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Network Localization of Fatigue in Multiple Sclerosis

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ABSTRACT

Background: Fatigue is among the most common symptoms and one of the main factors determining the quality of life in multiple sclerosis (MS). However, the neurobiological mechanisms underlying fatigue are not fully understood. Here we studied lesion locations and their connections in individuals with MS, aiming to identify brain networks associated with fatigue.

Methods: 38 MS patients with and 21 without fatigue were included in the study. Association between fatigue and lesion locations was investigated using voxel-lesion symptom mapping and lesion connectivity using lesion network mapping. The findings were tested in two independent datasets, including (1) MS patients scanned using resting-state functional connectivity MRI (rs-fcMRI) ($n = 199$) and (2) individuals with stroke lesions ($n = 85$).

Results: There were no specific anatomical MS lesion locations significantly associated with fatigue, but lesions associated with fatigue were connected to a common network with peak positive connectivity to the right premotor cortex and negative connectivity to the left temporal pole ($p_{FWE} < 0.05$). Of the two identified network nodes, connectivity from the premotor cortex to multiple other brain regions was significantly associated with MS fatigue severity in the independent dataset of MS patients ($p < 0.05$). The MS fatigue network was also reproducible in poststroke fatigue (spatial correlation $r = 0.57$, permutation test $p = 0.02$), again showing that lesion connectivity to the premotor cortex, but not the temporal pole, was associated with fatigue ($p = 0.04$).

Conclusions: Our results show that fatigue in MS localizes to a brain network, lending insight into the neural substrates of fatigue.

1 | Introduction

Multiple sclerosis (MS) is the most common chronic demyelinating inflammatory disease of the central nervous system. The prevalence is increasing with over 2 million people affected worldwide in 2015 while the latest global prevalence estimate is 2.8 million [1, 2]. MS can cause a variety of motor and non-motor symptoms, associated with inflammation and resulting damage in the central nervous system [3]. Fatigue is one of the most common non-motor symptoms, reported by 53%–83% of people with MS, and a large proportion of MS patients consider fatigue as their most disabling symptom [3–5].

Fatigue is common in many disorders affecting the immune system [6] but it also occurs in patients without immunological disorders, such as patients with focal stroke lesions [7]. Recent

studies have demonstrated that there is no clear relationship between MS fatigue and inflammation per se [8, 9]. Fatigue in MS also seems to be a persistent symptom after it has appeared [10, 11], suggesting that fatigue is, at least partly, caused by damage to specific neural structures.

Localization of MS-related fatigue, however, has proven difficult with no lesion locations or structural brain abnormalities consistently associated with fatigue [12]. Instead, several new models propose that prolonged fatigue more likely arises from dysfunction of brain networks [5, 8]. This view is supported by the findings from structural and functional connectivity imaging studies, highlighting widespread connectivity abnormalities in MS. The networks underlying fatigue have been hypothesized to include cortico-striato-thalamo-cortical circuits or sensory networks but have not yet been identified in detail [12–15]. One of the reasons for this gap is that identifying the networks disrupted by lesions is difficult because the tissue in the lesion locations and their connections is already lost [12].

Lesion network mapping is a relatively recently developed technique, which can be used to identify brain intrinsic connections disrupted by lesions by using an external connectome, a "wiring diagram" of the human brain [16]. This technique was originally developed for incidental focal brain lesions (e.g., caused by strokes or tumors) and has successfully been used to localize networks of several different neurological and psychiatric symptoms [17]. For example, lesions causing hemichorea-hemiballismus localize to a network, defined by connectivity to the posterolateral putamen, the exact same structure that becomes hyperintense in metabolic chorea-ballism [18]. Lesions causing visual hallucinations are located in heterogeneous brain regions but are connected to the visual cortex [19]. Many studies have demonstrated that networks identified by lesion network mapping are also abnormal in other disorders causing the same symptom, suggesting generalizability across etiologies [20–22]. In addition, networks identified based on causal lesions seem to converge with efficacious treatment targets, also suggesting relevance for therapy. More recently, this technique has also been shown to be applicable on MS white matter lesions [23]. Importantly, brain networks derived from white matter lesions of MS patients with depression compared to those without depression aligned with brain networks identified from focal gray matter lesions causing depression, demonstrating feasibility of this approach [23]. Furthermore, functional magnetic resonance signal of white matter has recently been shown to represent tract-specific responses to neural activity, supporting the rationale of utilizing white matter lesions in connectivity analysis [24].

Here we use lesion network mapping to identify the brain network associated with fatigue in MS, comparing patients with vs. without fatigue. The relevance of the identified network for fatigue was tested in an independent, unselected sample of MS patients with assessment of fatigue, scanned using rs-fcMRI. Finally, to control for immunological mechanisms and test for potential generalizability outside MS, the relevance of the fatigue network was further tested in patients with a recent stroke and fatigue assessment.

2 | Materials and Methods

2.1 | Patients With Multiple Sclerosis With and Without Fatigue (Dataset 1)

2.1.1 | Patients

MS patients with and without fatigue in the Hospital District of Southwest Finland were identified from the national MS register [25]. The data collection was approved by the Hospital District of Southwest Finland with a waiver for a separate ethical board review because of the retrospective nature of the data, in accordance with the current research legislation in Finland.

Two groups of patients were included into this study: MS patients with fatigue and matched control MS patients without fatigue. The inclusion criteria for both of these groups were (i) age at least 18 years old, (ii) had early MS (less than 5 years from the diagnosis), (iii) good quality MRI within 12 months from the clinical evaluation and (iv) no relapses within three months prior the clinical evaluation. For relapsing remitting MS (RRMS), only patients with Expanded Disability Status Scale (EDSS) ≤ 3 were included, to minimize the confounding effect of more advanced disability on fatigue. Higher EDSS scores were accepted in primary progressive MS (PPMS), because PPMS causes hemiparesis more often at an early phase of the disease, increasing the EDSS score earlier compared to RRMS [26, 27]. However, the main analyses were repeated with RRMS patients only to ensure that the results are not driven by patients with progressive MS.

