

# Scavenger receptor class B type I expression and elemental analysis in cerebellum and parietal cortex regions of the Alzheimer's disease brain<sup>☆</sup>

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## Abstract

Metal ions play an important role in health and disease by influencing cellular biochemical pathways. The increased concentrations of some metal ions may have cytotoxic effects through their ability to oxidatively modify biomolecules, which may cause oxidative stress-induced brain cell death leading to neurodegenerative disorders observed in Alzheimer's disease (AD). We therefore performed elemental analysis of human brain tissues by a sophisticated method of inductively coupled plasma mass spectrometry (ICP-MS) in two regions of the AD brain, the parietal cortex and cerebellum, and compared them with the age-matched control. Our analysis shows the differential distribution of some metal ions in the two regions of the brain. Most importantly, Si, Sn, Al and Mn showed significantly higher levels in the parietal cortex of the AD brain compared to the control. The other metal ions showing moderate increases in the parietal cortex were Na, Te, Cr, Fe and B. Since these metal ions can modify lipoproteins in the brain and modified lipoproteins are taken up by scavenger receptors class B type I (SR-BI), we also determined the presence of SR-BI in the parietal cortex and cerebellum regions of the control and AD brains using a sensitive method, the reverse transcriptase-polymerase chain reaction. Our results suggest that SR-BI are present in the parietal cortex as well as in the cerebellum of the control and AD brains, suggesting that the presence of SR-BI may be involved in the uptake of oxidatively modified lipoproteins and  $\beta$ -amyloid (A $\beta$ ) protein complexed with apoE, suggesting implications in the progression of late onset AD and other neurodegenerative disorders characterized by the deposition of insoluble aggregates observed in the AD brain. © 2002 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Among several factors contributing to the pathophysiology of neurodegenerative disorders leading to diseases like Alzheimer's and dementia, the oxidative stress-induced cytotoxicity appears to play an important role in this process. Alzheimer's disease (AD) is characterized by neuritic plaques and neurofibrillary tangles [1]. Neuritic or senile plaques contain, among other components, amyloid- $\beta$  (A $\beta$ ) which is a hydrophobic peptide [2], and apolipoprotein E, a

protein component of several classes of lipoproteins [3]. A number of hypotheses have been proposed regarding the cause of AD. One of them is the metal ion-induced fragmentation of  $\beta$ -amyloid protein and the accumulation of reactive oxygen species in the neurons [4]. A strong correlation between the radical generation by  $\beta$ -amyloid peptide and neurotoxicity has been observed [5,6]. A recent study [7] suggested that the neurotoxic A $\beta$  peptide increases oxidative stress in vivo through mechanisms that involve NMDA receptors and nitric oxide synthase. Under physiological conditions, the superoxide is readily converted to hydrogen peroxide by superoxide dismutase. Hydrogen peroxide generates toxic hydroxyl radicals by transition metals like Cu and Fe [5,6]. Indeed, iron binding protein p97 is found in elevated amounts in the serum of AD patients compared with that of healthy controls [8]. Metal ions have been reported to be present at altered levels in the AD brain compared to the brains of individuals with no symptoms of AD [9–14]. Among others, aluminum salts have been detected in the

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senile plaques and neurofibrillary tangles, suggesting aluminum as a risk factor for AD [13]. Other metal ions that have been suggested to be associated in the pathogenesis of AD are Se, Zn, Sn, Fe, Si and Te [9–14]. These metal ions are suspected to be involved in the pathogenesis of neurodegenerative disorders either through their oxidant properties or via cytotoxic effects. Metal ions are also known to oxidatively modify lipoproteins [15], and scavenger receptors class B type I (SR-BI) are involved in the uptake of native HDL particles as well as modified lipoprotein particles [16,17]. Therefore, in addition to other lipoprotein receptors like the LDL receptor [18] and LDL receptor-related protein (LRP) [19,20], it is possible that SR-BI are present in the human brain to participate in the uptake of modified lipoprotein particles. A recent study did indicate that SR-BI are expressed in the porcine brain and that they participate in the uptake of HDL-associated vitamin E into cells constituting the blood–brain barrier [21]. Paresce et al. [22] showed that murine microglia internalizes microaggregates of A $\beta$ , which was reduced by scavenger receptor ligands. They also reported that both class A and B scavenger receptors were involved. However, the presence of SR-BI in the human brain has not been reported. We therefore isolated RNA from two regions of the human brain, the parietal cortex and cerebellum, and investigated the presence of SR-BI by RT-PCR.

