# Microglial activation and tau propagate jointly across Braak stages

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**ABSTRACT**

Compelling experimental evidence suggests that microglial activation is involved in the spread of tau tangles over the neocortex in Alzheimer's disease (AD). We tested the hypothesis that the spatial propagation of microglial activation and tau accumulation colocalize in a Braak-like pattern in the living human brain. We studied 130 individuals across the aging and AD clinical spectrum with positron emission tomography brain imaging for microglial activation ([11C]PBR28), amyloid-β (Aβ) ([18F]AZD4694) and tau ([18F]MK-6240) pathologies. We further assessed microglial triggering receptor expressed on myeloid cells 2 (TREM2) cerebrospinal fluid (CSF) concentrations and brain gene expression patterns. We found that [11C]PBR28 correlated with CSF soluble TREM2 and showed regional distribution resembling TREM2 gene expression. Network analysis revealed that microglial activation and tau correlated hierarchically with each other following Braak-like stages. Regression analysis revealed that the longitudinal tau propagation pathways depended on the baseline microglia network rather than the tau network circuits. The co-occurrence of Aβ, tau and microglia abnormalities was the strongest predictor of cognitive impairment in our study population. Our findings support a model where an interaction between Aβ and activated microglia sets the pace for tau spread across Braak stages.

## Introduction

Microglial activation is part of the repertoire of immune responses in the human brain and is a key element associated with the development of Alzheimer's disease (AD) [1-3]. Microglia coexist in the brain with the hallmark pathological features of AD, amyloid-β (Aβ) plaques and tau neurofibrillary tangles [4,5]. Rather than merely being an inflammatory epiphenomenon, recent studies demonstrate that microglial activation drives tau pathology [2,6-8]. These animal studies predict that microglial activation may be a key player in the advance of tau pathology in the living human brain leading to dementia.

Postmortem studies suggest that the accumulation of tau tangles in AD patients follows a stereotypical pattern known as Braak stages [9-12]. The deposition of tau tangles in early Braak stages in the mesial temporal cortex is a typical finding in cognitively healthy populations [9-12], whereas the presence of neocortical tau is normally associated with cognitive impairment. In fact, the propagation of tau over the neocortex has been postulated as the culprit of dementia symptoms [13]. Although it has been proposed that Aβ triggers the spread of tau from transentorhinal/entorhinal structures to the neocortex [14], the low concentrations of Aβ in mesial temporal regions challenge this framework [9,12,15,16]. Thus, understanding the mechanisms by which tau tangles spatially spread, leading to dementia, is still a pressing issue in AD.

Although animal studies support the role of microglial activation in the development of tau pathology and human studies suggest that tau progresses in Braak stages, it is unknown whether microglia play a role in the stereotyped spread of tau in AD patients. Applying recent advances in brain molecular networks [17], using a large group of genetically screened individuals to optimize the signal of microglia activation brain imaging [18,19], and a novel tau imaging agent capable of capturing early and late Braak stages with high sensitivity [20,21], we studied the association between microglial activation and tau propagation. Specifically, we used positron emission tomography (PET) imaging agents selective for Aβ aggregates, tau tangles, and microglial activation, as well as post-mortem triggering receptor expressed on myeloid cells 2 (*TREM2*) gene expression brain maps and cerebrospinal fluid (CSF) concentrations to test whether signatures of AD-related microglial activation are associated with the stages of tau spread. Inspired by experimental literature showing that microglial activation precedes tau pathology in a similarly topographical fashion [4,5,7,22-25], we hypothesized that microglial activation sets the stage for tau propagation in a Braak-like pattern in AD. The characterization of microglial activation as the basis of tau spread in Braak stages would provide critical understanding of an elementary mechanism associated with the development of AD dementia and could lead to novel therapeutic strategies aiming to contain disease progression.

