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## **Longitudinal Network Changes and Conversion to Cognitive Impairment in Multiple Sclerosis**

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## Abstract

**OBJECTIVE:** To characterize functional network changes related to conversion to cognitive impairment in a large sample of MS patients over a period of 5 years.

**METHODS:** 227 MS patients and 59 healthy controls (HCs) of the Amsterdam MS cohort underwent neuropsychological testing and resting-state fMRI at two time points (time-interval  $4.9 \pm 0.9$  years). At both baseline and follow-up, patients were categorized as cognitively preserved (CP, N=123), mildly impaired (MCI,  $Z < -1.5$  on  $\geq 2$  cognitive tests, N=32) or impaired (CI,  $Z < -2$  on  $\geq 2$  tests, N=72) and longitudinal conversion between groups was determined. Network function was quantified using eigenvector centrality, a measure of regional network importance, which was computed for individual resting-state networks at both time-points.

**RESULTS:** Over time, 18.9% of patients converted to a worse phenotype; 22/123 CP patients (17.9%) converted from CP to MCI, 10/123 from CP to CI (8.1%) and 12/32 MCI patients converted to CI (37.5%). At baseline, DMN centrality was higher in CI compared to controls ( $P=.05$ ). Longitudinally, ventral attention network (VAN) importance increased in CP, driven by stable CP and CP-to-MCI converters ( $P < .05$ ).

**CONCLUSIONS:** Of all patients, 19% worsened in their cognitive status over five years. Conversion from intact cognition to impairment is related to an initial disturbed functioning of the VAN, then shifting towards DMN dysfunction in CI. As the VAN normally relays information to the DMN, these results could indicate that in MS, normal processes crucial for maintaining overall network stability are progressively disrupted as patients clinically progress.

## Introduction

Cognitive impairment (CI) occurs in 40-70% of multiple sclerosis (MS) patients and has severe consequences for daily life.<sup>1</sup> Despite recent efforts to characterize the course of cognitive decline, it is still unknown which mechanisms constitute the conversion from preserved cognition to mild or severe CI, and who is at risk, hampering the provision of adequate and timely care.<sup>2,3</sup> While longitudinal studies remain scarce, grey and white matter damage are known to relate to cognitive decline. In addition, a major cause of these deficits is thought to reside within functional network dysfunction.<sup>2,4,5</sup>

Recent work has implicated the default-mode network (DMN) as one of the key networks of interest for cognition. Normally the DMN is suppressed during cognitive tasks. However, in CI-MS the DMN is seemingly “stuck” in a hyperconnected state and cannot be suppressed sufficiently, possibly precluding cognitive networks to become engaged.<sup>6</sup> One particular network that regulates the DMN is the ventral attention network (VAN), which functions as a ‘switch’ between task-negative (e.g. DMN) and task-active networks (e.g. frontoparietal network, FPN, and dorsal attention network, DAN).<sup>7,8</sup> The VAN consists of the anterior cingulate and insular cortices, which are among the regions most affected by cortical pathology in MS.<sup>9</sup> It remains unclear, however, which part of this organization of the VAN, DAN, FPN and DMN shows dysfunction during conversion to CI.

In order to better understand conversion to cognitive impairment in MS, we measured longitudinal cognition in a large sample of MS patients and characterized how this relates to longitudinal network changes.

## Methods

### *Participants*

All participants were part of the Amsterdam Multiple Sclerosis cohort.<sup>2,4,10</sup> Previous work on this cohort has identified cross-sectional patterns of network dysfunction in patients with CI, but this is the first study to describe longitudinal network changes in relation to cognitive performance. Participants were included if both neuropsychological and neuroimaging data were available at the baseline and five year follow-up measurements, resulting in a total of 227 MS patients (67.4% women, mean age:  $47.6 \pm 11.0$  years, mean disease duration:  $14.8 \pm 8.5$  years) and 59 healthy controls (HCs; 52.5% female, mean age  $46.0 \pm 9.9$  years). At baseline, the MS group consisted of 177 patients with relapsing-remitting MS (RRMS), 18 primary progressive MS (PPMS) and 32 secondary progressive MS (SPMS). The mean time-interval between baseline and follow-up visits was  $5.4 \pm 1.1$  years for HCs and  $4.8 \pm 0.8$  years for MS patients. At follow-up, 22 RRMS patients had converted to SPMS.