## 2. Materials and methods

### 2.1. Human brain tissues

Human brain tissues were obtained through the archive of the Alzheimer's Disease Research Center, Washington University, Saint Louis, MO 63110. From eight deceased brains (four control and four Alzheimer's disease), we obtained eight samples of cerebellum and eight samples of parietal cortex. These brain tissues were post mortem autopsy samples from females. The age of the deceased at the time of death ranged from 78 to 88 years. Slices of various regions of the brain samples were cryopreserved immediately after excising, stored and frozen until analysis. Since the brain samples were limited, we performed three repeats of the elemental analysis to eliminate variability due to the analytical method.

### 2.2. Elemental analysis

Human brain tissues obtained as above were divided into two parts. One part was used for the isolation of total RNA, and the other part (100–200 mg) was thawed and placed in a 15-ml pre-cleaned screw-capped Teflon beaker. Five milliliters of concentrated HNO<sub>3</sub> was added to the sample, and the beaker was placed on a hot plate maintained at a temperature of 100 °C and incubated overnight, followed by evaporation to dryness. To the dried mass, 2 ml of HNO<sub>3</sub> followed by 25 drops of H<sub>2</sub>O<sub>2</sub> were added drop-wise to minimize foaming

and then evaporated to near dryness. After three repetitions of the HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> procedure, the samples were allowed to cool and were made up gravimetrically with 2% HNO<sub>3</sub> to ca. 20 g.

A VG Elemental PlasmaQuad model PQ2 inductively coupled plasma mass spectrometry (ICP-MS) was used for all data acquisition according to the operating conditions described by Takahashi et al. [23]. Single element standard solutions purchased from Inorganic Ventures (Lakewood, NJ) were utilized to prepare calibration and internal standard solutions. Analyses were performed using an external calibration procedure, and internal standards were included for matrix and instrumental drift corrections. For data reduction, the raw intensities were corrected for background counts, instrumental drift, matrix effects and wherever applicable for molecular interferences. Procedural blanks were analyzed to check for any contribution from the reagents. The reference materials SRM-8414 (bovine muscle) and SRM-1577b (bovine liver) were digested and analyzed to be identical to the unknowns for quality control purposes.

### 2.3. Preparation of RNA

Total RNA was prepared by the one-step isolation method described in Ref. [24]. One microgram of RNA from each sample was examined on the RNA agarose gel for their integrity. A 1.2% agarose gel containing 6% formamide and 0.2 M formaldehyde were prepared and 1  $\mu$ g total RNA was run at 100 V for 1.5 h. After the electrophoresis, the gel was stained with ethidium bromide (5 mg/ml) followed by destaining [25]. Visualization of 28S and 18S ribosomal RNA under the UV illuminator showed the RNA preparation to be partially degraded. The RNA preparation was therefore not suitable for quantitative analysis by Northern blotting [21] or by RNase protection assay [26,27]. We therefore carried out reverse transcription-polymerase chain reaction to examine the presence of the desired messenger RNA.

### 2.4. Scavenger receptor class B type I (SR-BI) detection

To detect the presence of SR-BI in the two regions of the human brain tissues, one-tube reverse transcription followed by polymerase chain reaction was carried out as described in Ref. [28]. Oligomeric primers corresponding to SR-BI nucleotides from 731 to 754 and from 1200 to 1177 for 5' and 3' sequences, respectively, were designed based on the published sequences of SR-BI from Gene Bank. The following profile was used for RT-PCR: one cycle—94 °C, 30 s; one cycle—50 °C, 50 min; 30 cycles—94 °C, 30 s/58 °C, 30 s/72 °C, 60 s; one cycle—72 °C, 10 min. After the RT-PCR cycles were completed, 10  $\mu$ l of the product was analyzed in 1% agarose gel containing ethidium bromide and visualized under UV lamp. Appropriate positive and negative controls were run to ensure that there is no contamination of genomic DNA. To confirm that the amplified PCR product was indeed SR-BI, we cloned the RT-PCR product in the pGEMT vector

(Promega) and performed dideoxy DNA sequencing [28], which matched the published sequences of SR-BI.

