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Verification

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Highlights:

- Study if grey matter networks can predict decline over time in cognition in MCI.
- Reduced grey matter connectivity predicts steeper decline in cognitive functioning.
- Network measures might detect progression to dementia in MCI patients.

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Abstract

Grey matter networks are disrupted in Alzheimer's disease and related to cognitive impairment. However, it is still unclear whether these disruptions are associated with cognitive decline over time. Here, we studied this question in a large sample of patients with mild cognitive impairment with extensive longitudinal neuropsychological assessments. Grey matter networks were extracted from baseline structural MRI and we tested associations of network measures and cognitive decline in MMSE and five cognitive domains (i.e., memory, attention, executive function, visuospatial and language). Disrupted network properties were cross-sectionally related to worse cognitive impairment. Longitudinally, lower small-world coefficient values were associated with a steeper decline in almost all domains. Lower betweenness centrality values correlated with a faster decline in MMSE and memory and, at a regional level, these associations were specific for the precuneus, medial frontal and temporal cortex. Furthermore, network measures showed additive value over established biomarkers in predicting cognitive decline. Our results suggest that grey matter network measures might have use in identifying patients who will show fast disease progression.

Keywords: Alzheimer's disease; cognitive decline; grey matter networks; mild cognitive impairment; single-subject; graph theory

1. Introduction

Therapies targeted to treat Alzheimer's disease (AD) are probably most effective when administered at very early stages of the disease, before the clinical syndrome of dementia has become evident (Scheltens et al., 2016). Patients with mild cognitive impairment (MCI) have an increased risk to develop dementia. Being able to identify those MCI patients that will show fast cognitive decline could increase potential treatment effects in clinical trials. However, this is challenging for individual patients, as subjects with MCI show considerable variability in cognitive decline (Jack et al., 2013; Scheltens, 2013). In addition, the biological substrate associated with decline in specific cognitive domains is not well understood. Increasing evidence indicates that measures of brain networks change during the course of AD (Pereira et al., 2016; Yao et al., 2010), already starting at early, pre-clinical stages (Tijms et al., 2016). Therefore, measures of brain networks might have promise as prognostic biomarkers for future cognitive decline (Tijms et al., 2013b).

Brain networks can be determined based on similarity in grey matter structure between brain areas as measured with structural MRI (Mechelli et al., 2005; Tijms et al., 2012). Such patterns of grey matter similarity have been associated with coordinated growth of grey matter during development (Alexander-Bloch et al., 2013a), functional co-activation (Alexander-Bloch et al., 2013b) and/or axonal connectivity (Gong et al., 2012). Several studies have shown that grey matter networks are disrupted in AD, as indicated by a more random network organization (He et al., 2009; Li et al., 2012; Tijms et al., 2013a; Tijms et al., 2013b; Yao et al., 2010). Furthermore, a more random network topology has been cross-sectionally related to worse cognitive impairment in AD patients (Tijms et al., 2013a; Tijms et

al., 2014). In MCI, the network topology seems to lie in between those of cognitively healthy older subjects and AD patients (Pereira et al., 2016; Yao et al., 2010). Therefore, it could be hypothesized that MCI patients who have a more randomly organized network will show faster decline in cognitive functioning over time. However, previous studies investigated cross-sectional effects and/or used a methodology that results in one network for a group of subjects. Thus, this hypothesis has not been tested yet as it is not possible to relate group-based networks to inter-individual measures of decline. Therefore, it remains unclear whether a more random network topology may provide a biological substrate to explain cognitive decline in single patients with MCI, and if so, whether this can be attributed to specific cognitive domains.

In this study, we assessed whether baseline single-subject grey matter network measures could explain differences among MCI subjects in their rates of cognitive decline for specific cognitive domains. We further tested whether grey matter network measures have additive value over established markers for Alzheimer's disease (i.e., hippocampal volume, CSF amyloid β 1-42 and total tau levels) in predicting which patients will show increased cognitive decline.

2. Materials and Methods

2.1. Participants

Two-hundred and fifty-eight MCI patients (mean age 67±8 years, MMSE 27±2) with available baseline structural MRI, and at least one year of follow-up including repeated neuropsychological testing were selected from the Amsterdam Dementia

Cohort of the Alzheimer Center of the VU University Medical Center. Patients initially visited our memory clinic between 2000 and 2013. Most subjects received a standard dementia screening that often included a medical history, physiological and neurological examination, extensive neuropsychological screening, blood testing, lumbar puncture, an EEG and an MRI scan (Van Der Flier et al., 2014). Patients were diagnosed with MCI during a multidisciplinary consensus meeting based on international consensus criteria: patients initially visiting our memory clinic before 2012 were diagnosed with MCI according to Petersen criteria (Petersen et al., 1999); after that the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria were used (Albert et al., 2011). Follow-up visits were scheduled approximately annually as part of the clinical routine and often included standardized neuropsychological testing. During a multidisciplinary consensus meeting at follow-up, a diagnosis of AD or another type of dementia was made when subjects met the corresponding international research and/or clinical consensus criteria (Gorno-Tempini et al., 2011; Mckeith et al., 2005; Mckhann et al., 1984; Mckhann et al., 2011; Neary et al., 1998; Rascovsky et al., 2011; Roman et al., 1993). Over a median follow-up time of 2.3 years, 115 (45%) patients progressed to dementia. Ninety-eight (85%) out of the progressing patients received a diagnosis of probable or possible AD during follow-up and 17 patients received another diagnosis (n=7 vascular dementia; n=3 dementia with Lewy bodies; n=4 frontotemporal lobar degeneration; n=1 primary progressive aphasia; n=2 unspecified dementia). The medical ethics committee of the VU University Medical Center approved the study and all subjects provided written informed consent.

