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Loss of functional connectivity is greater outside the default mode network in non-familial early-onset Alzheimer's disease variants

Manja Lehmann[a,b,c,*], **Cindee Madison**[b], **Pia M. Ghosh**[a,b], **Zachary A. Miller**[a], **Michael D. Greicius**[d], **Joel H. Kramer**[a], **Giovanni Coppola**[e], **Bruce L. Miller**[a], **William J. Jagust**[a,b,f], **Maria L. Gorno-Tempini**[a], **William W. Seeley**[a], and **Gil D. Rabinovici**[a,b,f]

[a]Memory & Aging Center, Department of Neurology, University of California San Francisco, CA 94158, US

[b]Helen Wills Neuroscience Institute, University of California Berkeley, CA 94720, US

[c]Dementia Research Centre, University College London, Institute of Neurology, Queen Square, London, WC1N 3BG, UK

[d]Functional Imaging in Neuropsychiatric Disorders (FIND) Laboratory, Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305, US

[e]Semel Institute for Neuroscience and Human Behavior, Departments of Psychiatry and Neurology, David Geffen School of Medicine, UC Los Angeles, US

[f]Lawrence Berkeley National Laboratory, CA 94720, US

Abstract

The common and specific involvement of brain networks in clinical variants of Alzheimer's disease (AD) is not well understood. We performed task-free ("resting-state") functional imaging in 60 non-familial AD patients, including 20 early-onset AD (EOAD, age at onset <65 years, amnestic/dysexecutive deficits), 24 logopenic aphasia (lvPPA, language deficits) and 16 posterior cortical atrophy patients (PCA, visual deficits), as well as 60 healthy controls. Seed-based connectivity analyses were conducted to assess differences between groups in 3 default mode network (DMN) components (anterior, posterior and ventral) and four additional non-DMN networks: left and right executive-control, language and higher visual networks. Significant decreases in connectivity were found across AD variants compared with controls in the non-DMN networks. Within the DMN components, patients showed higher connectivity in the anterior DMN, in particular in lvPPA. No significant differences were found for the posterior and ventral DMN. Our findings suggest that loss of functional connectivity is greatest in networks outside the DMN in early-onset and non-amnestic AD variants, and may thus be a better biomarker in these patients.

Corresponding author Manja Lehmann, Dementia Research Centre, University College London, Queen Square, London WC1N 3BG, United Kingdom, m.lehmann@ucl.ac.uk, Tel: +44 2034483676.

Keywords

Networks; intrinsic connectivity; functional magnetic resonance imaging; Alzheimer's disease; posterior cortical atrophy; logopenic-variant primary progressive aphasia

1. Introduction

Although Alzheimer's disease (AD) is typically associated with the presence of memory deficits, non-amnestic syndromes have been described in up to 15% of patients with AD (Snowden, et al., 2007). Non-memory presentations are particularly common in patients with early-onset AD (EOAD, onset <65 years) who typically show a more heterogeneous cognitive profile, including greater attention and executive deficits than late-onset patients (Frisoni, et al., 2007, Koedam, et al., 2010). Focal non-amnestic AD syndromes have also been described, including patients with the logopenic variant of primary progressive aphasia (lvPPA) (Gorno-Tempini, et al., 2008, Mesulam, et al., 2008), a condition characterized by predominant language deficits, and posterior cortical atrophy (PCA), associated with predominant visuospatial and visuoperceptual dysfunction (Crutch, et al., 2012). Although progress has been made in characterizing these syndromes, it is currently unclear what drives the clinical and anatomical heterogeneity in AD.

Findings from studies using task-free functional neuroimaging suggest that disease may spread via distinct functional networks (Seeley, et al., 2009, Zhou, et al., 2012). The differential involvement of functional networks may therefore represent a possible mechanism for the clinico-anatomical heterogeneity in AD. One network suggested to be particularly vulnerable and affected in AD is the default mode network (DMN) (Buckner, et al., 2005, Greicius, et al., 2004). Changes in DMN connectivity have been reported even in preclinical stages (Mormino, et al., 2011, Petrella, et al., 2011), making it a potential biomarker for the early detection of AD. The DMN has been shown to be preferentially activated during internal tasks such as daydreaming, envisioning the future, and retrieving episodic memories, while it is deactivated during externally focused and engaging cognitive tasks (Buckner, et al., 2008). The DMN has further been divided into 2–3 functional sub-networks suggested to serve different functions in the brain: a ventral component (including retrosplenial cortex and medial temporal lobe) and a dorsal component that can be further divided into anterior (prefrontal-predominant) and posterior (parietal-predominant) modules (Damoiseaux, et al., 2012). These have further been shown to be differentially affected during the progression of AD, with the posterior DMN showing reduced connectivity in the early symptomatic stages, whilst the anterior and ventral DMN components show increased connectivity which diminishes with disease progression (Damoiseaux, et al., 2012).

