Clinical relevance of cortical network dynamics in early primary progressive MS

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ABSTRACT

Background

Structural cortical networks (SCNs) reflect the covariance between the cortical thickness of different brain regions, which may share common functions and a common developmental evolution. SCNs appear abnormal in neurodegenerative conditions such as Alzheimer's and Parkinson's diseases, but have never been assessed in primary progressive multiple sclerosis (PPMS).

Objective

To test whether SCNs are abnormal in early PPMS and change over five years, and correlate with disability worsening.

Methods

Twenty-nine PPMS patients and 13 healthy controls underwent clinical and brain MRI assessments for five years. Baseline and five-year follow-up cortical thickness values were obtained and used to build correlation matrices, considered as weighted graphs to obtain network metrics. Bootstrap-based statistics assessed SCN differences between patients and controls and between patients with fast and slow progression.

Results

At baseline, patients showed features of lower connectivity ($p=0.02$) and efficiency ($p<0.001$) than controls. Over five years, patients, especially those with fastest clinical progression, showed significant changes suggesting an increase in network connectivity ($p<0.001$) and efficiency ($p<0.02$), not observed in controls.

Conclusion

SCNs are abnormal in early PPMS. Longitudinal SCN changes demonstrated a switch from low- to high-efficiency networks especially among fast progressors, indicating their clinical relevance.

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Key words: Primary progressive multiple sclerosis, structural covariance networks, cortical thickness, grey matter damage, robust statistical methods, bootstrapping.

INTRODUCTION

In primary progressive multiple sclerosis (PPMS), grey matter (GM) damage occurs from the earliest stages of the condition[1-4] and is associated with greater risk of disability accumulation.[1-3] Whole brain GM volume loss is commonly used as marker of GM damage in PPMS.[5] However, the strength of the correlation between GM damage and clinical deterioration in PPMS is only moderate. A possible explanation for that could be that the techniques used to assess GM volume loss are frequently based on averaged values over the whole brain, failing to capture the spatial variability of GM volume loss. Instead, innovative approaches such as covariance network analysis applied to cortical thickness data can account for the complexity of the spatial distribution of such GM damage, providing new insights into the pathogenic mechanisms underlying disability progression.[6]

Covariance networks allow the assessment of the interdependencies across variables of a system –generally a *biological system*, e.g. the brain GM volume– on a specific outcome. Moreover, they do so in a straightforward fashion, through the use of graph theory principles. In the brain, covariance networks have been successfully applied to cortical thickness data, where they are called *structural cortical networks* (SCNs).[7] SCNs are based on the covariance of GM thickness of different regions, being therefore able to capture important aspects of the spatial complexity of GM data. SCNs use the correlations between the GM thickness of the different areas as the main predictors of the clinical outcome and can potentially provide complementary information to more conventional analysis methods of structural data. Additionally, it has been shown that cortical areas with similar thickness or volumes share common functions and a common developmental evolution.[7]

In the healthy brain, SCNs have proved to behave as small-world networks, where transmission of information across the nodes of the network is done fast and efficiently thanks to a particularly small mean length of the shortest path, also known as *mean shortest path*,[6, 8] an estimate of the shortest distance between any pair of nodes in the network.[9] Thus, healthy SCNs can be considered as high efficiency networks. In neurodegenerative conditions such as MS, SCNs show disruptive patterns early in the disease course,[10-13] leading to suboptimal topological organisations.[14, 15] However, the behaviour of SCNs in progressive MS has never been assessed.

Here we tested the hypotheses that there are abnormalities in SCNs in PPMS patients when compared with controls and that the main characteristics of the SCNs, such as mean shortest path, local and global efficiency and nodal connectivity, significantly change over five years of follow-up. To understand whether SCNs changes reflect mechanisms that contribute to disability, the differences in SCNs characteristics between patients who showed a rapid worsening of disability and those who showed a slow worsening of disability were investigated.

METHODS

Subjects

All subjects included in this study belong to a prospectively followed-up cohort of 44 patients with a diagnosis of *early* PPMS (i.e. and less than five years from symptom onset) and 20 healthy controls (HCs). The clinical and demographic features of this cohort have been previously reported.[1, 16-18] Because of the nature of this study, where covariance networks were obtained with cortical thickness values and where such

networks had to have the same size at baseline and at follow-up to be comparable, we excluded 15 patients and seven HCs who had missed two or three time points. Patients with only one time point missing were included and the missing value was imputed using simple linear regression, as explained below.

