

# Influence of the APOE $\epsilon$ 4 Allele and Mild Cognitive Impairment Diagnosis in the Disruption of the MEG Resting State Functional Connectivity in Sources Space

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Handling Associate Editor: Susana Cid Fernández

Accepted 2 September 2014

**Abstract.** The apolipoprotein E (APOE)  $\epsilon$ 4 allele constitutes the major genetic risk for the development of late onset Alzheimer's disease (AD). However, its influence on the neurodegeneration that occurs in early AD remains unresolved. In this study, the resting state magnetoencephalography (MEG) recordings were obtained from 27 aged healthy controls and 36 mild cognitive impairment (MCI) patients. All participants were divided into carriers and non-carriers of the  $\epsilon$ 4 allele. We have calculated the functional connectivity (FC) in the source space along brain regions estimated using the Harvard-Oxford atlas and in the classical bands. Then, a two way ANOVA analysis (diagnosis and APOE) was performed in each frequency band. The diagnosis effect consisted of a diminished FC within the high frequency bands in the MCI patients, affecting medial temporal and parietal regions. The APOE effect produced a decreased long range FC in delta band in  $\epsilon$ 4 carriers. Finally, the interaction effect showed that the FC pattern of the right frontal-temporal region could be reflecting a compensatory/disruption process within the  $\epsilon$ 4

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allele carriers. Several of these results correlated with cognitive decline and neuropsychological performance. The present study characterizes how the APOE  $\epsilon 4$  allele and MCI status affect the brain's functional organization by analyzing the FC patterns in MEG resting state in the sources space. Therefore a combination of genetic, neuropsychological, and neurophysiological information might help to detect MCI patients at higher risk of conversion to AD and asymptomatic subjects at higher risk of developing a manifest cognitive deterioration.

Keywords: Aging, APOE  $\epsilon 4$ , functional connectivity, magnetoencephalography, mild cognitive impairment, source analysis

## INTRODUCTION

The apolipoprotein E (APOE)  $\epsilon 4$  allele may induce neuropathology through several cellular pathways, not only through amyloid- $\beta$  ( $A\beta$ ) aggregation [1]. This allele has two major effects: 1) an increased risk of late-onset Alzheimer's disease (AD) in the Caucasian population by a factor of 3 to 12; and 2) a reduction of the age of AD onset by 10 to 20 years in APOE  $\epsilon 4$  heterozygote and homozygote, respectively [2]. In addition, carrying the  $\epsilon 4$  allele produces an increased risk of conversion to AD in patients at earlier stages of the degenerative process (e.g., mild cognitive impairment (MCI)) [3]. However, in addition to its influence on AD, the  $\epsilon 4$  allele may have intrinsic effects on brain function [4].

The functional connectivity (FC) [5] considers the brain to be a complex neural network and brain diseases to be a network disruption process. Thus, the progressive disturbance of synaptic transmission, the loss of neurons, or the  $A\beta$  accumulation are some of the biological processes present in AD that affect the neural network. In resting state brain activity, there is a set of regions strongly connected representing the locations in which  $A\beta$  first accumulates; the default mode network (DMN) [6]. The analysis of the DMN in AD and MCI by means of functional magnetic resonance imaging (fMRI) has shown a reduced FC in both groups [7–10]. In addition, magnetoencephalography (MEG)/electroencephalography (EEG) studies have reported neural network disruption in AD and MCI [11–14], especially in alpha and beta bands. These results agree with the view of AD as a “disconnection syndrome [15]” and stress the role of MEG/EEG as an important tool to study the synaptic disruption present in the preclinical stage of AD [16].

Regarding the  $\epsilon 4$  allele, in healthy aged population, a recent fMRI study [17] has shown diminished FC in posterior regions of the DMN and enhanced FC in frontal-parietal regions within  $\epsilon 4$  carriers. These subjects showed a decreased DMN FC even in the absence

of amyloid deposition or cognitive symptoms [18]. To the best of our knowledge, there is not any study including healthy controls (HC)  $\epsilon 4$  carriers and only one [13], comparing the FC of amnesic MCI (aMCI) carriers and non-carriers of the  $\epsilon 4$  allele. Considering the above, our study is the first neurophysiological investigation that analyzes the FC differences in different frequency bands in source space. Our study has been carried out with HC and aMCI patients divided into carriers and non-carriers of the  $\epsilon 4$  allele. We hypothesize that the  $\epsilon 4$  allele will induce changes in the network configuration with a different profile in HC and MCI patients.

## MATERIALS AND METHODS

### *Subjects*

63 Caucasian subjects (27 controls (HC) and 36 MCI patients) were recruited from the “Hospital Universitario San Carlos”, the “Memory Decline Prevention Center”, and the “Seniors Center of Chamartin District” of Madrid. All of them were right-handed and native Spanish speakers. No significant differences ( $p > 0.05$ ) were found in educational level, gender, and age among groups. See Table 1 for the demographic description.

### *Diagnostic criteria*

All participants were screened by means of a variety of standardized diagnostic instruments and received a neuropsychological assessment as described in Cuesta et al. [19]. The MCI diagnosis was established following NIA-AA criteria [20], which includes: (a) self- or informant-reported cognitive complaint; (b) objective evidence of impairment in one or more cognitive domains; (c) preserved independence in functional abilities; and (d) not demented. Besides from meeting the Core Clinical Criteria for MCI, MCI patients had a positive biomarker reflecting neuronal injury:

Table 1

Demographic description. APOE  $\epsilon$ 3, APOE 3/3 genotype; APOE  $\epsilon$ 4, APOE 3/4 genotype; M, males; F, females; MMSE, Mini-Mental State Examination; n trials, number of clean 4 seconds segments employed in the analysis. RHV (right hippocampal volume) and LHV (left hippocampal volume) were normalized with the overall intracranial volume. Educational level was grouped into five levels: 1) Illiterate, 2) Primary studies, 3) Elemental studies, 4) High school studies, and 5) University studies

