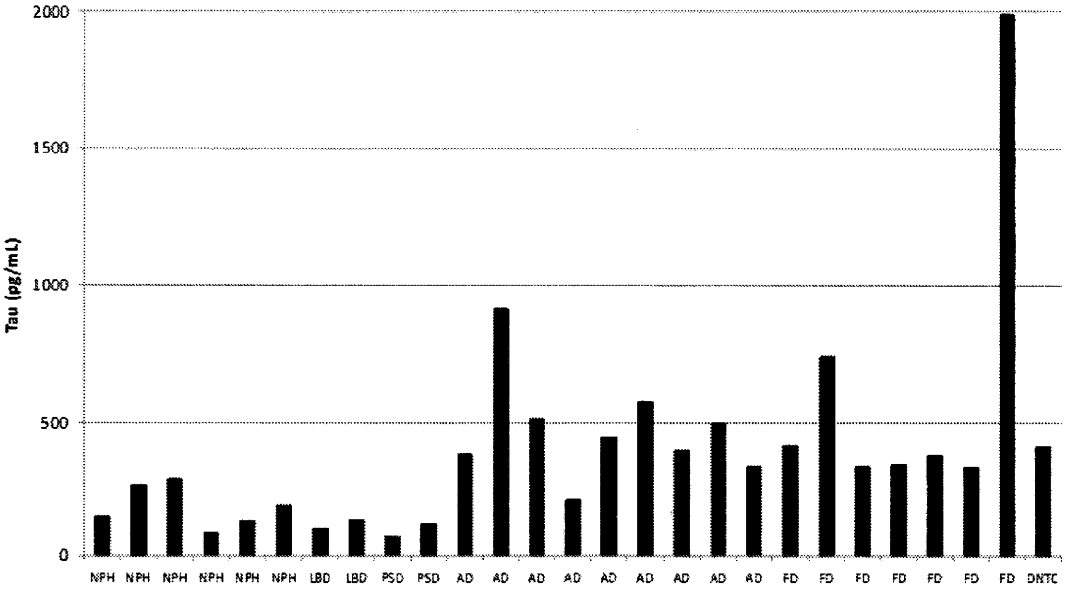

図1

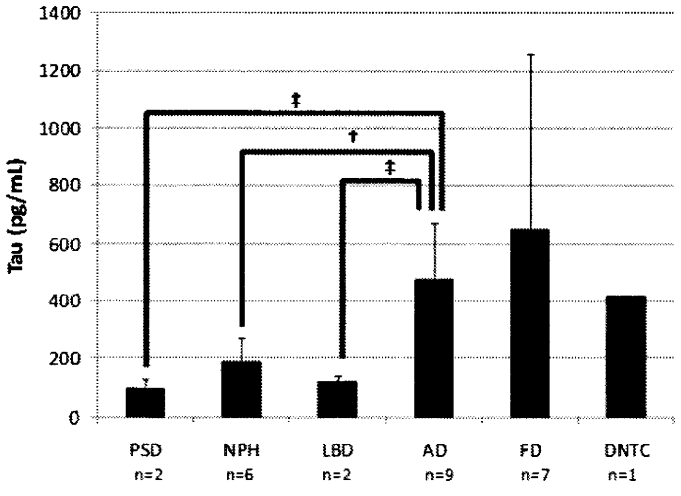

図2

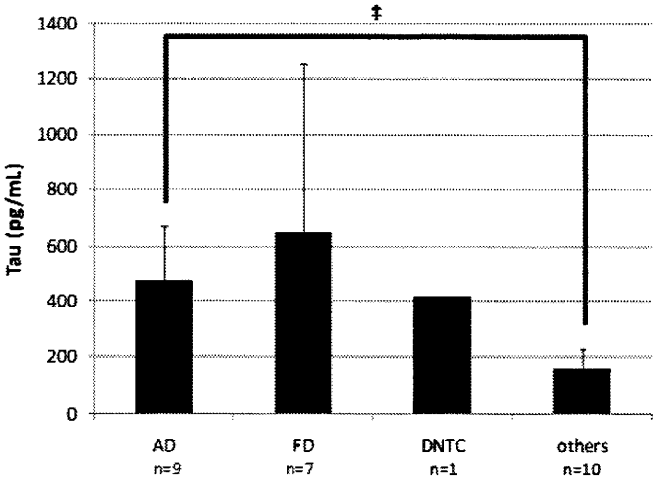

図3

Student's t-test †:p<0.005 ‡:p<0.001

別紙４　研究成果の刊行に関する一覧表

書籍：なし

雑誌：下記

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hozumi I, Kohmura A, Kimura A, Hasegawa T, Honda A, Hayashi Y, Hashimoto K, Yamada M, Sakurai T, Tanaka Y, Satoh M, Inuzauka T.	High levels of copper, zinc, iron and magnesium, but not calcium, in the cerebrospinal fluid of patients with Fahr's disease.	Case Rep in Neurol	2	46-51	2010
Hozumi I, Hasegawa T, Honda A, Ozawa K, Hayashi Y, Hashimoto K, Yamada M, Koumura A, Sakurai T, Kimura A, TanakaY, Satoh M, Inuzauka T.	Patterns of high levels of heavy metals in the CSF are different among neurodegenerative diseases.	J Neurol Sci	303	95-99	2011