There are currently only limited data on how the DMN and networks outside the DMN are affected in early-onset and non-amnestic variants of AD. A recent study found similar reductions in DMN connectivity in EOAD and late-onset AD patients compared with controls, whereas a double dissociation was found for networks outside the DMN, with EOAD showing reduced connectivity in a dorsolateral prefrontal network and increased connectivity in an anterior temporal network, and LOAD showing the reverse pattern (Gour,

et al., 2014). Another study showed reduced connectivity in left language and working memory networks in lvPPA (Whitwell, et al., 2014). In a previous study, we found that functional connectivity maps in normal controls derived by seeding brain regions *specifically* involved in each AD variant closely resembled cognitive networks linked to the patients' predominant clinical deficits (Lehmann, et al., 2013b). For example, seeding the peak atrophy region found in PCA compared with lvPPA and EOAD (right middle occipital gyrus) yielded a connectivity map that closely resembled the higher visual network. Analogously, the connectivity map derived from seeding the peak atrophy region specific to lvPPA closely fit the language network, and the connectivity map based on peak atrophy specific to EOAD closely matched the right executive-control network (converging with the prominent executive deficits seen in this variant). In contrast, seeding regions that were *commonly* atrophied across AD variants yielded close matches with the posterior DMN. Similar results were obtained in another study examining covariance patterns in glucose hypometabolism in a heterogeneous cohort of AD patients (Lehmann, et al., 2013a). These results have led us to hypothesize that the posterior DMN is a core network involved across AD clinical variants, whereas the relative involvement of cognitive networks outside the DMN drives the phenotype in specific AD variants.

The aim of the current study was to assess functional connectivity networks in different variants of AD, including early-onset and non-amnestic syndromes, to assess how network dysfunction relates to clinical heterogeneity in a diverse patient sample. A secondary objective was to evaluate the potential utility of functional connectivity (within and outside the DMN) as a biomarker across a range of AD phenotypes. We predicted a marked reduction in connectivity in networks outside the DMN, namely the executive-control, language and higher visual networks, reflecting the distinct clinico-anatomical phenotypes of the different AD syndromes.

2. Methods

2.1. Subjects

Subjects were recruited from research cohorts at the University of California San Francisco (UCSF) Memory and Aging Center. All subjects or their assigned surrogate decision-makers provided informed consent and the study was approved by the UCSF institutional review board for human research. All patients underwent a history and physical examination by a neurologist, a structured caregiver interview by a nurse, and a battery of neuropsychological tests (Kramer, et al., 2003). Clinical diagnosis was assigned by consensus at a multidisciplinary conference.

The cohort consisted of 60 controls and 60 patients with non-autosomal dominant AD, which included 20 EOAD, 24 lvPPA, and 16 PCA patients. Demographics and clinical data are summarized in Table 1. All subjects had at least one usable structural and functional MRI scans. All patients fulfilled criteria for probable AD according to the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria (McKhann, et al., 2011). PET scans with the amyloid β-specific tracer Pittsburgh compound B (PIB) were available in 68% (10 EOAD, 18 lvPPA and 13 PCA), and with ^{18}F-labeled florbetapir in 7% of all patients (2 EOAD and 2 PCA), with all of them rated amyloid-positive on visual interpretation. Patients

were excluded if they presented with core clinical features of other dementias (e.g. dementia with Lewy bodies, vascular dementia) to reduce the likelihood of underlying co-pathologies. PCA and lvPPA patients were initially selected based on their clinical diagnosis. Clinical and neuropsychological reports were then reviewed to assess whether patients fulfilled specific diagnostic criteria (lvPPA: Gorno-Tempini, et al., 2011, PCA: Mendez, et al., 2002, Tang-Wai, et al., 2004). EOAD patients had an age-of-onset <65 years and did not meet criteria for PCA and lvPPA. Since most of the lvPPA and PCA patients have an early age at onset, including the EOAD group means that groups are relatively well matched for age at onset. Please note that we refer to groups as early-onset and non-amnestic AD throughout the paper reflecting the early age at onset in all EOAD and most lvPPA and PCA patients, and the non-amnestic profile of the lvPPA and PCA patients. A group of late-onset patients was not included due to insufficient functional MRI data. Control subjects were deemed eligible if they had a Clinical Dementia Rating (CDR) Scale total score of 0, a Mini Mental State Examination (MMSE) score of 28 or higher, no significant history of neurological disease, and no evidence of conversion to a dementia syndrome at follow-up where available. Subjects further had to have an age at time of scan below 70 years in order to match them to the relatively young AD patients. Amyloid-PET imaging was not available in the control subjects.

2.2. Image acquisition and processing

Functional images were acquired on a 3T Siemens MRI scanner at the Neuroscience Imaging Center, UCSF. A detailed description of acquisition parameters, processing steps and motion assessment can be found in the supplementary material. In brief, functional MRI scans were acquired using a T2*-weighted echo planar sequence and co-registered to a volumetric T1-weighted image. Functional images were realigned and unwarped, slice-time corrected, co-registered to the skull-stripped structural T1-weighted image, normalized, and smoothed with a 4mm full-width at half-maximum Gaussian kernel. The first 5 frames were discarded to allow for magnetic field stabilization, and the waveform of each brain voxel was filtered using a bandpass filter ($0.0083/s < f < 0.15/s$). Functional images were reviewed for excessive motion and subjects with greater than 3 mm of translational movement or 3° of rotational movement were excluded. A general linear model (GLM) was used to regress out the time series of 3 nuisance ROIs (global, white matter, CSF), and 6 motion parameters.