	Control APOE $\epsilon$ 3	Control APOE $\epsilon$ 4	MCI APOE $\epsilon$ 3	MCI APOE $\epsilon$ 4	ANOVA p value
n	19	8	20	16	
Age	70 $\pm$ 4	70 $\pm$ 4	72 $\pm$ 5	72 $\pm$ 3	0.28
Genderratio (M/F)	8/11	3/5	8/12	8/8	0.92
MMSE score	29.4 $\pm$ 0.7	29.5 $\pm$ 0.5	27.4 $\pm$ 2.4	27.4 $\pm$ 3.0	0.0014
Educational level	3.7 $\pm$ 1.3	3.9 $\pm$ 0.9	3.3 $\pm$ 1.2	3.0 $\pm$ 1.1	0.21
RHV	0.0025 $\pm$ 0.0003	0.0026 $\pm$ 0.0003	0.0022 $\pm$ 0.0005	0.0021 $\pm$ 0.0005	0.0037
LHV	0.0025 $\pm$ 0.0004	0.0026 $\pm$ 0.0003	0.0022 $\pm$ 0.0005	0.0021 $\pm$ 0.0005	0.0071
n trials	31 $\pm$ 6	31 $\pm$ 7	30 $\pm$ 8	32 $\pm$ 6	0.94

Hippocampal atrophy measured by MRI. Thus, all of them may be considered MCI due to AD-intermediate likelihood.

Finally, according to their clinical and neuropsychological profile, all MCI patients were diagnosed as aMCI, as they exhibited isolated memory impairments. All participants were free of significant medical, neurologic, and/or psychiatric diseases (other than MCI), and none of them were using drugs which could affect MEG activity (including cholinesterase inhibitors). Inclusion criteria included the absence of significant cerebral-vascular disease (modified Hachinski score  $\leq$ 4) or depressive symptomatology (Yesavage's Depression Scale scores  $>$ 9), an age between 65 and 85 years. In addition, a T2-weighted MRI within 12 months before MEG screening without indication of infection, infarction, or focal lesions (rated by two independent experienced radiologists) [21].

Prior to the MEG recording, all subjects signed an informed consent that explained the technical and ethical considerations of the investigation. The study was approved by the local Ethics Committee.

#### APOE genotype test

Genomic DNA was extracted from whole-blood samples of MCI patients and controls. APOE haplotype was determined by analyzing SNPs rs7412 and rs429358 genotypes with TaqMan assays using an Applied Biosystems 7900 HT Fast Real Time PCR machine (Applied Biosystems, Foster City, CA). A genotyping call rate over 90% per plate, sample controls for each genotype and negative sample controls were included in each assay. Three well-differentiated genotyping clusters for each SNP were required to validate results. Intra and interplate duplicates of several DNA samples were included.

#### MRI Acquisition

3D T1 weighted anatomical brain MRI scans were collected with a General Electric 1.5T MRI scanner, using a high-resolution antenna and a homogenization PURE filter (Fast Spoiled Gradient Echo (FSPGR) sequence with parameters: TR/TE/TI = 11.2/4.2/450 ms; flip angle 12°; 1 mm slice thickness, a 256  $\times$  256 matrix and FOV 25 cm). Freesurfer (v5.1.0) was used for sub-cortical segmentation [22]. Hippocampal volumes were normalized with the overall intracranial volume.

#### MEG Acquisition

MEG methods have been published elsewhere [19] and will be summarized here. Three minutes of resting state with eyes closed were recorded at 1000 Hz sampling rate (online bandpass filtering at 0.1–330 Hz) with an ElektaNeuromag MEG system. The MEG system was housed in a magnetically shielded room (VacuumSchmelze GmbH, Hanua, Germany). The head movement was controlled by means of four head-position indicator (HPI) coils attached to the scalp. The position of HPI coils and subject's head-shape relative to three anatomical locations (nasion and both preauricular points) were defined using a 3D digitizer (FastrakPolhemus). Ocular movements were monitored by two bipolar electrodes. MEG signals were filtered and corrected for head movements by means of a temporal signal space separation filter (Maxfilter Software 2.2) [23].

#### Source Analysis

##### MEG preprocessing

Magnetometer data was automatically scanned for artifacts through Fieldtrip software [24]. Artifact-free

data were segmented in continuous 4-s fragments (trials). The power spectra of the survival trials were visually inspected by an experienced technician. Only MEG recordings with at least 15 clean trials were included. The number of clean trials did not differ significantly among groups (see Table 1).

The present study has considered the following frequency bands: Delta (2–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta1 (12–20 Hz), beta2 (20–30 Hz), and gamma (31–45 Hz).

#### *Headmodels and beamforming*

A regular grid of 302 nodes, with 2 cm spacing, was created in the template Montreal Neurological Institute (MNI) brain. This set of nodes was transformed to the subject's space using a non-linear normalization between the native T1 image (whose coordinate system was previously converted to match the MEG coordinate system) and a standard T1 in MNI space. The forward model was solved with the realistic single-shell model introduced by Nolte [25]. Source reconstruction was performed with a Linearly Constrained Minimum Variance beamformer [26]. For each subject, the covariance matrix was first averaged over trials to compute the spatial filter's coefficients, and then these coefficients were applied to individual trials, obtaining a time series per segment and source location.

#### *Functional connectivity and atlas based analysis*

As a measure of the phase synchrony, the Phase Locking value (PLV) [27] was calculated, per each frequency band, among all pairs of 302 nodes. The 302 nodes data set was converted in 30 regions of interest (ROIs) using the Harvard-Oxford probabilistic atlas [28] as described in [19]. The original  $302 \times 302$  PLV matrices were transformed into  $30 \times 30$  matrices. The values in the main diagonal contained the intra-ROI phase coupling.

#### *Statistics*

The statistical procedure consisted of a two level analysis.

#### *Level 1 analysis*

The first level analyzed the ROI's average FC in each frequency band. This average FC value was called *strength*, following the nomenclature of Graphs Theory. Therefore, each subject had, in each frequency band, 30 strength values.