All patients were clinically assessed using the Expanded Disability Status Scale (EDSS)[19] at baseline, and at 12, 24, 36 and 60 months, as reported elsewhere.[1, 16-18] An EDSS increase over the five-year follow-up period greater than 0.2 points/year (which was the median in the whole PPMS group) was considered as *fast* progression. We also included a group of healthy controls (HCs). All participants underwent MRI scans at all time points.

The study was approved by the local Ethics Committee and all participants provided informed written consent.

MRI analysis

All MRI scans were performed with a 1.5 T GE Signa Echospeed MRI (Milwaukee, WI) scanner. The scanner maximum gradient strength was 33 mT m^{-1}.

Acquisition of brain structural scans

For all subjects and for the purpose of this study, the following images at baseline and five-year follow-up were analysed: axial oblique, proton-density (PD), dual echo, fast spin echo images were acquired, as previously described;[1, 16-18] axial three-dimensional fast prepared spoiled gradient recall (3D-FSPGR, 3D T1-weighted) (resolution:

1.7×1.7×1.5mm^3). In April 2004 there was a scanner upgrade, which was taken into account in all subsequent analyses (see *statistical analysis* section).

We manually outlined T2 hyperintense WM lesions on the PD-weighted images using the semi-automated edge finding tool in JIM (JIM v6.0, Xinapse systems, Aldwincle, UK, http://www.xinapse.com). We co-registered PD-weighted lesion masks to the 3D-T1 images using a pseudo-T1 image generated by subtracting the PD from the T2-weighted image.[20] We transformed lesion masks from native space to 3DT1 space and the 3DT1 images were filled using a non-local patch match lesion filling technique.[21]

Measurement of cortical thickness

We calculated cortical thicknesses for 68 bilateral brain cortical areas using the FreeSurfer version 5.3 longitudinal stream.[22-24] Briefly, this included skull-stripping, intensity normalisation, non-linear registration to Talairach space, segmentation, estimation of brain surfaces, and surface parcellation. We visually assessed the final segmentation, and re-ran the pipeline after manual correction in cases of incorrect surface estimation. We performed an unbiased longitudinal image analysis creating a symmetric within-subject template.[25] Afterwards, in order to increase reliability and statistical power all the steps were re-initialised for each time point using the common information.[22] We extracted cortical thickness values for each cortical parcellation according to Desikan-Killiany atlas.[24]

Structural covariance network analysis

Construction of weighted structural covariance networks

We built SCNs for all subjects, at baseline and five-year follow-up, as follows:

1) Networks were built using the same number of subjects at both time points. Thus, any missing values in cortical thickness data at five-year follow-up were imputed, for each subject, applying a single imputation technique that used simple linear regression, where the values at the remaining time points were used to estimate the slope and hence the missing point. To minimise the introduction of bias, such single imputation technique was only applied if patients had missed, at most, one visit.

2) Any variability in the cortical thickness data related to lesion load, age or gender, for all subjects and time points, was removed regressing at once cortical thickness data over lesion load at baseline, mean cortical thickness at baseline, age and gender. Additionally, a variable indicating whether the images had been acquired before or after the upgrade (April 2004) was also included as a covariate. Controls were assigned a lesion load equal to zero mL, as previously done.[13, 26] In subsequent steps, we used the residuals of these regression models as the new, *adjusted*, cortical thickness values.[7]

3) For each group and time point, i.e. all PPMS patients, HCs, PPMS patients with fast disability worsening and PPMS with slow worsening, at baseline and five-year follow-up, pairwise Pearson's correlation matrices using *adjusted* cortical thickness values were obtained using MATLAB (The MathWorks, Inc., Natick, Massachusetts, USA).