### *Standard Protocol Approvals, Registrations, and Patient Consents*

The study protocol was approved by the institutional ethics review board of Amsterdam UMC (location VUmc) and all participants gave written informed consent prior to participation.

### *Neuropsychological assessment*

Extensive neuropsychological assessment was performed at both time-points as described previously.<sup>10</sup> In short, all participants underwent neuropsychological testing on the day of scanning, using an expanded Brief Repeatable Battery of Neuropsychological tests. This test battery consisted of the following tests (and the domains usually associated with each test in previous work): the Selective Reminding Test (SRT; verbal memory), the Symbol Digit Modalities Test (SDMT; information processing speed), the Memory Comparison Test (MCT; working memory), the Stroop Color-Word test (attention), the Spatial Recall Test (SPART; visuospatial memory), the Word List Generation (WLG; verbal fluency) and the Concept Shifting Test (CST; executive functioning). Regression-based analyses were used to correct test scores of all subjects for normal effects of age, sex and education as present in HCs.<sup>2</sup> Cognitive scores of each test were converted to Z-scores, based on means and standard deviations (SDs) of the HCs in this study, at each time-point. Subsequently, patients were classified as cognitively impaired (CI; at least 2 tests with  $Z \leq -2$ ), mildly cognitively impaired (MCI; at least 2 tests with  $Z \leq -1.5$ , but not fulfilling CI criteria) and cognitively preserved (CP; not being CI or MCI). To deal with learning effects, all Z-scores were determined using the control sample at each respective time-point only. Conversion to (mild) cognitive impairment was defined as the change in cognitive phenotypes between the two visits. CP patients at baseline could convert to MCI (CP→MCI), to CI (CP→CI) or remain preserved (CP→CP). Likewise, MCI and CI patients at baseline could convert to overt impairment (MCI→CI) or remain stable (MCI→MCI or CI→CI). Finally, reversion from (mild) impairment to no or less impairment (i.e. CI→MCI, CI→CP, MCI→CP) was also quantified.

## *Magnetic Resonance Imaging*

MRI scanning was performed as reported previously.<sup>4</sup> In short, at both time-points all participants were scanned on a 3 T whole-body magnetic resonance system (GE Signa-HDxt, General Electric, Milwaukee, WI) using the same 8-channel phased-array head coil. Between both time-points, a partial hardware update was installed (e.g. gradient system update), the effects of which were corrected for as described below. The sequences acquired included a high-resolution, 3D T1-weighted fast spoiled gradient-echo sequence for volumetric measurements (repetition time 8 ms, echo time 3 ms, inversion time 450 ms, flip angle 12°, 1.0 mm sagittal slices, 0.9 x 0.9 mm in-plane resolution), a 3D T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence for white matter lesion segmentation (repetition time 8000 ms, echo time 125 ms, inversion time 2350 ms, 1.2 mm sagittal slices, 1.0 x 1.0 mm in-plane resolution) and, finally, a resting-state fMRI sequence was acquired for eigenvector centrality mapping (whole brain coverage, 202 volumes of which the first two were discarded, echo-planar imaging, repetition time 220 ms, echo time 35 ms, flip angle 20°, 3 mm contiguous axial slices, 3.3 x 3.3 mm in-plane resolution).

## *MRI preprocessing*

Following previously published methods<sup>11,12</sup>, calculation of lesion volumes at each visit was done by automatic segmentation of white matter (WM) lesions on the FLAIR scans and, subsequently, by lesion filling on the 3D T1-weighted scans. For subsequent volumetric analyses SIENAX and FIRST, both part of FSL5 (fsl.fmrib.ox.ac.uk), were used at both time-points in combination with a correction for the effects of the MRI hardware update.<sup>13</sup> Using this method, brain volume, cortical grey matter (GM) volume, WM and deep GM volume, all normalized for head size, were calculated. Resting-state fMRI scans were preprocessed with the MELODIC pipeline of FSL5 in combination with an independent component analysis-based approach for Automatic Removal of Motion Artifacts (ICA-AROMA<sup>14</sup>) combined with the regression-based removal of WM and CSF signals. Then, boundary-based registration was used to automatically register functional MR images to lesion-filled, 3D T1-weighted images and finally, images were nonlinearly registered to Montreal Neurologic Institute standard space.

