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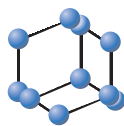
The Association between Polygenic Hazard and Markers of Alzheimer's Disease Following Stratification for APOE Genotype

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

Objective: Given the "genetic centrality" of the APOE gene, we tested whether this held true for both APOE-ε4 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 (Aβ+/Aβ-). Linear models were devised in each sub-cohort and for each APOE-ε4 status to test the association between PHS and memory, executive functioning and grey-matter volumetric maps.

Results: PHS predicted memory and executive functioning in ε4ε3 MCI patients, memory in ε3ε3 MCI patients, and memory in ε4ε3 Aβ$^+$ participants. PHS also predicted volume in sensorimotor regions in ε3ε3 Aβ$^+$ participants.

Conclusion: The link between polygenic hazard and neurocognitive variables varies depending on APOE-ε4 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, amyloid-beta (Aβ) 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 gene 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 [15-17].

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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 statistical comparison of observed and expected frequencies [21] are illustrated in Fig. (**1a**). To safeguard the statistical robustness of the analyses, genotypes including the ε_2 allele (having a relatively small sample size) were not analysed in this study. Therefore, only $\varepsilon_3\varepsilon_3$ and $\varepsilon_4\varepsilon_3/\varepsilon_4\varepsilon_4$ unimpaired

adults ($n = 270$), $\varepsilon_3\varepsilon_3$, $\varepsilon_4\varepsilon_3$ and $\varepsilon_4\varepsilon_4$ MCI patients ($n = 514$) were thus retained for analysis (total $n = 784$ of the original 875). While $\varepsilon_4\varepsilon_4$ MCI patients were analysed as a separate group, $\varepsilon_4\varepsilon_3$ and $\varepsilon_4\varepsilon_4$ 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 did self-identify as being of white racial background.

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 $p < 10\text{-}5$) 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) within 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 were associated 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
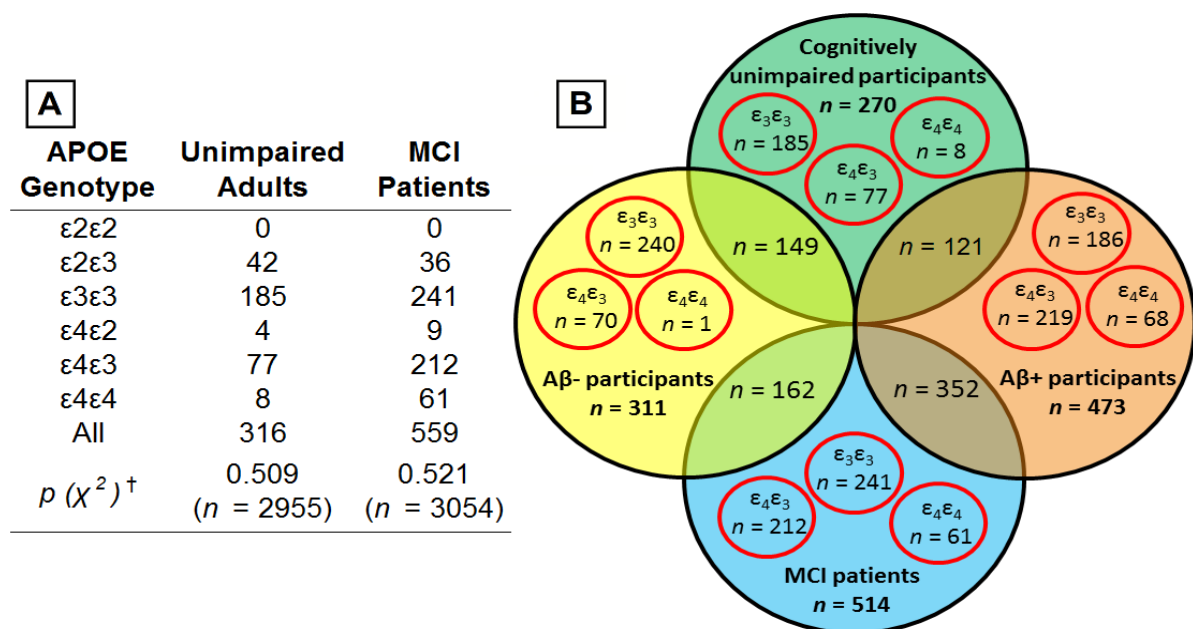
A

