LONGITUDINAL ASSESSMENT OF LARGE-SCALE BRAIN FUNCTIONAL NETWORKS IN PATIENTS WITH MULTIPLE SCLEROSIS: RELATIONSHIP WITH CLINICAL DISABILITY AND COGNITIVE IMPAIRMENT

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INTRODUCTION AND PURPOSE

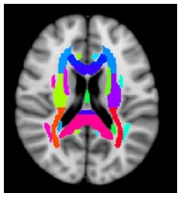
The human brain is a complex network of interacting regions connected by white matter (WM) tracts. The characterization of the structural and functional features of such a network has the potential to improve our understanding of the mechanisms leading to irreversible clinical disability and cognitive impairment in MS. Resting state (RS) fMRI allows to investigate intrinsic, synchronized brain activity across the whole brain, and to measure the degree of functional correlations between different cerebral regions [1]. The study of RS functional connectivity (FC) data of patients with relapse-onset MS, enrolled at our Unit, has demonstrated diffuse abnormalities of global network properties, which contributed to distinguish cognitively impaired MS patients from controls. Compared to normal subjects, MS patients showed a redistribution of cortical and subcortical hubs (defined as brain regions that interact with many other regions), especially in patients with secondary progressive MS. However, little is known about the temporal evolution of functional network RS FC and the association between network abnormalities and the concomitant development of physical and cognitive disability.

Aim of this longitudinal study is to investigate the temporal evolution of RS FC in patients with MS and its correlation with clinical and cognitive worsening.
The predictive value of baseline global and regional functional network measures on the worsening of clinical disability and cognitive impairment in MS patients was also explored.

METHODS

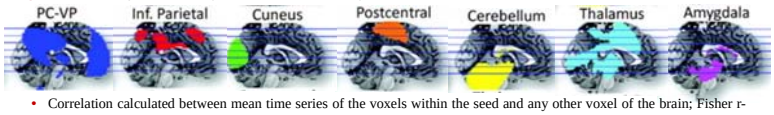
- Clinical, neuropsychological and MRI evaluations were obtained from 56 MS patients and 24 healthy controls (HC) at baseline and after a median follow-up (FU) of 3.6 years.
- **Clinical evaluation:** rating of the EDSS score [2]. At FU, MS patients were considered clinically worsened if they had an EDSS score increase ≥1.0 when baseline EDSS was <6.0, or an EDSS score increase ≥0.5 when baseline EDSS was ≥6.0.
- **Neuropsychological evaluation:** Brief Repeatable Battery of Neuropsychological Tests (BRB-N) [3]. Patients with at least two abnormal tests (≤2 SD below Italian normative values) were considered as cognitively impaired (CI). During the study, 2 alternate versions of the BRB (A at baseline, B at FU) were used to minimize possible practice effects. Evolution of cognitive performance was assessed on each test using the Reliable Change Index (RCI) [4], which allows corrections for both measurement error and practice effects. RCI scores for each domain were obtained by averaging RCI scores of the tests of a given domain. A global RCI score (obtained by averaging RCI scores of all domains) was also calculated.
- **MRI acquisition (Philips Achieva, 3.0 T):**
  - T2*-weighted single-shot EPI scans for RS fMRI;
  - Structural MRI: dual-echo TSE, 3D T1-weighted FFE, pulsed-gradient SE EPI sequence with SENSE and diffusion gradients applied in 35 non-collinear directions.
- **Structural MRI analysis:**
  - Quantification of T2 hyperintense and T1-hypointense lesion volumes (LV) at baseline and FU (Jim 6.0 software);
  - Count of the number of new T2 hyperintense and T1 hypointense lesions at FU;
  - Estimation of baseline normalized brain volumes (SIENAx software), after T1-hypointense lesion refilling, and of the percentage brain volume change (PBVC) between baseline and FU (SIENA software).
- **Diffusion MRI analysis:**
  - Eddy current correction and DT estimation;
  - Calculation of average fractional anisotropy (FA) and mean diffusivity (MD) values within lesions, normal appearing (NA) WM and MD in the GM;
  - Derivation of average FA and MD values from the 48 WM regions of the ICBM-DTI-81 white-matter label atlas (**Figure 1**).

