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Featured Article

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Abstract

Introduction

The longitudinal trajectories of functional brain dynamics and the impact of genetic risk factors in individuals at risk for Alzheimer's disease are poorly understood.

Methods

In a large-scale monocentric cohort of 224 amyloid stratified individuals at risk for Alzheimer's disease, default mode network (DMN) resting state functional connectivity (FC) was investigated between two serial time points across 2 years.

Results

Widespread DMN FC changes were shown in frontal and posterior areas, as well as in the right hippocampus. There were no cross-sectional differences, however, apolipoprotein E ε4 (*APOE* ε4) carriers demonstrated slower increase in FC in frontal lobes. There was no impact of individual brain amyloid load status.

Discussion

For the first time, we demonstrated that the pleiotropic biological effect of the *APOE* ε4 allele impacts the dynamic trajectory of the DMN during aging. Dynamic functional biomarkers may become useful surrogate outcomes for the development of preclinical targeted therapeutic interventions.

Keywords: Brain functional dynamics; Longitudinal study; Precision medicine; fMRI; Alzheimer's disease; Subjective memory complaints; APOE; Default mode network; Amyloid β

1 Background

Individuals at risk for complex, nonlinear dynamic diseases, such as Alzheimer's (AD), have active adaptive responses and compensatory mechanisms to maintain homeostasis [1]., which results from theseThese mechanisms are triggered and driven by an interplay between genetic/epigenetic and environmental factors. Stratifying individuals according to the genetic risk factors for AD is a crucial step to attain reliable measures essential for identifying distinctive endophenotypes. Breakthrough technological advances have facilitated synergistic research alliances between geneticists and neuroscientists to determine how genomic factors affect brain structure, function, and metabolism. Overall, robust evidence is mounting, indicating several genes associated with an increased risk of AD [2]. The *apolipoprotein E* (*APOE*) ε4 allele is considered the most relevant genetic risk factor for late-onset AD and is observed in up to 50% of all cases of sporadic AD [3]. *APOE* gene is involved in many different processes: it regulates amyloid β (Aβ) oligomerization, aggregation, and receptor-mediated clearance, brain lipid transport, glucose metabolism, neuronal signaling, and neuroinflammation [4–6]. In vivo pathophysiological or topographic biomarkers (e.g., reduced metabolism [7], hippocampal atrophy [8], and cortical thickness [9]) provide supportive evidence for the altering impact of the *APOE* ε4 allele on brain changes occurring across a continuum from physiological conditions to AD pathophysiology.

In the past decade, brain functional connectivity (FC) has become a promising candidate biomarker for early identification of brain functional changes related to AD pathophysiology [10]. Functional neuroimaging genetics offers an efficient strategy for characterizing intermediate phenotypes of AD and improving the understanding of genetic pathways that interact with brain functioning [11]. Although the field is relatively young, the number of studies investigating possible downstream effects of risk genes on brain

functional dynamics is rapidly growing. Particularly, *APOE* ε4 carriers show specific and consistent alterations in the default mode network (DMN) [12] connectivity at rest, including decreased connectivity in the middle and posterior regions, and increased connectivity in the frontal and lateral structures [13–21]. Increases in resting state FC (rsFC) at the early stages of AD have been interpreted as either upstream or downstream brain compensatory mechanisms between brain regions typically affected in AD [22], mostly in the DMN [23–25]. Whether different aging trajectories occur in the DMN in cognitively intact *APOE* ε4 carriers and noncarriers, however, is currently unknown and requires longitudinal investigations ~~of the DMN~~ at the individual level. This will be an essential step forward for the functional neuroimaging genetic field because, to date, studies have been almost exclusively conducted using cross-sectional designs, exploring DMN alterations over time by comparing cross-sectional groups with increased genetic risk across different severity stages [2].

Longitudinal methods are particularly sensitive in detecting individual functional changes in neural networks. ~~Longitudinal~~Long-term follow-up studies performed on cognitively intact individuals at risk for AD ~~individuals~~ would enable ~~clinical~~ researchers to trace pathophysiological trajectories from health to late dementia stages and identify critical windows for failing compensatory mechanisms. This is of special interest for older individuals with subjective memory complaints (SMC~~s~~), a condition at increased risk of developing AD [26,27]. Such memory complaints may represent early signs of a wider cognitive impairment and be considered as a valid measure of self-reported memory decline [27]. Both memory complaints and the presence of at least one *APOE* ε4 allele predict cognitive decline at an early stage of AD [28]. However, no evidence of longitudinal alterations in functional networks has been established so far in asymptomatic individuals at risk for AD.

We compared longitudinal DMN ~~resting-state functional connectivity (~~rsFC~~)~~ between two time points (24 months apart) in a large-scale standardized observational university expert center-based monocentric cohort of 224 cognitively intact individuals with SMC, devoid of any objective cognitive impairment. Given (1) the critical role of *APOE* in regulating Aβ-related processes [4], (2) the strong prevalence of the *APOE* ε4 allele in Aβ positive individuals across the spectrum of AD, and (3) the massive effect of brain amyloid on brain structural [22,29] and functional [22,30,31] connectivity, we evaluated the impact of the individual amyloid status on brain aging trajectories related to *APOE* ε4 status.

