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Reduced clinical connectome fingerprinting in multiple sclerosis predicts fatigue severity

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ABSTRACT

Background: Brain connectome fingerprinting is progressively gaining ground in the field of brain network analysis. It represents a valid approach in assessing the subject-specific connectivity and, according to recent studies, in predicting clinical impairment in some neurodegenerative diseases. Nevertheless, its performance, and clinical utility, in the Multiple Sclerosis (MS) field has not yet been investigated.
Methods: We conducted the Clinical Connectome Fingerprint (CCF) analysis on source-reconstructed magnetoencephalography signals in a cohort of 50 subjects: twenty-five MS patients and twenty-five healthy controls.
Results: All the parameters of identifiability, in the alpha band, were reduced in patients as compared to controls. These results implied a lower similarity between functional connectomes (FCs) of the same patient and a reduced homogeneity among FCs in the MS group. We also demonstrated that in MS patients, reduced identifiability was able to predict, fatigue level (assessed by the Fatigue Severity Scale).
Conclusion: These results confirm the clinical usefulness of the CCF in both identifying MS patients and predicting clinical impairment. We hope that the present study provides future prospects for treatment personalization on the basis of individual brain connectome.

1. Introduction

The last twenty years have represented a new era of Multiple Sclerosis (MS), not only in the therapeutic field but also in the growing relevance that several non-motor symptoms have progressively acquired (Chiaravalloti and DeLuca, 2008; Silveira et al., 2019). Among these, fatigue is the most common (Van Der Vuurst De Vries et al., 2018) and disabling (Hadjimichael et al., 2008) one, with a prevalence of up to 90% of MS patients (Sandry et al., 2014).

Fatigue assessment is biased by subjective elements, such as emotions, experiences, personality, and mood (Jakimovski et al., 2020; Rooney et al., 2019). Hence, while clinical management cannot overlook fatigue, objective means of assessment is still lacking. This is partly due to the poor understanding of the pathophysiological changes that lead to fatigue. In particular, it appears that fatigue is not associated with lesions in a specific region but, rather, it emerges from diffuse damage at the whole-brain level (Manjaly et al., 2019).

In the last two decades, neuroimaging studies highlighted changes in large-scale brain features (either structural or functional), thereby contributing to elucidate the mechanisms leading to fatigue (ARM et al., 2019; Manjaly et al., 2019). To this regard, structural MRI studies showed an association of fatigue severity with global atrophy (Marrie et al., 2005; Sander et al., 2016), cortico-subcortical white matter (WM), and gray matter (GM) volume reduction (Marrie et al., 2005; Riccitelli

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et al., 2011). Moreover, other studies revealed the involvement of multiple areas in the occipital (Gobbi et al., 2013), frontal (Gobbi et al., 2013; Rocca et al., 2014), and parietal (Andreasen et al., 2010; Gobbi et al., 2013) lobes, as well as in the thalamic areas, and even in the basal ganglia (Bernitsas et al., 2017).

From the functional standpoint, task-related fMRI studies showed a relationship between fatigue and increased cerebral activation within the frontal lobes, parietal and occipital regions, basal ganglia, thalamus, substantia nigra and cerebellum during cognitive performance (Engström et al., 2013; Genova et al., 2013; Spiteri et al., 2017). During resting-state, fatigue was also associated to altered interactions between brain areas, as captured by altered co-fluctuations (averaged over-time) in the corresponding signals (i.e., functional connectivity). On the one hand, fatigue severity was positively related with increased functional connectivity between basal ganglia/thalamic subregions and the frontal (dorsolateral prefrontal, middle frontal, sensory motor cortex (SMC)), parietal, insular and even cerebellar cortices (Hidalgo de la Cruz et al., 2017; Jaeger et al., 2019), as well as with increased functional connectivity in the posterior cingulate cortex (PCC), in default mode network (DMN) -associated areas, and in the primary motor cortex (and SMC) of SMN (Bisecco et al., 2017). On the other hand, fatigue was negatively correlated with reduced functional connectivity between the basal ganglia and some DMN-related structures (Finke et al., 2014) and with decreased DMN functional connectivity in the anterior cingulate cortex (ACC), supplementary motor area (SMA) and associative somatosensory cortex (Cruz Gómez et al., 2013).

Hyperconnectivity has been traditionally interpreted as a compensatory mechanism, whereby more regions would be recruited in order to maintain the optimal functional output. Such compensatory recruitment of additional brain regions might provide a defense mechanism against MS-induced injuries, that in turn may increase the metabolic cost causing fatigue (Manjaly et al., 2019). These results suggest that a neuroimaging signature of fatigue should not be researched in a specific brain region or network but, rather, in the context of a functional remodeling of the whole brain functional connectome.

As known, MS affects the brain with multiple pathophysiological mechanisms and lesion loads that change (by site and entity) among patients, making each MS patient virtually unique. Hence, the need for customized diagnostic approaches detecting the clinical heterogeneity of the disease. Fingerprinting analysis, by considering whole functional connectomes (FC), defines brain network characteristics of each individual (Amico and Goñi, 2018; Finn et al., 2015; Sareen et al., 2021). Such approach has been tested in both health and disease, revealing that people suffering from neurological diseases show reduced identifiability with respect to healthy subject ((Romano et al., 2022; Sorrentino et al., 2021)). Interestingly, the reduced identifiability was predictive of individual clinical features, leading to the concept of the Clinical Connectome Fingerprint (CCF) (Romano et al., 2022; Sorrentino et al., 2021).

Based on our previous work, we believe that the CCF could be exploited to extract subject-specific connectome features whose characteristics may differ between MS and healthy populations and, more importantly, even within the patient population itself. Furthermore, we hypothesize that the CCF may be predictive of patient-specific symptoms, not attributable to certain brain areas, such as fatigue.

