



Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/170128/>

Version: Accepted Version

Article:

De Marco, M., Manca, R., Kirby, J. et al. (2020) The association between polygenic hazard and markers of Alzheimer's disease following stratification for APOE genotype. *Current Alzheimer Research*, 17 (7). pp. 667-679. ISSN: 1567-2050

<https://doi.org/10.2174/1567205017666201006161800>

The published manuscript is available at EurekaSelect via
<http://www.eurekaselect.com/openurl/content.php?genre=article&doi=10.2174/1567205017666201006161800>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

**The Association between Polygenic Hazard and Markers of Alzheimer's Disease Following Stratification for APOE
Genotype**

Matteo *De Marco*, Riccardo *Manca*, Janine *Kirby*, Guillaume M *Hautbergue*, Daniel J *Blackburn*, Stephen B *Wharton*,
Annalena *Venneri*^{*}, for the Alzheimer's Disease Neuroimaging Initiative[#]

Department of Neuroscience, University of Sheffield, Sheffield, UK

***Corresponding author:** Annalena Venneri: Department of Neuroscience, University of Sheffield, Medical School, Beech Hill Road, Sheffield, South Yorkshire, UK, S10 2RX, , email: a.venneri@sheffield.ac.uk

[#]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

Running title

Polygenic Hazard and Markers of AD after APOE Stratification

Acknowledgements

This study was supported by an award to MDM and AV from Alzheimer's Research UK Yorkshire Network Centre under the Small Grant Scheme. This is a summary of independent research carried out at the NIHR Sheffield Biomedical Research Centre (Translational Neuroscience). The views expressed are those of the authors and not necessarily those of the National Health Service, the NIHR or the Department of Health.

Author contributions: Matteo De Marco: Funding acquisition, Conceptualisation, Methodology, Formal analysis, Writing - Review & Editing; Riccardo Manca: Data Curation, Formal analysis, Writing - Review & Editing; Janine Kirby: Writing - Review & Editing; Guillaume M Hautbergue: Writing - Review & Editing; Daniel J Blackburn: Writing - Review & Editing; Stephen B Wharton: Writing - Review & Editing; Annalena Venneri: Funding acquisition, Conceptualisation, Writing - Review & Editing.

Abstract

Background: Research indicates that polygenic indices of Alzheimer's disease's risk are linked to clinical profiles.

Objective: Given the “genetic centrality” of the APOE gene, we tested whether this held true for both APOE- ϵ 4 carriers and non-carriers.

Methods: A polygenic hazard score (PHS) was extracted from 784 non-demented participants recruited in the Alzheimer's Disease Neuroimaging Initiative and stratified by APOE ϵ 4 status. Datasets were split into sub-cohorts defined by clinical (unimpaired/MCI) and amyloid status ($A\beta^+$ / $A\beta^-$). Linear models were devised in each sub-cohort and for each APOE- ϵ 4 status to test the association between PHS and memory, executive functioning and grey-matter volumetric maps.

Results: PHS predicted memory and executive functioning in ϵ 4 ϵ 3 MCI patients, memory in ϵ 3 ϵ 3 MCI patients, and memory in ϵ 4 ϵ 3 $A\beta^+$ participants. PHS also predicted volume in sensorimotor regions in ϵ 3 ϵ 3 $A\beta^+$ participants.

Conclusion: The link between polygenic hazard and neurocognitive variables varies depending on APOE- ϵ 4 allele status. This suggests that clinical phenotypes might be influenced by complex genetic interactions.

Keywords:

Mild Cognitive Impairment, Apolipoprotein, Memory, Executive Function, Polygenic Traits, Amyloid

1. Introduction

Alzheimer's disease (AD) is a complex neurodegenerative disorder characterised by multiple pathophysiological features including, among others, beta amyloid (A β) and tau protein deposits, reactive gliosis and metallo-dyshomeostasis [1]. Pathological complexity is mirrored by a large heterogeneity of neuroimaging and clinical presentations [2]. Variability of genetic expression may play a role in this heterogeneity. Although the forms of AD due to autosomal dominant mutations are estimated to be only about 0.25% of all cases [3], there is mounting evidence that normal genetic variability of single-nucleotide polymorphisms (SNPs) plays a major role in altering the predisposition and the neurological profiles of AD.

Apolipoprotein E (APOE) is the gene with, by far, the best established connection with sporadic AD, with its two SNPs (differentiating ϵ_4 and ϵ_2 alleles from ϵ_3) being associated with increased and decreased risk, respectively. Genome-wide association studies, however, have identified additional SNPs that show statistically significant associations with AD [4] and these have suggested potential new pathophysiological mechanisms [5,6]. To characterise their effect on pathological, neuroimaging and clinical markers of AD, these supplementary "non-APOE" SNPs have either been studied as stand-alone variables, e.g., [7-9], or as global combinatory indices. Depending on the computational procedure such indices are called polygenic risk scores or polygenic hazard scores (PHS) and have been the object of experimental interest for a more detailed characterisation of AD [10]. Specifically, polygenic risk scores are based on the combination of SNP weights expressed as log odds ratios, and PHS are based on the combination of SNP weights expressed as log hazard ratios [11]. Higher polygenic scores have been shown to be associated with reduced regional brain volumes [12,13] and cognitive decline [14] in healthy adults. This has been found in multiple cohorts as a function of different combinations of SNPs, but usually including the APOE ϵ_4 allele among the pool of genes of interest, e.g., [15-17].

It is not clear, however, to what extent polygenic scores can be informative of clinical profiles beyond the APOE genotype. Although it is known that the statistical association between APOE SNPs and AD is considerably stronger than that of any other non-APOE SNPs and AD [4,18], studies that have investigated samples stratified for APOE genotype have been mostly limited to describing diagnostic and prognostic accuracy of polygenic scores [19,20], without exploring clinical phenotypes in more detail. On this note, we hypothesised that the combination of non-APOE SNPs will be particularly informative of clinical markers of AD (and give rise to significant associations) when APOE-related risk is controlled for and minimal (i.e., in ϵ_4 non-carriers). To address this experimental question in an exploratory way, we tested the association between whole-brain neurovolumetric and cognitive profiles and a PHS based on 33 AD-related SNPs [18] in a large cohort of pathology-informed participants enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI), and stratified by APOE ϵ_4 allele status.

2. Materials and Methods

2.1. Participants

Data used in the preparation of this article were obtained from the ADNI database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. All ADNI participants provided written informed consent, and study protocols were approved by each participating site's institutional review board. For research governance and compliance with ethical standards and informed consent please consult the ADNI website at www.adni-info.org and associated material. Additional local ethical approval was not required since the ADNI database contains only anonymised data that are publicly available for download.

The complete repository was consulted to identify datasets acquired on participants classified as healthy unimpaired adults or MCI. One single dataset per participant was used. Each individual dataset had to include CSF biomarker information, a T1-weighted MRI image, cognitive testing and genetic information on SNPs for the calculation of AD-related PHS. These criteria resulted in 879 datasets (317 cognitively unimpaired adults, 562 MCI patients) being considered for analyses. Four datasets (one unimpaired adult, three MCI patients) were discarded because of MRI signal artefacts, leaving a cohort of 875.

2.2. APOE genotype

The observed frequencies of all APOE genotypes and a statistical comparison of observed and expected frequencies [21] are illustrated in **Figure 1a**. To safeguard the statistical robustness of the analyses, genotypes including the ϵ_2 allele (having a relative small sample size) were not analysed in this study. Therefore, only $\epsilon_3\epsilon_3$ and $\epsilon_4\epsilon_3/\epsilon_4\epsilon_4$ unimpaired adults ($n = 270$), $\epsilon_3\epsilon_3$, $\epsilon_4\epsilon_3$ and $\epsilon_4\epsilon_4$ MCI patients ($n = 514$) were thus retained for analysis (total $n = 784$ of the original 875). While $\epsilon_4\epsilon_4$ MCI patients were analysed as a separate groups, $\epsilon_4\epsilon_3$ and $\epsilon_4\epsilon_4$ unimpaired adults were instead grouped together, as the small number of homozygous datasets ($n = 8$) in this latter group would not warrant independent analysis. The two diagnostic sub-cohorts are characterised in **Table 1**. All participants self-identified as being of white racial background.

