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## The association of essential metals with *APOE* genotype in Alzheimer's disease

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## **Abstract**

The major confirmed genetic risk factor for late-onset, sporadic Alzheimer's disease (AD) is variant  $\epsilon 4$  of apolipoprotein E gene (*APOE*). It is proposed that ApoE, a protein involved in transport of cholesterol to neurons can cause neurodegeneration in AD through interaction with metals. Previous studies mostly associated copper, iron, zinc and calcium with ApoE4-mediated toxicity. We tested the association of other essential metals with ApoE. We compared plasma and cerebrospinal fluid (CSF) levels of copper, zinc, iron, sodium, magnesium, calcium, cobalt, molybdenum, manganese, boron and chromium, and CSF ferritin levels among AD, mild cognitive impairment (MCI) patients and healthy controls (HC) with different *APOE* genotype. Sodium, copper and magnesium levels were increased in carriers of  $\epsilon 4$  allele. Additionally, the increase in sodium, calcium and cobalt plasma levels was observed in carriers of  $\epsilon 4\epsilon x$  genotype. The decrease in boron plasma levels was observed in carriers of  $\epsilon 4$  allele and  $\epsilon 4\epsilon 4$  genotype. Additionally, CSF zinc levels as well as plasma sodium levels were increased in AD patients compared to HC. These results indicate that the molecular underpinnings of association of essential metals and metalloids with ApoE should be further tested and clarified *in vivo* and *in vitro*.

**Key words:** Alzheimer's disease; apolipoprotein E; metals; copper; zinc; mild cognitive impairment.

## **Abbreviations**

A $\beta$ , amyloid  $\beta$ ; AD, Alzheimer's disease; AP, amyloid plaques; ApoE, apolipoprotein E protein; *APOE*, Apolipoprotein E gene; CSF, cerebrospinal fluid; DMN, default mode network; EEG, electroencephalography; HC, healthy controls; ICP-MS, inductively coupled plasma mass spectroscopy; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; NMDAR, *N*-methyl-D-aspartate receptor; QSM, quantitative susceptibility mapping; SNPs, single nucleotide polymorphisms; ZnT3, Zinc transporter 3.

## 1. Introduction

Homeostasis of essential metals is altered in Alzheimer's disease (AD) [1–3], and it has been proposed that such changes are directly related to AD pathology [1]. This primarily refers to essential metals that are normally present in organisms and are crucial for normal functioning of many proteins and enzymes. Altered metal homeostasis in the AD brain has mainly been related to copper, zinc and iron [1].

Apolipoprotein E (ApoE) is a protein involved in transport of cholesterol to neurons, and is mainly produced by astrocytes [4]. The apolipoprotein E gene (*APOE*) variant  $\epsilon 4$  is the major confirmed genetic risk factor for late-onset, sporadic AD that comprises over 99% of all AD cases [5,6]. There are three common ApoE variants (ApoE2, ApoE3 and ApoE4). Two single nucleotide polymorphisms (SNPs) present in *APOE* gene determine which amino acid will be present at the protein level at positions 112 and 158. Thus, ApoE2 variant has Cys112 and Cys158, ApoE3 has Cys112 and Arg158 and ApoE4 has Arg112 and Arg158 [7]. *APOE*  $\epsilon 4$  heterozygotes have a 5 times increased risk, while *APOE*  $\epsilon 4$  homozygotes have a 20 times increased risk for developing AD [8]. ApoE2 is considered to have protective effect in AD [9]. Association of metals with ApoE was observed in AD. Xu and collaborators proposed three mechanisms through which metal ions may interact with ApoE: 1) copper, zinc, and iron accumulate in amyloid plaques (AP); AP cause metal dyshomeostasis that leads to decrease in ApoE levels in AD; 2) metal dyshomeostasis in AD decreases *APOE* transcription and translation, which may promote toxicity of amyloid  $\beta$  ( $A\beta$ ) as ApoE promotes  $A\beta$  clearance; and 3) the ApoE proteolysis that occurs in AD is more prominent for ApoE4 isoforms whose fragments disrupt mitochondrial and cytoskeletal functions and lead to neurodegeneration [10]. Stability of ApoE isoforms could be mediated by metal binding [11] and as metals stabilize ApoE isoforms in the order  $\epsilon 2 > \epsilon 3 > \epsilon 4$ , this could be the cause of higher vulnerability of the ApoE4 isoform for proteolysis. However, as these authors emphasized, these assumptions should be further tested [10].

Previous studies associated copper, iron, zinc and calcium with ApoE4-mediated toxicity [12–15]. In the current study we further analyzed the association of other essential metals like sodium, magnesium, cobalt, molybdenum, manganese and chromium and the metalloid boron with ApoE. We also measured cerebrospinal fluid (CSF) ferritin levels as it likely reflects the levels of iron in the brain. The scope of this study was to compare plasma and CSF levels of Cu, Zn, Fe, Na, Mg, Ca, Co, Mo, Mn, B and Cr, and CSF levels of ferritin in AD, mild cognitive

impairment (MCI) patients and healthy controls (HC) with different *APOE* genotype. The investigation of a possible association between essential metals and *APOE* permitted us to assess the existence of altered metal homeostasis in AD.

## **2. Materials and Methods**

### **2.1. Cerebrospinal fluid and blood collection**

This study included 197 patients recruited at the University Hospital Centre, Zagreb and General Hospital Varaždin of whom 126 fulfilled NINCDS-ADRDA criteria for AD, 52 suffered from MCI [16,17], and 19 were HC. Patients were neurologically tested, as described previously [18]. The examination included the Mini-Mental State Examination (MMSE), VDRL testing for syphilis, complete blood tests including thyroid function, albumin levels, and levels of vitamin B12 and electrolytes. None of the subjects included in this study suffered from renal diseases. It should be however noted that a limitation of this study is that although we had information on medication regimens for the majority of the included patients (summarized in **Supplementary Table 1**), we did not have information on use of supplements (since levels of boron, copper and magnesium may be affected by supplements). CSF was obtained between the L3/L4 or L4/L5 intervertebral spaces by lumbar puncture, always performed in the morning between 9 a.m. and 11 a.m. After centrifugation for 10 min at 2000 g, samples were aliquoted and stored at -80°C. Venous blood samples (4 ml) were collected into plastic syringes with 1 ml of acid citrate dextrose as an anticoagulant. Blood samples were consistently obtained in the morning on an empty stomach. Thrombocyte-free plasma samples were collected by centrifugation (1100 g for 3 min and then 5087 g for 15 min), and stored at -20°C. Ferritin levels in CSF were determined by electrochemiluminescence (ECL) using a Roche Cobas E601 instrument (Roche, Basel, Switzerland). All procedures were implemented in accordance with the approval of the Central Ethical Committee of the University of Zagreb Medical School (case no. 380-59-10106-18-111/126, class 641-01/18-02/01 from June 20, 2018) and Ethical Committee of the Clinical Hospital Centre Zagreb (case no. 02/21 AG, class 8.1-18/82-2 from April 24, 2018).

### **2.2. Genotyping**

Genomic DNA was extracted from peripheral blood using the salting-out method [19]. *APOE* polymorphisms (rs7412 and rs429358) were determined in 122 AD and 52 MCI patients and

15 HCs by ABI Prism 7300 Real Time PCR System apparatus (Applied Biosystems, Foster city, CA, USA) using primers and probes purchased from Applied Biosystems as TaqMan® SNP Genotyping Assay (C\_904973\_10 ND C\_3084793\_20). All genotyping procedures were done by a researcher who was blind to all clinical data according to the procedures described by Applied Biosystems. Out of 189, 54 samples (29%) were genotyped again as a quality control for genotyping analyses. The three common variants of *APOE* ( $\epsilon$ 2,  $\epsilon$ 3, and  $\epsilon$ 4) were determined by two SNPs (rs429358 and rs7412).

### **2.3. Analysis of metals by inductively coupled plasma mass spectroscopy (ICP-MS)**

CSF and plasma Cu, Zn, Fe, Na, Mg, Ca, Co, Mo, Mn, B and Cr levels were determined using inductively coupled plasma mass spectroscopy (ICP-MS) on Agilent 7500cx (Agilent Technologies, Tokyo, Japan) (**Table 1**). Before analysis CSF and plasma samples were prepared by dilution (1:10 for CSF and 1:20 for plasma) with solution containing 0.7 mM ammonia, 0.01 mM EDTA, 0.07% (v/v) Triton X-100 and 2 $\mu$ g/l of internal standards (Ge, Rh, Tb, Lu and Ir) in ultrapure water. MicroMist nebulizer combined with a Peltier standard quartz spray chamber (Scott-type) cooled at 2°C and a quartz torch with a 2.5 mm diameter injector with a Shield Plate system and Ni sampler and skimmer cones were used. Tune solution of 1 $\mu$ g/l  $^7$ Li,  $^{59}$ Co,  $^{89}$ Y,  $^{140}$ Ce, and  $^{205}$ Tl was used for daily optimization of ICP-MS working conditions. Samples preparation and analysis were done in a laboratory with HVAC system (Heating, Ventilating and Air Conditioning) combined with HEPA filters. Standard addition method (i.e. matrix-matched calibration) was used for the quantification of elements concentration in CSF and plasma samples. To confirm the accuracy of the measurements, commercially available reference materials were used: ClinChek® Plasma Controls (Level I and II) and ClinChek® Serum Controls (Level I and II) from RECIPE (Munich, Germany); Seronorm™ Trace Elements Serum (Level I and II) (Sero AS, Billingstad, Norway). Since there is a possibility for contamination of the samples with chromium from the needles used for sample collection, chromium was removed from statistical analysis.

