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## FEATURED ARTICLE

# Menopause hormone therapy significantly alters pathophysiological biomarkers of Alzheimer's disease

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[Correction added on 21 September 2022, after first online publication: The order of the first and last names of the authors had been reversed and this has been corrected in this version.]

## Abstract

**Introduction:** This increasing body of literature indicates that menopause hormonal replacement therapy (MHT) may substantially mitigate the risk of developing late-life cognitive decline due to progressive Alzheimer's disease (AD) pathophysiology. For the first time, we investigated the question whether MHT impacts AD biomarker-informed pathophysiological dynamics in de-novo diagnosed menopausal women.

**Methods:** We analyzed baseline and longitudinal differences between MHT-taking and -not women in terms of concentrations of core pathophysiological AD plasma biomarkers, validated in symptomatic and cognitively healthy individuals, including biomarkers of (1) the amyloid- $\beta$  ( $A\beta$ ) pathway, (2) tau pathophysiology, (3) neuronal loss, and (4) axonal damage and neurodegeneration.

**Results:** We report a prominent and significant treatment response at the  $A\beta$  pathway biomarker level. Women at genetic risk for AD (APOE e4 allele carriers) have particularly shown favorable results from treatment.

**Discussion:** To our knowledge, we present first prospective clinical evidence on effects of MHT on AD pathophysiology during menopause.

Andrea Vergallo and Pablo Lemercier contributed equally to this work.

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## 1 | INTRODUCTION

Decades of failing late-stage neuropharmacological trials and highly variable therapeutic outcomes in current healthcare suggest that merely adjusting statistical regression models for sex may not be sufficient when it comes to an appropriate understanding of the question why sex-based biological differences may account for substantially different risk rates of disease.<sup>1-3</sup> Evidence clearly indicates that there is an increased age-independent prevalence of Alzheimer's disease (AD) in women compared to men (average odds ratio is 1.6) and that it is consistent with sexual dimorphism in AD pathophysiology, spanning molecular pathways of brain proteinopathies, neurodegeneration, neuroinflammation, and large-scale high-order organization of the brain.<sup>4-12</sup> In addition, women who experience early menopause, either naturally or iatrogenic, represent the highest risk cluster within the menopause population of late-life cognitive decline and dementia.<sup>13,14</sup>

The higher vulnerability of perimenopausal (transition phase) and early-menopause women than men has been established by large-scale neuropsychological studies, post-mortem investigations, biomarker-based studies, data-driven multi-variate analysis, and experimental models of aging and AD. Multi-modal imaging studies corroborate the evidence of sexual dimorphism in AD biology and specifically indicate a positive association of (peri)menopause status with AD proteinopathies, neuronal loss and neurodegeneration, and cognitive outcomes.<sup>6,15-17</sup> In all these inter-sex comparison studies, the reported sexual dimorphism related to AD risk is clearly not significantly impacted by age, generating and supporting the hypothesis that midlife endocrinological, hormonal change patterns-rather than aging-related metabolic pathways-may drive/trigger/facilitate AD related pathophysiological events. This evidence-driven model is consistent with the established effects of sexual steroids on physiological neurodevelopment, which also account for the sexual dimorphism of global brain volume, regional gray- and white-matter density, shape, and volume of regional anatomical structures.<sup>4,18,19</sup>

Systematic and single-center studies provide indirect evidence of an endocrine-mediated AD risk in menopause since the chronic use of menopausal hormone therapy (MHT)-a therapeutic approach to mitigate menopause associated signs, symptoms, and complications-reduces the incidence of AD.<sup>2,5,20</sup>

The timing of MHT commencement is an additional significant factor concerning the AD-risk and long-term clinical outcomes.<sup>2,5,20</sup> Longer exposure to MHT (estrogen/progestogen) may trigger and facilitate neuroprotective pathways, as suggested by the calculated risk reduction of AD equal to 0.5% for every extra-month of steroids intake.<sup>21,22</sup>

While initiating MHT early during menopause seems significantly protective against development of AD, a delayed therapeutic program may less likely be effective.<sup>5,22,23</sup> Moreover, sub-analysis of the large-scale randomized Women's Health Initiative (WHI) clinical trial show that delayed MHT may even enhance the risk of vascular incidents and worsen an established dementia syndrome.<sup>23-25</sup>

In the present university-based expert outpatient menopause clinic prospective study, we recruited cognitively healthy women who had

## RESEARCH IN CONTEXT

- 1. Systematic Review:** Women in menopause have a 1,67 higher risk to develop Alzheimer's disease. Menopausal hormone treatment (MHT) and related timing of initiation significantly reduces the risk of AD in this population.
- 2. Interpretation:** We report a prominent MHT response at the A $\beta$  pathway biomarker level, more pronounced in APOE  $\epsilon$ 4 allele carrier women. This is the first prospective clinical evidence on the effects of MHT on Alzheimer's disease pathophysiology during menopause.
- 3. Future Directions:** If corroborated, these data hold the potential to support menopause- Alzheimer's disease joint screening for timely and precision medicine interventions.

recently transitioned to menopause and met the indication criteria and were prescribed MHT (i.e., natural estrogens and progesterone).

