Interactions between apolipoprotein E, sex, and amyloid-beta on cerebrospinal fluid p-tau levels in the European prevention of Alzheimer's dementia longitudinal cohort study (EPAD LCS)

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Summary

Background Alzheimer's Disease, the leading cause of dementia, is over-represented in females. The apolipoprotein E (*APOE*) ε4 allele is the strongest genetic risk factor for late-onset AD and is associated with aberrant cerebrospinal fluid levels (CSF) of total tau (t-tau), phosphorylated tau (p-tau), and amyloid-β (Aβ). There is some evidence that sex may mediate the relationship between *APOE* status and CSF tau, however, evidence is mixed.

Methods We aimed to examine the interaction between sex, *APOE* ε4 status, CSF Aβ on t-tau and p-tau in 1599 mid-to-late life individuals without a diagnosis of dementia in the European Prevention of Alzheimer's Dementia (EPAD) longitudinal cohort study.

Findings We found a significant interaction between *APOE* status, sex, and CSF Aβ on CSF p-tau levels (β = 0·18, p = 0·04). Specifically, there was a stronger association between *APOE* status and CSF $A\beta_{42}$ on CSF p-tau in males compared to females. Further, in females with high Aβ levels (reflecting less cortical deposition), ε4 carriers had significantly elevated p-tau levels relative to non-carriers (W = 39663, p = 0·01). However, there were no significant differences in p-tau between male ε4 carriers and non-carriers with high Aβ (W = 23523, p = 0·64).

Interpretation An interaction between sex and cerebrospinal fluid Aβ may mediate the relationship between *APOE* status and CSF p-tau. These data suggest tau accumulation may be independent of Aβ in females, but not males.

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Introduction

An estimated 50 million people are currently living with dementia, which is expected to increase to 82 million by the year 2030(1). Alzheimer's Disease (AD) is the leading cause of dementia, accounting for approximately 60-70% of all cases.[1] The key risk factors associated with late-onset AD development are age, apolipoprotein E (*APOE*) ε4 allele, and female sex. Females account for an estimated 60% of those diagnosed with AD[2,3] and while increased life expectancy may in-part explain this over-representation, there may also exist other biological factors driving these sex differences in AD.[2,3]

It is widely recognised that *APOE* ε4 allele is the strongest genetic risk factor for late-onset AD. This risk increases in a dose-dependent manner[4] and varies by sex, with meta-analytic evidence of a stronger effect in females aged 55-70.[5,6] The *APOE* ε4 allele is associated

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Research in context

Evidence before this study

We searched PubMed and Web of Science from database inception to Feb 1 2022, without language restrictions, for studies published in English using the search terms "apolipoprotein E" OR "APOE" AND "sex" AND "cerebrospinal fluid tau". Some studies suggest female *APOE* ε4 carriers have significantly elevated CSF p-tau and t-tau, with two studies suggesting this difference is specific to those with low levels of CSF Aβ. However, three studies report no significant differences between female and male ε4 carriers on CSF biomarkers and so evidence is still mixed. Many of the existing studies draw samples from the same cohort and so replication in other mid-life, healthy cohorts is needed.

Added value of this study

We report a significant interaction between sex, *APOE* status, and CSF Aβ on CSF p-tau in healthy, mid-life individuals. Specifically, CSF p-tau levels in female ε4 carriers were independent of CSF Aβ. We showed that the *APOE*-sex interaction may need to be considered in the context of Aβ.

Implications of all the available evidence

Findings from this study support the evidence of sex differences in the interaction between *APOE* genotype, CSF Aβ, and CSF p-tau. This has future implications for the implementation of CSF AD biomarkers in clinical practice, as well as pharmacological interventions which target of cortical Aβ such as aducanumab.

with the pathological hallmarks of AD such as accelerated Aβ deposition,[7] disruption of Aβ clearance,[8] and acceleration of tau spread.[9] These pathological hallmarks can also be measured *in vivo* in the cerebrospinal fluid (CSF), with reduced levels of Aβ (reflecting deposition in the brain) and increased levels of phosphorylated tau (p-tau) and total tau (t-tau) indicating an AD-like CSF biomarker profile.[10] *APOE* ε4 carriers have lower CSF $A\beta_{42}$ levels, explained by increased amyloid deposition in carriers[11] and increased levels of CSF p-tau and t-tau.[12] The effects of *APOE* genotype on CSF markers may also differ by sex. Some studies report significant elevations of CSF p-tau and t-tau in female ε4 carriers with mild cognitive impairment, subjective cognitive impairment, and in those who are cognitively unimpaired.[13–16] Evidence from a number of studies suggests this difference in cognitively unimpaired females is specific to those with low levels of CSF Aβ, suggesting a sex-specific risk of AD may be downstream of Aβ.[13,17] However, a number of studies also report no significant differences between cognitively unimpaired female and male ε4 carriers on CSF AD biomarkers.[14,15,18] While there is increasing research around *APOE* and sex interactions on CSF tau, evidence appears to still be mixed.