## *Fast eigenvector centrality mapping analysis*

To assess network changes over time, we used fast eigenvector centrality mapping (fECM, [github.com/amwink/bias/tree/master/matlab/fastECM](https://github.com/amwink/bias/tree/master/matlab/fastECM)), a measure of relative functional 'importance' for each voxel in the brain which was previously validated in MS.<sup>6</sup> Eigenvector centrality provides a score for each voxel based on the strength of connections with other voxels and the importance of these other voxels themselves. To ensure a reliable signal for longitudinal ECM analysis, two subject-specific separate masks for GM and fMRI were constructed at each time-point. The GM mask was based on a combination of SIENAX-based cortical GM segmentation and FIRST to exclude any WM voxels, and the fMRI mask excluded voxels with unreliable signal (e.g. in orbitofrontal cortex), based on the robust

range of signal intensity as described before.<sup>6</sup> Both masks were then non-linearly registered to standard space and multiplied for the entire population, forming one mask to ensure comparable node positioning for all subjects. Masks were made for both time points, and also multiplied, to ensure that all subjects had GM voxels in this mask at both visits. Voxel-wise eigenvector centrality mapping was then performed within this mask and eigenvector centrality scores were averaged over the different brain networks (i.e. visual, sensorimotor, ventral attention, dorsal attention, default mode and frontoparietal networks defined by Yeo et al.<sup>15</sup>) as well as the deep grey matter and cerebellum, for which FIRST segmentations and the Harvard Oxford-based mask of the cerebellum (part of FSL) were used. In order to minimize any residual effects of the scanner hardware update, network ECM values were finally converted to Z-scores based on the means and standard deviations of the HCs at each time point, similar to cognitive scores.

### *Functional connectivity analysis*

Networks that showed significant effects in the ECM analysis were explored in more detail by looking at functional connectivity between these networks and our cognition-related networks of interest as described in the introduction (i.e. VAN, DMN, DAN and FPN). We used the Brainnetome atlas<sup>16</sup> to parcellate the brain in 210 cortical areas, which was non-linearly registered to 3DT1 together with the Harvard Oxford-based cerebellum and the 14 FIRST-based deep grey matter regions, resulting in 225 regions. This atlas was multiplied with the aforementioned individualized GM mask and brought to fMRI space using boundary-based registration and 33 regions with unreliable signal were excluded (mainly orbitofrontal and inferior temporal areas, as well as the nucleus accumbens). Next, time series were extracted and imported into Matlab R2018b (Natick, Massachusetts). Per individual, a connectivity matrix was constructed by calculating the Pearson's correlation coefficients between all 192 regions, which was subsequently corrected for whole brain connectivity (i.e. the average of all connections in the matrix). Next, similar to the ECM analysis, averaged functional connectivity (FC) scores were determined between each brain network. Finally, as with cognition and ECM, FC values were converted to Z-scores using the means and SDs of healthy controls at both time points.

### *Statistical analysis*

Demographic, clinical and structural MRI measures were checked for normality using Kolmogorov-Smirnov tests and histogram inspection. These variables were subsequently compared between HC, CP, MCI and CI groups at baseline using one-way ANOVAs for continuous measures (corrected for age, sex and education) or chi-square tests for categorical data in IBM SPSS version 26 (Chicago, IL). To replicate previously found cross-sectional network differences between cognitive groups (i.e. HC, CP, MCI and CI<sup>6</sup>), baseline ECM network values were assessed and post-hoc tests were performed between CI and CP groups. Over time, ECM and FC values were analyzed using repeated measures ANOVAs with group as between-subjects factor and time as within-subjects factor, corrected for age, sex, education and time-interval. Longitudinal network evolution was first assessed using group-status at baseline (i.e. HC, CP, MCI and CI) and repeated using the converter groups (e.g. CP converting to



MCI), separately for the cognitively worsening and improving groups. Finally, in the total patient group we correlated significant networks in the ECM analysis to performance on cognitive tests and the amount of structural damage using partial Pearson correlations (controlled for age, sex and education and time-interval in longitudinal analyses). Correlations, post-hoc ANOVAs or t-tests were false discovery rate (FDR) corrected to reduce type-1 errors. *P*-values of <0.05 were considered statistically significant.

*Data availability*

Anonymized data, not published in the article, can be shared upon reasonable request from a qualified investigator.