Figure 1. ICBM-DTI-81 white-matter label atlas [5].



- **RS fMRI analysis:**
  - Standard pre-processing (SPM12 and REST);
  - Network identification using a seed-based approach with the following seeds (spherical volumes having radius=6 mm) as identified by Tomasi *et al.* [5]: right posterior cingulate cortex (MNI coordinates: 4 -52 29) for the default mode network (DMN); left inferior parietal cortex (MNI coordinates: -38 -53 39) for the dorsal attention network (DAN); right postcentral gyrus (MNI coordinates: 20 -44 57) for the somatosensory network; left cuneus (MNI coordinates: -24 -80 18) for the visual network; left cerebellum (MNI coordinates: -9 -56 -20) for the cerebellum network; left thalamus, medial dorsal nucleus (MNI coordinates: -12 -19 8) for the thalamic network; right amygdala (MNI coordinates: 24 -6 -15) for the reward-emotion network (**Figure 2**).

Figure 2. Seven major connectivity networks as identified by Tomasi *et al.* [5].



  - Correlation calculated between mean time series of the voxels within the seed and any other voxel of the brain; Fisher r-to-z transform used to improve gaussianity of the obtained correlation maps.

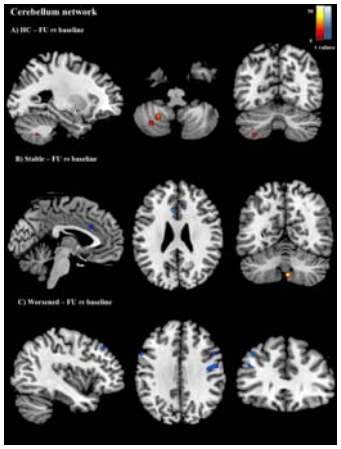
RESULTS

Table 1. Demographic, clinical and structural MRI characteristics of HCs and MS patients, main MS clinical phenotypes and patients with and without cognitive impairment at baseline.

	Healthy subjects	MS patients	p	RRMS patients	BMS patients	SPMS patients	p	CP MS patients	CI MS patients	p
Men/Women	14/10	22/34	ns^{++}	15/14	3/8	4/12	ns^{++}	15/17	2/7	ns^{++}
Mean age [years] (range) at baseline	33.4 (19.9-59.5)	41.3 (18.0-69.4)	0.004*	36.0 (18.0-48.7)	43.2 (32.9-54.9)	49.4 (30.7-68.4)	<0.001**	40.2 (22.2-63.5)	51.3 (37.4-68.4)	<0.001*
Mean DD [years] (range) at baseline	-	14.2 (1.2-32.5)	-	9.4 (1.2-17.0)	19.1 (15.2-25.0)	19.6 (12.0-32.5)	<0.001**	13.9 (3.2-25.0)	18.1 (3.2-32.5)	ns*
Median EDSS (range) at baseline	-	2.0 (0.0-8.0)	-	1.5 (0.0-4.5)	2.0 (1.0-3.0)	6.0 (4.0-8.0)	<0.001§	2.0 (0.0-8.0)	3.0 (1.0-6.5)	ns^{+}
T2 LV at baseline [ml] (SD)	-	10.7 (11.5)	-	4.2 (3.5)	11.6 (10.8)	21.8 (13.3)	<0.001**	9.0 (9.3)	16.6 (13.7)	ns*
T1 LV at baseline [ml] (SD)	-	7.5 (8.4)	-	2.8 (2.5)	8.1 (7.4)	15.5 (19.9)	<0.001**	6.1 (6.5)	13.8 (13.4)	ns*
NBV at baseline [ml] (SD)	1595 (88)	1473 (101)	<0.001*	1527 (89)	1451 (81)	1393 (74)	<0.001**	1479 (95)	1435 (111)	<0.001*
PBVC [%] (SD)	-0.72 (0.78)	-1.26 (1.05)	0.03*	-1.06 (1.03)	-0.69 (0.91)	-1.96 (1.30)	<0.001**	-1.28 (1.19)	-1.22 (0.65)	ns*

*Two-sample t test; **ANOVA model; $^{+}$Mann-Whitney test; §Kruskal and Wallis test $^{++}$Chi-square test.

