*Acta Neuropsychiatrica*

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# Intrinsic functional connectivity, CSF biomarker profiles and their relation to cognitive function in mild cognitive impairment

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## Original Article

**Cite this article:** Matura S, Köhler J, Reif A, Fusser F, Karakaya T, Scheibe M, Ehret F, Hartmann D, Kang J-S, Mayer C, Prvulovic D, and Pantel J. (2020) Intrinsic functional connectivity, CSF biomarker profiles and their relation to cognitive function in mild cognitive impairment. *Acta Neuropsychiatrica* **32**:206–213. doi: 10.1017/neu.2019.49

Received: 10 July 2019
Revised: 8 November 2019
Accepted: 19 November 2019
First published online: 5 December 2019

**Key words:**
magnetic resonance imaging; cognition; amyloid; resting state fMRI

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## Abstract

Mild cognitive impairment (MCI) often precedes Alzheimer's Dementia (AD), and in a high proportion of individuals affected by MCI, there are already neuropathological processes ongoing that become more evident when patients progress to AD. Accordingly, there is a need for reliable biomarkers to distinguish between normal aging and incipient AD. Recent research suggests that, in addition to established biomarkers such as CSF Aß42, total tau and hyperphosphorylated tau, resting state connectivity established by functional magnetic resonance imaging might also be a feasible biomarker for prodromal stages of AD. In order to explore this possibility, we investigated resting state functional connectivity as well as cerebrospinal fluid (CSF) biomarker profiles in patients with MCI ($n = 30$; age 66.43 ± 7.06 years) and cognitively healthy controls ($n = 38$; age 66.89 ± 7.12 years). CSF Aß42, total tau and hyperphosphorylated tau concentrations were correlated with measures of cognitive performance (immediate and delayed recall, global cognition, processing speed). Moreover, MCI-related alterations in intrinsic functional connectivity within the default mode network were investigated using functional resting state MRI. As expected, MCI patients showed decreased CSF Aß42 and increased total tau concentrations. These alterations were associated with cognitive performance. However, there were no differences between MCI patients and cognitively healthy controls regarding intrinsic functional connectivity. In conclusion, our results indicate that CSF protein profiles seem to be more closely related to cognitive decline than alterations in resting state activity. Thus, resting state connectivity might not be a reliable biomarker for early stages of AD.

### Significant outcomes

- Decreased CSF Aß42 levels were associated with impaired cognitive performance.
- Intrinsic functional connectivity was not altered in mild cognitive impairment patients.
- CSF biomarkers seem to be more closely related to cognitive function than resting state functional magnetic resonance imaging.

### Limitations

- Since there are no longitudinal data available, the prognostic value of the biomarkers assessed in this study is limited.

## Introduction

Alzheimer's Dementia (AD) is a neurodegenerative condition that results in a progressive clinical syndrome that is characterised by an impairment in memory and other cognitive functions (Hodges, 2006). Amnestic mild cognitive impairment (MCI) is considered as the prodromal phase of AD (Dubois & Albert, 2004). This prodromal phase is characterised by the accumulation of beta-amyloid (Aß) plaques and neurofibrillary tangles that lead to neuronal death and deteriorating neurocognitive function (Jack & Holtzman, 2013). Cerebrospinal fluid (CSF) biomarkers of these neurodegenerative processes are the 42 amino acid form of amyloid protein ($Aß_{42}$), total tau (T-tau) and hyperphosphorylated tau (p-tau) (Andreasen & Blennow, 2005).

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The effect of these three CSF biomarkers on neurocognitive function has been investigated by several studies. It has been shown that lower levels of cerebral $A\beta_{42}$ are closely associated with impaired memory performance in MCI patients (Hedden *et al.*, 2012; Nathan *et al.*, 2017). Elevated levels of total tau and hyperphosphorylated tau seem to be related to impaired memory (Malpas *et al.*, 2015; Nathan *et al.*, 2017) and executive function (Nathan *et al.*, 2017).

