Sex differences in CSF biomarkers vary by Alzheimer's disease stage and APOE ε4 genotype

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ABSTRACT

Objective: To evaluate sex-differences in cerebrospinal fluid (CSF) biomarkers, taking the potential modifying role of clinical disease stage and APOEe4 genotype into account.

Method: We included participants (n=1801) with probable AD dementia (n=937), Mild Cognitive Impairment (MCI; n=437) and Subjective Cognitive Decline (SCD; n=427). Main outcomes were CSF amyloid β1-42 (Aβ42), total tau (Tau) and tau phosphorylated at threonine 181 (pTau) levels. Age corrected three-way interactions between sex, disease stage (i.e. syndrome diagnosis at baseline) and APOEe4 were tested with linear regression analyses for each outcome measure. In case of significant interactions ($p<0.05$), sex-differences were further evaluated by stratifying analyses for clinical disease stage and APOEe4 genotype including age as a covariate. ~~Covariates included age (model 1), and additionally MMSE and educational level (model 2).~~

Results: Three-way interactions were significant for Tau ($p<0.001$) and pTau ($p<0.01$), but not Aβ42. In APOE carriers, women showed higher (p)Tau concentrations than men in SCD (Tau:β±se= 0.25±0.08, $p=0.002$; pTau:β±se= 0.16±0.06, $p=0.009$) and MCI (Tau:β±se=0.29±0.07, $p<0.001$; pTau:β±se=0.21±0.06, $p<0.001$), but not AD dementia. In APOE non-carriers, women showed higher (p)Tau concentrations in MCI (Tau:β±se= 0.22±0.09, $p=0.012$; pTau:β±se= 0.19±0.08, $p=0.013$) and AD dementia (Tau: β±se= 0.20±0.07, $p=0.006$; pTau:β±se= 0.14±0.06, $p=0.014$), but not in SCD.

Conclusions: Within APOEe4 carriers, sex-differences in CSF (p)Tau are more evident in early disease stages, whereas for APOEe4 non carriers sex-differences are more evident in advanced disease stages. These findings suggest that the effect of APOE e4 on sex-differences in CSF biomarkers depends on disease stage in AD.

Introduction

Alzheimer's Disease (AD) is a global health care challenge due to the rapidly growing disease prevalence and the lack of preventive or curative treatment.[1] Therefore, knowledge regarding the underlying pathophysiological process of AD that could potentially contribute to the development of treatments is needed.[2] Current knowledge indicates that AD is characterized by initial brain depositions of amyloid-β (Aβ), followed by accumulation of neurofibrillary tangles (NFT).[3–5] A growing body of literature is pointing towards sex-differences in AD neuropathology, with women showing a higher NFT burden, while differences in Aβ are less apparent.[6–10] Additionally, there are indications that sex-differences in AD biomarkers are modified by the presence of the Apolipoprotein (APOE) e4 allele, the major genetic risk factor for sporadic AD.[6,9] *In-vivo* studies have shown that female APOEe4 allele carriers have higher cerebrospinal fluid (CSF) total Tau and phosphorylated Tau (pTau) concentrations than male APOEe4 carriers.[6,9,10] However, post-mortem studies do not show this sex-specific association.[6,7] A possible explanation for these seemingly discrepant results is that *in-vivo* studies mostly included pre-dementia subjects (i.e. normal cognition or Mild Cognitive Impairment (MCI)), whereas post-mortem studies largely included end-stage dementia patients. It could be hypothesized that the association between APOEe4 genotype and sex-differences in AD neuropathology may be present in initial phases of the disease, but diminishes as the disease progresses, and is no longer seen in end-stage AD dementia during post-mortem examinations. Therefore, in the present study, we investigated whether sex-differences in CSF Aβ42, Tau and pTau are modified by APOEe4 genotype and clinical disease stage.

Methods

Subjects

We selected 1801 subjects who visited our outpatient clinic between October 2000 and July 2015. Selection from the Amsterdam Dementia Cohort was based on a clinical diagnosis of probable AD dementia (*n*=937), MCI (*n*=437) or Subjective Cognitive Decline (SCD; *n*=427, and the availability of CSF biomarker results and APOE genotype.[11,12] There were no exclusion criteria. All participants underwent a standardized dementia screening at baseline that included physical and neurological examination, a neuropsychological test battery including a Mini Mental State Examination (MMSE), Electroencephalogram (EEG), Magnetic Resonance Imaging (MRI), and laboratory tests. Clinical diagnosis was given by consensus in a multidisciplinary team according to international research and clinical criteria. Subjects were labeled as having SCD when results of clinical examinations and test results were normal (i.e. criteria for MCI or dementia were not fulfilled, and no psychiatric diagnosis was given). MCI subjects were labeled according to the criteria by Petersen et al. and the National Institute on Aging and Alzheimer's Association (NIA-AA) clinical criteria.[13,14] The core clinical NIA-AA criteria were met for all probable AD patients.[15,16] Clinical diagnosis at time of lumbar puncture, that is at baseline visit, was used to reflect clinical AD disease stage (i.e. syndrome diagnosis). Sex was self-reported and defined as a biological characteristic that discriminate women from men.[17] Educational levels were reported according to the Verhage scoring system.[18]

Patient consents and Data availability statement

All subjects gave written informed consent and the study was approved by the local ethical review board.[11,12] Anonymized data will be shared by request from any qualified investigator.

