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Effect of anti-P ribosomal and anti-NR2 antibodies on depression and cognitive processes in SLE: an integrated clinical and functional MRI study

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ABSTRACT

Objectives To explore the effects of anti-ribosomal P protein (anti-P) and anti-N-methyl-D-aspartic acid receptor subunit 2 (anti-NR2) autoantibodies on depression and cognitive dysfunction and their relationships with functional brain connectivity in SLE.

Methods This cross-sectional study included adult patients who fulfilled the American College of Rheumatology/European Alliance of Associations for Rheumatology 2019 SLE criteria. Anti-P and anti-NR2 were quantified using ELISA. A 1-hour battery of neuropsychological testing interpreted by a neuropsychologist explored depressive symptoms (Center for Epidemiologic Studies Depression Scale, CES-D), cognitive domains and quality of life (SF-12). Resting-state functional connectivity (rs-fc) MRI analysis was performed within 1 month, and region-of-interest to region-of-interest (ROI-to-ROI) analyses with the graph theory were performed.

Results Thirty-three patients with SLE (9% male) were enrolled, mean age (SD) of 43.5 (14) years and median disease duration of 10.4 years (2.9–25.4). Anti-P was positive in 6 (18.2%) and anti-NR2 in 14 (42.4%) patients. Depressive symptoms were found in 14 (42.4%) patients using the CES-D (range 0–51). After correction for age, disease duration, disease activity and white matter lesion load, the CES-D score was independently associated with anti-P serum level (β=0.32; p=0.049) and prednisone daily dose (β=0.38; p=0.023). Nineteen patients (57.6%) showed at least a cognitive test alteration, but no significant association with autoantibodies was found. The rs-fc MRI analysis revealed an independent association between the anti-P serum levels and many altered brain ROI properties but no anti-NR2 and prednisone effects on the cerebral network.

Conclusions Anti-P was associated with brain network perturbation, which may be responsible for depressive symptoms in patients with SLE.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Antineuronal antibodies are blamed for contributing to the pathogenesis of neuropsychiatric SLE, but until now, their role has not been fully clarified yet.

WHAT THIS STUDY ADDS

⇒ Anti-ribosomal P and daily prednisone dose are associated with depressive symptoms in SLE.

⇒ Anti-ribosomal P antibodies exert a perturbation effect on functional MRI brain network properties in patients with SLE.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study may help disentangle the multifactorial pathogenesis of SLE neuropsychiatric manifestations, suggesting the need to monitor anti-ribosomal P levels and supporting the increasing awareness of reducing glucocorticoids in patients with SLE.

INTRODUCTION

SLE is a chronic autoimmune disease that can potentially involve any organ and system. Neuropsychiatric (NP) manifestations are common, and although their severity may range from mild symptoms to life-threatening complications, NPSLE is generally associated with higher hospitalisation rates and mortality.[1 2]

Cognitive dysfunction (CD) and mood disorder (MD) are among the most frequent NPSLE events, with a significant impact on socioeconomic, employment and health-related quality of life (QoL). They are more prevalent in SLE than in other chronic conditions, with CD afflicting up to 81% of patients with SLE,[3] MD up to 92%[4] and the major depressive-like episode being reported in about 25% of patients with SLE.[4] When primarily attributed to SLE activity, CDs and MDs are deemed consequent to a neuroinflammatory brain injury mediated by undetermined mechanisms potentially involving

autoantibodies, cytokines and complement activation.[5 6] However, CDs and MDs in patients with SLE can also be related to associated factors (eg, organ damage, pain and drug adverse effects) or completely unrelated to the disease (eg, relationship issues, primary NP disorders). Therefore, attributing CD and MD to SLE or unrelated co-occurring disorders is as challenging as pivotal to defining the correct therapeutic interventions.[7]Anti-ribosomal P protein (anti-P) and anti-N-methyl-D-aspartic acid receptor subunit 2 (anti-NR2) antibodies are blamed for contributing to the pathogenesis of CDs and MDs in patients with SLE. The positive rate of anti-P in SLE ranges between 10% and 40%, with high specificity for SLE (95–100%).[8] Anti-NR2 showed a similar prevalence ranging from 25% to 35%,[9 10] with lower specificity (70–80%).[11] In 1987, Bonfa *et al*[12] first described an association of the anti-P with lupus psychosis, lately corroborated by several studies. Through the binding with a neuronal surface protein antigen, an integral plasma membrane protein that exposes P antigenicity in neuronal cells, anti-P elicits a rapid increase in calcium influx and glutamatergic transmission by activating both AMPAR and NMDAR, which leads to apoptosis in cortical and hippocampal neurons and induces memory impairment and psychotic symptoms in rabbits.[13] Moreover, mice injected intracerebroventricularly with anti-P displayed depression-like behaviour.[14] In 2000, DeGiorgio *et al*[15] demonstrated that a murine monoclonal anti-double-stranded DNA antibody (anti-dsDNA) directed against the DWEYS peptides sequence in the extracellular, ligand-binding domain of the NR2 subunit induced neuronal damage and death by excitotoxicity in mice. In the mouse model, behavioural abnormalities and neuronal apoptosis were induced after breaking down the blood–brain barrier (BBB). Many human studies aimed to demonstrate how anti-P and anti-NR2 could be responsible for NPSLE manifestations found controversial results.

Some researchers investigated the mechanisms underlying behavioural disorders in SLE by examining brain abnormalities with functional MRI (fMRI), an imaging technique that measures the variations in blood flow to study brain activity following a task or at rest. A decreased hippocampus/parahippocampal gyrus activation during a spatial working memory task and the resting state has been observed in patients with SLE.[16] A few preliminary studies with fMRI also noted that patients with SLE use compensatory brain mechanisms to maintain cognitive function, leading to overuse that causes cognitive fatigue.[17] Nevertheless, the contribution of anti-P and anti-NR2 antibodies to cognitive dysfunction and depression in SLE and their relationship with structural and brain fMRI alterations have not been fully clarified yet, and neither has the pathogenesis hidden behind them.

