



OPEN Post-mortem human Alzheimer's brain metallome depends on Braak stages and brain regions

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Limited studies have evaluated the influence of redox-related metals on Alzheimer's brain. We determined 15 metals in 241 human brain samples obtained from five biobanks. We examined the levels of metals in the frontal cortex (CTX) and the basal ganglia (BG) of Alzheimer's brains of different stages, ranging from Braak stages I and II to Braak stages V and VI. ICP-MS. Imbalances measured metals in the Alzheimer's brain metallome of different Braak stages and different regions compared to controls and associations in the other areas were estimated by the Krustall-Wallis Test and Spearman correlations, respectively. The metallomes of brain regions compared to the controls were significantly different for most of the metals in the CTX and BG samples. We observed higher levels of Mn, Cu, Mo, and Se and lower levels of Co in Alzheimer's CTX compared to the controls and higher levels of V and Cr and lower levels of Co in BG. Different associations were found in the Braak stages compared to the control group in CTX and BG samples. Metals may differ in Braak stages across different human brain regions.

Although the etiology of Alzheimer's disease (AD) remains unclear, the amyloid cascade hypothesis is the most widely accepted. Thus, the accumulation of amyloid beta ($A\beta_{42}$) in the brain would be the primary event in the cascade that led to p-tau hyperphosphorylation, neurofibrillary tangle (NFT) formation, synaptic dysfunction, and neuronal death. Although it is clear that $A\beta_{42}$ plays an important role in the neurodegenerative process, various findings, such as the abundant presence of plaques in cognitively normal elderly individuals or the limited success of therapeutic approaches based solely on this peptide, raise doubts about whether it is the sole responsible¹. In this context, the evident link between AD and redox metal dysregulation could be the basis for using metal chelators as an alternative. Research has shown that these metals cause $A\beta$ aggregation, and both result in the production of hydrogen peroxide, which in turn causes oxidative damage. Clotrimazole, an antibiotic that acts as a chelating agent facilitates the removal of metal ions and has the potential to slow the progression of AD and modify disease pathology². Likewise, metal ions, mainly Cu and Zn ions bound to $A\beta$ are involved in the catalytic generation of reactive oxygen species (ROS) that participate in oxidative stress, the most important mechanism in the onset of AD. Higher accumulation of Cu, Fe, and Zn has been observed in AD brains than in controls³. The role of metals in the tau protein phosphorylation and aggregation in the form of NFT has also been pointed out⁴. Likewise, Zn competes with Cu for amyloid coordination⁵ and supplementation has been reported to influence Cu distribution in the brain⁵. The Zn^{2+} ion is also a substrate of ferroportin and amyloid precursor protein (APP) is essential for ferroportin persistence. The loss of ferroportin induces memory impairment by promoting ferroptosis in AD⁶. Furthermore, Al-overloading in the brain can lead to the apoptotic death of neurons and glial cells⁷ and might disturb Fe homeostasis⁸. Interestingly, AD patients tend to accumulate Fe in their nervous system⁹. Additionally, dysregulation of Mg may lead to synaptic dysfunction, impaired plasticity, and neural degradation¹⁰. Moreover, exposure to toxic metals such as As, Cd, and Pb may increase the risk of neurodegeneration in later life due to their ability to cross the blood-brain barrier (BBB) and accumulate in

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the brain¹¹. Additionally, overexposure to Mn can have toxic effects, causing oxidative stress and disrupting mitochondrial energy metabolism, which are known to contribute to AD¹². Lastly, Se is an antioxidant element that is a component of selenoproteins. Se deficiency may lead to irreversible changes in neuronal cells, potentially causing cognitive impairment and AD¹³. It has been found a negative correlation between cognitive impairment, Se levels, and the activity of several selenoproteins in AD patients¹⁴. Due to this disease can only be definitively diagnosed at post-mortem examination recording the extensive deposition of A β and tau protein in typical brain regions, the study of metals dyshomeostasis in the AD pathology could shed light to their potential role in the onset and development of the pathology.