We assessed the baseline and longitudinal concentrations of validated core pathophysiological AD plasma biomarkers, including biomarkers of (1) the amyloid- $\beta$  (A $\beta$ ) pathway, (2) tau pathophysiology, (3) neuronal loss, and (4) axonal damage and neurodegeneration.

The A $\beta$  pathway, currently known as the earliest biochemical and pathophysiological cycle occurring in the AD biological continuum by<sup>26</sup> analyzing A $\beta$  peptide 42 over A $\beta$  peptide 40 (A $\beta$ -42/A $\beta$ -40 ratio)<sup>27</sup> and protein concentrations of the  $\beta$ -site amyloid precursor protein cleaving enzyme 1 (also known as  $\beta$ -secretase 1 or BACE1).<sup>28,29</sup>

Plasma A $\beta$ 1-42/A $\beta$ 1-40 ratio has close concordance with amyloid-PET status.<sup>27</sup> BACE1 plasma concentration is hypothesized to reflect levels of gene expression of BACE1, and are associated with brain A $\beta$  concentrations, axonal damage, and neurodegeneration biomarkers, volumetric loss in the cholinergic circuitry, and cognitive scores.<sup>12,28,30,31</sup>

We further implemented tau protein phosphorylated at threonine 231 (p-tau<sub>231</sub>), a particularly early indicator of AD related tau protein phosphorylation and neurofibrillary pathology,<sup>32</sup> rate of hippocampal atrophy<sup>33</sup> and rate of regional brain atrophy, longitudinal patterns of brain networks functional decline, and indirectly reflects A $\beta$  plaque rates of deposition when combined with A $\beta$ 1-42 in a composite value (A $\beta$ 1-42/p-tau<sub>231</sub> ratio).<sup>34,35</sup> Plasma NfL and to a lesser extent total tau (t-tau) are surrogate biomarkers of axonal damage, neurodegeneration, and neuronal loss, respectively.<sup>28,36</sup>

The primary objective of the present study was to investigate whether women receiving MHT show significantly different longitudinal changes of core pathophysiological AD plasma biomarkers compared with women who opted out of MHT. The second objective was to address the question whether age and the presence of at least one APOE  $\epsilon$ 4 allele may influence biomarker expression and trajectories and partially explain potential significant inter-group differences (treated vs. untreated menopausal women).

## 2 | MATERIALS AND METHODS

Women consulting the University-based academic expert Breast and Menopause Clinic, at the Ghent University Hospital and the Coupure Menopause Clinic in Ghent were proposed to participate in the present prospective, interventional study. The study was approved by the ethical board of the University of Ghent (Belgian registration number: B670201835724/clinicaltrials.gov number is NCT04312399). The core eligibility criteria were the established diagnosis of menopause, the condition of drug-naïve for MHT, no medical contraindications to MHT, the preservation of normal global cognition and the absence of risk factors for acute onset of cognitive impairment (see section "Global cognition").

Additional inclusion criteria were: normal blood pressure at study baseline, normal thyroid function parameters; normal values of urea, glucose, electrolytes, ALAT, homocysteine, vitamin B12, vitamin B9, cholesterol, and triglycerides. Moreover APOE genotyping was performed. The use of pharmacological treatments such as antihypertensives, thyroid medication, lipid/cholesterol lowering medication, antidiabetic drugs, psychotropic medications were exclusion criteria to participate in the study. This was done to exclude comorbidity that could influence the risk of AD development. Body mass index (BMI) and adherence to the Mediterranean diet were also quantified and recorded.

### 2.1 | Treatment assignment

Given the multi-dimensional (medical, psychological, and social) burden of menopause on the individual experiencing it,<sup>37</sup> we opted to give the women the possibility to decide which treatment/control arm to be allocated in, that is, whether starting an MHT program.

MHT was prescribed in accordance with the guidelines of international societies IMS, EMAS, NAMS, and NICE.<sup>38</sup> Women with a history of hysterectomy or in women with a levonorgestrel-releasing intrauterine system (LNG-IUS),<sup>39</sup> natural oral or transdermal estrogen was prescribed. All other women were prescribed a combination of estrogen and natural progesterone. Natural progesterone was used, since it offers a well-documented protection for the endometrium,<sup>40</sup> is safe for the breast,<sup>41</sup> and does not interfere with the beneficial effects of estrogens on the serum lipid profile.<sup>42</sup>

Oral or transdermal route of estrogen supplementation was prescribed according to the woman's preference. If a woman opted for an oral estrogen, estradiol valerate 1 mg (Progynova 1 mg once daily) was prescribed. If a woman opted for a transdermal intake of hormones, an estrogen gel (Oestrogel two doses per day) or spray (Lenzetto two spray applications per day) was used. If women had menopausal complaints, but after extensive counselling about the advantages and risks of MHT, opted not to take hormonal therapy, they were asked to participate in this trial as controls. Women without menopausal complaints, who met inclusion criteria for the study, were equally asked to participate in this trial as controls.

Hereafter, women who underwent a therapeutic MHT program are identified in the "MHT" arm, whereas women who opted out for any MHT treatment are identified in the "control" arm.