4) We obtained correlation matrices with the absolute value of Pearson's correlation coefficients, which corresponded to weighted networks that reflected the strength of the association regardless of its sign.[7]

Obtaining network topological metrics

Each weighted matrix was considered as the numerical representation of a network with 68 nodes (i.e. 68 cortical areas) and edges that indicated the strength of the connection between two cortical areas. Thus, we obtained network topological metrics for each network, i.e. for each group and time point, using the freely available Brain Connectivity Toolbox[9] (https://sites.google.com/site/bctnet/) in MATLAB (The MathWorks, Inc., Natick, Massachusetts, USA). These metrics were:

1. Mean nodal strength, the average, across all nodes, of the nodal strength, defined as the sum of the correlation coefficients of the edges emerging from a given node. A node with a high strength indicates that the node is very well connected and a network with high mean nodal strength indicates a very well connected network. In this context, highly connected networks indicate a high degree of similarity across cortical regions.

2. Nodal clustering coefficient, which reflects the connectivity among the neighbours of a given node and can be understood as the probability that each two nodes that are connected to a given node are also connected among themselves. Therefore, the mean clustering coefficient is the average clustering coefficient across all nodes of the network.[27] In this context, a network with a high mean clustering coefficient would indicate that cortical regions have, in general, strong similarities regarding cortical thickness with neighbouring regions (in the network).

3. Mean shortest path (or *characteristic path length*, *L*), the average of the shortest path lengths between all pairs of nodes in the network.[9] Smaller values of mean shortest path infer more efficient information transfer between nodes and greater information integration within the network.[28]

4. Global efficiency, the reciprocal of the harmonic mean of the shortest path lengths of the entire network.[28]

5. Local efficiency, the reciprocal of the harmonic mean of the shortest path lengths of the *subgraph* (i.e. *subnetwork*) defined by the neighbours of a given node.[28] Mean local efficiency is the across-node average of local efficiency values of the nodes of the network.[9] Higher global and local efficiency values indicate greater ability to integrate information, globally and locally, respectively.

6. Modularity coefficient, which describes how well a network can be subdivided into groups of nodes (i.e. modules) highly correlated with each other.

Statistical analysis

Descriptive and structural imaging data analyses

We report descriptive statistics as mean (standard deviation [SD]) or median (range), depending on the nature of the variable. Changes in mean cortical thickness over five years were assessed using linear mixed-effects models. The dependent variable was 'cortical thickness' and the main explanatory variables were 'time', a 'categorical group indicator': patient/control, and an interaction term: 'time X group indicator', which assessed differences in the rates of change in cortical thickness between groups. All models were adjusted for age, gender, lesion load at baseline (HCs were assigned lesion load equal to zero) and upgrade status (i.e. before or after the scanner upgrade). In patients only, changes in lesion load over five years were assessed using similar linear mixed-effects models. The dependent variable was 'lesion load' and the main explanatory variable was 'time'. When patients with fast and slow progression were compared, an interaction term: 'time X group indicator' was also included as a covariate. Mixed-effects models allowed for three hierarchical levels: cortical thickness measurements at each time point, cortical area and subject. These models were run using all available data: baseline, 12, 24, 36 and 60 months, as explained in the

Supplementary methods. All the above described statistical analyses were carried out with Stata 14.2 (Copyright 1985-2015 StataCorp LLC).

SCN analysis in all PPMS patients and HCs

Given that all network parameters were obtained at the network level, i.e. we had a value for each network, classical statistical approaches could not be used to compute the 95% confidence intervals (95% CI) of the metric values at baseline and their changes over time. We used, instead, a bootstrap-based approach which has been previously applied[13] and is described in detail in the **Supplementary methods**.

SCN analysis in PPMS patients with fast and slow progression

The same steps described above were also applied to assess the 95% CIs of baseline, follow-up and change values for the network metrics in the groups of PPMS patients with fast and with slow progression.

Data availability

The models and data sets that have been generated during the current study are available, on reasonable request, from the corresponding author.

RESULTS

For this study, we included 29 patients (11 female) and 13 healthy controls (HCs). During the five-year follow-up, 13 patients were classified as showing *fast* disability progression and the remaining 16 *slow* progression. Patients had a greater decrease in cortical thickness over time than HCs ($p<0.001$), whereas no differences were seen between fast and slow progressors (**Table 1**).

Structural cortical network analysis

All PPMS patients vs HCs

At baseline, patients showed lower mean nodal strength (p=0.02) and longer mean shortest path (p<0.001) of their SCNs when compared to controls (**Table 2**; **Figure 1**). No differences were observed in other network metrics.

