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# *APOE4* homozygosity represents a distinct genetic form of Alzheimer's disease

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# *Supplementary Material*

**APOE4 homozygozity represents a distinct genetic form of Alzheimer's disease**

Author names: Juan Fortea, MD;[1,2,3*] Jordi Pegueroles, PhD;[1,2] Daniel Alcolea, MD;[1,2] Olivia Belbin, PhD;[1,2] Oriol Dols, PhD;[1,2] Lídia Vaqué-Alcázar, PhD; [1,2,4] Laura Videla, PhD;[1,2,3] Juan Domingo Gispert, PhD;[5] Marc Suárez, MD;[5] Sterling Johnson, MD;[6] Reisa Sperling, MD;[7] Alexandre Bejanin, PhD;[1,2] Alberto Lleó MD;[1,2] Víctor Montal, PhD.[1,2,8*]

Affiliations:
1. Sant Pau Memory Unit, Hospital de la Santa Creu i Sant Pau - Biomedical Research Institute Sant Pau, Barcelona, Spain.
2. Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas. CIBERNED, Spain.
3. Barcelona Down Medical Center, Fundació Catalana Síndrome de Down, Barcelona, Spain.
4. Department of Medicine, Faculty of Medicine and Health Sciences, Institute of Neurosciences, University of Barcelona, Barcelona, Spain.
5. BarcelonaBeta Brain Research Center (BBRC), Pasqual Maragall Foundation, Wellington 30, 08005, Barcelona, Spain.
6. Wisconsin Alzheimer's Institute, University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI 53726, USA.
7. Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.
8. Barcelona Supercomputing Center, Plaça d'Eusebi Güell, 1-3, 08034 Barcelona, Spain

* These authors contributed equally to the manuscript

**Co-corresponding authors:**
Juan Fortea Ormaechea, MD PhD
Memory Unit, Department of Neurology, Hospital de la Santa Creu y Sant Pau.
Sant Antoni María Claret, 167. 08025. Barcelona. Spain.
Phone number: (34) 935565986. Fax: (34) 935565602.
E-mail address: jfortea@santpau.cat

Víctor Montal, PhD
Memory Unit, Department of Neurology, Hospital de la Santa Creu y Sant Pau.
Sant Antoni María Claret, 167. 08025. Barcelona. Spain.
Phone number: (34) 935565986. Fax: (34) 935565602.
E-mail address: victor.montal@protonmail.com

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## SUPPLEMENTARY METHODS

Each of the cohorts included in this work focuses on various stages of Alzheimer's disease (AD), ranging from preclinical to symptomatic phases, and they employ multimodal assessments, including clinical evaluations, neuroimaging, cognitive testing, and biological sampling. Here is a summary of each cohort:

- **NACC (National Alzheimer's Coordinating Center)**:

Multicenter, USA. The NACC cohort involves both symptomatic participants, including those with mild cognitive impairment (MCI) and AD dementia, and cognitively normal individuals. The age range of participants includes older adults, typically over the age of 60. The NACC database contains data from multiple ADRCs that conduct multimodal assessments (Contact email: NACCmail@uw.edu).

- **Alfa plus (Alzheimer's and Families cohort)**:

Unicenter, Barcelona, Spain. Alfa plus primarily focuses on the preclinical phase of Alzheimer's disease in cognitively healthy individuals, specifically targeting descendants of AD dementia patients. Participants in the Alfa plus study are generally middle-aged to older adults. The study includes a wide array of multimodal assessments such as neuroimaging (MRI and PET scans), blood sample analysis, and cognitive testing (Contact person: Juan Domingo Gispert -jdgispert@barcelonabeta.org).

- **A4 Clinical Trial**:

Multicenter. The A4 Clinical Trial is specifically designed for the preclinical study of Alzheimer's disease, targeting individuals who are asymptomatic but have evidence of amyloid plaque buildup in the brain. The trial includes older adults who are over the age of 65. As part of its multimodal approach, the A4 trial utilizes neuroimaging, cognitive assessments, and other biomarker evaluations (Contact person: Reisa Sperling – reisa@bwh.harvard.edu).

- **OASIS (Open Access Series of Imaging Studies)**:

Multicenter, USA. OASIS includes a mix of demented and non-demented older adults, thus covering a spectrum from normal aging to symptomatic AD. The age range of participants is broad, including adults typically over the age of 60. Multimodal assessments in OASIS primarily consist of neuroimaging studies (Contact Person: Pamela LaMontagne pjlamontagne@wustl.edu).

- **ADNI (Alzheimer's Disease Neuroimaging Initiative)**:

Multicenter, USA and Canada. ADNI studies individuals across the spectrum of Alzheimer's disease, from cognitively normal to those with MCI and mild AD dementia. Participants are generally in the age range of 55 to 90 years old. The initiative includes multimodal assessments such as MRI and PET imaging, blood biomarker analyses, genetic testing, and cognitive assessments. (Contact Person: Dr. Michael W. Weiner – Michael.weiner@ucsf.edu)

- **WRAP (Wisconsin Registry for Alzheimer's Prevention)**:

  Unicenter, Wisconsin, USA. While WRAP initially focused on the preclinical understanding of AD, particularly in individuals with a family history of the disease, it has broadened to include participants with symptoms of MCI. The participants are generally middle-aged adults, with most individuals recruited in their 40s and 50s and followed longitudinally. WRAP employs multimodal assessments, including cognitive tests, health assessments, and biological sample collection. (Contact Person: Sterling Johnson – SCJ@MEDICINE.WISC.EDU)

These cohorts are integral to AD research, offering valuable insights into the disease's progression from its earliest asymptomatic stages to symptomatic phases. Their multimodal assessment approaches ensure a comprehensive evaluation of potential biomarkers, cognitive changes, and other factors that contribute to the understanding of AD and related disorders. One limitation is the fact the clinical cohorts are convenience cohorts. However, these cohorts are currently the most viable means of gathering substantial data on APOE4 homozygotes. These cohorts are prospective in design and employ rigorous, state-of-the-art diagnostic protocols for AD.

Indeed, it is important to note that these are not population-based cohorts and present different biases. One hand, the NACC dataset tends to have a higher prevalence of symptomatic (mild cognitive impairment and dementia) cases, particularly in neuropathological analyses, showing almost complete clinical and pathological penetrance in APOE4 homozygotes. The individuals included in the clinical cohorts are skewed towards high-risk individuals for dementia as they are enriched by family history, subjective memory concerns and, in the case of the A4 study, exclusion of individuals with high cognitive performance. On the other hand, our convenience cohorts lean towards asymptomatic individuals due to their inclusion criteria, underestimating the true burden of AD as all symptomatic individuals at all ages are excluded from the clinical cohorts included (with the exception of ADNI and OASIS; whose proportion of asymptomatic and asymptomatic individuals does not reflect the prevalence of the disease). This suggests that our results, which show an ultra-high (88%) penetrance of biological AD penetrance by age 80 in living subjects, likely underestimate AD burden in APOE homozygotes due to opposing selection biases, but more impactful bias of excluding APOE4 homozygotes with clinical AD. Finally, we would like to stress that in order to address potential heterogeneity arising from the inclusion of different cohorts, this supplementary material also has sensitivity analyses.

Despite these methodological constraints, the fact that we still observe a near full penetrance of biological AD among APOE4 homozygotes in the clinical cohorts that underestimate AD burden strongly suggests an ultra-high risk or near-full penetrance of AD in this population. This finding, we believe, underscores the robustness of our conclusions even when considering the acknowledged limitations.