### **2.4. Statistical analysis**

CSF and plasma Cu, Zn, Fe, Na, Mg, Ca, Co, Mo, Mn and B levels were compared between two groups using Mann-Whitney U tests, while non-parametric Kruskal-Wallis tests were used

for comparison across three or more groups. A *post-hoc* non-parametric Dunn test to correct *p* values was used for pairwise comparisons. Statistical analysis was also done after introduction of age and sex as covariates. When analysing sodium plasma and CSF levels, presence of hypertension and cardiovascular diseases were also introduced as additional covariates (**Table 2**). Covariate analysis was performed using non-parametric Quade's ANCOVA. The genotype and allele distributions were determined by a  $\chi^2$  test. Statistical analyses were performed with SPSS 19.0.1 (SPSS, Chicago, IL, USA), with the  $\alpha$  value set at 0.05 for statistical significance.

### 3. Results

#### 3.1. Levels of metals in plasma and CSF of AD, MCI patients and HC

Significant difference in the levels of zinc measured in CSF was observed among AD, MCI patients and HC (H test=9.317, df=2,  $p=0.009$ ; **Fig. 1A**). This association was preserved after correction for age and sex ( $F=3.766$ ; df=2, 190;  $p=0.025$ ). The levels of zinc were significantly increased in CSF of AD patients compared to HC ( $p=0.027$ ). The levels of sodium measured in plasma were significantly different between AD, MCI patients and HC (H test=10.567, df=2,  $p=0.005$ ; **Fig. 1B**). Sodium levels were significantly increased in plasma of AD patients compared to HC ( $p=0.004$ ). This association was preserved after correction for age and sex ( $F=6.228$ ; df=2, 141;  $p=0.003$ ). When introducing hypertension in addition to age and sex as a covariate, the levels of sodium plasma levels remained significantly different between the groups ( $F_{(2,113)}=4.724$ ;  $p=0.011$ ). However, when introducing existence of cardiovascular diseases as covariate in addition to age and sex, the significance in sodium plasma levels between the groups was lost ( $F_{(2,110)}=2.384$ ;  $p=0.097$ ). Also, introduction of hypertension and cardiovascular diseases as covariates in addition to age and sex, resulted in loss of significance in plasma sodium levels between the groups ( $F_{(2,110)}=2.288$ ;  $p=0.106$ ). There was no significant difference in the levels of zinc measured in plasma, sodium measured in CSF and copper, iron, calcium, magnesium, cobalt, molybdenum, manganese and boron measured in both plasma and CSF among AD, MCI patients and HC (**Table 1**).

#### 3.2. Levels of metals in AD, MCI patients and HC with different *APOE* genotypes

No significant difference in distribution of *APOE* genotypes and alleles was observed among AD, MCI patients, and HC (**Table 3**).

A significant increase in sodium plasma levels was observed in AD, MCI patients and HC carriers of  $\epsilon 4$  *APOE* genotype ( $\epsilon 4\epsilon 4 + \epsilon 4\epsilon x$ ) ( $F_{(1,90)}=4.354$ ;  $p=0.040$ ) (**Fig. 2; Table 4**). Sodium plasma levels were also significantly increased in AD, MCI patients and HC carriers of  $\epsilon 4\epsilon x$  *APOE* genotype compared to carriers of  $\epsilon x\epsilon x$  *APOE* genotype ( $F_{(2,90)}=3.414$ ;  $p=0.037$ ;  $p=0.037$ ) (**Fig. 2; Table 4**). Additionally, sodium plasma levels were significantly increased in carriers of  $\epsilon 4\epsilon 3$  *APOE* genotype compared to carriers of  $\epsilon 3\epsilon 3$  *APOE* genotype in AD, MCI patients and HC (H test=12.530,  $df=4$ ,  $p=0.014$ ;  $p=0.020$ ) and AD and MCI patients (H test=10.427,  $df=4$ ,  $p=0.034$ ;  $p=0.050$ ; **Fig. 2G-H**), but this association was lost after introduction of covariates (hypertension and cardiovascular diseases) (**Table 4**).

Plasma boron levels were significantly decreased in AD and MCI patients carrying  $\epsilon 4\epsilon 4$  *APOE* genotype compared to patients carrying  $\epsilon x\epsilon x$  *APOE* genotype ( $F_{(2,105)}=3.998$ ,  $p=0.021$ ;  $p=0.020$ ; **Fig. 3A**). Decrease in boron plasma levels was also observed in AD and MCI patients carriers of  $\epsilon 4$  allele ( $F_{(1,105)}=4.077$ ,  $p=0.046$ ) (**Fig. 3**).

Copper plasma levels ( $U=456$ ,  $Z=-1.987$ ,  $p=0.047$ ; **Fig. 4A**) and magnesium plasma levels ( $F_{(1,69)}=6.041$ ,  $p=0.016$ ; **Fig. 4B**) were increased in AD patients carrying  $\epsilon 4$  allele [for copper, significance was lost after introduction of covariates (age and sex) ( $F_{(1,69)}=3.665$ ,  $p=0.060$ )]. Additionally, zinc plasma levels ( $F_{(2,68)}=3.556$ ,  $p=0.034$ ; **Fig. 5A**), calcium plasma levels ( $F_{(2,69)}=4.155$ ,  $p=0.020$ ;  $p=0.028$ ; **Fig. 5B**) and cobalt plasma levels ( $F_{(2,116)}=3.069$ ,  $p=0.050$ ;  $p=0.040$ ; **Fig. 5C**) were increased in patients carrying  $\epsilon 4\epsilon x$  *APOE* genotype compared to patients carrying  $\epsilon 4\epsilon 4$  *APOE* genotype.

Plasma levels of iron, molybdenum, manganese and CSF levels of copper, zinc, iron, sodium, magnesium, calcium, cobalt, molybdenum, manganese and boron did not differ significantly among patients carrying different *APOE* genotype. No significant difference in the levels of CSF ferritin was observed among patients with different *APOE* genotype.



## 4. Discussion

In this study we demonstrate increases in plasma levels of sodium, copper and magnesium in carriers of  $\epsilon 4$  allele of *APOE*. Additionally, sodium, calcium and cobalt plasma levels were increased in carriers of  $\epsilon 4\epsilon x$  genotype. Conversely, boron plasma levels were decreased in carriers of  $\epsilon 4$  allele and  $\epsilon 4\epsilon 4$  genotype. We also observed an increase in CSF zinc levels and plasma sodium levels in AD patients compared to HC.

Squitti and collaborators were among the first to observe an increase of serum copper in *APOE*  $\epsilon 4$  carriers compared to *APOE*  $\epsilon 4$  non-carriers [12,20,21]. Zappasodi *et al.* tested the correlation of “free” serum copper and electroencephalographic (EEG) activity in AD and reported stronger correlation between serum copper and temporal  $\alpha 1$  EEG activity in *APOE*  $\epsilon 4$  carriers compared to *APOE*  $\epsilon 4$  non-carriers [22]. Gonzalez *et al.* reported higher levels of serum zinc and copper in AD patients *APOE*  $\epsilon 4$  carriers compared to non-carriers [13]. Miyata and Smith proposed that ApoE antioxidative activity might be mediated by sequestration of copper in an isoform-dependent manner [11]. In fact, copper can even affect transcription of the *APOE* gene [23]. Additionally, patients carrying the *APOE*  $\epsilon 4$  genotype have earlier onset of symptoms of Wilson disease [24], a rare genetic disorder characterized by copper overload [25]. These results support our observation of increased copper plasma levels in AD patients carrying *APOE*  $\epsilon 4$  allele.

Increased risk for AD in *APOE*  $\epsilon 4$  carriers could be at least in part due to zinc dyshomeostasis in AD brains [10]. These authors tested ApoE proteolysis in the presence of zinc and concluded that ApoE4 isoform is the most sensitive to proteolysis compared to ApoE2 and ApoE3 isoforms [10]. In addition to the fact that metal-induced aggregation of A $\beta$  (either by Zn or Cu) is highest in the presence of ApoE4 [26], Oh *et al.* showed that zinc promotes ApoE and A $\beta$  aggregation into larger ApoE/A $\beta$  complexes, making A $\beta$  more resistant to A $\beta$ -degrading proteases [27]. In turn, ApoE can regulate zinc homeostasis, as reduction in synaptic zinc levels and reduced expression of ZnT3 (a zinc transporter required for accumulation of zinc in synaptic vesicles) was observed in *APOE* knockout mice [28]. Because synaptic zinc is important for long-time potentiation [29], decrease in its levels can lead to cognitive impairment. A study of *APOE*-targeted gene replacement mice showed no difference in the levels of zinc, copper and iron measured in the liver of mice with different *APOE* genotypes [30]. However, a study in humans showed higher levels of serum zinc in *APOE*  $\epsilon 4$  carriers with AD compared to non-carriers [13]. The present study shows significantly elevated zinc plasma levels in  $\epsilon 4\epsilon x$  heterozygotes compared to  $\epsilon 4\epsilon 4$  homozygotes and a significant increase in zinc

CSF levels in AD patients compared to HC, while there was no difference in zinc plasma levels in AD vs MCI, and MCI vs HC groups.