During the five years of follow-up, patients showed a significantly greater decrease in mean shortest path, denoting a more efficient network, than HCs (p=0.03). Patients also showed a significant increase in mean nodal strength (p<0.001) and mean clustering coefficient (p=0.01), which was not observed in HCs (**Table 3**; **Figure 2**).

When analysing regional changes in metrics that admit a node-level analysis, i.e. clustering coefficient and local efficiency, the most prominent changes in the SCN of patients with PPMS appeared in the post- and pre-central gyri and the entorhinal cortex. This regional pattern was not clearly observed in HCs' network (**Figure 3**).

PPMS patients with fast vs slow progression

At baseline, fast progressors showed a significantly shorter mean shortest path than slow progressors: 3.14 vs. 3.63 (p=0.04), indicating higher network efficiency (**Table 4**; **Figure 4**).

Over the five-year follow-up period, fast progressors showed a significant increase in mean nodal strength and mean clustering coefficient (p=0.02 and p=0.03, respectively), whereas slow progressors only showed changes in the nodal strength. Yet the

differences between patient groups in the rates of change in these metrics did not reach statistical significance. Fast progressors also showed significant changes in all nodal distance metrics indicating a progressive increase in network efficiency over time: mean shortest path decreased ($p<0.001$) and global and local efficiency increased ($p=0.02$ for both). Slow progressors, instead, only showed a decrease in mean shortest path ($p=0.01$) (**Table 5**; **Figure 5**).

A visual inspection of the changes in the SCN parameters that admit a node-level analysis, the most prominent changes in fast progressors mirrored the regional pattern of changes observed in the whole PPMS cohort. Instead, changes in slow progressors did not show any clear regional predominance (**Figure 3**).

DISCUSSION

In this longitudinal study, we describe, for the first time, the dynamics of SCNs in progressive MS. Our finding of an overall lower connectivity and efficiency of the patients' network as compared to the controls' network is in line with previous studies, which showed that cortical networks were disrupted in established MS.[10, 11, 15] In our study, as in He et al.'s study, we used cortical thickness data *adjusted* for lesion load and mean cortical thickness at baseline. Thus, although possible confounding effects derived from early brain damage had already been removed before obtaining our correlation matrices, we could still see a less efficient network in patients than in controls. As pointed out by He et al. (2009), these findings reflect a disrupted harmonisation of cortical thickness among brain cortical regions in our patients. Interestingly, neither at baseline nor at follow-up did patients and controls significantly differ in terms of mean cortical thickness. This means that SCN analysis is likely to

have detected morphological changes not seen using conventional mean-based cortical thickness metrics.[15]

When temporal network dynamics were analysed, all patients' networks showed a significant increase in both connectivity and efficiency metrics, which was not observed in the controls' network. This phenomenon was also –and especially– observed in the network of patients with the fastest progression, whereas slow progressors' network only showed minimal changes. Importantly, the hints of different behaviour between fast and slow progressors happened in the absence of significant differences between groups in the rates of change in cortical thickness over time, highlighting the fact that cortical thickness and cortical network analyses provide at least partly independent pieces of information. Additionally, when patients with fast and slow progression were compared, the fast progressors' network showed overall higher connectivity and efficiency values than the slow progressors' network at both baseline and five-year follow-up, in line with the observed longitudinal changes. However, this finding would be in apparent contradiction with the overall lower connectivity and efficiency of patients' network when compared to the controls' one and suggests a biphasic behaviour of the SCNs in patients: an initial efficiency loss would be followed by a gain in efficiency. Thus, whereas the initial stages of cortical atrophy may cause the initial disorganisation among cortical thickness of different brain regions leading to a less efficient network, the latest stages of atrophy may imply increasing homogenisation of cortical thickness across areas, leading to increased cortical network connectivity and efficiency.

Longitudinal changes observed in network topological properties in patients could be considered as network fingerprints in progressive MS. These changes describe a shortening of the mean shortest paths in the whole network and the local subnetworks formed of each cortical area and its neighbouring areas. Neighbourhood in the context of SCNs is determined by the presence of a statistical correlation between the cortical thickness of each pair of areas. Therefore, an increase in local efficiency would reflect an increased correlation among the cortical thickness of those areas that were already highly correlated. Conversely an increase in global efficiency would reflect a general strengthening in the correlations between the cortical thickness of each pair of areas, independently of whether they had already shown a high correlation or not, in line with the observed increase in network connectivity. Whether these changes are supporting a preservation of function or are actually maladaptive is a challenging question that is still unresolved in the context of connectivity analysis.