**National Alzheimer Coordinating Center Participants**

We included participants with both a neuropathological evaluation and APOE haplotype information. Out of the 12,320 brains available in the NACC (data accessed on August 1st, 2022), 8,992 participants were excluded from our analysis because of missing neuropathological evaluation data. We also excluded 31 subjects who had a known pathogenic mutation in the PSEN1, PSEN2, or APP genes. Ultimately, a total of 3,297 data from brain donors were included in our analysis. Each brain donor had undergone an antemortem clinical evaluation, which included a Clinical Dementia Rating (CDR) score. Most participants also had information on the age at disease onset (symptom onset, MCI, and/or dementia).

**APOE groups**

We classified every participant as APOE4 homozygote (two copies of APOE4), APOE4 heterozygote (one copy of APOE4 and one copy of APOE3) and APOE3 homozygotes (two copies APOE3). For APOE2, we grouped together all the participants with one copy of APOE2, and an extra copy of APOE2 or APOE3. We decided to not include the subgroup of APOE-2/4 into this APOE-2/X group based on multiple reports that have shown its differential behavior compared to APOE-2/2 and APOE-2/3. The low number of APOE-2/4 participants with available biofluids and imaging markers made impossible to perform reliable statistical analyses in that specific population and were further excluded along the manuscript analyses.

**Diagnosis of dementia**

In the NACC dataset, AD dementia using either NINCDS/ADRDA or NIA-AA guidelines, depending on the year of enrollment. For the clinical cohorts, diagnoses are confirmed through consensus conferences and adhere to the most current NIA-AA criteria.

**Neuropathological scoring**

We used the AD neuropathologic change (ADNC) score, which is provided by NACC and which categorizes individuals into non-AD, low, intermediate, or high AD pathology based on the ABC (Amyloid/Braak/Cerad) score[1]. A full description of the NACC procedures can be found elsewhere[2]. We dichotomized NACC participants into a low AD neuropathology group (low-NP) that included those with non-AD and low ADNC scores, and a high AD neuropathology group (high-NP) that included those with intermediate and high ADNC scores.

**Neuropathological analyses**

For the neuropathological analyses, we employed ggstream (v0.1.0-R) to examine the proportion of neuropathological changes (ADNC no, low, middle, and high) with age at death.

**Tau-PET preprocessing**

Each tau-PET volume was average at the native space. Then, we computed an affine registration matrix between the tau-PET in the native space and the T1w image of each subject. Afterwards, using ANTS SyN registration tool, we computed the registration displacement volume between the subject-specific T1w image and the MNI152

template. Finally, we concatenated the affine and the non-linear transformation to normalize the tau-PET volume to the standard space. Using the Desikan atlas parallelization at the MNI space, we computed the SUVr scores normalizing by the grey matter cerebellum tau uptake. Finally, we average the tau-PET SUVr within the set of ROIs that do encompass each Braak stage to end up with a single scalar value for Braak-I/II, Braak-III/IV and Braak-V/VI.

**Biomarkers inter-site differences**

To minimize the putative inter-site differences for both biofluids and imaging markers, we pulled together data acquired only from similar protocols. Concretely, we used CSF data only quantified in Roche Elecsys and Simoa for plasma. Importantly, we only pulled together quantifications done with the same antibody to reduce the assay-dependence artefacts. For imaging, we used the ratio of hippocampal volume to total intracranial volume to mitigate possible MRI acquisition differences and processing pipelines, as previously done. On top of the aforementioned considerations, in the comparison of biomarkers levels at different age intervals (Main text Figure 1B), we did also include clinical cohort as a nuisance factor to our statistical analyses.

## SUPPLEMENTARY RESULTS

The primary figures presented in the manuscript are centered on APOE4 homozygotes, aligning with our primary objective to examine the high penetrance of AD biology and its predictable age of onset within this group, in accordance with the revised NIA-AA criteria. Nevertheless, in this supplementary material we have extended our analyses to show the sex-stratified analyses and to include the results of the APOE4 heterozygotes.

**Neuropathological and biomarker analyses by sex**

Supplementary Figures 1 and 4 present the sex-stratified analyses of our primary data sets. We observed consistent results across both sexes in neuropathological examinations and biomarker analyses. Notably, women exhibited significantly smaller hippocampal volumes compared to men across all age groups, both in APOE3 and APOE4 homozygotes. We also noted an earlier decline in Abeta42 levels in men compared to women. However, this observation warrants caution due to the limited number of younger participants in our study. Additionally, sex differences in amyloid PET uptake were not evident.

**APOE4 gene dose effect on AD neuropathology and biomarker levels**

We found a gene dose effect for the APOE4 haplotype, with heterozygotes displaying intermediate neuropathological and biomarker characteristics that fall between those of APOE3 homozygotes and APOE4 homozygotes.

The neuropathological analyses showed an APOE4 gene dose effect on the prevalence of high or intermediate AD neuropathological changes, which were present in over 95% of APOE4 homozygotes, 85% of heterozygotes, 57% of APOE33 carriers, and 40% of APOE2 carriers in the entire sample, regardless of clinical diagnosis (Supplementary Figure 2). Notably, the diagnosis of AD dementia was associated with nearly universal

intermediate to high AD neuropathological changes in APOE4 homozygotes and heterozygotes but not in APOE3 homozygotes (79.8%) or APOE-2/X carriers (59.8%). Interestingly, we observed a negative association between age and AD pathology in those with an AD dementia diagnosis in APOE4 noncarriers (Main text Fig 5A). In fact, 22.2% of individuals older than 70 years with an AD dementia diagnosis had no or low AD neuropathological changes. In short, AD pathology was near universal in APOE4 homozygotes irrespective of clinical symptoms and in APOE4 heterozygotes in patients with AD dementia. However, non-carriers showed an increasing percentage of individuals with no or low AD neuropathological changes with increasing age even in AD dementia individuals. These results were concordant with those found in our clinical cohort with amyloid PET and in agreement with the data in the Bapineuzumab trial or a large metanalysis on amyloid PET in dementia syndromes[3,4].

Similarly, we found a similar APOE4 gene dose effect on the age of symptoms onset and both MCI and dementia diagnosis as well as age at death (Supplementary Figure 5).

Finally, Supplementary Figure 6, which presents the progression of biomarker changes with age groups stratified by APOE haplotype under the AT(N) classification framework, delineates a clear gene dose effect. APOE4 heterozygotes exhibit intermediate biomarker levels that rest between the values for APOE3 and APOE4 homozygotes, across all biomarkers. This pattern provides robust evidence of the gene dose effect in the biology of AD.

**Extra analyses and Post-hoc sensitivity analyses in the clinical cohorts**

We performed several post-hoc sensitivity analyses to investigate the possibility of site-specific variations in the clinical cohort analyses, which may have been caused by differences in imaging protocols or biochemical biomarker analysis protocols. Concretely, we assessed cohort-specific differences for APOE4 homozygotes, APOE4 heterozygotes and APOE3 homozygotes participants (Supplementary Figure 7). As expected, and in line with Table2 of main text, there were slight differences in the overall quantifications and dynamics, which might naturally arise from the difference in enrolment criteria.

## Bibliography:


1 Montine TJ, Phelps CH, Beach TG, *et al.* National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol* 2012; **123**: 1–11.

2 Beekly DL, Ramos EM, van Belle G, *et al.* The National Alzheimer's Coordinating Center (NACC) Database: an Alzheimer disease database. *Alzheimer Dis Assoc Disord* 2004; **18**: 270–7.

3 Salloway S, Sperling R, Fox NC, *et al.* Two Phase 3 Trials of Bapineuzumab in Mild-to-Moderate Alzheimer's Disease. *N Engl J Med* 2014; **370**: 322–33.

