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Abnormal Regional and Global Connectivity Measures in Subjective Cognitive Decline Depending on Cerebral Amyloid Status

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Running Title: Abnormal Connectivity in SCD with Amyloidosis

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ABSTRACT

Background: Amyloid- β accumulation was found to alter precuneus-based functional connectivity (FC) in MCI and AD dementia, but its impact is less clear in Subjective Cognitive Decline (SCD), which in combination with AD pathologic change is theorized to correspond to stage 2 of the Alzheimer's continuum in the 2018 NIA-AA research framework.

Objective: This study addresses how amyloid pathology relates to resting-state fMRI FC in SCD, especially focusing on the precuneus.

Methods: From the DELCODE cohort, two groups of 24 age- and gender-matched amyloid-positive ($SCD_{A\beta+}$) and amyloid-negative SCD ($SCD_{A\beta-}$) patients were selected according to visual [18F]-Florbetaben (FBB) PET readings, and studied with resting-state fMRI. Local (Regional Homogeneity [ReHo], fractional Amplitude of Low-Frequency Fluctuations [fALFF]) and global (degree centrality [DC], precuneus seed-based FC) measures were compared between groups. Follow-up correlation analyses probed relationships of group differences with global and precuneal amyloid load, as measured by FBB standard uptake value ratios ($SUVR_{FBB}$).

Results: ReHo was significantly higher (voxel-wise $p < .01$, cluster-level $p < .05$) in the bilateral precuneus for $SCD_{A\beta+}$ patients, whereas fALFF was not altered between groups. Relatively higher precuneus-based FC with occipital areas (but no altered DC) was observed in $SCD_{A\beta+}$ patients. In this latter cluster, precuneus-occipital FC correlated positively with global ($SCD_{A\beta+}$) and precuneus $SUVR_{FBB}$ (both groups).

Conclusions: While partial confounding influences due to a higher APOE $\epsilon 4$ carrier ratio among $SCD_{A\beta+}$ patients cannot be excluded, exploratory results indicate

functional alterations in the precuneus hub region that were related to amyloid- β load, highlighting incipient pathology in stage 2 of the AD continuum.

KEYWORDS

PET;

amyloid;

Alzheimer's Disease;

prodromal symptoms;

precuneus;

occipital cortex;

functional Magnetic Resonance Imaging;

subjective cognitive decline

BACKGROUND

Subjective cognitive decline (SCD) is increasingly acknowledged as the earliest symptomatic manifestation of Alzheimer's disease (AD) [1, 2]. According to the conceptual framework for SCD research, subjective complaints include 'self-experienced persistent decline in cognitive capacity in comparison with a previously normal status and unrelated to an acute event', along with 'normal age-, gender-, and education-adjusted performance on standardized cognitive tests, which are used to classify mild cognitive impairment (MCI) or prodromal AD' (p. 847) [2]. Although SCD is not specific for the presence of underlying AD pathology, an increased prevalence of preclinical AD pathology was reported in SCD complainers [3-5], and linked with an increased risk of future cognitive decline, progression to MCI and, ultimately, AD dementia [6-10]. SCD with additional biomarker evidence of underlying AD pathologic change is thought to correspond to stage 2 of the clinical AD continuum of the novel NIA-AA (National Institute on Aging and Alzheimer's Association) working group criteria [11, 12]. Accordingly, these SCD patients should show an increased risk of incipient AD-related functional brain alterations.

Functional connectivity (FC) analyses, using resting-state functional magnetic resonance imaging (rs-fMRI) to measure synchronous low-frequency brain activity fluctuations in spatially distinct brain areas, are a sensitive tool for interrogating intrinsic FC properties of the brain on both local and global scale, and for determining functional network disruptions as a consequence of underlying AD pathology [13]. While FC alterations in manifest AD dementia and MCI are also observed in other heteromodal brain networks [14-17], the most consistent abnormalities are found in the Default Mode network (DMN), especially its posterior aspects [18-22]. Some

studies report linear associations between the amount of brain amyloid depositions and severity of FC alterations [15, 23, 24] that suggest possible causal links, although the direction of this causality remains a matter of debate [25-27]. This is also supported by studies showing that FC alterations already emerge during the preclinical stages of AD [28-30], where significant brain amyloid accumulations are already detectable, but not yet coupled with subjective or objective cognitive impairments (NIA-AA stage 1): The involvement of DMN structures is of special interest here, as its hub regions, like the posterior cingulate cortex (PCC) and precuneus, appear particularly vulnerable to amyloid accumulation, and are among the first brain structures that show increasing amyloid tracer uptake in early amyloidosis [31, 32]. Various studies have described reductions of DMN connectivity in cognitively normal elderly with amyloid plaque depositions, with some studies also reporting linear relationships between amyloid load and FC alterations [18, 33, 34]. Meanwhile, there are studies that indicate DMN FC increases [35, 36], and often show mixed patterns of both regional FC decreases and increases [29, 34, 37, see also: 38], which may reflect dynamic functional shifts within and between FC networks (e.g. from posterior to anterior) that are linked with amyloid accumulation [27, 39].

Some of the observed variability may relate to the multitude of local and global FC metrics in the available literature, which examine different aspects of connectivity and may therefore be measure complementary physiological responses to AD pathology. Regarding *global* FC metrics, seed-based FC analyses that identify brain regions whose blood-oxygen-level-dependent (BOLD) time course is correlated with predefined regions of interest are long-established, showing altered FC patterns especially for precuneus/PCC seed regions, not only in prodromal or clinical AD [14, 40], but already in cognitively normal individuals with amyloid depositions [18]. While

identifying FC changes with specific brain regions, these traditional FC metrics provide limited insights into the role of these AD-vulnerable regions within the global brain network. Graph-theory based analyses of Degree Centrality (DC), defined by the number of direct connections (i.e. significant correlations) between a given voxel or region (node) and the rest of the brain, indicate that the precuneus/PCC areas are among the “hub” areas with highest DC values, indicating an important role in the global brain network [41]. Previous studies in prodromal [19] and clinical AD patients [16] show gradual degradation of this hubness, with preliminary evidence also in amyloid-positive cognitively normal individuals [19]. Gradual breakdown of FC networks may be complemented by more circumscribed functional segregation on the regional level, as measured by *local* connectivity metrics like Regional Homogeneity (ReHo) and Amplitude of Low-Frequency Fluctuations (ALFF) [42]: While ReHo examines synchronization of resting-state BOLD fluctuations in neighboring voxels (i.e. local coherence) [43], ALFF focuses on measuring the voxel-level magnitude of regional BOLD fluctuations in the low-frequency range (0.01-0.08 Hz) which is considered proportional to neural activity [44]. Fractional ALFF (fALFF) is a normalized index of ALFF which is regarded as less sensitive to physiological noise [45]. Various studies in prodromal and clinical AD patients [20, 21, 24, 46-48] observe ReHo and ALFF/fALFF decreases especially in DMN areas, but also parallel increases (e.g. in cuneus, lingual or fusiform areas) that may reflect compensatory adaptations via increased local synchronization or neural activity, respectively. While showing some spatial convergence, especially in the precuneus/PCC region, the anatomical distributions of ReHo/ALFF/fALFF changes are not completely overlapping, both within single studies [21], and comparing meta-analytic findings [47, 49], suggesting that they actually tap into complementary physiological changes which may show

differential onset and time courses in AD pathogenesis. To date, there is only preliminary evidence that similar changes already emerge in amyloid-positive cognitively normal elderly [24, 29].

