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Cortical analysis using 7T MR phase images reveals regional differences between early and late onset Alzheimer's disease

This chapter was adapted from:

S. van Rooden*, **N. T. Doan***, M. J. Versluis, J. D. C. Goos, A. G. Webb, A. M. Oleksik, W. M. van der Flier, P. Scheltens, A. W. E. Weverling - Rynsburger, F. Barkhof, G. J. Blauw, J. H. C. Reiber, M. A. van Buchem, J. Milles, and J. van der Grond, "Cortical analysis using 7T MR phase images reveals regional differences between early and late onset Alzheimer's disease," Submitted, * share first authorship

Abstract

The overall aim of the present study is to explore regional iron related differences possibly indicative for AD pathology in the cerebral cortex between early (EOAD) and late onset Alzheimer's disease (LOAD) patients using 7T MR phase images. High resolution T_2^*-weighted scans were acquired in 12 EOAD and 17 LOAD patients at 7T MRI. Lobar peak phase shifts, regional phase contrasts, and cortical profiles were computed from the phase images in different cortical regions to compare the EOAD and LOAD groups. An increased peak phase shift was found for the whole brain in EOAD patients compared to LOAD patients ($p = 0.005$). The regional phase contrast in EOAD patients was higher than in LOAD patients in the middle frontal gyrus, postcentral gyrus, superior parietal gyrus, inferior parietal gyrus and precuneus ($0.002 < p < 0.042$). EOAD patients had a different cortical layer pattern in the middle frontal gyrus, precentral gyrus, postcentral gyrus, superior parietal gyrus, and precuneus compared to LOAD patients ($0.005 < p < 0.050$). These data suggest that patients with EOAD have an increased iron accumulation possibly related to increased amyloid deposition in specific cortical regions as compared to LOAD patients.

5.1 Introduction

Alzheimer's disease (AD) is a very common form of dementia and prevalence is increasing rapidly [145, 146]. Although age is one of the main risk factors for the development of AD, AD may also develop in younger patients, often referred to as early onset AD (EOAD). Previous studies have demonstrated that EOAD patients show a different profile of cognitive impairment and more rapid cognitive decline compared to late onset AD (LOAD) patients [147–152]. EOAD patients exhibit atypical non-amnestic symptoms more often than LOAD patients, leading to an incorrect clinical diagnosis in one-third of the EOAD cases [153, 154]. Although EOAD patients share multiple characteristics with LOAD patients, several studies have shown differences between the two groups with respect to the pattern and severity of atrophy [155, 156] as well as the pattern and severity of disturbances in metabolism [157–159], any or all of which might account for the differences in cognition [160, 161].

Neuropathological studies have shown that EOAD patients show more neuritic plaques and neurofibrillary tangles than LOAD patients [162–164]. However, these findings have not been substantiated in clinical studies. Studies using Positron Emission Tomography (PET) with carbon-11-labelled Pittsburgh compound-B (PiB) were not conclusive in replicating the post-mortem findings. In two studies, the reported overall amyloid burden did not differ between EOAD and LOAD patients [158, 159], whereas in another study, higher amyloid burden was shown in EOAD patients compared to LOAD patients [165]. A more recent PET study showed a regional increased amount of amyloid burden within the parietal cortex as opposed to an overall increase in the whole brain in EOAD patients in comparison to LOAD patients [158].

Recently, high resolution T_2^*-weighted high field MRI was used to highlight indirect cortical changes due to differences in iron accumulation related to amyloid deposition in vivo [125, 166]. A previous study suggested that iron accumulation, which may reflect AD pathology and more specifically amyloid deposition, is reflected by the phase information from a T_2^*-weighted MRI sequence at 7T MRI and measurement of the relative phase in regions of interest demonstrated a high specificity with respect to AD detection [125]. In contrast to PiB-PET, which is based on an in-plane resolution of 2 x 2 mm^2, the high resolution high field MRI approach uses images with an in-plane resolution of 0.24 x 0.24 mm^2, allowing accurate and detailed detection of phase changes, even within cortical layers [167].