To test our hypotheses, we utilized CCF in a cohort of twenty-five MS patients and twenty-five healthy controls (HC). We performed two separate magnetoencephalography (MEG) recordings for each subject of both groups. After filtering the source-reconstructed signals in the five canonical frequency bands, we used the phase linearity measurement (PLM) (Baselice et al., 2019), a phase-based metric of synchronization between pairs of MEG signals, to build the FCs. Firstly, we assessed the identifiability rate for both patients and controls. Then, we compared the similarity of each patient FC, with the healthy group's FCs obtaining, for each patient, a "clinical fingerprinting'' score (I-*clinical*). Finally, to test the hypothesis that the I-*clinical* score can be predictive of fatigue, we designed a multilinear regression model, to predict individual fatigue levels (as measured by the Fatigue Severity Scale, FSS) from the I-*clinical* score of each subject.

2. Methods

2.1. Participants

Twenty-five MS patients (5 males and 20 females) and twenty-five age-, sex- and education-matched healthy controls were recruited. MS was diagnosed in accordance with the 2017 revision of the McDonald criteria (Thompson et al., 2018). MS individuals were further classified in Relapsing-Remitting MS (RRMS) and Secondary-Progressive MS (SPMS). The eligibility of the patients was defined according to the following exclusion criteria: 1) use of illicit drugs, stimulants, amphetamines, barbiturates, and cannabis; 2) a history of central nervous system (CNS) disorder other than MS; 3) severe mental illness; 4) other systemic disorders with possible secondary involvement of the CNS.

MS patients underwent clinical examination performed by an experienced neurologist. The Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) was used to evaluate the disease-related disability, whereas fatigue was assessed by the Fatigue Severity Scale (FSS) (Krupp et al., 1989). The study protocol was approved by the Local Ethics Committee (University of Campania "Luigi Vanvitelli") with protocol number 591/2018. All participants provided written informed consent in accordance with the Declaration of Helsinki.

2.2. MEG and MRI acquisition, preprocessing and source reconstruction

MEG acquisition, preprocessing, source reconstruction and synchrony estimation have been performed according to our previous studies (Romano et al., 2022; Sorrentino et al., 2021; Troisi Lopez et al., 2023), see also supplementary data). All the patients and healthy controls underwent magnetic resonance imaging (MRI); thalamic volume estimation has been obtained by using the Computational Anatomy Toolbox 12 (CAT12) built in Statistical Parametric Mapping 12 (SPM12). A detailed description is provided in supplementary data.

2.3. Fingerprint analysis

Based on the FCs (Fig. 1), we estimated the brain-fingerprinting of both patients and controls. We started by creating frequency-specific identifiability matrices (IM) for each group, using the Pearson's correlation coefficient between the test and re-test FCs. Specifically, the test FC of each subject was correlated to the retest FCs of all the subjects belonging to the same group (including her/himself) (Amico and Goñi, 2018). So, the resultant IM embodies, in the main diagonal, the information inherent to homo-similarity (I-self, the similarity between FCs of the same individual), whilst data about hetero-similarity (I-others, i.e., the similarity of that subject's FC with the whole group) are represented by the off-diagonal elements. Then, we extracted the differential identifiability (I-diff), a score that estimates the subject-specific fingerprint level of a specific brain dataset, by subtracting the I-others value from the I-self value (Amico and Goñi, 2018). Moreover, to define the probability of correctly identifying a specific subject, we calculated the success rate (SR) of subject recognition within a specific group. The SR was computed on the number of times (expressed as percentage) that each subject showed an I-self higher than the I-others (i.e., how many times an individual was more similar to themselves than to another individual of the same group).

Finally, we set out to measure how much each patient's FC was similar to healthy controls FCs by computing the I-*clinical* score. Similarly to Sorrentino et al., (Sorrentino et al., 2021) we built two block identifiability matrices by crossing the patients and the HC FCs test and re-test respectively. Specifically, the first block derived from the correlation of the controls' FCs test and the patients FCs retest; the second

