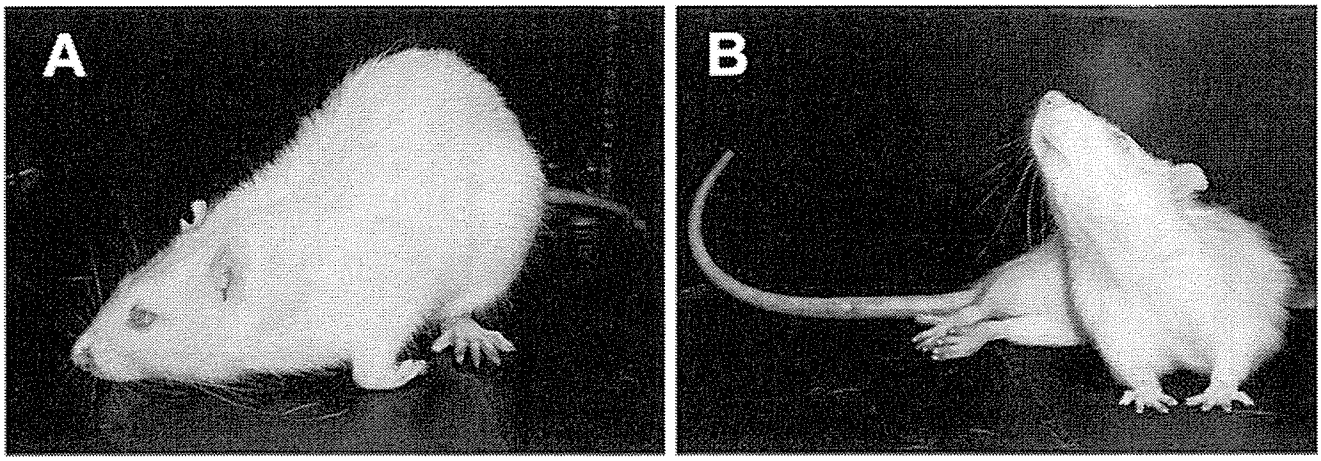

Fig. 2. Characteristic appearance of hSOD1 (G93A) transgenic rats. **A:** Forelimb type. The rat was unable to raise its head and was obligated to take a posture of raising the lumbar region, as indicated, because of the paralyzed forelimbs. **B:** Hindlimb type. The rat showed paraplegia, but was able to raise its head and upper trunk with its non-paralyzed forelimbs.

mogen. Immunohistochemical images were examined with a Zeiss-AxioCam microscope system.

Motor neurons bearing ChAT-immunoreactivity in laminae VII, VIII, and IX of the ventral horn were counted in every tenth section (5 sections total for each segment) for each of the C6, T5, and L3 segments. Only the neurons that showed labeling above background level and were larger than 20 μm in diameter were counted. The numbers of motor neurons in all segments (C6, T5, and L3) were summed for each animal to evaluate not only the local motor neuron loss, but the generalized loss of motor neurons throughout the spinal cord of each animal ($n = 3$ for each genotype at each time point). We next examined the correlation between the number of residual motor neurons and the results of the functional analyses described in this study. Statistical analysis was carried out with two-tailed unpaired Student's *t*-test. A *P*-value of <0.05 was considered statistically significant.

RESULTS

Clinical Types of hSOD1 (G93A) Transgenic Rats

Because we noticed variations in the disease phenotypes expressed by the G93A rats, we classified 49 rats into three clinical categories according to the location of initial paralysis. The clinical types were: the forelimb type, hindlimb type, and general type (Table III). Rats whose paralysis started in the forelimbs and progressed to the hindlimbs were defined as the "forelimb type." In contrast, rats whose paralysis started from the hindlimbs and progressed to the forelimbs were defined as the "hindlimb type." A typical appearance for the forelimb and hindlimb types is shown in Figure 2. Other rats, which showed simultaneous paralysis in the forelimbs and hindlimbs, were categorized as the "general type".

In addition, we classified the forelimb- and hindlimb-type rats into two subtypes, the pure and eventual types, based on the timing of the initial paralysis (Table III). Rats of the pure type showed paralysis that was limited to one or more of the four limbs as the initial observable deficit. Those of the eventual type initially showed symptoms of general muscle weakness (e.g., walking with a limp, sluggish movement), but without unequivocal limb paralysis. In the eventual type animals, paralysis of one of the limbs became apparent later. The ratio of each subtype is shown in Table III.

Evaluation of Disease Progression in the hSOD1 (G93A) Transgenic Rats

Although the transgenic rats varied in their clinical types, all four measures of disease progression (body weight, inclined plane test, cage activity, and SCANET) showed significant differences between the transgenic and wild-type rats (Fig. 3).

In contrast to the continuous weight gain in wild-type rats, the body weight in the affected rats ceased to increase and gradually decreased, with peak body weight attained around 110–120 days of age ($P < 0.05$, after 112 days of age) (Fig. 3A).

In the inclined plane test, initially both the transgenic and wild-type rats uniformly scored 75–80 degrees, after several training trials. However, the transgenic rats showed a significant decline in performance compared to their wild-type littermates from 120 days of age (Fig. 3B).

In the cage activity measurement, the movements of the wild-type rats remained stable, whereas those of the transgenic rats declined rapidly after 125 days of age (Fig. 3C).

In the SCANET test, even the wild-type rats showed decreased movements for all parameters (M1, M2, RG) in the late observation period, though they showed no abnormality in their motor functions. This might be because they had acclimated to the SCANET cage. The movement score of the transgenic rats was consistently worse than that of the wild-type rats after

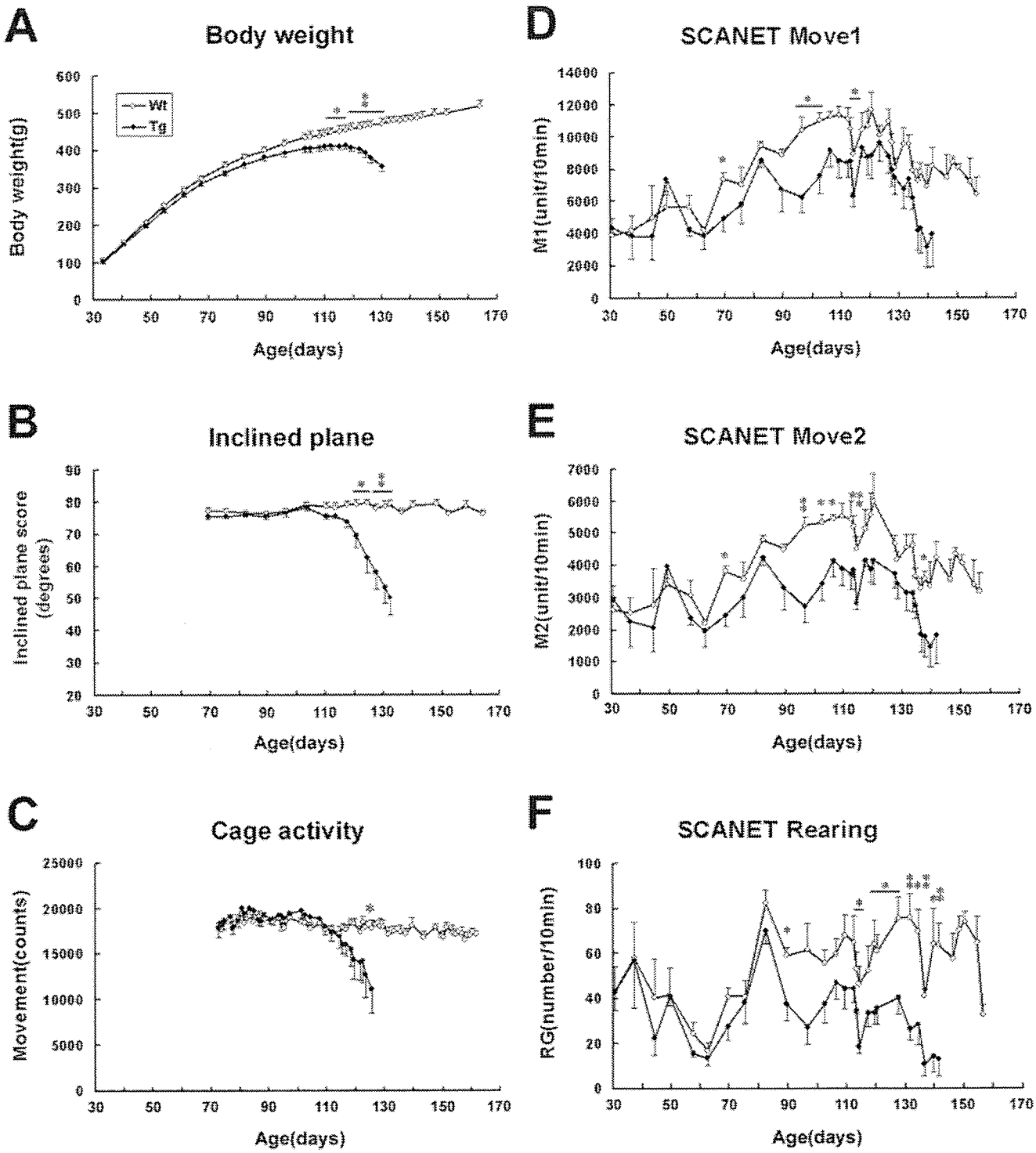

Fig. 3. Disease progression in hSOD1 (G93A) transgenic rats monitored by four effective measures. **A:** Body weight. The weight gain of the transgenic group stopped at around 110–120 days. The difference became statistically significant at 112 days of age ($n = 9$ for each genotype). **B:** Inclined plane. The wild-type group scored 75–80° throughout the period, whereas the score of the transgenic group declined. The difference became statistically significant at 120 days of age ($n = 9$ for each genotype). **C:** Cage activity. The movements of the wild-type group were stable, whereas the scores of the transgenic group declined. Significance was reached at 125 days of age ($n = 8$ for each genotype). **D–F:** SCANET. For all parameters (M1, M2, RG), the movement scores of the transgenic group became constantly worse than those of the wild-type group after 60 days of age. The differences between the groups increased markedly after 90 days of age. Significance was attained beginning at 67 days of age for M1 and M2, and at 87 days of age for RG ($n = 4$ for each genotype). The comparison between the wild-type and transgenic groups was stopped when the first of the transgenic rats reached the end-stage of the disease and was sacrificed. Mean ± SEM. $^{*}P < 0.05$. $^{**}P < 0.01$; two-tailed unpaired Student's t-test.