APOE Genotype	Unimpaired Adults	MCI Patients
ε2ε2	0	0
ε2ε3	42	36
ε3ε3	185	241
ε4ε2	4	9
ε4ε3	77	212
ε4ε4	8	61
All	316	559
$p\,(x^2)^\dagger$	0.509 (n = 2955)	0.521 (n = 3054)

B

Cognitively unimpaired participants n = 270
$\varepsilon_3\varepsilon_3$ n = 185
$\varepsilon_4\varepsilon_4$ n = 8
$\varepsilon_4\varepsilon_3$ n = 77
$\varepsilon_3\varepsilon_3$ n = 240
n = 149
n = 121
$\varepsilon_3\varepsilon_3$ n = 186
$\varepsilon_4\varepsilon_3$ n = 70
$\varepsilon_4\varepsilon_4$ n = 1
$\varepsilon_4\varepsilon_3$ n = 219
$\varepsilon_4\varepsilon_4$ n = 68
Aβ- participants n = 311
n = 162
n = 352
Aβ+ participants n = 473
$\varepsilon_3\varepsilon_3$ n = 241
$\varepsilon_4\varepsilon_3$ n = 212
$\varepsilon_4\varepsilon_4$ n = 61
MCI patients n = 514

Fig. (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. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

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 $A\beta_{1-42}$. The presence of Aβ pathology is a necessary requisite to make a diagnosis of AD, 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 (Aβ+) and amyloid-negative (Aβ-). A complete characterisation of the cohort split into Aβ+ and Aβ- participants is included in Table **2**. Of the 270 unimpaired adults 121 were Aβ+ and 149 were Aβ-. Of the 514 MCI patients, 352 were Aβ+ and 162 were Aβ-. There was only one $\varepsilon_4\varepsilon_4$ Aβ- participant who was thus excluded from the analyses of pathology-defined sub-cohorts. Fig. (**1b**) illustrates the distribution of Aβ- and Aβ+ MCI patients and unimpaired participants and their APOE genotype.

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 the MRI and 11 days from the 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 Dis-

ease Assessment Scale [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 $A\beta_{1-42}$ 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 a predictor. The 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 ISBM SPSS Statistics 23 and hierarchical regression models. Age, educational attainment (in years) and gender were included as part of the 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.,* MPRAGE or IG-SPGR). A cluster-forming threshold of $p < 0.001$ was applied and clusters were

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

Variable	Unimpaired Adults $\varepsilon_3\varepsilon_3$ (n = 185)	Unimpaired Adults $\varepsilon_4\varepsilon_3/\varepsilon_4\varepsilon_3$ (n = 85)	p(difference)	MCI Patients $\varepsilon_3\varepsilon_3$ (n = 241)	MCI Patients $\varepsilon_4\varepsilon_3$ (n = 212)	MCI Patients $\varepsilon_4\varepsilon_4$ (n = 61)	p (difference) [†]
Demographic Characteristics							
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
Global Neurostructural Indices							
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
Cerebrospinal Fluid Biomarkers							
Aβ (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 **
Genetic Characterisation							
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 ***
Composite Neurocognitive Indices							
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 *