The primary aims of this study were to explore the association of anti-P and anti-NR2 antibodies with depressive symptoms and cognitive performance in patients affected by SLE and to investigate the associated brain fMRI changes.

MATERIALS AND METHODS

Patient selection

Patients with SLE at the Lupus Clinic of Cagliari were enrolled in a cross-sectional study between April 2019 and February 2020. Study inclusion criteria were: (a) fulfilment of the American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology 2019 criteria, (b) age ≥18 years old and (c) being capable of giving consent.

Demographics, serological and clinical data, and ongoing medications, including prednisone (PDN) equivalent daily dose, were recorded. Disease activity was assessed by the SLE Disease Activity Index 2000 (SLEDAI-2K)[18] and Physician Global Assessment (PGA).[19] Organ damage was measured according to the Systemic Lupus International Collaborating Clinics/ACR Damage Index (SDI).[20] The SF-12 questionnaire, consisting of 12 questions combined in physical (12-PCS) and mental (12-MCS) components, was used to evaluate perceived QoL, with higher scores reflecting better perceived QoL. SF-12 mean values from a reference population were considered normal.

NP assessment

All patients performed a 1-hour battery of neuropsychological testing interpreted by a neuropsychologist (EP, AP). Neuropsychological testing explored memory (Rey Figure, Rey Words), psychomotor speed (Trail Making Test (TMT), Digit Symbol Test), visual-spatial processing (Rey Figure, Digit Symbol Test), reasoning/problem-solving (Digit Symbol Test), simple attention (TMT A–B), complex attention (Stroop Test), language (FAS) and executive functions (Rey Words, TMT, Stroop Test, Frontal Assessment Battery) whose results were corrected for education level and age. Depressive symptoms were screened using the Center for Epidemiologic Studies Depression (CES-D) Scale, and a high probability of the presence of a depressive episode[21] was defined in patients scoring ≥16.[4]

Exploratory biomarkers

Serum anti-P antibodies (cut-off 17 U/mL) were quantified using ELISA kits (IBL International, RE70141) for in vitro diagnosis per the manufacturer's instructions.

The quantification of serum anti-NR2 antibodies was performed by homemade ELISA using the DWEYSVWLSN peptide (ThermoFisher, UK), as described by Putterman and Diamond.[22] We used 10 healthy controls as a background and 2 SDs from the mean of healthy control optical density (OD) for selecting anti-NR2-positive sera. The OD was monitored at 405 nm using a multilabel plate reader (Chameleon: Hidex, Turku, Finland).

Magnetic resonance

MRI data

MRI examinations were performed within 1 month since the neuropsychological assessment using a Vantage Titan

3 Tesla scanner (Canon Medical Systems Corporation, Ōtawara, Japan) with a 32-channel head coil. Patients with a history of cerebrovascular diseases and those with other significant psychiatric, neurological or other primary central nervous system diseases, left-handed and claustrophobic were excluded from the imaging assessment.

The MRI scan protocol included: (a) structural three-dimensional T1-weighted fast field echo (3D-T1-FFE) sequences; (b) 3D fluid-attenuated inversion recovery (3D-FLAIR) imaging sequences; and (c) T2*-weighted field-echo echo planar imaging (T2*-FE-EPI) sequences. The 3D-T1-FFE and 3D-FLAIR sequences were used for white matter lesion load (WMLl) calculation, whereas 3D-T1-FFE and T2*-FE-EPI sequences were used for fMRI analysis.

WMLl calculation

In analogy to previous studies,[23–25] the WMLl calculation was performed in periventricular, juxtacortical and deep white matter (WM) using an automated brain lesion online tool.[26] First, for each subject, the 3D-T1-FFE and the 3D-FLAIR sequences were processed using the default pipeline to obtain the WM absolute volume (WMv) and the WML absolute volume (WMLv), expressed in cm^3. Then, the WMLl was calculated according to the following formula:

$$\text{WMLl} = \frac{\text{WMLv}}{\text{WMv}}$$

The WMLl results were expressed in percentages (%).

The accuracy of the analyses was verified by two blind expert neuroradiologists (MPo, LS), and the subjects whose analyses were judged inadequate by at least one of the neuroradiologists were excluded from the study.

Resting-state functional connectivity MRI analyses

The resting-state functional connectivity (rs-fc) MRI analysis was performed on the Matlab platform V.R2020b (Mathworks, California, USA) by using the CONN-fMRI toolbox V.20b[27 28] based on the SPM V.12 software package (Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm/). We performed two region-of-interest to region-of-interest (ROI-to-ROI) analyses with the graph theory,[27] using the CONN's default atlas for mapping the brain regions. Cortical and subcortical ROIs were extracted from the Harvard-Oxford atlas,[29] whereas the cerebellar regions were from the Automated Anatomical Labelling atlas.[30] In addition, the following network properties were calculated: average path length (the mean number of links that join one node to another), global efficiency and local efficiency (the property of a node of the network to exchange data among the other nodes at the global and local level, respectively), betweenness centrality (a measure of the centrality of a node within a graph), degree (the number of connections that join a single node to the other nodes of the network), cost (the actual number of links of the network as a proportion of the total number of possible links) and clustering coefficient (the nearest neighbours of a given node as a proportion of the maximum number of potential links).

Statistical analysis

Continuous variables with normal distribution are reported as mean (SD), and those with non-normal distribution are reported as median with 25th–75th IQR; categorical variables are absolute numbers and percentages. Differences between groups and subgroups were investigated using the Mann-Whitney test for continuous variables and the X^2 or the Fisher's test for categorical variables. In addition, linear regression evaluated the association between a single psychometric test scoring and anti-P, anti-NR2, anti-dsDNA, antiphospholipid antibodies (aPL), age, gender, disease duration, SLEDAI, PGA, SDI and ongoing treatments, including daily PDN dose. Variables showing p values of <0.10 were included in multiple regression models. Results are presented as partial correlation coefficient (r) and regression beta coefficient. The statistical significance was set for $p<0.05$.