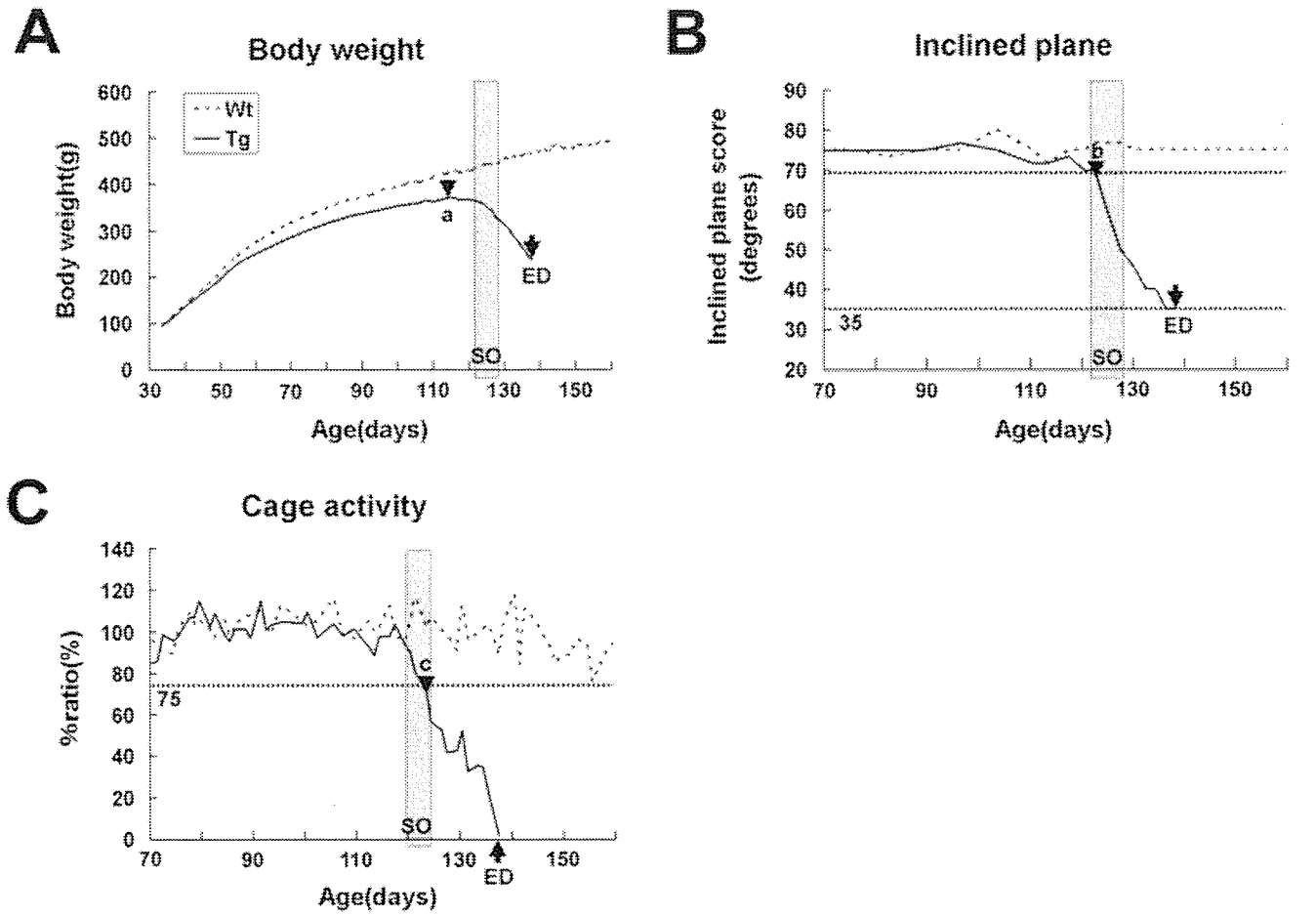

Fig. 4. Schematic presentation of the results from the body weight (**A**), inclined plane test (**B**), and cage activity (**C**) assessments. The onset defined by each measure (black arrowheads) and the end-stage of the disease (ED, black arrows) are indicated in the figures. a, pre-symptomatic onset: the day the transgenic rats scored their maximum body weight. b, muscle weakness onset: the earliest day the transgenic rats scored <70° in the inclined plane test. c, hypo-activity onset: the earliest day the transgenic rats scored <75% of the mean movements from 70–90 days of age in the cage activity measure. SO, subjective onset: the earliest day that observable functional deficits such as paralysis of the limbs or symptoms of general muscle weakness were observed subjectively in the open field (the gray shaded region in A–C).

60 days of age for all parameters (M1, M2, RG), however, even after the wild-type animals showed the decrease in their movement scores. The differences between the two groups increased markedly after 90 days of age for M1, M2, and RG (Fig. 3D–F). The performance of each rat fluctuated so markedly that the SCANET test seems to be inappropriate for statistical analysis.

Onset, End-Stage, and Duration of Disease in hSOD1 (G93A) Transgenic Rats

Using the quantitative analysis of disease progression by body-weight measurement, the inclined plane test, and cage activity, as described above, we defined three time points of "objective onset," as shown in Figure 4. The SCANET results did not allow us to define a time of objective onset, because we could not establish a stable baseline level using the data from the highly variable measurements we obtained, even for wild-type rats. The righting reflex failure was useful for detecting the time point of end-stage disease, which we defined as the generalized loss of motor activity in affected rats. A total of 20 transgenic rats assessed by body weight and the inclined plane test were analyzed for the day of objective onset, end-stage, and duration of the disease. The cage activity data from the eight transgenic rats were obtained simultaneously. The results are shown in Table IV.

The day the transgenic rats reached their maximum body weight was defined as pre-symptomatic onset (113.6 ± 4.8 days of age, black arrowhead in Fig. 4A, Table IV). This onset was judged retrospectively and always preceded the subjective onset (gray shaded region, Fig. 4A), which was determined by observable functional deficits in the open field, such as paralysis of limbs and symptoms of general muscle weakness. The

TABLE IV. Onset, End-Stage, and Duration in Days of Disease in hSOD1 (G93A) Transgenic Rats

Evaluation methods	Body weight and inclined plane (n = 20)	Cage activity (n = 8)
Objective onset		
Pre-symptomatic onset[a]	113.6 ± 4.8 (103–124)	
Muscle weakness onset[b]	125.2 ± 7.4 (110–144)	
Hypo-activity onset[c]		122.8 ± 9.2 (109–139)[c]
Subjective onset (SO)[d]	126.5 ± 7.1 (113–147)	121.3 ± 9.8 (109–140)
End-stage disease (ED)[e]	137.8 ± 7.1 (128–155)	134.1 ± 8.2 (122–149)
Duration[f]		
ED-a[g]	24.3 ± 6.5	
ED-b[h]	12.6 ± 3.5	
ED-c[i]		11.4 ± 1.3

Values are means ± SD.
[a] Maximum of body weight.
[b] Less than 70 degrees in the inclined plane test.
[c] Less than 75% in the mean movements of 70–90 days in the cage activity.
[d] Observable functional deficits.
[e] Righting reflex failure.
[f] Difference in days between ED and each onset;
[g] between ED and pre-symptomatic onset,
[h] between ED and muscle weakness onset,
[i] between ED and hypo-activity onset.

TABLE V. Comparison of the Onset, End-stage, and Duration in Days of Disease in the Forelimb-type and the Hindlimb-type Rats

	Forelimb type (n = 4)	Hindlimb type (n = 14)	General type* (n = 2)
Pre-symptomatic onset[a]	112.5 ± 6.7	114.6 ± 4.3	(108.5)
Muscle weakness onset[b]	125.8 ± 2.8	126.7 ± 7.3	(113.5)
End-stage disease (ED)[c]	134.0 ± 2.4	140.1 ± 7.1	(129.5)
Duration[d]			
ED-a[e]	21.5 ± 8.5	25.5 ± 6.2	(21)
ED-b[f]	8.3 ± 1.0	13.4 ± 3.0	(16)

Values are mean ± SD.
* Values of general-type rats are listed in parenthesis for reference.
[a] Maximum of body weight.
[b] Less than 70 degrees in the inclined plane test.
[c] Righting reflex failure.
[d] Difference in days between ED and each onset;
[e] between ED and pre-symptomatic onset,
[f] between ED and muscle weakness onset.

pre-symptomatic onset was the most sensitive of all the onset measures described in this study (Table IV).

The first day the transgenic rats scored <70° in the inclined plane test was defined as the muscle weakness onset (black arrowhead, Fig. 4B). We could judge this onset prospectively. Muscle weakness onset (125.2 ± 7.4 days of age, Table IV) was usually recorded before or at almost the same time as the subjective onset (8 days before to 1 day after, gray shaded region, Fig. 4B and 126.5 ± 7.1 days of age, Table IV). The day the transgenic rats scored 35° or less on the inclined plane test coincided with the day of righting reflex failure (black arrow, Fig. 4B).

The first day the transgenic rats scored <75% of their baseline movements in the cage activity test was defined as hypo-activity onset (black arrowhead, Fig. 4C and 122.8 ± 9.2 days of age, Table IV). We could also judge this onset prospectively. Hypo-activity onset was recorded 1 day before to 4 days after the subjective onset (SO, shown as the gray shaded region in Fig. 4C and 121.3 ± 9.8 days of age, Table IV). A 0% movement score for cage activity was seen at almost the same time as righting reflex failure (black arrow, Fig. 4C). Although disease onset and end-stage could be objectively defined with these methods, they had a wide range, of about 1 month, because of the diversity of the phenotypes (Table IV).

