Decreased CSF levels of L-carnitine in non-apolipoprotein E4 carriers at early stages of Alzheimer’s disease.

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Running title: CSF L-carnitine levels along AD progression.

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ABSTRACT

Increasing evidences suggest that Alzheimer’s disease (AD) is a heterogeneous disorder that includes several subtypes with different aetiology and progression. Cerebrospinal fluid (CSF) is being used to find new biomarkers reflecting the complexity of the disease’s pathological pathways. We used CSF and clinical data from patients to investigate the status of asymmetric dimethyl-L-arginine, creatine, suberylglycine and L-carnitine along AD progression. These molecules play important roles on mitochondrial function and dysfunctions in mitochondrial metabolism are involved in AD pathology. We found that non-apolipoprotein E4 carriers show lower levels of L-carnitine in CSF early in AD. L-carnitine levels correlate with Aβ42 levels and MMSE score, but do not add specificity or sensitivity to the classical AD CSF biomarkers, Aβ42, phospho-Tau and total-Tau. Our results suggest apoE genotype-depending differences in L-carnitine synthesis or metabolism along AD, and insinuate that L-carnitine treatments would be more beneficial for AD patients not carrying the apoE4 isoform.

Key words: ApoE4; L-carnitine; biomarkers; CSF; Alzheimer’s disease.
INTRODUCTION

Alzheimer’s disease (AD) is a chronic progressive neurodegenerative disorder characterized by cognitive and psychiatric symptoms, being the main cause of dementia [1]. AD currently affects nearly 2% of the population in industrialized countries and the risk dramatically increases in individuals beyond the age of 70; indeed, it is diagnosed to more than 50% of people above the age of 85 [2, 3]. It is predicted that the incidence of AD will triple within the next 50 years [4] which, among other things, supposes a high economic burden. Increasing evidences suggest that AD is a heterogeneous and multifactorial disorder resulting from interactions between the genetic susceptibility and environmental factors [5]. The most important genetic susceptibility factor is the presence of the e4 allele of apolipoprotein E (apoE) that accounts for approximately half of late onset AD [6].

The lack of homogeneity would imply the existence of several subtypes with different aetiology and progression. This concept has not been properly investigated, although it could be of great importance for a proper diagnosis, evaluation of disease progression and adjusting therapeutics strategies. During recent years, a great effort has been made in improving early diagnosis and in disease modifying treatments for AD. However, until now, all the therapeutical approaches based on regulating or eliminating Aβ from the brain have failed [7], perhaps due to variations in the aetiology of the disease among the patients on the trials.

Cerebrospinal fluid (CSF) represents an important source for the searching and discovering of new biomarkers, as its composition is directly related to metabolite production in the brain [8, 9]. Up to date, three biomarkers in CSF (total Tau protein, Aβ protein and phosphorylated Tau protein) fulfil the criteria required by the consensus group [10]. The ratio between T-tau and Aβ42 levels has been proposed as the most sensitive for diagnostic purposes [11]. However, the sensitivity and specificity of these biomarkers permit the discrimination between controls and AD patients in late stages, but it is still poor in early stages of the disease. Thus, novel, and
specifically early biomarkers, will aid in diagnosis, monitor disease progression and response to treatment.

We recently used a non-targeted metabolomic approach to analyze differences in the metabolic pattern of CSF samples from subjects with different cognitive status related to AD progression. We found a high prediction power (98.7% of accuracy and specificity and sensitivity values above of 95%) using a combination of novel metabolites and classical AD biomarkers [12, 13]. Abnormalities in cerebral metabolism have been documented widely over the past 50 years in neurodegenerative diseases, including AD, with decreased cerebral metabolism preceding clinical or neuroanatomical development of the disease [14]. Thus, some of these metabolites contributing to an enhanced diagnostic power of AD may reflect early abnormalities in the cerebral metabolism leading to disease progression. Of particular interest could be those ones reflecting dysfunctions in mitochondrial metabolism, since it is believed that mitochondrial alterations are involved in loss of cognitive function, as well as in the exacerbation of structural changes in the pattern of AD pathology [14]. From the group of metabolites contributing to an enhanced diagnostic power [12, 13], here we focused in investigating those reflecting mitochondrial alterations, namely asymmetric dimethyl–L-arginine (ADMA), creatine, suberylglycine and L-carnitine [15-18]. We used CSF and clinical data from patients with mild AD, subjective cognitive impairment (SCI), and mild cognitive impairment (MCI), a state considered of high risk for AD. We analysed if levels of these metabolites were abnormal in AD or MCI and their possible association with AD hallmarks, apoE genotype, gender and mini mental score (MMSE). We report that the levels of L-carnitine were significantly decreased in the CSF of MCI and AD patients that were non-apoE4 carriers, showing positive correlations with CSF Aβ42 levels and MMSE score. The implications of these findings for mitochondrial functioning, early diagnosis and L-carnitine supplementation treatment approaches in AD are discussed.
MATERIAL AND METHODS