### 3. Results and discussion

The aim of the present study was to measure the elemental content in two regions of the brain, the parietal cortex and cerebellum, obtained from healthy and Alzheimer’s disease brains. It is important to evaluate the elemental contents in the two brain regions because the cerebellum is minimally affected in AD [14]. Elements like phosphorus and sodium did not show variations in the two regions of the brain when the normal and AD results were combined (Fig. 1). However, sodium appeared to show increased levels in the parietal cortex of AD compared to the age-matched control group. Similarly, although magnesium, calcium, silicon and zinc did not show any variation in the two regions of the brain when

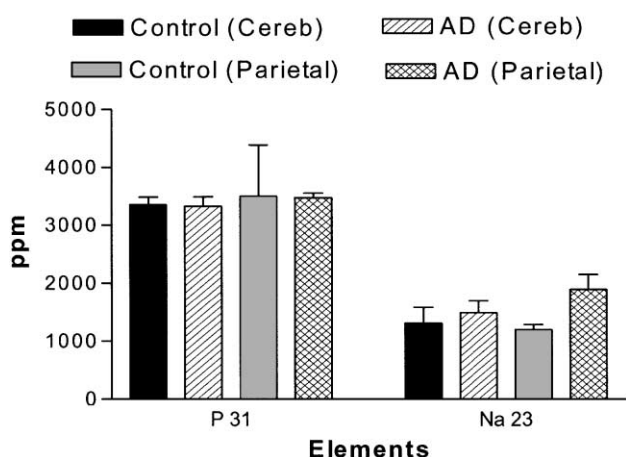
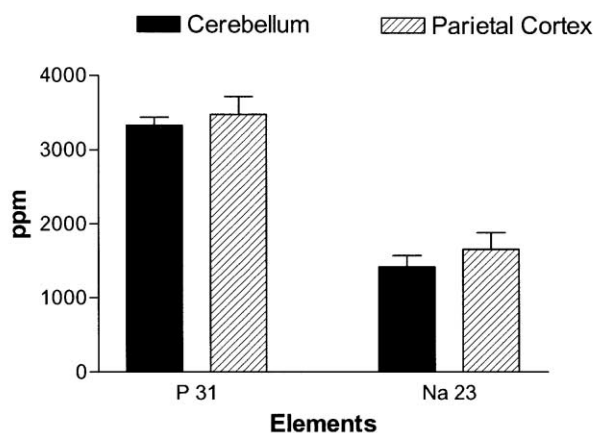


Fig. 1. Levels of phosphorus and sodium in the two regions of the brain, the parietal cortex and cerebellum of the control and AD brains, as determined by ICP-MS. The upper panel shows a comparison between two regions of the brain, the parietal cortex and cerebellum, using the combined results from control and AD brain tissues. The bottom panel shows a comparison of metal ions in the two regions of the brain from control and AD. Cereb, cerebellum; Parietal, parietal cortex.