Note: [†]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 Aβ and PHS. As for age, the sole difference indicated that $\varepsilon_3\varepsilon_3$ patients were significantly older than $\varepsilon_4\varepsilon_4$ patients. As for ADNI-MEM, total TAU and phosphorylated TAU, $\varepsilon_3\varepsilon_3$ differed from the other two groups. Finally, as for ADNI-EF, $\varepsilon_3\varepsilon_3$ patients had better performance than $\varepsilon_4\varepsilon_3$ patients. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

considered significant when surviving Family-Wise Error correction. All peak coordinates were converted into 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 and Aβ+ participants. The presence of the ε_4 allele was also associated with lower Aβ and higher TAU levels in cognitively unimpaired participants, MCI patients and Aβ+ participants (in these two latter sub-cohorts a dose-dependent effect was also observed). Lower Aβ levels were also observed in Aβ- ε_4 carriers compared to Aβ-$\varepsilon_3\varepsilon_3$ participants. Differences among APOE

genotypes were also found for the MMSE and ADNI-MEM in Aβ+ 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 (Fig. **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.

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

Variable	Aβ- $\varepsilon_3\varepsilon_3$ (n = 240)	Aβ- $\varepsilon_4\varepsilon_3$ (n = 70)	p(difference)	Aβ+ $\varepsilon_3\varepsilon_3$ (n = 186)	Aβ+ $\varepsilon_4\varepsilon_3$ (n = 227)	Aβ+ $\varepsilon_4\varepsilon_4$ (n = 60)	p(difference) [†]
Demographic Characteristics							
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 ***
Global Neurostructural Indices							
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
Cerebrospinal Fluid Biomarkers							
Aβ (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 ***
Genetic Characterisation							
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 ***
Composite Neurocognitive Indices							
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

Note: [†]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, Aβ, PHS and ADNI-MEM. As for the Mini-Mental State Examination, $\varepsilon_4\varepsilon_4$ participants scored significantly lower than the other two groups. As for Total and Phosphorylated TAU, $\varepsilon_3\varepsilon_3$ participants had significantly lower levels compared to the other two groups. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