For the fatigue group, inclusion criteria were clinically significant fatigue symptoms without another explanation for their fatigue and Fatigue Severity Scale (FSS) score at least four points per item on average on a scale from 1 to 7 [28]. FSS scores were obtained as part of the clinical routine. For the control group, we included patients without clinically significant fatigue and FSS scores less than four points. The exclusion criteria for both groups were (i) any diagnosed depression or antidepressant medication use for mood disorders, (ii) sleep disorders (iii) any focal brain lesions not caused by MS, or (iv) recent MS relapse (within three months from the clinical evaluation). Current disease-modifying therapy information was collected and classified as high-efficacy (heDMT; natalizumab, rituximab, ocrelizumab, alemtuzumab, cladribine, fingolimod) and moderate-efficacy (meDMT; dimethyl fumarate, teriflunomide, glatiramer acetate, interferon beta-1a, peginterferon beta-1a). In addition, current use of medications used to treat fatigue (venlafaxine, bupropion, modafinil or amantadine) was collected.

2.1.2 | Multiple Sclerosis Lesions

White matter lesions of the MS patients were identified from available clinical MRI scans from the fluid-attenuated inversion recovery (FLAIR) images using a CE marked and FDA 510(k) cleared cNeuro cMRI software (version 1.9.0) [29] that is based on the expectation–maximization algorithm, as described in detail previously [30]. A single lesion mask combining all MS lesions was created for each patient. These lesion masks were then registered to Montreal Neurological Institute (MNI) space using the spatial information of T1-weighted MR images with

FSL software (version 6.0.3): First, brain extraction tool (BET) was used to remove all non-brain tissue from images. Second, the extracted brain images were registered to MNI space using linear registration (FLIRT) followed by non-linear registration (FNIRT) [31]. The resulting registration results and accurate representation of the lesion locations were carefully evaluated visually. Based on the visual evaluation of the normalized lesion masks, some of them extended to adjacent gray matter regions. To investigate which type of lesions drive the observed effects, the connectivity analysis was repeated separating the lesion voxels in each case into white matter, cortical, and basal ganglia lesions according to the SPM12 templates (https://fil.ion.ucl.ac.uk/spm/). The total volumes of MS lesions in the whole brain and white matter were calculated from the lesion masks registered to MNI space [31].

2.1.3 | Voxel-Lesion Symptom Mapping (VLSM)

Lesion locations of the MS lesion dataset's patients with and without fatigue were compared using voxel-based lesion symptom mapping (VLSM) using NiiStat software (https://github.com/neurolabusc/NiiStat) according to the published recommendations [32]. The analyses were conducted across the whole brain voxels affected in at least 10% ($n \geq 6$) of the patients, as recommended by the published VLSM guidelines [32, 33] using age, EDSS score and lesion load as covariates. Statistical significance was set at false discovery rate (FDR) corrected $p < 0.05$.

2.1.4 | Lesion Network Mapping

The network of brain regions functionally connected to each MS lesion dataset's lesions location was identified as described previously [34]. Briefly, lesion masks in MNI space were used as seeds and functional connectivity to all brain voxels was computed using a rs-fcMRI dataset collected from 1000 neurologically healthy volunteers [34], resulting in a single whole-brain connectivity map per patient. These connectivity maps of the MS patients with ($n = 38$) and without ($n = 21$) fatigue were then compared using non-parametric permutation interference with threshold-free cluster enhancement implemented in FSL software [31]. EDSS, age, and overall lesion load were used as nuisance covariates. Family-wise error (FWE) corrected p values below 0.05 were considered significant. To ensure the results were not biased by MS type heterogeneity, the main analysis was repeated with RRMS patients only, and also using sex, MRI field strength or disease-modifying therapies as additional covariates. The similarity of the connectivity maps derived from all MS patients and from RRMS only was evaluated using spatial correlation coefficients and permutation analysis (1-tailed test, testing the hypothesis that the maps are more similar than what would be expected by chance), as described earlier [35]. Correlation between FSS score and the nuisance covariates (EDSS, age, and overall lesion load) in the fatigue group was tested using Spearman's rank order correlation coefficients.

Eventually, a whole brain connectivity profile (referred to as the MS fatigue map) and significant regions of positive and negative functional connectivity specific to MS patients compared to patients without fatigue were obtained. The relevance of this network for fatigue was then tested using the two independent datasets: (1) MS patients who underwent rs-fcMRI, and (2) stroke patients with poststroke fatigue assessment, as detailed below. To investigate the relationship of the findings to known large-scale functional networks, we used the 7-network cortical parcellation defined by Yeo et al. (2011) [36]. First, the mean t-value in each individual's connectivity map for each of the seven networks was computed. Then, for every network, the mean t-values were compared between MS patients with and without fatigue using an independent samples t-test.

2.2 | Confirmation of the Relevance of the Lesion Network Mapping Findings in an Independent Sample of MS Patients With Resting State Functional MRI (Dataset 2)

To test whether the regions significantly associated with fatigue using lesion network mapping are abnormally connected in MS patients with fatigue, we investigated the association between functional connectivity from these regions and fatigue in an independent, large sample of unselected MS patients scanned with rs-fcMRI.

2.2.1 | Patients

Patients with MS were recruited by the Department of Human Neuroscience of Sapienza University and the study protocol was approved by the ethical committees of Policlinico Umberto I/Sapienza University (Rome, Italy). All subjects provided written informed consent. Patients with MS were retrospectively selected from the databases of the recruiting site, according to the following selection criteria: diagnosis of MS in compliance with McDonald's criteria [37, 38]: age between 18 and 70 years; baseline clinical assessment and MRI examination not more than one month apart; absence of relapses and/or steroid treatment in the 30 days preceding the MRI; absence of neurological diseases other than MS; and available FSS data.

