

I. Introduction

- Alzheimer's disease (AD) affects gray matter (GM) and white matter (WM) as a connected structural system, but most diffusion MRI studies evaluate GM and WM abnormalities separately.
- Free water (FW) is sensitive to extracellular water and tissue microstructural disruption, and may capture coordinated GM-WM vulnerability across disease stages.
- We tested **whether regional GM FW and anatomically adjacent WM FW become more tightly coupled across clinically defined stages of the AD continuum.**

II. Methods

Participants

- A total of 496 participants** were recruited from the dementia clinic at Seoul National University Bundang Hospital (SNUBH) and from individuals enrolled in the Korean Longitudinal Study on Cognitive Aging and Dementia (KLOSCAD)
- Clinical diagnosis groups were defined following the diagnostic staging framework used in a prior JKMS free-water study: cognitively normal amyloid-negative participants (CN A-), cognitively normal amyloid-positive participants corresponding to preclinical AD (CN A+), amyloid-positive mild cognitive impairment corresponding to prodromal AD (MCI A+), and amyloid-positive dementia due to AD (ADD).

Study design

- The **primary analysis used a 3-group definition by combining the two non-demented amyloid-positive stages:** CN A- (n=191), non-demented A+(n=154; 46 CN A+ and 108 MCI A+), and ADD (n=151). Sensitivity analysis retained the 4-group clinical diagnosis definition: CN A-, CN A+, MCI A+, and ADD.
- FW was estimated using a bi-tensor model from diffusion MRI data. Structural covariance was operationally defined as the covariate-adjusted partial correlation between regional GM FW and anatomically adjacent WM FW across individuals within each group.
- We analyzed three region pairs: **PCC-Cingulum, Temporal-Entorhinal, and Precentral-WM**, controlling for age, sex, education, white matter hyperintensity volume, and global amyloid PET SUVR.
- Group differences in coupling were tested using moderated regression (WM FW ~ GM FW * group + covariates), with bootstrap confidence intervals and permutation testing.

III. Results

Demographic and clinical characteristics

Table 1. Demographic and clinical characteristics				
Variable	CN A- (n=191)	non-demented A+ (n=154)	Demented A+ (n=151)	p
Age, years	72.7 ± 6.5	75.5 ± 6.7	75.8 ± 7.8	<0.001
Education, years	12.9 ± 4.3	11.4 ± 5.1	11.4 ± 4.9	0.002
WMH/ICV	0.0045 ± 0.0057	0.0089 ± 0.0106	0.0095 ± 0.0095	<0.001
Global amyloid SUVR	0.63 ± 0.11	1.39 ± 0.26	1.50 ± 0.24	<0.001
MMSE	28.2 ± 1.7	25.1 ± 3.7	19.0 ± 4.8	<0.001
Female (sex=1), n (%)	104 (54.5%)	91 (59.1%)	100 (66.2%)	0.087
APOE ε4 carrier, n/N (%)	40/183 (21.9%)	81/152 (53.3%)	83/149 (55.7%)	<0.001

Continuous variables were compared using one-way ANOVA; categorical variables were compared using chi-square tests. All p values are two-sided; p < 0.05 was considered statistically significant.

Stage effects on individual GM and WM FW levels

- Adjusted FW levels increased across clinical stages in several GM and WM regions, especially in the temporal-entorhinal pair.**

Table 2. Stage effects on individual GM and WM Free Water levels						
ROI pair	Tissue	CN A-	non-demented A+	demented A+	p	Bonf p
PCC-Cingulum	GM	0.3706	0.4134	0.4405	0.002	0.014
	WM	0.2936	0.3305	0.3518	0.023	0.140
Temporal-Entorhinal	GM	0.4191	0.4766	0.5218	<0.001	0.002
	WM	0.2349	0.274	0.3142	<0.001	<0.001
Precentral-WM	GM	0.4203	0.4327	0.4478	0.012	0.074
	WM	0.1777	0.1849	0.1902	0.173	1

ANCOVA model: FW level ~ group + age + sex + education + WMH/ICV + global amyloid SUVR. Bonf p indicates Bonferroni correction across 6 FW-level tests (3 ROI pairs x 2 tissues). Statistical significance was defined as p < 0.05