Using the European Prevention of Alzheimer's Dementia longitudinal cohort study (EPAD LCS), we investigated the interaction between *APOE* status, sex, and CSF Aβ on CSF p-tau and t-tau levels.

Methods

Study design

Participants included in the current study were enrolled in EPAD LCS. The background and aims are described in detail elsewhere.[19] In summary, EPAD is a multi-site pan-European project with participants recruited from parent cohorts across 21 European sites from May 2016 to December 2019.[20]

CSF biomarkers

Cerebrospinal fluid samples were collected via lumbar puncture and all samples were shipped from study sites and stored centrally at the EPAD BioBank at the University of Edinburgh. CSF samples were measured for $A\beta_{42}$, t-tau, and p-tau181 in a single laboratory at the Clinical Neurochemistry Laboratory at University of Gothenburg, Sweden, using the Roche cobas Elecsys System.

APOE genotyping

APOE genotyping was determined from Taqman Genotyping of blood samples, analysed in a single laboratory at the University of Edinburgh using QuantStudio 12KL Flex. *APOE* ε4 carriers were defined as having at least one ε4 allele.

Other information

Socio-demographic data were self-reported at baseline visit. The term "sex" is used to refer to biological sex, in contrast to "gender" which refers to identity, psychosocial, and culture factors. Data were collected for sex, but not gender. The Clinical Dementia Rating scale (CDR)[21] is comprised of two semi-structured face-to-face interviews, one with the participants and another with a reliable collateral source. Six domains are assessed (memory, orientation, judgement, and problem solving, community affairs, home and hobbies, and personal care), and rated accordingly on a 5-point scale: 0 = no impairment, 0.5 = questionable impairment, 1 = mild dementia, 2 = moderate dementia, 3 = severe dementia. CDR raters were blinded to other cognitive and clinical assessments, except if biomarker status was disclosed. At present, it is not possible to include medical comorbidities in analyses as records have not yet been fully processed for the entire sample and checks are being

conducted to investigate how medications and conditions align.

Statistics

All analyses were conducted in R (version 4·0·4). Inclusion criteria included: individuals aged 50 years or older, having at least seven years of formal education, and availability of a study partner willing to provide function and behavioural corroborative information. Exclusion criteria included: inability to consent to the study, a known genetic mutation associated with autosomal-dominant AD, significant physical or mental illness, or cancer within the past five years (except for localised prostate cancer and basal or squamous carcinoma). Participants were also not eligible for inclusion in the current study if they received a dementia diagnosis, a CDR score of one or more (indicating mild dementia), or a score below 20 on the Mini Mental State Examination. Investigators were blinded to results of CSF and *APOE* data collected to limit biases in clinical assessments.

Normality of data was assessed by visual inspection of histograms, qqplots, and Shapiro-Wilk tests. Where data were not normally distributed, non-parametic tests were used to examine group differences. Group differences were analysed using chi-square test for categorical variables, Wilcoxon rank sum tests for continuous variables, and correlations were performed using Spearman's correlations. Power analysis to determine sample size was not conducted as all eligible participants in the cohort were included in the current analyses. Linear regression models were used to examine interactions between *APOE,* sex, and CSF Aβ_{42} on CSF p-tau181 and CSF t-tau. Model building was guided by Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) values, indices of model fit and complexity that favour most parsimonious models. A series of models of increasing complexity were fitted. Terms were selected by researchers. The first model included age and sex. Model two added *APOE* status, CDR score, and an interaction term between *APOE* status and sex. Model three added CSF Aβ_{42} and an interaction term between *APOE* status, sex, and CSF Aβ_{42}. *APOE* status was entered as a binary variable (ε4 carrier vs non-carrier), where carriers had at least one ε4 allele and non-carriers had no ε4 alleles (ε2/ ε2, ε2/ ε3, ε3/3). CSF markers were log-transformed, and all linear regression assumptions were met. Stratified analyses were also conducted where the sample was stratified by *APOE* status (carrier or non-carrier), by sex, CDR rating (0 or 0.5), and Aβ status (positive or negative). Amyloid-positivity (Aβ+) was defined as < 1000 pg/mL[22,23] and amyloid-negative (Aβ-) as > 1000pg/mL. *Post*-hoc analyses were conducted using spearman correlations and Wilcoxon rank sum tests. Multilevel modelling was considered to adjust for study site and explore possible sources of heterogeneity across sites, however, the number of participants in each visit site is highly unbalanced. Some sites have as few as two participants, while others have over 100 and so it would not be possible to adequately model between-site heterogeneity or add covariates to explain this heterogeneity. Study protocols were identical in all sites and so differences would not be expected between study sites.