Differences in Disease Courses Between the Forelimb- and Hindlimb-Type Rats

Because we noticed variability in disease courses among different clinical types of hSOD1 (G93A) rats, we next assessed disease progression in 20 transgenic rats with forelimb- (n = 4), hindlimb- (n = 14), and general- (n = 2) type, using the probability of objective

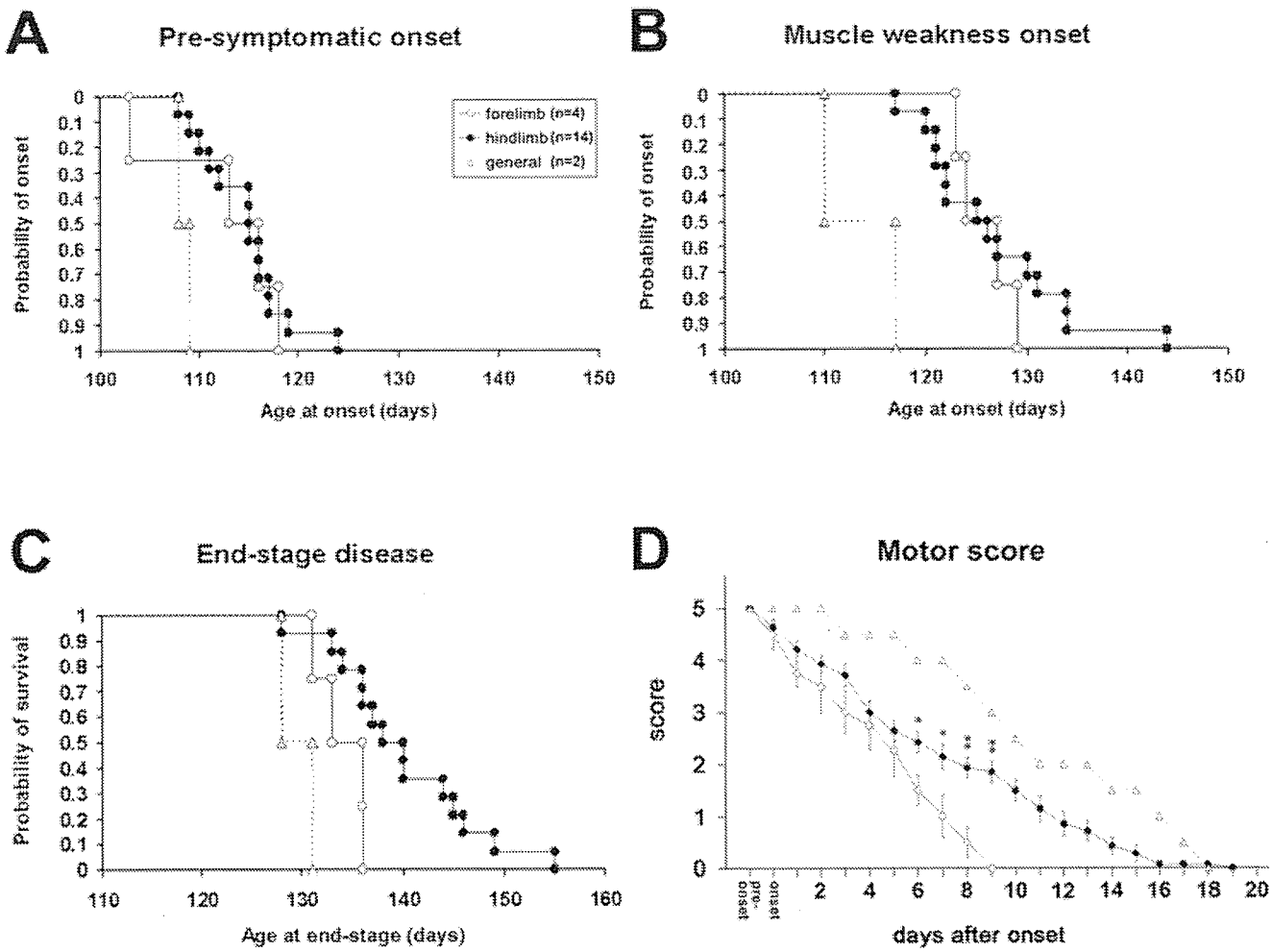

Fig. 5. Comparison of onset, end-stage, and disease progression in the forelimb-type ($n = 4$), and the hindlimb-type ($n = 14$) rats. Data from the general-type rats are also shown as dotted lines. **A,B:** The probability of the objective onsets. We did not see any differences in the probability of the objective onsets defined by body weight measurement (pre-symptomatic onset) and the inclined plane test (muscle weakness onset) between the forelimb- and hindlimb-type rats. **C:** The probability of survival as defined by end-stage disease. Survival was significantly shorter in the forelimb-type than in the hindlimb-type rats ($P < 0.05$, Log-rank test). **D:** Assessment of disease progression using the Motor score. Affected rats were evaluated after muscle weakness onset. The forelimb type worsened more quickly than the hindlimb type. Score decline correlated well with the exacerbation of symptoms in both clinical types, clearly and objectively. Bars = means ± SEM. Statistically significant differences between forelimb and hindlimb types are indicated in the figures. $^{*}P < 0.05$. $^{**}P < 0.01$; two-tailed unpaired Student's t-test.

onsets (pre-symptomatic onset and muscle weakness onset), the probability of survival defined by end-stage disease (failure in righting reflex), and the Motor score (Table V, Fig. 5). We did not see any differences in the objective onsets between the forelimb- and hindlimb-type rats (Fig. 5AB, Table V). However, survival as defined by end-stage disease was significantly shorter in the forelimb-type than in the hindlimb-type rats ($P < 0.05$, Log-rank test, Fig. 5C). Moreover, the duration of the disease calculated from the muscle weakness onset was also significantly shorter in the forelimb-type (8.3 ± 1.0 days) than in the hindlimb-type rats (13.4 ± 3.0 days) (see ED — b, $P < 0.01$, two-tailed unpaired Student's t-test, Table V).

The courses of functional deterioration evaluated by the Motor score after onset (muscle weakness onset) for each clinical type were well represented by the declines in their scores (Fig. 5D). The assessment by the Motor score also showed that disease progression in the forelimb type was more rapid than that in the hindlimb type (Fig. 5D).

Our results raise the question of why this variability in the disease course of each clinical type was observed. We speculated that there might be correlation between clinical type in G93A rats and the amount of locally expressed mutant hSOD1 (G93A) gene product. Therefore, we next investigated expression of the mutant hSOD1 gene in each segment of the spinal cord (cervical, thoracic, and lumbar) in the forelimb- and

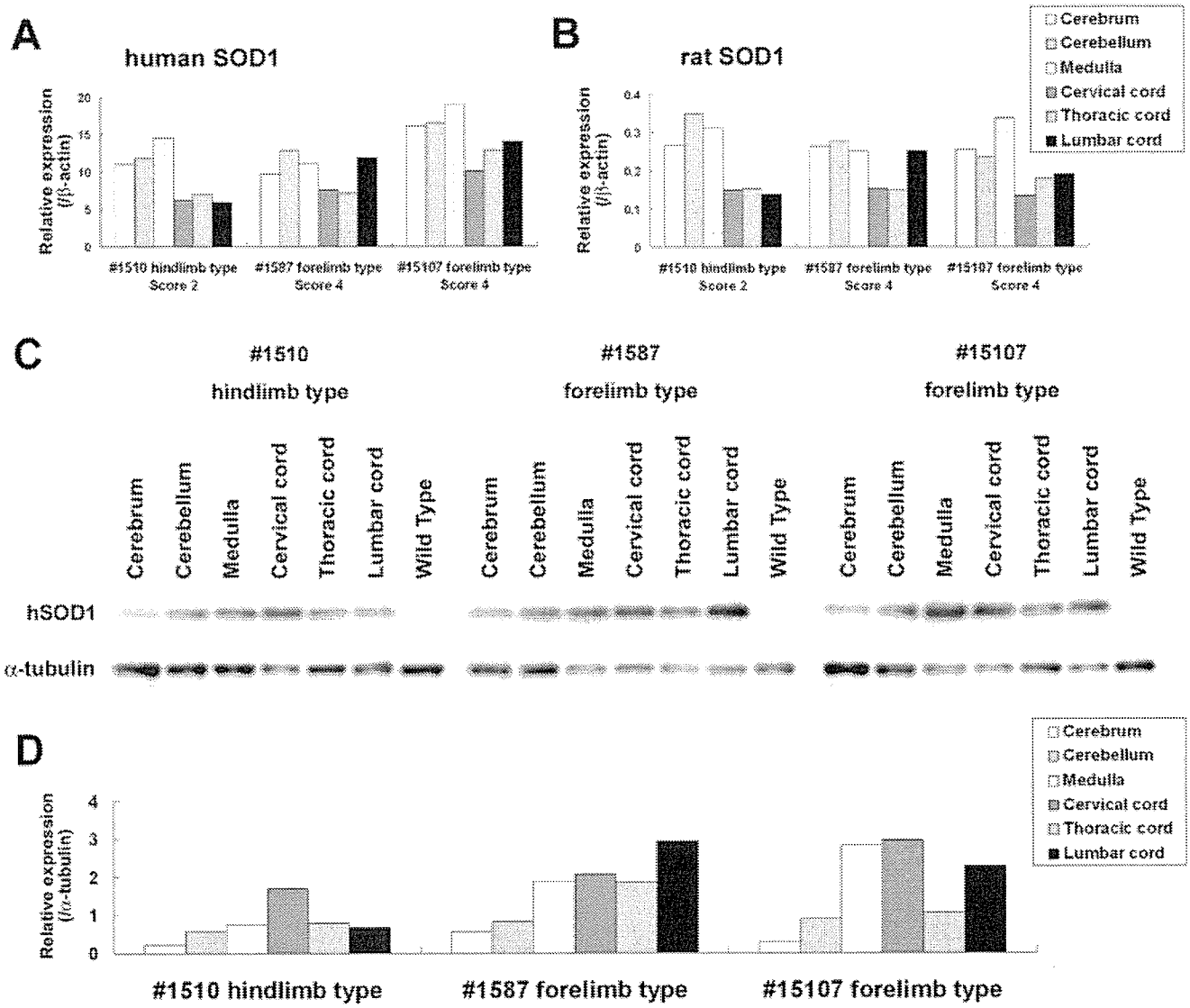

Fig. 6. The expression of mutant hSOD1 mRNA and protein in the cerebral cortex, cerebellum, medulla, and spinal cord (cervical, thoracic, and lumbar) of forelimb- and hindlimb-type rats. **A,B:** The amounts of human (A) and endogenous rat (B) SOD1 mRNA normalized to those of *β-actin* were quantified by real time RT-PCR analysis. **C,D:** Western blot analysis of the mutant hSOD1 protein was carried out in the same rats. Quantitative analysis was carried out with a Scion Image. The amounts of proteins were normalized to those of α-tubulin (D).

hindlimb-type rats by real time RT-PCR and Western blot analysis. However, at least at the stages after the apparent onset of muscle weakness, neither forelimb-type (#1587, Score 4 and #15107, Score 4) nor hindlimb-type rats (#1510, Score 2) necessarily expressed larger amounts of the mutant hSOD1 (G93A) transgene in the cervical cord or in the lumbar cord, respectively, at the mRNA and the protein level (Fig. 6). We also investigated the expression of endogenous rat SOD1 mRNA in the same rats by REAL TIME RT-PCR (Fig. 6B). Distribution of endogenous rat SOD1 mRNA expressed in each segment of the spinal cord showed almost the same pattern as that of mutant hSOD1 mRNA. The expression of endogenous rat SOD1 mRNA was lower than that of mutant hSOD1 mRNA. Thus, we could not detect any definite correlation between the hSOD1 (G93A) transgene local expression profile in the spinal cord and the phenotypes of G93A rats for either the forelimb-type or the hindlimb-type rats (Fig. 6).