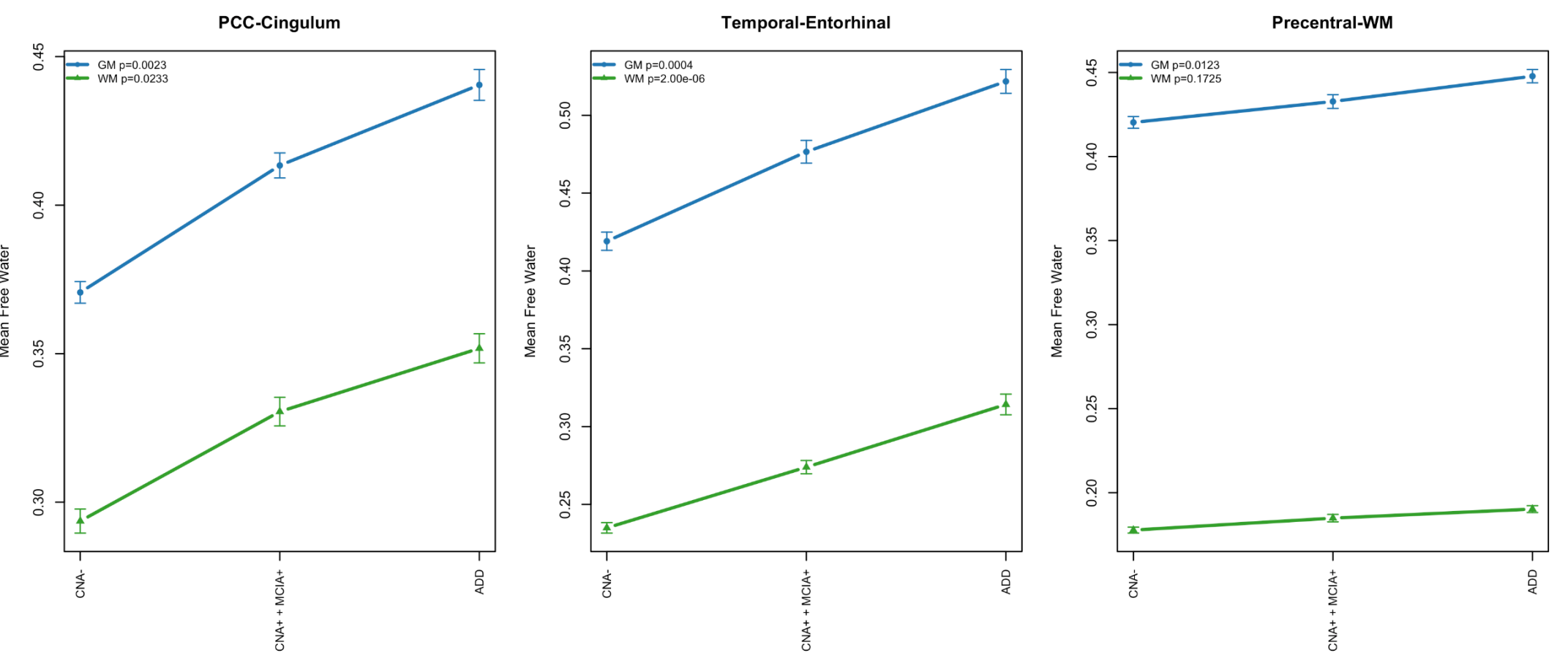


Figure 1. FW Levels by Group
Mean GM and WM Free Water (FW) levels are shown for each ROI pair across the primary clinical groups.

GM-WM FW coupling and stage interaction

- Stage-dependent gray-white FW coupling was **specific to the temporal-entorhinal system.**

Table 3. GM-WM FW coupling and stage interaction						
ROI pair	CN A- r [95% CI]	non-demented A+ r [95% CI]	demented A+ r [95% CI]	Slopes	p	Bonf p
PCC-Cingulum	0.61 [0.50, 0.69]	0.58 [0.47, 0.67]	0.60 [0.48, 0.69]	0.69/0.65/0.56	0.470	1
Temporal-Entorhinal	0.55 [0.41, 0.68]	0.57 [0.40, 0.70]	0.74 [0.63, 0.82]	0.48/0.47/0.90	<0.001	<0.001
Precentral-WM	0.66 [0.57, 0.73]	0.61 [0.49, 0.71]	0.55 [0.44, 0.65]	0.68/0.63/0.57	0.779	1

r indicates covariate-adjusted within-group partial correlation between regional GM FW and adjacent WM FW. 95% CI was estimated using bootstrap resampling (B=2000). Bonf p indicates Bonferroni correction across 3 ROI pairs. Statistical significance was defined as p < 0.05; corrected significance was defined as Bonf p < 0.05.

