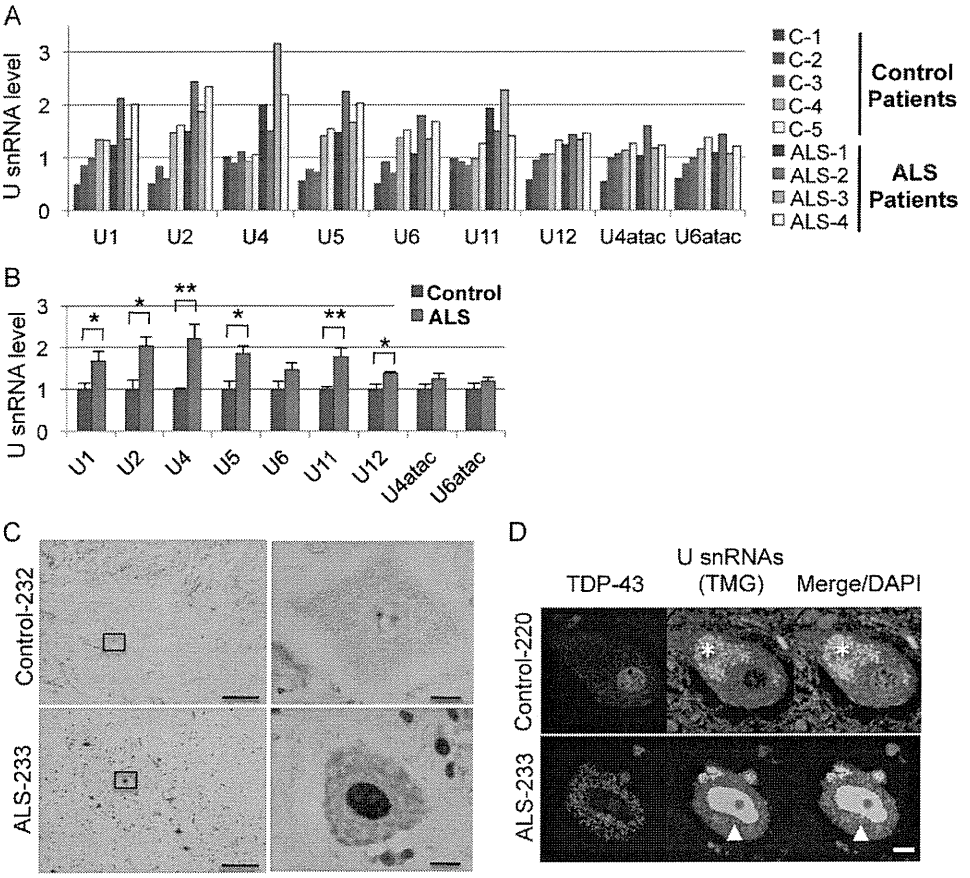


**Figure 5. Expression levels of U snRNAs are up-regulated in cervical spinal cords of ALS patients.**

**A.** The RNAs were isolated from cervical spinal cords of four ALS patients (ALS-1 to ALS-4) or five control patients with other neurological disease (C-1 to C-5), and U snRNA levels were determined by quantitative RT-PCR as in Fig 4. Detailed clinical information is listed in Supporting Information Table S1.

**B.** Mean U snRNA levels of control and ALS patients determined in **A** were plotted. Average amounts of U snRNAs from the five control patients were used for normalization. Robust U snRNA misregulation was observed in ALS patients. (Bars: standard errors, $^{*}p < 0.05$, $^{**}p < 0.01$, Student's *t*-test).

**C.** Immunohistochemistry of spinal cords from patients with control disease or ALS using an anti-2,2,7-trimethylguanosine (TMG) antibody, which recognized the 5′ cap structure of snRNA. Boxed areas were shown as the magnified images (Right panels). Bars: 500 μm (left), 20 μm (right).

**D.** Immunofluorescence staining of spinal motor neurons using anti-TMG and anti-TDP-43 antibodies. Note that strong accumulation of U snRNAs in nucleus of motor neurons from ALS patients (arrowheads). Asterisks show autofluorescence derived from lipofuscin in the cytoplasm. Bars: 10 μm.

## DISCUSSION

In this study, we show that TDP-43 localizes to Gems through an association with an SMN/FUS complex and is critically involved in Gem formation and spliceosome maintenance by controlling U snRNA levels. Dysfunction of these proteins impairs the spliceosome resulting in abnormal splicing in motor neurons and neurodegeneration (Fig 6G). We also show that TDP-43 and SMN-dependent spliceosome dysregulation is common to the motor neuron diseases ALS and SMA, respectively, but not FTLD-TDP. In tissue from sporadic ALS patients, or following TDP-43 knockdown in cells, nuclear Gems collapsed and expression levels of U snRNAs spliceosome components were aberrantly up-regulated. Furthermore, U snRNPs aberrantly accumulated in motor neuron nuclei from ALS patients, but not in temporal cortex neurons from FTLD-TDP patients. Our findings strongly indicate that abnormal U snRNP level, which can cause abnormal RNA splicing and/or isoform expression (Berg et al, 2012), is an important mechanism resulting in neurodegeneration common to the motor neuron diseases ALS and SMA (Fig 6G).

The most provocative findings in our study revealed that U snRNA misregulation was observed in several distinct contexts: cells with a decreased expression of TDP-43 (Fig 4B–D) and spinal cord tissue samples of ALS patients (Fig 5), but not in the affected brain regions of FTLD-TDP (Supporting Information Fig S5A and B). Cell type- or tissue- specific alterations in U snRNA repertoires were identified in cells with low levels of SMN and in SMA mouse tissues (Gabanella et al, 2007; Zhang et al, 2008). Similarly, we observed that different sets of U snRNAs were misregulated in neuronal and non-neuronal cells following TDP-43 depletion. Although the direction of U snRNAs misregulation is different between the two diseases; upregulated U snRNAs in ALS, and downregulated U snRNAs

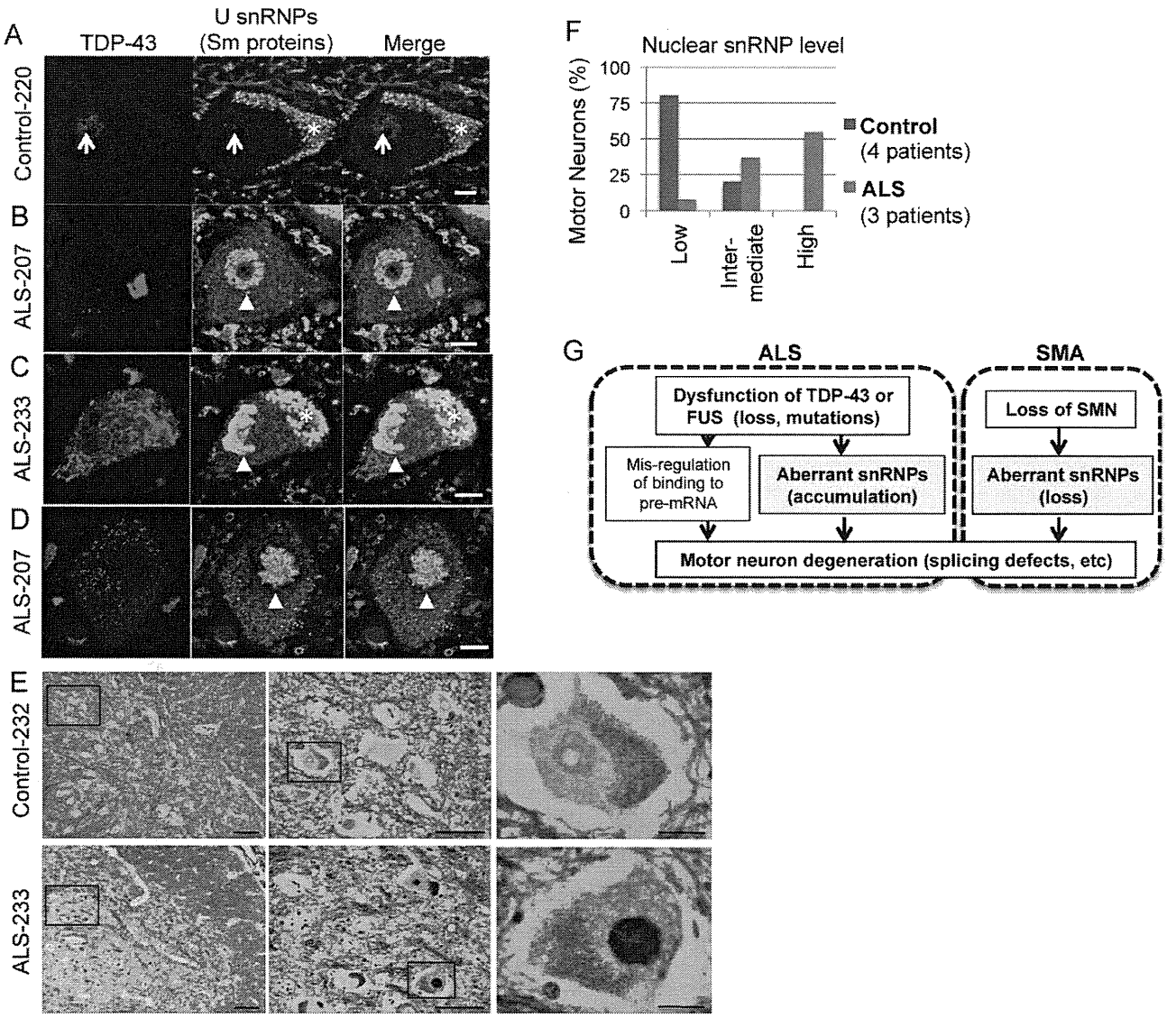


**Figure 6. Abnormal accumulation of U snRNPs in motor neuron nuclei of ALS patients.**

**A–D.** Immunofluorescent staining of TDP-43 and U snRNPs using an anti-Sm proteins antibody (Y12) in spinal cord motor neurons from patients with control diseases (**A**) or ALS (**B–D**). Arrow shows colocalization of the Y12 antigen and TDP-43 (**A**). The Y12 antigen accumulated in nuclei of ALS motor neurons with TDP-43 mislocalization (**B–D**). Arrowheads (**B–D**) show accumulated U snRNPs in motor neuron nuclei from ALS patients. Asterisks show autofluorescence derived from lipofuscin in the cytoplasm. Bars: 10 μm.

**E.** Immunohistochemistry of U snRNPs using an anti-Sm proteins antibody (Y12) in spinal cord motor neurons from patients with control diseases or ALS. Boxed areas in the left and middle panels were shown as the magnified images in the middle and right panels, respectively. Bars: 500 μm (left), 100 μm (middle), 20 μm (right).