Our study involved analyzing the concentrations of eighteen toxic and essential elements in two regions of post-mortem human brains, such as the frontal cortex (CTX) and basal ganglia (BG). We conducted this analysis on AD human brains with Braak stages ranging from I to VI and compared the metal levels to those of controls to identify any significant alterations and correlations between elements in different brain regions. Our analysis was performed using the inductively coupled plasma mass spectrometry equipped with a triple quadrupole (ICP-QQQ-MS).

Results

The average concentration heatmap of the elemental distribution in human brain regions in the different study groups is illustrated in Fig. 1.

Table S1 of the Supplementary Material shows the concentration of various elements in CTX and BG regions of postmortem AD human brains with varying Braak stages and healthy controls. To investigate the potential dyshomeostasis of elements and group clustering in different regions of the human brain, we utilized Partial Least Square discriminant analysis (PLS-DA) on our dataset. The PLS-DA results for the pairwise comparison between the control and the groups Braak I-II (Figure S1), Braak III-IV (Figure S2), and Braak VI-V (Figure S3) AD brains showed group clustering mainly in the BG region, while the PLS-DA of CTX samples yielded minimal clustering. The fold changes and p-values are reported in the Supplementary Material (Tables S3 and S4). In addition, the element distribution for control (Figure S4), Braak I-II (Figure S5), Braak III-IV (Figure S6), and Braak VI-V (Figure S7) are described in Supplementary Material. Generally, considering all the study groups, the CTX and BG showed higher concentrations of Mg and Fe, with levels around 44% and 43% respectively. Conversely, Cd, Tl, V, and Pb were present in lower concentrations, constituting less than 0.001% in the brain regions. Tables S2 (CTX) and S3 (BG) provide the fold change of each element between groups in these two different regions, along with the statistical p-value from the Kruskal-Wallis test. We observed several significant differences in CTX and BG among the study groups. In this sense, our research revealed a significant increase in the CTX levels of Mg (FC = 1.11, $p < 0.001$), Mn (FC = 1.41, $p < 0.001$), Fe (FC = 1.60, $p < 0.001$), Cu (FC = 1.61, $p < 0.001$), Mo (FC = 1.45, $p < 0.02$), and Se (FC = 1.20, $p < 0.001$), in the Braak I-II group when compared to control levels. In Braak III-IV, V (FC = 1.90, $p < 0.02$), Co (FC = 0.64, $p < 0.001$), Se (FC = 1.12, $p < 0.02$), and Cu