2.2.2 | MRI Protocol

Images were acquired on a 3.0T scanner (12-channel head coil for parallel imaging, Verio, Siemens AG). The MRI protocol included the following sequences for all the subjects: (a) High-resolution 3D, T1-weighted (T1w) MPRAGE with 176 sagittal slices, 1-mm-thick slice, no gap (TR = 1900 ms, TE = 2.93 ms, flip angle = 9°, matrix = 256 × 256, FOV = 260 mm); (b) Dual-echo, Proton Density (PD) and T2-weighted images (TR = 3320 ms, TE = 10/103 ms, FOV = 220 mm, 384 × 384 matrix, 25 4-mm-thick slices, 30% gap); (c) Blood-oxygenation-level-dependent (BOLD) single-shot echo-planar images (TR = 3000 ms, TE = 30 ms, flip angle = 89°, 64 × 64 matrix, voxel size $3 \times 3 \times 3\,mm^3$, 50 contiguous slices, 140 volumes, acquisition time = 7 min). The patients were instructed to close their eyes and stay awake during the rs-fMRI acquisitions.

Structural and Functional images were pre-processed and analyzed using fMRIprep v20.2.3 and FSL v6.0.1 (FMRIB's Software Library http://fsl.fmrib.ox.ac.uk/fsl). Lesions were

identified on Proton Density (PD)-weighted images (Jim 5.0, Xinapse System, Leicester, UK; http://www.xinapse.com) by experienced neuroscientists and used to fill the lesions of the T1w images prior to further preprocessing. The lesions were filled to avoid misidentification of the lesioned white matter as gray matter when using the tissue-segmentation tool. Structural images were then bias-corrected and skull stripped. Functional preprocessing included motion correction, slice time correction, and co-registration onto anatomical scans and MNI standard space. Automatic removal of motion artifacts was performed using non-aggressive regression of the components obtained via independent component analysis (ICA-AROMA) and spatial smoothing with a kernel of 6 mm was applied. Finally, the corrected time series were high-pass filtered at 0.1 Hz and regressed by the cerebrospinal fluid signal.

2.2.3 | Functional Connectivity Analyses

The seed-based analysis of the rs-fcMRI dataset was performed in MNI space. The positive and negative nodes of the MS fatigue map were used as seeds to perform the connectivity analysis. The mean time series of both of these seeds was extracted from the corrected functional image of each subject and the voxel-wise maps of functional connectivity were individually calculated between each seed and the rest of the brain separately via a general linear model.

Association between voxel-wise functional connectivity maps with FSS was calculated non-parametrically for both positive and negative nodes of the MS fatigue map using FSL randomize, setting 5000 permutations and TFCE (Threshold-Free Cluster Enhancement) family-wise error correction with significance $p < 0.05$. Age and sex were included as covariates of no interest.

2.3 | Confirmation of the Relevance of the Lesion Network Mapping Findings for Fatigue in an Independent Sample of Stroke Patients (Dataset 3)

Finally, to control for immunological mechanisms and investigate potential generalizability across lesion etiologies, we tested whether the lesion network mapping findings are reproducible in patients with poststroke fatigue, as stroke is primarily not an immunological disorder.

2.3.1 | Patients

Patients with stroke (ischemic or hemorrhagic) were obtained from an ongoing prospective study at the Turku University Hospital Neurocenter between 25th of February 2020 and 30th of August 2021. The subjects in this dataset were identified and enrolled at the stroke ward, including all patients who had suffered a stroke within a month at the time of enrollment and were willing to participate in the study. At baseline, the subjects were screened for pre-existing brain disorders and clinically interviewed and examined by a study physician in consultation with a consultant neurologist. The subjects were invited for a follow-up visit approximately three months after the stroke. Fatigue was assessed using FSS [28]. To minimize the confounding effects of stroke severity, we only included patients who had suffered a minor stroke (NIHSS < 5) with poststroke FSS scores available.

The study protocol for the prospective data collection was approved by the local ethics committee and received approval from the Hospital District of Southwest Finland. All subjects signed an informed consent before participating in this study, and the study was conducted according to the principles of the Declaration of Helsinki.

2.3.2 | Stroke Lesions

Lesions of the stroke patients were delineated on the clinical T1 weighted images (T1w) or computed tomography (CT) images based on available imaging sequences for each patient. Stroke lesions were then manually segmented to a mask image by a trained researcher or a clinical investigator with expertise in neurology and neuroimaging. The lesions were manually drawn using ITK-SNAP [39]. If the lesion masks were done by the trained researcher, they were double-checked by the study physician. Lesion masks were again registered to MNI 152 space: First, anatomical T1-weighted MRIs were skull stripped using the brain extraction tool implemented in the Advanced Normalization Tools package (ANTs) [40] and CT images were skull stripped using the FSL pipeline (FMRIB Software Library v6.0, Analysis Group, FMRIB, Oxford, UK), as described previously [41]. Next, the skull-stripped images were normalized to MNI space using rigid, affine, and diffeomorphic (SyN) registration methods from the ANTs software package [40]. The binary lesion masks traced in anatomical space were used as inputs on normalization with the skull-stripped T1 and CT images to avoid distortion near brain lesions. Finally, the obtained transformations from native to MNI space were applied to the binary lesion masks, as described previously [42]. Volumes of the stroke patients' lesions were calculated similarly as with the MS lesions.

2.3.3 | Lesion Network Mapping in Stroke Patients

To test the connectivity findings in an independent cohort and to investigate potential generalizability in other etiologies, connectivity analyses were conducted similarly to the MS lesion dataset lesions. We computed voxelwise correlation between lesion connectivity and post stroke FSS scores in the stroke cohort ($n = 85$). Age and lesion volume were used as nuisance covariates. The outcome from this analysis was a connectivity map showing connections associated with poststroke fatigue. The similarity of the connectivity maps derived from MS and the stroke lesion sample was calculated using spatial correlation and tested using a permutation analysis (1-tailed test, testing the hypothesis that the maps are more similar than would be expected by chance) [35].