A neuroimaging study [15] revealed that cortical iron (measured by quantitative susceptibility mapping magnetic resonance imaging) and *APOE*  $\epsilon$ 4 allele synergistically interact with the default mode network (DMN) activity. DMN function is altered early in AD [31-33]. Kagerer *et al.* proposed that *APOE*  $\epsilon$ 4 allele could accelerate the accumulation of iron in the brain that could contribute to DMN dysfunction [15]. Moreover, Van Bergen *et al.* observed increased brain iron levels (measured by quantitative susceptibility mapping) in carriers of *APOE*  $\epsilon$ 4 allele [34]. Ayton and collaborators also reported an increase in CSF ferritin levels in carriers of *APOE*  $\epsilon$ 4 allele [35,36]. As CSF ferritin levels likely reflect the levels of iron in the brain, they proposed that elevation of the brain iron could be a possible mechanism through which *APOE*  $\epsilon$ 4 allele contributes to the increased risk for AD [35]. Additionally, Tisato *et al.* observed that presence of certain variants in genes responsible for iron metabolism (such as *HFE* 282Y allele) can reduce the *APOE*  $\epsilon$ 4-associated risk for AD [37]. It has been shown that ApoE binds iron [11] and another study revealed that iron upregulates ApoE levels in cultured neurons and astrocytes [38]. However, no changes were found in brain iron levels, measured postmortem in gray matter in carriers of *APOE*  $\epsilon$ 4 allele [39]. Also, as mentioned above, no difference in the levels of zinc, copper and iron measured in the liver of mice with different *APOE* genotypes [30]. These two studies support our results as we did not observe the difference in CSF and plasma iron levels in patients with different *APOE* genotypes. We also did not observe a difference in CSF levels of ferritin in patients with different *APOE* genotypes.

In addition, the present investigation reveals a significant increase in sodium plasma levels in carriers of *APOE*  $\epsilon$ 4 allele and in patients carrying  $\epsilon$ 4 $\epsilon$ x and  $\epsilon$ 4 $\epsilon$ 3 *APOE* genotypes. Also, sodium plasma levels were significantly increased in AD patients compared to HC. However, an increase in sodium CSF levels was observed in hypertensive patients with history of familial AD [40]. Also, quantitative sodium imaging using ultrahigh-field MRI revealed increase in tissue sodium concentrations in many AD brain regions [41,42]. Moreover, an increase in sodium levels in frontal and parietal cortex was detected in postmortem AD brains [43]. These authors did not observe any changes in sodium CSF levels, as in our study. They also showed that treatment of astrocytes with A $\beta$  leads to an increase in intracellular levels of sodium, suggesting that imbalance in cell ion homeostasis in AD brain can be triggered by A $\beta$  and thus could contribute to the pathophysiology of AD [43]. Some studies indicate that higher dietary sodium intake might be associated with impaired cognitive function, although with mixed

results (for a systematic review, see [44]). When analyzing sodium levels among groups, we introduced hypertension and cardiovascular diseases as additional covariates. Sodium levels show a positive association with blood pressure [45], while hypertension is a major risk factor for development of cardiovascular diseases [46,47]. In addition, various cardiovascular pathologies are observed in AD (cerebral amyloid angiopathy, cerebral arteriosclerosis, small blood vessel disease, microvascular degeneration and dysfunction of blood-brain barrier) [48,49]. By introducing covariates, we wanted to exclude the possibility that difference in sodium levels between the groups with different *APOE* genotype and diagnoses is the consequence of cardiovascular pathology rather than AD pathology. Although after introduction of these covariates, statistical significance was lost in some groups, but when including all cases (AD, MCI patients and HC), statistical significance remained. Thus, we concluded that the observed alterations in sodium levels between patients with different *APOE* genotype and patients with different diagnoses is the consequence of AD pathology, not cardiovascular pathology.

Boron deprivation can affect cognitive performance and lead to poorer performance on tasks for short-term memory [50–52]. The results of our study support these findings as we observed a significant decrease in boron plasma levels in carriers of  $\epsilon 4$  allele and  $\epsilon 4\epsilon 4$  *APOE* genotypes.

Impaired calcium signalling is a hallmark of many neurodegenerative disorders, including AD [53]. There is ample evidence of ApoE interaction with calcium. A study in primary neurons collected in *APOE* wild-type and knockout mice showed that after a mechanical injury, rates of apoptosis and intercellular calcium levels were higher in ApoE4 neurons [14]. These authors hypothesized that *APOE* polymorphisms can influence calcium levels. Our results support this view. Compared to carriers of  $\epsilon 4\epsilon 4$  genotype, we observed increased calcium plasma levels in patients carrying  $\epsilon 4\epsilon x$  *APOE* genotype. The results of Tolar and collaborators, who showed that ApoE and truncated ApoE peptide lead to increased intracellular calcium levels in embryonic rat hippocampal neurons and cause neuronal death [54]. Also, treatment of primary cerebral cortical neurons isolated from *APOE* knockout mice with ApoE4 lead to calcium overload through *N*-methyl-D-aspartate receptor and CaMK II signaling pathway [55], offer further support. Other studies based on different approaches have also shown that ApoE4 disrupts calcium homeostasis [56–60].

Treatment of streptozotocin-induced rat models of sporadic AD with magnesium sulfate decreased tau protein phosphorylation and had positive effect on cognitive functions and

synaptic plasticity [61]. Magnesium deficiency in diet was also associated with impaired memory [62], while magnesium supplementation improved memory [63–65]. Decreased levels of magnesium were detected in the brain and blood cells of AD patients [66,67]. Zhu *et al.* recently showed that optimal dietary magnesium intake improves cognitive function at least in part through modification of *APOE* methylation [68]. However, in apparent contrast to these data, we observed increased levels of plasma magnesium in carriers of  $\epsilon 4$  *APOE* allele. Interestingly, we observed a significant increase in cobalt plasma levels in patients carrying  $\epsilon 4\epsilon x$  *APOE* genotype compared to carriers of  $\epsilon 4\epsilon 4$  genotype. Increased levels of cobalt have been reported in brains of AD patients, especially in nucleus basalis of Meynert [69].

In conclusion, our study reveals a strong association between copper, zinc, sodium, magnesium, calcium and cobalt, as well as the metalloid boron, with *APOE* genotype in AD and MCI patients. As previous studies addressed mostly the association of calcium, copper, iron and zinc with ApoE4-mediated toxicity, our findings indicate that additional *in vivo* and *in vitro* studies into the molecular basis of the association of other essential metals and metalloids with ApoE-dependent mechanisms are warranted. The most notable finding of this study is the increase of sodium plasma levels and decrease in boron plasma levels in carriers of risk alleles in *APOE* gene that to our knowledge had not been previously observed. Additionally, variation in the plasma levels of magnesium and cobalt in patients with different *APOE* genotype should be further tested on larger cohorts.

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### **Conflict of interest**

The authors declare no conflict of interest.

## References

- [1] Bush AI (2013) The metal theory of Alzheimer's disease. *J Alzheimer's Dis* **33 Suppl 1**, S277-281.
- [2] Zubčić K, Radovanović V, Vlainić J, Hof PR, Oršolić N, Šimić G, Jazvinščak Jembrek M (2020) PI3K/Akt and ERK1/2 signalling are involved in quercetin-mediated neuroprotection against copper-induced Injury. *Oxid Med Cell Longev* **2020**, 9834742.
- [3] Zubčić K, Hof PR, Šimić G, Jazvinščak Jembrek M (2020) The role of copper in tau-related pathology in Alzheimer's disease. *Front Mol Neurosci* **13**, 572308.
- [4] Bu G (2009) Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nat Rev Neurosci* **10**, 333–344.
- [5] Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: high-avidity binding to  $\beta$ -amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A* **90**, 1977–1981.
- [6] Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ (1993) Association of apolipoprotein E allele  $\epsilon$ 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* **43**, 1467–1472.
- [7] Mahley RW, Rall SC (2000) Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* **1**, 507–537.
- [8] Strittmatter WJ (2012) Medicine. Old drug, new hope for Alzheimer's disease. *Science* **335**, 1447–1448.
- [9] Conejero-Goldberg C, Gomar JJ, Bobes-Bascaran T, Hyde TM, Kleinman JE, Herman MM, Chen S, Davies P, Goldberg TE (2014) *APOE2* enhances neuroprotection against Alzheimer's disease through multiple molecular mechanisms. *Mol Psychiatry* **19**, 1243–1250.
- [10] Xu H, Finkelstein DI, Adlard PA (2014) Interactions of metals and apolipoprotein E in Alzheimer's disease. *Front Aging Neurosci* **6**, 121.
- [11] Miyata M, Smith JD (1996) Apolipoprotein E allele-specific antioxidant activity and

- effects on cytotoxicity by oxidative insults and  $\beta$ -amyloid peptides. *Nat Genet* **14**, 55–61.
- [12] Squitti R, Bressi F, Pasqualetti P, Bonomini C, Ghidoni R, Binetti G, Cassetta E, Moffa F, Ventriglia M, Vernieri F, Rossini PM (2009) Longitudinal prognostic value of serum ‘free’ copper in patients with Alzheimer disease. *Neurology* **72**, 50–55.
- [13] González C, Martín T, Cacho J, Breñas MT, Arroyo T, García-Berrocal B, Navajo JA, González-Buitrago JM (1999) Serum zinc, copper, insulin and lipids in Alzheimer’s disease epsilon 4 apolipoprotein E allele carriers. *Eur J Clin Invest* **29**, 637–642.
- [14] Jiang L, Zhong J, Dou X, Cheng C, Huang Z, Sun X (2015) Effects of ApoE on intracellular calcium levels and apoptosis of neurons after mechanical injury. *Neuroscience* **301**, 375–383.
- [15] Kagerer SM, van Bergen JMG, Li X, Quevenco FC, Gietl AF, Studer S, Treyer V, Meyer R, Kaufmann PA, Nitsch RM, van Zijl PCM, Hock C, Unschuld PG (2020) APOE4 moderates effects of cortical iron on synchronized default mode network activity in cognitively healthy old-aged adults. *Alzheimer’s Dement Diagnosis, Assess Dis Monit* **12**,.
- [16] Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E (1999) Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* **56**, 303–308.
- [17] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer’s disease: recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. *Alzheimer’s Dement* **7**, 263–269.
- [18] Boban M, Malojčić B, Mimica N, Vuković S, Zrilić I, Hof PR, Šimić G (2012) The reliability and validity of the mini-mental state examination in the elderly Croatian population. *Dement Geriatr Cogn Disord* **33**, 385–392.
- [19] Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* **16**, 1215.
- [20] Squitti R, Lupoi D, Pasqualetti P, Dal Forno G, Vernieri F, Chioyenda P, Rossi L,