Ethics

All procedures were followed in accordance with the declaration of Helsinki and the EPAD LCS (www.clinicaltrials.gov:NCT02804789) protocol and materials were approved by the Independent Ethics Committees local to each site. EPAD LCS has received ethical approval from numerous institutional review boards across Europe. All participants provided informed consent. The study is designed and conducted in accordance with the guidelines for Good Clinical Practice (GCP).

Role of funders

The funding source was not involved in the study design, collection, analysis, or interpretation of the data, or in the writing of the report and decision to submit the paper for publication.

Data statement

Data from EPAD are open-access and are available upon application from www.ep-ad.org.

Results

Demographics

Sample characteristics are provided in Table 1. In brief, 1599 participants (mean age = 65·94, *SD* = 7·29) were included in analyses. Of the total sample, 1217 (76.11%) participants were Caucasian/White, 6 participants (0.38%) were Hispanic, 5 participants (0.31%) were Asian (Chinese, mixed Asian, South East Asian), and 2 participants (0.13%) were Black. Data on ethnicity were not available for 368 participants (23.01%). Group comparisons for sex and *APOE* status are listed in Table 1. In summary, males were significantly older than females (Wilcoxon rank sum, $W = 348598$, $p < 0{\cdot}001$) and there were no significant differences in *APOE* carriership between men and women (Chi-square, $\chi^2 = 10{\cdot}14$, df = 5, $p = 0{\cdot}07$). There was a significant sex difference in CDR scores ($\chi^2 = 8.61$, df = 1, $p = 0.01$), with males having a larger proportion of CDR scores of 0.5 than females. There was also a significant difference between *APOE* ε4 carriers and non-carriers in CDR score ($\chi^2 = 6.40$, df = 1, $p = 0.01$), with a larger proportion of ε4 with a CDR score of 0.5.

	Females (*n* = 905)	Males (*n* = 694)	*p*	ε4 carriers (*n* = 623)	Non-carriers (*n* = 976)	*p*	Total (*n* = 1599)
APOE status, *n* (%)			0.07	..	..	..	..
2/2	*4 (0.22%)*	*2 (0.29%)*		..	..	..	6 (0.38%)
2/3	*75 (8.29%)*	*68 (9.80%)*		..	..	..	143 (8.94%)
2/4	*21 (2.32%)*	*25 (3.60%)*		..	..	..	46 (2.88%)
3/3	*481 (53.15%)*	*346 (49.86%)*		..	..	..	827 (51.72%)
3/4	*291 (32.15%)*	*210 (30.26%)*		..	..	..	*501 (31.33%)*
4/4	*33 (3.65%)*	*43 (6.20%)*		..	..	..	76 (4.75%)
Age (years), mean (SD)	65.30 (7.28)	66.77 (7.23)	<**0.001**	65.62 (7.06)	66.14 (7.44)	0.19	65 .94 (7.29)
BMI (kg/m^2), mean (SD)	25.58 (4.65)	26.83 (3.73)	<**0.001**	26.12 (4.17)	26.13 (4.41)	0.62	26.12 (4.32)
CDR global score			**0.01**			**0.01**	
0, *n* (%)	685 (75.69%)	479 (69.02%)		431 (69.18%)	733 (75.10%)		1164 (72.80%)
0.5, *n* (%)	216 (23.87%)	212 (30.55%)		189 (30.34%)	239 (24.49%)		428 (26.77%)
Missing, *n* (%)	4 (0.44%)	3 (0.43%)		3 (0.48%)	4 (0.41%)		7 (0.44%)
CSF Aβ (pg/ml)	1433.65 (769.97)	1300.56 (660.75)	<**0.001**	1135.12 (590.52)	1530.98 (762.97)	< **0.001**	1374.79 (726.52)
CSF p-tau (pg/ml)	19.87 (10.49)	20.52 (10.58)	0.14	22.41 (12.13)	18.70 (9.17)	< **0.001**	20.15 (10.54)
CSF t-tau (pg/ml)	226.12 (98.40)	227.64 (100.16)	0.9	246.96 (112.08)	214.11 (88.39)	< **0.001**	226.79 (99.16)

Table 1*: Sample demographics *P-values were computed using chi-square analyses for categorical variables and wilcoxon rank sum tests for continuous variable. APOE = apolipoprotein E, CDR = clinical dementia rating, CSF = cerebrospinal fluid, Aβ = amyloid-beta, p-tau = phosphorylated tau, t-tau = total tau.