In-vivo markers of AD pathology

CSF Aβ42, Tau and pTau concentrations were used as *in-vivo* markers for the presence of AD pathology. CSF samples were collected and processed according to international consensus protocols as previously described.[19,20] Commercially available ELISAs were employed to measure baseline Aβ42, Tau and pTau (Innotest β-amyloid(1-42), Innotest hTAU-Ag and Innotest Phosphotau(181P); Fujirebio, Ghent, Belgium) concentrations. Intra- and inter-assay variations for all analyses were below 3.2% and 10.9% respectively.[21] The team performing the CSF analyses was not aware of the clinical diagnosis. To correct for the drift in CSF Aβ42 concentrations throughout the analysis-years we used adjusted Aβ42 concentrations. [22,23] Cut-offs to determine abnormality were <813 pg/ml for Aβ42 [23] and >375 pg/ml for t-tau[24].

MRI measurements

MRI measurements were acquired on 3T whole-body MR system (Discovery; GE Medical Systems Milwaukee, WI, USA), using an eight-channel head coil at the Amsterdam UMC, location VUmc. Medial temporal lobe atrophy (MTA) scores ranged from 0-4, and were rated on coronal reconstructions of T1-weighted images.[25] Posterior cortical atrophy (PCA) and global cortical atrophy (GCA) scores ranged from 0-3, and were rated on the combination of T1-weighted and FLAIR sequences (PCA), or FLAIR sequences alone (GCA).[26] White matter hyperintensities (WMH) were rated on FLAIR images using the Fazekas scale, with scores ranging from 0-3.[27] The imaging took about 40 minutes in total. There was no intravenous contrast administration. All scans were evaluated by an experienced neuroradiologist.

Apolipoprotein E genotyping

DNA was isolated from 10 ml vacutainer tubes containing EDTA using the QIAamp DNA blood isolation kit from Qiagen (Venlo, The Netherlands). Followed by genotype

159 determination using the LightCycler ApoE mutation Detection Kit (Roche Diagnostics,
160 GmbH, Mannheim, Germany). Subjects with at least one APOEe4 allele were defined as
161 APOEe4 carriers, whereas no e4 allele defined subjects as non-carriers.

162 *Statistical analysis*

163 Statistical analyses were completed using R studio (version: 3.3.2; "sincere Pumpkin Patch").
164 Prior to performing statistical analyses, Tau and pTau were log transformed as they were not
165 normally distributed. Demographical and clinical data were compared between groups using
166 independent t-tests, chi-square tests and Mann-Whitney U tests as appropriate. To assess how
167 sex-differences in biomarkers depend on APOEe4 genotype and clinical disease stage, we
168 used General Linear Models (GLM) with factors sex, APOEe4 genotype and clinical disease
169 stage, their 2-way interactions and 3-way interactions, and age was included as a covariate
170 (see table 3 for full models). In case of a significant interaction between sex, APOEe4
171 genotype and clinical disease stage, we performed GLM in CSF biomarker concentrations
172 stratified for APOE genotype and clinical disease stage as shown in figure 1. These analyses
173 included sex as a factor and age as covariate. ~~and additionally MMSE and education (model~~
174 ~~2).~~ We repeated analyses restricted to subjects with abnormal CSF Aβ42 concentrations (<813
175 pg/ml) to study the specificity of the findings for the AD spectrum. Reported Effect Sizes
176 were calculated as the difference of the means of two groups divided by the weighted pooled
177 standard deviations of these groups according to Cohen's d statistics. We adjusted for multiple
178 testing by multiplying *p*-values with the number of tests (i.e. 12) according to the Bonferroni
179 method. In an additional set of sensitivity analyses, three-way interactions were repeated in
180 the total sample stratified by age (cut-off: median 67 years) to assess the effects of age. Three-
181 way interactions included factors sex, clinical disease stage and APOEe4 genotype, their 2-
182 way interactions, and main factors. Separate models were run for CSF Tau and pTau. Finally,
183 we performed two additional analyses to test APOE genotype dose effects: 1) we repeated

analyses after excluding APOE e2e4 carriers (n=43), as the conveyed risk of APOE e2e4 carriers for AD is not fully known; 2) We evaluated the effect of e2 ((i.e. e2e2 = 4; e2e3= 111)) and e4 allele carriers (i.e. e3e4 =690; e4e4 = 298) against the e3 allele for sex effects on CSF biomarkers. $p<0.05$ was considered significant for main and interaction effects.

Results

Within SCD, the majority was male (61%), and females and males showed a similar frequency of APOEe4 (F: 38% vs M: 37%, $p>0.05$). Within MCI, the majority was male (62%), females showed a higher frequency of APOEe4 than males (F: 65% vs M: 50%, $p=0.002$), and females had less atrophy than males (table 1). Within AD dementia the percentage of females and males was similar (52% vs 48%), as was the frequency of APOEe4 carriers between females and males (F: 67% vs. M: 68%, $p>0.05$). Females had less medial temporal atrophy than males (F:1.32 vs M:1.52, $p>0.01$). Females and males did not differ in age within clinical disease stages. In MCI and AD dementia, males had higher educational levels and higher MMSE scores compared to females. Subject characteristics of the CSF amyloid positive cohort was largely comparable to the total cohort (table 2).

For CSF Aβ42, age adjusted general linear models including sex, APOEe4 genotype, diagnosis and all interactions showed main effects for diagnosis and APOEe4 genotype, but not for sex. There were no significant interactions. For CSF Tau and pTau there was a three-way interaction between sex, APOEe4 genotype, and clinical diagnosis (full models shown in table 3). Therefore, we further stratified these analyses for APOEe4 and clinical diagnosis as shown in figure 1.

