Wild-type Neuro2A cells or NSC34 cells and NSC34 cells stably expressing the human AR24Q or AR97Q were plated in six-well dishes in 5 ml of DMEM/10% fetal bovine serum (FBS) (Dulbecco's modified Eagle's medium supplemented with 10% FBS) with penicillin and streptomycin. Each dish was transfected with 4 μg of DNA plasmids and siRNA or control (mock) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. NSC34 cells were cultured in differentiation medium (DMEM/2% FBS) containing dihydrotestosterone (DHT; 1 nM) after transfection. The transfection efficiency was 60–70%. Neuro2A cells were cultured in differentiation medium (DMEM/2% FBS) containing retinoic acid (20 μM) and DHT (1 nM) after transfection. The transfection efficiency was 60–70%.

Promoter assay

GoClone and the pGL4 Luciferase Reporter Vector encoding the NF-YA, TFEB, Hsp70 and CHIP promoter (SwitchGear Genomics) were transfected into NSC34 cells with Lipofectamine 2000 (Invitrogen). The Steady-Glo Luciferase Assay System (Promega) was used to measure expression with POWERSCAN 4 (DS Pharma Biomedical).

Generation and maintenance of transgenic mice

We used AR-24Q and AR-97Q male mice in this study (21). The AR-97Q (Line #7–8) male mice showed progressive muscular atrophy and weakness accompanied by diffuse nuclear staining and NIs of the mutant AR. Similar to SBMA patients, these phenotypes were very pronounced in the male transgenic mice. The littermates were also used for phenotypic analyses. All of the animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and under the approval of the Nagoya University Animal Experiment Committee.

Therapeutic agents and protocol for administration

PF, purchased from Wako Pure Chemical Industries (Japan) (molecular weight: 480.45, 10 mg), was dissolved in 1 ml of PBS, diluted for use in culture medium and stored in the freezer until use. For the SBMA mouse model, we stored 10 mg/ml stock solutions of PF dissolved in a 0.9% NaCl solution at −20°C. To demonstrate that PF slows the onset and progression of the disease, PF treatments were initiated when the mice reached 5 weeks of age and continued until they were 32 weeks old. The stock solution was diluted to 1 mg/ml with saline solution just before the experiment, and 6.7 or 13.4 mg/kg of PF was administered intraperitoneally every day. The control mice received saline.

Neurological and behavioral assessments of the SBMA mouse model

Mice of varying ages were assessed with two different behavioral tests by investigators blind to the genotype and treatment. The rotarod task (Ugo Basile) was performed on a weekly basis, and cage activity was measured weekly with the AB system (Neuroscience) (21). Spontaneous motor activity was monitored for periods of 24 h; all spontaneous movements, both vertical and horizontal, including locomotion, rearing and head movements, were counted and automatically totaled.

Immunohistochemistry and histopathology

Mice were deeply anesthetized with pentobarbital sodium. The spinal cord and skeletal muscle tissues were removed, fixed overnight in 10% phosphate-buffered formalin and processed for paraffin embedding. Sections (6 μM thick) of tissue were deparaffinized, dehydrated with alcohol and treated in formic acid for 5 min at room temperature. For the immunohistochemical studies, the paraffin sections were preheated in a microwave oven for 10 min and blocked with normal animal serum (1:20). The sections were then incubated with anti-expanded polyQ antibody (1:10 000; 1C2; Millipore), anti-NF-YA antibody (1:1000; H-209; Santa Cruz), anti-TFEB antibody (1:1000; Medical and Biological Laboratories) and anti-GFAP antibody (1:1000; Roche Diagnostics). The primary antibodies were probed with a biotinylated anti-species-specific IgG (Vector), and the immune complexes were visualized using streptavidin–horseradish peroxidase (HRP) (Dako) and 3,3-diaminobenzidine (Dojindo) as a substrate. The sections were counterstained with Mayer's hematoxylin. Paraffin-embedded sections (6 μm thick) of the gastrocnemius muscles were air-dried and stained with hematoxylin and eosin. For double-immunofluorescence staining, the sections were blocked with TSA Blocking Reagent (PerkinElmer) and then sequentially incubated with anti-NF-YA antibody (1:1000; H-209; Santa Cruz) and 1C2 antibody (1:10000; Millipore) at 4°C overnight. The sections were then incubated with Alexa 488-conjugated goat anti-rabbit IgG (1:1000; Invitrogen) and Alexa 546-conjugated goat anti-mouse IgG (1:1300; Invitrogen) for 8 h at 4°C. The stained sections were examined and imaged with a confocal laser-scanning microscope (LSM 710; Carl Zeiss).