-- Please insert **Figure 1** and **Table 1** about here --

2.3. Polygenic hazard scores

The PHS were constructed upon the analysis of data collected as part of the International Genomics of Alzheimer's Project initiative [4]. All SNPs associated with a significant increase or decrease in AD risk (at a $p < 10e-5$) were included as predictors into a Cox Hazard Regression model, to identify those leading to a significant change in the log hazard for every unit increase in the predicted variable. This procedure identified 33 SNPs (including the two APOE SNPs) located in 25 genes (sorted by chromosome number: CR1, BIN1, INPP5D, HLA-DRB5, GPR115, BC043356, ZCWPW1, AL833583, PTK2B, CHRNA2, CLU, CR595071, SPI1, MS4A6A, PICALM, SORL1, FERMT2, SLC24A4, abParts, TRIP4, BZRAP1, C19orf6, APOE, ABCA7 and CASS4) of which 19 SNPs were associated with a significantly higher and 14 SNPs with a significantly lower log hazard ratio [18]. An individual PHS was then calculated as a matrix product between the vector matrix of individual SNPs and the matrix of group-level log hazard ratios estimated by the statistical model [18]. The resulting scores ranged between -0.915 and 1.441 among unimpaired adults and between -0.875 and 2.937 among MCI patients. Since ϵ_4 is part of the PHS equation, ϵ_4 carriers tended to have higher PHS in a dose-dependent manner. Although ϵ_4 status did affect the absolute value of PHS, stratification by APOE enabled us to eliminate the effect of APOE SNPs on PHS variability within each specific APOE genotype.

2.4. MRI processing

One brain MRI acquisition per participant was used. This was the MRI acquired in the closest proximity to a CSF sample and to a cognitive assessment (see Sections 2.5 and 2.6 for more details). T1-weighted sequences were acquired following the specifications illustrated in the ADNI MRI protocol [22]. All processing routines were run with Matlab (Mathworks Inc., UK) and Statistical Parametric Mapping, version 12 (Wellcome Centre for Human Neuroimaging, London, UK). Each image was initially reoriented to its bicommissural axis and subjected to a quality check. Four datasets were discarded at this stage because of signal artefacts. All retained images were then processed in compliance with the standard voxel-based morphometry protocol [23]. A probabilistic segmentation was carried out to separate three tissue maps (grey matter, white matter, CSF). Grey-matter maps were then registered to the Montreal Neurological Institute space and smoothed with an 8 mm full-width at half maximum Gaussian kernel. The global native-space volume of each tissue class was quantified with the procedure described by Malone and colleagues [24]. Total intracranial volumes (obtained by summing all three tissue classes) were also extracted.

2.5. Amyloid positivity

CSF samples obtained in close proximity to the MRI scan (at an average of 24 days apart) were processed with the automated Roche Elecsys immunoassay kit to analyse and quantify levels of A β ₁₋₄₂. The presence of A β pathology is a necessary requisite to make a diagnosis, as per criteria published by the U.S. National Institute of Aging [25]. A cut-off of 1100 pg/ml [26] was used to classify participants into amyloid-positive (A β ⁺) and amyloid-negative (A β ⁻). A complete characterisation of the cohort split into A β ⁺ and A β ⁻ participants is included in **Table 2**. Of the 270 unimpaired adults 121 were A β ⁺ and 149 were A β ⁻. Of the 514 MCI patients, 352 were A β ⁺ and 162 were A β ⁻. There was only one $\epsilon_4\epsilon_4$ A β ⁻ participant who was thus excluded from the analyses of pathology-defined sub-cohorts. **Figure 1b** illustrates the distribution of A β ⁻ and A β ⁺ MCI patients and unimpaired participants and their APOE genotype.

-- Please insert **Table 2** about here --

2.6. Cognitive profiling

Composite cognitive scores made available by ADNI were included in the analyses. These were derived from tests administered at an average distance of 23 days from MRI and 11 days from lumbar puncture. The memory composite (ADNI-MEM) was constructed based on the immediate and delayed recall of the Logical Memory Test, the recall of the three words of the Mini Mental State Examination, and the fifteen measures of learning, recall and recognition included as part of the Rey Auditory Verbal Learning Test and as part of the cognitive subscale of the Alzheimer's Disease Assessment Schedule [27]. The executive function composite (ADNI-EF) was calculated based on the Digit Symbol Substitution task of the Wechsler Adult Intelligence Scale (Revised), Digit Span Backwards, Trail Making Test - Parts A and B, Category Fluency Test and five indices from the Clock Drawing Test [28].

2.7. Statistical analysis

Two sets of analyses were run separating the cohort first into two diagnostic sub-cohorts according to clinical diagnosis (unimpaired adults, MCI patients) and then into two pathology-informed sub-cohorts based on CSF A β ₁₋₄₂ positivity. This was to address the bias that may result from the choice of one single set of diagnostic criteria.

MRI images and cognitive indices were analysed with linear-association models, using genetic information as predictor. Presence of an association with PHS was tested within each entire sub-cohort (regardless of APOE) and, subsequently, within each APOE ϵ_4 allele status in a stratified way.

Models analysing cognitive composites were run with IBM SPSS Statistics 23 and hierarchical regression models. Age, educational attainment (in years) and gender were included as part of a first block of nuisance regressors, while PHS was added in a second block.

Statistical Parametric Mapping and multiple regression models were used to analyse the association between PHS and grey-matter maps. Models were corrected for age, gender, total intracranial volume, magnetic-field strength and T1 sequence type (i.e., MPAGE or IG-SPGR). A cluster-forming threshold of $p < 0.001$ was applied and clusters were considered significant when surviving Family-Wise Error correction. All peak coordinates were converted to Talairach space using a non-linear transform (imaging.mrc-cbu.cam.ac.uk/downloads/MNI2tal/mni2tal-m) and interpreted via the Talairach Daemon Client [29].

3. Results

Of the 784 individuals analysed in this study, ϵ_4 carriers were significantly younger in each sub-cohort, with a further dose-dependent effect in MCI A β + participants. Presence of the ϵ_4 allele was also associated with lower A β and higher TAU levels in cognitively unimpaired participants, MCI patients and A β + participants (in these two latter sub-cohorts a dose-dependent effect was also observed). Lower A β levels were also observed in A β - ϵ_4 carriers compared to A β - $\epsilon_3\epsilon_3$ participants. Differences among APOE genotypes were also found for the MMSE and ADNI-MEM in A β + participants with ϵ_4 carriers scoring worse in a dose-dependent manner. All these findings with exact p values are reported in **Tables 1-2**.

3.1. Association between PHS and brain structure

3.1.1. Clinically-defined sub-cohorts

In the entire sub-cohort of MCI patients ($n = 514$), PHS was negatively associated with grey matter in mediotemporal clusters encompassing the anterior hippocampus, amygdala and dorsal entorhinal cortex bilaterally, and the left posterior

hippocampus (**Figure 2a**). When the MCI sub-cohort was stratified by APOE ϵ_4 status, no significant results were found. A trend was present for a cluster located in the right somatosensory cortex ($p = 0.087$). No significant results were found in the sub-cohort of clinically unimpaired adults.

--- Please insert **Figure 2** about here ---

3.1.2. Pathology-defined sub-cohorts

In the entire sub-cohort of $A\beta^+$ participants ($n = 473$) a significant negative association between PHS and grey matter volumes was found in the left anterior hippocampus and amygdala (**Figure 2b**), with a similar, yet non-significant trend observed in the contralateral territory. After APOE stratification, a significant negative association was found between PHS and grey matter in $\epsilon_3\epsilon_3$ participants. This was located in the primary sensorimotor cortex in a bilateral vertex cluster located in proximity of the inter-hemispheric sulcus and overlapped with the non-significant trend found among $\epsilon_3\epsilon_3$ MCI patients (**Figure 2c**). No significant association was found in the sub-cohort of $A\beta^-$ participants.