	CSF p-tau		CSF t-tau	
	β (SE)	p	β (SE)	p
Model 1				
Sex (Female)	0.01 (0.02)	0.99	0.03 (0.02)	0.17
Age	0.35 (0.01)	< **0.001**	0.37 (0.01)	< **0.001**
R^2	0.12	< **0.001**	0.13	< **0.001**
Model 2				
Sex (Female)	0.01 (0.01)	0.61	0.05 (0.02)	0.07
Age	0.31 (0.01)	< **0.001**	0.33 (0.01)	< **0.001**
CDR global score (0.5)	0.18	< **0.001**	0.17 (0.02)	< **0.001**
APOE (ε4)	0.17 (0.03)	< **0.001**	0.16 (0.03)	< **0.001**
APOE * Sex	−0.01 (0.04)	0.92	−0.02 (0.03)	0.67
R^2	0.18	< **0.001**	0.18	< **0.001**
Model 3				
Sex (Female)	0.05 (0.05)	0.41	0.12 (0.05)	0.06
Age	0.31 (0.01)	< **0.001**	0.33 (0.01)	< **0.001**
CDR global score (0.5)	0.20 (0.02)	< **0.001**	0.20 (0.02)	< **0.001**
CSF Aβ	0.32 (0.01)	< **0.001**	0.43 (0.01)	< **0.001**
APOE (ε4)	0.57 (0.07)	< **0.001**	0.53 (0.06)	< **0.001**
APOE * Sex	−0.15 (0.09)	0.09	−0.15 (0.07)	0.07
APOE * CSF Aβ	−0.40 (0.01)	< **0.001**	−0.33 (0.01)	< **0.001**
CSF Aβ * Sex	−0.07 (0.01)	0.33	−0.11 (0.01)	0.10
CSF Aβ * *APOE* * Sex	0.18 (0.01)	**0.04**	0.15 (0.01)	0.06
R^2	0.23	< 0.001	0.28	< **0.001**

***Table 2*: Results from linear regression models examining CSF tau.**
Bold represents where $p < 0.05$. β coefficients are standardised coefficients. APOE = apolipoprotein E, CDR = clinical dementia rating, CSF = cerebrospinal fluid, Aβ = amyloid-beta, p-tau = phosphorylated tau, t-tau = total tau.

CSF biomarkers

Biomarker concentrations and group differences are provided in Table 1. Males had significantly lower baseline levels of CSF $A\beta_{42}$ (Wilcoxon rank sum, $W = 350278$ $p < 0{\cdot}001$). There were no significant differences in CSF p-tau or t-tau levels between males and females (p-tau: Wilcoxon rank sum, $W = 377960$, $p = 0{\cdot}14$, t-tau: Wilcoxon rank sum, $W = 384928$, $p = 0{\cdot}90$). Conversely, *APOE* ε4 carriers had elevated CSF p-tau (Wilcoxon rank sum, $W = 268102$, $p < 0{\cdot}001$), t-tau (Wilcoxon rank sum, $W = 291374$, $p < 0{\cdot}001$), and lower CSF $A\beta_{42}$ levels (Wilcoxon rank sum, $W = 486188$, $p < 0{\cdot}001$) relative to non-carriers. 1599 participants had data available for CSF p-tau, 1597 for t-tau, and 1599 for $A\beta_{42}$.

Main interaction

Standardised regression coefficients are provided in Table 2 and model selection statistics (AIC and BIC) are provided in Table 3. In models 2 and 3, *APOE* status was a significant predictor of both CSF p-tau (linear regression, $\beta = 0{\cdot}57$, $p < 0{\cdot}001$) and t-tau (linear regression, $\beta = 0{\cdot}53$, $p < 0{\cdot}001$). In model 3, there was a significant interaction between sex, *APOE* and CSF $A\beta_{42}$ on CSF p-tau (linear regression, $\beta = 0{\cdot}18$, $p = 0{\cdot}04$) but not CSF t-tau, although this association was approaching significance (linear regression, $\beta = 0{\cdot}15$, $p = 0{\cdot}06$). There was no significant interaction between sex and *APOE* status in either model 2 or 3 on CSF p-tau (linear regression, $\beta = -0{\cdot}15$, $p = 0{\cdot}09$) or t-tau levels (linear regression, $\beta = -0{\cdot}15$, $p = 0{\cdot}07$).

Stratified analyses

APOE status. Stratified analyses by *APOE* status revealed no significant interactions between sex and CSF $A\beta_{42}$ on CSF p-tau or t-tau in either ε4 carriers (linear regression, p-tau: $\beta = 0{\cdot}16$, $p = 0{\cdot}17$, t-tau: $\beta = 0{\cdot}09$,

Model	AIC	BIC
Model 1 (p-tau ~ sex + age)	1524.70	1546.29
Model 2 (p-tau ~ sex**APOE* + age + CDR)	1385.41	1423.03
Model 3 (p-tau ~ sex**APOE** Aβ + age + CDR)	1274.61	1333.71
Model 1 (t-tau ~ sex + age)	1231.79	1253.49
Model 2 (t-tau ~ sex**APOE* + age + CDR)	1112.47	1150.27
Model 3 (t-tau ~ sex**APOE** Aβ + age + CDR)	902.16	961.56

***Table 3*: Model selection statistics.**
AIC = Akaike Information Criteria, BIC = Bayesian Information Criterion.

p = 0·36) or non-carriers (linear regression, p-tau: $\beta = -0{\cdot}07$, $p = 0{\cdot}36$, t-tau: $\beta = -0{\cdot}12$, $p = 0{\cdot}11$).

