Frequency and longitudinal clinical outcomes of Alzheimer's AT(N) biomarker profiles:

a longitudinal study

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Abstract

INTRODUCTION: We aimed to estimate the proportion of each AT(N) (β-amyloid deposition [A], pathologic tau [T] and neurodegeneration [N]) profile in different clinical diagnosis groups and to describe the longitudinal change in clinical outcomes of individuals in each group.

METHODS: Longitudinal change in clinical outcomes and conversion risk of AT(N) profiles are assessed using linear mixed effects models and multivariate Cox proportional hazard models, respectively.

RESULTS: Participants with A+T+N+ showed faster clinical progression than those with A-T-N- and A+T±N-. Compared with A-T-N-, participants with A+T+N± had an increased risk of conversion from CN to incident prodromal stage of AD, and from MCI to AD dementia. A+T+N+ showed an increased conversion risk when compared with A+T±N-.

DISCUSSION: The 2018 research framework may provide prognostic information of clinical change and progression. It may also be useful for targeted recruitment of AD participants into clinical trials.

Keywords: Alzheimer's disease; research framework; biomarker; prognosis

1. Background

Alzheimer's disease (AD) is characterized by amyloid plaques, tau tangles, synapse loss, neurodegeneration leading to impairments on memory and other cognitive domains and subsequently dementia syndrome. Prior to the use of biomarkers, including Magnetic Resonance Imaging (MRI) to detect atrophy, amyloid Positron Emission Tomography (PET) scans and cerebrospinal fluid (CSF) measurements to measure amyloid, and tau, AD could only be diagnosed with certainty at autopsy. In 2011, the National Institute on Aging–Alzheimer's Association (NIA-AA) published research criteria for AD diagnosis [1] : Dementia due to Alzheimer's disease, prodromal AD (mild cognitive impairment (MCI) due to AD), and preclinical AD (individuals with normal cognition who have AD pathology). Similarly, other criteria including the International Working Group-2 (IWG-2) [2], and Dubois criteria [3] were reported.

Recently, the NIA-AA published an updated research framework defining AD biologically by neuropathological biomarkers which are independent from clinical symptoms. By updating the 2011 guidelines [4], the new 2018 research framework grouped biomarkers into three categories: biomarkers of Aβ plaques (labeled "A"): cortical amyloid PET ligand binding or low CSF $A\beta_{42}$; biomarkers of paired helical filament tau (labeled "T"): elevated CSF phosphorylated tau (p-tau) and cortical tau PET ligand binding; biomarkers of neurodegeneration or neuronal injury (labeled "N"): elevated CSF total tau (t-tau), 18F-fluorodeoxyglucose (FDG) PET, and brain atrophy on MRI. Dichotomizing these biomarkers as normal or abnormal results in eight AT(N) profiles. The idea of AT(N) biomarker grouping did not originate with the NIA-AA research framework. It was first proposed by an international group of investigators in 2016[5]. The research framework indicated that if an individual presents with both biomarker evidence of Aβ and pathological tau, the term "Alzheimer's disease" would be applied. Symptoms of AD are treated as a phase of an "Alzheimer's continuum" and can be used to stage severity of the disease.

This recommendation is labeled a "research framework" since its intended use is for observational and interventional research, but not for clinical use. Applying the framework in a large longitudinal cohort would help researchers to modify this framework if needed before it being adopted into actual clinical practice. Recent cross-sectional studies from the Mayo Clinic and H70 Gothenburg Birth Cohort reported the prevalence of each AT(N) profile in cognitively unimpaired individuals [6, 7]. However, this study is limited by its cross-sectional design, and longitudinal data is hence necessary and urgently needed to provide important information on the clinical/cognitive outcomes of these AT(N) profiles. The Alzheimer's Disease Neuroimaging Initiative (ADNI) is a very large multisite longitudinal observational study with the objective of validating biomarkers for AD clinical trials [8-10]. ADNI makes all data available without embargo to all qualified scientists, leading to over 1500 publications [11, 12]. To date all ADNI publications either use purely clinical classifications (dementia, MCI, subjective memory complaints, or cognitively normal), or the previous NIA-AA classifications (described above). The objective of the present study was to estimate the proportion of AT(N) profiles in clinical diagnosis groups and to describe the longitudinal change in clinical outcomes of individuals in each group.

2. Methods

2.1 ADNI Study Design

We undertook cross-sectional and longitudinal analyses of participants enrolled in the ADNI database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership with the primary goal testing whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI and early AD. For up-to-date information on ADNI, see www.adni-info.org. ADNI was approved by the institutional review boards of all participating institutions. Written informed consent was obtained from all participants at each site.

2.2 Participants

Individuals from the ADNI were included in our study if they underwent amyloid PET or CSF Aβ analysis (A), CSF p-tau examination (T) and FDG-PET (N) at baseline. Detail information of the included participants were presented in the eMethods in the Supplement. Amyloid abnormal (A+) and normal (A-) were determined by applying a cutoff value of 1.11 for the florbetapir standardized uptake value ratio (SUVR) and 192pg/ml for CSF $A\beta_{42}$[13]. Whether tau pathology was abnormal (T+) or normal (T-) using 23 pg/ml for CSF p-tau level[13]. The cutoff point for FDG PET (N) (average of angular, temporal, and posterior cingulate) was 1.21[14] . As a secondary analysis, abnormal N was defined as hippocampal volume adjusted for total intracranial volume (HVa) of less than 6723 mm^3 [13, 15]. (eMethods in the Supplement) For the current study, we excluded borderline cases and reset the cut-offs that were ±5% from the original cutoffs to avoid drawing conclusions based on borderline cases (supplementary table 1). In our study, we stratified the MCI group into stable MCI (sMCI) with no progression to AD dementia during at least 2 follow-up years, and progressive MCI (pMCI) with progression to AD dementia during at least 2 follow-up years. Controls had MMSE scores of 24 or higher and a CDR score of 0.

2.3 CSF Measurements

CSF $A\beta_{42}$ and p-tau were measured at the ADNI Biomarker Core Laboratory (University of Pennsylvania) using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use-only reagents) immunoassay kit-based reagents. All CSF biomarker assays were performed in duplicate and averaged.

2.4 Neuroimaging and Cognition

Amyloid PET imaging was measured with florbetapir. Florbetapir binding images were averaged, spatially aligned, interpolated to a common voxel size (1.5 mm^3), and smoothed to a common resolution of 8mm full width at half maximum. The global 18F-florbetapir SUVR was calculated by averaging the 18F-florbetapir retention ratio from four large cortical grey matter regions (frontal, anterior cingulate, precuneus, and parietal cortex) using the cerebellum as a reference region.

FDG-PET data were acquired and reconstructed according to a standardized protocol (http://adni.loni.ucla.edu/). Spatial normalization of each individual's PET image to the standard template was conducted using SPM529. For FDG-PET, we averaged counts of angular, temporal, and posterior cingulate regions.

Structural MRI was performed using a Siemens Trio 3.0T scanner (n=507) or Vision 1.5T scanner (n=131) (GE, Siemens and Philips). Regional volume estimates were processed using Free-surfer software package version 4.3 and 5.1 image processing framework for the 1.5 and 3.0T MRI images, respectively. ROIs included the hippocampus and ventricles. Estimated intracranial volume (ICV) was used to adjust ROIs for head size variation based on covariance.

General cognition was assessed by MMSE and Alzheimer Disease Assessment Scale–cognitive subscale (ADAS-Cog) 11 score. (eMethods in the Supplement)

2.5 Statistical analysis

We summarized binomial distributions with percentages and calculated 95% confidence intervals for percentages using the Wilson method. Differences across the eight biomarker profiles were tested by the Kruskal-Wallis tests for continuous variables and chi-square tests for categorical data. To evaluate how clinical outcomes changed overtime, we included A-T-N-, A+T-N-, A+T+N- and A+T+N+ using linear mixed-effects models. To access the risk of progression from no cognitive impairment to incident prodromal stage of AD indicated by the Clinical

Dementia Rating scale (CDR)–Global score of 0.5 or greater, and from MCI to incident AD dementia, we constructed un-adjusted Kaplan-Meier plots. Additionally, we ran multivariate Cox proportional hazard models (eMethod in the Supplement). All statistical analyses were performed using the R statistical software (version 3.4.4).

3. Results

Of the 645 (198 CN, 310 MCI and 137 AD dementia) were assessed at enrolment. 541 participants had follow-up data of at least one year. 283 participants were also assessed at 3 years, 238 participants at 4 years, and 80 participants at 5 years. The mean (SD) duration of follow-up for each cognitive status group were presented in supplementary table 2. The demographic, clinical, and imaging characteristics of the included participants are shown in Table 1 and supplementary table 3. We added a flow chart to demonstrate the participant screening process (supplementary figure1). The mean (SD) age of the participants was 72.7 (7.3) years; 53.6% were men; 98.6% had more than 12 years of education; 47.0% had an *APOE* ε4 allele.

3.1 Proportion of AT(N) profiles based on four traditional clinical diagnostic groups

The proportion of abnormal amyloid was 41.4% (95% CI 34.5% to 48.6%) in CN group, 58.2% (95% CI 51.5% to 64.7%) in sMCI group, 96.5% (95% CI 90.0% to 99.3%) in pMCI group, and 94.9% (95% CI 89.8% to 97.9%) in AD dementia group. A+T+N± accounted for 32.5% in CN group, 42.0% in sMCI group, compared to 93.0% in pMCI group and 92.0% in AD dementia group. The proportion of A+T+N+ was 2.5% in CN group, 15.1% in sMCI group, 70.7% in pMCI group, and 87.6% in AD dementia group. Suspected non-AD pathophysiology (SNAP) (A-T+N-, A-T-N+ and A-T+N+) accounted for 39.4% in CN group, 22.7% in sMCI group, compared to only 3.5% in pMCI group and 3.6% in AD dementia group (Figure 1). AT(N) proportion by traditional clinical

diagnosis was also calculated using HVa as the N measure (supplementary figure 2). We found the proportion of N in the entire group varied with the methods we used (7% using FDG-PET and 41.46% using HVa). Moreover, there were no participants that had the biomarker combinations A+T-N+ and A-T-N+.

3.2 Clinical and demographic characteristics of individuals in each AT(N) profile

Among "Alzheimer's continuum" profiles (A+T-N-, A+T+N-, A+T-N+ and A+T+N+), 30.6% were clinically diagnosed as "AD dementia". For those with SNAP, 3.6% were clinically diagnosed as "AD dementia". In the A-T-N- profile, only 2.4% were clinically diagnosed as "AD dementia" (supplementary figure 3). Proportion of diagnosis in each AT(N) profile was also calculated using HVa as the N measure (supplementary figure 4). All MRI (hippocampal and ventricular volumes) and cognitive (ADAS-COG 11 and MMSE) measures were different among A-T-N-, A+T-N-, A+T+N- and A+T+N+ profiles at baseline in either CN or MCI patients after adjustment for age, gender, ICV (for MRI) and years of education (for cognitive measures) (supplementary figure 5).

3.3 Longitudinal clinical outcomes in each AT(N) profiles

In CN individuals, only A+T+N+ individuals showed changes in MMSE score. Furthermore, changes in hippocampal and ventricular volumes were observed in all four biomarker profiles (A-T-N-, A+T-N-, A+T+N- and A+T+N+) (Figure 2). As expected, CN individuals with A+T+N+ showed faster clinical progression than the remaining three profiles (A-T-N-, A+T-N- and A+T+N-). However, no significant differences were detected between A+T-N- vs A-T-N- and A+T+N- vs A+T-N- (supplementary table 4).

Among MCI patients, cognitive changes were observed in A+T+N±. However, changes in hippocampal and ventricular volumes were observed in all four profiles (Figure 2). MCI patients with A+T+N+ also showed faster clinical progression than the remaining three profiles. However, no significant differences were detected between

A+T-N- vs A-T-N- (supplementary table 4). In addition, longitudinal analysis for cognitive decline adjusted for age, gender, *APOE* ε4 status and education years, and analyses for brain atrophy adjusted for age, gender, *APOE* ε4 status, total intracranial volume and field strength (1.5T vs 3.0T) are presented in supplementary figure 6. Because of the general unavailability of amyloid PET or FDG-PET for routine clinical use in many geographic locations, we added a longitudinal analysis of the data based only on CSF and MRI measures (supplementary figure 7).

We further accessed the changes in clinical outcomes of subgroups stratified by gender and *APOE* ε4 status (supplementary figure 8-9). In addition, we examined the differences in the change rates of clinical outcomes in female versus male and *APOE* ε4 carriers versus *APOE* ε4 non-carriers (supplementary figure 10-11). In the CN group, females with A+T-N- showed faster change rates of MMSE score than males. In the MCI group, females with A+T+N+ showed faster change rates of ventricular volume than males. In CN group, *APOE* ε4 carriers showed faster rates of hippocampal atrophy than did those with *APOE* ε4 non-carriers in A+T+N+ individuals. MCI patients who carried *APOE* ε4 showed faster ADAS-COG and ventricular volume change in A+T+N- and A+T+N+ individuals respectively than *APOE* ε4 non-carriers.