2.2. Neuropsychological assessment

Neuropsychological examinations consisted of a standardized test battery (Van Der Flier et al., 2014) and included the Dutch version of the Rey Auditory Verbal Learning Test (RAVLT) total immediate recall and delayed recognition and correct words of the visual association test (VAT) for the memory domain; the trail making test part A (TMT), the forward subtest of the Digit Span, the Stroop test part 1 and 2 for the attention domain; the backward subtest of the Digit Span, the trail making test part B, the Stroop test part 3, the letter fluency test (DAT) and the frontal assessment battery test (FAB) for executive functioning; category fluency (animals) and the VAT naming subtest for the language domain and the dot counting and fragmented letters test for the visuospatial domain. General cognitive function was assessed with the mini-mental state examination (MMSE). A total number of 922 neuropsychological evaluations were available (median number of follow-ups: 3, range: 1-11). The percentage of missing values over all follow-up visits in any neuropsychological test ranged from 1 to 37% (see also table 2). We combined tests into cognitive domains in order to reduce the number of tests, and therefore we estimated missing values using multiple imputation as implemented in SPSS (version 22) to obtain unbiased estimates of cognitive functioning. Age, sex and education were included as predictors. Imputation was repeated for 15 times. After imputation, test scores of the Stroop and TMT tests were inverted so that lower scores reflect more impairment. All baseline test-scores were z-transformed and follow-up z-scores were determined relative to baseline scores. Per time point the z-transformed scores were averaged across tests per cognitive domain.

2.3. MRI acquisition & pre-processing

Due to the long period of time that subjects were included, imaging was acquired from 7 different systems using spoiled gradient-echo sequences. Acquisition details for the different systems are listed in the Supplementary Material. All structural T1-weighted MRI scans were reviewed for brain pathology other than neurodegeneration by an experienced radiologist. Images were preprocessed using SPM12 as implemented in Matlab 7.12. First, the structural T1 weighted images were segmented into grey matter, white matter and cerebrospinal fluid with the default settings for all parameters. The native space grey matter segmented images were then resliced to a voxel size of 2×2×2 mm, in order to standardize voxel sizes and to reduce dimensionality. Next, 90 anatomical areas in subject space were identified using the automated anatomical labelling atlas (AAL; Tzourio-Mazoyer et al., 2002) which was warped from standard space to native space using subject specific inversed normalization parameters. Total intracranial volume (TIV) was computed as the sum of grey and white matter and cerebrospinal fluid volumes in cm^3. Normalized grey matter was defined as the ratio of grey matter to total intracranial volume. Single-subject grey matter networks were extracted from native space grey matter segmentations using an automated method that has been published previously (https://github.com/bettytijms/Single_Subject_Grey_Matter_Networks; Tijms et al., 2012).

2.4. Grey matter network measures

The obtained networks were binarized after determining a threshold that ensured a similar chance for all subjects to include on average 5% spurious correlations in the

network. We then calculated graph theoretical measures for the obtained grey matter networks that quantify the amount of connectivity (i.e., degree and connectivity density) and the network topology (i.e., clustering, path length, betweenness centrality; Rubinov and Sporns, 2010). For each network, the network measures size, degree, connectivity density, clustering coefficient, path length and betweenness centrality were calculated. Connectivity density is defined as the ratio of existing connections to the maximum number of connections possible in the network. The clustering coefficient indicates the interconnectedness of neighboring nodes. The path length quantifies the number of connections between two nodes along the shortest path. Betweenness centrality measures the number of shortest paths that pass through a node and is indicative of the importance of a node in a network (Rubinov and Sporns, 2010). In order to estimate how the network topology deviates from randomly organized networks, we also calculated measures of the small-world property. Normalized versions of global clustering coefficient (gamma) and path length (lambda) were calculated by dividing the unnormalized measures with the corresponding average of clustering or path length values of 20 randomized reference networks that kept the degree distribution intact (Maslov and Sneppen, 2002). The small-world coefficient is defined as the ratio of gamma to lambda (Humphries and Gurney, 2008) with values >1 indicating an optimal balance between information segregation (greater than random clustering) and integration (similar to random path length; Rubinov and Sporns, 2010). At a regional level, we averaged local values across nodes that were labeled according to the AAL atlas to enable comparison across subjects and global network measures were obtained by averaging the local network measures across all nodes. All network measures were computed with

functions from the Brain Connectivity Toolbox adjusted for large-sized networks (https://sites.google.com/site/bctnet/; Rubinov and Sporns, 2010).

2.5. Cerebrospinal fluid analysis

CSF samples were obtained with a lumbar puncture between the L3/L4, L4/L5 or L5/S1 intravertebral space using a 25-gauge needle and syringe and collected in polypropylene tubes. Concentrations of amyloid β 1-42 (Aβ42) and total tau were determined with sandwich ELISAs (Innotest, Fujirebio, Belgium) (Mulder et al., 2010) at the Neurochemistry Laboratory of the Department of Clinical Chemistry of the VUmc.

2.6. Statistical analysis

Comparisons of clinical characteristics between stable MCI subjects and those patients who progressed during follow-up were performed with Student's t-tests, Kruskal tests or chi-square tests where appropriate. We tested associations of baseline grey matter network measures (predictor variables) and decline over time in each cognitive domain (outcome variables) with linear mixed models, including grey matter network measures and time as main terms to assess baseline effects and an interaction term of grey matter network measures × time to assess annual change effects. We estimated random slopes and intercepts for subjects with the lme4 package (Bates et al., 2015) in R (version 3.3.0, 2016-05-03). Results were pooled over imputed datasets using Rubin's rules as implemented in the package MICE (Van Buuren and Groothuis-Oudshoorn, 2011). Sex, age, education, scanner type and TIV were included as covariates. For the network size we excluded TIV as a covariate due

to the high correlation between these measures. First size, degree and connectivity density were tested and if any of these measures showed a significant association they were included as an additional covariate in the respective model, since they influence other network property values (Van Wijk et al., 2010). Analyses of annual change effects were repeated at a local level for each of the 90 AAL areas including local grey matter atrophy as an additional covariate. Local analyses were corrected for multiple testing with the FDR-procedure (Benjamini and Hochberg, 1995) with $p_{FDR} < 0.05$ indicating statistical significance. We used logistic regression modeling to study whether network properties could predict which subjects would show fast progression. Per cognitive domain, we classified patients based on whether their slope was higher (i.e., slow decline) or lower (i.e., fast decline) than the median slope of the total group corrected for education. Logistic regression analyses were employed for slow / fast cognitive decline (outcome variable) with network measures as the predictor variable and age, sex, total intracranial volume and scanner included as covariates (Model 1). To study whether network measures could explain variance beyond more established biomarkers, we repeated the logistic regression analyses subsequently adding hippocampal volume (Model 2), CSF Aβ42 levels (Model 3) and CSF total tau levels (Model 4) as covariates. All statistical analyses were performed in R (version 3.3.0, 2016-05-03) and brainviewer (Version 1.53; Xia et al., 2013) was used to visualize regional results.