Aß42, total tau and hyperphosphorylated tau have been proven useful in distinguishing MCI patients with a high risk of conversion to AD from those with a low risk (Riemenschneider *et al.*, 2002; Zetterberg *et al.*, 2003). Additionally to the well-established CSF biomarkers, resting state connectivity has also been proposed as a possible biomarker for the prodromal stages of AD (Sorg *et al.*, 2007). Resting state functional magnetic resonance imaging (rs-fMRI) allows analysing regional interactions of brain regions in a resting state when no task is performed. Previous rs-fMRI studies have shown that functional connectivity is already altered in the early stage of AD (Greicius *et al.*, 2004; Rombouts *et al.*, 2009) as well as in MCI patients (Ishii *et al.*, 2003). One of the most investigated resting state networks is the default mode network (DMN). The DMN is a set of interacting brain regions that are typically active during rest, while being deactivated when engaged in a cognitively demanding task (Raichle *et al.*, 2001). Evidence from functional neuroimaging studies suggests substantial topographic overlap between the DMN and networks underpinning episodic memory processing (Buckner *et al.*, 2005; Spreng *et al.*, 2009; Sestieri *et al.*, 2011). Activity in the DMN during resting state seems to predict episodic memory performance in healthy adults (Yang *et al.*, 2012) and in neurological patients (McCormick *et al.*, 2013). Besides their role for episodic memory processing, the regions of the DMN also show a striking overlap with regions with high amyloid depositions (Buckner *et al.*, 2005; Sperling *et al.*, 2009; Sheline *et al.*, 2010). $A\beta_{42}$ load has been proposed to have a direct effect on functional connectivity. Clinically healthy subjects with high $A\beta_{42}$ load have been shown to have decreased connectivity in the DMN (Sperling *et al.*, 2009). This has also been shown in patients with MCI (Drzezga *et al.*, 2011). On the contrary, Adriaanse *et al.* (2014) did not find an association between amyloid load and DMN connectivity in healthy elderly, MCI patients and AD patients. Thus, the role of $A\beta_{42}$ load in the brain on DMN connectivity is still unclear. For the current study, we investigated alterations in intrinsic functional connectivity associated with MCI. Furthermore, we investigated the association between the three major CSF biomarkers (Aß42, total tau and hyperphosphorylated tau) and cognitive function in MCI patients. We hypothesised that lower concentrations of CSF $A\beta_{42}$ and higher concentrations of CSF total tau (T-tau) and phosphorylated tau (p-tau) would be associated with greater cognitive decline and that DMN connectivity would be altered in MCI patients.

## Materials and methods

### Participants

We examined a subset of 68 participants (MCI group: mean age 66.9 ± 7.1 years; control group: mean age 66.4 ± 4.1 years) drawn from a larger cohort of 250 subjects. The larger cohort includes 117 young (age 20–39 years) and 153 old participants (age 55–80 years) without any history of neurological or psychiatric disease. Participants were recruited through various advertisements in local and national newspapers. MCI patients were recruited via cooperation with the Memory Clinic at Goethe University Frankfurt am Main. The selection of the 68 participants analysed here was based on the cognitive status. 38 participants fulfilling the diagnosis of an MCI were selected together with 30 cognitively healthy controls, matched for age and education. The local ethics committee of Goethe University Frankfurt approved the study. All subjects declared that they understood the experimental procedure and signed a written informed consent. The study was undertaken in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki; Rickham, 1964). The CSF samples were collected by lumbar puncture. The samples were stored in polypropylene tubes at −80°C until analyses. The measurement of CSF, $A\beta_{42}$, T-tau and p-tau was done by the laboratory of Dr. Fenner and colleagues. Commercially available colorimetric enzyme immunoassays (Innogenetics, Ghent, Belgium) were used for the quantitative determination of $A\beta_{42}$, T-tau and p-tau following the manufacturer's protocol. The measurements were done blinded to the diagnosis. Verbal learning and memory were assessed using the German Version of the California Verbal Learning Test (CVLT, Delis *et al.*, 1987; German version by Niemann *et al.*, 2008). Additionally, attention was assessed using the Trail Making Test A (Spreen & Strauss, 1998). The CERAD–NP (Consortium to Establish a Registry for AD) (Morris *et al.*, 1988) was done to capture cognitive decline. MCI was diagnosed, if participants met the following criteria: (1) subjective memory impairment and (2) a score of at least 1.5 standard deviations below the norm (adjusted for sex, age and education) in any of the CERAD's subtests. Depressive symptoms were assessed using the German Version of the Beck Depression Inventory (Beck *et al.*, 1996, German adaptation by Hautzinger *et al.*, 2006). Each participant was assessed by an experienced psychiatrist or a psychologist to rule out concurrent causes for cognitive deficits such as sleep disorders, depression or other psychiatric disorders (e.g. alcoholism and psychosis).