3.2. Association between PHS and cognitive functioning

3.2.1. Clinically-defined sub-cohorts

In the entire sub-cohort of MCI patients ($n = 514$), PHS was negatively correlated with memory ($r^2 = 0.203$, r^2 change = 0.087 , $p = 3.69 \cdot 10^{-13}$) and executive functioning ($r^2 = 0.179$, r^2 change = 0.019 , $p = 0.001$). After APOE stratification, an association was found between PHS and memory in $\epsilon_3\epsilon_3$ patients ($r^2 = 0.180$, r^2 change = 0.022 , $p = 0.012$) and in $\epsilon_4\epsilon_3$ patients ($r^2 = 0.209$, r^2 change = 0.031 , $p = 0.005$). In $\epsilon_4\epsilon_3$ patients, a significant association was also found between PHS and executive functioning ($r^2 = 0.246$, r^2 change = 0.026 , $p = 0.008$). In the entire sub-cohort of healthy adults ($n = 270$), PHS was not associated with cognition. Similarly, no results emerged after stratifying for APOE. These findings are illustrated in **Figure 3a, c, d**.

--- Please insert **Figure 3** about here ---

3.2.2. Pathology-defined sub-cohorts

In the entire sub-cohort of A β + participants ($n = 473$), a negative association was found between PHS and memory ($r^2 = 0.146$, r^2 change = 0.069, $p = 1.53 \cdot 10^{-9}$) but not between PHS and executive functioning. When the sub-cohorts were stratified according to APOE genotype, the sole significant association was that between PHS and memory in $\epsilon_4\epsilon_3$ A β + participants ($r^2 = 0.094$, r^2 change = 0.016, $p = 0.048$). No significant associations were found in the sub-cohort of A β - adults. All these findings are illustrated in **Figure 3b, e, f**.

3.3. Post hoc analysis of the sensorimotor cortex

To describe the association between PHS and the size of motor and sensory areas more precisely, we extracted the volume (in ml) of a series of cortical patches from each individual normalised MRI image. The specific sensory and motor sub-regions were selected from the precentral gyrus, the paracentral lobule and the postcentral gyrus of the Brainnetome atlas [30]. This atlas divides the brain into 210 cortical and 36 subcortical regions giving an optimal level of detail to separate sensory and motor cortex according to the body district subjected to cortical control (i.e., ‘head and face’, ‘upper limb’, ‘trunk’, ‘tongue and larynx’ and ‘lower limb’) for a total of 24 patches. Linear hierarchical regression models were run (using the same set of covariates as with whole-brain models) to test the association between PHS and the volume in each of the 24 sub-regions within the sub-cohort of $\epsilon_3\epsilon_3$ A β + participants. A Bonferroni-corrected $p < 0.00208$ (correcting for 24 independent contrasts) was used as threshold of significance. Associations surviving statistical significance were found in the right Brodmann area 1/2/3, specifically in the ‘lower limb’ ($r^2 = 0.242$, r^2 change = 0.044, $p = 0.002$) and ‘trunk’ region ($r^2 = 0.290$, r^2 change = 0.050, $p < 0.001$). The location of the two sub-regions where a significant association was found is shown in **Figure 4**.

--- Please insert **Figure 4** about here ---

3.4. Post hoc gender-stratified re-analysis of significant findings

All significant findings described in Sections 3.1-3.3 were explored at *post hoc* after stratification by gender. This was carried out to rule out the presence of discrepant trends in the pattern of findings between males and females.

Although not consistently retaining statistical significance (arguably due to a decrease in power), comparable trends in patterns of findings were observed for both genders (**Figure 5**).

--- Please insert **Figure 5** about here ---

4. Discussion

The statistical association linking an AD-related PHS, brain structure and cognitive indices was investigated in 784 individuals selected based on their APOE genotype from the original cohort of 875 individuals fulfilling the study criteria. These were divided into clinically- and pathology-defined sub-cohorts. We found no significant effect of any of the genetic variables when cognitively unimpaired or A β - participants were analysed. Conversely, in MCI patients and, in an analogous way, in A β + participants, PHS was associated with regional grey-matter volumes in the mediotemporal complex. When the sub-cohorts were stratified by APOE ϵ_4 status, a significant association emerged in $\epsilon_3\epsilon_3$ A β + participants. This was mirrored by a qualitatively similar non-significant trend among $\epsilon_3\epsilon_3$ MCI patients. The findings were centred in a large pericentral cluster including regions involved in sensorimotor processing, particularly, homuncular regions known to control gross sensorimotor processing. *Post hoc* models analysing global volumes of atlas-based sub-regions of this territory confirmed these findings, highlighting that the core of the effect was located in the right sensorimotor region controlling the left lower-limb region and the left part of the trunk. The link between APOE alleles and motor function in the elderly is well established. In fact, evidence from large cohort studies indicates that carrying the ϵ_4 allele confers an increased risk of mobility reduction [31] and motor decline [32]. Moreover, regional accumulation of A β is associated with gait speed reduction, and APOE genotype modulates this association in the sensorimotor cortex [33]. In addition, evidence suggests that the sensorimotor cortex is involved in memory processing in ϵ_4 carriers [34]. This is compelling evidence indicating that the function of the sensorimotor cortex is statistically linked to the ϵ_4 allele. In the context of the evidence reported in these studies, our findings indicate that other genetic variables account for the volumetric variability of sensorimotor cortex when the ϵ_4 allele is absent, in support of our initial hypothesis.

PHS was also associated with cognitive functioning. When participants were split according to clinical criteria, PHS was associated with executive function among $\epsilon_4\epsilon_3$ MCI patients and with memory in both $\epsilon_4\epsilon_3$ and $\epsilon_3\epsilon_3$ MCI patients. When participants were split according to pathological criteria, PHS was solely associated with memory in $\epsilon_4\epsilon_3$ A β + participants. This indicates that cognitive functioning is influenced by multiple genetic variables, and that this influence is more robust among ϵ_4 carriers at risk for established sporadic AD (against our initial hypothesis). We argue that the absence of an association in the $\epsilon_4\epsilon_4$ is mainly due to lack of statistical power. In these groups, in fact, the sample size was considerable

smaller ($\epsilon_4\epsilon_4$ MCI patients: $n = 61$; $\epsilon_4\epsilon_4$ A β^+ participants: $n = 60$) when compared to $\epsilon_4\epsilon_3$ MCI patients ($n = 212$) or $\epsilon_4\epsilon_3$ A β^+ participants ($n = 219$).

These findings expand the literature on the link between PHS and APOE. Previous studies have reported that APOE and PHS predict distinct aspects of cognition [35], and that PHS accounts for cognitive and regional volumetric decline even when APOE ϵ_4 status is used as a control variable [20]. In this study, based on APOE stratification, we found separate statistical associations of PHS in ϵ_4 carriers and non-carriers, suggesting an interactive effect. The multiplicity of genetic mechanisms conveyed by PHS is likely to be associated with the clinical markers of AD in complex ways. Non-APOE SNPs are generally involved in various processes that are crucial for brain and cognitive functioning, such as neural development, axonal transport and immune response [36]. Clinical profiles of patients depend on the interplay of these pathways. When APOE is singled-out and used for stratification, this interplay predicts profiles of individuals at risk for AD in ways that are ϵ_4 -dependent, indicating a role of APOE in this interaction. Furthermore, the pattern of findings is similar in both males and females as emerged from analyses following additional stratification by gender.

The polygenic scores used in this study reflect the combination of statistical indices (the log hazard ratios) associated with an increase or decrease in the risk of developing AD. The log hazard ratio for the APOE ϵ_4 allele used to calculate these PHS was 1.03 and it was considerably larger than the other SNPs associated with increased risk, which ranged between 0.07 and 0.3 [18]. This evidence is also supported by the study of polygenic risk scores: the *odds-ratio* coefficient for the ϵ_4 allele (~ 0.2) is considerably smaller (indicating more risk) than that of the other SNPs, which ranges instead between 0.7 and 0.9 [37]. Although these numerical differences indicate that the APOE ϵ_4 allele plays a leading role within a polygenic score, the combination of the other SNPs is still informative independently of APOE. In a recent study, in fact, it was found that a polygenic risk score was a significant predictor of AD in pathologically-confirmed cases with an APOE $\epsilon_3\epsilon_3$ genotype [38].

