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The effects of normal aging and ApoE genotype on the levels of CSF biomarkers for Alzheimer's disease

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Abstract

While cerebrospinal fluid (CSF) biomarkers are of use in the prediction and diagnosis of Alzheimer's disease our understanding of the background effects of age and the ApoE genotype is limited. Seventy-eight community-based normal volunteers (mean age 60 ± 10 years, range 36–86) were examined to determine the relationships between CSF measures of total tau (T-tau), hyperphosphorylated tau (P-tau 231), amyloid beta ($A\beta_{42}/A\beta_{40}$ ratio), and isoprostane (IP) with age and ApoE genotype. The results showed that age by $\epsilon 4$ genotype interactions were found for P-tau231 ($\beta = 1.82$; $p < 0.05$) and IP ($\beta = 1.6$; $p < 0.05$). T-tau CSF concentration increased with age. The increasing CSF concentrations of P-tau and IP in $\epsilon 4$ carriers suggest that early tauopathy and oxidative stress may be related to the increased risk for AD. The data also suggest that T-tau changes are more age dependent than $A\beta$ changes. The evidence that P-tau231 and IP are the earliest markers for the neuronal damage related to AD awaits longitudinal study. © 2007 Elsevier Inc. All rights reserved.

Keywords

Biomarkers; Cerebrospinal fluid; Alzheimer's disease; Normal aging

1. Introduction

Age is major risk factor for AD (Bachman et al., 1993). Neuropathology (Bennett et al., 2006) and imaging studies [for review (Glodzik-Sobanska et al., 2005)] have shown that Alzheimer pathology begins years before clinical presentation. Both neurofibrillary tangles (NFT) and amyloid plaques are present in the brains of clinically normal subjects (Knopman

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et al., 2003), as are volume (Rusinek et al., 2003) and metabolic losses (de Leon et al., 2001) in limbic regions in aging subjects that predict cognitive decline.

Several CSF assays have been developed to estimate *in vivo* tau and amyloid pathology. These have proven useful in the diagnosis of AD and in the prediction of AD from the MCI stage (Blennow et al., 2006), and for prediction of decline among normal to MCI (Fagan et al., 2007). The total CSF tau (T-tau) concentration mirrors neuronal and axonal loss (Mori et al., 1995). However, the increased CSF T-tau level is not specific for AD as levels are also elevated in other neurodegenerative diseases (Arai et al., 1997; Mollenhauer et al., 2005). Overall, CSF T-tau reflects both the normal metabolism of tau, and the non-specific release of tau following neuronal damage, whereas P-tau231 reflects abnormal tau metabolism that is specific for tangles and for AD (Mitchell and Brindle, 2003). Several isoforms for hyperphosphorylated tau (P-tau) exist, all reflecting NFT pathology (Blennow, 2004). Meta-analysis indicated a specificity (AD vs. control) of 94% for P-tau and 82% for T-tau; sensitivity was 70% and 83%, respectively. (Blennow and Vanmechelen, 2003).

The decrease in CSF A β 42 concentration is a reliable finding in AD samples as compared to controls, with both sensitivity and specificity fluctuating around 85% (Blennow and Vanmechelen, 2003). Recent studies suggested that the A β 42/A β 40 ratio instead of A β 42 increases the overall correct classification (AD patients and controls) (Lewczuk et al., 2004a,b). The changes in A β 42 may result not only from AD but also can be a consequence of total A β peptides concentrations (A β load). The load is best estimated by A β 40 concentration, therefore the use of A β 42/A β 40 is recommended (Wiltfang et al., 2007).

CSF isoprostane, a marker of oxidative stress and inflammation has been also shown to be increased in AD (Pratico et al., 2002) and to predict AD at the MCI stage (Brys et al., 2007; de Leon et al., 2007).

The apolipoprotein E (ApoE) polymorphism is the most widely accepted genetic factor increasing the risk for sporadic AD (Farrer et al., 1997). The presence of an ApoE ϵ 4 allele is associated with a reduced age of clinical onset of AD, an increased extent of AD pathology in *post mortem* studies (Ohm et al., 1999) and altered CSF T-tau and A β 42 biomarkers levels in AD patients (Galasko et al., 1998).

