INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a heterogeneous motor neuron disease that results from selective death of motor neurons in the brain and spinal cord (1). The predominant clinical feature of ALS is progressive wasting and weakness of limb, bulbar and respiratory muscles. The mean survival of patients after onset of symptoms is 3–5 years. Its worldwide incidence and prevalence are 0.3–2.4 and 0.7–7.0 per 100 000 each year (2). The heritability of ALS is high, with twin studies estimating it at 0.61 and the unshared environment component at 0.39 (3).

Approximately 10% of ALS cases are familial (fALS), and the remaining 90% are sporadic (sALS). Genetic factors have been reported in ALS. Detailed information regarding ALS-related genes is available via amyotrophic lateral sclerosis online genetics database and the ALS mutation database (4,5). Most fALS is monogenic in origin. At least 15 fALS loci, under various modes of inheritance, have been identified by linkage studies, and pathogenic mutations have been described in 11 genes, *SOD1*, *NEFH*, *ALS2*, *DCTN1*, *VAPB*, *SETX*, *ANG*, *TARDBP*, *FUS*, *OPTN* and *DAO*, in fALS (6–19). Despite the abundance of genes and loci identified in fALS, mutations in these genes explain only a small minority of sALS (20).

Regarding susceptibility genes for sALS, >30 association studies based on the candidate-gene approach have been reported (21,22). Among them, *NEFH*, *APEX* and *ANG* have the most evidence; associations of these genes have been found in Caucasians (23–25) and replicated in several studies (7,22,26). However, many of the reported genes are still controversial. For example, the association of non-synonymous substitution (P413L) in the chromogranin B gene (*CHGB*) is reported in French, French-Canadian and Scandinavian ALS populations (27), but has not been found in a Dutch and another French population (28,29).

The genome-wide association study (GWAS) has identified five ALS-susceptibility genes (*FGGY*, *ITPR2*, *DPP6*, *KIFAP3* and *UNC13A*) and two loci (9p21.2 and 10q26.3) in Caucasian (30–35). These results are promising, but remain slightly controversial (36–39). The association of the 9p21.2 locus has been independently replicated in three studies (34,40,41), but is not found in all populations, including those from Japan and China (42). More studies are necessary to evaluate and confirm these previously reported ALS-susceptibility genes.

To identify novel susceptibility genes for ALS, we conducted a large-scale genetic association study in Japanese ALS patients using gene-based single-nucleotide polymorphisms (SNPs) (43). We identified a functional SNP that was significantly associated with ALS. The SNP was located in an enhancer region of *ZNF512B*, a previously uncharacterized transcription factor, and the susceptibility allele of the SNP had decreased enhancer activity for the *ZNF512B* promoter and decreased binding capacity to nuclear proteins. We found that in neuron cells, ZNF512B acts as a positive regulator of transforming growth factor-β (TGF-β) signaling, which is known to be neuroprotective and critical for maintenance and/or survival of neurons (44–46). We demonstrated the localization of ZNF512B in the spinal cord of ALS patients and it showed enhanced expression in motor neuron cells of the anterior horn when compared with controls.

RESULTS

Genome screening

We carried out a stepwise case–control association study (Supplementary Material, Fig. S1) as previously described (47–51). In stage 1 of the discovery series, 92 ALS and 233 control subjects were analyzed at 52 608 gene-based SNP loci selected from the JSNP database (43). Genotype information was successfully obtained for 48 939 SNPs on autosomal chromosomes passed after the quality control. Either the Chi-square test or Fisher's exact test was performed for three genetic models: dominant, recessive and allelic. Comparison of observed and expected distributions showed no evidence for inflation of the trend test statistics (inflation factor, lambda = 1.04; Supplementary Material, Fig. S2). Also, principal component analysis (52) in stage 1 and HapMap samples showed no evidence of population stratification between the case and control groups (Supplementary Material, Fig. S3). In stage 2 of the discovery series, 893 SNPs that showed *P*-values of ≤ 0.01 in stage 1 were genotyped for an additional 1087 subjects (362 ALS cases and 725 controls). Subsequently, 10 SNPs with *P*-values <0.001 were identified by the Chi-square test for the three models (Supplementary Material, Table S1).

Identification of genetic association between rs2275294 and ALS

We validated the association of these SNPs using independent subjects from Biobank Japan (sample set 1). In all, 249 ALS cases and 1030 controls were genotyped and validated the association in rs2275294 (allele model, $P = 1.8 \times 10^{-3}$). The SNP was then genotyped in an independent Japanese population consisting of 602 ALS cases and 2256 controls (sample set 2). Significant association was replicated in this population (allele model $P = 5.6 \times 10^{-5}$). The combined *P*-values for the stepwise association study calculated by the Mantel–Haenszel method and the joint analysis were 9.3×10^{-10} and 6.7×10^{-10}, respectively (Table 1). The combined *P*-values remained significant after Bonferroni correction ($9.3 \times 10^{-10} \times 52\,608 \times 3 = 1.47 \times 10^{-4}$). The *P*-values from the Mantel–Haenszel method and the joint analysis were very similar, supporting the fact that there is no hidden confounder in our population. The minor allele frequency (MAF) of rs2275294 in 744 samples of the Japan Biological Informatics Consortium (JBIC)-genotyping data deposited in the dbSNP database was similar to that of our controls (0.414).

Evaluation of rs2275294

We assessed the stratification using principal component analysis (52). The top six principal components were associated with case–control status. The association of rs2275294 with the top six principal components included as covariates (trend model $P = 0.00287$) was similar to that in stage 1 (trend model $P = 0.00246$), suggesting no stratification. Population stratification was also assessed by evaluating differences in population structure among all case and control sample sets using Wright's F statistics (53). There was no difference in the population structure among these groups (Supplementary Material, Table S2). Potential confounding factors were also

Table 1. Association of rs2275294 in *ZNF512B* with ALS

	No. of subjects		Risk allele frequency			
	Case	Control	Case	Control	*P*-value	Odds ratio (95% CI)
Discovery series	454	958	0.491	0.422	6.3×10^{-4}	1.32 (1.13–1.55)
Sample set 1	249	1030	0.512	0.434	1.8×10^{-3}	1.37 (1.12–1.66)
Sample set 2	602	2256	0.481	0.416	5.6×10^{-5}	1.30 (1.14–1.48)
Combined	1305	4244				
Meta-analysis[a]					9.3×10^{-10}	1.32 (1.21–1.44)
Joint analysis					6.7×10^{-10}	1.32 (1.21–1.44)

[a]By the Mantel–Haenszel method.

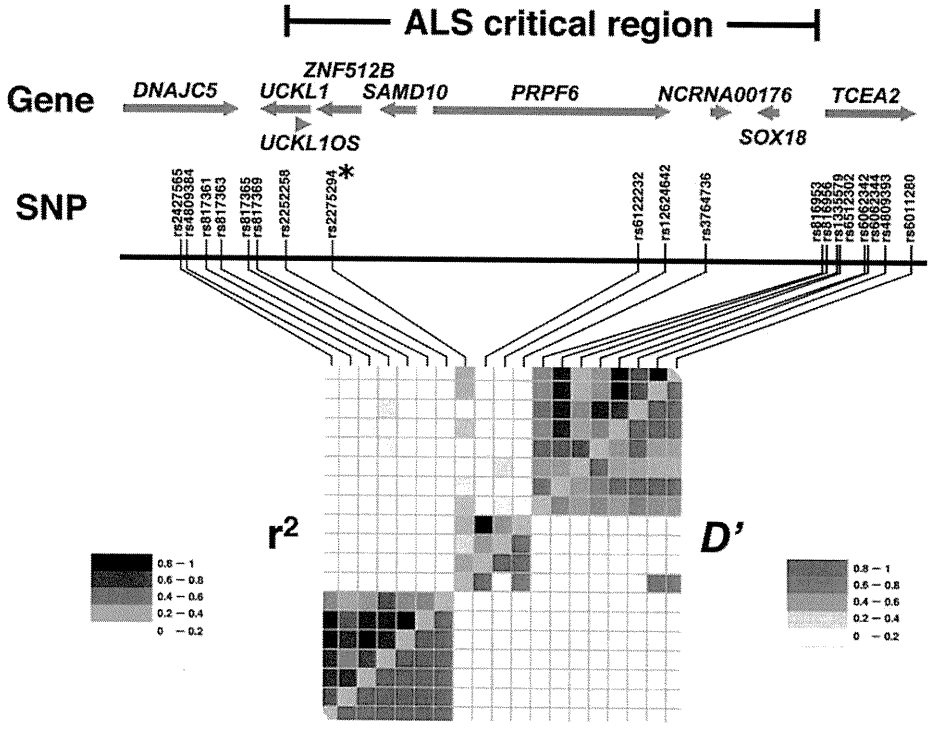

Figure 1. Genomic structure and linkage disequilibrium (LD) map in the ALS critical region. Top, an SNP map of a 111 kb genomic region containing *ZNF512B*. The orientation of each gene is indicated by a green arrow. An asterisk shows the landmark SNP. Bottom, an LD map as measured by D' (lower right triangle) and r^2 (upper left triangle).

examined and no significant differences in age and gender distribution were found among rs2275294 genotyped. The associations with rs2275294 were significant in two sample sets ($P = 4.1 \times 10^{-3}$ and 1.4×10^{-4}), even after adjusting for age and gender in a logistic regression analysis.

Genome analysis of the ALS critical region containing rs2275294

We constructed a linkage disequilibrium (LD) map around rs2275294 on the basis of the genotyping data for Japanese subjects used in HapMap (HapMap JPT). Because rs2275294 was unmapped in the HapMap data, we genotyped the SNP for the HapMap JPT samples and integrated the data with the HapMap JPT data. We found that rs2275294 was in strong LD with the two SNPs rs6122232 and rs3764736 ($D' > 0.85$). Subsequently, the critical region could be confined to a 111 kb interval flanked by rs2252258 and rs816953 on chromosome 20q13.33 (Fig. 1). This region included four genes (*ZNF512B, SAMD10, PRPF6* and *SOX18*) and a part of *UCKL1*, as well as two non-protein-coding RNAs (*UCKL1OS* and *NCRNA00176*). In order to identify a more significantly associated SNP, we searched for SNPs in each gene by re-sequencing genomic DNA of 48 ALS subjects. A total of 24 SNPs were identified and their level of association was examined using 455 cases and 452 controls, but rs2275294 remained the most significantly associated (Supplementary Material, Table S3).