We also assessed how subjects move between the different ATN groups from baseline to each follow-up examination. Individuals were included if they underwent amyloid PET or CSF Aβ analysis (A), CSF p-tau examination (T) and FDG-PET (N) at baseline and with at least 2 years' follow-up data of each biomarker group. However, as time went on, the number of individuals in each group became extremely small. It is hard to conclude how subjects moved between ATN categories and how clinical outcomes changed (supplementary figure 12).

3.4 Prediction of disease progression for each biomarker profile

Figure 3 exhibits the results of a Kaplan-Meier analysis and the log-rank test. Cox proportional hazards models

were developed to estimate the conversion risk from no cognitive impairment to incident prodromal stage of AD indicated by a CDR–Global score of 0.5 or greater, and from MCI to incident AD dementia for each biomarker profile, controlling for baseline age, gender and years of education. Covariates of both models met PH assumptions using Schoenfeld residuals technique (Global Schoenfeld Test p =0.23 and 0.51, respectively). CN individuals with A+T+N+ and A+T+N- had an increased risk of conversion to the prodromal stage of AD (CDR-GS ≥ 0.5) compared with A-T-N-. CN individuals with A+T+N+ also had an increased risk of conversion to prodromal stage of AD compared with A+T-N- and A+T+N-. However, we did not detect any differences in conversion risk among CN individuals between A+T+N- and A+T-N-, and between A+T-N- and A-T-N-.

In MCI patients, compared with A-T-N-, participants with A+T+N+ and A+T+N- had an elevated risk of conversion to AD dementia. MCI patients with A+T+N+ also had an increased risk of conversion to AD dementia compared with A+T-N- and A+T+N-. However, we did not detect any differences in conversion risk among MCI individuals between A+T+N- and A+T-N-, and between A+T-N- and A-T-N- (Table 2). We also accessed the changes in clinical outcomes and the conversion risk by using HVa to define N (supplementary figure 13-14).

4. Discussion

Our findings suggest that as the disease progresses, abnormal A/T/N biomarkers accumulate. Cognitive decline was observed only in A+T+N±. However, brain atrophy was observed in A-T-N-, A+T-N- and A+T+N±, in both MCI and CN elders. Individuals with abnormal amyloid had faster change rates of clinical outcomes and faster progression rates if they had abnormal phospho-tau, with or without neurodegeneration. Brain β-amyloidosis alone (without tauopathy and neurodegeneration) did not predict clinic outcomes change and disease progression. A faster change rate of clinical outcomes was observed in female versus male and *APOE* ε4 carriers versus *APOE* ε4 non-carriers but only in amyloid positive profiles.

In CN group, the proportion of A-T-N- was only 19.2%. This proportion is consistent with other publications that have shown that biomarkers change years before symptom onset [16]. A recent study from the Mayo Clinic reported the proportion of each AT(N) profile in CN individuals [6]. Unlike the Mayo clinic study, which is likely to be representative of a community-type sample of older people, the ADNI samples exclude people with comorbidities such as stroke and other neurodegenerative disease. However, the proportions of N+ were highly similar when we used HVa to define N (37% from Mayo vs 41.46% from our study), and were consistent with a recent ADNI study [17]. Furthermore, it is of interest that SNAP accounts for a sizeable proportion of the CN group. How the pathology of individuals with this biomarker category developed needs further scrutiny in longitudinal studies. In CN group, proportion of A/N scheme were also conducted in Knight Alzheimer's Disease Research Center[18]. Proportion of A/N scheme were also performed previously in ADNI and Mayo Clinic Study of Aging in MCI patients[19]. The results were nearly identical with ours. In AD dementia patients, the proportion of A+T+N±, which could be termed "Alzheimer's disease" according to the NIA-AA research framework, reached 92.0%. The "AD diagnosis" of the remaining 8% individuals was pathologically proven to be false.

There is face validity to the observation that the proportion of A+T+N+ was increasing and the proportion of A-T-N- was decreasing from CN to AD dementia. A+T+N- increased from 30.3% (in CN group) to 36.9% (in sMCI group) and then decreased to 4.4% (in AD dementia group), and A+T-N- were concentrated in CN and sMCI groups. This supports that A+T−N− to A+T+N− to A+T+N+ is the temporal sequence of biomarkers towards AD. We postulate SNAP consist of primary age-related tauopathy (PART), non-Alzheimer's degeneration and their combination. From our findings, SNAP, especially PART, usually range from normal to mild cognitive changes.

By using different methods to define "N", we detected that the proportion of "N" was 7% using FDG-PET and 41.46% using HVa. A previous study also detected a poor agreement between FDG-PET and hippocampal volume in the ADNI database [20], reflecting discordance among biomarkers in the N group. In fact, the two methods for

defining N track distinct aspects of the AD pathophysiological process: Atrophy on MRI reflects dendritic and neuronal losses and FDG PET likely indicates synaptic activity and loss of synapses[21, 22]. Beyond that, the discordance may be partly explained by the dynamic character of biomarker changes and suboptimal cutoff values. While FDG may (and almost certainly does) reflect something about progression, its group differences even in much younger normals makes its meaning less clear than structural volume changes.

In the longitudinal analyses, changes in hippocampal and ventricular volumes were observed in all the four profiles in both CN and MCI groups. This might partly relate to aging not captured by these biomarkers. However, general cognition (MMSE score and ADAS-COG 11 score) decline was only seen in A+T+N±. This might be partly interpreted as brain atrophy occurring earlier than cognitive decline [23, 24]. However, one limitation should be noted that MMSE and ADAS-Cog may not be the most sensitive markers for change (vs longer, more difficult episodic memory tests). Non-cognitive functions such as function (Alzheimer's disease cooperative study-activities of daily living) and behavior (neuropsychiatric inventory) could also be accessed in the future which be informative for clinical trials. In comparing the change rates of clinical outcomes, it is reasonable that A+T+N+ showed the fastest and A-T-N- showed the slowest clinical progression among the four profiles. Note that previous work with CN individuals adopting a two-class biomarker construct, A+N- vs A-N- [15], found no differences in cognitive decline. However, an another A/N study reported contrary results[25]. CSF p-tau specifically reflects the phosphorylation state of tau, a different form of t-tau, which in turn reflects nonspecific neurodegeneration or neuronal injury. Therefore, the 2018 research framework separates biomarkers for pathologic tau from measures of neurodegeneration or neuronal injury. However, no significant differences were detected between A+T-N- vs A-T-N-. In line with our findings, there was no association between CSF $A\beta_{42}$ status and cognitive decline or volume loss among CSF p-tau negative individuals. This association only occurred among CSF p-tau positive individuals [26, 27]. To further examine this result, comparisons of clinical outcomes between A-T+N± and

A+T+N± would be warranted in future studies. There is considerable prior work indicating that cerebral amyloidosis is associated with longitudinal clinical decline before the clinical diagnosis of AD[28, 29]. However, whether there were interactions between Aβ and tau is not clear, although the evidence points towards interactions between Aβ and tau being implicated in AD-related clinical decline [30]. Longitudinal analyses of Alzheimer's continuum profiles could facilitate participant selection and prediction of trial outcomes [31]. Moreover, our longitudinal study examined the extent to which relationships of signs/symptoms and biomarkers improved study validity if they were to be adopted into clinical practice.

Overall, females had a faster change rate of clinical outcomes than males, but only in amyloid positive profiles according to our findings. These results were consistent with previous studies, which found that females with AD pathology are more likely to be expressed clinically as dementia than males[32]. Our findings indicated that the increased change rate of clinical outcomes in females might be Alzheimer's continuum-specific. Whether the same results are observed amongst SNAP individuals will need further study.

Many studies have reported that *APOE* ε4 carriage increases the rate of Aβ-related cognitive decline occurring in the preclinical AD [33]. We found *APOE* ε4 carriage could accelerate clinical decline among Aβ positive individuals. However, this effect only existed in the presence of phospho-tau with or without neurodegeneration (A+T+N- or A+T+N+). Again, these findings point to phospho-tau as an important marker of Aβ-associated clinical decline. We also confirmed that this effect is greater in *APOE* ε4 carriers.

In line with our findings, a recent study reported that among cognitively healthy elderly participants with subjective memory complaints, brain β-amyloidosis alone (negative for raised tau protein concentrations) did not predict progression to prodromal Alzheimer's disease [34]. Our present findings are also consistent with our previous meta-analysis, which indicated that the combination of low CSF Aβ and high CSF tau levels could significantly predict the progression from MCI to AD dementia, whereas abnormal CSF Aβ alone had no

significant association with the progression to AD dementia in patients with MCI.[35] Our findings support the hypothesis that Aβ accumulation is necessary, but not sufficient to produce the clinical decline of AD [4].

Our study had limitations. The number of individuals within each profile was relatively small as eight possible AT(N) combinations exist. The numbers were smaller still when stratifying by gender and *APOE* ε4. Focusing on the biomarker sequence of preclinical AD, small samples sizes precluded A-T-N+, A-T+N-, A-T+N+ and A+T-N+ groups in longitudinal and survival analyses. The cut-off points we used should be thoroughly examined using the methods described previously [36]. A single cut point approach lacks accuracy when research questions require high diagnostic certainty. Last, the present study did not analyze how vascular factors (e.g. small vessel disease), which commonly co-occur in elderly, may affect the clinical manifestation and rate of cognitive decline in this framework. Reproducibility of findings in different patient groups from different centers would be benefit for the clinical applicability of these biomarkers.

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Competing interests

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biomarker cut points for brain aging and Alzheimer's disease. Alzheimers Dement. 2017;13:205-16.

Figure legends

Figure 1: Proportion of each AT(N) profile in different clinical diagnosis group. AD=Alzheimer disease dementia. MCI=mild cognitive impairment. CN=cognitively normal. A−=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−, tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

Figure 2: Change in clinical outcomes among the four AT(N) profiles based on linear mixed effects regression models. Analyses of cognitive decline were adjusted for age, gender and education years. Analyses of brain atrophy were adjusted for age, gender, total intracranial volume and field strength (1.5T vs 3T). Change in clinical outcomes is expressed as an annual percentage of cognitive function scores and volume change, with 95% CIs and p value. MCI=mild cognitive impairment. CN=cognitively normal. A−=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−, tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

Figure 3: Kaplan-Meier curves showing cumulative probability of disease progression. (A) Progression from cognitively normal participants to incident prodromal stage of AD indicated by a CDR–Global score of 0.5. (B) Progression from mild cognitive impairment to incident AD dementia. AD=Alzheimer disease dementia. MCI=mild cognitive impairment. CN=cognitively normal. A−=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−, tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

Table1. Characteristics of 645 participants by AT(N) biomarker classification

	A-T-N-	A-T+N-	A-T-N+	A-T+N+	A+T-N-	A+T+N-	A+T-N+	A+T+N+	P value *
n	83	114	15	8	25	168	13	219	
AGE, mean (sd), years	71.03 (6.68)	70.62 (6.97)	76.09 (8.61)	78.19 (5.31)	71.38 (6.83)	73.41 (6.80)	76.31 (6.47)	73.40 (7.58)	<0.001
Male, No. (%)	38 (45.8)	60 (52.6)	10 (66.7)	5 (62.5)	15 (60.0)	78 (46.4)	11 (84.6)	129 (58.9)	0.04
APOE ε4 genotype carriers, No. (%)	9 (10.8%)	24 (21.1%)	1 (6.7%)	3 (37.5%)	8 (32.0%)	99 (58.9%)	6 (46.2%)	153 (69.9%)	<0.001
Education, mean (sd), years	16.51 (2.67)	16.63 (2.60)	16.33 (3.15)	15.12 (1.46)	15.48 (3.15)	15.86 (2.60)	16.15 (3.29)	15.89 (2.91)	0.143
MMSE score, mean (sd))	28.66 (1.59)	28.74 (1.48)	28.13 (2.39)	26.50 (2.98)	28.84 (1.07)	28.23 (1.80)	26.54 (3.57)	24.78 (2.70)	<0.001
Hippocampus, mean (sd), mm^3	7405.01 (811.68)	7579.93 (987.22)	7065.33 (911.39)	5789.17 (1802.62)	7696.72 (769.82)	7136.62 (980.27)	6026.30 (1031.97)	6131.52 (1012.89)	<0.001
Amyloid PET, SUVR, mean (sd)	1.01 (0.06)	1.02 (0.05)	1.01 (0.06)	1.01 (0.08)	1.14 (0.21)	1.33 (0.19)	1.17 (0.14)	1.43 (0.17)	<0.001
FDG, mean (sd)	1.38 (0.08)	1.36 (0.08)	1.10 (0.05)	1.07 (0.04)	1.39 (0.08)	1.37 (0.09)	1.04 (0.10)	1.04 (0.09)	<0.001

Data are mean (SD) or number (%) unless otherwise stated. MMSE=Mini-Mental State Examination. FDG=18F-fluorodeoxyglucose. A−=amyloid normal

using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+, tau abnormal using CSF p-tau. N−, neurodegeneration or neuronal injury normal using FDG. N+, neurodegeneration or neuronal injury abnormal using FDG.

* P values are from the Kruskal-Wallis test or Fisher exact test.