3. **Results**

3.1. Sample description

Baseline demographical, clinical and grey matter connectivity measures are summarized in Table 1. Subjects were on average 67±8 years of age and 105 (41%) were female. Follow-up information was available over a median of 2.3 (1.4-3.1) years. Over follow-up, patients showed decline in all cognitive domains examined (all $p < 0.01$), and this was most pronounced for the MMSE and the memory domain (Table 2). Progressing patients had lower normalized grey matter and hippocampal volumes and CSF Aβ42, and higher CSF total tau and p-tau levels (all $p < 0.05$). All networks had an average connectivity density of 16.31% (± 1.33) and were small-world. None of the networks had disconnected nodes. Compared to subjects who remained stable, subjects who progressed to dementia showed significantly lower values of gamma and the small-world coefficient ($p < 0.05$) and a trend for lower betweenness centrality values ($p = 0.053$) at baseline.

========== Please insert Table 1 about here ==========

========== Please insert Table 2 about here ==========

3.2. Baseline and annual change effects of global network measures

Table 3 shows estimated baseline and annual change effects of global network measures on cognitive impairment. We found several baseline effects of global network measures on cognition: for global cognitive function, lower values of the degree (β±SE; 0.2±0.09) and betweenness centrality (β±SE =0.33±0.15) were associated with worse performance in the MMSE ($p < 0.05$). Patients with higher

values of the characteristic path length (β±SE = -0.11±0.05) showed worse memory performance at baseline ($p < 0.05$). Lower values of betweenness centrality were associated with worse performance in attention (β±SE = 0.45±0.13; $p < 0.001$), executive (β±SE = 0.38±0.1; $p < 0.001$) and language functioning (β±SE = 0.29±0.13; $p < 0.05$). Lower values of the characteristic path length (β±SE = 0.1±0.04) and lambda (β±SE = 0.1±0.5) were additionally associated with worse executive functioning (all $p < 0.05$).

Longitudinal analyses showed that lower small-world coefficient values at baseline were associated with increased decline in memory (β±SE = 0.05±0.02), attention (β±SE = 0.04±0.02) and executive functioning (β±SE = 0.04±0.02; all $p<0.05$). Patients with lower gamma values at baseline showed faster decline in memory (β±SE = 0.04±0.02), attention (β±SE = 0.04±0.02) and executive functioning (β±SE = 0.04±0.02; all $p < 0.05$). Smaller network size (β±SE = 0.07±0.03) and lower betweenness centrality values (β±SE = 0.08±0.03) were additionally related to steeper decline in MMSE, while lower values of network size (β±SE = 0.05±0.02), degree (β±SE = 0.05±0.02) and betweenness centrality (β±SE = 0.06±0.02) were associated with steeper decline in memory (all $p < 0.01$). No associations were found for grey matter network properties and change over time in language and visuospatial functioning (all $p > 0.05$).

========== Please insert Table 3 about here ==========

When we restricted analyses to patients who remained stable and those who progressed to AD-type dementia (n=98) the observed associations between size and

betweenness centrality and cognitive decline over time became slightly stronger for memory and MMSE (Supplementary Table 1a). Effect sizes for attention and executive functioning remained similar, but were no longer significant. In this subsample higher connectivity density values were related to steeper decline in language functioning (β±SE = -0.05±0.03; $p < 0.05$; Supplementary Table 1a). When restricting analyses to patients who remained stable and those who received a diagnosis other than AD during follow-up (n=17), effect sizes for the associations of network measures and decline in the MMSE decreased (Supplementary Table 1b). Effect sizes of network measures remained similar for decline in memory and attention and became slightly stronger for executive functioning. In this sub-group we found additional associations of lower connectivity density (β±SE = 0.05±0.02) and clustering coefficient values (β±SE = 0.05±0.02) with steeper decline in visuospatial functioning (all $p < 0.05$).

3.3. Anatomical specificity of associations between grey matter network measures and cognitive decline

We found several effects of local network measures on cognitive decline over time for global cognitive functioning and memory: lower values of the network degree in the temporal lobes and prefrontal areas showed the largest effect sizes for decline in memory (Fig. 1A and Supplementary Table 2). Lower baseline betweenness centrality values were related to faster decline in both memory and MMSE for several brain areas, including the left superior medial orbito-frontal and the bilateral precentral gyrus (all $p_{FDR} < 0.05$; Fig. 1). The strongest effects were found for the associations of lower betweenness centrality in the right precuneus and faster decline in memory (β±SE = 0.07±0.02) and MMSE (β±SE = 0.11±0.03; all $p_{FDR} < 0.01$). Lower values

of the betweenness centrality in the right supramarginal, middle occipital, superior parietal, middle temporal, parahippocampal gyrus and bilateral inferior temporal gyri were specifically associated with increased decline in memory functioning over time (all $p_{FDR} < 0.05$; Fig. 1B). For the MMSE, the associations additionally involved the left anterior cingulate, right lingual gyrus, right fusiform gyrus, right hippocampus, left parahippocampal gyrus and right thalamus (all $p_{FDR} < 0.05$; Fig. 1C; see also Supplementary Table 2 and 3 for all local effects for MMSE and memory).