Functional analysis of rs2275294

To gain insight into the biological significance of rs2275294, luciferase reporter plasmids corresponding to a genomic

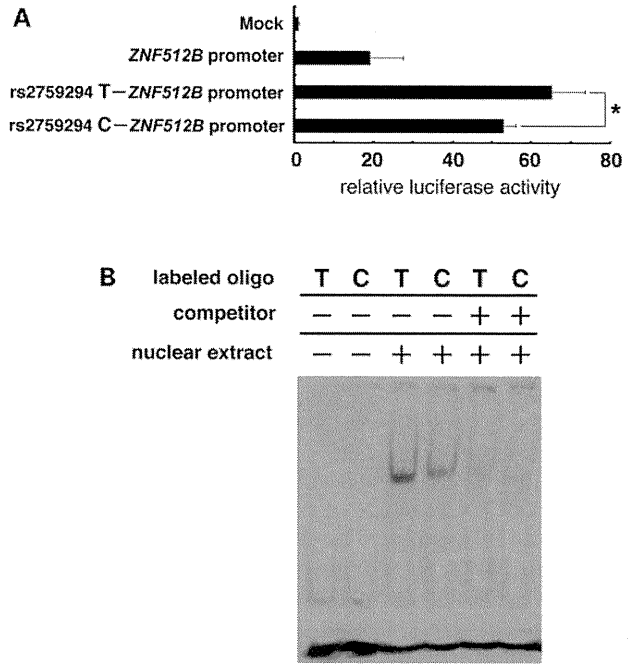

Figure 2. Functional analysis for rs2275294 in *ZNF512B*. (**A**) Difference in the enhancer activity of genomic DNA segments containing rs2275294. Luciferase assay in SK_N_Be(2)C cells. Enhancer activity was lower in the ALS-susceptibility allele (C allele). *ZNF512B* promoter:native promoter (nts −820 to −74) of *ZNF512B*. Data represent the mean ± SEM ($n = 6$). $^{*}P < 0.01$ (Student's t-test). (**B**) Difference in binding of nuclear proteins to a cis-element containing rs2275294. An EMSA using nuclear extracts from SK_N_AS cells. The specific band was weaker in the ALS-susceptibility allele (C allele).

DNA fragment containing rs2275294 were constructed and a luciferase assay using the human neuroblastoma cell line SK_N_Be(2)C was performed. Constructs containing the ALS-susceptibility allele (C allele) of rs2275294 showed lower enhancer activity than those containing the non-susceptibility allele, indicating that the SNP affects the *ZNF512B* transcription level (Fig. 2A). We then examined the allelic difference in the binding of genomic DNA containing rs2275294 to nuclear proteins by the electrophoretic mobility shift assay (EMSA). The DNA–protein complex from the C allele showed weaker binding (Fig. 2B). Thus, it is feasible that the presence of the susceptibility allele leads to lower *ZNF512B* levels as a consequence of decreased enhancer activity.

ZNF512B is a positive regulator in the TGF-β signaling pathway

Proteomics analysis has suggested that ZNF512B functions as a regulator of the TGF-β signaling pathway (54). We examined the effect of ZNF512B on TGF-β signaling using the TGF-β-dependent SMAD2/3-specific luciferase assay (55) in a HepG2 cell (data not shown). SMAD2/3-mediated reporter activity after TGF-β stimulation was enhanced by ZNF512B over-expression. The TGF-β-dependent reporter activity was activated by ZNF512B over-expression in a neuroblastoma

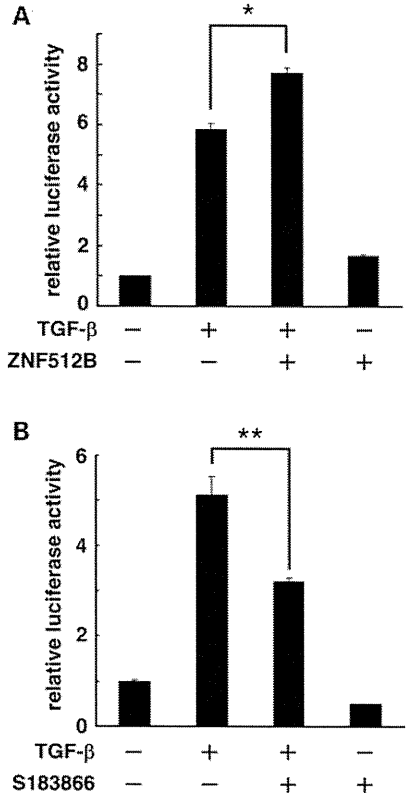

Figure 3. ZNF512B is a positive regulator of the TGF-β signal. (**A**) Luciferase assay using SBE4-luciferase. ZNF512B trans-activated the TGF-β-induced SMAD transcriptional activity in the SK_N_AS cell line ($^{*}P < 0.0005$). (**B**) S183866, a *ZNF512B*-targeting siRNA oligonucleotide repressed the TGF-β-dependent SBE4-luciferase activity ($^{**}P < 0.005$).

cell line SK_N_AS (Fig. 3A) and a glioblastoma cell line U87MG (Supplementary Material, Fig. S4). Next, we knocked down expression of the endogenous *ZNF512B* in SK_N_AS by using the short-interfering RNA (siRNA) technique. Real time polymerase chain reaction (PCR) showed that *ZNF512B* siRNA significantly reduced *ZNF512B* transcription, and TGF-β-dependent reporter activity was repressed by the siRNA (Fig. 3B).

ZNF512B expression in the spinal cord of ALS

The localization of ZNF512B in the spinal cord of ALS patients was investigated by immuno-histochemical studies. The immuno-reactivity for an anti-ZNF512B polyclonal antibody was intense in motor neuron cells in the anterior horn of the spinal cords of ALS patients, while it was barely detectable in those of controls (Fig. 4A–D). Glial cells in the anterior horn did not show ZNF512B immuno-reactivity.

DISCUSSION

By a large-scale case–control association study using gene-based SNPs and enrolling a total of more than 5500 subjects, we identified *ZNF512B* at chromosome 20q13.33 as a new susceptibility gene for ALS. rs2275294 in *ZNF512B* had

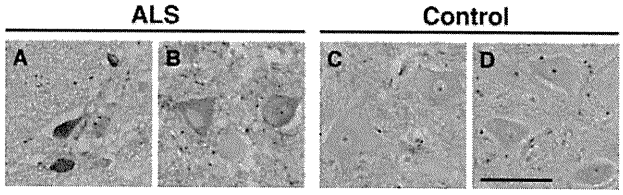

Figure 4. Immuno-histochemical localization of ZNF512B in the spinal cord of ALS patients (**A** and **B**) and controls (**C** and **D**). The ZNF512B immuno-reactivity was intense in motor neuron cells in the anterior horn of ALS patients, while it was hardly detectable in those of controls. Glial cells in the anterior horn did not show ZNF512B immuno-reactivity. Scale bar, 100 μm.

significant association that satisfied a genome-wide significance level ($P = 9.3 \times 10^{-10}$). rs2275294 affected *ZNF512B* transcription *in vitro*, and the ALS-susceptibility allele (C allele) showed lower enhancer activity for the *ZNF512B* promoter. Therefore, *ZNF512B* is presumably lower in those who have the susceptibility allele than in those who have the non-susceptibility allele. *ZNF512B* over-expression enhanced TGF-β signaling, while its knockdown decreased the signal. Our findings suggest that ZNF512B is an important positive regulator of TGF-β and that lowered ZNF512B expression is implicated in the pathogenesis of ALS susceptibility via decreased TGF-β signal.

In this study, we screened the genic regions using >52 000 gene-based SNPs from the JSNP database. The number of SNPs and their coverage are not sufficient to screen the entire genome. Our study must have many false negatives. Current commercial GWAS platforms are considered superior to ours in terms of the study power and the coverage of SNPs in the human genome. In contrast, the false-positive association of rs2275294 is unlikely. The inflation factor was low and principal component analysis showed no evidence of population stratification. We validated the association in independent Japanese panels. The statistical significance of the association for the combined *P*-values by two different methods fulfilled criteria of the genome-wide significance level. The results of the two analyses were very similar, which further shows that a hidden confounder in our population is unlikely. In addition, there was no difference in the population structures among the case–control sets by Wright's F statistics (53) throughout the study. The MAF of rs2275294 in 744 Japanese samples deposited in dbSNP is similar to that of our controls (0.414).

In spite of its very significant association in our study, rs2275294 in *ZNF512B* has not been found in the previous GWASs. Several explanations can be considered. The main reason is that rs2275294 was not included in the platforms of the previous GWASs. Only 15 SNPs in Illumina 610K SNP Array were mapped to the 111 kb genomic region (1 SNP/7.4 kb) corresponding to the ALS critical region we determined. Also, only 10 SNPs in Affymetrix SNP Array 6.0 were mapped to the genomic region (1 SNP/11.1 kb). In addition, rs2275294 is not even mapped in the HapMap JPT database, nor included in the CEU and YRI HapMap data sets. In the Illumina and Affymetrix SNP arrays, the numbers of SNPs in the *ZNF512B* locus are only two and one, respectively. Their coverages of *ZNF512B*-SNPs in the ALS critical region were very low, 2/15 (13%) and 1/10 (10%), respectively. The low coverage of the region might have led to the false-negative association in the previous GWASs. No SNP was in strong LD ($r^2 > 0.8$) with rs2275294 in CHB-JPT, CEU and YRI in the 1000 Genomes data (Supplementary Material, Table S4). Hence, we speculate that rs2275294 has been identified by virtue of our platform. Still another explanation is the ethnic difference of ALS susceptibility.

A number of GWASs in ALS have been performed recently. They report the identification of five candidate genes and two candidate loci (30–35). Among them, only five gene loci (*DPP6*, *ITPR2*, *FLJ10986*, *KIFAP3* and *UNC13A*) were included in our platform. We checked 16 SNPs in *DPP6*, 23 in *ITPR2*, 2 in *FLJ10986*, 9 in *KIFAP3* and 4 in *UNC13A*; however, their associations were not replicated in our study (Supplementary Material, Table S5). The small number of samples and the low coverage of SNPs in our platform may have resulted in false-negative association. Ethnic differences may be another reason for no replication. The 9p21.2 SNP that has been reported in the previous study (42) was not included in the present study. The tested SNPs for previous associations were negative, but no evidence can be provided for the chromosome 9p21.2 locus. Because the powers of Japanese and Chinese were only 0.37 and 0.11, respectively (42), the negative association may be due to a lack of power in the study. More extensive association studies using larger panels of Japanese samples will be required to conclude the associations between previous candidate genes and ALS.