2 Materials and methods

2.1 Participants

Data used in the preparation of this article were obtained from the “INveStIGation of AlzHeimer'sPredicTors in Subjective Memory Complainers” (INSIGHT-preAD) study [detailed method is available in [24]]. The INSIGHT-preAD study includes 318 cognitively intact Caucasian older adults recruited from the community in the wider Paris area, France, aged 70 to 85 years, with SMC, defined as follows: (1) Participants answered “YES” to both questions “Are you complaining about your memory?” and “Is it a regular complaint that has lasted now more than 6 months?”; (2) participants present intact cognitive functions based on Mini-Mental State Examination (≥27), Clinical Dementia Rating scale (0), and Free and Cued Selective Rating Test (total score ≥41). Written informed consent was obtained from all participants. The study was approved by the local Institutional Review Board and has been conducted in accordance with the Helsinki Declaration of 1975.

For the present study, we had considered only participants who underwent the resting state functional magnetic resonance imaging (rs-fMRI) acquisition both at the first time point (M0) and after 24 months (M24). Demographic characteristics, cognitive performance, and *APOE* genotype of the final subset (224 participants) are shown in Table 1.

Table 1 Demographic characteristics, global cognitive performance, and *APOE* genotype of the final subset, including 224 cognitively intact individuals with SMC

		All	APOE ε4 noncarriers	APOE ε4 carriers
Participants (female)		224 (133)	180 (107)	44 (26)
Education		6.3 ± 2.0	6.1 ± 2.1	6.4 ± 2.2
Age ± SD	M0	75.5 ± 3.4	75.5 ± 3.4	75.6 ± 3.5
	M24	77.5 ± 3.5	77.5 ± 3.5	77.6 ± 3.5
MMSE ± SD	M0	28.6 ± 1.0	28.6 ± 1.0	28.5 ± 1.0
	M24	28.8 ± 1.3	28.7 ± 1.4	28.8 ± 1.3

Abbreviations: APOE, apolipoprotein E; MMSE, Mini-Mental State Examination; SD, standard deviation; SMC, subjective memory complaint.

NOTE. Education was measured in a range from 1 (no diploma) to 8 (bachelor, master's degree, or doctorate).

2.2 Clinical and neuropsychological assessments

A comprehensive neuropsychological battery was administered to all participants of the INSIGHT-preAD cohort to assess they were cognitively intact in all relevant cognitive domains [27].

2.3 *APOE* genotype

Participants were divided into two groups based on the *APOE* status: individuals carrying at least one *APOE* ε4 allele (genotype ε4/ε4 and ε4/ε3) were classified as *APOE* ε4 carriers (*APOE* ε4+); the others (genotype ε2/ε2, ε2/ε3, and ε3/ε3) were classified as *APOE* ε4 noncarriers (*APOE* ε4−) [detailed method is available in [24]].

2.4 Amyloid status definition

Reconstructed ~~PET~~positron emission tomography images were analyzed with a pipeline developed by the ~~CATI~~Centre Acquisition et Traitement des Images (CATI), a neuroimaging platform funded by the French Plan Alzheimer (http://cati-neuroimaging.com~~);~~ detailed method is available in [24]~~)~~).

Our cohort was divided into two subgroups based on their cortical ~~SUVR~~standardized uptake value ratio value, that is, amyloid positive (Amy+) and amyloid negative (Amy−) individuals [32]. The ~~SUVR~~standardized uptake value ratio threshold of 0.79 to determine abnormality uptake was extracted performing linear correlation between our method [32] and the method used by Besson et al. [33] using 53 ~~PET~~positron emission tomography scans from another French study, the ~~IMAP~~Imagerie Multimodale de la maladie de l'Alzheimer à un stade Précoce (IMAP) cohort (multimodal neuroimaging project on early Alzheimer's disease).

2.5 MRI acquisition and preprocessing

MRI images were acquired using a Siemens MagnetomVerio (Erlangen, Germany) 3~~-~~T scanner, equipped with a quadrature detection head coil with 12 channels (transmit-receive circularly polarized–head coil). Three-dimensional (3D) TurboFLASH sequences were performed (orientation sagittal; repetition time 2300 ms; echo time 2.98 ms; inversion time 900 ms; flip angle 9°; 176 slices; slice thickness 1 mm; field of view 256 × 240 mm; matrix 256 × 240; bandwidth 240 Hz/Px).

2.6 rs-fMRI acquisition and preprocessing

Both baseline and follow-up scanning were performed on a 3 T MRI scanner qualified by the central MRI analysis core at the Cogimage Team, ICM (Paris, France). During the rs-fMRI scan, participants were instructed to keep their eyes closed and stay as still as possible. The rs-fMRI images were collected by using an echo-planar imaging sequence (repetition time 2460 ms, echo time 30 ms, slice thickness 3 mm, matrix 64 × 64, voxel size 3 × 3 × 3 mm^3, number of volumes 250, number of slices 45, run 1) sensitive to blood oxygenation level–dependent contrast (T2* weighting).