Building on the assumption that SCD patients are at-risk for preclinical AD, a variety of studies started to explore whether AD-related patterns of FC alterations (especially in DMN areas) are already observable in these patients, with mixed evidence: While FC decreases in the DMN, but also additional (e.g. visual) resting-state networks are reported [50-54], data showing only FC increases [55-59] or null findings [60, 61] also exist. Seed-based FC studies in SCD populations that examine precuneus (or more generally: posterior DMN) connectivity present both decreased [51] and increased (or unchanged) connectivity patterns [52, 58]. While one study [57] found an absolute decrease, but relative increase of DC in posterior cingulate cortex/precuneus areas for SCD compared to healthy controls [59], another study observed increased DC for the bilateral hippocampus and left fusiform area in subjective memory complainers, as compared with normal controls, to be positively correlated with cerebrospinal fluid (CSF) total and phosphorylated tau (but not amyloid) levels, providing preliminary, yet variable evidence for changing regional involvement in the global brain network. Turning to local connectivity measures, while ALFF/fALFF reductions in the precuneus were observed [62], there is also opposing evidence for ALFF increases in occipital and lateral parietal regions [56]. Meanwhile, there is a lack of data on possible ReHo alterations in SCD patients.

In general, most studies (except: [63]) examined SCD patients without stratifying by AD biomarker status, i.e. actual FC changes related to advancing preclinical AD pathology may be obscured by varying proportions of participants with

SCD due to non-AD mechanisms in the study samples. For example, one study [51] reported that only 1 of 13 SCD participants with available amyloid PET was amyloid-positive. Likewise, there are very limited data examining linear associations between the severity of amyloid burden and FC measures in SCD populations, reporting no [51, 59] or negative correlations [64].

Here, we report data from DELCODE (DZNE – Longitudinal Cognitive Impairment and Dementia Study), which is an ongoing observational longitudinal multicenter study focusing on SCD [65]. Considering that few studies in the SCD stage had AD biomarker information available to probe its influence on resting-state connectivity, this study specifically addresses the question how amyloid pathology relates to rs-fMRI FC in SCD patients, with a special focus on the precuneus. We used qualitative ratings from amyloid PET examinations to directly compare 24 SCD patients with significant amyloid pathology ($SCD_{A\beta+}$) with 24 age- and sex-matched SCD patients without significant amyloid pathology ($SCD_{A\beta-}$). We compared these biomarker-stratified samples regarding both local (fALFF and, for the first time in SCD patients, ReHo) and global FC measures (seed-based connectivity of the precuneus and DC). We hypothesized (i) differences of precuneus-based FC and regional measures between $SCD_{A\beta+}$ and $SCD_{A\beta-}$ groups, and (ii) these differences to correlate with the severity of global cortical and regional precuneus $A\beta$ load which we examined in additional, exploratory analyses. The direction of respective differences was not predicted *a priori*, based on equivocal findings in the available SCD literature.

METHODS

General procedures

All participants in this study were taken from the PET sub-cohort of the multi-centric DELCODE study, whose overall study design and detailed inclusion and exclusion criteria have been described elsewhere [65]. The reported sample included SCD participants from 7 of the 10 participating DELCODE sites. The SCD patients were recruited via referrals or self-referrals from the university-based memory clinics of these sites (i.e. only individuals seeking medical help because of their SCD [12]), and were included in DELCODE if they reported subjective experience of decline in cognitive functioning with an onset within the last six months to 5 years, but performed above -1.5 standard deviations of the age-, sex-, and education-adjusted mean on all subtests of the *Consortium to Establish a Registry for Alzheimer's Disease* (CERAD) [66] neuropsychological battery. Moreover, age ≥ 60 years, fluent German language skills, capacity to provide informed consent, and presence of a study partner were mandatory. Main exclusion criteria for DELCODE participation were past or present neurological disorders; significant medical diseases; any other detectable cause of memory impairment; and current and lifetime psychiatric disorders. Additionally, PET examinations were not allowed for patients who had radiation exposure for therapeutic or research purposes within the last 10 years.

All participants provided written informed consent. DELCODE and DELCODE-PET were conducted in accordance with the currently effective version of the Declaration of Helsinki, and approved by the institutional review boards of all participating sites (Charité Berlin, Bonn, Cologne, Ludwig-Maximilians-University Munich, Rostock, and Tübingen), and coordinated by the ethics committee of the medical faculty of the University of Bonn, under the registration numbers: 117/13

(DELCODE) and 221/13 (DELCODE-PET). DELCODE-PET was also approved by the federal radiation protection authority (Bundesamt für Strahlenschutz). All procedures were performed in accordance with the relevant guidelines and regulations.

Patient selection

Based on qualitative visual readings of [18F]-Florbetaben (FBB, Neuraceq™: Life Radiopharma Berlin GmbH) PET scans, the first 24 available SCD_{Aβ+} individuals with significant cerebral amyloid depositions and valid rs-fMRI data were selected for analysis, and compared with a respective group of 24 age- and sex-matched SCD_{Aβ-} participants without significant cerebral amyloid depositions from the ongoing data collection, after excluding 3 participants with PET reconstruction and 2 participants with MR segmentation issues.

Sample characteristics

Further details about general sample characterization in DELCODE can be found elsewhere [65]. In brief, all DELCODE study participants complete extensive neuropsychological testing during the yearly study visits, including the Mini Mental State Examination (MMSE: [67]), and a modified version of the Alzheimer's Disease Assessment Scale-Cognitive-Plus (ADAS-Cog-13: [68]). Apolipoprotein (APOE) genotyping was performed using commercially available TaqMan® SNP Genotyping Assay (ThermoFisher Scientific).