Finally, to test if connectivity to the nodes of the MS fatigue map is associated with poststroke fatigue, stroke lesion to MS

fatigue map node connectivity was computed using a seed-to-seed analysis. The resulting R values were z-transformed and used as dependent variables in multiple linear regression models investigating the association between connectivity and poststroke fatigue including age and lesion volume as nuisance covariates. Correlation analyses between significant cluster average connectivity values and demographic and clinical characteristics were investigated using Pearson's correlation coefficient or Spearman's rank order correlation coefficient, as appropriate.

2.3.4 | Statistical Analyses

Differences in demographical and clinical characteristics and lesion load between MS patients with or without fatigue were compared using independent samples t-test or Mann–Whitney U-test (continuous variables) and Fisher Exact test (categorical variables), as appropriate. For clarity, all other statistical tests associated with specific datasets are described in the corresponding methods sections.

3 | Results

3.1 | Localization of Fatigue in the MS Lesion Dataset (Dataset 1)

We identified 38 MS patients with fatigue and 21 MS patients without fatigue. There were no significant differences between the groups in sex, age, EDSS score, medications (use of heDMT, meDMT, or interferons), MRI field strength, time between clinical evaluation and MRI, or overall lesion load (Table 1). As expected, medications used to treat fatigue were more common in the fatigue group compared to the control group (Table 1). FSS scores did not correlate significantly with EDSS, age or lesion load in the fatigue group ($p \geq 0.22$). Overall lesion load was not significantly associated with MRI field strength ($p = 0.97$), heDMT ($p = 0.42$), or meDMT ($p = 0.95$). All lesions were white matter lesions, but some extended to the adjacent gray matter with 87.6% of the lesion load within the white matter. There was no significant difference in white matter lesion load between MS patients with and without fatigue ($p = 0.46$).

In the voxelwise whole brain VLSM analysis, there were no anatomical lesion locations significantly associated with fatigue (Figure 1). However, MS lesion locations associated with fatigue had significantly higher connectivity to the right premotor cortex and lower connectivity to the left temporal pole when compared to the MS lesion locations not associated with fatigue ($p_{FWE} < 0.05$, Figure 2A–C, Table S1). The significance of the right premotor node did not change when adding sex, MRI field strength, use of heDMT/meDMT, or use of interferons as a covariate (Figure S1). However, the left temporal pole node was not significant across all these analyses (Figure S1). Connectivity from the MS lesions to the significant nodes also was not significantly associated with MRI field strength, EDSS, overall lesion load, heDMT, or meDMT ($p \geq 0.16$). As all statistical thresholds can be considered somewhat arbitrary, the unthresholded whole brain MS fatigue map associated with fatigue is shown in Figure 2D. To ensure that the results were not driven by disease type, the MS fatigue map was recomputed with RRMS patients only. The resulting RRMS fatigue map ($n = 47$) was almost identical to the fatigue map computed from the whole sample (spatial correlation $r = 0.89$) (Figure S2). The results also remained similar when analyzing white matter, but not cortical or basal ganglia, lesions only with identical peaks (spatial correlation with the main map $r = 0.96$, which

TABLE 1 | Demographic and clinical characteristics of patients with multiple sclerosis (MS).

	MS with fatigue	MS without fatigue	*p*
Sample size	38	21	
Female, *n* (%)	29 (76%)	14 (67%)	0.54
Age y, mean ± SD	42.4 ± 10.2	38.2 ± 10.2	0.13
Disease duration y, mean ± SD	1.2 ± 1.4	1.8 ± 1.5	0.11
Time between clinical evaluation and MRI y, mean ± SD	0.3 ± 0.3	0.4 ± 0.3	0.54
EDSS score, median [IQR]	2.0 (1.0, 2.5)	1.5 (1.3, 3.0)	0.24
RRMS, *n* (%)	30 (79%)	17 (81%)	0.45
Lesion load, mean (ml) ± SD	8.6 ± 6.1	11.2 ± 6.8	0.61
3T MRI, *n* (%)	29 (76%)	16 (76%)	1.00
Medications for fatigue	9 (24%)	0 (0%)	0.01
DMT, *n* (%)	32 (84%)	20 (95%)	0.40
heDMT, *n* (%)	18 (47%)	10 (48%)	1.00
meDMT, *n* (%)	14 (37%)	10 (48%)	0.58
Interferon, *n* (%)	6 (16%)	4 (19%)	0.73

Abbreviations: DMT, disease modifying therapy; EDSS, Expanded Disability Status Scale; heDMT, high efficacy DMT; meDMT, moderate efficacy DMT; RRMS, relapsing–remitting multiple sclerosis.

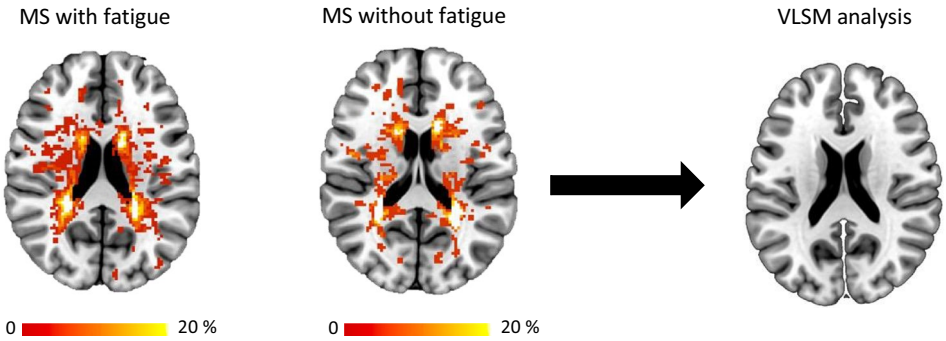

FIGURE 1 | **White matter lesion locations in MS patients.** Lesion overlap in multiple sclerosis (MS) patients with (left) and without fatigue (middle). The maximum overlap of lesions at any voxel was 53% and 67% of the sample in the periventricular white matter, respectively. There were no lesion locations significantly different between patients with and without fatigue in Voxel-Based Lesion Symptom Mapping (VLSM) analysis.