Reduction in the Number of Spinal Cord Motor Neurons at Different Disease Stages

We examined histo-pathological changes in the spinal cords of the transgenic rats in comparison with those

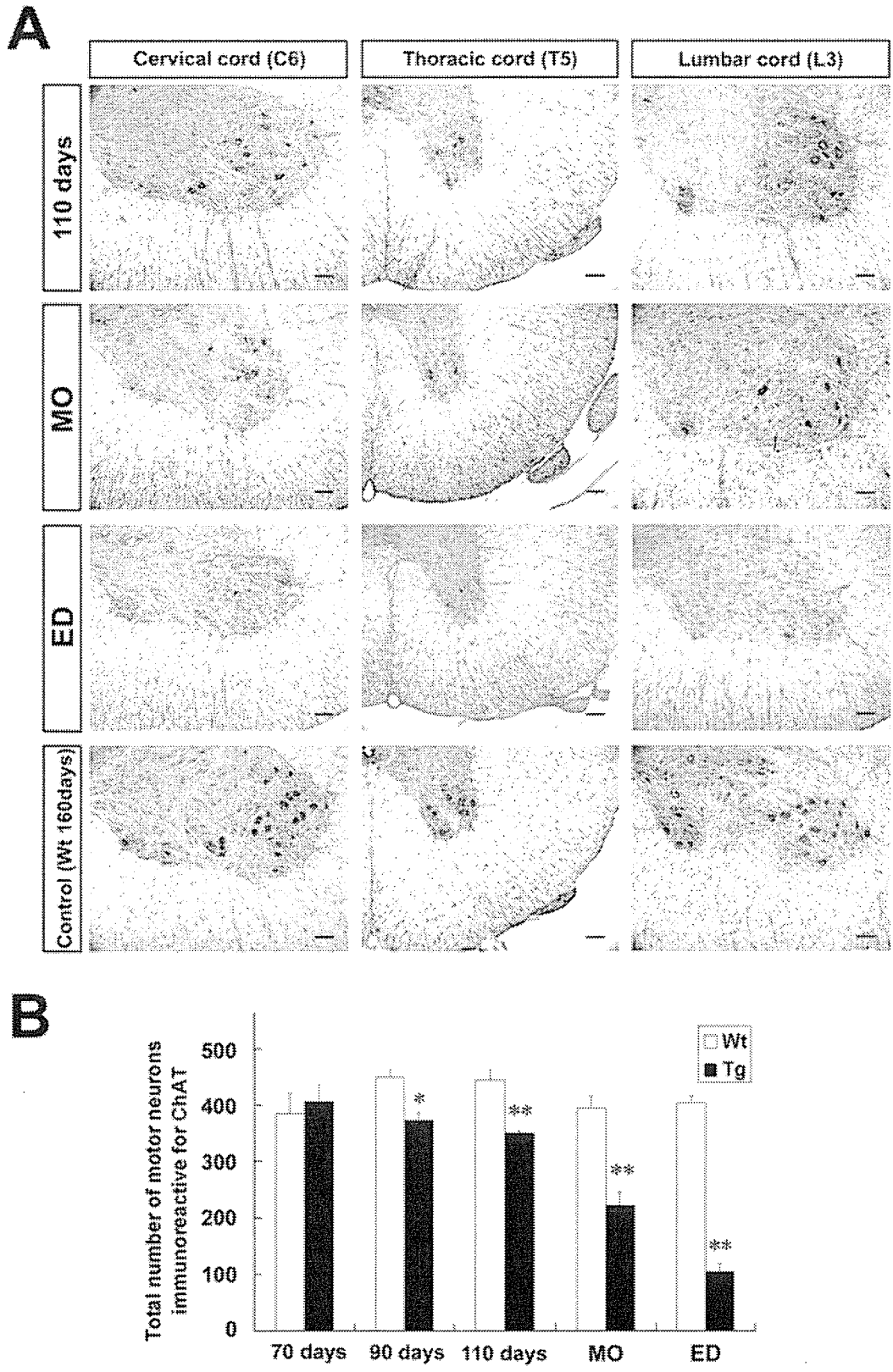

Fig. 7. The loss of motor neurons in the spinal cord of hSOD1 (G93A) transgenic rats at different stages. **A:** Immunohistochemical analysis of the spinal cord of transgenic rats. Transverse sections of the cervical (C6), thoracic (T5), and lumbar (L3) spinal cord of the transgenic rats and their wild-type littermates were stained with an anti-ChAT antibody to label viable motor neurons at the indicated stages (Scale bars = 100 μm). **B:** The number of ChAT immunoreactive motor neurons was counted and is shown in the histograms as the total number of motor neurons in the C6, T5, and L3 segments. This number began to decrease in the transgenic rats at 90 days of age, rapidly declined after 110 days of age, and fell to about 50% and 25% of wild-type rats at the muscle weakness onset (MO, around 125 days) and at end-stage disease (ED, around 140 days), respectively. Bars = means ± SEM ($n = 3$ for each genotype). $^{*}P < 0.05$. $^{**}P < 0.01$; two-tailed unpaired Student's *t*-test.

of their wild-type littermates at 70, 90, and 110 days of age, when the transgenic rats scored <70° in the inclined plane test (muscle weakness onset), and failed the righting reflex. To quantify the number of spinal motor neurons, we stained spinal cord sections of both groups with an anti-ChAT antibody.

As shown in Figure 7A, the numbers of ChAT immunoreactive motor neurons in the cervical (C6), thoracic (T5), and lumbar (L3) segments of the spinal cord decreased with disease progression. Quantitative analysis of the residual motor neurons showed that the total number of motor neurons in the transgenic rats began to decrease at 90 days of age, rapidly declined after 110 days of age, and fell to about 50% and 25% of the numbers in age-matched wild-type littermates at the time the score was <70° in the inclined plane test (muscle weakness onset) and of righting reflex failure, respectively (Fig. 7B).

DISCUSSION

Factors Underlying the Variability in Phenotypes of hSOD1 (G93A) Transgenic Rats

In previous studies of this G93A rat, only the hindlimb-type has been described, and the variety of phenotypes and variable clinical courses have not yet been mentioned (Nagai et al., 2001). Recently, however, another line of G93A rats backcrossed onto a Wistar background ($SOD1^{G93A/HWr}$ rats) was reported to present two phenotypes, including forelimb-type, and a large inter-litter variability in disease onset (Storkebaum et al., 2005). In the same way, commonly used FALS model mice harboring hSOD1 (G93A) gene have been reported to have clinical variability to some extent, and some of them dominantly show forelimb paralysis (Gurney et al., 1994). In this study, we recognized various clinical types, including forelimb-, hindlimb-, and general-type and established quantitative methods to evaluate disease progression that can be applied to any of the clinical types of this ALS model. We have also shown the variability in disease progression to depend on clinical types, that is, disease progression after the onset was faster in forelimb-type than in hindlimb-type rats. This difference may be due to the aggressiveness of the disease per se because we evaluated the time point of "death" (end-stage disease) according to righting reflex failure (Howland et al., 2002) to exclude the influence of feeding problems (bulbar region) and respiratory failure (level C2–C4).

These findings give rise to the next question; why is this variety of phenotypes and variability in the clinical course observed in the same transgenic line? There are at least three possible explanations. One is that the variation is due to the heterogeneous genetic background of the Sprague-Dawley (SD) rat (i.e., the strain used to generate this transgenic line), which might have led to different phenotypes. This idea is supported by the fact that the SD strain shows a large inter-individual disease variability in other models of neurodegenerative disorders, such as Huntington's disease (Ouary et al., 2000). Similar phenotypic variability takes place in human FALS carrying the same mutations in hSOD1 gene (Abe et al., 1996; Watanabe et al., 1997; Kato et al., 2001), which could be explained by heterogeneous genetic backgrounds. Thus, the present transgenic ALS model rats may be highly useful to understand the mechanisms of bulbar onset, arm onset, or leg onset that are seen in human disease. There may be modifier genes of these phenotypes, which should be identified in the future study.

TABLE VI. Adequacy of Evaluation Methods in Regard to Practical Use*

	Body weight	Inclined plane	Cage activity	SCANET	Motor score
Objectivity	A	B	A	A	B
Sensitivity	A	B	C	(A)	-
Specificity	C	B	C	C	A
Motivation independence	A	B	B	D	B
Skill requirements	A	B	A	A	B
Cost of apparatus	B	B	D	D	A

*A, more appropriate; B, appropriate; C, less appropriate; D, inappropriate.

The second is that there is variability in the expression of the mutant hSOD1 protein. The transcriptional regulation of this exogenous gene could be affected by one or more unknown factors, such as epigenetic regulation, and may not be expressed uniformly throughout the spinal cord of each animal. Therefore, some rats might express mutant proteins more in the cervical spinal cord and others might express more in the lumber cord, possibly resulting in the forelimb type and hindlimb type, respectively. However, we found no definite correlation between local expression levels of the mutant hSOD1 mRNA/protein in the spinal cord and the phenotypes of these animals, using real time RT-PCR and western blot analysis after the onset of muscle weakness, when the clinical type of the transgenic rats could be defined (Fig. 6). Moreover, the pathological analysis showed no correlation between the number of residual motor neurons in each segment and the phenotypes of end-stage animals. However, because >50% of spinal motor neurons have already degenerated at the stage of muscle weakness onset, whether local expression of the mutant hSOD1 gene and segmental loss of motor neurons correlate with the clinical types of G93A rats should be further investigated by analyzing younger animals at a stage when motor neuron loss has not progressed as much.