ZNF512B was originally identified as a *KIAA1196* in the course of the Kazusa Human cDNA project (56). The *ZNF512B* cDNA is 5919 bp long and encodes an 893 amino-acid protein that is ubiquitously expressed in various tissues, including the brain and spinal cord (56). Our immunohistochemical studies confirmed its localization in the spinal cord. The ZNF512B protein showed no significant homology with any proteins in the public database. It contains six C2H2-type zinc finger domains and is predicted to act as a transcription factor. The ALS-susceptibility SNP rs2275294 was localized to intron 12 of *ZNF512B*. We have demonstrated that the genomic region containing rs2275294 can act as an enhancer of the *ZNF512B* promoter and that the susceptibility allele of rs2275294 had reduced transcriptional activity, which was likely due to its decreased binding capacity to trans-factors. Further studies for the upstream factors of *ZNF512B* are necessary to clarify the molecular pathogenesis of ALS related to *ZNF512B*.

We showed that ZNF512B is a positive regulator of the canonical TGF-β signaling pathway through SMAD2/3. TGF-β signal is essential for the survival of neurons (44–46). Upregulation of PAI-1 by SMAD3-dependent induction in astrocytes mediates the neuroprotective activity of TGF-β against NMDA receptor-mediated excitotoxicity (57). TGF-β signal has been implicated in the pathogenesis of ALS. Plasma TGF-β1 level is significantly increased in ALS patients compared with healthy controls, and there is a significant positive correlation between TGF-β1 concentration in ALS patients and duration of their disease (58). A microarray analysis showed a 4.8-fold increased expression of *SMAD4* in sALS compared with neurologically normal controls (59).

Also, phosphorylated SMAD2/3 immuno-reactivity is increased in the remaining spinal motor neurons and glial cells in sporadic and familial ALSs, as well as in *Sod1* transgenic mice (60). These findings suggest that the TGF-β signal is increased in ALS.

Several studies have shown an association between duration of ALS and TGF-β levels. Houi *et al.* (58) found a positive correlation between the plasma concentration of TGF-β1 in ALS patients and the duration of disease. Another group reported that TGF-β1 concentrations in serum and cerebrospinal fluid did not differ between ALS patients and controls, but were higher in ALS patients with a terminal clinical status than in controls (61). These data suggest that TGF-β is increased in the motor neuron cells of ALS patients during the disease process. As ZNF512B is a critical enhancer of TGF-β signaling, its genetic association may be related to the progression of the disease rather than its onset.

We have demonstrated the localization of ZNF512B in the spinal cord of ALS patients, and that ZNF512B expression in the motor neurons of ALS patients was significantly increased compared with that of controls (Fig. 4). It is biologically plausible that ZNF512B is a positive regulator (co-activator) of neuroprotective TGF-β signaling (Fig. 3) and may act as a protector against ALS. Taken together with the results of luciferase assay and EMSA that showed allelic differences in *ZNF512B* expression level (Fig. 2), a patient harboring the susceptibility allele would have decreased *ZNF512B* expression level compared with a patient harboring non-susceptibility alleles. The decreased *ZNF512B* enhancer activity by the susceptibility allele leads to insufficient increase in ZNF512B, which leads to insufficient increase in the TGF-β signal that results in decreased potential for survival and/or recovery of motor neurons. The discovery of this ALS-susceptibility gene and its pathway should shed light on ALS pathogenesis and facilitate development of targeted therapies.

MATERIALS AND METHODS

Subjects

A total of 1305 ALS patients diagnosed as having probable, probable and laboratory-supported, or definite ALS according to the El Escorial revised criteria (62) were included in the study. All subjects were unrelated Japanese individuals. We obtained a total of 703 DNA samples from the Biobank Japan project (63). All patients were screened for mutations in *SOD1*, *TARDBP* and *ANG* and none was detected. The mean age of cases was 60.8 years (range: 28–82 years), and 66.1% were male. 74.4% had a spinal onset, 19.6% a bulbar one and 6% a multiple and the others. We obtained a total of 602 DNA samples from the Japanese Consortium for Amyotrophic Lateral Sclerosis Research (JaCALS), Jichi Medical University and The University of Tokyo. The mean age was 61.5 years (range: 27–89 years), and 62.0% were male. 70.4% of the patients had a spinal onset and the remaining had a bulbar one. We recruited 4244 controls through several medical institutes in Japan. Their mean age was 66.8 years (range: 18–98 years), and 48.0% were male. All controls had negative medical and family histories for neurodegenerative disorders. Written informed consent was obtained from all the subjects. The ethical committees at the participating institutions approved this project.

SNP genotyping

Using standard protocols, genomic DNA was extracted from the peripheral blood leukocytes. SNPs were genotyped using the multiplex PCR-based invader assay (Third Wave Technologies) as described previously (64). A total of 52 608 gene-based SNPs were selected from the JSNP database on the basis of the haplotype block structure reported previously (43,65). We calculated the total number of independent SNPs in this study to be 43 052 (the SNPs in LD: $r^2 > 0.80$ were considered as one SNP). We checked the cryptic relatedness for each pair of samples by identity-by-state by estimating the average number of shared alleles between two individuals (V_I) using 48 884 autosomal SNPs. Six individuals in controls were related ($V_I > 1.65$). They were excluded from the analysis. A stepwise screening method was adopted to increase the statistical power (66). In stage 1, 92 ALS and 233 control subjects were analyzed. We applied the SNP quality control filters of call rate of ≥ 0.95 in both cases and controls and *P*-value of Hardy–Weinberg equilibrium (HWE) test of $\geq 1.0 \times 10^{-2}$ in controls. A total of 48 939 SNPs on autosomal chromosomes passed the quality control filters and were analyzed for the association. The data of this study are available at the JSNP database (http://snp.ims.u-tokyo.ac.jp/). Among the SNPs analyzed in stage 1, 893 SNPs showing the smallest *P*-values (0.01 or smaller) were selected for stage 2. Three models (i.e. allelic, dominant and recessive) were tested for the association. Since these three models are not independent, 893 SNPs were isolated. In stage 2, we genotyped an additional 1087 subjects consisting of 362 ALS cases and 725 controls. Stage 1 and stage 2 were defined as the discovery series of this research and the following sample sets were defined as sample set 1 and sample set 2.

SNP discovery

Appropriate genome sequences were extracted from the UCSC Genome Bioinformatics website. The critical region contained five genes (*ZNF512B, SAMD10, PRPF6, SOX18* and part of *UCKL1*) and two non-protein-coding RNAs (*UCKL1OS* and *NCRNA00176*). We defined the exon–intron boundaries of each gene and designed PCR primer sets for the critical region except for repetitive sequence regions. Each PCR was performed with 5 ng of mixed genomic DNA derived from three ALS subjects; 16 mixed samples were amplified in the GeneAmp PCR system 9700 (PE Applied Biosystems) under the following conditions: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 96°C for 30 s, annealing at 60–65°C for 30 s, extension at 72°C for 2 min and post-extension at 72°C for 7 min. PCR products served as templates for direct sequencing by the fluorescent dye-terminator cycle sequencing method.

Statistical analysis

For general statistical analyses, we used R statistical environment version 2.6.1 and programs created by our group. The Chi-square test or Fisher's exact test was applied to a two-by-two contingency table in three genetic models: an allele frequency model, a dominant-effect model and a recessive-effect model. Principal component analysis was performed using the smartpca program (52). We calculated the association in case–control status of stage 1 by using a twstats of EIGENSOFT (52). The top six principal components were associated with case–control status. Genotype data from the HapMap project were used (67) to estimate the population structure. The significance of stratification was determined using the Wright method (53). The Mantel–Haenszel method was used for meta-analysis. An automated laboratory system and bar-coding were employed to reduce clerical errors. The accuracy of our system has been guaranteed in data of the HapMap project (67). We checked HWE and personally retyped some SNPs from genome screening in duplicated samples. We also obtained age- and gender-adjusted odds ratios by logistic regression analysis by program R. Haploview 4.1 was used to infer the LD structure of the ALS critical region. An LD pattern was created based on the JPT HapMap data. Luciferase assay data were analyzed by Student's *t*-test.

Luciferase assay

We cloned DNA fragments containing rs2275294, nucleotides (nts) 190–208 of intron 12 of *ZNF512B*. The fragments for both alleles as three tandem copies were inserted into pGL3-Basic vector (Promega) upstream of its luciferase gene in 5′–>3′ orientation together with the *ZNF512B* core promoter of nts −820 to −74 of its 5′ flanking region. We transfected SK_N_Be(2)C cells with 400 ng of each reporter construct using FuGene 6 transfection reagent (Roche) together with 8 ng of pRL-TK vector (Promega) as a control. After 24 h, the cells were lysed in a passive lysis buffer and luciferase activities were measured using Dual-Luciferase Reporter Assay System (Toyo Ink). The entire coding sequence of ZNF512B was cloned into pcDNA3.1, which had a Myc-tag sequence. We also co-transfected with SBE4 (four copies of Smad Binding Element) luciferase reporter vector (55)/Myc-tagged ZNF512B or SBE_4-luciferase reporter vector/Myc-tagged pcDNA3.1, and pRL-TK vector using Trans-IT LT reagent (TAKARA Bio). After 24 h, we treated the cells with 10 ng/ml of TGF-β for 24 h. The cells were lysed in a passive lysis buffer and luciferase activities were measured using Dual-Luciferase Reporter Assay System (Toyo Ink).

Electrophoretic mobility shift assay

A nuclear extract from SK_N_AS cells was prepared as previously described (68) and incubated with oligonucleotides (nts 184–203 of intron 12 of *ZNF512B*) that were labeled with digoxigenin-11-ddUTP using the Dig Gel Shift Kit (Roche). The reaction was carried out at a room temperature with an additional 1 mg/ml of poly[d(I-C)]. For the competition assay, the nuclear extract was pre-incubated with unlabeled oligonucleotides (200-fold molar excess) before adding digoxigenin-labeled oligonucleotide. The protein–DNA complex was separated on a non-denaturing 6% polyacrylamide gel in 0.25× Tris-borate-EDTA buffer. We transferred the gel to membrane and detected the signal with a chemiluminescent detection system (Roche) according to the manufacturer's instructions.

RNAi experiment

Double-strand stealth RNAi oligonucleotides (ZNF512B-S183866 for ZNF512B and negative universal control medium GC duplex for negative control) were purchased from Invitrogen. The RNAi oligonucleotides were transfected into a cell line using Lipofectamine RNAiMAX reagent according to the manufacturer's instructions (Invitrogen). After 24 h, we also transfected with SBE_4-luciferase reporter vector and pRL-TK vector. We treated the cells with TGF-β (10 ng/ml) for 24 h, collected the cells and measured luciferase activity using the Dual-Luciferase Reporter Assay System (Toyo Ink).