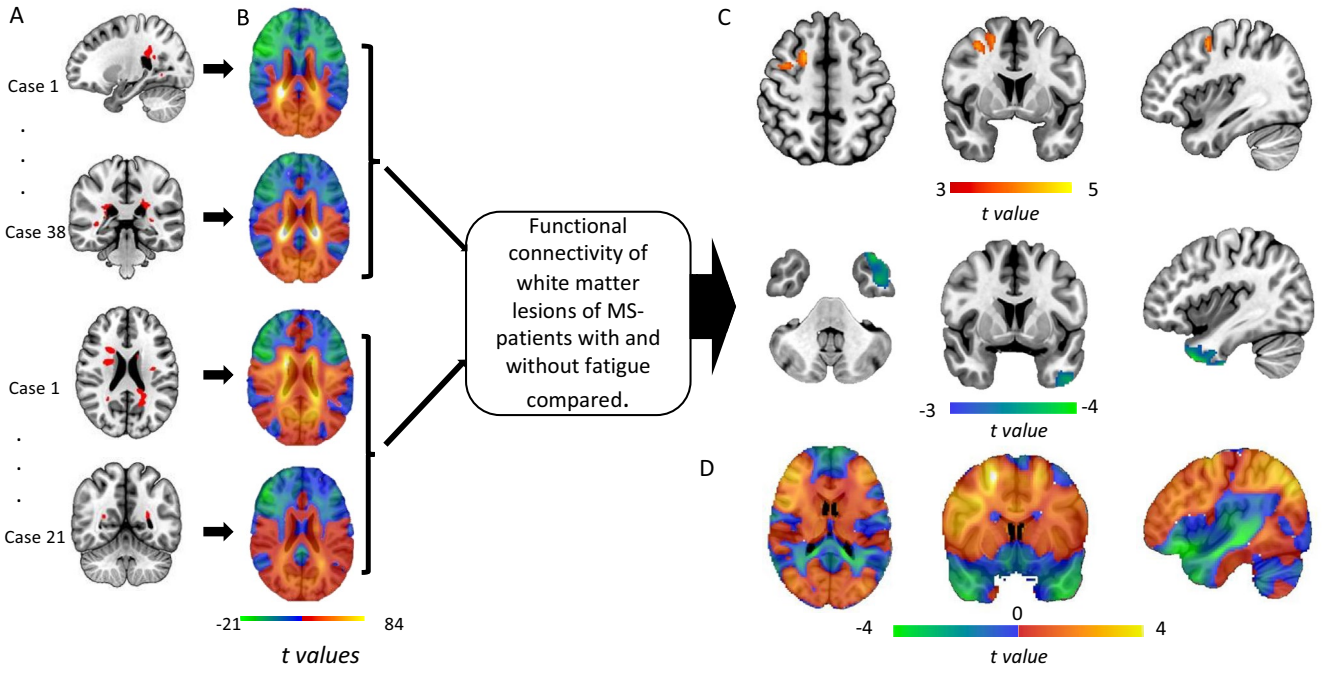

FIGURE 2 | **Lesion network mapping of fatigue in MS.** First, white matter lesions of multiple sclerosis (MS) patients with and without fatigue were transferred to MNI space (A). Second, functional connectivity maps were created for each lesion location and compared between MS patients with and without fatigue (B). Lesion locations in patients with fatigue showed significantly higher connectivity to the premotor cortex and lower to the temporal pole ($p_{FWE} < 0.05$, C). The untresholded whole brain MS fatigue map is shown in panel D. FWE, family wise error.

was higher than what would be expected by chance, $p < 0.001$) (Figures S3 and S4). When examining the contribution of each of the seven large-scale networks, mean *t*-values were higher in patients with compared to without fatigue in the dorsal attention network, and lower in the default mode network and the limbic network (Figure S5 and Table S2).

3.2 | Relevance of the Lesion Network Mapping Findings in an Independent Sample of MS Patients (Dataset 2)

The demographic and clinical information of patients with MS of the connectivity analysis dataset ($n = 199$) is reported in Table 2. In this sample, FSS score was negatively associated with connectivity from the right premotor cortex to multiple regions, including somatosensory, motor, prefrontal and cingulate cortex, and precuneus (Figure 3). However, there was no significant association between FSS and connectivity from the left temporal pole to any brain regions (Figure 3).

3.3 | Reproducibility of the Findings in Poststroke Fatigue (Dataset 3)

The demographic and clinical information of patients with minor stroke and poststroke fatigue assessment ($n = 85$) is shown in Table 3. Poststroke fatigue severity map computed from the stroke patients was similar to the MS fatigue map (spatial correlation $r = 0.57$, permutation test $p = 0.02$, Figure 4A,B). In the lesion-to-ROI connectivity analyses, more positive connectivity between the lesion location and right premotor cortex, but not

the left temporal pole, was associated with post-stroke fatigue severity ($n = 85$) (Figure 4C).

4 | Discussion

There are several important findings in the present study. First, overall lesion load or specific anatomical lesion locations was not associated with fatigue in patients with MS, consistent with the findings from previous studies [12]. However, lesions in MS patients with fatigue localized to a common network, with peaks in the right premotor cortex and left temporal pole. Second, the association between connectivity from the premotor cortex and fatigue severity was confirmed in a large, independent dataset of MS patients scanned with resting state fcMRI. Finally, the MS fatigue network was shown to be reproducible in poststroke fatigue, confirming the findings in an independent dataset with a more focal lesion type that is not caused by an immunological mechanism. Overall, our findings support the hypothesis of a network dysfunction underlying fatigue.

Fatigue in MS has been hypothesized to originate from dysfunction of neural networks, including cortico-striato-thalamo-cortical circuits [8, 14]. However, the networks underlying fatigue have not yet been identified in detail [8, 14]. For example, rs-fcMRI studies have shown multiple connectivity abnormalities in MS patients with fatigue, such as abnormal

TABLE 2 | Demographic and clinical characteristics of MS patients with resting state functional connectivity data.