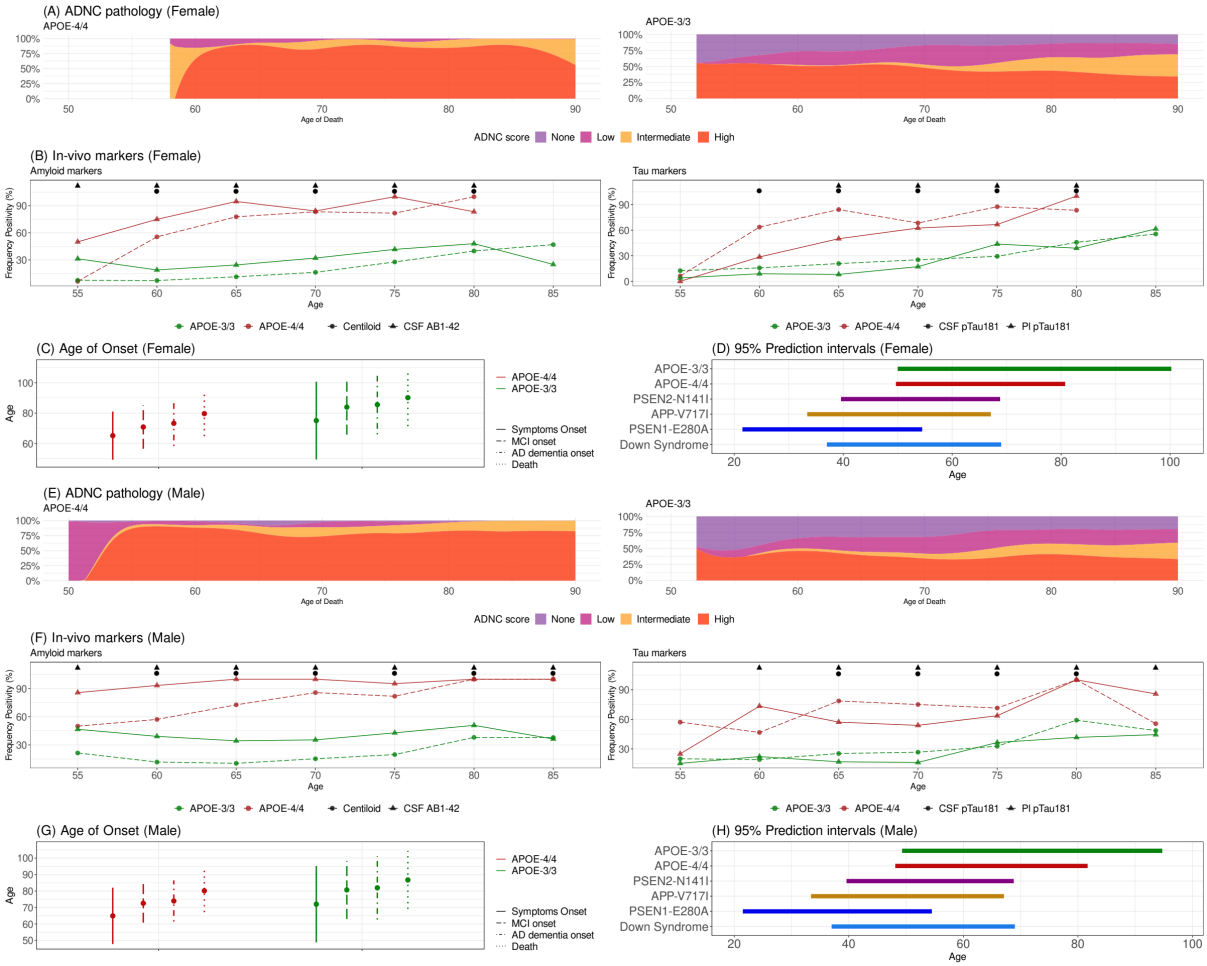
4 Ossenkoppele R, Jansen WJWJ, Rabinovici GDGD, *et al.* Prevalence of amyloid PET positivity in dementia syndromes: a meta-analysis. *JAMA* 2015; **313**: 1939–49.

## SUPPLEMENTARY FIGURES

High resolution versions of the supplementary figures can be found in:

https://gitlab.com/vmontalb/apoe4-asdad

**Supplementary Figure 1. Penetrance and Predictive Value by APOE haplotype and by sex in Alzheimer's Disease Neuropathological Findings in the NACC Database.**




Distribution of ADNC Scores by Age-of-Death for female (A) and male (E): This panel depicts the distribution of Alzheimer's Disease Neuropathologic Change (ADNC) scores across different ages at death, comparing APOE4 homozygotes with APOE3 homozygotes.

Age-Stratified Biomarker Levels for female (B) and male (F): This panel illustrates the frequency of positive amyloid and tau biomarkers across 5-year age intervals for both APOE4 and APOE3 homozygotes and the statistical significance. It demonstrates that APOE4 homozygotes consistently exhibit higher levels of abnormal biomarkers; by age 65, nearly all subjects in APOE4 homozygotes show abnormal levels of CSF abeta. Asterisk mark biomarker levels significant differences between APOE4 homozygotes and APOE3 homozygotes in that age interval.

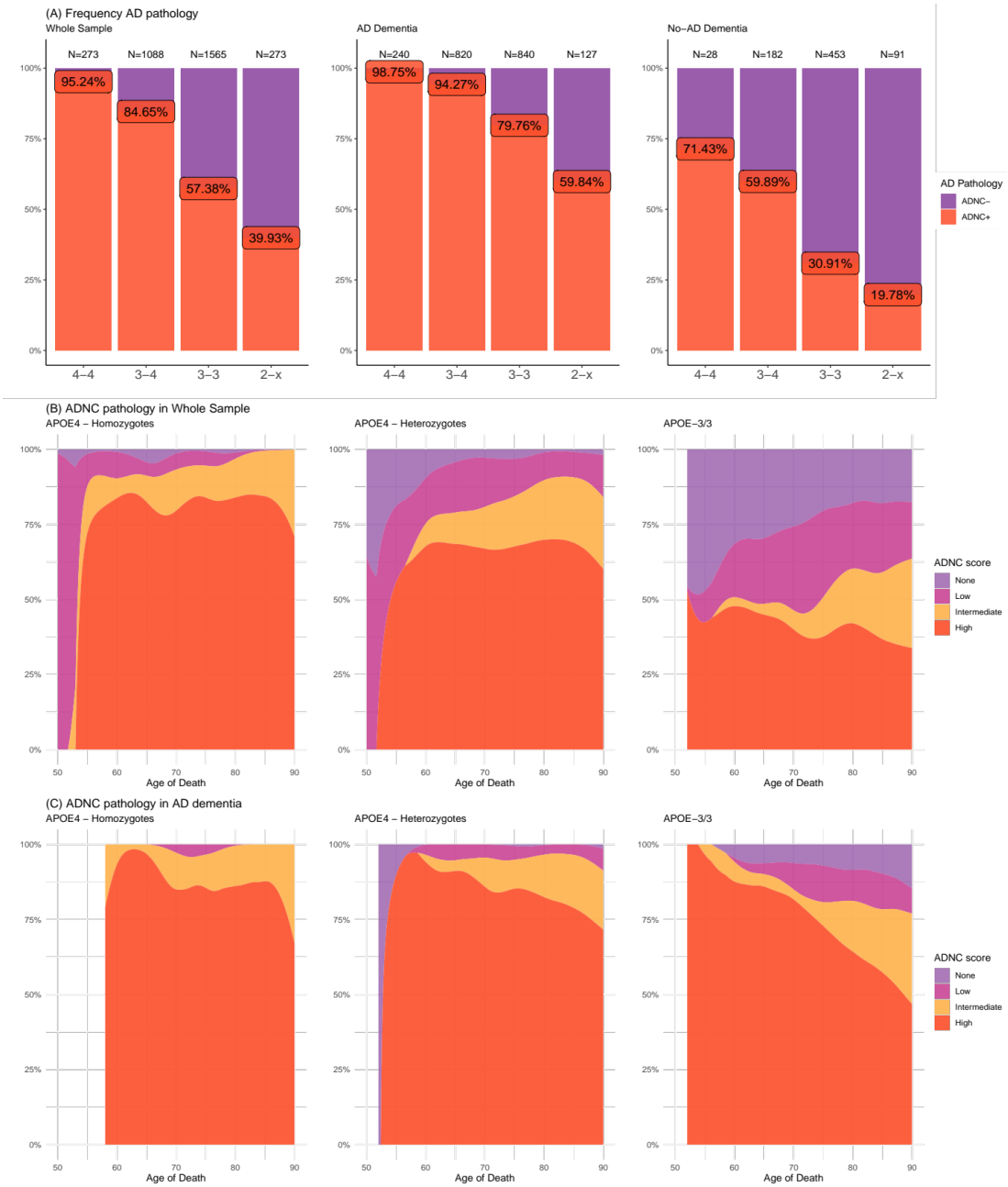
Onset Age of Symptoms and Disease Milestones for female (C) and male (G): This panel provides an overview of the ages at which symptoms, Mild Cognitive Impairment (MCI), and dementia manifest in APOE4 homozygotes compared to APOE3 homozygotes. It highlights that APOE4 homozygotes experience significantly earlier onset ages and have narrower 95% confidence intervals for these milestones than APOE3 homozygotes.