Given the exploratory nature of this study, a number of limitations are noticed. First, polygenic indices have been specifically designed to capture the interplay of multiple mechanisms of risk, therefore it is not possible to interpret these findings by referring to a specific pathophysiological pathway. In addition, the generation of the PHS published by ADNI was based on methodological criteria (i.e., including all SNPs associated with a significant increase in the statistical prediction), not theory-based criteria. As a consequence, different procedural choices in the construction of polygenic indices may result in slightly different polygenic variables. Following the seminal study by Lambert and colleagues [4], further genome-wide investigation of AD have been further pursued [39]. As a result, the list of genetic loci statistically associated with AD is in constant evolution. On these grounds, the future definition and use of novel polygenic indices is

destined to lead to the design of variables that are increasingly accurate at capturing the holistic genetic risk (or hazard) associated with AD. Furthermore, certain established polygenic scores are more parsimonious (i.e., based on fewer SNPs) than the PHS used in this study [40]. This, however, does not undermine the validity of the PHS, but is certainly a methodological aspect to be taken into consideration in the pathway of clinical translation. Finally, although we classified APOE genotypes based on the three main allelic isoforms, we did not take into consideration promoter polymorphisms. The risk of developing AD is significantly influenced by APOE promoter SNPs in ϵ_4 non-carriers only [41]. Future hypotheses may address more in detail the association between APOE alleles and polygenic scores in relation to APOE promoter SNPs.

5. Conclusion

In conclusion, the PHS defined by Desikan and coworkers [18] predicts mediotemporal volumes and cognitive performance in $A\beta^+$ or MCI adults. In these sub-cohorts, PHS robustly predicts memory performance in the presence of an APOE $\epsilon_4\epsilon_3$ genotype and volumetric properties of the sensorimotor cortex in the presence of an APOE $\epsilon_3\epsilon_3$ genotype.

Ethics Approval and Consent to Participate

All data were collected in compliance with the appropriate ethics approval and each participant signed a written informed consent. For more precise information, visit www.adni-info.org.

Human and Animal Rights

All procedures were carried out in compliance with the Declaration of Helsinki and subsequent amendments. For more precise information, visit www.adni-info.org.

List of Abbreviations

A β : Amyloid Beta; AD: Alzheimer's disease; ADNI: Alzheimer's Disease Neuroimaging Initiative; MCI: mild cognitive impairment; MRI: magnetic resonance imaging; PHS: polygenic hazard score; SNP: single nucleotide polymorphism

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding information

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California. The GPU card used for the procedures of hippocampal segmentation included in this research was donated by the NVIDIA Corporation.

References

- [1] Lahiri DK. Lessons from Alzheimer's Disease (AD) Clinical Trials: Instead of "A-Drug", AD-D prevention to Avert AD. *Curr Alzheimer Res* 16: 279-80 (2019).
- [2] Lam B, Masellis M, Freedman M, Stuss DT, Black SE. Clinical, imaging, and pathological heterogeneity of the Alzheimer's disease syndrome. *Alzheimers Res Ther* 5: 1 (2013).
- [3] Hartmann S, Ledur Kist TB. A review of biomarkers of Alzheimer's disease in noninvasive samples. *Biomark Med* 12: 677-90 (2018).
- [4] Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, *et al.* Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 45:1452–8 (2013).
- [5] Medway C, Morgan K. Review: The genetics of Alzheimer's disease; putting flesh on the bones. *Neuropathol Appl Neurobiol* 40: 97-105 (2014).
- [6] Cuyvers E, Sleegers K. Genetic variations underlying Alzheimer's disease: Evidence from genome-wide association studies and beyond. *Lancet Neurol* 15: 857-68 (2016).
- [7] Zhang P, Qin W, Wang D, Liu B, Zhang Y, Jiang T, *et al.* Impacts of PICALM and CLU variants associated with Alzheimer's disease on the functional connectivity of the hippocampus in healthy young adults. *Brain Struct Funct* 220: 1463-75 (2015).
- [8] Zhang X, Yu JT, Li J, Wang C, Tan L, Liu B, *et al.* Bridging Integrator 1 (BIN1) genotype effects on working memory, hippocampal volume, and functional connectivity in young healthy individuals. *Neuropsychopharmacology* 40: 1794-803 (2015).
- [9] Zhu XC, Wang HF, Jiang T, Lu H, Tan MS, Tan CC, *et al.* Effect of CR1 genetic variants on cerebrospinal fluid and neuroimaging biomarkers in healthy, mild cognitive impairment and Alzheimer's disease cohorts. *Mol Neurobiol* 54: 551-62 (2017).
- [10] Chasioti D, Yan J, Nho K, Saykin AJ. Progress in polygenic composite scores in Alzheimer's and other complex diseases. *Trends Genet* 35:371-82 (2019).
- [11] Leonenko G, Sims R, Shoai M, Frizzati A, Bossù P, Spalletta G, *et al.* Polygenic risk and hazard scores for Alzheimer's disease prediction. *Ann Clin Transl Neurol* 6: 456-65 (2019).

- [12] Axelrud LK, Santoro ML, Pine DS, Talarico F, Gadelha A, Manfro GG, *et al.* Polygenic risk score for Alzheimer's disease: Implications for memory performance and hippocampal volumes in early life. *Am J Psychiatry* 175: 555-63 (2018).
- [13] Li J, Zhang X, Li A, Liu S, Qin W, Yu C, *et al.* Polygenic risk for Alzheimer's disease influences precuneal volume in two independent general populations. *Neurobiol Aging* 64: 116-22 (2018).
- [14] Tan CH, Bonham LW, Fan CC, Mormino EC, Sugrue LP, Broce IJ, *et al.* Polygenic hazard score, amyloid deposition and Alzheimer's neurodegeneration. *Brain* 142: 460-70 (2019).
- [15] Verhaaren BF, Vernooij MW, Koudstaal PJ, Uitterlinden AG, van Duijn CM, Hofman A, *et al.* Alzheimer's disease genes and cognition in the nondemented general population. *Biol Psychiatry* 73: 429-34 (2013).
- [16] Adams HH, de Bruijn RF, Hofman A, Uitterlinden AG, van Duijn CM, Vernooij MW, *et al.* Genetic risk of neurodegenerative diseases is associated with mild cognitive impairment and conversion to dementia. *Alzheimers Dement* 11: 1277-85 (2015).
- [17] Andrews SJ, Das D, Cherbuin N, Anstey KJ, Eastaer S. Association of genetic risk factors with cognitive decline: the PATH through life project. *Neurobiol Aging* 41: 150-8 (2016).
- [18] Desikan RS, Fan CC, Wang Y, Schork AJ, Cabral HJ, Cupples LA, *et al.* Genetic assessment of age-associated Alzheimer disease risk: Development and validation of a polygenic hazard score. *PLoS Med* 14: e1002258 (2017).
- [19] Kauppi K, Fan CC, McEvoy LK, Holland D, Tan CH, Chen CH, *et al.* Combining polygenic hazard score With volumetric MRI and cognitive measures improves prediction of progression from mild cognitive impairment to Alzheimer's disease. *Front Neurosci* 12: 260 (2018).
- [20] Tan CH, Fan CC, Mormino EC, Sugrue LP, Broce IJ, Hess CP, *et al.* Polygenic hazard score: An enrichment marker for Alzheimer's associated amyloid and tau deposition. *Acta Neuropathol* 13: 85-93 (2018).
- [21] Mattsson N, Groot C, Jansen WJ, Landau SM, Villemagne VL, Engelborghs S, *et al.* Prevalence of the apolipoprotein E ϵ 4 allele in amyloid β positive subjects across the spectrum of Alzheimer's disease. *Alzheimers Dement* 14: 913-24 (2018).
- [22] Jack Jr CR, Bernstein MA, Fox NC, Thompson P, Alexander G, Harvey D, *et al.* The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. *J Magn Reson Imaging* 27: 685-91 (2008).
- [23] Ashburner J, Friston KJ. Voxel-based morphometry -- The methods. *Neuroimage* 11: 805-21 (2000).