Study population

The patients included in the study (n=73) were from the Memory Clinic at the Karolinska University Hospital in Huddinge, Sweden: 19 had subjective cognitive impairment (SCI), 31 mild cognitive impairment (MCI) and 23 mild AD. These patients were all living independently in the community. They were evaluated according to a standard comprehensive assessment protocol including clinical examination, brain imaging, electroencephalography, analysis of blood and CSF (including total Tau (T-tau), phospho-Tau (P-tau) and Aβ42) and a detailed neuropsychological evaluation. Dementia and AD were diagnosed according to DSM-IV and NINCDS-ADRDA criteria. MCI patients were (1) not demented, (2) had (self and/or an informant) reported cognitive decline and impairment on objective cognitive tasks, and (3) had preserved basic ADL/minimal impairment in complex instrumental functions. SCI patients had cognitive complaints without impairment on objective cognitive tasks. Women under hormonal replacement therapy, as well as patients with psychiatric disorders (i.e., depression, alcohol abuse) or other conditions (i.e., diabetes, brain tumors, normal pressure hydrocephalus) were not included. The study was conducted under the guidelines of the Declaration of Helsinki and approved by the ethics committee of the Karolinska Institutet.

CSF measurements

CSF was collected for diagnostic purpose by lumbar puncture as previously described [19, 20]. CSF samples were obtained by lumbar puncture performed in the sitting position. CSF extraction is routinely performed at the Karolinska University Hospital Memory Clinic in Huddinge (Sweden) as part of the medical examination. The extractions were performed in the mornings in fasting patients. CSF samples were obtained from L3/L4 or L4/L5 interspaces after local anesthetic infiltration on the skin. After disposal of the first ml. the following 10 ml were collected in polypropylene tubes. No sample containing more than 500 erythrocytes/μl CSF samples was used. Samples were gently mixed to avoid gradient effects and centrifuged at
2000xg for 10 minutes to eliminate cells and insoluble material. Supernatants were aliquoted, immediately frozen and stored at -80°C pending biochemical analysis. Total-Tau was determined using a sandwich enzyme-linked immunoabsorbent assay (ELISA) [21]. P-Tau (P-Thr 181) was determined using a sandwich ELISA with monoclonal antibody HT7 (recognizing all forms of Tau) used as capturing antibody, and AT270 (specific to P(Thr181)-Tau used as a detection antibody. [22] Aβ_{42} was determined using a sandwich ELISA as previously described[19]. All kits were purchased from Innogenetics NV, Ghent, Belgium.

Metabolite levels were measured using a new metabolomic approach based on capillary electrophoresis-mass spectrometry (CE-MS) [12]. CE analyses were carried out in a P/ACE 5500 CE apparatus from Beckman Instruments (Fullerton, CA, USA). The instrument was controlled by a PC running the System Gold software from Beckman. Uncoated fused-silica capillaries (50 μm id and 87 cm total length) from Composite Metal Services (Worcester, England) were coupled to the mass spectrometer through an orthogonal electrospray ionization (ESI) interface model G1607A from Agilent Technologies (Palo Alto, CA, USA). Electrical contact at the electrospray needle tip was established via a sheath liquid. A time of flight (TOF) MS instrument (micrOTOF) from Bruker Daltonics (Bremen, Germany) was employed. The instrument was controlled by a PC running the micrOTOF control software from Bruker Daltonics. Injections were made at the anodic end using N_{2} pressure of 0.5 psi (34.5 mbar) for 80 s. The electrophoretic separation was achieved applying +25 kV as running voltage at a constant temperature of 25 °C. The micrOTOF was operated to acquire spectra in the m/z range of 50-500 [12].