ACCEPTED

## Results

### *Clinical and cognitive data*

For the HC, CP, MCI and CI groups, demographic, clinical and neuropsychological data at baseline and follow-up visits can be seen in Table 1. At baseline, cognitive groups consisted of 123 CP (54.2%), 32 MCI (14.1%) and 72 CI patients (31.7%). Zooming in on patients that showed impairment on only a single test showed that the SPART and MCT were most frequently affected. Overall, the MCI and CI participants were more frequently male and had a lower educational level, longer disease duration and higher EDSS scores than CP patients. In addition, all brain volumes were lower and lesion volumes were higher in the cognitively affected groups. At follow-up, the distribution over the cognitive groups changed, resulting in a total of 104 CP (45.8%), 51 MCI (22.5%) and 72 CI (31.7%) patients. In total, 32 CP patients (26.0%) deteriorated and converted to MCI (N=22) or to CI (N=10). The exact numbers of MS patients remaining stable or converting between cognitive phenotypes are presented in figure 1b. The 10 people that converted from CP to CI became impaired most frequently on two (N=4) or three (N=3) tests, but impairment also developed on one (N=2) or four (N=1) tests. The most frequently affected test was the CST (N=5), followed by the SDMT and the Stroop (N=4). The 11 people that converted from MCI to CI declined most frequently on two tests (N=5) and on one and three tests (N=3). One person declined on six tests. Performance declined most often on the SDMT (N=8), followed by the SPART (N=5). Several people improved and reverted from MCI to CP (N=9), from CI to MCI (N=18) or from CI to CP (N=4).

### *Functional network centrality: differences between CP, MCI and CI patients at baseline and over time*

Despite the slightly smaller group due to only including longitudinal samples, our baseline results confirmed previously found network differences between cognitive groups (main effect of group  $F(24, 792.4) = 2.25, P = .001$ ).<sup>6</sup> Post-hoc tests showed significant differences between CI and CP patients in the visual network (i.e. lower in CI than in CP,  $P = 0.013$ ) and in the DMN (i.e. higher in CI than in CP,  $P = 0.05$ ). In the total MS sample, large network deviations (i.e.  $Z < -1.5$  or  $Z > 1.5$ ) were observed most frequently in the visual and FPN networks (N=42 and N=36, respectively). Over time, a group\*time interaction effect was noted in the VAN ( $F(3, 277) = 3.60, P = 0.014$ ) and in the cerebellum ( $F(3, 277) = 2.81, P = 0.040$ ). Post-hoc tests demonstrated an opposite effect over time only in the CP patients: an increasing VAN centrality ( $P = 0.001$ ), but decreasing cerebellum centrality ( $P < 0.001$ ). None of the other groups defined at baseline showed significant changes in the VAN or cerebellum.

### *Post-hoc explorations of functional connectivity using groups defined at baseline*

Next, we further explored the VAN at baseline by calculating functional connectivity strength with the other a-priori defined networks of interest (DMN, FPN and DAN), all of which showed group differences at baseline ( $F(3, 289) = 4.55, P = 0.004$ ;  $F(3, 289) = 3.66, P = 0.013$  and  $F(3, 289) = 2.99, P = 0.032$ , respectively), but not over time. At baseline, VAN-DMN and VAN-FPN connectivity was only increased in the CI group as compared to the CP and HC groups (VAN-DMN:  $P = 0.032$  and  $P =$

0.018; VAN-FPN:  $P = 0.010$  and  $P = 0.034$ , respectively), while VAN-DAN connectivity was only higher in CI compared to the HC group ( $P = 0.026$ ).

#### *Centrality and connectivity in cognitively converting CP patients*

Finally, as only the CP group showed a significant increase in VAN centrality over time, we investigated this change in more detail in the stable and converting CP groups (i.e. CP→CP, CP→MCI and CP→CI). Moreover, we studied the functional connectivity of the VAN with the other a-priori defined networks. In patients that remained cognitively preserved (CP→CP) and in those converting to MCI (CP→MCI) VAN centrality increased over time ( $P = 0.017$  and  $P = 0.008$ , respectively), whereas no change was observed in the CP→CI group. As thalamic volume is an important correlate of cognitive dysfunction in MS, we repeated this analysis with thalamic volume as covariate, which did not affect the results. Longitudinal changes for all groups are shown in figure 1. No significant effects were observed in connectivity between the VAN and the other cognitive networks. Finally, no longitudinal changes were observed in the back converters (i.e. MCI→CP, CI→MCI and CI→CP) in any of the networks.

