ApoE-3/4. The ApoE genotype also affected the increase in aerobic capacity produced by exercise training possibly via undefined effects on nerve and skeletal muscle function.[168] In another study, no association was found between ApoE and maximal oxygen uptake levels either in the sedentary state or in response to exercise training.[169] In summary, all these studies globally indicate that ApoE-related polymorphic variants, especially the ApoE-4 allele, represent a biological disadvantage for brain function and lipid metabolism.

GENOTYPE-PHENOTYPE CORRELATIONS

Different ApoE genotypes confer specific phenotypic profiles to AD patients. Some of these profiles may add risk or benefit when the patients are treated with conventional drugs, and in many instances the clinical phenotype demands the administration of additional drugs, which increase the complexity of therapeutic protocols. From studies designed to define ApoE-related AD phenotypes,[29,37,72,170–175] several confirmed conclusions can be drawn: (i) the age-at-onset is 5–10 years earlier in approximately 80% of AD cases harboring the ApoE-4/4 genotype; (ii) the serum levels of ApoE are lowest in ApoE-4/4, intermediate in ApoE-3/3 and ApoE-3/4, and highest in ApoE-2/3 and ApoE-2/4; (iii) serum cholesterol levels are higher in ApoE-4/4 than in the other genotypes; (iv) HDL-cholesterol levels tend to be lower in ApoE-3 homozygotes than in ApoE-4 allele carriers; (v) LDL-cholesterol levels are systematically higher in ApoE-4/4 than in any other genotype; (vi) triglyceride levels are significantly lower in ApoE-4/4; (vii) nitric oxide levels are slightly lower in ApoE-4/4; (viii) serum Aβ levels do not differ between ApoE-4/4 and the other most frequent genotypes (ApoE-3/3, ApoE-3/4); (ix) blood histamine levels are dramatically reduced in ApoE-4/4 as compared with the other genotypes; (x) brain atrophy is markedly increased in ApoE-4/4>ApoE-3/4>ApoE-3/3; (xi) brain mapping activity shows a significant increase in slow wave activity in ApoE-4/4 from early stages of the disease; (xii) brain hemodynamics, as reflected by reduced brain blood flow velocity and increased pulsatility and resistance indices, is significantly worse in ApoE-4/4 (and in ApoE-4 carriers, in general, as compared with ApoE-3 carriers); (xiii) lymphocyte apoptosis is markedly enhanced in ApoE-4 carriers; (xiv) cognitive deterioration is faster in ApoE-4/4 patients than in carriers of any other ApoE genotype; (xv) occasionally, in approximately 3–8% of the AD cases, the presence of some dementia-related metabolic dysfunctions (e.g. iron, folic acid, vitamin B_{12} deficiencies) accumulate more in ApoE-4 carriers than in ApoE-3 carriers; (xvi) some behavioral disturbances (bizarre behaviors, psychotic symptoms), alterations in circadian rhythm patterns (e.g. sleep disorders), and mood disorders (anxiety, depression) are slightly more frequent in ApoE-4 carriers; (xvii) aortic and systemic atherosclerosis is also more frequent in ApoE-4 carriers; (xviii) liver metabolism and transaminase activity also differ in ApoE-4/4 with respect to other genotypes; (xix) blood pressure (hypertension) and other cardiovascular risk factors also accumulate in ApoE-4; and (xx) ApoE-4/4 carriers are the poorest responders to conventional drugs. These 20 major phenotypic features clearly illustrate the biological disadvantage of ApoE-4 homozygotes and the potential consequences that these patients may experience when they receive pharmacological treatment.[170–176]

CONCLUSION

AD is a multifactorial and complex disorder in which over 150 different genes distributed across the human genome may be involved. Among AD-causing genes, APP, PS1, and PS2 mutations in part explain AD pathogenesis, however Mendelian mutations in those three genes only account for less than 10% of AD cases, indicating that many other networking mechanisms must be involved in neurodegeneration and premature neuronal death in AD. ApoE-related polymorphic variants (ApoE-4 allele) represent the most significant susceptibility genetic defect in AD, contributing to neuronal dysfunction in approximately 30–40% of AD cases. The precise mechanism by which ApoE affects neurodegeneration is still unclear. ApoE-4 is a genetic risk factor of cognitive impairment in many neurodegenerative disorders, including AD and other types of dementia.

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Caffeine Modulates Tau Phosphorylation and Affects Akt Signaling in Postmitotic Neurons

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Abstract Neuronal cell cycle reentry, which is associated with aberrant tau phosphorylation, is thought to be a mechanism of neurodegeneration in AD. Caffeine is a neuroprotective drug known to inhibit the cell cycle, suggesting that its neuroprotective nature may rely, at least in part, on preventing tau abnormalities secondary to its inhibitory effect on neuronal cell cycle-related pathways. Accordingly, we have explored in the present study the impact of caffeine on cell cycle-linked parameters and tau phosphorylation patterns in an attempt to identify molecular clues to its neuroprotective effect. We show that caffeine blocks the cell cycle at G1 phase in neuroblastoma cells and leads to a decrease in tau phosphorylation; similarly, exposure of postmitotic neurons to caffeine led to changes in tau phosphorylation concomitantly with downregulation of Akt signaling. Taken together, our results show a unique impact of caffeine on tau phosphorylation and warrant further investigation to address whether caffeine may help prevent neuronal death by preventing tau abnormalities secondary to aberrant entry into the cell cycle.

Keywords Neurodegeneration · Alzheimer's · Tau · Caffeine · Cell cycle · Akt

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Introduction

Alzheimer's disease (AD) is characterized neuropathologically by extracellular neuritic plaques of beta-amyloid (Aβ), intracellular aggregation of hyperphosphorylated tau into neurofibrillary tangles (NFTs), and neuronal degeneration in vulnerable areas (Tanzi and Bertram 2005).

In addition, a broad set of cell cycle abnormalities has been consistently described in postmitotic neurons of AD postmortem brains. This includes the ectopic expression of cell cycle proteins as well as evidence of DNA replication (reviewed in Currais et al. 2009), consistent with the notion that neurons in AD, under stress stimuli, may attempt to reenter the cell cycle. Importantly, reactivation of the cell cycle is lethal (Kruman et al. 2004; Park et al. 2007), which has led to the proposal that neuronal cell cycle reentry may be a specific mechanism of neurodegeneration in AD.

Indeed, NFTs and cell cycle regulators co-localize in the AD brain (Hoozemans et al. 2004; Evans et al. 2007), and in vivo induction of the cell cycle in postmitotic neurons shows that the instigation of cell cycle in quiescent neuronal cells results in a phenotype that shares features of degenerative neurons in AD, including hyperphosphorylated tau (McShea et al. 2007; Park et al. 2007). The evidence is consistent with a mechanism of neurodegeneration in

which cell cycle reentry in AD may contribute to tau dysregulation and, eventually, neuronal death.