Sex. Next, the sample was split by sex. There was a significant interaction between *APOE* status and CSF Aβ_{42} on CSF p-tau in both males (linear regression, $\beta = -0{\cdot}37$, $p < 0{\cdot}01$) and females $\beta = -0{\cdot}20$, $p = < 0{\cdot}01$), although the standardised coefficients (β) show a stronger association in males. A similar pattern was found on CSF t-tau where the interaction between *APOE* status and CSF Aβ_{42} significantly predicted CSF t-tau levels in males (linear regression, $\beta = -0{\cdot}30$, $p < 0{\cdot}01$) and females (linear regression, $\beta = -0{\cdot}17$, $p < 0{\cdot}01$) and the coefficients demonstrate this relationship is stronger in males (Figure 1).

CDR score. Next, the sample was split by CDR score (0 reflecting no impairment and 0.5 reflecting questionable impairment). In participants with CDR 0, there was a significant sex**APOE** Aβ_{42} interaction on both CSF p-tau (linear regression, $\beta = 0{\cdot}28$, $p < 0{\cdot}01$) and CSF t-tau (linear regression, $\beta = 0{\cdot}21$, $p = 0{\cdot}04$). However, in CDR 0.5 participants, there was no significant interaction on either CSF p-tau (linear regression, β = 0·03, p = 0·84) or t-tau (linear regression, β = 0·07, $p = 0{\cdot}67$).

Aβ status. Finally, participants were split into amyloid-positive (Aβ+) and amyloid-negative (Aβ-) groups (with positivity being defined as < 1000 pg/mL[22,23]). Both Aβ + male and female *APOE* ε4 carriers had significantly higher CSF p-tau levels relative to non-carriers (males: Wilcoxon rank sum, W = 5399·5, $p < 0{\cdot}001$; females: Wilcoxon rank sum, $W = 7768{\cdot}5$, $p < 0{\cdot}001$). In Aβ- participants, there were no significant differences in CSF p-tau levels between male ε4 carriers and non-carriers in (Wilcoxon rank sum, $W = 23523$, $p = 0{\cdot}64$). However, in Aβ- females, ε4 carriers had significantly elevated CSF p-tau relative to non-carriers (Wilcoxon rank sum, $W = 39663$, $p = 0{\cdot}01$), suggesting elevated CSF p-tau is driven by ε4 carriage regardless of Aβ in females.

Sensitivity analysis

Identical linear regression models were run with the inclusion of years of education to examine whether educational attainment influences these interactions. While years of education was a significant predictor of both CSF p-tau (linear regression, $\beta = -0{\cdot}05$, p =0.02), and t-tau (linear regression, β = -0·05, p = 0.01), interactions between sex, *APOE*, and CSF Aβ_{42} remained significant.

To examine a potential dose-response effect of the *APOE* ε4 allele, we repeated analyses with *APOE* status entered as a variable with three levels: ε4 homozygotes (two copies of the ε4 allele; n = 76), ε4 heterozygotes (one copy of the ε4; n = 547), and non-carriers (n = 976). Both homozygote (linear regression, $\beta = 0{\cdot}41$, $p < 0{\cdot}001$) and heterozygote ε4 (linear regression, $\beta = 0{\cdot}43$, $p < 0{\cdot}001$) status were significant predictors of CSF p-tau. Furthermore, the interaction between sex, *APOE* and CSF Aβ_{42} remained significant, but only in ε4 homozygotes (linear regression, $\beta = 0{\cdot}17$, $p = 0{\cdot}03$). When examining CSF t-tau as an outcome, both ε4 homozygosity (linear regression, $\beta = 0{\cdot}39$, $p < 0{\cdot}001$) and heterozygosity (linear regression, $\beta = 0{\cdot}38$, $p < 0{\cdot}001$) were significant predictors of elevated CSF t-tau. However, there was no significant interaction between sex, *APOE* ε4 homozygosity and CSF Aβ_{42} on CSF t-tau levels, although this was approaching significance (linear regression, $\beta = 0{\cdot}15$, $p = 0{\cdot}05$).