#### *Partial correlations with cognition and structural MRI*

Next, baseline and longitudinal VAN importance was related to average cognition, all individual cognitive tests and structural damage using partial correlations (controlling for age, sex and education and time-interval for longitudinal analyses). At baseline, a negative correlation was noted between average cognition and VAN importance both in the HC and MS groups ( $r = -0.33$ ,  $P = .014$ ;  $r = -0.14$ ,  $P = .045$  respectively), indicating that a lower VAN importance is related to a higher average cognition. Interestingly, in MS a positive relationship was noted between delta VAN importance and average cognition at baseline ( $r = 0.18$ ,  $P = .006$ , figure 2), indicating that higher average cognition at baseline correlated to a stronger increase in VAN importance over time. However, this could not be explored in HCs over time, as all functional data was normalized based on HC fluctuations, which sets mean HC ECM values to zero by definition. Subsequent correlations with individual cognitive tests at baseline in MS demonstrated a positive relationship only with the SPART ( $r = 0.15$ ,  $P = .024$ ). To further examine the relation between delta VAN and SPART performance, we compared patients with high delta VAN (i.e.  $>1.5$ ) to the HC group. The patients with high delta VAN ( $N=29$ ) showed a significantly worse deterioration of SPART performance ( $P = .042$ ) and average cognition ( $P = .005$ ) than HCs.

For structural damage, VAN change was positively related to deep gray matter volume at baseline ( $r = 0.15$ ,  $P = 0.025$ ) indicating that people with less severe structural damage at baseline, showed stronger increases in VAN centrality over time.

## Discussion

The current study showed that cognitive conversion (i.e. the shift between preserved cognitive status to mild or severe impairment) over a period of 5 years occurred in 18.9% of MS patients and is related to ventral attention network changes. The direction of this change depended on whether patients were already impaired to some degree or not. The CP group at baseline demonstrated an increase in VAN importance over time. When this was further disentangled in the converter groups, this increase was still present in the CP→CP and CP→MCI phenotypes, but not in the CP→CI group. The MCI group (both MCI→MCI and MCI→CI) showed no significant change over time. At baseline, CI patients demonstrated higher importance of the DMN and higher functional connectivity of the VAN with both the DMN and the FPN. In the entire MS group, the change in VAN importance was positively related to average baseline cognition and deep gray matter volume. Finally, the correlations with individual cognitive tests showed that patients with larger increases in VAN importance over time had higher SPART scores at baseline, but also faster decline over time.

The increased VAN importance in the converting CP patients may be the result of structural damage to WM tracts, which leads to a reduced diversity of structural connections over which information transfer can occur.<sup>17</sup> As a result, flexibility in functional pathways goes down and the remaining patterns of activity will occur more frequently, leading to a more rigid functional backbone that becomes more important.<sup>17</sup> The significant positive relationship between deep gray matter volume and longitudinal change in VAN importance is in line with this reasoning, as it indicates that patients with less structural damage will show a stronger increase in VAN importance. One longitudinal study in clinically isolated syndrome (CIS) patients found a weakened relationship between structural and functional connectivity in the VAN in CP CIS patients, also indicating that longitudinal VAN changes occur in CP CIS patients, who often progress to developing MS.<sup>18</sup> With more longstanding disease and progressive neurodegeneration, it has been suggested that the remaining functional highways are compromised as well, eventually culminating in an overall reduction of functional connectivity.<sup>17,19</sup> This may explain the levels of VAN importance that were seen in the CI groups with more severe structural damage, where no increase or even a small decrease was observed (i.e. in the CI→CI group), although this did not survive FDR-correction. Future replication in longitudinal as well as modeling studies is warranted, but this theory offers an explanation for what appears to be an initial increase in VAN importance during early stages of cognitive conversion, followed by a decrease during more severe cognitive decline.

The ventral attention or salience network, of which the main components are the anterior cingulate cortex (ACC) and the anterior insula, has a crucial function of regulating and providing input into networks involved in cognition such as the DMN, DAN and FPN.<sup>20</sup> Several studies have even suggested that the VAN functions as a switch between these networks and is crucial in directing the flow of information.<sup>7,8</sup> This regulatory function arises from the sensitivity of the VAN for detecting salient environmental stimuli.<sup>21,22</sup> In order to make the information about these stimuli accessible for subsequent (cognitive) processing, the VAN connects extensively with networks such as the DAN, DMN and FPN.<sup>20</sup> In MS, pathological studies have shown that the insular and cingulate cortices are predilection sites for cortical pathology.<sup>9</sup> This may result in abnormal VAN connectivity as cross-sectional studies have found increased functional connectivity between the VAN and FPN in RRMS<sup>23</sup> and decreased dynamic functional connectivity in the VAN, which usually also indicates an increase in