Predictive Intervals for Genetically Determined Forms of Alzheimer's Disease (AD) In female (D) and male (H):

This panel displays the 95% predictive intervals for various genetically determined forms of AD. The panel highlights a similar variability (or predictability) for disease onset in APOE4 homozygotes and both ADAD mutation carriers or individuals with DS, but a significantly wider predictive interval in the APOE3 homozygotes compared to all other examined groups.
Used sample sizes: n=119 APOE4 homozygotes females for symptoms onset, n=26 for MCI, n=24 for AD and n=24 for death ; n=387 APOE3 homozygotes females for symptoms onset, n=172 for MCI, n=119 for AD and n=118 for death; n=121 APOE4 homozygotes males for symptoms onset, n=29 for MCI, n=24 for AD and n=24 for death ; n=445 APOE3 homozygotes males for symptoms onset, n=197 for MCI, n=146 for AD and n=144 for death

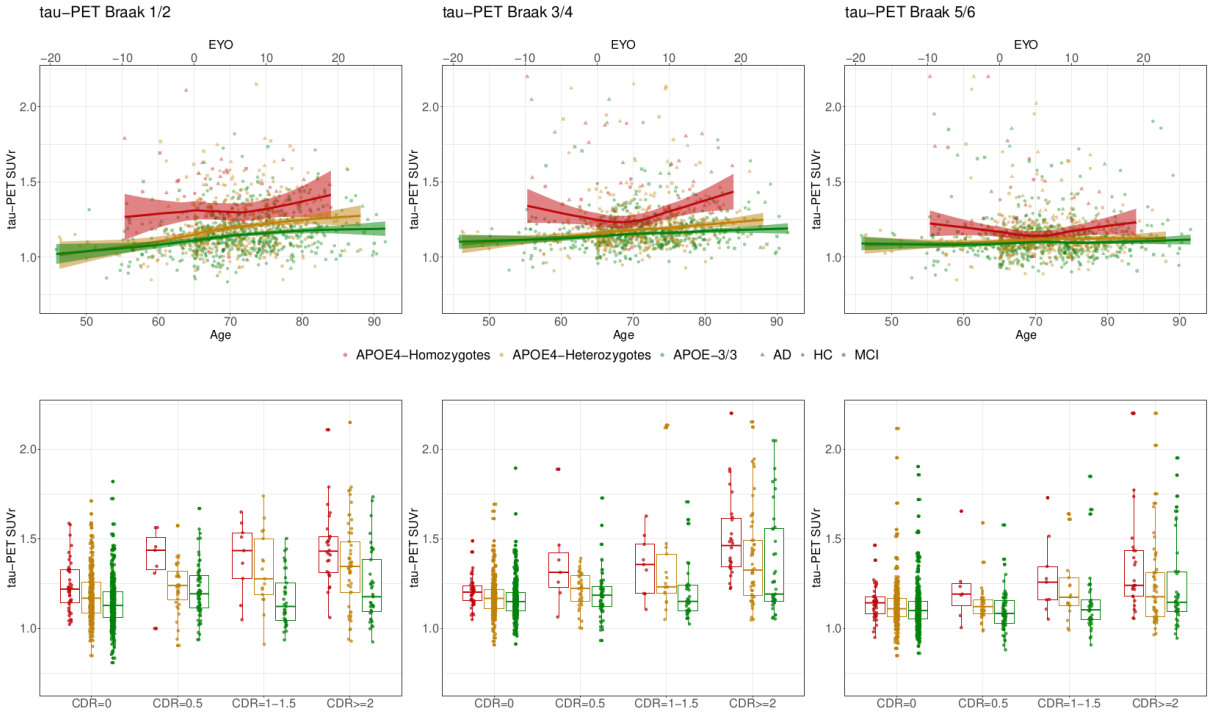
The data on autosomal dominant AD (ADAD) and Down syndrome (DS) has been taken from Iulita et al. (JAMA Network Open 2022).

**Supplementary Figure 2. Neuropathological Findings in the NACC Database including APOE heterozygotes to show the APOE4 gene dose effect.**




This figure displays the neuropathological findings related to Alzheimer's disease (AD) in the National Alzheimer's Coordinating Center (NACC) database. AD pathology was defined by the Alzheimer's Disease Neuropathologic Changes (ADNC) classification. Panel A shows the frequency of AD neuropathology categorized as positive (high-moderate) or negative (no or low) by APOE haplotype (44, 34, 33, and carriers of an APOE2 allele) in the entire sample, as well as in those who received a diagnosis of AD dementia in life and those who did not. Bar plots are used to visualize these frequencies. Panel B shows the proportion of ADNC scores with age in APOE4 homozygotes, heterozygotes, and APOE33 carriers in the whole sample. ADNC scores are plotted against age to investigate the relationship between APOE haplotype and AD pathology over time. Panel C shows the proportion of ADNC scores with age in APOE4 homozygotes, heterozygotes, and APOE33 carriers in individuals who received a diagnosis of AD dementia in life. This panel explores the relationship between APOE haplotype and AD pathology in those who developed AD dementia during their lifetime.