The third explanation involves a structural property of the mutant hSOD1 (G93A) protein itself. It is now thought that mutations in the hSOD1 gene may alter the 3-D conformation of the enzyme and, in turn, result in the SOD1 protein acquiring toxic properties that cause ALS (Deng et al., 1993; Hand and Rouleau 2002). For instance, the hSOD1 (G93A) mutant protein has been reported to be susceptible to nonnative protein–protein interactions because of its mutation site and unfolded structure (Shipp et al., 2003; Furukawa and

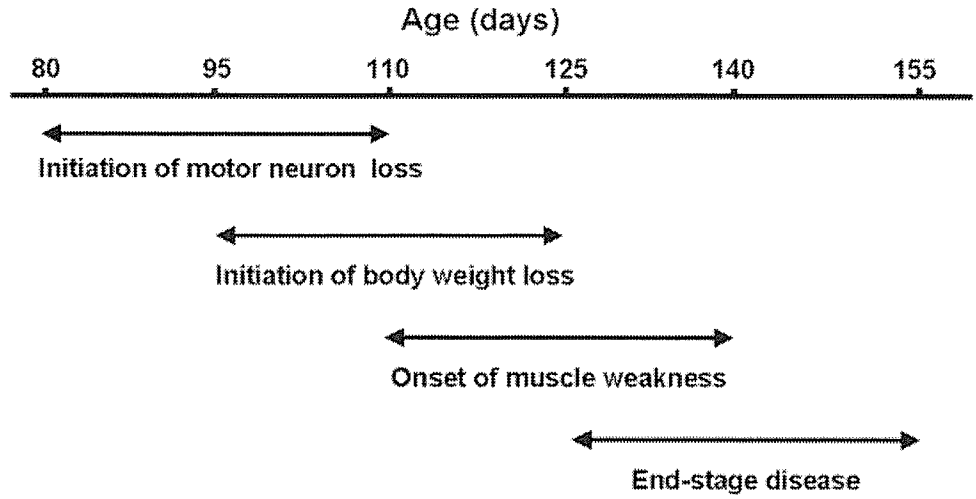

Fig. 8. Four stages of disease progression in hSOD1 (G93A) transgenic rats. The disease progression can be classified into four stages as shown. The range for each stage is about 1 month and overlaps approximately 2 weeks with the next stage.

O'Halloran, 2005), suggesting that the G93A mutation might accelerate the formation of SOD1 protein aggregates, which may ultimately sequester heat-shock proteins and molecular chaperones, disturb axonal transport or protein degradation machineries, including the ubiquitin-proteasome system (Borchelt et al., 1998; Bruening et al., 1999; Williamson and Cleveland 1999; Okado-Matsumoto and Fridovich 2002; Urushitani et al., 2002). Curiously, the mutated hSOD1 (G93A) protein is more susceptible to degradation by the ubiquitin-proteasome system and has a shorter half-life than other mutants (Fujiwara et al., 2005), suggesting that it may cause more unstable toxic aggregates in the spinal cord than other mutations. The degradation rate is also affected by environmental factors unique to each animal, such as the progressive decline of proteasome function with age (Keller et al., 2000), and these factors could contribute to the variability of the clinical course of G93A rats.

Taking all these findings into consideration, the mutated hSOD1 (G93A) protein may gain properties that are responsible for a variety of phenotypes and variability in the clinical course of the affected animals.

Characteristics of Different Methods for Assessing hSOD1 (G93A) Transgenic Rats

The ideal measure is not influenced by the judgment of the observer, sensitive to small abnormalities, specific to detect pathologic events that are related to pathogenesis of the ALS-like disease, not influenced by the motivational factors of rats, minimal in the requirements for skill in the observer, and inexpensive to carry out. We assessed each evaluation method by the categories in regard to practical use as shown in the Table 6.

The initiation of body weight loss seems to be an excellent marker to detect the onset and should be highly recommended. Muscle volume might have already started to decrease, even in the period of continuous weight gain, as reported for hSOD1 (G93A) transgenic mice (Brooks et al., 2004). As a result, it could detect an abnormality relatively earlier than subjective onset. The inclined plane test is considered to be the least defective method of all. It could objectively and specifically detect the decline in the muscle strength of these ALS model rats as a muscle weakness onset almost at the same time of the subjective onset. The cage activity measurement and SCANET require very expensive apparatus, and are limited by the availability of funds and space for making the measurements. Although SCANET test was most sensitive among these measures, it seems inappropriate for the statistical analysis, and does not add any more information than that obtained through simple observation of the rats because the performances of the rats might be severely affected by the extent of their motivation to explore. Motor score can specifically assess disease progression of each clinical type and is valuable in keeping the experimental costs at a minimum.

Correlation Between the Loss of Spinal Motor Neurons and Disease Stages

This study clearly shows the variable clinical course of G93A rats. According to our behavioral and histological analyses, we can divide the disease course of this transgenic model into four stages, whose durations have a range of about 1 month, as shown in Figure 8. Furthermore, we have established the pathological validity of the performance deficits detected by each measure of disease progression. "Initiation of motor neuron loss" was defined as a statistically significant decrease in the number of spinal motor neurons, which was found at around 90 days of age, but not 70 days of age (Fig. 7B). This coincides with, and seems to be sensitively detected by the marked difference in SCANET scores that begins at around 90 days of age (Fig. 3D–F). The "initiation of body weight loss" was usually detected at around 110 days of age as the peak body weight (pre-symptomatic onset, 113.6 ± 4.8 days of age, range = 103–124, Table IV). This stage coincides with the initiation of a rapid decline in the number of motor neurons at around 110 days of age (Fig. 7B). "Onset of muscle weakness" was detected at around 125 days of age, as assessed by the

inclined plane test (muscle weakness onset, 125.2 ± 7.4 days of age, range = 110–144, Table IV). This coincides with the number of spinal motor neurons in the transgenic rats being reduced to about 50% of the number in wild-type rats (Fig. 7B). We presume that transgenic rats do not present obvious muscle weakness until the number of motor neurons has been reduced to approximately half the number found in the healthy state. "End-stage disease" as defined by righting reflex failure was recorded at around 140 days of age (137.8 ± 7.1 days of age, range = 122–155, Table IV). At this stage, the affected rats had only about 25% of the spinal motor neurons of age- and gender-matched wild-type rats (Fig. 7B), and showed a generalized loss of motor activity. Thus, our findings allow us to estimate the extent of spinal motor neuron loss by evaluating the disease stage with the measures described in this study.

In summary, we have described the variable phenotypes of mutant hSOD1 (G93A) transgenic rats and established an evaluation system applicable to all clinical types of these rats. Disease stages defined by this evaluation system correlated well pathologically with the reduction of motor neurons. Our evaluation system of this animal model should be a valuable tool for future preclinical experiments aimed at developing novel treatments for ALS.

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Clinical features of chromosome 16q22.1 linked autosomal dominant cerebellar ataxia in Japanese

Abstract—Chromosome 16q22.1-linked autosomal dominant cerebellar ataxia (16q-ADCA) is strongly associated with a substitution in the puratrophin-1 gene. This locus overlaps with spinocerebellar ataxia type 4 (SCA4) which shows ataxia with prominent sensory axonal neuropathy. We found that 16q-ADCA is a common ADCA subtype in the Tohoku District of Japan. The clinical feature of Japanese 16q-ADCA is characterized as late-onset pure cerebellar ataxia.

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According to Harding's classification, autosomal dominant cerebellar ataxia (ADCA) is clinically classified as follows: ADCAI is associated with additional features related to the optic nerve, (extra) pyramidal system, cerebral cortex, and peripheral nerves; ADCAII is associated with pigmentary macular dystrophy; ADCAIII is late onset pure cerebellar syndrome; and ADCAIV is associated with myoclonus and deafness.[1] Recently, genetic classification of the ADCAs has been carried out worldwide. The mutations causing 12 types of ADCA subtypes have already been identified. In addition, a single nucleotide C→T substitution in 5′ UTR of the puratrophin-1 gene is strongly associated with 16q-ADCA.[2] Although the locus of 16q-ADCA overlaps with SCA4, the phenotype of 16q-ADCA in Japan differs from that of SCA4 in Utah[3] and Germany.[4] Interestingly, it was described that 16q-ADCA is pure cerebellar ataxia which belongs to ADCAIII and may be associated with hearing impairment.[5]

We studied 218 unrelated ADCA families and 129 sporadic spinocerebellar ataxia cases from the Tohoku District of Japan. We found that 16q-ADCA is a common subtype of hereditary ataxia in the Tohoku District. We also describe the clinical features of 32 patients with the C→T substitution in the 5′ UTR of the puratrophin-1 gene. All of these patients showed late-onset pure cerebellar ataxia.

Additional material related to this article can be found on the *Neurology* Web site. Go to www.neurology.org and scroll down the Table of Contents for the October 10 issue to find the title link for this article.

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Methods. We studied cases with dominantly inherited or sporadic ataxia collected from January 1991 to August 2005 in the Tohoku District. The Tohoku District is in the northernmost part of Honshu Island which is the main island of Japan (figure 1). All of the patients had been neurologically and radiologically diagnosed with spinocerebellar ataxia. We excluded the families and patients who had been considered as recessive inherited ataxia from the present study. No attempts were made to classify or select patients with specific clinical subtypes of ataxia. We examined a total of 218 unrelated families (279 affected members) and 128 sporadic cases.

Peripheral blood samples of the patients were obtained with informed consent. Genomic DNA was extracted from peripheral blood leukocytes, as previously described.[3] Genetic analysis was performed to estimate the number of triplet repeats at nine disease loci corresponding to SCA-1, 2, 3 (Machado-Joseph disease [MJD]), 6-8, 12, 17, and dentatorubral-pallidoluysian atrophy (DRPLA).