- Cortesi M, Cassetta E, Rossini PM (2002) Elevation of serum copper levels in Alzheimer's disease. *Neurology* **59**, 1153–1161.
- [21] Squitti R, Ventriglia M, Barbati G, Cassetta E, Ferreri F, Dal Forno G, Ramires S, Zappasodi F, Rossini PM (2007) 'Free' copper in serum of Alzheimer's disease patients correlates with markers of liver function. *J Neural Transm* **114**, 1589–1594.
- [22] Zappasodi F, Salustri C, Babiloni C, Cassetta E, Del Percio C, Ercolani M, Rossini PM, Squitti R (2008) An observational study on the influence of the *APOE-ε4* allele on the correlation between 'free' copper toxicosis and EEG activity in Alzheimer's disease. *Brain Res* **1215**, 183–189.
- [23] Jo DW, Leren TP, Yang ZY, Chung YH, Taylor JM, Paik YK (1995) Characterization of an upstream regulatory element of the human apolipoprotein E gene, and purification of its binding protein from the human placenta. *J Biochem* **117**, 915–922.
- [24] Litwin T, Gromadzka G, Członkowska A (2012) Wilson's disease: does iron metabolism impact phenotypic presentation? *Liver Int* **32**, 869–870.
- [25] Burkhead JL, Gray LW, Lutsenko S (2011) Systems biology approach to Wilson's disease. In *BioMetals* Springer, pp. 455–466.
- [26] Moir RD, Atwood CS, Romano DM, Laurans MH, Huang X, Bush AI, Smith JD, Tanzi RE (1999) Differential effects of apolipoprotein E isoforms on metal-induced aggregation of Aβ using physiological concentrations. *Biochemistry* **38**, 4595–4603.
- [27] Oh SB, Kim JA, Park S, Lee J-Y (2020) Associative interactions among zinc, apolipoprotein E, and amyloid-β in the amyloid pathology. *Int J Mol Sci* **21**, 802.
- [28] Lee JY, Cho E, Kim TY, Kim DK, Palmiter RD, Volitakis I, Kim JS, Bush AI, Koh JY (2010) Apolipoprotein E ablation decreases synaptic vesicular zinc in the brain. *BioMetals* **23**, 1085–1095.
- [29] Pan E, Zhang X an, Huang Z, Krezel A, Zhao M, Tinberg CE, Lippard SJ, McNamara JO (2011) Vesicular zinc promotes presynaptic and inhibits postsynaptic long-term potentiation of mossy fiber-CA3 synapse. *Neuron* **71**, 1116–1126.
- [30] Graeser AC, Huebbe P, Storm N, Höppner W, Döring F, Wagner AE, Rimbach G (2012) Apolipoprotein E genotype affects tissue metallothionein levels: studies in targeted gene replacement mice. *Genes Nutr* **7**, 247–255.

- [31] Greicius MD, Srivastava G, Reiss AL, Menon V (2004) Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *Proc Natl Acad Sci U S A* **101**, 4637-4642.
- [32] Šimić G, Babić M, Borovečki F, Hof PR (2014) Early failure of the default-mode network and the pathogenesis of Alzheimer's disease. *CNS Neurosci Ther* **20**, 692–698.
- [33] Liang L, Yuan Y, Wei Y, Yu B, Mai W, Duan G, Nong X, Li C, Su J, Zhao L, Zhang Z, Deng D (2021) Recurrent and concurrent patterns of regional BOLD dynamics and functional connectivity dynamics in cognitive decline. *Alzheimers Res Ther* **13**, 28.
- [34] Van Bergen JMG, Li X, Hua J, Schreiner SJ, Steininger SC, Quevenco FC, Wyss M, Gietl AF, Treyer V, Leh SE, Buck F, Nitsch RM, Pruessmann KP, Van Zijl PCM, Hock C, Unschuld PG (2016) Colocalization of cerebral iron with amyloid beta in mild cognitive impairment. *Sci Rep* **6**, 35514.
- [35] Ayton S, Faux NG, Bush AI, Weiner MW, Aisen P, Petersen R, Jack CR, Jagust W, Trojanowki JQ, Toga AW, Beckett L, Green RC, Saykin AJ, Morris J, Shaw LM, Khachaturian Z, Sorensen G, Kuller L, Raichle M, Paul S, Davies P, Fillit H, Hefti F, Holtzman D, Mesulam MM, Potter W, Snyder P, Schwartz A, Montine T, Thomas RG, Donohue M, Walter S, Gessert D, Sather T, Jiminez G, Harvey D, Bernstein M, Fox N, Thompson P, Schuff N, Borowski B, Gunter J, Senjem M, Vemuri P, Jones D, Kantarci K, Ward C, Koeppe RA, Foster N, Reiman EM, Chen K, Mathis C, Landau S, Cairns NJ, Householder E, Taylor-Reinwald L, Lee V, Korecka M, Figurski M, Crawford K, Neu S, Foroud TM, Potkin S, Shen L, Faber K, Kim S, Nho K, Thal L, Buckholtz N, Albert M, Frank R, Hsiao J, Kaye J, Quinn J, Lind B, Carter R, Dolen S, Schneider LS, Pawluczyk S, Beccera M, Teodoro L, Spann BM, Brewer J, Vanderswag H, Fleisher A, Heidebrink JL, Lord JL, Mason SS, Albers CS, Knopman D, Johnson K, Doody RS, Villanueva-Meyer J, Chowdhury M, Rountree S, Dang M, Stern Y, Honig LS, Bell KL, Ances B, Carroll M, Leon S, Mintun MA, Schneider S, Oliver A, Marson D, Griffith R, Clark D, Geldmacher D, Brockington J, Roberson E, Grossman H, Mitsis E, De Toledo-Morrell L, Shah RC, Duara R, Varon D, Greig MT, Roberts P, Albert M, Onyike C, D'Agostino D, Kielb S, Galvin JE, Cerbone B, Michel CA, Rusinek H, De Leon MJ, Glodzik L, De Santi S, Doraiswamy PM, Petrella JR, Wong TZ, Arnold SE, Karlawish JH, Wolk D, Smith CD, Jicha G, Hardy P, Sinha P, Oates E, Conrad G, Lopez OL, Oakley MA, Simpson DM, Porsteinsson AP, Goldstein BS, Martin K,



Makino KM, Ismail MS, Brand C, Mulnard RA, Thai G, Mc-Adams-Ortiz C, Womack K, Mathews D, Quiceno M, Diaz-Arrastia R, King R, Weiner M, Martin-Cook K, DeVous M, Levey AI, Lah JJ, Cellar JS, Burns JM, Anderson HS, Swerdlow RH, Apostolova L, Tingus K, Woo E, Silverman DHS, Lu PH, Bartzokis G, Graff-Radford NR, Parfitt F, Kendall T, Johnson H, Farlow MR, Hake AM, Matthews BR, Herring S, Hunt C, Van Dyck CH, Carson RE, MacAvoy MG, Chertkow H, Bergman H, Hosein C, Black S, Stefanovic B, Caldwell C, Hsiung GYR, Feldman H, Mudge B, Assaly M, Kertesz A, Rogers J, Bernick C, Munic D, Kerwin D, Mesulam MM, Lipowski K, Wu CK, Johnson N, Sadowsky C, Martinez W, Villena T, Turner RS, Johnson K, Reynolds B, Sperling RA, Johnson KA, Marshall G, Frey M, Lane B, Rosen A, Tinklenberg J, Sabbagh MN, Belden CM, Jacobson SA, Sirrel SA, Kowall N, Killiany R, Budson AE, Norbash A, Johnson PL, Allard J, Lerner A, Ogrocki P, Hudson L, Fletcher E, Carmichael O, Olichney J, DeCarli C, Kittur S, Borrie M, Lee TY, Bartha R, Johnson S, Asthana S, Carlsson CM, Potkin SG, Preda A, Nguyen D, Tariot P, Reeder S, Bates V, Capote H, Rainka M, Scharre DW, Kataki M, Adeli A, Zimmerman EA, Celmins D, Brown AD, Pearlson GD, Blank K, Anderson K, Santulli RB, Kitzmiller TJ, Schwartz ES, Sink KM, Williamson JD, Garg P, Watkins F, Ott BR, Querfurth H, Tremont G, Salloway S, Malloy P, Correia S, Rosen HJ, Miller BL, Mintzer J, Spicer K, Bachman D, Finger E, Pasternak S, Rachinsky I, Drost D, Pomara N, Hernando R, Sarrael A, Schultz SK, Boles Ponto LL, Shim H, Smith KE, Relkin N, Chaing G, Raudin L, Smith A, Fargher K, Raj BA, Neylan T, Grafman J, Davis M, Morrison R, Hayes J, Finley S, Friedl K, Fleischman D, Arfanakis K, James O, Massoglia D, Fruehling JJ, Harding S, Peskind ER, Petrie EC, Li G, Yesavage JA, Taylor JL, Furst AJ (2015) Ferritin levels in the cerebrospinal fluid predict Alzheimer's disease outcomes and are regulated by APOE. *Nat Commun* **6**, 6760.