### 2.2 | Global cognition

Medical exclusion criteria were a diagnosis of mild cognitive impairment/dementia, any type of disease potentially affecting cognition in the short- or long-term, including severe cardiac comorbidities (severe congestive heart failure -NYHA III-IV-; severe arrhythmias),<sup>43</sup> severe psychiatric comorbidities, alcohol and/or substances abuse (excluding nicotine) according to the Diagnostic and Statistical Manual of Mental Disorders-fifth edition (DSM-V),<sup>44</sup> uncompensated hepatic/renal/metabolic, endocrine disorders. These clinical data were based on information provided by patient and caregiver, and all the available medical records.

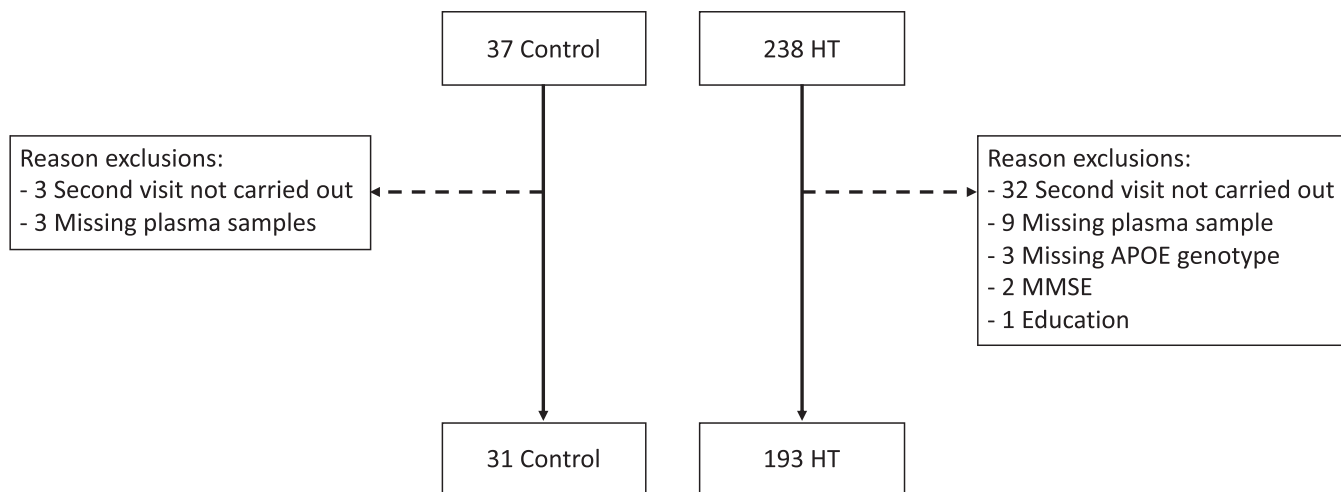
All individuals had achieved the high-school education level. The neuropsychometric-based baseline inclusion criterion was a Mini Mental State Examination (MMSE), a measure of global cognition,<sup>45</sup> with a score below 25 out of 30 (raw values). This cutoff was used to rule out individuals who may exhibit objective cognitive impairment in one or more cognitive domains if tested with an extensive psychometric battery.<sup>46</sup> We did not assess MMSE at V2 since we do not expect significant changes in the global cognition status over the 6-month follow-up.

### 2.3 | Blood collection, pre-analytical processing, and AD plasma biomarkers assessment: Analytical protocols

Blood withdrawal was performed at V1 and V2 following the same pre-analytical protocol. BD vacutainers n#368861 (K2 EDTA as anticoagulant) were used for blood collection. All blood samples were handled in a standardized way and centrifuged at 1200 × g during 15 min. After that, the plasma was extracted, aliquoted per 1 ml, and frozen at -80°C within 1 h. Aliquoting was done in Sarstedt PP tube 1.5 ml (Cat No 72.694.105) using PP tips Greiner Cat No 741050. Six months later, participating women had another blood draw. Plasma and whole blood were subsequently stored at -80°C. The subsequent neurological plasma parameters were all determined in one run to exclude inter-assay variation.

Plasma t-tau and NfL concentrations were measured using the Single molecule array (Simoa) Tau2.0 and the NF-Light Advantage assays, respectively (Quanterix, Billerica, MA). The p-tau231 concentration was measured using an in-house Simoa assay on an HD-X Analyzer (Quanterix, Billerica, MA), as previously described.<sup>34</sup>

Plasma BACE1, A $\beta$ 1-42, and A $\beta$ 1-40 were measured at ADx NeuroSciences, Ghent, Belgium. The previously described amyblood assay<sup>47</sup> was used to measure plasma A $\beta$ 1-42 and A $\beta$ 1-40 was used to measure plasma A $\beta$ 1-42 and A $\beta$ 1-40.



**FIGURE 1** Study design overview of the study inclusion / exclusion flowchart

See Supplementary Methods for more details about the AD plasma biomarkers assessment.

## 2.4 | APOE genotyping

APOE variants were genotyped by sequencing at the Genetic Service Facility (GSF, [www.vibgeneticservicefacility.be](http://www.vibgeneticservicefacility.be)) of the VIB Department of Molecular Genetics, in line with previous works carried out by the same group.<sup>48</sup>

## 3 | STATISTICAL ANALYSIS

The statistical analyses were conducted on the 224 participants who had no missing data on plasma biomarkers collected at the two time-points, education, MMSE, and APOE genotype (see study flow-chart illustrated in Figure 1). The Gaussian distribution and homoskedasticity were checked visually through histograms and density plots with normal probability density function curve overlaid as well as Q-Q plots. To perform group-wise comparisons for categorical and continuous variable, we used Pearson's chi-squared test and Student's *t*-test, respectively.