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High Levels of Copper, Zinc, Iron and Magnesium, but not Calcium, in the Cerebrospinal Fluid of Patients with Fahr's Disease

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Key Words
Fahr's disease · Calcification · Copper · Zinc · Dementia · Parkinsonism

Abstract
Patients with marked calcification of the basal ganglia and cerebellum have traditionally been referred to as having Fahr's disease, but the nomenclature has been criticized for including heterogeneous etiology. We describe 3 patients with idiopathic bilateral striatopallidodentate calcinosis (IBSPDC). The patients were a 24-year-old man with mental deterioration, a 57-year-old man with parkinsonism and dementia, and a 76-year-old woman with dementia and mild parkinsonism. The former 2 patients showed severe calcification of the basal ganglia and cerebellum, and the latter patient showed severe calcification of the cerebellum. We found significantly increased levels of copper (Cu), zinc (Zn), iron (Fe) and magnesium (Mg), using inductively coupled plasma mass spectrometry in the CSF of all these 3 patients. The increased levels of Cu, Zn, Fe and Mg reflect the involvement of metabolism of several metals and/or metal-binding proteins during the progression of IBSPDC. More numerous patients with IBSPDC should be examined in other races to clarify the common mechanism of the disease and to investigate the specific treatment.

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Introduction

Mild calcification of the basal ganglia is sometimes seen, especially in the elderly. Some patients with marked calcification of the basal ganglia and cerebellum have been reported to be associated with hypoparathyroidism. Most other idiopathic cases have traditionally been referred to as having Fahr's disease, but the nomenclature has been criticized for including a heterogeneous etiology and the disease has presented as a clinically complex syndrome. The patients have not been clearly demonstrated to exhibit any endocrine, metabolic or genetic disorder [1, 2]. The pathophysiological mechanism remains to be elucidated and there is no clue for the treatment. The disease is thus being referred to by some as idiopathic bilateral striatopallidodentate calcinosis (IBSPDC). Inductively coupled plasma mass spectrometry (ICP-MS) can measure the levels of several metals in a small amount of CSF [3]. We have measured those of Japanese patients with IBSPDC to clarify the pathophysiological features of the disease.

Case Reports

Patient 1

A 24-year-old man was hospitalized for gait and speech disturbance. He had been diagnosed with Fahr's disease when 15 years old in a hospital and his IQ was 79. On admission, neurological examination revealed mental deterioration (IQ 69), exaggerated deep tendon reflexes, mild rigidity on the right, and limb and truncal ataxia. CT showed a striking high density area in the basal ganglia and dentate nuclei and revealed progression with age (fig. 1a). No abnormal findings were detected in the blood tests including metals [calcium (Ca), iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg) and manganese (Mn)], in Ca metabolism including parathyroid hormone and the Ellsworth-Howard test, and in routine CSF studies.

Patient 2

A 57-year-old man was hospitalized for dementia, bradykinesia, and gait disturbance. He showed parkinsonism at age 50 and mental deterioration since age 55. Neurological examination revealed dementia, slurred speech, limb ataxia, rigidity, bradykinesia and truncal ataxia. Interestingly, L-DOPA led to a slight improvement in symptoms. He showed similar CT findings as patient 1 (fig. 1b), diabetes mellitus, and no other abnormal findings either in the above-mentioned tests.

Patient 3

A 76-year-old woman came to our hospital for dementia. Neurological examination revealed dementia and mild parkisonism. CT showed a striking high density area in the dentate nuclei, and a moderate area in the basal ganglia and border of the cortex and white matter of the parietal lobe (fig. 1c). No abnormal findings were detected in the above-mentioned tests.

None of the 3 patients had a skeletal structural abnormality or a family history of IBSPDC. Analysis of the levels of Ca, Fe, Cu, Zn, Mg, and Mn in the scalp hair showed no specific findings in the 3 patients using a commercially-available ICP-MS method (La Belle Vie Inc., Tokyo, Japan).

Metals in CSF Analysis

CSF samples were obtained from 3 patients with IBSPDC and 15 controls (9 females and 6 males, age from 22 to 81 years with a mean of 52 years). CSF samples were nebulized with perhydroxyl-nitrate, and the levels of metals (Fe, Cu, Zn, Mg, and Mn) were measured using ICP-MS (HP4500, Agilent Technologies, Japan). Scandium (Sc), yttrium (Y) and thallium (Tl) were added to samples as internal standards. The concentrations of the elements were normalized by the internal standards. The level of

Ca in the CSF was measured by colorimetry using o-cresolphthalein-complexone (o-CPC) for appropriate means. This study was approved by the Ethics Committee of the Gifu University Graduate School of Medicine.