- [24] Malone IB, Leung KK, Clegg S, Barnes J, Whitwell JL, Ashburner J, et al. Accurate automatic estimation of total intracranial volume: A nuisance variable with less nuisance. *Neuroimage* 104: 366-72 (2015).
- [25] Jack Jr CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* 14: 535-62 (2018).
- [26] Hansson O, Seibyl J, Stomrud E, Zetterberg H, Trojanowski JQ, Bittner T, et al. CSF biomarkers of Alzheimer's disease concord with amyloid- β PET and predict clinical progression: A study of fully automated immunoassays in BioFINDER and ADNI cohorts. *Alzheimers Dement* 14: 1470-81 (2018).
- [27] Crane PK, Carle A, Gibbons LE, Insel P, Mackin RS, Gross A, et al. Development and assessment of a composite score for memory in the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Brain Imaging Behav* 6: 502-16 (2012).
- [28] Gibbons LE, Carle AC, Mackin RS, Harvey D, Mukherjee S, Insel P, et al. A composite score for executive functioning, validated in Alzheimer's Disease Neuroimaging Initiative (ADNI) participants with baseline mild cognitive impairment. *Brain Imaging Behav* 6: 517-27 (2012).
- [29] Lancaster JL, Woldorff MG, Parsons LM, Liotti M, Freitas CS, Rainey L, et al. Automated Talairach atlas labels for functional brain mapping. *Hum Brain Mapp* 10: 120-31 (2003).
- [30] Fan L, Li H, Zhuo J, Zhang Y, Wang J, Chen L, et al. The Human Brainnetome Atlas: A new brain atlas based on connectional architecture. *Cereb Cortex* 26: 3508-26 (2016).
- [31] Melzer D, Dik MG, van Kamp GJ, Jonker C, Deeg DJ. The apolipoprotein E e4 polymorphism is strongly associated with poor mobility performance test results but not self-reported limitation in older people. *J Gerontol A Biol Sci Med Sci* 60: 1319-23 (2005).
- [32] Buchman AS, Boyle PA, Wilson RS, Beck TL, Kelly JF, Bennett DA. Apolipoprotein E e4 allele is associated with more rapid motor decline in older persons. *Alzheimer Dis Assoc Disord* 23: 63-9 (2009).
- [33] Nadkarni NK, Perera S, Snitz BE, Mathis CA, Price J, Williamson JD, et al. Association of brain amyloid- β with slow gait in elderly individuals without dementia: Influence of cognition and apolipoprotein E ϵ 4 genotype. *JAMA Neurol* 74: 82-90 (2017).
- [34] Shu H, Shi Y, Chen G, Wang Z, Liu D, Yue C, et al. Distinct neural correlates of episodic memory among apolipoprotein E alleles in cognitively normal elderly. *Brain Imaging Behav* 13: 255-69 (2019).

- [35] Ge T, Sabuncu MR, Smoller JW, Sperling RA, Mormino EC; Alzheimer's Disease Neuroimaging Initiative. Dissociable influences of APOE ϵ 4 and polygenic risk of AD dementia on amyloid and cognition. *Neurology* 90: e1605-12 (2018).
- [36] Karch CM, Cruchaga C, Goate AM. Alzheimer's disease genetics: From the bench to the clinic. *Neuron* 83: 11-26 [2014].
- [37] Escott-Price V, Myers AJ, Huentelman M, Hardy J. Polygenic risk score analysis of pathologically confirmed Alzheimer disease. *Ann Neurol* 82: 311-4 (2017).
- [38] Escott-Price V, Myers AJ, Huentelman M, Shoai M, Hardy J. Polygenic risk score analysis of Alzheimer's disease in cases without APOE4 or APOE2 alleles. *J Prev Alzheimers Dis* 6: 16-9 (2019).
- [39] Andrews SJ, Fulton-Howard B, Goate A. Interpretation of risk loci from genome-wide association studies of Alzheimer's disease. *Lancet Neurol* 19: 326-35 (2020).
- [40] Altmann A, Scelsi MA, Shoai M, de Silva E, Aksman LM, Cash DM, *et al.* A comprehensive analysis of methods for assessing polygenic burden on Alzheimer's disease pathology and risk beyond APOE. *Brain Commun* 2:fcz047 (2020).
- [41] Maloney B, Ge YW, Petersen RC, Hardy J, Rogers JT, Pérez-Tur J, *et al.* Functional characterization of three single-nucleotide polymorphisms present in the human APOE promoter sequence: Differential effects in neuronal cells and on DNA-protein interactions. *Am J Med Genet B Neuropsychiatr Genet* 153B: 185-201 (2010).

Figure Legends

Figure 1. (A) Frequency distribution of the six APOE genotypes across the two diagnostic sub-cohorts. † The distribution of genotype frequencies in the two diagnostic sub-cohorts was compared statistically with the APOE genotypes of the diagnostic cohorts (n is indicated in brackets) of cognitively normal and MCI individuals described in Mattsson et al. (2018). (B) Venn diagram illustrating the distribution of datasets included in the study according to clinical diagnosis, amyloid positivity and APOE genotype.

Figure 2. Voxel-by-voxel associations found in the whole-brain map of grey-matter (colour intensity reflects t scores shown at the top). (A): Association between PHS and grey matter across the entire clinically-defined sub-cohort of MCI patients. Slices in the Montreal Neurological Institute space are: $y = -8$, $x = -27$. (B): Association between PHS and grey matter across the entire pathologically defined cohort of $A\beta+$ participants. Slices in the Montreal Neurological Institute space are: $y = -8$, $x = -27$. (C): Association between PHS and grey matter in the pathology-defined sub-cohort of $A\beta+$ participants with an APOE $\epsilon_3\epsilon_3$ genotype. Slices in the Montreal Neurological Institute space are: $x = 4$, $y = -38$, $x = 10$. All slices are in neurological visualisation.

Figure 3 Associations investigated in relation to memory and executive functions composite indices in clinically-defined (C,D) or pathology-defined (E,F) sub-cohorts stratified by APOE ϵ_4 status. The distribution of scores within each APOE genotype is illustrated at the top (A,B; the error bar indicates the standard error of the mean). The association between cognitive indices and PHS within each sub-cohort and genotype is reported within each graph: uncorrected slope (b) and Block 1 to Block 2 r^2 change. *: $p < 0.05$; **: $p < 0.01$.

Figure 4. The sensorimotor sub-regions from the Brainnetome atlas where a significant association between PHS and cortical volume was found in $A\beta+$ participants with an APOE $\epsilon_3\epsilon_3$ genotype. ‘Brodmann area 1/2/3: lower limb region’ is shown in yellow and ‘Brodmann area 1/2/3: trunk region’ is shown in blue. Slices in the Montreal Neurological Institute space are: $x = 12$ and $y = -34$ (A). The graphs showing the linear association between PHS and regional volumes in the two sub-cohorts is illustrated below (B). Exact statistics (slope and r^2 change) are indicated in text.

Figure 5. *Post hoc* analyses after stratification by gender. The inferential models showing a significant effect of PHS were reanalysed separately in male and female participants to verify whether trends differed between males and females. The models testing the association between PHS and grey matter in $A\beta+$ participants with an APOE $\epsilon_3\epsilon_3$ genotype (see Section 3.1.2) were thresholded at a cluster-forming threshold of 0.01 and showed similar results among males and females when compared visually with one another and with the whole sample [A]. Likewise, the association between PHS and ADNI-

MEM [B,D} and ADNI-EF [C] calculated based on the statistical findings described in Sections 3.2.1 and 3.2.2 was very similar in males and females.