The overall aim of the present study was to explore regional differences in AD based on phase information, which may reflect AD pathology, in the cerebral cortex between EOAD and LOAD patients using 7T MR phase images. In particular, group differences in lobar phase shift, cortical contrast and the layer pattern within the cortex at local cortical regions were investigated.

5.2 Materials and methods

5.2.1 Participants

This study was approved by the local institutional review board. In all cases, informed consent was obtained according to the declaration of Helsinki [168]. In total 17 LOAD patients (age > 67 years) with a mean age of 76.4 years (range 68 to 86 years, 11 male/6 female) and 12 EOAD patients with a mean age of 62.2 years (range 51 to 67 years, 7 male/5 female) were included.

The AD patients were recruited from the memory clinic of the Leiden University Medical Center, the VU University Medical Center in Amsterdam, the Bronovo Hospital in The Hague and the Diaconessen Hospital in Leiden. Memory clinic patients were referred to the hospital by their general practitioner or a medical specialist. Prior to the 7T study, all patients underwent a routine clinical protocol, comprising a whole brain MRI, a battery of neuropsychological tests, and a general medical and neurological examination performed by a neurologist, psychiatrist or internist-geriatrician. The diagnosis was made in a multidisciplinary consensus meeting using the NINCDS-ADRDA criteria for diagnosing probable Alzheimer's disease [169]. Participants with the diagnosis "probable AD", who were capable of giving informed consent (Mini Mental State Examination (MMSE) ≥ 19) were selected for inclusion in the 7T study either retrospectively within one year after attending the memory clinic, or prospectively.

5.2.2 MR acquisition

MRI was performed on a whole body human 7T MR system (Philips Healthcare, Best, the Netherlands) using a quadrature transmit and 16-channel receive head coil (Nova Medical, Wilmington, MA, USA). Participants were scanned using a 2D flow-compensated transverse T_2^*-weighted gradient-echo scan including the frontal and parietal regions, which are most prone for amyloid deposition, with a total imaging duration of 10 minutes. Positioning of this stack was performed using the sagittal plane of the survey within the frontal and parietal region above the occipital lobe. The middle of the stack was positioned through the corpus callosum, just above the thalamus. Imaging parameters were: repetition time (TR)/echo time (TE) 794/25 ms, flip angle 45^o , slice thickness 1.0 mm with a 0.1 mm interslice gap, 20 slices, 240 x 180 x 22 mm^3 field of view, 1000 x 1024 matrix size, resulting in an in-plane nominal spatial resolution of 0.24 x 0.24 mm^2. The band width per pixel was 46 Hz, corresponding to a readout length of approximately 22 ms. The frequency and phase encoding directions were along the anterior-posterior and right-left axes, respectively. These sequences are very sensitive to image artifacts arising from resonance frequency fluctuations within the brain caused by slight patient movements, even in areas significantly away from the head. A navigator echo was included to correct for these artifacts [114]. Shimming up to third order was performed using an image based shimming approach [170]. The phase images were subsequently unwrapped by high-pass filtering with a 92x92 kernel size [171].

5.2.3 Image analysis

5.2.3.1 Lobar peak phase shift measurements

As in a previous study, phase values in the cortex were determined using the transverse 2D T_2^*-weighted gradient echo scans. The peak phase values of the cortical gray matter (GM) were determined on the unwrapped phase images in regions of interest (ROIs) in four different areas of the brain: frontal, left temporoparietal, right temporoparietal and parietal [125]. The overall peak-to-peak phase shift (expressed in radians) between cortical gray and subcortical white matter (WM) (lobar cortical phase shift) was calculated for each region in each subject.

5.2.3.2 Regional GM/WM phase contrast

The regional GM/WM phase contrast was measured in more localized cortical regions using the method presented in [167]. The cerebral cortex was segmented using a

combination of T_2^*-weighted magnitude and phase information as described in [123]. An atlas with labels of predefined cortical ROIs (AAL atlas [128]) was then registered to the subject T_2^*-weighted image for cortical anatomical parcellation (Figure 5.1b). For each atlas label (Figure 5.1c), cortical GM and subcortical WM ROIs on the phase images were identified with the GM ROI being the intersection between the segmented cortex and the binary mask of the label, and the WM ROI located within the label and adjacent to the GM ROI. The regional GM/WM contrast was computed as the difference between the mean phase value of the GM and WM ROIs.