Results

The levels of Cu, Zn, Fe, and Mg were significantly increased by 3.7, 2.5, 1.9, and 1.6 times of control levels, respectively. Statistical analysis using Mann-Whitney U test showed significant difference ($p < 0.01$) in the levels of Cu, Fe and Mg, and significant difference ($p < 0.05$) in that of Zn, but the levels of Ca (1.1 times) and Mn (0.9 times) in the CSF of all 3 cases with IBSPDC were not significantly different from those of controls (table 1 and fig. 2)

Discussion

Chemical analyses of brain stones in the striopallidodental system has shown high levels of Ca and other metals, such as Fe, Mg, Cu, Zn, Mn, lead, and aluminium [4, 5]. However, there is no apparent explanation for the accumulation of calcium and other metals. The pathophysiological features of Fahr's disease thus remain to be elucidated. The term 'Fahr's disease' has various entities including familial and secondary cases. As the concept of Fahr's disease may encompass diseases derived from different genetic or environmental etiologies in the region, we prefer the term 'IBSPDC' to 'Fahr's disease'. In Japan, elderly patients with dementia and calcification of the basal ganglia were reported to show diffuse neurofibrillary tangles and absence of senile plaques in the pathology [6, 7]. Patients 2 and 3 are considered to be included in this category. We presented 3 clinically idiopathic cases of IBSPDC with variable clinical characteristics and ages.

ICP-MS can measure the level of several metals in a small amount of CSF (less than 1 ml). ICP-MS is more sensitive and accurate than traditional colorimetery and the atomic absorption spectrophotometry method for the measurement of several metals such as Cu, Zn, Mg, except for that of Ca.

Generally, the high density of the basal ganglia and cerebellum in CT images has been thought to be mainly associated with calcification. However, a disorder of Ca metabolism has not been demonstrated in IBSPDC. Only one preliminary study reported rather decreased levels of Ca in the CSF in Fahr's disease, contrary to our expectations [8]. Our 3 cases with IBSPDC showed various ages and clinical presentation, but a similar and significant increase in Cu, Zn, Fe and Mg. This suggests that some cases with IBSPDC are associated with a disorder including heavy metals, especially Cu, Zn, and Fe metabolism, and some metal-binding proteins. Even at low levels, Fe and Cu can catalyze a Fenton reaction, producing highly reactive hydroxyl radicals. Excessive amounts of Cu can be a directly neurotoxic factor and also damage neurons by producing reactive oxygen in neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis [9–11].

Pathological and biochemical analyses at autopsy are needed for further evaluation. In the study we could not recognize whether metals in the CSF are free or are derived from metal-binding proteins such as superoxide dismutase-1 and metallothioneins (MT). The high levels of metals in the CSF do not necessarily reflect correctly the pathophysiological mechanisms in the brain; however, this feature of the CSF provides some novel aspects of

the diseases. CSF of more numerous and clinically variable cases with IBSPDC should be examined in other races to clarify the common pathophysiological features.

We have detected high levels of Cu, Zn, Fe and Mg in the CSF of 3 patients with IBSPDC in Japan. There is no specific and effective treatment for IBSPDC at present, and the progression of the disease is accelerated with age. MT is a small (7 kDa), metal-binding (4 Cu and 3 Zn per molecule) protein that scavenges reactive oxygen species [10]. The study of CSF may provide a clue regarding a common pathway of IBSPDC including the metabolism of Cu, Zn, Fe and Mg and appropriate treatments including metal-chelating agents such as ammonium tetrathiomolybdate, a Cu-chelating agent [11], and metal-binding proteins such as MT [10].

Disclosure

Dr. Hozumi has received research support from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Basic Research (B) 19390151) and Mitsui Sumitomo Insurance Welfare Foundation, Japan.

Table 1. Levels of metals in CSF

	Age	Ca (mg/l)	Mg (mg/l)**	Fe (μg/l)**	Cu (μg/l)**	Zn (μg/l)*	Mn (μg/l)
Patient 1	26	45.0	49.1	418	33.9	8.00	2.10
Patient 2	58	42.0	47.3	461	38.0	10.0	1.00
Patient 3	76	49.0	48.2	458	40.1	22.2	2.10
Average ± SD	53.3 ± 25.3	45.3 ± 3.51	48.2 ± 0.90	446 ± 23.7	37.3 ± 3.15	13.4 ± 7.69	1.73 ± 0.635
Control (n = 15)							
Average ± SD	48.4 ± 22.2	41.1 ± 4.64	29.6 ± 6.52	238 ± 54.7	10.2 ± 2.07	5.30 ± 3.31	1.90 ± 0.971

The levels of Ca, Fe, Cu, Zn, Mg, and Mn in CSF of patients and controls (n = 13). Statistical analysis was performed using Mann-Whitney U test.

* Significant difference, $p < 0.05$. ** Significant difference, $p < 0.01$.

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Fig. 1. CT findings in patients. **a** CT findings in patient 1. A sagittal view shows a striking high density area in the basal ganglia and the dentate nuclei of the cerebellum. **b** CT findings in patient 2. An axial view shows a marked high density area in the basal ganglia and spots at various sites such as the pulvinar thalami, the subcortical area in the frontal lobe, and the border area of the cortex and white matter in the occipital lobe. **c** CT findings in patient 3. An axial view shows a striking high density area in the dentate nuclei of the cerebellum.