Figure 1

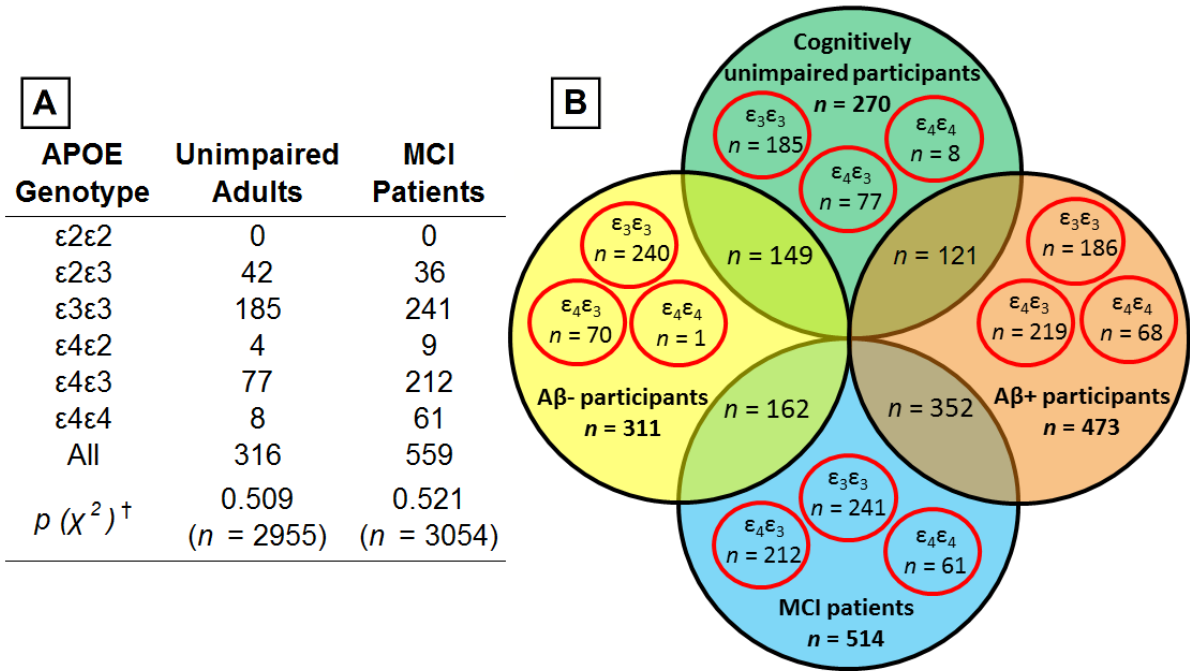


Figure 2

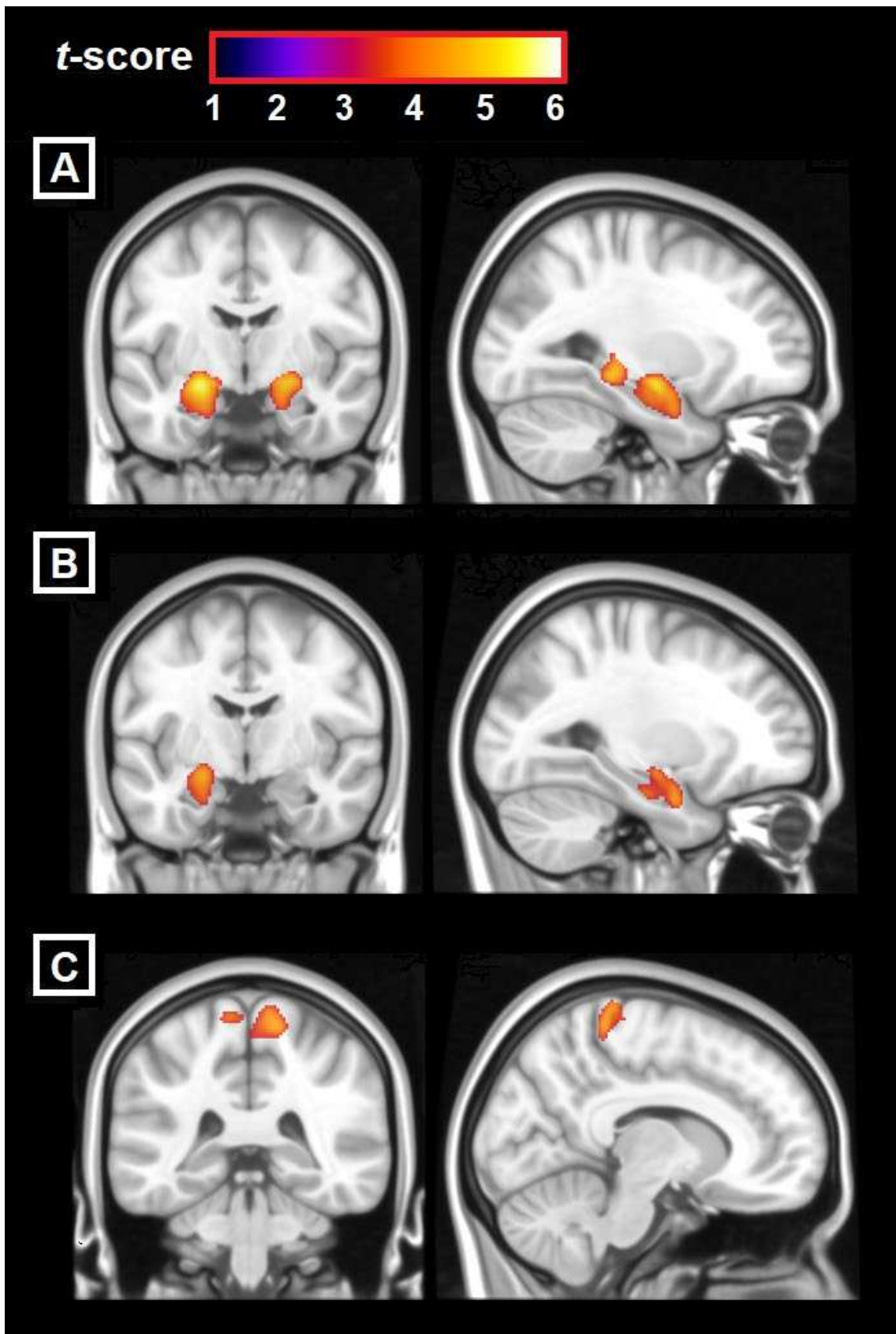


Figure 3

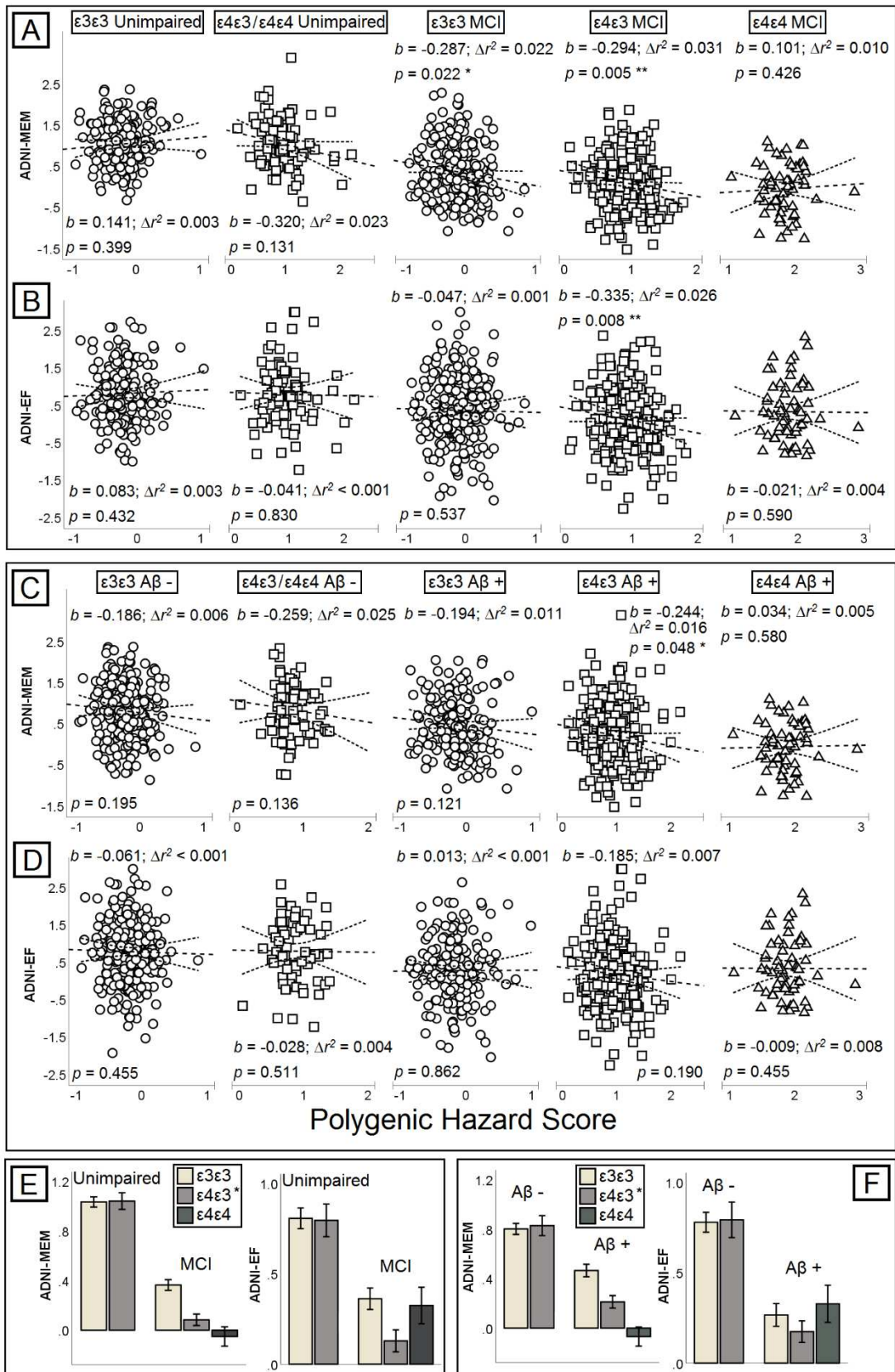


Figure 4

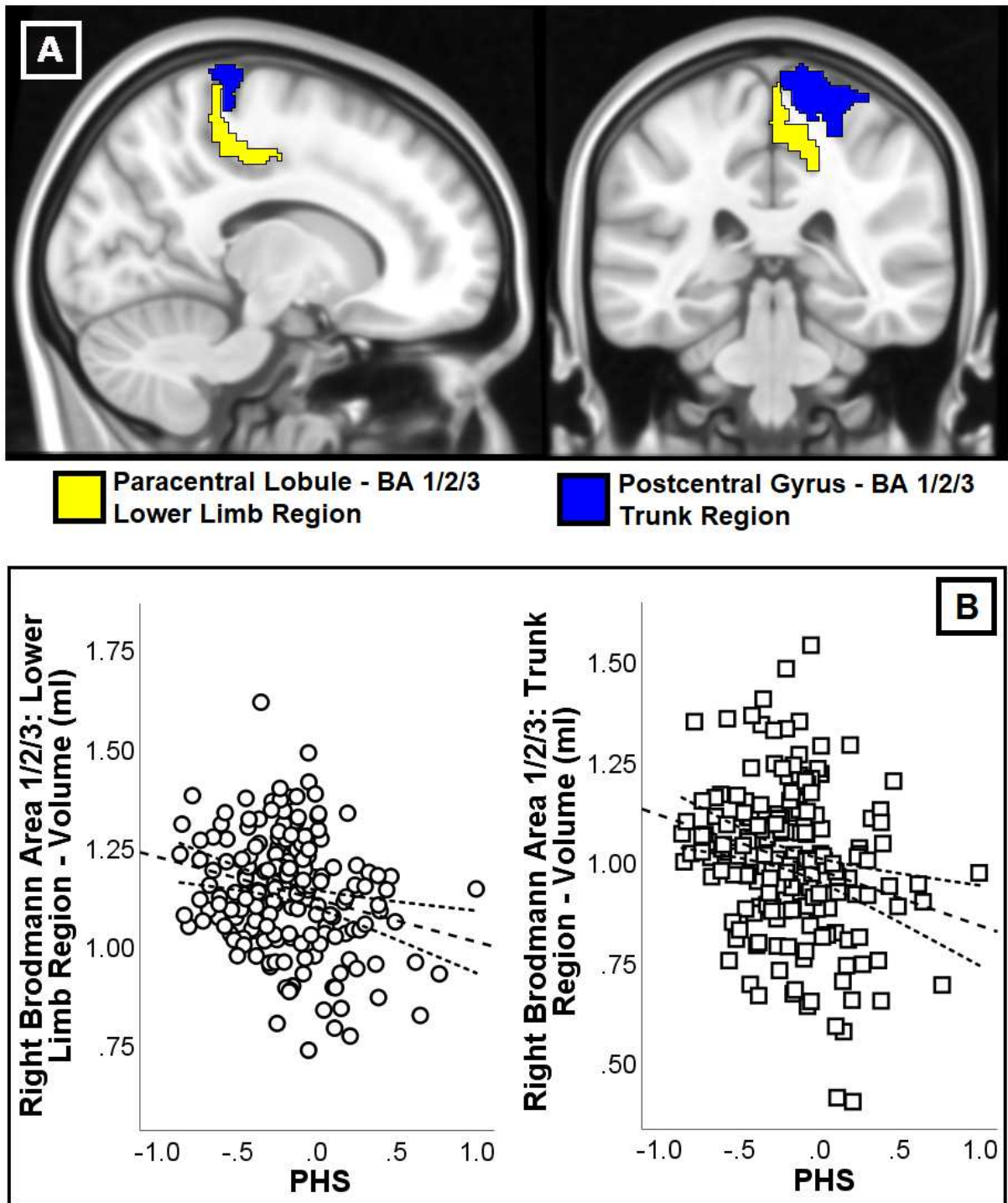


Figure 5

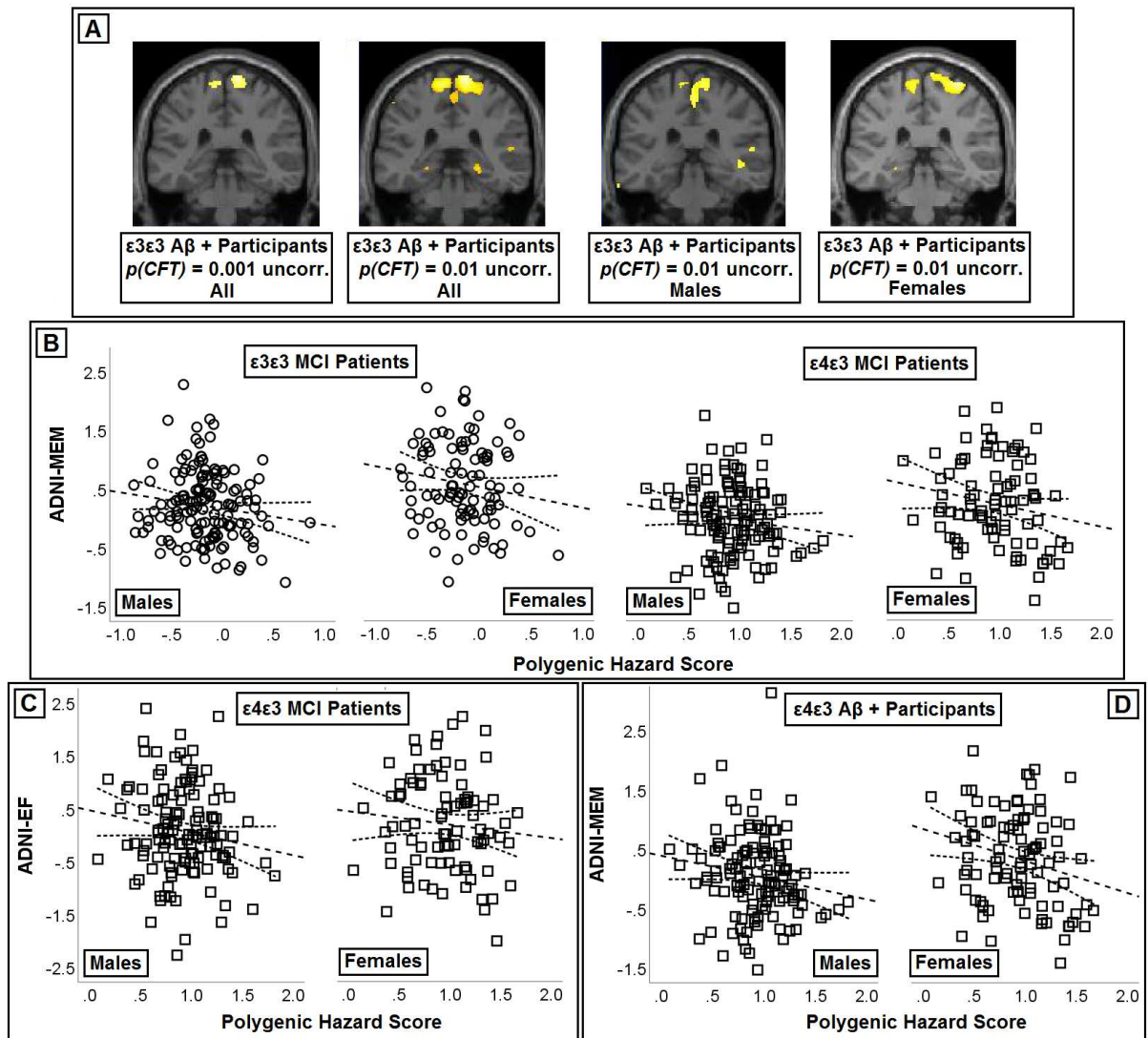


Table 1. Characterisation of the cohort divided according to clinical diagnosis

Variable	Unimpaired Adults	Unimpaired Adults	<i>p</i> (difference)	MCI Patients	MCI Patients	MCI Patients	<i>p</i> (difference) [†]
	£³£³ (<i>n</i> = 185)	£4£³/£4£³ (<i>n</i> = 85)		£³£³ (<i>n</i> = 241)	£4£³ (<i>n</i> = 212)	£4£4 (<i>n</i> = 61)	
<i>Demographic Characteristics</i>							
Gender (f/m)	93/92	41/36	0.661	98/143	87/125	23/38	0.893
Age (years)	74.85 (5.67)	72.86 (5.98)	0.009 **	73.37 (7.64)	71.87 (7.22)	69.84 (6.82)	0.022 *
Education (years)	16.50 (2.54)	16.47 (2.53)	0.923	16.40 (2.69)	15.97 (2.86)	16.26 (2.76)	0.822
MMSE	29.07 (1.19)	29.07 (1.09)	0.998	28.09 (1.67)	27.61 (1.84)	27.15 (1.86)	0.900
<i>Global Neurostructural Indices</i>							
Total Intracranial vol (ml)	1443.85 (143.42)	1432.14 (136.66)	0.528	1462.28 (138.53)	1460.52 (154.92)	1450.29 (154.88)	0.850
Grey Matter vol (ml)	610.35 (65.77)	620.17 (60.08)	0.243	601.75 (63.90)	608.53 (73.70)	614.33 (74.67)	0.357
White Matter vol (ml)	415.09 (55.18)	421.10 (53.77)	0.356	423.62 (54.55)	423.57 (58.22)	425.36 (55.69)	0.914
<i>Cerebrospinal Fluid Biomarkers</i>							
A β (pg/ml)	1445.49 (643.67)	983.04 (574.25)	< 0.001 ***	1222.20 (600.46)	858.27 (424.30)	572.38 (194.90)	< 0.001 ***

Total TAU (pg/ml)	239.63 (86.32)	266.76 (99.66)	0.023 *	251.27 (112.52)	315.91 (119.82)	351.97 (152.22)	0.030 *
Phosphorylated TAU (pg/ml)	21.67 (8.53)	25.51 (10.81)	0.005 **	23.60 (12.53)	31.23 (13.48)	35.39 (17.25)	0.003 **