Caffeine, an antagonist of A_1 and A_{2A} adenosine receptors, has been shown to be neuroprotective against AD-related stress insults, including Aβ (Dall'Igna et al. 2007; Arendash et al. 2009). Long-term consumption of caffeine has been reported to improve cognition, reduce decline of both cognitive function and memory (Ritchie et al. 2007), and, crucially, to delay onset of AD (Lindsay et al. 2002; Maia and de Mendonca 2002). In addition, and of particular relevance to neuronal cell cycle-linked mechanisms of neurodegeneration in AD, caffeine has been reported to inhibit cell cycle progression in numerous cell lines (Bode and Dong 2006).

Based on the available evidence, we have reasoned that the neuroprotective nature of caffeine in AD may rely, at least in part, on its inhibitory effect on neuronal cell cycle-related pathways, thus preventing associated deleterious changes in tau. Accordingly, we have explored the impact of caffeine on cell cycle-linked parameters and tau phosphorylation patterns.

Materials and Methods

Cell Culture

SH-SY5Y cells were cultured in minimum essential medium Eagle (Sigma) and F(12)HAM (Gibco) at a ratio of 1:1, supplemented with 15% (*v/v*) fetal calf serum (Sera Labs), 2 mM L-glutamine, 1% (*v/v*) non-essential amino acids (Sigma), 400 nM sodium pyruvate (Sigma), and 1% (*v/v*) penicillin streptomycin (Gibco), and grown at 37°C with 5% CO_2.

Primary cultures of rat cerebral cortices were prepared from embryonic day 17 rat embryos as described previously (Williamson et al. 2002). Cultures were maintained at 37°C in 5% CO_2 and allowed to differentiate for 7 days before use. Rat handling and husbandry was carried out according to the Animals (Scientific Procedures) Act 1986 under the Personal License (PIL) no. 70/20946.

Experimental Treatments

Caffeine (Invitrogen) was used at a maximum concentration of 20 mM, as described previously (Kruman et al. 2004), LiCl (Sigma) was used at 10 mM (Hong et al. 1997), and aphidicolin (Biomol International) was prepared in DMSO (Invitrogen) and used at a concentration of 10 μM (Wilcox and Johnson 1988). SH-SY5Y cells were treated with 0.5 μM staurosporine as a positive control for cell death. All treatments were carried out for 4 h in both primary cultures and SH-SY5Y cells.

Assessment of Cell Viability

Cell viability was measured by LDH release using CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega, UK) according to the manufacturer's protocol.

Levels of cleaved caspase 3 were assessed by Western blot and are indicative of apoptosis-mediated toxicity. The antibody recognizes only the active form of the enzyme cleaved at Asp175 (1:1,000, Cell Signaling Technology, UK). It does not recognize the full sequence or other cleaved caspases.

Cell Lysis and Western Blotting

Primary cortical neurons and SH-SY5Y cells were collected and lysed in ice-cold lysis buffer (100 mM Tris–HCl, pH 8.0, 150 mM NaCl, and 1% (*v/v*) Triton X-100) with protease and phosphatase inhibitors (Complete™ Protease and Phosphatase inhibitor cocktail tablets, Roche Diagnostics). Lysates were clarified by centrifugation and total protein content was quantified by bicinchoninic acid assay (BCA, Novagen, UK). Equal amounts of protein were loaded in 10% SDS-PAGE gel and Western blot analysis was performed according to standard protocols. The following primary antibodies were used: cyclin D1 (DCS6, aa. 151-170; 1:1,000, Cell Signaling Technology, UK); Retinoblastoma (pSer780; 1:500, Santa Cruz Biotechnology, UK); cyclin A (H-432, aa. 1-432; 1:1,000, Santa Cruz Biotechnology); cyclin B1 (V152; 1:2,000, Cell Signaling Technology, UK); PCNA (PC10; 1:1,000, Cell Signaling Technology, UK); total tau (1:50,000, DAKO, Cambridge, UK); AT8 (1:1,000, Pierce Biotechnology, Rockford, USA); PHF-1 (1:2,000, courtesy of Peter Davies); Tau-1 (1:1,000, courtesy of Peter Davies); ptau Ser214 (1:1,000, Invitrogen, Japan); ptau Thr231 (1:1,000, Invitrogen); pGSK3 α/β Ser21/9 (1:1,000, Cell Signaling Technology, Japan); pAkt Ser473 (1:1,000, Cell Signaling Technology, Japan); total Akt (1:1,000, Cell Signaling Technology, Japan); and β-actin (AC-15; 1:40,000, Abcam, USA). Blots were incubated with IRDye™800 conjugated secondary antibody and immunoreactive protein analysis was carried out using the Odyssey Infrared Imaging System (Li-Cor Bioscience, UK)

BrdU Incorporation Assay

To detect SH-SY5Y cells in S phase of the cell cycle, 10 μM of 5′-bromo-2′-doxyuridine (BrdU, Sigma) was added to the culture medium for 2 h 30 min, cells fixed, and assessed for BrdU incorporation as previously described (Malik et al. 2008). Nuclei were counterstained with Hoechst 33,342 (Invitrogen) to assay for total cell numbers. Values shown are the mean ± SEM. One hundred fifty or

more cells per coverslip were counted for the different treatments.

Statistical Analysis

Statistical analysis was carried out by one-way ANOVA followed by Student's Newman–Keuls and Tukey's honestly significant difference post hoc tests in SPSS v16.0. The data shown are from at least three independent experiments. Sampling errors are reported as the standard error of the mean, and significance of difference is indicated as $*p<0.05$, $**p<0.01$, and $***p<0.001$.

Results

We initially aimed to characterize the impact of caffeine on the cell cycle progression and overall patterns of tau phosphorylation in SH-SY5Y neuroblastoma cells. The rationale for the use of these cells was twofold. First, they are mitotically competent, allowing for a thorough investigation of the impact of caffeine at all stages of the cell cycle; second, being of neuronal origin, they express endogenous tau, thereby allowing us to measure caffeine-induced changes in tau phosphorylation. We monitored the cell cycle status of cells by measuring the incorporation rates of BrdU and changes in the levels of specific cell cycle markers.