In our study, the increase in connectivity and local efficiency did not occur homogeneously throughout the whole brain. It mainly occurred in the post- and pre-central gyri, the sites for primary sensory and motor cortices, respectively, and also the entorhinal cortex, a medial temporal region largely involved in memory networks, as shown in **Figure 3**. Therefore, it could be possible that the clinical relevance of such abnormal cortical network changes was in fact related to the location of the regional changes in network connectivity. Damage in these regions has been repeatedly observed in neurodegenerative conditions.[29, 30] A recent post-mortem study showed extensive fibrinogen deposition in the motor cortex of people with progressive MS, which was not present in the healthy control cortex and which correlated with the degree of neurodegeneration.[30] Moreover, these regions have also been considered as

hubs, i.e. highly connected areas or nodes, in networks defined through diffusion-weighted MRI techniques.[31] Therefore, they may be especially vulnerable to damage in neurodegenerative conditions.

Our study has some limitations, in particular the relatively small sample size, especially in the control group. On the other hand, the homogeneity of this unique cohort, i.e. PPMS patients evaluated within five years of disease onset, the long follow-up and our robust statistical approach offset the impact of the small sample size. Yet our statistical approach was not primarily designed to address the problem of small sample size but to ensure unbiased statistical inferences, given the group-level nature of network metrics. The small sample size in the control group might have contributed to the lack of significant changes over time in controls. However, the size of the healthy control group was similar to the size of both patient groups, i.e. those with fast and with slow progression, and the fast progressors did show significant changes over time despite the small numbers. Thus, it is unlikely that the smaller size of the control group was responsible for the lack of significant changes over time observed in this group. Nonetheless, future studies with larger cohorts are necessary to confirm our findings.

Another potential limitation is that possible confounders were not always equally distributed across groups. Although we built our SCNs using cortical thickness values adjusting for covariates, it might be possible that some confounding effect of these variables remained after the adjustment. Additionally, to predict the missing cortical thickness values we assumed that cortical thickness dynamics over time varied in a linear fashion and the presence of non-linear behaviours of our cortical thickness values over time were not explored, given the relatively small sample size of our cohort. Future

studies with larger cohorts using more complex models to impute missing cortical thickness data and to further minimise the effect of potential confounders are warranted.

Finally, in our study we did not include deep GM regions to compute our networks. Nonetheless, it is possible that deep GM plays a major role in the complex biological system defined by the brain GM, especially in MS patients,[2] and its inclusion in future SCN studies in progressive MS should be considered.

In conclusion, we present results of the first longitudinal cortical network analysis in primary progressive MS, revealing aspects of cortical atrophy dynamics beyond data obtained from the conventional analysis of cortical thickness. In particular, we found lower connectivity and efficiency in PPMS patients' networks as compared to controls. For the first time we demonstrate an increase in connectivity and efficiency in patients with progressive MS over time, suggesting that SCN dynamics may have a biphasic behaviour. These changes, which were not observed in healthy controls, were driven by changes in patients with faster clinical deterioration over time, possibly reflecting their clinical relevance.

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Table 1. Demographic, clinical and neuroimaging data at baseline and over time