Immuno-histochemistry

Autopsy specimens of lumbar spinal cord were obtained from clinically and histopathologically diagnosed ALS patients (13 males and 9 females, age 41–79 years) and from neurologically normal patients (4 males and 3 females, age 42–76 years). The autopsy times in relation to death for the cases and controls (average ± SD) were 4.0 ± 2.8 h and 4.5 ± 5.2 h, respectively. 6-μm-thick sections were prepared from paraffin-embedded tissues. The sections were microwaved for 20 min in 50 mM citrate buffer (pH 6.0) and then treated with a TNB blocking buffer (PerkinElmer) before incubation with an anti-ZNF512B antibody (Santa Cruz Biotechnology, 1:200). The immuno-reactivity was detected using EnVision+ System-HRP (Dako). The sections were photographed with an optical microscope (BX51, Olympus).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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原　著
Original Article

若年性認知症2剖検例の臨床病理学的検討

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A Clinicopathological Study of Young-Onset Dementia: Report of 2 Autopsied Cases

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Abstract

We retrospectively examined the clinical features and the neuroradiological findings on autopsy of 2 cases of young-onset dementia.

The patient in case 1 was a 43-year-old woman who was unable to determine the time on the clock and who made frivolous remarks. Neuropsychological test batteries demonstrated memory impairment and frontal lobe dysfunction. T_2-weighted magnetic resonance imaging (MRI) of the head revealed abnormal high-intensity signals around the lateral ventricles and thinning of the corpus callosum. Single photon emission computed tomography (SPECT) revealed patchy reduction in the accumulation of tracers in both the frontal lobes. Her neurological condition gradually deteriorated, and she died 13 years after the onset of the disease. She was clinically diagnosed with atypical Alzheimer's disease on the basis of visual cognitive impairment and memory impairment observed in the initial phase. However, the neuropathological diagnosis was adult-onset leukodystrophy with axonal spheroids.

The patient in case 2 was a 43-year-old man who had gradually started behaving selfishly and had become ill-tempered and apathetic. He was admitted to a hospital. He was anosognosic and showed frontal lobe dysfunction. T_2-weighted MRI scan of the brain showed abnormal high-intensity signals around the lateral ventricles; atrophy of the frontal and temporal lobes, hippocampus, and brainstem; and thinning of the corpus callosum. SPECT revealed patchy reduction in the accumulation of tracers in both the frontal lobes and the cerebellum. His neurological condition gradually deteriorated, and he died after being clinically ill for 7 years. The patient was clinically diagnosed with frontotemporal dementia on the basis of the clinical features and MRI findings. However, the neuropathological diagnosis was chronic meningoencephalitis.

The frequency of neurological metabolic and inflammatory diseases is significantly high although it is not as high as that of degenerative diseases in young-onset dementia. Since such diseases may respond to therapy, they should be considered in the differential diagnosis of young-onset dementia, especially in patients presenting with atypical clinical features. Neuroradiological examination may contribute to the differential diagnosis of atypical dementia at young age.

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はじめに

65歳以下の早期発症例についても，認知症をきたす原因疾患としては，Alzheimer病や前頭側頭葉変性症などの変性性疾患，脳血管性認知症が多いことが各国から報告されている[1-5]。これらの疾患は典型的な症状，画像所見を示す場合には臨床診断も困難ではないが，非典型的な症状，経過を示す症例では診断に難渋することも少なくない。われわれは当初変性性疾患と診断した40歳代発症の認知症剖検例2例を対象に，臨床症状および経過，画像所見を後方視的に検討し，鑑別診断で留意すべき点について考察した。

Ⅰ．臨床経過

症例1　死亡時54歳，女性，右利き，高校卒。家族内に類症の発生はない。

高校卒業後も両親と同居し，37歳時までは学習塾の先生をしていた。41歳時より時計の時刻を読み間違えるようになった。近医で施行された頭部CT，MRIでは異常はみられなかった。その後，買い物の際に計算を間違える，頼まれたものとは別の，自分が欲しいものを買って帰る，という症状も出現した。また何を尋ねても「はいはい」と返答するようになった。発症半年後の頭部CTでは前頭骨の肥厚および脳室拡大，頭部MRIで脳室前角・後角周囲のT_2高信号と脳梁の菲薄化がみられた。脳血流SPECTでは異常はみられなかった。42歳時に当院を受診し，精査加療目的で入院した。

現症として，一般身体所見は異常なかった。神経学的には，病識がなく，また質問に対して深く考えずに簡単に「わかりません」と即答することが多く，軽薄な性格という印象を受けた。また，採血や腰椎穿刺など疼痛を伴う検査時にも，にこにこ笑いながら「痛くありません。大丈夫です」と反応した。話し方は早口であったが，小声ではなく，抑揚も正常であった。自伝的記憶は比較的保持されていたが，三宅式記銘検査で有関係対3-4-5，無関係対0-0-0，仮名拾いテストは0/61，Stroopテストは色丸呼称時間2分7秒，時間差2分32秒であり，Wisconsin Card Sorting Testでも達成カテゴリー数は0と，記銘力低下，前頭葉機能低下がみられた。また，ことわざの理解が不良であり，字義どおりに解釈した。改訂長谷川式認知症スケール(HDS-R)は22/30，WAIS-RはVIQ 68，PIQ 48，IQ 55であり，いずれも低下していた。そのほか運動系，感覚系，自律神経系に異常は認めなかった。

検査所見では，尿検査，血液一般，血液凝固系に異常なく，血液生化学では中性脂肪188 mg/dL，総コレステロール266 mg/dLと上昇していたが，そのほかには異常なかった。内分泌検査では甲状腺機能，下垂体機能，副腎機能に異常を認めなかった。血清極長鎖脂肪酸，アリルスルファターゼA，スフィンゴミエリナーゼなどライソゾーム酵素活性は検索しなかった。髄液検査では異常は認めなかった。

Alzheimer病の暫定診断にて，退院後も定期的に経過を観察した。発症5年後より歩行障害が出現し，また以前よりも多幸的となった。頭部MRIでは脳室拡大と脳室周囲異常信号が経時的に進行し，脳幹の萎縮もみられた（Fig. 1）。海馬の萎縮は認められなかった。脳血流SPECTでは大脳で前頭葉に優位な斑状の取り込み低下がみられた（Fig. 2）。発症6年後の時点で自発話がほとんどなく，認知機能評価は不能であった。また歩行は不可能であり，四肢の筋緊張亢進および筋力低下，筋萎縮を認めた。この後も認知機能障害は進行し，発症約8年後に無動性無言，食事摂取困難，栄養状態悪化のため経内視鏡的胃瘻造設を行った。尿路感染症あるいは肺炎を併発し，何度か入院したが，抗生剤治療および全身管理により症状は軽快したため，在宅管理を継続した。発症13年後に呼吸器感染を契機として呼吸不全となり死亡した。

症例2　死亡時50歳，男性，右利き，4年制大学卒。家族内に類症の発生はない。

大学卒業（22歳時）後は定職に就かずに，趣味のサーフィンをしながらアルバイトなどで生計を立てていた。35歳時に運転手としてタクシー会社に就職したが，勤務の状況や同僚との人間関係には問題なかった。しかし43歳頃より自分勝手な行動，易興奮性が目立つようになった。その後，理髪店で女性店員の身体に触ったことを契機に，他院精神科に入院し，躁病の診断で加療開始された。退院後は抑うつ状態となり，45歳時に睡眠薬の大量服用による薬物中毒を2回起こした。46歳時に退職し，その後は自宅で療養していた。47歳時に他院で施行した頭部画像検査で脳萎縮を認めたため，当院を紹介受診した。当院で施行した頭部MRIでは前頭葉および側頭葉前方，海馬，扁桃体，脳幹の萎縮および大脳半球深部白質にT_2強調およびFLAIR高信号を認めた(Fig. 3)。脳血流SPECTでは両側前頭葉および側頭葉前方部の血流低下を認めたが，斑状に血流が保持されている部位もあった（Fig. 4）。48歳頃より，一日中テレビを見続け，歯磨きや洗顔は親にいわれるまでしない，声が小さく何

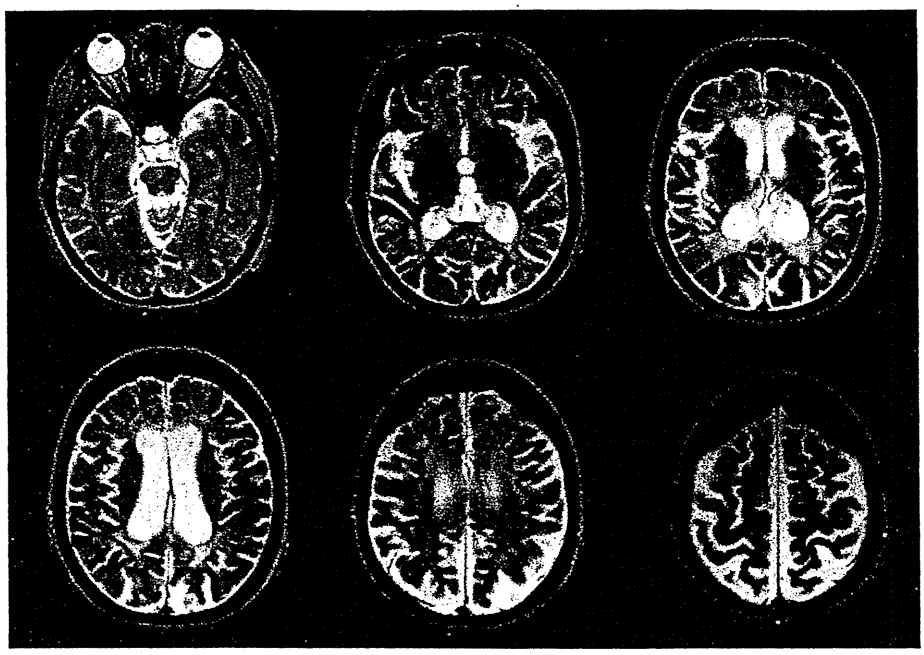

Fig. 1
Magnetic resonance imaging (MRI) of case 1, performed approximately 4 years after the onset of the disease (T_2-weighted images). The MRI scan shows abnormal high-intensity signals around both the lateral ventricles and subcortical white matter. Atrophy of both the frontal and parietal lobes, corpus callosum, and brainstem are observed. Both the hippocampi are not atrophied.