Patients

Tissue was obtained from three patients with clinicopathologically and genetically confirmed SBMA (51–77 years of age; mean, 65.7 years) and three non-neurological controls (51–78 years of age; mean, 64.0 years). The patients were hospitalized and evaluated at Nagoya University Hospital and affiliated hospitals. The collection of human tissues and their use for this study were approved by the Ethics Committee of Nagoya University Graduate School of Medicine. Informed consent was obtained to use the tissues for research purposes. Paraffin-embedded sections of the spinal cord were processed and examined in the same manner as those of the transgenic mice.

Quantification of 1C2-positive cells

To assess 1C2-positive cells in the ventral horn of the spinal cord, 50 consecutive transverse sections of the thoracic spinal cord were prepared from each individual mouse. 1C2-positive cells within the ventral horn of every fifth section were counted (55). Populations of 1C2-positive cells were expressed as the number/mm^2 (Fig. 5B). To examine 1C2-positive cells in the muscle, the number of 1C2-positive cells was calculated from counts of 500 fibers in randomly selected areas and expressed

as the number per 100 muscle fibers (Fig. 5B). The quantitative data of six individual mice were expressed as the mean ± SEM.

Protein expression analysis

Forty-eight hours after transfection, the cells were lysed in CelLytic-M Mammalian Cell Lysis/Extraction Reagent (Sigma), supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 6 μg/ml aprotinin and centrifuged at 1000*g* for 15 min at 4°C. NE-PER Nuclear Cytoplasmic Reagents (Thermo Scientific) were used for the analysis of the nuclear/cytoplasmic translocation of TFEB. Sixteen-week-old mice were exsanguinated under pentobarbital sodium anesthesia, and the tissues were snap-frozen with powdered CO_2 in acetone. The tissues were homogenized in CelLytic-M Mammalian Cell Lysis/Extraction Reagent with 1 mM PMSF and 6 μg/ml aprotinin and centrifuged at 2500*g* for 15 min at 4°C. The supernatant protein concentrations were determined using the DC protein assay (Bio-Rad). Aliquots of the supernatant fractions were loaded on 5–20% SDS–PAGE gels, where each lane contained 7 μg of cell protein and 40 μg of neural and muscle tissue. The gels were then transferred to Hybond-P membranes (GE Healthcare) using transfer buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, 10% methanol). The following antibodies were used in our studies: anti-AR (1:1000; N20; Santa Cruz), anti-AR (1:1000; H280; Santa Cruz), anti-NF-YA (1:1000; H-209; Santa Cruz), anti-TFEB (1:5000; ab2636; Abcam), anti-HSP70 (1:10 000; SPA-810; Enzo Life Sciences), anti-HSP70 (1:1000; #4872; Cell Signaling Technology), anti-HSP40 (1:10 000; SPA-400; Enzo Life Sciences), anti-HSP40 (1:1000; #4868; Cell Signaling Technology), anti-CHIP (1:1000; #C3B6; Cell Signaling Technology), mouse anti-GAPDH (1:10000; MAB374; Millipore), mouse anti-α-tubulin (1:5000, T9026; Sigma), anti-LC3 (1:1000; #D11; Cell Signaling Technology), anti-V5 (1:5000; PM003; Medical & Biological Laboratories), anti-hexosaminidase A (1:1000; N-19; Santa Cruz), anti-Cathepsin B (1:2000; ab33538; Abcam), anti-Cathepsin D (1:2000; ab6313; Abcam), anti-tripeptidyl peptidase 1 (1:1000; G-16; Santa Cruz), anti-V-ATPase H (1:2000; H-300; Santa Cruz), anti-mTOR (1:2000; #2983; Cell Signaling Technology), anti-Phospho-mTOR (1:2000; #2974; Cell Signaling Technology), anti-p70 S6 Kinase (1:2000; #2708; Cell Signaling Technology), anti-Phospho-p70 S6 Kinase (1:2000; #9234; Cell Signaling Technology), anti-eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) (1:2000; #9644; Cell Signaling Technology), anti-Phospho-4E-BP1 (Thr37/46; 1:2000; #2855; Cell Signaling Technology) and anti-Beclin-1 (1:2000; #3495; Cell Signaling Technology). The primary antibodies were probed using HRP-conjugated anti-rabbit IgG F(ab′)2 and anti-mouse IgG F(ab′)2 (1:5000; GE Healthcare) secondary antibodies and were detected with the ECL Prime kit (GE Healthcare). An LAS-3000 imaging system was used to produce digital images and to quantify the band intensities, which were then analyzed with the Image Gauge software version 4.22 (Fujifilm). Densitometric values of ARs were normalized to endogenous GAPDH.