Table 2. Progression risk from CN to prodromal stage of AD and from MCI to AD

Biomarkers	5-year progression rate	Hazard ratio (95% CI) *	P-value	Hazard ratio (95% CI) *	P-value	Hazard ratio (95% CI) *	P-value
Progression from CN to prodromal stage of AD							
A-T-N-	10.7%	Reference		/	/	/	/
A+T-N-	45.4%	1.84(0.50-6.8)	0.36	Reference		/	/
A+T+N-	44.4%	2.79(1.14-6.9)	0.03	1.50(0.52-4.4)	0.46	Reference	
A+T+N+	100%	11.21(2.83,44.4)	<0.001	6.90(1.16-29.6)	0.009	4.68(1.39,15.7)	0.01
Progression from MCI to AD							
A-T-N-	10%	Reference		/	/	/	/
A+T-N-	11.1%	2.92(0.18-47.3)	0.45	Reference		/	/
A+T+N-	24.5%	9.89(1.30-75.2)	0.03	3.33(0.44-25.2)	0.24	Reference	
A+T+N+	85.95%	53.66(7.22,398.8)	<0.001	17.34(2.33-128.9)	0.005	5.20(3.13-8.6)	<0.001

CN=cognitively normal. MCI=mild cognitive impairment. AD=Alzheimer's disease. A−=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid

abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

* Hazard ratios (95% CI) calculated using Cox regression analyses and corrected for baseline age, gender, *APOE* ε4 status and years of education.

Figure 1

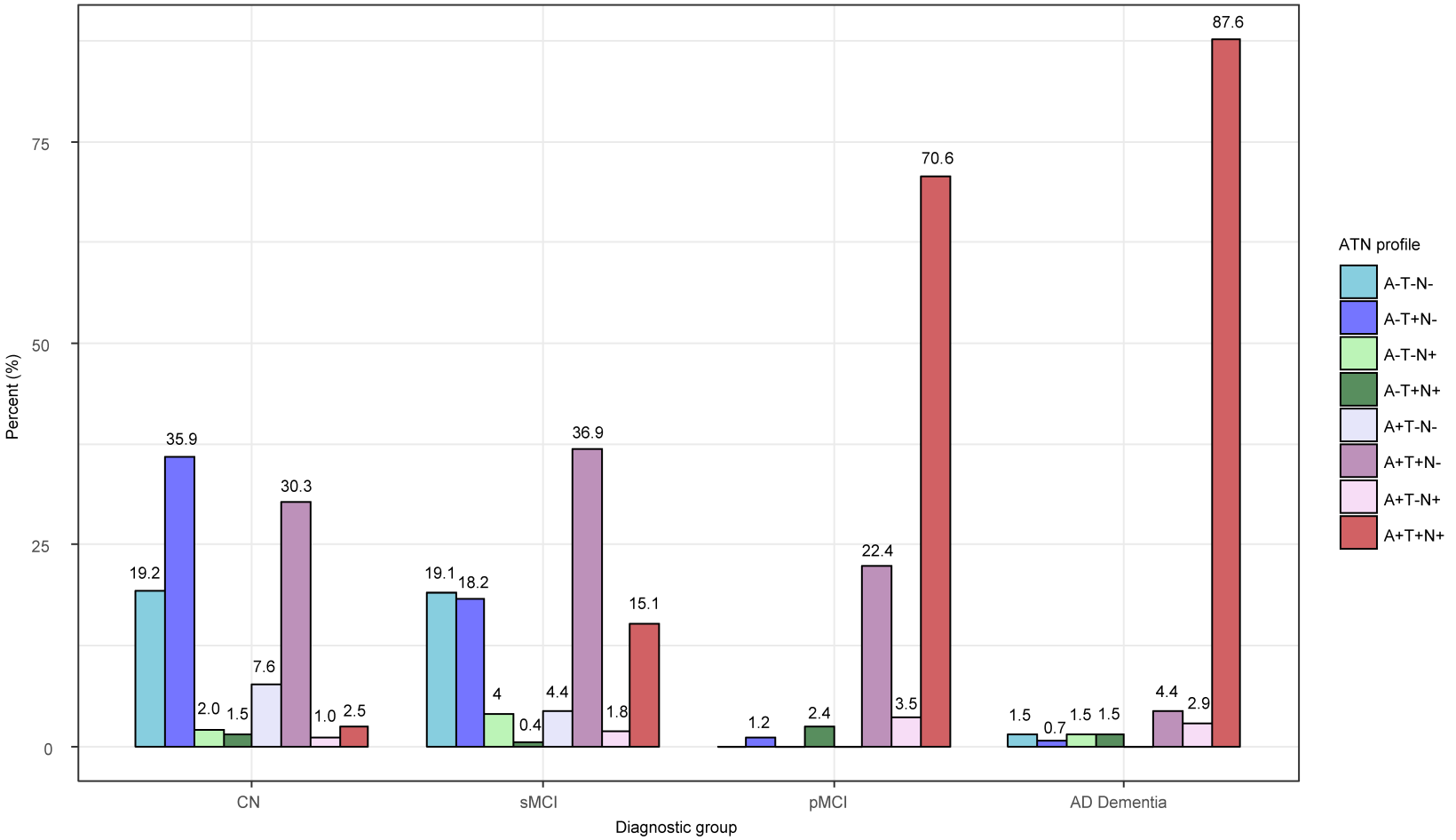

Percent (%)
75
50
25
0
19.2
35.9
2.0
1.5
7.6
30.3
1.0
2.5
19.1
18.2
4
0.4
4.4
36.9
1.8
15.1
1.2
2.4
22.4
70.6
3.5
1.5
0.7
1.5
1.5
4.4
2.9
87.6
CN
sMCI
pMCI
AD Dementia
Diagnostic group
ATN profile
A-T-N-
A-T+N-
A-T-N+
A-T+N+
A+T-N-
A+T+N-
A+T-N+
A+T+N+

Figure 2

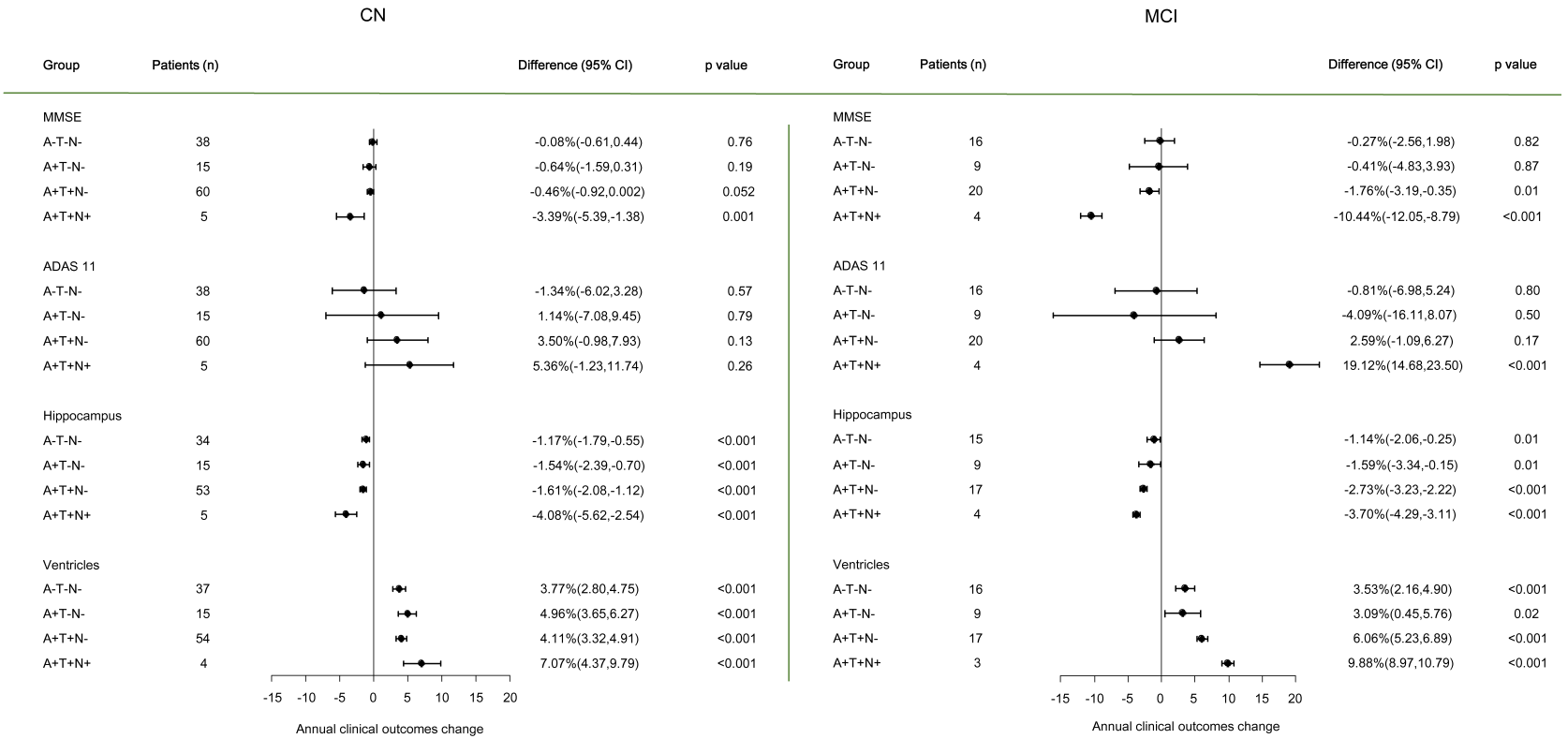
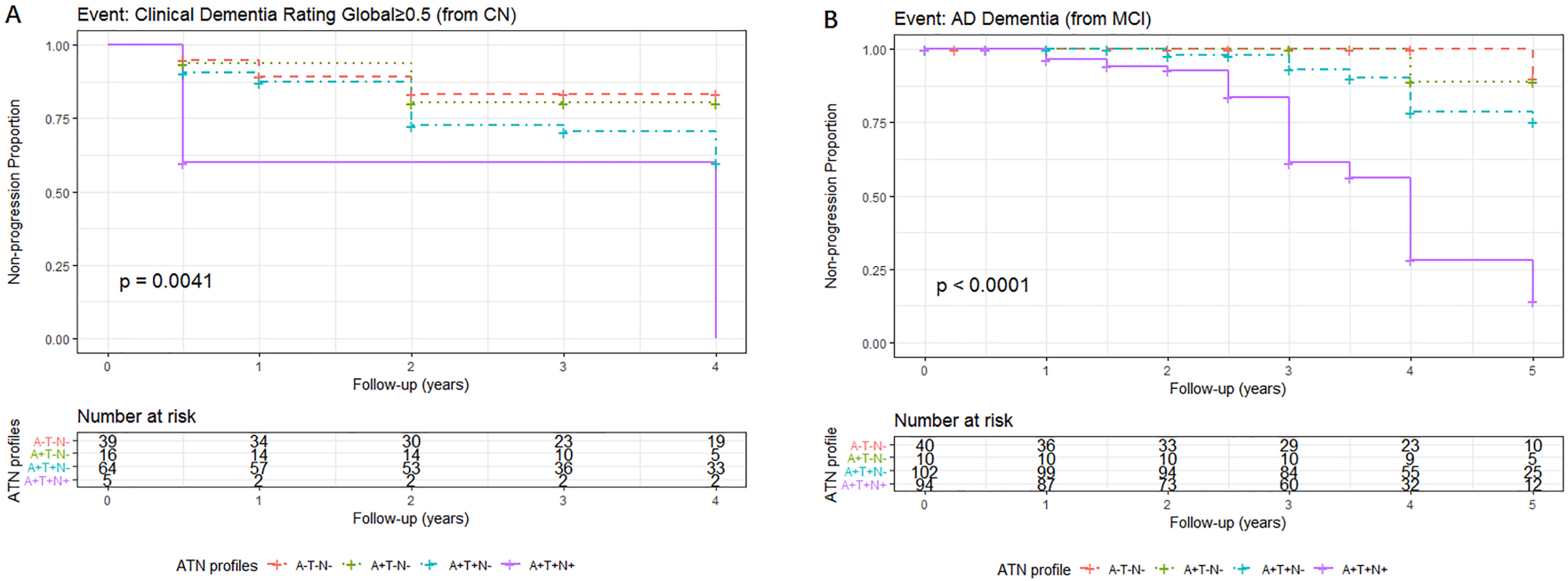