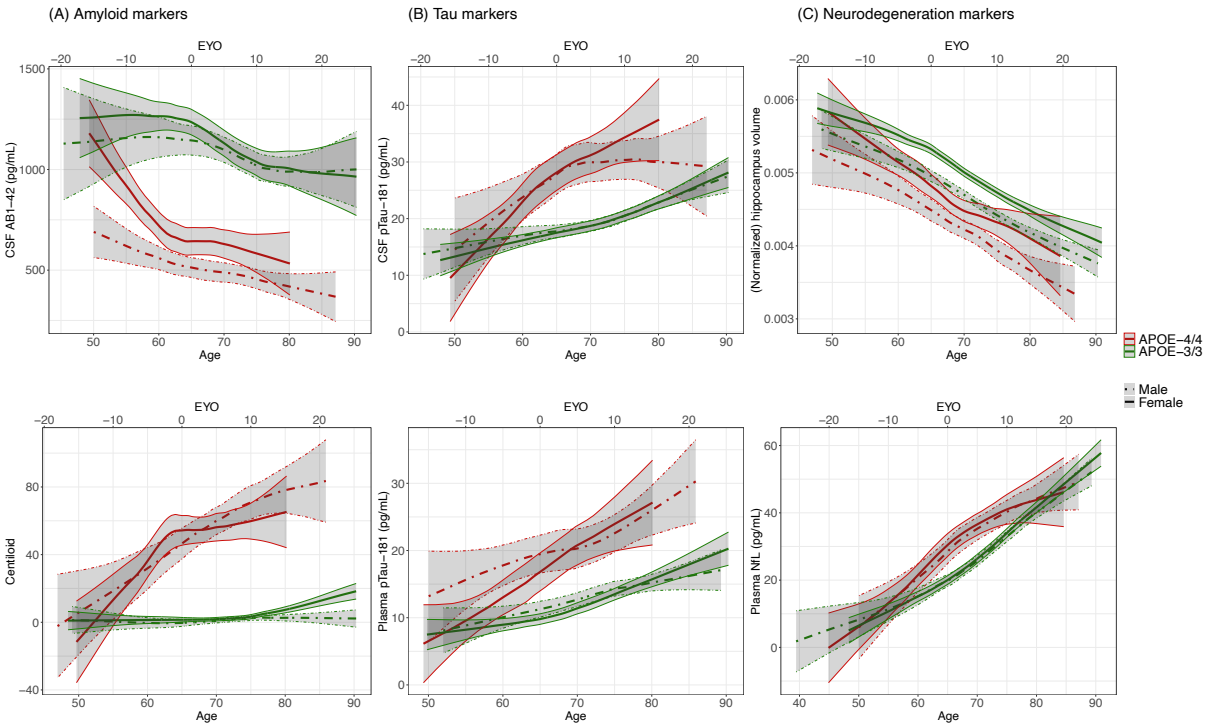
**Supplementary Figure 3. Tau-PET SUVr by age and CDR score**




Top panel: tau-PET SUVr along age, by haplotype. Bottom panel: tau-PET SUVr by CDR score. APOE homozygotes are shown in red, heterozygotes in orange and APOE33 in green.

The initial analyses shown in this figure suggested a higher tau burden in APOE4 homozygotes, which was particularly pronounced when only age was considered. This finding was visualized in the first row. However, when we stratified solely by clinical status, the tau burden also seemed elevated, as shown in the figure's lower row. It is only when we account for both age and clinical status that we observe the tau burden align more closely across different haplotypes as shown in Figure 5 of the manuscript. This substantiates the critical importance of a dual-stratified approach, as overlooking either factor can lead to misleading interpretations of tau pathology progression.

**Supplementary Figure 4. Changes in Alzheimer's disease biomarkers in APOE3 and APOE4 homozygotes with age by sex.**

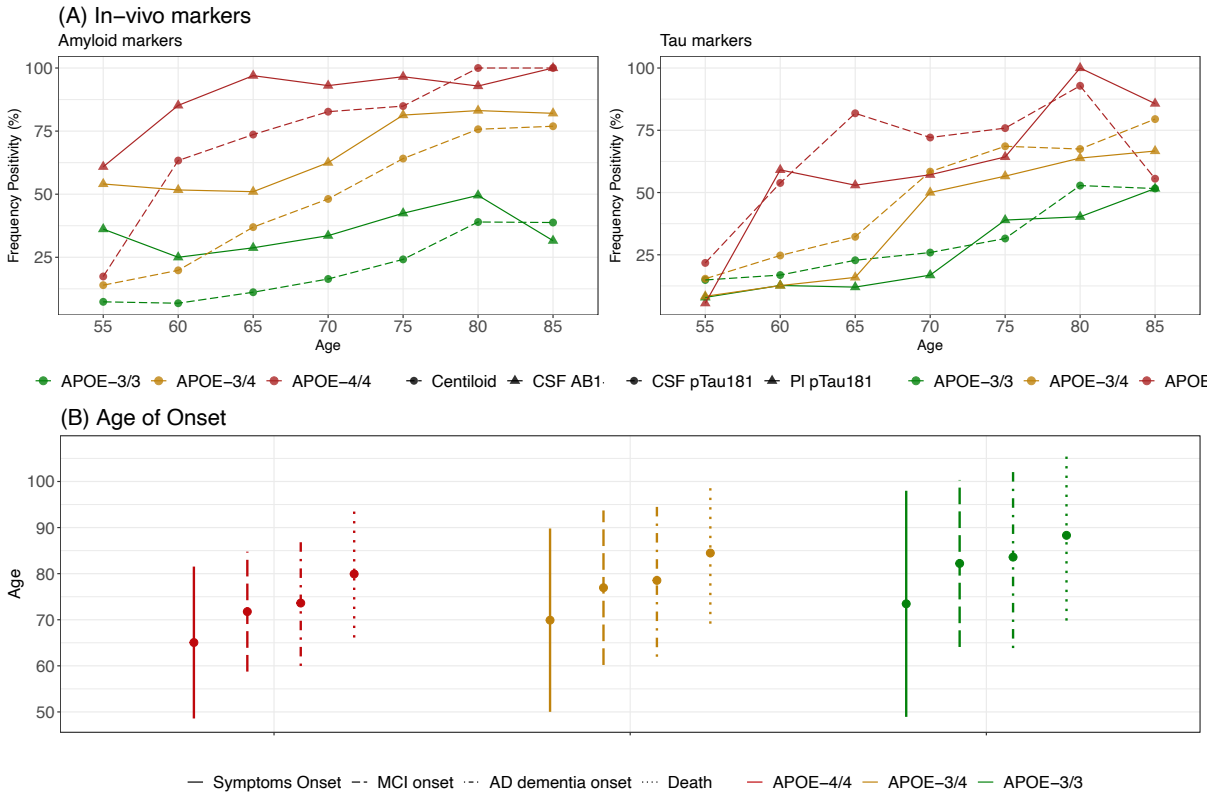



Biomarker changes with age by APOE haplotype using the AT(N) classification system are shown separately in men and women. APOE4 homozygotes are shown in red and APOE3 homozygotes in green.

In both men and women, APOE4 homozygotes exhibit a consistent and sequential progression of biomarker changes, with a notable alignment in the pattern of amyloid (Panel A), tau (Panel B), and neurodegeneration (Panel C) biomarker alterations. This similarity underscores a gender-independent trajectory in the development of AD pathology. The only consistent disparity observed was in the hippocampal volumes, where women showed significantly reduced sizes compared to men across all age groups for both APOE3 and APOE4 homozygotes. Additionally, while an earlier decrease in Abeta42 levels was noted in men, this finding should be considered with caution due to the limited number of younger participants and because amyloid PET scans did not reveal any sex differences, suggesting that the observed Abeta42 trend may not reflect broader amyloid pathology differences between sexes. Shading represents 95% confidence intervals. AD: Alzheimer´s disease.

CSF: cerebrospinal fluid. Aβ1–42=amyloid β peptide 1–42. pTau-181=phosphorylated tau at residue 181. NFL=neurofilament light chain. SUVR=standardized uptake value ratio. Outliers have been visually truncated for ease of interpretation, while all statistical analyses were performed using the complete data set.