5.2.3.3 Regional cortical phase profile

A cortical phase profile (Figure 5.1d) representing the distribution of the phase value across the cortical layer [167] was calculated for each atlas label. The outer mask, bordered by the outer cortical contour, was iteratively eroded. For each iteration, the mean phase value of voxels positioned both in the contour of the eroded mask and the particular label was computed, resulting in one node of the profile (see Figure 5.1). The length of the profile was determined as the local distance between the cerebral spinal fluid (CSF)/GM and GM/WM interfaces on the phase images. The mean phase value of the corresponding WM ROI was subtracted from the profile to correct for local RF coil inhomogeneity.

To minimize the effect of noise on the measurements, the regional GM/WM contrast and cortical profile for labels were computed only when both GM and WM ROIs satisfied a size criterion ($\geq 1\text{cm}^3$). Finally, statistical testing was only performed on labels having data from at least 10 subjects to ensure sufficient statistical power.

5.2.4 Statistics

A Mann-Whitney U-test was used to assess the difference in MMSE and a chi-square test was used to assess the difference in gender between groups. In this study, we used MMSE score as a measure of the disease severity. Linear regression analysis was used to assess the association between early and late age-at-onset and lobar peak phase shift measurements in the four different lobar regions of the cortex, adjusted for gender and disease severity (MMSE).

Linear regression analysis adjusted for gender and disease severity (MMSE) was also used to assess group differences in regional GM/WM contrast. To compare the cortical profiles, two different permutation tests were used. Due to possible differences between subjects in cortical thickness, the cortical profiles were normalized to have the same length prior to the permutation tests. The first permutation test checked whether there was a difference between two groups considering the entire profile, resulting in one p value per comparison. The second permutation test was more localized. It was performed at each cortical depth, resulting in one p value per node of the profile. The resulting p values were then adjusted for the total number of nodes using the False Discovery Rate (FDR) procedure.

Permutation tests were carried out using Matlab (Natick, MA, version 7.14). All other statistical analyses were performed with the Statistical Package of Social Sciences (SPSS, version 17.0.1; SPSS, Chicago, Ill). A significance level of 0.05 was used.

5.3 Results

Characteristics of the participants are shown in Table 5.1. Age was 76.4 ± 5.9 years in the LOAD group and 62.2 ± 5.2 years in the EOAD group. No difference in gender and MMSE was present between the EOAD and LOAD groups.

Figure 5.1 shows a representative transverse T_2^*-weighted phase image of the cortex of a subject (Figure 5.1a) and an illustration of cortical parcellation (Figure 5.1b) as well as the computation of regional cortical profiles (Figure 5.1c-d). Table 5.2 shows the lobar peak phase shifts of the frontal, temporoparietal, parietal cortex and the whole brain. For all of these regions, a larger cortical phase shift ($p < 0.05$) was found in the EOAD patient group compared to the LOAD patient group which was associated with age-at-onset (adjusted for gender and disease severity).

Table 5.3 reports the mean regional GM/WM contrast computed from the EOAD and LOAD groups in 18 studied regions as well as the p values obtained from the statistical comparisons between the two groups in terms of regional GM/WM contrast and cortical profile. The p values obtained from linear regression analysis (adjusted for gender and disease severity) and from the permutation test using the entire profile are color-mapped on the cortex of an EOAD patient in Figure 5.2a-b. Linear regression analysis (adjusted for gender and disease severity) showed significant differences in regional GM/WM contrast in the left middle frontal gyrus, right postcentral gyrus, left and right superior parietal gyrus, right inferior parietal gyrus, and left and right precuneus. The EOAD group consistently showed higher mean GM/WM contrast than the LOAD group.