Outliers were visually inspected for each single biomarker and not for the composite ratio values.

We used linear models (LM) to assess the potential difference of plasma biomarkers concentrations at V1 (baseline) between the two study groups (treatment vs. non-treatment). Age and APOE  $\epsilon 4$  carrier status (presence vs. absence) were included as covariates, to rule out potential confounding effect and to better weight in on treatment effect size in case of significant results. For longitudinal analysis, we calculated the annual rate of change (ARC) as the difference in plasma concentration between the two visits (V1 and V2) divided by the delay in years.<sup>49</sup>

$$\text{ARC} = \frac{\text{Concentration at V1} - \text{Concentration at V2}}{\text{Delay in year}} \quad (1)$$

We used LM to test whether the two study groups may have a different ARC. Age and APOE  $\epsilon 4$  status were set as covariates.

To follow, we performed an additional subgroup analysis to examine whether APOE  $\epsilon 4$  status impacts the treatment effect on plasma biomarkers. We used a LM with an interaction term between the group (MHT arm or control arm) and APOE  $\epsilon 4$  status (APOE  $\epsilon 4$  status\*group). Eventually, we performed post-hoc analysis to breakdown significant results and capture the difference between each pair of subgroups created by the interaction term.

For all models, we reported regression coefficients ( $\beta$ ) and standard error (SE). In addition, effect sizes were estimated using partial Cohen's  $f^2$ , which represent the amount of variance of the response variables (outcome) that is explained by an explanatory variable (predictor) after accounting for other predictors in the regression model.<sup>50</sup> All tests were two-sided and *p*-values,  $p < .05$  were considered significant in all statistical elaboration. Influential data points on model outputs were inspected through Cook's distance. When the distance is equal or higher than 1, models were refitted with a robust regression to consider the presence of influential data point.<sup>51</sup> Since the plasma biomarker investigated track partially independent molecular pathways, we ran separate LM for each biomarker and applied no correction of *p*.

Statistical analyses were performed using R software, version 4.0.5. Robust linear models, post-hoc analyses, and Cohen's  $f^2$  were carried with the libraries "MASS", "emmeans", and "effectsize", respectively, all available at <http://cran.r-project.org/web/packages>.

## 4 | RESULTS

### 4.1 | Group-wise comparison for socio-demographic and clinical features

V1 socio-demographic features, clinical measures and scores, APOE  $\epsilon 4$  status are reported in Table 1. In the 224 women participating in this study 67 (29.9%) were genotyped as carriers of at least one APOE  $\epsilon 4$  allele. The percentage of APOE  $\epsilon 4$  carriers was not significantly

**TABLE 1** Sociodemographic variables, MMSE, and APOE  $\epsilon$ 4 status at V1

	Control (n = 31)	MHT (n = 193)	Total (n = 224)	p value
Age, in years				
Mean (SD)	55.69 (4.94)	53.76 (4.63)	54.03 (4.71)	.03 <sup>a</sup>
APOE $\epsilon$ 4 status				
$\epsilon$ 4-negative	21 (67.7%)	136 (70.5%)	157 (70.1%)	.76 <sup>b</sup>
$\epsilon$ 4-positive	10 (32.3%)	57 (29.5%)	67 (29.9%)	
APOE genotype				
$\epsilon$ 2/ $\epsilon$ 2	0 (0.0%)	1 (0.5%)	1 (0.4%)	.83 <sup>b</sup>
$\epsilon$ 2/ $\epsilon$ 3	6 (19.4%)	24 (12.4%)	30 (13.4%)	
$\epsilon$ 2/ $\epsilon$ 4	0 (0.0%)	3 (1.6%)	3 (1.3%)	
$\epsilon$ 3/ $\epsilon$ 3	15 (48.4%)	111 (57.5%)	126 (56.2%)	
$\epsilon$ 3/ $\epsilon$ 4	8 (25.8%)	43 (22.3%)	51 (22.8%)	
$\epsilon$ 4/ $\epsilon$ 4	2 (6.5%)	11 (5.7%)	13 (5.8%)	
MMSE score				
Mean (SD)	29.77 (0.56)	29.52 (1.07)	29.55 (1.01)	.19 <sup>a</sup>
Familial AD				
No	24 (77.4%)	146 (75.6%)	170 (75.9%)	.83 <sup>b</sup>
Yes	7 (22.6%)	47 (24.4%)	54 (24.1%)	

Abbreviations: APOE, apolipoprotein E; Familial AD, familial history of Alzheimer's or other neurodegenerative diseases with prominent cognitive impairment, MHT, menopause hormone therapy, MMSE, Mini Mental State Examination.

<sup>a</sup>Student's t-test.