'static' FC.<sup>24</sup> Our data add to this by showing increased functional connectivity between the VAN and FPN and between the VAN and DMN in cognitively impaired patients, in addition to elevated DMN importance in CI. Increased importance signals a stronger connection to the hubs in the brain. This could indicate that the VAN is attempting to connect more to hub regions of the DMN and FPN and is inadequately trying to regulate these networks. Another possibility is that the increased communication of the VAN is dysfunctional and eventually leads to aberrations in connected networks. In order to pinpoint the specific effects of VAN dysfunction on individual cognitive tests, longitudinal VAN changes were related to cognitive decline. This analysis showed that VAN increases relate to decline in SPART function only, possibly because of the strong anatomical connections between the VAN and hippocampus.<sup>25-27</sup> Memory deficits were common in our patients not classified as cognitively impaired, possibly indicating an early involvement of memory deficits in MS, as has also been suggested previously.<sup>28</sup>

In our sample, almost 70% of CI patients at baseline remained CI and 19% of the entire sample showed cognitive worsening (i.e. CP→MCI, CP→CI or MCI→CI) indicating that approximately one in five MS patients deteriorate cognitively over a span of five years. These results also suggest that the concept of cognitive conversion holds value for clinical application. In addition, at baseline the 32% of patients that were CI were older, more often male and had more disability, confirming previous reports indicating that male MS patients are more susceptible to a more aggressive disease course.<sup>29</sup> However, before this concept of cognitive conversion can be applied beyond research, specific thresholds defining such conversion should be validated in other cohorts. Another point that follows from the overall cognitive stability in the sample, is that it may explain why DMN importance did not show longitudinal changes, as DMN dysfunction manifests when cognitive impairment becomes more overt. The relative cognitive stability of our sample emphasizes the need for longitudinal cohort studies with even longer follow-up durations. This will likely also result in more clearly defined cognitive states in which less people revert back from more to less impaired states. In our sample, this may be explained by isolated cognitive relapses, which have been described in literature, but are not well-understood.<sup>30</sup> The suggestion that VAN changes precede DMN dysfunction may also explain why in this study the VAN stood out in the longitudinal analysis, rather than the DMN. This early VAN increase was related to decline in SPART performance, which could indicate an imminent network destabilization as the VAN's regulatory 'switch' function is increasingly put under pressure. In later stages, this network dysfunction seems to shift towards the DMN, where more severe cognitive impairment becomes apparent. Interestingly, other networks showing strong deviations (i.e. the visual and FPN networks) were not related to cognitive decline in our sample. The balance between the VAN and DMN is currently also under investigation in other neurological disorders, such as Alzheimer's disease.<sup>31-33</sup> A second explanation as to why the DMN did not appear in our longitudinal analysis is that here the voxel-wise ECM values were averaged over networks to test longitudinal differences in a priori defined networks. By contrast, earlier work used a voxel-wise approach in which the posterior cingulate cortex (PCC) showed significant effects that were post-hoc assigned to the DMN. There are indications that in MS the anterior and posterior parts of the DMN show different functional behaviors and therefore considering the DMN as one network might have obscured regional effects in this study.<sup>5</sup> For future work, it would be highly interesting to investigate anterior and posterior (e.g. PCC)

DMN functioning over time, in addition to its connectivity with the VAN, which was outside the scope of the current study.

Several limitations apply to this work. First, we employed a longitudinal approach to characterize the evolution of cognitive impairment over five years only and at the first measurement our sample already had a mean disease duration of approximately 15 years. The disease processes and network alterations that already occurred in the first decade of MS could therefore not be assessed, which warrants studies to investigate this important first phase of the disease. Second, an upgrade of scanner hardware occurred in between measurements, which could have influenced volumetric and functional measurements. However, our group developed a method to successfully account for volumetric differences as a result of the upgrade.<sup>13</sup> Additionally, we minimized upgrade effects on functional measures by expressing ECM and FC values as Z-scores compared to HC values at each time point, which is common practice in our, and others', analyses of cognitive and functional connectivity data.<sup>34</sup> Third, although the initial sample was large, subgroups with converting phenotypes were substantially smaller, affecting statistical power to detect additional network changes. Future studies are encouraged in multicenter datasets also noting additional approaches to limit type 1 errors. Additionally, the initial sample showed a significant difference in gender distribution between the HC and MS groups (i.e. fewer women in the HC group), due to which gender was always entered in the analyses as covariate. And, finally, we adopted ECM as a measure of network importance, a robust and widely used method to define "hub" regions. Nonetheless, other metrics could offer complementary information, for instance betweenness centrality, which quantifies how many shortest paths flow through a network, which was not possible with the current approach.<sup>6,35-37</sup>