[18F]-Florbetaben PET acquisition

PET data were acquired on clinical PET/CT or PET/MR scanners at the nuclear medicine departments of the participating sites (Table S1). Data acquisition followed

established standard procedures for FBB scanning: After intravenous FBB tracer application of 282 ± 9 MBq, dynamic 3D-acquisition of list mode emission data started 90.9 ± 3.6 min post-injection, for a total duration of 20 minutes (which were subsequently reconstructed into 4 x 5 min time frames). Additionally, low-dose CT (or for PET/MR: 3D Dixon-VIBE sequences) were collected for calculation of attenuation correction maps. Iterative reconstruction was performed based on the established PET brain protocols at the local sites, including decay, random, scatter, dead time, normalization, and attenuation correction.

[18F]-Florbetaben PET analyses

Qualitative analysis: As the current gold-standard, visual readings of the [18F]-Florbetaben (FBB) scans were conducted by two experienced readers (HB, FG) according to manufacturer guidelines [69], resulting in a consensus rating of amyloid positivity ($SCD_{A\beta+}$) and amyloid negativity ($SCD_{A\beta-}$) which was used for group definition. In brief, readers evaluated whether significant cortical tracer binding was found across the majority of slices within any of the four predefined regions of interest (frontal, lateral temporal, parietal, and posterior cingulate/precuneus). Cases were rated amyloid positive if cortical tracer binding was observed in at least one of these regions.

Quantitative analysis: To explore potential linear relationships between the severity of cortical amyloid load and rs-fMRI measures, we conducted secondary quantitative analyses. Using the PNEURO Maximum Probability Atlas pipeline in PMOD 4.004 (PMOD Technologies LLC, Zurich, Switzerland), PET images series were motion-corrected and coregistered with a T1-weighted anatomical scan of the participant (see below) which was segmented using Unified Segmentation [70]. Normalization parameters from this segmentation were used to warp the AAL atlas

template [71] into participants' native PET space, where the transformed AAL volume of interest (VOI) definitions were additionally masked by thresholding the individual gray matter (GM) and cerebrospinal fluid (CSF) probability maps. Within each VOI, standard uptake values (SUV) were averaged across voxels and time frames. Similar to Barthel et al. [72], a volume-weighted average of bilateral frontal, lateral temporal, parietal, occipital and cingulate SUV was calculated, and scaled by tracer uptake in the cerebellar cortex to derive a global SUV ratio ($SUVR_{FBB}$) for measuring global A β load. The same procedure was applied to left and right precuneus VOI to derive regional $SUVR_{FBB}$ for the bilateral precuneus.

MRI acquisition

MRI data acquisition was performed on 3 Tesla Siemens scanners at the participating study sites (two TrioTim, three Verio, two Skyra, and one Prisma), using 32- (two scanners: 20-) channel head coils, with harmonized sequence parameters. The rs-fMRI data were acquired axially using an echo-planar imaging (EPI) sequence with the following sequence parameters: TR/TE = 2580/ 30 ms, flip angle: 80°, field of view = 224 x 224 mm², resolution = 64 x 64 matrix, number of slices = 47, slice thickness = 3.5 mm, total of 180 volumes, acquisition time approximately 8 min. During the examination, subjects were instructed to hold still, keep their eyes closed, not to fall asleep, and not to think of anything in particular. For registration purposes, T1-weighted magnetization-prepared rapid gradient echo sequence (MPRAGE) sequences were acquired (TR: 2500ms, TE: 4.37ms, flip angle: 7°, TI: 1100ms, GRAPPA = 2, 256 x 256 matrix, FOV: 256 x 256 mm², slice thickness: 1mm, 192 sagittal sections, no gap).

rs-fMRI data preprocessing

The rs-fMRI data preprocessing steps were conducted using the Data Processing & Analysis for Brain Imaging (DPABI) toolbox [73] in Matlab 2015a (MathWorks, Inc., Natick, MA). The first 5 EPI volumes were discarded to account for transient signal changes before magnetization reached a steady-state. The remaining 175 EPI volumes were corrected for different signal acquisition times and head movements. The T1-weighted images were co-registered to the mean of the realigned functional images series, and segmented into GM, white matter (WM) and CSF [70]. Nuisance covariates including 24 motion parameters [74], WM and CSF mean time course signals were regressed out. In addition, a motion scrubbing regressor method [75] was used for each bad time point (frame-wise displacement > 0.2 mm included as covariate). The images were normalized into ICBM-152 reference space using DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra: [76]). According to previous studies, spatial smoothing was performed with a Gaussian kernel of 6 mm full-width at half-maximum (FWHM) before fALFF and FC calculation [45], but after ReHo and degree centrality (DC) calculation [77]. Finally, temporal band-pass filtering (0.01–0.08 Hz) was adopted to reduce the effect of low-frequency drifts and high-frequency physiological noise before seed-based FC, ReHo and DC calculation, but after fALFF calculation.

Regional Homogeneity (ReHo) calculation

ReHo represents a voxel-based measure of brain activity which evaluates the local synchronization between the time series of a given voxel and its nearest neighbors [43]. We used DPABI to obtain each subject's ReHo map by calculating Kendall's coefficient of concordance for a given voxel time series with its nearest 26 neighboring

voxels. This analysis was based on the unsmoothed preprocessed images [43]. To improve comparability between subjects, standard normal z-transformation was applied to all ReHo maps. Finally, these 'zReHo maps' were spatially smoothed with a 6 mm FWHM Gaussian kernel for the following statistical analysis.

Fractional ALFF calculation

ALFF is defined as the total power within the low frequency range (0.01-0.08 Hz) and represents the strength or intensity of low frequency oscillations (LFO), while fALFF is defined as the power within the low-frequency range divided by the total power in the entire detectable frequency range, and represents the relative contribution of specific LFO to the whole frequency range [78]. Because fALFF is assumed to be less sensitive to physiological noise [45], we considered this metric as our primary outcome for this analysis. Individual fALFF maps were calculated using DPABI, as described previously [44]: The functional time series of each voxel was transformed to the frequency domain using a fast Fourier transform algorithm, and the power spectrum was obtained. As the power of a given frequency is proportional to the square of the amplitude of this frequency component, the square root was calculated at each frequency of the power spectrum and the averaged square root was obtained across 0.01–0.08 Hz at each voxel. fALFF was calculated using the ratio of power spectrum of low-frequency (0.01 Hz to 0.08 Hz) to that of the entire frequency range. For statistical analysis, standard normal z-transformation was applied to generate zfALFF maps.