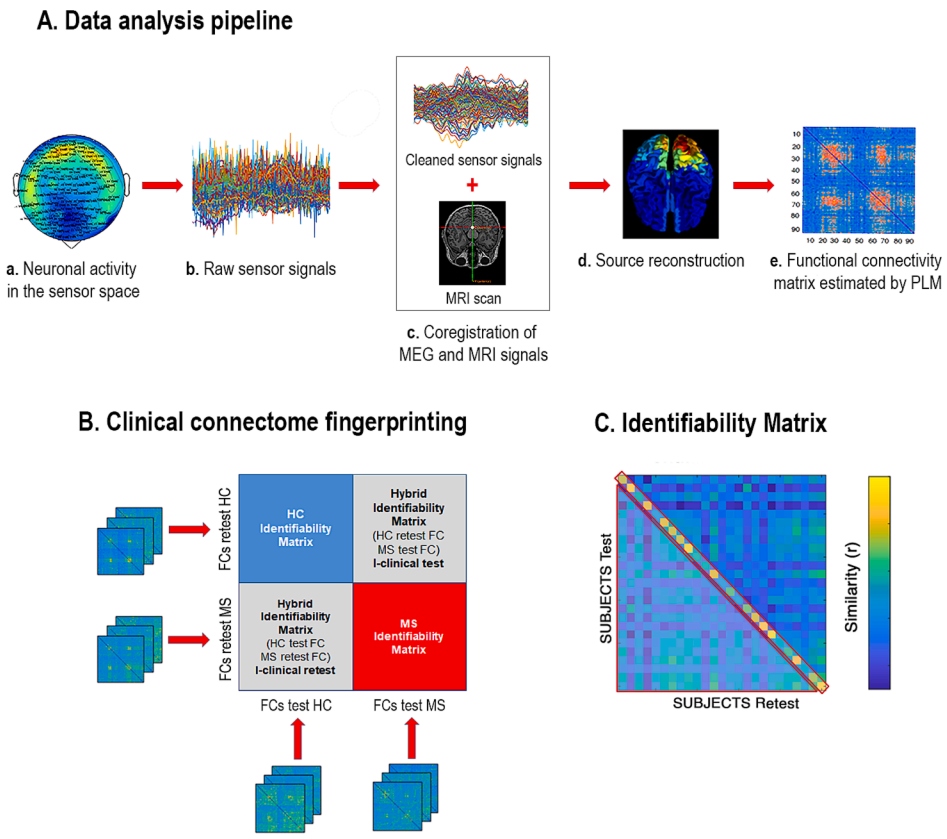

Fig. 1. FCs processing and fingerprint analysis. (A) a: the neuronal activity was recorded using a 154-sensors magnetoencephalography (MEG); b: noisy MEG signals, with cardiac and blinking artifacts; c: MEG-MRI signals coregistration after MEG signals cleaning by removing noise and artifacts; d: source reconstruction (beamforming); e: functional connectivity matrix estimation by PLM. (B) The blue and the red blocks represent the two identifiability matrices of HC and MS patients, resulting from the correlation of the test and re-test individual functional connectomes, in each group separately. Hybrid identifiability matrices (IMs) were created by crossing the FCs test of the HC with the FCs retest of the MS and vice-versa. The hybrid IMs return the I-clinical value of each patient (see methods). (C) Identifiability matrix representation, resulting from the correlation of the test and re-test functional connectomes of each subject. In the red triangle, the similarities between two recordings of each subject with respect to the others are shown (I-others score); the diagonal represents the similarity between two recordings of the same subject (I-self score). For details see supplementary materials.

block was obtained by correlating the controls' FCs retest with the patients' FCs test. Finally, the I-*clinical* (test) for each patient represents the similarity averaged between the FC of that patient in the test session and the FCs of each healthy control in the retest session. Conversely, the I-*clinical* (retest) for each patient represents the average similarity over the FCs of that patient in the retest session with the FCs of each healthy control in the test session. The I-*clinical* score was obtained by averaging the I-*clinical* (test) and the I-*clinical* (retest), thus providing information about the similarity of a patient with respect to the whole control group across test and retest sessions (for more details, see Sorrentino et al. 2021(Sorrentino et al., 2021)).

2.4. Edges of interest for fingerprint

To estimate the edgewise reliability of individual connectomes across the test and the re-test recordings, we used the intra-class correlation coefficient (ICC) (Koch, 2004), a measure that quantifies the similarity of the elements belonging to the same group. In our case, the higher the ICC values, the greater the stability of an edge over different recordings (Sorrentino et al., 2021). We hypothesized that, in a functional connectome, the edges with the higher ICC were the most stable ones over time and then, those that could contribute more to subject identifiability. So, we conducted a fingerprint analysis by sequentially adding them based on their ICC values. We conducted this analysis by adding 100 edges at each interaction (and computed the SR values) starting from the highest ICC value to the lowest. A null model was built by adding the edges in random order, 100 times at each iteration, to validate our findings.

2.5. Fingerprint clinical prediction

Since the I-*clinical* describes the similarity of a patient to the healthy subjects, we hypothesized that similarity would be lower for more severe cases. So, on the assumption that the I-*clinical* could predict clinical impairment, we built a multilinear regression model to predict FSS and EDSS scores based on the I-*clinical* scores and four other nuisance predictors: age, sex, schooling and disease duration (in months) (Shen et al., 2017). Multicollinearity was assessed through the variance inflation factor (VIF) (Belsley et al., 2004; Snee, 1983). Since the I-clinical can be computed of different subset of edges, similarly to the analysis described in methods 2.4, we used nested k-fold cross validation (k = 5) to select the appropriate model while preventing overfitting and information leakage (Parvandeh et al., 2020). In particular, we exploited the inner loop of the cross-validation, to perform the analysis within an iterative scenario where the I-*clinical* values of the validation set, were computed on different subset of edges (from 100 to 4500, with a 100-edges step), ranked according to the ICC values of the inner training set. Based on the standard cross-validation procedures, also the beta coefficients used to build the model for the validation set were computed on the inner training set. After the inner loop was completed, we evaluated the performance of each model by calculating the normalized root mean square error (NRMSE) between the actual and predicted FSS values. Then, in the outer loop, we calculated the I-*clinical* ranking the edges according to the ICC values of the training group and using the edge-threshold the showed the lowest NRMSE in the inner loop. Finally, the beta coefficients, and the same ICC order and threshold were used to calculate the I-*clinical* and predict the FSS in the test set. After the outer loop was completed, we calculated the NRMSE of our five best models. The model with the lowest NRMSE was selected, and a multilinear regression for FSS prediction was performed using k-fold cross validation on the chosen edge-threshold.

2.6. Statistics

Statistical analysis was performed in MATLAB 2021b. We analyzed all the comparisons among the I-self, the I-others and the I-diff values

using permutation testing, where the labels of the two groups were randomly allocated 10,000 times. Thus, we obtained a null distribution of the randomly determined differences by computing the absolute value of the difference of the group averages at each iteration (Nichols and Holmes, 2002). The relationship between variables was studied with Pearson's correlation and the results were corrected for multiple comparisons using the False Discovery Rate (FDR) (Benjamini and Hochberg, 1995), setting the significance level at p-value < 0.05.

3. Results

3.1. Connectome fingerprint

In our cohort, whose sociodemographic and clinical characteristics (including the thalamic volumes) are reported in Table 1, we firstly built the IM (Fig. 1) and then we generated block IMs to produce the I-*clinical* score. Furthermore, the relationships between I-*clinical* score and both fatigue severity (assessed with the FSS) and functional impairment (assessed with the EDSS) were investigated.