**Data analysis**

Normal distribution of protein data was checked by Shapiro-Wilks. Metabolites are presented as mean values ± standard error mean (SEM). CSF metabolite levels along the different groups were analyzed using ANOVA. Pairwise comparisons were performed by HSD Tukey´s post-hoc test. For interaction analysis, two-way ANOVA followed by Bonferroni post-hoc test was used.
Associations between Aβ42, Total-Tau or P-tau levels, MMSE score, apoE genotype and CSF carnitine levels were assessed by partial correlation analysis. Significance was defined as p ≤ 0.05.

CE-MS metabolite data was processed by using the open-source bioinformatics tool MZmine [12]. Briefly, this software was applied to the experimental information obtained by the mass spectrometer to obtain a final data matrix containing the relative metabolite concentration (related to MS peak areas) from each metabolite found in all the CSF samples. The relative concentrations of metabolites were compared among samples by statistical analysis and finally, a statistical validation was assessed by a leave-one-out cross-validation procedure [12].
RESULTS

General characteristics of the population

Demographic and clinical characteristics of study participants are shown in Table 1. Mild AD patients were significantly older than SCI and MCI (F(2,70)= 13.95, p < 0.001). MMSE score was lower in mild AD patients compared to SCI and MCI patients (F(2,70) = 32.33, p < 0.001).

Mild AD patients presented lower CSF levels of Aβ_{42} and higher T-tau and P-tau when compared to either SCI (F(2,66) = 13.29, p < 0.001; F(2,69) = 10.83, p < 0.001; F(2,58) = 9.02, p < 0.01) or MCI (p < 0.05; p < 0.001; p < 0.01, respectively).

L-Carnitine levels are decreased in CSF of AD patients and correlate with CSF Aβ_{42} levels and MMSE score.

Analysis of L-carnitine, suberylglycine, ADMA and creatine in CSF were performed and are shown in Figure 1A. No significant differences were found between groups in the levels of suberylglycine, ADMA or creatine. However, we found significant reductions of L-carnitine levels in CSF of patients with mild AD (0.0191 ± 0.0021) and MCI (0.0196 ± 0.0016) compared to SCI (0.0265 ± 0.0016) (one way ANOVA followed by HSD Tukey’s test, F(2,70) =4.42, p = 0.0155 and p<0.05, respectively; Figure 1A).

CSF levels of L-carnitine correlated directly with Aβ_{42} levels (r = 0.2875, n = 69, p = 0.0166) and MMSE score (r = 0.3130, n = 73, p = 0.0070) (Figure 1B). However, no correlation between L-carnitine levels and T-Tau (r= -0.2127, n = 72, p = 0.0728), P-Tau (r = -0.2121n = 61, p = 0.1007) or age (r=-0.08598, n=73, p = 0.4695) were found.

Decreased CSF L-carnitine levels in non-apoE4 carriers patients.
Figure 2A shows the gender–stratified distribution of CSF-L-carnitine levels along the three different groups. On average, women and men presented similar levels (0.02135 ± 0.0015 versus 0.02104 ± 0.0015 a.u.; p = 0.88). There was no interaction between groups and gender.

Average levels of CSF L-carnitine between ApoE4 carriers and non-carriers did not differ (0.0210 ± 0.0016 versus 0.0212 ± 0.0013 a.u.; p = 0.91. Figure 2B). An interaction between groups and genotype was found (F(2,64) = 3.64; p = 0.0318 two-way ANOVA followed by Bonferroni post-hoc test). A group effect was also shown, and differences were found among non-E4 carriers in the SCI compared to the mild-AD (F(2,64) = 3.70; p = 0.0301. Figure 2B).

When the Aβ42 and MMSE correlations were done taking into account the apoE4 genotype, we found only significant correlations in the group of non-E4 carriers (Figure 2C and D) for both Aβ42 (r = 0.4979, n = 35, p = 0.0023) and MMSE score (r = 0.5162, n = 36, p = 0.0013), but no for apoE4 carriers (Figures 2E and F).

L-carnitine did not improve the diagnostic power of classical AD biomarkers in non-apoE4 carriers.