## Materials and methods

### Participants

The study participants were enrolled in the Translational Biomarkers of Aging and Dementia (TRIAD) study, McGill University, Canada, and genotyped for the Ala147Thr polymorphism of the *TSPO* gene (rs6971, https://www.ncbi.nlm.nih.gov/snp/rs6971), which is a predictor of the binding affinity of the tracer [$^{11}$C]PBR28 to the 18-kDa translocator protein (*TSPO*) [18]. *TSPO* is overexpressed in the outer membrane of the mitochondria in activated microglia [26,27]. Based on this genotype, individuals can either be high-, mixed-, or low-affinity binders. Since [$^{11}$C]PBR28 signal in low-affinity binders is negligible, and mixed and high-affinity binders show inherently different signals, we used only high-affinity binders to increase the reliability of the results. All participants had clinical and cognitive assessments, including Clinical Dementia Rating (CDR) and Mini-Mental State Examination (MMSE). Cognitively unimpaired (CU) individuals had no objective cognitive impairment and a CDR score of 0. Mild cognitive impairment (MCI) subjects had subjective/objective cognitive impairment, a CDR score of 0.5, and relatively preserved activities of daily living. Mild-to-moderate sporadic AD dementia met the National Institute on Aging and the Alzheimer's Association criteria for probable AD as determined by a physician and had a CDR score between 0.5 and 2. We excluded participants with inadequately treated systemic conditions, active substance abuse, recent head trauma, major surgery, or presenting with magnetic resonance imaging (MRI) / PET safety contraindications. The study was approved by the Montreal Neurological Instituted PET Working Committee and the Douglas Mental Health University Institute Research Ethics Board, and written informed consent was obtained from all participants.

### Brain imaging methodology

Study participants underwent a 3D MRI (Siemens) anatomical (T1) study, as well as Aβ [$^{18}$F]AZD4694, tau [$^{18}$F]MK-6240, and microglia activation *TSPO* [$^{11}$C]PBR28 PET imaging in the same brain-dedicated scanner (Siemens High Resolution Research Tomograph). [$^{18}$F]AZD4694 images were acquired at 40-70 min after the intravenous bolus injection of the tracer and reconstructed with an OSEM algorithm on a 4D volume

with 3 frames (3 x 600s) [28]. [$^{18}$F]MK-6240 images were acquired at 90-110 min after the intravenous bolus injection of the tracer and reconstructed using the same OSEM algorithm on a 4D volume with 4 frames (4 x 300s) [21]. Finally, [$^{18}$F]PBR28 images were acquired at 60-90 min after the intravenous bolus injection of the tracer and reconstructed using the OSEM algorithm on a 4D volume with 6 frames (6 x 300s) [29]. A 6-min transmission scan with a rotating $^{137}$Cs point source was performed at the end of each PET emission acquisition for attenuation correction. PET images were also corrected for motion, dead time, decay, and scattered and random coincidences. In brief, PET images were automatically registered to the native T1-weighted MRI, and MRIs were linearly and non-linearly registered to the MNI space [30]. Then, PET images were registered to the MNI space using transformations from the PET to native MRI and from the native MRI to the MNI space. [$^{18}$F]MK-6240 Standardized Uptake Value Ratios (SUVRs) used the inferior cerebellum gray matter, whereas [$^{18}$F]AZD4694 SUVRs and [$^{18}$F]PBR28 SUVRs used the whole cerebellum gray matter as the reference region [21,28,29]. Previous studies suggested that simplified methods using the cerebellum as reference offer reliable estimates for [$^{18}$F]PBR28 binding, in fact with a possibly higher sensitivity than with absolute quantitation [29]. Finally, PET images were spatially smoothed to an 8-mm full-width at half maximum resolution. Abnormal PET uptake of tracers (positivity) was defined as SUVR values 2.5 standard deviations (SD) above the mean SUVR of CU young individuals, similar to what has been proposed in previous publications [20,31,32]. The Desikan-Killiany-Tourville atlas was used to define the regions-of-interest [33]. The transentorhinal cortex was defined based on an established segmentation procedure on a 1mm isotropic voxel matrix in stereotaxic space [20,34,35]. PET Braak-like cortical stages were determined based on results from prior autopsy studies [9-12]: Braak I (transentorhinal), Braak II (entorhinal and hippocampus), Braak III (amygdala, parahippocampal gyrus, fusiform gyrus, lingual gyrus), Braak IV (insula, inferior temporal, lateral temporal, posterior cingulate, and inferior parietal), Braak V (orbitofrontal, superior temporal, inferior frontal, cuneus, anterior cingulate, supramarginal gyrus, lateral occipital, precuneus, superior parietal, superior frontal, rostro medial frontal), and Braak VI (paracentral, postcentral, precentral, and pericalcarine). A *TREM2* gene expression distribution image was derived from microarray data obtained