Variable	All PPMS N=29	Healthy controls N=13	PPMS vs HCs, p-value (*)	PPMS with fast progression N=13	PPMS with slow progression N=16	Fast vs slow progressors, p-value (*)
Demographic data						
Age at study onset [years], mean (SD)	46.241 (10.077)	34.083 (6.640)	**p<0.001**	43.462 (0.445)	48.5 (9.494)	p=0.1853
Gender, no. females (%)	11 (38%)	5 (38%)	p=0.823	5 (38%)	6 (38%)	p=0.958
EDSS score						
Baseline score, median (range)	4.0 (3.5 to 6.5)	-	-	4.0 (3.5 to 6.5)	4.0 (3.5 to 6.5)	p=0.862
five-year score, median (range)	6.25 (2.0 to 8.0)	-	-	6.5 (5.5 to 8.0)	4.0 (2.0 to 7.0)	**p<0.001**
Yearly rate of change (95% CI), p-value	0.221 (0.121 to 0.320), **p<0.001**	-	-	0.438 (0.329 to 0.547), **p<0.001**	0.048 (-0.047 to 0.144), p=0.324	**p<0.001**
Cortical thickness [mm]						
Baseline mean (SD)	2.549 (0.189)	2.514 (0.125)	p=0.517	2.543 (0.157)	2.553 (0.159)	p=0.820
five-year mean (SD)	2.500 (0.190)	2.504 (0.126)	p=0.940	2.489 (0.158)	2.499 (0.160)	p=0.821
Yearly rate of change (95% CI), p-value	-0.010 (-0.012 to -0.008), **p<0.001**	-0.002 (-0.005 to 0.0004), p=0.102	**p<0.001**	-0.011 (-0.013 to -0.008), **p<0.001**	-0.011 (-0.013 to -0.009), **p<0.001**	p=0.994
T2 lesion volume [mL]						
Baseline mean (SD)	20.114 (15.948)	-	-	18.597 (17.911)	19.549 (20.155)	p=0.889
five-year mean (SD)	35.475 (35.019)	-	-	45.186 (31.299)	22.403 (32.519)	**p=0.025**
Yearly rate of change (95% CI), p-value	2.533 (0.563 to 4.503), **p=0.012**	-	-	5.318 (2.241 to 8.395), **p=0.001**	0.571(-2.213 to 3.354), p=0.688	**p=0.006**

Table 1 (footnote). (*) Adjusted for age, gender and lesion load at baseline. *Abbreviations: CI: confidence interval; EDSS: Expanded Disability Status Scale; HCs: healthy controls; N: number of subjects; PPMS: primary progressive multiple sclerosis; SD: standard deviation.*

Table 2. Estimated baseline and five-year follow-up values of network parameters in PPMS patients and controls

	PPMS patients (all)	HCs	Patients vs HCs, estimated p-value
1. Measures of nodal connectivity			
Mean nodal strength, estimated value (bootstrap-based 95% CI)			
Baseline	11.642 (10.087 to 13.832)	16.741 (13.255 to 21.421)	**0.02**
Follow-up	17.757 (13.819 to 22.199)	21.122 (17.106 to 28.412)	0.40
Mean clustering coefficient, estimated value (bootstrap-based 95% CI)			
Baseline	0.1798 (0.156 to 0.210)	0.221 (0.178 to 0.282)	0.20
Follow-up	0.2482 (0.205 to 0.304)	0.282 (0.232 to 0.388)	0.50
2. Measures of nodal distance			
Mean shortest path, estimated value (bootstrap-based 95% CI)			
Baseline	4.554 (4.192 to 4.893)	3.2828 (2.951 to 3.625)	**<0.001**
Follow-up	3.495 (3.129 to 3.938)	2.8596 (2.482 to 3.169)	**0.02**
Global efficiency, estimated value (bootstrap-based 95% CI)			
Baseline	0.309 (0.274 to 0.345)	0.3596 (0.314 to 0.414)	0.10
Follow-up	0.369 (0.330 to 0.414)	0.4093 (0.364 to 0.491)	0.25
Mean local efficiency, estimated value (bootstrap-based 95% CI)			
Baseline	0.213 (0.187 to 0.245)	0.2566 (0.214 to 0.315)	0.20
Follow-up	0.278 (0.237 to 0.331)	0.3151 (0.266 to 0.413)	0.40
3. Measures of network organisation			
Modularity coefficient (bootstrap-based 95% CI)			
Baseline	0.095 (0.082 to 0.111)	0.097 (0.082 to 0.119)	0.90
Follow-up	0.098 (0.073 to 0.118)	0.100 (0.072 to 0.123)	0.90

Table 2 (footnote). Comparison between PPMS patients and HCs was made looking at the 95% CIs. Significant p-values are indicated in bold. *Abbreviations:* CI: confidence interval; HCs: healthy controls; PPMS: primary progressive multiple sclerosis.