3.1.2. Pathology-defined Sub-cohorts

In the entire sub-cohort of Aβ+ participants (*n* = 473) a significant negative association between PHS and grey matter volumes was found in the left anterior hippocampus and amygdala (Fig. **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 $\varepsilon_3\varepsilon_3$ participants. This was located in the primary sensorimotor cortex in a bilateral vertex cluster located in the proximity of the inter-hemispheric sulcus and overlapped with the non-significant trend found among $\varepsilon_3\varepsilon_3$ MCI patients (Fig. **2c**). No significant association was found in the sub-cohort of Aβ- 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, $p = 3.69^{-13}$) 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 $\varepsilon_3\varepsilon_3$ patients ($r^2 = 0.180$, r^2 *change* = 0.022, $p = 0.012$) and in $\varepsilon_4\varepsilon_3$ patients ($r^2 = 0.209$, r^2 *change* = 0.031, $p = 0.005$). In $\varepsilon_4\varepsilon_3$ 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 cogni-
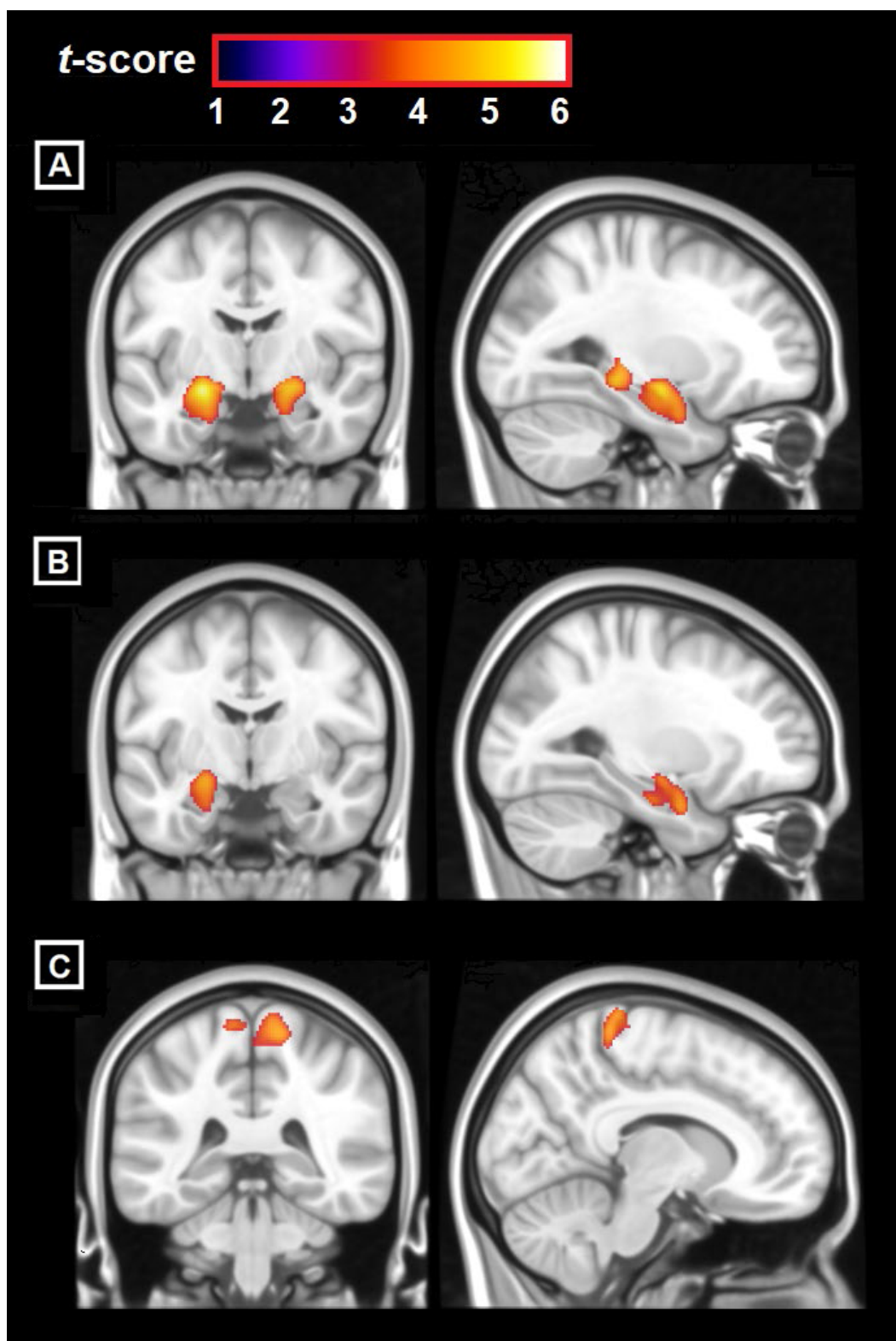
Fig. (2). Voxel-by-voxel associations found in the whole-brain map of grey matter (colour intensity reflects *t* scores shown at the top). (**A**): Association between PHS and grey matter across the entire clinically-defined sub-cohort of MCI patients. Slices in the Montreal Neurological Institute space are: y = -8, x =-27. (**B**): Association between PHS and grey matter across the entire pathology-defined cohort of Aβ+ 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 Aβ+ participants with an APOE ε₃ε₃ genotype. Slices in the Montreal Neurological Institute space are: x = 4, y = -38, x = 10. All slices are in neurological visualisation. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

tion. Similarly, no results emerged after stratifying for APOE. These findings are illustrated in Fig. **3**. (**3a, c, d**).

3.2.2. Pathology-defined Sub-cohorts

In the entire sub-cohort of Aβ+ participants (*n* = 473), a negative association was found between PHS and memory (r^2 = 0.146, r^2 *change* = 0.069, *p* = 1.53^{-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 $\varepsilon_4\varepsilon_3$ Aβ+ participants (r^2 = 0.094, r^2 *change* = 0.016, *p* = 0.048). No significant associations were found in the sub-cohort of Aβ- adults. All these findings are illustrated in Fig. (**3b, e, f**).