2.3. Seed-based analysis

2.3.1. ROI definitions—Seed ROIs were based on peak intensity voxels of network templates generated and published by the Stanford Functional Imaging in Neuropsychiatric Disorders Lab (http://findlab.stanford.edu/functional_ROIs.html). Based on our hypotheses, seeds were selected for the ventral DMN, left and right executive-control, language, higher visual (Shirer, et al., 2012) as well as anterior and posterior DMN components (Damoiseaux, et al., 2012). Peak intensity voxels were found in the left middle orbital gyrus for the anterior DMN (MNI −2 50 −4), right precuneus for the posterior DMN (MNI 2 −68 36), left precuneus for the ventral DMN (MNI −4 −58 56), left angular gyrus for the left executive-control network (MNI −38 −68 48), right supramarginal gyrus for the right executive-control network (MNI 52 −46 48), left middle temporal gyrus for the language network (MNI −54 −56 22), and right middle occipital gyrus for the higher visual network (MNI 36

−88 0). We also assessed connectivity in the sensorimotor network (left precentral gyrus seed, MNI −24 −20 72) which we predicted to show no significant differences between groups, to determine the specificity of our findings in the non-DMN in AD variants. Eight mm spheres were drawn around the peak intensity voxels to generate the seed ROIs. Seed regions are shown in Supplementary figure 1.

2.3.2. Seed-based correlations—The average time series for each seed ROI was used as a covariate of interest in a whole-brain regression analysis. The voxel-wise z-scores in the resulting subject-level intrinsic connectivity maps describe the correlation between each voxel's spontaneous blood-oxygen-level-dependent (BOLD) signal time series and the average time series of all voxels within the seed ROI. Connectivity maps were derived from each seed ROI in each individual subject. Supplementary figure 2 shows seed-based correlation maps for each group.

2.3.3. Z-score extractions—Z-scores for individual connectivity maps were extracted for each subject, using the binarized Stanford network templates as masks. Z-scores were then entered into STATA (version 11.2, STATA Corporation, College Station, TX, USA) to assess differences between groups using a linear regression model correcting for age, gender and education.

2.3.4. Voxel-wise comparisons—Unthresholded z-score maps were used to conduct voxel-wise comparisons between groups which were performed using the FSL randomise program (5000 random permutations), correcting for age, gender and education, using the Stanford templates as masks. Comparisons between controls and patients were visualized as p maps at $p<0.05$ uncorrected for multiple comparisons. For the comparison between patient groups, differences were relatively small at an uncorrected threshold of $p<0.05$. Results are therefore shown as raw t values (t-max=2) in the supplementary material to give an impression of the differences found between patient groups. None of the comparisons survived the most conservative statistical correction for multiple comparisons (family-wise error (FWE) at $p<0.05$).

2.3.5. Atrophy correction—In order to assess differences in network connectivity between groups independent of regional atrophy patterns, z-score comparisons were corrected for atrophy by including grey matter volume within the network mask region as a covariate in the regression model in STATA. Connectivity data for the voxel-wise comparisons were also corrected for atrophy by including grey matter images as voxel-dependent covariate in the regression model. Grey matter maps obtained by Statistical Parametric Mapping (SPM, see supplementary material) were modulated by their Jacobian determinants, and then resliced to the voxel resolution of the functional images.

3. Results

3.1. Subjects

AD groups were well matched for age, gender, education, age at onset, disease duration (as estimated by date of first reported symptom) and MMSE score (Table 1). Whilst there was no significant difference in ApoE4 status between groups, EOAD patients had a higher

proportion of ApoE4-carriers than controls, whereas the proportion of ApoE4-carriers in the lvPPA and PCA groups was similar to that in the controls. Patients had a significantly lower MMSE ($p<0.0001$) and education level ($p=0.02$) than controls. Detailed cognitive assessments within one year of the MRI scan were available in 19 EOAD, 22 lvPPA and 13 PCA patients. As expected, EOAD patients showed poor performance on visual memory (modified Rey figure delayed recall), whilst PCA patients performed significantly worse on visual and visuospatial tasks (modified Rey figure copy and delayed recall, VOSP number location, CATS face matching). The lvPPA group showed significantly worse performance on naming and letter fluency tasks as well as on digit span forward and sentence repetition tasks, both tests that involve phonological loop processing (Supplementary table 1). Patients also showed syndrome-typical patterns of atrophy with PCA showing atrophy predominantly in posterior regions, lvPPA patients showing asymmetric (L>R) atrophy in lateral and medial temporal as well as lateral parietal areas, and EOAD showing atrophy bilaterally in lateral temporal, hippocampus and posterior cingulate cortex (Supplementary figure 3, see supplementary methods for further details on the methods).