Table 3. Five-year changes in SCN parameters, in PPMS patients and HCs

	PPMS patients (all)	HCs	Patients vs HCs, estimated p-value
1. Measures of nodal connectivity			
Mean nodal strength, estimated value (bootstrap-based 95% CI), p-value			
Estimated change	6.116 (1.938 to 9.847), **p<0.001**	4.381 (-0.075 to 10.450), p=0.06	0.70
Mean clustering coefficient, estimated value (bootstrap-based 95% CI), p-value			
Estimated change	0.0684 (0.020 to 0.125), **p=0.01**	0.061 (-0.003 to 0.153), p=0.06	0.90
2. Measures of nodal distance			
Mean shortest path, estimated value (bootstrap-based 95% CI), p-value			
Estimated change	-1.059 (-1.431 to -0.578), **p<0.001**	-0.423 (-0.754 to -0.149) **p<0.001**	**0.03**
Global efficiency, estimated value (bootstrap-based 95% CI), p-value			
Estimated change	0.060 (0.011 to 0.115), **p=0.02**	0.050 (-0.006 to 0.121), p=0.10	0.90
Mean local efficiency, estimated value (bootstrap-based 95% CI), p-value			
Estimated change	0.066 (0.018 to 0.119), **p=0.01**	0.058 (-0.003 to 0.144), p=0.07	0.90
3. Measure of network organisation			
Modularity coefficient (bootstrap-based 95% CI), p-value			
Estimated change	0.002 (-0.024 to 0.025), p=0.90	0.002 (-0.039 to 0.027), p=0.95	>0.99

Table 3 (footnote). Comparison between PPMS patients and HCs was made looking at the 95% CIs. Significant p-values are indicated in bold. *Abbreviations:* CI: confidence interval; HCs: healthy controls; PPMS: primary progressive multiple sclerosis

Table 4. Estimated baseline and five-year follow-up values of SCN parameters in PPMS patients with fast and slow progression

	Patients with fast progression	Patients with slow progression	Patients with fast vs slow progression, estimated p-value
1. Measures of nodal connectivity			
Mean nodal strength, estimated value (bootstrap-based 95% CI)			
Baseline	17.831 (14.869 to 22.819)	15.076 (12.480 to 18.933)	0.30
Follow-up	24.586 (18.892 to 32.238)	17.977 (14.741 to 23.207)	0.10
Mean clustering coefficient, estimated value (bootstrap-based 95% CI)			
Baseline	0.234 (0.195 to 0.299)	0.234 (0.207 to 0.278)	>0.99
Follow-up	0.327 (0.251 to 0.440)	0.243 (0.208 to 0.309)	0.20
2. Measures of nodal distance			
Mean shortest path, estimated value (bootstrap-based 95% CI)			
Baseline	3.136 (2.803 to 3.418)	3.631 (3.275 to 3.989)	**0.04**
Follow-up	2.588 (2.230 to 2.951)	3.2589 (2.890 to 3.598)	**0.02**
Global efficiency, estimated value (bootstrap-based 95% CI)			
Baseline	0.372 (0.331 to 0.428)	0.384 (0.352 to 0.425)	0.70
Follow-up	0.451 (0.388 to 0.542)	0.381 (0.344 to 0.436)	0.20
Mean local efficiency, estimated value (bootstrap-based 95% CI)			
Baseline	0.270 (0.230 to 0.333)	0.273 (0.245 to 0.317)	0.99
Follow-up	0.359 (0.287 to 0.466)	0.278 (0.243 to 0.341)	0.10
3. Measures of network organisation			
Modularity coefficient, estimated value (bootstrap-based 95% CI)			
Baseline	0.091 (0.075 to 0.112)	0.092 (0.077 to 0.110)	0.99
Follow-up	0.084 (0.052 to 0.108)	0.110 (0.092 to 0.128)	0.10

Table 4 (footnote). Significant p-values are indicated in bold. *Abbreviations:* CI: confidence interval; PPMS: primary progressive multiple sclerosis.

Table 5. Five-year changes in SCN parameters, in PPMS patients with fast and slow progression