Aphidicolin, a specific inhibitor of DNA polymerase α and δ, was used as a control to assess modifications in cell cycle parameters with and without inhibition of S-phase. Treatment of cells with aphidicolin decreased BrdU incorporation, synchronizing cells at S phase (Fig. 1a, c; no quantitation for Fig. 1b was carried out due to the very low levels of BrdU incorporation in all S-phase cells after inhibition of DNA polymerase by aphidicolin). Aphidicolin treatment also led to lower levels of cyclin D1 (control 1±0.06, aphidicolin 0.52±0.08, $**p<0.01$), whose degradation is required prior to S phase, and an increase in the levels of cyclin A (control 1±0.03, aphidicolin 1.75±0.06, $**p<0.01$) and B1 (control 1±0.03, aphidicolin 1.88±0.06, $**p<0.01$; Fig. 1c), whose expression starts at S phase. pRb levels were not altered in the presence of aphidicolin (Fig. 1c). Rb is only found in its active hypophosphorylated form prior to the G1/S transition, and its levels remain constant thereafter. Similarly, levels of PCNA, a protein responsible for the processivity of DNA polymerase δ and whose expression peaks in late G1, were unchanged.

Exposure of SH-SY5Y cells to caffeine significantly reduced the rate of entry into S phase and decreased the levels of the different cell cycle proteins assessed (Fig. 1a–c; cyclin D1: control 1±0.06, caffeine 0.71±0.05, $*p<0.05$; pRb: control 1±0.01, caffeine 0.39±0.05, $**p<0.01$; cyclin A: control 1±0.03, caffeine 0.56±0.05, $**p<0.01$; cyclin B1: control 1±0.03, caffeine 0.68±0.07, $*p<0.05$; PCNA: control 1±0.02, caffeine 0.6±0.04, $**p<0.01$). The fact that both cyclin D1, a rate-limiting factor in the progression of cells through G1 phase, and cyclin B1, whose levels decrease at the end of mitosis, were downregulated (Fig. 1c), indicates that caffeine specifically blocked cell cycle progression at G1 phase.

Having established that caffeine blocks the cell cycle at G1 in SH-SY5Y cells, we next measured its impact on tau levels and phosphorylation patterns. Figure 1d shows that while there is no effect on the overall levels, there is a clear shift in the electrophoretic mobility of tau bands, indicating that exposure of cells to caffeine leads to tau dephosphorylation.

To assess the specificity of caffeine on cell cycle dynamics and tau modulation, we treated cells in parallel with LiCl, a potent inhibitor of tau kinase GSK3 known to cause extensive tau dephosphorylation, thus providing a suitable control to assess modifications in tau phosphorylation and their relation to the cell cycle. As expected, and in common with caffeine, exposure of SH-S5Y5 cells to LiCl also led to a shift in electrophoretic mobility (Fig. 1d), indicative of tau dephosphorylation. However, and in contrast with caffeine, no changes in cell cycle parameters were observed in cells treated with LiCl (Fig. 1a–c).

Assessment of potential drug-specific toxicity revealed no deleterious impact of caffeine or LiCl, as measured by levels of activated caspase 3 (Fig. 1c).

Since caffeine, but not LiCl, effectively inhibited entry into the cell cycle in SH-SY5Y cells, we next asked whether caffeine treatment led to changes in tau phosphorylation that could differ from those caused by LiCl as such potential changes might conceivably be linked to cell cycle dynamics. To test that notion, we firstly determined the levels of specific tau phosphoepitopes in postmitotic neurons in response to both LiCl and caffeine. The rationale for the use of these cells is that unlike SH-SY5Y cells, they constitute a model for differentiated neurons in which tau homeostasis more closely resembles what occurs in the adult brain.

Exposure of neurons to LiCl led to GSK3αβ inactivation, as measured by its phosphorylation at S21/9 (control 1±0.16, LiCl 1.76±0.17, $*p<0.05$; Fig. 2b). Accordingly, we detected tau dephosphorylation at epitopes S199/S202/T205 (AT8: control 1±0.03, LiCl 0.57±0.06, $*p<0.05$; Tau-1: control 1±0.03, LiCl 1.82±0.1, $**p<0.01$), S214 (control 1±0.12, LiCl 0.6±0.08, $*p<0.05$), T231 (the dephosphorylation is evident from a decrease of the upper band, although total levels did not change significantly), and S396/S404 (control 1±0.07, LiCl 0.43±0.02, $**p<0.01$; Fig. 2a), all known to be targets of GSK3β activity (Liu et al. 2002). Furthermore, tau dephosphorylation was also evident from the apparent shift in tau electrophoretic mobility measured with a pan-tau antibody (Fig. 2a).