The strength values were transformed with  $x = \log(x/1 - x)$  in order to obtain a normal distribution

[29] and subjected to a statistical analysis in each frequency band. The analysis consisted of a two-way analysis of variance (ANOVA) test, including diagnosis (MCI and HC) and APOE genotype ( $\epsilon 3$ ,  $\epsilon 4$ ). *Post Hoc* pairwise *t*-tests were performed in ROIs with significant ( $p < 0.05$ ) main or interaction effects.

#### *Level 2 analysis*

In this level, the ROIs with significant strength values were further inspected in order to characterize how their FC was affected. For each significant ROI, we analyzed the network composed by its entire dysfunctional links. This network (henceforth called associated network) was composed by the 29 links with the other 29 ROIs and the intra-ROI FC. In order to do this, each of these 30 FC values were transformed with  $x = \log(x/1 - x)$  and subjected to another two-way ANOVA test. In addition, *Post Hoc* pairwise *t*-tests were performed for each link with significant effect ( $p < 0.05$ ).

Besides the FC analysis, the relationship between significant FC values and neuropsychological performance was assessed through Pearson correlation tests, at both levels

In order to control the family-wise error caused by multiple comparisons, a permutation test procedure was utilized for *t*-tests and correlations. This procedure has been fully described elsewhere [19].

## RESULTS

### *Diagnosis Effect*

Significant differences were found only in alpha (Fig. 1) and beta1 (Fig. 2) bands. In both cases the MCI group showed decreased FC, mainly in parieto-temporal areas and hippocampi.

#### *Alpha band strength analysis (Fig. 1, top)*

The MCI group showed decreased strength values in four ROIs: Left supramarginal gyrus (lSMG), rSMG, and both hippocampi.

#### *Alpha band associate network analysis (Fig. 1, bottom)*

The rSMG had seven altered links: Four were bilateral (involving parietal and hippocampal areas) and three were ipsilateral (involving parietal-frontal regions). The lSMG showed four links: Three of them were localized within the left hemisphere (involving left prefrontal cortex (lPFC), left hippocampus (lHip) and Intra lSMG) and one linking the right precentral gyrus (rPreCG). The hippocampal networks showed

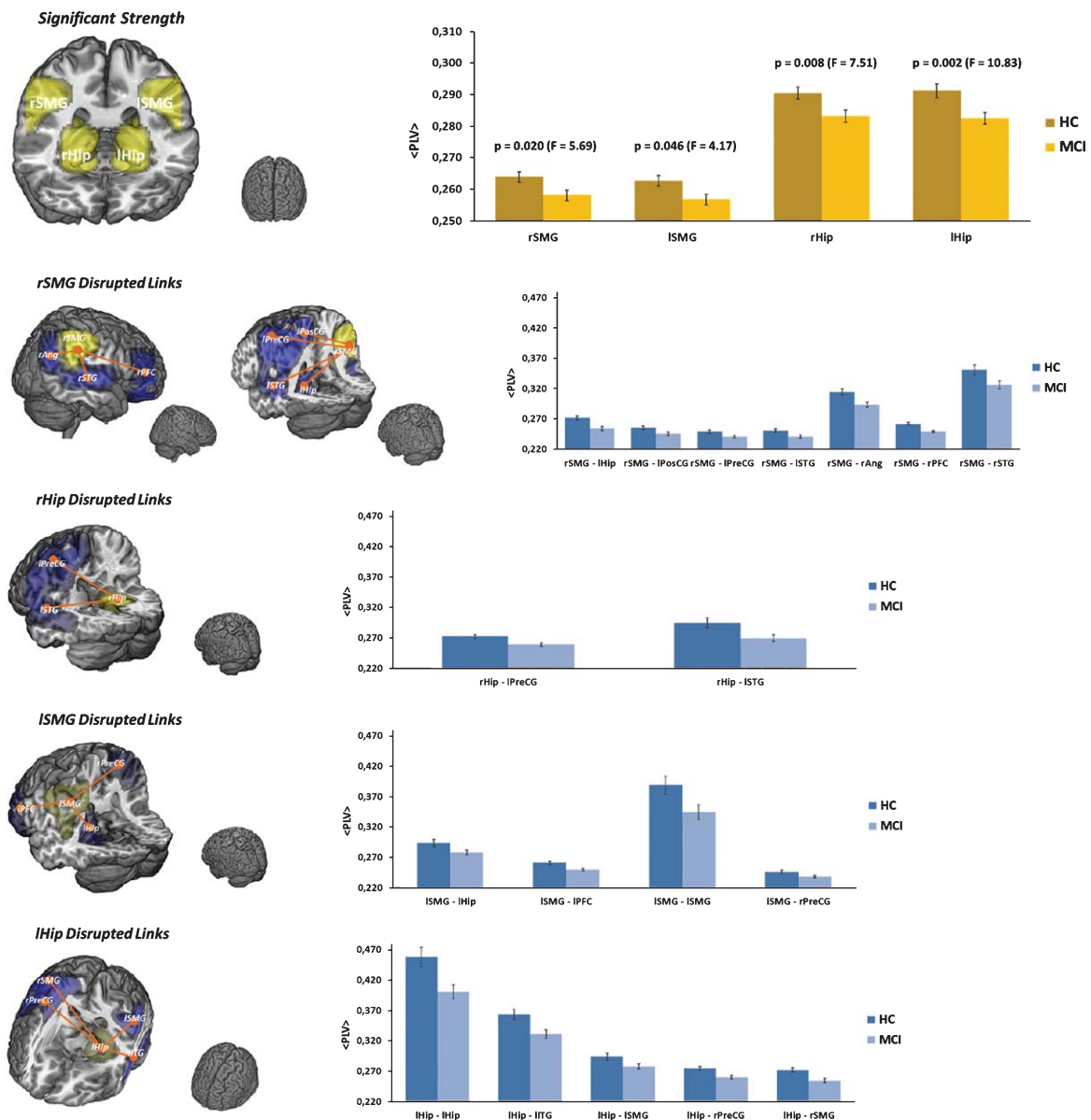


Fig. 1. Main effect of diagnosis in the alpha band. At the top, ROIs highlighted in yellow showed significant differences ( $p < 0.05$ ) in strength between healthy controls (HC) and MCI subjects. Yellow bar graphs show the corresponding ROI's average strength for each group. Just below the strength results, the links (among each yellow ROI and the rest of the network) with significant differences ( $p < 0.05$ , corrected) between HC and MCI subjects can be seen. The average PLVs for each group of these links are plotted in the blue bar graphs. The error lines of the bar graphs show standard error values.

asymmetric FC patterns. While rHip had only two links (involving lPreCG and left superior temporal gyrus (lSTG)), the lHip ROI had five involving both temporoparietal regions.