Degree Centrality (DC) calculation

DC represents the number of direct connections for a given voxel in voxel-based graphs [41] and has been widely used to represent the node property of large-scale brain networks. DC maps were calculated as described previously [79] using DPABI. Specifically, we calculated Pearson's correlations between the time course of any pair of voxels within the whole brain to generate the functional connectivity matrix. We obtained each subject's undirected adjacency matrix by thresholding each correlation at $r > 0.25$, which is the default setting for DC map calculation in DPABI, in order to exclude voxels that had low temporal correlation attributable to signal noise [41]. Previous studies reported that different threshold selections did not qualitatively change results [16, 41]. Moreover, only positive Pearson correlation coefficients were considered due to the uncertain interpretability of negative correlations. Standard normal z transformation was applied to each DC map to generate the zDC map. Finally, the zDC maps were spatially smoothed with a 6 mm FWHM Gaussian kernel for the following statistical analysis.

Voxel-based Bilateral Precuneus FC Analysis

FC analysis of the bilateral precuneus was conducted in voxel-wise manner. The seed region, bilateral precuneus, was generated from the AAL atlas [71], multiplied with the GM mask. Voxel-wise FC map was calculated using the correlations between the mean time series of the seed region and remaining voxels within the brain. To improve the normality of FC correlation coefficient maps, the correlation coefficients were converted to z values using Fisher's transformation.

Statistical analyses

Statistical analyses were performed with SPM12 (Wellcome Department of Cognitive Neurology, London) and SPSS 22.0 (IBM Corp.: Armonk, NY). For background characteristics of the sample, group differences were assessed using independent sample t-, Mann-Whitney U-, or chi-squared tests ($p < 0.05$). For the voxel-wise FC, ReHo, ALFF/fALFF and DC differences between groups, a second-level two-sample t-test was performed on the individual maps in a voxel-by-voxel manner with an explicit GM mask. The GM mask is generated by thresholding (cutoff = 0.2) the GM probability map in SPM12. Considering the small sample size of this novel study, which limits statistical power and increases the risk of false negative findings [80], we supplemented basic analyses (using a stringent cluster-defining voxel threshold of $p < .001$, with $p < 0.05$, family-wise error (FWE) correction at cluster level) with an exploratory follow-up analysis using a more liberal statistical threshold (cluster-defining voxel-wise $p < .01$, $p < 0.05$, FWE at cluster level). Based on the results of the voxel-wise analysis, we extracted the mean FC value from significant clusters showing significant group differences. Then we calculated partial correlations between mean FC values in clusters showing significant group differences between $SCD_{A\beta+}$ and $SCD_{A\beta-}$ and (i) precuneus and (ii) global $SUVR_{FBB}$, respectively. Finally, to explore the potential functional relevance of FC group differences in these clusters, we calculated analogous partial correlations with MMSE and ADASCog performance.

RESULTS

Sample characteristics and Amyloid SUVRs

Descriptive statistics of background characteristics for the $SCD_{A\beta+}$ and $SCD_{A\beta-}$ samples are presented in Table 1, showing no significant group differences, except for a higher proportion of APOE $\epsilon 4$ carriers in the $SCD_{A\beta+}$ (see also: Supplementary Table 2). As expected, the global $SUVR_{FBB}$ and the regional precuneus $SUVR_{FBB}$ was significantly higher in the $SCD_{A\beta+}$ compared to the $SCD_{A\beta-}$ group. Yet, we note that several participants in both subgroups showed global SUVR values borderline to previous FBB SUVR cut-off definitions for amyloid positivity, resulting in slightly varying amyloid-positivity classifications. Using $SUVR_{global} = 1.39$ from Barthel et al. [72], $N=1$ $SCD_{A\beta+}$ would be classified amyloid-negative, while $N=5$ $SCD_{A\beta-}$ would be classified amyloid-positive.

[Place Table 1 about here]

Group comparison: Local rs-fMRI metrics

The voxel-based analysis revealed no significant differences on any of the local metrics between the two groups using the stringent statistical threshold. Under the exploratory liberal threshold (voxel-wise $p < .01$, $p < .05$ FWE cluster-level), the voxel-based ReHo analysis revealed significantly higher ReHo in the bilateral precuneus and the adjacent superior parietal lobule in the $SCD_{A\beta+}$, as compared to the $SCD_{A\beta-}$ group (Table 2, Figure 1). On the other hand, no significant group differences were found in fALFF.

[Place Table 2, Figure 1 about here]

Group comparison: Global rs-fMRI metrics

The voxel-based analysis revealed no significant differences on any of the global metrics between the two groups using the stringent statistical thresholding. Under the exploratory liberal threshold (voxel-wise $p < .01$, $p < .05$ FWE cluster-level), voxel-wise FC analysis revealed that the $SCD_{A\beta+}$ group showed a higher FC between the precuneus mean time course and occipital regions, including the superior occipital gyrus and the bilateral cuneus (Table 3, Figure 2). No significant group differences were found in DC measures.

[Place Table 3, Figure 2 about here]

Possible influence of nuisance variables:

To test for possible effects of (a) different MR sites on rs-fMRI measures in this multi-center study and (b) the highly significant group differences in APOE genotype, additional second-level two-sample t-tests with MR scanners (dummy-coded) and APOE status ($\epsilon 4+/\epsilon 4-$) as additional covariates were performed. For ReHo, these analyses yielded slightly weaker findings (likely due to the reduced degrees of freedom), but left-sided group differences were still significant (Figure S1) with the liberal statistical threshold (voxel-wise $p < .01$, $p < .05$ FWE cluster-level), indicating that pertinent findings were not primarily influenced by scanner effects or APOE status. As for the precuneus-FC findings, this analysis rendered the reported group differences nonsignificant with the liberal statistical threshold, suggesting that confounding influence of APOE genotype cannot be excluded, as significant group differences were still evident in a separate model controlling for MR scanners only (Figure S2).

Exploratory analyses: Associations of FC differences with quantitative amyloid load

When examining correlations between amyloid SUVR values and FC metrics in the significant clusters, we therefore additionally controlled for APOE status ($\epsilon 4+/\epsilon 4-$) and MR scanners. No significant association was found between the global and/or local precuneus $SUVR_{FBB}$ and the mean z-score of the ReHo extracted from the precuneus cluster that showed the significant group difference between the $SCD_{A\beta+}$ (global $SUVR_{FBB}$: $r_{A\beta+} = -0.14$, $p=0.60$; precuneus $SUVR_{FBB}$: $r_{A\beta+} = 0.05$, $p=0.85$) and $SCD_{A\beta-}$ (global $SUVR_{FBB}$: $r_{A\beta-} = -0.04$, $p=0.89$; precuneus $SUVR_{FBB}$: $r_{A\beta-} = 0.05$, $p = 0.86$) groups.