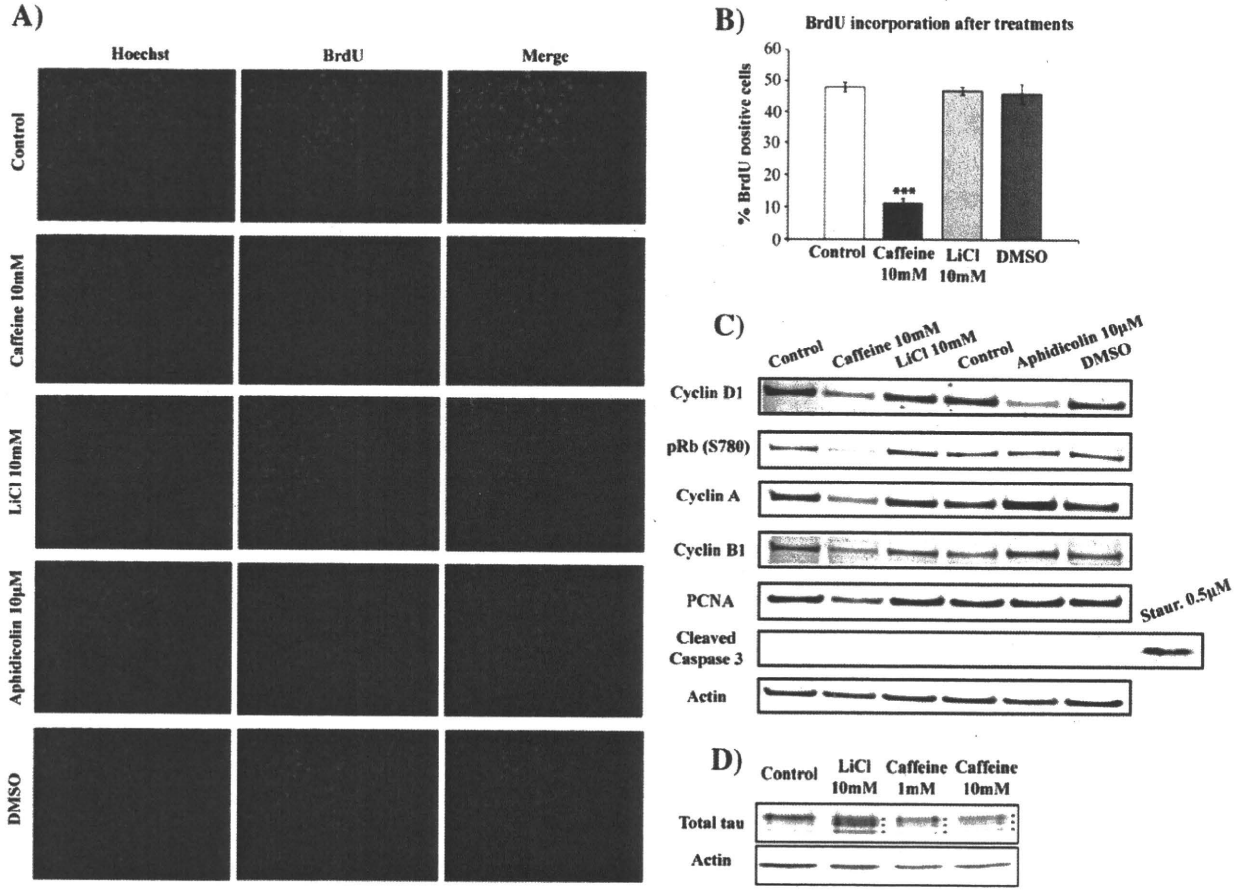

Fig. 1 Impact of caffeine on cell cycle kinetics and tau homeostasis in SH-SY5Y cells. **a** Representative fields showing total number of cells (Hoechst) and cells in S phase (BrdU) after 4 h of treatment with 10 mM caffeine, 10 mM LiCl, 10 μM aphidicolin, and DMSO (as control for aphidicolin). **b** Quantitation of SH-SY5Y cells in S phase. Data expressed as the mean ± SEM (n=6). ***P<0.001. Because aphidicolin reduced BrdU incorporation in all cells, quantitation was not carried out. **c** Western blot analysis of TX-100 soluble cell lysates with antibodies for cyclin D1 (G1 phase), pRb (S780, G1/S transition), cyclin A (S and G2 phases), cyclin B1 (G2 phase), proliferating cell nuclear antigen (*PCNA*, marker of S phase), cleaved caspase 3 (apoptotic marker), and actin as a loading control. Cells were treated with 0.5 μM staurosporine (*Staur.*) for 4 h as a positive control for cell death. **d** Western blot analysis of SH-SY5Y cells treated with 1 or 10 mM caffeine or 10 mM LiCl for 4 h with antibodies for total tau and actin. *Asterisks* mark the different tau bands and highlight a shift in the electrophoretic mobility, indicative of changes in phosphorylation

Similar to LiCl, treatment of postmitotic neurons with caffeine led to tau dephosphorylation at sites S199/S202/T205 (AT8: control 1±0.03, caffeine 20 mM 0.44±0.04, **p<0.01; Tau-1: control 1±0.03, caffeine 20 mM 1.67±0.1, **p<0.01) and T231 (Fig. 2a). In contrast with LiCl, however, there were no significant differences in the levels of active GSK3αβ or phosphorylation at S396/S404 (Fig. 2a, b). Strikingly, caffeine exposure also led to a dramatic increase in tau phosphorylation at S214 (control 1±0.15, caffeine 20 mM 3.95±0.05, ***p<0.001; Fig. 2a).

Having established that caffeine does have a measurable specific effect on neuronal tau phosphorylation patterns, we then explored the possible mechanisms involved. Caffeine acts as an antagonist of adenosine A_1 and A_{2A} receptors, and it has been shown that the specific A_{2A} subtype receptors can positively regulate cell cycle when over-stimulated (Merighi et al. 2002). In this regard, activation of A_{2A} receptors has been reported to activate the PI3K/Akt survival/proliferation pathway (Mori et al. 2004), which has been associated with pathological Aβ (Bhaskar et al. 2009) and found upregulated in AD brain (Griffin et al. 2005). Importantly, the spatial and temporal patterns of Akt overexpression in the AD brain coincide with those of early pathological tau changes, consistent with the notion that Akt could be involved in tau metabolism in vivo and play a protective role in cellular responses in AD-related neurodegeneration (Stein and Johnson 2002). In view of the link between Akt activity, tau phosphorylation, and AD pathology, we analyzed the effect of caffeine on Akt activation, measured by phosphorylation at its residue S473. Akt was found strongly dephosphorylated at S473 after treatment with caffeine (levels too low to quantify at