- [36] Ayton S, Faux NG, Bush AI (2017) Association of cerebrospinal fluid ferritin level with preclinical cognitive decline in *APOE-ε4* carriers. *JAMA Neurol* **74**, 122–125.
- [37] Tisato V, Zuliani G, Vigliano M, Longo G, Franchini E, Secchiero P, Zauli G, Paraboschi EM, Singh AV, Serino ML, Ortolani B, Zurlo A, Bosi C, Greco A, Seripa D, Asselta R, Gemmati D (2018) Gene-gene interactions among coding genes of iron-homeostasis proteins and *APOE*-alleles in cognitive impairment diseases. *PLoS One* **13**, e0193867.
- [38] Xu H, Perreau VM, Dent KA, Bush AI, Finkelstein DI, Adlard PA (2016) Iron

- regulates apolipoprotein e expression and secretion in neurons and astrocytes. *J Alzheimer's Dis* **51**, 471–487.
- [39] Ayton S, Wang Y, Diouf I, Schneider JA, Brockman J, Morris MC, Bush AI (2020) Brain iron is associated with accelerated cognitive decline in people with Alzheimer pathology. *Mol Psychiatry* **25**, 2932–2941.
- [40] Souza LAC, Trebak F, Kumar V, Satou R, Kehoe PG, Yang W, Wharton W, Earley YF (2020) Elevated cerebrospinal fluid sodium in hypertensive human subjects with a family history of Alzheimer's disease. *Physiol Genomics* **52**, 133–142.
- [41] Mellon EA, Pilkinton DT, Clark CM, Elliott MA, Witschey WR, Borthakur A, Reddy R (2009) Sodium MR imaging detection of mild Alzheimer disease: preliminary study. *Am J Neuroradiol* **30**, 978–984.
- [42] Haeger A, Coste A, Lerman-Rabrait C, Lagarde J, Schulz JB, Vignaud A, Sarazin M, Bottlaender M, Reetz K, Romanzetti S, Boumezbeur F (2020) Quantitative sodium imaging using ultra-high field magnetic resonance imaging in patients with Alzheimer's disease. *Alzheimer's Dement* **16**, e042107.
- [43] Vitvitsky VM, Garg SK, Keep RF, Albin RL, Banerjee R (2012) Na<sup>+</sup> and K<sup>+</sup> ion imbalances in Alzheimer's disease. *Biochim Biophys Acta - Mol Basis Dis* **1822**, 1671–1681.
- [44] Mohan D, Yap KH, Reidpath D, Soh YC, McGrattan A, Stephan BCM, Robinson L, Chaiyakunapruk N, Siervo M, Pase M (2020) Link between dietary sodium intake, cognitive function, and dementia risk in middle-aged and older adults: a systematic review. *J Alzheimer's Dis* **76**, 1347–1373.
- [45] Law MR, Frost CD, Wald NJ (1991) By how much does dietary salt reduction lower blood pressure? III - Analysis of data from trials of salt reduction. *Br Med J* **302**, 819–824.
- [46] Yusuf PS, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanans F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L (2004) Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* **364**, 937–952.
- [47] O'Donnell MJ, Denis X, Liu L, Zhang H, Chin SL, Rao-Melacini P, Rangarajan S,

- Islam S, Pais P, McQueen MJ, Mondo C, Damasceno A, Lopez-Jaramillo P, Hankey GJ, Dans AL, Yusoff K, Truelsen T, Diener HC, Sacco RL, Ryglewicz D, Czlonkowska A, Weimar C, Wang X, Yusuf S (2010) Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. *Lancet* **376**, 112–123.
- [48] Attems J, Jellinger KA (2014) The overlap between vascular disease and Alzheimer's disease - lessons from pathology. *BMC Med* **12**, 206.
- [49] Šimić G, Španić E, Langer Horvat L, Hof PR (2019) Blood-brain barrier and innate immunity in the pathogenesis of Alzheimer's disease. *Prog Mol Biol Transl Sci* **168**, 99–145.
- [50] Penland JG (1994) Dietary boron, brain function, and cognitive performance. In *Environmental Health Perspectives* Public Health Services, US Dept of Health and Human Services, pp. 65–72.
- [51] Penland JG (1998) The importance of boron nutrition for brain and psychological function. In *Biological Trace Element Research* Humana Press, pp. 299–317.
- [52] Pizzorno L (2015) Nothing boring about boron. *Integr Med* **14**, 35–48.
- [53] Tong BCK, Wu AJ, Li M, Cheung KH (2018) Calcium signaling in Alzheimer's disease & therapies. *Biochim Biophys Acta - Mol Cell Res* **1865**, 1745–1760.
- [54] Tolar M, Keller JN, Chan S, Mattson MP, Marques MA, Crutcher KA (1999) Truncated apolipoprotein E (ApoE) causes increased intracellular calcium and may mediate ApoE neurotoxicity. *J Neurosci* **19**, 7100–7110.
- [55] Xu D, Peng Y (2017) Apolipoprotein E4 triggers multiple pathway-mediated Ca<sup>2+</sup> overload, causes CaMK II phosphorylation abnormality and aggravates oxidative stress caused cerebral cortical neuron damage. *Eur Rev Med Pharmacol Sci* **21**, 5717–5728.
- [56] Hartmann H, Eckert A, Förstl H, Müller WE (1994) Similar age-related changes of free intracellular calcium in lymphocytes and central neurons: effects of Alzheimer's disease. *Eur Arch Psychiatry Clin Neurosci* **243**, 218–223.
- [57] Wang XS, Gruenstein E (1997) Rapid elevation of neuronal cytoplasmic calcium by apolipoprotein E peptide. *J Cell Physiol* **173**, 73–83.

- [58] Müller W, Meske V, Berlin K, Scharnagl H, März W, Ohm TG (1998) Apolipoprotein E isoforms increase intracellular  $\text{Ca}^{2+}$  differentially through a  $\omega$ -agatoxin IVa-sensitive  $\text{Ca}^{2+}$ -channel. *Brain Pathol* **8**, 641–653.
- [59] Veinbergs I, Everson A, Sagara Y, Masliah E (2002) Neurotoxic effects of apolipoprotein E4 are mediated via dysregulation of calcium homeostasis. *J Neurosci Res* **67**, 379–387.
- [60] Larramona-Arcas R, González-Arias C, Perea G, Gutiérrez A, Vitorica J, García-Barrera T, Gómez-Ariza JL, Pascua-Maestro R, Ganfornina MD, Kara E, Hudry E, Martínez-Vicente M, Vila M, Galea E, Masgrau R (2020) Sex-dependent calcium hyperactivity due to lysosomal-related dysfunction in astrocytes from *APOE4* versus *APOE3* gene targeted replacement mice. *Mol Neurodegener* **15**, 1–23.
- [61] Xu Z-P, Li L, Bao J, Wang Z-H, Zeng J, Liu E-J, Li X-G, Huang R-X, Gao D, Li M-Z, Zhang Y, Liu G-P, Wang J-Z (2014) Magnesium protects cognitive functions and synaptic plasticity in streptozotocin-induced sporadic Alzheimer's model. *PLoS One* **9**, e108645.
- [62] Bardgett ME, Schultheis PJ, McGill DL, Richmond RE, Wagge JR (2005) Magnesium deficiency impairs fear conditioning in mice. *Brain Res* **1038**, 100–106.
- [63] Cherbuin N, Kumar R, Sachdev PS, Anstey KJ (2014) Dietary mineral intake and risk of mild cognitive impairment: the PATH through life project. *Front Aging Neurosci* **6**, 4.
- [64] Glick J (1990) Use of magnesium in the management of dementias. *Med Sci Res* **18**, 831–833.
- [65] Ozturk S, Cillier AE (2006) Magnesium supplementation in the treatment of dementia patients. *Med Hypotheses* **67**, 1223–1225.
- [66] András E, Igaz S, Molnár Z, Makó S (2000) Disturbances of magnesium concentrations in various brain areas in Alzheimer's disease. *Magnes Res* **13**, 189–196.
- [67] Vural H, Demirin H, Kara Y, Eren I, Delibas N (2010) Alterations of plasma magnesium, copper, zinc, iron and selenium concentrations and some related erythrocyte antioxidant enzyme activities in patients with Alzheimer's disease. *J Trace Elem Med Biol* **24**, 169–173.

- [68] Zhu X, Borenstein AR, Zheng Y, Zhang W, Seidner DL, Ness R, Murff HJ, Li B, Shrubsole MJ, Yu C, Hou L, Dai Q (2020) Ca:Mg ratio, *APOE* cytosine modifications, and cognitive function: results from a randomized trial. *J Alzheimer's Dis* **75**, 85–98.
- [69] Thompson CM, Markesbery WR, Ehmann WD, Mao YX, Vance DE (1988) Regional brain trace-element studies in Alzheimer's disease. *Neurotoxicology* **9**, 1–7.