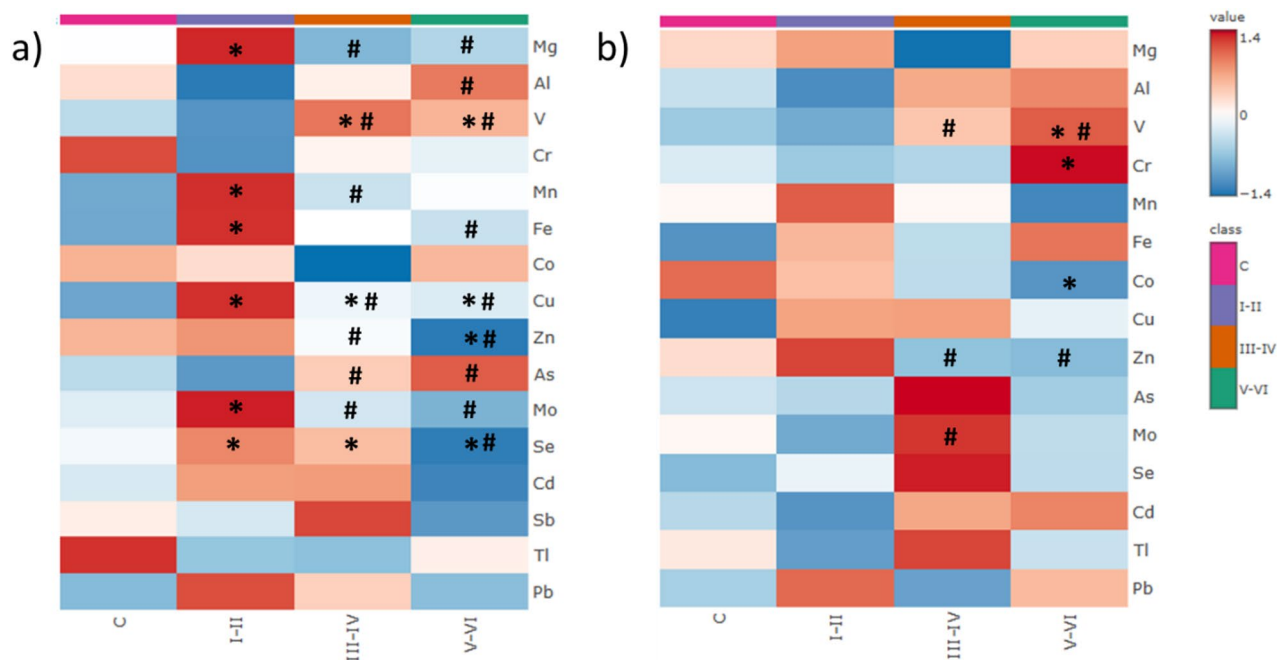


Fig. 1. Average concentration Heatmap of the abundance of elements in (a) CTX and (b) BG from control (C), and groups of AD with Braak I-II, Braak III-IV, and Braak V-VI. *Significant values compared to the control group. # Significant values compared to the Braak I-II group. Red color means increased levels while blue color means decreased levels. Pink color represents the control group, purple color represents the Braak I-II group, orange color represents the Braak III-IV group, and green color represents the Braak V-VI group.

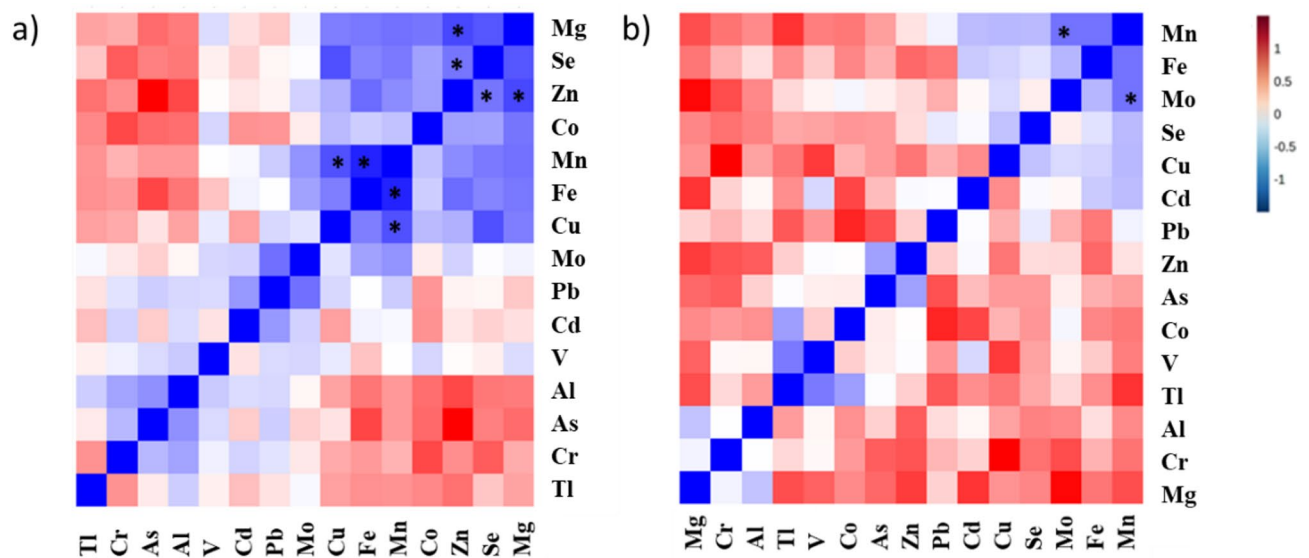


Fig. 2. Correlation heatmap between elements in (a) CTX and (b) BG in Braak V-VI stages. *Significant correlation ($p < 0.05$). Red color means a positive correlation, and blue color means a negative correlation.