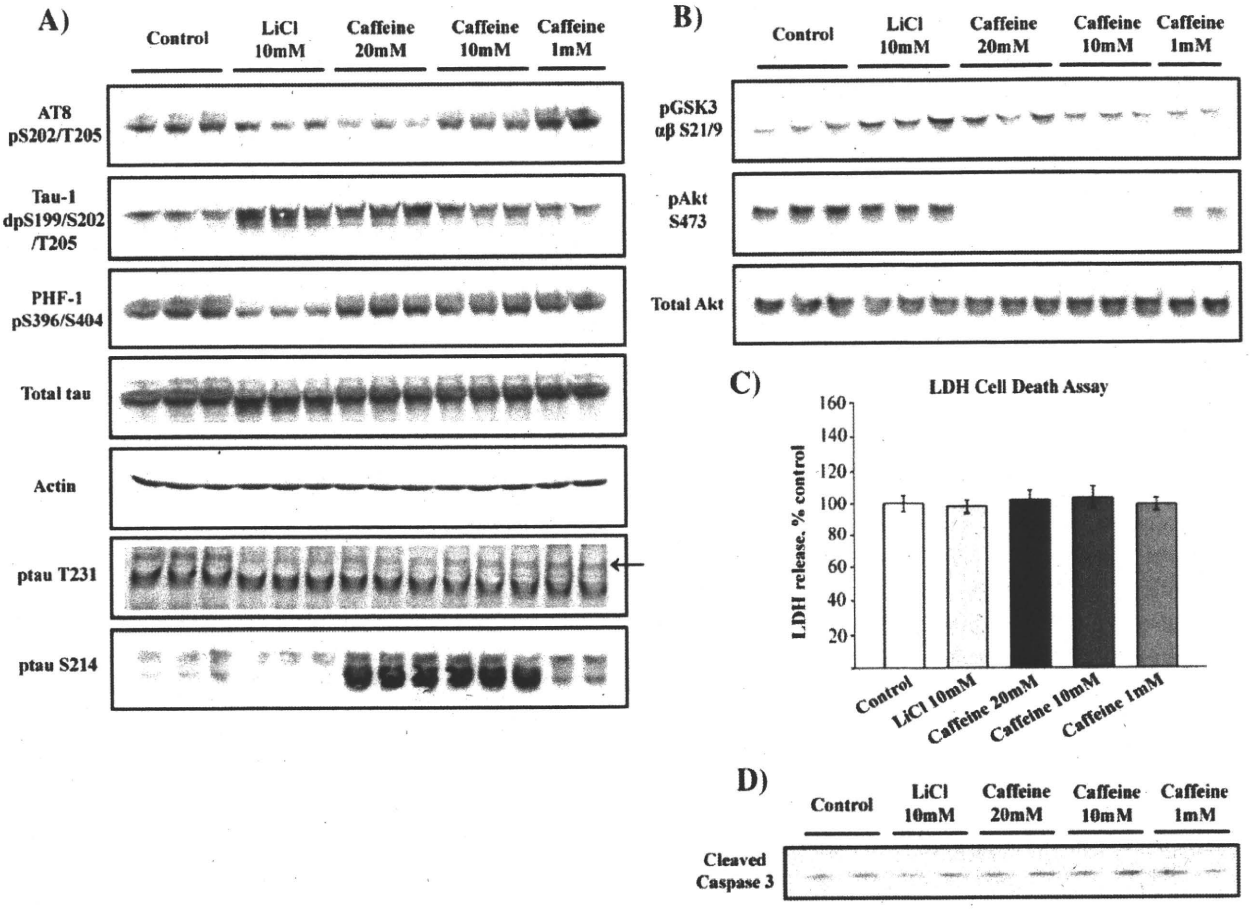

Fig. 2 Analysis of caffeine-associated tau phosphorylation and toxicity in postmitotic neurons. Primary neurons were treated with 1, 10, or 20 mM caffeine and 10 mM LiCl for 4 h and Western blot analysis of TX-100 soluble lysates carried out for the following proteins: **a** Total tau levels; tau phosphorylation at S202/T205 (AT8), S396/S404 (PHF-1), T231 (ptau T231; *arrow* indicates visible changes in phosphorylation), S214 (ptau S214); tau dephosphorylation at S199/S202/T205 (Tau-1). **b** Phosphorylated GSK3αβ; Akt phosphorylated at S473 (*pAkt*), and total Akt. Treatment toxicity was assessed by measuring: the release of LDH in culture media (data expressed as the mean ± SEM, $n=6$) (**c**) and the expression levels of cleaved caspase 3 (apoptotic marker) (**d**) by Western blot

caffeine 20 mM; Fig. 2b) and correlated with the increase in tau phosphorylation at S214 (Fig. 2a).

Treatment of primary cortical neurons with caffeine and LiCl for a period of 4 h revealed no toxicity, as assessed by LDH levels (Fig. 2c) and by protein levels of the apoptotic marker activated caspase 3, similar throughout all treatments (Fig. 2d), indicating that our results are not a consequence of cell death activation.

Discussion

Cell cycle dysregulation in postmitotic neurons is thought to be a mechanism of neurodegeneration in AD. The fact that aberrant tau phosphorylation is strongly associated with cell cycle abnormalities, including co-localization with cell cycle markers in AD brain (Hoozemans et al. 2004; Evans et al. 2007), is consistent with a role for tau dysregulation in abortive cell cycle reentry in susceptible neurons. Caffeine is a neuroprotective compound which has been shown to inhibit cell cycle progression (Bode and Dong 2006); thus, it is possible that caffeine might protect stressed neurons by inhibiting neuronal cell cycle-related pathways and preventing associated deleterious changes in tau. Here, we have explored the impact of caffeine on cell cycle parameters and associated tau phosphorylation patterns.

Our results show that in SH-SY5Y cells, caffeine is a potent inhibitor of cell cycle progression at G1 stage (Fig. 1a–c) and that it modulates tau phosphorylation (Fig. 1d). Assessment of caffeine in postmitotic neurons confirmed its impact on the phosphorylation patterns of tau (Fig. 2a) and revealed modulation of Akt signaling (Fig. 2b) as a possible mechanism of action of caffeine neuroprotection.

Tau phosphorylation is upregulated during cellular division in a wide variety of cells (Preuss and Mandelkow

1998; Delobel et al. 2002). Treatment of SH-SY5Y cells with caffeine inhibited the cell cycle at G1 (Fig. 1a–c) and also led to a decrease in tau phosphorylation (Fig. 1d). However, it is noteworthy that LiCl, while also leading to widespread tau dephosphorylation (Fig. 1d), did not affect the cell cycle (Fig. 1a–c). Accordingly, we sought to identify changes in tau phosphorylation that were specific to caffeine in postmitotic neurons where cell cycle reentry has been shown to cause cell death (Kruman et al. 2004; Park et al. 2007). We found differences between LiCl and caffeine regarding their impact on tau phosphorylation, in particular at the epitope S214 which was highly phosphorylated after caffeine treatment but did not change in response to LiCl. S214 phosphorylation correlated with the inactivation of Akt signaling (Fig. 2a, b), a pathway affected by pathological Aβ (Bhaskar et al. 2009) and found upregulated in the AD brain co-localizing with tau pathology (Griffin et al. 2005). Akt is involved in tau metabolism, and residues S212 and S214 have been reported to be major Akt-dependent tau phosphorylation target residues (Ksiezak-Reding et al. 2003). More recently, Zhou et al. (2009) have shown that both overexpression and knockdown of Akt can lead to high S214 phosphorylation in N2a neuroblastoma cells. Although future work will be needed to determine the exact impact of Akt on tau, our results show a clear correlation between low levels of pAkt and high levels of pS214 upon caffeine exposure (Fig. 2a, b). Interestingly, phosphorylation at S214 has been shown to detach tau from microtubules, protecting it against pathological aggregation (Schneider et al. 1999; Sadik et al. 2009). Thus, it is tempting to speculate that tau phosphorylation at S214 may be part of a neuroprotective mechanism through an inhibitory effect on tau aggregation.

Although dephosphorylation of tau at S199/S202/T205 occurred both in response to LiCl and caffeine, GSK3 activity was only reduced in response to the former (Fig. 2a, b). Caffeine-induced changes in these epitopes are also unlikely to reflect changes in Akt activity since Akt does not appear to phosphorylate tau at these sites, as demonstrated in vitro (Ksiezak-Reding et al. 2003). Thus, the kinases and/or phosphatases that may be involved in tau changes at S199/S202/T205 remain to be elucidated. Nevertheless, caffeine-induced changes at these sites may not be of specific relevance to cell cycle dynamics in neurons given that they also occur in response to LiCl.

In conclusion, our work is, to our knowledge, the first to identify tau modulation and the Akt signaling pathway, both of which are altered in AD, as potential targets of the neuroprotective mechanism of caffeine. Our results are consistent with a functional link between the Akt pathway and tau phosphorylation in postmitotic neurons and warrant further research to establish whether the neuroprotective role of caffeine may stem from its prevention of tau abnormalities secondary to aberrant neuronal entry into the cell cycle.