Numerous studies have explored the use of the CSF concentration of classical AD hallmarks as biomarkers for an early diagnosis. The ratio between T-tau and Aβ42 levels has been proposed as the most sensitive for diagnostic proposes [23]. To explore the possibility of using L-carnitine values in combination with T-tau and Aβ42 for improving diagnose accuracy in non-apoE4 carriers, we calculated the area under the curve (AUC) for the T-Tau/ (Aβ42 *L-carnitine). In all patients of the study, the AUC for the T-Tau/ Aβ42 ratio was 0.86 . This was not improved by L-carnitine (AUC= 0.87; Figure 3A). When we performed the same analysis only among the apoE4 non-carriers patients, the AUC values changed from 0.80 to 0.89 respectively (Figure 3B). This change was not statistically significant (p=0.19).
DISCUSSION

Since it is suggested that AD is a heterogeneous disorder that includes several aetiologies, a better characterization of causes and subtypes would help to improve diagnosis and treatment efficiency. Several other reports indicate differences in AD biomarkers, progression or pathogenesis related to gender or to the apoE genotype [24]. For example, we previously showed that levels of insulin in CSF are decreased among women, but no men, in prodomal AD. This decrease was associated with lower Aβ_{42} levels and cognitive score [25].

From the complexity of AD pathology, there is an increasing consensus pointing that disruption of mitochondrial function could be an early event in the pathological cascade [15, 26]. Mitochondria are highly dynamic organelles that not only supply the main energy to the cell but also regulate apoptosis [27]. The changes suffered by mitochondria during AD pathology include: morphology, increase in permeability of mitochondrial membranes, reduction of mass, decrease in the activities of enzymes, increase in mtDNA fragmentation, deregulation in fission/fusion processes and transport, alteration of oxidative stress with a overproduction of ROS and a deregulation of apoptosis. Therefore, decline in mitochondrial function impacts many cellular processes, such as inflammation, autophagy, and apoptosis, needed for the proper maintenance of the cellular function [26, 28].

We recently showed changes in the metabolite pattern in CSF during the progression of AD that could help to an early diagnosis of the disease. Four of them, ADMA, creatine, suberylglucose and L-carnitine, have well reported roles in mitochondrial function [15-18].

Here, we report that levels of L-carnitine in CSF were decreased in patients in the MCI and mild AD groups compared to SCI. Moreover, CSF L-carnitine levels directly correlated to CSF Aβ_{42} levels and MMSE score, but not to the other classical AD biomarkers (T-Tau, P-Tau). On average, women and men presented similar levels, but when we stratified the study population for the apoE genotype, we found that decreased CSF L-carnitine was seen only in non-apoE4 carriers, with more pronounced correlations with Aβ_{42} levels and MMSE score. Despite these
differences, not improvements in either sensitivity or specificity were found when combining L-carnitine CSF measurements with the classical AD biomarkers (T-tau/Aβ42 ratio) both in the general population and in the non-apoE4 carriers.

Carnitine-acetyl-O transferase converts L-carnitine and acetyl-Coenzyme A (acetyl-CoA) in acetyl-L-carnitine (ALC) and CoA in the mitochondria. ALC is transported also outside of the mitochondria were it is converted back to its precursors. This process i) facilitates the uptake of acetyl CoA into the mitochondria during fatty acid oxidation, ii) enhances the acetylcholine production and iii) stimulates protein and membrane phospholipid synthesis. The mechanism of action of ALC includes enhance neuronal metabolism in the mitochondria, ability to stabilize cell membrane fluidity, and prevention of cell death and neuronal damage. In addition, it also promotes an increase in the hippocampal binding of glucocorticoids and nerve growth factor and reduces the oxidative stress. [29].

In a previous study, no significant differences were found in free carnitine (FC) and acyl-carnitine esters between CSF samples from patients with AD and controls and they did not correlate with age, age at onset of AD, duration of AD or scores in the MMSE [30]. On the other hand the beneficial effects of ALC in preventing the loss of age-related brain function and neurodegenerative disorders have been widely studied [31-34]. Regarding AD, it has been described that ALC treatment improves cognitive performance in patients [35]. Several reports showed positive effects in vitro of ALC on Aβ toxicity, through the prevention of Aβ-induced mitochondrial damage and ATP depletion [36]; on Aβ deposition, through the up-regulation of kinesin light-chain 1, a protein involved in the fast anterograde axonal transport of Aβ peptide [37]; and on Aβ production, through stimulation of α-secretase activity (ADAM 10) [38]. Besides, ALC has also many neuromodulatory and neurotrophic actions on neuronal activity helping to maintain mitochondrial bioenergetics and lowering oxidative stress associated with aging [31, 32]. An increasing number of studies suggest that other mechanisms than the antioxidant activities could be involved in the neuroprotective effect of carnitine such as the activation of heat-shock proteins, which can exert neuroprotective effects against oxidative
stress-related injury [31, 39]. In animal models, L-carnitine was shown to reverse the homocysteine-induced tau hyperphosphorylation, Aβ accumulation and memory deficits in rats [40]. Besides, L-carnitine supplementation was shown to improve cognition and to reduce oxidative and mitochondrial damage in the brain of aged rats [33, 41]. These evidences support the possibility that L-carnitine may provide therapeutic benefits in oxidative stress-associated neurodegenerative diseases such as AD.