Finally, two separated multiple regression models were built to evaluate the effects of anti-P and anti-NR2 serum levels on cerebral networks, including age, gender, disease activity, PDN daily dose and WMLl as covariates. Further analysis included the PDN daily dose as the dependent variable and age, gender, disease activity, and WMLl as covariates. In analogy to Porcu *et al*,[23] we used a conventional two-sided p value corrected for false discovery rate (p-FDR)<0.05 for identifying statistically significant correlations between ROIs and a one-sided positive cost value=0.15 as adjacency matrix threshold for network edges.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

RESULTS

Demographic, clinical and therapeutic features of the 33 enrolled patients with SLE are presented in table 1. Anti-P antibodies were positive in 6 (18.2%) and anti-NR2 in 14 (42.4%) patients; among them, 3 (9.1%) patients were positive for both.

Nineteen out of 33 patients (57.6%) showed at least a cognitive test alteration, 12 (36.4%) were classified as cognitive deficit according to the ACR nomenclature.[31] The most frequently involved domains were memory (27.3%), executive functions (21.9%), psychomotor speed/problem-solving (18.2%) and attention (15.2%) (table 2). Overall, cognitive tests scored worse in older patients (data not shown). No association was found between cognitive test results and disease duration, anti-P, anti-NR2, anti-dsDNA, aPL, PDN daily dose, disease activity and SDI.

A high probability of a depressive episode was found in 14 (42.4%) patients using the CES-D as a screening instrument, 11 (33.3%) were classifiable as depression

Table 1 Characteristics of the whole SLE cohort and of the anti-P-positive and anti-NR2-positive patients

	Whole SLE cohort (N=33)	Anti-P positive (N=6)	P value*	Anti-NR2 positive (N=14)	P value†
Age, years, mean (SD)	43.5 (14.0)	40.6 (12.2)	0.591	39.1 (14.2)	0.113
Gender, male	3 (9%)	0	0.392	0	0.119
Disease duration, months, median (IQR)	124.4 (34.7–305)	282.5 (188.5–414.1)	0.129	241.1 (81.2–332.4)	0.316
SLEDAI-2K, median (IQR)	4 (0–14)	7 (6–12.5)	0.420	6 (2–13.5)	0.394
SLICC-DI, median (IQR)	0 (0–1)	0 (0–1)	0.958	0 (0–1)	0.934
C3, mean (SD)	89.9 (20.8)	87.4 (19.9)	0.724	84.7 (20.3)	0.285
C4, mean (SD)	13.7 (6.6)	12.4 (5.2)	0.681	13.2 (6.0)	0.706
Antiphospholipid‡ antibodies	11 (33.3%)	2 (33.3%)	1	4 (26.7%)	0.618
anti-dsDNA	18 (54.5%)	5 (83.3%)	0.117	9 (60%)	0.335
Anti-Ro/SSA	16 (48.5%)	3 (50%)	0.935	8 (53.3%)	0.393
Anti-La/SSB	4 (12.1%)	0	0.315	2 (13.3%)	0.744
Anti-RNP	11 (33.3%)	3 (50%)	0.338	4 (26.7%)	0.618
anti-Sm	10 (30.3%)	2 (33.3%)	0.858	5 (33.3%)	0.561
SF-12 questionnaire, median (IQR)	35.0 (31.0–37.0)	31.5 (21.0–38.0)	0.467	32.0 (28.0–37.0)	0.486
SF-12 MCS, median (IQR)	19.0 (21.0–16.5)	16.5 (13.0–18.0)	0.159	18.0 (16.0–19.0)	0.134
SF-12 PCS, median (IQR)	14.5 (13.0–17.0)	15.0 (12.0–18.0)	0.979	16.0 (14.0–19.0)	0.167
Dose PDN, mg/daily, median (IQR)	6.4 (3.8–13.5)	10.8 (8.2)	0.640	8.8 (4.4–12.7)	0.412

Unless otherwise specified, values are absolute numbers and values in brackets are percentages.
*P values obtained comparing anti-P-positive and anti-P-negative patients.
†P values obtained comparing anti-NR2-positive and anti-NR2-negative patients.
‡Lupus anticoagulant and/or anticardiolipin IgM/IgG and/or anti-β2-glycoprotein-1 IgM/IgG.
anti-NR2, anti-N-methyl-D-aspartic acid receptor subunit 2; anti-P, anti-ribosomal P protein; dsDNA, double-stranded DNA; MCS, mental component summary; PCS, physical component summary; PDN, prednisone; SLEDAI-2K, SLE Disease Activity Index 2000; SLICC-DI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

according to the ACR nomenclature.[31] The CES-D scoring correlated with anti-P serum levels (r=0.355, p=0.043) and with PDN daily dose (r=0.405, p=0.019) (figure 1). No significant association was found between CES-D and age, disease duration, anti-NR2 titre, anti-dsDNA titre, aPL, disease activity and SDI (data not shown). When evaluated after correction for age, disease duration and disease activity, the CES-D scores confirmed an independent association with anti-P serum levels (β=0.32 per U/mL; p=0.049) and PDN daily dose (β=0.38 per mg/day; p=0.023).

QoL measured by the SF-12 was impaired in 25 (78.1%) patients and negatively correlated with CES-D score (r=−0.691; p<0.001) and PDN daily dose (r=−0.561, p<0.001). When evaluated after correction for age, disease duration, disease activity and SDI, the SF-12 scores confirmed an independent association with CES-D scores (β=−0.36 per point; p<0.001) and PDN daily dose (β=−0.25 per mg/day; p=0.017). In addition, the SF-12-MCS correlated with both the CES-D score (r=−0.722; p<0.001) and PDN daily dose (r=−0.467, p<0.001), whereas the SF-12-PCS showed an exclusive correlation with CES-D score (r=−0.403; p=0.022).

MRI results

Twenty patients were eligible for brain MRI volumetric assessment, and 17 were deemed adequate by the two neuroradiologists for rs-fc MRI analysis. Online supplemental table 1 reports attrition analysis, with descriptive data comparing the whole cohort of patients with SLE with the subgroup of those who performed brain MRI, showing no relevant differences between groups.