When comparing the cortical phase profile, significant differences were found in the left middle frontal gyrus, right precentral gyrus, right postcentral gyrus, left and right superior parietal gyrus, and left and right precuneus (Table 5.3, Figure 5.2b). Figure 5.3 presents the mean cortical phase profile of each group at each region studied (Figure 5.3a-b) and the p values obtained at each node before and after the FDR correction for the total number of nodes (Figure 5.3c-d). As can be observed from Figure 5.3a-b, the cortical phase profiles of both groups are bell shaped. The profile of the EOAD group however exhibits larger amplitudes than the LOAD group. The p values presented in Figure 5.3c-d specify the cortical depths where the EOAD group shows significantly larger phase values than the LOAD group. Before multiple comparison correction, differences were shown at multiple nodes on the cortical profile of the left middle frontal gyrus, left inferior frontal gyrus opercular part, right precentral gyrus, right postcentral gyrus, left and right superior parietal gyrus, right inferior parietal gyrus and left and right precuneus. In most of these structures, the differences were most significant at the cortical layers between the CSF/GM interface and the middle of the cortex. After correction, significant differences were shown in the left and right superior parietal gyrus as well as left and right precuneus.

TABLE 5.1: Characteristics of early and late onset AD patients. EOAD = early onset Alzheimer's disease, LOAD = late onset Alzheimer's disease, MMSE = Mini Mental State Examination.

	EOAD (n = 12)	LOAD (n = 17)
Mean age, years (range)	62.2 (51–67)	76.4 (68–86)
Male/female	7/5	11/6
Median MMSE, points (range)	22 (19–26)	23 (19–26)

TABLE 5.2: Mean lobar phase shifts (in radians) and SDs of the brain lobes in early and late onset Alzheimer's disease patients. p values were obtained using linear regression analysis adjusted for gender and disease severity. EOAD = early onset Alzheimer's disease, LOAD = late onset Alzheimer's disease, asterisks indicate significant differences.

	EOAD (n = 12)	LOAD (n = 17)	p value
Frontal	1.15 ± 0.09	1.04 ± 0.11	0.014*
Temporoparietal left	1.25 ± 0.05	1.18 ± 0.09	0.029*
Temporoparietal right	1.31 ± 0.09	1.23 ± 0.10	0.045*
Parietal	1.27 ± 0.08	1.16 ± 0.10	0.006*
Total	1.25 ± 0.06	1.15 ± 0.09	0.005*

TABLE 5.3: The mean regional gray matter/white matter phase contrast computed for both groups and the p values obtained by linear regression analysis (adjusted for gender and disease severity) using this measure and permutation test using the entire cortical profile. GM = gray matter, WM = white matter, L = left, R = right, EOAD = early onset Alzheimer's disease, LOAD = late onset Alzheimer's disease, asterisks indicate significant differences.

Lobe	Structure	GM/WM contrast (rad)		p value Linear regression	p value Permutation test
		EOAD	LOAD		
Frontal	Superior frontal gyrus L	0.165 ± 0.057	0.166 ± 0.090	0.844	0.720
	Superior frontal gyrus R	0.167 ± 0.078	0.149 ± 0.087	0.688	0.849
	Middle frontal gyrus L	0.199 ± 0.035	0.144 ± 0.049	0.005*	0.050*
	Middle frontal gyrus R	0.197 ± 0.045	0.160 ± 0.049	0.072	0.203
	Inferior frontal gyrus, opercular part L	0.225 ± 0.044	0.180 ± 0.074	0.169	0.114
	Inferior frontal gyrus, opercular part R	0.237 ± 0.031	0.227 ± 0.048	0.656	0.425
	Inferior frontal gyrus, triangular part L	0.196 ± 0.035	0.160 ± 0.051	0.051	0.164
	Inferior frontal gyrus, triangular part R	0.199 ± 0.028	0.169 ± 0.058	0.126	0.186
Temporoparietal	Precentral gyrus L	0.274 ± 0.047	0.236 ± 0.086	0.220	0.170
	Precentral gyrus R	0.310 ± 0.041	0.287 ± 0.072	0.415	0.035*
	Postcentral gyrus L	0.277 ± 0.052	0.247 ± 0.052	0.165	0.156
	Postcentral gyrus R	0.288 ± 0.030	0.252 ± 0.044	0.025*	0.028*
Parietal	**Superior parietal gyrus L**	0.305 ± 0.045	0.245 ± 0.035	0.004*	0.005*
	Superior parietal gyrus R	0.284 ± 0.031	0.248 ± 0.034	0.020*	0.037*
	Inferior parietal gyrus L	0.273 ± 0.033	0.255 ± 0.038	0.210	0.405
	Inferior parietal gyrus R	0.293 ± 0.041	0.246 ± 0.043	0.042*	0.063
	Precuneus L	0.249 ± 0.052	0.191 ± 0.044	0.007*	0.008*
	Precuneus R	0.241 ± 0.026	0.190 ± 0.036	0.002*	0.007*