Within APOEe4 carriers, females showed higher Tau and pTau concentrations than males in SCD (Cohens *d* (95%CI): Tau= 0.52 (0.19-0.84), p_{adj}= .008; pTau=0.44 (0.11-0.77) p_{adj}=.05)

208 and MCI (Cohens *d* (95%CI): Tau= 0.54 (0.28-0.80), p_{adj}=.0006; pTau=0.52 (0.26-0.77)
209 p_{adj}=.001), but not in AD dementia (figure 1~~; table 2~~). Within APOEe4 non-carriers, females
210 showed higher Tau and pTau concentrations than males in MCI (Cohens *d* (95%CI): Tau=
211 0.49 (0.17-0.80), p_{adj}=.02; pTau=0.47 (0.16-0.78) p_{adj}=.04) and AD dementia (Cohens *d*
212 (95%CI): Tau= 0.42 (0.19-0.65), p_{adj}=.006; pTau=0.38 (0.15-0.61) p_{adj}=.02), but not in SCD
213 (figure 1~~; table 2~~). When restricting analyses to individuals with abnormal CSF Aβ42, results
214 for Tau and pTau were largely comparable with that of the total cohort, albeit significance
215 was overall somewhat attenuated for Tau and pTau, and was lost for Tau in APOE e4 carriers
216 in the SCD stage and for pTau in non-carriers in the MCI stage due to a smaller effect size
217 (table 4).

218 In an additional analysis, we stratified for age and found a significant three way interaction
219 between sex, APOE genotype and diagnosis for CSF (p)Tau in older individuals (median
220 [IQR]: 72.2 [69.5-76.0]), but not in younger individuals (median [IQR]: 61.3 [58.4-64.1])
221 (full models shown in table 5). When we repeated the analyses in the sample excluding APOE
222 e2e4 carriers (n=43), results remained essentially unchanged (full models shown in table 6).
223 Finally, we studied dose effects for APOE genotypes and found that APOEe2 carriers
224 behaved similar to APOEe3 carriers for all clinical disease stages (supplementary table 1).

Discussion

Our findings suggest that APOE differentially affects sex-differences in CSF biomarkers throughout the AD spectrum. Within APOEe4 carriers, females show higher Tau and pTau concentrations in early disease stages (i.e. SCD and MCI) which equalized in the later dementia stage. Within APOE non-carriers, we observed an opposite pattern, with females showing higher Tau and pTau concentrations in later disease stages (i.e. AD dementia and MCI), but not in the early disease stage of SCD. We did not find sex-differences in Aβ concentrations between females and males for any disease stage or APOEe4 genotype. Although derived from cross-sectional data our findings suggest that within APOEe4 carriers sex-differences in Tau and pTau become less evident in advanced disease stages, whereas for APOEe4 non-carriers sex-differences in Tau and pTau become more evident in advanced disease stages.

Our results seem to be in line with previous studies who reported higher CSF Tau and pTau concentrations for female APOEe4 carriers.[6,9,10,28,29] We further expand on those studies by showing that the sex-specific interaction of APOEe4 on CSF biomarkers depends also on disease stage. Here we observed that female APOE e4 carriers had higher Tau and pTau concentrations than male APOE e4 carriers in the SCD and MCI stage of AD, which is in line with previous work in cognitively normal older adults and MCI subjects. [9,10,28,30] Additionally, a recent other study showed that within cognitively normal amyloid positive adults, especially female APOE e4 carriers exhibited accelerated rates of longitudinal CSF (p)Tau concentrations.[31] We further show that at the AD dementia stage these sex-differences within APOEe4 carriers seem to diminish. This could possibly explain why multi-cohort autopsy studies did not find an interaction between sex and APOEe4.[6,7]

249 It has been suggested that the sex-difference in APOEe4 carriers diminishes with increasing
250 age, as previous studies only found a sex-specific interaction with APOE e4 in younger
251 individuals (<75 years). [6,32] Seemingly in contrast to these previous studies, stratification for
252 age in our study revealed that results were largely attributable to older elderly individuals
253 (>67 years). However, closer inspection of the data in fact shows that our 'older' participants
254 fall within the same age range as the 'younger' participants in former studies (i.e. 65-75
255 years). [6,32] Another explanation for these age dependent sex-differences could be that younger
256 and older participants might have different underlying AD pathological mechanisms. For
257 instance, younger participants with a similar cognitive status as older individuals, may not
258 reflect an earlier phase of AD than older participants and vice versa. Therefore, it could be
259 possible that younger individuals might have more (unknown) genetic risk factors for AD,
260 which in turn influence Tau accumulation and sex-differences in Tau concentrations.[33] Taken
261 together, our results support the idea that the sex by APOEe4 interaction depends on age, and
262 we further show that this interaction effect depends also on clinical disease stage as well.
263
264 In the latest framework for AD, Tau is considered a marker for neuronal injury [5]. As such,
265 higher Tau and pTau concentrations in female APOEe4 carriers may imply an initial steeper
266 pathological disease course, and more neurodegenerative change compared to male APOEe4
267 carriers. Increased neurodegenerative changes in female APOEe4 carriers was also implied by
268 previous studies who reported increased hypometabolism and (hippocampal) atrophy [34], and a
269 decreased hippocampal connectivity. [35] However, other studies have shown discordant
270 findings. Previous population studies reported lower hippocampal volume in males compared
271 to females [29], and more rapid parahippocampal atrophy in amyloid positive males compared
272 to amyloid negative females.[36] In the current study, we observed more atrophy in males than
273 females in both APOEe4 carriers and non-carriers, which seems in contrast to the higher tau

levels we found in females. This suggests that discrepant findings between CSF Tau and atrophy may reflect different pathological processes, and should perhaps not be used interchangeably. Similar discrepant findings between tau and MRI have been reported previously, which may depend on sex as well.[37] Future research combining *in-vivo* CSF biomarker and MRI data with pathology data is needed to examine the relationship with each other and neuropathology.