from the open-source Allen Human Brain Atlas (www.brain-map.org) [36], which is composed of mRNA expression intensity values measured on 3702 samples from 6 healthy human brains (4 males, mean age = 42.5 (13.4), postmortem delay= 20.6 (7) hours). *TREM2* mRNA expression was derived from a Gaussian process [37], and downloaded from www.meduniwien.ac.at/neuroimaging/mRNA.html.

**Cerebrospinal fluid measurements**

CSF soluble TREM2 (sTREM2) concentration was measured using a Meso-Scale Discovery assay, as previously described [38]. Additionally, CSF samples were analyzed using a multiplex immunoassay analysis (Inflammation panel; Olink, Uppsala, Sweden) (https://www.olink.com/products/inflammation/) for 92 inflammation-related markers[39]. The list of the 92 proteins may be found in the Supplementary Table 1.

**Statistical methods**

Statistical models were generated using the R statistical software version 3.1.2 (http://www.r-project.org/); voxel-wise statistics were carried out with MATLAB software version 9.2 (http://www.mathworks.com) and VoxelStats package [40]. Voxel-wise receiver operating characteristic curves contrasting groups provided the area under the curve for disease diagnosis. The linear associations between biomarkers were tested using Pearson correlations. Voxel-wise associations between biomarkers were tested using linear regressions accounting for age, gender, education, and *APOE ε4* status. Network analysis for tau tangles ([$^{18}$F]MK-6240 ) and microglia ([$^{18}$F]PBR28) was performed using uptake values from composite brain regions corresponding to Braak histopathological stages (Braak I-VI), derived from regions-of-interest in the Desikan-Killiany-Tourville atlas [33]. The edge values used as the matrix elements were equated to the correlation coefficients between regions-of-interest. The interregional correlation coefficients were calculated using Pearson partial correlation accounting for age, gender, education, *APOE ε4* status, and remaining Braak regions. We verified the adequacy of these associations by verifying that the residuals of the same aforementioned associations tested using linear regressions had a normal distribution, as previously described [17]. Networks were evaluated with a symmetric matrix, showing the strength of the

correlations between regions. Tau and microglia matrices elements were further correlated with each other using Pearson's correlation. Matrices correlations were corrected for multiple comparisons using Bonferroni at $P < 0.05$. A voxel-wise interaction model was built to test the interactive and main effects of Aβ and microglia on tau pathology. Statistical parametric maps were corrected for multiple comparisons using a fast discovery rate threshold of $P < 0.05$. To asses individuals' percentage of abnormal voxels, we created a voxel-wise frequency of tau positivity maps. First, we built z-score parametric maps for each subject, anchored on the normative data of CU young. Then, we generated binary maps of tau positivity for each individual as 2.5 SD higher than the mean SUVR of CU young and averaged these maps. Finally, we calculated the percentage of abnormal voxels for each subject.

**Data availability**

The data presented in this study are available from the corresponding author on a request. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

# Results

We genotyped 503 individuals for *TSPO* polymorphism (rs6971). Two hundred and sixty three (52.4%) were identified as *TSPO* high-affinity binders. Following the inclusion/exclusion criteria, we studied 130 *TSPO* high-affinity binder individuals across the aging and AD clinical spectrum (22 CU young, 64 CU elderly, 28 MCI, 16 AD) who had complete cognition, MRI, and PET data (**Fig. 1**). Table 1 shows the demographic characteristics of the population.