After False Discovery Rate (FDR) correction, statistically significant differences were found in the alpha band (Fig. 2). Healthy subjects showed a significantly higher I-self (pFDR = 0.015), I-others (pFDR = 0.015) and I-diff (pFDR = 0.042).

3.2. Edge-based identifiability

We used the one-way random-effects intra-class correlation coefficient (ICC) to test the edgewise reliability of the individual FCs. The edges with higher ICC values are those that contributed the most to the identifiability. Our results showed different contributions of the edges in the identifiability of HC and MS respectively. (Fig. 3). Specifically, healthy controls showed higher ICC value compared to MS patients. Hence, MS patients displayed lower edge stability in the test–retest FCs.

Moreover, we studied the distribution of SR values extracted from the fingerprint analysis by adding 100 edges per iteration, from the most to the least stable ones, based on ICC values. The control group quickly reached a complete SR (100%) (Fig. 4). By using the same method, the MS group rapidly approached the complete SR, but without ever reaching it. To demonstrate the robustness of our approach, we created a null model by adding edges in random order and extracting the corresponding expected SR values (Fig. 4). The ROIs with the greatest nodal strength (i. e. the regions with the most highly contributing edges hinging upon them) were mostly located in the frontal and parietal lobes of the left hemisphere and in the temporal lobe of the right hemisphere (Fig. 3).

Table 1
Demographic and clinical characteristics.

Demographic data	MS (25)	HC (25)	*P value*
Age	45.68 ± 9.47	45.8 ± 11.83	0.94
Gender (M/F)	7/18	7/18	1
Education	13.36 ± 4.28	13.93 ± 3.91	0.68
MS-specific clinical characteristics			
MS type (RRMS/SPMS)	10/15		
DD (months)	173.24 ± 144.97		
EDSS (mean/range)	4.56/1.5–7		
FSS (mean, SD/range)	37.60 ± 14.19/9–59		
BDI (mean, SD)	11.20 ± 8.75		
BDI (range)	0–36		
Thalamic volumes			
Left thalamus	3.057 ± 1.14 ml	4.247 ± 0.44	***0.008***
Right thalamus	2.831 ± 1.13 ml	4.009 ± 0.38	***0.008***

Abbreviations/acronyms: RRMS = Relapsing Remitting MS, SPMS = Secondary Progressive MS, DD = Disease Duration, EDSS = Expanded Disability Status Scale, FSS = Fatigue Severity Scale, BDI = Beck Depression Inventory.

3.3. Multilinear regression analysis

We used the I-*clinical* values to predict the clinical condition assessing both the impairment of specific brain systems (through the EDSS) and the severity of fatigue (through the FSS) which, in contrast, is a poorly localizable and highly subjective symptom. We performed two different edge-based multilinear regression models using the *I-clinical* (alongside with age, education level, disease duration and gender) as a predictor. In the first model, through a nested k-fold cross validation we selected the best edge-threshold for computing the I-*clinical* by ranking the edges in ascending order of stability. As showed in Fig. 5A, the best thresholding was obtained at 300 edges. The resulting multilinear regression model significantly explained 41% of the FSS variance ($R2 = 0.414$, $f(5,19) = 3.9$, $p = 0.013$), and the *I-clinical* resulted as a significant predictor (beta coefficient $= -0.519$, $p = 0.032$) (Fig. 5B). Comparison between actual and predicted values, and residuals distribution are showed in panel C and D, respectively The I-clinical was also calculated by adding the same number of edges (100) in descending order of stability, with loss of predictive capacity over fatigue severity. By setting the EDSS as a dependent variable in the same multilinear model, the I-*clinical* did not show any statistically significant contribution to the EDSS prediction (data not shown).

To verify the confounding effect of the clinical form (RRMS and SPMS) on the ability of *I-clinical* to predict FSS we performed a multilinear model including, in addition to the aforementioned variables, MS clinical form as one more independent variable. The only independent variable that showed predictive power was the *I-clinical* in the alpha band (data not shown). To evaluate the relationship between the EDSS and FSS, we used a multilinear regression model (see supplementary material) setting the EDSS as an adjunctive independent variable. In that case EDSS was significantly able to predict FSS but with lower predictive power than I-Clinical in the alpha band (Fig. S2).

3.4. Clinical features of fingerprinting

Furthermore, we performed Pearson's correlation to assess whether there was a relationship between the *I-clinical* and the clinical scales of the disease. We found a significant negative correlation between *I-clinical* in the alpha band and FSS ($p = 0.0057$, $\rho = -0.537$) (Fig. 6). Furthermore, Pearson's correlation between FSS and I-*clinical* was performed separately in the RRMS and SPMS groups, confirming in both cases the significant relationship. The same analyses were carried out using EDSS as a variable of interest without finding any significant relationship between EDSS and I-*clinical* in the alpha band.

4. Discussion

In the present study we set out to investigate whether the functional connectivity of MS patients shows reduced identifiability, and whether such reduction relates to the levels of fatigue, as assessed by the FSS. To this end, we used source reconstructed MEG data from two subsequent scans in order to build frequency-specific functional connectomes of both patients and healthy subjects, based on the PLM. Using the clinical connectome fingerprint approach (Sorrentino et al., 2021), we developed an identifiability matrix from which we extracted the I-*self*, the I-*others* and the I-*diff* values of each individual. All these measures of identifiability showed a statistically significant lower score in MS patients as compared to HC, specifically in the alpha band. These results implied lower similarity between two FCs of the same patient and reduced homogeneity among the FCs in the MS group.