**Table 1.** Levels of Cu, Zn, Fe, Na, Mg, Ca, Co, Mo, Mn and B measured in CSF and plasma of AD, MCI patients and HC.

Metal		AD		MCI		HC	
		Mean $\pm$ SD (Number of patients)	Median (25-75 <sup>th</sup> percentile)	Mean $\pm$ SD (Number of patients)	Median (25-75 <sup>th</sup> percentile)	Mean $\pm$ SD (Number of patients)	Median (25-75 <sup>th</sup> percentile)
Cu	CSF ( $\mu\text{g/l}$ )	16.8 $\pm$ 6.4 (125)	16.5 (11.9-20.4)	15.4 $\pm$ 6.3 (50)	14.4 (10.8-19.6)	15.3 $\pm$ 8.4 (19)	13.1 (8.5-20.4)
	Plasma ( $\mu\text{g/l}$ )	914.9 $\pm$ 177.9 (93)	910 (775-1023)	972.35 $\pm$ 216.4 (37)	958.0 (817.5-1103.5)	1004.4 $\pm$ 304.4 (14)	1008 (848.8-1209)
Zn	CSF ( $\mu\text{g/l}$ )	94.9 $\pm$ 44.8 (125)	88.8 (58.4-121.4)	83.4 $\pm$ 52.7 (50)	67.9 (48.2-107.1)	66.8 $\pm$ 45.6 (19)	47.2 (26.7-97.8)
	Plasma ( $\mu\text{g/l}$ )	690.8 $\pm$ 101 (92)	675.5 (628.3-757.3)	703.49 $\pm$ 110.4 (37)	681.0 (634.5-754.0)	762 $\pm$ 154.7 (14)	760.5 (630.8-838.5)
Fe	CSF ( $\mu\text{g/l}$ )	36.9 $\pm$ 20.6 (126)	33.7 (21.6-47.9)	35.2 $\pm$ 18.2 (50)	29.9 (22-44.4)	30.9 $\pm$ 13 (19)	30.8 (20.6-39.9)
	Plasma ( $\mu\text{g/l}$ )	1074.1 $\pm$ 429.4 (80)	981 (845.3-1257.8)	1150.7 $\pm$ 418.8 (35)	1149 (849-1345)	1189 $\pm$ 1028.4 (12)	835 (559.3-1252.3)
Ca	CSF (mg/l)	44.9 $\pm$ 14 (126)	44.4 (34.5-55)	40.3 $\pm$ 11.7 (50)	37.9 (32-47.7)	40.5 $\pm$ 13.1 (19)	39 (31.5-49.7)
	Plasma (mg/l)	78.3 $\pm$ 6.7 (93)	78.4 (74.4-82.7)	78.67 $\pm$ 7.9 (37)	77.7 (73.4-81.2)	79.6 $\pm$ 8 (14)	82.45 (75.52-85.33)
Na	CSF (mg/l)	3531.5 $\pm$ 879.2 (126)	3547 (2997.5-4266)	3200.6 $\pm$ 982 (50)	3108 (2540.5-3927)	3231.8 $\pm$ 708.6 (19)	3164 (2816-3637)
	Plasma (mg/l)	3677.23 $\pm$ 286 (93)	3691 (3540.5-3864.5)	3636.2 $\pm$ 313.9 (37)	3649 (3398.5-3822.5)	3387.9 $\pm$ 345.5 (14)	3457.5 (3145-3644.5)
Mg	CSF (mg/l)	30.2 $\pm$ 8.4 (126)	29.9 (24.6-35.9)	27.9 $\pm$ 8.6 (50)	27.8 (21.6-34.2)	27.8 $\pm$ 8.3 (19)	24.8 (23.5-30.1)
	Plasma (mg/l)	24.3 $\pm$ 2.7 (93)	24.1 (22.6-25.9)	24.6 $\pm$ 2.9 (37)	23.8 (22.9-25.6)	24.1 $\pm$ 6.8 (14)	23 (20.2-26.7)
Mo	CSF ( $\mu\text{g/l}$ )	0.745 $\pm$ 0.511 (126)	0.595 (0.410-0.880)	1.1 $\pm$ 0.7 (50)	1.1 (0.4-1.6)	0.716 $\pm$ 0.833 (19)	0.470 (0.200-0.660)
	Plasma ( $\mu\text{g/l}$ )	1.409 $\pm$ 1.292 (93)	1.180 (0.935-1.505)	1.6 $\pm$ 1.2 (37)	1.2 (1.0-1.6)	1.142 $\pm$ 0.480 (14)	1.060 (0.865-1.588)
Mn	CSF ( $\mu\text{g/l}$ )	1.330 $\pm$ 0.789 (126)	1.220 (0.773-1.613)	1.2 $\pm$ 0.8 (50)	1.0 (0.6-1.5)	1.240 $\pm$ 0.841 (19)	1.030 (0.680-1.380)
	Plasma ( $\mu\text{g/l}$ )	1.158 $\pm$ 0.448 (93)	1.080 (0.940-1.240)	1.1 $\pm$ 0.3 (37)	1.1 (0.9-1.3)	1.142 $\pm$ 0.480 (14)	1.060 (0.865-1.588)
B	CSF ( $\mu\text{g/l}$ )	29.7 $\pm$ 17.6 (126)	27.4 (16.3-41.8)	34.9 $\pm$ 43.6 (50)	28.2 (15-37.6)	23.7 $\pm$ 18.5 (19)	21.1 (11-30.6)
	Plasma ( $\mu\text{g/l}$ )	30.8 $\pm$ 14.4 (93)	27.1 (21.6-34.9)	35.9 $\pm$ 32.9 (37)	28.6 (21.5-40.2)	22.9 $\pm$ 9.5 (14)	22 (14.5-28.9)
Co	CSF ( $\mu\text{g/l}$ )	0.142 $\pm$ 0.073 (126)	0.122 (0.092-0.178)	0.151 $\pm$ 0.142 (50)	0.115 (0.081-0.174)	0.122 $\pm$ 0.081 (18)	0.099 (0.068-0.151)
	Plasma ( $\mu\text{g/l}$ )	0.428 $\pm$ 0.089 (93)	0.430 (0.370-0.490)	0.453 $\pm$ 0.158 (37)	0.44 (0.335-0.525)	0.393 $\pm$ 0.242 (14)	0.33 (0.218-0.445)
Ferritin	CSF ( $\mu\text{g/l}$ )	9.48 $\pm$ 3.63 (64)	8.76 (7.11-11.18)	8.38 $\pm$ 3.24 (29)	7.61 (6.11-9.9)	9.83 (1)	

AD, Alzheimer's disease; CSF, cerebrospinal fluid; HC, healthy control; MCI, mild cognitive impairment; SD, standard deviation.

**Table 2.** Demographic data and information on the presence of hypertension and cardiovascular diseases in AD and MCI patients and HC.

	Age	Sex	Hypertension	Cardiovascular diseases
	Median (25–75th percentile)	F/M	Yes/No	Yes/No
<b>AD</b>	72 (65-78)	68/58	35/39	12/62
<b>MCI</b>	65 (60-73)	25/27	19/12	11/20
<b>HC</b>	61 (52-75)	9/10	7/4	1/7

AD, Alzheimer’s disease; F, female; HC, healthy controls; M, male; MCI, mild cognitive impairment

**Table 3.** Count (N) and frequencies (%) of *APOE* gene polymorphism (rs7412 and rs429358) genotypes and alleles in AD and MCI patients and HCs.

<i>APOE</i>	AD (N=122) N (%)	MCI (N=52) N (%)	HC (N=15) N (%)
<b>Genotype</b>			
ε3ε2	10 (8.2)	1 (1.9)	2 (13.3)
ε3ε3	67 (54.9)	33 (63.5)	11 (73.3)
ε4ε2	4 (3.3)	0 (0.0)	0 (0.0)
ε4ε3	33 (27.0)	16 (30.8)	1 (6.7)
ε4ε4	8 (6.6)	2 (3.8)	1 (6.7)
$\chi^2 = 9.398$ ; df = 8; p = 0.154			
<b>Genotype</b>			
εxεx	77 (63.1)	34 (65.4)	13 (86.7)
ε4εx	37 (30.3)	16 (30.8)	1 (6.7)
ε4ε4	8 (6.6)	2 (3.8)	1 (6.7)
$\chi^2 = 4.349$ ; df = 4; p = 0.183			
<b>Allele</b>			
ε4 non-carriers	77 (63.1)	34 (65.4)	13 (86.7)
ε4 carriers	45 (36.9)	18 (34.6)	2 (13.3)
$\chi^2 = 3.285$ ; df = 2; p = 0.136			

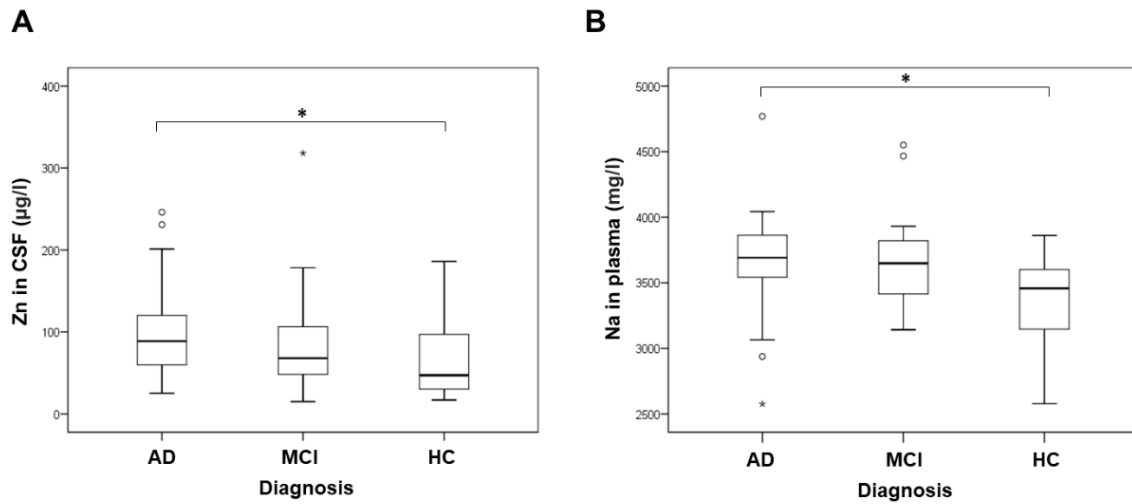
AD, Alzheimer's disease; APOE, apolipoprotein E; HC, healthy control; MCI, mild cognitive impairment.