($FC = 1.23$, $p < 0.03$), were significantly altered in this region. Moreover, Braak V-VI showed significant alterations in the levels of Se ($FC = 0.87$, $p < 0.02$), Cu ($FC = 1.21$, $p < 0.03$), Zn ($FC = 0.78$, $p < 0.001$), and V ($FC = 1.64$, $p < 0.02$). Otherwise, significant alterations in the levels of Mg ($FC = 0.87$, $p < 0.001$), V ($FC = 2.05$, $p < 0.03$), Cu ($FC = 0.75$, $p < 0.03$), Zn ($FC = 0.76$, $p < 0.001$), Se ($FC = 0.73$, $p < 0.001$), and Mo ($FC = 0.59$, $p < 0.001$) were found in the CTX of AD brains with Braak III-IV and V-VI compared to those with Braak I-II. The levels of V were notably higher in stages Braak III-IV ($FC = 2.54$, $p < 0.001$), and Braak V-VI ($FC = 3.49$, $p < 0.001$), in BG, in comparison to control levels. Furthermore, the levels of V in Braak V-VI ($FC = 6.22$, $p < 0.001$), and Braak III-IV ($FC = 4.53$, $p < 0.001$), were significantly elevated in comparison to Braak I-II stages. No significant differences were observed between Braak III-IV and Braak V-VI, or between Braak I-II and controls. Moreover, the concentration of Co was significantly decreased ($FC = 0.67$, $p < 0.002$) in Braak V-VI patients compared to the control group. Furthermore, the levels of Cr were significantly higher in Braak V-VI compared to Braak III-IV ($FC = 1.61$, $p < 0.01$), Braak I-II ($FC = 1.57$, $p < 0.05$), and control levels ($FC = 1.63$, $p < 0.05$). Additionally, we found significantly diminished levels of Zn ($FC = 0.89$, $p < 0.002$) and increased levels of Mo ($FC = 1.49$, $p < 0.02$) in Braak V-VI compared to Braak I-II. In addition, the concentration of Mo ($FC = 1.27$, $p < 0.03$) was also higher in Braak III-IV compared to Braak I-II patients.

Moreover, we applied the Spearman correlation analysis to the dataset of all the study groups in CTX and BG samples. Figure 2 shows the correlation between elements in the most affected AD brains with the Braak IV-V group. Correlations heatmap for C (Figure S8), Braak I-II (Figure S9), and Braak III-IV (Figure S10) are described in the Supplementary Material as well as correlation coefficients for CTX (Table S4) and BG (Table S5). In CTX samples from the Braak I-II group, we observed several significant positive correlations between several oligo elements. For instance, we found significant correlations between Mn and Cu ($\rho = 0.72$, $p = 0.03$), Fe and Zn ($\rho = 0.77$, $p = 0.02$), Cu and Mo ($\rho = 0.8$, $p = 0.01$), and Se and Zn ($\rho = 0.73$, $p = 0.02$). Additionally, we found positive associations between non-essential elements such as As with Pb ($\rho = 0.83$, $p = 0.01$) and V ($\rho = 0.73$, $p = 0.02$). Further correlations were found between Al and non-essential elements, including V ($\rho = 0.83$, $p = 0.01$), Pb ($\rho = 0.76$, $p = 0.01$), and Sb ($\rho = 0.87$, $p = 0.00$). Moreover, we identified increased and significant associations between Mn with Fe and Mo in CTX in Braak III-IV, and Braak V-VI stages. In our analysis, we found notable correlations in BG samples. Specifically, we mainly observed correlations between Se and Mn ($\rho = 0.9$, $p < 0.001$), Mg ($\rho = 0.9$, $p < 0.001$), Fe ($\rho = 0.8$, $p < 0.001$), Cu ($\rho = 0.7$, $p < 0.001$) in brains with Braak stages I-II. Similarly, we observed associations between Mn and Mo in Braak III-IV ($\rho = 0.7$, $p < 0.001$), and Braak V-VI ($\rho = 0.7$, $p < 0.001$) stages.