Possible explanatory biological mechanisms for higher levels of Tau and pTau in females might be related to abrupt hormonal changes that have occurred in post-menopausal women.[17] A drastic drop of estradiol levels in post-menopausal women, has shown to lead to an increased activity of enzymes involved in Tau phosphorylation (GSK3-β and Protein Kinase A), thus resulting in a higher pTau concentrations.[38,39] In addition, post-menopausal women show indications of increased oxidative stress and mitochondrial dysfunction, which through cell death and apoptosis mechanisms may lead to increases in CSF Tau concentrations.[5,39] APOE e4 has also been associated with higher levels of CSF Tau and NFT's,[2] and it was shown to stimulate Tau phosphorylation as a result of impaired cholesterol exchange between neuronal and non-neuronal cells.[6,31,41,42] Therefore, it is conceivable that the lack of neuroprotective effects of estrogen together with the presence of APOE e4 might act synergistically, leading to increased Tau concentrations in female APOE e4 carriers. ~~Further supporting this hypothesis is the observation that post-menopausal female APOE e4 carriers on estrogen replacement therapy show more signs of neuroprotection compared to non-treated female carriers.~~ Another factor that may influence tau levels is cerebrovascular injury. [43–45] Therefore, it could be that the sex-difference in CSF Tau concentrations is caused by a difference in the amount cerebrovascular injury between both sexes. However, we did not observe a difference between sexes in Fazekas scores, which are considered a marker of

cerebrovascular injury .[46] Further (fundamental) research is needed to discover the true underlying cause of the sex-differences seen in AD.

Other possible explanations for higher Tau and pTau concentrations in female APOEe4 carriers in the earlier stages of the disease spectrum, could be a difference in survival between females and males. A faster disease progression in males or a higher mortality rate of the "very sick" men dying from comorbidities at young ages could possibly cause an overrepresentation of "healthier" males with lower levels of neuropathology (i.e. CSF Tau concentrations) therefore making it seem as though women have higher Tau concentrations. However, in our cohort this seems less likely since men had more atrophy in general.

~~In our MMSE and education adjusted analyses, we found that females with similar MMSE scores and educational level to men, had higher Tau and pTau levels. Moreover, despite having higher Tau levels, and, possibly more neurodegeneration, females were given the same clinical diagnosis as men. These findings seemingly imply that the females in our cohort have more cognitive reserve than males. Cognitive reserve has been defined as the difference between individuals in their ability to preserve cognitive function in the presence of neuropathology.[47] Previous ADNI studies have shown that the female advantage in verbal memory is maintained despite similar levels of temporal hypometabolism and moderate hippocampal atrophy between females and males.[48,49] This would imply that women at first better compensate for neuropathology and maintain cognitive function, thus have a greater cognitive reserve.~~

In our study population, females and males showed similar Aβ42 concentrations within both APOEe4 genotypes throughout clinical stages of AD. Our findings align with previous work, which suggest that sex-differences in AD pathology mainly occur in Tau and pTau

concentrations, downstream from amyloid deposition.[6,7,9,10,29–31,50] Interestingly, within the current NIA-AA criteria[5] CSF total tau and pTau are seen as markers for separate pathological mechanisms, where one represents neuronal injury (Tau) and the other is a specific marker for Alzheimer's disease pathology (pTau). However, in our cohort Tau and pTau are highly correlated ($r= 0.93$, $p<0.001$), and sex-differences in both APOEe4 carriers and non-carriers were similar for Tau and pTau. This suggests that Tau and pTau to some extent reflect similar or overlapping aspects of neuronal injury.

Among the limitations of our study is that to adequately assess the temporal order of AD pathology markers, longitudinal data are needed. Moreover, our data are derived from a tertiary memory clinic cohort which consist of a relatively young population, and may result in a lack of generalizability of our findings. Among the strengths of this study is the use of a large well-defined cohort, and the careful clinical work-up that was used to diagnose all participants. Moreover, as our findings were largely replicable in an Aβ42 positive subset, we were able to show that our findings were specific for the AD spectrum. Our data show that a woman's brain can be more susceptible to Tau pathology depending on disease stage and APOEe4 genotype. The effect sizes we found for (p)Tau concentrations between women and men were moderate, and therefore not large enough for clinical use, for instance by developing sex-specific cut-offs for Tau or pTau. However, a moderate difference in the underlying pathology of AD between women and men is large enough to be taken into consideration when developing disease modifying therapies.

In conclusion, within APOEe4 carriers sex-differences in Tau and pTau become less evident in advanced disease stages, whereas in APOEe4 non-carriers sex-differences in Tau and pTau become more evident in advanced disease stages. These findings largely remain for the amyloid positive subgroup. Our findings imply a difference in neuropathological trajectories

for women and men depending on APOEe4 genotype, and add to a growing body of evidence of sex-differences in the underlying mechanism of AD.