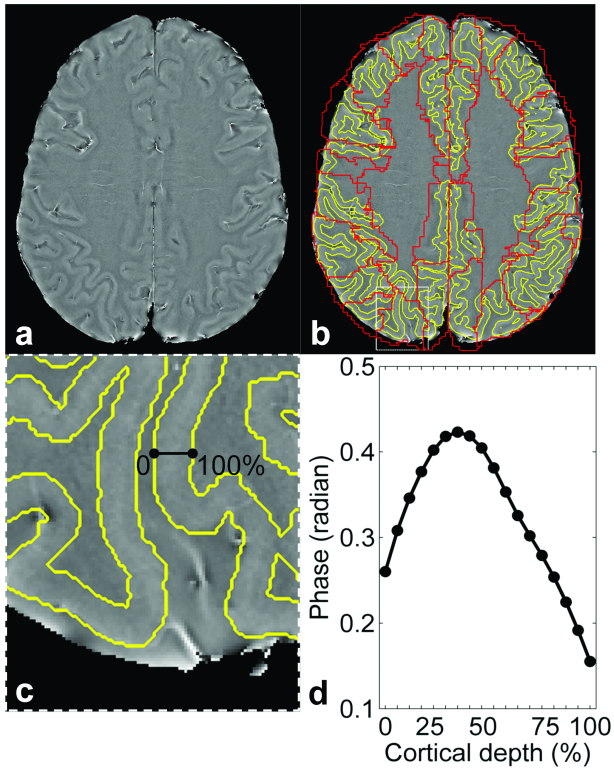

FIGURE 5.1: Illustrative example of cortical parcellation and the computation of regional cortical profiles: (a) representative transverse 2D T_2^*- weighted gradient echo phase image (in-plane spatial resolution is 0.24 x 0.24 mm^2); (b) contours of the segmented cortex (in yellow) and of the labels as defined in the AAL atlas (in red) overlaid on the phase image; (c) magnified cortical ROI; (d) the cortical phase profile computed for the superior parietal region located within the ROI presented in (c).

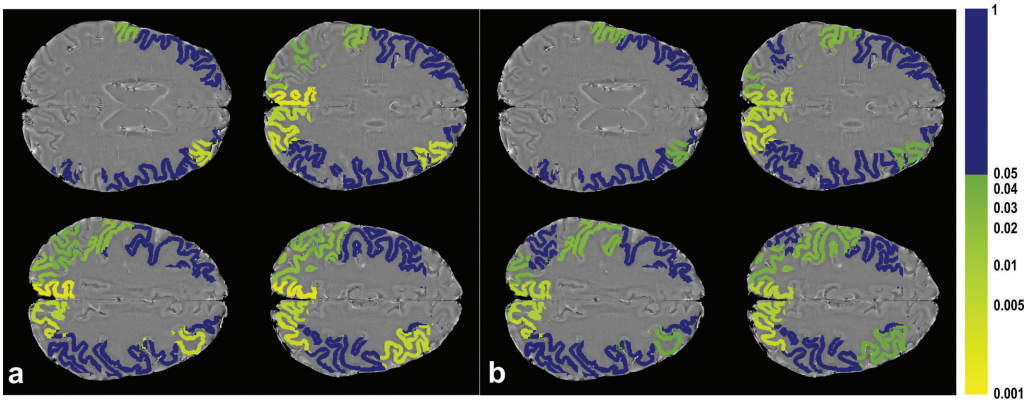

FIGURE 5.2: Maps of p values on the cortex of an early onset AD patient: (a) linear regression analysis (adjusted for gender and disease severity) on regional GM/WM contrast, and (b) permutation test on entire cortical profile. All p values > 0.05 are presented in blue, and the color bar is scaled using $-\log_{10}(p \text{ values})$ otherwise. Only the cortical regions included in this study were presented.