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症例報告
Case Report

経時的に詳細な言語機能評価をした運動ニューロン疾患を伴う意味性認知症の1例

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Language and Semantic Memory Impairment in a Patient with Motor Neuron Disease and Semantic Dementia — A Case Report

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Abstract

We report the rare case of a 59-year-old man with motor neuron disease and semantic dementia (SD-MND); SD-MND was in a very early stage, and its clinical progression, especially with regard to language impairment, and abnormalities on neuroimages were evaluated for 3 years. The patient complained only of difficulties in recalling names of acquaintances and in writing kanji characters. After 1 year, he experienced difficulty in describing common objects. He developed two-way anomia only in some words, which varied from day to day. His anomia was not category-specific and was noted even with respect to words that describe color. In addition to experiencing difficulty in writing kanji characters, he experienced difficulty in writing kana characters. Muscle atrophy was observed, and he experienced weakness in his limbs, especially in the right upper limb; however, bulbar symptoms were not observed. At this point, he fulfilled the diagnostic criteria for MND. In the next year, semantic memory impairment became apparent, and he was subsequently diagnosed with SD. Deterioration in his ability to name objects in all categories, except body parts, was noted. Further, the ability of writing both kana and kanji characters was increasingly impaired. He developed bulbar symptoms and experienced increased muscle weakness. The characteristics of this patient differed from those of SD patients without MND with regard to the difficulty in writing kana characters and naming colors even though the SD-MND was in the early stage. Further, the pattern of brain hypoperfusion was different from that observed for SD patients without MND. In the case of this patient, brain hypoperfusion was found not only in the left anterior temporal lobe but also in the frontal lobe. The characteristics of his language symptoms might be related to the specific pattern of brain hypoperfusion, which might be commonly observed in patients with dementia and MND.

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Key words：semantic dementia (SD), motor neuron disease (MND), frontotemporal lobar degeneration (FTLD), three dimensional-stereotactic surface projections (3D-SSP)

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はじめに

前頭側頭葉変性症（frontotemporal lobar degeneration：FTLD）は前頭葉・側頭葉の萎縮を伴う初老期発症・緩徐進行性の臨床症候群としてNearyら[1]によって定義され，行動異常などを前景とする前頭側頭型認知症（frontotemporal dementia：FTD）と，失語症状を主とする意味性認知症（semantic dementia：SD）および進行性非流暢性失語に分けられる。

FTLDにはmotor neuron disease（MND）を伴う症例が存在することが知られている。MNDを伴う臨床型は，MND with dementia（D-MND）として報告された症例を含めてもFTDがほとんど[2,3]で，MNDを伴うSD（SD-MND）の報告は日本から2例[4,5]，海外から1例[6]にとどまる。しかし，病理学的検討からはSD-MND例が一定数存在することが明らかにされている[7]。

FTD-MNDはMNDを伴わないFTDと比べ生存期間が短く，障害が前頭葉に限局するなど症状や画像所見が異なると報告されている[8]。SD-MNDも仮名の錯書を初期から有するなどMNDを伴わないSDと異なる徴候を持つ可能性が示唆されているが詳細は不明である。

今回われわれはSD-MNDの1例を経験し，言語を含む神経心理学的評価・神経画像学的評価を2年にわたり施行したので報告する。

Ⅰ．症　　例

〈患　者〉 60歳，右利き，男性

主　訴　人の名前や漢字が思い出しにくい

既往歴・家族歴　精神神経疾患なし

現病歴　58歳頃から物忘れを自覚。特に人名が想起しにくくなった。その後悪化を自覚し，200X年2月，59歳時に当院当科を初診。漢字の字形想起困難の訴えもあった。半年後には日常物品の名前が想起困難となった。同時期から箸が持ちにくいなど右手母指と前腕の筋力低下と筋萎縮を自覚し翌200X＋1年5月に精査入院となった（初回評価）。日常生活動作は自立し，明らかな性格変化もなく診察に協力的だった。

神経学的所見　脳神経系に明らかな異常を認めなかった。舌の萎縮や線維束攣縮は認めず嚥下障害もなかった。運動系では右優位四肢の筋力低下，右上肢優位の四肢の筋萎縮を認めた。両側上腕・右肋間に誘発による筋攣縮，両側大腿に自発性筋攣縮を認めた。四肢深部腱反射に減弱亢進はなかったが下顎反射の亢進を認めた。

一般検査所見　動脈血液ガス検査，髄液検査を含め異常なし。

針筋電図検査　上肢，下肢，頸部，舌の4カ所で神経原性変化を認めた。

臨床経過　初回評価から1年後の200X＋2年5月に再度評価を行った（1年後評価）が，Mini-Mental State Examination（MMSE）は27点から24点，Alzheimer's Disease Assessment Scale-cognitive component-Japanese Version（ADAS J-Cog.）は17.33点から23.33点に悪化した（Table）。初回評価時の失点は語彙の低下によると考えられる記憶課題での失点に限られたが，1年後には長文の把持や教示の理解が困難になったことによる失点も加わった。

1年後評価時には嚥下障害が出現，上肢の筋萎縮と筋力低下が進行，日常生活に支障が生じ始めた。毎日定時に，特定のテレビ番組をみる，特定の場所へ外出するなど，時刻表的生活も認めた。軽度の脱抑制も認めたが，診察場面の礼容は保たれ検査にまじめに取り組んだ。エピソード記憶，視空間認知機能は保たれていた。

言語症状・意味記憶障害（Fig. 1）　初回評価時，自発語は流暢でアナルトリーは認めず。喚語困難は明らかで日常物品の呼称も顕著に障害され，指示語が目立った。「豆腐」を「納豆」と言うような意味性錯語を認めた。復唱は20音節まで可能。文レベルの了解は良好だったが一部の単語の理解は不良だった。書字では「新聞」を「新分」と書く類音的錯書と，字形想起困難を認めた。仮名より漢字で障害が目立ったが，「いぬ」を「いめ」と書く字性錯書を認めた。仮名の読字は保たれていたが漢字は「海老」を「かいろう」と読むなど熟字訓で類音的錯読を認めた。

ことわざの補完課題[9]は20個のうち4個のみで可能で，他は既知感もなかった。補完できたことわざについて意味を問うと，3つは無回答であった。残りの1つは「ちりも積もれば山となる」で，「いろいろやるとこういう風になる」と手で山の形を作るという，字義的な回答であった。