Interestingly, we found significant results only in the alpha band. It is widely accepted that the thalamus represents a key region in coordinating the cortical alpha band activity (Cifelli et al., 2002; Hughes and Crunelli, 2007). In the last few years, several studies have highlighted

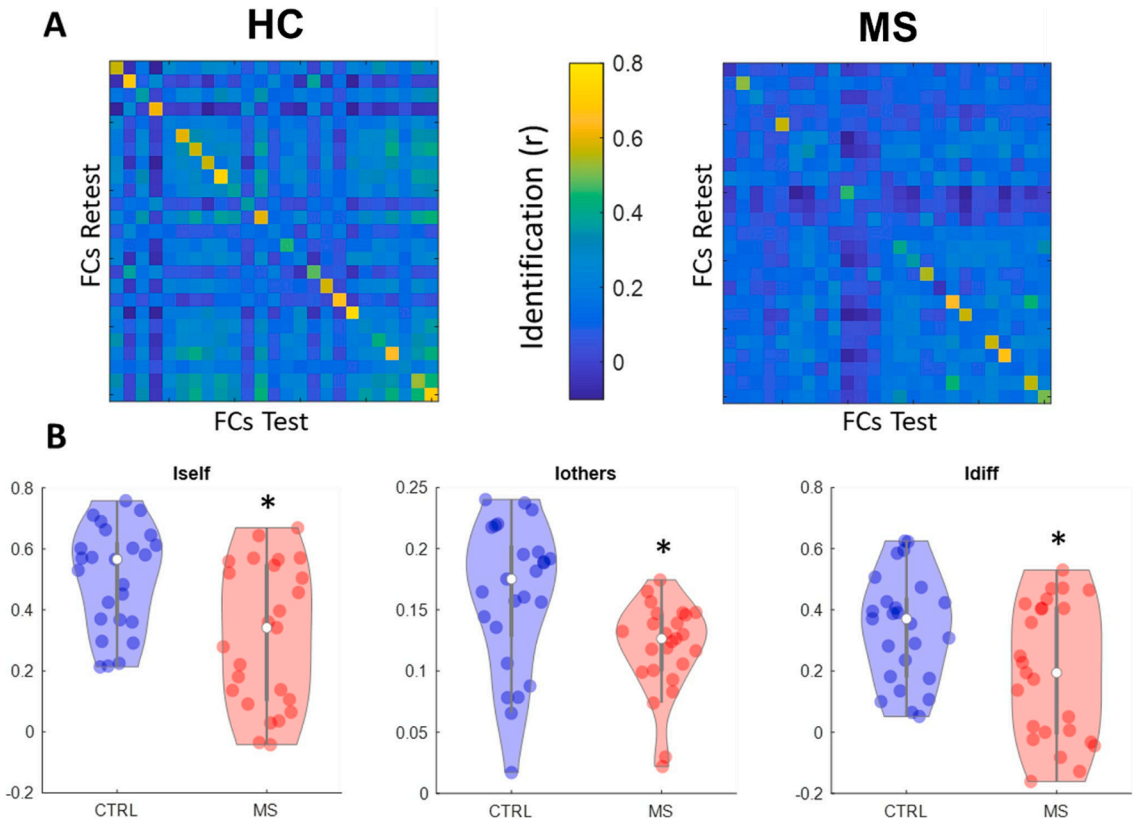

Fig. 2. Brain identification in healthy subjects and MS patients in the alpha band. (A) Identifiability matrices of healthy controls (HC) and patients with Multiple Sclerosis (MS). The FCs test and retest are displayed on rows and columns respectively, while the entries represent the level of similarity between two different recordings. The main diagonal represents self-identifiability (I-self), while off-diagonal elements outline the similarity among different individuals (I-others). The I-diff, resulting from the difference between I-self and I-others, gives an estimation of the fingerprinting level of a group. The more the main diagonal is evident (towards yellow), the more the subjects are identifiable. (B) Statistical comparison between fingerprint parameters calculated on the identifiability matrices of HC and MS. HS show greater identifiability compared to MS patients; all results, reported here, were statistically significant (I-self pFDR = *0.015*, I-others pFDR = *0.015*, I-diff pFDR = *0.042*).

reduced volume of the thalamus in the earliest phases of the disease, which might be related to cellular damage and, in turn, induce changes in the functional connectivity (Amin and Ontaneda, 2021; Cifelli et al., 2002). For example, Tewarie et al. have shown that thalamic volume was positively related to a more random cortical functional network topology and that this result was mostly evident in the alpha band (Tewarie et al., 2015). It is noteworthy that the reduced identifiability of our MS cohort occurs in the alpha band and that there is a statistically significant reduction of the volumes of the thalami as compared to the healthy population (Table 1).

Furthermore, we investigated the relevance of subsets of edges toward subject identification within each group (i.e., patients/controls). In the control group, a few hundred edges led to subject identification, suggesting that subject-specific information is contained preferentially in definite functional patterns. Adopting the same method in the MS group, a few hundred edges were sufficient to obtain a precise subject's identification, but with a slightly lower identifiability power compared to controls. In short, few stable edges were sufficient to identify MS patients and controls. However, the highest identifiability was reached in both groups only when considering a subset of edges and not when the entire functional connectome was used. The progressive addition of more edges yielded worse performance; this might be due to the inclusion of edges whose values are shared by multiple individuals and, therefore, with low power in subject identification. This is in line with recent literature, focused on brain connections and inter-subject variability, that describe the co-existence across individuals of a common structure along with highly subject-specific patterns (Gratton et al., 2018; Laumann et al., 2015). In line with this evidence, when we studied the ROI-specific contributions to individual identification, we found that the most connected ROIs (i.e. with the most edges with the highest stability) were located in the frontal, parietal and temporal lobes. Most of these ROIs are part of the medial frontal and frontoparietal networks, both of which have previously been described (Finn et al., 2015) as the most relevant towards subject identification in fMRI brain connectivity studies. Of note, the identifiability performance drops rapidly as more edges are added in the MS group. This might be interpreted in the light of the fact that each MS patient possesses a specific pattern of lesions. As such, the ICC matrix might not be optimally representative of each subject and, hence, stable edges across whole patients will be fewer.