*Alpha band correlation analysis (Table 2)*

The strength values of the rSMG, ISMG, and lHip showed positive correlations with neuropsychologi-

cal scores. Moreover, several links followed the same tendency. Particularly, three of them correlated with all neuropsychological tests: rSMG-lSTG, rSMG-right angular gyrus (rAng), and ISMG-rPreCG.

*Beta1 band strength analysis (Fig. 2, top)*

Four ROIs showed diminished FC in the MCI group: rSMG, rPreCG, rSTG, and rHip.

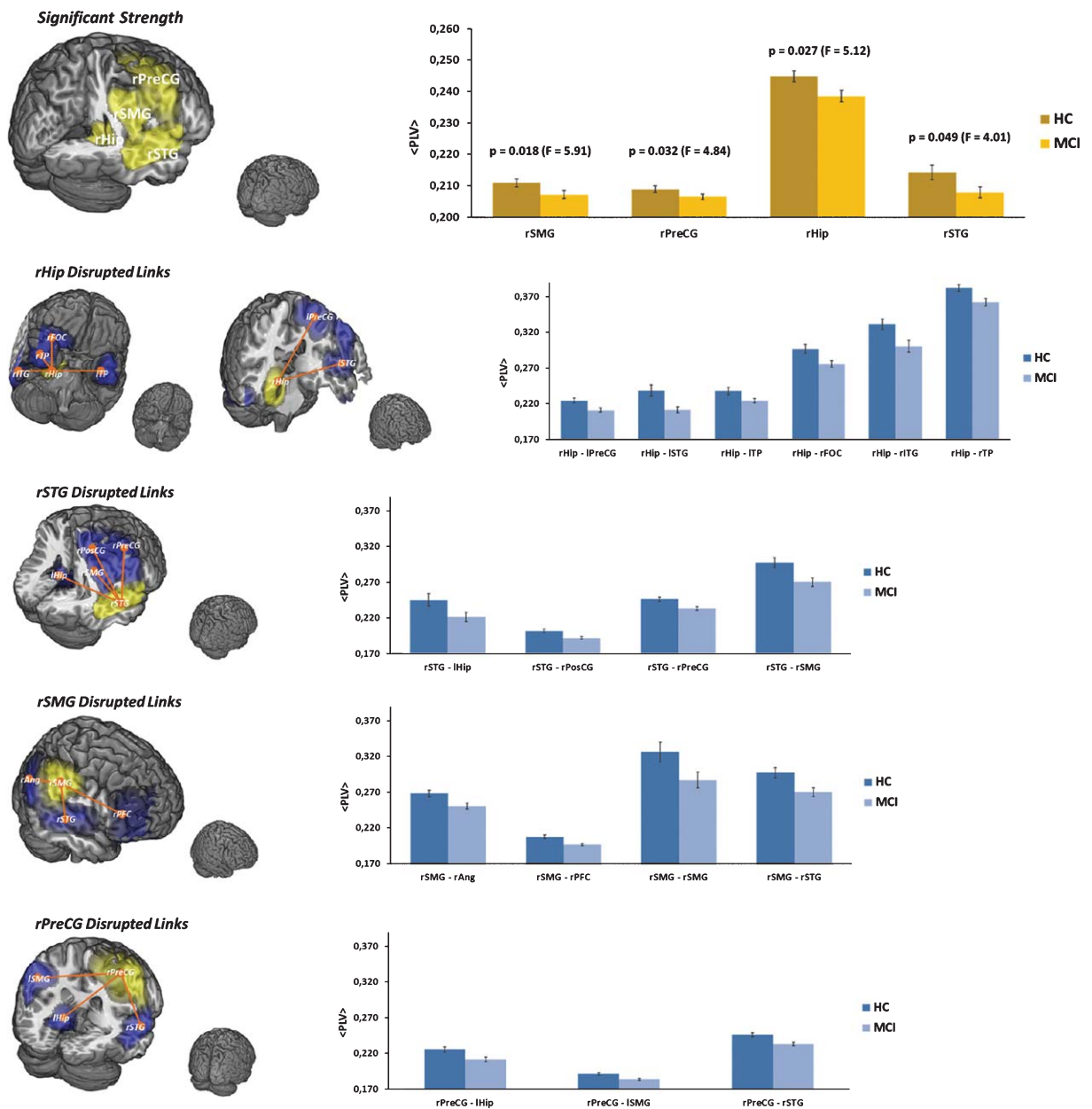


Fig. 2. Main effect of diagnosis in the beta1 band (12–20 Hz). At the top, ROIs highlighted in yellow showed significant differences ( $p < 0.05$ ) in strength between healthy controls (HC) and MCI subjects. Yellow bar graphs show the corresponding ROI's average strength for each group. Just below the strength results, the links (among each yellow ROI and the rest of the network) with significant differences ( $p < 0.05$ , corrected) between HC and MCI subjects can be seen. The average PLVs for each group of these links are plotted in the blue bar graphs. The error lines of the bar graphs show standard error values.

*Beta1 band associate network analysis (Fig. 2, bottom)*

The rHip was the brain region with more altered links (involving right frontal orbital cortex (rFOC), right inferior temporal gyrus (rITG), right temporal pole (rTP), lTP, lPreCG, and lSTG). The rSTG and rSMG altered links were almost completely

contained within the right parietal-temporal region (involving rAng, rSTG, and rPFC for the rSMG and right post-central gyrus (rPosCG), rSMG, and rPreCG for the rSTG), with the only exception of lHip-rSTG. Finally, the rPreCG had three links with significant FC differences, involving lHip, lSMG, and rSTG.