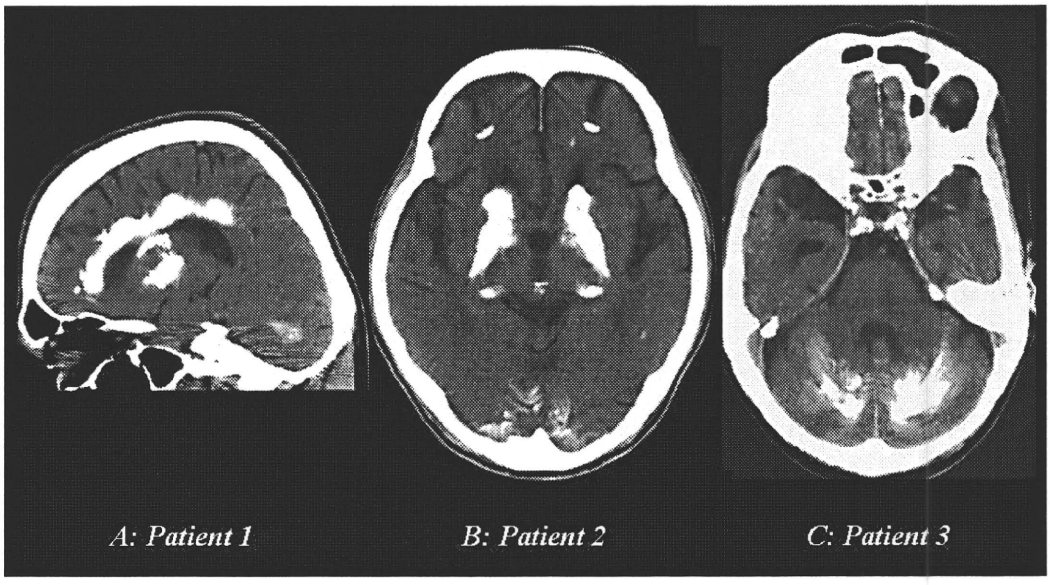
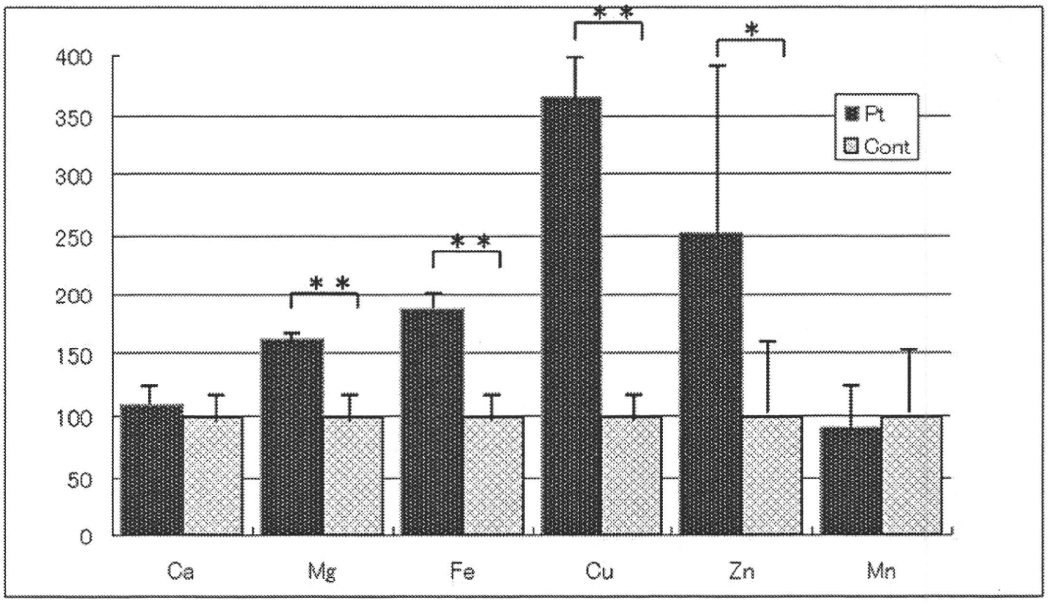


Fig. 2. Comparative values of metals in the CSF. The average levels of Ca, Fe, Cu, Zn, Mg, and Mn in the CSF of patients and controls are shown to be set at the value of 100 (%) in the figure. Especially the values of Cu and Zn in patients are markedly higher compared to those of controls. * Significant difference, $p < 0.05$. ** significant difference, $p < 0.01$.



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Patterns of levels of biological metals in CSF differ among neurodegenerative diseases

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ABSTRACT

We measured the levels of some biological metals: copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn) in the cerebrospinal fluid (CSF) in patients with neurodegenerative diseases (52 patients with amyotrophic lateral sclerosis (ALS)), 21 patients with Alzheimer's disease (AD), and 20 patients with Parkinson's disease (PD) by inductively coupled plasma mass spectrometry (ICP-MS). The diagnoses were additionally supported by neuroimaging techniques for AD and PD. In ALS, the levels of Mg ($p<0.01$ significant difference), Fe, Cu ($p<0.05$), and Zn ($p<0.10$) in CSF were higher than those in controls. Some patients showed very high levels of Cu and Zn before the critical deterioration of the disease. In AD, the levels of Cu and Zn in CSF were significantly higher in patients with late-onset AD ($p<0.01$). In PD, we found significantly increased levels of especially Cu and Zn in particular ($p<0.01$) and Mn ($p<0.05$) in CSF. A multiple comparison test suggested that the increased level of Mg in ALS and that of Mn in PD were the pathognomonic features. These findings suggest that Cu and Zn in particular play important roles in the onset and/or progression of ALS, AD, and PD. Therefore, Cu-chelating agents and modulators of Cu and Zn such as metallothionein (MT) can be new candidates for the treatment of ALS, AD, and PD.