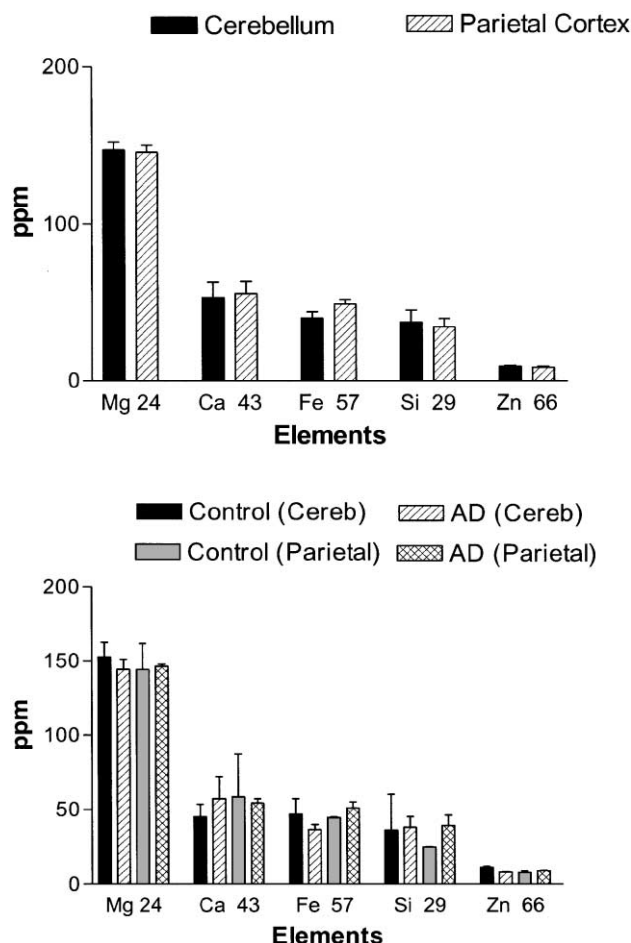


Fig. 2. Levels of magnesium, calcium, iron, silicon and zinc. The upper panel represents a comparison between two regions of the brain regardless of the course (control and AD). The lower panel shows a comparison between the control and AD brain tissues as described in the legends of Fig. 1.

the control and AD results were combined (Fig. 2), but when the two regions were analyzed separately, it was clear that silicon showed elevated levels in the parietal cortex of AD brain compared to the control group although no changes were noticed in the cerebellum region. The role of silicon in AD has been discussed by several groups. Silicon has been suggested to retard the blood–brain barrier function in elderly persons [29]. Possibly, silicon is contained in AD brain as aluminosilicates [30] since aluminum species interact with silicic acid,  $\text{Si}(\text{OH})_4$ , a normal component of plasma to form aluminosilicate [31]. Indeed, aluminum and silicon were found to colocalize in the central region of the senile plaques [32]. Thus, the elevated levels of silicon found in the present study in the AD parietal cortex region compared to the control group are of significance, given the interaction of silicon and aluminum, and suggested the role of aluminum in the pathogenesis of AD. Elevated levels of silicon have been reported in the central spinal fluid of Alzheimer’s-type dementia patients [33]. We also noticed increased levels of tin in the parietal cortex region of the AD brain compared to

the control group (Fig. 3) although copper did not show significant changes. Despite the paucity of information on the role of tin in the pathogenesis of AD, one study did provide a link between the levels of tin and lipid metabolism [34]. The same group also reported elevated levels of tin in patients with AD. Our results on the elevation of tin in AD brain appear to be consistent with the reported elevation of plasma tin levels [35]. Calcium did not appear to have changed in the AD brain although iron showed a moderate increase in the parietal cortex region of the AD brain when compared to the control group. Although elevated levels of tin were reported in the AD blood [34], Corrigan et al. [35] did not find significant increases of tin in the AD brain. However, the levels of aluminum and silicon were found to be elevated in the AD brain. We also observed elevated levels of aluminum (Fig. 4) and silicon (Fig. 2) in the parietal cortex region of the AD brain compared to the control group. A number of studies have linked brain aluminum levels to the pathogenesis or

etiology of AD [36]. The neurotoxicity of aluminum has been suggested via several mechanisms including the binding of aluminum to the hyper-phosphorylated tau in AD [37]. Indeed, aluminum concentration in the serum was found to increase with aging [37]. Aluminum concentrations measured by ICP-MS showed elevated levels in the hippocampal tissues in AD compared to controls [13]. Those elevated aluminum levels indeed correlate with the pathogenesis of AD that was further corroborated by our studies showing almost twofold increases in the parietal cortex region of AD compared to the control group. Interestingly, the levels of aluminum in the cerebellum region of the AD brain showed no changes compared to the control group (Fig. 4). This finding is significant given the minimal abnormalities in the cerebellum region of the AD brain. Our results are of significance since we showed for the first time that the aluminum concentrations in the AD brain are elevated specifically in the cortex region and not in the cerebellum region. Several other metal ions measured in this study also show a differential distribution in the two regions of the brain.