CN
Group
Patients (n)
Difference (95% CI)
p value
MMSE
A-T-N- 38 -0.08%(-0.61,0.44) 0.76
A+T-N- 15 -0.64%(-1.59,0.31) 0.19
A+T+N- 60 -0.46%(-0.92,0.002) 0.052
A+T+N+ 5 -3.39%(-5.39,-1.38) 0.001
ADAS 11
A-T-N- 38 -1.34%(-6.02,3.28) 0.57
A+T-N- 15 1.14%(-7.08,9.45) 0.79
A+T+N- 60 3.50%(-0.98,7.93) 0.13
A+T+N+ 5 5.36%(-1.23,11.74) 0.26
Hippocampus
A-T-N- 34 -1.17%(-1.79,-0.55) <0.001
A+T-N- 15 -1.54%(-2.39,-0.70) <0.001
A+T+N- 53 -1.61%(-2.08,-1.12) <0.001
A+T+N+ 5 -4.08%(-5.62,-2.54) <0.001
Ventricles
A-T-N- 37 3.77%(2.80,4.75) <0.001
A+T-N- 15 4.96%(3.65,6.27) <0.001
A+T+N- 54 4.11%(3.32,4.91) <0.001
A+T+N+ 4 7.07%(4.37,9.79) <0.001
-15 -10 -5 0 5 10 15 20
Annual clinical outcomes change
MCI
Group
Patients (n)
Difference (95% CI)
p value
MMSE
A-T-N- 16 -0.27%(-2.56,1.98) 0.82
A+T-N- 9 -0.41%(-4.83,3.93) 0.87
A+T+N- 20 -1.76%(-3.19,-0.35) 0.01
A+T+N+ 4 -10.44%(-12.05,-8.79) <0.001
ADAS 11
A-T-N- 16 -0.81%(-6.98,5.24) 0.80
A+T-N- 9 -4.09%(-16.11,8.07) 0.50
A+T+N- 20 2.59%(-1.09,6.27) 0.17
A+T+N+ 4 19.12%(14.68,23.50) <0.001
Hippocampus
A-T-N- 15 -1.14%(-2.06,-0.25) 0.01
A+T-N- 9 -1.59%(-3.34,-0.15) 0.01
A+T+N- 17 -2.73%(-3.23,-2.22) <0.001
A+T+N+ 4 -3.70%(-4.29,-3.11) <0.001
Ventricles
A-T-N- 16 3.53%(2.16,4.90) <0.001
A+T-N- 9 3.09%(0.45,5.76) 0.02
A+T+N- 17 6.06%(5.23,6.89) <0.001
A+T+N+ 3 9.88%(8.97,10.79) <0.001
-15 -10 -5 0 5 10 15 20
Annual clinical outcomes change

Figure 3


A
Event: Clinical Dementia Rating Global≥0.5 (from CN)
Non-progression Proportion
1.00
0.75
0.50
0.25
0.00
p = 0.0041
0
1
2
3
4
Follow-up (years)
Number at risk
ATN profiles
A-T-N- 39 34 30 23 19
A+T-N- 16 14 14 10 5
A+T+N- 64 57 53 36 33
A+T+N+ 5 2 2 2 2
Follow-up (years)
ATN profiles A-T-N- A+T-N- A+T+N- A+T+N+
B
Event: AD Dementia (from MCI)
Non-progression Proportion
1.00
0.75
0.50
0.25
0.00
p < 0.0001
0
1
2
3
4
5
Follow-up (years)
Number at risk
ATN profile
A-T-N- 40 36 33 29 23 10
A+T-N- 10 10 10 10 9 5
A+T+N- 102 99 94 84 55 25
A+T+N+ 94 87 73 60 32 12
Follow-up (years)
ATN profile A-T-N- A+T-N- A+T+N- A+T+N+

Research in context

Systematic review

We reviewed available English language literature in PubMed for studies related to A/T/N classification. Recent cross-sectional studies reported the prevalence of each ATN profile in cognitively unimpaired individuals. However, this study is limited by its cross-sectional design, and longitudinal data is hence necessary and urgently needed to provide important information on the clinical/cognitive outcomes of these ATN profiles.

Interpretation

Participants with A+T+N+ showed faster clinical progression than those with A-T-N- and A+T±N-. Compared with A-T-N-, participants with A+T+N± had an increased risk of conversion from CN to incident prodromal stage of AD, and from MCI to AD dementia. A+T+N+ showed an increased conversion risk when compared with A+T±N-.

Future directions

Applying the 2018 research framework in participants screening for AD clinical trials may be beneficial.

Highlights

1. The prevalence of pathologic AD markers was very common even in CN individuals.
2. Participants with A+T+N+ showed faster clinical progression than those with A-T-N- and A+T±N-.
3. Faster clinical progression was observed in females versus males, and *APOE* ε4 carriers versus non-carriers in A+ groups.
4. Compared with A-T-N-, participants with A+T+N± had an increased risk of conversion from CN to incident prodromal stage of AD, and from MCI to AD dementia. A+T+N+ showed an increased conversion risk when compared with A+T±N-.

Supplementary materials

catalogue

eMethod

Method for calculating adjusted HV (HVa)

Hippocampus volume (HV) and estimated intracranial volume (ICV) was performed using a Siemens Trio 3.0T scanner. Volume estimates were processed using Free-surfer software.

HV was adjusted for eICV using the following equation:

$$\text{Adjusted HV (HVa)} = \text{Raw HV- b(eICV – Mean eICV)}$$

where b indicates the regression coefficient when HV is regressed against eICV. All statistical analyses were performed using a software program (R, version 3.4.4).

Detailed information of the included participants

Patients with AD dementia fulfilled the National Institute of Neurological and Communicative Disorders and Stroke (NINDS) – Alzheimer's Disease and Related Disorders Association (ADRDA) criteria for probable AD, had Mini Mental State Examination (MMSE) scores of 20 to 26, and had Clinical Dementia Rating (CDR) global scores of between 0.5-1.0. MCI patients had MMSE scores of 24 or higher, a CDR score of 0.5, objective memory loss tested by delayed recall of the Wechsler Memory Scale (WMS) logical memory II (>1 SD below the normal mean), preserved activities of daily living, and absence of dementia.

Information on the cognitive assessment scales

1. The Mini–Mental State Examination (MMSE) is a 30-point questionnaire that is used extensively in clinical and research settings to measure cognitive impairment. It briefly measures orientation to time and place, immediate recall, short-term verbal memory, calculation, language, and construct ability. It is commonly used in medicine and allied health to screen for dementia. It is also used to estimate the severity and progression of cognitive impairment and to follow the course of cognitive changes in an individual over time; thus making it an effective way to document an individual's response to treatment.

2. The Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-COG) test is one of the most frequently used tests to measure cognition in research studies and clinical trials for new drugs and other interventions. It primarily measures language and memory. The ADAS-Cog consists of 11 parts and takes approximately 30 minutes to administer.[1] Total scores range from 0 to 70, with higher scores (⩾18) indicating poor cognitive function.

Detailed statistical methods for liner mixed effects model and survival analysis

The model allowed an individual's change rate of clinical outcomes to depend on his or her pathophysiological stage by fitting a model with an interaction between time and "ATN" profiles. Subject specific intercepts and slopes were included in random-effects models that allow for heterogeneity among subjects accounting for repeated measures on the same subject. Analyses for cognitive decline were adjusted for age, gender and education years. Analyses for brain atrophy were adjusted for age, gender, total intracranial volume and field strength (1·5T vs 3T). Each clinical outcome measurement was log-transformed so that the rate could be interpreted on an annual percentage scale. Estimates and 95% confidence intervals (CIs) were calculated using parametric bootstrap method in the arm package with 10000 replicates[2].

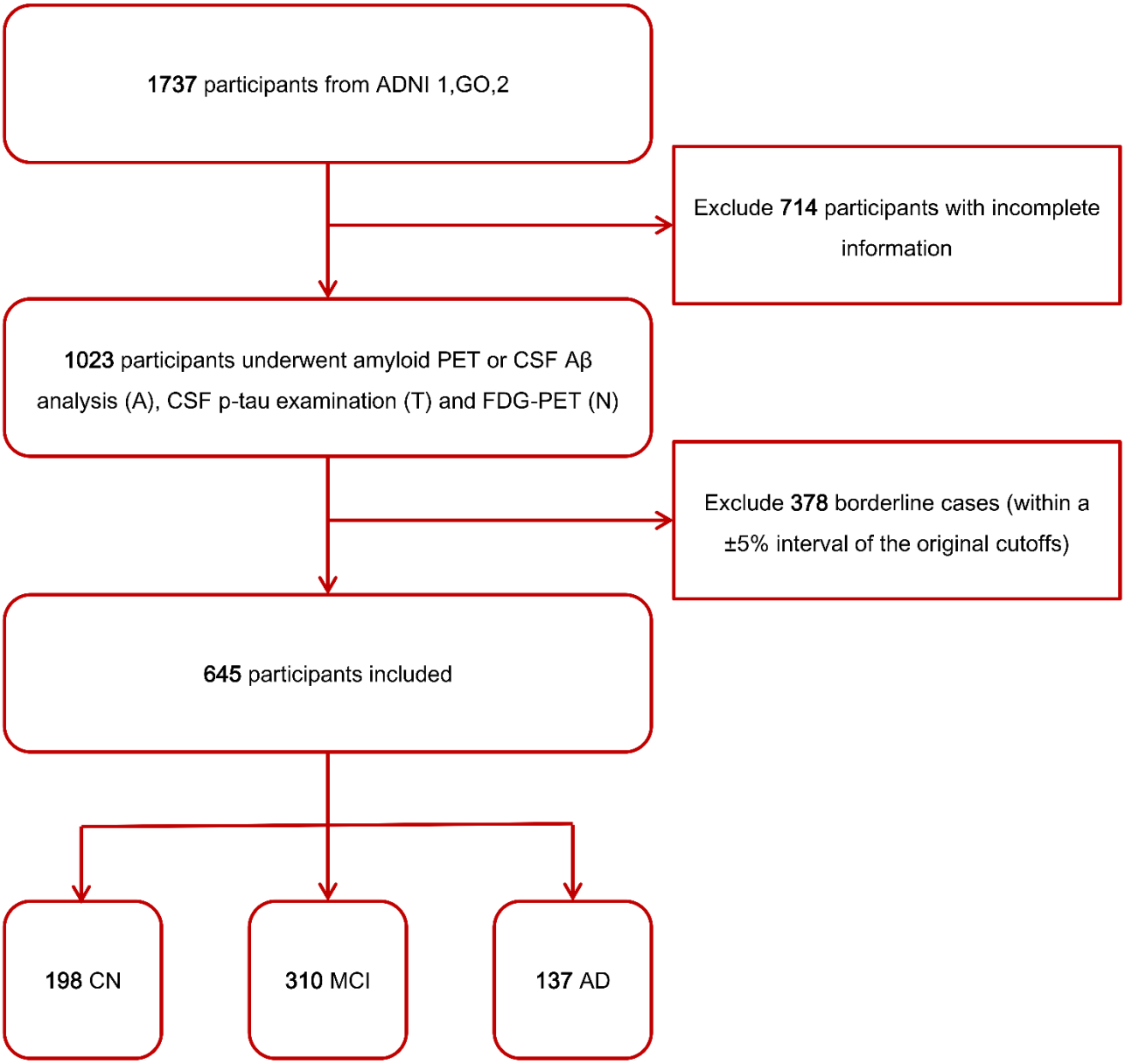
The conversion was defined as the first detection of AD dementia and those who had not converted at their last recorded visit were considered censored. The proportionality of hazards assumption was assessed using the Schoenfeld residuals. Further, the model was adjusted for baseline age, gender and years of education. Hazard ratios (HR) along with their 95% CIs were reported.

Reference

1. Kolibas E, Korinkova V, Novotny V, Vajdickova K, Hunakova D. ADAS-cog (Alzheimer's Disease Assessment Scale-cognitive subscale)--validation of the Slovak version. *Bratisl Lek Listy* 2000; **101**(11): 598-602.

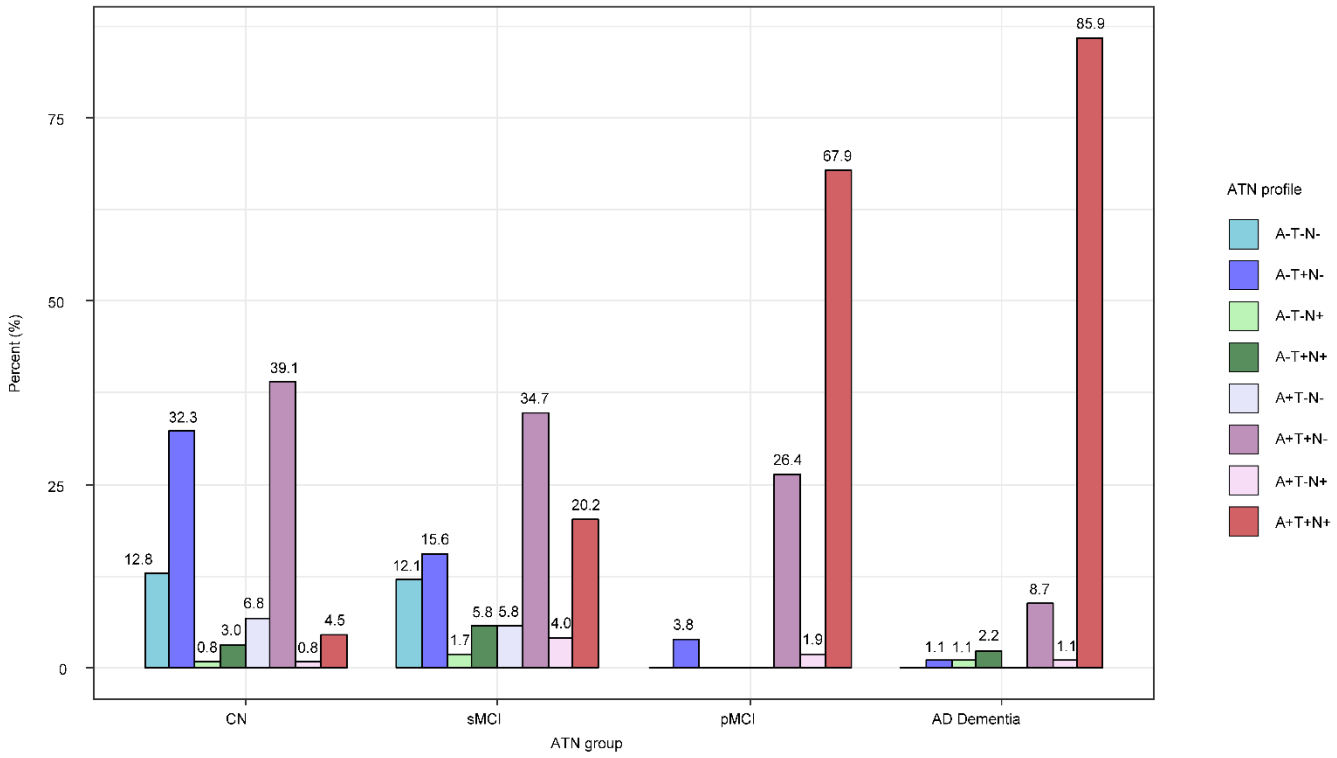
2. Hilbe JM. Data Analysis Using Regression and Multilevel/Hierarchical Models. *Journal of Statistical Software* 2015; **30**(b03): 94-7.

eFigure 1: Flow chart of study participants.