1. Introduction

Biological metals such as copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn) have been considered to play very important roles in the progression of some neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) [1–3]. However, the roles and the metabolisms of such metals remain to be elucidated. Not only the direct toxicity of metals but also the oxidative stress via metals, and metal-associated enzymes and transcription factors modify the progression and diversity of the neurodegenerative diseases. Recently, we have found significantly increased levels of Cu, Zn, Fe, and Mg in the cerebrospinal fluid (CSF) of three patients with 'Fahr's disease' (idiopathic bilateral striato-pallido-dentate calcinosis (IBSPDC), its nomenclature remains controversial) by highly sensitive inductively coupled plasma mass spectrometry (ICP-MS) [4].

Recently, the diagnoses for neurodegenerative diseases such as AD and PD have been more accurate than before using the neuroimage techniques such as magnetic resonance imaging (MRI) with a quantitative analytical method [5], positron emission tomography (PET) or ^{99m}Tc-ethyl cysteinate dimmer-(ECD)-single photon emission computed tomography (SPECT) with quantitative analyses [6], and metaiodobenzylguanidine (MIBG)-cardioscintigraphy with quantitative measurements [7].

Some metals have been thought to be associated with the onset and/or progress of neurodegenerative diseases; Cu, Zn, and Fe for AD, Fe for PD, and Cu and Zn for familial ALS [1]. The mutations of superoxide dismutase 1 (SOD 1) including Cu and Zn in mice cause ALS [8]. Recently, the development of methods of measuring metals has progressed such as ICP-MS [4,9]. With this development, it is possible to clarify the molecular mechanisms underlying the development of neurodegenerative diseases and identify implicated metalloproteins and enzymes. In this situation, it is important to measure accurately the levels of metals in CSF of patients with ALS, AD, and PD using ICP-MS. We speculate on the molecular mechanisms and the roles of metals in neurodegenerative diseases, and develop new therapeutic strategies on the basis of the metal metabolism.

We measured the levels of some biological metals including Cu, Fe, Mg, Mn, and Zn in the CSF of 52 patients with ALS using ICP-MS. We compared the measured values with other clinical data including the subtypes, duration, and the levels of the metals in the serum in the patients with ALS. In addition, we had examined the levels of the

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heavy metals in the CSF of patients with typical features of AD and PD using neuroimaging techniques, and the pathognomonic patterns of neurodegenerative diseases were analyzed by a multiple comparison test.

2. Methods

2.1. CSF sample collection

We obtained samples of the CSF from 52 patients with ALS, 21 patients with AD, and 20 patients with PD. All the patients with ALS fulfilled the revised El Escorial criteria [10] for clinically definite and probable ALS (mean age 65.1 ± 1.6, 28 cases, classical type, 22 cases, bulbar type; and 2 cases, familial type; 17 females and 35 males). We chose samples from patients with AD diagnosed on the basis of the Diagnostic and Statistical Manual for Mental Disorders (4th ed. DSM-IV) [11]. Patients were selected on the basis of both MRI and SPECT findings (n = 21; 7 early-onset type and 14 late-onset type; 8 females and 13 males) to rule out other dementia such as vascular dementia and frontotemporal dementia [5,6]. We excluded patients with abnormal MIBG findings from the AD group to rule out Lewy body disease. We chose 20 patients (11 females and 9 males) with PD diagnosed on the basis of the criteria of British Brain Bank [12] and supported by MRI, ECD-SPECT, and MIBG-cardioscintigraphy to rule out other types of parkinsonism, such as drug-induced parkinsonism and progressive supranuclear palsy. Fifteen patients (9 females and 6 males) with unspecific neurological diseases were used as controls in the study. The first CSF samples that were obtained after the diagnosis were analyzed in this study. The study was approved by the Ethics Committee of the Gifu University Graduate School of Medicine.

2.2. Metals in CSF analysis

The CSF samples were moistly powdered to ash with perhydroxyl-nitrate, and the levels of metals (Cu, Fe, Mg, Mn, and Zn) were measured at least twice using ICP-MS (HP4500, Agilent Technologies, Japan) as previously described [4].

2.3. Statistical analyses

Data were statistically analyzed between disease groups and the control group using the Student's *t*-test. The correlations between the levels of metals in the CSF and other clinical data were analyzed using Pearson Product Moment correlation. Clinical data include age, time between the CSF examination and the disease onset, serum Cu and Zn levels, severity (mini-mental state examination (MMSE) in AD), and the clinical disease subtypes. Correlation coefficients >0.70 were considered significant. Multiple comparisons among disease groups were analyzed using Tukey's honestly significant difference (HSD) test. A significant level of 0.05 was used for all statistical tests (two-tailed). Statistical analyses were performed using IBM SPSS Statistics Base 18.

3. Results

The levels of Cu, Zn, Fe, and Mg in the CSF in patients with ALS, AD, and PD, and controls are shown in Table 1.