Group characteristics are summarised in Table 1.

### Analysis of neuropsychological and CSF biomarker data

Statistical analysis of behavioural and CSF data was carried out using SPSS 25 (SPSS, Chicago, IL, USA). A Kolgomorov–Smirnov test was used on all cognitive variables to test for the assumption of normality. To test for group differences (MCI vs. healthy controls) in normally distributed variables, a two-sample *t*-test was used. Group differences in variables that did not meet the assumption of normality were tested with a non-parametric test, the Mann–Whitney *U*-test. For all neuropsychological test scores that were significantly different between MCI patients and healthy controls, we examined the relationship between these scores and $A\beta_{42}$, T-tau and p-tau concentrations in cerebrospinal fluid. Associations between CSF biomarkers and cognition were investigated with Spearman's partial correlation as variables were not normally distributed. In the first step, a Spearman partial correlation between neuropsychological test scores and CSF biomarker concentrations, adjusted for age and years of education, was calculated for a pooled total group consisting of both MCI patients and healthy controls. In the second step, the partial correlation coefficients were computed for each group separately. Initially, we had aimed to analyse the association between CSF biomarkers and intrinsic functional connectivity in the DMN. Since there were no significant differences in posterior cingulate cortex

https://doi.org/10.1017/neu.2019.49 Published online by Cambridge University Press

**Table 1.** Demographic and clinical characteristics

| Variables | MCI patients | Healthy controls | *p* |
|---|---|---|---|
| N | 30 | 38 | |
| Age | 66.43 (7.06) | 66.89 (7.12) | 0.80[a] |
| Education | 15.83 (4.01) | 15.24 (2.99) | 0.75[a] |
| Gender (% male) | 53% | 37% | 0.17[b] |
| Aß42 (pg/ml) | 672.8 (292.01) | 859.89 (240.32) | 0.00[a] |
| p-tau (pg/ml) | 62.27 (32.78) | 45.79 (18.79) | 0.04[a] |
| T-tau (pg/ml) | 420.5 (280.17) | 253.79 (110.15) | 0.02[a] |
| T-tau/Aß42 | 0.344 (0.249) | 0.798 (0.705) | 0.00[a] |
| CVLT early recall | 8.26 (4.63) | 11.84 (3.22) | 0.00[a] |
| CVLT late recall | 8.85 (5.12) | 12.53 (3.14) | 0.00[a] |
| MMSE | 27.43 (2.27) | 29.18 (0.86) | 0.00[a] |
| TMT-A | 58.57 (45.25) | 36.78 (10.63) | 0.00[a] |

Values denote mean raw scores (s.d.). MCI, mild cognitive impairment; p-tau, phospho-tau; T-tau, total tau. CVLT, California Verbal Learning Test; MMSE, Mini-Mental State Examination; TMT-A, Trail Making Test A.
[a]Mann–Whitney *U*-test.
[b]Chi-squared test.

(PCC) resting connectivity between MCI patients and healthy controls, this analysis was not performed.

### MRI hardware and procedure

All MR images were acquired using a Trio 3-T scanner (Siemens, Erlangen, Germany) with a standard head coil for radiofrequency transmission and signal reception. Participants were outfitted with protective earplugs to reduce scanner noise. For T1-weighted structural brain imaging, an optimised 3D modified driven equilibrium Fourier transform sequence (Deichmann *et al.*, 2004) with the following parameters was conducted: acquisition matrix = 256 x 256, repetition time (TR) = 7.92 ms, echo time (TE) = 2.48 ms, field of view (FOV) = 256 mm, 176 slices and 1.0-mm slice thickness. Functional resting state images were acquired using a blood oxygen level-dependent-sensitive echo-planar imaging sequence comprising the following parameters: 300 volumes, voxel size: $3 \times 3 \times 3$ mm$^3$, TR = 2000 ms, TE = 30 ms, 30 slices, slice thickness = 3 mm, distance factor = 20%, flip angle = 90°, and FOV = 192 mm. The resting state measurements were part of a larger fMRI study on episodic memory. After participants had to encode and recognise face–name pairs in the scanner, the resting state measurements were subsequently assessed. For the resting state measurements, all participants were instructed to keep their eyes open, to lie still, not to engage in any speech, to think of nothing special and to look at a white fixation cross presented in the centre of the visual field during the whole scan.