Discussion

In the current study, we investigated whether there was an interaction between sex, *APOE* ε4 carriage, and CSF Aβ_{42} on CSF tau. We found no significant interaction between *APOE* status and sex on CSF p-tau or t-tau. We failed to replicate previous findings of an *APOE*-sex interaction on baseline CSF tau levels. Much of the extant literature draws samples from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort[14,15,17,18] which are slightly older than the current sample. Where the mean age of the current EPAD sample is 65.94 years, previous work has examined samples ranging from 73 to 74.4 years. This interaction may fail to replicate in younger cohorts, although a large multicohort study did report a significant interaction on CSF t-tau after excluding ADNI participants and with cohorts similar in age to the EPAD cohort.[13] Future work would benefit from replicating the current study in cohorts similar in age to the EPAD cohort to understand discrepancies in the literature.

We identified a significant interaction between *APOE* status, sex, and CSF Aβ_{42} on CSF p-tau. Stratified analyses revealed a stronger association between *APOE* status and CSF Aβ_{42} on CSF p-tau in males compared to females. A similar pattern was found on CSF t-tau. Further, sex**APOE**CSF Aβ_{42} interactions remained significant for participants with no cognitive impairment, but not those with a CDR score of 0.5 (indicating questionable impairment). When participants were stratified by amyloid-positivity into Aβ+ (< 1000pg/mL) and Aβ- (>1000pg/mL), in male Aβ- participants, there were no significant differences in CSF p-tau levels between ε4 carriers and non-carriers. However, Aβ- female ε4 carriers had greater CSF p-tau levels than Aβ- female non-carriers (see Figure 2). Taken together, our results suggest that the effect of *APOE* ε4 carriership on the association between tau and Aβ_{42} is stronger in males than in females. That is, CSF p-tau accumulation in *APOE* ε4 females is somewhat

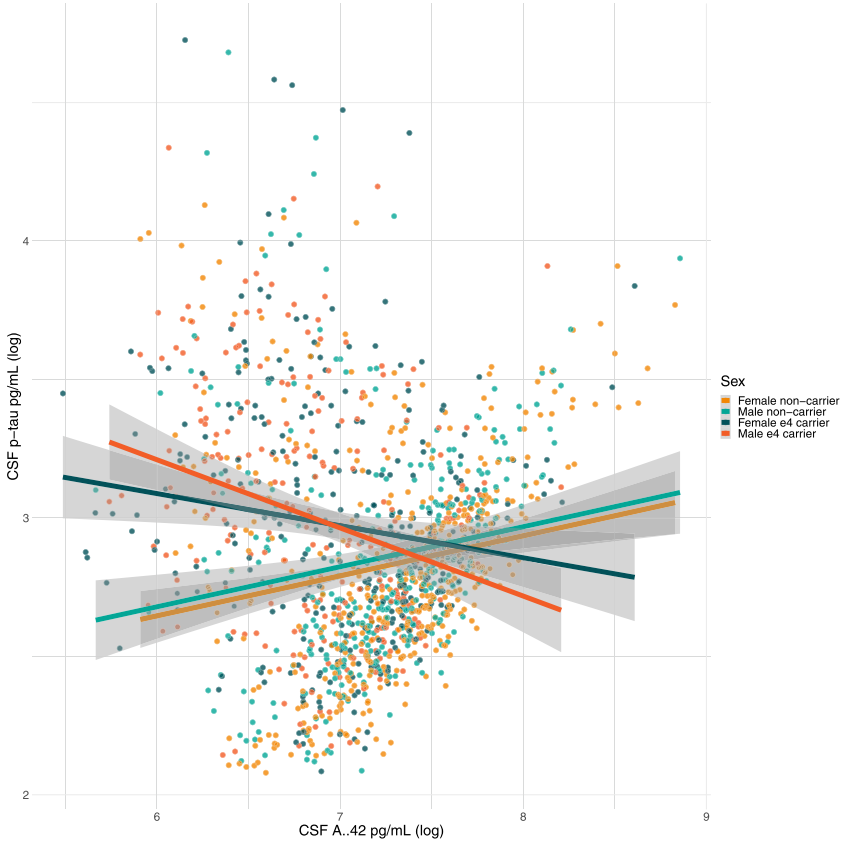

Figure 1. Scatterplot showing the relationship between CSF p-tau (log) and CSF Aβ (log) in male ε4 carriers (n = 278; green), male non-carriers (n = 416; orange), female ε4 carriers (n = 345; blue), and female non-carriers (n = 560; yellow). Grey shading indicates 95% confidence interval, formula (CSF p-tau ~ CSF Aβ).

independent of Aβ levels. Whereas in males, Aβ is more tightly related to *APOE* status and CSF p-tau accumulation. Sensitivity analyses indicate this is only present in in ε4 homozygotes. These findings contrast with previous work,[13] including those reported by Buckley and colleagues[17] where they reported female ε4 carriers with lower baseline CSF Aβ_{42} had greater tau accumulation than males. However, where Buckley and colleagues had a sample comprised of cognitive unimpaired individuals, the sample in the current study included those with a CDR of both 0 and 0.5, indicating questionnaire impairment. Further, the authors do not that there was a strong female outlier possibly influencing results, results were significant at a trend-level only, and the sample is somewhat older in age than the EPAD sample. The study conducted by Buckley and colleagues was longitudinal in design, therefore examining the rate of change in CSF tau, while the current study is cross-sectional and so concerns CSF tau levels at one time point. The discrepant results between the two studies may be in part due to differences between the rate of change in CSF tau and a snapshot measure of CSF tau.