**[$^{11}$C]PBR28 as a proxy of microglial activation in AD**

We found that *in vivo* regional mean [$^{11}$C]PBR28 SUVR was correlated with the postmortem regional mean *TREM2* mRNA expression obtained from the Allen Human Brain Atlas in the respective brain regions (**Fig. 2**), indicating that microglia activation TREM2 gene expression was partially colocalized with *in vivo* [$^{11}$C]PBR28 SUVR uptake in the human brain. In addition, we found that [$^{11}$C]PBR28 SUVR correlated with CSF

sTREM2 concentration (**Fig. 3A**). We also found that CSF sTREM2 concentration was more highly associated with [¹¹C]PBR28 uptake, tau pathology, and other AD-related processes than most of other 92 inflammation-related proteins tested (**Fig. 3B, C**), supporting a stronger link of our results with TREM2 microglial activation compared to other inflammation pathways (see **Supplementary Table 1 and Fig. 1**).

**[¹¹C]PBR28 microglial activation and AD pathophysiology**
[¹¹C]PBR28 SUVR was progressively higher from CU young to CU elderly, MCI, and AD dementia individuals in posterior cingulate/precuneus, inferior parietal, and lateral temporal cortices (**Supplementary Fig. 2**). Voxel-wise receiver operating characteristic curves revealed that [¹¹C]PBR28 uptake differentiated CU young from CU elderly with the highest accuracy in the posterior cingulate, precuneus, lateral temporal, and inferior parietal cortices. [¹¹C]PBR28 uptake differentiate CU elderly from MCI and MCI from AD dementia with the highest accuracy in posterior cingulate/precuneus, inferior parietal, and occipital cortices (**Supplementary Fig. 3**).

Microglial activation measured with [¹⁸F]PBR28 SUVR and CSF sTREM2 correlated with elevated brain levels of Aβ (CSF Aβ42/40 ratio and [¹⁸F]AZD4694) and tau (CSF p-tau181 and [¹⁸F]MK-6240) pathologies (**Fig. 4A-D**). CSF sTREM2 levels in CU and MCI were associated with [¹⁸F]MK-6240 tau load in early Braak regions (transentorhinal, entorhinal, and hippocampus cortices) and later Braak regions, respectively, suggesting a role for TREM2 microglial activation in the spread of tau across the disease spectrum (**Fig. 4E, F**). The prevalence of individuals with abnormal [¹¹C]PBR28 was higher in Aβ-positive than Aβ-negative subjects, and progressively higher according to the individuals' tau Braak stage (**Supplementary Fig. 4**).

**Microglia and tau networks are closely linked with one another**
Partial correlation matrices revealed that microglia levels in regions comprising Braak histopathological stages hierarchically correlated with each other from Braak I to Braak VI. Similarly, partial correlation matrices revealed that tau tangle levels correlated with each other following the hierarchical stages proposed by Braak (**Fig. 5A, B**). The

elements of the microglia-microglia and tau-tau matrices were highly correlated with one another ($r = 0.8574$, $P < 0.0001$), supporting a strong link between the patterns of microglial activation and tau accumulation in the human brain (**Fig. 5C**). Notably, we found that [$^{11}$C]PBR28 SUVR values in the transentorhinal cortex (Braak I) independently predicted short term 1-year tau propagation over regions comprising Braak II-VI accounting for age, sex, *APOE ε4* carrier status, and Aβ (**Fig. 5D**). [$^{11}$C]PBR28 SUVR in other brain regions was not associated with tau propagation over 1 year.

**Aβ potentiates the effect of microglia on tau spreading and dementia**

Interaction models showed that high levels of global Aβ burden and high levels of microglial activation in the transentorhinal cortex synergistically associated with tau load predominantly confined to allocortical regions (**Fig. 6A**). Similarly, high levels of global Aβ burden and microglial activation in neocortical regions comprising Braak IV synergistically determine neocortical tau (**Fig. 6B**), supporting the effect of Aβ in the hierarchical propagation of tau. Also, we found a synergistic interaction between global Aβ deposition and microglial activation on the topographical spread of tau pathology (percentage of abnormal voxels for tau pathology in the brain) (**Fig. 6C**). Together, these models suggested that high levels of both Aβ and microglial activation are required to potentiate tau spread.