The rs-fMRI data were preprocessed using ~~BCB~~the Brain Connectivity and Behaviour toolkit v4.0.0 (available at http://toolkit.bcblab.com) [34] based on ~~FSL~~FMRIB Software Library (FSL) (available at https://fsl.fmrib.ox.ac.uk), which has previously been used successfully to identify the DMN [35]. Each participant's first 10 volumes were excluded to avoid potential noise related to the equilibrium of the magnet and participant's adaptation to the scanner. After individual correction for movement, the remaining 240 volumes were first motion corrected using ~~MCFLIRT~~a motion-correction tool based on FMRIB's Linear Image Registration Tool, then corrected for slice timing, smoothed with a full half width maximum equal to 1.5 times the largest voxel dimension, and finally filtered for low temporal frequencies using a Gaussian-weighted local fit to a straight line. These steps are available in Feat as part of ~~FSL~~FMRIB Software Library package. fMRI images are linearly registered to the T1 structural images. Estimated motion parameters, the first eigenvariate of the ~~WM~~white matter and ~~CSF~~cerebrospinal fluid and their first derivatives were regressed out from the functional data. We used masks based on the T1 derived three-class segmentation thresholded to a probability value of .9, registered to the rs-fMRI images and binarized. Finally, the first derivative of the motion parameters, ~~WM~~white matter, and ~~CSF~~cerebrospinal fluid signal was calculated by linear convolution between their time course and a [−1 0 1] vector.

Given that rs-fMRI signal can be severely affected by motion, ~~an independent component analysis (ICA)-AROMA procedure was used to~~a strategy based on the independent component analysis for Automatic Removal of Motion Artifacts (ICA-AROMA) was used to estimate the signal fluctuation associated with motion and then to regress it out from the fMRI data [36]. ICA-AROMA performs an ICA decomposition of the data and estimates which components reflect motion-related noise in the fMRI signal on the basis of a robust set of spatial and temporal features because of the distinctiveness of the motion-related components [37]. This approach outperforms other methods, such as the regression of the motion parameter estimates, while limiting at~~in~~ the same time the loss in degrees of freedom [36]. Compared with spike removal methods such as scrubbing [38], ICA-AROMA has the advantage of preserving the temporal structure of the fMRI signal.

2.7 rs-fMRI independent component analysis

Analysis of the rs-fMRI data was carried out using probabilistic ICA as implemented in Multivariate Exploratory Linear Optimized Decomposition into Independent Components, part of FMRIB's Software Library. Multivariate Exploratory Linear Optimized Decomposition into Independent Components was used to decompose the original concatenated 4D rs-fMRI data set into different spatial and temporal components [39]. The preprocessed functional data for each participant were temporally concatenated into a single 4D data set, which was analyzed using ICA.

In this analysis, the data set was decomposed into 20 independent components. However, it should be noted that there is no consensus yet on how to choose the optimal number of components [40]. The dual regression analysis was performed to identify subject-specific time course~~s~~ and spatial maps [41]. In the first step, the 20 components were used in a linear model fit against each individual subject's fMRI data set to create the average time course within each network for every subject (spatial regression), ultimately resulting in 20 *z*-score maps per individual per visit. In the second step, the personalized time courses were regressed back onto that participant's fMRI data set to create personal spatial maps (temporal regression). This gives 20 3D images per individual per visit, with voxelwise the *z* scores of FC to each of the templates.

The first step (group ICA) was performed using the first time point (M0) of all individuals. To keep the spatial patterns consistent across analyses, the resulting 20 independent components were used in the next dual regression steps to analyze the second data point (M24).

The DMN was finally selected by visual inspection based on the previous literature [42].

2.8 Statistical analyses

To perform longitudinal analyses, we calculated the change in rsFC (i.e., ΔFC) after 24 months (M24) as the change in rsFC from the first acquisition (M0).

The FSL's randomized permutation-testing tool (5000 permutations) was used to detect statistically significant differences within the DMN, correcting for age. Familywise error (FWE) correction for multiple comparisons was performed, implementing threshold-free cluster enhancement (TFCE) using a significance threshold of $P < .05$.

2.8.1 Effect of time

The resulting ΔFC values were compared with the null hypothesis of no change in DMN FC after 24 months by using one simple *t* test on ΔFC.

2.8.2 Effect of APOE

Given the pattern of results described in previous works [2], we were primarily interested in the comparison of *APOE* ε4 carriers (*APOE* ε4+) versus *APOE* ε4 noncarriers (*APOE* ε4−). Cross-sectional group differences between *APOE* ε4+ and *APOE* ε4− participants were firstly identified using two sample *t* tests in FSL for M0. Then, we evaluated the longitudinal impact of being *APOE* ε4+ versus *APOE* ε4−, using a two independent *t* test on ΔFC.

2.8.3 Impact of the amyloid status

Finally, we investigated any potential effect of amyloid on the longitudinal brain dynamics changes. To this aim, a 2 (amyloid status, Amy+ vs. Amy−) × 2 (APOE genetic risk, *APOE* ε4+ vs. *APOE* ε4−) repeated measure analysis of variance was performed in ~~FEAT~~fMRI Expert Analysis Tool (available at https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT) on ΔFC. Results were corrected for multiple comparisons using correction for FWE rate and TFCE, which is similar to cluster-based thresholding, but generally more robust and without the identification of the arbitrary initial cluster-forming threshold. TFCE is recommended when randomization is performed [43].