### Selection of seed region

To analyse DMN connectivity, we used a seed region-based approach. Because we were specifically interested in DMN resting state activity and its relation to CSF biomarker concentrations, we investigated the intrinsic functional connectivity of a region anatomically co-localised with the major posterior hub of the DMN (PCC). The PCC is an anatomical/computational hub in the DMN and brain in general (Hagmann *et al.*, 2008; Greicius *et al.*, 2009). Additionally to being an important component of the DMN, the PCC seems to be especially vulnerable towards AD pathology. Studies using fluorodeoxyglucose positron emission tomography (FDG-PET) have consistently shown diminished resting state glucose metabolism in the PCC of patients with early AD (Buckner *et al.*, 2005), MCI (Ishii *et al.*, 2003) and also in cognitively intact (Reiman *et al.*, 1996; Small *et al.*, 2000; Reiman *et al.*, 2004; Chen *et al.*, 2012) elderly persons, possibly reflecting synaptic decline in this region. The vulnerability of the PCC towards AD pathology and the fact that it is a key component of the DMN made it an ideal candidate region for investigating associations between intrinsic functional connectivity and its relation to CSF biomarkers of AD.

### Image processing

Image processing Brain Voyager QX 2.3 (Brain Innovation, Maastricht, the Netherlands) was used to analyse the fMRI data (Goebel *et al.*, 2006). Anatomical data were pre-processed with intensity inhomogeneity correction and transformed into Talairach space. Pre-processing of functional data included 3D motion correction to overcome minor head movements during the scan, slice scan time correction, spatial smoothing with a 4-mm Gaussian kernel (full-width at half-maximum) to accommodate inter-subject anatomical variability, temporal high-pass filtering to remove low-frequency non-linear drifts of three or fewer cycles per time course (cut-off = 0.0075 Hz) and linear trend removal. Quality assurance measures of head motion showed no group differences. The complete set of functional data of each subject was co-registered to the anatomical scans, transformed into Talairach space and resampled to an iso-voxel size of $3 \times 3 \times 3$ mm$^3$. We computed a seed-correlation analysis with a seed region localised in the left PCC. The seed location that was used for the resting state analysis (Fig. 1(A); Talairach coordinates: $x = -2$, $y = -56$, $z = 18$, 134 anatomical voxels) was derived from an event-related fMRI analysis of the same participants: it showed the strongest task-related deactivation during retrieval of face–name pairs and has been reported in the literature as a major hub of the DMN (Hagmann *et al.*, 2008; Buckner *et al.*, 2009; Greicius *et al.*, 2009). The analysis of intrinsic functional connectivity with the PCC as seed region was done using MATLAB software (MathWorks, Natick, MA, USA) version 2016a and freely available toolboxes and custom-written routines (https://vincentvandeven.weebly.com/my-own-software.html). The computation of group differences of intrinsic PCC functional connectivity comprised a two-level random effects analysis (Fox *et al.*, 2005; Oertel-Knochel *et al.*, 2013). At the first level, we first regressed out fMRI nuisance signals from the functional time series, which included the z-normalised signal sampled from the lateral ventricles, white matter, six motion correction parameters (Birn *et al.*, 2006) and the global brain signal. We then sampled and z-normalised the temporal profile from the PCC seed region and correlated (Pearson's r) it with the nuisance-corrected time series (residuals) in a voxel-by-voxel manner. The correlation values were normalised using Fisher's Z and were then entered into the second level of the random effects analysis using an analysis of covariance with group as between-subjects factor, and age, sex and education as subject-level covariates. We performed a one-sample *t*-test to obtain a multi-subject map of overlapping spatial distributions of PCC-related connectivity across all subjects (one-sided to include only positive effects), and the results were visualised using a false discovery rate (FDR) of $q = 0.05$ (Genovese *et al.*, 2002). Nuisance-corrected between-group effects were also corrected for multiple comparisons using an FDR of 0.05. FDR corrects for the