In ALS patients, the levels of Cu, Fe ($p<0.05$), and particularly Mg ($p<0.01$) were significantly higher in the CSF of the patients with ALS, and those of Zn were slightly elevated ($p<0.10$) than those in the controls. The data on Cu and Zn in ALS patients, were very widely scattered, because 2 patients had very high levels of Cu (>49.1 ng/ml: > the mean level in ALS + 2 SD) and 3 patients had very high levels of Zn (> 33.5 ng/ml: > the mean level in ALS + 2 SD) in the study. Interestingly these 5 patients with very high levels of Cu and Zn had underwent gastrostomy or tracheostomy within 6 months after the spinal tap in the clinical follow-up research, although all the patients who underwent gastrostomy or tracheostomy within 6 months after the spinal tap did not necessarily show high levels of Cu or Zn. A follow-up study revealed that the patients showed transiently very high levels of Cu or Zinc in CSF (data not shown). Then, we classified 50 ALS patients (exclusion of 2 patients with the familial type) according to the clinical subtypes: classical type (n = 28) and bulbar type (n = 22), and rapidly progressive types (n = 25) and slowly progressive types (n = 25) (data not shown). The patients with the rapid progressive type are those who underwent gastrostomy or tracheostomy, or who died within 2 years of the onset of the disease. No significant correlation was detected between two types. The analyses using Pearson's chi-square test supports the notion that the bulbar type is also generally the rapidly-progressive type ($p<0.01$). We show the levels of biological metals in the CSF in ALS patients in Fig. 1.

Table 1
Levels of metals in the CSF of patients with ALS, AD, and PD. The levels of Cu, Fe, Mg, Mn, and Zn in CSF of patients and controls (n = 15). Fifty patients with ALS (except familial type (n = 2)) are divided into classical type (n = 28) and bulbar type (n = 22). The patients with AD are divided into two groups: early-onset type (the onset is below 65 years) (n = 7), and late-onset type (the onset is at 65 and over 65 years) (n = 14). Statistical analysis was performed using the Student's *t*-test. Significant difference, ** $p<0.01$, * $p<0.05$, + $p<0.10$.

Cont and Pt		Age	Cu	Fe	Mg	Mn	Zn
		years	ng/ml	ng/ml	μg/ml	ng/ml	ng/ml
Cont	Av	48.4	10.2	238.0	29.6	1.9	5.3
(n = 15)	S.D	22.2	2.1	54.7	6.5	1.0	3.3
ALS	Av	65	19.5*	282.5*	35.9**	2.2	11.1 + 11.2
(n = 52)	S.D	11.7	14.8	74.9	4.8	1.5	
Classical	Av	64.6	18.3	276.8	35.2	2.2	12.7
(n = 28)	S.D	10.6	9.3	74.7	5.1	1.4	13.0
Bulbar	Av	67.7	21.0	285.9	36.6	2.3	9.3
(n = 22)	S.D	10.7	19.8	78.9	4.7	1.6	8.7
AD	Av	65.4	17.4*	238.6	31.8	1.8	8.4
(n = 21)	S.D	13.1	10.4	38.7	4.0	0.9	6.4
Early-onset AD	Av	49.6	10.3	221.6	33.8	1.2	3.9
(n = 7)	S.D	8.1	5.4	16.5	4.8	0.3	3.3
Late-onset AD	Av	73.3	20.9**	247.2	30.8	2.1	10.7**
(n = 14)	S.D	5.6	10.7	44.1	3.3	1.0	6.5
PD	Av	68.7	18.8**	263.9	31.6	3.3*	14.5**
(n = 20)	S.D	5.8	6.9	112.9	3.6	2.1	7.6

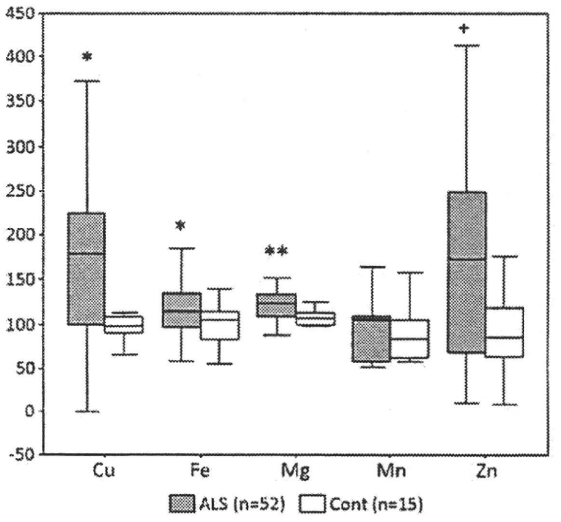

Fig. 1. The levels of the biological metals in the CSF of patients with ALS. The levels of Cu, Fe, Mg, Mn, and Zn in the CSF in patients and controls are shown in the box-and whisker type figure using SPSS. The levels of Mg (** significant difference: $p<0.01$), Fe, Cu (* $P<0.05$) and Zn (+ $p<0.10$), were higher in ALS patients than in controls.

In AD patients, we found significantly increased levels of Cu in the CSF ($p<0.05$). Then, we classified the AD patients according to the clinical subtype; early-onset AD (Alzheimer's disease with the onset under 65 years) ($n=7$) and late-onset AD (senile dementia of Alzheimer type (SDAT), onset at 65 and over 65 years) ($n=14$) (Table 1). The levels of Cu and Zn in the CSF were significantly higher in the patients with late-onset AD than in the controls. Correlation between the levels of Cu and Zn in the CSF was clearly recognized in patients with AD ($\sigma=0.812$) as well as in the controls ($\sigma=0.725$), but not in patients with ALS or PD. Although the ages of AD patients were significantly higher than those of the controls, the level of each metal did not correlate with the ages of the controls and AD patients. No other significant correlations could be observed between the levels of metals in the CSF and clinical manifestations such as MMSE, and serum Cu and Zn levels in this study. We show the levels of the biological metals in the CSF only in late-onset AD in Fig. 2.