- **Baseline DT MRI findings:**
  - Compared with HC, MS patients had significantly lower FA and higher MD in the NAWM and in all main WM tracts. The same trend was found in clinically worsened *vs* stable MS patients (p ranging from <0.001 to 0.05 for both comparisons).
- **Baseline RS FC findings:**
  - MS patients showed a widespread reduction of global RS FC in all networks *vs* HC (p ranging from <0.001 to 0.01).
  - At a regional level, a complex pattern of abnormalities, characterized by the concomitant presence of regions with decreased and increased RS FC was found (p<0.001). Clinically worsened patients showed higher RS FC in DAN, sensorimotor and visual networks compared to stable patients. They also experienced lower RS FC in DMN, cerebellar and thalamic networks.
- **Follow-up clinical, neuropsychological and structural MRI findings:**
  - At FU, 11 (20%) MS patients were clinically worsened. Six MS patients developed cognitive impairment. During the FU period, 21 patients had clinical relapses and 20 patients changed disease-modifying treatment. Two patients evolved from RRMS to SPMS, whereas 10 patients evolved from RRMS to BMS.
  - At FU, MS patients formed on average 1.9 new T2 hyperintense lesions (range=0-12 lesions) and 1.1 new T1 hypointense lesions (range=0-7 lesions). T1 LV was significantly increased in MS patients at FU compared to baseline (p<0.001).
  - Clinically worsened MS patients had a higher EDSS (p=0.02) and lower NBV (p=0.02) at baseline than clinically stable MS patients. Clinically worsened MS patients had also a higher rate of new T2 (p=0.02) and T1 (p=0.03) lesion formation and a higher rate of brain atrophy development (p=0.01) than clinically stable MS patients.
  - PBVC changes during the FU were significantly more pronounced in MS patients than HC (p=0.03).
  - Longitudinal changes of T1 LV were significantly different among disease clinical phenotypes (time *x* group interaction, p=0.01) and between CI and CP MS patients (time *x* group interaction, p=0.004)
  - In both clinically stable and worsened MS patients, DT MRI abnormalities tended to worsen over time, with a significant reduction of FA values in T2-lesions and NAWM (p ranging from p<0.001 to 0.05).
- **Modifications of network RS FC:**
  - *Global network RS FC* remained stable over time in HC and MS patients for the cerebellar, DMN, reward-emotion, and thalamic networks, whereas it increased significantly, in both groups, in the DAN, sensorimotor and visual networks.
  - *Regional network analysis* detected no significant RS FC changes in HC. MS patients showed a complex pattern of longitudinal changes in the different networks. In particular:
    - In the cerebellar network, worsened MS patients had a widespread decrease of RS FC in regions of the sensorimotor system, while clinically stable MS patients showed a decreased RS FC in the anterior cingulate cortex (ACC) and an increased RS FC in temporal and cerebellar regions (**Figure 3**);
    - In the DMN, both clinically stable and worsened MS patients had decreased RS FC of fronto-temporal regions. In stable MS, increased RS FC of the bilateral precuneus was also detected;
    - In the DAN, stable MS patients showed increased RS FC of frontal, parietal and cerebellar regions, while no significant RS FC changes were found in clinically worsened MS patients;
    - In the sensorimotor and visual networks, a significant increase of RS FC was found in regions of the temporal and parietal lobes in clinically stable MS (**Figure 3**), while clinically worsened MS patients showed a decreased RS FC of the precentral gyrus and cerebellum;
    - In the reward-emotion network, clinically stable MS patients showed increased RS FC mainly in frontal, insular and parietal regions, while no changes were detected in clinically worsened MS patients;
    - In the thalamic network, significantly decreased RS FC of frontal, temporal and subcortical regions was found in both clinically stable and worsened MS.