- In Temporal-Entorhinal pair, **demented A+ showed stronger coupling** than CN A- (p=0.004) and than the non-demented A+ group by FDR correction (p=0.010).
- In contrast, PCC-Cingulum and Precentral-WM showed no significant group-related divergence.

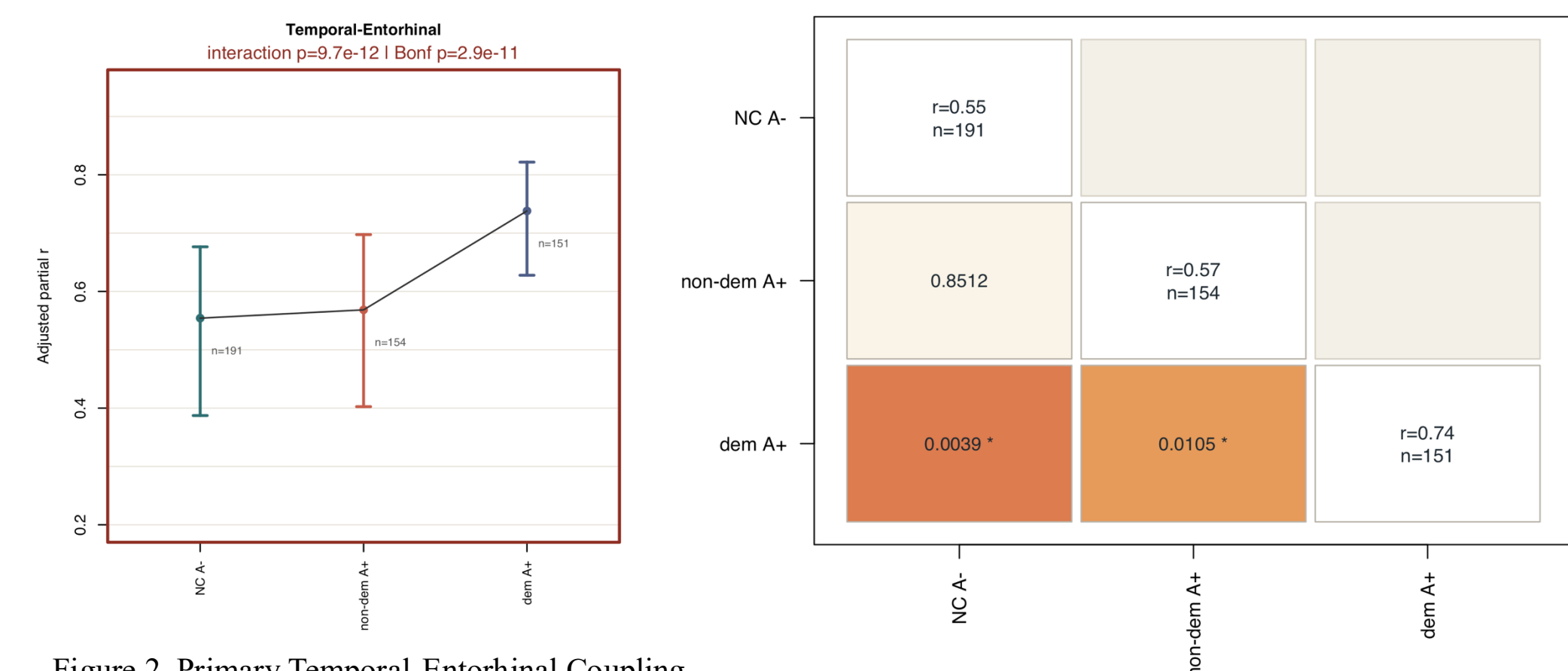


Figure 2. Primary Temporal-Entorhinal Coupling
Adjusted partial correlations between entorhinal GM FW and adjacent temporal WM FW are shown across the primary 3-group clinical definition. Error bars indicate bootstrap 95% confidence intervals. The group interaction was tested using moderated regression on adjusted for age, sex, education, WMH/ICV, and global amyloid SUVR. Pairwise p values compare covariate-adjusted partial correlations using Fisher r-to-z tests. * p < 0.05.

4-group sensitivity analysis

- The Temporal-Entorhinal interaction remained robust, with partial correlations of r=0.55 in CN A-, r=0.67 in CN A+, r=0.46 in MCI A+(MCI), and r=0.74 in demented A+(ADD).