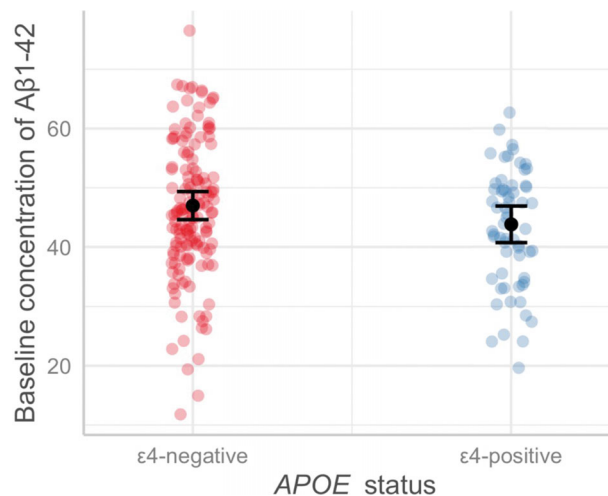
<sup>b</sup>Pearson's chi-squared test.

different between the control and the MHT study arms (Table 1). There was no group-wise difference in terms of MMSE scores. Women enrolled in the control group were significantly older than participants enrolled in the MHT arm ( $p = .033$ ). No significant differences but a trend was present in education levels with the control group having lower levels ( $p = .055$ ). The mean delay between V1 and V2 was  $7.2 \pm 2.4$  months in the control group and  $6.7 \pm 1.6$  months in the MHT group, with no significant between-group difference.

#### 4.2 | Baseline and longitudinal comparison between MHT and control arms for AD biomarkers

Description of plasma biomarkers at baseline and follow-up, and the ARC are reported in Table S1. We report no significant differences for plasma concentrations of AD biomarkers result from the V1 and V2 comparison of women enrolled in the MHT arm versus untreated women. As expected, LM shows that age is positively associated with a few AD biological pathways, as indicated by the  $A\beta$ 1-42/p-tau<sub>231</sub> ratio, BACE1, and NfL (all  $p < .05$ ). Such an age-wise significant association faded out at the follow-up (longitudinal changes of biomarkers).

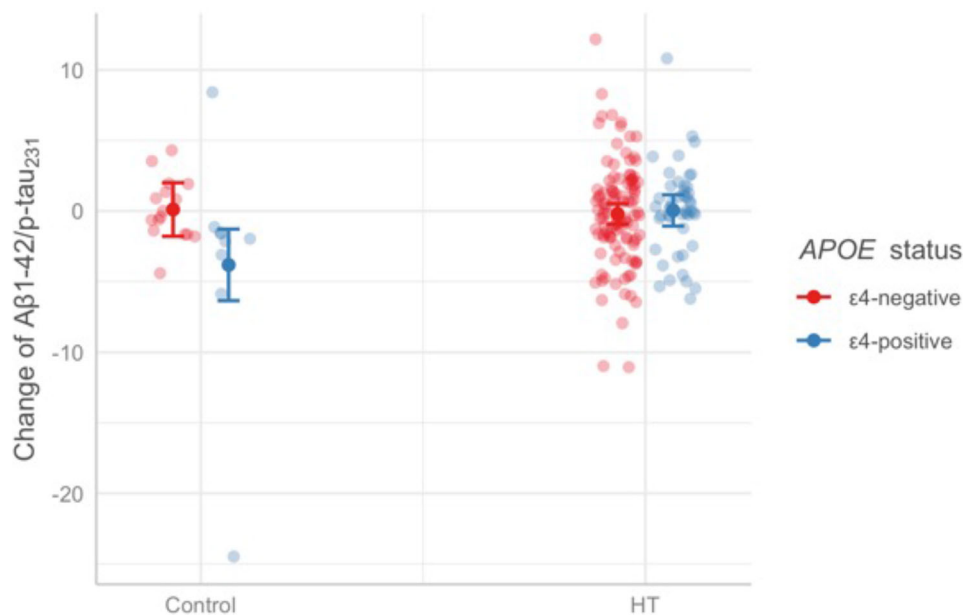
In addition, we observe an expected cross-sectional and longitudinal trend in the APOE  $\epsilon$ 4 positive group, exhibiting lower levels of plasma  $A\beta$ 1-42 concentrations at baseline compared to the APOE  $\epsilon$ 4 negative group (baseline: Figure 2,  $\beta = -3.18$ , SE = 1.65,  $p = .055$ , Cohen  $f^2 = 0.02$ , and longitudinal:  $\beta = 4.32$ , SE = 2.35,  $p = .067$ , Cohen  $f^2 = 0.02$ ).



**FIGURE 2** Plasma  $A\beta$ 1-42 concentrations at V1 according to APOE  $\epsilon$ 4 allele carrier status (positive or negative). Notes: The values represented refer to the estimated marginal means computed based on the linear model to account for the effect of age and treatment groups.  $A\beta$ , amyloid- $\beta$ ; APOE, apolipoprotein E; V1, baseline visit

#### 4.3 | Longitudinal APOE-wise comparison between MHT and control arms for AD biomarkers

The MHT-control groups difference for  $A\beta$ 1-42/p-tau<sub>231</sub> ratio is significant at the longitudinal analysis. At the 6-month follow-up evaluation