をいっているか聴き取れない，歩こうとしないなど，意欲低下，自発性低下がみられるようになった。49歳時に認知症精査目的で入院した。

現症として，一般身体所見では，陰部に多発する皮膚潰瘍と右膝関節痛を認めた。神経学的所見として，意識は清明であるが病識はなく，入院の目的も理解していなかった。小声で発話速度が速く，会話の聴取が困難であった。脳神経領域では軽度の麻痺性構音障害，運動系では四肢で抵抗症，腱反射の全体的な亢進を認めたが，筋力は正常であり，病的反射も認めなかった。感覚系に異常はなかった。高次脳機能では言語面で漢字の錯書を認めたが，物品呼称，聴理解は正常であった。構成課題，計算課題では異常なし。数唱は順唱6桁，逆唱4桁であった。また動物名の想起が1分間に2個と低下しており，両手の病的把握，模倣行為を認めるなど，前頭葉機能の低下がみられた。MMSEは22点，WAIS-RはVIQ 74，PIQ 62，IQ 65といずれも低下していた。

検査所見では，血液一般，血液生化学に異常なく，甲状腺機能は正常範囲であり，血清梅毒反応，抗HIV抗体，抗核抗体，リウマチ因子も陰性であった。検尿，検便も異常なかった。髄液検査および脳波は施行していなかった。頭部MRIでは，脳萎縮の程度は前回と変化なかったが，T_2強調およびFLAIR高信号が拡大し，側脳室後角周囲にも明らかであった。

入院後も，自分が気に入ったタレントが出演するテレビを見続けながら寝転んでいる，自分からは歩こうとしない，入浴せず髭を剃らないなど清潔についての意識が

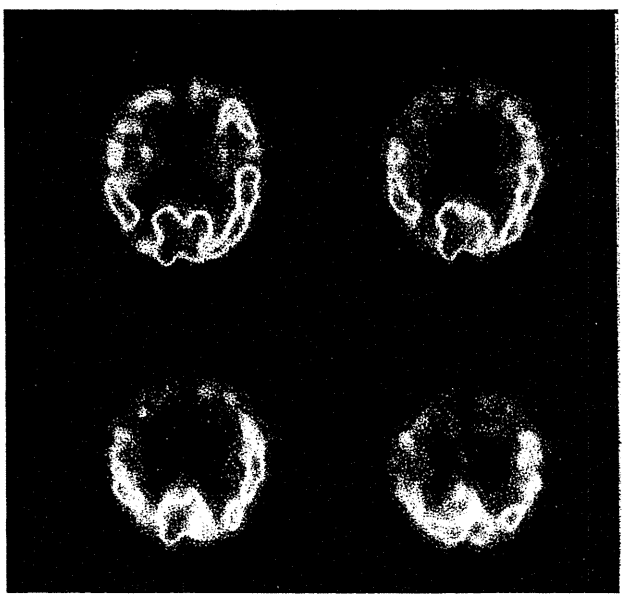

Fig. 2
^{99m}Tc-ECD single photon emission computed tomography (SPECT) of case 1, performed approximately 5 years after the onset of the disease. The image shows that accumulation of the tracers is partially reduced in both the frontal lobes.

低い，食事は好き嫌いが強くパンばかりをゆっくりと食べる，などの症状がみられた。また入院中に発熱がみられ，血液検査で炎症所見陽性であったが，抗生剤投与後には症状，検査所見ともに軽快した。右膝関節痛は安静によって，また陰部潰瘍も清潔ケアを続けることで軽快した。前頭側頭型認知症と診断し，退院後は主として在宅介護を受けていたが，徐々に嚥下障害，歩行障害が進

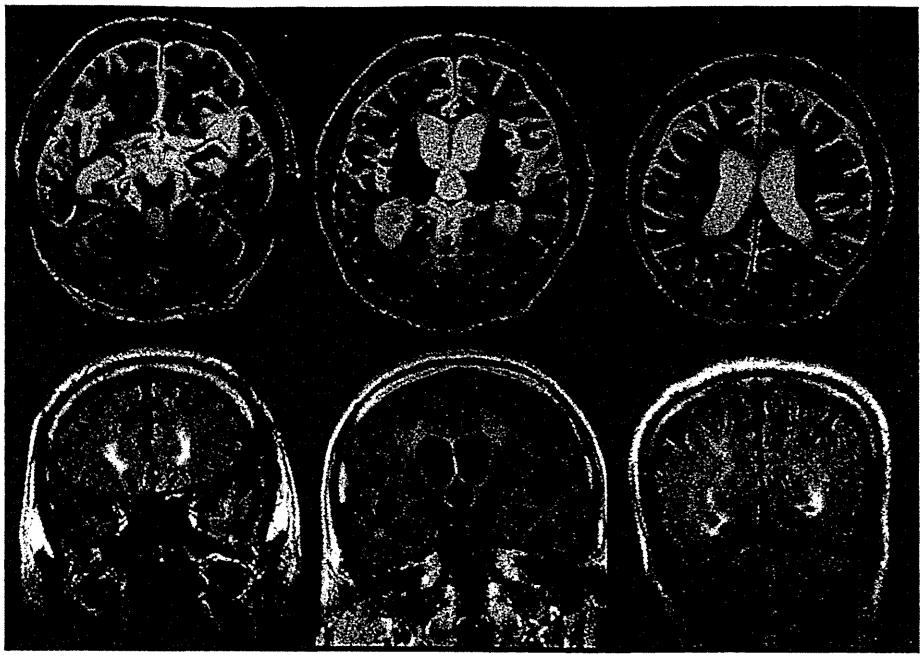

Fig. 3
MRI of case 2, performed approximately 4 years after the onset of the disease (T_2-weighted images). The MRI scan shows abnormal high-intensity signals around both the lateral ventricles and atrophy of both frontal lobes and parietal lobes, hippocampi, corpus callosum, brainstem, and cerebellum.

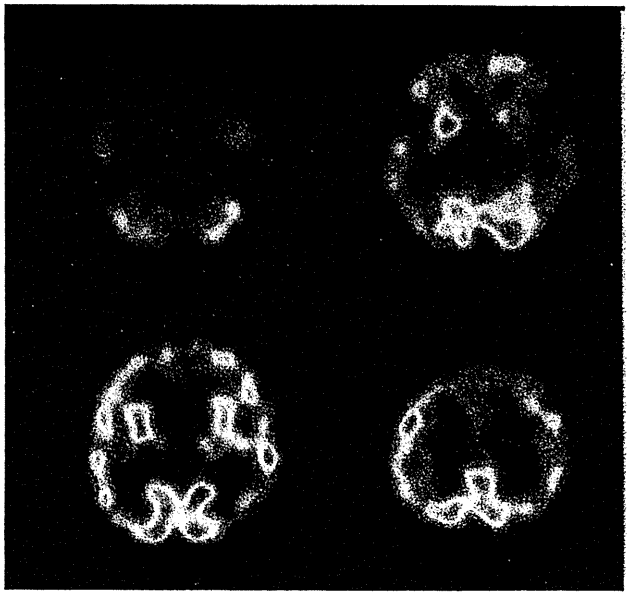

Fig. 4
^{99m}Tc-ECD SPECT of case 2, performed approximately 6 years after the onset of the disease. The image shows that uptake of tracers is partially reduced in both the frontal lobes and cerebellum.

行し，また両便失禁状態となった。発症7年後，自宅で誤嚥によって窒息し，当院に救急搬送されたが死亡確認された。

II. 病理所見

症例1　一般病理所見には，検索した範囲では特記すべき異常はみられなかった。神経病理所見として，脳重は910 g（固定後）。脳は全体的に小さく，動脈硬化性変化は軽度であった。割面では大脳半球白質の白色調が消失し，褐色調を呈していた。白質の容積が減少していたが，皮質直下のU線維は全体的に保たれていた。脳梁が全長にわたり菲薄化していたが，脳弓，前交連は保たれていた。中脳割面では大脳脚，橋割面では底部が，それぞれ小造りであった。小脳白質は保たれていた。

組織所見として，大脳皮質には異常はみられなかったが，大脳白質は広範に髄鞘崩壊と軸索脱落を呈していた（Fig. 5）。皮質直下のU線維は保たれていた。病変内には残存する軸索の腫大やスフェロイドを認めた。また少数ではあるがマクロファージも認めた。これらの白質病変は前頭葉に強かったが，頭頂後頭葉にもみられた。血管周囲のリンパ球浸潤，多核の大型マクロファージ，Rosenthal線維は，いずれも認めなかった。基底核・海馬・扁桃体には異常を認めなかった。視床はMD核に明らかな神経細胞脱落とグリオーシスを，またVL核の一部に髄鞘染色性低下と多数のスフェロイドを認めた。脳梁は全長にわたり，高度の髄鞘淡明化と軸索脱落，線維性グリオーシスを呈していた。大脳脚の中央1/3，橋底部の縦走線維，延髄錐体は両側で髄鞘染色性が低下していた。以上の病理所見から，本例は成人発症型の軸索スフェロイドを伴う白質ジストロフィー[6]と診断した。