With few exceptions the effects of age and ApoE genotype on CSF levels of AD biomarkers in normal individuals have not been addressed (Peskind et al., 2006; Sunderland et al., 2004). This paper examines these relationships in cross-section for five commonly used biomarkers.

2. Methods

2.1. Participants

Seventy eight healthy subjects (mean age 60.4 ± 10.4 years, range 36–86) were recruited to participate in longitudinal research studies at the Center for Brain Health (CBH) of the New York University School of Medicine NIH-NIA funded Alzheimer's Disease Center. Volunteers were recruited through advertisement about the research program, or were the spouses and caregivers of impaired patients being evaluated at the center. Education ranged from 10 to 20 years with a mean of 16.5 ± 2 years. The study was approved by the NYU Institutional Review Board. All the subjects signed informed consent form allowing for lumbar puncture and the analysis of CSF.

2.2. Clinical and neuropsychological evaluations

All subjects received complete clinical assessments (medical, neurological, psychiatric, radiological and laboratory examinations). It was shown that cholesterol reducing medications

(e.g. statins) (De Caterina et al., 2002) and antioxidant agents (i.e. vitamin E) (Sutherland et al., 2007) can affect IP levels in urine and plasma. As knowledge on what drugs may effect biomarker levels in the CSF is limited, specific information was gathered concerning the use of medications. Participants received a comprehensive blood work-up (including lipid profile, blood count, homocysteine levels) and MRI was used to exclude possible confounding brain pathology (e.g. tumor, infarction etc.). Individuals with medical conditions or history of significant conditions that might affect brain integrity (e.g. stroke, head trauma, other neurodegenerative disease, or depression) were excluded.

All participants received a Global Deterioration Scale (GDS) rating of 1 or 2 (Reisberg et al., 1993). Both GDS 1 and 2 represent normal functioning, where GDS = 1 indicates no subjective memory complaint, and GDS = 2 indicates awareness of memory change over the lifespan, in the absence of objective evidence of memory or functional problems on clinical interview. A semi-structured interview based on the brief cognitive rating scale (BCRS) (Reisberg et al., 1983) was used to query the subject and in most cases, also an informant for the subject's recent memory performance and global functioning. In addition to the clinical evaluation, in every case, a neuropsychological test battery was administered but was not used to determine normal functioning. Rather cognitive tests were used for descriptive purposes. The measures include subtests of the Guild Memory Scale (Gilbert et al., 1968) assessing delayed and immediate recall of orally presented paragraphs (initial: PARI, and delayed: PARD); verbal paired associates (initial: PRDI, and delayed: PRDD); visual/ verbal paired associates with numbers (DESN). Subtests of the Wechsler Intelligence Scale Revised (WAIS-R) (Wechsler, 1981) were used to assess working memory (digits forward: (WAISDIG-F, and backward: WAISDIG-B), and attention (digit symbol substitution subtest: DSST).

2.3. Lumbar puncture and CSF collection

After 12 h fasting, at 11 a.m., 15 ml of clear CSF was collected into three polypropylene tubes using a fine 25G LP needle guided by fluoroscopy. All CSF samples were kept on ice for a maximum of 1 h until being centrifuged for 10 min at 1500 g at 4 °C. Samples were aliquoted to 0.25 ml and were stored in polypropylene tubes at -80 °C. All samples were blindly analyzed in batches. The subject distributions for genotype and age did not differ between the batches. For all the analytes the intra-observer and inter-observer variabilities were calculated by the collaborating labs.

2.3.1. 3.1.CSF total tau (T-tau)—CSF T-tau measurements were determined using the commercially available INNOTEST hTAU Antigen kit from Innogenetics (Hansson et al., 2006). The detection limit is 60 pg/ml for T-tau (INNOTEST hTau) and the coefficients of variability are 5.5% (intra-assay) and 11.6% (inter-assay).

2.3.2. CSF phospho-tau 231 (P-tau231)—A sandwich ELISA assay (Applied NeuroSolutions Vernon Hills, IL, USA) was used to detect tau phosphorylated at threonine 231 in CSF. In this assay, tau is captured with two backbone-directed antibodies, tau-1 and CP-27. The captured tau is then detected by CP9, which is specific for P-tau231 (Kohnken et al., 2000). The detection limits for this assay was 9 pg/ml. The coefficients of variation ranged from 6.0 to 10.3% (intra-assay) and 11.6 to 14.4% (inter-assay).