Our study brings evidence for a different metabolism of L-carnitine in AD brain, depending upon the apoE genotype. The presence of apoE4 is the most prevalent risk factor for late onset or sporadic AD and some of the pathological features of AD, such as impairment of energy metabolism and mitochondrial dysfunctions are seen earlier in apoE4 homozygotitic patients [42]. In a transgenic mice model for human apoE4, it was reported that acetyl-L-carnitine treatment eliminated the neuronal mitochondrial damage and also prevented the formation of lipofuscin and/or myelin-like structures in neurons. Moreover, an improvement of the cognitive function was also observed [34].

However, the presence of apoE4 has been also associated with increased risk for atherosclerosis and for coronary artery disease [43], diseases where some negative effects of carnitine have been also reported. A recent paper has shown that the metabolism of carnitine, through a specific gut microbiota, produces an intermediate metabolite which accelerates atherosclerosis in mice [44]. Other authors have reported that in ApoE/LDLR⁻/⁻ mice, treatment with mildronate, an inhibitor of L-carnitine biosynthesis and transport that is used as a cardioprotective drug, decreased the size of atherosclerotic plaques and L-carnitine concentrations of aortic tissues [45]. A report from the carotid ultrasound disease assessment study (CUDAS), found a significant association between apoE4 and increased incidence of carotid plaque but not of intima-media thickening (IMT)[46]. However, Altamura et al., have reported an increase of the IMT values in AD patients carrying the apoE4 allele respect to non-apoE4 AD-carriers, proposing that apoE4 might promote IMT interacting with other factors contributing to AD development [47].
The efficacy of ALC in cognitive decline or AD has been investigated in several clinical trials, yet with unclear results. Cochrane reviewed in 2003 of 11 ALC clinical trials in AD showed benefits of ALC in clinical global impression but not in cognition [48]. In recent years, still conflicting results have been reported [35, 49-52]. To our knowledge, these studies were never stratified for the apoE genotype. In view of our results, where AD patients carrying apoE4 do not appear to have deficits in brain ALC, while non-apoE4 carriers do, and considering that both, increased ALC and the presence of apoE4, increase the risk for atherosclerosis, we propose that dietary L-carnitine supplementation will be more beneficial for non-apoE4 carriers with AD even at MCI stages.

In summary, we report that during the progression of AD, non-apoE4 carriers show lower levels of L-carnitine in CSF. However, this difference does not add specificity or sensitivity to the classical AD CSF biomarkers, T-Tau/Aβ42 ratio. The mechanisms behind the apoE isoform differences on L-carnitine synthesis or metabolism in brain should be investigated. Since both, high L-carnitine levels and apoE4 genotype confer higher risk for atherosclerosis and for coronary artery disease, our results suggest that L-carnitine treatments would be more beneficial for AD patients do not carrying the apoE4 isoform.
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Disclosure statement

The authors report no conflict of interest including financial or personal.
REFERENCES


## Tables

### Table 1: Demographic and clinical characteristics of study participants.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>SCI</th>
<th>MCI</th>
<th>Mild-AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>31</td>
<td>23</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>(7/12)</td>
<td>(18/13)</td>
<td>(5/18)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.3 (1.44)</td>
<td>60 (1.6)</td>
<td>70 (1.9)</td>
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<tr>
<td>Education (years)</td>
<td>14.6 (0.7)</td>
<td>12.8 (0.6)</td>
<td>9.3 (0.6)</td>
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<tr>
<td>ApoE carriers (%)</td>
<td>7/19 (36,84%)</td>
<td>16/31 (51,6%)</td>
<td>11/23 (47,82%)</td>
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<tr>
<td>MMSE (points)</td>
<td>28.9 (0.3)</td>
<td>28 (0.3)</td>
<td>23.2 (0.8)</td>
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</tbody>
</table>