Discussion

Our results showed that the most abundant elements in the different brain regions were Mg, Fe, and Zn. It is known that alterations in the levels of Fe, and Zn, called neuro-metals due to their important role in the maintenance of function of the central nervous system¹⁵ may contribute to AD neuropathology¹⁶. Some authors have suggested that Fe and Zn are enriched within amyloid plaques¹⁷. However, decreased levels of these elements have been found in AD brain regions, suggesting disturbances and a decrease in the bioavailability of these metals. We found elevated levels of these metals in AD CTX regions compared to controls, being significant in Braak I-II. Many authors have reported higher levels of Fe in AD brains¹⁸ although this increase depends on the particular region. In addition, according to our results, Szabo et al. found¹⁹ a significant increase in the Fe concentration of CTX from AD brains compared to control. Moreover, Ramos et al. also reported higher concentrations of Fe

in the BG in the AD human brain⁹. Increased Fe levels in the brain are related to lipid peroxidation, oxidative stress, and severe tissue damage²⁰. Similarly, we mostly found decreased levels of Cu in the BG region, but increased levels in CTX in AD brains (Braak I to VI) compared to controls. Several authors have found that Cu levels in brain tissue including the hippocampus, cerebellum, and CTX decreased in AD brains^{19,21–23}. However, according to our results, Loeffler et al. found increased levels of Cu in CTX from AD brains compared to controls²⁴. Impairments in Cu homeostasis have been described in AD brains²⁵ inducing the formation of the β -sheet and the fibrillary form of A β and the tau hyperphosphorylation and aggregation in the brain²⁶. Not too many significant differences were found in the Zn levels of the different regions of AD brains compared to the control. Specifically, we observe a significant diminution in BG of AD Braak III–IV and Braak V–VI brains compared to Braak I–II samples. Decreased levels of Zn are associated with loss of synapses near plaques²⁷. However, there is a discrepancy between the levels of Zn in AD brains. Corrigan et al. reported diminished levels of Zn in the hippocampus of AD brains²⁸ while Andrási et al. increased levels of Zn in the cortex of AD cases²⁹. According to our results, De Wilde et al. did not find significant changes in Zn levels in the brain, and Szabo et al. determined similar levels of Zn CTX from AD cases and controls¹⁹. According to our results Mg levels in AD brains appear to be decreased in the brain tissue compared to physiological levels^{22,30–33}. Specifically, we found decreased and significant levels in the CTX in the more advanced AD stages (Braak III–IV and V–VI) compared to early stage Braak I–II. Some studies have demonstrated that Mg deficiency affects memory and dementia³⁴. The effect of Mg supplementation in AD patients has improved memory in dementia patients³⁵ and increased the maintenance of cognitive function in animals³². Al, Mn, and Se were also abundant in the different brain regions and higher than other metals. Al has been extensively studied in AD patients^{12,28,36–40}. We found elevated levels, although not significant, of Al in the BG of AD brains, being higher in advanced stages of the disease. Several authors have found increased levels of Al in brain tissue, including the entorhinal cortex, hippocampus, and amygdala⁴⁰ suggesting that Al plays an important role in the neuropathology of AD since Al co-deposits with A β in amyloid plaques in human AD brain tissue and induces the apoptotic death of neurons and glial cells¹¹. On the other hand, we found decreased levels of Mn in BG, but higher levels in CTX from AD advanced staged patients compared to control and early AD stages. Some authors have reported increased levels of Mn in the postmortem AD brain^{19,22,23}. Increased levels of Mn are related to abnormal tau aggregation and hyperphosphorylation⁴¹ and it is linked to decreased cognitive performance⁴² contributing to the etiology of AD. On the contrary, diminished levels of Mn have been found in CTX¹⁹ samples from AD. On the other hand, higher levels of Se were mostly found in the different brain regions of AD brains compared to controls. Specifically, we observed significantly increased levels in CTX of Braak I–II and III–IV stages compared to controls. However, we also observed a significant reduction in Se levels of Braak V–VI brains compared to Braak I–II and III–IV. Moreover, the levels of Se in BG were also slightly higher in Braak I–II, Braak III–IV, and Braak V–VI compared to controls, although they did not show significant differences. The levels of Se in different brain regions of AD patients have been little described. In general, the levels of Se have been reported to be increased in AD brain tissue, with exceptions^{43–45}. Ramos et al. reported the Se concentration of different AD human brain regions including CTX. According to our results, they found increased levels of Se in CTX from AD cases compared to an age-match control group²⁰. Se is part of selenoproteins, which have antioxidant activity and it is known to play important roles in the maintenance of brain function and the protection of neurons from oxidative stress⁴⁶. In addition, disturbances in Se levels may contribute to cognitive impairment, seizures, and AD disease⁴⁷.