In conclusion, we showed that one in five MS patients converts to (mild) cognitive impairment over a period of five years and that this is related to an initial disturbed functioning of the ventral attention network, which shifts towards DMN dysfunction as overt cognitive impairment manifests. As such, our results could indicate that in MS, normal processes crucial for maintaining overall network stability are progressively disrupted as patients clinically progress. This stresses the importance of future longitudinal studies in MS, in order to confirm our hypothesis on the role of the VAN in early stages of clinical progression, as well as the DMN and other attention/executive networks in later stages.

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Table 1 | Demographic, clinical and structural MRI data at both time-points of the HC and cognitive MS groups. Values are mean (SD) unless specified otherwise.

	HC (N = 59)	CP (N = 123)	MCI (N = 32)	CI (N = 72)	P-value
Female (%)	33 (53.2)	89 (72.4)	24 (75.0)	40 (55.6)	0.016*
Age (years)	46.3 (9.8)	45.1 (9.6)	49.6 (12.2)	50.9 (11.8)	0.001*
Level of education (1-7)	5.4 (1.7)	5.2 (1.4)	4.3 (2.0)	4.5 (1.7)	<0.001*
Disease duration (years)	-	13.4 (7.7)	16.4 (8.3)	16.6 (9.5)	0.021*
MS-type (RR/SP/PP)	-	106/13/4	25/4/3	46/15/11	0.01*
Medication (IF- $\beta$ /GA/NTZ/other/none)	-	28/6/7/3/79	10/1/1/0/19	17/6/3/1/44	0.963 <sup>a</sup>
EDSS, median [range]	-	2.5 [0-8]	3.5 [0-8]	4.0 [1.5-7.5]	<0.001*
Average cognition, z-scores	0.00 (0.48)	-0.28 (0.47)	-1.09 (0.29)	-1.86 (0.74)	<0.001*
Normalized white matter lesion volume (ml)	-	13.0 (1.0)	19.5 (12.0)	24.1 (19.7)	<0.001*
NBV (ml)	1513.5 (64.0)	1484.6 (60.7)	1437.7 (82.2)	1413.6 (84.2)	<0.001*

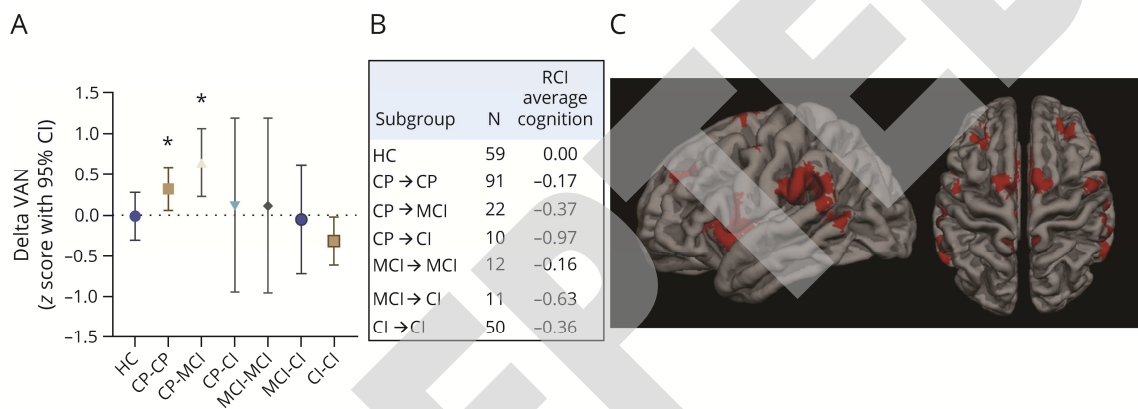
NWMV (ml)	697. (32.9)	676.4 (33.2)	661.5 (37.9)	660.2 (36.6)	<0.001*
NCGMV (ml)	777.2 (47.4)	771.6 (43.3)	741.9 (55.0)	720.4 (57.7)	<0.001*
NDGMV (ml)	62.7 (3.5)	58.9 (4.9)	55.2 (6.2)	53.1 (7.1)	<0.001*