3 Results

3.1 Participants

From the INSIGHT-preAD cohort, we included only 242 participants. See Fig. 1 for details.

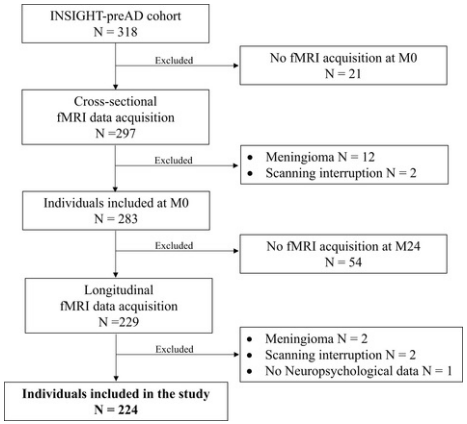

Fig. 1 Flow diagram shows participant inclusion process for the present study from the initial INSIGHT-preAD cohort. Abbreviation: fMRI, functional magnetic resonance imaging.

3.2 DMN extraction

After individual correction for movement, an ICA identified the group level DMN, as depicted in Fig. 1 of the ~~Supplemental Material~~Supplementary Material, where classical hubs can be seen, such as the Precuneus (Pcu), the inferior parietal lobe, the anterior and posterior cingulate cortices (ACC and PCC), as well as the medial frontal and prefrontal cortices [12]. Individual maps w~~h~~ere subsequently extracted using a dual regression approach [41].

3.3 Main longitudinal effects

Fig. 2 illustrates rsFC longitudinal changes in the DMN for more than a time period of 24 months. The widespread increase in rsFC concerned the medial prefrontal, frontal, and posterior DMN regions, including the ACC extending to the anterior medial frontal cortex, the PCC, the Pcu, and the left hippocampus and parahippocampus (Fig. 2, green-red scale). Significant decrease in rsFC was minimal involving the most dorsal portion of the Pcu (Fig. 2, blue scale).

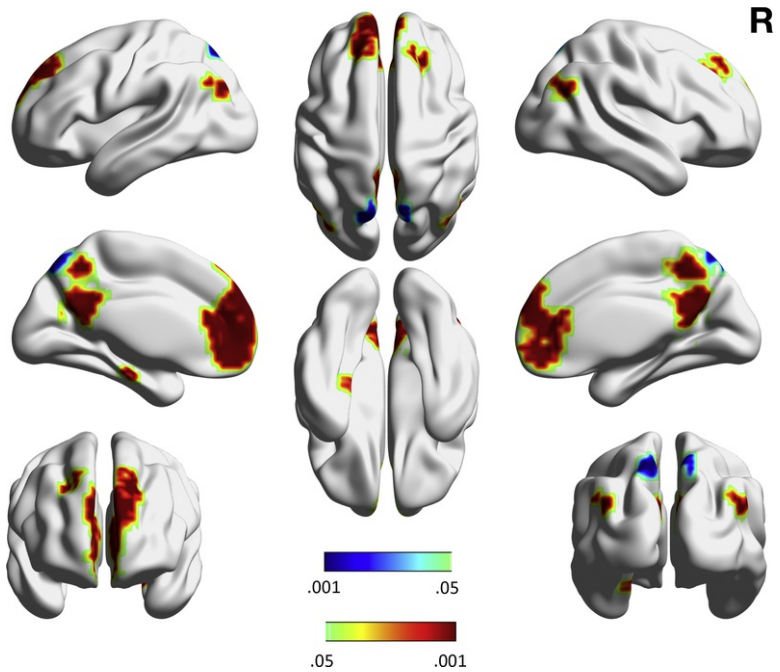

Fig. 2 DMN longitudinal changes in 224 participants for more than a time period of 24 months. Widespread increased (green-red voxels) DMN rsFC was found in our participants, including bilateral frontal and prefrontal regions, ACC, PCC, Pcu, angular gyrus, left hippocampus, and parahippocampus. Pcu also showed decreased rsFC in time (blue voxels). The statistical maps were thresholded using TFCE and $P < .05$ familywise error corrected, cluster >15 voxels. All results are described in Table 2 with their coordinates in the ~~MNI~~Montreal Neurological Institute space.

Table 2 Peak of maximal increased and decreased connectivity in the DMN related to the longitudinal differences at M24 with respect to M0 in all 224 participants

Brain region	BA	Voxels	*t* value	P_{FWE}	Peak		
					X (mm)	*Y* (mm)	*Z* (mm)
Increased connectivity	10	602	14.2	<.001	−2	62	20
	23	330	10.8	<.001	−6	−54	28
	39	48	9.53	<.001	50	−70	32
	39	32	9.15	<.001	−46	−70	36
	9	29	7.63	<.001	22	42	40
	30	16	6.25	<.001	−26	−22	−24
Decreased connectivity	7	61	8.36	<.001	−14	−74	56

Abbreviations: BA, Brodmann area; DMN, default mode network; FWE, familywise error.