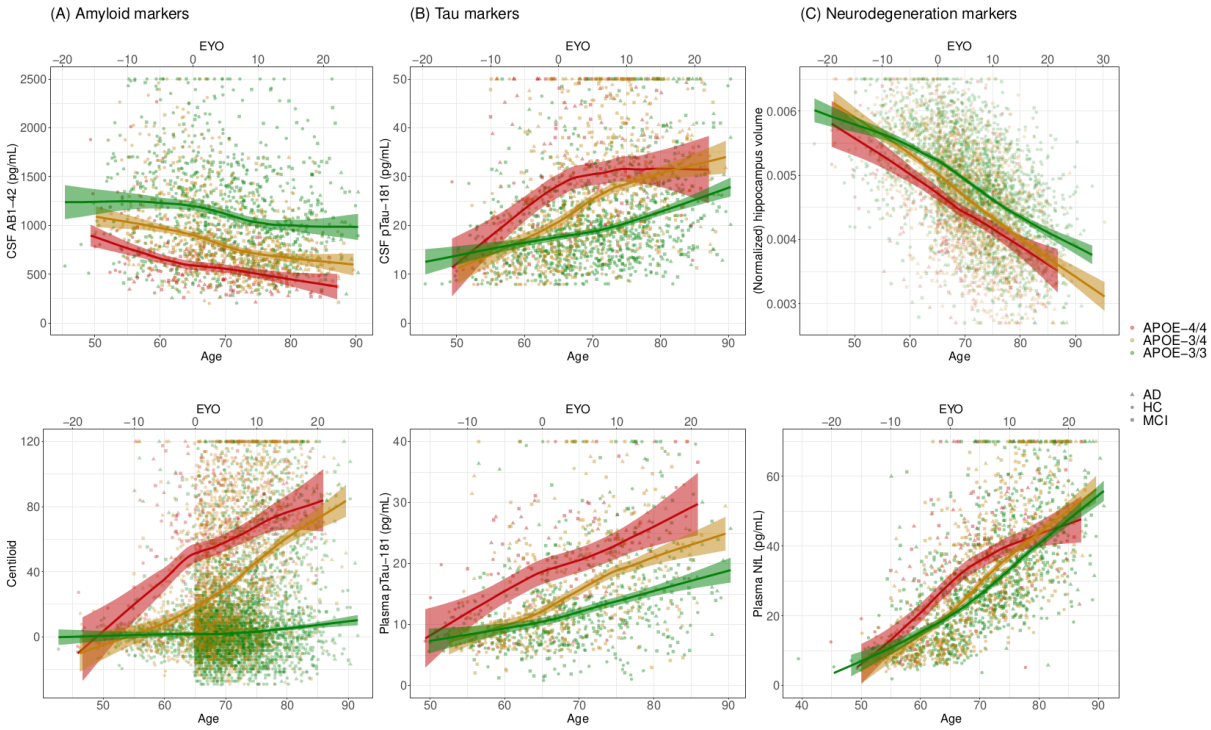
**Supplementary Figure 5. Age-stratified biomarker levels. including APOE heterozygotes to show the APOE4 gene dose effect and Age of symptoms, MCI, dementia onset and death by APOE haplotype including APOE heterozygotes to show the APOE4 gene dose effect.**




Top panel illustrate the frequency of positive amyloid and tau biomarkers across 5-year age intervals by APOE haplotype including APOE heterozygotes. It shows a clear APOE4 gene dose effect on the biological penetrance of AD with age. It highlights that APOE4 heterozygotes have intermediate levels of abnormal biomarkers between APOE4 and APOE3 homozygotes.

Bottom panel provides an overview of the mean and 95% confidence intervals ages at which symptoms, Mild Cognitive Impairment (MCI), and dementia manifest by APOE haplotype including APOE heterozygotes and APOE2 carriers. There is a clear APOE4 gene dose effect on the age of symptoms onset and its predictability. It highlights that APOE4 homozygotes experience significantly earlier onset ages and have narrower 95% confidence intervals for these milestones than APOE3 homozygotes while APOE4 heterozygotes show intermediate results. Sample size: n=240 APOE4 homozygotes for symptoms onset, n=55 for MCI, n=48 for AD and n=48 for death ; n=832 APOE3 homozygotes for symptoms onset, n=369 for MCI, n=265 for AD and n=268 for death; n=814 APOE4 heterozygotes for symptoms onset, n=257 for MCI, n=214 for AD and n=214 for death and n=125 APOE2-X for symptoms onset, n=56 for MCI, n=38 for AD and n=37 for death.

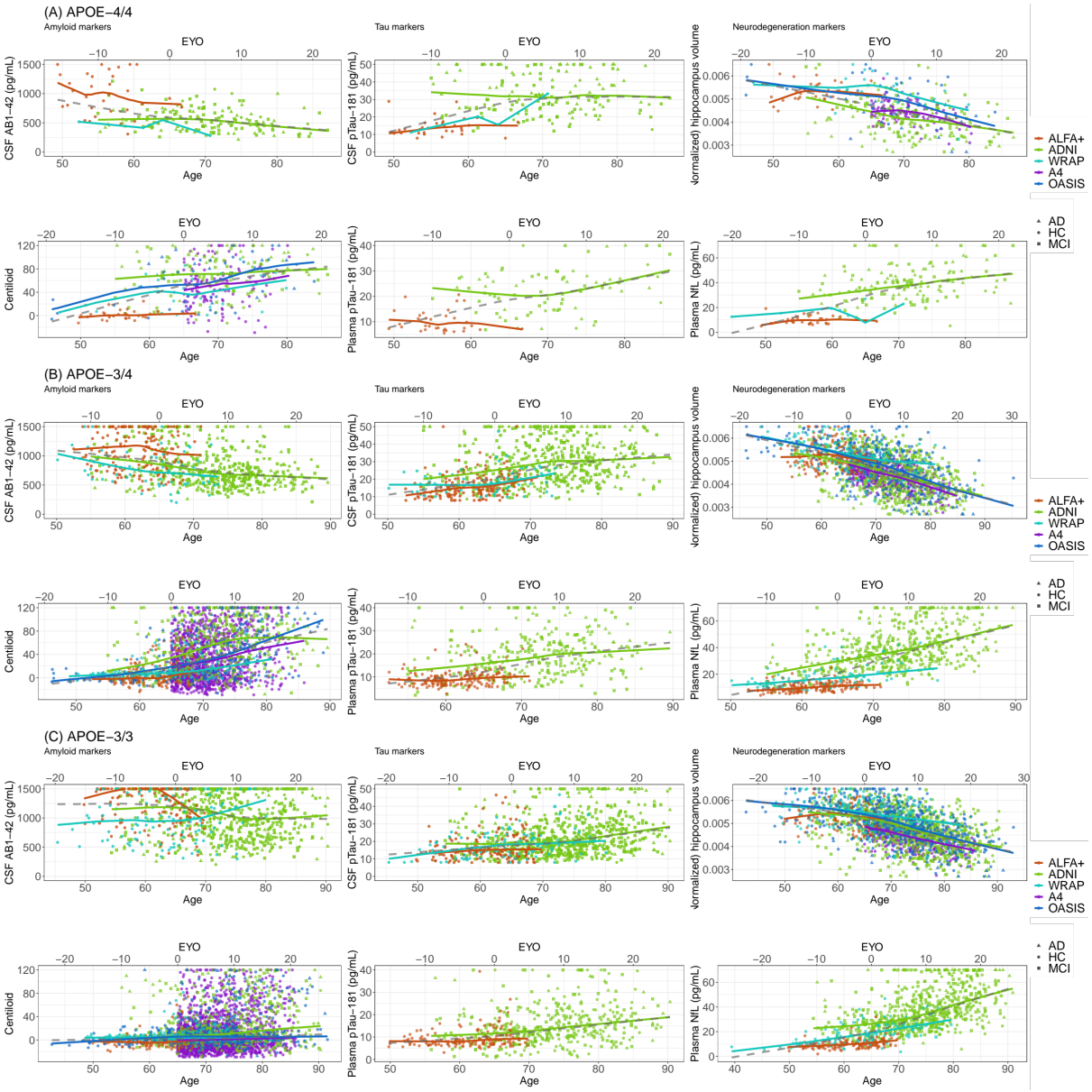
**Supplementary Figure 6. Changes in biomarkers by APOE haplotype and age in Alzheimer's disease.**




Biomarker changes with age by APOE haplotype using the AT(N) classification system for amyloid (A), tau (B) and neurodegeneration (C) biomarkers. APOE4 homozygotes are shown in red, heterozygotes in orange and APOE3 homozygotes in green. Circles represent cognitively unimpaired participants, triangles represent patients with mild cognitive impairment (MCI), and squares represent patients with AD dementia. Shading represents 95% confidence intervals.