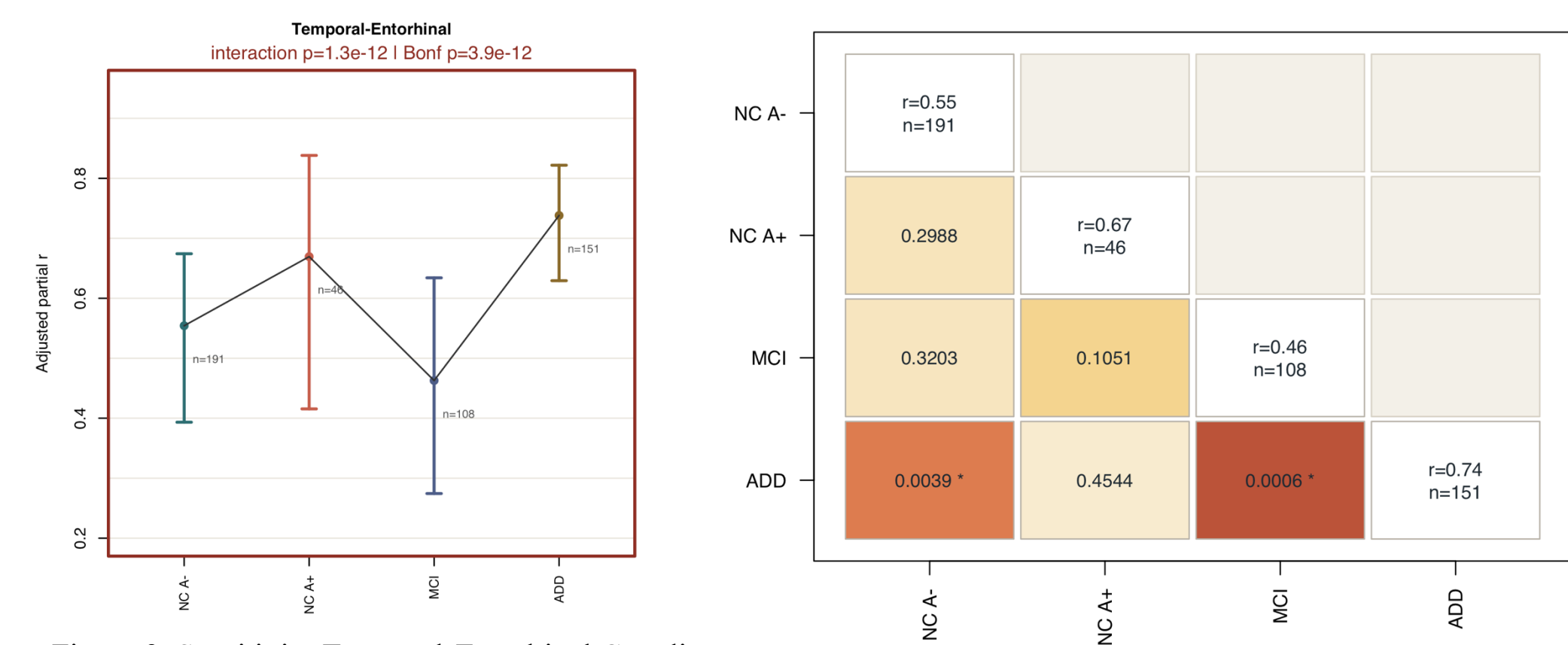


Figure 3. Sensitivity Temporal-Entorhinal Coupling
Sensitivity analysis separating the non-demented amyloid-positive group into CN A+ and MCI. Adjusted partial correlations between entorhinal GM FW and adjacent temporal WM FW are shown for the 4 clinical groups. Error bars indicate bootstrap 95% confidence intervals. The group interaction was tested using moderated regression adjusted for age, sex, education, WMH/ICV, and global amyloid SUVR. Pairwise p values compare covariate-adjusted partial correlations using Fisher r-to-z tests. * p < 0.05.

IV. Conclusions

- Our findings demonstrate **accelerated GM-WM pathological synchronization in the entorhinal cortex specifically at the dementia stage.**
- This hyper-coupling remained evident after adjustment for global amyloid burden and was preserved when the non-demented amyloid-positive group was separated into CN A+ and MCI A+ stages.
- The absence of comparable coupling changes in PCC-Cingulum and Precentral-WM suggests that **this phenomenon reflects a regionally selective medial temporal GM-WM coupling abnormality** rather than a global increase in FW levels.
- Temporal-Entorhinal FW coupling may serve as an imaging marker for tracking clinical progression in AD.

Acknowledgement

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