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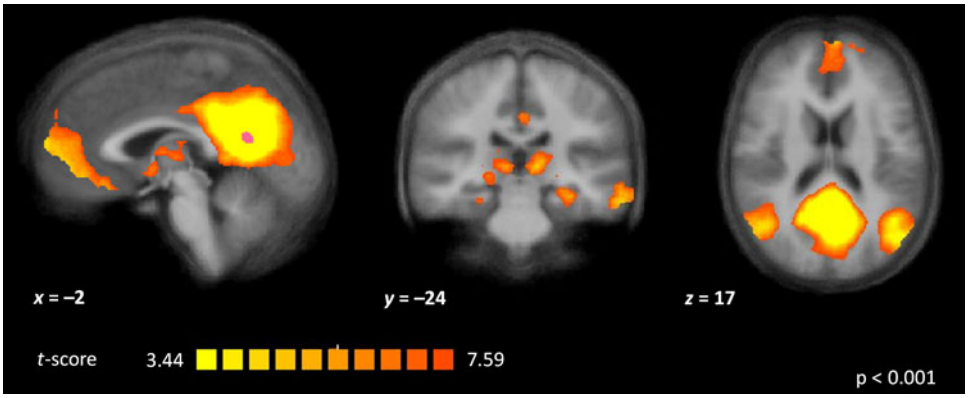



**Fig. 1.** Functional connectivity maps of the entire cohort ($n = 68$) showing blood oxygen level-dependent fluctuations during rest correlated with the left posterior cingulate cortex seed region (depicted in violet) demonstrating the default mode network ($p < 0.001$).

expected proportion of false positives among those tests for which the null hypothesis was rejected. It has been shown to reliably reject false positives, albeit being less conservative than family-wise error correction (Genovese *et al.*, 2002).

## Results

### Demographic and clinical characteristics

The demographic and clinical characteristics of the MCI group and healthy controls are shown in Table 1. The Mann–Whitney *U*-test revealed no significant group differences in age and years of education ($p > 0.05$). There were also no significant group differences with regard to gender distribution (chi-squared test, $p > 0.05$). With regard to different subtypes of MCI, the group showed the following distribution: 15 patients with amnestic MCI, multiple domain (performing 1.5 standard deviations below the norm in two different cognitive domains, one of them memory), 13 patients with amnestic MCI, single domain (performing 1.5 standard deviations below the norm only in the memory domain) and 2 patients with non-amnestic MCI (performing 1.5 standard deviations below the norm in at least one domain and no memory impairment). MCI patients showed significantly lower concentrations of CSF $Aß_{42}$ ($p < 0.001$) and higher concentrations of p-tau ($p = 0.04$) as well as T-tau ($p = 0.02$) than controls. Furthermore, MCI patients showed a much higher T-tau/$Aß_{42}$ ratio than healthy controls ($p = 0.004$). The MCI group performed significantly worse than healthy controls in all neuropsychological tests.

### Relationship between cognitive performance and CSF biomarkers

There were significant associations between CSF biomarker concentrations and cognitive performance in the MCI group (Table 2). Lower levels of $Aß_{42}$ concentrations were associated with worse performance in verbal episodic memory (CVLT early recall $r_s = 0.51$, $p = 0.013$, CVLT late recall $r_s = 0.44$, $p = 0.37$, Fig. 2) and global cognitive functioning [Mini-Mental State Examination (MMSE) $r_s = 0.54$, $p = 0.008$]. Similarly, a higher T-tau/$Aß_{42}$ ratio was also associated with worse performance in verbal episodic memory (CVLT early recall $r_s = -0.52$, $p = 0.011$; CVLT late recall $r_s = -0.48$, $p = 0.022$, Fig. 1) and global cognitive function (MMSE $r_s = -0.55$, $p = 0.006$). Higher levels of T-tau were associated with lower MMSE scores ($r_s = -0.47$, $p = 0.026$). We did not find a relationship between CSF biomarker levels and cognitive performance in healthy controls (see Table 2).