失語症語彙検査の意味カテゴリー別名詞検査では，線画を提示し名前を言わせる呼称課題と，名前を聴覚的に提示し対応する線画を10個から選択させる指示課題を行った（Table）。呼称と指示の2方向性の障害を認める語が200語中13語あり，これらの語には既知感もなかった。呼称できなかった78語中39語で語頭音効果を認めず，語頭音自体を単語ととらえてしまうこともあった。線画に既知感がなかったのは「タツノオトシゴ」だけであった。

1週間後に再び同検査を行うと，2方向性の障害を200語中12語で認めた。このうち1回目も2方向性の障害を認めた単語は3語のみで，障害は一貫せず浮動的であった。道具のカテゴリーに属する線画をみて使用法を説明できるか否かについては，20個の課題すべてで言語かジェスチャーで説明可能であった。

1年後評価時には単語理解がより悪化した。食べ物をみるとなんでも「納豆」と言うなど意味性錯語が頻繁に出現した。失語症語彙検査では2方向性の障害が65/200語，既知感のない線画が10個になり，障害は一貫するようになった。道具の使用法の説明は，20個中，アイロン，ちりとりなど8個でできなくなった。

カテゴリー別の成績では，初回評価時はどのカテゴリーでも呼称障害を認めたが，1年後には身体部位がほかよりも保たれていた。書字障害も進行し仮名でも目立った。

画像検査　初回評価時には頭部magnetic resonance images (MRI) (**Fig. 2**) では両側前頭葉と左側頭葉前方部の軽度の萎縮を認め，1年後には若干の進行を認めた。N-isopropyl-p-[^{123}I] iodoamphetamine (IMP)-single photon emission computed tomography (SPECT) を，自施設健常者データを用いたthree dimensional-stereotactic surface projections (3D-SSP)[10] で解析すると，初回評価時には萎縮部位を中心とした血流低下があり，1年後に進行を認めた (**Fig. 3**)。

stereotactic extraction estimation (SEE) 法[11] を用いて左上・中・下側頭回それぞれの脳回内で有意な血流低下を認めた領域の割合(extent)を測定したところ，初回評価時では，30.2%，36.0%，57.0%であった。そしてそれぞれの脳回内の血流低下部位におけるZスコアのピクセルごとの平均値 (severity) は，それぞれ2.41±0.44，2.30±0.39，2.54±0.45であった。

1年後評価時は，extentが各々37.7%，51.9%，65.1%，severityが各々2.79±0.54，2.42±0.49，3.10±0.88と進行した。また，左上・中・下前頭回の血流低下も，初回評価時で各々11.8%，8.7%，59.7%，severityは各々1.88±0.24，1.98±0.30，2.19±0.40，であり，1年後評価時でextentが68.0%，36.0%，88.5%，severityが2.24±0.46，2.14±0.39，2.71±0.49と進行した。

II. 考　　察

本症例は，筋萎縮性側索硬化症 (amyotrophic lateral sclerosis：ALS) の診断基準[12] から初回評価時に上肢優位四肢型のclinically probable-laboratory-supported ALSと診断した。1年後には球症状の出現や四肢の筋萎縮の進行を認めた。認知機能障害は，初回評価時は2方向性の障害を認める語彙は少なく浮動的であったが1年後に明らかな意味記憶障害を呈しNearyら[1] の診断基準からSDと診断した。過去のSD-MNDの症例[4-6] と認知機能障害が先行する点で共通していたが，MNDが四肢型である点で異なっていた。

Table Neuropsychological test scores obtained during the first assessment and those obtained after one-year follow-up

	first assessment	one-year follow-up
MMSE (/30)	27	24
ADAS-J cog. (/70)	17.3	23.3
TLPA		
naming total (/200)	78	37
color (/20)	12	5
body parts (/20)	10	10
interiors (/20)	9	5
buildings (/20)	12	5
vehicles (/20)	8	3
tools (/20)	5	1
processed food (/20)	8	4
fruits and vegetables (/20)	7	2
plants (/20)	2	0
animals (/20)	5	2
two-way anomia (/200)	13	65
line-drawings without familiarity (/200)	1	10

〔Abbreviation〕 MMSE: Mini-Mental State Examination, ADAS-J cog.: Alzheimer's Disease Assessment Scale-cognitive component-Japanese Version, TLPA: Test of Lexical Processing in Aphasia

本症例では早期から仮名書字障害と色の呼称障害を認めた。通常，SDでは表意文字でもある漢字書字に障害が認められるが表音文字である仮名書字は保たれるとされる[13]。しかし過去に報告されたSD-MNDの日本人症例2例のうち1例[4] で本症例と同様に「ねこ」を「ぬこ」と書くような置換による錯書を認めている。FTD-MND患者のうち書字評価が可能であった6例中4例で，置換，脱字，文法障害による錯書を認めたという報告[14] や，ALS 15例のうち認知機能障害を認めなかった5例を含む13例で仮名書字障害を認めたという報告[15] がなされている。さらに，認知機能障害を伴わないALS患者16例で検討された報告においても，3例で仮名の脱字が認められたことが報告されている[16]。このことから，仮名書字障害は認知機能障害の有無やその臨床病型によらず，

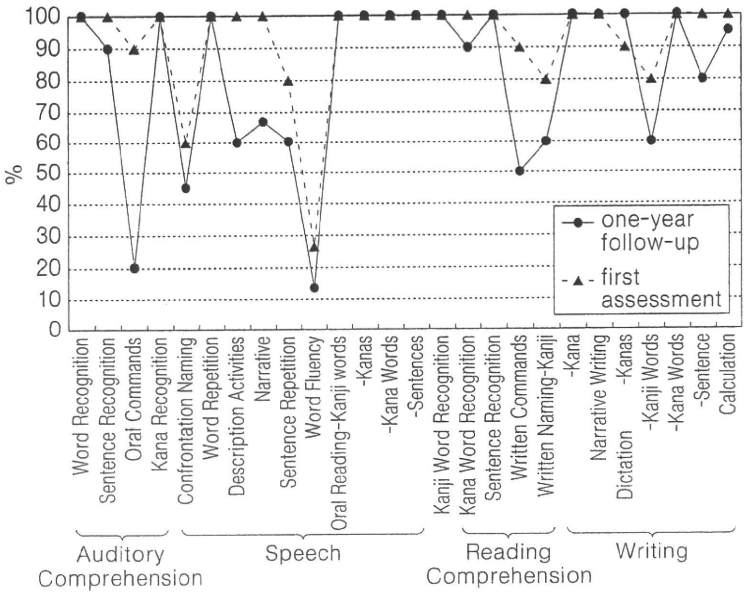
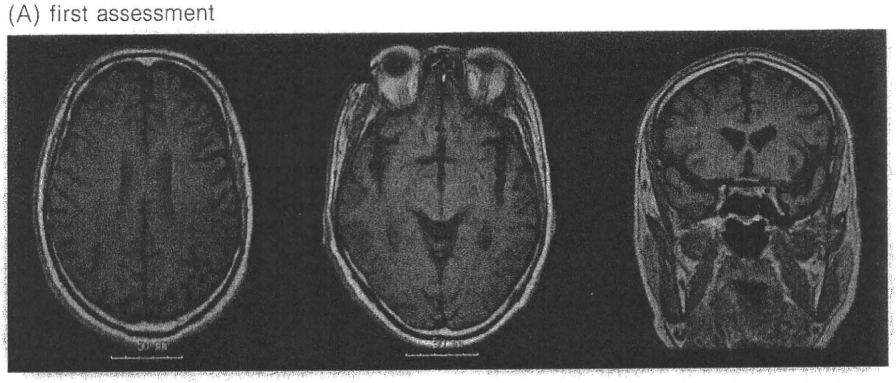