In PD patients, we found significantly increased levels of Cu and Zn in particular ($p<0.01$), and Mn ($p<0.05$) in CSF (Table 1). We show the levels of the biological metals in the CSF in PD in Fig. 3.

In addition, to clarify the pathognomonic features, we performed a multiple comparison using Tukey's HSD test. The level of Mg in ALS was significantly higher than those in AD and PD ($p<0.01$). The level of Mn in PD was significantly higher than those in ALS and AD ($p<0.01$) (Fig. 4).

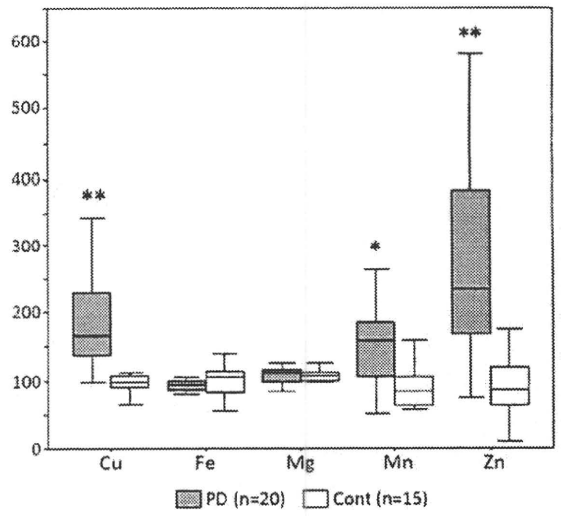

Fig. 3. The levels of biological metals in the CSF of patients with PD. The average levels of Cu, Fe, Mg, Mn, and Zn in the CSF in patients and controls are shown in the box-and whisker type figure using SPSS. The average levels of Mg, Fe, Cu, Zn, and Mn in the CSF in controls are shown to be set at 100 (%) in the figure. The levels of Cu and Zn (** $p<0.01$, respectively) and Mn (* $P<0.05$) in CSF were higher in PD patients than in controls.

4. Discussion

We measured the levels of some important metals (Cu, Fe, Mg, Mn, and Zn) in the CSF of patients with neurodegenerative diseases (AD, PD and ALS). We were able to find some pathognomonic patterns in the levels of the biological metals in the neurodegenerative diseases. Several remarkable studies on metals in the CSF of patients with neurodegenerative diseases have been published and we discuss some important metals for each disease.

In ALS, Kanias and Kapaki reported that the levels of Cu and Zn in CSF were higher in patients with ALS (age >40) than in older controls (age >40) as determined by atomic absorption spectrophotometry [13]. This is compatible with our finding. In particular, Cu and Zn are considered to play pivotal roles in the onset and/or progression of ALS. Studies on the spinal cord of G93A SOD-1 transgenic mice revealed high levels of Cu and labile Zn [14,15]. In this study, intriguingly, 5 patients showed very high levels of Cu and Zn before their critical deterioration. A researcher had observed that some patients with ALS showed transiently high levels of Zn in the urine during the course of the disease (personal communication). The mechanism remains to be elucidated and it remains to be clarified whether these phenomena are a harbinger or a result. In our study the levels of Mg were also significantly elevated ($P<0.01$) and the levels of Fe are also increased than those in the controls ($p<0.10$). Glutamate excitotoxity is suspected to cause motor neuron damage [16] and Mg ions inhibit the opening of NMDA receptors [17]. Taken together, the findings

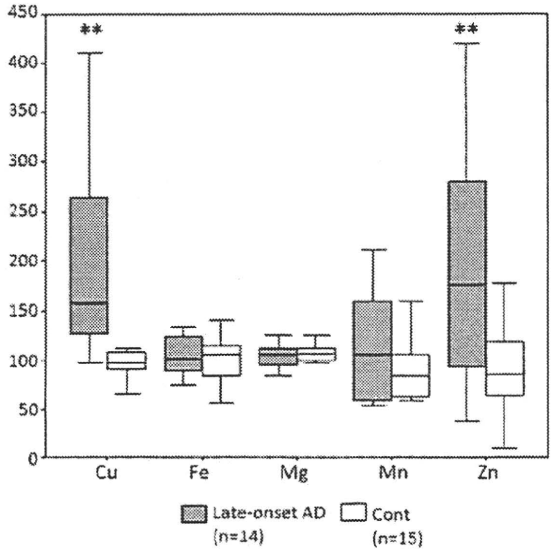

Fig. 2. The levels of biological metals in the CSF of patients with late-onset AD. The average levels of Cu, Fe, Mg, Mn, and Zn in the CSF in patients and controls are shown in the box-and whisker type figure using SPSS. The average levels of Mg, Fe, Cu, Zn, and Mn in the CSF in controls are shown to be set at 100 (%) in the figure. The levels of Cu and Zn (** $p<0.01$, respectively) in CSF were higher in late-onset AD than in controls.