Finally, we verified our hypothesis that changes in the individual connectome could be reflected in fatigue severity. We used the individual I-*clinical* scores to predict fatigue severity, as assessed by the FSS. We also searched for possible relationships between the I-*clinical* score and the EDSS scale to support the hypothesis that our approach is suitable to recognize clinical symptoms dependent on large-scale network dysfunction rather than those mainly dependent on focal damage (i.e., those considered by the EDSS scale). The I-*clinical*, as calculated by adding the edges from the least stable to the most stable ones, predicted fatigue. This seems to be in contrast with our previous work on degenerative diseases (Alzheimer's and Parkinson's disease (Rucco et al., 2022; Sorrentino et al., 2021)) in which the best prediction of the clinical parameter of interest was obtained by adding the edges in descending order (from more stable to less stable). A speculative hypothesis to explain these results might lie in the different underlying neuropathological processes. While in neurodegenerative diseases the tissue damage follows a well-defined neuropathological pattern that

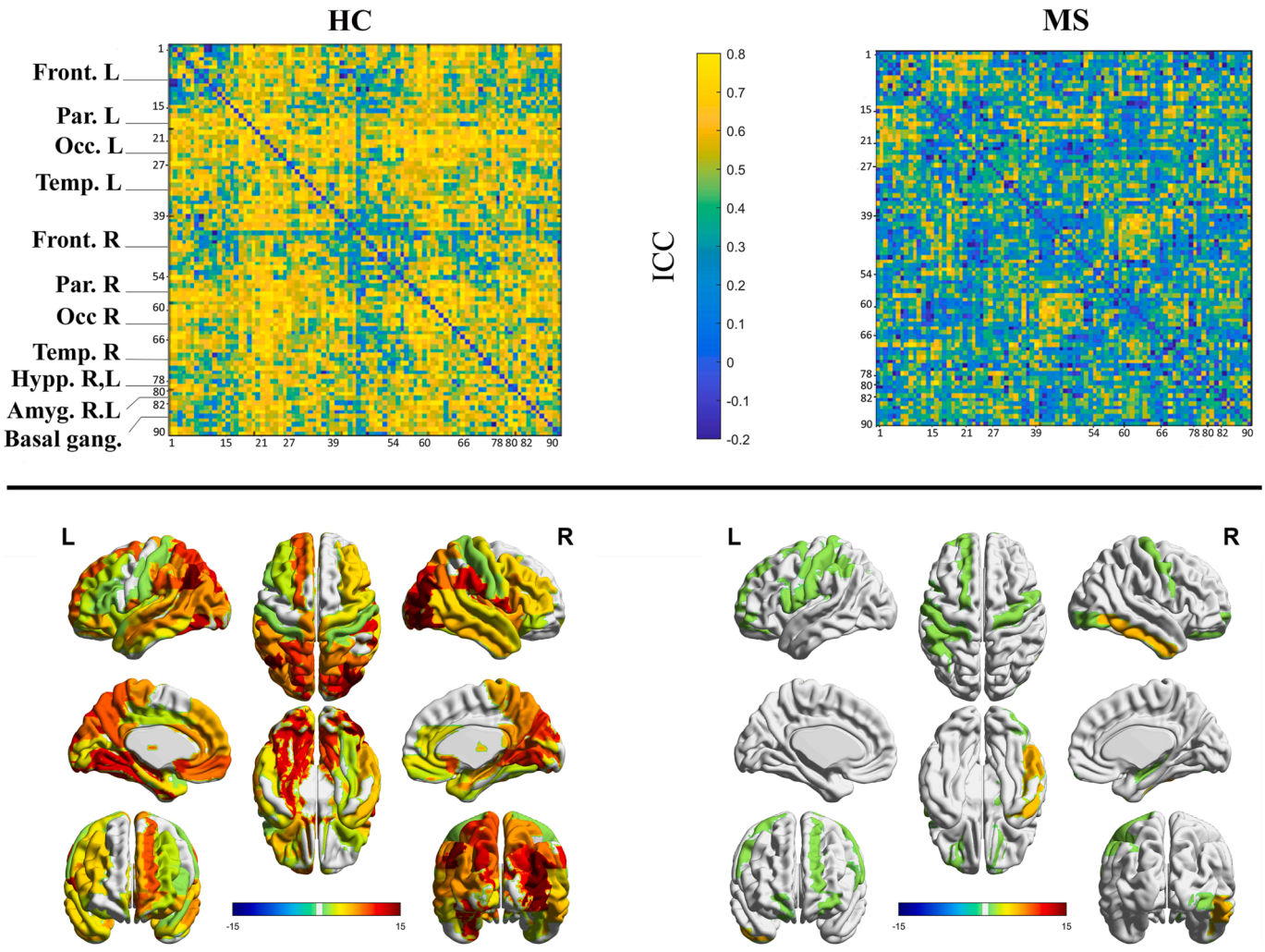

Fig. 3. Edge contribution to connectome fingerprint. The intra-class-correlation (ICC) matrices in the alpha band show the contribution of each edge to the identifiability. The MS patients exhibited lower ICC values. On the bottom, the brains show the nodal strength of the most reliable edges (ICC > 0.75).