**F.** Quantification of U snRNP immunofluorescence levels in motor neuron nuclei. Motor neurons from four control patients (blue, $N = 35$) and three ALS patients (red, $N = 105$) were analysed for U snRNP fluorescence intensity in their nuclei. The level of fluorescence intensity of U snRNPs in nuclei compared with cytoplasm were classified as low (nuclear U snRNP level is lower than cytosol), intermediate (nuclear U snRNP level is equal to cytosol), or high (nuclear U snRNP level is higher than cytosol), and plotted. Representative images of motor neurons showing low, intermediate or high nuclear U snRNP levels are shown in Supporting Information Fig S7.

**G.** Model for mechanism underlying neurodegeneration in ALS with dysfunction of TDP-43 in comparison to SMA. In this study, we show that TDP-43 localizes in nuclear Gem through association with SMN complex, and is involved in maintenance of spliceosome through controlling the levels of U snRNAs. In ALS patients and SMA mice, U snRNA levels are misregulated in spinal cord. Intriguingly, accumulation of U snRNA is seen in ALS, while loss of U snRNAs is reported in SMA. Moreover, Gems are lost and spliceosomal U snRNPs abnormally accumulate in nuclei of motor neurons from ALS patients. These findings indicate collapse of spliceosome integrity is the critical process common to motor neuron degeneration in ALS and SMA, and may explain cell-type specific vulnerability in motor neurons.

in SMA, our work is the first to imply that motor neurons may be sensitive to the collapse of spliceosome, resulting in abnormal splicing through alteration of U snRNP levels (Fig 6). Interestingly, transgenic mice overexpressing poly-Q binding protein-1, which binds to the U5 snRNP component, under a ubiquitous promoter show a late-onset motor neuron disease-like phenotype (Okuda et al, 2003; Waragai et al, 2000). Therefore, motor neurons might be sensitive to snRNP alterations, which would be a potential target for the therapy of motor neuron diseases.

Our results clearly indicate that TDP-43 and SMN might function in a common pathway, namely the regulation of splicing through the maintenance of U snRNP repertoires. Previous studies have demonstrated a genetic link between ALS and SMA. Aberrant copy numbers of SMN1 or SMN2 genes increase the risk of sporadic ALS and disease severity (Andersen & Al-Chalabi, 2011; Blauw et al, 2012; Corcia et al, 2006; Veldink et al, 2001, 2005). Our observation that TDP-43 regulates U snRNAs and associates SMN complex suggests a major function shared between TDP-43 and SMN and supporting a genetic link between ALS and SMA.

Recent studies identifying RNA targets of TDP-43 (Polymenidou et al, 2011; Sephton et al, 2011; Tollervey et al, 2011; Xiao et al, 2011) demonstrated that TDP-43 directly binds to some pre-mRNAs to regulate RNA splicing. However, not all TDP-43 binding sites in pre-mRNAs (as determined by CLIP-seq) are located close to splicing sites, suggesting that TDP-43 might regulate splicing through both direct binding to pre-mRNA and other mechanisms. Our study suggests that TDP-43 also regulates spliceosomal U snRNP biogenesis, which would provide another mechanism of TDP-43-mediated regulation of splicing. Abnormal splicing in motor neurons from ALS patients was reported before (Rabin et al, 2010), however, we are not able to correlate abnormally spliced genes in ALS with abnormal U snRNA repertories at present. The genes indispensable for motor neuron survival may be identified in the future by comparing the numerous abnormally spliced genes reported in SMA with the same genes in ALS.

Although U snRNAs were upregulated only about twofold in the whole spinal cord by quantitative RT-PCR, intense U snRNA staining in ALS motor nuclei indicates much higher U snRNAs upregulation in ALS motor neuron nuclei (presumably more than 100-fold, considering the number of motor neuron much smaller than that of the other spinal cord cell types; Fig 5C and D). Moreover, U snRNAs accumulate and sometimes form aggregates with proteins (Fig 6A–E). Therefore, we speculate that the abnormal accumulation of U snRNPs (they are likely to be non-functional) in ALS motor nuclei could have a substantial impact on RNA splicing and metabolism in motor neurons.

We discovered that TDP-43 and FUS/TLS localizes to nuclear Gems and that Gems are lost in motor neurons of spinal cords from ALS patients as well as TDP-43 depleted or FUS/TLS knockout cells. This is similar to observations in SMA patient-derived cells and SMA mouse models. The number of Gems is correlated with SMA disease severity in fibroblast from SMA patients (Coovert et al, 1997). Furthermore, a recent study showing a reduced number of Gems in the fibroblasts from familial ALS cases with TDP-43 or FUS mutations (Yamazaki et al, 2012) strengthens our findings on the importance of Gem in motor neuron survival. U snRNPs are thought to be stored in Gems for recycling; therefore, TDP-43 might be important for the maintenance of U snRNPs in Gems. The relationship between Gems and TDP-43 has been investigated in several studies. One study demonstrated that the alternatively spliced minor form of mouse TDP-43, which is lacking the C-terminal portion, interacted with SMN (Wang et al, 2002). Another study demonstrated the co-localization of full-length TDP-43 and SMN in human non-neuronal cells (Fiesel et al, 2010), however, additional studies claimed that the colocalization of TDP-43 and SMN was not detected in rat and mouse neurons (Casafont et al, 2009; Shan et al, 2010). In contrast, our study clearly demonstrated that endogenous human TDP-43 was localized in Gems of cultured cells and human motor neurons by coimmunostaining with TDP-43, SMN and Gemin8 (Fig 1A–E and 3). The loss of Gems seen in motor neurons of ALS patients (Fig 3), coupled with the fact that eliminating TDP-43 from mouse neurons *in vivo* leads to the loss of Gems (Shan et al, 2010), clearly supports our findings that TDP-43 and perhaps FUS/TLS is important for Gem formation and U snRNPs biogenesis, as observed before in a similar way with SMN. Furthermore, SMA mutations in the tudor domain of SMN, which is crucial for binding to Sm proteins, abolished SMN association with TDP-43 and FUS/TLS (Fig 2G), supporting an importance of SMN/TDP-43/FUS complex in the biogenesis of spliceosome and in motor neuron degeneration. Moreover, profilin1, which binds to SMN and localizes to Gem (Giesemann et al, 1999), was recently discovered as an ALS causative gene product (Wu et al, 2012). Overexpression of an ALS causing-mutant SOD1 prevents the formation of Gem in the motor neurons of mice (Kariya et al, 2012). Therefore, abnormal Gem formation and/or abnormal U snRNPs formation may underlie the mechanisms of motor neuron degeneration.

The importance of the C-terminal region of TDP-43 was demonstrated through the identification of a domain required for the proper targeting of TDP-43 to Gems and association with SMN (Fig 2A–D), and also by the identification of interactions with several proteins implicated in RNA metabolism (Supporting Information Fig S4). TDP-43 associated with various proteins implicated in RNA metabolism, including proteins involved in pre-mRNA splicing, translational control and the miRNA pathway. Considering that most ALS-linked mutations reside in the C-terminus of TDP-43 (Lagier-Tourenne & Cleveland, 2009), C-terminal region-mediated regulation of RNA metabolism may be disturbed in motor neuron diseases. The proteins we identified could therefore be important to analyse for further potential contributions to motor neuron degeneration.

It is intriguing that TDP-43 localized not only to Gems but also to paraspeckles and nuclear speckles. The long non-coding RNA (lncRNA) NEAT1 (also called Men ε/β) is indispensable for the formation of paraspeckles, where highly edited mRNAs are stored (Bond & Fox, 2009). Furthermore, nuclear speckles are enriched with spliceosomal U snRNPs, other splicing regulators important for RNA splicing such as SR proteins and Malat1

lncRNA (Mao et al, 2011). The expression of NEAT1 and Malat1 lncRNA, both of which have multiple TDP-43 binding sites, is elevated in FTLD-TDP brain (Tollervey et al, 2011). Our study with FTLD-TDP patients also demonstrated an increased expression level of NEAT1 (Supporting Information Fig S6B and D). However, NEAT1 was not significantly altered in ALS spinal cord and TDP-43 depleted cells (Supporting Information Fig S6A and C), suggesting distinct regulations of this lncRNA in different disease conditions. Nevertheless, the enrichment of TDP-43 in paraspeckles and speckles should be examined further to determine any potential role in RNA metabolism in these nuclear subdomains. Taken together, the expression of U snRNA spliceosome components was aberrant and long non-coding RNAs were normal in ALS spinal cords, but these profiles were reversed in FTLD. These results suggest that while neurodegenerative diseases with distinct causal genes (ALS, SMA) can have disruptions in a common biochemical pathway, diseases with the same causal gene (ALS, FTLD) can also have disruptions in distinct pathways.

In conclusion, we show here that TDP-43 and SMN share a common function in spliceosomal U snRNP biogenesis. Dysfunction of these distinct proteins in ALS and SMA leads to collapse of spliceosome integrity and abnormal splicing in motor neurons. We expect that further investigation of defects in RNA metabolism common to these motor neuron diseases but different from a related brain disease should provide explanation to the cell-type specific vulnerability observed in neurodegenerative diseases. In addition, targeting spliceosome and/or Gem stability in motor neurons may represent a new class of candidate therapeutics for motor neuron diseases.

## MATERIALS AND METHODS

### Expression vectors

The open reading frame of human *TARDBP* was inserted into p3XFLAG-CMV14 vector (Sigma), resulting in the insertion of an 18 amino acid spacer between the TDP-43 C-terminus and 3XFLAG peptides. Coding regions of TDP-43 fused with 3xFLAG were subcloned into pF5K-CMV-neo vector (Promega). For FUS/TLS expression, the open reading frame of human *FUS/TLS* fused with 3XFLAG on its N-terminus were subcloned into pF5K-CMV-neo or pF5A-CMV-neo vector (Promega).

### Cell culture and immunofluorescence

Hela cells were maintained in DMEM with high glucose (Gibco) supplemented with L-glutamine, and 10% foetal bovine serum (Gibco). SH-SY5Y cells were maintained in Advanced DMEM/F12 (Gibco) with non-essential amino acids, sodium pyruvate, GlutaMAX (Gibco), and 10% foetal bovine serum. Hippocampal neurons were isolated from E16.5 C57BL6 or *FUS*$^{-/-}$ mouse embryos and cultured essentially as described (Huang et al, 2007). Cells were cultured on chamber slides (Lab-Tek, Nunc), fixed with 4% paraformaldehyde for 10 min and permeabilized with 0.1% Triton X-100. For paraspeckle staining with mouse anti-p54 (BD transduction), cells were fixed with cold methanol. Non-specific binding was blocked by incubation with 1% normal goat serum prior to the application of primary antibody. Antibodies used were as follows: rabbit anti-TDP-43 (ProteinTech), anti-coilin (Sigma, clone pδ), mouse anti-p54 (BD transduction), anti-SRSF2 (Sigma, clone SC-35), mouse anti-SMN (BD transduction, 610646), rabbit anti-SMN (Santa Cruz, sc-15320), mouse anti-Gemin8 (Santa Cruz, sc-130669), rabbit anti-FUS/TLS (Abcam, 70381), anti-dimethylated Sm proteins (Lab Vision Corp./Thermo Scientific, clone Y12), anti-TMG (Santa Cruz, clone K121), mouse anti-FLAG (M2), rabbit anti-FLAG (Sigma) and rabbit anti-GFP (MBL).