3.2. Comparison of connectivity scores

AD patients, when pooled together, showed significantly lower connectivity scores in the bilateral executive-control ($p<0.0001$) and language network ($p=0.001$) compared with controls, with a trend towards lower connectivity also found in the higher visual network ($p=0.06$) (Figure 1). Individual patient groups showed significantly lower connectivity compared with controls in the bilateral executive-control and language networks. For the higher visual network, PCA patients showed lower connectivity compared with controls ($p=0.02$). Connectivity in the anterior DMN was significantly higher across AD patients compared with controls ($p=0.006$) which was mainly driven by the lvPPA patients ($p=0.02$) and to some extent also by the PCA patients ($p=0.05$). Higher connectivity in the anterior DMN across all AD patients remained borderline significant ($p=0.05$) after removing the 3 outliers above the upper fence of the whisker in the box plot in Figure 1. No significant differences were found for the posterior, ventral DMN and sensorimotor network. No differences were detected between patient groups for any of the networks. After including grey matter volume to correct for atrophy, overall differences between patients and controls were less significant, with differences in the higher visual network no longer significant (Supplementary table 2).

3.3. Voxel-wise group comparisons

The results from the voxel-wise analysis largely support the findings from the connectivity score comparison, and provide some additional information about region-specific differences between groups. As with the connectivity scores, differences between patients and controls in the DMN components were relatively small (Figure 2), whereas differences in the non-DMN components were more pronounced (Figure 3). Connectivity in the prefrontal cortex of the anterior DMN was higher in lvPPA and PCA compared with controls. Only subtle differences were found between patient groups for the DMN components (Supplementary figure 4), with decreased connectivity in lateral and medial parietal regions in the posterior DMN in PCA compared with EOAD and lvPPA, and EOAD

compared with lvPPA. Increased connectivity in the prefrontal cortex regions included in the anterior DMN was also found in PCA and lvPPA compared with EOAD.

Connectivity in the left and right executive-control networks was reduced in middle and superior frontal and angular gyri and superior parietal lobules in each patient group compared with controls (Figure 3), with connectivity in anterior regions being particularly reduced in the EOAD and lvPPA patients (Supplementary figure 4). Whilst only subtle differences between groups were found in the language network, the higher visual network showed reduced connectivity in PCA compared with EOAD and lvPPA, and EOAD compared with lvPPA.

Using atrophy-corrected data, differences between controls and patient groups almost disappeared for the DMN components and were altered for the non-DMN networks with the spatial extent of the differences reduced, in particular in anterior regions (Supplementary figure 5). In contrast, similar patterns were found for the between-patient group comparisons using atrophy-corrected data (Supplementary figure 6).

4. Discussion

In this study, we assessed network connectivity in different variants of AD, with a particular focus on the involvement of different components of the DMN vs. specific cognitive networks outside the DMN. Whilst the DMN has been shown to be critically involved in AD, making it a potential biomarker for the early detection of AD, a growing number of studies suggest that networks outside the DMN are particularly affected in early-onset and non-amnestic AD variants. In this study, we found reduced connectivity in early-onset and non-amnestic AD patients in executive-control, language and higher visual networks compared with controls. In contrast, within DMN sub-networks, only the anterior DMN showed altered connectivity, with AD patients (in particular patients with lvPPA) showing higher connectivity compared with controls. Together, our data suggest that functional networks outside the DMN are more affected than the DMN in early-onset and non-amnestic AD variants, and may therefore represent a better biomarker in these patients.

Studies that assessed connectivity in mild late-onset AD patients emphasize the importance of networks outside the DMN including antero-medial temporal and executive-control networks (Agosta, et al., 2012, Brier, et al., 2012). Functional connectivity studies in EOAD have shown similar involvement of the DMN in early and late-onset AD patients, whereas EOAD patients showed reduced connectivity in a dorsolateral prefrontal network and enhanced connectivity in an anterior temporal network (Gour, et al., 2014). A recent study investigating functional connectivity networks in lvPPA found lower connectivity in the left temporal language network and inferior parietal and prefrontal regions of the left working memory network compared with controls and AD patients (Whitwell, et al., 2014). Whilst there are no reports on functional connectivity in PCA, tractography studies suggest that the clinical features in PCA might not result from cortical atrophy alone, but by damage along visual white matter pathways (including inferior longitudinal fasciculus) in particular in the right hemisphere (Migliaccio, et al., 2012).

The marked reduction in connectivity in networks outside the DMN in the AD patients is consistent with the distinct clinical and anatomical characteristics, with prominent dysexecutive and amnestic deficits in EOAD, language deficits in lvPPA and visual deficits in PCA. The sensorimotor network is not clinically affected until the most advanced clinical stages of AD, and sparing of connectivity in our early-stage patients supports the notion that changes in connectivity are specific to clinically involved cognitive networks, at least at early disease stages. Whilst differences between network connectivity scores were not significant across AD variant groups, the voxel-wise comparisons do indicate some (albeit subtle) regional differences which are consistent with the clinical profiles of the patients, with PCA patients showing greater involvement of the higher visual network, and EOAD patients particularly low connectivity in anterior parts of the left and right executive-control networks. On the other hand, it is noteworthy that different clinical presentations in AD have been suggested to represent a spectrum rather than categorical subtypes (Lehmann, et al., 2011), which means that, whilst some patients present with very focal, syndrome-typical clinical deficits, there is also overlap between these syndromes, with language deficits found in PCA (Crutch, et al., 2013) and visuospatial and language deficits reported in EOAD (Koedam, et al., 2010), with increasing overlap seen with progression in each of the variants (see below). This overlap may have limited our ability to detect differences between syndromes in the non-DMN networks. It is also possible that connectivity in syndrome-specific networks attains an early floor effect, making differences between involved networks less apparent in our patients who are already well advanced in their disease course (average disease duration 5–6 years). Non-linear changes of functional connectivity over space and time may complicate matters further, as shown by the increased connectivity found in the anterior DMN, and may contribute to the poor correlation between network connectivity and clinical symptoms.