A major finding of the present study is the correlation of the levels of manganese in the parietal cortex and AD. The parietal cortex and cerebellum regions appeared to have similar levels of manganese when the results of the AD and control were combined (Fig. 4). However, when the results were analyzed separately, it was clear that the levels of manganese in the cerebellum region of the AD brain were similar to the levels of the control group. Interestingly, when the levels of manganese were compared in the parietal cortex, it was evident that the levels of manganese showed elevated levels in AD compared to the control group, similar to the observations obtained with aluminum. This finding is of great importance given the role of manganese in the oxidative stress-induced biologic activities in the brain. Manganese is an essential element for the normal functioning of the brain. Thus, the stringent regulation of manganese levels in the brain is essential. Manganese-superoxide dismutase (Mn-SOD) is involved in the detoxification of reactive oxygen species generated during oxidative modification of biomolecules. Therefore, the levels of Mn-SOD as well as manganese provide an index of the level of oxidative stress in the brain. The higher levels of manganese would suggest higher levels of oxidative modification undergoing in a given tissue. Thus, elevated levels of manganese in the parietal cortex region of the AD brain compared to the control group suggest an oxidant-mediated damage in the pathogenesis of AD [38]. Indeed, increased free radical production has been suggested in AD [39], and Mn-SOD has been found to be localized in the cerebral cortex and hippocampus regions of Alzheimer's-type senile dementia [40]. The other metal ions reported to be relevant in the pathogenesis of AD are tellurium and selenium [41]. Tellurium can damage the mitochondria leading to defects in mitochondrial energy metabolism and pathogenesis of neurodegenerative disease. Thus, higher levels of tellurium can be neurotoxic. Although the basal levels of tellurium were

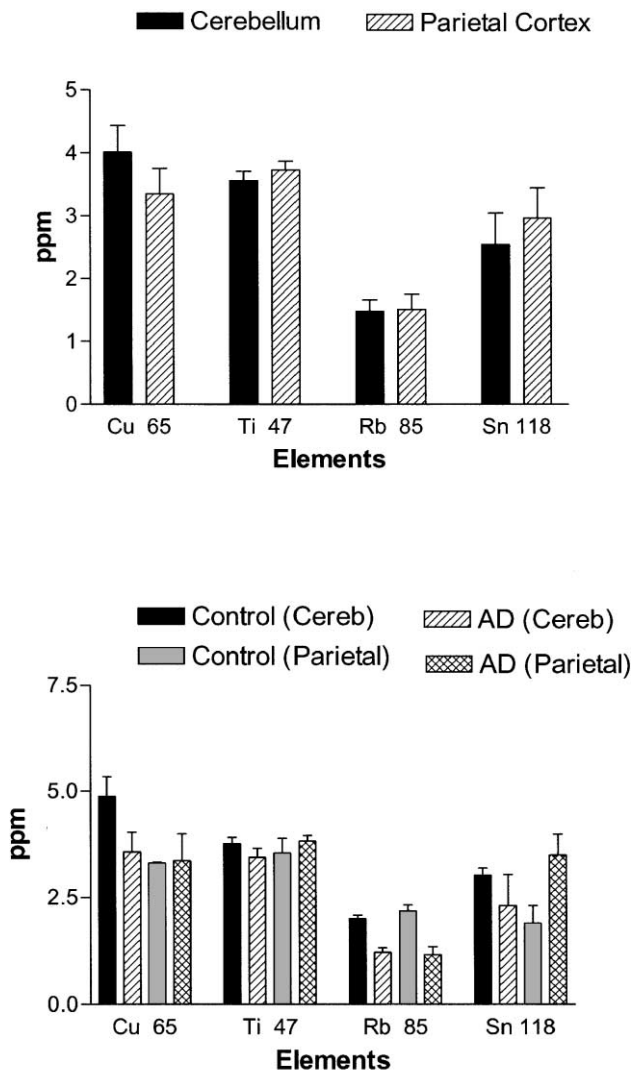


Fig. 3. Levels of copper, titanium, rubidium and tin. The upper and lower panels are the same as those described in the legends of Fig. 2.