The volumetric brain MRI results are reported in table 3. No correlation was found between volumetric brain MRI and sex, disease duration, anti-P, anti-NR2, aPL, anti-dsDNA, PDN daily dose and disease activity.

The rs-fc MRI analysis of the effects of anti-P serum levels on cerebral networks revealed several statistically significant changes in many properties of the ROI of the brain (figure 2). The average path length was increased in 22 ROIs (12 cerebellar and 10 supratentorial ROIs) and especially in the left parahippocampal gyrus (p-FDR=0.001), right accumbens (p-FDR=0.001), left temporal gyrus (p-FDR=0.002), vermis (p-FDR=0.004), frontal medial (p-FDR=0.004) and subcallosal (p-FDR=0.004) cortex. The global efficiency resulted reduced in nine ROIs (three cerebellar

Table 2 Neuropsychological testing results reported according to the cognitive domain investigated by each test

Cognitive domains	Tests	N=33
Executive functions, memory, visual-spatial processing, praxis skills	Rey Figure Copy, mean (SD)	32.4 (3.1)
	Rey Figure Copy, altered	0
	Rey Figure Immediate, mean (SD)	13.5 (7.5)
	Rey Figure Immediate, altered	5 (15.6%)
	Rey Figure Delayed, mean (SD)	12 (7.6)
	Rey Figure Delayed, altered	8 (24.2%)
Memory	Rey Words Immediate, mean (SD)	35.6 (9.8)
	Rey Words Immediate, altered	6 (18.2%)
	Rey Words Delayed, mean (SD)	7.5 (2.7)
	Rey Words Delayed, altered	2 (6.1%)
	Rey Words Recognition, mean (SD)	0.9 (0.1)
	Rey Words Recognition, altered	9 (27.3%)
Simple attention, shifting (B-A), executive functions, psychomotor speed	TMT A, mean (SD)	63.5 (24.8)
	TMT A, altered	5 (15.2%)
	TMT B, mean (SD)	126.7 (50.8)
	TMT B, altered	0
	TMT B-A, mean (SD)	62.3 (45.9)
	TMT B-A, altered	1 (3%)
Complex attention, executive functions	Stroop Test errors, mean (SD)	1.3 (0.)
	Stroop Test errors, altered	0
	Stroop Test time, mean (SD)	26.7 (12.5)
	Stroop Test time, altered	4 (12.1%)
Language (eg, verbal fluency, semantic skills)	FAS fl verbal, mean (SD)	33.6 (8.7)
	FAS fl verbal, altered	2 (6.3%)
	FAS fl semantic, mean (SD)	17.1 (3.6)
	FAS fl semantic, altered	1 (3.1%)
Executive functions	FAB, mean (SD)	16.2 (1.9)
	FAB, altered	7 (21.9%)
Psychomotor speed, visual-spatial processing, reasoning/problem-solving	Digit Symbol Test, mean (SD)	42.6 (12.4)
	Digit Symbol Test, altered	6 (18.2%)
Depressive symptoms	CES-D total, mean (SD)	15.2 (10.7)
	CES-D, altered	14 (42.4%)

Unless otherwise specified, values are absolute numbers and values in brackets are percentages.
CES-D, Center for Epidemiologic Studies Depression; FAB, Frontal Assessment Battery; TMT, Trail Making Test.

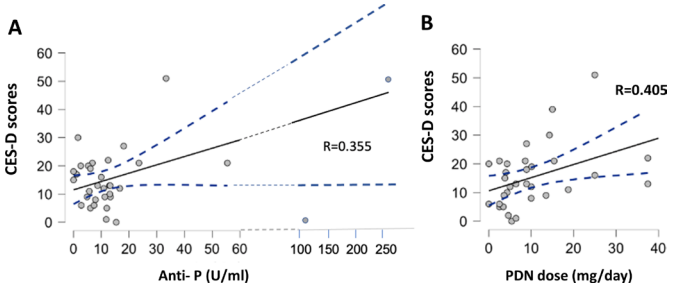

Figure 1 Correlation between CES-D scores and (A) anti-P titre or (B) PDN daily dose (scatter plot). anti-P, anti-ribosomal P protein; CES-D, Center for Epidemiologic Studies Depression; PDN, prednisone.

and six supratentorial ROIs), including the left parahippocampal gyrus (p-FDR=0.034), right accumbens (p-FDR=0.03), left temporal gyrus (p-FDR=0.034), vermis (p-FDR=0.034), medial frontal (p-FDR=0.047) and subcallosal (p-FDR=0.034) cortex. The local efficiency was reduced in the medial frontal cortex (p-FDR=0.018) and the vermis (p-FDR=0.018). The betweenness centrality was increased in seven ROIs (one cerebellar and six supratentorial ROIs); degree and cost resulted increased in four supratentorial ROIs and reduced in vermis 4 and 5; no ROI showed significant statistical modification in terms of clustering coefficient (detailed results in online supplemental table 2).

Table 3 Volumetric MRI data calculated in periventricular, juxtacortical and deep white matter areas

Volumetric MRI data	(N=20)
Sex, male (%)	3 (15)
Age, mean (SD)	42.2 (12.5)
Cerebral volume, cm^3, mean (SD)	1298.0 (145.6)
Total lesion count, median (IQR)	10 (7–13)
Total lesion volume (absolute), cm^3, median (IQR)	0.95 (0.29–1.68)
Total lesion volume (normalised), %, median (IQR)	0.07 (0.02–0.14)
Total lesion burden, median (IQR)	0.18 (0.06–0.37)
Periventricular lesion count, median (IQR)	6 (5–8)
Periventricular lesion volume (absolute), cm^3, median (IQR)	0.75 (0.19–1.36)
Periventricular lesion volume (normalised), %, median (IQR)	0.06 (0.02–0.1)
Periventricular lesion burden, median (IQR)	0.15 (0.04–0.24)
Juxtacortical lesion count, median (IQR)	1 (1–3)
Juxtacortical lesion volume (absolute), cm^3, median (IQR)	0.12 (0.01–0.31)
Juxtacortical lesion volume (normalised), %, median (IQR)	0.01 (0–0.02)
Juxtacortical lesion burden, median (IQR)	0.02 (0–0.06)
Deep white lesion count, median (IQR)	2 (1–6)
Deep white lesion volume (absolute), cm^3, median (IQR)	0.01 (0–0.05)
Deep white lesion volume (normalised), %, median (IQR)	0
Deep white lesion burden, median (IQR)	0

The rs-fc MRI analysis of the effects of anti-NR2, anti-dsDNA and PDN doses on cerebral networks did not find any statistically significant change.