### Knockdown of protein expression in cells

To eliminate TDP-43 expression, Hela cells were transfected with 4 nM Stealth siRNA for *TARDBP* (Invitrogen, ID#HSS177422 or originally designed oligos with the sequences listed in Supporting Information Table S2) or control siRNA (Invitrogen, LoGC#2 or #3) in suspension at $1.5 \times 10^5$ cells/ml using Lipofectamine RNAiMax (Invitrogen). After an overnight culture, cells were then transfected with siRNA once more, and then cultured for two more days. For SH-SY5Y cells, cells were transfected in suspension at $3 \times 10^5$ cells/ml. After 3 days of culture, cells were divided into three dishes, transfected with siRNAs again and cultured for three more days.

### Immunoprecipitation

Cells were transfected with pF5K-TDP-43-3xFlag constructs using X-tremeGENE HP (Roche). Cells were harvested and washed with PBS 3 times. TBS supplemented with 0.2% Triton X-100, protease inhibitors (Nakalai, Japan), and RNase inhibitor SUPERase-In (Ambion) was used as a Lysis buffer. The cell pellet was then lysed in the same volume of Lysis buffer on ice for 10 min. The nuclear membrane was disrupted by passage through a 28 G needle and then centrifuged at 14,000*g* for 15 min. Supernatants were collected as total cell extracts. After the protein concentration of cell extracts was adjusted to 5–8 mg/ml with Lysis buffer, cell extracts were mixed with agarose beads conjugated with anti-FLAG antibody (M2-agarose, Sigma) and incubated overnight at 4°C. After washing with Lysis buffer for three times, non-specific protein binding to the anti-FLAG agarose beads was washed out by incubating with FLAG peptide at 50 μg/ml for 15 min at 4°C. This step was critical to wash out non-specific or weak binding to the anti-FLAG agarose beads. To elute the protein complex with 3XFLAG-tagged protein, 3XFLAG peptides were added to the agarose beads at 500 μg/ml and incubated for 1 h at 4°C. Eluted proteins were then analysed by immunoblotting. For immunoprecipitation of HA-tagged protein, anti-HA-agarose (Sigma) was used instead of anti-FLAG agarose, and the precipitated proteins were eluted with SDS-sample buffer.

For immunoprecipitation of mature U snRNPs, a nuclear pellet was obtained from suspension cells using Buffer A (50 mM Hepes pH 7.5/1 mM $MgCl_2$/1 mM EGTA/1 mM DTT/protease inhibitors) on ice for 10 min followed by centrifugation at 3000*g* for 5 min. The nuclear pellet was lysed in Buffer A supplemented with 150 mM KCl, 1% NP-40, 10% glycerol, and RNase inhibitor SUPERase-In (Ambion) and then the nuclear membrane was disrupted by passage through a 27 G syringe 10 times and a repeated freeze/thaw cycle 3 times. Nuclear extract was obtained after the removal of cell debris by centrifugation at 20,000*g* for 10 min. Anti-Sm proteins monoclonal antibody (Y12) and mouse immunoglobulin (as negative control) were used for immunoprecipitation. Antibodies used for Western

## The paper explained

### PROBLEM:

The motor neuron diseases (ALS) and spinal muscular atrophy (SMA) are caused by dysfunction of proteins involved in RNA metabolism. For ALS, the RNA-binding proteins TDP-43 and FUS/TLS, have been implicated, while in SMA the protein SMN, essential for biogenesis of spliceosomal component snRNPs, is critical. A key question is whether there is a shared defective mechanism in RNA metabolism common to these two diseases.

### RESULT:

We report a convergent function for TDP-43, FUS/TLS and SMN by showing that the genes for these diseases share a common mechanism: maintenance of nuclear Gems and controlling the level of U snRNA spliceosomal complex. In ALS spinal motor neurons as well as TDP-43 depleted neurons, we observed disruption of Gems and abnormal accumulation of U snRNAs/snRNPs. Together, our findings indicate that TDP-43, FUS/TLS and SMN are important for spliceosome integrity, and that collapse of the spliceosome is the critical mechanism that must underlie the neurodegenerative process in both ALS and SMA.

### IMPACT:

Our study reveals the important role of nuclear Gems and spliceosomal U snRNPs in motor neuron survival. Although it requires more investigation, our work substantially contributes to understanding the molecular mechanism of motor neuron disease by providing evidence linking for the first time the selective vulnerability of motor neurons to spliceosome breakdown in Gems of ALS and SMA. Furthermore, targeting spliceosome and/or Gem stability in motor neurons may represent a new class of candidate therapeutics for motor neuron diseases.

blot were as follows: mouse anti-FUS/TLS (Santa Cruz, sc-47711), rat anti-PRPF3 (MBL, clone 4E3), goat anti-U1-70K (Santa Cruz), mouse anti-PABP (Sigma, clone 10E10) and rabbit anti-eIF4G (Cell Signaling, #2498).

#### FUS/TLS knockout mice

ES cell clone (HMA274) with β-Gal-neo cassette inserted between exon 2 and 3 of *FUS/TLS* gene were obtained from mutant mouse regional resource centers at University of California, Davis, and used to generate FUS/TLS heterozygous mice with support by Research Resource Center of RIKEN Brain Science Institute. Genotyping of mice with disrupted FUS/TLS allele was performed using RT-PCR, and FUS/TLS protein levels were confirmed by Western blot analysis. Heterozygote mice (F3) were intercrossed to generate $FUS^{-/-}$ mice.

#### Postmortem human tissues

Specimens of spinal cords from five patients with sporadic ALS and seven other neurological disease patients as controls, as well as temporal lobes from three patients with FTLD-TDP and four other neurological disease patients as controls, were obtained by autopsy with informed consent (Supporting Information Table S1). The diagnosis of ALS was confirmed by El Escorial diagnostic criteria as defined by the World Federation of Neurology and with the presence of TDP-43 pathology, as detected by histopathology. For the diagnosis of FTLD-TDP, selective sections were immunostained with antibodies against phosphorylated tau, ubiquitin, phosphorylated TDP-43 and FUS/TLS to select FTLD-TDP (Cairns et al, 2007). All patients with ALS and FTLD-TDP showed no hereditary traits. The collection of tissues and their use in this study was approved by the ethics committee of Nagoya University Graduate School of Medicine, Fukushimura Hospital, Tokyo Metropolitan Institute, and RIKEN. Tissues for RNA analysis were immediately frozen using liquid nitrogen and stored at −80°C until use. For immunofluorescent staining, 6 μm sections were prepared from paraffin-embedded tissues, deparaffinized, microwaved for 20 min in 50 mM citrate buffer (pH 6.0), treated with TNB blocking buffer (PerkinElmer, Boston, MA) and then incubated with primary antibodies. After washing, sections were incubated with Alexa-546-conjugated goat anti-rabbit IgG (1:1000; Invitrogen) and Alexa-488-conjugated goat anti-mouse IgG (1:1000; Invitrogen) for 30 min, mounted with Prolong gold antifade reagent (Invitrogen) and then imaged with a laser confocal microscope (LSM710, Carl Zeiss, Jena, Germany). The position of the nucleus was confirmed by TO-PRO-3 Iodidel (Invitorgen). For immunohistochemistry, sections were deparaffinized and boiled for 20 min in 50 mM citrate buffer (pH 6.0), treated with 3% goat serum/0.5% tween-20/PBS supplemented with Avidin solution (Vector, Avidin/Biotin Blocking kit, #SP-2001), and then incubated with primary antibodies in 3% goat serum/0.5% tween-20/PBS supplemented with Biotin solution (Vector, Avidin/Biotin Blocking kit). After washing, sections were incubated with Biotin-conjugated anti-mouse IgG or anti-rabbit IgG (1:400, Vector) in 0.05%Tween-20/PBS. Signals were visualized with Vectastain ABC kit (Elite, #PK-6100) and Metal Enhanced DAB substrate kit (Thermo Scientific, #34065).

#### Quantitative RT-PCR

Prior to RNA extraction, cultured cells were harvested and stored in RNAlater (Ambion), and frozen tissue samples were stored in RNAlater ICE (Ambion). Total RNA containing a small RNA fraction was extracted with a *mir*Vana miRNA Isolation Kit (Ambion) according to the manufacturer's instructions, and then treated with DNase (TURBO DNA-free Kit, Ambion) for either 20 min or 1 h depending on whether the source was cultured cells or tissue samples, respectively. U snRNAs were transcribed with specific primers as described previously (Zhang et al, 2008), and RNA levels were quantified with specific primers as described previously (Zhang et al, 2008) using the Syber Green system (Applied Biosystems). The primers we used are listed in Supporting

Information Table S3. All PCR reactions were performed in triplicate. RNA levels in samples were normalized with GAPDH for mRNA, and average of 5S and 5.8S for small RNA.

For more detailed Materials and Methods see the Supporting Information.

## Author contributions

HT and KY designed the study; HT, YI, AF and AK performed the experiments; YI, HH, NA, FT, YH, HA, SM and GS obtained the patient autopsy samples and performed neuropathological and clinical diagnosis; HH and SM advised the staining of human sample; YI, NA, FT and GS provided critical inputs for the manuscript; HT analysed the data and KY provided inputs to analysis; HT and KY wrote the manuscript. All authors approved the manuscript.

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Supporting Information is available at EMBO Molecular Medicine online.

The authors declare that they have no conflict of interest.

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RESEARCH PAPER

# Neck weakness is a potent prognostic factor in sporadic amyotrophic lateral sclerosis patients

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## ABSTRACT

**Objective** To clarify the emergence of muscle weakness in regions of the body that affect survival, and deterioration in activities of daily living (ADL) in amyotrophic lateral sclerosis (ALS) patients.

**Methods** We conducted a multicentre-based prospective cohort study of patients with ALS. We enrolled 401 sporadic patients with ALS. Death or the introduction of invasive ventilation was defined as the primary endpoint, and the time to five clinical markers of ADL deterioration associated with bulbar paralysis or limb weakness were defined as ADL milestones. Muscle weakness was assessed in the neck flexor muscles; the bilateral abductors of the shoulders; the bilateral wrist extensor muscles; the bilateral flexor muscles of the hips; and the bilateral ankle dorsiflexion muscles. We performed Cox proportional hazards regression analyses for the primary endpoint and the five ADL milestones, adjusting for known covariate prognostic factors for ALS.