<i>Genetic Characterisation</i>							
Polygenic Hazard Score	-0.21 (0.28)	0.92 (0.38)	< 0.001 ***	-0.16 (0.30)	0.93 (0.33)	1.88 (0.27)	< 0.001 ***
<i>Composite Neurocognitive Indices</i>							
Memory (ADNI-MEM)	1.03 (0.56)	1.04 (0.62)	0.931	0.36 (0.67)	0.08 (0.67)	-0.05 (0.61)	< 0.001 ***
Executive Functions (ADNI-EF)	0.81 (0.78)	0.80 (0.83)	0.914	0.36 (0.67)	0.13 (0.89)	0.32 (0.79)	0.019 *

† For each significant group difference MCI patients, Bonferroni-corrected *t*-tests were run at *post hoc* to compare the three genotypes. All groups differed from one another for A β and PHS. As for age, the sole difference indicated that $\epsilon_3\epsilon_3$ patients were significantly older than $\epsilon_4\epsilon_4$ patients. As for ADNI-MEM, total TAU and phosphorylated TAU, $\epsilon_3\epsilon_3$ differed from the other two groups. Finally, as for ADNI-EF, $\epsilon_3\epsilon_3$ patients had better performance than $\epsilon_4\epsilon_3$ patients. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Table 2. Characterisation of the cohort divided according to amyloid positivity

Variable	A β - $\epsilon 3\epsilon 3$ (n = 240)	A β - $\epsilon 4\epsilon 3$ (n = 70)	<i>p</i> (difference)	A β + $\epsilon 3\epsilon 3$ (n = 186)	A β + $\epsilon 4\epsilon 3$ (n = 227)	A β + $\epsilon 4\epsilon 4$ (n = 60)	<i>p</i> (difference) †
<i>Demographic Characteristics</i>							
Gender (f/m)	118/132	33/40	0.764	97/137	135/175	41/71	0.441
Age (years)	73.26 (6.95)	68.75 (7.46)	< 0.001 ***	74.98 (6.70)	73.20 (6.37)	70.06 (6.67)	< 0.001 ***
Education (years)	16.52 (2.48)	16.01 (2.86)	0.145	16.34 (2.81)	16.15 (2.76)	16.20 (2.74)	0.777
MMSE	28.78 (1.26)	28.61 (1.64)	0.450	28.18 (1.83)	27.85 (1.80)	27.13 (1.87)	< 0.001 ***