It is also important to note that a network can fail due to the breakdown of distinct hub regions (Buckner, et al., 2009), which may explain the differential involvement of regions within a network in AD variants in the voxel-wise analysis, whilst less apparent differences in the z-score analysis which assessed connectivity across whole networks. The finding of elevated connectivity in the anterior DMN is consistent with a previous study that showed a similar pattern in AD patients compared with controls (Damoiseaux, et al., 2012), possibly indicating an early compensatory mechanism for reduced connectivity in more posterior networks which is later ameliorated with disease progression. Similar differences in anterior vs. posterior networks have been reported in AD (Jones, et al., 2011, Machulda, et al., 2011, Zhou, et al., 2010). On the other hand, the lack of a difference in the posterior DMN is perhaps more surprising. Since amyloid-PET was not available in the control subjects, it is possible that some of the controls have preclinical AD, which may affect the DMN in particular, masking differences between patients and controls. If the DMN is more affected in preclinical AD than other networks, this may confound our results towards larger differences in networks outside the DMN. It is also noteworthy that controls had a relatively high prevalence of ApoE4-carriers which may have also contributed to the lack of a difference in the posterior DMN. However, whilst reduced connectivity in the posterior DMN in controls may have some effect on the results, the striking reduction in connectivity in networks outside DMN in the patient groups suggests that non-DMN networks are more

affected at this stage of the disease. Whilst differences between patients and controls were diminished after atrophy-correction in both the z-score and voxel-wise comparisons, differences between patient groups were very similar after atrophy correction, indicating that differences in functional connectivity between patient groups are not merely driven by atrophy.

Putting our data in the context of disease progression, the greater involvement of specific "off-target" networks outside the DMN may indicate that these are networks involved early in the disease course and that the posterior DMN is a region where the disease converges in the different syndromes. Support for the central role of posterior DMN regions (including precuneus and posterior cingulate cortex) comes from structural MRI studies that have shown that these are core regions commonly affected across different variants of AD (Migliaccio, et al., 2009). Reductions in DMN connectivity have also been shown in cognitively healthy individuals with high amyloid burden (Hedden, et al., 2009, Mormino, et al., 2011). However, there is currently only limited data on the direction of disease spread in AD, i.e. whether the disease starts in the DMN and spreads into specific networks outside the DMN or vice versa. A small number of studies that have assessed changes over time in different AD variants suggest that atrophy is initially concentrated in regions outside the DMN (which is particularly apparent in cases of prodromal PCA (Chan, et al., 2015, Kennedy, et al., 2012), and later spreads more widely across the brain, indicating that these different clinical variants of AD might converge anatomically over time (Chan, et al., 2015, Cho, et al., 2013, Kennedy, et al., 2012, Lehmann, et al., 2012, Leyton, et al., 2013, Rohrer, et al., 2013). However, these studies are often based on small sample sizes and include patients that were relatively advanced, therefore providing limited information about the early stages. Further studies using longitudinal data and milder cases are required to obtain a better understanding of the origin and spread of pathological changes in AD variants.

Whilst the mechanisms by which AD spreads through neural networks is not well understood, converging data from *in vitro* and *in vivo* studies in animals and humans support the hypothesis that AD pathology spreads through neural networks (Clavaguera, et al., 2009, de Calignon, et al., 2012, Jucker and Walker, 2011, Liu, et al., 2012, Sepulcre, et al., 2013, Zhou, et al., 2012). Whilst amyloid deposition on PET has been shown to correlate with reduced network connectivity in patients with prodromal AD (Myers, et al., 2014), other studies suggest that tau pathology plays a key role in the differential involvement of functional networks in clinical variants of AD. PET studies have shown that the distribution of fibrillar amyloid in AD is largely overlapping and is indistinguishable across clinical variants (de Souza, et al., 2011, Lehmann, et al., 2013a, Leyton, et al., 2011, Rabinovici, et al., 2010, Rosenbloom, et al., 2011), suggesting that other factors, such as oligomeric Aβ (not imaged by current PET ligands) or tau neurofibrillary tangles may drive network-based degeneration. The distribution of tangle pathology has also been shown to correlate much better with clinical phenotype than amyloid pathology (Gefen, et al., 2012, Mesulam, et al., 2008, Renner, et al., 2004, Tang-Wai, et al., 2004). Together, these data suggest that tau pathology drives clinicoanatomical heterogeneity in AD, but how the distribution and spread of tau relates to amyloid and network dysfunction is not understood. Development of PET tracers that bind specifically to tau will allow the assessment of these relationships in future

studies (Xia, et al., 2013), with first insights provided by a single case study that showed a good correspondence between tau burden, clinical symptoms and neurodegeneration in PCA (Ossenkoppele, et al., 2015).