CSF: cerebrospinal fluid. Aβ1–42=amyloid β peptide 1–42. pTau-181=phosphorylated tau at residue 181. NFL=neurofilament light chain. SUVR=standardized uptake value ratio.

**Supplementary Figure 7. Changes in Biomarkers in APOE4 homozygotes, APOE4 heterogyzotes and APOE# homozygotes, by cohort, and Age in Alzheimer's Disease.**




Biomarker changes with age by site using the AT(N) classification system for amyloid (A), tau (B) and neurodegeneration (C) biomarkers. Panel A show APOE4 homozygotes. Panel B show APOE4 heterozygotes and Panel C display APOE3 homozygotes. Circles represent cognitively unimpaired participants, triangles represent patients with mild cognitive impairment (MCI), and squares represent patients with AD dementia. Shading represents 95% confidence intervals.

CSF: cerebrospinal fluid. Aβ1–42=amyloid β peptide 1–42. pTau-181=phosphorylated tau at residue 181. NFL=neurofilament light chain. SUVR=standardized uptake value ratio.

**Supplementary Table 1.**
Demographic, clinical and biomarker data of from the multi-site clinical cohort and neuropathological cohort by sex.

| Characteristic | APOE4 Homozygotes | | APOE4 Heterozygotes | | APOE3 Homozygotes | | APOE-2/X | |
|---|---|---|---|---|---|---|---|---|
| | Female N=240[1] | Male N=204[1] | Female N=1,527[1] | Male N=1,186[1] | Female N=2,595[1] | Male N=1,922[1] | Female N=577[1] | Male N=470[1] |
| **Clinical Cohort** | | | | | | | | |
| **Age** | 68 (7) | 70 (7) | 69 (6) | 71 (6) | 71 (6) | 72 (6) | 71 (6) | 72 (6) |
| **DX** | | | | | | | | |
| HC | 159 (67%) | 93 (46%) | 1,291 (85%) | 862 (73%) | 2,352 (91%) | 1,582 (82%) | 529 (92%) | 410 (87%) |
| MCI | 50 (21%) | 63 (31%) | 158 (10%) | 226 (19%) | 188 (7.3%) | 276 (14%) | 35 (6.1%) | 51 (11%) |
| AD | 30 (13%) | 48 (24%) | 78 (5.1%) | 98 (8.3%) | 53 (2.0%) | 63 (3.3%) | 12 (2.1%) | 9 (1.9%) |
| **CSF_AB42_Elecsys** | 755 (375) | 524 (210) | 940 (446) | 854 (440) | 1,233 (610) | 1,116 (518) | 1,192 (550) | 1,226 (573) |
| **CSF_pTau_Elecsys** | 32 (20) | 30 (14) | 27 (15) | 26 (13) | 21 (11) | 22 (12) | 21 (12) | 20 (11) |
| **Hippo_normali-zed_icv** | 0.0047 (0.0008) | 0.0042 (0.0007) | 0.0048 (0.0007) | 0.0043 (0.0007) | 0.0050 (0.0007) | 0.0045 (0.0007) | 0.0050 (0.0007) | 0.0046 (0.0007) |
| **PET_Centiloid** | 52 (41) | 58 (40) | 34 (41) | 35 (43) | 11 (30) | 11 (32) | 10 (28) | 7 (27) |
| **Plasma_pTau_Si-moa** | 17 (12) | 22 (11) | 15 (9) | 17 (11) | 13 (9) | 17 (30) | 13 (7) | 15 (11) |
| **Plasma_NfL** | 31 (19) | 36 (19) | 29 (22) | 34 (23) | 31 (26) | 34 (22) | 30 (21) | 33 (30) |
| **PET_tau_Bk12** | 1.29 (0.18) | 1.34 (0.21) | 1.23 (0.17) | 1.23 (0.15) | 1.15 (0.13) | 1.19 (0.14) | 1.15 (0.14) | 1.13 (0.11) |
| **PET_tau_Bk34** | 1.26 (0.14) | 1.43 (0.30) | 1.22 (0.19) | 1.22 (0.13) | 1.17 (0.11) | 1.20 (0.15) | 1.16 (0.08) | 1.15 (0.10) |
| **PET_tau_Bk56** | 1.20 (0.16) | 1.30 (0.36) | 1.15 (0.17) | 1.14 (0.14) | 1.12 (0.15) | 1.12 (0.14) | 1.10 (0.10) | 1.09 (0.15) |
| **Neuropath cohort** | | | | | | | | |
| | **Female, N = 130[1]** | **Male, N = 143[1]** | **Female, N = 511[1]** | **Male, N = 577[1]** | **Female, N = 723[1]** | **Male, N = 842[1]** | **Female, N = 189[1]** | **Male, N = 182[1]** |
| **ADNC** | | | | | | | | |
| High-ADNC | 109 (84%) | 116 (81%) | 338 (66%) | 358 (62%) | 259 (36%) | 292 (35%) | 60 (32%) | 58 (32%) |
| Intermediate-ADNC | 17 (13%) | 18 (13%) | 101 (20%) | 124 (21%) | 185 (26%) | 162 (19%) | 41 (22%) | 27 (15%) |
| Low-ADNC | 4 (3.1%) | 6 (4.2%) | 61 (12%) | 76 (13%) | 160 (22%) | 200 (24%) | 48 (25%) | 43 (24%) |
| not-AD | 0 (0%) | 3 (2.1%) | 11 (2.2%) | 19 (3.3%) | 119 (16%) | 188 (22%) | 40 (21%) | 54 (30%) |
| **Alzheimer Dementia** | 119 (92%) | 121 (85%) | 399 (78%) | 421 (73%) | 391 (54%) | 449 (53%) | 102 (54%) | 97 (53%) |
| **Age Death** | 77 (8) | 76 (9) | 82 (10) | 79 (10) | 84 (12) | 81 (11) | 84 (12) | 81 (12) |

[1] Mean (SD); n (%)

**Supplementary Table 2.**

Demographic, clinical and biomarker data for the different clinical cohorts.