### Clinical data

<table>
<thead>
<tr>
<th></th>
<th>SCI</th>
<th>MCI</th>
<th>Mild-AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q ALB</td>
<td>6.7 (0.7)</td>
<td>6.5 (0.6)</td>
<td>6.8 (0.5)</td>
</tr>
<tr>
<td>CSF Aβ42 (ng/L)</td>
<td>830.3 (43.1)</td>
<td>651.2 (43.8)</td>
<td>516.5 (26.8)</td>
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<tr>
<td>CSF T-Tau (ng/L)</td>
<td>290.9 (34.6)</td>
<td>332.3 (34.9)</td>
<td>602.7 (72.3)</td>
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<tr>
<td>CSF P-Tau (ng/L)</td>
<td>50.8 (3.8)</td>
<td>52.9 (5.2)</td>
<td>86.8 (8.2)</td>
</tr>
</tbody>
</table>

Numeric values are presented as number of patients, percentage or mean (±SEM).

SCI: subjective cognitive impairment
MCI: mild cognitive impairment
Mild AD: mild Alzheimer's disease
MMSE: Mini-Mental State Examination
CSF: cerebrospinal fluid
Alb: albumin
FIGURE LEGENDS

**Figure 1: L-carnitine is decreased in CSF from MCI and AD patients and correlates with Aβ42 and MMSE score.** Figure 1A shows CSF levels of L-carnitine, suberylglycine, assimetric dimethyl-L-arginine (ADMA) and creatine. Only L-carnitine determinations showed statistically significant changes along AD progression (*p<0.05, one-way ANOVA, Tukey post-hoc test). Figure 1B shows correlation analysis between CSF levels of L-carnitine and CSF Aβ42 levels (r=0.2875, p=0.0166; left figure) and MMSE score (r=0.3130; p=0.007; right figure).

**Figure 2: L-carnitine is decreased in CSF of apoE4 non-carriers in AD.** Figure 2A shows CSF L-carnitine levels stratified by gender. No significant statically differences were found between men and women. Figure 2B represents CSF L-carnitine levels in apoE4 carriers or non-carriers in the different diagnosed groups. Levels of L-carnitine were significantly lower on apoE4-non carriers with AD (*p=0.0301; two-way ANOVA followed by Bonferroni post-hoc analysis). Figure 2C and 2D shows correlations of CSF L-carnitine and CSF Aβ42 levels (Fig.C; r=0.4979, p=0.0023) or MMSE score (Fig.D; r=0.5162, p=0.0013). In apoE4 carriers, no correlations were found between CSF L-carnitine and CSF Aβ42 levels (Fig.2E) or MMSE score (Fig.2F).

**Figure 3: L-carnitine does not improve the sensitivity and specificity of classical AD biomarkers.** The plot shows a ROC curve analysis for all patients from the groups of the study (MCI and mild AD) versus controls (SCI). In the general population of the study, the AUC was 0.86 for T-Tau/Aβ42 ratio (Fig.3A, red line) and 0.87 for T-Tau/(Aβ42*L-carnitine) (Fig.3A, black line). Figure 3B shows the same analysis for apoE4 non carriers; the AUC was 0.80 for T-Tau/Aβ42 ratio (Fig.3B, red line) and 0.89 for T-Tau/(Aβ42*L-carnitine) (Fig.3B, black line). L-carnitine did not improved significantly the diagnostic power of the T-Tau/Aβ42 ratio (p=0.19).
Figure 1

A

![Graph showing CSF carnitine levels](image1)

![Graph showing CSF Aβ1-42 levels](image2)

B

![Graph showing CSF suberylglycine levels](image3)

![Graph showing CSF creatine levels](image4)

![Graph showing CSF ADMA levels](image5)

![Graph showing CSF MMSE score](image6)
Figure 2

A

B

C

D

E

F

CSF carnitine levels vs. CSF Aβ1-42 levels for different groups: SCI, MCI, Mild AD, ApoE4 carriers, ApoE4 non-carriers, men, women. CSF carnitine levels are shown with error bars. MMSE score is plotted against CSF Aβ1-42 levels and CSF carnitine levels with a linear regression line.
Figure 3

A

All patients

B

ApoE4 Non-carriers

0.00 0.25 0.50 0.75 1.00

Sensitivity

1 - Specificity

T-tau/\(A\beta_{1-42}\)

T-tau/(\(A\beta_{1-42}\) * L-carnitine)

T-tau/\(A\beta_{1-42}\)

T-tau/(\(A\beta_{1-42}\) * L-carnitine)