DISCUSSION

This study assessed a wide range of cognitive function areas and depressive symptoms, investigating their association with anti-P and anti-NR2 autoantibodies in a cohort of patients with SLE. The most important finding to emerge from this study is the possible independent correlation between anti-P serum levels and depressive symptoms, potentially due to the perturbation effect of anti-P on the cerebral network, as evinced by rs-fc MRI analysis. Furthermore, we provided data on the independent association between CES-D score, PDN daily dose and low patient-perceived QoL.

A meta-analysis of 12 studies reported the association between anti-P and depression (pooled OR 3.03; 95% CI 1.32 to 6.95) in patients with SLE,[32] even if no association with NPSLE flares was reported.[33] The mechanisms underlying the association with depression have not been clarified yet. Our rs-fc MRI analysis of ROI-to-ROI connectivity showed that anti-P antibodies are independently associated with several significant deranging effects on the cerebral network supporting their role as responsible for mood disorders in patients with SLE. According to the graph theory,[34] a random and complex network, such as a brain network, should have short mean path lengths and high efficiency in interaction between nodes. In contrast, we observed an opposite situation in our patients' brains, with increased average path length and reduced global and local efficiency. The decreased efficiency reflected the reduction in effective interaction and neural information transmission across both remote (global) and neighbouring (local) cortical regions.[35] As already observed by Mackay *et al*,[36] these alterations in the network efficiencies mirror a disruption in the topological organisation because of the disease. In our cohort, ROI alterations were mainly located in the temporal and frontal medial cortex, parahippocampal gyrus and cerebellar structures, with a predilection for the vermis. Our results establish a link between previous fMRI studies in patients with primary depression, showing neural circuitry alterations in the medial frontal cortex, temporal gyrus, hippocampus, thalamus[37–39] and cerebellum,[40] and immunohistological studies proving that anti-P bound to neurons in specific areas of mice brains, including the cingulate cortex, the dentate gyrus of the hippocampus and the piriform cortex.[14]

Preziosa *et al*[41] showed that more severe structural global and nodal abnormalities were found in patients with SLE with anti-dsDNA positivity, irrespective of the serum levels. Nevertheless, another study by the same authors described similar network alterations in patients with multiple sclerosis.[42] The potential role of anti-dsDNA in brain damage is still unclear. The most accredited hypothesis assumed that a subset of circulating anti-dsDNA cross-reacts with NR2, causing neuronal damage and eventually death.[43] Although we found no association between NP manifestations, altered functional networks on rs-fc MRI analysis and anti-dsDNA or anti-NR2 serum levels, it might still be possible that they play a role in NPSLE pathogenesis. On the other hand, our results support the role of anti-P in causing cerebral network derangement resulting in mood and behavioural disorders.

The most accredited theory assumes that SLE-related autoantibodies cross the BBB to access neuronal tissue and play their pathogenetic role.[5] BBB permeability can increase in several conditions including in patients with SLE with high disease activity, which is also associated with higher serum levels of anti-P.[32 44] Moreover, a growing body of scientific evidence supports that autoantibodies found in the blood of patients with NPSLE are first produced in the brain parenchyma and then enter the systemic circulation by reverse crossing the BBB.[45] Unfortunately, we could not check for autoantibodies in cerebrospinal fluid (CSF), which represents a limitation of this study.