2.3.3. CSF amyloid beta assays (A β 40 and A β 42)—Using ELISA, CSF A β levels of A β 40 and A β 42 were measured using the monoclonal antibody 6E10 (specific to an epitope present on Ab-16) and rabbit antisera to A β 1–40 and A β 1–42, respectively, in a double antibody sandwich ELISA (Metha et al., 2000). The detection limit for A β 1–40 and A β 1–42 was 10pg/mL. The coefficients of variability ranged from 8–14% (intra-assay) and 10–18% (inter-assay). The amyloid beta assays were not N-terminus specific. The epitopes used were

x-40 and x-42. Consistent with recent reports, we used the ratio of these two species of A β —A β 42/A β 40 (Lewczuk et al., 2004a,b).

2.3.4. Isoprostanes-8,12-iso-iPF2a-VI (IP)—CSF samples were spiked with a fixed amount of internal standard ((d4)8,12-iso-iPF2a-VI) and extracted on a C18 cartridge column. The eluate was purified by thin layer chromatography and finally assayed by negative ion chemical ionization gas chromatography/mass spectrometry (Pratico et al., 2000). The coefficient of variation ranged 4–7% (intraassay) and 4.5–6.5% (inter-assay).

2.4. ApoE genotyping

Genotyping was conducted using methods previously published by Main et al. (1991). Study subjects were classified as ApoE ϵ 4 positive if they had one or two ϵ 4 alleles and otherwise ϵ 4 negative.

2.5. Statistical analysis

To compare clinical measures, demographic characteristics, and medication histories between ApoE genotype groups we used χ^2 and t -test. Cognitive variables were studied using MANOVA (general linear models). To examine the relationships between biomarker concentrations and age and ApoE genotype we used linear regression models. We determined the statistical power for the reported CSF models. Altogether for different analytes it ranged from around 80–98%. When appropriate the biomarker data was square root or log transformed to a normal distribution and analyses were repeated to insure that deviation from normality did not affect the results. For regression models, the standardized β are reported. To examine the relationships between biomarkers the Pearson-product correlation coefficient was used. The proportions of patients taking different classes of drugs in ApoE groups were compared with χ^2 test. Lipid profile (cholesterol, LDL, HDL, triglycerides) and blood pressure (diastolic and systolic) were examined in two separate MANOVA models, with ApoE group as the grouping factor.

All reported p values were declared statistically significant when $p \leq 0.05$. The analyses were performed.

3. Results

3.1. Demographic characteristics of the ApoE groups

There were no differences between ApoE ϵ 4 negative ($n = 48$) and ApoE ϵ 4 positive ($n = 30$) groups for age, education, gender or GDS score. The ApoE groups also did not differ in their neuropsychological test performances ($F = 1.6$, $df = 9.65$, $p > 0.05$, see Table 1). The percentages of subjects using different types of drugs in two ApoE groups did not differ (see Table 2).

Only BMI showed genotype related effects with lower BMI in the ApoE ϵ 4 positive group ($t = 3.2$, $df = 1.72$, $p < 0.01$). For lipid profile only a trend was found (MANOVA, $F = 2.3$, $df = 65$, $p = 0.07$) and further analysis revealed that it was due to higher triglyceride levels in ApoE ϵ 4 negative group ($F = 8.9$, $p < 0.05$).

3.2. Genotype and age interactions

3.2.1. Total tau—Significant age ($\beta = 0.50$, $p < 0.001$) and genotype ($\beta = 0.26$, $p < 0.01$) effects were observed for T-tau concentrations (for the whole model: $F = 15.5$, $df=2$, $p < 0.001$).

Higher T-tau was associated with older age and the ApoE ϵ 4 positive group (means: ϵ 4 positive = 344.4 ± 152.8 ; ϵ 4 negative = 277.1 ± 135.6). No age by genotype interaction effect was

observed (see Fig. 1 A). Fig. 1A shows that the regression lines for T-tau by age as a function of ApoE group are parallel. The results remained unchanged after square root transformation of the T-tau concentrations.

