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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REFERENCES

Abe K, Aoki M, Ikeda M, Watanabe M, Hirai S, Itoyama Y. 1996. Clinical characteristics of familial amyotrophic lateral sclerosis with Cu/Zn superoxide dismutase gene mutations. J Neurol Sci 136:108–116.

Azzouz M, Ralph GS, Storkebaum E, Walmsley LE, Mitrophanous KA, Kingsman SM, Carmeliet P, Mazarakis ND. 2004. VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. Nature 429:413–417.

Barneoud P, Lolivier J, Sanger DJ, Scatton B, Moser P. 1997. Quantitative motor assessment in FALS mice: a longitudinal study. Neuroreport 8:2861–2865.

Borchelt DR, Wong PC, Becher MW, Pardo CA, Lee MK, Xu ZS, Thinakaran G, Jenkins NA, Copeland NG, Sisodia SS, Cleveland DW, Price DL, Hoffman PN. 1998. Axonal transport of mutant superoxide dismutase 1 and focal axonal abnormalities in the proximal axons of transgenic mice. Neurobiol Dis 5:27–35.

Brooks KJ, Hill MD, Hockings PD, Reid DG. 2004. MRI detects early hindlimb muscle atrophy in Gly93Ala superoxide dismutase-1 (G93A SOD1) transgenic mice, an animal model of familial amyotrophic lateral sclerosis. NMR Biomed 17:28–32.

Brown RH Jr. 1995. Amyotrophic lateral sclerosis: recent insights from genetics and transgenic mice. Cell 80:687–692.

Bruening W, Roy J, Giasson B, Figlewicz DA, Mushynski WE, Durham HD. 1999. Up-regulation of protein chaperones preserves viability of cells expressing toxic Cu/Zn-superoxide dismutase mutants associated with amyotrophic lateral sclerosis. J Neurochem 72:693–699.

Chiu AY, Zhai P, Dal Canto MC, Peters TM, Kwon YW, Prattis SM, Gurney ME. 1995. Age-dependent penetrance of disease in a transgenic mouse model of familial amyotrophic lateral sclerosis. Mol Cell Neurosci 6:349–362.

de Belleroche J, Orrell R, King A. 1995. Familial amyotrophic lateral sclerosis/motor neurone disease (FALS): a review of current developments. J Med Genet 32:841–847.

Deng HX, Hentati A, Tainer JA, Iqbal Z, Cayabyab A, Hung WY, Getzoff ED, Hu P, Herzfeldt B, Roos RP, Warner C, Deng G, Soriano E, Smyth C, Parge HE, Ahmed A, Roses AD, Hallewell RA, Pericak-Vance MA, Siddique T. 1993. Amyotrophic lateral sclerosis and structural defects in Cu, Zn superoxide dismutase. Science 261:1047–1051.

Fujiwara N, Miyamoto Y, Ogasahara K, Takahashi M, Ikegami T, Takamiya R, Suzuki K, Taniguchi N. 2005. Different immunoreactivity against monoclonal antibodies between wild-type and mutant copper/zinc superoxide dismutase linked to amyotrophic lateral sclerosis. J Biol Chem 280:5061–5070.

Furukawa Y, O'Halloran TV. 2005. Amyotrophic lateral sclerosis mutations have the greatest destabilizing effect on the Apo- and reduced form of SOD1, leading to unfolding and oxidative aggregation. J Biol Chem 280:17266–17274.

Gale K, Kerasidis H, Wrathall JR. 1985. Spinal cord contusion in the rat: behavioral analysis of functional neurologic impairment. Exp Neurol 88:123–134.

Garbuzova-Davis S, Willing AE, Milliken M, Saporta S, Zigova T, Cahill DW, Sanberg PR. 2002. Positive effect of transplantation of hNT neurons (NTera 2/D1 cell-line) in a model of familial amyotrophic lateral sclerosis. Exp Neurol 174:169–180.

Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, Caliendo J, Hentati A, Kwon YW, Deng HX, Chen W, Zhai F, Sufit RL, Siddique T. 1994. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 264:1772–1775.

Hand CK, Rouleau GA. 2002. Familial amyotrophic lateral sclerosis. Muscle Nerve 25:135–159.

Howland DS, Liu J, She Y, Goad B, Maragakis NJ, Kim B, Erickson J, Kulik J, DeVito L, Psaltis G, DeGennaro LJ, Cleveland DW, Rothstein JD. 2002. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). Proc Natl Acad Sci USA 99:1604–1609.

Inoue H, Tsukita K, Iwasato T, Suzuki Y, Tomioka M, Tateno M, Nagao M, Kawata A, Saido TC, Miura M, Misawa H, Itohara S, Takahashi R. 2003. The crucial role of caspase-9 in the disease progression of a transgenic ALS mouse model. EMBO J 22:6665–6674.

Kaspar BK, Llado J, Sherkat N, Rothstein JD, Gage FH. 2003. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. Science 301:839–842.