Fig. 1 The results of the Standard Language Test of Aphasia (SLTA)

In the first assessment, low scores of confrontation naming, word fluency, and kanji writing were obtained. Repetition was not considered, when sentences were short.

At the one-year follow-up, his vocabulary had further reduced and severe semantic memory impairment was noted. Lower scores of confrontation naming, word fluency, and kanji writing were obtained during one-year follow-up examination than during the first assessment. A considerably low score of oral command was obtained. However his syntactic comprehension appeared to be preserved, because he could understand our commands when we used words that he could recognize. He had difficulty in writing both kana and kanji characters.

(A) first assessment



(B) one-year follow-up

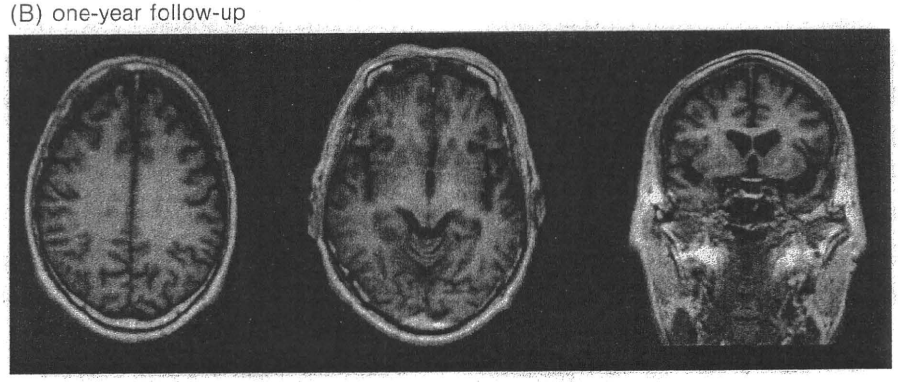


Fig. 2 Brain magnetic resonance (MR) imaging T_1 weighted images

A: T_1-weighted MR images in the axial and coronal planes obtained during the first assessment. Atrophy in both the frontal lobes and in the anterior aspects of both the temporal lobes (more conspicuous on the left side) can be seen. B: T_1-weighted MR images in the axial and coronal planes obtained during the one-year follow-up examination. Progression of atrophy in both the frontal lobes and in the left anterior temporal lobe can be seen.

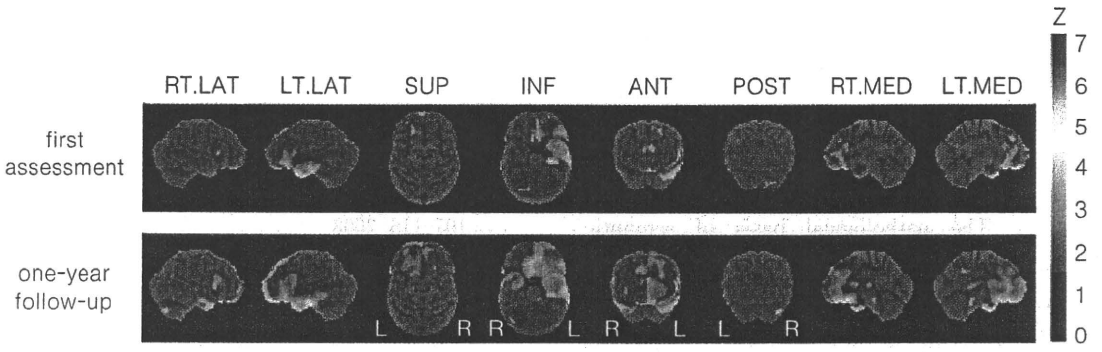


Fig. 3 Regions of reduced regional cerebral blood flow (rCBF) detected using three dimensional-stereotactic surface projections (3D-SSP) analysis of *N*-isopropyl-p-[123I] iodoamphetamine-single photon emission computed tomography IMP-SPECT images

Decrease in the rCBF was adjusted according to the cerebellar blood flow and compared with that in age-matched normal individuals recruited in Osaka University Hospital. Z-score was calculated as (mean of normal subject group-value for an individual patient) / (standard deviation of normal subject group). In this study, a Z-score of >1.64 was considered to be significant. Images were constructed in 8 views. In each line, from left to right, right lateral (RT. LAT), left lateral (LT. LAT), superior (SUP), inferior (INF), anterior (ANT), posterior (POST), right medial (RT. MED), and left medial (LT. MED). The Z-score images constructed during the first assessment show a significant reduction in the rCBF in both the frontal lobes, especially in the left frontal lobe and in the left anterior temporal lobe. The one-year follow-up examination revealed a greater degree of rCBF reduction and an increase in the number of areas with decreased rCBF.

ALS/MNDで高率に認める特徴である可能性がある。

D-MND患者におけるカテゴリー別呼称障害は過去に報告がなく今回初めての知見である。MNDを伴わないSDでは，色と身体部位の呼称が保存されやすいことが知られている[17]。本症例では身体部位は比較的保たれていたが，色名呼称はほかと同程度に障害されており，SD-MNDの特徴の1つである可能性が考えられた。

仮名書字障害は左中前頭回[18]，色の呼称は左中・下前頭回[19]の障害が関連すると過去に報告されている。通常のSDでは初期の障害範囲は一側の側頭葉前方部に限られ前頭葉には及ばない[20]。一方FTD-MNDでは前頭葉に広範囲な機能障害を認めると報告されている[8]。前述の仮名書字障害を認めたALS患者においても，前頭葉の血流低下が確認されている[12-14]。本症例では意味記憶障害がごく軽度であった初回評価時から前頭葉にも血流低下があり，1年後には顕著となった。MNDを伴うために，早期から左側頭葉前方部に加えて同側前頭葉が侵され，仮名書字障害や色の呼称障害を呈した可能性があると考えられた。

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