| | A4 | | ADNI | | ALFA | OASIS | | WRAP | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | HC, N = 5,477 | HC, N = 799 | MCI, N = 1,021 | AD, N = 389 | HC, N = 418 | HC, N = 940 | AD, N = 227 | HC, N = 584 | MCI, N = 26 | AD, N = 2 |
| **Age** | 70·4 (67·6, 74·5) | 73 (68, 77) | 74 (68, 78) | 75 (70, 80) | 61·7 (58·1, 64·8) | 71 (65, 76) | 75 (70, 80) | 65 (59, 70) | 71 (68, 76) | 67 (66, 68) |
| **Sex** | | | | | | | | | | |
| Female | 3,219 (59%) | 446 (56%) | 416 (41%) | 171 (44%) | 254 (61%) | 548 (58%) | 104 (46%) | 412 (71%) | 15 (58%) | 2 (100%) |
| Male | 2,258 (41%) | 353 (44%) | 605 (59%) | 218 (56%) | 164 (39%) | 392 (42%) | 123 (54%) | 172 (29%) | 11 (42%) | 0 (0%) |
| **APOE** | | | | | | | | | | |
| APOE4/4 | 165 (3·0%) | 22 (2·8%) | 110 (11%) | 78 (20%) | 36 (8·6%) | 37 (3·9%) | 27 (12%) | 29 (5·0%) | 3 (12%) | 0 (0%) |
| APOE3/4 | 1,582 (29%) | 211 (26%) | 373 (37%) | 174 (45%) | 184 (44%) | 265 (28%) | 114 (50%) | 176 (30%) | 11 (42%) | 2 (100%) |
| APOE-3/3 | 3,010 (55%) | 460 (58%) | 455 (45%) | 116 (30%) | 163 (39%) | 492 (52%) | 68 (30%) | 301 (52%) | 9 (35%) | 0 (0%) |
| APOE-2/3 | 559 (10%) | 91 (11%) | 58 (5·7%) | 11 (2·8%) | 25 (6·0%) | 108 (11%) | 11 (4·8%) | 56 (9·6%) | 1 (3·8%) | 0 (0%) |
| APOE-2/2 | 29 (0·5%) | 3 (0·4%) | 1 (<0·1%) | 1 (0·3%) | 0 (0%) | 6 (0·6%) | 0 (0%) | 3 (0·5%) | 0 (0%) | 0 (0%) |
| APOE-2/4 | 132 (2·4%) | 12 (1·5%) | 22 (2·2%) | 9 (2·3%) | 10 (2·4%) | 32 (3·4%) | 7 (3·1%) | 19 (3·3%) | 2 (7·7%) | 0 (0%) |
| **CSF AB42** | - | 1,129 (779, 1,503) | 765 (584, 1,129) | 588 (454, 750) | 1,232 (886, 1,660) | - | - | 843 (592, 1,147) | 1,396 (689, 1,517) | - |
| **CSF pTau** | - | 19 (15, 26) | 24 (18, 34) | 33 (26, 44) | 14 (11, 19) | - | - | 17 (13, 22) | 27 (21, 31) | - |
| **Hippo Vol** | 0·0045 (0·0041, 0·0048) | 0·0050 (0·0046, 0·0055) | 0·0045 (0·0039, 0·0051) | 0·0037 (0·0034, 0·0042) | 0·0052 (0·0049, 0·0057) | 0·0052 (0·0046, 0·0057) | 0·0040 (0·0035, 0·0046) | 0·0054 (0·0050, 0·0058) | 0·0048 (0·0040, 0·0051) | 0·0043 (0·0043, 0·0043) |
| **AB Centiloid** | 5 (-7, 31) | 10 (1, 30) | 29 (4, 72) | 81 (50, 108) | -1 (-6, 5) | 6 (-1, 23) | 84 (46, 106) | 5 (2, 11) | 12 (9, 58) | 76 (74, 78) |
| **Plasma pTau** | - | 13 (9, 18) | 15 (11, 23) | 23 (17, 28) | 8·6 (7·0, 11·0) | - | - | - | - | - |
| **Plasma NfL** | - | 30 (24, 40) | 35 (26, 46) | 44 (33, 59) | 10·0 (7·9, 12·4) | - | - | 17 (13, 24)- | 18 (17, 30) | - |
| **TauPET Braak 1/2** | 1·18 (1·10, 1·27) | 1·15 (1·08, 1·22) | 1·23 (1·07, 1·38) | 1·31 (1·17, 1·45) | - | 1·11 (1·05, 1·19) | 1·37 (1·20, 1·51) | - | - | - |
| **TauPET Braak 3/4** | 1·16 (1·11, 1·22) | 1·16 (1·12, 1·23) | 1·20 (1·12, 1·31) | 1·37 (1·21, 1·57) | - | 1·16 (1·10, 1·21) | 1·37 (1·21, 1·60) | - | - | - |
| **TauPET Braak 5/6** | 1·09 (1·05, 1·14) | 1·11 (1·07, 1·17) | 1·11 (1·05, 1·19) | 1·20 (1·10, 1·36) | - | 1·12 (1·07, 1·17) | 1·24 (1·13, 1·40) | - | - | - |

Median (IQR); n (%)

ADNC refers to the degree of amyloid plaque and neurofibrillary tangle burden in the brain. The data is broken down into four categories: High-ADNC: Refers to individuals with high levels of amyloid plaque and neurofibrillary tangle burden. Interm-ADNC: Refers to individuals with intermediate levels of amyloid plaque and neurofibrillary tangle burden. Low-ADNC: Refers to individuals with low levels of amyloid plaque and neurofibrillary tangle burden. not-AD: Refers to individuals who do not have Alzheimer's disease pathology. The age at death is reported as a median (IQR).

**Supplementary Table 3. Frequency of positive amyloid and tau biomarkers by age in APOE4 homozygotes and APOE3 homozygotes.**

| | AGE INTERVALS | | | | | | |
|---|---|---|---|---|---|---|---|
| | 52.5 – 57.5 | 57.5 – 62.5 | 62.5 – 67.5 | 67.5 – 72.5 | 72.5 – 77.5 | 77.5 – 82.5 | 82.5 – 87.5 |
| **APOE-4/4** | | | | | | | |
| Centoiloid | 4/19 (17.4%) | 19/11 (63.3%) | 67/24 (73.6%) | 86/18 (82.7%) | 45/8 (85%) | 21/0 (100%) | 9/0 (100%) |
| CSF AB1-42 | 14/9 (60.9%) | 23/4 (85.1%) | 32/1 (96.7%) | 40/3 (93%) | 28/1 (96.6%) | 13/1 (92.9%) | 9/0 (100%) |
| CSF pTau181 | 5/18 (21.7%) | 14/12 (53.8%) | 27/6 (81.8%) | 31/12 (72%) | 22/7 (75.9%) | 13/1 (92.8%) | 5/4 (55.6%) |
| Plasma pTau181 | 1/17 (5.6%) | 13/9 (59%) | 9/8 (52.9%) | 12/9 (57.1%) | 9/5 (64.3%) | 7/0 (100%) | 6/1 (85.7%) |
| **APOE-3/3** | | | | | | | |
| Centoiloid | 5/63 (7.4%) | 7/96 (6.8%) | 93/741 (11.2%) | 197/1004 (16.4%) | 204/640 (24.2%) | 161/256 (39%) | 57/90 (38.8%) |
| CSF AB1-42 | 17/30 (36.1%) | 19/57 (25%) | 44/109 (28.8%) | 38/95 (33.6%) | 59/80 (42.4%) | 54/55 (49.5%) | 18/39 (31.6%) |
| CSF pTau181 | 7/40 (14.9%) | 14/69 (16.9%) | 39/132 (22.8%) | 43/123 (25.9%) | 53/115 (31.5%) | 66/59 (52.8%) | 32/30 (51.6%) |
| Plasma pTau181 | 3/35 (7.9%) | 8/55 (12.7%) | 16/117 (12%) | 17/84 (16.8%) | 37/58 (38.9%) | 29/43 (40.2%) | 16/15 (51.6%) |

Positive / negative cases (percentage positive%)