On the other hand, we mostly observed increased levels of toxic metals such as Cr, Pb, As, and Cd in advanced AD stages compared to control and Braak I–II groups. Exposure to these toxic metals has been associated with increased A β levels and tau phosphorylation contributing to AD etiology¹¹. Significant and elevated Cr levels have been noted in CTX¹⁹ in AD cases. Our findings align with this, as we predominantly observed increased levels of Cr in the BG and CTX in advanced AD stages compared to those in the early stages of the disease. Some research studies have suggested that exposure to Pb may contribute to cognitive impairments and be associated with AD pathological changes. This is because lead interacts with A β and tau proteins, leading to increased aggregation¹¹. Our study observed higher, though not statistically significant, levels of Pb in AD brains compared to control samples, particularly in the CTX and BG. Other studies analyzing blood samples from AD patients have also found elevated levels of Pb, indicating a potential risk factor for AD⁴⁸. However, Szabo et al. did not find significant differences between AD cases and control samples in the cortex¹⁹. We observed significantly higher levels of As CTX of Braak V–VI stages compared to the control and Braak I–II groups. While there are few studies on brain tissue, most studies on other samples such as hair, nails, blood, and urine from AD patients have not found significant differences in As levels compared to the control group¹¹. However, some authors have reported that As exposure is associated with cognitive impairments, increased A β levels, tau phosphorylation, oxidative stress, and neuronal apoptosis¹¹. Elevated levels of Cd have been linked to cognitive decline and the death of cholinergic neurons in the basal forebrain⁴⁹. Moreover, some studies have suggested a correlation between increased Cd levels and higher mortality from AD⁵⁰. Our observations revealed higher levels of Cd in the BG advanced Braak stages III–IV and V–VI compared to Braak I–II, although these differences were not statistically significant. According to our findings, augmented levels of Cd have been detected in the hippocampus and A in AD cases compared to control²³.

We have also reported the concentrations of the minority elements Co, V, Ti, and Mo in CTX and BG regions. Limited information is available regarding these elements in brain tissue. Our findings revealed reduced levels of Co, an essential cofactor of Vitamin B12, in the BG and CTX from AD cases. Specifically, significantly decreased Co levels were observed in the BG from AD Braak V–VI brains compared to the control group. Similarly, these diminished levels were also notable in CTX from Braak III–IV. Studies involving mice have shown that exposure to Co is associated with age-related neurodegeneration⁵¹. Moreover, we observed significantly elevated levels of V in CTX and BG of Braak III–IV and V–VI brains as compared to control and Braak I–II. According to our findings, Szabo et al. noted higher levels of V in CTX from AD cases compared to control¹⁹. V is considered

an essential micronutrient in many organisms⁵² with protective effects on cognitive impairment in AD⁵³. The levels of Tl were found to be statistically insignificant in our study. However, Tl is widely recognized as a toxic heavy metal that can cause damage to the human central, peripheral, and autonomic nervous systems⁵⁴. Several studies have demonstrated the neurotoxic effects of this metal on neuronal cells⁵⁵. Although limited information is available regarding Mo in tissue from AD cases, studies have examined the concentration of Mo in plasma^{56,57} serum^{58,59} cerebrospinal fluid (CSF)⁵⁶ and whole blood⁶⁰. Our findings revealed significantly increased levels of Mo in BG of Braak III-IV compared to Braak I-II stage. Mo plays multiple roles in neurodegenerative diseases, particularly in AD, including its potential as a diagnostic agent and its ability to inhibit β -amyloid and regulate oxidative stress⁶¹.