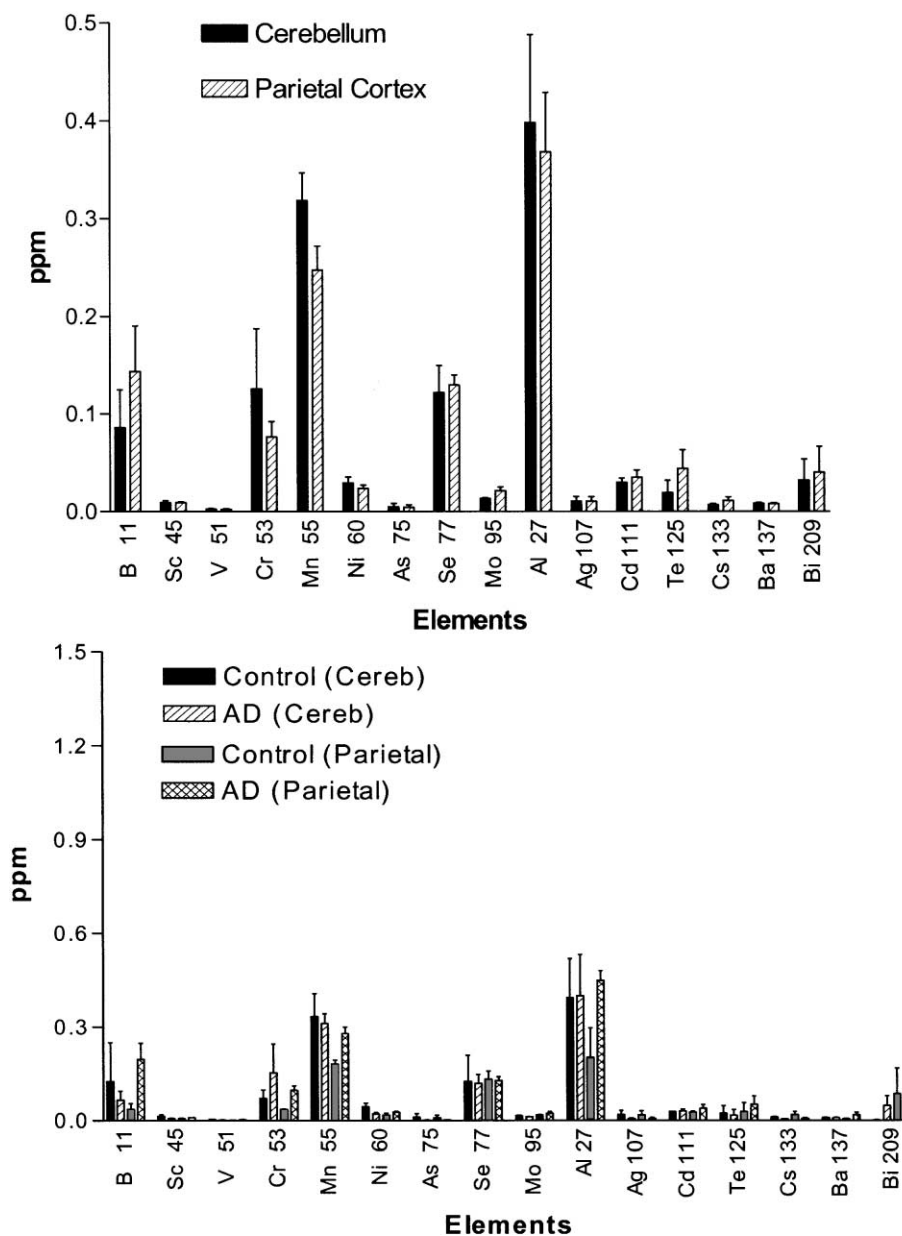


Fig. 4. Levels of metal ions in the two regions of the brain as indicated. The upper panel shows the comparison of the distribution of metal ions in the two regions of the brain, the cerebellum and parietal cortex, when the AD and normal brain data were combined. The lower panel shows the comparison of the elements between the control and AD brains in two regions of the brain as shown in the figure.

found to be very low in the brain, the parietal cortex region, not the cerebellum region, appeared to have higher levels of tellurium in the AD brain (Fig. 4), while selenium did not show any changes.

Since the oxidative stress caused by the elevated levels of certain metal ions in the brain may ultimately result to the initiation of oxidative modification of lipoproteins in the brain, we hypothesized the presence of a lipoprotein receptor involved in the uptake of oxidatively modified lipoproteins. Lipoprotein receptors like the LDL receptor [26] and LDL receptor-related protein (LRP) [19] are present in the brain, but these receptors are not involved in the uptake of all oxidatively modified lipoproteins. We

therefore reasoned that a different receptor is involved in the uptake of oxidized lipoprotein particles. Scavenger receptor class B type I (SR-BI) is a receptor involved in the uptake of oxidized lipoprotein particles [17], as well as in the transport of HDL particles for the delivery of lipid-soluble vitamins to the brain [21]. Since the presence of SR-BI, an HDL receptor, in the human brain has not been established, we first attempted to detect SR-BI in the brain RNA sample using a sensitive method of detection, the reverse transcription-polymerase chain reaction. As shown in Fig. 5, we detected an appropriate size of RT-PCR product in the brain RNA sample signifying the presence of SR-BI in the brain. This DNA fragment, when sub-cloned in the pGEMT