### Resting state correlation analysis

Seed-correlation analysis of resting state signal fluctuations for a pooled total group consisting of both MCI patients and healthy controls (one-sample *t*-test, $n = 68$) revealed regions of significant connectivity [$q$ (FDR) < 0.05], with the PCC seed region comprising extended areas of bilateral PCC and precuneus, bilateral medial frontal gyrus, bilateral superior frontal gyrus, bilateral parahippocampal gyrus/hippocampus, bilateral nucleus mediodorsalis and bilateral medial temporal gyrus (Fig. 1). This pattern largely resembles cortical regions of the DMN in cognitively normal elderly individuals (Greicius *et al.*, 2004; Spreng *et al.*, 2009). The nuisance-corrected between-group comparison showed no significant differences in intrinsic functional connectivity between the PCC seed region and other areas of the brain [$q$ (FDR) > 0.05].

## Discussion

Extending the findings of previous studies on the relationship between altered AD specific biomarker profiles and cognitive performance, we found that decreased $Aß_{42}$ levels and elevated T-tau levels are closely associated with cognitive decline. MCI patients did not differ from cognitively normal controls with regard to intrinsic functional connectivity.

Our finding that $Aß_{42}$ concentrations are closely associated with cognitive decline is in line with previous studies on the relationship between CSF biomarkers and cognitive performance. Several studies also found worse performance in different measures of memory to be associated with $Aß_{42}$ burden in MCI patients (Hedden *et al.*, 2012; Lim *et al.*, 2013; Nathan *et al.*, 2017). Unlike previous studies (Malpas *et al.*, 2015; Nathan *et al.*, 2017), we did not find a significant association between T-tau levels, nor p-tau levels and memory performance. This finding might indicate that MCI patients who were included in our study were just at the beginning of the disease trajectory. It is assumed that amyloid accumulation precedes neocortical T-tau accumulation and neurodegeneration (Andreasen & Blennow, 2005).

Thus, at the beginning of the AD trajectory, there might be stronger associations between amyloid levels and cognitive performance in comparison to other CSF biomarkers (T-tau and p-tau). Since accumulation of $Aß_{42}$ in the brain already begins before any cognitive symptoms are present and is closely associated with accelerated cognitive decline, $Aß_{42}$ has been suggested to be a sensitive CSF biomarker for prodromal AD (Doraiswamy *et al.*, 2012; Rowe *et al.*, 2013; Lim *et al.*, 2018). We found the T-tau/$Aß_{42}$ ratio to show the strongest associations with memory decline and global

https://doi.org/10.1017/neu.2019.49 Published online by Cambridge University Press

**Table 2.** Relationship between cognitive performance and CSF biomarkers

| | | *MCI group* | | | | *Control group* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Episodic memory | | Attention | | Verbal memory | | Attention | |
| | | CVLT early recall | CVLT late recall | TMT-A | MMSE | CVLT early recall | CVLT late recall | TMT-A | MMSE |
| $A\beta_{42}$ | $r_s$ | 0.507 | 0.437 | −0.382 | 0.541 | 0.172 | 0.179 | −0.108 | 0.059 |
| | p | **0.013*** | **0.037*** | 0.072 | **0.008**** | 0.336 | 0.318 | 0.547 | 0.742 |
| p-tau | $r_s$ | −0.262 | −0.230 | 0.164 | −0.321 | −0.064 | −0.002 | 0.030 | −0.185 |
| | p | 0.227 | 0.289 | 0.453 | 0.135 | 0.720 | 0.989 | 0.867 | 0.302 |
| T-tau | $r_s$ | −0.389 | −0.357 | 0.097 | −0.464 | −0.065 | −0.016 | 0.140 | −0.139 |
| | p | 0.066 | 0.094 | 0.658 | **0.026*** | 0.717 | 0.926 | 0.437 | 0.440 |
| T-tau/$A\beta_{42}$ | $r_s$ | −0.520 | −0.457 | −0.239 | −0.550 | −0.110 | −0.114 | −0.141 | −0.266 |
| | p | **0.011*** | **0.022*** | 0.270 | **0.006**** | 0.544 | 0.529 | 0.434 | 0.134 |