Pulse-chase labeling assay

NSC34 cells stably expressing the human AR24Q or AR97Q were plated in 6-cm dishes in 5 ml of DMEM/10% FBS containing DHT (1 nM) supplemented with penicillin and streptomycin. Each dish was treated with 10 μM of PF or PBS as a control. The cells were starved for 1 h in methionine- and cysteine-free DMEM/10% FBS and then labeled for 1 h with 150 Ci/ml of Redivue Pro-Mix L-[35S] *in vitro* cell-labeling mix (GE Healthcare). After washing in PBS, the cells were chased for the indicated time intervals in complete medium containing 10 μM of PF or saline. PF-treated cells were also treated with 20 nM bafilomycin A1 (11707; Sigma) or 2.5 μM lactacystin (PEPTIDE INSTITUTE, Inc.). Immunoprecipitation was performed using equivalent amounts of protein lysates, 50 μl of protein G Dynabeads (Invitrogen) and 5 μl of anti-AR antibody (N20; Santa Cruz) for 10 min at room temperature. Each sample was separated by 5–20% SDS–PAGE and analyzed by phosphorimaging (Typhoon LFA 9000 PhosphorImager; GE Healthcare) and Image Gauge software version 4.22 (Fujifilm).

Quantitative real-time reverse transcription–PCR

Total RNA was isolated from the NSC34 cells using the RNeasy Mini kit (Qiagen) and from mouse tissues using the PureLink RNA Mini kit (Invitrogen) according to the manufacturer's instructions. Total RNA from cells (3 μg) and mouse tissues (2.5 μg) was transcribed using the SuperScript VILO cDNA Synthesis Kit (Invitrogen). Real-time RT–PCR was performed in a total volume of 25 μl containing 12.5 μl of 2× QuantiFast SYBR Green PCR Master Mix (Qiagen) and 1 μM of each primer. The PCR products were detected by the iCycler system (Bio-Rad). The reaction conditions were 95°C for 5 min, 40 cycles of 10 s at 95°C and 30 s at 60°C. As an internal standard control, the expression level of β2 microglobulin was simultaneously quantified. The primer sequences are listed in Supplementary Material, Table S1.

Cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS)-based cell proliferation assay was performed in triplicate in NSC34 cells stably expressing the human AR24Q or AR97Q using the Cell Proliferation Reagent WST-1 (Roche Applied Science) and the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega) 48 h after incubation with PF.