In conclusion, a significant dyshomeostasis of metals has been found in the CTX and BG brain regions that strongly depend on the Braak stages. The neuro-metals Mg, Fe, Cu, and Zn were the most abundant elements in the different brain regions, but they decreased in some regions of the AD brain tissue compared to physiological levels (e.g. Mg in the CTX of higher Braak stages). Al, Mn, and Se were abundant in the different brain regions, but we also observed a significant reduction in Se levels in Braak V-VI and elevated levels of Al especially in de BG of de AD patients, being higher in advanced stages of the disease. Increased levels of toxic metals (e.g. Cr, Pb, As, and Cd) and minority elements (e.g. V) were found in advanced AD patients, while other minority elements (e.g. Co and Mo) decreased in the brain regions. Thus, while our findings should be confirmed in additional studies, public health interventions to prevent uncontrolled exposure to these metals and the modulation of the essential elements levels may contribute to mitigating the neurotoxicity in the population.

Methods

Sample collection

Samples and data from patients included in this study were provided by the Principado de Asturias BioBank (PT20/0161 and PT23/0077), financed jointly by Health Service of the Principality of Asturias, Carlos III Health Institute, and Cajastur Banking Foundation and integrated into the Spanish National Biobanks and Biomodels Network; the Biobank HUB-ICOIDIBELL (PT20/00171), integrated into the ISCIII Biobanks and Biomodels Platform and Xarxa Catalonia Bank of Tumours (XBTC); the Biobank Network of the Region of Murcia, BIOBANC-MUR, registered on the National Registry of Biobanks with registration number B.0000859 and supported by the “Instituto de Salud Carlos III (project PT20/00109), by “Murcian Institute of Biosanitary Research Virgin of Arrixaca, IMIB” and by “Ministry of Health of the Autonomous Community of the Region of Murcia”; the Biobank of tissues CIEN and the Biobank University Hospital Alcorcón Foundation. Samples were processed following standard operating procedures with the appropriate approval of the Ethics. Informed consent was obtained from all subjects and/or their legal guardian(s). The manuscript does not include information nor images that could lead to the identification of a study participant. Patients names are not present in any part of the manuscript and samples are anonymized. Research has been performed in accordance with the Declaration of Helsinki. The experimental protocol was approved by a named institutional and/or licensing committees. Therefore, the study was conducted in accordance with relevant ethical guidelines and regulations. Prior to sample collection and analysis, the experimental protocol was reviewed and approved by the institutional research ethics committee of each biobank: Principality of Asturias (CEImPA), Biobank of the University Hospital of the Alcorcón Foundation, Biobank HUB-ICOIDIBELL, Murcian Institute of Biosanitary Research (IMIB) and Tissue Biobank of the CIEN Foundation. Scientific Committees Neuropathological severity was determined by the assignment of the Braak stage⁶². The brain regions obtained for the analysis were CTX (n = 119) and BG (n = 122) (Figure S9). The samples were divided into 4 groups: a control group of healthy people (25 CTX and 25 BG), and three AD groups with different Braak stages: Braak I-II (9 CTX and 14 BG), Braak III-IV (30 CTX and 33 BG), and Braak V-VI (55 CTX and 51 BG). Clinical parameters of post-mortem brains and the evaluation of the effect of different clinical parameters were described in the Supplementary Material (Table S6, Supplementary Material).