MCI, mild cognitive impairment; CVLT, California Verbal Learning Test; TMT-A, Trail Making Test A; MMSE, Mini-Mental State Examination; p-tau, phospho-tau; T-tau, total tau; $r_s$, Spearman's correlation coefficient.*: $p < 0.05$; **: $p < 0.01$

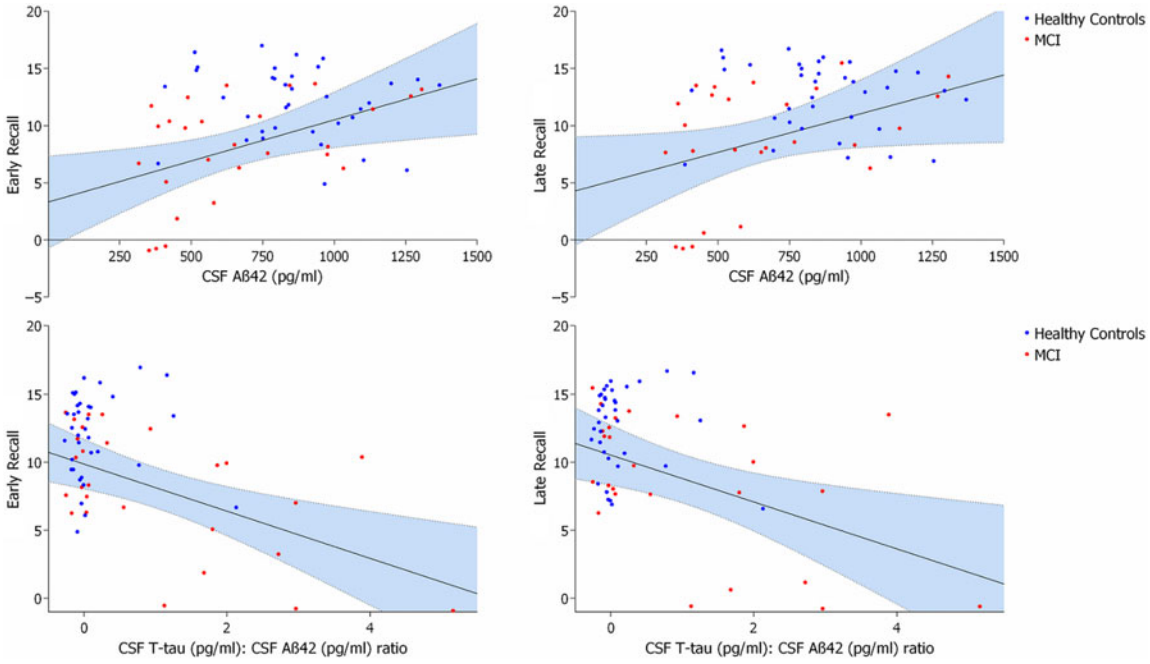



**Fig. 2.** Relationship between CSF biomarkers and cognitive performance. Model predicted scores adjusted for age and education. MCI, mild cognitive impairment. Early recall and Late recall were assessed with the California Verbal Learning Test.

cognitive functioning. This is in line with a study by Nathan *et al.* (2017) that also found associations between CSF biomarkers and cognition to become stronger, affecting broader cognitive domains when levels of both CSF tau and $A\beta_{42}$ are considered together. Thus, the T-tau/$A\beta_{42}$ ratio might be an even more sensitive biomarker for prodromal AD than $A\beta_{42}$ alone. However, this hypothesis should be further investigated using a much larger prospectively followed sample before definite conclusions can be drawn.

Our finding that intrinsic functional connectivity was not significantly different between MCI patients and cognitively healthy controls contradicts a number of studies that either found hyperconnectivity (Jin *et al.*, 2012; Esposito *et al.*, 2013) or hypo-connectivity (Sorg *et al.*, 2007; Cha *et al.*, 2013; Wang *et al.*, 2013) in the DMN of MCI subjects. However, consistent with our study, a number of studies have also shown no alterations in resting state functional connectivity in the DMN of MCI patients (Binnewijzend *et al.*, 2012; Zamboni *et al.*, 2013; Adriaanse *et al.*, 2014).