3.2.2. P-tau231—Significant age ($\beta = 0.48, p < 0.001$) and genotype ($\beta = 0.25, p < 0.05$) effects were observed for P-tau231 concentrations (for the whole model: $F = 14.0, df = 2, p < 0.001$). Higher P-tau231 was associated with older age and the ApoE $\epsilon 4$ positive group (means: $\epsilon 4$ positive = 13.7 ± 12.6 ; $\epsilon 4$ negative = 9.4 ± 7.5). The age by genotype interaction was significant ($\beta = 1.82; p < 0.05$, (the change for the interaction step: $F = 10.7, df = 1, p < 0.05$). The results remained similar after the square root transformation of P-tau231 (for interaction $\beta = 1.20; p = 0.05$, the change in the interaction step: $F = 4.1, df = 1, p < 0.05$). Overall, the results indicated that the P-tau231 concentration increased with age in both genotype groups, and that this increase was greater in the ApoE $\epsilon 4$ positive group (Fig. 1B).

3.2.3. Isoprostanes—Significant age ($\beta = 0.21, p < 0.01$) and genotype ($\beta = 0.32, p < 0.01$) effects were observed for IP (for the whole model: $F = 6.1, df = 2, p < 0.01$). Higher IP was associated with older age and with the $\epsilon 4$ positive groups (means: $\epsilon 4$ positive = 35.1 ± 16.2 ; $\epsilon 4$ negative = 27.4 ± 7.9). The age by genotype interaction was significant ($\beta = 1.60; p < 0.05$, the change for the interaction step: $F = 7.2, df = 1, p < 0.05$). However with inclusion of the age by genotype interaction, age alone was no longer significant in the model. These results remained unchanged after the log transformation of the IP levels (interaction $\beta = 1.40; p < 0.05$, the change for the interaction step was $F = 4.0, df = 1, p < 0.05$). We repeated the regression analysis in the two genotype groups separately. For the ApoE $\epsilon 4$ positive group there was an age effect ($\beta = 0.43, p < 0.01$), which was not found in the ApoE $\epsilon 4$ negative group ($\beta = 0.03, p = 0.80$). Overall, the results indicated that the CSF isoprostane level did not change with age in the ApoE $\epsilon 4$ negative group, while it increased linearly in the ApoE $\epsilon 4$ positive group (Fig. 1C).

3.2.4. A β peptides—The A β 42/A β 40 ratio decreased with age, but only a statistical trend was observed ($\beta = -0.21, p = 0.06$) (for the whole model: $F = 2.6, df = 2, p = 0.08$). The ApoE genotype effect and age interaction were not significant (Fig. 1D). Mean A β 42/A β 40 values for the ApoE $\epsilon 4$ positive and negative groups were: 11.5 ± 3.0 and 12.6 ± 3.9 , respectively). The results remained unchanged after square root transformation.

No significant age, genotype or interaction effects were found for A β 40 or A β 42.

For the $\epsilon 4$ positive and negative groups the values for A β 40 were 8552.90 ± 2420.04 and 8634.23 ± 3418.51 , respectively. The values for A β 42 were 980.40 ± 406.04 and 1109.2 ± 566.0 for $\epsilon 4$ positive and negative groups, respectively.

No gender effects for any of the biomarkers were found.

3.2.5. Relationships between biomarkers—The correlation coefficients for biomarkers pairs are given in Table 3 and Fig. 2A–E. The transformation of variables did not change relationships between them.

4. Discussion

To our knowledge, this is the first report describing normal lifespan changes in multiple CSF biomarkers used for Alzheimer's disease. We examined, in normal subjects, the relationship between age and ApoE genotype for five CSF AD biomarkers. The results showed age by genotype interactions, for some analytes and for others only age effects. No CSF biomarker marker showed effect restricted to genotype. Specifically, both P-tau231 and IP showed age

by ApoE genotype interactions in the direction consistent with the known risk that carriers have for AD. Separate age and genotype effects were found for T-tau, no effects were found for the A β ratio or the individual A β peptide fragments studied. Because other work suggests that P-tau231 and IP are excellent univariate discriminators of AD (Brys et al., 2007), these biological data highlight the importance of increasing age and the ApoE ϵ 4 genotype in modifying the risk for AD in normal subjects.