	MS patients with fatigue
Sample size	199
Female, *n* (%)	160 (80%)
Age y, mean ± SD	42.6 ± 9.6
Disease duration y, mean ± SD	11.8 (9.2)
Phenotype (RRMS/SPMS)	185/14
EDSS, median [IQR]	2 [0–7.5]
FSS, mean ± SD	4.3 (1.8)

Abbreviations: EDSS, Expanded Disability Status Scale; FSS, Fatigue Severity Scale; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.

TABLE 3 | Demographic and clinical characteristics of stroke patients.

	Stroke patient cohort
Sample size	85
Female, *n* (%)	28 (33%)
Age y, mean ± SD	66.8 ± 11.3
NIHSS score, median [IQR]	0 (0.0, 1.0)
Ischemic stroke, *n* (%)	76 (89%)
Lesion volume, mean (mL) ± SD	8.4 ± 17.1
MRI/CT, *n* (%)	64/21 (75/25%)

Abbreviation: NIHSS, National Institute of Health Stroke Scale.

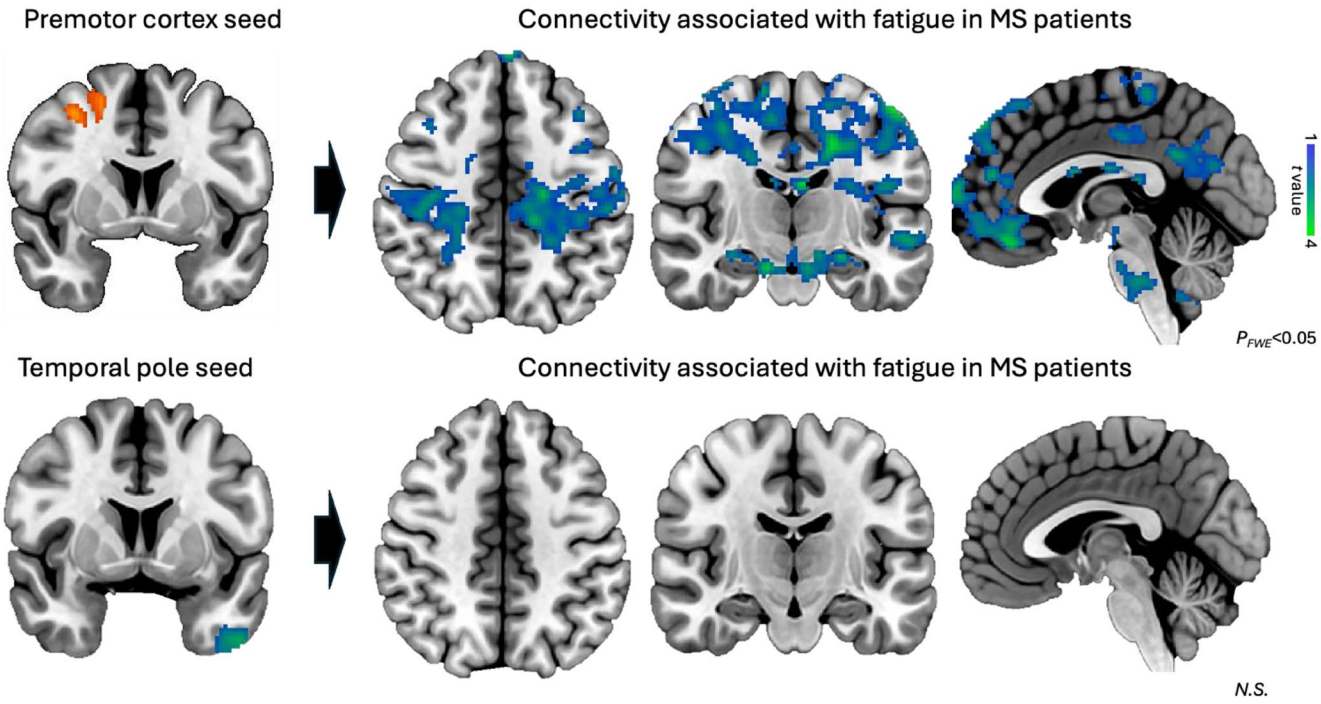

FIGURE 3 | **Resting-state functional connectivity to right premotor cortex of MS patients with fatigue.** The previously defined MS fatigue network nodes at the right premotor cortex and left temporal pole (Figure 2C) were leveraged in a region-of-interest analysis, where the correlation between resting-state functional connectivity and Fatigue Severity Scale scores was studied among all MS patients in the connectivity analysis group. The right premotor cortex showed more negative connectivity to multiple regions, with emphasis on the somatomotor cortex, orbitofrontal and anterior cingulate cortices, precuneus, and posterior cingulate cortex. ($p < 0.05$). FWE, family wise error; MS, multiple sclerosis.