3.3. Post hoc Analysis of the Sensorimotor Cortex

To describe the association between PHS and the size of the 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 $\varepsilon_3\varepsilon_3$ Aβ+ participants. A Bonferroni-corrected *p* < 0.00208 (correcting for 24 independent contrasts) was used as a 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 Fig. (**4**).

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 (Fig. **5**).

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 Aβ- participants were analysed. Conversely, in MCI patients and, in an analogous way, in Aβ+ 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 $\varepsilon_3\varepsilon_3$ Aβ+ participants. This was mirrored by a qualitatively similar non-significant trend among $\varepsilon_3\varepsilon_3$ 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. 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 Aβ 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 $\varepsilon_4\varepsilon_3$ MCI patients and with memory in both $\varepsilon_4\varepsilon_3$ and $\varepsilon_3\varepsilon_3$ MCI patients. When participants were split according to pathological criteria, PHS was solely associated with memory in $\varepsilon_4\varepsilon_3$ Aβ+ 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 $\varepsilon_4\varepsilon_4$ is mainly due to a lack of statistical power. In these groups, in fact, the sample size was considerably smaller ($\varepsilon_4\varepsilon_4$ MCI patients: *n* = 61; $\varepsilon_4\varepsilon_4$ Aβ+ participants: *n* = 60) when compared to $\varepsilon_4\varepsilon_3$ MCI patients (*n* = 212) or $\varepsilon_4\varepsilon_3$ Aβ+ 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 the role of APOE in this interaction. Furthermore, the pattern of findings was similar in both males and females as emerged from
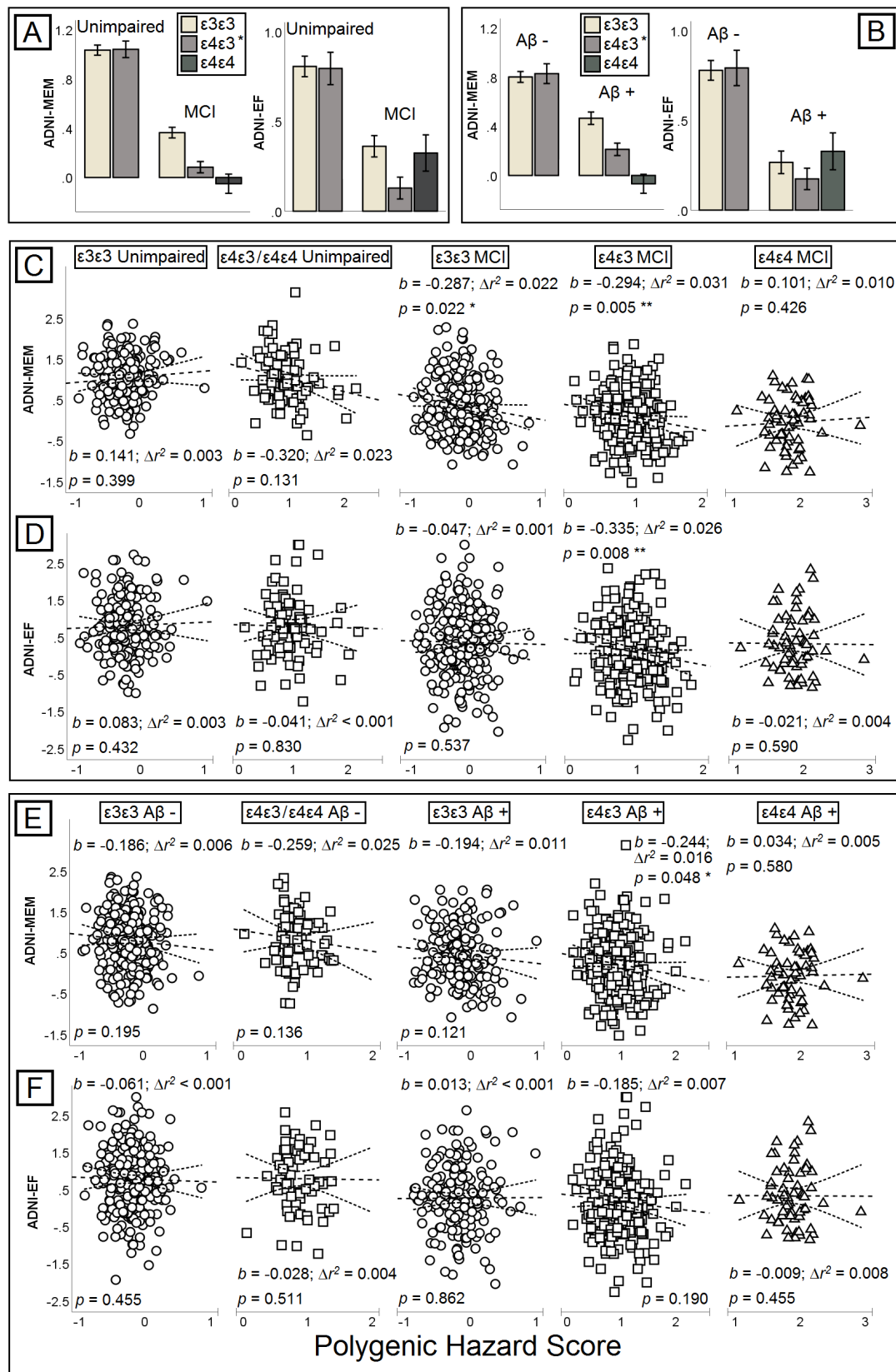
Fig. (3). Associations investigated in relation to memory and executive functions composite indices in clinically-defined (**A, B**) or pathology-defined (**C, D**) sub-cohorts stratified by APOE ε 4 status. The distribution of scores within each APOE genotype is illustrated at the bottom (**E, F**; 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. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).
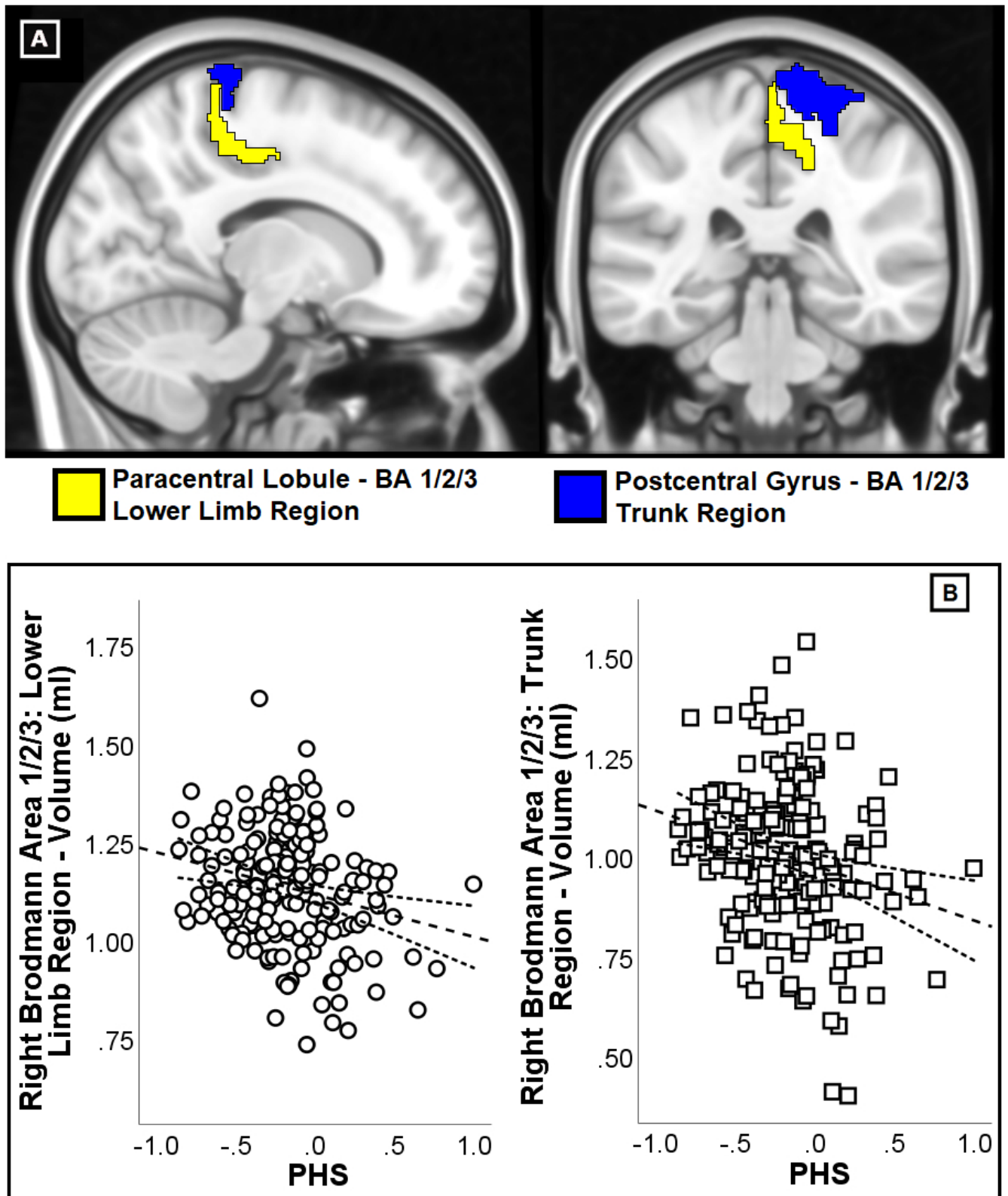