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Tables

Table 1. Subject characteristics for the total cohort, stratified per APOE e4 genotype and clinical disease stage.

		Total cohort		APOE e4 carrier		APOE e4 non-carrier	
		females	males	females	males	females	males
SCD	n, (%)	167 (39)	260 (61)	63 (40)	95 (60)	104 (39)	165 (61)
	Age, mean (SD), y	64.9 (6.5)	64.4 (6.2)	64.6 (6.2)	64.6 (6.4)	64.8 (6.7)	64.0 (6.1)
	MMSE, mean (SD)	28 (1.6)	28 (1.8)	28 (1.8)	28 (1.7)	28 (1.5)	28 (1.7)
	Education, mean (SD) [a]	5.3 (1.3)	5.4 (1.3)	5.4 (1.3)	5.5 (1.2)	5.3 (1.3)	5.5 (1.3)
	CSF Aβ42, pg/ml, mean (SD)	1032 (252)	1070 (263)	908 (245)	964 (265)	1114 (224)	1131 (232)
	Abnormal CSF Aβ42, n (% within sex) [b]	39 (23)	52 (19)	26 (41)	29 (31)	12 (12)	17 (11)
	CSF LN Tau, pg/ml, mean (SD)	5.7 (0.5)	5.6 (0.5)	5.9 (0.5)	5.6 (0.5)**	5.5 (0.5)	5.6 (0.5)
	Abnormal CSF Tau, n (% within sex) [b]	42 (24)	60 (22)	25 (40)	18 (19)*	15 (15)	33 (20)
	Fazekas, mean (SD) [c]	0.69 (0.76)	0.74 (0.74)	0.56 (0.82)	0.75 (0.82)	0.75 (0.74)	0.75 (0.75)
	MTA, mean (SD) [c]	0.35 (0.51)	0.38 (0.50)	0.33 (0.59)	0.45 (0.59)	0.36 (0.47)	0.34 (0.48)

	PCA, mean (SD) [c]	0.54 (0.70)	0.62 (0.68)	0.49 (0.79)	0.60 (0.76)	0.55 (0.67)	0.65 (0.68)
	GCA, mean (SD) [c]	0.38 (0.60)	0.47 (0.59)	0.32 (0.63)	0.49 (0.63)	0.41 (0.59)	0.49 (0.60)
MCI	n, (%)	168 (38)	269 (62)	109 (45)	134 (55)	59 (30)	135 (70)
	Age, mean (SD), y	68.5 (6.9)	68.3 (7.1)	68.1 (6.4)	67.7 (6.5)	68.9 (7.6)	68.1 (7.2)
	MMSE, mean (SD)	26 (2.4)	27 (2.4)*	26 (2.5)	27 (2.6)	26 (2.6)	27 (2.2)*
	Education, mean (SD)[a]	4.8 (1.3)	5.2 (1.4)*	4.9 (1.3)	5.3 (1.4)*	4.7 (1.4)	5.2 (1.4)*
	CSF Aβ42, pg/ml, mean (SD)	774 (247)	867 (290)***	688 (156)	757 (249)*	925 (311)	977 (283)
	Abnormal CSF Aβ42, n (% within sex)[b]	127 (72)	163 (53)***	89 (84)	94 (71)*	28 (48)	46 (34)
	CSF LN Tau, pg/ml, mean (SD)	6.2 (0.6)	5.9 (0.6)***	6.3 (0.5)	6.0 (0.6)***	6.0 (0.6)	5.7 (0.5)**
	Abnormal CSF Tau, n (% within sex)[b]	124 (70)	142 (46)***	82 (77)	79 (59)*	33 (57)	41 (30)**
	Fazekas, mean (SD)[c]	1.16 (0.98)	1.01 (0.93)	1.18 (0.99)	0.88 (0.93)*	1.12 (1.03)	1.15 (1.00)
	MTA, mean (SD)[c]	0.64 (0.85)	1.03 (0.81)***	0.62 (0.82)	0.94 (0.77)**	0.67 (0.95)	1.14 (0.94)**
	PCA, mean (SD)[c]	0.71 (0.74)	0.93 (0.70)**	0.73 (0.76)	0.92 (0.73)	0.68 (0.77)	0.93 (0.76)*
	GCA, mean (SD)[c]	0.68 (0.68)	0.90 (0.65)***	0.66 (0.68)	0.89 (0.64)**	0.72 (0.76)	0.93 (0.75)