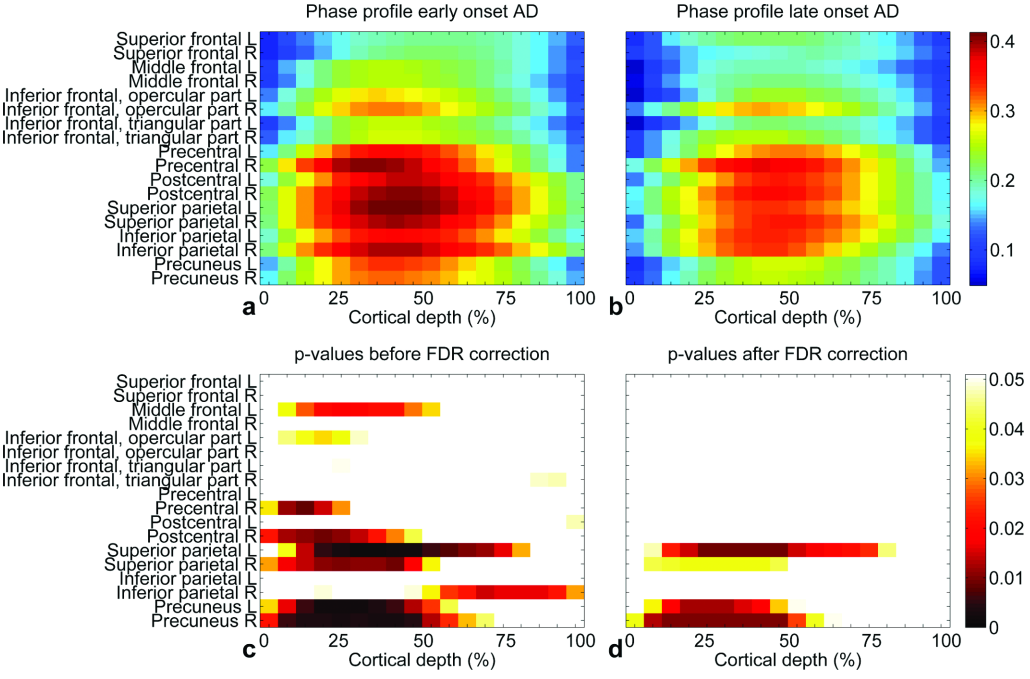

FIGURE 5.3: Cortical phase profiles and the p values obtained using a permutation test at each cortical depth: (a) profile of early onset AD, (b) profile of late onset AD, (c) p value before FDR correction for the number of nodes on the profile, and (d) p values after the FDR correction. The color map in (a) and (b) presents the phase values in radians. The hotter the color, the higher the phase value and vice versa. A cortical depth of 0% corresponds to the CSF/GM interface and 100% the GM/WM interface.

5.4 Discussion

This study shows that using 7T MRI phase images, global (i.e. per lobe) and regional differences in the cortex, as well as differences within the cortical layers, can be detected between EOAD and LOAD patients in great detail. Measurement of the lobar peak phase shift demonstrated a higher phase shift throughout the whole brain in EOAD patients compared to LOAD patients independent of the disease severity. Within the four lobar cortical regions studied, a higher cortical phase shift of the frontal, temporoparietal and parietal regions was associated with an early age-at-onset. Previous studies in brain specimens of AD patients have shown that amyloid deposition and neurofibrillary tangles as well as tau deficiency co-localize with neuronal iron accumulation [98, 172–174]. The relative phase in cortical regions of interest can be used as an indicator of iron in the brain [26, 124, 175, 176], which opens up the possibility to measure neuronal iron co-localized with AD pathology. Previously, it was demonstrated that AD pathology and more specifically amyloid deposition is indirectly reflected by the phase information from a T_2^*-weighted MRI sequence [125]. In this respect, the in-vivo data presented in this study confirm the previous histological and in-vivo studies which reported that EOAD patients show more amyloid plaques and tangles than LOAD patients [162–165]. Therefore, the finding of an overall increased phase shift in EOAD patients compared to LOAD patients suggests that AD pathology, and more specifically amyloid deposition, is more severe in the EOAD patients. Measurement of the regional phase contrast showed higher phase values in EOAD patients compared to LOAD patients in the middle frontal gyrus, postcentral gyrus, superior parietal gyrus, inferior parietal gyrus and precuneus independent of disease severity. The finding of an increased regional phase contrast mainly in the parietal sub-regions is in accordance with a recent PET-PiB study which shows a higher amyloid burden in the parietal lobe in EOAD patients in comparison with LOAD patients [158]. Moreover, we also found an increased regional phase contrast in the middle frontal gyrus which confirms an earlier neuropathological study showing a higher amount of amyloid plaques in EOAD patients compared to LOAD patients in the parietal and frontal regions [162]. Previous studies have shown that the precuneus, posterior cingulate gyrus, parietal and frontoparietal regions in EOAD patients also show more atrophy than in LOAD patients [155, 156, 160, 177–179]. This might imply a direct association between severity of AD pathology and atrophy in EOAD patients.