3.4 *APOE* ε4 genotype effect

We asked the question whether carrying the *APOE* ε4 allele affected the DMN rsFC. First, we compared the rsFC at the initial time point (M0): cross-sectional comparisons between *APOE* ε4 carriers (*APOE* ε4+) and *APOE* ε4 noncarriers (*APOE* ε4−) revealed that the presence of the ε4 allele did not significantly modulate the DMN rsFC. Second, we assessed the genetic impact of the *APOE* ε4 allele on the rate of individual aging of the DMN rsFC: the longitudinal analysis indicated that individuals carrying the ε4 allele presented a slower increase in rsFC in the frontal lobes ($P < .05$ TFCE corrected), specifically in ~~BA~~Brodmann area (BA) 9, BA10, BA46, bilaterally (Fig. 3). Hence, although the level of FC between *APOE* ε4+ and *APOE* ε4− is comparable, the rate of increase in FC in *APOE* ε4+ is significantly slower than *APOE* ε4−.

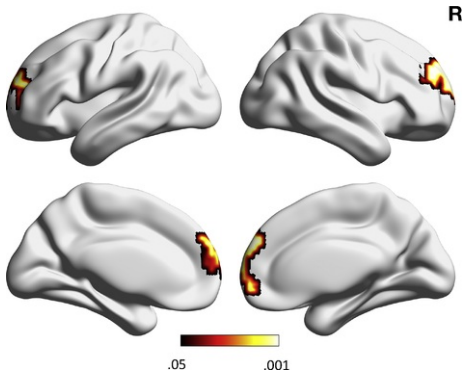

Fig. 3 Effect of APOE on the longitudinal DMN changes. The comparison of the DMN changes over time between APOE ε4− and APOE ε4+ showed a significant increased rsFC in the frontal and prefrontal areas over time [~~(A)~~ 245 voxels, [30 50 28], $P_{FWE} < .001$]. No effects of global amyloid load or an interaction effect between the amyloid status and APOE were found. The statistical maps were thresholded using TFCE and $P < .05$ familywise error corrected.

3.5 Impact of amyloid status on functional dynamics of the *APOE* ε4 genotype

Whether individuals' amyloid status may further influence connectivity dynamics changes linked to the presence of *APOE* ε4 allele is still unknown. Therefore, we perform a 2 × 2 repeated measures analysis of variance to evaluate the presence of an interaction effect between *APOE* and amyloid status. Results showed that neither the amyloid nor the APOE × amyloid interaction were significant on the longitudinal changes ($P < .05$ FWE corrected).

4 Discussion

In summary, brain network dynamics were examined in a standardized large-scale, observational monocentric university expert center cohort of 224 cognitively intact individuals with SMC, devoid of any objective cognitive impairment. Consistent with our a priori hypothesis, rsFC changes within the DMN were found 2 years after the first rs-fMRI evaluation. In addition, we provided in vivo evidence that the *APOE* ε4 genotype leads to ~~different~~ distinct DMN functional alterations regardless of the individual amyloid status.

At the group level, DMN changes were particularly evident in the posterior, frontal, and prefrontal cortices, as well as in the right hippocampus. Consistently with previous findings [44–47], there might have been a reorganization of brain rsFC induced by age-related mechanisms and/or disease progression. The increased brain activity during aging and at early stages of AD may serve as a compensatory mechanism and may reflect the enhanced metabolic demand engaged by an adaptive brain to cope with disruptive signals affecting specific vulnerable neural regions. This increased ~~compensatory~~ resilient response has frequently been observed in the frontal lobes [48,49], which may play a key role for cognitive reserve [50]. Overall, in our population, compensatory mechanisms seem to involve a posterior-anterior shift [51], which is characterized by reduced activation in the posterior midline cortex along with increased activity in medial frontal cortex [48,49]. Consistent with this model, we found decreased rsFC in the most dorsal part of the Pcu. However, we also showed the presence of increased activity in the more ventral Pcu extending to the PCC. The presence of ~~different~~ distinct expression of posterior alterations might originate from ~~different~~many sources. First, owing to the functional heterogeneity of the Pcu [52,53], each subregion might be differently damaged at the early stages of AD. Here, we found decreased rsFC in the dorsal Pcu, which showed positive rsFC with the superior parietal cortex, and negative rsFC with the orbital and frontal cortices, the amygdala, and the cerebellum [52]. Additional functional studies are needed to clarify whether specific Pcu subregions are differently affected across the whole AD continuum. Second, rsFC might start decreasing in a narrow subregion of the Pcu and slowly perturbing other adjacent neurons before spreading the functional impairment to the rest of the brain through a mechanism of diaschisis [54]. We hypothesize that spatiotemporal changes across brain networks, in the context of complex and nonlinear dynamic diseases such as AD, may have a stepwise evolution from an “intra-network phase” to a whole-network and, ultimately, a multinetwork compensation phase. Spreading of pathophysiological processes during brain disease phases are mediated by complex mechanisms [54], which are not fully understood yet. However, two longitudinal data points are insufficient to investigate the potential spreading of the functional impairment in time. Future ~~longitudinal data~~ follow-up acquisitions of the INSIGHT-preAD cohort may provide us with key evidence in this regard. ~~In the incoming future, advanced~~ The implementation of multimodal approaches, such as neuroimaging genetics integrated with ~~the implementation of~~ computational biology~~chemical~~ ~~analyses~~, will provide the basis to establish whether the trajectories of molecular/cellular

pathways are linked to dynamic patterns of neural network reorganization ~~in our population~~, thus potentially mapping~~classifying~~ compensatory or adaptive strategies.