Repeating analyses after excluding subjects who progressed to non-AD type dementia, we found similar effects for the local betweenness centrality on memory decline over time while the weakest associations were no longer significant (see Supplementary Fig. 1B). Effects for local betweenness centrality values and decline in the MMSE were slightly stronger and additionally included the right paracentral lobule (see Supplementary Fig. 1C). For this subsample, we additionally found associations for lower values of the local degree and increased decline in memory functioning for regions that included the right olfactory gyrus, left precuneus, bilateral putamen and left superior temporal pole (see Supplementary Fig. 1A). After restricting analyses to stable patients and those with a follow-up diagnosis other than AD, lower values of the betweenness centrality in the right supramarginal gyrus (β±SE = 0.07±0.02) and left inferior temporal gyrus (β±SE = 0.06±0.02) were associated with steeper memory decline over time (all $p_{FDR} = 0.02$). Local associations of the degree with increased memory decline largely overlapped with those seen for the total group (see Supplementary Fig. 2). Additionally, we found a significant association of lower betweenness centrality in the right olfactory gyrus with increased decline in attention over time (β±SE = -0.09±0.02; $p_{FDR} = 0.004$).

========== Please insert Fig. 1 about here ==========

3.4. Comparison of network measures with other biomarkers to predict which subjects will show fast cognitive decline

We further investigated whether those network measures that showed the largest effect sizes in the mixed model analyses would show additive value to established biomarkers to predict which patients show faster than median rate of cognitive decline. Across the domains, subjects classified as fast progressors showed similar proportions of progression to clinical AD dementia (Supplementary Table 4). For the MMSE and memory functioning, gamma and the small-world coefficient were predictive for fast decline, and this effect remained after correcting for hippocampal volume and CSF Aβ42 levels (Table 4). These effects lost significance, however, when CSF total tau levels were added to the model. For attention and executive functioning, the betweenness centrality, gamma and small-world coefficient showed the strongest predictive effects, which remained stable after correcting for hippocampal volume, CSF Aβ42 and total tau levels (all $p < 0.05$).

========== Please insert Table 4 about here ==========

4. Discussion

Our main finding is that MCI patients who had grey matter network measures that are indicative of a more random network organization at the time of first visit showed a

steeper rate of decline in cognitive functioning. These results suggest that grey matter networks might contain information that could help in discriminating MCI subjects who will show fast cognitive decline in specific cognitive domains.

Grey matter network properties showed the strongest associations with decline in global cognitive functioning and memory compared to other cognitive domains. Memory is among the first cognitive functions to be affected in AD, while other cognitive domains usually become impaired at later stages of the disease (Jack et al., 2013). Lower values of gamma and the small-world property are indicative of a more random network organization, which has often been reported for AD patients (Tijms et al., 2013a; Tijms et al., 2013b; Yao et al., 2010). We have previously shown that a more random network topology was associated with worse cognitive impairment when comparing patients with AD dementia with controls cross-sectionally, and within AD (Tijms et al., 2013a; Tijms et al., 2014). Previous studies investigating grey matter networks in MCI have reported intermediate values of the small-world coefficient relative to cognitively normal controls and AD subjects (Pereira et al., 2016; Yao et al., 2010), suggesting that lower small-world values in these MCI patients may herald prodromal AD. Our findings seem to support this idea, since MCI subjects who progressed to dementia showed lower betweenness centrality, gamma and small-world coefficient values, suggesting that their networks seems to be similarly organized to networks we previously observed in subjects with AD dementia. Still, previous studies have reported conflicting results concerning the directionality of network measures when comparing patient groups to healthy controls. For example, both increased and decreased clustering coefficient and path length values (He et al., 2008; Pereira et al., 2016; Tijms et al., 2013a; Yao et al.,

2010), have been reported for AD patients compared to controls. A potential explanation for these conflicting results might lie in the different network reconstruction methods that have been used (Tijms et al., 2013b). Alternatively, the changes in network measures due to illness might not follow a linear path (Seo et al., 2013). Future studies should further investigate trajectories of network changes over time.

Lower betweenness centrality values were associated with a steeper decline in the MMSE and the memory domain. Betweenness centrality measures the importance of a node in a network and identifies hubs (Rubinov and Sporns, 2010). Hub regions are thought to control the information flow between functionally segregated areas and seem to be especially vulnerable in AD-related pathology (Tijms et al., 2013a; Tijms et al., 2013b; Yao et al., 2010). We hypothesize that the loss of hubs impedes the information flow between functionally distinct areas, resulting in impaired cognitive functioning as suggested by our findings. Our results showed that decreased betweenness centrality values in several distinct brain areas, including the precuneus, the superior frontal gyrus and the supramarginal gyrus, were associated with a steeper cognitive decline. These brain areas have previously been implied in the progression to AD as showing increased cortical thinning (Dickerson et al., 2009). Our analyses were adjusted for local grey matter atrophy, and so these results suggest that grey matter network measures explain variance in cognitive decline beyond atrophy. The precuneus and superior frontal gyrus have previously also been implicated with early amyloid aggregation (Rowe et al., 2007; Thal et al., 2002), suggesting that grey matter networks might reflect subtle structural alterations in the brain caused by amyloid deposits. We additionally found associations for the bilateral precentral gyri

and cognitive decline. Decreased clustering coefficient values in the precentral gyri have been associated with lower Aβ42 levels in a study of cognitively healthy adults (Tijms et al., 2016), suggesting that network alterations in this area might be affected in the very early phases of AD.

After we restricted analyses to subjects who progressed to Alzheimer's dementia during follow-up, associations of betweenness centrality on cognitive decline in global cognition and memory slightly increased, suggesting that MCI subjects with lower betweenness centrality values are on the path towards AD dementia. Analyses restricted to patients that developed non-AD dementia during follow-up showed decreased effect sizes of network measures for decline in the MMSE, and slightly increased effect sizes for executive functioning, suggesting that domain-specific associations of network measures depend on the composition of the patient groups.

In contrast to previous studies that used methods that result in one network for an entire group of subjects, our approach to extract networks for single-subjects allowed us to further extend the literature by showing that network alterations are related to cognitive decline, and showed additive value in predicting which subject showed worse than median decline. Gamma, e.g., could predict which subjects showed fast decline in memory and MMSE, even when corrected for hippocampal volume, suggesting that this measure contains additive value over this more established biomarker for memory functioning. Results remained similar when adding CSF Aβ42 levels to the model, but did not have additive value over total tau levels. Thus, when CSF is available, tau would be the biomarker of preference to select subjects who will show fast decline in memory. For attention and executive functioning network

measures were the best predictors for fast decline, beyond all other established biomarkers. These results suggest that grey matter network measures contain information beyond established biomarkers that could further aid in identifying those patients that will show increased cognitive decline and in specifying which specific cognitive domains will be affected over time.