症例2　剖検時に全身のるいそうがみられた。気管内に喀痰が貯留しており，直接の死因は痰詰まりによるも

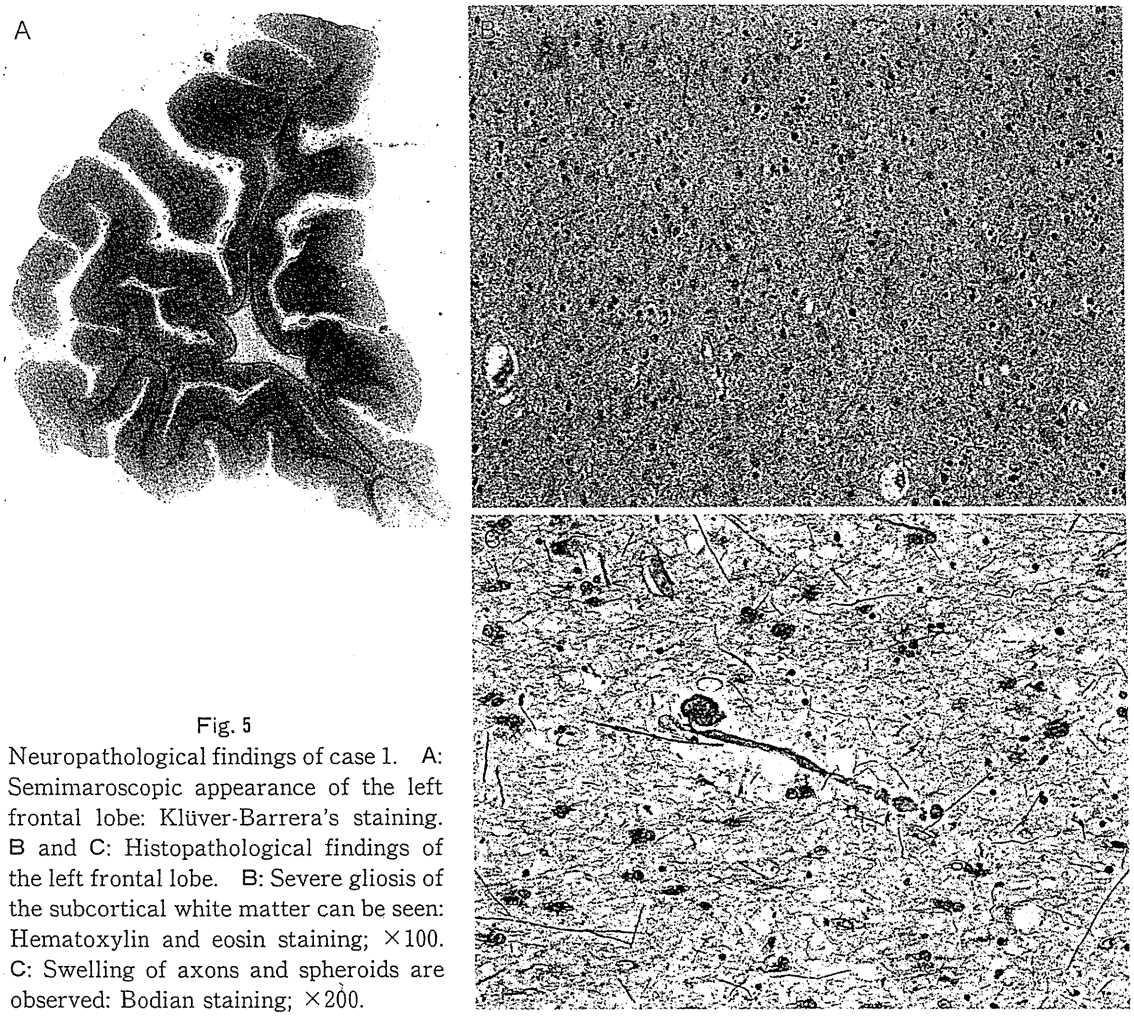

Fig. 5
Neuropathological findings of case 1. A: Semimaroscopic appearance of the left frontal lobe: Klüver-Barrera's staining. B and C: Histopathological findings of the left frontal lobe. B: Severe gliosis of the subcortical white matter can be seen: Hematoxylin and eosin staining; ×100. C: Swelling of axons and spheroids are observed: Bodian staining; ×200.

のと考えられた。そのほかには，両肺の軽度うっ血水腫と気腫性変化，慢性気管支炎，右室の拡張，大動脈の粥状硬化を認めたが，腸管，肝臓，脾臓，腎臓には，検索し得た範囲では，特記すべき所見はなかった。神経病理所見として，脳重は 950 g。脳は全体的に小さいが，脳溝の開大は認めなかった。割面では視床および尾状核の萎縮，側脳室拡大，脳幹の全体的な萎縮を認めた。また大脳半球白質に斑状の淡褐色病変が散在していた。軟膜は軽度に混濁していたが，硬膜には異常を認めなかった。

組織所見として，大脳白質では，MRI で認められた異常信号域に概ね対応する部位で髄鞘の脱落と軸索密度の減少がみられた。また生地の粗鬆化，軽度のグリオーシス，血管周囲の炎症細胞浸潤(主にリンパ球，部位によってはマクロファージの集簇)，少数の小壊死巣を認めた(Fig. 6)。これらの病変は前頭葉，側頭葉に多かったが，後頭葉にも認められた。髄膜でも複数箇所で血管周囲のリンパ球浸潤を認めた。皮質は運動野も含めて著変なく，海馬錐体細胞は保持されていた。視床および基底核は異常なかった。脳幹では上丘，橋底部，延髄に血管周囲リンパ球浸潤を認めた。黒質，青斑，舌下神経核には異常を認めなかった。延髄錐体では髄鞘染色性低下を認めたが，軸索は保持されていた。小脳は異常なかった。脊髄では血管周囲炎症細胞浸潤を認めたが，前角神経細胞は保持されていた。以上の所見から，本例は病理学的に慢性髄膜脳脊髄炎と診断した。鑑別診断として神経 Behçet 病の可能性が考えられたが，血液や凍結脳組織が保存されていなかったため，HLA の検索などの診断確定に必要な検査を施行し得ず，炎症の原因となった疾患は特定できなかった。

Ⅲ. 考　　察

症例 1 は 41 歳時に時計を読めないという症状，性格変化で発症しており，約 5 年で無動性無言に至る進行性の経過を示した。発症初期には記銘力障害のほかに，Alzheimer 病などの後方型認知症を示唆する「時計を読めな

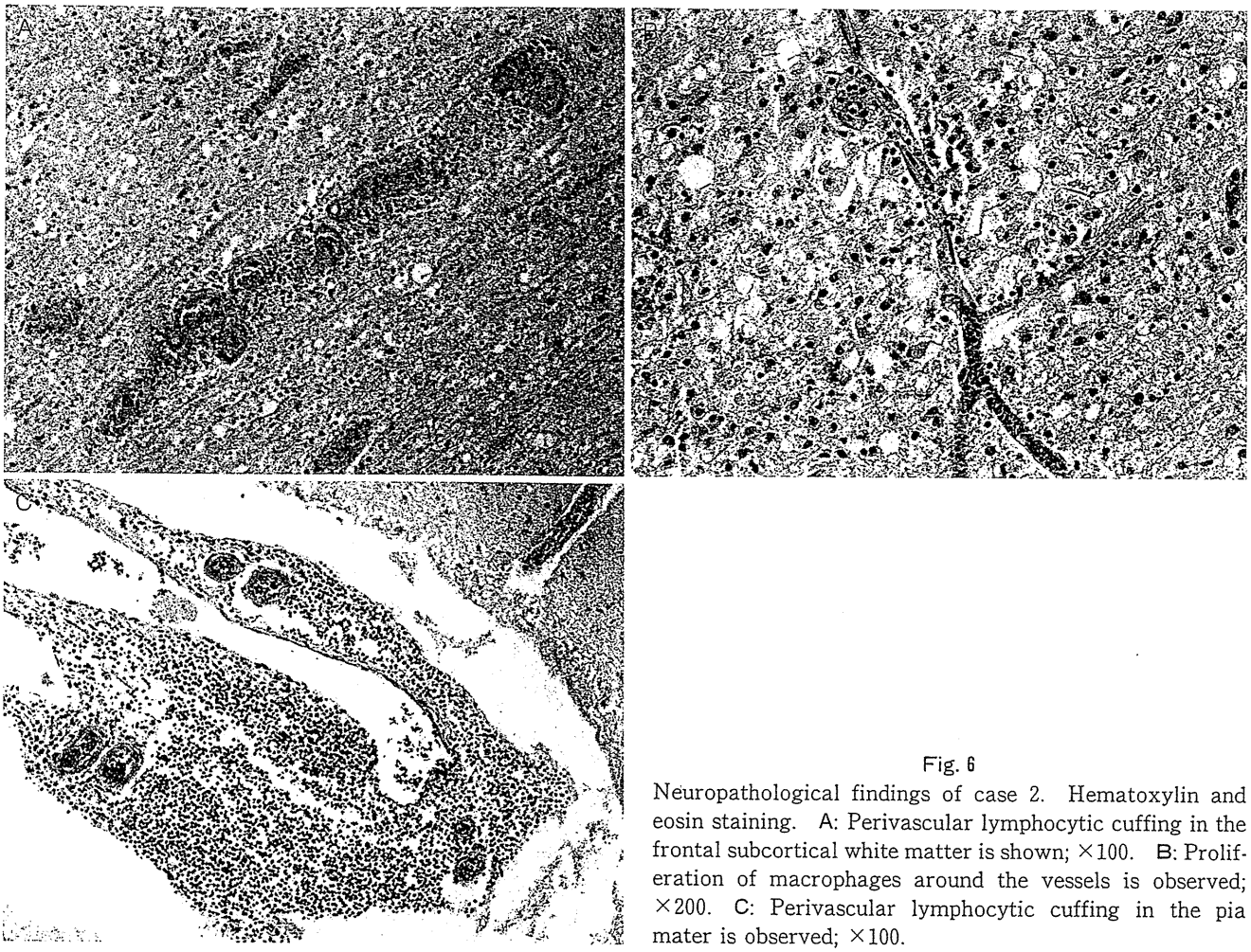

Fig. 6
Neuropathological findings of case 2. Hematoxylin and eosin staining. A: Perivascular lymphocytic cuffing in the frontal subcortical white matter is shown; ×100. B: Proliferation of macrophages around the vessels is observed; ×200. C: Perivascular lymphocytic cuffing in the pia mater is observed; ×100.

い」という症状，前頭側頭型認知症を示唆する性格変化，前頭葉機能低下がともにみられたため，診断に苦慮した。質問に対して深く考えず「わかりません」と即答する症状は，前頭側頭型認知症でみられる思考怠惰に相当すると考えられるが，笑いながら返答している様子は，進行性皮質下神経膠症でみられるような「へらへらとした印象」[7]にも該当すると考えられる。

症例 2 にみられた進行性の認知症症状，すなわち病識欠如，自発性低下などは，前頭葉機能低下を反映するものであり，頭部 MRI で両側前頭側頭葉の萎縮を認めたことと併せて，前頭側頭型認知症を示唆すると考えた。また，衛生に関する認識の欠如や嗜好食品の変化，自発話の減少もみられたが，これらも前頭側頭型認知症の診断基準で支持項目に含まれている[8]。しかし，臨床診断にあたっては，髄液検査や脳波など，認知症の診断に際して除外診断を行う必要があるいくつかの項目について，検索していなかった。さらに脳血流 SPECT 所見は，前頭側頭葉における一様な血流低下を示唆するものではなく，この点からも前頭側頭型認知症の診断について，慎重であるべきであった。

症例 1，**症例 2** ともに，病理学的には大脳皮質に変性所見を認めず，認知症症状は白質病変によるものと考えられるが，「忘れっぽさ，思考過程の緩徐化，人格変化（無欲あるいは抑うつ傾向），獲得知識の利用障害」を特徴とする皮質下性認知症の特徴に一致するものではなく，鑑別が難しい。画像所見としては，頭部 MRI では発症早期から脳室周囲の白質に異常信号がみられたこと，脳梁菲薄化，脳幹萎縮がみられたことは，Alzheimer 病としても，前頭側頭型認知症としても一致しない。また脳血流 SPECT で，発症早期には局所的な集積低下を認めず，進行期には大脳で斑状に集積低下がみられた点も，変性疾患とは異なる。