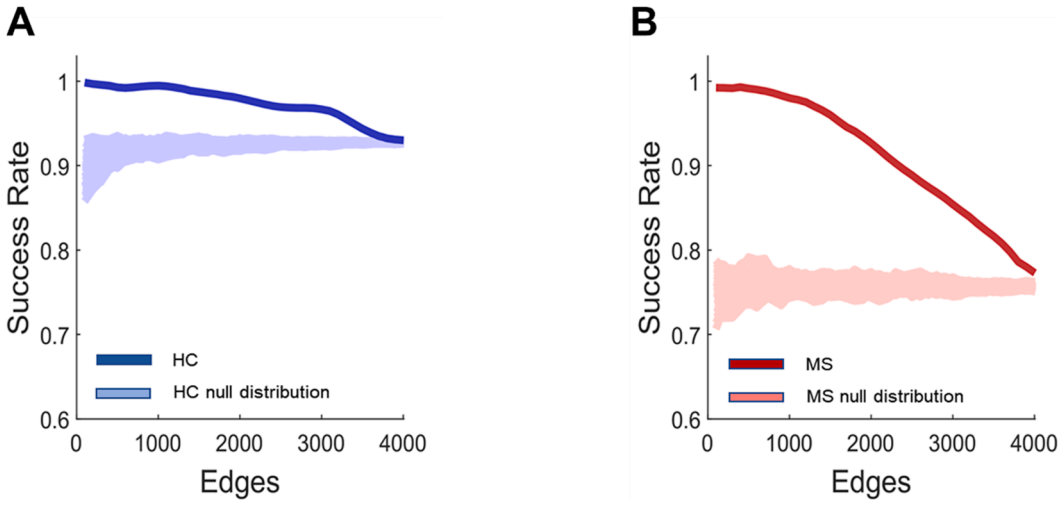

Fig. 4. Iterative model of edgewise subject identification. The success rate (SR) distributions of HC (blue) and MS (red) obtained by adding 100 edges at each step from the most to the least contributing ones, according to the intraclass correlation (ICC) values. The control group (A) quickly reached a complete SR (100%), with a plateau at 1200 edges, and then a progressive decline up to 3000 edges, and a drop beginning at ~3100 edges. The MS group (B) did not reach the complete SR and, in the absence of a real plateau, started a marked drop after ~1000 edges. The shaded areas (4A and 4B) represent the null distributions obtained by adding 100 edges at a time in a random order. The SR based on the ICC-ordered edges displays higher values compared to the null model distributions for both MS patients and HC.

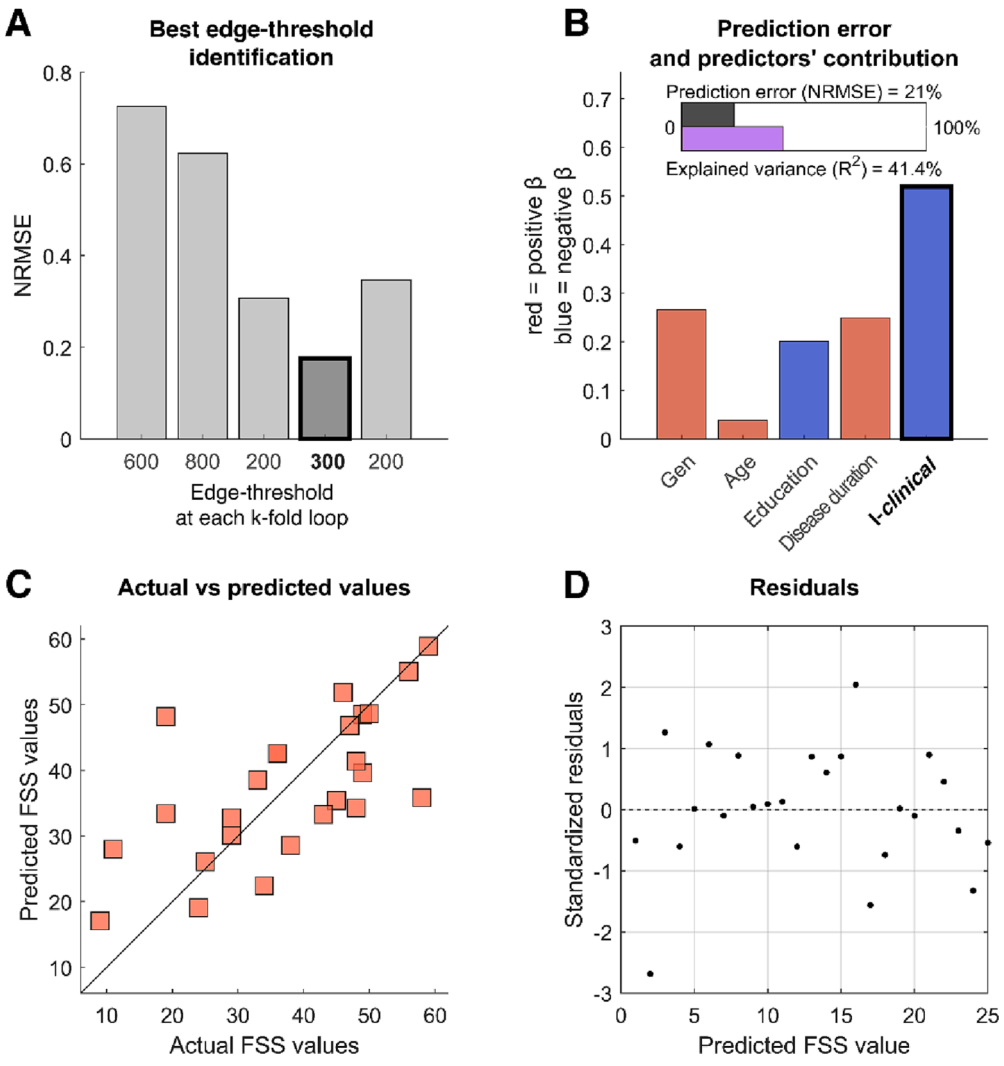

Fig. 5. Multilinear regression for Fatigue severity scale prediction (FSS): features selection and model evaluation. (A) Normalized root mean square error (NRMSE) of the prediction of the best regression models, built within the nested k-fold cross validation framework. Models were built using I-*clinical* values tested on different edge subsets, according to the stability ranking of the training set. After validation, the same edge-threshold and ranking was considered to evaluate the performance on the test set. The characteristics of the model with the lowest NRMSE were used to build the multilinear regression model. (B) Model and predictors evaluation. Significant predictor displays bold bar and text. Blue and red bars represent negative and positive coefficient of the predictor, respectively. (C) Comparison between actual and predicted FSS values after cross-validation. (D) Standardized residuals after cross-validation.

