN AAV2-GAD: PDRP assortativity was elevated at baseline in the gene therapy group (Table S2E) compared to healthy control (HC) subjects but declined by

12 months after gene therapy. (C) Sham surgery: In this group (Table S2E), PDRP assortativity increased relative to baseline at 12 months, but did not differ significantly from HC at either time point. [Levels 1 to 7 correspond to cutoff graph thresholds of $r=0.3$ to 0.6 in increments of 0.05 . *** $p<0.001$ for baseline or 12 months vs. HC. $\dagger p<0.05$ $\dagger\dagger\dagger p<0.001$ for baseline vs. 12 months.]

Figure 6

Figure 6

Changes in the GADRP connectivity pattern with treatment versus sham surgery. (A) The GADRP connectivity pattern was assortative at baseline but transitioned to a less assortative, neutral configuration 12 months after treatment (Table S2E). (B) In the sham surgery group, the baseline pattern was assortative and remained so 12 months later. [Top: Threshold levels 1 to 7 correspond to cutoff thresholds of $r=0.3$ to 0.6 in increments of 0.05 . $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ for baseline or 12 months vs. HC; $\dagger p < 0.05$, $\dagger\dagger p < 0.01$ for baseline vs. 12 months. Middle, Bottom: Core nodes are represented by discs, with radius proportional to the corresponding degree centrality. High, low and intermediate degree nodes (top, bottom, middle quartile) are depicted in yellow, blue and gray, respectively. Connections were thresholded at $|r| \geq 0.3$ and displayed as gray lines. N=number of nodes; D=degree centrality; ρ =assortativity).]

Figure 7

A.

B.

Figure 7

Increase in connections linking PDRP core modules: A shared adaptive response. (A) Map of functional connections between PDRP core nodes in low-assortativity configurations associated with genotype (PD-LRRK28) or treatment (12 months after subthalamic (STN) AAV2-GAD4). In both groups, novel connections were observed between two previously identified modules (dashed circles) in the PDRP core zone22. [The PDRP network is composed of 38 nodes (Table S3A)22, represented by circles with radius

proportional to the corresponding degree centrality. The core zone is composed of 20 metabolically active regions (red circles) and 18 relatively underactive nodes (blue circles) which form the network periphery. PDRP node-to-node connections were displayed at minimum threshold ($|r|=0.3$) and represented by thin gray lines for PD-LRRK2. (B) In the PDRP core, both PD-LRRK2 and STN AAV2-GAD patients 12 months after gene therapy showed lower modularity than healthy controls (HC) subjects and PD patients scanned at baseline. [*** $p<0.001$ for the PD groups (gene therapy or PD-LRRK2) relative to HC. ††† $p<0.001$ for gene therapy baseline vs. 12 months or for gene therapy baseline vs. PD-LRRK2.]

Supplementary Files

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