**Table 4.** Analysis of sodium levels between patients with different *APOE* genotype with covariate analysis (with age, sex, hypertension and cardiovascular diseases as covariates).

<b>AD, MCI, HC</b>	<b><i>APOE</i> 3 groups - εxεx, ε4εx, ε4ε4</b>	<b><i>APOE</i> 2 groups - ε4+ vs ε4-</b>	<b><i>APOE</i> 5 groups - ε3ε2, ε3ε3, ε4ε3, ε4ε4, ε4ε2</b>
Uncorrected	H test=11.867, df=2, p=0.003* εxεx vs ε4εx, p=0.002*	U=1119, Z=-3.056, p=0.002*	H test=12.530, df=4, p=0.014* ε4ε3 vs ε3ε3, p=0.020*
Corrected for age and sex	F <sub>(2,116)</sub> =6.474; p=0.002* εxεx vs ε4εx, p=0.002*	F <sub>(1,116)</sub> =9.868; p=0.002*	F <sub>(4,116)</sub> =3.479; p=0.010 ε4ε3 vs ε3ε3, p=0.009*
Corrected for hypertension, age and sex	F <sub>(2,90)</sub> =3.335; p=0.040* εxεx vs ε4εx, p=0.040*	F <sub>(1,90)</sub> =4.263; p=0.042*	F <sub>(4,90)</sub> =2.258; p=0.069
Corrected for cardiovascular diseases, age and sex	F <sub>(2,90)</sub> =3.524; p=0.034* εxεx vs ε4εx, p=0.034*	F <sub>(1,90)</sub> =4.440; p=0.038*	F <sub>(4,90)</sub> =2.267; p=0.068
Corrected for hypertension, cardiovascular diseases, age and sex	F <sub>(2,90)</sub> =3.414; p=0.037* εxεx vs ε4εx, p=0.037*	F <sub>(1,90)</sub> =4.354; p=0.040*	F <sub>(4,90)</sub> =2.287; p=0.066
<b>AD, MCI</b>	<b><i>APOE</i> 3 groups - εxεx, ε4εx, ε4ε4</b>	<b><i>APOE</i> 2 groups - ε4+ vs ε4-</b>	<b><i>APOE</i> 5 groups - ε3ε2, ε3ε3, ε4ε3, ε4ε4, ε4ε2</b>
Uncorrected	H test=9.613, df=2, p=0.006* εxεx vs ε4εx, p=0.006*	U=984, Z=-2.755, p=0.006*	H test=10.427, df=4, p=0.034* ε4ε3 vs ε3ε3, p=0.050*
Corrected for age and sex	F <sub>(2,105)</sub> =4.790; p=0.010* εxεx vs ε4εx, p=0.008*	F <sub>(1,105)</sub> =7.165; p=0.009*	F <sub>(4,105)</sub> =2.703; p=0.035 ε4ε3 vs ε3ε3, p=0.038*
Corrected for hypertension, age and sex	F <sub>(2,82)</sub> =2.379; p=0.099	F <sub>(1,82)</sub> =3.184; p=0.078	F <sub>(4,82)</sub> =1.983; p=0.105
Corrected for cardiovascular diseases, age and sex	F <sub>(2,82)</sub> =2.492; p=0.089	F <sub>(1,82)</sub> =3.355; p=0.071	F <sub>(4,82)</sub> =2.004; p=0.102
Corrected for hypertension, cardiovascular diseases, age and sex	F <sub>(2,82)</sub> =2.434; p=0.094	F <sub>(1,82)</sub> =3.269; p=0.074	F <sub>(4,82)</sub> =2.036; p=0.097
<b>AD</b>	<b><i>APOE</i> 3 groups - εxεx, ε4εx, ε4ε4</b>	<b><i>APOE</i> 2 groups - ε4+ vs ε4-</b>	
Uncorrected	H test=8.971, df=2, p=0.011* εxεx vs ε4εx, p=0.014*	U=429, Z=-2.571, p=0.010*	
Corrected for age and sex	F <sub>(2,69)</sub> =4.332; p=0.017* εxεx vs ε4εx, p=0.026*	F <sub>(1,69)</sub> =3.967; p=0.050*	
Corrected for hypertension, age and sex	F <sub>(2,52)</sub> =1.829; p=0.171	F <sub>(1,52)</sub> =0.882; p=0.352	
Corrected for cardiovascular diseases, age and sex	F <sub>(2,52)</sub> =2.049; p=0.139	F <sub>(1,52)</sub> =0.726; p=0.398	
Corrected for hypertension, cardiovascular diseases, age and sex	F <sub>(2,52)</sub> =2.062; p=0.137	F <sub>(1,52)</sub> =0.727; p=0.398	

AD, Alzheimer's disease; APOE, apolipoprotein E; HC, healthy control; MCI, mild cognitive impairment.



**Figure 1.** Levels of A) zinc measured in CSF (<sup>1</sup>p=0.027\*, <sup>2</sup>p=0.051) and B) sodium measured in plasma of AD, MCI patients and HC (<sup>1</sup>p=0.004\*, <sup>2</sup>p=0.002\*, <sup>3</sup>p=0.013\*, <sup>4</sup>p=0.097, <sup>5</sup>p=0.106).

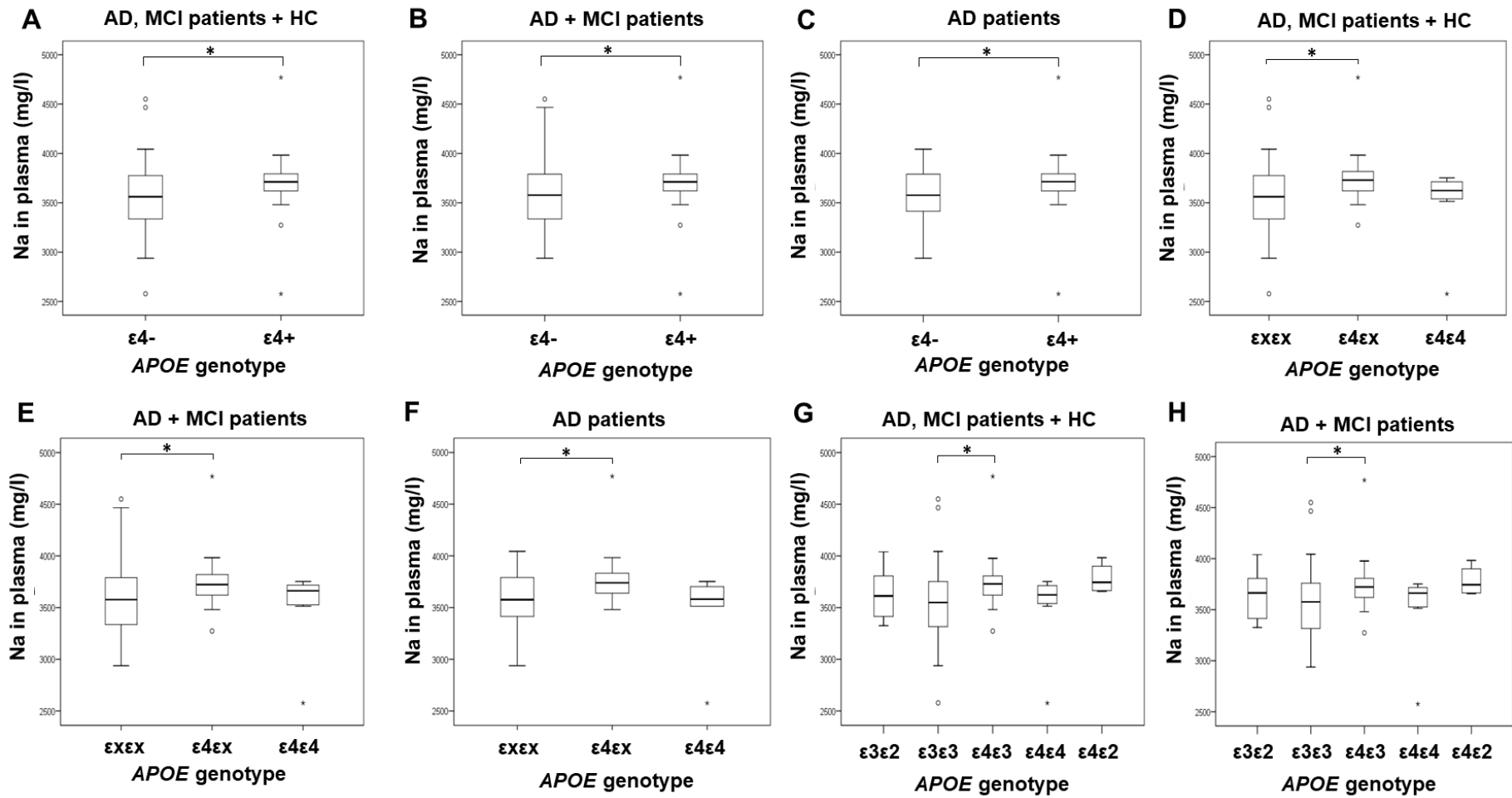
<sup>1</sup> Uncorrected

<sup>2</sup> Corrected for age and sex

<sup>3</sup> Corrected for hypertension, age and sex

<sup>4</sup> Corrected for cardiovascular diseases, age and sex

<sup>5</sup> Corrected for hypertension, cardiovascular diseases, age and sex



**Figure 2.** Levels of sodium measured in plasma of AD, MCI patients and HC with different *APOE* genotypes. A) <sup>1</sup>p=0.002\*, <sup>2</sup>p=0.002\*, <sup>3</sup>p=0.042\*, <sup>4</sup>p=0.038\*, <sup>5</sup>p=0.040\*, B) <sup>1</sup>p=0.006\*, <sup>2</sup>p=0.009\*, <sup>3</sup>p=0.078, <sup>4</sup>p=0.071, <sup>5</sup>p=0.074, C) <sup>1</sup>p=0.010\*, <sup>2</sup>p=0.050\*, <sup>3</sup>p=0.352, <sup>4</sup>p=0.398, <sup>5</sup>p=0.398, D)