Our study has limitations. Whilst pathological confirmation of AD is not available in our subjects, our inclusion criteria are aimed to maximize the likelihood of underlying AD pathology by only including clinical phenotypes that are strongly associated with AD pathology, and by excluding patients with core features of other neurodegenerative diseases. Furthermore, a substantial majority of our patients had evidence of amyloid deposition on amyloid PET scans. As mentioned above, including an early age-at-onset AD group controlled for the effects of age since lvPPA and PCA are typically associated with an early disease onset. However, this may have limited our ability to detect differences between "typical" EOAD syndrome and lvPPA/PCA. Since our study did not include a late-onset AD group due to insufficient functional MRI data, our results cannot yet be generalized to the late-onset AD population. It is possible that early functional alterations occur in DMN and non-DMN networks in late and early-onset AD, respectively. Nevertheless, although early-onset AD variants are less common than the late-onset AD phenotype, studying early-onset and non-amnestic AD variants still provides important insights into the factors that drive clinico-anatomical heterogeneity in AD, and can help us get a better understanding of the mechanisms underlying disease pathogenesis which may also be applicable to the more common, late-onset AD phenotype. Furthermore, although subject numbers in the different AD groups are relatively large given the frequency of the syndromes, they may have been too small to detect significant differences in connectivity between AD variants. None of our results met rigorous statistical thresholds correcting for multiple comparisons. It is also worth noting that matching AD variants for disease severity based on cognitive or functional scales can be difficult given the distinct nature of their clinical symptoms. However, patient groups were matched for disease duration and MMSE score, increasing the likelihood that patients had similar disease severities.

With regard to the functional connectivity analysis, motion correction is an ongoing concern in the resting-state fMRI field. Whilst several measures were undertaken in this study to correct for motion, we cannot rule out the possibility that residual motion had an effect on the data. It is also important to note that definitions and taxonomy of networks vary across studies and centers, for example the ventral DMN partly overlaps with the dorsal attention network (as defined by Allen, et al., 2011). Attribution of connectivity changes to specific canonical networks should be interpreted with this caveat in mind. Furthermore, using a seed-based approach to extract networks is typically a useful and reliable approach. Seed-based methods have the advantage of being hypothesis-driven, and the interpretation of the resulting maps is relatively straightforward. However, disadvantages of the seed-based approach include the arbitrary choice of the size of the seed region which may bias connectivity results towards smaller or overlapping sub-systems (Cole, et al., 2010). An alternative method is independent component analysis (ICA) which is more data-driven and avoids spatial assumptions about the size and location of seed regions. However, ICA also has its limitations, including run-to-run variability, potential bias introduced by setting a priori criteria for the number of components which can result in "under-splitting" or "over-splitting" of networks, and potential error in assigning functional meaning to data-driven