**Results** The Medical Research Council (MRC) score for the neck flexors was the most significant prognostic factor for the primary endpoint (HR 0.74, p<0.001), *loss of speech* (HR 0.66, p<0.001), and *loss of swallowing function* (HR 0.73, p<0.001), and was one of the significant prognostic factors for *loss of upper limb function*, *difficulty turning in bed*, and *loss of walking ability* (p=0.001, 0.002, and 0.008, respectively). The MRC score for the neck flexors was also a significant prognostic factor for covariates of the previously reported prognostic factors.

**Conclusions** Neck weakness is an independent prognostic factor for survival and deterioration in ADL in Patients with ALS.

## INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterised by progressive upper and lower motor neuron loss, which leads to limb and bulbar paralysis and respiratory failure.[1] Symptoms develop at a progressive rate, and the median survival time from disease onset is 2–4 years.[2–4] However, patients with ALS show extensive variability in clinical courses, with durations ranging from a few months to more than 10 years. Furthermore, major symptoms that differentially affect activities of daily living (ADL) and prognosis also show variability among patients with different disease forms.[5] A better understanding of the factors influencing deterioration in ADL and prognosis would help physicians and patients determine whether and when to introduce non-invasive positive pressure ventilation, tube feeding, tracheostomy and artificial ventilation, and would lead to effective stratification strategies in clinical trials. Several studies have shown that age,[6–10] bulbar symptom onset,[6 7] respiratory function,[3 8 11 12] time from symptom onset to diagnosis,[2 6 10 13 14] functional score[2 14] and rate of disease progression[2 15–17] are predictors of survival. Muscle weakness in particular regions of the body affect the prognosis of ALS, although it has not been sufficiently determined which regions are most predictive.[18] To investigate the longitudinal course of patients with ALS and clarify the emergence of muscle weakness, which affects deterioration in ADL and ALS prognosis, we conducted a prospective, multicentre study.

## METHODS

### Patient registry and follow-up system

We constructed a multicentre registration and follow-up system called the Japanese Consortium for Amyotrophic Lateral Sclerosis research (JaCALS), which consists of 21 neurology facilities in Japan. Patients with ALS diagnosed in these facilities were consecutively registered with written informed consent. The ethics committees of all the participating institutions approved the study. Full clinical examinations were conducted at registration by neurologists in each of the respective institutions. Muscle strength was manually tested and scored with the scale of the Medical Research Council (six points, range: 0–5)[19] in nine muscle groups as follows: neck flexors; bilateral abductors of shoulders as representatives of proximal upper extremity muscles; wrist extensors muscles as representatives of distal upper extremity muscles; bilateral flexors of hips as representatives of proximal lower extremity muscles; and ankle dorsiflexion muscles as representatives of distal lower extremity muscles. All manual muscle testing was performed with standard positioning and procedures by certified neurologists.[20] The MRC score of the neck

flexors was determined with the patient in the supine position. We confirmed the inter-rater reliability of the manual muscle testing method employed in this study using 23 patients with neuromuscular disease. The values of the kappa statistics of each muscle ranged from 0.65 to 0.93. To standardise the procedures and the examinations, the three organising doctors (NA, RN, HaW) visited each participating facility and ascertained the evaluation methods for this study.

Disease onset was defined as when the patients became initially aware of muscle weakness or impairment of swallowing, speech, or respiration. We enrolled patients who fulfilled the revised El Escorial criteria.[21] The diagnostic accuracy of the enrolled patients was then assessed by members of the steering committee of the JaCALS. Included patients were then followed-up with telephone surveys conducted by clinical research coordinators (CRC) or with examinations by neurologists every 3 months, and the degree of deterioration in ADL was determined at each time point. We employed the Japanese version of the ALSFRS-R as a scale for ADL, which was validated by Ohashi *et al*, using a telephone survey system.[22] We confirmed the reliability of the telephone survey system for the Japanese version of the ALSFRS-R previously,[23] and the English version of the telephone survey system has been confirmed in several previous studies.[24–26] Prior to the study, we informed and trained the CRCs of the study plan, procedures for the telephone survey, ethical issues relevant to the study, and requisite considerations for patients with ALS and caregivers, and then provided them with a general knowledge of ALS.

We defined a primary endpoint and ADL milestones in the disease course of the patients with ALS and determined their occurrence by telephone survey or examinations by neurologists. The introduction of tracheostomy positive pressure ventilation (TPPV) or death of the patient was defined as the primary endpoint, and TPPV-free survival was defined as survival. *Loss of speech function*, *loss of swallowing function*, *loss of upper limb function*, *difficulty turning in bed*, and *loss of walking ability* were set as ADL milestones. The time at which each ADL milestone occurred was defined as follows: *loss of speech function* was determined to have occurred when non-vocal communication became needed; *loss of swallowing function* was determined to have occurred when parenteral or enteral feeding became needed exclusively; *loss of upper limb function* occurred when the patient needed to be fed and became unable to grip a pen; *difficulty turning in bed* occurred when the patient became unable to turn in bed alone; *loss of walking ability* occurred when the patient became unable to walk without assistance.

### Patients

A total of 520 patients with ALS were initially registered in the JaCALS from January 2006 to June 2011. We excluded 26 patients with known gene mutations: 17 patients with SOD-1 mutations, two patients with TDP-43 mutations, two patients with FUS/TLS mutations, three patients with angiogenin mutations, and two patients with C9ORF72 repeat expansions. We also excluded 13 patients with family histories of ALS and 40 patients who were categorised as clinically possible or suspected according to the revised El Escorial criteria. An additional 20 patients for whom we could not obtain follow-up information to their refusal to participate in the telephone survey were also excluded. Twenty patients were excluded due to invalid data. The study finally included 401 sporadic patients with ALS diagnosed as clinically definite, probable, or probable laboratory-supported. Of these, 382 patients were followed for more than a year or died within a year of registration, and 19 patients were censored within a year from registration. Eleven patients declined the telephone survey during the course of the study, and we lost contact with eight patients during the survey.

### Statistical analysis

The clinical data of the registered patients were anonymised in each participating facility of the JaCALS and assigned unique patient numbers. The data were then sent to the clinical data centre located at the Nagoya University Graduate School of Medicine and inputted into the JaCALS database.

We performed Cox proportional hazards regression analyses for the time of registration to the primary endpoint or onset of each ADL milestone to evaluate the impact of muscle weakness on the time to the primary endpoint and each decline in ADL. Specifically, for the primary endpoint and each ADL, we evaluated the HR for the MRC scores in nine muscle groups (ie, neck flexors, left and right abductors of shoulders, wrist extensor muscles, flexors of hips and ankle dorsiflexion muscles) at registration, identifying the muscles groups associated with the primary endpoint and five common ADL milestones. Additionally, we examined the HR for each muscle group after adjusting for known prognostic factors as follows: age at registration,[6–10] gender (male vs female),[6 27] disease duration,[2 6 10 13 14] percent vital capacity (%VC),[3 8 11 12] ALSFRS-R score,[14] riluzole use (yes vs no),[28] bulbar symptom,[6 7] and classification according to the revised El Escorial criteria (definite vs probable or probable laboratory-supported).[7 8 10 14] We compared the time from registration to the primary endpoint or each of the previously defined ADL milestones in the patients divided by their degree of muscle weakness using the Kaplan–Meier method. The log-rank test was used to test the null hypothesis that all the Kaplan–Meier curves were equal. A two-sided $p<0.05$ was considered statistically significant. All statistical analyses were conducted using the PASW V.18.0 program (SPSS Inc, Chicago, Illinois, USA).

## RESULTS

### Demographic characteristics of the registered patients

The patient sample comprised 244 men and 157 women. The median age at disease onset was 62.2 years (IQR: 53.5–68.5 years), and the mean follow-up period was 2.1±1.5 years. The follow-up rate at 1 year after registration was 95.3%. As initial symptoms, 47.4% of the patients showed upper limb weakness, 31.4% lower limb weakness, 22.9% dysarthria, 5.5% dysphagia and 2.0% cervical weakness. At registration, the median score on the ALSFRS-R was 38 (IQR: 32–42). (see online supplementary table S1).

### Identification of weakened muscle groups that affect survival and progression to the ADL milestone

#### Cox proportional hazard regression analyses for the primary endpoint and the ADL milestones

Table 1 shows the results of Cox proportional hazard regression analyses for the primary endpoint and the five ADL milestones, including the MRC scores of the nine muscle groups. The MRC score for the neck flexors was the most significant negative prognostic factor for the primary endpoint, *loss of speech*, and *loss of swallowing function* (HR 0.74, $p<0.001$, HR 0.66, $p<0.001$, HR 0.73, $p<0.001$, respectively). For the *loss of upper limb function*, *difficulty turning in bed* and *loss of walking ability*, the MRC score for the neck flexors was a significant negative prognostic factor (HR 0.77, $p=0.001$, HR 0.77, $p=0.002$, and HR 0.80, $p=0.008$, respectively). Whereas, the MRC score for the left wrist extensors was a significant positive prognostic factor for the primary endpoint and each ADL milestone except for *difficulty turning in bed*.

**Table 1** Multivariate Cox regression analyses for the primary endpoint and each activity of daily living milestone using the MRC score of each muscle group at registration