We ~~may~~can argue that methodological choices may also have affected the results. For instance, although SMC represents per se a condition at risk of developing AD [26,28], the different endophenotypes included in such heterogeneous group may range from physiological brain aging to AD-related signatures (i.e., preclinical AD). Moreover, although the application of ICA in rs-fMRI studies is a powerful analytic method to separate bold signal from physiological noise induced by the cardiac pulse and respiration, some residual noise might still be present in the components [55]. Finally, we focused our attention on the DMN; however, early dysfunctions may involve further large-scale networks [56]. More complex methods will allow us to extend the analyses to all the brain networks that are normally affected in neurologic disorders, including AD. Finally, it is worth to note that the INSIGHT-preAD cohort includes well-educated individuals, which may be able to compensate more effectively. As a consequence, our cohort may be affected by a selection bias that comes from errors in selecting the study participants and/or from factors affecting the study participation.

Successively, the impact of the *APOE* ε4 genotype was investigated. In the present study, no significant results were found between *APOE* ε4− and *APOE* ε4+ at the first time point ~~the first time point~~ (M0). The reason why cross-sectional findings on the DMN connectivity do not consistently demonstrate differences between *APOE* ε4− and *APOE* ε4+ is still debated in the literature [2]. It has been previously shown that the inclusion of a wide age range in the same sample generated conflicting results: both decreased [13] and increased [14,15] connectivity were found cross-sectionally in a number of DMN nodes. In this regard, Staffaroni et al. [21] did not find any significant difference between *APOE* ε4+ and *APOE* ε4− ~~in~~at baseline or in the longitudinal trajectory of DMN rsFC by including individuals at different aging stages (ages 49–87 years~~)~~ with only 23 older adults). Our data might be similarly affected by the inclusion of different endophenotypes whose differences are exacerbated later in time. It has been also demonstrated that different neural dynamics are detectable in mutation carriers of several additional genes, such as phosphatidylinositol binding clathrin assembly protein (PICALM), clusterin (CLU), and bridging integrator 1 (BIN1) genes across the lifespan [2]. Liu et al. [57] showed that the outer mitochondria membrane 40 homolog (TOMM40) genotype may modulate regional spontaneous brain activity and is related to the progression of individuals in prodromal phase of AD. Several additional pathophysiological mechanisms, epigenetic factors, and interactions with other genes might need to be considered and integrated in future studies.

For the first time, we demonstrated in vivo evidence that carrying the *APOE* ε4 allele leads to a different aging trajectory of DMN. According to our results, we suggest that carrying the *APOE* ε4 allele leads to different longitudinal changes: *APOE* ε4+ showed a slower increase in rsFC in the frontal and prefrontal areas compared with *APOE* ε4−. Future studies including neuropsychological outcome measures will disclose whether such dynamics represent compensatory mechanisms to cope with pathophysiological processes driven by *APOE* ε4 genotype.

Given the established association between *APOE* ε4 genotype and the impairment of Aβ metabolism with subsequent extracellular accumulation [58], we further investigated whether the functional changes related to *APOE* ε4 might be partially explained by the presence of increased fibrillary Aβ deposition. Our data suggest that *APOE* ε4 leads to DMN rsFC changes regardless of the Aβ brain accumulation, indicating that other biological mechanisms, besides Aβ, are driven by the *APOE* ε4 allele. Such finding is in line with both animal and human studies reporting a pleiotropic effect of *APOE* ε4 on several molecular pathways, including, among others, tau hyperphosphorylation, inhibition of neurite outgrowth, and axonal sprouting.

Interestingly, a recent study showed that brain Aβ deposition seems to be associated with specific network dysfunction even in the absence of neural hypometabolism [59]. However, individuals were not genotyped for *APOE*, thus leading to potential ambiguous results. Given that *APOE* ε4 has a powerful effect on cerebral Aβ metabolism [58], the presence of neural dysfunctions may be linked to the *APOE* ε4 allele, which downstream promotes Aβ deposition. Consequently, the DMN dysfunction might be a cause, rather than a consequence, of Aβ aberrant pathways [60]. It has been previously shown that the neuronal components of the DMN have an increased vulnerability to bioenergetic failure because of their highly persistent activation [61]. Thus, a reorganizational pattern of connectivity within the DMN may ensure adaptation for bioenergetic cellular stress.

The dramatic rate of failures of large-scale clinical trials has suggested that novel target and outcome measures are needed to overcome insufficient clinical trial~~s~~' design [62–64]. The new challenge in pharmacologic Research and Development programs is to reconsider previous traditional hypotheses and to better define how preclinical data can support novel data-driven hypotheses, how to perform a convincing and evidence-driven "preclinical to clinical" translation, how to ~~longitudinally~~ chart the biological effect of a candidate drug over time ~~during investigation~~, and, finally, how to assess whether such a biological effect is ~~really~~ truly correlated with functional and ~~later~~ clinical improvement.