Also, we found that a synergistic interaction between Aβ, tau, and microglia activation PET concentrations rather than their independent or grouped by two effects was highly associated with a worse MMSE score ($P = 0.0003$). Analysis of variance strongly supported that the model with the triple interaction best described this relationship than the possible reduced models ($P < 0.0001$). In addition, we found a progressive increase in the percentage of individuals with the combination of abnormal Aβ (A), tau (T), and microglial activation (MA) from CU young (0%), to CU elderly (0%), MCI (27%), and AD dementia (67%). On the other hand, the prevalence of microglia activation abnormality alone or together with only Aβ or tau abnormalities were not different between clinical groups, suggesting that the presence of both Aβ and tau is necessary for microglial activation associates with cognitive impairment (**Fig. 6D**). The concomitant

presence of A/T/MA was the factor most highly associated with cognitive impairment in our population (**Fig. 6E**).

## Discussion

Our results suggest that microglial activation and tau accumulation spatially spread in parallel following brain circuits predicted by postmortem series from the transentorhinal/entorhinal to sensorimotor cortices. In addition, our results support that Aβ potentiates the effect of microglial activation on tau spread. We also found evidence suggesting that when these three pathologies are present in the human brain concomitantly, they interact with each other to determine dementia.

The spatial distribution of microglial activation and tau accumulation followed a similar patterns of progression, which were previously described for tau pathology in postmortem series [9-12]. These findings support established *in vitro* literature suggesting that microglial activation occurs in a similarly stereotyped fashion as tau pathology [7,22-25]. Although it has been demonstrated that microglia are able to degrade tau [41], one may speculate that the mechanism by which this joint propagation occurs rests on the fact that microglia may phagocytize and release exosomes containing inefficiently degraded tau, which would contaminate surrounding cells with tau pathology [42-45]. Indeed, recent studies confirm that microglia release tau seeds, which is the tau conformation capable of inducing tau aggregation in affected cells [42-46], as well as increased levels of exosomes containing tau in AD [47,48]. Expanding upon these *in vitro* studies, we showed here that this join propagation of microglia and tau hierarchically follows Braak-like stages in the human brain. Our results bring together two important concepts in AD, Braak-like tau propagation [9-12] and the emerging concept of the effects of microglial activation on tau spread [2]. Understanding the relationship between microglia and the spatial spread of tau may prove crucial to designing new therapies targeting the interplay between these pathologies.

Although there is compelling evidence supporting that Aβ is associated with neocortical tau [14], the mechanisms by which Aβ triggers tau spread is poorly understood. Indeed, the

lack of a strong correlation between Aβ plaques and tau and the relatively low concentrations of Aβ in the allocortex contest a direct link between Aβ and tau spread from allocortical to neocortical regions [9,12,15,16]. Here, we found that Aβ pathology leads to tau spread by potentiating the effects of microglial activation on tau. Our interaction model suggested that Aβ pathology in the absence of activated microglia was associated with modest levels of neocortical tau. These results are in line with recent experimental literature, supporting that Aβ potentiates tau propagation in the presence of activated microglia [2]. Together, our findings support the notion that microglial activation links the deleterious effects of Aβ on tau aggregation. Interestingly, similar to previous postmortem literature [49], we also showed neocortical tau in the absence of activated microglia in a small number of AD subjects, suggesting that other pathways may also potentiate tau propagation.

We found that $[^{11}C]$PBR28 uptake was closely related to the microglial activation marker TREM2 [50]. In our analyses, *in vivo* $[^{11}C]$PBR28 SUVR correlated with CSF sTREM2 concentration, and it at least partially overlapped with the distribution of *TREM2* gene expression obtained from the Allen Human Brain Atlas [36]. These results offer further evidence support $[^{11}C]$PBR28 as a proxy of activated microglia in the brain, and suggest that $[^{11}C]$PBR28 uptake may be closed related to TREM2 microglial activation pathways in AD [50]. In fact, several previous publications suggest a link between the microglial marker TREM2 and AD [50]. sTREM2 has already been shown to have a positive correlation with tau biomarkers [51]. Interestingly, silencing *TREM2* in a model presenting tau pathology in the absence of Aβ exacerbates tau pathology [52], which supports the idea that Aβ is crucial for the deleterious association between microglia and tau. We also measured other 92 inflammation-related proteins in CSF, under the assumptions that microglial activation sTREM2 better represent our imaging results than other inflammation pathways. We found that sTREM2 was among the inflammation proteins most strongly associated with $[^{18}F]$PBR28 uptake in key AD regions, cognitive impairment, hippocampal atrophy, tau pathology, and white matter disease. On the other hand, several other proteins showed a stronger association with Aβ than sTREM2. Together, these results support a link of our $[^{18}F]$PBR28 results with TREM2 microglial

activation pathways and the notion that Aβ and microglial activation may be two partially independent processes that, when converge, synergistically potentiate tau pathology.