| | Primary endpoint | | Loss of speech | | Loss of swallowing function | | Loss of upper limb function | | Difficulty turning in bed | | Loss of walking ability | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Muscle group | HR (95% CI) | p Value | HR (95% CI) | p Value | HR (95% CI) | p Value | HR (95% CI) | p Value | HR (95% CI) | p Value | HR (95% CI) | p Value |
| Neck flexors | 0.74 (0.65to 0.86) | <0.001 | 0.66 (0.56 to 0.76) | <0.001 | 0.73 (0.63 to 0.85) | <0.001 | 0.77 (0.66 to 0.89) | 0.001 | 0.77 (0.66 to 0.91) | 0.002 | 0.80 (0.67 to 0.94) | 0.008 |
| Left shoulder abductors | 0.87 (0.69 to 1.11) | 0.266 | 0.89 (0.69 to 1.14) | 0.363 | 0.89 (0.71 to 1.12) | 0.309 | 0.62 (0.49 to 0.79) | <0.001 | 0.72 (0.56 to 0.93) | 0.012 | 0.75 (0.57 to 1.00) | 0.049 |
| Right shoulder abductors | 0.98 (0.77 to 1.25) | 0.890 | 1.11 (0.87 to 1.43) | 0.399 | 1.02 (0.81 to 1.29) | 0.867 | 1.19 (0.94 to 1.50) | 0.159 | 1.08 (0.85 to 1.39) | 0.529 | 0.99 (0.75 to 1.30) | 0.917 |
| Left wrist extensors | 1.29 (1.04 to 1.59) | 0.018 | 1.28 (1.03 to 1.59) | 0.026 | 1.24 (1.02 to 1.51) | 0.034 | 1.42 (1.14 to 1.77) | 0.002 | 1.24 (1.00 to 1.55) | 0.054 | 1.39 (1.08 to 1.79) | 0.010 |
| Right wrist extensors | 0.90 (0.74 to 1.08) | 0.254 | 0.88 (0.73 to 1.07) | 0.193 | 0.92 (0.77 to 1.11) | 0.380 | 0.73 (0.60 to 0.88) | 0.001 | 0.80 (0.66 to 0.98) | 0.029 | 0.98 (0.79 to 1.22) | 0.884 |
| Left hip flexors | 0.99 (0.72 to 1.36) | 0.964 | 0.96 (0.73 to 1.28) | 0.791 | 0.85 (0.62 to 1.15) | 0.284 | 0.74 (0.55 to 0.99) | 0.040 | 0.77 (0.56 to 1.06) | 0.115 | 0.90 (0.61 to 1.32) | 0.585 |
| Right hip flexors | 0.96 (0.69 to 1.34) | 0.830 | 0.95 (0.70 to 1.28) | 0.719 | 1.09 (0.79 to 1.50) | 0.613 | 1.18 (0.87 to 1.62) | 0.290 | 1.18 (0.84 to 1.66) | 0.331 | 1.06 (0.69 to 1.64) | 0.788 |
| Left ankle extensors | 1.14 (0.93 to 1.40) | 0.214 | 1.13 (0.94 to 1.34) | 0.185 | 1.14 (0.95 to 1.37) | 0.166 | 1.26 (1.04 to 1.52) | 0.021 | 1.09 (0.91 to 1.30) | 0.367 | 0.93 (0.71 to 1.21) | 0.583 |
| Right ankle extensors | 0.94 (0.76 to 1.15) | 0.530 | 0.95 (0.79 to 1.14) | 0.564 | 0.94 (0.77 to 1.14) | 0.539 | 0.85 (0.70 to 1.04) | 0.125 | 0.81 (0.68 to 0.97) | 0.023 | 0.72 (0.57 to 0.91) | 0.007 |

According to table 1, the MRC score for the neck flexors was commonly identified as a possible prognostic factor for the primary endpoint and the five ADL milestones. We further examined its impact after adjusting for the other established or potential risk factors, that is, age at registration, gender, disease duration from onset to registration, percent vital capacity (% VC) at registration, ALSFRS-R score at registration, classification according to revised El Escorial criteria, riluzole use and bulbar symptom at registration (table 2). As seen in table 2, the MRC score for the neck flexors was an independent and significant prognostic factor for the primary endpoint, *loss of speech*, *loss of swallowing*, *loss of upper-limb function* and *difficulty turning in bed* in patients with ALS except for *loss of walking ability*. ($p<0.001$, $p=0.001$, $p=0.003$, $p<0.001$, $p=0.027$, respectively). At registration, there were moderate and significant correlations between the MRC score for the neck flexors and the % VC or the ALSFRS-R score. Pearson's correlation coefficients were 0.367 ($p<0.001$) and 0.496 ($p<0.001$), respectively.

### Differences in survival time and time to ADL milestones in patients in terms of the MRC score grade for the neck flexors

We divided the registered patients into four categories according to their MRC score for the neck flexors (ie, 5, 4, 3 and ≤2). Figure 1 shows the Kaplan–Meier curves for the four categories for the primary endpoint and each ADL milestone. All the differences between the curves were significant according to a log-rank test ($p<0.001$).

## DISCUSSION

In a prospective and multicentre cohort study, we identified that weakness of the neck flexors is a potent factor for the prediction of survival and for the deterioration of ADL, such as speech, swallowing, upper limb function, turning in bed, and walking, in sporadic patients with ALS.

The neck flexors consist of the sternocleidomastoid muscle (SCM), the platysma muscle, hyoid muscle, longus capitis muscle, longus colli and scalenus. These muscles are innervated by motor neurons in the cervical cord (C1–8) and accessory nerve nuclei,[29 30] primarily the C2–4 segments. By contrast, respiratory muscles consist of the diaphragm and the internal and external intercostals muscles, which are innervated by motor neurons of the upper cervical cord (C3–5) and thoracic cord (Th1–Th12), respectively.[30] Thus, the muscles for neck flexion and those for respiration partially share spinal segments of the motor neuron column for their motor innervations. Furthermore, significant correlations are present between compound muscle action potentials of the SCM and those of the diaphragm in patients with ALS,[31] suggesting that neck muscle weakness is correlated with weakness of the diaphragm to some extent in ALS. Because the main cause of death in patients with ALS is respiratory insufficiency, it is reasonable that neck flexor weakness was associated with respiratory impairments and, eventually, survival time. The motor response amplitude of the phrenic nerve motor neurons which are located in the C3–5 segments has been shown to be a significant prognostic factor for survival in patients with ALS.[32] This supports our findings.

Why then is weakness of the neck flexors a determinant factor for the deterioration of ADL for speech, swallowing, upper limb function, truncal turning and walking ability? Recently, some studies have suggested that the degeneration of motor neurons is initially a focal process in ALS that later spreads contiguously throughout the three-dimensional anatomy of connected or neighbouring neurons.[33–36] Dysfunction of speech and swallowing involves the impairment of motor

**Table 2** Multivariate Cox regression analyses with the adjustments of the covariates that we selected for the primary endpoint and each activity of daily living milestone using known predictive factors

| | Primary endpoint | | Loss of speech | | Loss of swallowing function | | Loss of upper limb function | | Difficulty turning in bed | | Loss of walking ability | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HR (95% CI) | p Value | HR (95% CI) | p Value | HR (95% CI) | p Value | HR (95% CI) | p Value | HR (95% CI) | p Value | HR (95% CI) | p Value |
| MRC score of neck flexors at registration | 0.72 (0.62 to 0.83) | <0.001 | 0.78 (0.67 to 0.90) | 0.001 | 0.80 (0.69 to 0.93) | 0.003 | 0.76 (0.65 to 0.88) | <0.001 | 0.83 (0.70 to 0.98) | 0.027 | 0.95 (0.79 to 1.15) | 0.601 |
| Age at registration (years) | 1.03 (1.02 to 1.04) | <0.001 | 1.02 (1.01 to 1.03) | 0.002 | 1.03 (1.01 to 1.04) | <0.001 | 1.01 (0.99 to 1.02) | 0.264 | 1.01 (0.99 to 1.02) | 0.265 | 1.00 (0.98 to 1.01) | 0.890 |
| Gender (male vs female) | 1.14 (0.85 to 1.52) | 0.381 | 0.85 (0.65 to 1.12) | 0.247 | 1.13 (0.85 to 1.49) | 0.398 | 1.27 (0.97 to 1.68) | 0.088 | 1.01 (0.76 to 1.33) | 0.947 | 0.85 (0.61 to 1.17) | 0.309 |
| Duration from onset to registration (years) | 0.64 (0.57 to 0.72) | <0.001 | 0.72 (0.64 to 0.80) | <0.001 | 0.69 (0.62 to 0.77) | <0.001 | 0.82 (0.75 to 0.9) | <0.001 | 0.74 (0.65 to 0.85) | <0.001 | 0.75 (0.66 to 0.87) | <0.001 |
| %VC at registration | 0.98 (0.98 to 0.99) | <0.001 | 0.98 (0.97 to 0.99) | <0.001 | 0.98 (0.98 to 0.99) | <0.001 | 0.99 (0.98 to 1.00) | 0.001 | 0.99 (0.99 to 1.00) | 0.007 | 1.00 (0.99 to 1.00) | 0.491 |
| ALSFRS-R at registration | 0.97 (0.94 to 0.99) | 0.008 | 0.99 (0.97 to 1.02) | 0.483 | 0.96 (0.93 to 0.98) | 0.001 | 0.96 (0.94 to 0.98) | 0.001 | 0.89 (0.86 to 0.92) | <0.001 | 0.91 (0.88 to 0.94) | <0.001 |
| El Escorial criteria (probable or probable laboratory-supported) | 0.72 (0.53 to 0.99) | 0.043 | 0.61 (0.45 to 0.82) | 0.001 | 0.76 (0.56 to 1.04) | 0.087 | 0.67 (0.50 to 0.90) | 0.007 | 0.71 (0.52 to 0.97) | 0.031 | 0.63 (0.44 to 0.88) | 0.008 |
| Riluzole administration | 1.02 (0.75 to 1.37) | 0.916 | 1.09 (0.82 to 1.44) | 0.551 | 0.97 (0.73 to 1.29) | 0.843 | 0.95 (0.72 to 1.25) | 0.694 | 0.84 (0.63 to 1.13) | 0.258 | 0.94 (0.68 to 1.31) | 0.721 |
| Bulbar symptom at registration | 0.91 (0.67 to 1.22) | 0.524 | 2.04 (1.52 to 2.73) | <0.001 | 1.41 (1.06 to 1.86) | 0.018 | 0.68 (0.50 to 0.93) | 0.015 | 0.63 (0.47 to 0.84) | 0.002 | 0.68 (0.49 to 0.96) | 0.028 |

%VC, percent vital capacity; ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale.

neurons relayed via the glossopharyngeal, vagus, accessory and hypoglossal nerves to the medulla oblongata.[30] The medulla oblongata and cervical cord motor neurons innervating the neck flexion muscles are anatomically different in their three-dimensional layering, while these two groups of neurons are rather contiguously located. Thus, it may be speculated that if the contiguous spreading of motor neuron degeneration occurs according to the local spreading hypothesis, neck flexion impairment may eventually affect speech and swallowing functions. Furthermore, motor neurons for the neck flexion muscles, which are located in the C1–8 segments,[29 30] are also contiguous or overlapping with those for the upper limb muscles in the C5–Th1 segments, particularly the proximal upper limb muscles.[29 30] Neck flexion and upper limb function may be correlated with disease progression through the local spreading view of motor neuron degeneration. Truncal turning and walking require not only lower limb muscle activities but also power in a broad area of the chest, abdominal and back muscles, which are innervated by the cervical to lumbar cord.[37–39] Therefore, propagation of weakness from the cervical and lumbar areas may affect truncal turning or walking. We need, however, further investigations to demonstrate the underlying mechanisms of the correlation between the neck muscles and other muscles of the body that together determine ADL.

In this study, the MRC score for the left wrist extensors shows a positive prognostic factor for the primary endpoint and some ADL milestones, the reason for which might be that the weakness of the distal muscle in the non-dominant arm was least relevant to survival, or ADL declines so that it was shown to be a positive factor in the multivariate analyses.