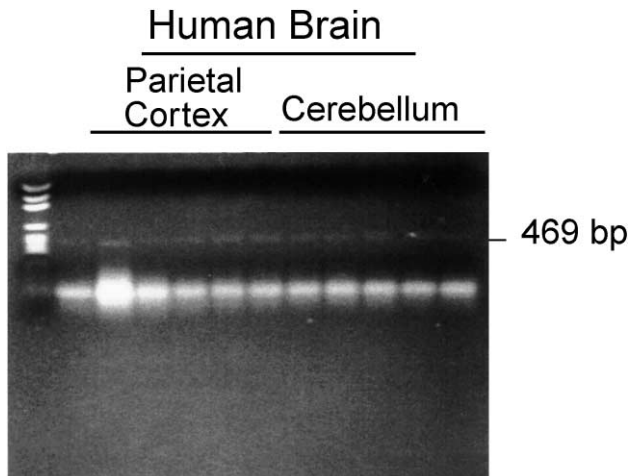


Fig. 5. Detection of scavenger receptor class B type I (SR-BI) in the parietal cortex and cerebellum of RNA samples from the control and AD brains. One microgram of the total brain RNA from the cerebellum and parietal cortex was used for the reverse transcriptase-polymerase chain reaction. The 469-bp PCR product indicates the presence of scavenger receptor class B type I (SR-BI) transcript. This PCR product (469 bp) was sub-cloned in the pGEMT vector and sequenced (data not shown), which matched 100% of the published sequence of SR-BI.

vector and sequenced by the dideoxy DNA sequencing method, showed a complete match with the published sequences of the human SR-BI (data not shown). To further confirm that SR-BI is indeed expressed in the mammalian brain, we precisely detected SR-BI mRNA in the mouse brain RNA by RNase protection assay (Srivastava, unpublished results). Another lipoprotein receptor, glycoprotein-330/megalin, is reported to be present in the cerebral microvessels [42]. Using megalin-specific primers, we performed RT-PCR on the same samples of RNA used for the detection of SR-BI. In addition, we also performed RT-PCR for megalin using kidney RNA since megalins are abundant in the kidney. As shown in Fig. 6, megalin transcripts were found to be abundant in the kidney but could not be detected in the liver and in the brain RNA samples. However, using the same liver and brain RNA samples, we could detect SR-BI transcript in the human liver and brain RNA. Others have reported very low levels of megalin in brain capillary RNA [43]. Since we did not use brain RNA prepared exclusively from the brain capillaries, it is possible that the numbers of megalin transcripts are too low to be detected in the total brain RNA. Nevertheless, the presence of SR-BI transcripts in the brain does suggest a role for SR-BI in the uptake of oxidatively modified lipoproteins. Recently, ATP binding cassette transporter A1 (ABCA1) was identified as an important player in the cellular cholesterol trafficking [44–46]. It was recently shown that ABCA1 also participates in the uptake of vitamin E [47], which is consistent with the deficiency of vitamin E and other fat-soluble vitamins in mice lacking ABCA1 [48]. Given the role of HDL in vitamin E delivery to brain [21] and in ABCA1-mediated cholesterol trafficking, we reasoned that ABCA1