Table 2

Pearson's "r" values of all significant correlation ( $p < 0.05$ , corrected) among neuropsychological test and functional connectivity values. MMSE, Mini-Mental State Examination score; Del. R., delayed recall; Imm.R., Immediate recall; Sem. F., semantic fluency; Phon. F., phonemic fluency

Test	MMSE	Del. R.	Imm. R.	Sem. F.	Phon. F.
<i>Alpha Diagnosis Effect</i>					
Strength rSMG	ns	0.30	0.30	0.31	ns
Strength lSMG	ns	0.38	0.34	0.30	ns
Strength lHIP	ns	0.33	0.31	ns	ns
rSMG-lHIP	ns	0.30	ns	ns	ns
rSMG - lPosCG	0.31	ns	ns	0.30	ns
rSMG - lPreCG	ns	0.36	0.38	ns	ns
rSMG - lSTG	0.31	0.39	0.39	0.32	0.42
rSMG - rAng	0.32	0.43	0.38	0.32	0.31
rSMG - rPFC	ns	0.35	0.35	0.32	ns
rSMG - rSTG	ns	0.30	0.31	0.30	ns
lSMG - lHIP	ns	0.31	0.35	0.37	0.32
lSMG - lPFC	0.38	0.32	0.30	ns	ns
lSMG - lSMG	0.30	0.34	0.33	ns	ns
lSMG - rPreCG	0.31	0.39	0.35	0.36	0.33
rHip - lPreCG	0.32	0.47	0.44	ns	ns
rHip - lSTG	ns	0.31	0.32	0.30	ns
lHip - lHIP	0.30	0.38	0.31	ns	ns
lHip - lITG	0.31	0.40	0.35	ns	ns
lHip - lSMG	ns	0.32	0.35	0.37	0.32
lHip - rPreCG	0.32	0.40	0.32	0.31	ns
lHip - rSMG	ns	0.30	ns	ns	ns
<i>Betal Diagnosis Effect</i>					
rSMG - rAng	ns	ns	ns	ns	0.30
rSMG - rSMG	ns	0.31	0.30	ns	ns
rPreCG-lHIP	0.30	0.40	0.41	ns	ns
rPreCG - lSMG	ns	0.30	0.31	ns	ns
rPreCG - rSTG	ns	0.33	0.33	ns	0.31
rHip - rTP	0.33	0.34	0.33	ns	ns
rHip - lPreCG	0.36	0.51	0.44	ns	ns
rSTG - rPosCG	ns	0.31	0.32	ns	0.30
rSTG - rPreCG	ns	0.33	0.33	ns	0.31
<i>Delta Interaction Effect</i>					
Strength rFOC	ns	0.37	0.34	ns	ns

#### Betal band correlation analysis (Table 2)

In this case, only some links depicted positive correlations with neuropsychological scores. The highest correlation score corresponded with the FC between rHip and lPreCG:  $r = 0.51$  and  $r = 0.44$  for Delayed and Immediate Recall, respectively.

#### APOE Genotype Effect

A significant difference in strength value was found only in delta band and in the left lateral inferior occipital cortex (lLIOC) ROI. Carriers of the  $\epsilon 4$  allele showed diminished strength value (see Fig. 3, left). This decrement was mediated by three long range links (lLIOC with lPFC, mPFC and rTP) (Fig. 3, right).

#### Interaction (Diagnostic – APOE Genotype) Effect

Only the strength values within delta and theta bands of rFOC and rTP ROIs presented a significant interaction effect.

#### Delta band strength analysis (Fig. 4, top)

The MCI $\epsilon 4$  group showed diminished FC in comparison to the other three groups. The relation among the strength values of the four groups followed the pattern: HC  $\epsilon 4 >$  HC  $\epsilon 3$  MCI  $\epsilon 3 >$  MCI  $\epsilon 4$ .

#### Delta band associate network analysis (Fig. 4, middle & bottom)

The rFOC had six links altered: Both hippocampi and four temporo-parietal in the left hemisphere (lPreCG, lPosCG, lTP, and lSTG). The rTP ROI had two altered links: lPreCG and lSTG.

#### Delta band correlation analysis (Table 2)

The rFOC strength value showed significant positive correlations with episodic memory scores.

#### Theta band strength analysis (Fig. 5, top)

In this case, the tendency of the strength values was HC  $\epsilon 4 >$  MCI  $\epsilon 3 >$  HC  $\epsilon 3 >$  MCI  $\epsilon 4$  with significant differences intra MCI groups.

#### Theta band associate network analysis (Fig. 5, middle & bottom)

All altered links were contained within the right hemisphere. The rTP had 5 links: rAng, rSMG, rLIOC, rPFC, and rFOC while rFOC had two: rTP and mPFC.

## DISCUSSION

This study aimed to explore the resting state functional network in aMCI patients and HC carriers and non-carriers of the APOE  $\epsilon 4$  allele. The MCI group presented a decreased FC in alpha and beta bands. Additionally, the  $\epsilon 4$  carriers showed a diminished FC in a frontal-posterior network within delta band. Regarding the interaction between diagnosis and APOE genotype, our results clearly indicated two opposite effects: 1) the HC  $\epsilon 4$  group showed an enhanced FC in the frontal-temporal regions (rFOC and rTP) in delta and theta bands, as compared to the rest of groups (HC  $\epsilon 3$ , MCI  $\epsilon 3$ , and MCI  $\epsilon 4$ ); 2) the MCI  $\epsilon 4$  group showed reduced FC in the same regions and bands when compared to the remaining three groups (HC  $\epsilon 3$ , HC  $\epsilon 4$ , and MCI  $\epsilon 3$ ).