The lack of significant differences in DMN connectivity between MCI patients and healthy controls might be due to the heterogeneity of the MCI group investigated in this study. MCI patients differed with regard to their CSF profiles. Thus, 50% ($n = 15$) of MCI patients in this study showed a CSF $A\beta_{42}$ profile that was in line with the assumption of prodromal AD (<560 pg/ml), whereas $A\beta_{42}$ CSF profiles of the other MCI patients were within normal range. There is some evidence that amyloid load in the brain rather than MCI diagnosis is associated with alterations in intrinsic connectivity within the DMN. For instance, Adriaanse *et al.* (2014) found that functional connectivity within the DMN was reduced in subjects with high cerebral amyloid load,

https://doi.org/10.1017/neu.2019.49 Published online by Cambridge University Press

independent of diagnosis (AD, MCI or healthy controls). Similarly, Yi *et al.* (2015) found reduced DMN functional connectivity in MCI subjects with high amyloid load compared to cognitive healthy controls. Interestingly, this pattern was reversed in MCI subjects with low cerebral amyloid load. Thus, MCI subjects with very little or low $A\beta_{42}$ deposition in the brain (measured with Carbon-11-labelled PiB ($^{11}$C-PiB) PET) showed increased functional connectivity within the DMN compared to cognitively healthy controls and MCI subjects with high $A\beta_{42}$ deposition in the brain.

The big variation in resting neuronal activation patterns within the DMN depending on amyloid load might explain the lack of significant findings regarding DMN connectivity in our study. Since the MCI subjects in our study showed no consistent pattern of amyloid load in the brain (only 50% showing a pathological reduction of $A\beta_{42}$ in CSF), there might have been hypoconnectivity as well as hyperconnectivity within the DMN of our MCI subjects, thus showing no consistent pattern when the group was analysed altogether. In order to investigate the hypothesis of reduced DMN connectivity in MCI subjects with high amyloid load, we performed an additional analysis of resting state fMRI data. Only MCI subjects showing pathological $A\beta_{42}$ levels were included into this analysis. Moreover, cognitively healthy controls showing pathological $A\beta_{42}$ levels ($n = 3$) were excluded from the analysis. In contradiction to previous studies (e.g. Adriaanse *et al.* 2014; Yi *et al.*, 2015), subjects with high amyloid load in the brain (low CSF $A\beta_{42}$ concentrations) did not significantly differ from subjects with low $A\beta_{42}$ concentrations with respect to DMN functional connectivity. The lack of significant results [$q$ (FDR) > 0.05] might have been due to the reduction of statistical power by analysing only half of the MCI group ($n = 15$).

A possible limitation of this study is the lack of an adjustment for multiple comparisons. We decided against an adjustment for multiple comparisons since we had a priori hypotheses regarding the CSF biomarkers for AD and their relationship with cognitive outcomes reported here.

A clear limitation of this study is the lack of longitudinal data. Our results indicate that CSF biomarkers are more closely related to cognitive impairment than alterations in resting state activity. However, longitudinal data would be needed to make assumptions about the ability of CSF biomarkers to predict cognitive decline. In the end, information on the progression of the cognitive impairment would be needed to state with certainty that the aberrant CSF biomarker profiles of our patients were a sign of incipient AD.

Taken together, our findings indicate that a decline in CSF $A\beta_{42}$ and an elevation of T-tau are related to changes in cognitive performance in MCI and might be a more reliable indicator of neurodegenerative processes than intrinsic functional connectivity. This assumption is in line with a study by Bertens *et al.* (2015) which showed that $A\beta_{42}$ levels are abnormal already in incipient stages of AD before any cognitive symptoms are present. Their study also included measures of hippocampal volume and glucose metabolism (FDG-PET) that did not show any abnormalities in incipient AD.

## Conclusion

We suggest that CSF biomarker profiles constitute a more robust marker of neurodegenerative processes underlying cognitive decline than intrinsic functional connectivity. Alterations in resting state functional connectivity do not seem to be consistent in MCI patients and are not likely to be closely related to cognitive function.

**Acknowledgements.** This work was supported by the Neurodegeneration & Alzheimer's disease research grant of the LOEWE program 'Neuronal Coordination Research Focus Frankfurt' (NeFF), awarded to J.P and D.P. The authors certify that there is no actual or potential conflict of interest in relation to this article.

**Conflict of interest.** The authors have no conflict of interest to report.

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