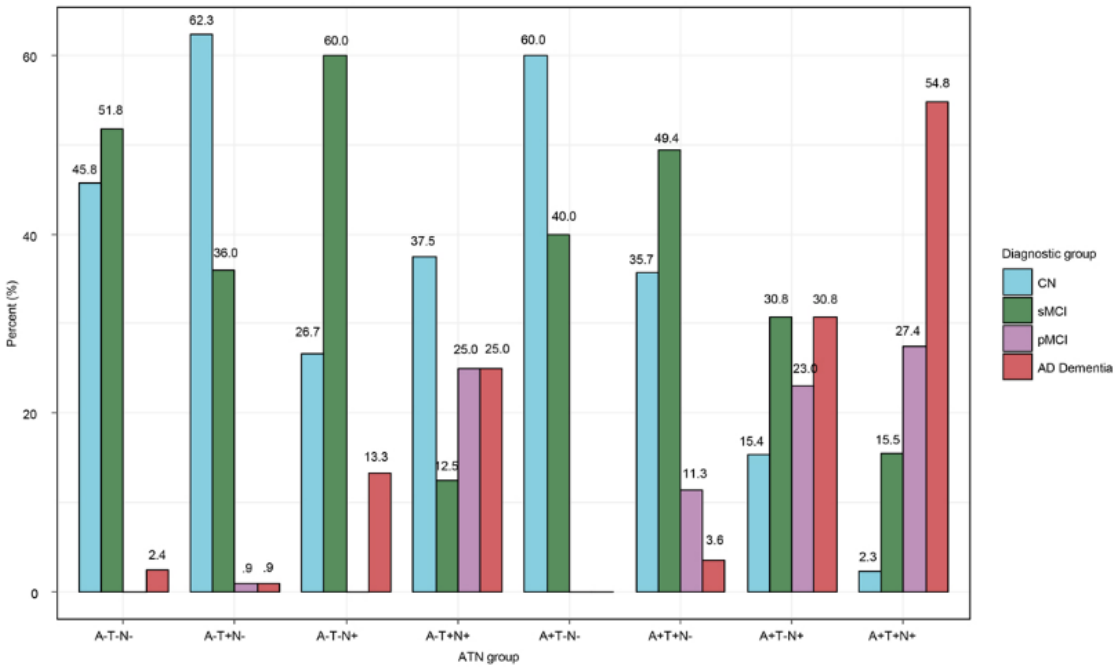
ADNI=Alzheimer's Disease Neuroimaging Initiative. AD=Alzheimer disease dementia. MCI=mild cognitive impairment. CN=cognitively normal. FDG=18F-fluorodeoxyglucose. PET=Positron Emission Tomography. CSF= cerebrospinal fluid.

eFigure 2 Prevalence of each ATN profile in different clinical diagnosis group (defining N by HVa)



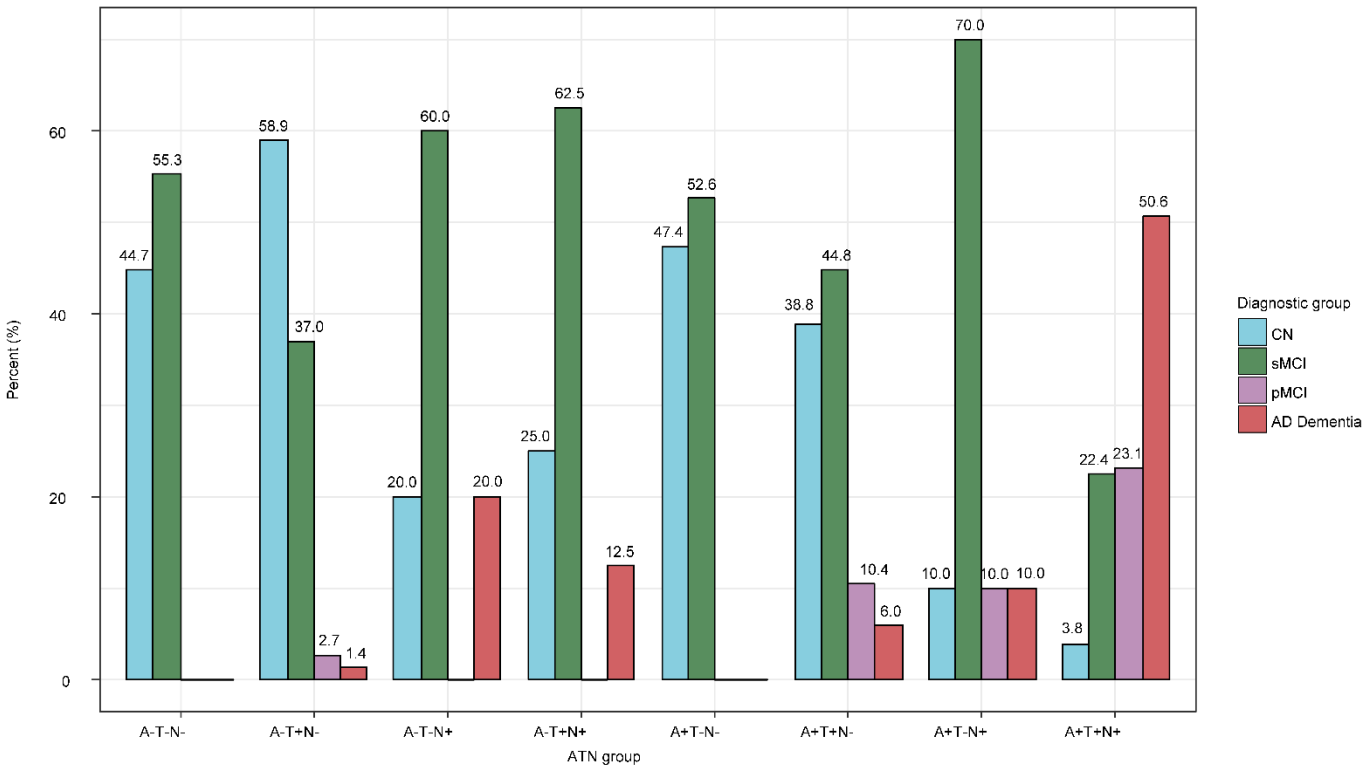
AD dementia=Alzheimer disease dementia. pMCI= progressive mild cognitive impairment. sMCI= stable mild cognitive impairment. CN=cognitively normal. A-=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using HVa. N+=neurodegeneration or neuronal injury abnormal using HVa.

eFigure 3 Prevalence of diagnosis in each ATN profile (defining N by FDG)



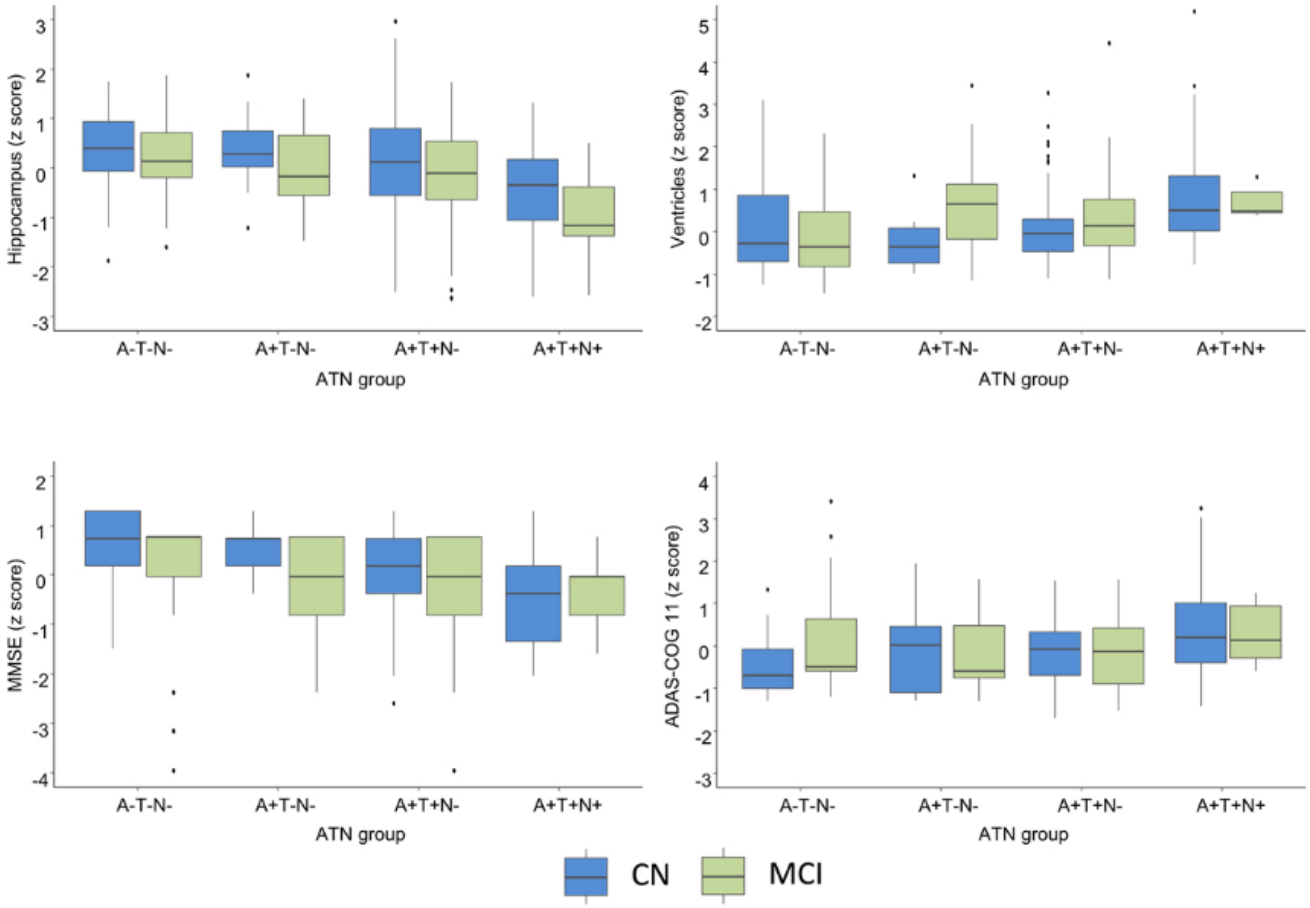
AD dementia indicates Alzheimer disease dementia; pMCI= progressive mild cognitive impairment. sMCI= stable mild cognitive impairment; CN, cognitively normal; A−, amyloid normal using amyloid PET or CSF Aβ; A+, amyloid abnormal using amyloid PET or CSF Aβ; T−, tau normal using CSF p-tau; T+, tau abnormal using CSF p-tau; N−, neurodegeneration or neuronal injury normal using FDG; N+, neurodegeneration or neuronal injury abnormal using FDG.

eFigure 4 Prevalence of diagnosis in each ATN profile (defining N by HVa)