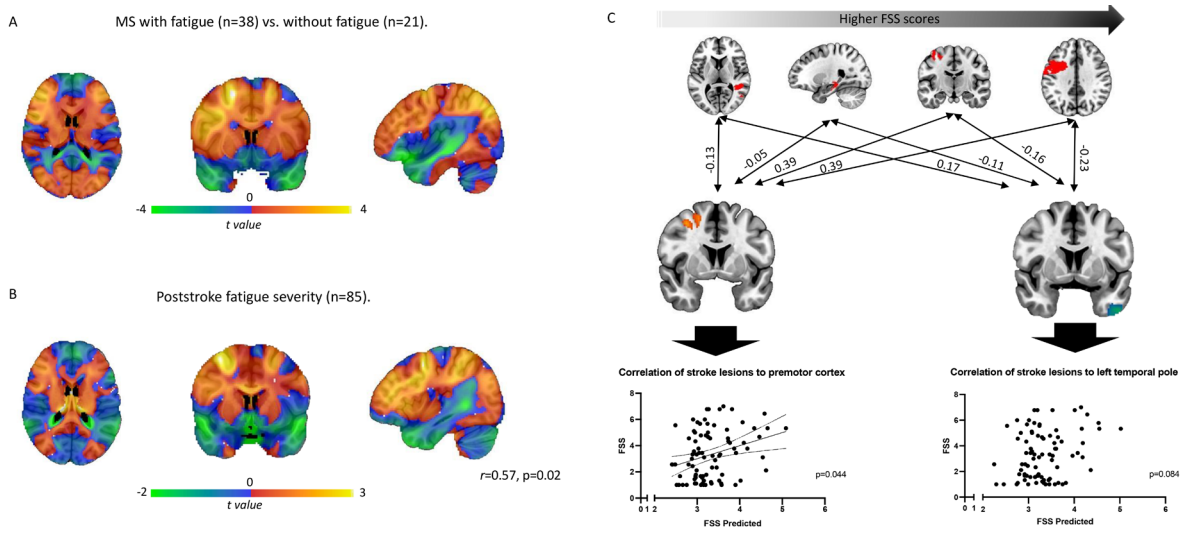

FIGURE 4 | **Similarity between MS and poststroke fatigue maps, and stroke lesion connectivity to the MS fatigue map node.** Multiple sclerosis (MS) fatigue map (A, also shown in Figure 2D) and poststroke fatigue severity map (B). Poststroke fatigue severity map (B) was computed with 85 poststroke patients with fatigue assessment after their stroke. This map was more similar to the MS fatigue map than what would be expected by chance (permutation test $p < 0.05$). Spatial correlation coefficient with the MS fatigue map and permutation test p-values are shown. Connectivity between each stroke lesion and MS fatigue map nodes were computed and used to investigate association between poststroke fatigue severity (based on FSS score). Connectivity to the premotor cortex, but not to the temporal pole, was significantly associated with fatigue ($p < 0.05$) (C). FSS, Fatigue Severity Scale; MS, multiple sclerosis.

connectivity between basal ganglia and cortical sensorimotor or default mode network (DMN) regions [43, 44]. MS fatigue has also been shown to be associated with fronto-striatal and parieto-striatal microstructural white matter abnormalities [12], and widespread abnormalities in cortical metabolism and neurotransmitter function, including gamma-aminobutyric acid and glutamate [45]. However, findings from these case–control studies are somewhat heterogeneous and correlational in nature, lacking causal inference between the observed brain abnormalities and fatigue.

Studying brain lesions can help to address this limitation [46]. For example, new-onset symptoms following an acute focal brain lesion, such as those caused by a stroke, provide a likely causal link from the focal brain damage to clinical symptoms. Studying lesion-induced deficits has allowed for localizing memory to the hippocampi and speech production to the inferior frontal lobe [47]. However, locations of lesions causing similar symptoms often do not overlap anatomically but are still part of a common network, demonstrating that the symptoms originate from a network rather than a single anatomical brain region, as already demonstrated with several neurological and psychiatric symptoms [17].

Studying MS lesions however is more complicated compared to stroke lesions, as most patients with MS have multiple lesions and it may be impossible to identify the lesion(s) causing the symptom, especially with complex symptoms, such as fatigue. Assuming that fatigue is caused by lesions, MS patients with fatigue have lesions causing fatigue whereas MS patients without fatigue don't have any. MS patients with fatigue likely also have lesions not associated with fatigue, which will reduce the statistical power to detect lesion locations or networks associated with fatigue. However, contrasting MS patients with and without fatigue with otherwise similar disease severity, as in the present study, can still be used to disentangle the lesion locations and networks associated with fatigue [23].

The association between fatigue and connectivity from the premotor cortex, but not from the temporal pole, was confirmed in the independent, large rs-fcMRI dataset of MS patients. Premotor connectivity abnormalities were widespread, including sensory, motor, limbic and higher-order cortical areas, possibly reflecting the multifaceted nature of fatigue [48]. Combined with our findings in stroke patients, these results suggest that fatigue localizes to a brain network connected to the right premotor cortex. However, the premotor cortex likely represents only "a tip of the iceberg" of the underlying broader brain network, as demonstrated by the extension of the significant clusters when analyzing white matter lesions only, involvement of large-scale functional networks, and widespread connectivity abnormalities from the premotor cluster in the second MS dataset.

Potential for generalizability to other lesion etiologies of the identified network to fatigue was tested in an independent cohort of stroke patients with poststroke fatigue assessments. Remarkably, this poststroke fatigue severity map was similar to the MS fatigue map, even though the lesion etiology, demographic and clinical characteristics, and imaging modalities were clearly different between the MS and stroke samples. These findings are in line with the previously proposed disease etiology-independent involvement of motor networks in the pathophysiology of fatigue [49]. Similar to the rs-fcMRI analyses in MS, fatigue after stroke lesions was associated with connectivity to the premotor cortex, but not the temporal pole. As the premotor cortex is highly involved in planning voluntary movements, premotor connectivity abnormalities could explain

previously demonstrated alterations in movement preparation among MS and stroke patients with fatigue associated with increased perceived effort of motor acitivity [50, 51], which has been proposed to be part of the pathomechanism of fatigue [8]. However, replication of the entire MS fatigue network topography in stroke strongly supports involvement of a wider network beyond a single node, best aligning spatially with the dorsal attention, limbic and default mode networks. Although previous MS fatigue studies show considerable variability, for example, MS fatigue has been shown to be associated with default mode network alterations and functional connectivity abnormalities from the supplementary motor cortex [52]. Interestingly, a recent study suggests that the limbic network may be a subcomponent of the default mode network [53], and the dorsal attention network is involved in multiple functions, including attention control and working memory, which are impaired in fatigue [54].