Interestingly, we also report a positive correlation between CSF Aβ_{42} and CSF p-tau in non-carriers of the *APOE* ε4 allele. As low CSF Aβ_{42} is reflective of more deposition in the brain, a negative correlation would be expected and has been reported in the literature,[17,24] although some studies have reported a positive correlation or lack of association between the two markers in controls.[25,26] As *APOE* ε4 non-carriers had higher CSF Aβ_{42} than carriers (reflecting less deposition in the brain), there may be other mediating factors, such as lifestyle, driving tau accumulation in the absence of Aβ pathology. Future work would benefit from examining *APOE* and sex differences in CSF tau rate of change in the EPAD sample.

Our findings contribute to the literature regarding the interaction between sex, *APOE* status, and tau accumulation- and a possible role of Aβ_{42}. Any exploration of the mechanisms behind this interaction is speculative, however, it has been proposed that Aβ plaque accumulation is thought to initiate a pathological cascade of hyperphosphorylated tau[27]- highlighting the association between CSF Aβ_{42} and CSF p-tau in the current study. However, in female *APOE* ε4 homozygotes, this p-tau accumulation appears to be independent of CSF Aβ_{42}. The loss of hormones such as oestrogen and progesterone as a result of aging has been shown to be associated

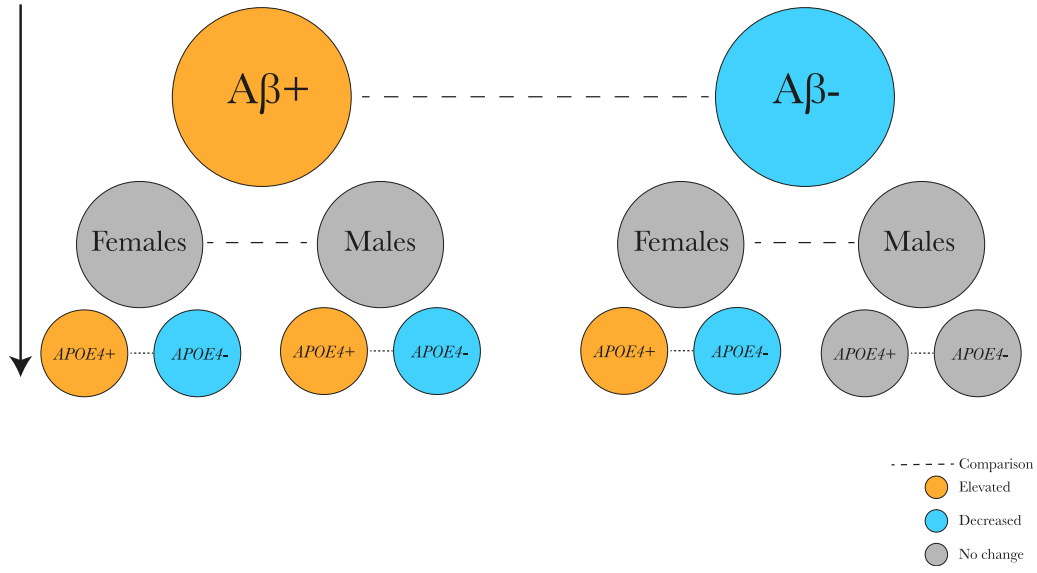

Figure 2. Figure showing comparisons in CSF p-tau between Aβ+ and Aβ- participants, males and females, and APOE ε4 carriers and noncarriers. Dotted line represents the level of comparison, orange represents significantly elevated p-tau (Wilcoxon rank sum) compared to the comparison group (blue). No significant difference is shown in grey.