AD dementia	n, (%)	488 (52)	449 (48)	325 (51)	307 (49)	163 (53)	142 (47)
	Age, mean (SD), y	67.4 (7.2)	67.3 (7.2)	66.7 (6.7)	67.5 (6.8)	67.7 (7.5)	67.0 (7.5)
	MMSE, mean (SD)	20 (4.8)	21 (5.1)***	20 (4.8)	21 (8.1)*	19 (5.0)	22 (4.7)***
	Education, mean (SD)[a]	4.6 (1.3)	5.0 (1.4)***	4.7 (1.2)	5.0 (1.4)**	4.5 (1.3)	5.1 (1.4)***
	CSF Aβ42, pg/ml, mean (SD)	663 (173)	649 (164)	638 (132)	626 (129)	709 (221)	705 (214)
	Abnormal CSF Aβ42, n (% within sex)[b]	460 (90)	447 (91)	296 (82)	286 (82)	130 (82)	112 (82)
	CSF LN Tau, pg/ml, mean (SD)	6.5 (0.5)	6.4 (0.5)***	6.5 (0.5)	6.4 (0.5)	6.5 (0.5)	6.2 (0.6)***
	Abnormal CSF Tau, n (% within sex)[b]	437 (85)	390 (79)*	268 (85)	248 (82)	135 (85)	103 (75)*
	Fazekas, mean (SD)[c]	1.0 (0.88)	0.95 (0.85)	1.04 (0.90)	0.94 (0.91)	0.95 (0.87)	0.95 (0.82)
	MTA, mean (SD) [c]	1.32 (0.90)	1.51 (0.88)**	1.32 (0.93)	1.53 (0.93)**	1.28 (0.90)	1.45 (0.85)
	PCA, mean (SD) [c]	1.26 (0.83)	1.33 (0.81)	1.17 (0.83)	1.32 (0.84)*	1.45 (0.87)	1.37 (0.83)
	GCA, mean (SD) [c]	1.11 (0.73)	1.20 (0.71)	1.03 (0.74)	1.21 (0.74)**	1.27 (0.78)	1.14 (0.72)

Table shows mean (SD), unless otherwise specified. Independent t-test. chi-square test and Mann-Whitney U test were applied where applicable. *P* <0.05 is considered significant: *

p<0.05, ** < 0.01, *** p<0.001. [a] Education according to Verhage score. [b] Cut-off for Aβ42: 813 pg/ml, and Tau: 375 pg/ml. [c] adjusted for age. Abbreviations: APOE, Apolipoprotein E4; SCD, Subjective Cognitive Decline; MCI, Mild Cognitive Impairment; AD, Alzheimer's Disease; MMSE, Mini Mental State Exam; CSF, Cerebrospinal Fluid; Aβ42, Amyloid-β 1-42; Tau, total tau, MTA, Medial-Temporal Atrophy; PCA, Posterior Cortical Atrophy; GCA, Global Cortical Atrophy.

Table 2. Subject characteristics CSF Aβ42 positive subgroup stratified per APOE e4 genotype and clinical disease stage.

		APOE e4 carrier		APOE e4 non-carrier	
		Females	Males	Females	Males
SCD	n, (%)	26 (47)	29 (53)	12 (41)	17 (59)
	Age, mean (SD), y	66.7 (5.7)	66.8 (6.1)	67 (8.1)	70 (7.5)
	MMSE, mean (SD)	28 (1.5)	28 (2.0)	29 (1.1)	29(0.8)
	Education, mean (SD) [a]	5.5 (1.1)	5.1 (1.3)	4.8 (1.4)	5.6 (1.4)
	CSF Aβ42, pg/ml, mean (SD)	688 (82)	657 (113)	711 (85)	687 (114)
	CSF LN Tau, pg/ml, mean (SD)	6.1 (0.5)	5.9 (0.6)	5.8 (0.8)	5.8 (0.7)
	CSF Tau positive, n (% within sex)[b]	14 (58)	10 (42)	5 (36)	9 (64)
MCI	n, (%)	89 (49)	94 (51)	28 (38)	46 (62)
	Age, mean (SD), y	68.7 (6.0)	68.5 (6.7)	70.8 (7.7)	69.5 (7.2)
	MMSE, mean (SD)	26 (2.5)	26 (2.5)	26 (2.9)	27 (2.5)
	Education, mean (SD) [a]	4.9 (1.4)	5.4 (1.4)*	4.9 (1.3)	5.2 (1.6)
	CSF Aβ42, pg/ml, mean (SD)	638 (98)	629 (106)	686 (87)	659 (110)
	CSF LN Tau, pg/ml, mean (SD)	6.4 (0.4)	6.2 (0.5)**	6.3 (0.6)	6.0 (0.6)*
	CSF Tau positive, n (% within sex)[b]	74 (51)	72 (49)	22 (48)	24 (52)
AD	n, (%)	296 (51)	286 (49)	130 (54)	112 (46)
	Age, mean (SD), Y	66.6 (6.7)	67.3 (6.7)	66.9 (7.6)	66.7 (7.3)
	MMSE, mean (SD)	20 (4.9)	21 (5.0)	19 (5.0)	21 (4.6) ***

	Education, mean (SD)[a]	4.8 (1.2)	5.0 (1.4)**	4.6 (1.3)	5.2 (1.4)**
	CSF Aβ42, pg/ml, mean (SD)	618 (98)	609 (99)	624 (96)	620 (100)
	CSF LN Tau, pg/ml, mean (SD)	6.5 (0.5)	6.4 (0.5)	6.6 (0.5)	6.3 (0.6)
	CSF Tau positive, n (% within sex)[b]	256 (52)	238 (48)	122 (58)	90 (42)

Subject characteristics for the amyloid positive subgroup shown per clinical disease stage and APOE e4 genotype. Independent t-test, chi-square test and Mann Whitney U test were applied were applicable. *P* <0.05 is considered significant: * p<0.05, ** p<0.01, *** p<0.001. [a] Education according to Verhage score. [b] Cut-off Tau: 375 pg/ml.

Abbreviations: APOE, Apolipoprotein E4; SCD, Subjective Cognitive Decline; MCI, Mild Cognitive Impairment; AD, Alzheimer's Disease; MMSE, Mini Mental State Exam; IQR, Interquartile Range.