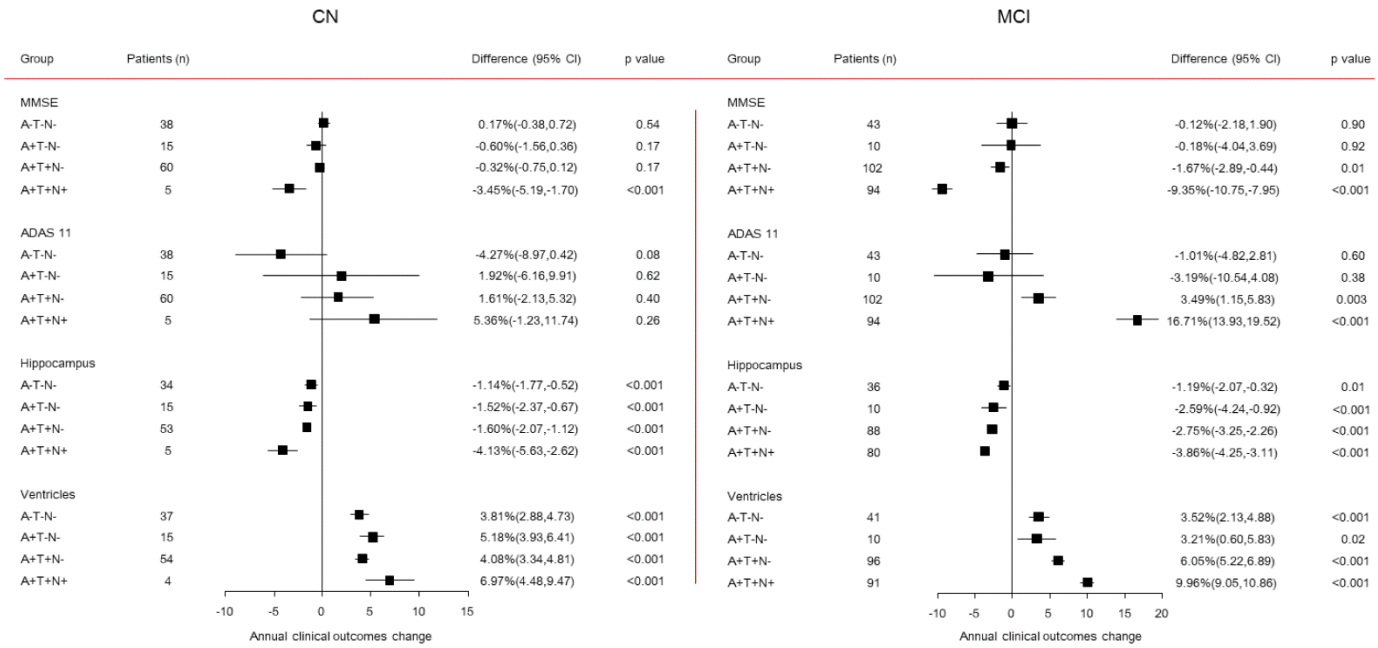
AD dementia=Alzheimer disease dementia. pMCI= progressive mild cognitive impairment. sMCI= stable mild cognitive impairment. CN=cognitively normal. A-=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using HVa. N+=neurodegeneration or neuronal injury abnormal using HVa.

eFigure 5 MRI and cognitive measures in ATN profiles at baseline among cognitively normals and MCI patients.



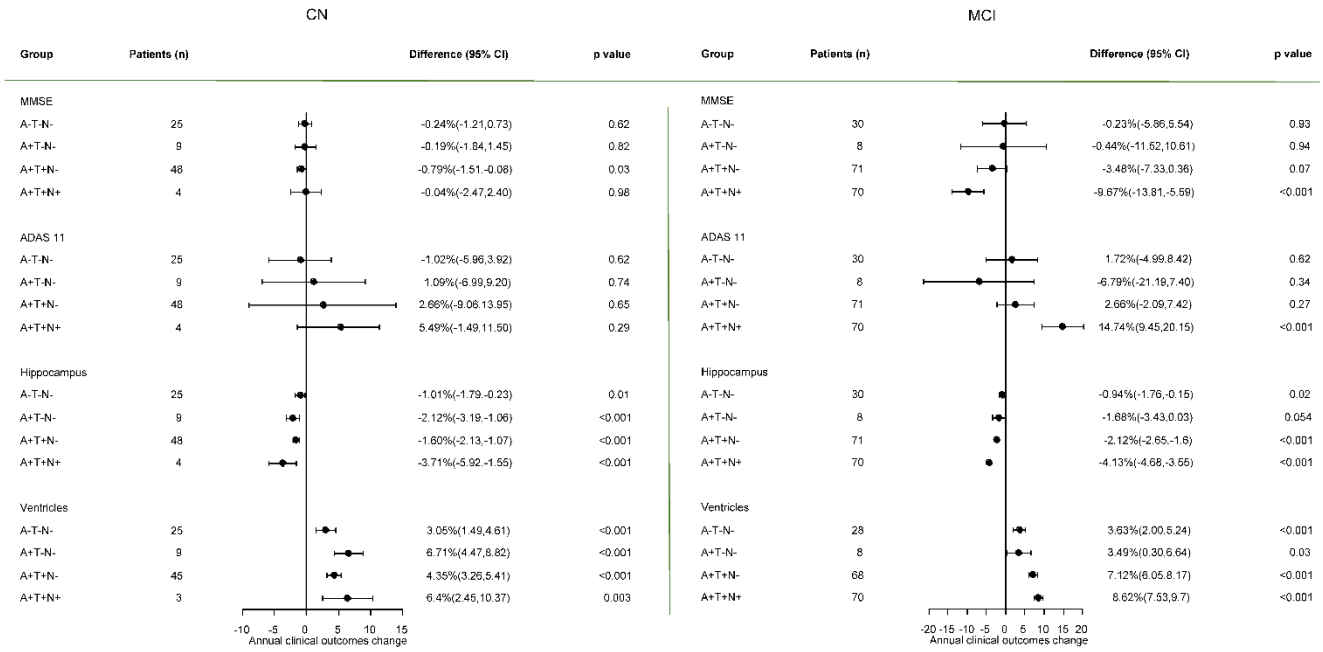
The box plot whiskers extend to the lowest and highest data points within 1·5 times the IQR from the lower and upper quartiles. The dots represent individual points that fall outside this range. P values test for any difference in each measure among the four ATN profiles. MCI=mild cognitive impairment. CN=cognitively normal. A-=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

eFigure 6 Change in clinical outcomes among the four ATN profiles based on linear mixed effects regression models.



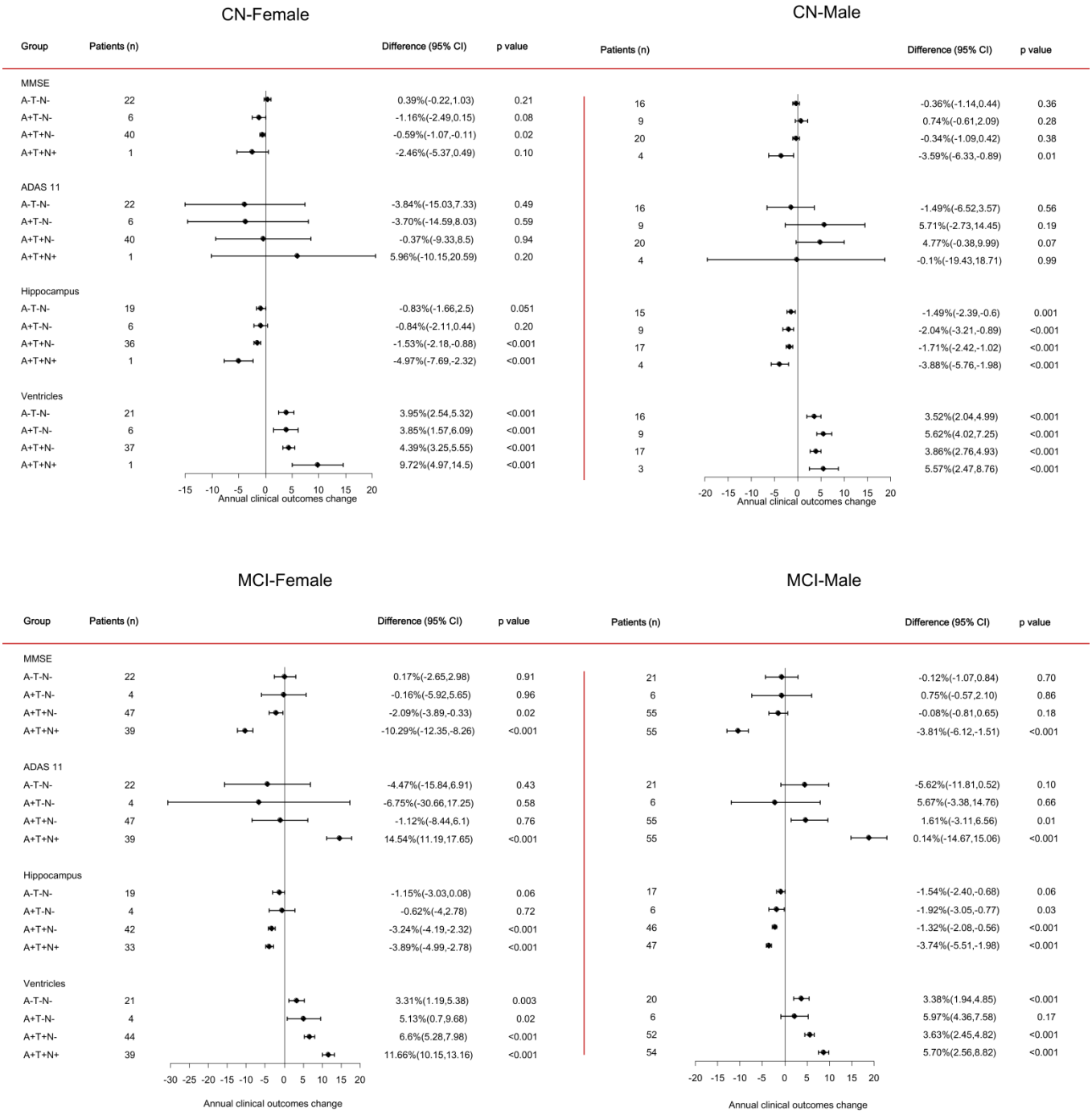
Analyses for cognitive decline were adjusted for age, gender, APOEε4 status and education years. Analyses for brain atrophy were adjusted for age, gender, APOEε4 status, total intracranial volume and field strength (1·5T vs 3T). Change in clinical outcomes expressed as an annual percentage cognitive function scores and volume change, with 95% CIs and p value. MCI=mild cognitive impairment. CN=cognitively normal. A-=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

eFigure 7 Change in clinical outcomes among the four ATN profiles based on linear mixed effects regression models (based only on CSF and MRI).



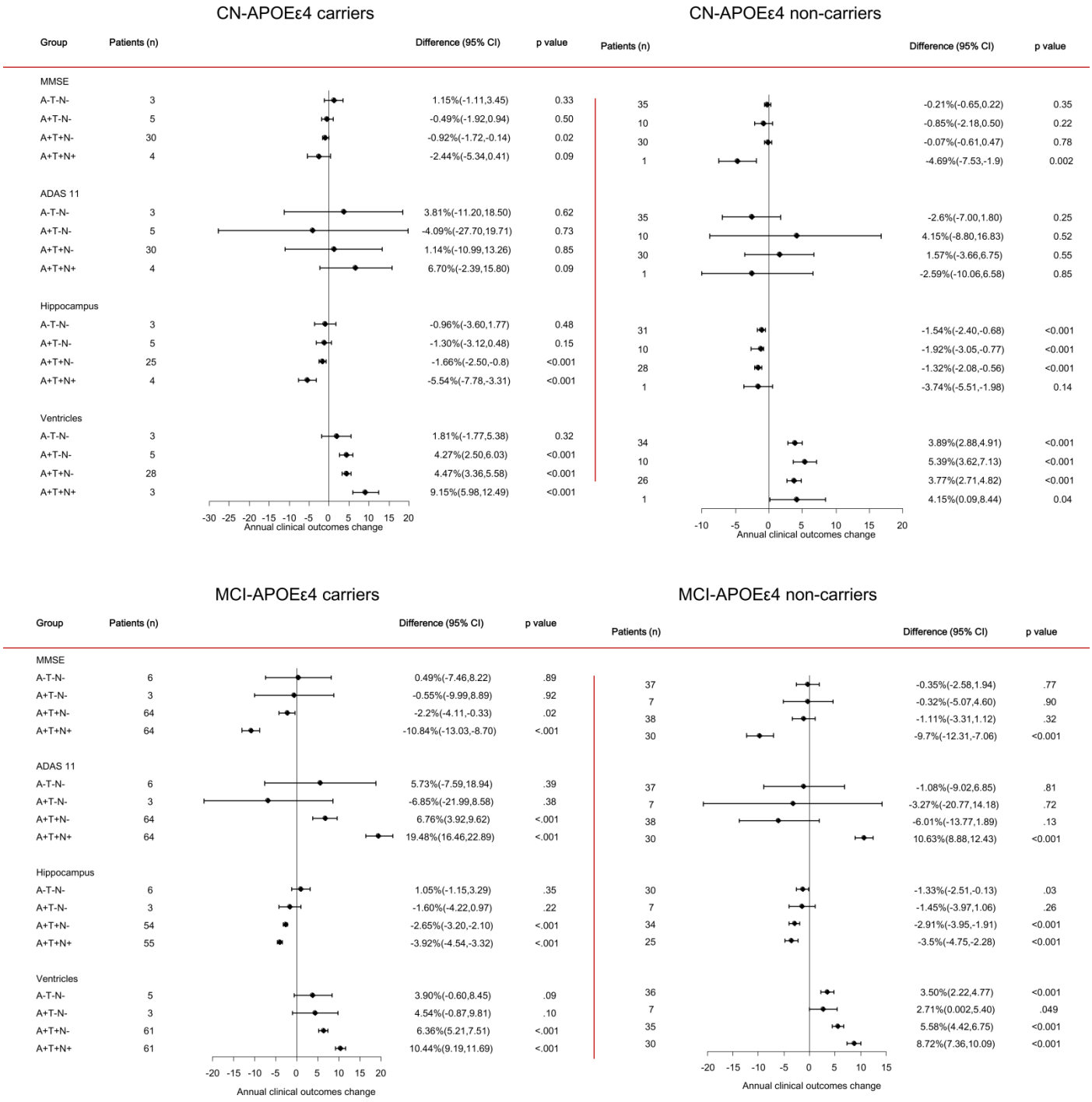
Analyses for cognitive decline were adjusted for age, gender, APOEε4 status and education years. Analyses for brain atrophy were adjusted for age, gender, APOEε4 status and total intracranial volume. Change in clinical outcomes expressed as an annual percentage cognitive function scores and volume change, with 95% CIs and p value. MCI=mild cognitive impairment. CN=cognitively normal. A-=amyloid normal using CSF Aβ. A+=amyloid abnormal using CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using HVa. N+=neurodegeneration or neuronal injury abnormal using HVa.