A number of studies have demonstrated survival curves for patients with ALS and some factors that influence these survival curves.[18] The majority of these studies have found that older age is a strong risk factor for shorter survival in patients with ALS,[6–10] and the onset of bulbar symptoms is associated with a worse prognosis than the onset of spinal symptoms.[6 7] Several studies have found that a longer diagnostic delay correlates with a better prognosis,[2 6 10 13 14] and that a lower %VC or a percent forced vital capacity (%FVC) is correlated with shorter survival for patients with ALS.[3 8 11 12] The progression rate of the ALSFRS-R at the time of diagnosis was also related to ALS prognosis.[17] Neck flexor weakness has not been listed as a prognostic factor for patients with ALS, and most of these studies evaluated survival alone as an endpoint, and did not determine the onset of loss of speech, swallowing, limb and truncal function. In this study, we showed that neck flexor weakness was not only a novel prognostic factor for survival but also a significant prognostic marker for non-survival events related to ADL decline for patients with ALS.

In the course of ALS, patients must make some difficult decisions, including the use of gastrostomy for tube feeding, the use of assisted ventilation, and end-of-life planning, which require the support of the attending physician and a multidisciplinary team. All patients with ALS should be provided with sufficient information concerning these interventions and given sufficient opportunity for the careful consideration of their decision. In the medical, nursing and social care of patients with ALS, simple and robust indicators for predicting the status of each patient for several months or a year after diagnosis are necessary for patient management. Medical staff and caregivers need to have a predictor of the patient's status in the near future, including survival prognosis and also estimates for the loss of speech, swallowing, limb and truncal function. Our findings may contribute to such prediction.

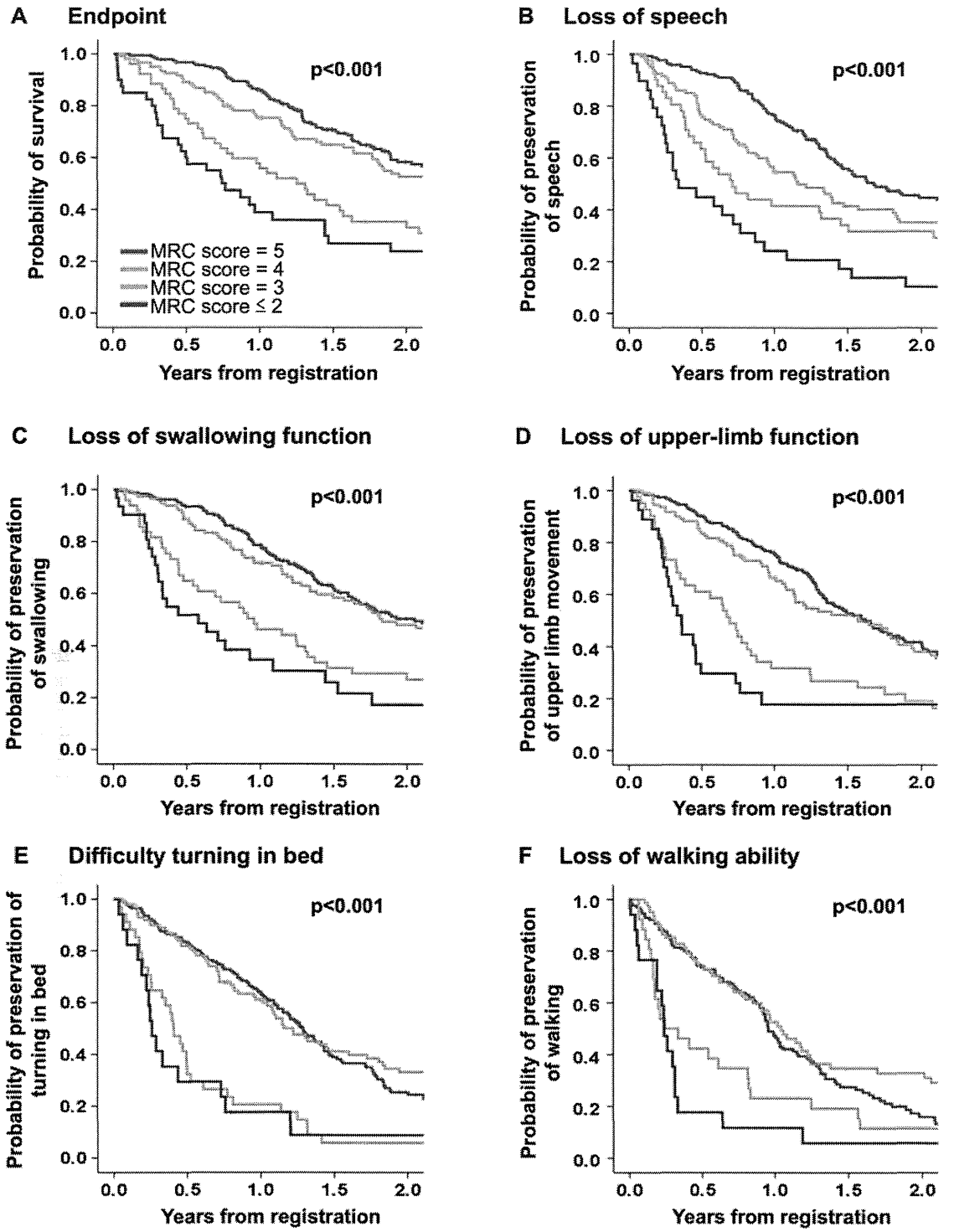


**Figure 1** Kaplan–Meier curves according to the MRC score for the neck flexors. The Kaplan–Meier curves for the primary endpoint and each activity of daily living milestone among four categories divided according to the MRC score for the neck flexors were compared by the log-rank test. Curves are shown for the MRC score 5 (blue), the MRC score 4 (green), the MRC score 3 (orange), and the MRC score ≤2 (purple) groups. All the differences of the curves were significant (p<0.001).

The course of ALS is highly variable between patients,[5] which is one of the major factors limiting the power of ALS clinical trials.[40 41] Therefore, robust stratification factors that could divide ALS patient groups depending upon prognosis are needed for designing trials. Compared with known prognostic factors for patients with ALS, such as age, duration from onset to registration, ALSFRS-R at registration, and presence of bulbar symptom, weakness of the neck flexors was a potent and independent prognostic factor. Thus, the MRC score for the neck flexors might be used for stratification factor in a future clinical trial.

Neck extensor muscle weakness with head drop as an early symptom has been reported in a few patients with ALS.[42 43] However, Katz *et al*[44] wrote that neck flexor weakness is typically observed. We assert that neck flexor weakness is commonly observed in patients with ALS, and is useful for the prediction of prognosis.

The limitations of this study are as follows: registered patients were followed-up by telephone survey, and we did not examine longitudinal changes in the strength of multiple muscles. As we demonstrated, the relationship between the involved muscle groups and survival prognosis and estimates of ADL deterioration would offer insights into the modalities of progression in patients with ALS. However, to examine the pattern of spread more precisely, a cohort study that observes longitudinal changes in the strength of muscle groups and extensions of muscle weakness will be required.

This study analysed a multicentre cohort of patients with ALS in a single nation, Japan. Although the clinical profiles of ALS are broadly similar among countries in previous reports, the outcome of our study would be better confirmed in cohorts of patients with ALS in multiple countries.

In conclusion, we showed that neck weakness is an independent prognostic factor for survival and deterioration in ADL in patients with ALS. We hope that our report will be helpful for clinicians who want to provide medical, social and nursing care to patients with ALS with proper timing, and to researchers as they plan clinical trials for ALS.

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ORIGINAL ARTICLE

# Tracheostomy with invasive ventilation for ALS patients: Neurologists' roles in the US and Japan

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**Abstract**

Our objective was to determine whether substantial differences in rates of TIV utilization in the U.S. and Japan are associated with the role of the treating neurologist. Questionnaires in English and Japanese were sent to neurologists who treated ALS patients in both countries. Questions included queries about rates of TIV use in their practices, level of encouragement of TIV use, the role of the neurologist in TIV decision making, management of patient/family requests to discontinue TIV once initiated, and personal choices if neurologists themselves had ALS. Results showed that 84% of American neurologists reported fewer than 10% of their patients had TIV, compared to 32% of Japanese. Americans less often encouraged TIV use (79% of American and 36% of Japanese seldom or never suggested or encouraged TIV). Finally, neurologists were asked whether they would choose TIV for themselves in the hypothetical scenario where they had ALS: over 70% of both groups declined TIV for themselves. In conclusion, consistent with past findings, Japanese neurologists were more likely to recommend TIV and more of their patients received TIV. Both groups believed their recommendations influence patient decisions. While Americans seldom recommended TIV to patients and most would not choose TIV for themselves, Japanese neurologists' recommendations and personal choices diverged.

**Key words:** *TIV, tracheostomy, ALS, end-of-life decisions*

## Introduction

ALS is a progressive, incurable, irreversible neurodegenerative disease that inexorably progresses to a point where respiratory capacity fails, even with non-invasive ventilatory support. At this point, a choice must be made between tracheostomy with invasive (long-term) mechanical ventilation (TIV or LTMV, we use here TIV), and palliative care with subsequent respiratory failure in the short-term future. With TIV, ALS continues to progress to the point where some patients may reach a 'locked-in' state in which they cannot communicate effectively, but breathing is maintained indefinitely. Survival after TIV varies widely, from months (1) to several years (2).

Substantial disparities in rates of TIV utilization by ALS patients have been noted for some time (3). Rates vary widely between countries, regions within countries, and even within institutions (3,4). Among American studies, TIV rates of 2% to 6% have been reported (5,6). In European studies, TIV use rates have ranged from 0% to 10.6% (1,7). In contrast, Japanese studies report rates of TIV use ranging from 25% to 46% (8–10).

Several explanations have been offered to account for this variation in TIV use, including different conceptualizations of the physician's role in medical decision-making, attitudinal differences of health care professionals, national variations in health insurance coverage and availability of institutional facilities, varying degrees of commitment to a life-prolonging approach (8), and availability of alternatives such as hospice.

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We conducted a survey of American and Japanese neurologists who treat ALS patients in order to determine whether these differences in rate of TIV utilization still persist, and to identify possible contributing factors.

## Methods

### *Sample*

In the United States, we (HM) sent e-mails to 324 neurologists who were directors of ALS clinics and adult MDA clinics and their affiliates. Recipients could respond on the internet (using Survey Monkey) or by returning questionnaires as e-mail attachments. In Japan, questionnaires were mailed to 210 neurologists known to see ALS patients (selected by Ogino et al.).

### *Questionnaire*

The questionnaire was written by the New York team (HM, JR with input from colleagues), with 24 fully structured multiple-choice items, several with subscales. Areas covered are shown in Table I. Questions were based on previous findings as well as clinical observations and published suggestions of other investigators (1,8). In order to preserve anonymity, minimal personal information was sought. The instrument was translated into Japanese and then reviewed and edited by MO and the Japanese TIV Study Group members. Questions about withdrawal of tracheostomy were modified to be appropriate in Japan. The protocol was approved by the New York State Psychiatric Institute – Columbia University Department of Psychiatry IRB. Data were collected during 2011.