The co-occurrence of Aβ, tau, and microglia activation abnormalities was synergistically associated with cognitive impairment and dementia. These results support models suggesting a double hit of Aβ on AD progression, first potentiating the effect of microglial activation on tau accumulation and then interacting with these pathologies to determine dementia [17]. Additionally, these results suggest a close link between microglial activation, AD hallmark biomarkers, and dementia, suggesting that microglial activation may be a key element associated with AD pathophysiology with the potential to be incorporated in the biological definitions of the disease [53].

Our results may have implications for clinical trials. Since microglial activation in transentorhinal cortex was potentially involved in "unleashing" mesial temporal tau over the neocortex, we may predict that individuals with tau deposition confined to transentorhinal/entorhinal regions could benefit from preventive therapies targeting transentorhinal/entorhinal microglial activation. These trials could have advantages from using validated fluid and brain imaging markers of microglial activation to assess drugs' target engagement and efficacy. In fact, previous studies have already associated microglia dysfunction in the entorhinal cortex to AD progression [54,55].

The strengths of the present study include the use of only high-affinity binders for [11C]PBR28 tracer, avoiding the need for corrections associated with mixed-affinity cases. The use of only high-affinity binders allowed to perform methods highly sensitive to noise associated with artificial uptake variation, such as network analyses. All PET scans were performed using a single high-resolution brain-dedicated camera to avoid site-specific variabilities. This study has methodological limitations. It would be highly desirable to replicate our results in studies using long-term sequential biomarkers measurements at multiple time points to evaluate the longitudinal progression of pathological processes and better understand their temporal interrelationships. However, due to the limited availability of large-scale studies including microglia activation PET

imaging, we will probably have to wait for the cohort to mature before being able to make such observations. Although it would be desirable to replicate our findings in larger populations, it is important to emphasize that, to the best of our knowledge, this is the largest study available using microglia activation PET imaging across a disease spectrum. *TSPO* expression indirectly captures microglia [56]. Still, there is no technology available that has been better validated for assessing cerebral microglial activation than *TSPO* PET ligands. One challenge for *TSPO* and numerous other PET tracers targeting physiological processes is the lack of an appropriate reference region for measuring non-displaceable binding because no brain area is devoid of *specific* binding for those molecules. However, previous studies have suggested that the cerebellum may be a useful reference region that can substitute, with possible advantages, absolute [$^{18}$F]PBR28 quantitation associated with complex set-ups for arterial blood sampling and HPLC measurements [29]. In fact, the use of a reference region as opposed to arterial sampling allows the design of large-scale studies, such as the one presented here. Finally, due to self-selection bias, our participants may not represent the general population.

To conclude, our results support that microglial activation is a key element linking the effects of Aβ to tau spread and ultimately dementia.

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## Disclosure statement

KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). The other authors declare no competing financial interests.

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**Table**

**Table 1. Demographics and key characteristics of the clinical population.**

| Characteristic | CU Young | CU Elderly | MCI | AD dementia |
|---|---|---|---|---|
| **Number** | 22 | 64 | 28 | 16 |
| **Age, years, mean (SD)** | 23 (2.4) | 72 (5.5) | 73 (8.6) | 70 (7.7) |
| **Male, number (%)** | 8 (36) | 14 (22) | 17 (61) | 6 (538) |
| **Education, years, mean (SD)** | 16.6 (1.7) | 15.5 (3.7) | 14.9 (3.4) | 14.4 (3.3) |
| **MMSE score, mean (SD)** | 29.8 (0.5) | 29.2 (1) | 27 (4) | 21 (6.3) |
| **APOE ε4, number (%)** | 3 (14) | 18 (28) | 13 (46) | 7 (44) |