Sample preparation

The samples were first cryohomogenized using liquid N₂ and a ceramic mortar until fully pulverized. Then, 100 μ g of the cryohomogenized brain sample from the different regions were weighed and mineralized. After that, 600 μ l of a 4:1, v/v HNO₃:H₂O₂ (trace metal grade, Leicestershire, UK) were added to each sample placed into a PTFE vessel. Microwave-assisted digestion was then performed in a MARS 6 model microwave oven (CEM Matthews, NC, USA) under specific parameters: a 15-minute ramp to 160 °C, followed by a 40-minute hold at 160 °C, with a power setting of 400 W. Once completed, ultrapure water (Milli-Q gradient system, Millipore, Watford, UK) was added and the samples were filtered, using 0.45 μ m nylon filters before being injected into the ICP-QQQ-MS.

Multielemental analysis

We utilized an Agilent 8800 Triple Quad (Agilent Technologies, Tokyo, Japan) inductively coupled plasma mass spectrometry with a triple quadrupole, alongside high-purity grade helium and oxygen (>99.999%) and pure hydrogen gas (>95%). Our instrumental settings were optimized using a tuning aqueous Li, Co, Y, and Tl solution at 1 μ g L⁻¹ (Agilent Technologies). We employed nickel sampling and skimmer cones, with a sampling depth of 10 mm. The forward power was set at 1550 W, and the gas flow rates were fixed at 15 L min⁻¹ (plasma gas), and 1.08 L min⁻¹ (carrier gas). We used a flow of 4.5 mL min⁻¹ of helium for most metals as collision gas to eliminate interferences. For Se and As, we used a flow of 2 mL min⁻¹ of H₂ with 40% of O₂ in MS/MS mode. A mixed solution containing 100 ng g⁻¹ of rhodium (Rh), bismuth (Bi), and scandium (Sc) was added to the samples as an internal standard. Isotopes monitored were ²⁴Mg, ²⁷Al, ⁴⁵Sc, ⁵¹V, ⁵³Cr, ⁵⁵Mn, ⁵⁷Fe, ⁶³Cu, ⁶⁶Zn, ⁷⁵As, ⁸⁰Se, ⁹⁵Mo, ¹⁰³Rh, ¹¹¹Cd, ²⁰⁵Tl, ²⁰⁸Pb and ²⁰⁹Bi, with a dwell time of 0.3 s per isotope. The Dogfish Liver Certified

Reference Material for Trace Metals (DORM-2) and Clinchek Serum Reference Material, Trace Metal Level II were used to secure the analysis's quality regarding reproducibility (Tabla S7). The limits of detection (LOD) and quantification (LOQ) for all the metals were also described in Table S7.

Statistical analysis

The results were analyzed using various statistical tests including Levene's Test, Krustal-Wallis Test, Partial Least Square Discriminant Analysis (PLS-DA), and Spearman correlation. These tests were conducted through the web tool MetaboAnalyst 6.0 (<https://www.metaboanalyst.ca/>), STATISTICA 8.0 (StatSoft), and SSPS (IBM SPSS Statistics for Windows, Version 27.0). The level of statistical significance was set at $p < 0.05$ for all tests.

Limitations

One of the primary limitations of this study is its sample size, which may impact the generalizability of the findings. Additionally, there is a notable imbalance in the distribution of samples across groups, with significantly fewer cases represented in Braak stages I-II compared to stages III-IV and V-VI. This asymmetry could influence the interpretation of the results, as a reduced number of cases in certain categories may hinder the detection of significant differences and increase data variability. While the inclusion of a control group enables comparisons with subjects not affected by pathology, the interindividual variability in the concentration of metals within brain tissue could still affect the outcomes. These limitations underscore the necessity for further studies with a larger sample size and a more balanced distribution among the various stages of the disease to enhance the robustness of the results.

Data availability

Data will be available from the corresponding author upon reasonable request.

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C.P.M.: formal analysis, investigation, data curation, writing—original draft, writing—review and editing, visualization. B.C.L.: conceptualization, methodology, investigation, data curation, software, writing original draft—review and editing, visualization, supervision. H.R.L., I.F.V., M.C.G.M., A.R.: resources, investigation, writing—review and editing. T.G.B.: conceptualization, methodology, investigation, resources, writing—original draft, writing—review and editing, visualization, supervision, funding acquisition, project administration.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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