T-tau levels increased with age and were higher in ApoE ϵ 4 carriers. The finding of positive association between age and T-tau is in agreement with other previously published data (Blomberg et al., 2001; Briani et al., 2002; Itoh et al., 2001; Shoji et al., 1998; Sjogren et al., 2001). However, there are also contrary findings (Andreasen et al., 1998; Blomberg et al., 1996; Burkhard et al., 2004; Jensen et al., 1995; Lewczuk et al., 2004a,b). However, in some of the published negative reports, the samples were much smaller than in our study. T-tau reflects the non-specific neuronal and axonal turnover and damage due to any pathological condition (for review (Blennow and Vanmechelen, 2003)). The increase in T-tau with age is not unexpected as age is related to multiple sources of neuronal damage and pruning (for review (Uylings and de Brabander, 2002)). We observed that over the lifespan, the ApoE ϵ 4 positive group had higher overall T-tau levels. This result is in agreement with the report by Golombowski et al. (1997), who described this ApoE ϵ 4 effect in group of three healthy ApoE ϵ 4 carriers and nine non-carriers. However, two other studies showed negative results (Blomberg et al., 2001; Sunderland et al., 2004). The current study examined risk factors for atherosclerosis in the sample. We hypothesized that the E4 positive carriers might be predisposed to vascular diseases which in turn could contribute to age-related brain damage and therefore to elevated T-tau levels. Our ApoE groups were similar with respect to the distribution of medical (atherosclerosis and medication related) factors. It remains a possibility that our careful screening, which removed from the sample those individuals who had clinical evidence for brain damage due to vascular causes differentially reduced the prevalence associated with the ApoE genotype and diminished the age and interaction effects.

The P-tau231 concentration increased with age in both ApoE groups; and the increase was significantly steeper in the ApoE ϵ 4 positive group. There is not much information about age effects on P-tau231 levels in normal controls, but our results are at variance with three other published reports, which did not show age-dependent changes either in P-tau181 (Lewczuk et al., 2004a,b), P-tau199 (Itoh et al., 2001) or P-tau231 (Buerger et al., 2002). Greater age-related P-tau231 elevations in the ϵ 4 positive group are consistent with the increased risk of this group for AD and for an earlier accumulation of AD pathology. Several reasons led us to use P-tau231. (1) Neuropathological data shows that P-tau231 is a marker for the earliest stages of the disease. Augustinack et al. (2002) showed that TG3 antibody (for thr231 epitope) was one of three, which stained predominantly pre-neurofibrillary tangles stage while staining with the antibody specific for thr175/181 was more prominent in the later stage of NFT evolution. This distinction is potentially of great value while assessing first, disease-related signal in normal population (as our study group). (2) Antemortem CSF P-tau231 correlates with *post mortem* NFT pathology in neocortical regions (Buerger et al., 2006) and P-tau181 does not (Buerger et al., 2007; Engelborghs et al., 2007). (3) Excellent diagnostic specificity data exist for P-tau231 (Hampel et al., 2004).

Interestingly, across the lifespan and in relationship to ApoE genotype, the P-tau231 data parallels the results observed for IP. IP levels also showed steep age-related slope in ϵ 4 carriers. This may reflect the possible link between oxidative stress and tau phosphorylation and is consistent with the hypothesis of an early role for oxidative damage in AD (Zhu et al., 2003). Examination of the relationship between these two analytes showed they were associated ($r = .48, p < .01$). We are not aware of other studies addressing the effect of ApoE genotype on CSF isoprostanes levels in healthy subjects. There is nonetheless, evidence of their higher

levels in plasma in healthy $\epsilon 4$ carriers (Dietrich et al., 2005). Oxidative damage to peptidyl prolyl-*cis*, *trans*-isomerase (Pin), an enzyme regulating phosphorylation-dephosphorylation of tau has been suggested as an initial event prompting tangle formation (Sultana et al., 2006). Oxidative stress results in the elevation of stress-activated kinases (SAPK) (Zhu et al., 2003), which in turn are crucial for the phosphorylation of tau protein. The correlations between IP and P-tau levels in our sample, speaks in favor for the hypothesis linking oxidative stress and tau phosphorylation. Our data, however, does not address the issue of temporal and causative relationships between biomarkers; to fully answer this question longitudinal and animal model studies are needed. Nevertheless, the association between P-tau231 and IP suggest the early diagnostic potential for these biomarkers.