Statistical tests were run between groups at baseline using one-way ANOVAs or Chi-square tests where appropriate. Significant tests between individual groups: % **Female** (HC vs. CP,  $P=0.01$ ; CP vs. CI,  $P=0.025$ ), **Age** (HC vs. CI,  $P=0.012$ ; CP vs. CI,  $P<0.001$ ; CP vs. MCI,  $P=0.049$ ), **Level of education** (HC vs. MCI,  $P=0.003$ ; HC vs. CI,  $P=0.001$ ; CP vs. MCI,  $P=0.014$ ; CP vs. CI,  $P=0.007$ ), **disease duration** (CP vs. CI,  $P=0.013$ ; CP vs. MCI,  $P=0.042$ ), **MS-type** (CP vs. CI,  $P=0.001$ ), **EDSS** (CP vs. CI,  $P<0.001$ ; CP vs. MCI,  $P=0.003$ ), **Average cognition** (HC vs. all MS groups,  $P<0.001$ ; CP vs. MCI and CI,  $P<0.001$ ; MCI vs. CI,  $P<0.001$ ), **Normalized white matter lesion volume** (CP vs. MCI,  $P=0.002$ ; CP vs. CI,  $P<0.001$ ), **NBV** (HC vs. CP,  $P=0.006$ ; HC vs. MCI,  $P<0.001$ ; HC vs. CI,  $P<0.001$ ; CP vs. MCI,  $P=0.003$ ; CP vs. CI,  $P<0.001$ ), **NWMV** (HC vs. all MS groups,  $P<0.001$ ; CP vs. MCI,  $P=0.025$ ; CP vs. CI,  $P=0.001$ ), **NCGMV** (HC vs. MCI,  $P=0.001$ ; HC vs. CI,  $P<0.001$ ; CP vs. MCI,  $P=0.002$ ), **NDGMV** (HC vs. all MS groups,  $P<0.001$ ; CP vs. MCI and CI,  $P<0.001$ ).

<sup>a</sup> Medication variable was dichotomized (yes/no MS medication used) and tested with chi-square. RR = relapsing remitting, SP = secondary progressive, PP = primary progressive, IF- $\beta$  = interferon-beta, GA = glatiramer acetate, NTZ = natalizumab, NBV = normalized brain volume, NWMV = normalized white matter volume, NCGMV = normalized cortical grey matter volume, NDGMV = normalized deep gray matter volume.

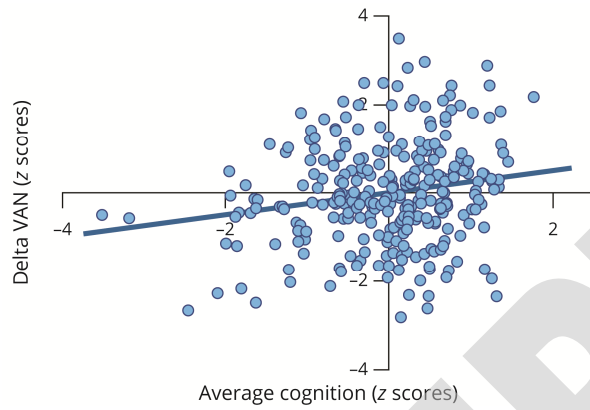
Longitudinal change in VAN importance is related to cognitive conversion in MS.

Fig. 1 | A. Delta VAN Z-scores (means and 95% confidence intervals) for each of the cognitive converter groups showing a significant increase in VAN importance over time in the CP-CP and CP-MCI groups (FDR-corrected). B. Sample size and reliable change index for the average cognition score per converter group. C. Lateral and top view of the VAN, projected on a standard brain.



Positive relationship between baseline average cognition and longitudinal VAN change in MS.

Fig. 2 | Residual scatterplot of average cognition at baseline (Z-scores) and the change from baseline to follow-up in VAN centrality (delta, also Z-scores). The positive relationship indicates that individuals with higher average cognition at baseline generally showed a stronger increase in VAN importance over time. Partial correlation coefficient ( $r = 0.18$ ,  $P = 0.006$ ) was corrected for age, sex, education and time-interval between visits.



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## Longitudinal Network Changes and Conversion to Cognitive Impairment in Multiple Sclerosis

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