networks when applying either subjective or objective classification methods (Cole, et al., 2010). Nevertheless, ICA may provide further insights into the dysfunction of brain networks in AD and should be subject for future studies including larger samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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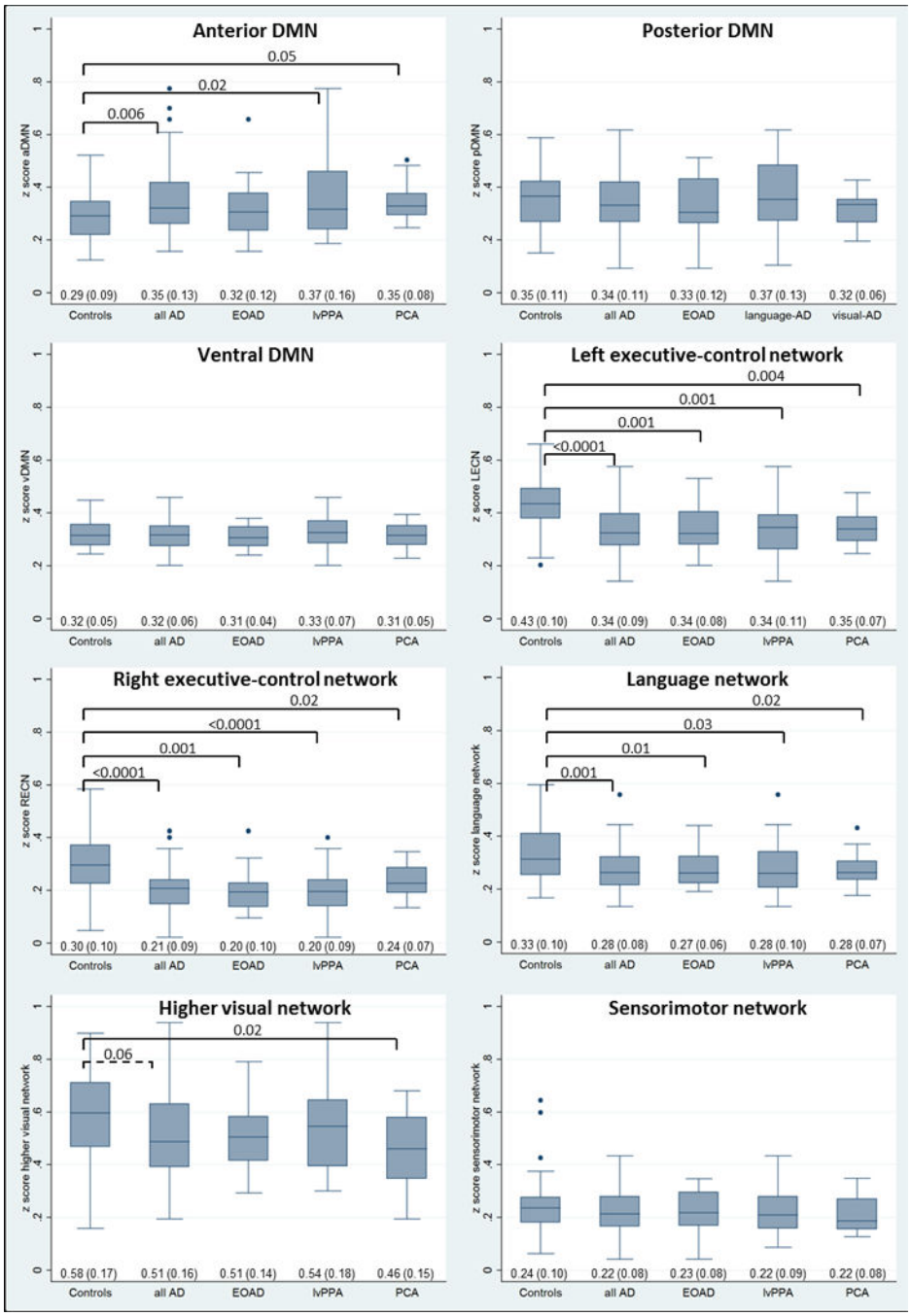

Figure 1.

Connectivity z-scores for different networks in each group and p values for group comparisons. Means and standard deviations for each group are shown at the bottom of each graph.

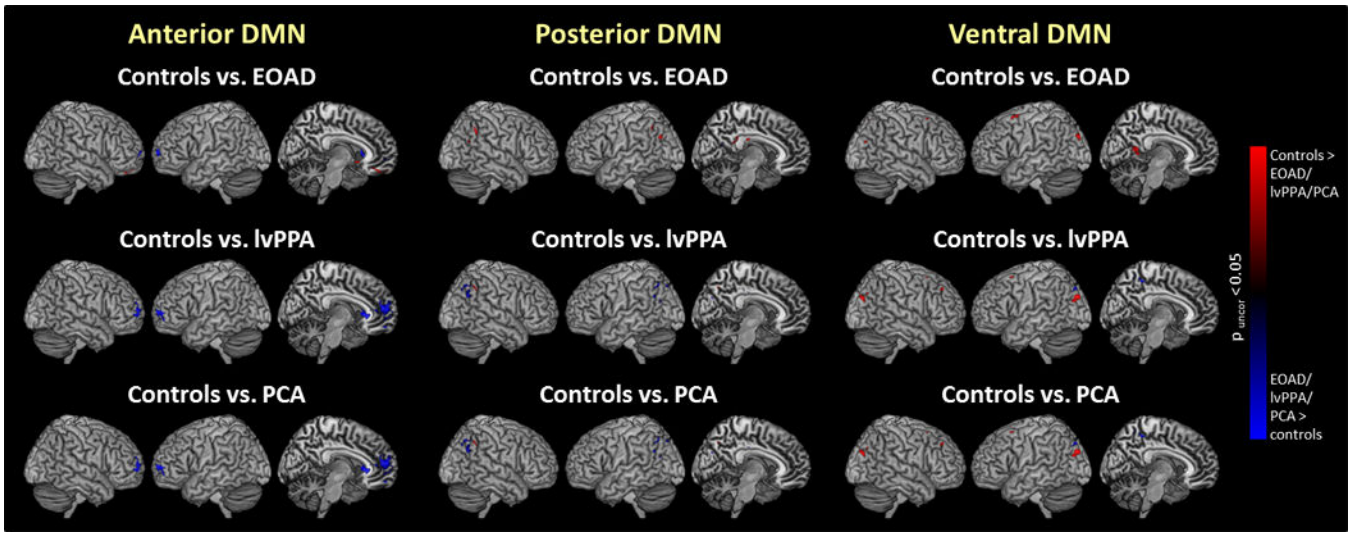

Figure 2.

Differences in connectivity in patients compared with controls in anterior, posterior and ventral DMN components. Shown are p-maps ($p<0.05$ uncorrected) with red indicating lower connectivity in the patients, whereas blue indicates higher connectivity compared with controls.