The reports about lifespan changes in the CSF A β levels of normal aging subjects are controversial. In a recent study, Peskind et al. (2006) found an age effect for A β 42 and A β 42/A β 40. The authors showed a decrease in CSF A β 42 after the age of 60, the decrease in A β 42/A β 40 occurred somewhat earlier. Significantly more pronounced age-related A β reductions were observed in subjects with an ApoE $\epsilon 4$ allele. However, others have not reported age effects for the A β ratio (Shoji et al., 1998). In our sample only a statistical trend for lower A β 42/40 ratio with increasing age was found and we did not find an ApoE effect nor an interaction contrary to other reports (Peskind et al., 2006; Sunderland et al., 2004). We believe the discrepancy might have come from different assays used. Moreover, the Fagan et al. (2000) findings support ours, as she found significantly elevated A β 40/A β 42 ratios (inverse of A β 42/A β 40) between $\epsilon 4$ groups only in the lipoprotein fraction of CSF, but not in the unfractionated CSF (as was ours). Additionally, the possibility exists CSF levels may not be exclusively brain derived, as the platelets are the biggest source of the soluble amyloid in the blood (Chen et al., 1995). Thus, the interpretation of level changes may be problematic.

As different ApoE isoforms differentially affect lipid metabolism (Xhignesse et al., 1991), atherosclerosis (Greenow et al., 2005) and subsequent lipid peroxidation, it is possible that this influence accounts for our observed increase in the IP of $\epsilon 4$ positive subjects. Similarly, drugs may influence the levels of some biomarkers (De Caterina et al., 2002; Sutherland et al., 2007). This later issues are not likely to be a major confounding factors in our study, as the $\epsilon 4$ groups did not differ in variables associated with atherosclerosis, lipid metabolism, or the use of lipid modifying, antioxidant or other drugs. Although we did find a difference in BMI values, in neither group the means were in the obesity range. Similarly, there was a trend toward more favorable lipid profile in $\epsilon 4$ positive individuals, but this was limited only to triglycerides. We do not think the slightly higher number of subjects taking statins among the $\epsilon 4$ positive individuals, could be an explanation. Statins are known to have only negligible influence on triglyceride levels. None of our subjects were taking fibrates. However, the $\epsilon 4$ positive group was a little (but not significantly) younger, this could influence the triglyceride levels. Nevertheless, our analysis may be also compromised by the lack of information pertaining the types and dosages of statins and antioxidants.

The presence of an ApoE $\epsilon 4$ allele modifies the age of onset of AD (Khachaturian et al., 2004). Cognitively intact ApoE $\epsilon 4$ positive individuals display brain glucose metabolic patterns similar to those observed in AD patients (Reiman et al., 1996) and more recently it was reported that $\epsilon 4$ carriers with subjective memory complaints showed elevated IP and P-tau231 levels in association with reduced medial temporal lobe glucose metabolism (Mosconi, 2007). Our CSF findings are in agreement with PET and other data indicating biological differences between ApoE $\epsilon 4$ positive and $\epsilon 4$ negative subjects that point to an AD predisposition or possibly to already existing preclinical pathology. Overall, this suggests a mechanism for their greater risk for cognitive decline and an early age of AD onset. However the observation of symptom development predicted by lower metabolism or altered biomarkers remains unexamined. The ApoE influence on CSF biomarkers may be exerted on different pathways:

Lipid metabolism, neurite outgrowth, tau phosphorylation, mitochondrial alteration (Mahley et al., 2006) or oxidative stress (Ramassamy et al., 2000). It remains unclear whether the CSF differences between $\epsilon 4$ carriers and non-carriers are a reflection of increased sub-clinical disease or whether there is a life long trait of biomarker differences found in association with the APOE genotype. This important question can only be answered with longitudinal studies with post-mortem or other validations, assessing the impact of both genotype and biomarkers levels on progression rates from normal to MCI and to AD.