The C→T substitution in the 5′ UTR was detected by amplifying genomic DNA with forward primer UK1-E1F1 (5′-CAGCGGTTCACACTGAGA-3′) and reverse primer UK1-E1R1N (5′-GGCCCTTTCTGACAGGACTGA-3′), which yielded a specific product of 360 bp. This amplicon harbors two XagI sites, one of which is destroyed by the C→T substitution.[2] This C→T substitution was not seen in 45 healthy Japanese individuals and 11 Japanese patients with muscular disease (112 chromosomes).

Results. We identified 218 families (included affected 279 members) with ADCA and 128 sporadic cases with spinocerebellar ataxia. For both the autosomal dominant ataxia families and sporadic cases, the relative prevalence of mutations causing ADCAs is summarized in the table.

The clinical features of 32 patients with 16q-ADCA are summarized in table E-1 on the *Neurology* Web site at www.neurology.org. The mean age at onset was 57.7 ± 7.1 years. In all of the cases, the initial symptoms were ataxia, gait disturbance, or dysarthria. During the clinical course, ataxia and dysarthria were evident in almost all cases (≥94%). Other symptoms were not common regardless of the duration of the disease. Only 2 (6%) of 32 cases with 16q-ADCA had mild sensory disturbance, and only one case showed hearing impairment.

Case 1 in table E-1 had at least four affected relatives: his elder and younger brother, his mother, and her younger brother. He first noticed clumsiness of his legs at age 44 years. He had been able to walk until age 50 years without a walking stick. As soon as he was wheelchair bound at the age of 52 years, dysarthria appeared. Because of his difficulty in mobility and conversation, he retired from his job as an office worker at age 52 years. Thereafter, the symptoms progressed very slowly. When he was age 74 years, brain MRI revealed cerebellar atrophy without obvious brainstem involvement (figure 2).

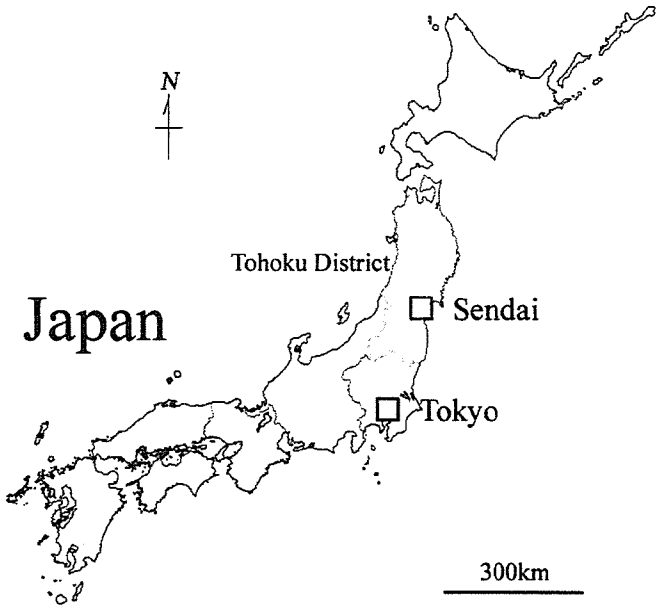

Figure 1. Our hospital is in Sendai City, and is located near the center of the Tohoku District. The Tohoku District is in the northernmost part of Honshu Island, which is the main island of Japan. Its population is 10.7 million, representing 8.4% of the Japanese population (127.7 million). In the present study, the addresses of all the cases were geographically limited to this district.

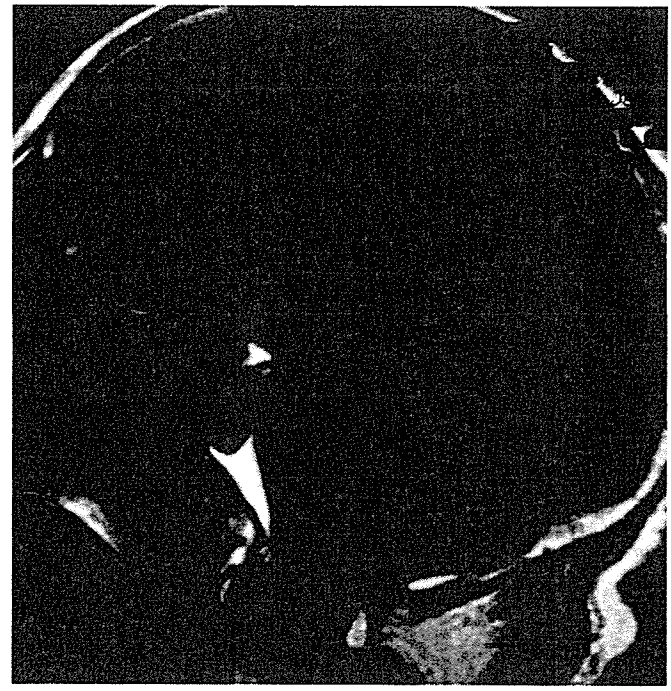

Figure 2. MRI showed cerebellar atrophy without obvious brainstem involvement in Case 1 (table E-1).

Discussion. We found that 16q-ADCA is a common subtype (12.0%) of ADCA in the Tohoku District of Japan. In addition, we identified 7 cases with 16q-ADCA (5.4%) in the sporadic cases.

In Tohoku District, SCA-1 is the most common ADCA and showed an exceedingly strong founder effect.[6,7] In the present study, however, the addresses and birthplaces of the families with 16q-ADCA were not geographically clustered as in the case of SCA-1 families (data not shown).

Although the single nucleotide C→T substitution in 5′ UTR of the puratrophin-1 gene may not be pathogenic, the puratrophin-1 gene which is considered to be responsible for 16q-ADCA is located in the region linked to SCA4.[2] However, it remains to be determined whether 16q-ADCA in Japan is allelic with SCA4 in Utah[3] and Germany.[4] In a previous study in Japan, the phenotype of 16q-ADCA was distinguished from that of SCA4.[8] A prominent feature of SCA4 is axonal neuropathy, which is observed in all cases with SCA4. Furthermore, preservation of eye movements is also characteristic of SCA4 pa-

Table *Genotype frequencies of autosomal dominant and sporadic cerebellar ataxia in the Tohoku District*

	Mode of inheritance								
	Autosomal dominant					Sporadic			
	No. of families	%	No. of patients	Male	Female	No. of patients	%	Male	Female
SCA1	45	20.7	68	41	27	0	0	0	0
SCA2	1	0.5	6	1	5	0	0	0	0
SCA3/MJD	38	17.4	48	29	19	4	3.1	2	2
SCA6	32	14.7	37	22	15	7	5.4	4	3
SCA7	2	0.9	6	2	4	0	0	0	0
SCA8	2	0.9	2	1	1	0	0	0	0
SCA12	0	0	0	0	0	0	0	0	0
SCA17	0	0	0	0	0	0	0	0	0
DRPLA	24	11.0	30	9	21	0	0	0	0
16q-ADCA	26	12.0	31	13	18	7	5.4	2	5
Unknown	48	22.0	51	27	24	110	85.9	58	52
Total	218	100	279	145	134	128	100	66	62

tients. In contrast, the leading symptom of 16q-ADCA in Japan is pure cerebellar ataxia, and neuropathy was absent in the previously reported cases of 16q-ADCA from Japan.[8] Moreover, the mean age at onset is older (55.9 years)[8] than that of SCA4 (39.3 years).[3]

In the present study, almost all the cases with 16q-ADCA showed late-onset pure cerebellar ataxia including limb ataxia (97%), truncal ataxia (94%), and dysarthria (97%). In contrast, other symptoms which are commonly seen in SCA4 were rare, with only two patients (6%) presenting mild sensory disturbance. The mean age at onset in our cases was 57.7 ± 7.1 years, which is compatible with the previous report.[8]

Our patients with 16q-ADCA showed a phenotype similar to patients with SCA6[9,10] rather than those with SCA4.[3] However, patients with SCA6 vary in terms of the age at onset and the severity, because these are based on the number of CAG repeats in the responsible gene.[9] However, although nystagmus is a common sign in all subtypes of ADCA including SCA6, only 5 (15%) of our cases of 16q-ADCA showed nystagmus. Furthermore, although juvenile cases (<25 years) were reported in SCA6, we found no juvenile cases of 16q-ADCA in the present study (table E-1). These findings indicate that the cardinal feature of our cases of 16q-ADCA was late-onset pure cerebellar ataxia and the cerebellar ataxia was not compatible with that of SCA6.

Interestingly, it was previously described that 42.9% of the affected members of a large 16q-ADCA pedigree had hearing impairment.[2] In contrast, only one patient (3%) showed hearing loss in the present study.

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特集 第41回脳のシンポジウム

運動ニューロン疾患をめぐる最近の進歩

運動ニューロン疾患，特にALSの治療戦略*

糸山泰人**

筋萎縮性側索硬化症（ALS）は運動ニューロンが選択的に傷害されて，体全体に筋萎縮と脱力が進行する予後不良な疾患である。病因は不明であるが，多くの病因論の中でも変異Cu/Zn superoxide dismutase（SOD1）説が最も有力である。有効な治療薬はなく，多くの患者は新たな治療薬の開発を待望している。筆者らは変異SOD1を導入した新たなトランスジェニックラットを作製し，そのALSラットに対し日本で発見された新規の神経栄養因子であり，運動ニューロンに栄養作用の強い肝細胞増殖因子（hepatocyte growth factor：HGF）の髄腔内投与実験を行った。その結果，HGFを投与したALSラットでは，非投与ALSラットに較べて寿命を約1.6倍も延長することができ，腰髄の運動ニューロン数の減少を抑えることを認めた。このHGFの髄腔内投与は新たなALSの治療として最も期待されるものであり，現在，ALS患者への臨床応用を目指して霊長類に対して安全試験を行っている。また，神経幹細胞移植療法や遺伝子治療など，将来的なALS治療研究の現状を紹介した。

キーワード：筋萎縮性側索硬化症（ALS），Cu/Zn superoxide dismutase（SOD1），肝細胞増殖因子（HGF），ALSトランスジェニックラット，神経幹細胞移植

はじめに

筋萎縮性側索硬化症（ALS）は神経難病の中でも最も苛酷な疾患と考えられており，上位および下位運動ニューロンが選択的に障害される神経変性疾患である。多くは中年以降に発症し，呼吸筋を含む体全身にわたる進行性の筋萎縮と脱力が起こる，極めて予後不良の疾患である。現状では本疾患の原因・病態は不明であり，その治療法も確立されたものはなく，リルゾール（リルテック）以外には治療薬として認められたものはない。世界中の多くの患者・家族にとって新たな治療薬の開発が望まれている。

この難病ALSの治療法の開発には，①ALSの臨床および病理像を忠実に再現する動物モデルを作製する，②その動物モデルを用いて病態の解明と治療法の開発研究をすることが重要と考えられる。この考えに基づき筆者らは，世界に先駆けてラットにおけるALS動物モデルを作製し，そのモデルを用いて新規の神経栄養因子として注目されている肝細胞増殖因子（hepatocyte growth factor：HGF）がALSの有用な治療薬となりうる研究を行っている。これらの研究の現状と次世代のALSに対する治療法開発の研究として，遺伝子治療や神経幹細胞移植を紹介する。

Ⅰ．ALSの病因研究—SOD1病因論の重要性—

ALSの病因に関する研究は，すでに100年以上の歴

2006年9月20日受稿

* New therapeutic strategy for amyotrophic lateral sclerosis.