Kato M, Aoki M, Ohta M, Nagai M, Ishizaki F, Nakamura S, Itoyama Y. 2001. Marked reduction of the Cu/Zn superoxide dismutase polypeptide in a case of familial amyotrophic lateral sclerosis with the homozygous mutation. Neurosci Lett 312:165–168.

Keller JN, Huang FF, Zhu H, Yu J, Ho YS, Kindy TS. 2000. Oxidative stress-associated impairment of proteasome activity during ischemia-reperfusion injury. J Cereb Blood Flow Metab 20:1467–1473.

Landis JR, Koch GG. 1977. The measurement of observer agreement for categorical data. Biometrics 33:159–174.

Mikami Y, Toda M, Watanabe M, Nakamura M, Toyama Y, Kawakami Y. 2002. A simple and reliable behavioral analysis of locomotor function after spinal cord injury in mice. Technical note. J Neurosurg Spine 97:142–147.

Mulder DW, Kurland LT, Offord KP, Beard CM. 1986. Familial adult motor neuron disease: amyotrophic lateral sclerosis. Neurology 36:511–517.

Nagai M, Aoki M, Miyoshi I, Kato M, Pasinelli P, Kasai N, Brown RH, Jr., Itoyama Y. 2001. Rats expressing human cytosolic copper-zinc superoxide dismutase transgenes with amyotrophic lateral sclerosis: associated mutations develop motor neuron disease. J Neurosci 21:9246–9254.

Ohki-Hamazaki H, Sakai Y, Kamata K, Ogura H, Okuyama S, Watase K, Yamada K, Wada K. 1999. Functional properties of two bombesin-like peptide receptors revealed by the analysis of mice lacking neuromedin B receptor. J Neurosci 19:948–954.

Okada Y, Shimazaki T, Sobue G, Okano H. 2004. Retinoic-acid-concentration-dependent acquisition of neural cell identity during in vitro differentiation of mouse embryonic stem cells. Dev Biol 275:124–142.

Okado-Matsumoto A, Fridovich I. 2002. Amyotrophic lateral sclerosis: a proposed mechanism. Proc Natl Acad Sci USA 99:9010–9014.

Ouary S, Bizat N, Altairac S, Menetrat H, Mittoux V, Conde F, Hantraye P, Brouillet E. 2000. Major strain differences in response to chronic systemic administration of the mitochondrial toxin 3-nitropropionic acid in rats: implications for neuroprotection studies. Neuroscience 97:521–530.

Rivlin AS, Tator CH. 1977. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. J Neurosurg 47: 577–581.

Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX, Rahmani Z, Krizus A, McKenna-Yasek D, Cayabyab A, Gasten SM, Berger R, Tanzi RE, Halperin JJ, Herzfeldt B, van den Bergh R, Hung WY, Bird T, Deng G, Mulder DW, Smyth C, Laing NG, Soriano E, Pericak-Vance MA, Haines J, Reuleau GA, Gusella JS, Horvitz HR, Brown RH Jr. 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362:59–62.

Shipp EL, Cantini F, Bertini I, Valentine JS, Banci L. 2003. Dynamic properties of the G93A mutant of copper-zinc superoxide dismutase as detected by NMR spectroscopy: implications for the pathology of familial amyotrophic lateral sclerosis. Biochemistry 42:1890–1899.

Storkebaum E, Lambrechts D, Dewerchin M, Moreno-Murciano MP, Appelmans S, Oh H, Van Damme P, Rutten B, Man WY, De Mol M, Wyns S, Manka D, Vermeulen K, Van Den Bosch L, Mertens N, Schmitz C, Robberecht W, Conway EM, Collen D, Moons L, Carmeliet P. 2005. Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. Nat Neurosci 8:85–92.

Sun W, Funakoshi H, Nakamura T. 2002. Overexpression of HGF retards disease progression and prolongs life span in a transgenic mouse model of ALS. J Neurosci 22:6537–6548.

Urushitani M, Kurisu J, Tsukita K, Takahashi R. 2002. Proteasomal inhibition by misfolded mutant superoxide dismutase 1 induces selective motor neuron death in familial amyotrophic lateral sclerosis. J Neurochem 83:1030–1042.

Wang LJ, Lu YY, Muramatsu S, Ikeguchi K, Fujimoto K, Okada T, Mizukami H, Matsushita T, Hanazono Y, Kume A, Nagatsu T, Ozawa K, Nakano I. 2002. Neuroprotective effects of glial cell line-derived neurotrophic factor mediated by an adeno-associated virus vector in a transgenic animal model of amyotrophic lateral sclerosis. J Neurosci 22: 6920–6928.

Watanabe M, Aoki M, Abe K, Shoji M, Iizuka T, Ikeda Y, Hirai S, Kurokawa K, Kato T, Sasaki H, Itoyama Y. 1997. A novel missense point mutation (S134N) of the Cu/Zn superoxide dismutase gene in a patient with familial motor neuron disease. Hum Mutat 9:69–71.

Weydt P, Hong SY, Kliot M, Moller T. 2003. Assessing disease onset and progression in the SOD1 mouse model of ALS. Neuroreport 14: 1051–1054.

Williamson TL, Cleveland DW. 1999. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. Nat Neurosci 2:50–56.

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