On the other hand, the mean z-score of the FC extracted from the superior occipital gyrus and the bilateral cuneus (showing significant group differences) showed positive partial correlations with global $SUVR_{FBB}$ in the $SCD_{A\beta+}$ ($r_{A\beta+} = 0.49$, $p=0.03$) but not in the $SCD_{A\beta-}$ ($r_{A\beta-} = 0.20$, $p=0.48$) group, without a significant difference between the correlation coefficients for the two groups ($z = 1.05$, $p = 0.15$), as well as with the regional precuneus $SUVR_{FBB}$ in both $SCD_{A\beta+}$ ($r_{A\beta+} = 0.45$, $p = 0.05$) and $SCD_{A\beta-}$ ($r_{A\beta-} = 0.49$, $p = 0.03$) individuals, which was not significantly different between groups ($z = 0.16$, $p = 0.44$). Figure 3 provides scatterplots of these associations for the raw values, while additionally visualizing the APOE status of the participants.

[Place Figure 3 about here]

Exploratory analyses: Partial correlations with cognitive performance

Testing possible associations with cognitive performance, the zReHo values in the $SCD_{A\beta-}$ group were negatively correlated with MMSE ($r_{A\beta-} = -0.74$, $p = 0.003$), and

moderately, but non-significantly, with ADAScog-13 scores ($r_{A\beta^-} = 0.41$, $p = 0.13$), indicating that higher ReHo was linked with worse performance, while only weak and nonsignificant partial correlations ($r < .25$, $p > 0.4$) were found for precuneus-occipital zFC. The complementary partial correlations for the $SCD_{A\beta^+}$ group were weak and non-significant ($r_{A\beta^+} \leq |0.1|$, $p \geq 0.7$), except for a moderate, but nonsignificant partial correlation of precuneus-occipital zFC with MMSE ($r_{A\beta^+} = -0.37$, $p = 0.18$).

DISCUSSION

Considering that few studies in the SCD stage had AD biomarker information available to probe its influence on precuneus resting-state connectivity, this study specifically addressed the question how amyloid pathology relates to rs-fMRI FC in SCD patients. Although a variety of publications examined rs-fMRI alterations in SCD populations, the present work is among the first studies which do not only provide background information about the AD neuropathological biomarker status [51], but also used this information for sample stratification [63] and correlational analyses [59, 64]. Based on amyloid PET, we compared precuneus-seed whole-brain FC and additional regional and global rs-fMRI metrics in matched $SCD_{A\beta^+}$ and $SCD_{A\beta^-}$ samples from the PET subcohort of the DELCODE study, and additionally explored potential linear relationships between the rs-fMRI measures and global/ precuneus A β load. It must be noted that significant results only emerged at liberal statistical thresholds, which may relate to the limited statistical power of the available sample size. Considering the local rs-fMRI metrics, a key observation was higher ReHo in bilateral precuneus and adjacent superior parietal areas for the $SCD_{A\beta^+}$ group, as compared to the $SCD_{A\beta^-}$ group (Figure 1), but no significant differences regarding fALFF. Regarding global FC

measures, $SCD_{A\beta+}$, as compared to $SCD_{A\beta-}$ participants, were characterized by higher precuneus FC with occipital regions (Figure 2), but showed no differences for DC measures. Confounding influences of group differences regarding APOE genotype cannot be excluded for the precuneus-occipital FC differences. Yet, the additional observation (Figure 3) that FC between these precuneus and occipital areas showed positive linear associations with global ($SCD_{A\beta+}$) as well as local precuneus amyloid load ($SCD_{A\beta+}$ and $SCD_{A\beta-}$) argues for gradual connectivity changes in SCD populations that are partially related to incipient accumulation of amyloid pathology.

To date, studies examining ReHo in SCD populations were lacking. Therefore, our observation that amyloid-positive SCD patients show higher ReHo in precuneus and superior parietal areas than amyloid-negative SCD patients is a novel finding, suggesting that amyloidosis in this stage is already linked with alterations of this local connectivity marker. The only available study that examined cognitively normal individuals depending on amyloid status [29] reported ReHo decreases in the left precuneus, and increases in the left fusiform gyrus, consistent e.g. with studies in prodromal AD patients [47], but in contrast to our analyses that found higher ReHo in more dorsal aspects of the precuneus. We cannot exclude that our $SCD_{A\beta-}$ group is not completely equivalent with the cognitively normal, but non-SCD $A\beta-$ control subjects in the previous study, i.e. ReHo changes due to other (i.e. non-amyloid) factors in our control group may disguise latent impairments in the $SCD_{A\beta+}$ group. Moreover, the present sample was older, included more male (instead of female) participants, and showed a higher educational level, which may influence resilience mechanisms against amyloidosis. Considering these discrepancies, future studies on local ReHo changes in biomarker-stratified SCD samples should be undertaken.

Our negative fALFF results in AD biomarker-stratified SCD patients are generally consistent with most previous studies in SCD populations without biomarker stratification which provided only limited evidence for replicable changes, especially for ALFF/fALFF reductions in the PCC/precuneus region [56, 60, 62]. This may suggest that reliable patterns of ALFF/fALFF alterations (especially reductions) only emerge later in AD pathogenesis. While ALFF/fALFF alterations show some topographical convergence with ReHo alterations, especially in the precuneus/PCC region, during AD progression, their patterns are not completely overlapping [21, 47, 49], which may indicate varying time courses of the underlying physiological changes, or differential sensitivity e.g. due to dependence on different hemodynamic components of the BOLD response [21], although further research is needed on this issue. Meanwhile, a recent study observed that cognitively normal individuals with pathological CSF amyloid *and* tau levels showed fALFF reductions in PCC/precuneus areas that were significantly impaired in MCI and AD dementia patients [24]. Since we could not clarify whether our participants also showed both AD neuropathological features, this may have contributed to our negative finding.

Previous studies have reported DC alterations in SCD compared to healthy controls [57, 59]. The fact that our biomarker-stratified comparison between SCD_{Aβ+} and SCD_{Aβ-} patients did confirm neither of these DC alteration patterns may suggest that these previous observations are not related to amyloid-specific mechanisms: At least one of these SCD studies suggested a role of CSF tau (instead of amyloid) biomarkers [59], which were not available here. Yet, further replications in larger samples are needed.