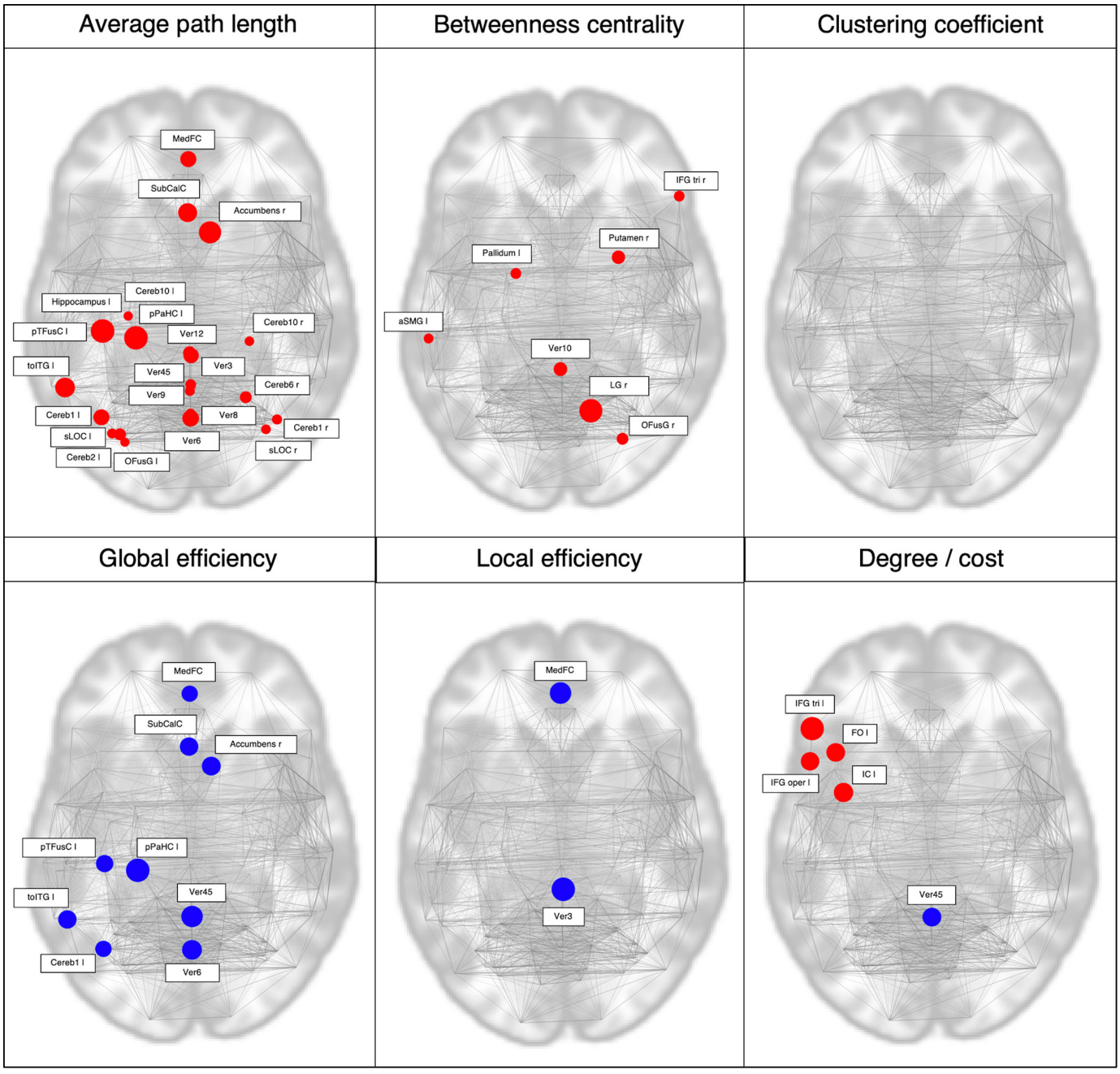

Figure 2 Results of resting-state functional connectivity MRI analysis on the effect of anti-P serum levels on cerebral networks. The regions with decreased and increased properties are shown in blue and red nodes, respectively (p-FDR<0.05). The node size represents the statistical significance magnitude of the between-group differences in the nodal degree. anti-P, anti-ribosomal P protein; aSMG, supramarginal gyrus anterior division; Cereb, cerebellum; FO, frontal operculum cortex; IC, insular cortex; IFG oper, inferior frontal gyrus pars opercularis; IFG tri, inferior frontal gyrus pars triangularis; l, left; LG, lingual gyrus; MedFC, medial frontal cortex; OFusG, occipital fusiform gyrus; p-FDR, p value corrected for false discovery rate; pPaHC, parahippocampal gyrus; pTFusC, temporal fusiform cortex posterior division; r, right; sLOC, lateral occipital cortex superior division; SubCalC, subcallosal cortex; tolTG, inferior temporal gyrus temporo-occipital part; Ver, vermis.

Along with these new observations, we confirmed previous reports of an independent correlation between the degree of depressive symptoms evaluated using the CES-D and PDN daily dose.[42] The independent association of anti-P and PDN daily dose with depressive symptoms corroborated the clinical observation that depression in SLE may be related to different mechanisms. Indeed, depression may depend on the disease itself (eg, anti-P) or may depend on factors secondary to SLE (eg, PDN daily dose, reactive depression, psychosocial stress), which also explains how challenging could be the process of attributing depression in patients with SLE.[46] It is universally recognised that high dosages of glucocorticoids can independently contribute to mood disorders, especially mania and psychosis,[47] but a recent study showed that PDN ≥7.5 mg/day was independently associated with depression over time.[48] Less is known about

the effect of lower doses of PDN, but our results add some novelty showing that the CES-D score accrued 1 point for every 0.4 mg/day of PDN, suggesting that even a low dose of glucocorticoids may influence the development of depressive symptoms in SLE. As glucocorticoid dose is a modifiable factor, our results would support the increasingly widespread awareness of reducing and withdrawing glucocorticoids in patients with SLE after achieving remission or low disease activity as the treatment target.[49]

Finally, we found that more than half of our patients with SLE presented with CDs in at least one domain according to a 1-hour neuropsychological test battery. Nevertheless, we found no correlation with autoantibodies or other disease-related factors such as duration, activity or brain damage. These negative results may have several potential explanations, including the small sample size. Therefore, other neuropathogenic factors must be explored to explain better the disease's potential role in developing CD.

Our study has some limitations. First, the sample size should be increased in future research to improve the statistical power. Moreover, the cross-sectionally reported data do not provide answers on long-term outcomes. Unfortunately, our study was interrupted due to the COVID-19 pandemic, but a longitudinal study is ongoing to provide answers to the modifications on depressive symptoms according to fluctuating levels of anti-P serum levels and PDN daily dose. Second, given the observational design of this study, autoantibodies were not explored in CSF because there were no clinical reasons to perform a lumbar puncture in our patients. Third, this study was performed in patients with SLE with longstanding disease, with a potential accrual of structural brain damage that can lead to reduced cerebral performance or compensatory brain mechanisms. Nevertheless, adjusted regression models were built to minimise this risk, and functional cerebral network modifications were unrelated to disease duration, damage and WML1. Finally, we were not able to explore the effect of treatment other than PDN dose, including antidepressants, anticonvulsants, immunosuppressants and biologics on depressive symptoms and cognitive dysfunction in patients with SLE, which deserve further specific investigation in properly designed studies with adequate sample size.

In conclusion, our study may help in disentangling the pathogenetic effect of anti-P in patients with SLE suffering from depressive symptoms. In particular, anti-P might have a perturbation effect on brain network properties in patients with SLE, which suggests the need to monitor and investigate the effect of targeting anti-P levels on depressive symptoms but needs to be confirmed in prospective studies. These findings increase the evidence of multifactorial pathogenesis in neuropsychiatric manifestations and show how direct (eg, antineuronal antibodies) and indirect (eg, glucocorticoids) factors are involved in developing depression in patients with SLE.

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Contributors Study conception—EC, MPi and AM. Study design—EC, MPi, MGC, LS and AC. Acquisition, analysis or interpretation of the data—AP, EP, MPo, CS, MC, MMA and AF. Drafting the manuscript—EC and MPi. All authors provided substantial manuscript revision and read and approved the final manuscript. MPi has full responsibility for the work and/or the conduct of the study as guarantor, had access to the data and controlled the decision to publish.