**FIGURE 3** Longitudinal changes of the A $\beta$ 1-42/p-tau<sub>231</sub> ratio according to treatment group and APOE  $\epsilon$ 4 allele carrier status (positive or negative). Notes: The values represented refer to the estimated marginal means computed based on the linear model to account for the effect of age. Post-hoc pairwise group comparison: Control APOE  $\epsilon$ 4-negative - MHT APOE  $\epsilon$ 4-negative:  $p = 0.76$ , Control APOE  $\epsilon$ 4-negative - Control APOE  $\epsilon$ 4-positive:  $p = 0.02$ , Control APOE  $\epsilon$ 4-negative - MHT APOE  $\epsilon$ 4-positive:  $p = 0.95$ , MHT APOE  $\epsilon$ 4-negative - Control APOE  $\epsilon$ 4-positive:  $p = 0.008$ , MHT APOE  $\epsilon$ 4-negative - MHT APOE  $\epsilon$ 4-positive:  $p = 0.71$ , Control APOE  $\epsilon$ 4-positive - MHT APOE  $\epsilon$ 4-positive:  $p = 0.007$ . A $\beta$ , amyloid- $\beta$ ; APOE, apolipoprotein E; MHT, menopause hormone therapy; p-tau<sub>231</sub>, tau protein phosphorylated at site threonine 231

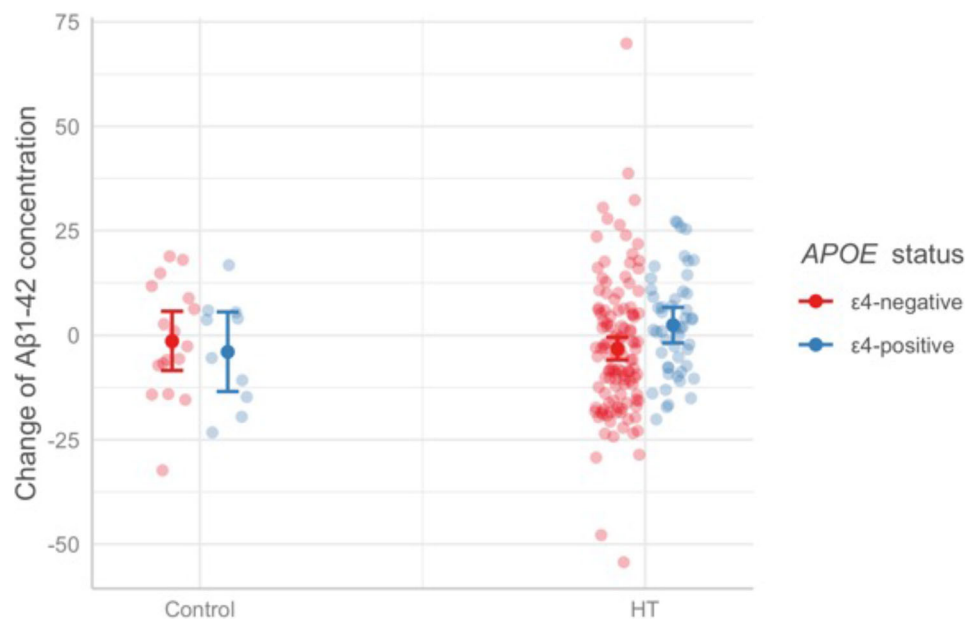
and biomarker assessment, the effect of treatment still differs according to APOE  $\epsilon$ 4 allele status ( $\beta = 4.18, 1.74, p = .02$ , Cohen  $f^2 = 0.03$ ), with the pairwise subgroups comparison indicating that controls with APOE  $\epsilon$ 4 showed greater reduction in A $\beta$ 1-42/p-tau<sub>231</sub> ratio levels than both MHT group individuals APOE  $\epsilon$ 4 allele carriers and non-carriers ( $p = .007$  and  $p = .008$  respectively, see Figure 3 and Table S2)). Although A $\beta$ 1-42/p-tau<sub>231</sub> ratio does not differ between the two MHT subgroups split by APOE  $\epsilon$ 4 status, the MHT APOE  $\epsilon$ 4-positive individuals exhibit a significantly greater reduction of A $\beta$ 1-42 levels than the MHT APOE  $\epsilon$ 4-negative individuals ( $p = .03$ , see Figure 4 and Table S2).

Moreover, APOE  $\epsilon$ 4 carrier control individuals have significantly different levels of A $\beta$ 1-42/p-tau<sub>231</sub> ratio than non-carriers ( $p = .02$ , Cohen  $f^2 = 2.65e-3$ , see Figure 3 and Table S2). MHT group individuals carrying at least one APOE  $\epsilon$ 4 allele have smaller reduction of A $\beta$ 1-42 than APOE  $\epsilon$ 4-negative treated women (see also Figure 4). Of note, HT APOE  $\epsilon$ 4-positive women have a mild within-group increment of plasma A $\beta$ 1-42 over time (2.41, 95% confidence interval [CI] -1.83 to 6.65;  $p = .26$ ), while HT APOE  $\epsilon$ 4-negative women show a significant longitudinal reduction of the same biomarker (-3.17, 95% CI -5.89 to 0.46,  $p = .02$ ). The two MHT subgroups show opposite directions, albeit with slight changes, in p-tau<sub>231</sub> levels with either an increase or a decrease in APOE  $\epsilon$ 4-positive and  $\epsilon$ 4-negative women, respectively. Notably, control APOE  $\epsilon$ 4-positive individuals display an average higher increment rate of p-tau<sub>231</sub> levels than each MHT subgroups (and the control APOE  $\epsilon$ 4-negative individuals as well). Hence, it is conceivable to infer that the effect of MHT in APOE  $\epsilon$ 4-positive women is on the A $\beta$  pathway.

