DYNAMIC FUNCTIONAL NETWORK CONNECTIVITY IN CIS PATIENTS: A LONGITUDINAL STUDY

M. Hidalgo de la Cruz[1], M.A. Rocca[1,2], P. Valsasina[1], S. Mesaros[3], J. Dackovic[3], I. Dujmovic-Basuroski[3], J. Drulovic[3], M. Filippi[1,2].

[1]Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, and [2]Dept. of Neurology, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy. [3]Clinic of Neurology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia.

INTRODUCTION and PURPOSE

Abnormal intra- and inter-network resting state (RS) functional connectivity (FC) has been described at the earliest stages of multiple sclerosis (MS) [1-2]. A recent technique, called dynamic functional network connectivity (dFNC), can identify changes in RS FC at a scale of seconds, offering a more precise characterization of brain dynamics [3-8].

The aim of this study was to define the trajectories of dFNC changes over two years of follow-up in patients with clinically isolated syndromes (CIS) suggestive of MS.

METHODS

MRI acquisition and analysis: Using a 3.0T scanner, axial DE TSE, sagittal 3D T_1-weighted FFE, pulsed-gradient SE EPI, and T_2*-weighted EPI sequences were acquired from **50 patients with CIS suggestive of MS** and **13** sex and age-matched healthy controls (**HC**). Brain T_2-hyperintense and T_1-hypointense lesion volume (*Jim 6*) were measured. After T_1-hypointense lesion refilling, normalized brain, gray matter, and white matter volumes, as well as percentage of brain volume changes, were calculated (*SIENAx, SIENA*).

Neurological and neuropsychological evaluation: In MS patients, a complete neurological examination with rating of the Expanded Disability Status Scale (EDSS) score [9] was performed at **baseline** (within 3 months from the first attack), **year 1** and **year 2**. At follow-up, patients who developed clinically-defined (CD) MS were identified [10].

Dynamic FNC analysis: Using the independent component analysis (ICA) (*GIFT*) [6], we identified 41 relevant intrinsic components, and classified them according with their functional system. Using a sliding window approach [7], the RS fMRI time courses of the intrinsic components were divided into short windows of 22 TR (66s) in steps of 1 TR (3s). Correlation matrices of RS FC were calculated for each window. Dynamic properties of RS FNC were assessed using the k-means clustering [7] and the "meta-states" analyses [11].

Statistical analysis: Between-group comparisons of demographic, clinical and structural MRI variables were assessed (*SPSS*). Between-group comparisons of dFNC (*MANCOVAN, GIFT dFNC)* [8,12], and group differences of the four metrics of global dynamism estimated by the meta-state analysis [11] were calculated (*GIFT dFNC*).

RESULTS

Clinical, neuropsychological and structural MRI measures are summarized in **Table 1**. By year 2, forty-seven (94%) patients developed CDMS.

Table 1	HC (n=13)	CIS (n=50)	p
Gender (Male/Female)	4/9	20/30	0.5[a]
Symptoms at clinical onset (n, %)			
Brain	-	22 (44.0)	-
Optic neuritis	-	8 (16.0)	-
Brainstem/cerebellum	-	12 (24.0)	-
Spinal cord	-	8 (16.0)	-
Baseline variables			
Age (years)	34.1 (7.8)	30.5 (7.7)	0.09[b]
EDSS [median (range)]	-	1.5 (0-3.0)	-
MRI dissemination in space (n, %)		44 (82.1)	
MRI dissemination in time (n, %)		19 (33.9)	
T2 lesion volume (ml)	-	3.5 (4.1)	-
T1 lesion volume (ml)	-	2.2 (2.7)	-
Normalized brain volume (ml)	1506 (93)	1495 (72)	0.9[b]
Normalized grey matter volume (ml)	786 (73)	789 (48)	0.8[b]
Normalized white matter volume (ml)	720 (33)	706 (38)	**0.01[b]**
Month 12 variables			
EDSS [median (range)]	-	1.5 (0-2.0)	-
T2 lesion volume (ml)	-	3.3 (3.8)	-
T1 lesion volume (ml)	-	2.2 (2.6)	-
Percentage of brain volume change year 1 vs baseline	-0.07 (0.64)	-0.43 (0.92)	0.2[b]
Month 24 variables			
EDSS [median (range)]	-	1.5 (0-4.5)	-
T2 lesion volume (ml)	-	3.6 (3.9)	-
T1 lesion volume (ml)	-	2.2 (2.7)	-
Percentage of brain volume change year 2 vs baseline	-0.67 (0.88)	-0.81 (0.94)	0.7[b]
Percentage of brain volume change year 2 vs year 1	-0.61 (0.60)	-0.36 (0.65)	0.2[b]

[a]Pearson chi-square test (HC *vs* MS patients), [b]Mann-Whitney-U test (HC *vs* MS patients).