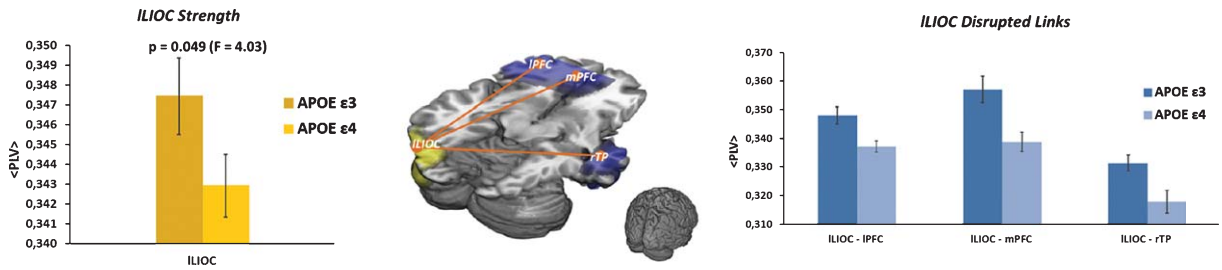


Fig. 3. Main effect of APOE genotype in the delta band. The left Lateral Inferior Occipital Cortex (ILIOC) ROI showed significant differences ( $p < 0.05$ ) in strength between APOE genotype  $\epsilon 3$  subjects (APOE  $\epsilon 3$ ) and APOE genotype  $\epsilon 4$  subjects (APOE  $\epsilon 4$ ). Yellow bar graphs show the corresponding ROI's average strength for each group. Orange lines represent links (which involved ILIOC) with significant differences between APOE  $\epsilon 3$  and APOE  $\epsilon 4$  groups ( $p < 0.05$ , corrected). The groups' average FC of these links are plotted in the blue bar graphs. The error lines of the bar graphics show standard error values.

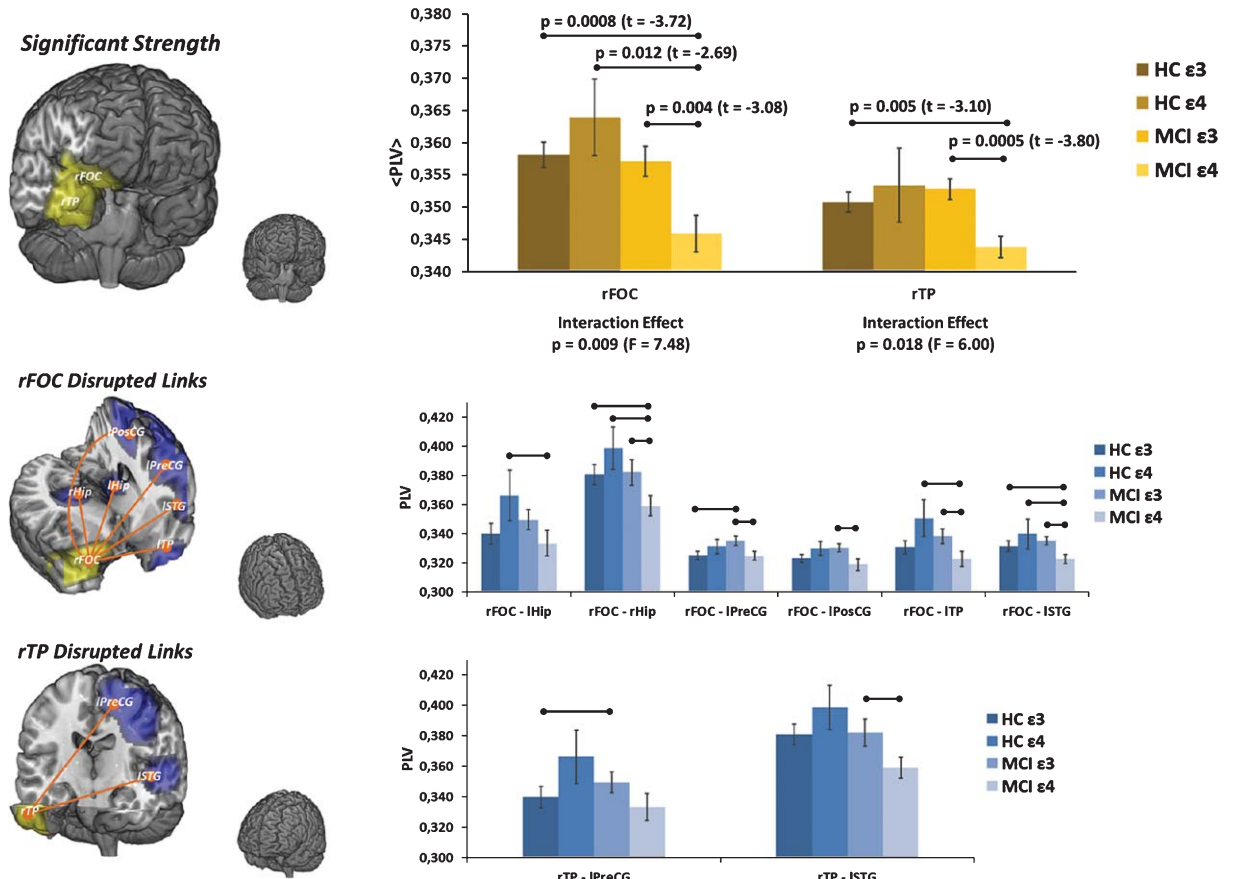


Fig. 4. Interaction (diagnosis- APOE genotype) effect in the delta band. Four groups have been considered in this analysis: Controls with APOE genotype 33 (HC $\epsilon 3$ ), controls with APOE genotype 34 (HC $\epsilon 4$ ), MCI subjects with APOE genotype 33 (MCI $\epsilon 3$ ) and MCI subjects with APOE genotype 34 (MCI $\epsilon 4$ ). At the top, the ROIs highlighted in yellow showed significant differences ( $p < 0.05$ ) in strength for ANOVA interaction effect. Yellow bar graphs show the corresponding ROI's average strength for each of the four groups. Horizontal lines display significant differences ( $p < 0.05$ , corrected) between pairs of groups. Just below the strength results, the links (among each yellow ROI and the rest of the network) with significant differences ( $p < 0.05$ , corrected) between the different pairs of groups can be seen. The groups' average FC of these links are plotted in the blue bar graphs. The error lines of the bar graphics show standard error values.