with an increased vulnerability to AD development and tau phosphorylation in humans.[28] Animal models also show that in female AD mice models the depletion of sex hormones is associated with elevated brain levels of Aβ and decreased memory performance.[29] Levels of tau in female participants undergoing hormone therapy (HT) are slightly reduced relative to those not taking HT,[30] and in female AD mouse models HT reduces tau phosphorylation.[29] The loss of any protective effects of oestrogen may contribute to tau accumulation even in the absence of low CSF Aβ_{42}. This has implications for the implementation of CSF biomarkers for AD in clinical practice. Further, pharmacological interventions targeting Aβ deposition, such as aducanumab, may have different outcomes between males and females based on *APOE* status. That is, clearing Aβ in female ε4 homozygotes may have less impact on tau accumulation which is thought to drive symptoms.[31] While there is some evidence that female sex hormones may be associated with tau accumulation, it is not a well-established relationship. Some studies report adverse effects of HT on cognition[32] and elevated CSF p-tau and low CSF Aβ_{42} with longer reproductive period (reflective of endogenous oestrogen).[33] Further, it is thought that the age-associated reduction of male sex hormones is also associated with increased AD risk.[32,34] However, as the current study is one of few to investigate sex differences in the relationship between *APOE*, CSF Aβ_{42}, and CSF tau, further research would benefit the field, particularly longitudinal studies to examine temporal relationships between CSF Aβ_{42} and CSF p-tau.

Sex differences in AD may also involve several other mediating factors which differentially affect males and females. For example, sex differences in the prevalence of vascular-related risk factors exist where males have more vascular risk factors and vascular events than females before the ages of 70-75,[35] which have been associated with levels of CSF p-tau and Aβ_{42}.[36–38] Sleep disturbances and increased wakefulness have been associated with Aβ and tau accumulation, as well as tau phosphorylation.[39,40] Insomnia is almost 1.5 times more common in females than males[41] and has been reported to be increased in *APOE* ε4 carriers,[42] although no known study has examined any sex-*APOE* interactions in association with sleep disturbances. Future work would benefit from investigating potential covariates of a sex-*APOE* interaction on CSF biomarkers, including hormonal changes and factors which disproportionately affect females such as sleep disorders.

The current study has several strengths, including the relatively large sample size and proportion of *APOE* ε4 carriers. However, the study is not without limitations; the sample was predominately Caucasian, with only 1.8% of the sample representing other ethnicities. Diverse sample populations are important for personalised medicine and in understanding how biomarker patterns may differ with sociodemographic factors such as ethnicity. Indeed, the risk of developing AD in *APOE* ε4 carriers has been found to differ with different ethnicities.[6,43] Overall, the EPAD LCS is not representative of the wider population. A balancing committee was responsible for ensuring the cohort was suitable for disease modelling and risk stratification, and so the proportions of individuals who carry an *APOE* ε4 allele and who are Aβ+ are likely to be greater than that of the general population. Second, the self-reporting of

sociodemographic factors creates the potential for self-report bias, where participants' responses may be altered by social desirability, memory, and survey conditions.[44] This may impact on the reporting of age, years of education, and sex. Further, the use of a single cohort means that replication of the *APOE,* sex, CSF $A\beta_{42}$ interaction was not possible. The cross-sectional analysis of data also means that it is difficult to draw conclusions about the temporal relationship between CSF $A\beta_{42}$ and CSF p-tau accumulation. Future work would benefit from replication the current analysis and the addition of longitudinal data, with diverse sample populations.

To conclude, the current study provides evidence of a sex difference in the interaction between CSF p-tau, *APOE* genotype, and CSF $A\beta_{42}$. We found evidence that *APOE* carriership $\varepsilon 4$ is more tightly coupled with CSF $A\beta_{42}$ and CSF p-tau in males than females, and that elevated CSF p-tau in *APOE* $\varepsilon 4$ females appears to be independent of CSF $A\beta_{42}$ levels.

Contributors

TS conducted formal analyses of data, wrote the original draft of the manuscript, and created figures. NJ was involved in data analysis/data cleaning and provided critical feedback of the manuscript. KB was responsible for cerebrospinal fluid assays and reviewed and provided critical feedback of the manuscript. CR is the chief investigator of the EPAD Longitudinal Cohort Study and provided clinical input, supervision, and reviewed and gave critical feedback of the manuscript. GM conceptualised the analyses, provided supervision, and gave statistical input and reviewed and gave critical feedback of the manuscript. All authors read and approved the final version of the manuscript. TS and GM verified the underlying data and code and CR also reviewed the data as the principal investigator of EPAD.

Data sharing statement

A publicly available dataset was analysed in this study. This dataset can be found at http://ep-ad.org/erap/, doi: 10.34688/epadlcs_v.imi_20.10.30 and is available by application.

Declaration of interests

KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, 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. KB has received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from GEECD, Rochie Diagnostics, and IFCC/SNIBE. CR has served as a consultant for Roche, Eli Lilly, Merck, Biogen, Eisai, Roche Diagnostics, and Actinogen. CR has participanted on a data safety monitory board/advisory board for the DESPIAD study (an NIHR funded project), and is the director for Brain Health Scotland, and the chair for the Scottish Dementia Research Consortium. TS, GM, NJ have no conflicts of interest to report.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104241.

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