Table 3. Full models for sex, APOE e4 genotype and clinical disease stage per CSF biomarker.

	aβ42		Tau [d]		pTau [d]	
	β (se)	*p*-value	*β* (se)	*p*-value	*β* (se)	*p*-value
Sex: male [a]	14.5 (26.3)	0.58	0.04 (0.07)	0.58	0.02 (0.05)	0.68
Diagnosis: MCI [b]	-180.9(34.2)	<0.001	0.45 (0.09)	<0.001	0.27 (0.07)	<0.001
Diagnosis: AD [b]	-398.9(26.5)	<0.001	0.91 (0.07)	<0.001	0.60 (0.05)	<0.001
APOE: carrier [c]	-206.7(33.2)	<0.001	0.34 (0.09)	<0.001	0.24 (0.07)	<0.001
Sex: male* diagnosis: MCI [ab]	35.5 (41.8)	0.40	-0.32 (0.11)	0.004	-0.25 (0.08)	0.004
Sex: male* diagnosis: AD [ab]	-18.2 (35.8)	0.61	-0.27 (0.09)	0.003	-0.18 (0.07)	0.01
Sex: male* APOE: carrier [ac]	41.3 (42.8)	0.33	-0.31 (0.11)	0.006	-0.20 (0.09)	0.03
Diagnosis: MCI* APOE: carrier [bc]	-31.5 (47.4)	0.51	0.009 (0.12)	0.98	0.05 (0.10)	0.62
Diagnosis: AD* APOE: carrier [bc]	133.4 (38.9)	<0.001	-0.33 (0.10)	<0.001	-0.22 (0.08)	0.004
Sex: male*diagnosis: MCI* APOE: carrier [abc]	-24.0 (60.2)	0.69	0.28 (0.15)	0.07	0.21 (0.12)	0.09
Sex: male*diagnosis: AD* APOE: carrier [abc]	-49.0 (52.0)	0.35	0.46 (0.13)	<0.001	0.33 (0.11)	0.002

We used General Linear Models (GLM) with factors sex, APOE e4 genotype and clinical disease stage, their 2-way interactions and 3-way interactions, and age was included as a covariate. This full model was run separately for each of the three biomarkers (i.e. aβ42, Tau and pTau). [a] Reference is female sex. [b] Reference is SCD. [c] Reference is APOE non-carrier. p <0.05 is considered significant. [d] The natural logarithm of Tau and pTau concentrations are shown. Abbreviations: APOE, Apolipoprotein E4; SCD, Subjective Cognitive Decline; MCI, Mild Cognitive Impairment; AD, Alzheimer's Disease.

Table 4. Differences in CSF Tau and pTau concentrations in men compared to women in the amyloid positive subgroup stratified per clinical diagnosis and APOE e4 genotype.

		Tau				pTau			
		Adjusted mean difference β(se)[a]	Effect size (95%CI)	*p*-value	Adjusted *p*-value	Adjusted mean difference β(se)[a]	Effect size (95%CI)	*p*-value	Adjusted *p*-value
APOE e4 carrier	SCD	-0.26 (0.14)	0.44 (-0.11-0.99)	0.07	0.84	-0.15 (0.11)	0.33 (-0.22–0.87)	0.18	2.16
	MCI	-0.20 (0.07)	0.42 (0.12-0.71)	0.006	0.07	-0.14 (0.06)	0.37 (0.08– 0.67)	0.01	0.12
	AD dementia	-0.07 (0.04)	0.13 (-0.03–0.29)	0.13	1.56	-0.03 (0.04)	0.06 (-0.10–0.23)	0.45	5.4
APOE e4 non-carrier	SCD	-0.15 (0.27)	0.02 (-0.76–0.79)	0.59	7.08	-0.01 (0.22)	-0.13(-0.90-0.65)	0.95	11.4
	MCI	-0.30 (0.15)	0.52 (0.04 – 1.0)	0.04	0.48	-0.24 (0.13)	0.46 (-0.03-0.94)	0.08	0.96
	AD dementia	-0.26 (0.07)	0.24 (0.24– 0.75)	<0.001	0.004	-0.18 (0.06)	0.44 (0.18-0.69)	0.002	0.02

Differences in CSF Tau and pTau for women and men in the amyloid positive subgroup stratified per APOE e4 genotype and clinical diagnosis. Tau and pTau were log transformed to meet assumptions of normality. We performed GLM in CSF biomarker concentrations stratified for APOE genotype and clinical disease stage as shown in figure 1. These analyses included sex as a factor and age ~~(model 1), and additionally MMSE and education (model 2)~~ as covariate. Cohen's d statistics were used to calculate effect sizes: small=0.2, medium= 0.5, large= 0.8. p <0.05 is considered significant. [a] Reference is female sex. [b] Adjustments for age at time of lumbar puncture. Abbreviations: APOE, Apolipoprotein E4; SCD, Subjective Cognitive Decline; MCI, Mild Cognitive Impairment; AD, Alzheimer's Disease; MMSE, Mini Mental State Exam; 95% CI, 95% Confidence Interval.

Table 5. Full models for sex, APOE e4 genotype and clinical disease stage for (p)Tau stratified per age group.