Table I. Components of TIV questionnaire.

| Domain | Number of items |
|---|---|
| 1. Neurologists' experience in general practice, and experience with ALS patients | 3 |
| 2. Doctor-patient communications: initial diagnosis; discussions of advance directives; assisted suicide | 10 |
| 3. Beliefs about the doctor's role in TIV decision-making; percentage of patients with TIV in own practice. How often they suggest and encourage TIV | 4 |
| 4. Attributes of patients and perceived patient preferences that influence their decision-making about TIV; practices regarding TIV discontinuation | 9* |
| 5. Personal choices if the neurologist him/herself had ALS (PEG, NIV, TIV) | 3 |

*Includes subscale queries about 16 patient/situational attributes and 12 reasons patients may or may not want TIV.

### *Statistical analysis*

Comparisons of responses of American and Japanese neurologists were first analyzed using $\chi^2$ tests or *t*-tests. Logistic and multiple regression analyses were also performed. For items with skewed response distributions, Mann-Whitney *U*-tests were used. For those with a 1–10 response format with anchors, ranging from 1 = 'almost never' to 10: 'almost always', responses of 1–3 were classified as 'low' or 'seldom/never'; scores of 4–7 = 'medium' or 'some of the time'; scores of 8–10 as 'high' or 'very often/almost always'. In those analyses regarding neurologists' degree of enthusiasm in recommending TIV to their patients, the 1–10 scale was dichotomized to reflect less (1–5) and more (6–10) enthusiasm. Because of the multiple comparisons examined, trend differences are not reported. Bonferroni corrections were not applied because this is an exploratory study. Alpha = .05, two-tailed.

## Results

### *Samples*

One hundred American neurologists in 44 states returned questionnaires, including 258 comments about various issues, a response rate of 31%. In Japan, 120 neurologists (57% response rate) living in all eight provinces, responded to mailed surveys, including 163 written comments. Respondents were highly experienced: over 85% had practiced neurology for >10 years (87% of U.S. and 90% of Japanese respondents), and over half of each group had practiced neurology 20+ years ($\chi^2 = 0.18$, 1df, $p = .672$). However, Americans were far more specialized regarding ALS treatment: 71% had seen more than 20 new patients in the past year, compared to 10% of Japanese ($\chi^2 = 87.79$, 1df, $p < .0001$). Similarly, 77% of American neurologists were currently treating >20 patients/year vs. 11% of Japanese neurologists ($\chi^2 = 100.43$, 1df, $p < .0001$).

### *Question 1. Do diagnostic styles (communication of initial diagnosis, discussion of advance directives, and frequency of requests for assisted suicide) vary by country?*

There were more similarities than differences in diagnostic styles and management of advance directives among American and Japanese neurologists. Most said they "tell the diagnosis as clearly as possible", although more Japanese neurologists said they "tell in different words, somewhat vaguely", and 6% of Japanese and no Americans said they defer telling at all (2×3 cross-tabulation analysis, FET = .017). Thus we focus on the data more directly associated with TIV. Regarding when neurologists raise the issue of future preferences, there

were no major differences in timing. However, while advance directives as independent documents are widely used in the U.S., they are not in Japan. Rather, Japanese patients who express preferences have their wishes noted in their charts.

*Assisted suicide.* Neurologists were asked whether any of their patients raised the issue of physician assisted suicide. Keeping in mind that Americans saw far more ALS patients than Japanese respondents did, and thus were more likely to hear such requests, 51% of their patients vs. 25% of Japanese patients had made such requests ($\chi^2 = 15.25$, 1df, $p < .0001$).

*Question 2. Do rates of TIV use in neurologists' practices vary by country? Do they differ in the extent to which they suggest and encourage use of TIV?*

Respondents were asked "in your own practice, about what % of patients get TIV?" Response options were "1–2%, 3–9%, 10–25%, 26–49%, 50+%". Eighty-four percent of American neurologists reported fewer than 10% of their patients had TIV, compared to 32% of Japanese. In contrast, 38% of Japanese but only 5% of Americans reported that more than 25% of their patients had TIV ($\chi^2 = 62.08$, 5df, $p < .0001$). Overall, TIV rates continue to show substantial differences between countries.

We then asked "While there are probably always exceptions, in general do you suggest and encourage the use of TIV?" Similarly large differences were seen between samples, with substantially higher rates of encouragement among Japanese neurologists. Classifying the 1–10 rating scale into never/seldom, moderately often, and very often/almost always, 79% of American and 36% of Japanese neurologists seldom or never encouraged and suggested TIV, 20% of American and 58% of Japanese said sometimes, and 1% of U.S. and 6% of Japanese said almost always ($\chi^2 = 39.36$, 2df, $p < .0001$).

*Question 3. The role of the neurologist in TIV decision making*

Three possible roles for physicians in medical decision-making were offered: 1) It is my responsibility to make decisions for the patient and inform them what will be done; 2) My role is to present treatment options along with my recommendations; 3) I present the options and let them decide.

American neurologists never endorsed the first (doctor decides) and overwhelmingly (70%) chose the second – present options and their recommendations, while Japanese neurologists either agreed that it is their responsibility to make decisions (18%) or present options without a recommendation to let the patient decide (58%). The overall $\chi^2 = 67.19$, 2df, $p < .0001$.

*Is there a relationship between size of ALS caseload and level of enthusiasm for TIV?* We examined number of patients currently followed and likelihood of recommending TIV. We used a cut-off of 1–10 patients vs. 11+ patients, and dichotomized the 1–10 rating scale, asking "In general do you suggest and encourage use of TIV?" (1–5 = less encouragement, 6–10 = more encouragement). Among neurologists seeing < 10 patients, TIV was not encouraged by 71% of American and 81% of Japanese neurologists ($\chi^2 = 0.21$, 1df, $p = .647$, NS). There was, however, a difference among those who saw 11+ patients: 95% of American neurologists did not recommend TIV compared to 68% of Japanese ($\chi^2 = 15.06$, 1df, $p = .0001$).

*Is neurologists' level of encouragement of TIV related to percent of their patients with TIV?* Our data show such a relationship. As shown in Table II, patients were more likely to have TIV if their doctors encouraged its use in both Japanese ($\chi^2 = 12.94$, 2df, $p = .002$) and American ($\chi^2 = 18.26$, 2df, $p < .0001$) samples.

Table II. Relationship between neurologist's level of encouragement in recommending TIV[1] and the percent of their patients who receive TIV.

| | Patients in practice receiving TIV | | | |
|---|---|---|---|---|
| Encouragement for TIV | 0–9 % | 10 – 25 % | 26+% | |
| U.S. | | | | |
| Less | 75 (87%) | 9 (10%) | 2 (2%) | $\chi^2 = 18.26$, df = 2, $p < .0001$ |
| More | 4 (50%) | 1 (13%) | 3 (38%) | |
| Japan | | | | |
| Less | 33 (38%) | 28 (33%) | 25 (29%) | $\chi^2 = 12.94$, df = 2, $p = .002$ |
| More | 3 (12%) | 5 (20%) | 17 (68%) | |

[1]Neurologists were asked "In general do you suggest and encourage the use of TIV rather than comfort care?" rated on a 1 (= almost never) to 10 (= almost always) scale which we dichotomized here.

Note: Based upon dichotomizing Encouragement for TIV, only eight of 94 (8.5%) U.S. neurologists were more encouraging of TIV compared to 25 of 111 (22.5%) of the Japanese neurologists. The 2 × 2 cross-tabulation for samples and the dichotomized encouragement exhibited a significant $\chi^2 = 7.40$, 1 df, $p < .01$.

*Question 4. Is neurologists' length of experience with ALS or their perception of patient characteristics related to their encouragement/advice about TIV?*

*Neurologists' experience and encouragement of TIV.* The dependent variable was percent of neurologists' own patients who have TIV. Predictor variables included years practicing neurology, number of new ALS patients seen in past year, and number of ALS patients currently followed. In both samples, only years of practice of neurology was positively associated with higher percent of patients with TIV (U.S.: Beta = .244, $t = 2.44$, $p = .017$; Japanese sample Beta = .234, $t = 2.52$, $p = .013$). Direct experience with ALS patients (new patients seen in past year, number currently followed) was unrelated to percent of own patients with TIV.

*Perceived patient attributes that influence neurologists' decision-making about TIV.* Neurologists were asked to rate the importance of each of 14 patient attributes in their decision-making and recommendations for TIV, on a 1−10 scale with higher numbers signifying greater importance. Scores on virtually all scales were skewed in the 'important' direction, but there were group differences. We performed a principal component analysis using varimax rotation with Kaiser normalization and identified four factors, as shown in Table III. The samples differed on three of the four factors: Japanese respondents considered Factor 1 (hope for the future) and Factor 3 (younger, young children, believe in cure) as more important than Americans, while U.S. respondents weighed Factor 4, patient functioning, as more important than Japanese respondents did. Regarding the influence of financial considerations on their recommendations, the majority of both American and Japanese neurologists agreed that they were highly important (55% vs. 63%, $\chi^2 = 1.59$, 1df, p = .451).

*Perceived reasons that patients decide against TIV.* Respondents were asked "When patients choose not to have TIV, what are their reasons?" Seven reasons were listed. As shown in Table IV, responses to five of the seven options differed by country. More American neurologists endorsed the reasons "patient so disabled that he/she is ready to go", "financial burden", and "can't be cared for at home and doesn't want to go to facility". More Japanese neurologists endorsed the items "because it is illegal to discontinue TIV once started", and "patient doesn't want to live any longer".

Table III. Patient attribute factors that may influence neurologists' decision-making about TIV (1−10 scale: higher= more important).

| Factor | U.S. ($n = 93$) Mean (SD) | Japan ($n = 120$) Mean (SD) | $t$-test, df, $p$ |
|---|---|---|---|
| 1. Hope | 7.38 (2.00) | 8.23 (1.45) | 3.42, 161, .001 |
| 2. Support | 7.74 (1.99) | 7.57 (1.71) | 0.66, 211, .509 |
| 3. Cure/Age | 5.29 (1.85) | 6.47 (1.97) | 4.43, 211, <.001 |
| 4. Function | 8.12 (2.27) | 7.28 (2.16) | 2.74, 211, .007 |

Factor 1: patient characteristics of hope for the future and capacity for enjoyment in the absence of depression and cognitive impairment.
Factor 2: external support (financial and familial).
Factor 3: having young children, being younger, and believing that an ALS cure is imminent.
Factor 4: poor patient functioning and medical comorbidities.