FNC meta-states: Dynamic FNC properties derived from the meta-states analysis are summarized in **Table 2.**

Table 2	HC (n=13)	CIS (n=50)	p[a]
Temporal-ICA – Baseline			
Number of meta-states	17.5 (5.4)	19.3 (8.1)	0.5
Number of changes between meta-states	37.5 (10.2)	36.5 (9.5)	0.7
Meta-state span distance	9.6 (1.9)	9.9 (2.8)	0.7
Total distance	46.6 (14.6)	47.8 (15.3)	0.8
Temporal-ICA - Year 1			
Number of meta-states	17.8 (7.4)	20.0 (7.0)	0.3
Number of changes between meta-states	36.2 (10.6)	37.9 (8.8)	0.5
Meta-state span distance	8.8 (2.7)	10.2 (2.6)	0.1
Total distance	45.4 (17.2)	49.9 (13.3)	0.3
Temporal-ICA - Year 2			
Number of meta-states	18.5 (5.4)	21.5 (6.0)	0.1
Number of changes between meta-states	36.5 (8.8)	40.6 (9.0)	0.1
Meta-state span distance	9.2 (2.2)	10.8 (2.6)	**0.04**
Total distance	46.1 (12.0)	54.8 (14.5)	**0.05**

[a]Two sample-t test (HC *vs* CIS patients). Results are expressed as mean (SD).

Dynamic FNC: The 41 intrinsic components were classified into the sensorimotor (5 components), default-mode (10), attention (5), executive (7), visual (6), auditory (2), temporal (2), and cerebellar (4) networks.

HC and CIS patients showed two d-FNC states. State 1 (frequency, 68%) was characterized by lower inter-network connectivity. State 2 (frequency, 32%) was characterized by higher inter-network connectivity. Correlation matrices across states (State 1 and State 2) in: **a)** all subjects and **b)** HC *vs* CIS patients are represented in **Figure 1.**