	<67 years				≥67 years			
	Tau [d]		pTau [d]		Tau [d]		pTau [d]	
	β (se)	*p*-value	β (se)	*p*-value	β (se)	*p*-value	β (se)	*p*-value
Sex: male [a]	0.05 (0.08)	0.57	0.01 (0.07)	0.84	0.04 (0.11)	0.73	0.04 (0.09)	0.66
Diagnosis: MCI [b]	0.40 (0.13)	0.002	0.30 (0.10)	0.005	0.37 (0.12)	0.003	0.24 (0.10)	0.02
Diagnosis: AD [b]	1.05 (0.09)	<0.001	0.71 (0.07)	<0.001	0.74 (0.11)	<0.001	0.49 (0.09)	<0.001
APOE: carrier [c]	0.20 (0.10)	0.05	0.13 (0.09)	0.12	0.57 (0.14)	<0.001	0.40 (0.11)	<0.001
Sex: male* diagnosis: MCI [a,b]	-0.35 (0.16)	0.03	-0.32 (0.13)	0.01	-0.28 (0.16)	0.08	-0.22 (0.13)	0.09
Sex: male* diagnosis: AD [a,b]	-0.29 (0.12)	0.02	-0.19 (0.09)	0.04	-0.26 (0.15)	0.07	-0.19 (0.12)	0.10
Sex: male* APOE: carrier [a,c]	-0.20 (0.13)	0.13	-0.11 (0.11)	0.30	-0.39 (0.19)	0.04	-0.29 (0.15)	0.06
Diagnosis: MCI* APOE: carrier [b,c]	0.28 (0.17)	0.10	0.21 (0.14)	0.13	-0.29 (0.18)	0.10	-0.14 (0.15)	0.33

Diagnosis: AD* APOE: carrier [b,c]	-0.14 (0.13)	0.26	-0.12 (0.10)	0.25	-0.62 (0.16)	<0.001	-0.40 (0.13)	0.002
Sex: male*diagnosis: MCI* APOE: carrier [a,b,c]	0.03 (0.21)	0.88	0.08 (0.17)	0.64	0.46 (0.24)	0.05	0.34 (0.19)	0.08
Sex: male*diagnosis: AD* APOE: carrier [a,b,c]	0.22 (0.17)	0.18	0.17 (0.14)	0.20	0.69 (0.22)	0.001	0.50 (0.18)	0.004

Full models: We used General Linear Models (GLM) with factors sex, APOE e4 genotype and clinical disease stage, their 2-way interactions and 3-way interactions, and age was included as a covariate. This full model was run separately for each of the three biomarkers. [a] Reference is female sex. [b] Reference is SCD. [c] Reference is APOE non-carrier. $p < 0.05$ is considered significant. [d] The natural logarithm of Tau and pTau concentrations are shown. Abbreviations: APOE, Apolipoprotein E4; SCD, Subjective Cognitive Decline; MCI, Mild Cognitive Impairment; AD, Alzheimer's Disease.

Table 6. Full models for sex, APOE e4 genotype and clinical disease stage for (p)Tau in APOE subgroups.

	Total cohort excluding APOE e2e4 carriers				Cohort containing only APOE e2 allele carriers			
	Tau [d]		pTau [d]		Tau [d]		pTau [d]	
	β (se)	*p*-value	*β* (se)	*p*-value	*β* (se)	*p*-value	*β* (se)	*p*-value
Sex: male [a]	0.04 (0.07)	0.58	0.02 (0.05)	0.76	0.09 (0.16)	0.59	0.09 (0.14)	0.54
Diagnosis: MCI [b]	0.45 (0.09)	<0.001	0.31 (0.07)	<0.001	0.37 (0.20)	0.06	0.27 (0.17)	0.11
Diagnosis: AD [b]	0.94 (0.09)	<0.001	0.63 (0.05)	<0.001	0.75 (0.18)	<0.001	0.44 (0.15)	0.004
APOE: carrier [c]	0.39 (0.07)	<0.001	0.27 (0.07)	<0.001	NA	NA	NA	NA
Sex: male* diagnosis: MCI [a,b]	-0.31 (0.11)	0.004	-0.24 (0.09)	0.004	-0.44 (0.25)	0.08	-0.49 (0.21)	0.02
Sex: male* diagnosis: AD [a,b]	-0.28 (0.09)	0.003	-0.19 (0.07)	0.01	-0.34 (0.25)	0.17	-0.24 (0.21)	0.26
Sex: male* APOE: carrier [a,c]	-0.36 (0.11)	0.002	-0.24 (0.09)	0.01	NA	NA	NA	NA
Diagnosis: MCI* APOE: carrier [b,c]	-0.05 (0.12)	0.71	0.02 (0.10)	0.85	NA	NA	NA	NA
Diagnosis: AD* APOE: carrier [b,c]	-0.38 (0.10)	<0.001	-0.26 (0.08)	0.002	NA	NA	NA	NA

Sex: male*diagnosis: MCI* APOE: carrier [a,b,c]	0.34 (0.16)	0.03	0.25 (0.13)	0.05	NA	NA	NA	NA
Sex: male*diagnosis: AD* APOE: carrier [a,b,c]	0.52 (0.14)	<0.001	0.28 (0.11)	<0.001	NA	NA	NA	NA

Full models: We used General Linear Models (GLM) with factors sex, APOE e4 genotype and clinical disease stage, their 2-way interactions and 3-way interactions, and age was included as a covariate. This full model was run separately for each of the biomarkers. [a] Reference is female sex. [b] Reference is SCD. [c] Reference is APOE non-carrier. $p < 0.05$ is considered significant. [d] The natural logarithm of Tau and pTau concentrations are shown. Abbreviations: APOE, Apolipoprotein E4; SCD, Subjective Cognitive Decline; MCI, Mild Cognitive Impairment; AD, Alzheimer's Disease; NA, Not Applicable.

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