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Patient consent for publication Not required.

Ethics approval This study involves human participants and the Independent Ethical Committee of AOU Cagliari (protocol number PG/2019/4522) approved the human experimental design and procedure. All participants gave their written informed consent.

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Supplementary table 1. Attrition analysis on the SLE patients performing the brain MRI analysis vs. SLE patients excluded from the brain MRI analysis showed no major differences between groups.

	Whole SLE cohort (N=33)	Brain MRI SLE cohort (N=20)	p
Age, years mean (DS)	43.5 (14.0)	41.9 (12.2)	0.577
Gender, male	3 (9%)	3 (15%)	0.406
Disease duration, months median (IQR)	124.4 (34.7-305)	176.3 (77.4-417.3)	0.886
SLEDAI-2k, median (IQR)	4 (0-14)	6 (2-10.8)	0.107
SLICC-DI, median (IQR)	0 (0-1)	0 (0-1)	0.078
Anti-P	6 (18.2%)	4 (21.1%)	0.504
Anti-NR2	14 (42.4%)	8 (42.1%)	0.782
C3, mg/dl mean (DS)	89.9 (20.8)	86.6 (24.1)	0.714
C4, mg/dl mean (DS)	13.7 (6.6)	13.4 (5.2)	0.974
Antiphospholipid#	11 (33.3%)	10 (52.6%)	0.126
anti-dsDNA	18 (54.5%)	10 (50%)	0.502
Anti-Ro/SSA	16 (48.5%)	6 (31.6%)	0.021
Anti-La/SSB	4 (12.1%)	2 (10.5%)	0.813
Anti-RNP	11 (33.3%)	4 (21.1%)	0.024
anti-Sm	10 (30.3%)	5 (26.3%)	0.635
SF-12 questionnaire, median (IQR)	35.0 (31.0-37.0)	32 (21.0-36.0)	0.835
SF-12 MCS, median (IQR)	19.0 (21.0-16.5)	18 (13.8-20.0)	0.014
SF-12 PCS, median (IQR)	14.5 (13.0-17.0)	14.5 (11.8-17.3)	0.059
Dose PDN mg/daily, median (IQR)	6.4 (3.8-13.5)	8.1 (3.9-14.5)	0.566

Unless otherwise specified, values are absolute numbers, and values in brackets are percentages. # Lupus anticoagulant and/or anticardiolipin IgM/IgG and/or anti-B2glicoprotein1 IgM/IgG. MCS : mental component summary PCS : physical component summary.

Supplementary table 2. Table reporting the results of the resting-state functional connectivity (rs-fc) MRI analysis of the effects of Anti-P serum levels on cerebral networks, according to brain theory, using the CONN's default atlas for mapping the regions. The significant results (p-FDR < 0.05) of the correlation between two region of interest to region of interest are reported for each property of the brain (as represented in figure 2).

Average path length					
ROI (according to the Conn's default atlas)	**beta**	**T**	**dof**	**p-unc**	**p-FDR**

Network	<0.01	2.99	8	0.017219	-
atlas.pTFusC l (Temporal Fusiform Cortex, posterior division Left) x,y,z = (-36,-30,-25) mm	0.01	9.13	8	0.000017	0.00111
atlas.pPaHC l (Parahippocampal Gyrus, posterior division Left) x,y,z = (-22,-32,-17) mm	<0.01	9.12	8	0.000017	0.00111
atlas.Accumbens r (Accumbens Right) x,y,z = (9,12,-7) mm	0.01	8.58	8	0.000026	0.001158
atlas.toITG l (Inferior **Temporal Gyrus**, temporooccipital part Left) x,y,z = (-52,-53,-17) mm	<0.01	7.64	8	0.000061	0.002013
atlas.SubCalC (Subcallosal Cortex) x,y,z = (-0,21,-15) mm	<0.01	7.32	8	0.000083	0.002179
atlas.Ver6 (Vermis 6) x,y,z = (1,-66,-16) mm	<0.01	6.44	8	0.0002	0.004308
atlas.MedFC (**Frontal Medial Cortex**) x,y,z = (0,43,-19) mm	<0.01	6.21	8	0.000257	0.004308
atlas.Cereb1 l (Cerebelum Crus1 Left) x,y,z = (-36,-66,-30) mm	<0.01	6.19	8	0.000261	0.004308
atlas.Ver3 (Vermis 3) x,y,z = (1,-40,-11) mm	<0.01	5.91	8	0.000356	0.005225
atlas.Ver12 (Vermis 1 2) x,y,z = (1,-39,-20) mm	0.01	5.09	8	0.000936	0.012361
atlas.Cereb2 l (Cerebelum Crus2 Left) x,y,z = (-29,-73,-38) mm	<0.01	4.64	8	0.001675	0.018863
atlas.Cereb6 r (Cerebelum 6 Right) x,y,z = (24,-58,-25) mm	<0.01	4.62	8	0.001715	0.018863
atlas.Ver45 (Vermis 4 5) x,y,z = (1,-52,-7) mm	<0.01	4.19	8	0.003027	0.030732
atlas.Cereb10 l (Cerebelum 10 Left) x,y,z = (-23,-34,-42) mm	0.01	4.29	7	0.003602	0.033959
atlas.Cereb1 r (Cerebelum Crus1 Right) x,y,z = (38,-67,-30) mm	<0.01	3.93	8	0.004362	0.036503
atlas.Ver9 (Vermis 9) x,y,z = (1,-55,-35) mm	<0.01	3.92	8	0.004431	0.036503
atlas.sLOC l (Lateral Occipital Cortex, superior division Left) x,y,z = (-32,-73,38) mm	<0.01	3.86	8	0.004779	0.036503
atlas.Ver8 (Vermis 8) x,y,z = (1,-64,-34) mm	<0.01	3.84	8	0.004978	0.036503
atlas.sLOC r (Lateral Occipital Cortex, superior division Right) x,y,z = (33,-71,39) mm	<0.01	3.79	8	0.005344	0.037124
atlas.Hippocampus l x,y,z = (-25,-23,-14) mm	<0.01	3.62	8	0.006794	0.044841
atlas.OFusG l (Occipital Fusiform Gyrus Left) x,y,z = (-27,-77,-14) mm	<0.01	3.58	8	0.007158	0.044993
atlas.Cereb10 r (Cerebelum 10 Right) x,y,z = (26,-34,-41) mm	<0.01	3.71	7	0.007572	0.045433