## 5 | DISCUSSION

To our knowledge, this is the first-ever prospective longitudinal study performed with healthy, MHT-naïve women during menopause who underwent two time point assessments of an extensive panel of blood-based biomarkers charting AD pathophysiological processes, including the A $\beta$  and tau pathways, axonal damage, neurodegeneration. We report that MHT is associated with smaller changes toward AD pathophysiology than no-therapy (control groups) and that APOE  $\epsilon$ 4 allele carrying condition is associated with an amplified treatment outcome. Specifically, the most prominent effect of MHT on AD pathophysiological biomarkers focuses on the A $\beta$  pathway, an early and central AD patho-biochemical cycle which defines and propagates progression in the disease continuum. Such a result is consistent with (a) the clinical features of the study cohort, cognitively healthy women with no familial history of AD; and (b) previous molecular imaging studies (A $\beta$ -PET) reporting that women in menopause have higher rates of brain A $\beta$  accumulation, especially in APOE  $\epsilon$ 4 carriers.<sup>4-12</sup>

Our findings are also in agreement with the large-scale, randomized WHI study showing that MHT can reduce the risk of late-life dementia and indicated that the timing of MHT influences this outcome. In this sense, our study provides preliminary biological context for the WHI clinical results.<sup>24,25</sup> Moreover, our results are in line with structural neuroimaging studies showing that perimenopausal women not treated with MHT have significantly greater AD-vulnerable regions



**FIGURE 4** Longitudinal changes of A $\beta$ 1-42 concentrations according to the treatment group and APOE  $\epsilon$ 4 allele carrier status (positive or negative). *Notes:* The values represented refer to the estimated marginal means computed based on the linear model to account for the effect of age. *Post-hoc* pairwise group comparison: Control APOE  $\epsilon$ 4-negative - MHT APOE  $\epsilon$ 4-negative:  $p = 0.64$ , Control APOE  $\epsilon$ 4-negative - Control APOE  $\epsilon$ 4-positive:  $p = 0.67$ , Control APOE  $\epsilon$ 4-negative - MHT APOE  $\epsilon$ 4-positive:  $p = 0.37$ , MHT APOE  $\epsilon$ 4-negative - Control APOE  $\epsilon$ 4-positive:  $p = 0.88$ , MHT APOE  $\epsilon$ 4-negative - MHT APOE  $\epsilon$ 4-positive:  $p = 0.03$ , Control APOE  $\epsilon$ 4-positive - MHT APOE  $\epsilon$ 4-positive:  $p = 0.23$ . A $\beta$ , amyloid- $\beta$ ; APOE, apolipoprotein E; MHT, menopause hormone therapy

volumetric reduction (a surrogate marker of regional neuronal loss and neurodegeneration) over time.<sup>17,52</sup>

We provide preliminary evidence of an MHT-associated effect on core biomarkers of AD pathophysiology, especially the central A $\beta$  pathway (currently the target of late-stage anti-A $\beta$  drug developments) and mostly in females carrying the APOE  $\epsilon$ 4 risk allele, the greatest genetic risk factor for late-onset AD.<sup>53</sup>

A recent study investigating sexual dimorphism in AD brain endophenotypes showed for the first time that the accumulation of soluble A $\beta$  aggregates was higher in women than men, especially in peri-menopausal and post-menopausal women carrying the APOE  $\epsilon$ 4 allele.<sup>54</sup>

The APOE  $\epsilon$ 4 allele-wise effect we found in the MHT arm is consistent with other therapeutic approaches for AD, either symptomatic or candidate disease-modifying drugs, reported to be associated with better cognitive-functional outcomes in APOE  $\epsilon$ 4 allele carriers.<sup>55-57</sup>

Clinical and animal models indicate the menopause-related risk of AD in women is non-linearly increased by the presence of the APOE  $\epsilon$ 4 allele.<sup>53,58-60</sup> Mouse models suggest that estrogen upregulates the APOE gene, whereas progesterone acts antagonistically to estrogen at the gene expression level. It is hypothesized that such balanced cross-talk is impaired during menopause.<sup>59</sup> Therefore, all these clinical-biological findings are backed by mouse models of aging and AD showing that induced estradiol deficiency is associated with cognitive worsening, synaptic decline, and AD biological signatures.<sup>58,61-64</sup>

Stratifying pharmacological clinical trial outcomes for female sex alongside concomitant intake of MHT may uncover specific population

clusters with selective response rates to pathways-targeting drugs.<sup>65</sup> This reasoning may apply to late stage-development/already approved anti-A $\beta$  treatments and developing anti-tau compounds, given the experimental and clinical evidence of a sex-APOE  $\epsilon$ 4 allele interaction effect on tau-mediated pathways related trajectories.<sup>7,11,55</sup>

We believe that an extensive and thorough elaboration of the limitation of this study is useful to facilitate future replication studies. Although a significant effect of MHT on AD neurobiology at 6-months is potentially clinically meaningful, data need to be handled with caution and call for studies with longer follow-up periods. Replication studies may also indicate that menopause-related and biomarker-guided dose adjustments of MHT may be beneficial and needed to maintain a stable significant effect on biomarker indicators of AD pathophysiology.