The precuneus was of particular interest for this study as this region is known to be vulnerable for early Aβ deposition [18, 31, 32, 41]. Our seed-based analyses

showed greater bilateral precuneus FC with adjacent occipital areas in $SCD_{A\beta+}$ which was also qualified by the positive association between FC in occipital regions and regional precuneus $A\beta$ load. Interestingly, the positive association between precuneus-occipital FC and global $SUVR_{FBB}$ values was only visible in the $SCD_{A\beta+}$ subgroup, while a positive correlation with the local precuneus $SUVR_{FBB}$ also emerged in the $SCD_{A\beta-}$ subgroup. This may indicate that these FC changes are mainly driven by incipient local (i.e., precuneus) amyloid accumulations which are masked in global composites. Complementary evidence for regional specificity comes from a mixed population of SCD and healthy controls [81] who had negative amyloid PET scans (according to visual reading and global cortical $SUVR$ cut-offs) but showed positive correlations between dynamic whole-brain connectivity measures and $SUVR$ values from brain areas with early amyloid accumulation (including PCC and precuneus: see [32]). These relationships were not evident when using complementary $SUVR$ values from an entire DMN, or global cortical mask.

The observed greater precuneus-occipital connectivity is partially consistent with a study [18] that observed stronger precuneus-occipital connectivity in cognitively normal $A\beta+$ participants without SCD. On the other hand, there are similarities with studies in SCD populations without amyloid stratification: For example, there are parallels with a previous study in patients with subjective memory complaints [55] that used independent component analysis (ICA) with dual regression, and observed higher FC within the DMN and medial visual network than healthy elderly without SCD symptoms. In cognitively normal individuals with a family history of AD [58], increased FC of the posterior DMN (i.e., PCC) with the medial temporal memory system was found in participants who were complaining about SCD (as compared to non-complainers). It is noteworthy that our study also found evidence for a positive

relationship with amyloid burden, drawing a closer link with AD pathology than previous SCD-related studies.

At first sight, the observed higher connectivity of the precuneus with the occipital gyrus (which was additionally qualified by the positive association with precuneus A β load), and precuneus ReHo increase, may reflect a local compensatory mechanism that helps to maintain normal behavioral performance in SCD_{A β +} patients, which corresponds to previous suggestions of elevated compensation in preclinical AD [37, 55, 58], where additional neuronal processing is required to balance the brain workload, thereby, maintaining normal cognitive functioning under the influence of increasing amyloid burden. Yet, caution is warranted with this interpretation since the only robust associations with cognitive performance were observed in the amyloid-negative group, and pointed into the opposite direction, i.e. higher connectivity was linked with worse performance, which would favor a dysfunctional upregulation. In a mechanistic sense, A β pathology may also be accelerated by increased metabolism and elevated intrinsic activity / connectivity [19, 25], with laboratory evidence indicating that neuronal activity directly increases production of A β peptides [82]. Considering that the precuneus is a prominent 'hub' in the intrinsic FC matrix of the human brain and metabolically active [83], our finding of higher connectivity and ReHo within this region might indeed trigger (or amplify) A β production in SCD_{A β +}. Meanwhile, it is possible that the causal relationships between resting-state connectivity and amyloid production vary during the course of AD progression [27].

In this study, we reported significant group differences at a liberal statistical threshold that have to be considered as exploratory findings and should therefore be interpreted cautiously. While the use of liberal voxel-level thresholds is not unusual, especially in the earlier fMRI literature [84], a seminal study [85] that examined the

efficiency of FWE control methods for task-fMRI designs in several software packages pointed out that this practice bears a substantial risk of inflated false-positive findings (i.e. type I errors), which also seems to be influenced by study-specific factors (e.g. physiological noise, voxel resolution, smoothness of data) [86]. Meanwhile, the sample size in this study was relatively small, which may have reduced statistical power to detect the true effects of interest at more stringent statistical thresholds. Some authors have advocated the exploratory use of liberal statistical thresholds to achieve a better balance between type I and type II errors [80], but not without emphasizing the need for replication (and meta-analytic) studies [80]. Thus, further studies with larger sample sizes are warranted.

Some further methodological limitations need to be considered. First, due the significantly higher rate of APOE ϵ 4 carriers in the amyloid-positive group, which is not unexpected given the frequently observed association between APOE genotype and amyloidosis [87], it is difficult to disentangle the relative contributions of amyloid positivity *per se* and APOE genotype, to FC group differences. For example, a recent study [63] did not observe cross-sectional, but widespread longitudinal DMN connectivity changes over 24 months (along with limited decreases in dorsal aspects of the precuneus) in a large cohort of SCD patients which were partly moderated by the APOE status of the patients, even when amyloid status was included as a covariate. While left-sided ReHo group differences in our study survived even after controlling for APOE status (Figure S1), precuneal-FC group differences were rendered non-significant. The available sample size precluded further stratifications according to APOE status. While APOE genotype was shown to have independent effects on FC measures, even in individuals whose amyloid PET was negative [27, 88], its influence may also be indirectly mediated by its effect on amyloid accumulation.

Actually, the observed linear association between quantitative amyloid load, especially in the precuneus, and precuneal FC with the occipital region (Figure 3), would support the latter interpretation. Ideally, the independent effect of APOE genotype would be tested by examining whether these FC alterations are already present in the amyloid-negative APOE ϵ 4 carriers, but the small number of these cases precluded systematic statistical evaluation, but highlights an important research question for future studies. Second, the relatively small sample size in this study limits statistical power. Future studies with larger sample sizes will have to be awaited to validate the findings in this study. Third, the present analyses were restricted to cross-sectional rs-fMRI data, precluding inferences about the longitudinal course of the amyloid-related FC changes [63]. Fourth, we have to acknowledge that the relationship between early brain functional alterations and longitudinal declines in neuropsychological test performance will need to be explored in future studies, as collection of the necessary follow-up data is still ongoing. For example, Buckley et al. [28] reported that lower FC particularly of DMN, salience, and control networks predicted more rapid cognitive decline in normal older adults with increased A β burden, suggesting that FC measures add predictive information about cognitive trajectories. Fifth, we had no access to a healthy control group with (PET-) proven amyloid-negativity to characterize relative FC differences in SCD_{A β +}, and to make clear statements whether FC increases reflect a state of true hyperconnectivity. Sixth, recent evidence for interaction effects regarding sex [89] could not be tested systematically due to the limited sample size. Yet, our comparison of SCD_{A β +} with sex-matched SCD_{A β -} participants assured that this potential confounds could not bias group comparisons. Seventh, visual reading vs. application of previously established global SUVR cut-offs does not result in entirely consistent amyloid status classifications. In general, recent data indicate that the correspondence

between FBB visual reading and classification with global SUVR cut-offs is high (>85%), yet not perfect [90]. Moreover, the global SUVR for a substantial number of participants were in proximity around the respective cut-off values, suggesting a continuum of intermediate amyloid deposition states which are more difficult to classify reliably. On the other hand, this observation provides further support for our exploratory linear correlation analyses with the SUVR measures. Finally, we did not have access to tau-PET data in this cohort and we are, thus, not able to unravel whether precuneus FC is affected additionally by elevated tau, as hypothesized by previous work [39]. Therefore, further studies will have to be awaited to further explore the FC changes in SCD with complementary amyloid and tau biomarker information.