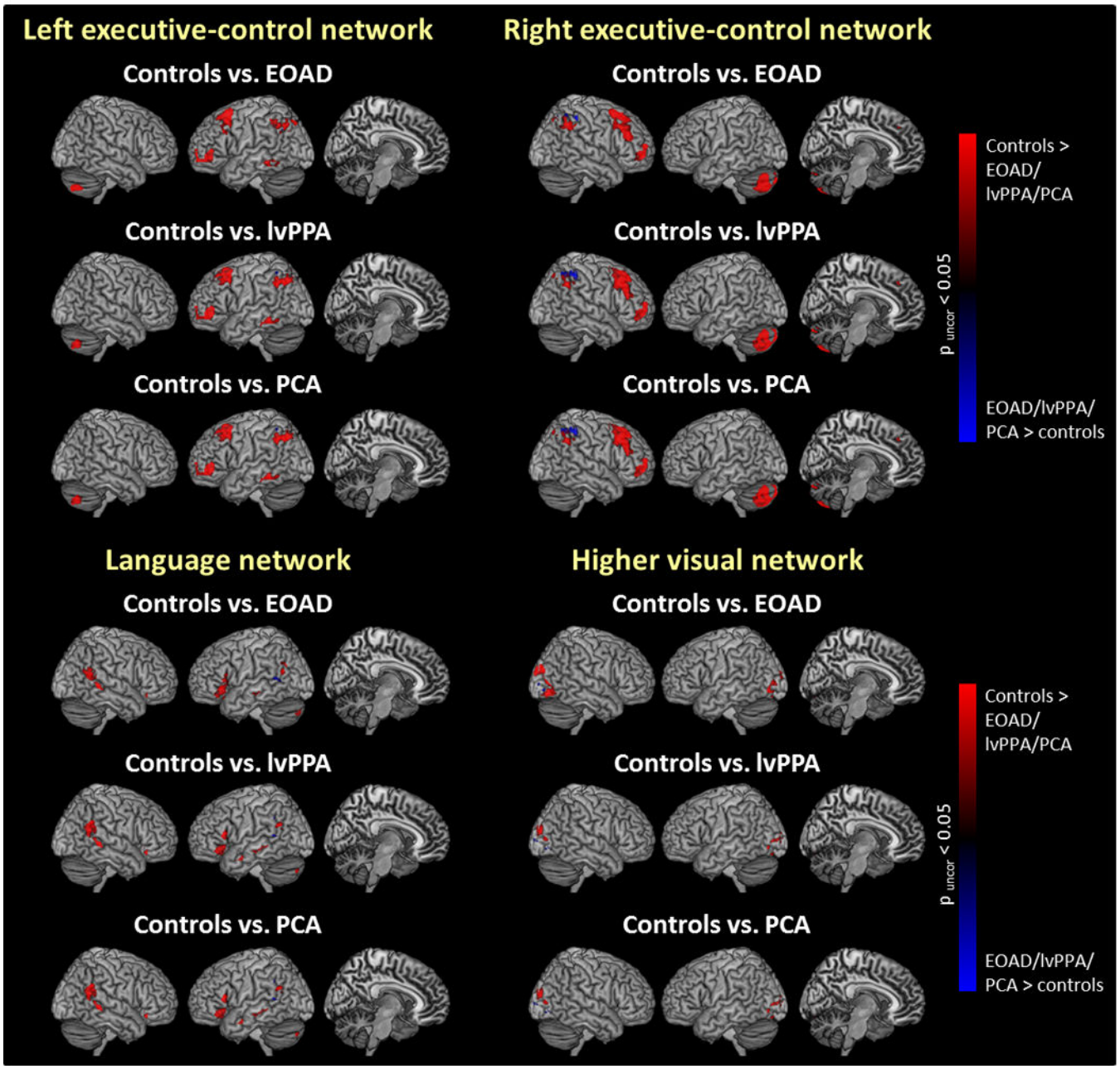

Figure 3.

Differences in connectivity in patients compared with controls in left and right executive-control, language and higher visual networks. Shown are p-maps ($p<0.05$ uncorrected) with red indicating lower connectivity in the patients, whereas blue indicates higher connectivity compared with controls.

Table 1

Subject demographics

	Controls	EOAD	lvPPA	PCA	p [a]
N	60	20	24	16	-
Age, years	62.2 (3.8)	61.0 (5.8)	62.0 (8.4)	62.2 (5.7)	0.9
Gender % male	43%	35%	46%	38%	0.8
Education, years	16.9 (1.8)	15.2 (4.3)	16.9 (3.2)	15.8 (2.0)	0.3
Age at onset, years	-	55.1 (5.1)	57.8 (7.9)	56.9 (6.5)	0.4
Disease duration, years	-	5.8 (3.5)	4.7 (2.0)	5.2 (2.3)	0.4
MMSE, /30	29.7 (0.5)	22.4 (4.5)	18.5 (7.5)	20.9 (5.2)	0.1
ApoE4, % positive	32%	56%	37%	33%	0.4

[a] for difference between AD groups; MMSE available in 59 controls, 19 EOAD, 15 PCA; ApoE available in 57 controls, 16 EOAD, 19 lvPPA, 12 PCA

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