Connectomics within the DMN have increasingly been recognized as established biomarkers for several contexts of use even in preclinical stages of the disease [10,47,65,66]. Alterations in connectivity, which extend beyond the DMN [18], are expected to be designated as further promising biomarker candidates or even as much needed surrogate biomarker candidates for clinical outcome [67]. Novel technologies and sophisticated computational analyses will soon enable integrating clinical, genetic/epigenetic, and neurodynamic/neuroimaging data, providing a full individual biological makeup. Biomarker-guided predictive trajectories will further permit researchers to identify and stratify preclinical biologically defined ''at risk'' populations. The~~A~~ new conceptual paradigm of ~~—~~precision medicine~~—~~operates~~ing~~ in this context and aims at tailoring medical treatments to the individual genetic drivers, pathophysiological, and clinical characteristics of the disease for the single patient [63]. In this perspective, the future precision medicine will ensure the successful achievement of innovative personalized therapeutic strategies to prevent, halt, and cure AD and other complex neurodegenerative diseases.

Research in Context

1. Systematic review: We reviewed the literature using traditional (e.g., PubMed) sources and meeting abstracts and presentations. Although the longitudinal brain functional trajectories of Alzheimer's disease (AD) have not been studied yet, recent publications describing the cross-sectional aspects of the brain functional dynamics are appropriately cited.

2. Interpretation: Consistently with previous findings, there might have been a reorganization of brain functional connectivity induced by age-related mechanisms and/or disease pathophysiological progression. In addition, we demonstrated that the pleiotropic biological effect of the *APOE* ε4 allele impacts the dynamic trajectory of the default

mode network over time

3. Future directions: These results may generate early and modifiable functional outcomes in the perspective of individualized and targeted therapeutic preclinical interventions. Further studies may evaluate (1) the functional changes in either physiological or pathophysiological conditions, such as AD, (2) the functional dynamics associated with conversion to AD, and (3) the inclusion of functional outcomes in clinical trials.

Acknowledgments

The study was promoted by INSERM in collaboration with ICM, IHU-A-ICM, and Pfizer and has received a support within the “Investissement d'Avenir” (ANR-10-AIHU-06). The study was promoted in collaboration with the “CHU de Bordeaux” (coordination CIC EC7), the promoter of Memento cohort, funded by the Foundation Plan-Alzheimer. The study was further supported by AVID/Lilly.

The research leading to these results was supported by the *Colam Initiatives* and the “*Fondation pour la Recherchesur Alzheimer*,” Paris, France.

This publication is within the framework of the Program “MIDAS” led by the Sorbonne University Foundation and sponsored by MSDAVENIR.

Michel Thiebaut de Schotten is supported by the “AgenceNationale de la Recherche” (grant number ANR-13- JSV4-0001-01) and a European Research Council Consolidator Investigator Award (ERC-2018-COG 818521).

H.H. is supported by the AXA Research Fund, the “Fondation partenariale Sorbonne Université” and the “Fondation pour la Recherchesur Alzheimer,” Paris, France. Ce travail a bénéficié d'une aide de l'Etat “Investissements d'avenir” ANR-10-IAIHU-06. The research leading to these results has received funding from the program “Investissements d'avenir” ANR-10-IAIHU-06 (Agence Nationale de la Recherche-10-IA Agence Institut Hospitalo-Universitaire-6).

Author contributions: P.A.C., E.C., B.D., H.H., and M.T.S. have contributed to the conception and design of the study. P.A.C., E.C., M.C.P., M.O.H., and M.T.S. have contributed to acquisition and analysis of data. P.A.C., A.V., E.C., S.L., M.T.S., and H.H. have contributed to drafting and revising a significant portion of the manuscript.

Competing financial interests: H.H. serves as a Senior Associate Editor for the Journal Alzheimer's & Dementia; he received lecture fees from Biogen and Roche, research grants from Pfizer, Avid, and MSD Avenir (paid to the institution), travel funding from Functional Neuromodulation, Axovant, Eli Lilly and company, Takeda and Zinfandel, GE-Healthcare, and Oryzon Genomics, consultancy fees from Qynapse, Jung Diagnostics, Cytox Ltd, Axovant, Anavex, Takeda and Zinfandel, GE Healthcare, and Oryzon Genomics, Functional Neuromodulation, and participated in scientific advisory boards of Functional Neuromodulation, Axovant, Eisai, Eli Lilly and Company, Cytox Ltd, GE Healthcare, Takeda and Zinfandel, Oryzon Genomics, and Roche Diagnostics. H.H. is a coinventor in the following patents as a scientific expert and has received no royalties:

- In vitro multiparameter determination method for the diagnosis and early diagnosis of neurodegenerative disorders. Patent number: 8916388.
- In vitro procedure for diagnosis and early diagnosis of neurodegenerative diseases. Patent number: 8298784.
- Neurodegenerative markers for psychiatric conditions. Publication number: 20120196300.
- In vitro multiparameter determination method for the diagnosis and early diagnosis of neurodegenerative disorders. Publication number: 20100062463.
- In vitro method for the diagnosis and early diagnosis of neurodegenerative disorders. Publication number: 20100035286.
- In vitro procedure for diagnosis and early diagnosis of neurodegenerative diseases. Publication number: 20090263822.
- In vitro method for the diagnosis of neurodegenerative diseases. Patent number: 7547553.
- ~~CSF~~Cerebrospinal fluid diagnostic in vitro method for diagnosis of dementias and neuroinflammatory diseases. Publication number: 20080206797.
- In vitro method for the diagnosis of neurodegenerative diseases. Publication number: 20080199966.
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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2019.03.006.