Fig. (4). The sensorimotor sub-regions from the Brainnetome atlas where a significant association between PHS and cortical volume was found in Aβ+ participants with an APOE ε₃ε₃ genotype. 'Brodmann area 1/2/3: lower limb region' is shown in yellow and 'Brodmann area 1/2/3: trunk region' is shown in blue. 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 the text. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*
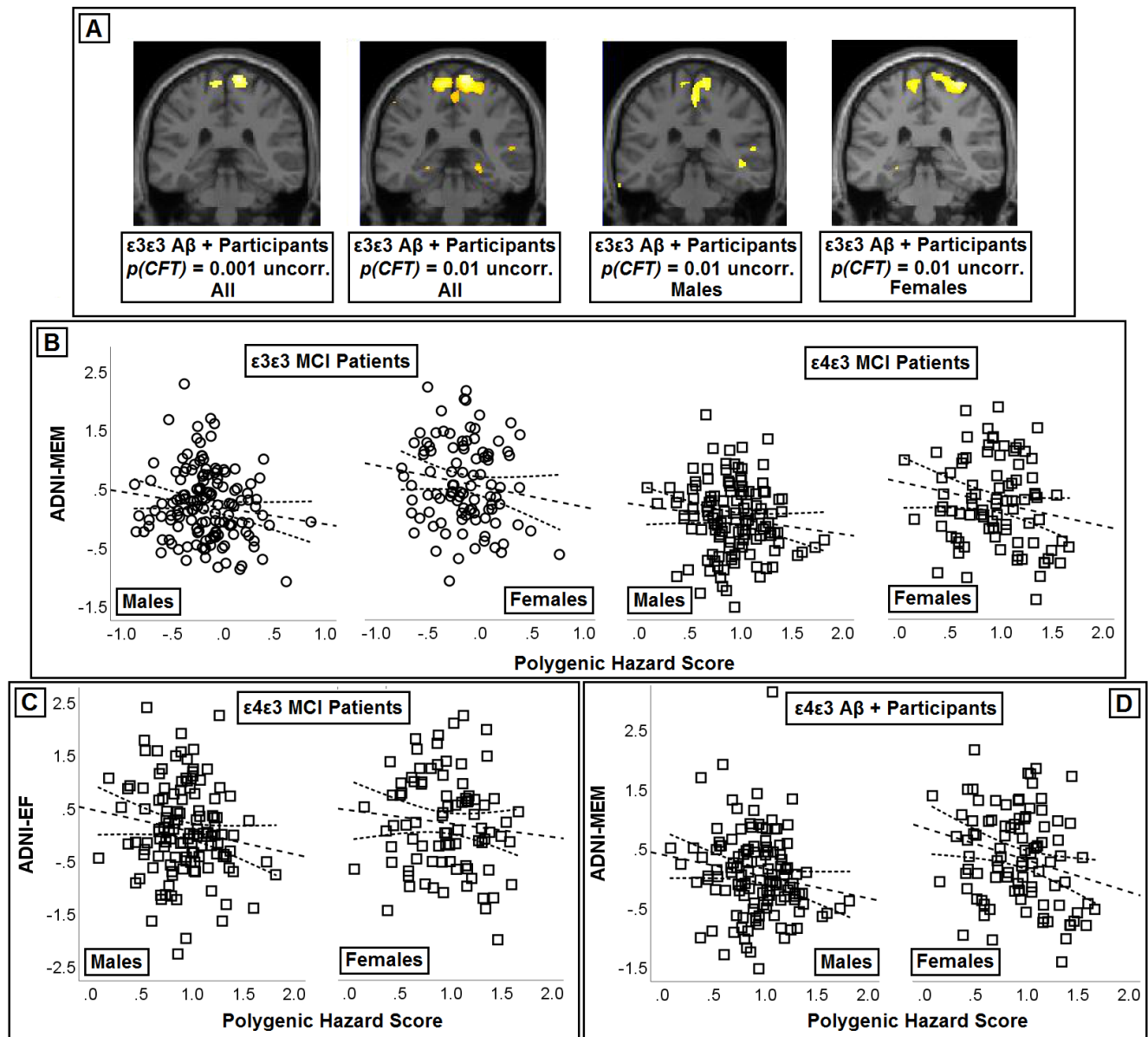
Fig. (5). *Post hoc* analyses after stratification by gender. The inferential models showing a significant effect of PHS were reanalysed separately in male and female participants to verify whether trends differed between males and females. The models testing the association between PHS and grey matter in Aβ+ participants with an APOE $\varepsilon_3\varepsilon_3$ genotype (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. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

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 pathology-confirmed cases with an APOE $\varepsilon_3\varepsilon_3$ genotype [38].

Given the exploratory nature of this study, a number of limitations are noticed. Firstly, 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 has been 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 are 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.

CONCLUSION

In conclusion, the PHS defined by Desikan and coworkers [18] predicts mediotemporal volumes and cognitive performance in $A\beta^+$/MCI adults. In these sub-cohorts, PHS robustly predicts memory performance in the presence of an APOE $\varepsilon_4\varepsilon_3$ genotype and volumetric properties of the sensorimotor cortex in the presence of an APOE $\varepsilon_3\varepsilon_3$ genotype.

LIST OF ABBREVIATIONS

Aβ =   Amyloid Beta

AD   =   Alzheimer's disease

ADNI =   Alzheimer's Disease Neuroimaging Initative

MCI   =   Mild cognitive impairment

MRI   =   Magnetic resonance imaging

PHS   =   Polygenic hazard score

SNP   =   Single nucleotide polymorphism

AUTHOR'S 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.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocols were approved by each participating site's institutional review board.

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. All human research procedures were followed in accordance with the guidelines of Helsinki.

CONSENT FOR PUBLICATION

All ADNI participants provided written informed consent.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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