白質ジストロフィーにみられる精神症状については，Shapiro ら[9]が晩期発症異染性白質ジストロフィーにみられる認知症の特徴を報告している。それによれば，注意障害，脱抑制，判断力低下，反社会的行動，視空間認

知機能低下，記憶障害が特徴とされている。これらは前方型認知症，後方型認知症の両者の症状を含んでいる。また，**症例 1** の病理診断に該当する成人発症型の軸索スフェロイドを伴う白質ジストロフィーとしては，Freeman ら[6]が報告した 4 例中，1 例では見当識障害，無為，記憶障害，視空間機能障害，気分の変動性，皮質盲，1 例では発話障害，記憶障害，1 例では性格変化，注意障害，集中力低下，視空間認知障害がみられた，とされている。これらはいずれも前方型認知症，後方型認知症の要素を含んでいる。そのほか，認知症の症状が記載されている成人発症の白質ジストロフィーの報告例[10-13]でも，前方型認知症と後方型認知症の要素が共存しているものが多い。

症例 2 と同様に，進行性の認知症症状を呈し，病理学的に炎症性髄膜脳炎と診断された症例しては，Caselli ら[14]による非血管炎性自己免疫性炎症性髄膜脳炎の報告がある。これは初老期以後に進行性認知機能低下，精神症状，歩行障害を呈し，ステロイド治療に反応した 5 例をまとめたものである。脳生検の結果，血管壁への浸潤を伴わない血管周囲リンパ球浸潤を認めたこと，髄液検査で IgG index および IgG 産生率の上昇を認めたことから，自己免疫性の機序による髄膜脳炎が想定されている。**症例 2** は病理学的に神経 Behçet 病の可能性が考えられたことからも，髄液所見が臨床診断に有用であった可能性が示唆される。

65 歳以下で発症する「早期発症」の認知症では，Alzheimer 病，前頭側頭葉変性症などの変性疾患，あるいは脳血管性認知症が多く，代謝性疾患，自己免疫性疾患の頻度については報告されていない[1-5]。しかし Kelley ら[15]の報告によれば，45 歳以下で発症する「若年発症」の認知症の原因疾患の頻度は，前頭側頭型認知症，多発性硬化症，代謝性疾患，自己免疫性疾患の順であったとされている。今回検討した症例のように，若年発症で前方型認知症と後方型認知症の症状を併せ持つ症例，脳梁を含む大脳白質の異常信号を MRI で認める症例では，治療の可否を含めて，代謝性疾患，炎症性疾患を念頭に精査を進める必要があると考えられる。

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進行性核上性麻痺(PSP)の発見から現在まで

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進行性核上性麻痺（PSP）の発見と概念の確立

カナダはトロントの病院（Toronto General HospitalとSunnybrook Military Hospital）に勤務していたJ Clifford Richardson（図1左）は、1955年彼自身の親友である52歳の男性から診察を依頼されました。親友の症状は、動作がぎこちなくなったこと、物を見にくくなったこと、そして少し忘れっぽくなったことでした。その症状は徐々に悪化して、目を上下に動かしにくくなり（垂直性核上性眼球運動障害）、飲み込みが悪くなり（偽性球麻痺）、呂律も回らなくなり（構音障害）、頸が固く反るようになって（頸部ジストニア）、軽い認知症もみられるようになりました。

Richardsonはその診断に苦慮していましたが、似たような症状の中年患者3人が彼の前に現れました。1人はジャマイカ人の労働者で、歩行が不安定になり、もう一人はトラック運転手で人柄が変わり、すぐに転ぶようになりました。最後の一人は物が見にくくなり、呂律が回らなくなって、飲み込みも悪くなりました。Richardsonは彼の親友を含めた4人の患者は同じ病気だと考えましたが、それがどのような病気なのか分かりませんでした。その後、同様の症状を示す症例が加わって合計8例になりました。

神経解剖学者でありかつ神経病理学者であったJezzy Olszewski（図1右）が1959年にトロントに赴任し、Richardsonの症例の神経病理を分担することになりました。次いで1962年には当時レジデントであったJohn Steeleが研究に参加しました[1]。

図1　Richardson（左）とOlszewski（右）

1963年、Richardsonは American Association of Neurology の年次学術集会において「核上性外眼筋麻痺、偽性球麻痺、項部ジストニアおよび認知症」のタイトルで8例の臨床報告を行い[2]、この内の6例の神経病理所見を同年 Atlantic City で開催されたアメリカ神経病理学会で Olszewski が報告しました[3]。そして、上記3名の名前で、翌1964年に7名の剖検例を含む9名の患者の臨床病理所見を「進行性核上性麻痺」（progressive supranuclear palsy: PSP）として論文発表しました[4]。神経症状として、眼球運動障害、顔の筋肉のこわばり、呂律不良、飲み込みの悪さ、頸の後方への反り返りおよび認知症に触れています。一方、神経病理学的には黒質、淡蒼球、視床下核、四丘体、脳幹被蓋、小脳歯状核の神経細胞脱落（変性）とこれらの箇所に神経原線維変化が出現することが述べられています。

Asao Hirano（平野朝雄）は1959年から1年間Guam島に滞在して、そこの原住民であるChamorro人に多発する Parkinsonism-dementia complex of Guam（PDG）の診療と神経病理研究に従事し、脳の黒質を初め多くの箇所の変性と神経原線維変化の出現を報告していました[5]。ニューヨークの Montefiore 病院に戻っていた Hiranoは、1963年、アメリカ神経病理学会の1月前に Olszewski か

らトロントに招かれて、PSP患者の一人を診察し、その標本を調べました[1)6)]。それを踏まえての学会でコメントし、「トロントの症例とGuamの症例には臨床的には、無表情、返答が遅いこと、寡動、呂律不良、四肢の筋強剛、振戦が見られないことで共通している。病理組織学的にも非常によく似ている[注1]」と述べています。この当時の学会抄録には発表演題の抄録のみでなく、質疑応答も記載されています。

進行性核上性麻痺の概念の変遷

1964年のSteele, Richardson, Olszewskiの報告では、PSPは臨床的には初期からの歩行不安定性と易転倒性、核上性注視麻痺、軽度の認知症を呈し、神経病理学的には淡蒼球、視床下核、黒質、四丘体、橋被蓋、小脳歯状核に特に目立つ、大脳深部と脳幹の広範な領域の変性（神経細胞脱落とグリオーシス）と神経原線維変化の出現を特徴としていました。

その後、PSPではグリアの病変（房状の星細胞：tuft-shaped astrocyte、オリゴデンドログリアに出現するcoiled body）も存在することが判明し、これらはいずれもタウ蛋白を構成成分とすることが分かりました（タウ蛋白症：tauopathy）。特にアストロサイトの異常であるtuft-shaped astrocytesはPSPにほぼ特異的な所見であることがわかりました。このような分子神経病理学的な進歩を背景にして、PSPと剖検診断された症例の臨床像を調べてみますと、最初に報告されたプロトタイプとは大きく異なる臨床像を呈する症例群が存在することが分かってきました[7)]。

この非定型的臨床像を呈するグループには、Parkinson病像（パーキンソニズム）を呈する群（PSP-P）、純粋無動症（pure akinesia with gait freezing: PAGF）を呈する群、大脳皮質基底核変性症（corticobasal degeneration: CBD）の臨床像を呈する群（PSP-CBS: CBSはcorticobasal syndromeでCBD擬きの意味です）、進行性非流暢性失語症（progressive non-fluent aphasia: PNFA）を示す群（PSP-PNFA）が含まれます[注2]。これに対して、上記のプロトタイプは最初に報告したRichardsonの名を冠して、Richardson症候群と呼ばれます[8)]（表）。

表

	Richardson症候群	PSP-P	PSP-PAGF	PSP-CBS	PSP-PNFA	Parkinson病
筋強剛	四肢より体幹に強	四肢は体幹で同じか、より強	体幹	在り	時々在り	体幹より四肢で顕著
寡動	軽度	中等度	中等度	在り	軽度	中等度
振戦	無し	+／−	−	−	−	+（静止時）
初期の転倒	+	−	−	時々	時々	−
初期の立ち直り反射障害	+	−	+	…	…	−
初期の認知障害	しばしば+	−	−	−	+	−
初期の眼球運動障害	+	−	−	−	時々	−
レボドパ反応性	−	しばしば	−	−	−	通常+
嗅覚低下	−	−	…	…	…	+
MIBG心筋シンチ	正常	正常		…	…	異常

ここで明確にしておく必要があるのは次の点です。1964年のSteele, Richardson, Olszewskiの報告のころには、PSPは臨床病理学的な概念でした。その臨床像は、歩行不安定性と易転倒性、核上性注視麻痺、軽度の認知症の組み合わせであり、神経病理像は淡蒼球、視床下核、黒質、四丘体、橋被蓋、小脳歯状核に特に目立つ広範な領域の変性（神経細胞脱落とグリオーシス）と神経原線維変化の出現であり、両者は一対一対応をしていると考えられていました。実際は、この関係がくずれることは当時から予言されていたのですが。今はこの関係が成り立たなくなり、臨床像と病理像が対応しなくなった時代です。現在使用されるPSPという言葉は、あくまで神経病理学的な診断名です。その病理像を背景にして上に述べたような種々の臨床病型を呈して来ます。言い換えれば、PSPの確定診断は病理診断を待たなければならず、臨床像からはPSPと診断できないのです。PSPであることを確実に示すバイオマーカーが見だされるまでは、この状況が続くものと予想されます。

同じ性質の病変を有しながら臨床像が異なるのは、病変の強さと広がり（トポグラフィー）に差が有るからだと考えられています。PSP-Pでは一般に病変の強さは軽く、広がりも限定的であるのに対して、Richardson症候群では病変が強く、広がりも大きいと言われています。そして、前者は罹病期間が

長く、後者は短いことが示されています[8]。これは、筋萎縮性側索硬化症において、同一性質の病理像を背景にしながら、その病変の分布から進行性球麻痺、脊髄性筋萎縮症、原発性側索硬化症の病像が現れ、病変の強さ（進行の遅速）により罹病期間に差が出るのに似ています。

終わりに

PSPは、1964年の報告当時は臨床像から病理像がほぼ確実に推定できると考えられた臨床病理学的概念でしたが、現在は神経病理学的診断名になっています。PSPの臨床診断が確実にできるようなバイオマーカーの発見が強く望まれます。