A potential limitation of the method we used to construct networks is that these result in subject specific network sizes and degrees. Van Wijk et al. (2010) have shown that those measures influence other network properties and, therefore, differences in network size and degree might impact the results. How to deal with networks of unequal size and/or degree is still an open question. Methods that enforce identical degree and size might introduce bias, especially in the case of patient populations that are known to show atrophy and/or changes in brain networks. By keeping intact patient-level information on the size and degree, we showed for which cognitive domains these basic network properties play a role. When necessary we controlled further analyses for these properties to assess whether more complex measures still explain additional variance in the data. A strong aspect of our study is that we were able to include a large sample of MCI patients who had extensive neuropsychological assessment over follow-up. This long period of time, however, also is a potential limitation, since MRI scans were acquired on seven different systems. Although we accounted for scanner system in the analyses by including this variable as a covariate, the possibility that this has introduced noise in the analyses cannot be excluded. Another potential limitation is that not all subjects had complete neuropsychological test data available. We have used a multiple imputation procedure to estimate missing values based on multivariate patterns of existing data, which at least enabled us to

avoid selection bias that might have been introduced if only complete cases were analyzed. Furthermore, cognitive tests that are used in clinical practice have not been designed to capture changes over time, and so might not adequately capture changes in cognition. We observed relatively little decline for both the visuospatial and the language domain, and cognitive tests used for the respective composite scores equally showed little variance, presumably due to ceiling effects. Future research should further investigate grey matter network measures in relation to cognitive tests that are more sensitive to detect decline over time.

5. Conclusion

MCI patients are at increased risk to develop dementia. However, they show considerable variability in symptom presentation and rate of decline. For clinical trial development, prognostic measures are needed that can be used to include those subjects who will show fast decline, in order to increase the chances to observe potential treatment effects. Here, we demonstrated that those MCI patients with more severe disruptions in baseline grey matter connectivity showed a steeper decline in MMSE and in memory, attention and executive functioning over time. Together our findings suggest that grey matter network measures might contain prognostic information about future cognitive decline in specific cognitive domains.

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Tables and Figures

Table 1. Demographics and grey matter network characteristics of the included sample.

	Total	Stable	Progression
N	258	100	115
Female	105 (41%)	40 (40%)	52 (45%)
Age, years	66.7 (7.96)	67.03 (6.85)	68.25 (8.05)
Education	5 (4-6)	5 (4-6)	5 (4-6)
Co-medication	38 (15%)	12 (12%)	18 (16%)
Follow up time, years	2.3 (1.4-3.1)	2.2 (1.2-3.1)	2.3 (1.7-3.3)
CSF Aβ42, pg/ml	589 (454-899)	639 (486.8-978.2)	511 (404-613) [c]
CSF total tau, pg/ml	405 (263.2-625)	345.5 (247.2-513.8)	550 (370-803) [c]
CSF p-tau, pg/ml	64 (45-84)	53 (41.2-74.8)	76 (59-107) [c]
Progression to AD-type dementia	98 (38%)	n.a.	98 (85%)
Progression to non-AD	17 (7%)	n.a.	17 (15%)
Nomalized grey matter volume, cm^3	410.48 (48.27)	411.26 (41.43)	396.75 (50.27) [b]
Hippocampal volume, cm^3	7.71 (1.28)	7.93 (1.19)	7.16 (1.2) [c]
Network size	7000.97 (669.22)	7033.14 (654.87)	6892.62 (672.31)
Network degree	1140.37 (135.18)	1130.65 (128.79)	1125.29 (137.94)
Connectivity density	16.31 (1.33)	16.09 (1.24)	16.34 (1.38)
Clustering coefficient	0.46 (0.02)	0.46 (0.02)	0.46 (0.03)
Path length	2.01 (0.02)	2.02 (0.02)	2.01 (0.02)
Betweenness centrality	7099.74 (700.32)	7140.93 (669)	6961.89 (697.79) [a]
Gamma	1.66 (0.1)	1.66 (0.09)	1.63 (0.1) [b]
Lambda	1.1 (0.01)	1.1 (0.01)	1.09 (0.01)
Small-world coefficient	1.51 (0.08)	1.51 (0.07)	1.49 (0.08) [b]

n.a. is not applicable. Data are presented as N (%), mean (SD) or median (IQR). Education was assessed with the Verhage classification system (Verhage, 1964).

[a] $p = 0.053$; [b] $p < 0.05$; [c] $p < 0.001$.

Table 2. Neuropsychological baseline data and annual change.

	Baseline score	Annual change	*p* Value (Annual change)	N missing (%)
MMSE	-0.24 (1.05)	-0.23 (0.03)	<0.001	
MMSE, raw score	26.68 (2.45)	-0.57 (0.07)	<0.001	105 (11.4)
Memory	-0.3 (0.64)	-0.16 (0.02)	<0.001	
RAVLT, immediate recall	30.95 (7.77)	-1.18 (0.2)	<0.001	78 (8.5)
RAVLT, correct	25.97 (2.96)	-0.56 (0.07)	<0.001	87 (9.4)
VAT a1 and a2	9.83 (2.74)	-0.67 (0.07)	<0.001	54 (5.9)
Attention	-0.14 (0.84)	-0.13 (0.02)	<0.001	
Digit span forward	12.28 (2.9)	-0.13 (0.05)	0.012	9 (1.0)
TMT A	-47.73 (19.98)	-4.15 (0.86)	<0.001	20 (2.2)
Stroop 1	-48.5 (11.48)	-2.03 (0.3)	<0.001	222 (24.1)
Stroop 2	-67.29 (16.33)	-2.95 (0.5)	<0.001	224 (24.3)
Executive function	-0.15 (0.73)	-0.11 (0.02)	<0.001	
Digit span backward	8.56 (2.87)	-0.07 (0.06)	0.265	9 (1.0)
Letter fluency (DAT)	33.56 (11.53)	-0.13 (0.21)	0.532	217 (23.5)
FAB	15.93 (1.86)	-0.27 (0.07)	<0.001	252 (27.3)
Stroop 3	-127.72 (42.43)	-11.85 (2.01)	<0.001	239 (25.9)
TMT B	-131.97 (71.88)	-13.02 (1.58)	<0.001	90 (9.8)
Language	-0.15 (0.88)	-0.15 (0.03)	<0.001	
VAT naming	11.88 (0.46)	-0.07 (0.02)	0.002	56 (6.1)
Category fluency (animals)	18.87 (5.21)	-0.96 (0.11)	<0.001	45 (4.9)
Visuospatial	-0.1 (0.67)	-0.07 (0.02)	<0.001	
Fragmented letters	18.67 (1.36)	-0.21 (0.07)	0.003	336 (36.4)
Dot counting	9.65 (0.64)	-0.06 (0.03)	0.041	340 (36.9)