Global efficiency					
ROI (according to the Conn's default atlas)	**beta**	**T**	**dof**	**p-unc**	**p-FDR**
Network	<0.01	-2.2	8	0.05756	-
atlas.pPaHC l (**Parahippocampal Gyrus, posterior** division Left) x,y,z = (-22,-32,-17) mm	<0.01	-6	8	0.000338	0.034656
atlas.Ver45 (Vermis 4 5) x,y,z = (1,-52,-7) mm	<0.01	-5.5	8	0.000602	0.034656
atlas.Ver6 (Vermis 6) x,y,z = (1,-66,-16) mm	<0.01	-5	8	0.001013	0.034656
atlas.Accumbens r (Accumbens Right) x,y,z = (9,12,-7) mm	<0.01	-4.8	8	0.001361	0.034656
atlas.SubCalC (Subcallosal Cortex) x,y,z = (-0,21,-15) mm	<0.01	-4.7	8	0.001559	0.034656
atlas.toITG l (Inferior Temporal Gyrus, temporooccipital part Left) x,y,z = (-52,-53,-17) mm	<0.01	-4.7	8	0.001575	0.034656
atlas.pTFusC l (Temporal Fusiform Cortex, posterior division Left) x,y,z = (-36,-30,-25) mm	<0.01	-4.4	8	0.002266	0.042735
atlas.Cereb1 l (Cerebelum Crus1 Left) x,y,z = (-36,-66,-30) mm	<0.01	-4.2	8	0.003186	0.047967
atlas.MedFC (Frontal Medial Cortex) x,y,z = (0,43,-19) mm	<0.01	-4.1	8	0.00327	0.047967

Local efficiency					
ROI (according to the Conn's default atlas)	**beta**	**T**	**dof**	**p-unc**	**p-FDR**
Network	<0.01	1.05	8	0.326157	-
atlas.Ver3 (Vermis 3) x,y,z = (1,-40,-11) mm	<0.01	-6.7	8	0.000153	0.018014
atlas.MedFC (**Frontal Medial Cortex)** x,y,z = (0,43,-19) mm	<0.01	-6.2	8	0.000273	0.018014

Betweenness centrality					
ROI (according to the Conn's default atlas)	**beta**	**T**	**dof**	**p-unc**	**p-FDR**
Network	<0.01	3.06	8	0.015592	-
atlas.LG r (Lingual Gyrus Right) x,y,z = (14,-63,-5) mm	<0.01	10.2	8	0.000007	0.000957
atlas.Ver10 (Vermis 10) x,y,z = (0,-46,-32) mm	<0.01	6.06	8	0.000304	0.015764
atlas.Putamen r (Putamen right) x,y,z = (25,2,0) mm	<0.01	5.91	8	0.000358	0.015764
atlas.OFusG r (Occipital Fusiform Gyrus Right) x,y,z = (27,-75,-12) mm	<0.01	5.18	8	0.000838	0.02766
atlas.Pallidum l (Pallidum Left) x,y,z = (-19,-5,-1) mm	<0.01	4.79	8	0.001381	0.03212
atlas.IFG tri r (Inferior Frontal Gyrus, pars triangularis Right) x,y,z = (52,28,8) mm	<0.01	4.74	8	0.00146	0.03212
atlas.aSMG l (Supramarginal Gyrus, anterior division Left) x,y,z = (-57,-33,37) mm	<0.01	4.36	8	0.002403	0.045322

Degree					
ROI (according to the Conn's default atlas)	**beta**	**T**	**dof**	**p-unc**	**p-FDR**
Network	<0.01	-0.3	8	0.774631	-
atlas.IFG tri l (Inferior Frontal Gyrus, pars triangularis Left) x,y,z = (-50,28,9) mm	0.14	5.86	8	0.000377	0.044299
atlas.IC l (Insular Cortex Left) x,y,z = (-36,1,0) mm	<0.01	4.87	8	0.001243	0.044299
atlas.Ver45 (Vermis 4 5) x,y,z = (1,-52,-7) mm	-0.11	-4.7	8	0.001548	0.044299
atlas.FO l (Frontal Operculum Cortex Left) x,y,z = (-40,18,5) mm	0.14	4.68	8	0.001576	0.044299
atlas.IFG oper l (Inferior Frontal Gyrus, pars opercularis Left) x,y,z = (-51,15,15) mm	0.16	4.63	8	0.001678	0.044299

Cost					
ROI (according to the Conn's default atlas)	**beta**	**T**	**dof**	**p-unc**	**p-FDR**
Network	<0.01	0	8	NaN	-
atlas.IFG tri l (Inferior Frontal Gyrus, pars triangularis Left) x,y,z = (-50,28,9) mm	<0.01	5.86	8	0.000377	0.044299
atlas.IC l (Insular Cortex Left) x,y,z = (-36,1,0) mm	<0.01	4.87	8	0.001243	0.044299
atlas.Ver45 (Vermis 4 5) x,y,z = (1,-52,-7) mm	<0.01	-4.7	8	0.001548	0.044299
atlas.FO l (Frontal Operculum Cortex Left) x,y,z = (-40,18,5) mm	<0.01	4.68	8	0.001576	0.044299
atlas.IFG oper l (Inferior Frontal Gyrus, pars opercularis Left) x,y,z = (-51,15,15) mm	<0.01	4.63	8	0.001678	0.044299

Clustering coefficient					
ROI (according to the Conn's default atlas)	**beta**	**T**	**dof**	**p-unc**	**p-FDR**
Network	<0.01	1.19	8	0.267329	-