*Perceived reasons that patients decide in favor of TIV.* Neurologists were asked "What factors do you think influence the patient's decision to choose TIV?" Five options were listed. Most neurologists (63% and 69%) agreed that "the way the doctor explains TIV" is influential ($\chi^2 = 0.93$, 1df, p= .335). Japanese neurologists were far more likely to think that patients are influenced by availability of facilities for ALS patients (72% vs. 36%, $\chi^2 = 24.36$, 1df, $p < .0001$). Questions about frequency of communication with patients who have TIV, and financial factors did not differ by country: about half of all neurologists thought communication with patients who had TIV was an influence ($\chi^2 = .021$, 1df, p= .647), and the majority thought financial factors were influential (67% vs. 55%, $\chi^2 = 2.59$, 1df, p= .108).

*Percent of patients with TIV having emergency intubation.* The range of reported rates was zero to 100%. We classified responses as 0−9%, 10−19%, and 20+%, based on skew in response distributions. Overall, about one-third of neurologists in both countries reported that more than 20% of their patients received TIV on an emergent basis without an advance directive requesting it ($\chi^2 = 5.71$, 2df, p= .058).

*TIV discontinuation.* Neurologists were queried whether they asked patients starting TIV to specify circumstances in which they would want it discontinued. Seventy-one percent of American and 8% of Japanese neurologists reported such conversations ($\chi^2 = 24.18$, 1df, $p < .0001$). They were asked whether patients or family had requested discontinuation of TIV, and if yes, whether they had done so. Seventy-one percent of American and 49% of Japanese neurologists reported being asked to discontinue TIV ($\chi^2 = 8.39$, 1df, $p = .004$). Of the 63 Americans asked, 78% had agreed to do so, usually after consulting their hospital ethics committee or conferring with their palliative care team. Others referred the family to hospice. Most Japanese told their patients that it is not supported legally to discontinue TIV, while 12% just said it should not be removed. Finally, the proportion of patients who actually had discontinued TIV differed substantially: 39% of Americans reported that more than a quarter of their patients had done so, compared to none in Japan (FET <.0001).

Table IV. Perceived determinants of patients' decisions regarding TIV; number (%) of neurologists who endorse each reason.

| | U.S. Yes (%) | Japanese Yes (%) | $\chi^2$ | $p$ |
|---|---|---|---|---|
| Reasons patients do not want TIV | | | | |
| Patient is so disabled that he/she is ready to go | 80 (87%) | 45 (38%) | 50.62 | <.0001 |
| Burden on family | 84 (91%) | 105 (88%) | 0.44 | .507 |
| No caregivers | 53 (58%) | 76 (63%) | 0.50 | .480 |
| Financial burden | 67 (73%) | 66 (55%) | 6.34 | .012 |
| Not able to discontinue TIV once initiated | 8 (9%) | 58 (48%) | 36.34 | <.0001 |
| Patient doesn't want to live any longer (including psychological factors) | 65 (71%) | 103 (86%) | 6.40 | .012 |
| Cannot be cared for at home and does not want institutional care | 70 (76%) | 48 (40%) | 26.04 | <.0001 |
| Influences on patient's decision in favor of TIV | | | | |
| The way the doctor explains TIV | 63 (72%) | 83 (69%) | 0.14 | .706 |
| Communication with patients who already have TIV | 44 (50%) | 65 (54%) | 0.21 | .647 |
| Home care availability 24/7 | 67 (76%) | 83 (69%) | 0.90 | .343 |
| Available facilities for TIV patients | 32 (36%) | 86 (72%) | 24.36 | <.0001 |
| Financial factors | 59 (67%) | 66 (55%) | 2.59 | .108 |

*Question 5. Would neurologists choose for themselves (in the hypothetical scenario where they have ALS) what they recommend to their patients?*

Respondents were asked whether they would choose to have three interventional procedures, if medically indicated, as shown on Table V. In this hypothetical situation, although the majority of both groups did agree to have either percutaneous endoscopy (PEG) or non-invasive ventilation (NIV), Japanese neurologists were less likely to want these procedures than American neurologists ($p$= .001 and <.0001, respectively). The groups did not differ with respect to TIV: over 70% of both groups would not choose TIV for themselves, and the remainder was divided between answering affirmatively and saying that they "can't tell now".

We then examined the correspondence between neurologists' choices for themselves and their degree of encouragement of TIV for their patients. If neurologists would not accept TIV for themselves, would they usually recommend it to their patients? Among American neurologists, 80% seldom or never encouraged TIV for their patients and 76% would decline it for themselves. In contrast, only 36% of Japanese neurologists seldom or never encouraged TIV for their patients while 72% declined to have it for themselves (see Table VI). We then examined level of encouragement for TIV for their own patients (using the dichotomous scale "Do you generally encourage TIV for your patients?") by Japanese neurologists who did not want TIV for themselves compared to those who did want TIV for themselves. We found that 89% of those who did not want TIV for themselves were less likely to recommend it to their patients, compared to only 33% who were less enthusiastic about TIV for their patients among neurologists who did personally want TIV (89% (75/84) vs. 33% (6/18), $\chi^2 = 28.38$, 1df, $p$<.0001).

## Discussion

While Japanese and American neurologists share many beliefs and practices about management of ALS, several consistent differences emerge. These must be considered in view of differences between countries in practice structure, government roles and laws. It is legal to discontinue TIV in the United States. In Japan, the situation is ambiguous. There is no specific law regarding TIV discontinuation, and there are Ministry of Health guidelines for doing so, but discontinuation is rarely considered. Even when a neurologist proposes this, hospital ethics committees and hospital administrators typically refuse, as there is no legal protection from prosecution.

Most American neurologists specialized in treating ALS patients saw them in major clinics with extensive staff. Government insurance (Medicare) is available for all ALS patients. Palliative care is the standard of care for patients approaching death, and

Table V. Neurologists' choices for themselves regarding interventional procedures.

| | PEG | NIV | TIV | | |
|---|---|---|---|---|---|
| Procedure | Yes (%) | Yes (%) | Yes (%) | No (%) | Cannot tell now (%) |
| U.S. | 80 (94%) | 82 (94%) | 7 (8%) | 68 (76%) | 14 (16%) |
| Japan | 85 (76%) | 74 (67%) | 18 (15%) | 84 (72%) | 15 (13%) |
| | $\chi^2 = 10.50$ df = 1 $p$ = .001 | $\chi^2 = 19.85$ df = 1 $p$ = <.0001 | | $\chi^2 = 2.80$ df = 2 $p$ = .247 | |

Table VI. Relationship between encouraging TIV for patients and personal choice of TIV.

| | | |
|---|---|---|
| U.S. | | |
| Encourage TIV | | |
| Seldom/Never | Sometimes | Often/Always |
| 69 (80%) | 16 (19%) | 1 (1%) |
| Choose TIV for self | | |
| No | Uncertain | Yes |
| 65 (76%) | 14 (16%) | 7 (8%) |
| Japan | | |
| Encourage TIV | | |
| Seldom/Never | Sometimes | Often/Always |
| 42 (36%) | 68 (58%) | 7 (6%) |
| Choose TIV for self | | |
| No | Uncertain | Yes |
| 84 (72%) | 15 (13%) | 18 (15%) |

Query 1: "In general, do you suggest and encourage the use of TIV rather than comfort care?".
Score on 1–10 scale: 1–3 = seldom/never, 4–7 = sometimes, 8–10 = often/always.
Query 2: "Should you be diagnosed with ALS, and had progressive dyspnea, would you accept TIV?".
Score: choice of yes/probably yes; cannot answer until I find myself in that position (= uncertain); no/probably no.
Note: Within the national samples, there was 65% agreement/consistency among the U.S. neurologists compared to 44% among the Japanese neurologists. The U.S. neurologists exhibited significantly higher agreement for encouraging and self-choosing TIV compared to the Japanese neurologists ($\chi^2 = 9.21$, df = 1, $p < .003$).

according to a large database from the 1990s, most ALS patients in the United States receive palliative care, hospice, and die at home (5). Long-term facilities are limited and generally not geared to ALS patients (11).

Japanese neurologists typically have individual practices, are less specialized and personally treat far fewer patients with ALS. They are, however, informed and knowledgeable about the disease and its prognosis. Patients with 'intractable diseases' including ALS receive all necessary medical care including hospitalization without charge, as well as long-term home care visits by personnel including physicians under the Long-term care Insurance Act law (12). A Japanese ALS patient voluntary organization vigorously promotes TIV (13), while American organizations do not take an advocacy position. Consistent with the literature and national policies, in our study Japanese neurologists were more likely to recommend TIV and more of their patients received TIV, while Americans seldom did so and few of their patients received TIV. Most (75%) Americans presented treatment options to patients along with their recommendations. Among Japanese neurologists, 58% said they presented options without recommendations (even though two-thirds said in response to another query that they moderately often encourage/suggest TIV). Both groups believed that their recommendations influence patient decisions, and our data support this. This may be one of the first studies to show a direct correspondence between neurologists' level of enthusiasm for TIV and the percentage of their patients who actually receive TIV.

In addition, American and Japanese neurologists largely agreed that most of the patient and family characteristics listed in the questionnaire did influence their decision-making, especially familial support and adequacy of financing. There were, however, some differences: Japanese neurologists weighed more heavily the patient's degree of hope for the future, their having young children and believing in an imminent cure, while Americans emphasized current patient functioning including medical comorbidities.

Our finding that two-thirds of Japanese neurologists moderately often encourage TIV for ALS patients is not universally seen among Japanese physicians. Aita et al. (14) queried 27 internists and surgeons about TIV use in older adults with stroke-caused profound impairment and no hope for recovery. Most discouraged TIV use, although artificial nutrition and hydration were considered essential. The difference between Aita's findings and ours may be due to the fact that most ALS patients retain substantial cognitive capacity, an attribute considered 'highly important' in recommending TIV by 87% of the Japanese neurologists we surveyed. Cognitive capacity was the most widely endorsed of the 14 attributes we asked about (listed in Table III). Thus, differences in level of consciousness and cognitive capacity, which are generally present in ALS patients and essentially absent in severely impaired stroke patients, likely account for these disparate attitudes.

An interesting finding concerns the association between what doctors recommend for their patients and what they would choose for themselves, should they have ALS. While Americans were largely consistent in not recommending TIV for patients and not wanting it for themselves, Japanese neurologists were far more likely to recommend TIV to their patients than choose it for themselves. This disparity is consistent with research showing that physicians are more likely to recommend invasive procedures with attendant risks of serious adverse sequelae to their patients than they would choose for themselves, given death as the alternative. For example, Ubel et al. (15) presented to physicians a colon cancer scenario with two options, varying in terms of survival rate with lower survival accompanied by fewer serious adverse events (i.e. length of life versus quality of life). Given two options, 38% of 242 internists chose for themselves the option with better quality of life despite a higher death rate, while only 25% chose this option to recommend to a hypothetical patient. In an invited commentary, Shaban et al. (16) observed that these data suggest physicians more often favor prolongation of life for their patients but place more emphasis on quality of life concerns when making decisions for themselves. Other investigators similarly have observed in differing