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Supplementary data

Multimedia Component 1

Supplemental Material

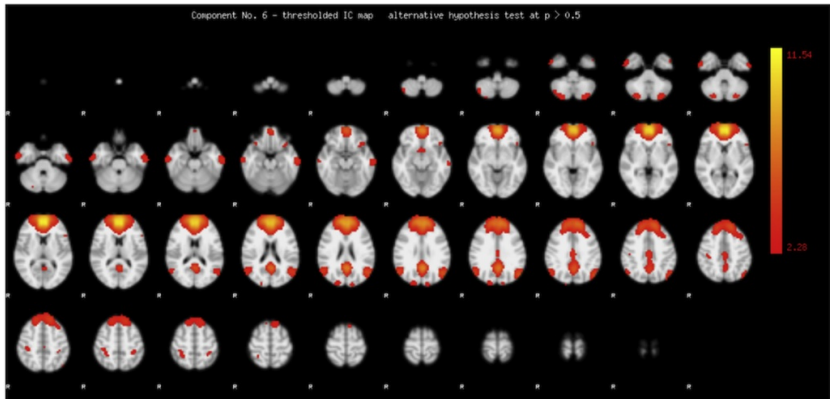

Figure S1

Highlights

- Widespread default mode network (DMN) functional connectivity changes were shown.
- *APOE* ε4 allele genotype leads to distinct DMN functional alterations.
- *APOE* ε4 allele leads to DMN changes regardless of the amyloid β brain deposition.

Queries and Answers

Query: If there are any drug dosages in your article, please verify them and indicate that you have done so by initialing this query

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Answer: keywords: Default Mode Network; Longitudinal study; Alzheimer's disease, APOE, Amyloid β

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Query: Please provide a significance for the † symbol presented for the author “Juan Castrillo” in the Acknowledgment section.
Answer: Juan Castrillo is deceased

Query: Please verify/clarify the use of this abbreviation throughout to conform to the following: 1. “apoE” is used for the protein distinction (all roman, capital E). 2. *“APOE”* is used to refer to the gene (all capitals, italic). 3. If describing a diploid *APOE* genotype, please specify as “*APOE*εX/εX” (where X = 2, 3, and/or 4).
Answer: OK

Query: Please note that “*APOE* ε4 noncarriers (*APOE* ε4+)” has been changed to “*APOE* ε4 noncarriers (*APOE* ε4−)” in the sentence “Participants were divided into…”. Kindly verify.
Answer: OK

Query: Please provide expansion for “PET and CATI” in the sentence “Reconstructed PET images were…”
Answer: Positron emission tomography (PET). CATI is the name of the platform (https://cati-neuroimaging.com)

Query: Please provide expansion for “SUVR” in the sentence “Our cohort was divided…”
Answer: Standardized uptake value ratio (SUVr)

Query: Please provide expansion for “IMAP” in the sentence “The SUVR threshold of …”
Answer: IMAP project: Multimodal neuroimaging project on early Alz- heimer’s disease (Imagerie Multimodale de la maladie de l'Alzheimer a un stade Precoce)

Query: Please provide expansion for “BCB and FSL” in the sentence “The rs-fMRI data were preprocessed…”
Answer: Brain Connectivity and Behaviour (BCB) toolkit; FMRIB Software Library (FSL)

Query: Please provide expansion for “MCFLIRT” in the sentence “After individual correction for movement…”
Answer: using a motion-correction tool based on FLIRT (**MCFLIRT)**

Query: Please provide expansion for “WM and CSF” in the sentence “Estimated motion parameters, the first…”
Answer: white matter (WM) and cerebrospinal fluid (CSF)

Query: Please provide expansion for “AROMA” in the sentence “Given that rs-fMRI signal can…”
Answer: a strategy based on the independent component analysis for Automatic Removal of Motion Artifacts (ICA-AROMA) was used to...

Query: Please note that “ANOVA” has been spelled out as “analysis of variance” in the sentence “To this aim, a 2 (amyloid status…”. Kindly verify. Also please provide expansion for “FEAT”.
Answer: fMRI Expert Analysis Tool (FEAT)

Query: Please provide expansion for “BA” at the first occurrence in the sentence “Second, we assessed the genetic impact…”
Answer: specifically in Brodmann area (BA) 9, BA10, BA46

Query: Please note that citation “Fig. 4” has been changed to “Fig. 3” as the text matches the caption. Also note that there are only three figures in this article.

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Answer: Montreal Neurological Institute (MNI) space

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