In summary, the present study examines the cross sectional effect of age (through the adult life-span) and ApoE genotype for five CSF analytes. The data show that CSF P-tau231 and IP are particularly sensitive to the interaction of age and genotype and therefore may be useful early predictors of the pathology leading to clinical recognition of AD.

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Fig. 1. T-tau (A), P-tau231 (B), IP (C) concentrations and A $\beta 42/A \beta 40$ (D) in CSF as a function of age and ApoE genotype. Solid line: $\epsilon 4$ positive group; dashed line: $\epsilon 4$ negative group.

Fig. 2. Significant correlations between CSF variables: (A) correlation between IP and Ttau; (B) correlation between IP and P-tau 231; (C) correlation between T-tau and A β 42/Afj40; (D) correlation between P-tau231 and A β 40/A β 42; (E) correlation between T-tau and P-tau231.

Table 1

Demographic and cognitive characteristics of ApoE ε4 negative and positive groups

	ApoE ε4 negative group N=48	ApoE ε4 positive group N=30
Age (36–86)	61.1 ± 10.8	59.2 ± 9.8
Sex (%F)	59	53
Education (years)	16.6 ± 2.0	16.3 ± 2.1
GDS 1/2	18/30	13/17
MMSE	29.3 ± 1.0	29.5 ± 0.8
PARD	9.7 ± 2.8	9.0 ± 3.0
PARI	7.3 ± 2.0	7.2 ± 2.2
PRDD	6.3 ± 3.1	5.7 ± 2.9
PRDI	5.7 ± 3.1	5.4 ± 2.7
DSST	56.9 ± 12.9	61.4 ± 11.2
DESN	7.2 ± 2.6	6.4 ± 2.5
WAISDIGF	7.3 ± 0.9	6.9 ± 1.2
WAISDIGB	5.7 ± 1.3	5.2 ± 0.9

Values represent means ± standard deviations unless indicated otherwise.

Table 2

Atherosclerosis risk factors and the frequency of drug use in ApoE ε 4 negative and positive groups

	ApoE ε4 negative group N=48	ApoE ε4 positive group N=30
Systolic blood pressure (95%)	123.2 ± 16.1	123.0 ± 11.9
Diastolic blood pressure (95%)	76.8 ± 10.4	75.3 ± 8.7
Cholesterol (89%)	188.5 ± 33.5	187.0 ± 32.8
HDL (89%)	54.6 ± 12.7	59.4 ± 16.6
LDL (89%)	111.8 ± 32.5	111.7 ± 26.6
Triglycerides (89%)	110.8 ± 49.6	79.6 ± 27.2
Homocysteine (59%)	9.6 ± 2.4	10.1 ± 2.5
Glucose (100%)	86.6 ± 19.1	81.1 ± 13.3
BMI (95%)	26.7 ± 5.0 ^a	23.5 ± 2.7 ^a
Antioxidants (%)	46.0	58.6
Statins (%)	21	31
Antihypertensive drugs (%)	16.7	10.3
Analgetics (%)	14.6	7.0
Anti-platelet drugs (%)	12.5	17.2
Antidepressant drugs (%)	14.6	10.3
Thyroid hormones (%)	6.3	6.9
Osteoporosis drugs (%)	10.4	27.6
Anti-ulcer drugs (%)	6.3	6.9

Values represent means ± standard deviations, except for medication data. Numbers in parentheses indicate the percentage of the total group for whom the information on a given variable was accessible. For the drug use information was available for all but one participant. Besides the most represented classes of drugs given in the table, three subjects were using inhaler (antiasthmatic), four men were taking drugs for benign prostate hypertrophy, two women estrogens, three women tamoxifen, three participants oral diabetes related drugs, two benzodiazepines, one person antiviral drugs and one person sulfasalazine.

^a $t = 3.2$, $df = 1.72$, $p < 0.01$.

Table 3

Pearson product correlations coefficients among CSF biomarkers

	T-tau	P-tau231	IP	Aβ42/Aβ40
T-tau		0.55**	0.54**	-0.26*
P-tau231			0.48**	-0.35**
IP				-0.15
Aβ42/40				

* Correlation significant at $p < 0.05$.

** Correlation significant at $p < 0.01$.