CONCLUSIONS

In summary, our study in well-matched SCD cohorts differing in PET-proven cerebral amyloid pathology provides an important addition to the previous literature, namely by providing preliminary evidence for higher local precuneus (ReHo) and global (precuneus-based FC with occipital areas) FC measures in SCD_{Aβ+} compared to SCD_{Aβ-} individuals. Our results indicate vulnerability of precuneal neural activity in the SCD stage with positive biomarker evidence for amyloid pathology (NIA-AA stage 2 of the clinical AD continuum) and suggest FC changes in this important hub region to be directly linked to concurrent amyloid pathology. Resting-state FC appears to be a useful neuroimaging biomarker for highlighting early brain functional consequences of early AD pathology.

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Conflict of Interest/Disclosure Statement

KB is advisor for Biogen GmbH. The other authors report no conflicts of interest relevant for this study.

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Table 1: Sample characteristics and regional amyloid tracer uptake for the amyloid-positive and -negative subgroups

	SCD _{Aβ+}	SCD _{Aβ-}	Test Statistics	P value
Age (yrs)	74.54 ± 4.40	74.04 ± 4.13	t (46) = 0.41	ns.
Gender (F/M)	9/15	9/15	χ ² (1) = 0.00	ns.
Education (yrs)	14.42 ± 2.78	14.96 ± 3.36	t (46) = 0.61	ns.
MMSE	29.13 ± 0.90*	29.00 ± 1.44*	U (46) = 247.50	ns.
ADAS-cog13	8.38 ± 3.68	7.25 ± 3.27	t (46) = 1.12	ns.
APOE(ε4/non-4)	17/6*	6/17*	χ ² (1) = 10.52	0.001
Global SUVR_{FBB}	1.79 ± 0.25	1.32 ± 0.10	t (46) = 8.38	<0.001
Precuneus SUVR_{FBB}	1.92 ± 0.31	1.29 ± 0.07	t (46) = 9.74	<0.001

*: Denotes 1 missing value.

Abbreviations: SCD_{Aβ+} = Amyloid-positive subjective cognitive decline group, SCD_{Aβ-} = amyloid-negative subjective cognitive decline group; ns. = Nonsignificant; U= Whitney U value; MMSE Mini-Mental -State Examination; ADAS-cog13 Alzheimer’s Disease Assessment Scale—cognitive part; APOE: Apolipoprotein; SUVR=Standard Uptake Value Ratio with cerebellar cortex as reference region; FBB= [18F]-Florbetaben. Continuous data are presented as means ± standard deviations.

Table 2: Brain regions showing increased regional homogeneity in amyloid-positive compared to amyloid-negative SCD participants

Cluster Size	P value (FWE)	Brain Regions	Peak MNI Coordinate			Peak T Value
			x	y	z	
270	0.034	Right precuneus	6	-54	69	4.34
		Left superior parietal lobule	-18	-54	72	3.75
		Right superior parietal lobule	15	-63	66	3.63

Abbreviations: FWE – Family-wise error. MNI – Montreal Neurological Institute.

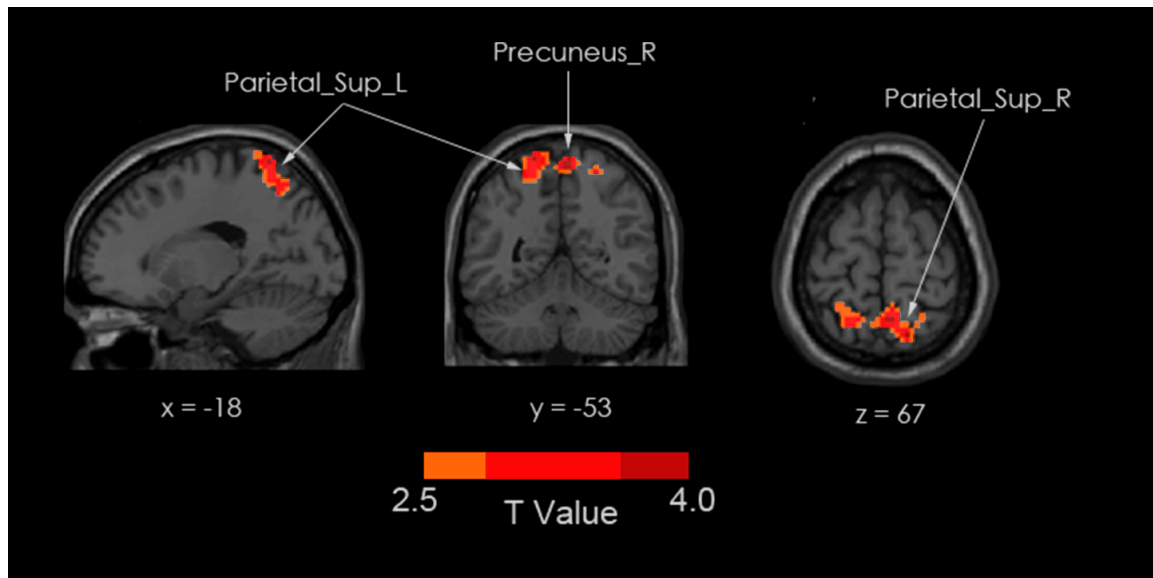
Table 3: Brain regions showing increased precuneus-based functional connectivity in amyloid-positive compared to amyloid-negative SCD participants

Cluster Size	P value (FWE)	Brain Regions	Peak MNI Coordinate			Peak T Value
			x	y	z	
296	< 0.001	Right middle occipital gyrus	27	-75	30	3.95
		Right cuneus	12	-87	42	3.62
		Right superior occipital_gyrus	27	-84	21	3.17

Abbreviations: FWE – Family-wise error. MNI – Montreal Neurological Institute.