注1：ここの原文は“These histological and cytological features (of PSP) are essentially similar to those found in all of the Guam cases.”であり、下線（筆者）のように非常に慎重な表現になっています。

注2：ここでの記載で、PSPは病理学的意味のPSPを指し、ハイフン以下の頭字語（acronym）は臨床像を指します。

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疫学，症候，神経病理

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疫 学

孤発性 ALS(sALS)の有病率と発病率は特殊な多発地域を除けば世界的にほぼ一定で，前者は 2〜7 人/10 万人，後者は約 1 人/10 万人である．男女比は約 2：1 と報告されてきた[1,2]が，徐々に 1：1 に近づきつつある[3]．これは女性の発症率が増えたことによると考えられており，その理由として女性の社会進出が進み，男性と同じ外因に晒される機会が増えたことによる可能性が指摘されている[3]．

発症年齢は 30〜80 歳代にわたり，最近のわが国の sALS の疫学研究では発症のピークは 70 歳代にあり[2]，かつ発症率の高さでは紀伊半島多発地域には及ばないが，それ以外に男女毎に異なる 2，3 の多発地域が見出されている[4]．sALS 発症のピークが 70 歳代にあり，それ以降では減少することに関して，① 高齢者では種々の疾患が合併するために sALS が見逃される，② 80 歳を超えた人では原因となる外因に対して遺伝的に感受性が低い，③ 発症するためにはある年齢に達するまでに外因に暴露される必要があり，それを超えての暴露は発症しない，が理由として考えられている[3]．近年，人口の高齢化とともに高齢発症の sALS を経験することが希でなく，われわれの施設の発症最高齢者は 90 歳の女性である．

sALS の発症因子を探る疫学研究では，激しい身体活動，金属や有機溶媒暴露，殺虫剤暴露等に加えて必ず外傷が候補としてあげられる．最近，慢性の頭部外傷に伴う TDP-43 proteinopathy が sALS の発症に関連するとの病理学的報告がなされた[5]，反論も寄せられている[6,7]．

症 候

sALS の症候は，基本的には下位運動ニューロン(LMN)症候(筋萎縮)と上位運動ニューロン(UMN)症候(側索硬化)とが併存する状態である．LMN 症候には筋萎縮，高度の筋力低下，筋弛緩，腱反射消失，線維束性収縮が含まれ，変性疾患での UMN 症候には痙縮，腱反射亢進，手指の巧緻運動障害，下肢の病的反射，特定の筋の軽度の筋力低下が認められる．軽微な UMN 障害(錐体路障害)に敏感な筋として，上肢では三角筋前部，総指伸筋，掌側骨間筋，下肢では腸腰筋，膝屈筋群，前脛骨筋があげられる．

LMN と UMN の障害には様々の組み合わせがあり，LMN のみ，あるいは UMN のみが侵される場合を両端とする一連のスペクトラムを示す．一方，LMN が最初に侵される箇所は上肢，球部，下肢と様々である．UMN が最初に侵される部位が下肢であれば痙性対麻痺，球部であれば偽性球麻痺となる．即ち，ALS では，LMN と UMN の侵され方の強弱，LMN と UMN の侵される箇所の組み合わせにより種々の症候型が出現することになる(表)[8](詳細は文献 9 参照)．

近年，Alzheimer 病，Parkinson 病，Huntington 病において蓄積蛋白がプリオン様性質を有して，細胞から細胞へ伝播するとの説があり，sALS の蓄積蛋白である TDP-43 もそのような特性を有することが推測されている[10]．この sALS のプリオン病様説に関連して興味深い病型が，昔から記載されている片麻痺型である．片麻痺型とは，筋萎縮と筋力低下が長期間にわたって著明な左右差を持って一側に目立つタイプである．これは TDP-43 の伝播説と相容れ

下位運動ニューロン，上位運動ニューロンの障害の部位と強弱に基づく臨床病型

	LMN のみ	UMN のみ	LMN＋UMN
球部	進行性球麻痺	偽性球麻痺	ALS (進行性球麻痺*1)
上肢	Flail arm syndrome 脊髄性筋萎縮症	(原発性側索硬化症)	ALS
下肢	偽性多発神経炎型	原発性側索硬化症 Mills 症候群*2	

*1) 球部で LMN と UMN の両者が侵された場合は，UMN 症候は捉えにくい．
*2) 原発性側索硬化症の片側型と考えられている．

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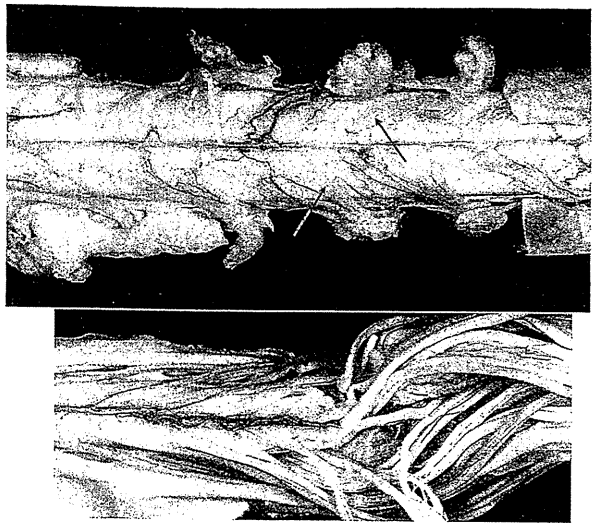

図 1　ALS の固定後脊髄
頸髄では前根(→)の変色と萎縮がみられる．腰髄では一般にこれらの変化は認めがたい．

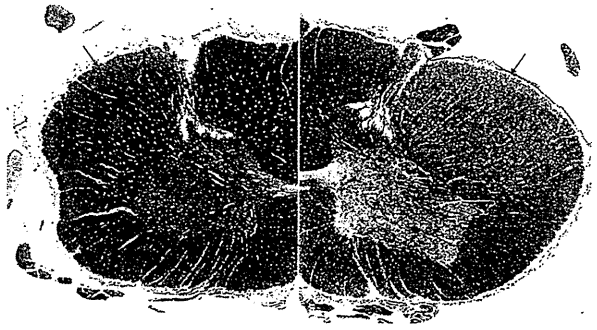

図 2　ALS(右)と正常対照(左)の頸髄 Klüver-Barrera 染色
ALS では錐体路の淡明化に加えて，前側索の他の部位も淡明になっている．正常では後脊髄小脳路(→)が錐体側索路よりも淡明であることに注意．

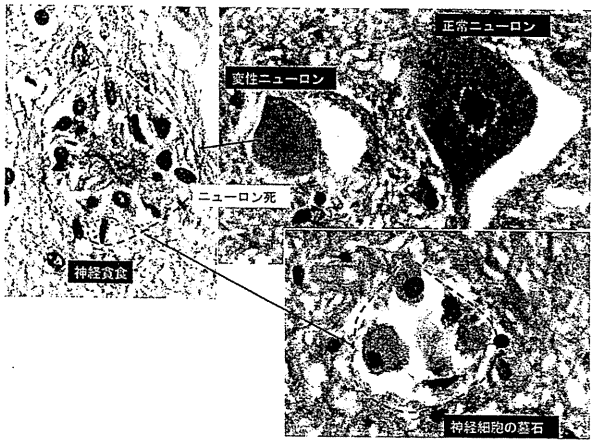

図 4　LMN の死にいたる過程と思われる像
いずれも HE 染色，400 倍

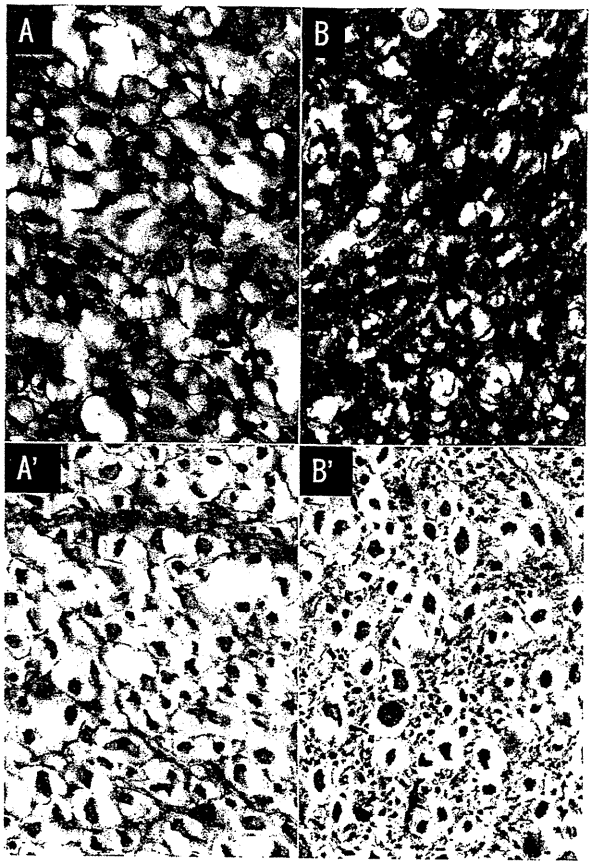

図 3　正常例頸髄の後脊髄小脳路(A，A')と錐体側索路(B，B')
後脊髄小脳路には錐体側索路よりも空隙が多く，鍍銀軸索染色により，これは後脊髄小脳路がほぼ大径有髄線維から構成されている(A')ことに由来することが分かる．錐体側索路(B')には非大径神経線維が多く含まれることが分かる．A，B：Klüver-Barrera 染色，A'，B'：Bodian 染色．いずれも 400 倍．

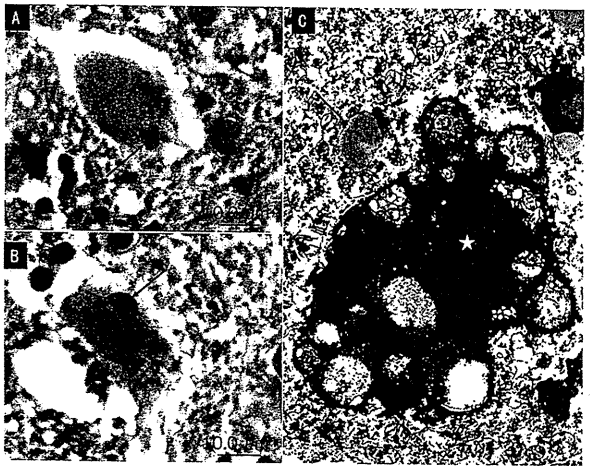

図 5　Bunina 小体
A, B) 好酸性で内部に小空隙(→)を有するのが特徴である．HE 染色，400 倍．C) 電顕像(★)．→はリポフスチン．8,000 倍