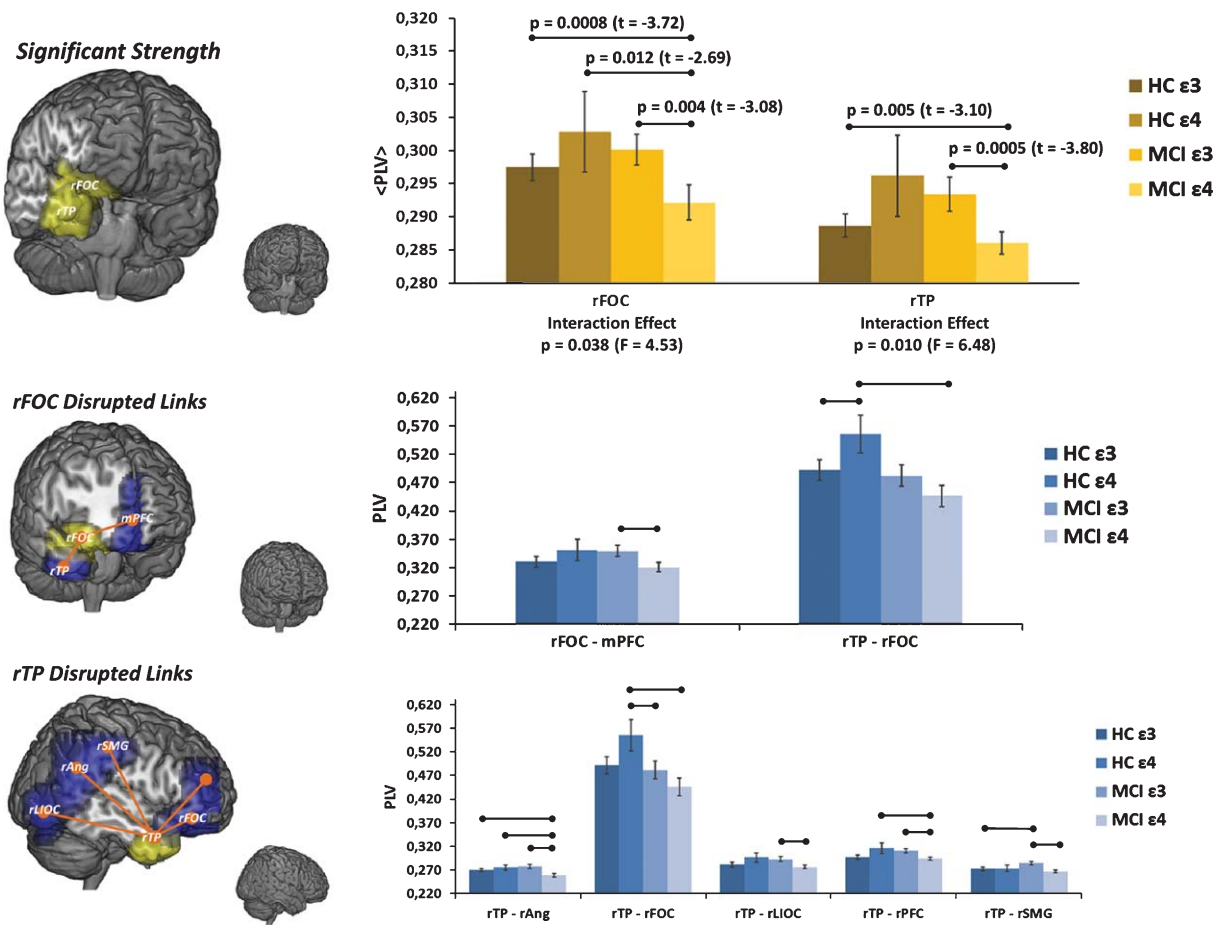


Fig. 5. Interaction (diagnosis- APOE genotype) effect in the theta band. Four groups have been considered in this analysis: Controls with APOE genotype 33 (HCε3), controls with APOE genotype 34 (HCε4), MCI subjects with APOE genotype 33 (MCIε3) and MCI subjects with APOE genotype 34 (MCIε4). At the top, the ROIs highlighted in yellow showed significant differences ( $p < 0.05$ ) in strength for ANOVA interaction effect. Yellow bar graphs show the corresponding ROI's average strength for each of the four groups. Horizontal lines display significant differences ( $p < 0.05$ , corrected) between pairs of groups. Just below the strength results, the links (among each yellow ROI and the rest of the network) with significant differences ( $p < 0.05$ , corrected) between the different pairs of groups can be seen. The groups' average FC of these links are plotted in the blue bar graphs. The error lines of the bar graphics show standard error values.

*Effect of diagnosis on functional network organization*

In concordance with previous resting state literature, FC in alpha and beta bands was diminished in both parietal and hippocampal areas in the MCI group when compared to controls [12, 13, 30, 31]. In addition, FC values correlated positively with cognitive performance [32]. In alpha band, the MCI group exhibited decreased FC in both supramarginal gyri and hippocampi. In the beta band, all the decreases were found only in the right hemisphere, involving the hippocampus and temporal-parietal regions. This decreased FC could be modulated by the impairment of cholinergic systems which affect the high frequency bands [33,

34]. Thus, the decreased FC might represent an altered basal forebrain cholinergic input to cortex and hippocampus [35, 36]. Our results in alpha band showed many changes in the frontal-MTL (medial temporal regions) and frontal-parietal networks. Recently, it has been stated [37] that the lateral entorhinal cortex is the first structure to be damaged in the preclinical stages of the AD, and this dysfunction may spread to the parietal cortex. This finding agreed with previous studies that reported significant differences in the hippocampal functional synchrony [38, 39] among MCI, AD, and controls. Moreover, in a fMRI study [40] that, analyzed resting state FC in aMCI subjects, the altered network showed decreased FC within several regions. Most of these regions were located in areas of the left

hemisphere, such as the left MTL or the left PFC. It is important to take note of the right parietal-temporal network disruption in the MCI group. These functional connections are part of the attentional system specialized in the detection of behaviorally relevant stimuli and it is largely lateralized to the right hemisphere [41]. This disruption could support previous findings of attentional deficits in the early stages of the disease [6].