Serum testosterone assay

Mice receiving PF or saline were deeply anesthetized with pentobarbital sodium at 16 weeks of age, 1 ml of blood was collected by cardiocentesis, and serum testosterone was assayed with the Coat-A-Count Total Testosterone radioimmunoassay (Roche) according to the manufacturer's instructions.

Statistical analysis

The data were analyzed using unpaired *t*-tests and *post hoc* tests (Tukey–Kramer *post hoc* tests) for multiple comparisons. The Kaplan–Meier and log-rank tests were used to assess the survival rate using StatView software version 5 (Hulinks).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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REFERENCES

1. Di Prospero, N.A. and Fischbeck, K.H. (2005) Therapeutics development for triplet repeat expansion diseases. *Nat. Rev. Genet.*, **6**, 756–765.
2. Adachi, H., Waza, M., Katsuno, M., Tanaka, F., Doyu, M. and Sobue, G. (2007) Pathogenesis and molecular targeted therapy of spinal and bulbar muscular atrophy. *Neuropathol. Appl. Neurobiol.*, **33**, 135–151.
3. Adachi, H., Katsuno, M., Minamiyama, M., Waza, M., Sang, C., Nakagomi, Y., Kobayashi, Y., Tanaka, F., Doyu, M., Inukai, A. *et al.* (2005) Widespread nuclear and cytoplasmic accumulation of mutant androgen receptor in SBMA patients. *Brain*, **128**, 659–670.
4. Sobue, G., Hashizume, Y., Mukai, E., Hirayama, M., Mitsuma, T. and Takahashi, A. (1989) X-linked recessive bulbospinal neuronopathy. A clinicopathological study. *Brain*, **112**, 209–232.
5. Soraru, G., D'Ascenzo, C., Polo, A., Palmieri, A., Baggio, L., Vergani, L., Gellera, C., Moretto, G., Pegoraro, E. and Angelini, C. (2008) Spinal and bulbar muscular atrophy: skeletal muscle pathology in male patients and heterozygous females. *J. Neurol. Sci.*, **264**, 100–105.
6. Atsuta, N., Watanabe, H., Ito, M., Banno, H., Suzuki, K., Katsuno, M., Tanaka, F., Tamakoshi, A. and Sobue, G. (2006) Natural history of spinal and bulbar muscular atrophy (SBMA): a study of 223 Japanese patients. *Brain*, **129**, 1446–1455.
7. Rhodes, L.E., Freeman, B.K., Auh, S., Kokkinis, A.D., La Pean, A., Chen, C., Lehky, T.J., Shrader, J.A., Levy, E.W., Harris-Love, M. *et al.* (2009) Clinical features of spinal and bulbar muscular atrophy. *Brain*, **132**, 3242–3251.
8. Buchberger, A., Bukau, B. and Sommer, T. (2010) Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. *Mol. Cell*, **40**, 238–252.
9. Adachi, H., Katsuno, M., Waza, M., Minamiyama, M., Tanaka, F. and Sobue, G. (2009) Heat shock proteins in neurodegenerative diseases: pathogenic roles and therapeutic implications. *Int. J. Hyperthermia*, **25**, 647–654.
10. Labbadia, J., Cunliffe, H., Weiss, A., Katsyuba, E., Sathasivam, K., Seredenina, T., Woodman, B., Moussaoui, S., Frentzel, S., Luthi-Carter, R. *et al.* (2011) Altered chromatin architecture underlies progressive impairment of the heat shock response in mouse models of Huntington disease. *J. Clin. Invest.*, **121**, 3306–3319.
11. Bailey, C.K., Andriola, I.F., Kampinga, H.H. and Merry, D.E. (2002) Molecular chaperones enhance the degradation of expanded polyglutamine repeat androgen receptor in a cellular model of spinal and bulbar muscular atrophy. *Hum. Mol. Genet.*, **11**, 515–523.