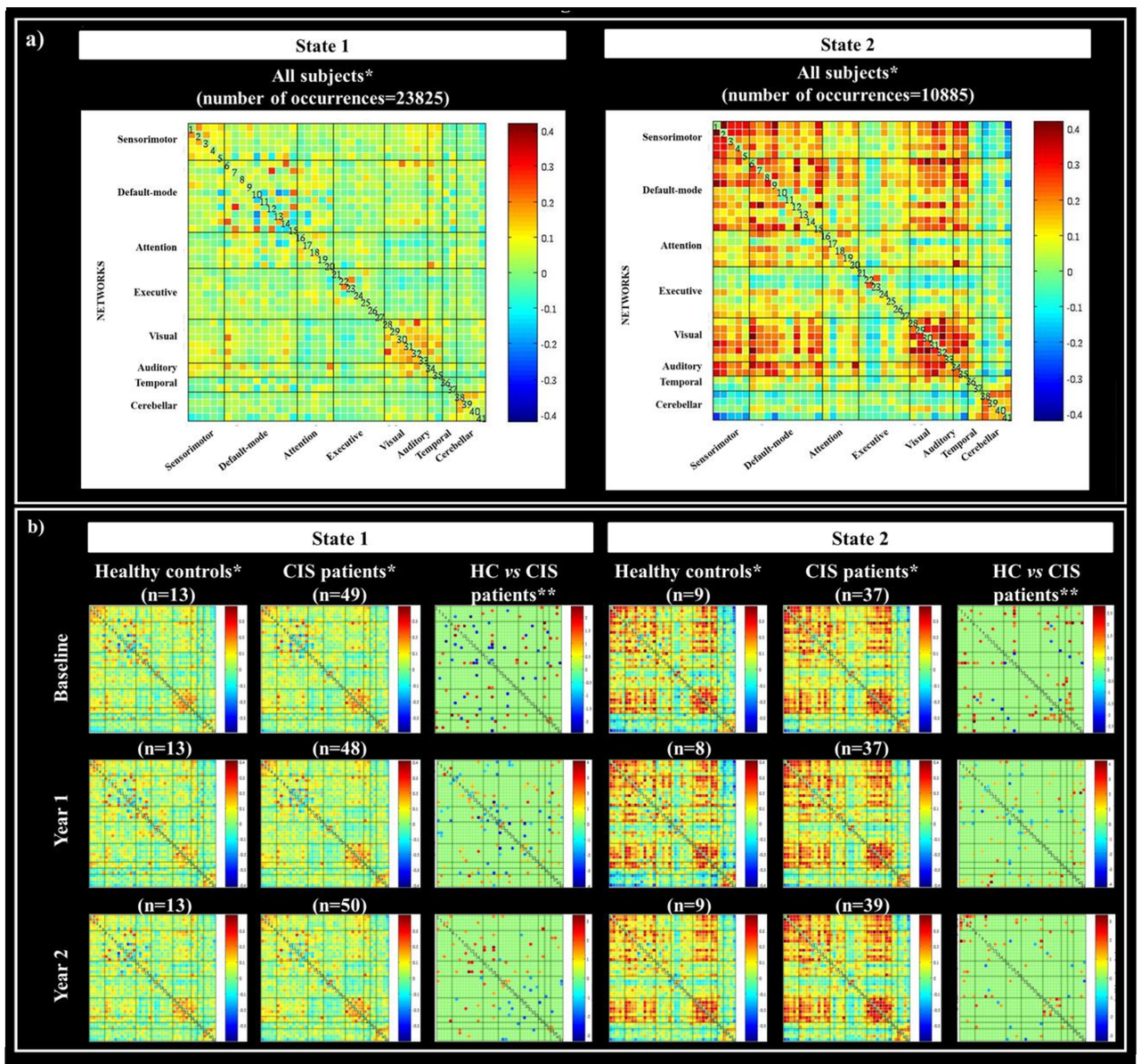

Figure 1. Correlation matrices across states (State1 and State2) in a) all subjects and b) HC *vs* CIS patients. *Mean normalized r value of the RS FNC correlations (p<0.05, uncorrected) (red-yellow: increased RS FNC correlation, blue: reduced RS FNC correlation). **Between-group statistically significant differences in RS FNC (p<0.05, uncorrected), colour-coded according with their p value (colour intensity) and group strength (i.e., red-yellow=CIS patients>HC; blue= CIS patients<HC). n=number of subjects who presented the indicated state.

CONCLUSIONS

By selecting a population of CIS patients, and decomposing the RS fMRI data on small temporal segments in order to analyse FNC strength and dynamism, this study provides novel insights into the pathophysiology of the early stages of MS. CIS patients showed a progressive increasing of FNC meta-states dynamism, along with abnormal dFNC strength patterns that changed significantly over time:

- At baseline, CIS patients showed increased RS inter-network FC in both states, mainly for the sensorimotor and default-mode networks in State 2, and reduced inter-network RS FC for the executive network and some areas of the default-mode network in State 1.
- At year 1, CIS patients showed increased inter-network connectivity in both states, mainly for the sensorimotor and default-mode network in State 2, along with reduced inter-network RS FC for the executive network in both states.
- At year 2, CIS patients showed increased inter-network connectivity, mainly for the sensorimotor and default-mode network, along with reduced inter-network RS FC for the executive network, in both states.

Such dFNC abnormalities help to explain the discrepancies that had been previously described in the literature [1-2, 13]. The progressive involvement of the default-mode, executive and sensorimotor networks highlights the relevant role that such networks play in the characterization of the first stages of MS. The analysis of time-varying RS FNC patterns in CIS patients may help to establish a potential target for MS early diagnosis, while targeting locations in which neurorehabilitation treatment might be used.

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DISCLOSURES

Dr. Hidalgo de la Cruz, Dr. Valsasina and Dr Dujmovic-Basuroski have nothing to declare. **Dr. Rocca** received speakers honoraria from Biogen Idec, Novartis and ExecMED and receives research support from the Italian Ministry of Health and Fondazione Italiana Sclerosi Multipla. Dr. Mesaros has received payment for lectures from Merck and Novartis; travel expenses from Bayer, Genzyme, Medis, and Sanofi. **Dr. Dackovic** has received payment for travel expenses from Teva. **Dr. Drulovic** has received personal fees from Bayer Schering, Medis, Merck Serono, Novartis, and Teva. **Prof. Filippi** serves on scientific advisory board for Teva Pharmaceutical Industries; has received compensation for consulting services and/or speaking activities from Biogen Idec, Excemed, Novartis, and Teva Pharmaceutical Industries; and receives research support from Biogen Idec, Teva Pharmaceutical Industries, Novartis, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, Cure PSP, Alzheimer's Drug Discovery Foundation (ADDF), the Jacques and GloriaGossweiler Foundation (Switzerland), and ARiSLA.

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