#### *Effect of APOE genotype on functional network organization*

The group of  $\epsilon 4$  carriers showed a diminished FC in an anterior-posterior network within delta band. This result agreed with recent findings which identified the loss of long range FC as a potential biomarker to track the progression of the disease [42]. This pointed to an enhanced vulnerability in the occipital region [43]. Such vulnerability could be associated with glucose metabolism reduction in  $\epsilon 4$  carriers when compared to non-carriers [44]. These findings can be interpreted as a marker of neurodegeneration because it is known that the  $\epsilon 4$  allele increases the risk of network malfunctioning [17], and increases the  $A\beta$  burden [1]. The alterations involved in the frontal lobe could be explained by the vulnerability of the frontal cortex to  $A\beta$  deposition and by the relationship between FC and  $A\beta$  deposition [10]. It is important to know whether controls and MCI  $\epsilon 4$  carriers are showing a similar functional disruption.

#### *Interaction effect of APOE genotype and diagnosis on functional network organization*

The interaction between the diagnostic group and APOE genotype revealed the main role of the low frequency bands (delta and theta) and the right fronto-temporal regions (rFOC and rTP, henceforth called rFT).

The MCI  $\epsilon 4$  group showed diminished FC in the rFT area in comparison to the rest of the groups, while the HC  $\epsilon 4$  group depicted enhanced FC in most of the cases (Figs. 4 and 5). In addition, the strength value in delta band in the rFOC correlated positively with episodic memory scores, which is in line with previous reports that have studied the role of the rFT in the encoding of information [45] and episodic memory deficits [46]. This result could be driven by a compensation/disruption process due to a specific effect of the  $\epsilon 4$  allele on the anterior hippocampal network [4]. Moreover, the FT regions have been described as locations in which  $A\beta$  accumulates [47] and hypometabolism

can be seen in the early stages of the disease [48]. It has been previously reported that  $\epsilon 4$  carriers (patients and controls) showed higher  $A\beta$  deposition over the orbitofrontal cortex than non-carriers [49]. Thus, it seems that the potential damage associated with  $A\beta$  accumulation could be caused by the following factors: 1) in the non-symptomatic stage,  $\epsilon 4$  carriers try to compensate by increasing FC; 2) in the stage of MCI, the network loses its ability to compensate, which leads to the disruption of the normal FC between rFT regions and the rest of the brain. It is interesting to note that the MCI  $\epsilon 3$  group usually had higher FC values than the HC  $\epsilon 3$  group. This group of patients may be using an alternative functional network to compensate for that potential malfunctioning [9]. This method is slightly different to the one used by the HC  $\epsilon 4$  group. Here, the results indicate that these differences were mainly due to a compensatory response of MCI non carriers rather than the MCI as a group.

Finally, we found that both MCI groups showed lower volume of the hippocampi in comparison to the control groups. Therefore, they should have reorganized their functional networks. There were no differences in hippocampal volume between both MCI groups. However, the differences obtained in FC, can be specifically explained by the greater effort of the non-carriers group in reorganizing the architecture of their functional networks and the inability of MCI  $\epsilon 4$  carriers to show a compensatory activity. In addition, it seems that the control  $\epsilon 4$  carriers were also compensating without showing hippocampi atrophy. This could indicate that the presence of the  $\epsilon 4$  allele leads to a loss of neural integrity [50], which result in the reorganization of the functional networks.

#### *Limitations*

The sample size is relatively small and this limitation affects the HC  $\epsilon 4$  group to a greater extent. However, all comparisons where this group was involved were treated with a very restrictive strategy in order to avoid the appearance of Type I errors. Unfortunately, we cannot state with complete confidence that Type II errors were totally avoided. Another important point which we could not address is that most of the literature is mainly focuses on grouping both  $\epsilon 4$  carriers. However, there is solid evidence about the differences in prevalence and risk between being homozygote or heterozygote [2]. Therefore, any future studies must address this issue by means of the inclusion of APOE genotype 4/4.

### General conclusions

Our investigation found FC alterations caused by diagnosis, APOE, and the interaction between both factors.

The MCI group showed DMN disruption in alpha and beta1 bands. This result agreed with previous studies of the alterations and progression (from MTL to cortical regions) of the neuropathology in AD [6, 37, 51]. In addition, as the hippocampus is among the last regions showing A $\beta$  deposition [52], this accelerated neuronal loss could be caused by factors such as tau protein. In fact, the potential impact of tau phosphorylation in white matter transmission, could lead to an anatomic-functional connectivity disruption [53]. In any case, the tau implication should be further analyzed in future studies. Meanwhile, the disruption in the hippocampal-parietal network, could offer, through the characterization of its FC pattern, an important noninvasive biomarker for the early stages of AD.

The different FC profile of the right frontal-temporal regions in the two groups of  $\epsilon$ 4 carriers (HC and MCI) could be associated with a tendency in both groups of accumulating A $\beta$ . This result indicates that there were representing two different stages of the disease. The FC anomalies could be explained by the direct relation between FC and A $\beta$  deposition [10, 54] and by the loss of gabaergic neurons around the amyloid plaques [55]. These facts could lead to hyperexcitability in the first term, followed by neuronal damage and neuronal death due to an increased calcium influx [56]. Thus, the increased FC showed by the HC  $\epsilon$ 4 group could be a consequence of synaptic disruption.

### ACKNOWLEDGMENTS

This study was supported by two projects, PSI2009-14415-C03-01 and PSI2012-38375-C03-01, from the Spanish Ministry of Science and Economy, one project from the National Institute of Health Carlos III PI10/01585. A predoctoral fellowship from the Spanish Ministry of Science and Innovation (BES-2010-036469), a predoctoral fellowship from the Ministry of Education (FPU AP-2008-00175), a PICATA predoctoral fellowship of the Moncloa Campus of International Excellence (UCM-UPM), a predoctoral fellow from the Basque Government and a predoctoral fellowship from the Ministry of Education (FPU AP2010-1317).

Authors' disclosures available online (<http://www.j-alz.com/disclosures/view.php?id=2539>).

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