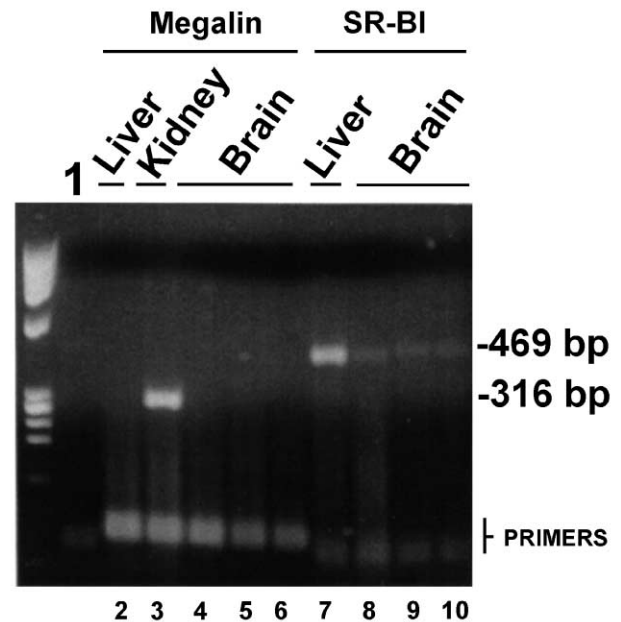


Fig. 6. Detection of glycoprotein-330/megalin and scavenger receptor class B type I in the liver and brain RNA samples of humans. Lane 1 shows the PCR of kidney RNA without reverse transcription. One microgram of liver, kidney and total brain RNA was used for RT-PCR using megalin-specific primers. An appropriate size of the RT-PCR product (316 bp) of megalin was present in the kidney but absent in the liver and brain RNA. Detection of the SR-BI transcript was also performed on the same human liver and brain RNA samples using SR-BI specific primers. Lanes 2–6 represent megalin and lanes 7–10 represent SR-BI. The liver appeared to express higher levels of SR-BI compared to the brain.

could be involved in the trafficking of vitamin E as well as cholesterol in the brain. We therefore attempted to find the presence of ABCA1 transcripts in the brain. As shown in Fig. 7, both human and mouse brain expressed ABCA1

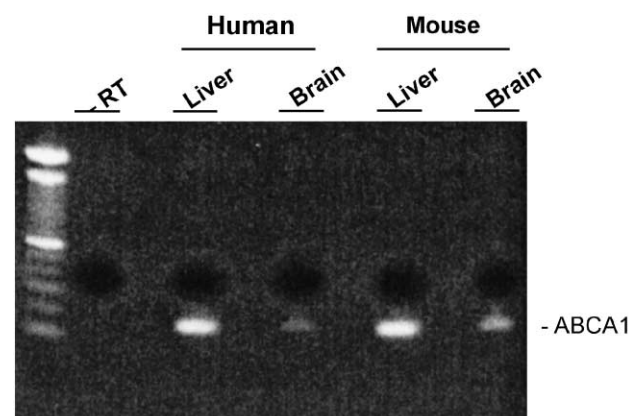


Fig. 7. Detection of the ATP-binding cassette transporter A1 transcripts by reverse transcription-polymerase chain reaction in the RNA samples prepared from human and mouse liver and brain. One microgram of total RNA was reverse-transcribed, and the resulting cDNA was amplified by polymerase chain reaction. The 142-bp RT-PCR product was resolved in 1.5% agarose gel. As shown, the liver expresses higher levels of ABCA1 compared to the brain. This amplified product was sub-cloned into the pGEM vector, and sequencing confirmed it to be ABCA1.

transcripts although the levels of ABCA1 expressed in the brain were much less compared to those in the liver.

Mononuclear phagocytes (monocytes, macrophages and microglia) are suggested to be involved in the pathogenesis of neurological disorders. Infiltration of the macrophages across the blood–brain barrier can generate superoxide dismutase and glutamate anions known to have neurotoxic effects [48]. The production of oxygen free radicals and glutamate was induced by  $\beta$ -amyloid protein in monocytes [48]. Therefore, macrophages and microglial cells impose the risk of initiating neurodegenerative processes through their proinflammatory [49] and oxidant [50] properties. Indeed, signs of neuronal degeneration are often accompanied by markers of microglial activation, inflammation and oxidant damage in the AD brain [51]. Celastrol has anti-inflammatory and antioxidant properties. Treating rats with Celastrol decreased induced NO production but not the constitutive NO production and improved their performance in memory, learning and psychomotor activity tests [51], suggesting that oxidant stress is an important factor in the pathogenesis of neurological disorders including AD. Thus, oxidant stress caused by metal ions may contribute to the progression and pathogenesis of AD.

In summary, we showed that some of the metal ions are increased in the AD brain, which may be involved in the pathogenesis of AD. We also showed the presence of a receptor, SR-BI, as well as ABCA1 in the human brain RNA, which may be involved in the transport of oxidized cholesterol-carrying particles generated by the oxidative stress caused by the elevated levels of metal ions. We further showed that some of the metal ions are specifically increased in the parietal cortex region but not in the cerebellum region, which are minimally affected in the AD pathogenesis.

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