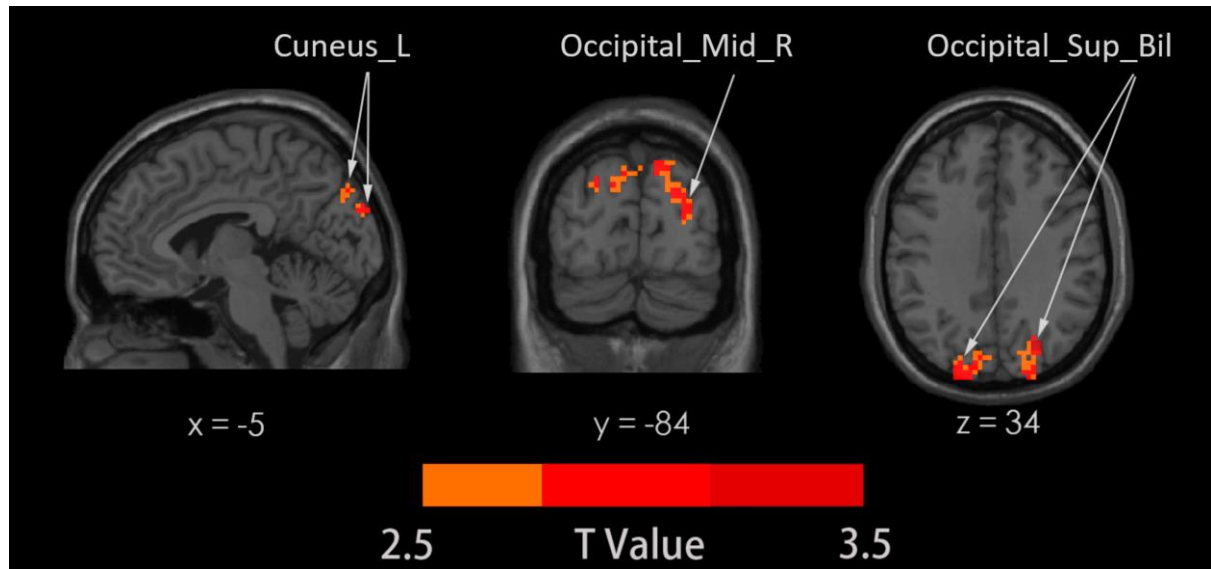
Figures:

Figure 1. Brain regions showing increased ReHo in SCD_{Aβ+} compared to SCD_{Aβ-}



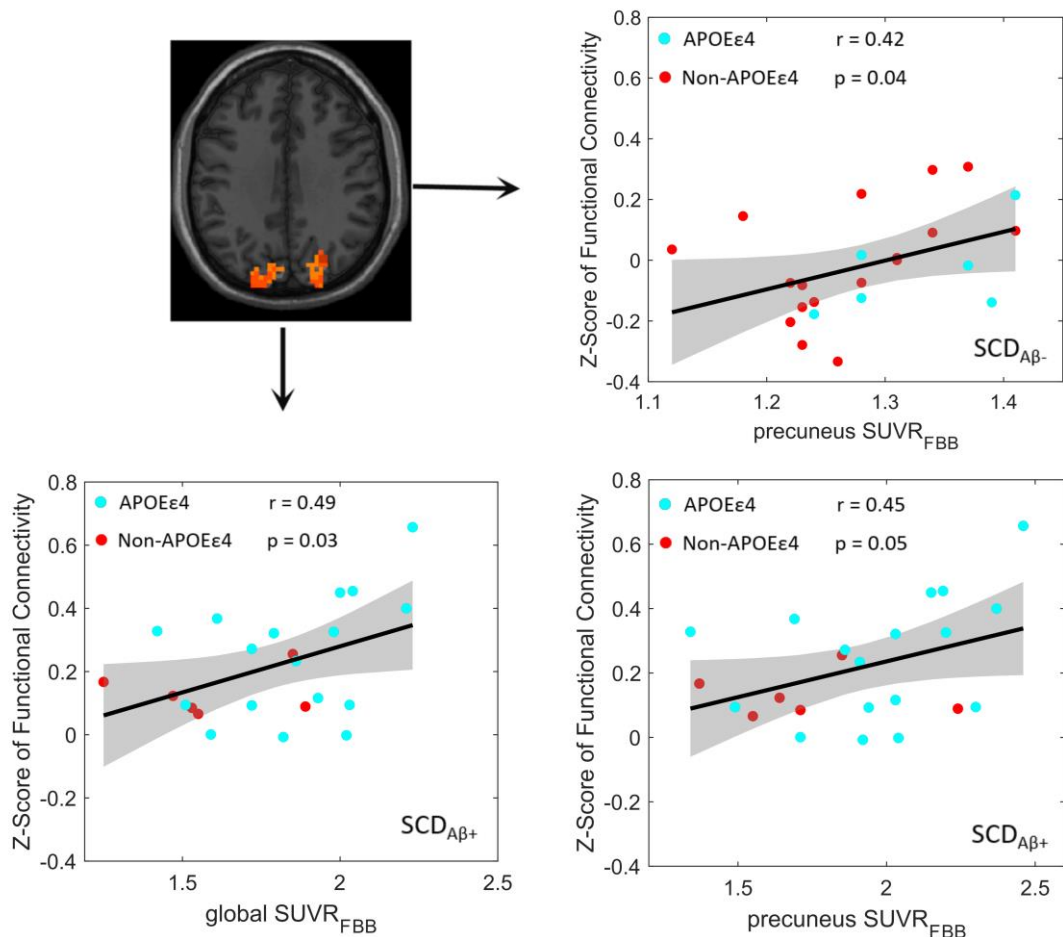
The figure shows the regions with significant increased ReHo value in SCD_{Aβ+} compared to SCD_{Aβ-} ($p < 0.05$, FWE cluster-level). The color bar represents the heights of suprathreshold t-values. L: Left; R: Right; Sup: Superior.

Figure 2. Brain regions showing increased precuneus-based FC in $SCD_{A\beta+}$ compared to $SCD_{A\beta-}$



The figure represents increased voxel-wise FC of the precuneus seed region with the cuneus and superior/middle occipital regions in the $SCD_{A\beta+}$ group ($p < 0.05$, FWE cluster-level). The color bar represents the height of suprathreshold t-values. L: Left; R: Right; Bil: Bilateral; Sup: Superior. Mid: Middle.

Figure 3. Positive association between precuneus-occipital FC and global SUVR_{FBB} and precuneus SUVR_{FBB}



The grey regions are 95% confidential intervals. The mean Z-Score of the FC was extracted from significant cluster within the red area. Abbreviations: SCD_{Aβ+} - amyloid-positive SCD; SCD_{Aβ-} - amyloid-negative SCD; SUVR=Standard Uptake Value Ratio (with cerebellar cortex as reference region). The scatterplots show raw data, additionally coded by APOE ε4 genotype to allow for visual inspection. Correlation coefficients represent partial correlations after controlling for APOE status and MR scanners. There are 23 datapoints in each group since one individual in each group miss APOE information.