Although further studies are needed to investigate the biological characteristics and clinical relevance of the identified network, it may have potential as a treatment target for fatigue. Previous studies have shown that networks identified by lesion network mapping align remarkably well with efficacious treatment targets [55, 56]. Even though cognitive behavioral therapy and physical exercise may alleviate fatigue [57, 58], none of the pharmacological [59, 60] or non-invasive brain stimulation [61, 62] interventions have proven efficacy for treatment of fatigue, highlighting the need for new treatment options. Zeroing in on the most promising treatment targets in the brain could facilitate development of brain stimulation treatments and also help to identify molecular targets for therapeutic trials. Although there are no prior studies investigating the efficacy of premotor cortex stimulation for MS fatigue [61], a recent sham-controlled randomized study demonstrated that deep transcranial magnetic stimulation to the primary motor and prefrontal cortices is safe and well-tolerated in MS patients with fatigue, demonstrating the feasibility of the treatment approach in this patient population [63].

5 | Limitations

There are some limitations in this study that should be considered when interpreting the results. First, the aim of the present study was specifically to investigate brain networks underlying fatigue caused by MS lesions and our findings provide direct support for the role of brain network dysfunction in fatigue [8, 14]. However, fatigue is a multifactorial symptom and there likely are also other biological and environmental factors (such as microstructural abnormalities in the normal appearing white matter, immunological mechanisms and psychosocial issues) that are highly relevant for the development of or protection from fatigue but were beyond the scope of the present study [5, 64]. Second, defining fatigue is complicated and there is considerable heterogeneity in this definition across different scales and studies [5]. In this study, we used the FSS, because it was specifically developed to assess fatigue in MS and it has demonstrated good psychometric properties and ability to detect change over time [28, 65]. In addition, FSS has previously been validated in Finnish MS patients and is currently used in standard clinical care in Finland [66]. Third, in addition to lesions, fatigue in MS can be caused by many health issues and other MS symptoms that can complicate identifying brain networks underlying fatigue. To control for these confounding factors, we excluded patients with other primary neurological or psychiatric disorders, such as depression and sleep disorders, patients with more advanced disease stages, and included a control group with matched disease severity and lesion load. However, this approach may limit the generalizability of the results to the broader MS population. Fourth, as it is practically impossible to identify the MS lesions causing fatigue from lesions that don't in patients with fatigue, all MS lesions in a patient were combined to a single lesion mask. However, specificity to fatigue was ensured by comparing these lesion connectivity profiles to those of MS patients without fatigue and confirmed in the independent datasets of MS patients with individual rs-fcMRI data and stroke patients to minimize the chances of nonspecific findings. Fifth, functional connectivity in the white matter has a weaker signal-to-noise ratio compared to gray matter [24]. However, white matter signal has been shown to be sufficient to detect relevant network neuroanatomy [24] and increased noise should mainly bias us against the present findings, demonstrating significant group differences between MS patients with and without fatigue. Finally, it should be noted that clinical imaging modality and scanning parameters in the MS lesion cohort (dataset 1) were variable, which may add noise to the data. However, the imaging modality did not differ significantly between MS patients with and without fatigue; the results did not change when controlling for the used imaging modality, demonstrating a significant group difference.

6 | Conclusions

The results of this study show that MS lesions associated with fatigue hit a common brain network. This network was reproducible in poststroke fatigue, supporting causal relevance and suggesting possible generalizability outside MS. These findings shed light on the neurobiological mechanisms of fatigue and may help to identify MS patients at risk and guide the search for new treatment options for fatigue. However, further studies are needed to characterize the resulting functional abnormalities and test the clinical relevance of these findings.

Author Contributions

Olli Likitalo: formal analysis, funding acquisition, investigation, writing – original draft preparation, review and editing, visualization. **Jaakko Kungshamn:** investigation, writing – review and editing. **Albert Bellmunt-Gil:** data curation, writing – review and editing. **Silvia Tommasin:** formal analysis, writing – review and editing. **Abhineet Ojha:** formal analysis, data curation, writing – review and editing. **Matias Viitala:** data curation, writing – review and editing. **Juho Aaltonen:** data curation, formal analysis, software, writing – review and editing. **Jyrki Lötjönen:** formal analysis, software, writing – review and editing. **Juha Koikkalainen:** formal analysis, software, writing – review and editing **Pauli Ylikotila:** investigation, writing – review and editing. **Patrizia Pantano:** investigation, writing – review and editing. **Merja Soilu-Hänninen:** conceptualization, investigation, writing – review and editing. **Juho Joutsa:** conceptualization, funding acquisition, supervision, writing – original draft preparation review and editing, methodology.

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Conflicts of Interest

O.L. has received grants from the Finnish Medical Foundation, Finnish Foundation for Alcohol Studies, University of Turku Foundation and Turku University Hospital (VTR funds), congress fees covered by Lundbeck and Teva. J.K. has received grants from the Finnish Parkinson Foundation, Maire Taponen Foundation, and the Finnish Medical Foundation, and conference travel support from the Finnish Neurological Society and Orion. A.B.-G., M.V., S.T., A.O. and P.P. report no conflicting interests. J.L. and J.Ko. are shareholders at Combinostics Ltd. M.S.-H. has received congress fees covering and lecture and consultation fees by Biogen, Cellgene, Merck, Novartis, Roche, Sanofi and Teva. J.J. has received grants from the Research Council of Finland, Sigrid Juselius Foundation, Signe and Ane Gyllenberg´s Foundation, Päivikki and Sakari Sohlberg´s Foundation, Finnish Medical Foundation, Instrumentarium Research Foundation, Finnish Foundation for Alcohol Studies, University of Turku (Sigrid Juselius Foundation, Private Donation) and Turku University Hospital (VTR funds); congress travel grants from Insightec, Abbott and Abbvie; lecturer honoraria from Insightec, Addiktum, Nordic Infucare Lundbeck and Novartis; consultancy fees from Summaryx, Adamant Health and TEVA Finland; is acting as an advisory board member for TEVA Finland; and owns stock of Neurologic Finland and Suomen Neurolaboratorio.

Data Availability Statement

Group level data are available from the corresponding author upon reasonable request. Individual data cannot be shared due to national and institutional regulations.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** acn370241-sup-0001-supinfo.pdf.