<i>Global Neurostructural Indices</i>							
Total Intracranial vol (ml)	1449.46 (146.05)	1455.42 (126.49)	0.757	1460.50 (133.86)	1451.47 (157.08)	1452.31 (155.37)	0.817
Grey Matter vol (ml)	614.37 (66.59)	638.30 (60.90)	0.007 **	594.02 (60.63)	603.71 (70.95)	614.60 (75.27)	0.094
White Matter vol (ml)	419.99 (56.36)	430.03 (53.75)	0.186	419.81 (53.16)	420.89 (57.78)	425.74 (56.09)	0.772
<i>Cerebrospinal Fluid Biomarkers</i>							
A β (pg/ml)	1752.91 (485.84)	1585.56 (466.51)	0.011 *	759.49 (209.98)	680.72 (185.91)	561.70 (177.65)	< 0.001 ***

Total TAU (pg/ml)	242.00 (87.06)	260.68 (94.09)	0.122	251.64 (118.61)	314.54 (119.81)	353.76 (152.86)	< 0.001 ***
Phosphorylated TAU (pg/ml)	21.32 (8.76)	23.24 (8.90)	0.110	24.62 (13.14)	31.55 (13.46)	35.63 (17.30)	< 0.001 ***
<i>Genetic Characterisation</i>							
Polygenic Hazard Score	-0.20 (0.27)	0.86 (0.27)	< 0.001 ***	-0.17 (0.32)	0.95 (0.36)	1.87 (0.27)	< 0.001 ***
<i>Composite Neurocognitive Indices</i>							
Memory (ADNI-MEM)	0.80 (0.69)	0.83 (0.67)	0.773	0.47 (0.69)	0.21 (0.76)	-0.07 (0.60)	< 0.001 ***
Executive Functions (ADNI-EF)	0.78 (0.85)	0.79 (0.82)	0.907	0.27 (0.86)	-0.17 (0.90)	0.33 (0.80)	0.365

† For each significant group-level difference in amyloid-positive participants, Bonferroni-corrected *t*-tests were run at *post hoc* to compare the three genotypes. All groups differed from one another for age, A β , PHS and ADNI-MEM. As for the Mini Mental State Examination, $\epsilon_4\epsilon_4$ participants scored significantly lower than the other two groups. As for Total and Phosphorylated TAU, $\epsilon_3\epsilon_3$ participants had significantly lower levels compared to the other two groups. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