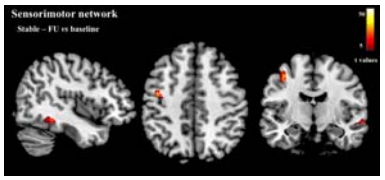


Figure 3. Within-group comparisons for cerebellar network (on the left) and sensorimotor network (on the right) RS FC between baseline and FU assessment (foci of increased RS FC at FU are indicated in red, foci of decreased FS FC are coded in blue) (p<0.001, uncorrected).

- **Follow-up RS FC findings:**

At FU, compared to HC, MS patients had a lower average RS FC in all analyzed networks (p values ranging from <0.001 to 0.05). Global network measures were lower in clinically worsened *vs* clinically stable MS patients and HC. Regional network analysis showed that, compared to clinically worsened MS patients, clinically stable ones had higher RS FC in several functional networks (**Figure 4**).
A significant time *x* group interaction (i.e., a significantly different behaviour of RS FC over time between clinically stable and clinically worsened MS patients) was found for the R supramarginal gyrus of the DAN and the left insula of the reward-emotion network (full factorial model, interaction analysis, p<0.001).

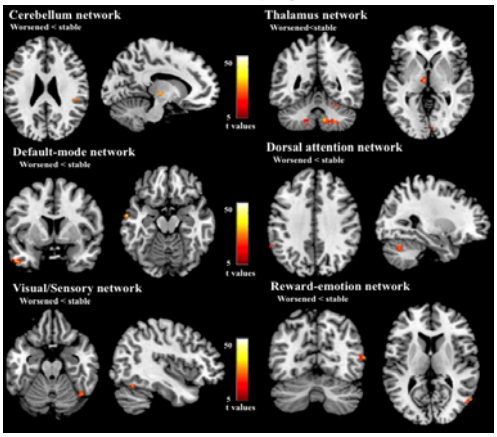


Figure 4. Comparison of RS FC at FU between clinically stable and clinically worsened MS patients (p<0.001, uncorrected).

Decreased RS FC between the cerebellum and the L postcentral gyrus, and decreased DMN RS FC of the left MTG correlated with worse performances at fluency and visual memory domains at FU.
Increased DAN RS FC of the left IFG correlated with a better performance at visual memory domain, as well as with a better global cognitive performance.
Increased RS FC in the reward-emotion network correlated with a better cognitive performance at FU.
Decreased thalamic RS FC with deep GM structures and frontal regions was correlated with worse cognitive performances at fluency and visual memory domains.

- **Analysis of prediction:**

A multivariable model with EDSS score deterioration as dependent variable, identified decreased RS FC of the left postcentral gyrus (p=0.03), average RS FC of the sensorimotor network (p=0.02), baseline GM atrophy (p=0.001), reduced FC of the right cingulum (p=0.003) and decreased RS FC of the right middle temporal gyrus of the DMN (p=0.01) as predictors of disability worsening (C-index=0.78).

CONCLUSIONS

- **Longitudinal modifications** of RS FC occur in MS patients and **differ from** those of **HC**.
- An **increased RS FC** plays an **adaptive role**, probably in response to accumulation of structural damage. Such an increased RS FC in a given brain network in MS patients might protect from the onset of clinical deficits and cognitive impairment.
- An **high RS FC at baseline** also contributes to identify MS patients with a **better clinical prognosis**.
- A distributed **reduction of RS FC** in clinically worsened and/or cognitively deteriorating MS patients is likely to reflect the **exhaustion of brain functional reserve**.
- Overall, the results of this medium-term study support the notion that **RS FC abnormalities** play a **key role** in the worsening of locomotor disability and cognitive impairment in patients with MS. The inclusion of these measures is desirable when monitoring the **evolution of MS** using MRI over time.

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DISCLOSURES **P. Valsasina, A. Colombi, F. Pirro, E. Pagani, E. De Meo, B. Colombo, P. Preziosa, V. Martinelli, A. Falini** report no actual or potential conflict of interest.
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