eFigure 8 Change in clinical outcomes in subgroups divided by gender



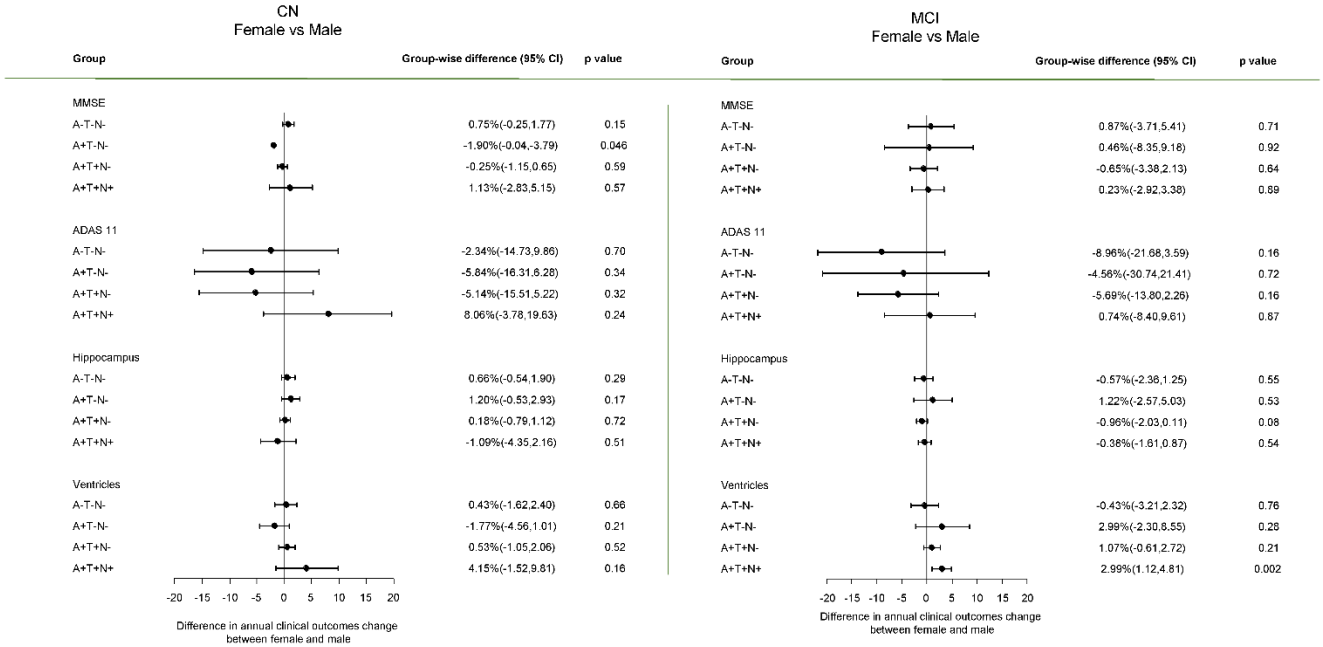
Change in clinical outcomes expressed as an annual percentage cognitive function scores and volume change, with 95% CIs and p values. MCI=mild cognitive impairment. CN=cognitively normal. A-=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

eFigure 9 Change in clinical outcomes in subgroups divided by APOE ε4 status



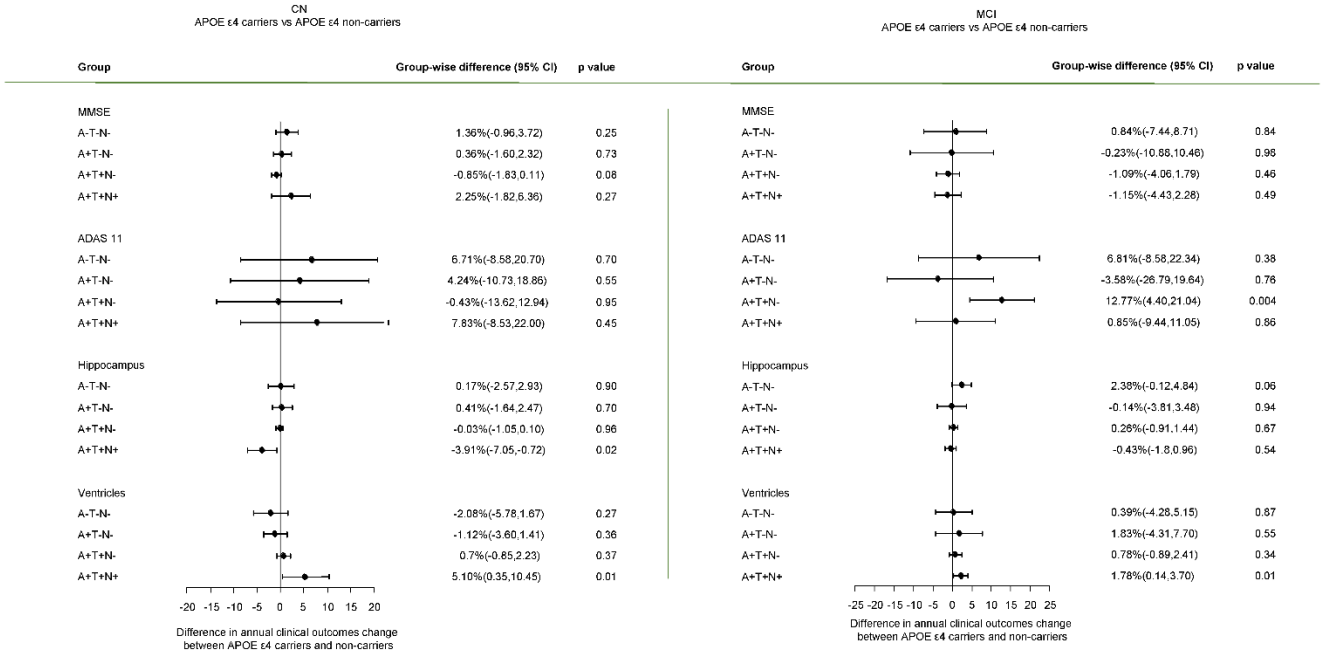
Change in clinical outcomes expressed as an annual percentage cognitive function scores and volume change, with 95% CIs and p values. MCI=mild cognitive impairment. CN=cognitively normal. A-=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

eFigure 10 Differences in the change rates of clinical outcomes in female versus male.



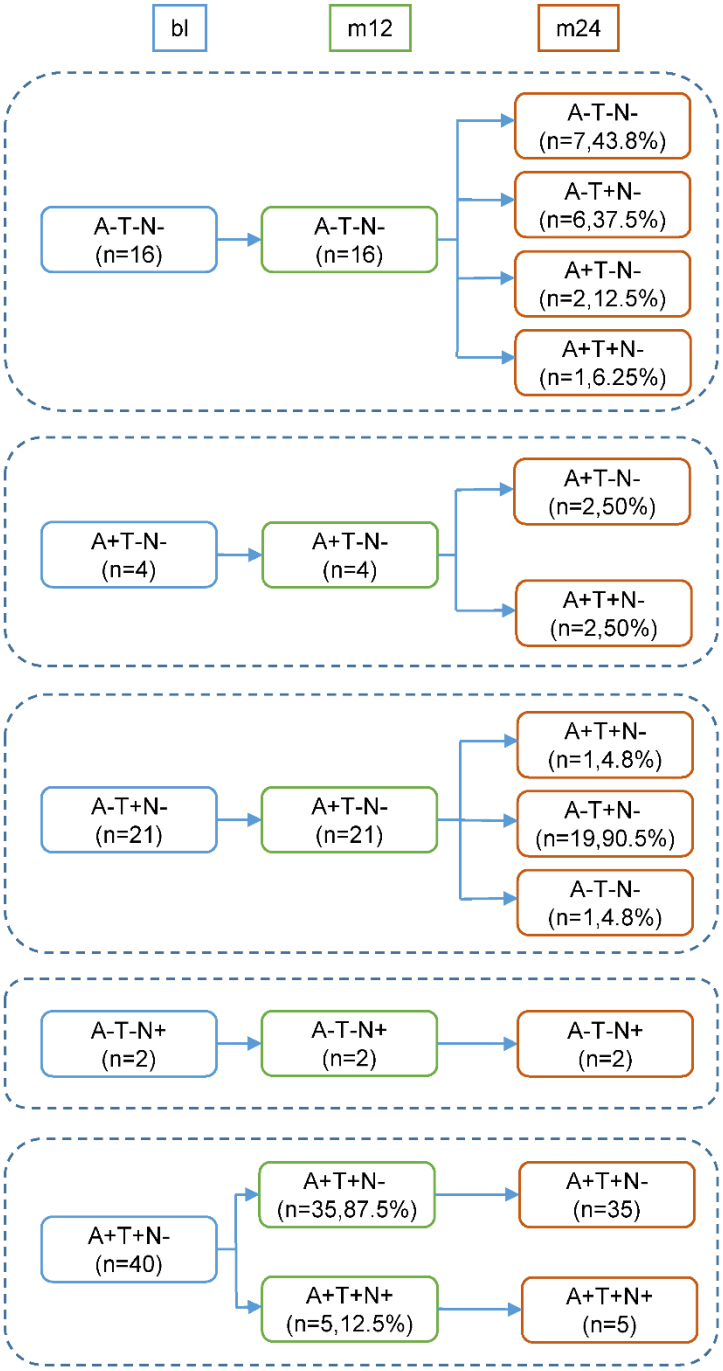
Comparison of change rates of clinical outcomes is expressed as differences in annual percentage volume change, with 95% CIs and p values, between gender. MCI=mild cognitive impairment. CN=cognitively normal. A−=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−, tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

eFigure 11 Differences in the change rates of clinical outcomes APOE ε4 carriers versus APOE ε4 non-carriers.



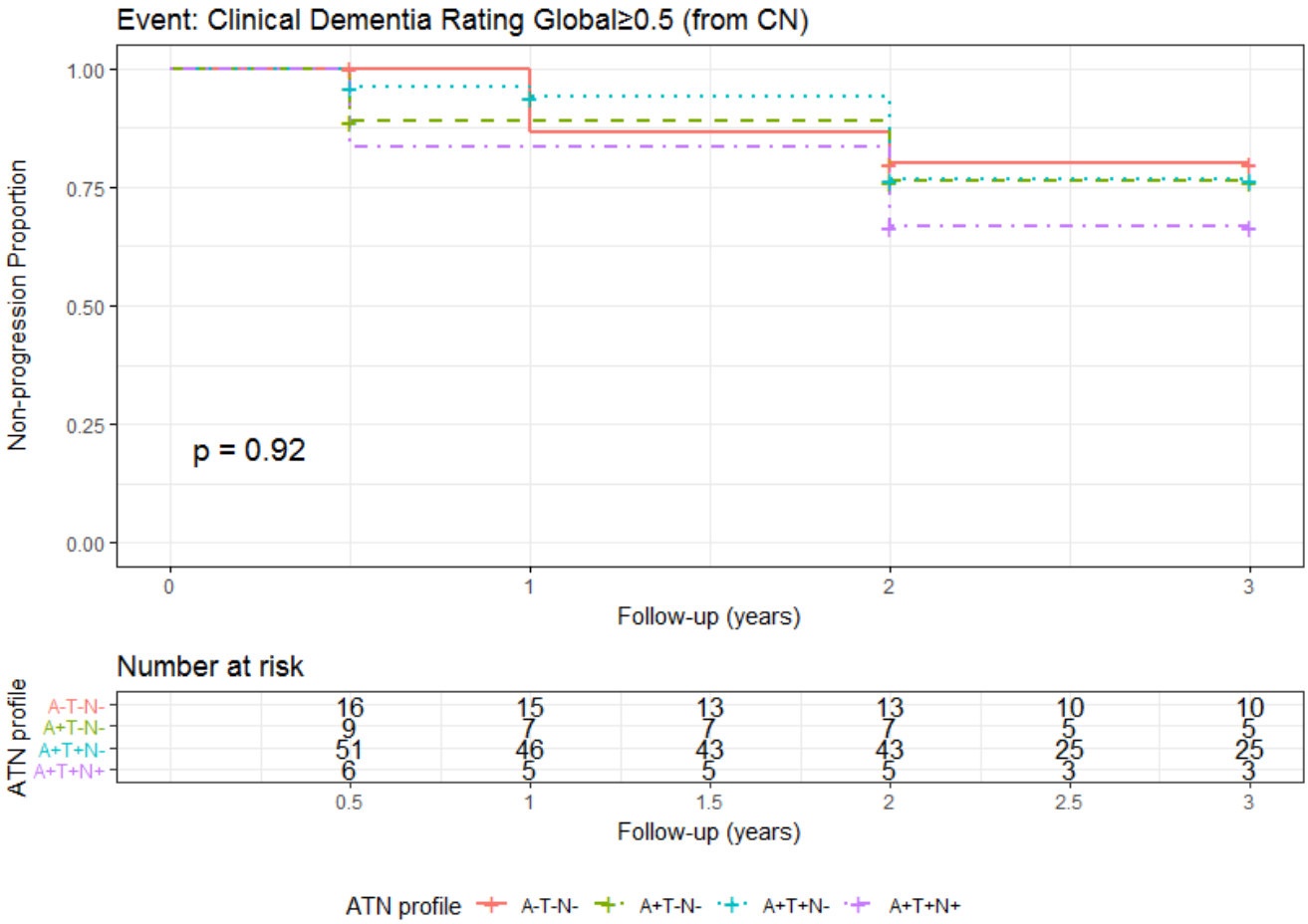
Comparison of change rates of clinical outcomes is expressed as differences in annual percentage volume change, with 95% CIs and p values, between APOE ε4 status. MCI=mild cognitive impairment. CN=cognitively normal. A−=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−, tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

eFigure 12 Specify the number of subjects from baseline to each follow-up examination.