<sup>1</sup>p=0.002\*, <sup>2</sup>p=0.002\*, <sup>3</sup>p=0.040\*, <sup>4</sup>p=0.034\*, <sup>5</sup>p=0.037\*, E) <sup>1</sup>p=0.006\*, <sup>2</sup>p=0.008\*, <sup>3</sup>p=0.099, <sup>4</sup>p=0.089, <sup>5</sup>p=0.094, F) <sup>1</sup>p=0.014\*, <sup>2</sup>p=0.026\*,  
<sup>3</sup>p=0.171, <sup>4</sup>p=0.139, <sup>5</sup>p=0.137, G) <sup>1</sup>p=0.020\*, <sup>2</sup>p=0.009\*, <sup>3</sup>p=0.069, <sup>4</sup>p=0.068, <sup>5</sup>p=0.066, H) <sup>1</sup>p=0.050\*, <sup>2</sup>p=0.038\*, <sup>3</sup>p=0.105, <sup>4</sup>p=0.102, <sup>5</sup>p=0.097.

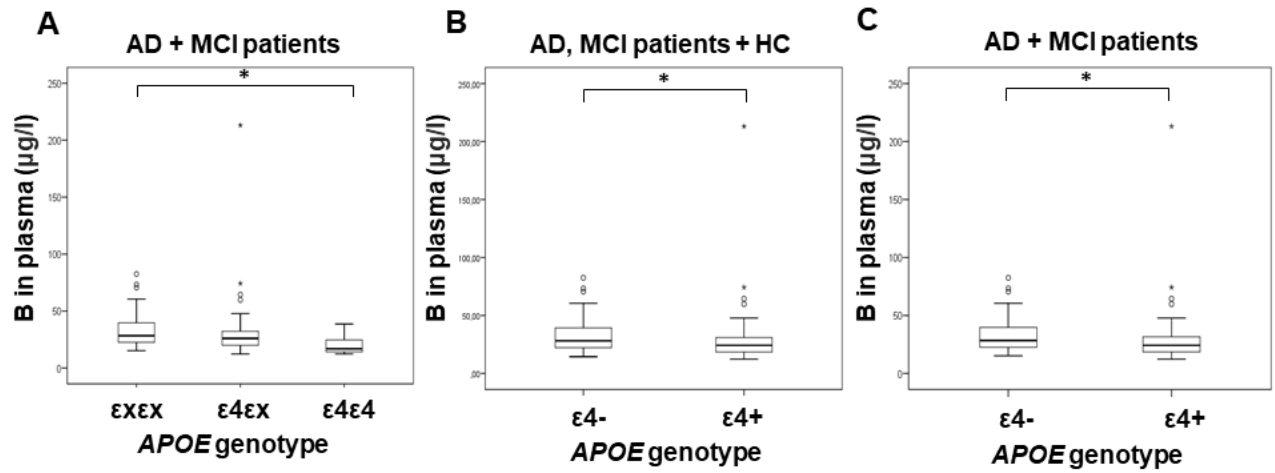
<sup>1</sup>Uncorrected

<sup>2</sup>Corrected for age and sex

<sup>3</sup>Corrected for hypertension, age and sex

<sup>4</sup>Corrected for cardiovascular diseases, age and sex

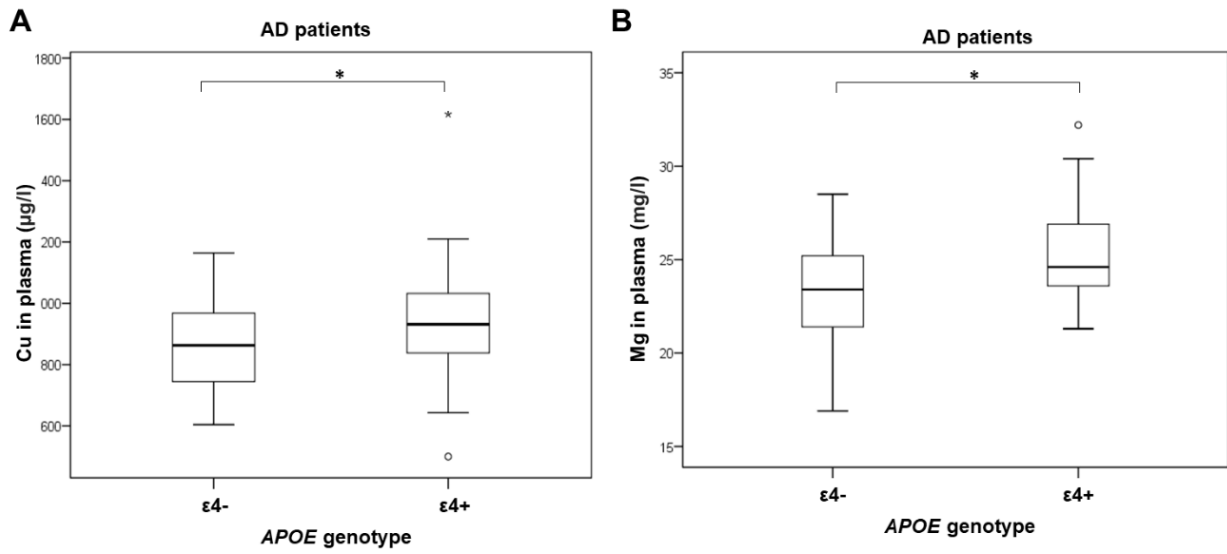
<sup>5</sup>Corrected for hypertension, cardiovascular diseases, age and sex



**Figure 3.** Levels of boron measured in plasma of AD, MCI patients and HC with different *APOE* genotypes. A) <sup>1</sup>p=0.012\*, <sup>2</sup>p=0.020\*, B) <sup>1</sup>p=0.045\*, <sup>2</sup>p=0.053, C) <sup>1</sup>p=0.043\*, <sup>2</sup>p=0.046\*.

<sup>1</sup>Uncorrected

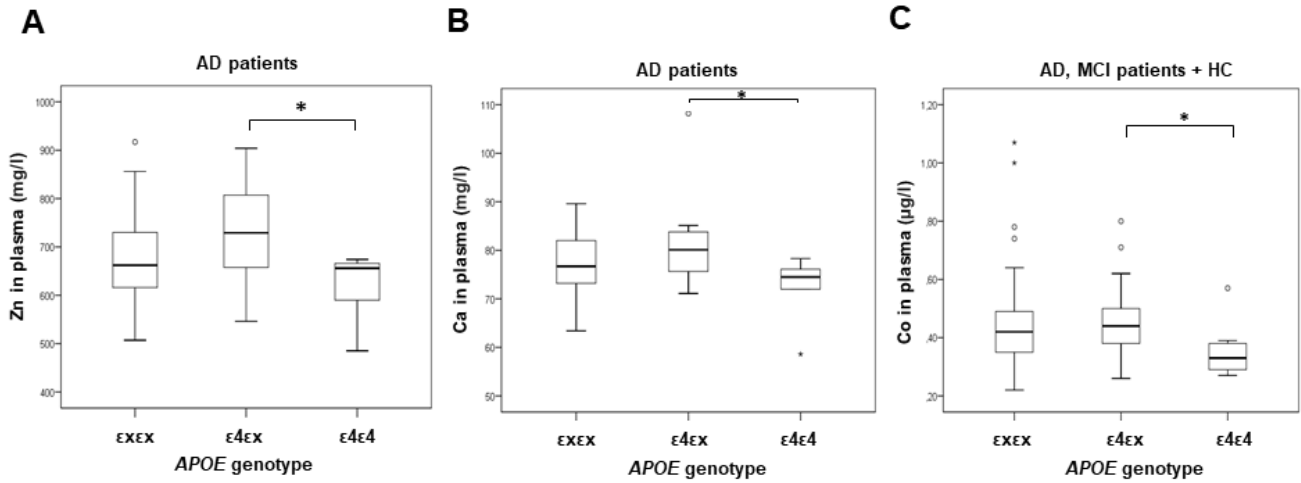
<sup>2</sup>Corrected for age and sex



**Figure 4.** Levels of A) copper (<sup>1</sup>p=0.047\*, <sup>2</sup>p=0.060) and B) magnesium (<sup>1</sup>p=0.010\*, <sup>2</sup>p=0.016\*) measured in plasma of AD patients with different *APOE* genotypes.

<sup>1</sup>Uncorrected

<sup>2</sup>Corrected for age and sex



**Figure 5.** Levels of A) zinc (<sup>1</sup>p=0.045\*, <sup>2</sup>p=0.034\*) and B) calcium (<sup>1</sup>p=0.044\*, <sup>2</sup>p=0.028\*) measured in plasma of AD patients and C) cobalt (<sup>1</sup>p=0.033\*, <sup>2</sup>p=0.040\*) measured in plasma of AD, MCI patients and HC with different *APOE* genotypes.

<sup>1</sup>Uncorrected

<sup>2</sup>Corrected for age and sex