develops over a very long period of time, and which is shared by almost all patients, in MS the tissue damage is extremely variable both with respect to both the site of lesion and the timing of appearance. For this reason, in neurodegenerative diseases, a damaged brain might potentially have potentially enough time to reorganize their brain connections and to adopt a compensatory pathway that progressively consolidates over time. Conversely, the dissemination in time and space, characteristic of MS, could force the affected brains to recruit alternative routes (in order to maintain a proper functional output), which would be more variable over time, hindering the consolidation of a unique, more stable compensatory pathway. This absence of a consolidated compensatory pathway, and the resulting variable recruitment of neuronal tissue, (Genova et al., 2013; White et al., 2009), could be reflected in an unfavorable cost-effectiveness ratio and/or in lower functional performances. As a consequence, MS patients would require more effort to perform even a simple task and, thence, become more readily fatigued (Hidalgo de la Cruz et al., 2017; Jaeger et al., 2019).

As mentioned above, we did not find any statistically significant relationship between the EDSS and the changes in FC. According to our hypothesis, the EDSS, which assesses the function of specific brain systems, might be ~~is~~ more sensitive to focal impairment than to functional changes induced by large-scale remodeling of the brain networks. Nevertheless, it cannot be excluded that the absence of statistical significance might be due to the small sample size, which represents the main limitation of our work. An additional shortcoming of the study is the application of only one test for fatigue severity assessment; it could be interesting to evaluate the reproducibility of these results using different scales for the same symptom.

In conclusion, in this study we applied the clinical fingerprint analysis in a cohort of MS patients, showing that MS individuals have lower identifiability than the healthy subjects and that the degree of patient's identifiability is related to the fatigue severity. We believe that this study could be an input to further investigate the impact of large-scale network remodeling on subject identifiability and its relation to clinical features.

5. Declarations

Author contributions: Concept and design: LC, ETL, PS. Data collection, analysis and interpretation: AR, MR, ML, AP, FC, MA, ETL, VJ, SB. Drafting of the manuscript: LC and GS. Critical revision of the manuscript for important intellectual content: GS, PS and SB.

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***Ethical statement*:** All procedures performed were in accordance with the ethical standards of the institutional research committee and with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent and consent to participate: Written informed consent has been obtained from all participants.

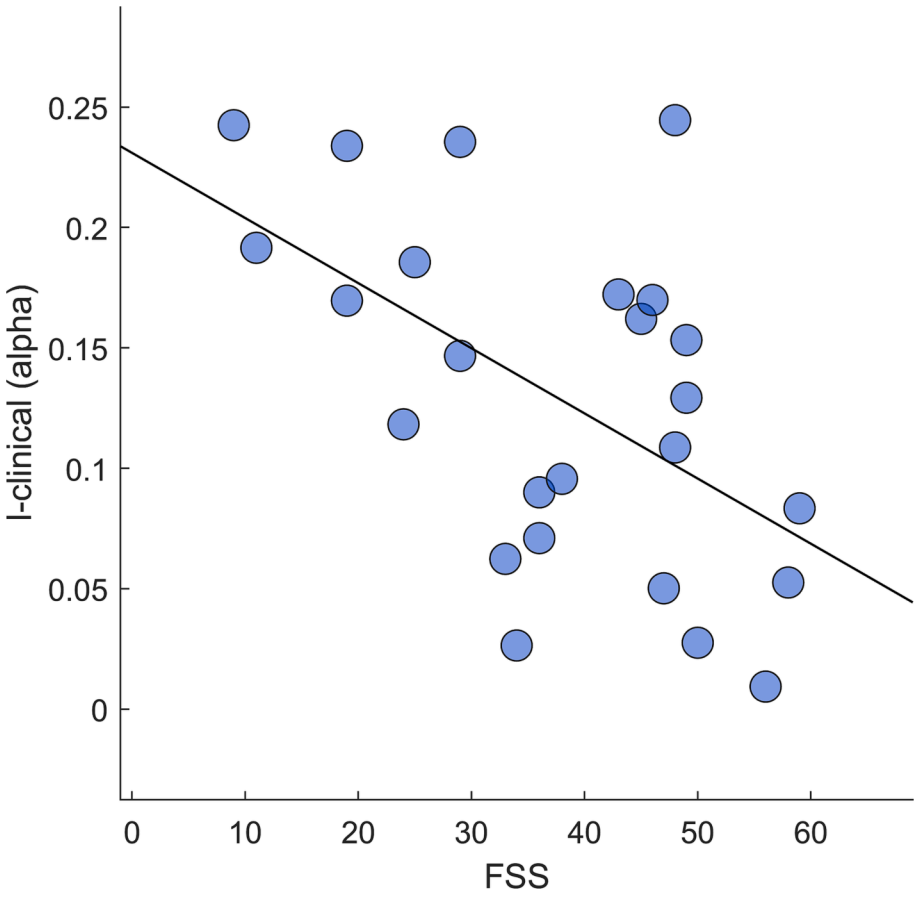

Fig. 6. Relationship between the fatigue severity scale and clinical identifiability. Pearson's correlation between FSS and clinical identifiability as measured by the *I-clinical* scores in the alpha band. The I-*clinical* was obtained by adding 300 edges in ascending order of stability according to k-fold cross validated ICC scores. An inverse relationship is shown between FSS and identifiability: the higher the fatigue symptoms, the least the patients' connectivity is similar to the one of the healthy individuals.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2023.103464.

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