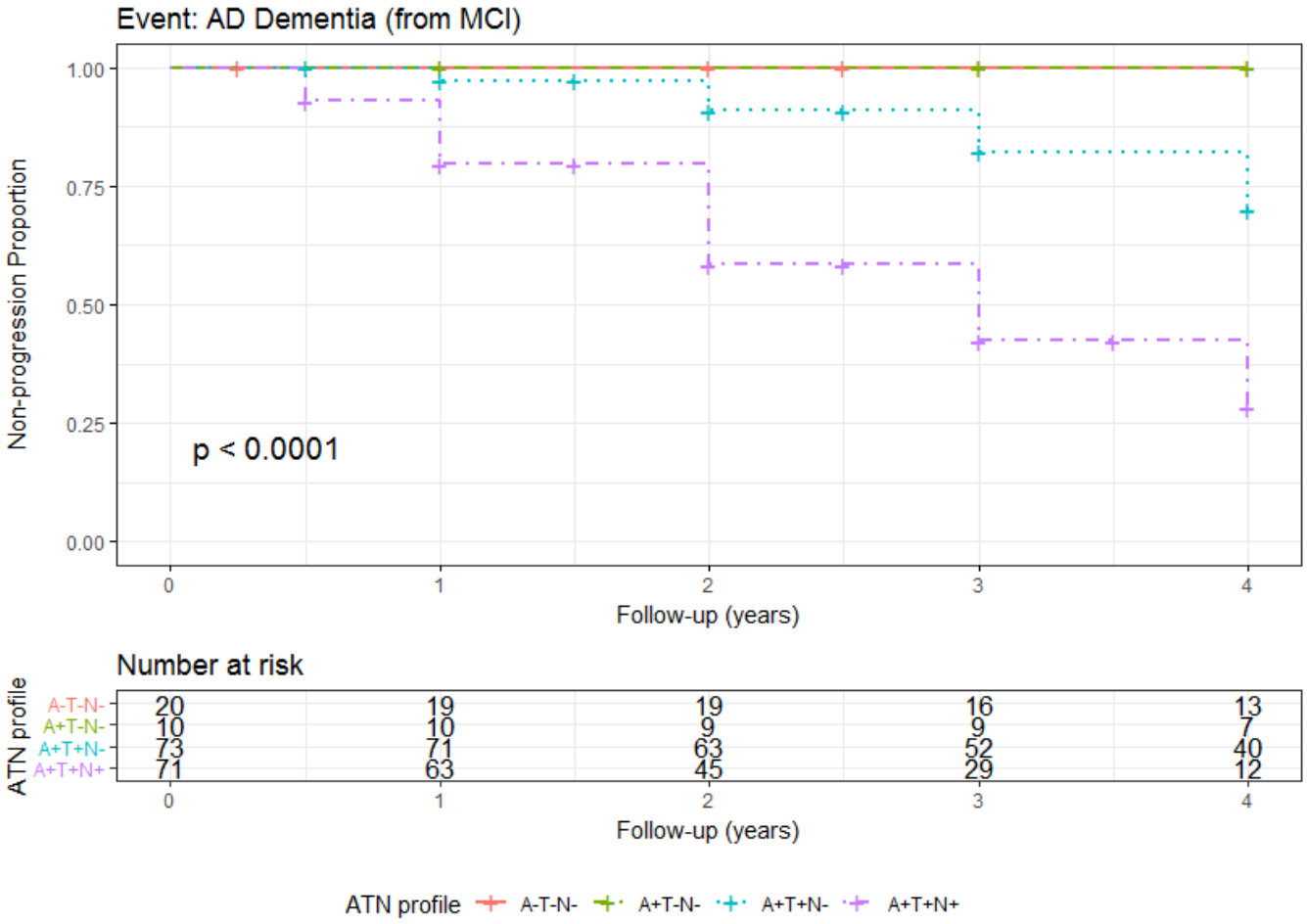
m=month. A−=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−, tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

eFigure 13 Kaplan-Meier curves showing cumulative probability of Progression from cognitively normal participants to incident prodromal stage of AD indicated by a CDR–Global score of 0.5 (Using HVa to define N).



CN=cognitively normal. HVa= hippocampal volume adjusted for total intracranial volume. A−=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using HVa. N+=neurodegeneration or neuronal injury abnormal using HVa.

eFigure 14 Kaplan-Meier curves showing cumulative probability of Progression from mild cognitive impairment to incident AD dementia (Using HVa to define N).



MCI= mild cognitive impairment. HVa= hippocampal volume adjusted for total intracranial volume. A−=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using HVa. N+=neurodegeneration or neuronal injury abnormal using HVa.

eTable1. Cutoff points used to define abnormality

	CSF Aβ42	Aβ PET	CSF p-tau	FDG-PET	HVa
Abnormal	< 182.4 pg/ml	> 1.17 SUVR	> 24.15 pg/ml	< 1.1495	< 6386.85 mm^3

Abbreviations: CSF, cerebrospinal fluid; Aβ, Amyloid-β; PET, Positron Emission Tomography; p-tau, phosphorylated tau; FDG, 18F-fluorodeoxyglucose; t-tau, total tau; SUVR, standardized uptake value ratio.

eTable 2. Follow-up time for each cognitive status group

	Mean (years)	Standard deviation
CN	3.34	1.97
sMCI	3.44	1.79
pMCI	3.69	1.72
AD Dementia	1.63	1.03

AD= Alzheimer Disease. pMCI= progressive mild cognitive impairment. sMCI= stable mild cognitive impairment. CN=cognitively normal.

eTable 3: Number of ATN profiles in clinical diagnosis groups.

	AD	pMCI	sMCI	CN
A−T−N− (n=83)	2	0	43	38
A−T+N− (n=114)	1	1	41	71
A−T−N+ (n=15)	2	0	9	4
A−T+N+ (n=8)	2	2	1	3
A+T−N− (n=25)	0	0	10	15
A+T+N− (n=168)	6	19	83	60
A+T−N+ (n=13)	4	3	4	2
A+T+N+ (n=219)	120	60	34	5

AD= Alzheimer Disease. pMCI= progressive mild cognitive impairment. sMCI= stable mild cognitive impairment. CN=cognitively normal. A-=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

eTable 4: Differences in clinical outcomes change rate among the four pathophysiological profiles

		MCI			CN		
		Difference (95% CI)	p value	Corrected-P value	Difference (95% CI)	p value	Corrected-P value
A+T-N- VS A-T-N-	MMSE	-0.14%(-5.15,4.85)	0.96		-0.56%(-1.62,0.5)	0.31	
	ADAS 11	-3.29%(-16.58,10.3)	0.64		2.48%(-7.12,11.83)	0.61	
	Hippocampus	-0.44%(-2.39,1.51)	0.66		-0.37%(-1.41,0.69)	0.49	
	Ventricles	-0.44%(-3.47,2.59)	0.77		1.19%(-0.45,2.82)	0.15	
A+T+N- VS A-T-N-	MMSE	-1.49%(-4.18,1.19)	0.28		-0.37%(-1.08,0.33)	0.29	
	ADAS 11	3.39%(-3.71,10.58)	0.35		10.13%(0.05,20.26)	0.049	0.99
	Hippocampus	-1.58%(-2.61,-0.54)	0.002	0.10	-0.44%(-1.22,0.34)	0.27	
	Ventricles	2.53%(0.91,4.13)	0.002	0.10	0.34%(-0.93,1.58)	0.59	
A+T+N+ VS A-T-N-	MMSE	-10.17%(-12.99,-7.37)	<0.001	<0.001	-3.31%(-5.42,-1.26)	0.002	0.10
	ADAS 11	19.93%(12.48,27.51)	<0.001	<0.001	10.90%(-9.92,31.36)	0.30	
	Hippocampus	-2.56%(-3.62,-1.49)	<0.001	<0.001	-2.91%(-4.57,-1.29)	<0.001	0.01
	Ventricles	6.34%(4.67,7.98)	<0.001	<0.001	3.3%(0.44,6.19)	0.002	0.10
A+T+N- VS A+T-N-	MMSE	-1.34%(-5.94,3.23)	0.57		0.18%(-0.86,1.24)	0.73	
	ADAS 11	6.68%(-6.01,19.16)	0.29		2.36%(-7.05,11.67)	0.63	
	Hippocampus	-1.15%(-2.95,0.71)	0.22		-0.07%(-1.04,0.92)	0.89	
	Ventricles	2.97%(0.2,5.74)	0.04	0.99	-0.85%(-2.38,0.69)	0.28	
A+T+	MMSE	-10.03%(-	<0.00	<0.001	-2.75%(-4.99,-	0.02	0.96

N+ VS A+T- N-		14.72,5.33)	1		0.58)		
	ADAS 11	23.21%(10.13,35.96)	<0.001	<0.001	8.42%(-13.69,30.01)	0.44	
	Hippocampus	-2.12%(-3.96,-0.26)	0.03	0.99	-2.54%(-4.29,-0.79)	0.005	0.24
	Ventricles	6.79%(3.99,9.58)	<0.001	<0.001	2.11%(-0.94,5.11)	0.17	
A+T+ N+ VS A+T+ N-	**MMSE**	-8.68%(-10.83,-6.55)	<0.001	<0.001	-2.93%(-4.99,-0.87)	0.003	0.14
	ADAS 11	16.53%(10.77,22.23)	<0.001	<0.001	6.06%(-14.43,26.25)	0.57	
	Hippocampus	-0.98%(-1.75,-0.2)	0.01	0.48	-2.47%(-4.09,-0.89)	0.003	0.14
	Ventricles	3.82%(2.58,5.04)	<0.001	<0.001	2.96%(0.13,5.83)	0.04	0.99

MCI=mild cognitive impairment. CN=cognitively normal. ADAS= Alzheimer Disease Assessment Scale–cognitive subscale. MMSE= Mental State Examination. A-=amyloid normal using amyloid PET or CSF Aβ. A+=amyloid abnormal using amyloid PET or CSF Aβ. T−=tau normal using CSF p-tau. T+=tau abnormal using CSF p-tau. N−=neurodegeneration or neuronal injury normal using FDG. N+=neurodegeneration or neuronal injury abnormal using FDG.

eTable 5: Differences in clinical outcomes change rate among the A+T+N+ groups by using two methods to define N.

		MCI		CN	
		Difference (95% CI)	p value	Difference (95% CI)	p value
FDG VS HVa	MMSE	0.83%(-0.85,2.51)	0.41	-1.73%(-6.52,3.06)	0.39
	ADAS 11	-1.32%(-3.86,1.22)	0.39	-5.26%(-16.53,6.01)	0.69
	Hippocampus	0.21%(-0.44,0.86)	0.55	-0.89%(-2.96,1.18)	0.51
	Ventricles	0.41%(-0.02,0.89)	0.08	-0.15%(-2.36,2.06)	0.92

MCI=mild cognitive impairment. CN=cognitively normal. ADAS=Alzheimer Disease Assessment Scale–cognitive subscale. MMSE= Mental State Examination. FDG=18F-fluorodeoxyglucose. HVa= hippocampal volume adjusted for total intracranial volume. t-tau=total tau. A+=amyloid abnormal using amyloid PET or CSF Aβ. T+=tau abnormal using CSF p-tau. N+=neurodegeneration or neuronal injury abnorma