Data are presented as mean (SD) for baseline test scores and annual change as β (SD) as estimated by linear mixed models. Domain scores are given in z-scores and are based on the averaged imputed z-scores of the respective subtests. Scores for subtests represent the unimputed, raw scores. Note that scores for TMT and Stroop were inverted, so that higher scores indicate better performance.

Key: FAB, Frontal Assessment Battery; MMSE, Mini-Mental State Examination; RAVLT, Rey Auditory Verbal Learning Test; TMT, Trail Making Test; VAT, Visual Association Test.

Table 3. Baseline and annual change effects of grey matter connectivity measures on cognition.

	MMSE		MEMORY		ATTENTION		EXECUTIVE FUNCTION		LANGUAGE		VISUOSPATIAL	
	Baseline effect	Annual change	Baseline effect	Annual change	Baseline effect	Annual change	Baseline effect	Annual change	Baseline effect	Annual change	Baseline effect	Annual change
Size	0.1 (0.07)	0.07 (0.03) [a]	0.03 (0.05)	0.05 (0.02) [b]	0.08 (0.06)	0.02 (0.02)	0.06 (0.05)	0.02 (0.02)	0.07 (0.06)	0.04 (0.03)	0.08 (0.05)	0.01 (0.02)
Degree	0.2 (0.09) [a]	0.03 (0.03)	0.08 (0.07)	0.05 (0.02) [b]	0.02 (0.07)	0.02 (0.02)	0.02 (0.06)	0.02 (0.02)	0.03 (0.08)	0.03 (0.03)	-0.05 (0.06)	0.03 (0.02)
ConDen	0.13 (0.07)	-0.05 (0.03)	0.09 (0.05)	0.01 (0.02)	-0.09 (0.06)	0.01 (0.02)	-0.05 (0.05)	0 (0.02)	-0.01 (0.06)	-0.01 (0.03)	-0.04 (0.05)	0.03 (0.02)
C	-0.21 (0.18)	-0.03 (0.03)	0.06 (0.05)	0.02 (0.02)	-0.04 (0.06)	0.03 (0.02)	-0.01 (0.05)	0.01 (0.02)	0.01 (0.06)	0.01 (0.03)	-0.02 (0.05)	0.03 (0.02)
L	0.06 (0.07)	0.04 (0.03)	-0.11 (0.05) [a]	0.02 (0.02)	0.1 (0.05)	0.02 (0.02)	0.1 (0.04) [a]	0.02 (0.02)	0.04 (0.06)	0.04 (0.03)	0.04 (0.04)	-0.01 (0.02)
BC	0.33 (0.15) [a]	0.08 (0.03) [b]	-0.17 (0.11)	0.06 (0.02) [b]	0.45 (0.13) [c]	0.02 (0.02)	0.38 (0.1) [c]	0.03 (0.02)	0.29 (0.13) [a]	0.05 (0.03)	-0.02 (0.1)	0.01 (0.02)
Gamma	0.04 (0.07)	0.04 (0.03)	-0.01 (0.05)	0.04 (0.02) [a]	0.02 (0.06)	0.04 (0.02) [a]	0.07 (0.04)	0.04 (0.02) [a]	0.05 (0.06)	0.05 (0.03)	0.03 (0.05)	0.01 (0.02)
Lambda	0.05 (0.07)	0.01 (0.03)	-0.09 (0.05)	0.03 (0.02)	0.08 (0.06)	0.03 (0.02)	0.1 (0.05) [a]	0.02 (0.02)	0.05 (0.06)	0.03 (0.03)	0.03 (0.05)	0.01 (0.02)
SW	0.04 (0.07)	0.05 (0.03)	0.01 (0.05)	0.05 (0.02) [b]	0 (0.05)	0.04 (0.02) [a]	0.06 (0.04)	0.04 (0.02) [a]	0.05 (0.06)	0.05 (0.03)	0.03 (0.05)	0.01 (0.02)

Data are presented as β (SE) as estimated by linear mixed models. The models included the covariates age, sex, education, MRI scanner type, TIV, network measure, follow-up time in years and the interaction term network measure × time. Size, Degree, ConDen were additionally corrected for when they showed a significant effect in the respective model.

[a] $p < 0.05$; [b] $p < 0.01$; [c] $p<0.0009$ (i.e., Bonferroni corrected)

Key: BC, Betweenness centrality; ConDen, Connectivity density; C, Clustering coefficient; L, Path length; MMSE, Mini-mental state examination; SW, Small-world coefficient.

Table 4. Odds ratio to predict which individual subjects will show faster than median decline for specific cognitive domains.