The overall cortical layer pattern also showed differences between EOAD and LOAD patients in the middle frontal gyrus, precentral gyrus, postcentral gyrus, superior parietal gyrus, and precuneus. The phase profiles showed higher phase values in the EOAD patients compared to LOAD patients. In the superior parietal gyrus and precuneus, significant differences within the cortical layers between the CSF/GM interface and the middle of the cortex could be detected between EOAD and LOAD patients. A previous study showed that the contrast between different cortical layers using high-field MRI T_2^*-weighted sequences and phase images is based on differences in iron and myelin content between different layers [72, 180]. The current results imply that the distribution of iron and/or myelin is different in the cortical layers close to the CSF/GM interface in the superior parietal gyrus and precuneus between the two groups, with the EOAD patients showing higher amounts of iron/ demyelination.

The precuneus and superior parietal gyrus were the only structures showing a significant difference in mean phase contrast and cortical profile at very specific cortical

depths. Previous studies have demonstrated that the precuneus and parietal cortex show more atrophy [156, 178, 179, 181] and more severely impaired metabolism [159, 182] in EOAD patients than in LOAD patients. Our results indicate that within the precuneus and the superior parietal gyrus more iron and/or less myelin is present, which may reflect increased AD pathology resulting in a change of the cortical layer pattern and phase contrast. This is in line with the notion that these structures play an important role in the development of EOAD, or alternatively that EOAD is a different subtype of AD characterized by pathological changes of the precuneus and superior parietal gyrus.

One overall limitation of the study is that phase measurements may be influenced by the geometry and orientation of the individual structures [26]. To limit such effects as much as possible, all participants were positioned in the same manner, and for every subject, the phase measurements were performed in the same way and were averaged to cancel out the possible effects of geometry and orientation. A second limitation is the small number of subjects and the absence of a healthy control group. However, it is very unlikely that our results were related to an age effect as with healthy aging an increased phase shift would be expected due to a slight increase in cortical iron and demyelination with aging [119, 183, 184]. Instead, we found an increased phase shift in the EOAD patients. Moreover, the source of the observed differences in phase contrast and cortical profile is not completely clear. Although it is most likely that the difference in phase contrast and profile between groups is mainly attributable to iron, other compounds may contribute to a change in phase contrast, such as deoxy-hemoglobin, myelin and proteins [185]. The effect of deoxy-hemoglobin is expected to be rather small in comparison with iron and myelin [72]. Myelin and proteins might explain part of the results in our study, although very few studies have investigated the differences in these substances between EOAD and LOAD patients [186, 187] and therefore it is difficult to verify what their influence has been. Future research should focus on studying the correlation between histology and MR images to confirm that the differences in phase contrast and profile between LOAD and EOAD patients is due to AD pathology.

In conclusion, in this study, using 7T MRI phase information, regional cortical differences in contrast and layer pattern were detected in EOAD versus LOAD patients, indicating regionally more severe iron accumulation possibly due to amyloid deposition in EOAD compared to LOAD patients.

5.5 Acknowledgement

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