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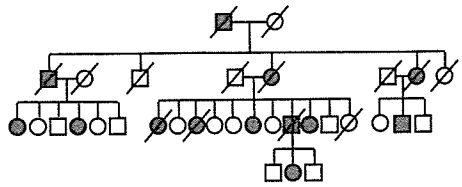
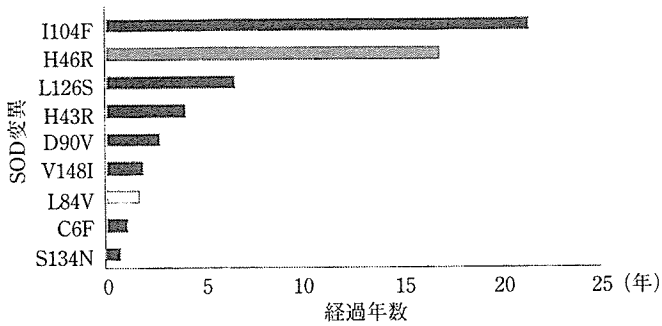
(1) 遺伝子異常が発症を規定



(2) 遺伝子変異と臨床経過の相関



(3) 遺伝子導入ALSモデルの作製

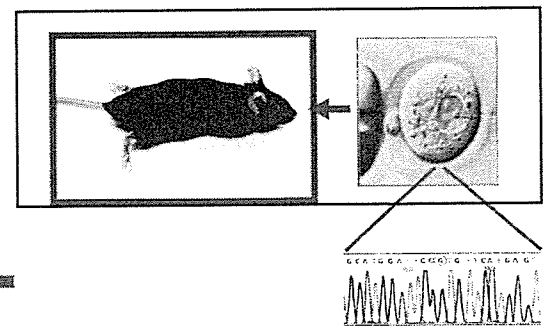


図 1　ALS の病因としての変異 Cu Zn SOD の関与

史があり，実に無数の病因論が報告されてきている。なかでも重金属による毒性説，グルタミン酸の毒性説，神経栄養因子の変調説，またウイルス感染説などが有力視されてきた。現在最も ALS の病因研究で重要視されているのは，銅・亜鉛スーパーオキサイド　ディスムターゼ (Cu/Zn superoxide dismutase：SOD1) 遺伝子変異である[1~3]。1993 年に ALS 患者の約 5〜10%を占める家族性 ALS の家系での遺伝学的解析研究から明らかにされた原因遺伝子の 1 つである。もっとも大半の ALS の患者は孤発例であるので，SOD1 病因説で全ての ALS の原因を説明することは出来ない。

しかし，この SOD1 が ALS の病因として重要視されている理由のいくつかを図 1 に示す。図 1-(1)に示すように家族性 ALS の家系の中において，SOD1 遺伝子の異常のある例（▨，◍）には ALS が発症し，遺伝子異常のない例（□，○）には病気は起こらず，SOD1 遺伝子の異常自体がその病気の発現を規定している事実がある。また，図 1-(2) で示すように，筆者らが検索した 9 家系の調査では，SOD1 の遺伝子異常の違いにより家系内の患者の臨床経過が規定されている[4]。すなわち，遺伝子異常が発症のみならず病態をも修飾する可能性が示された。加えて，家族性 ALS の患者に認められた SOD1 遺伝子変異をマウスの受精卵に導入することにより，臨床的および病理的に ALS に極めて類似した動物モデルが作製される[5]（図 1-(3)）。これらの事実は SOD1 遺伝子異常が，マウスの運動ニューロン死を引き起こす病態の鍵を握っていることを示唆している。

もちろん，ALS の病因を SOD1 遺伝子変異のみで解釈することには問題があるが，世界の多くの研究施設で再現性をもって共通の動物モデルが作製され，かつ利用出来ることは極めて重要なことと考える。このような理由から現状で科学的基盤に基づいて ALS の病因研究と治療法の開発研究を進めるには，SOD1 病因論に立って研究をすることが重要と考える。

II. ALS トランスジェニックラットの作製

変異 SOD1 遺伝子導入で作製された動物モデルは，現在のところマウスモデルとラットモデルの 2 種類があり，これらの動物モデルを使って行われている研究は主に 2 つの方向性がある。1 つは動物モデルに起こっている運動ニューロン傷害が，どのような機序で起こっていくのかの病態研究である。もう 1 つの方向性は，ALS 動物モデルを治療実験に使うことが挙げられる。従来からマウスによる動物モデルは，その個体の大きさによる研究上の様々な制約があったが，最近，東北大学で世界に先駆けて開発された ALS のラットモデルが，病態解明や治療法開発の点で注目されている。

ALS の治療実験には，患者に応用する際の薬物の投

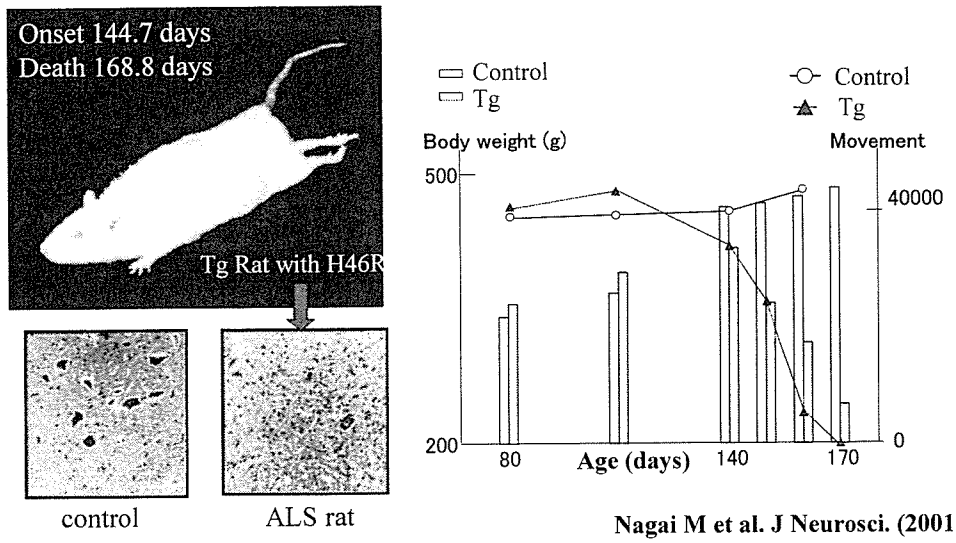


図2 新たなALSモデルラットの完成

与ルートとして髄腔内投与が注目されており，実際に米国ではALS患者への持続注入ポンプを用いた神経栄養因子の髄腔内投与治験が試みられている。そのような理由から東北大学では，動物モデルで薬剤の脊髄腔内投与に対する治療的なアプローチを可能にするために，変異Cu/Zn SOD1導入トランスジェニックALSラットを開発した（図2）[6]。このトランスジェニックラットの病理所見では，脊髄前角の運動ニューロンにおいて選択的な変性・消失がみられ，ヒトALS患者に特徴的に認められるLewy小体に類似した封入体も認められている。トランスジェニックラットは，従来のマウスに比較して約20倍の大きさがあるために，脳脊髄液の採取が可能であり，また薬剤や遺伝子治療用のベクターの髄腔内投与も極めて容易である。また電気生理学的に運動単位推定（motor unit number estimate：MUNE）も施行可能であり，治療法の評価にも有用である。将来的な遺伝子治療や外来の神経幹細胞の髄腔内投与や，脊髄への直接移植によるcell replacement therapyも含めた新しい治療法開発のために有用なモデルとなることも期待される。すなわち，このラットモデルを用いて各種の治療候補物を脊髄腔内に投入して，新しい治療法を開発することが可能になった。

Ⅲ．ALSの治療戦略

ALSの治療薬の開発は，ALSの病因・病態をまず明らかにし，次のステップとしてその病態に対しての対応治療手段を考えるのが理想である。しかし，ALSの病因論がまだ明確でないため，新しい治療法の開発は様々な考えに立って研究していかなければならない。その1つの立場としては，他の病気に対して使用されている既存の薬剤の中から，運動ニューロン死を防ぐ可能性の薬剤をみつける方法がある。これでみつかった薬はすでに安全性は確かめられているので，すぐにでもALS患者に用いることが出来る利点があり，今盛んに候補薬剤が選別されている。2つ目としては，いくつかのALSの病因・病態論から可能性のある薬剤を培養系の実験で確かめた後，動物モデルにてALSに対する有効性を確認して，患者への治療実験にもってゆく考えである。3つ目としては，今すぐには患者に対して治療応用は出来ないが，将来的に有用な治療法となりうるもの，すなわち遺伝子治療，あるいは神経幹細胞移植が挙げられる。この中で現在筆者らが最も力を入れている，肝細胞増殖因子（HGF）のALSの治療薬としての開発研究を紹介する。

1．肝細胞増殖因子を用いたALSの治療開発研究

ALSの病因は明確ではないが，運動ニューロン傷害の病態は様々な側面から研究されてきている。その病態を改善することにより運動ニューロン死を遅らせ，機能回復を与えるためには，神経栄養因子の一群がALSの治療薬として期待されている。この10～15年の間にCNTF，BDNF，GDNF，IGF-1をはじめとしたいくつかの神経栄養因子について，世界的な規模でALS患者に対して臨床試験が行われたが，有効性を示せない結果に終わっている。