	Model 1	Model 2	Model 3	Model 4
MMSE				
Size	0.32 (0.12-0.84) [b]	0.39 (0.14-1.08) [a]	0.38 (0.14-1.05) [a]	0.41 (0.15-1.14) [a]
Degree	0.56 (0.36-0.87) [b]	0.6 (0.38-0.97) [b]	0.61 (0.38-0.97) [b]	0.72 (0.45-1.14)
BC	0.59 (0.28-1.23)	0.73 (0.33-1.62)	0.7 (0.32-1.57)	0.7 (0.32-1.57)
Gamma	0.64 (0.46-0.9) [b]	0.68 (0.47-0.98) [b]	0.67 (0.47-0.98) [b]	0.78 (0.54-1.13)
Small world	0.63 (0.45-0.87) [c]	0.65 (0.46-0.94) [b]	0.65 (0.45-0.93) [b]	0.76 (0.53-1.09)
Memory				
Size	0.34 (0.13-0.89) [b]	0.5 (0.18-1.38)	0.47 (0.17-1.31)	0.51 (0.18-1.41)
Degree	0.67 (0.44-1.03) [a]	0.8 (0.51-1.24)	0.81 (0.52-1.25)	0.95 (0.61-1.48)
BC	0.44 (0.21-0.94) [b]	0.61 (0.27-1.37)	0.57 (0.25-1.29)	0.57 (0.25-1.3)
Gamma	0.63 (0.46-0.88) [c]	0.72 (0.5-1.04) [a]	0.71 (0.5-1.03) [a]	0.83 (0.58-1.19)
Small world	0.64 (0.46-0.88) [c]	0.73 (0.51-1.03) [a]	0.71 (0.5-1.01) [a]	0.83 (0.59-1.18)
Attention				
Size	0.43 (0.16-1.11) [a]	0.46 (0.17-1.29)	0.46 (0.16-1.27)	0.47 (0.17-1.3)
Degree	0.77 (0.49-1.21)	0.81 (0.5-1.3)	0.81 (0.5-1.31)	0.85 (0.53-1.38)
BC	0.34 (0.16-0.73) [c]	0.34 (0.15-0.77) [b]	0.33 (0.14-0.75) [c]	0.33 (0.14-0.75) [c]
Gamma	0.57 (0.4-0.81) [c]	0.54 (0.36-0.81) [c]	0.54 (0.36-0.81) [c]	0.56 (0.37-0.84) [c]
Small world	0.6 (0.43-0.85) [c]	0.59 (0.4-0.86) [c]	0.58 (0.4-0.86) [c]	0.6 (0.41-0.89) [b]
Executive functioning				
Size	0.26 (0.1-0.71) [c]	0.3 (0.1-0.89) [b]	0.3 (0.1-0.88) [b]	0.31 (0.11-0.93) [b]
Degree	0.79 (0.51-1.22)	0.88 (0.55-1.41)	0.89 (0.56-1.41)	1 (0.62-1.61)
BC	0.25 (0.11-0.55) [c]	0.27 (0.11-0.64) [c]	0.26 (0.11-0.62) [c]	0.26 (0.11-0.62) [c]
Gamma	0.56 (0.39-0.82) [c]	0.58 (0.38-0.88) [b]	0.57 (0.38-0.88) [b]	0.64 (0.42-0.96) [b]
Small world	0.59 (0.41-0.85) [c]	0.6 (0.4-0.91) [b]	0.6 (0.39-0.91) [b]	0.67 (0.45-1) [a]

Data is represented as OR (95% CI). Model 1 contains the respective network measure as predictor. Model 2 additionally includes hippocampal volume, Model 3 CSF Aβ42 levels and Model 4 CSF total tau levels as covariates. All models were corrected for age, gender age, total brain volume and scanner.

[a] $p < 0.1$; [b] $p < 0.05$, [c] $p < 0.01$.

Key: BC, betweenness centrality.

Figure 1. Regional associations of network measures with cognitive decline over time.

The colorbar indicates the level of significance after FDR-correction per AAL area. Analyses were adjusted for age, sex, education, local grey matter volume and the local degree. (A) For the memory domain, lower values of the degree in widespread areas, involving particularly the temporal lobes and prefrontal areas in the left hemisphere, were significantly associated with steeper decline over time. (B) Lower values of the betweenness centrality were associated with increased decline in memory functioning over time in the right precuneus, left superior medial orbito-frontal gyrus, right supramarginal gyrus, bilateral precentral gyrus, bilateral inferior temporal gyrus, left superior medial frontal gyrus, right superior parietal gyrus, right middle temporal gyrus, right parahippocampal gyrus, left superior temporal pole, right middle occipital gyrus and left thalamus. (C) Lower betweenness centrality values were associated with increased decline over time in MMSE, specifically for the right precuneus, left superior medial-orbito-frontal gyrus, bilateral precentral gyri, bilateral superior medial frontal gyri, bilateral thalami, left anterior cingulate, left parahippocampal gyrus, left superior temporal pole, right lingual gyrus, right fusiform gyrus and right hippocampus. Subcortical structures are plotted in ventricular areas as approximation. Estimated cross-sectional and annual change effects for all AAL areas are listed in Supplementary Table 2 and 3. Abbreviations: L, left hemisphere; R, right hemisphere.