	Patients with fast progression	Patients with slow progression	Patients with fast vs slow progression, estimated p-value
1. Measures of nodal connectivity			
Mean nodal strength, estimated value (bootstrap-based 95% CI), p-value			
Estimated change	6.755 (0.501 to 12.756), **p=0.02**	2.901 (0.213 to 6.936), **p=0.04**	0.40
Mean clustering coefficient, estimated value (bootstrap-based 95% CI), p-value			
Estimated change	0.093 (0.007 to 0.184), **p=0.03**	0.009 (-0.029 to 0.06), p=0.80	0.20
2. Measures of nodal distance			
Mean shortest path, estimated value (bootstrap-based 95% CI), p-value			
Estimated change	-0.548 (-0.843 to -0.179), **p<0.001**	-0.383 (-0.661 to -0.163), **p<0.001**	0.60
Global efficiency, estimated value (bootstrap-based 95% CI), p-value			
Estimated change	0.079 (0.010 to 0.147), **p=0.02**	-0.003 (-0.039 to 0.044), p=0.90	0.06
Mean local efficiency, estimated value (bootstrap-based 95% CI), p-value			
Estimated change	0.089 (0.008 to 0.172), **p=0.02**	0.0054 (-0.0320 to 0.0582), p=0.9	0.10
3. Measures of network organisation			
Modularity coefficient (bootstrap-based 95% CI), p-value			
Estimated change	-0.008 (-0.037 to 0.020), p=0.7	0.019 (-0.006 to 0.038), p=0.15	0.20

Table 5 (footnote). Significant p-values are indicated in bold. *Abbreviations:* CI: confidence interval; HCs: healthy controls; PPMS: primary progressive multiple sclerosis.

Figure legends

Figure 1. Network parameters at baseline in all PPMS patients and HCs

Figure 1. (Figure legend) This figure shows the point estimates for the baseline values of network parameters (thick vertical lines, in red for PPMS and black for HCs) and the bootstrap-based 95% CIs for those values, for all PPMS patients and HCs (thin vertical lines, in red for PPMS and black for HCs). The blue-purple histograms reflect the bootstrap distribution in PPMS patients, whereas the green histograms reflect the bootstrap distribution in HCs. Please look at the main text for more details. *Abbreviations:* CI: Confidence Interval; HCs: healthy controls; PPMS: primary progressive multiple sclerosis.

Figure 2. Five-year changes in network parameters in all PPMS patients and HCs

Figure 2. (Figure legend) This figure shows the point estimates for the five-year changes in network parameters (thick vertical lines, in red for PPMS and black for HCs) and the bootstrap-based 95% CIs for those changes, for all PPMS patients and HCs (thin vertical lines, in red for PPMS and black for HCs). The blue-purple histograms reflect the bootstrap distribution in PPMS patients, whereas the green histograms reflect the bootstrap distribution in HCs. Please look at the main text for more details. *Abbreviations:* CI: Confidence Interval; HCs: healthy controls; PPMS: primary progressive multiple sclerosis.

Figure 3. Five-year changes in clustering coefficient and local efficiency in all groups

Figure 3. (Figure legend) This figure shows the five-year changes in clustering coefficient and local efficiency for all four networks. **A**: all-PPMS network; **B**: HC network; **C**: fast-PPMS network; **D**: slow-PPMS network. As can be seen, all changes ranged from -0.11 to 0.11. Patients with fast clinical progression showed the greatest (positive) change in both clustering coefficient and local efficiency. *Abbreviations:* HCs: healthy controls; PPMS: primary progressive multiple sclerosis.

Figure 4. Network parameters at baseline in PPMS patients with fast and slow progression

Figure 4. (Figure legend) This figure shows the point estimates for the baseline values of network parameters (thick vertical lines, in red and black, for patients with fast and slow progression, respectively) and the bootstrap-based 95% CIs for those values, for PPMS patients with fast and slow progression (thin vertical lines, in red and black, for patients with fast and slow progression, respectively). The blue-purple histograms reflect the bootstrap distribution in PPMS patients, whereas the green histograms reflect the bootstrap distribution in HCs. Please look at the main text for more details. *Abbreviations:* CI: Confidence Interval; PPMS: primary progressive multiple sclerosis.

Figure 5. Five-year changes in network parameters in PPMS patients with fast and slow progression

Figure 5. (Figure legend) This figure shows the point estimates for the five-year changes in network parameters (thick vertical lines, in red and black, for patients with fast and slow progression, respectively) and the bootstrap-based 95% CIs for those changes, for PPMS patients with fast and slow progression (thin vertical lines, in red and black, for patients with fast and slow progression, respectively). The blue-purple histograms reflect the bootstrap distribution in PPMS patients, whereas the green histograms reflect the bootstrap distribution in HCs. Please look at the main text for more details. *Abbreviations:* CI: Confidence Interval; PPMS: primary progressive multiple sclerosis.