その神経栄養因子の中でALSの治療薬の可能性として現在最も注目されているのが肝細胞増殖因子

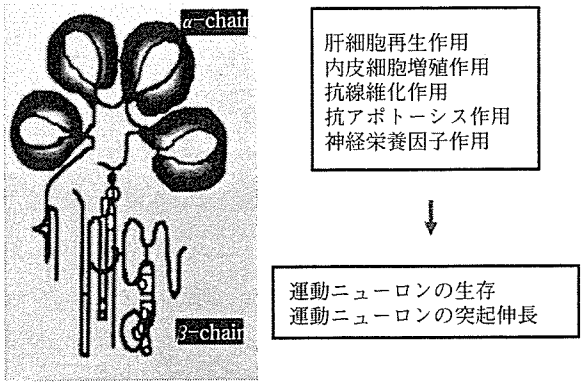


図3 肝細胞増殖因子(HGF)の生物学的作用

(hepatocyte growth factor：HGF)で，大阪大学の中村敏一らによってクローニングされた[7]。HGFは4つのクリングル構造を持つα鎖とセリンプロテアーゼ様構造を持つβ鎖からなるヘテロ2量体で，1本鎖の不活性型pro HGFとして産生され，その後プロセシングを受けて2本鎖活性型HGFとなる(図3)。活性型HGFはチロシンキナーゼ受容体(c-Met)に結合することにより細胞内シグナル伝達を行う。HGFは当初，培養肝細胞の増殖活性を指標に同定されたが，その後の研究の結果，実は神経細胞，なかでも運動ニューロンに対する神経栄養効果が強いことが注目されてきた。船越と中村らは，このHGFがALSの治療薬になりうる可能性を調べる目的のために，遺伝子工学的にHGF遺伝子を神経特異的に発現するトランスジェニックマウスを作製し，その後ALSトランスジェニックマウスと交配させダブルトランスジェニックマウスを作製した。すなわち，ALSトランスジェニックマウスの運動ニューロンに，長期間にわたってHGFを作用させることにより，HGFのALSに対する効果を検証する実験を行った。その結果，HGFを発現させることにより，麻痺の発症時期と寿命を約1カ月も延長させることが示された[8]。この結果はHGFがALSの治療薬として有望であることを示したものであり，現在進行中の「HGFをALS患者への治療薬として開発する」治療研究の基盤となっている。

2. ALSラットへのHGF髄腔内投与実験

薬物の投与ルートとしてALSの病態の主座である，脊髄の運動ニューロンに対して，効率よくしかも全身に対する副作用を回避できる髄腔内投与が注目されている。ALSトランスジェニックラットはマウスモデルに比べて，このルートによる薬剤供給が可能であり(図4)，これまで筆者らはALSラットに対して浸透圧ポンプを用いて髄腔内に遺伝子組み換え型ヒトHGFタンパクの投与実験を行ってきた。HGFを発症前の100日齢から1カ月にわたり投与し，腰髄1切片当たりの運動ニューロン数を定量した結果では，HGF投与群においては容量依存的に腰髄運動ニューロン数が保たれていることが示され，HGFの髄腔内投与はALSモデル動物の運動ニューロン傷害に対して保護効果があることが明瞭となった。

HGFのALSへの臨床応用を目指すには，動物モデルにおいてHGFの投与量，投与タイミングおよび投与期間の検討が重要である。それらの検討の中で200 μgのHGFをALSラットの症状が発症した時期から投与を始めても，明らかに寿命を延長させる結果が得られた。その効果は，HGFを投与しなかったALSラットモデルの寿命と比較すると約1.6倍も延長させるものであった。単純にラットの寿命とヒトの寿命を比較することは出来ないが，1.6倍の延命効果はヒトに換算するとALS患者の呼吸不全にいたる平均を3年とすると，それを5年にまで延ばす治療効果があることを意味しており，今後のALSに対するHGFの治療薬の開発にとって，大変期待できる結果と考えられる。

現在，ALSラットに対するHGFの有用性の機序の検討を行っている。その中でALSラットでは症状が発現する時期には，脊髄で内因性のHGFの発現量は正常対照に比べて増加しており，加えて神経細胞においてはHGFの受容体であるc-Metの発現も増加していることが確かめられており，病気の個体では疾患防御的機序が働いているものと考えられた。そこで治療実験とは逆にHGFの作用を抑制する目的で，ALSラットに抗HGF抗体(IgG)を発症前に投与する実験を行うとALSの発症時期は早まり，かつ症状の経過は急速に進行して死亡することが明らかになった。これらの結果を総合すると，ALSラットにおいては運動ニューロン傷害には内因性HGFは抑制的に作用しており，外因性HGFはその作用を増強しALSの症状進行に治療効果を示すことが示唆された。患者への臨床応用実験を可能にするために副作用の問題などを十分検討する必要があり，現在は霊長類を用いた安全試験を行っている。

Ⅳ. 将来的なALSの治療研究

1. 遺伝子治療の研究

将来的にALSの治療法として可能性があるものに，遺伝子治療が挙げられる。これには大きく分けて2つの方法があり，病気の根本的な原因にかかわる遺伝子異常に関して遺伝子治療を行う場合と，前述したよう

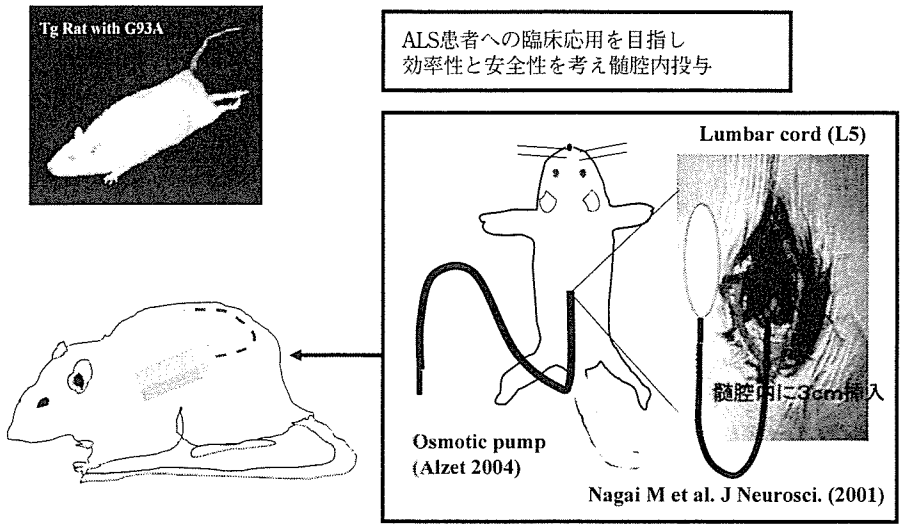


図4 ALSラットでのHGF髄腔内投与実験モデルの作製

な神経栄養因子の遺伝子をウイルスベクターを利用して，傷害されている運動ニューロンに作用させる治療法がある。なかでも神経栄養因子を運動ニューロンに作用させるウイルスベクターの研究が多く行われている。神経栄養因子の遺伝子を組み込ませて，目的の細胞へ運ぶウイルスベクターとしてAAVベクターやヘルペスウイルスベクターなどがあるが，それ以外で注目すべきベクターとしてポリオウイルスがあり，現在そのベクターの開発が研究されている。これはポリオウイルスが運動ニューロンに特異的に感染する性質を利用したもので，このベクターが実用化されるとALS患者で変性しかかっている運動ニューロンへ，集中的に治療薬剤を作用できるようになる[9]。このような考えで遺伝子治療を開発するには，ポリオウイルス自体が持つ毒性を消し去る必要があるとともに，またHGFのような外来の遺伝子を組み込ませる操作と，その後にもポリオウイルスの運動ニューロン感染性能力を維持することが要件であり，その点での研究が行われている。

2. 神経幹細胞移植治療

遺伝子治療と同じく将来的なALSの治療法として注目されているものに，神経幹細胞移植治療が挙げられる。運動ニューロンのように神経細胞として高度に分化した神経細胞は，いったん傷害されると再生されることはないと考えられてきたが，近年の研究によると，成人においても神経幹細胞が神経細胞やグリア細胞に分化する可能性が示されてきている。神経幹細胞を培養条件下にてあらかじめ多数の神経細胞を作り出し，それらを運動ニューロンにまで分化させて，傷害された患者の脊髄に投与して，傷害された運動ニューロンの代わりとして機能障害の補充が出来ないかという考えである。これに関しては現在，培養レベルで神経幹細胞から運動ニューロンにまで分化させることが可能になっている。今の研究課題は，分化した運動ニューロンを実験的にALSの動物の脊髄に導入して，神経突起を伸ばすことが出来るものかどうかという点である。この実験系においても，体の大きいALSラットモデルが用いられており，脊髄に導入した培養から得られた運動ニューロンが数週間にわたって生着していることが確認されている。今後は生着した運動ニューロンがいかに神経突起を伸展させて，どのような機能を果たすかの検討が必要である。

もう1つ違った方向からの神経幹細胞に関するALS治療の取り組みがある。ALSの動物モデルの脊髄を観察すると，脊髄の中心部分において神経前駆細胞が新たに増殖分化しており，場合によっては神経幹細胞，さらには運動ニューロンにまで分化する可能性を示している。この神経前駆細胞の増殖分化は，ALSという病気に対して1つの生体防御の反応ではないかとも考えられる。いずれにしてもALSの病気の過程でそのような神経前駆細胞が出現しているので，それらの細胞を効率よく神経幹細胞に分化させる方法の研究が現在進展中である。

おわりに

ALSの病因を明らかにし，そこから治療薬を開発す

るという基本的な治療法開発が容易ではない現状では，ALSの動物モデルを用いた各種の候補治療薬の開発研究が重要である。なかでも筆者らが開発したALSトランスジェニックラットの動物モデルを用いて，わが国で発見された神経栄養因子のHGFをALS治療薬として開発しようという研究は，最も期待されているものの1つである。一方，神経幹細胞移植療法や遺伝子治療など，将来的なALSの病因に基づいた治療法はまだ実験段階であるが，その方向性の研究は確実に進歩している。それらの研究の現状を紹介した。

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