Figure1

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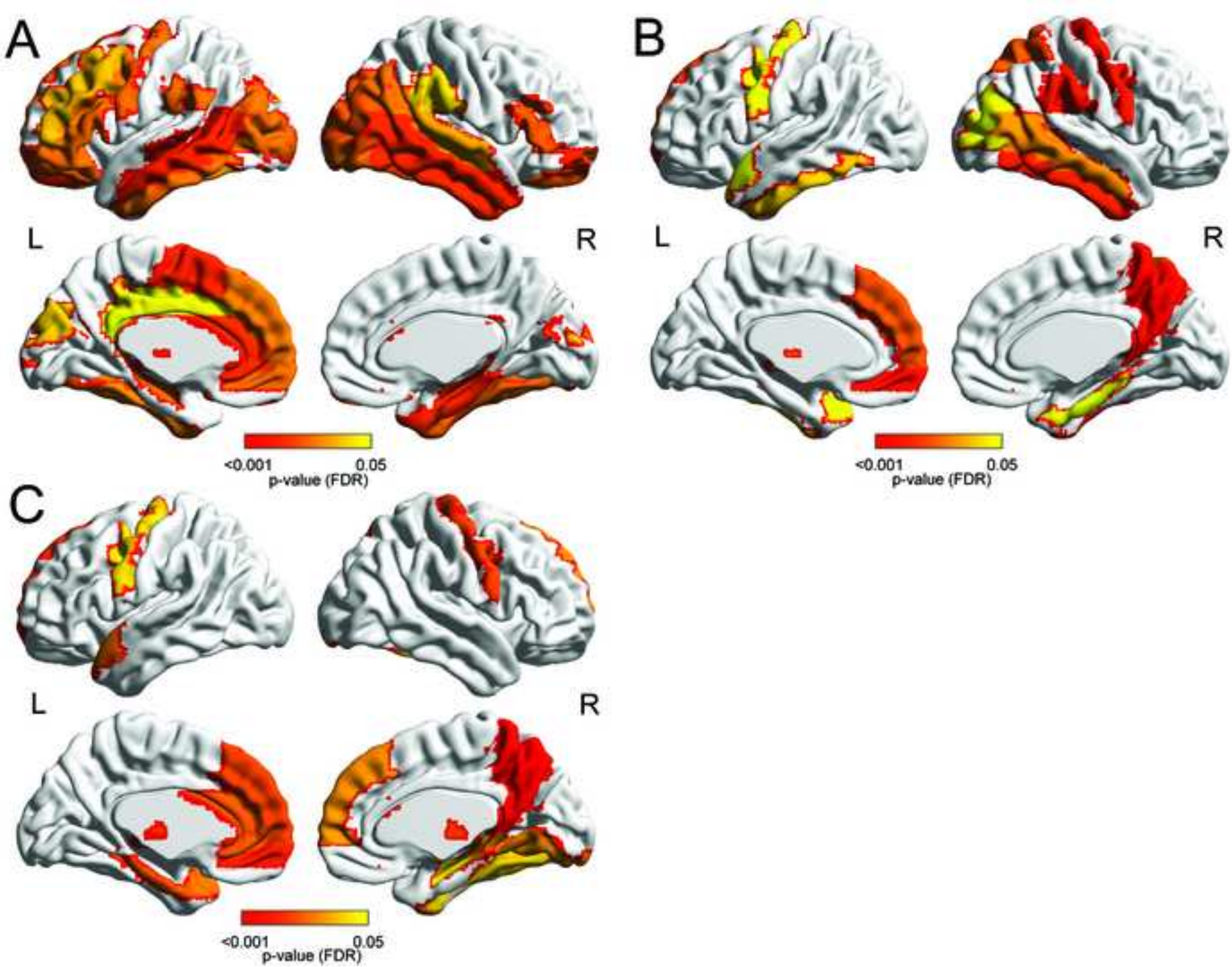

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SupplementaryFigure1

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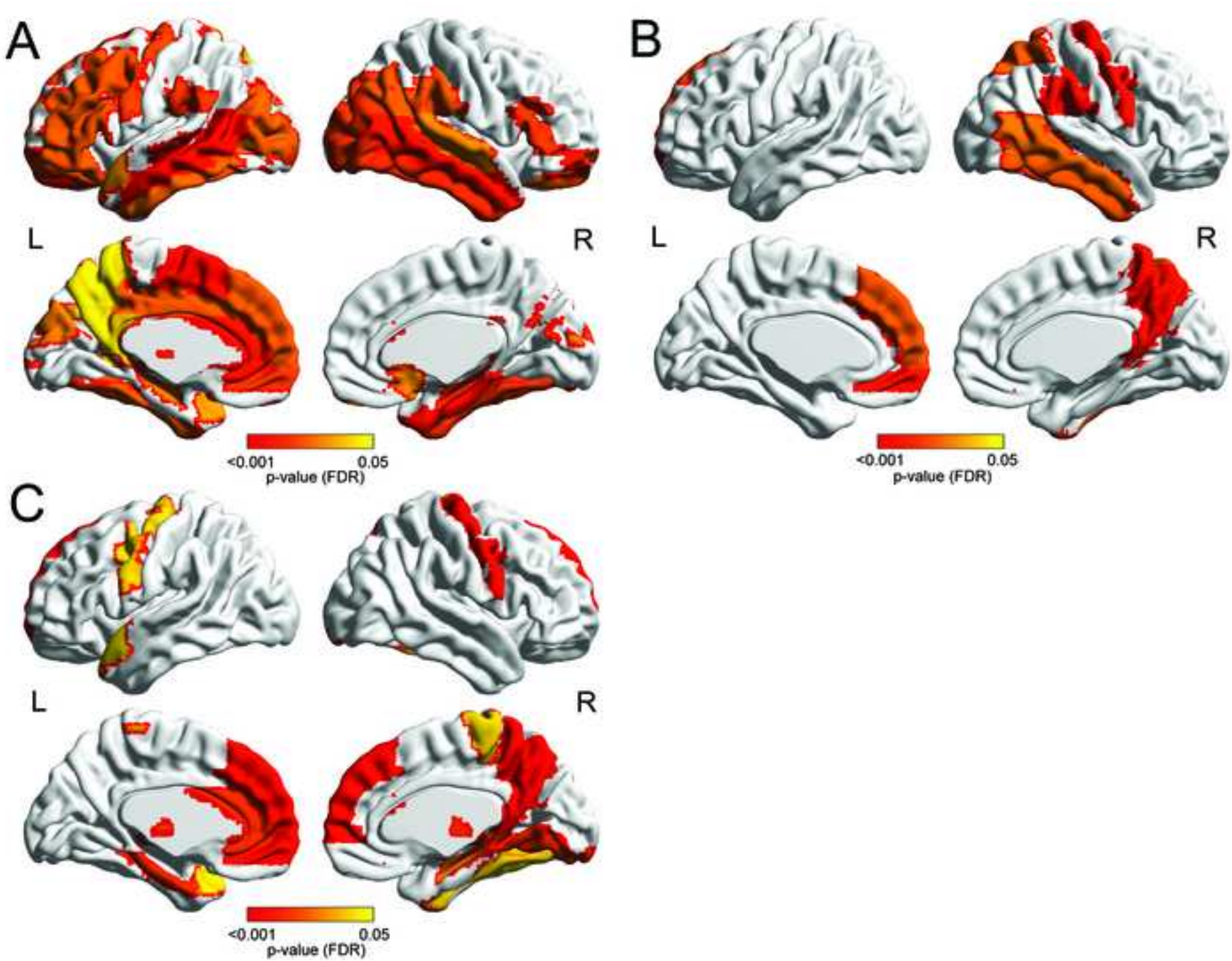

SupplementaryFigure2

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