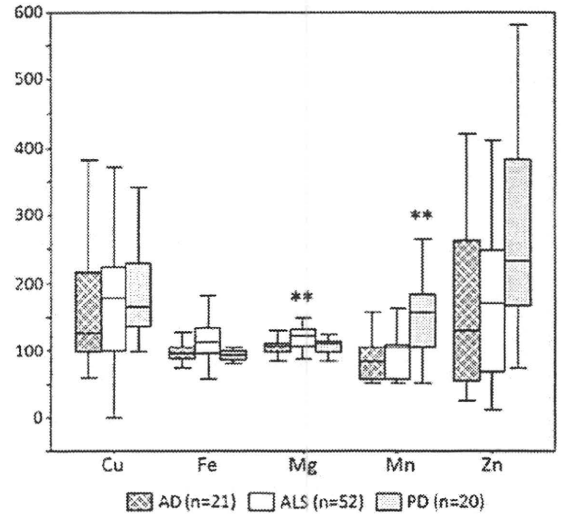

Fig. 4. The levels of the biological metals in the CSF among patients with ALS, AD and PD. The level of Mg in ALS patients was significantly higher than those in AD and PD patients (** $p<0.01$), and the levels of Mn were significantly higher than those in ALS and AD patients (** $p<0.01$) according to Tukey's HSD test.

suggest that multiple metals complexly contribute to the onset and/or progression of ALS.

We selected the patients with typical AD features using imaging studies, because the levels of Mg and Ca were reported to be increased in the CSF in patients with Levy body disease (LBD) than in those with AD [18]. Our study showed that the levels of Cu and Zn in CSF markedly higher in patients with late-onset AD. As similarly observed in ALS [13], markedly higher levels of Cu and Zn were also observed in late-onset AD patients in our study. However, no association of the levels of metals with age was found in both controls and patients with AD. A recent study showed that the serum copper level is associated with the MMSE score worsening in patients with AD [19]. Zn level was also reported to be increased in the human AD-affected cortex [20]. However, we found no association among the level of Cu in the CSF, the level of Cu in the serum, and the MMSE score in this study. A positive correlation between Cu and Zn levels in CSF was found in controls and patients with AD, although it is generally considered that there is a negative correlation between Cu and Zn levels in serum. However, the positive correlation between Cu and Zn levels in CSF in patients with AD was not observed in patients with ALS and PD. The mechanism underlying the correlation is unclear but some other pathognomonic factors may affect the levels of Cu or/and Zn in patients with ALS and PD. A study on Japanese American men suggested that Zn and Cu modulate Aβ-42 levels in CSF [21]. Therefore, both Cu and Zn are considered to be the main metals that are strongly associated with the onset and/or progression of AD, particularly late-onset AD.

In PD, our study showed that the levels of Cu and Zn in CSF were significantly ($p<0.01$) higher and the level of Mn was also higher ($p<0.05$) than those in the controls. Mn intoxication has been well known to cause parkinsonism. A survey suggested that chronic occupational exposure to Mn or Cu is associated with PD [22]. Low-level Mg intake over generations was shown to cause the degeneration of the substantial nigra in rats [23]. A study by ICP-AES showed lower Fe and Si levels in the CSF of 91 PD patients than in 18 controls in Italy and the levels of Mg concentration decreased in the CSF with the duration and severity of the disease [24]. The lower level of Fe and the decrease in the levels of Mg with time were not observed in our study. The reason is still unknown.

There are other studies on metals in the CSF of AD, PD and ALS patients. The important points are the methods of measurement of metals and the diagnosis of the diseases. ICP-MS is more sensitive and accurate than the conventional colorimetery and atomic absorption spectrophotometry methods for the simultaneous measurement of several biological metals such as Cu, Fe, Mg, Mn, and Zn [4,9]. We are able to accurately diagnose AD and PD by neuroimaging techniques [5–7]. However, there are some limitations in our study. The numbers of controls, and AD and PD patients were relatively small, and controls were significantly younger than the patients with ALS, AD, and PD ($p<0.01$). However, the levels of the metals in the CSF did not correlate with age. There may be several pathological factors that affect the levels of the metals in the CSF such as environmental factors including diet, drugs, life styles, the time of examination, and possibly races. We should examine the changes in the levels of metals in the CSF during the course of the diseases, particularly ALS. The levels of metals in the CSF only indicate the levels of metabolites similar to those in urine. We should examine the changes in the levels of metals and metal-transporting proteins in the causative parts for each disease to clarify the roles of metals in the brain and the spinal cord in the future.

Taken together, Cu and Zn are considered to play important roles in ALS, AD, and PD. Multiple metals seem to complexly contribute to the development of ALS and a surge of Cu or Zn level may be a harbinger of critical deterioration in ALS. The increased level of Cu and Zn in the CSF were prominent in the late-onset AD. The increased level of Mg in ALS and that of Mn in PD may be pathognomonic features. Cu and Zn may not be essential for the pathogenesis of neurodegenerative diseases but they probably promote the progression of the diseases through oxidative stress and conformational change of pivotal proteins. Cu-chelating agents [14], Zn-chelating agents [15], and, moreover, metallothioneins, which maintain Zn and Cu homeostasis [25,26], can be new candidates for the treatment of neurodegenerative diseases, based on the findings.

Acknowledgements

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