Given the multi-dimensional (medical, psychological, and social) consequences of menopause on the affected individual, we chose to give the study participants the option to decide which treatment/control arm to be allocated to, that is, whether starting a MHT program. The consequence of giving priority to this matter is represented by the absence of randomized treatment assignment, which also translates into unbalanced numbers of subgroups. On the flip side, the study enrolled more women than the number reported in the longitudinal results.

Another potential caveat of the present study is of pharmacological nature and refers to the impossibility to differentiate whether oral or transdermal estrogen as well as whether the concomitant use of progesterone yielded different effects on AD biomarkers.



Mono-centric studies have the intrinsic limitations of not adequately addressing inter-ethnic differences that may exist due to genetic polymorphism variations accounting for downstream differences in disease vulnerability and drug metabolism.

Finally, several lines of cross-disciplinary evidence have been published between menopause and the dysregulation of neuroinflammatory and neuroimmune responses,<sup>66-68</sup> two critical pathophysiological mechanisms occurring early in AD and involved in complex dynamic cross-links with other AD-related pathobiological changes.<sup>66-68</sup>

## 6 | CONCLUSIONS

Substantial progress in the analytical and clinical validation of blood-based biomarkers, alongside the definition of their context-of-use, have recently accelerated AD clinical diagnostic and therapy research. We provide the first prospective clinical evidence on the potential effect of MHT on core biomarkers associated to AD pathophysiology in menopause. In particular, we demonstrate a prominent response at the critical  $A\beta$  pathway biomarker level. Women at genetic risk for AD (carrying at least one APOE e4 allele) seem to be particularly benefiting from MHT.

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Andrea Vergallo: the present study and related article have been initiated and prepared as part of an academic position at Sorbonne University, Paris, France. At Sorbonne University he was supported by the translational research program "PHOENIX", administered by the Sorbonne University Foundation and sponsored by la Fondation pour la Recherche sur Alzheimer.

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Kaj Blennow is a co-founder of ADx NeuroSciences. Nele Dewit and Dirk Jacobs performed the amyloid and BACE1 assays.

### CONFLICTS OF INTEREST

H.Z. has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). K.B. has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, unrelated to the work presented in this paper. E.V.M. is a co-founder of ADx NeuroSciences. A.V. declares no competing financial interests related to the present article. The present study and related article have been initiated and prepared as part of an academic position at Sorbonne University, Paris, France and it reflects only and exclusively his own opinion and academic expertise on the matter. A.V. was an employee of Eisai Inc. (November 2019 - June 2021). A.V. does not receive any fees or honoraria since November 2019. Before November 2019 he had he received lecture honoraria from Roche, MagQu LLC, and Servier. S.L. received lecture honoraria from Roche and Servier. E.C. declares no competing financial interests related to the present article; her contribution to this article reflects only and exclusively his academic expertise on the matter. This work was initiated during her previous position at Sorbonne University, Paris, France. E.C. is currently an employee of Qynapse SAS. H.H. declares no competing financial interests related to the present article. He is currently an employee of Eisai Inc. The present study and related article have been initiated and prepared as part of an academic position at Sorbonne University, Paris, France and it reflects entirely and exclusively his own opinion. He serves as Senior Associate Editor for the Journal Alzheimer's & Dementia and does not receive any fees or honoraria since May 2019; before May 2019 he had received lecture fees from Servier, Biogen and Roche, research grants from Pfizer, Avid, and MSD Avenir (paid to the institution), travel funding from Eisai, Functional Neuromodulation, Axovant, Eli Lilly and company, Takeda and Zinfandel, GE-Healthcare and Oryzon Genomics, consultancy fees from Qynapse, Jung Diagnostics, Cytox Ltd., Axovant, Anavex, Takeda and Zinfandel, GE Healthcare, Oryzon Genomics, and Functional Neuromodulation, and participated in scientific advisory boards of Functional Neuromodulation, Axovant, Eisai,

Eli Lilly and company, Cytosx Ltd., GE Healthcare, Takeda and Zinfandel, Oryzon Genomics and Roche Diagnostics.

H.H. is inventor of 11 patents and has received no royalties:

In Vitro Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Patent Number: 8916388.

In Vitro Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Patent Number: 8298784.

Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20120196300.

In Vitro Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100062463.

In Vitro Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100035286.

In Vitro Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Publication Number: 20090263822.

In Vitro Method for The Diagnosis of Neurodegenerative Diseases Patent Number: 7547553.

CSF Diagnostic in Vitro Method for Diagnosis of Dementias and Neuroinflammatory Diseases Publication Number: 20080206797.

In Vitro Method for The Diagnosis of Neurodegenerative Diseases Publication Number: 20080199966.

Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20080131921 .

Method for diagnosis of dementias and neuroinflammatory diseases based on an increased level of procalcitonin in cerebrospinal fluid: Publication number: United States Patent 10921330.

Author disclosures are available in the [supporting information](#).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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