12. Stankiewicz, M., Nikolay, R., Rybin, V. and Mayer, M.P. (2010) CHIP participates in protein triage decisions by preferentially ubiquitinating Hsp70-bound substrates. *FEBS J*, **277**, 3353–3367.
13. Kabeya, Y., Mizushima, N., Ueno, T., Yamamoto, A., Kirisako, T., Noda, T., Kominami, E., Ohsumi, Y. and Yoshimori, T. (2000) LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J.*, **19**, 5720–5728.
14. Sardiello, M., Palmieri, M., di Ronza, A., Medina, D.L., Valenza, M., Gennarino, V.A., Di Malta, C., Donaudy, F., Embrione, V., Polishchuk, R.S. *et al.* (2009) A gene network regulating lysosomal biogenesis and function. *Science*, **325**, 473–477.
15. Settembre, C., Di Malta, C., Polito, V.A., Garcia Arencibia, M., Vetrini, F., Erdin, S., Erdin, S.U., Huynh, T., Medina, D., Colella, P. *et al.* (2011) TFEB links autophagy to lysosomal biogenesis. *Science*, **332**, 1429–1433.
16. Yan, D., Saito, K., Ohmi, Y., Fujie, N. and Ohtsuka, K. (2004) Paeoniflorin, a novel heat shock protein-inducing compound. *Cell Stress Chaperones*, **9**, 378–389.
17. Sun, X., Cao, Y.B., Hu, L.F., Yang, Y.P., Li, J., Wang, F. and Liu, C.F. (2011) ASICs mediate the modulatory effect by paeoniflorin on alpha-synuclein autophagic degradation. *Brain Res.*, **1396**, 77–87.
18. Dolfini, D., Gatta, R. and Mantovani, R. (2012) NF-Y and the transcriptional activation of CCAAT promoters. *Crit. Rev. Biochem. Mol. Biol.*, **47**, 29–49.
19. Lee, L.C., Chen, C.M., Wang, H.C., Hsieh, H.H., Chiu, I.S., Su, M.T., Hsieh-Li, H.M., Wu, C.H., Lee, G.C., Lee-Chen, G.J. *et al.* (2012) Role of the CCAAT-binding protein NFY in SCA17 pathogenesis. *PLoS ONE*, **7**, e35302.
20. Lieberman, A.P., Harmison, G., Strand, A.D., Olson, J.M. and Fischbeck, K.H. (2002) Altered transcriptional regulation in cells expressing the expanded polyglutamine androgen receptor. *Hum. Mol. Genet.*, **11**, 1967–1976.
21. Katsuno, M., Adachi, H., Kume, A., Li, M., Nakagomi, Y., Niwa, H., Sang, C., Kobayashi, Y., Doyu, M. and Sobue, G. (2002) Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. *Neuron*, **35**, 843–854.
22. Kondo, N., Katsuno, M., Adachi, H., Minamiyama, M., Doi, H., Matsumoto, S., Miyazaki, Y., Iida, M., Tohnai, G., Nakatsuji, H. *et al.* (2013) Heat shock factor-1 influences pathological lesion distribution of polyglutamine-induced neurodegeneration. *Nat. Commun.*, **4**. doi: 10.1038/ncomms2417.
23. Tagawa, K., Marubuchi, S., Qi, M.L., Enokido, Y., Tamura, T., Inagaki, R., Murata, M., Kanazawa, I., Wanker, E.E. and Okazawa, H. (2007) The induction levels of heat shock protein 70 differentiate the vulnerabilities to mutant huntingtin among neuronal subtypes. *J. Neurosci.*, **27**, 868–880.
24. Katsuno, M., Banno, H., Suzuki, K., Takeuchi, Y., Kawashima, M., Yabe, I., Sasaki, H., Aoki, M., Morita, M. and Nakano, I. (2010) Efficacy and safety of leuprorelin in patients with spinal and bulbar muscular atrophy (JASMITT study): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet Neurol.*, **9**, 875–884.
25. Fernandez-Rhodes, L.E., Kokkinis, A.D., White, M.J., Watts, C.A., Auh, S., Jeffries, N.O., Shrader, J.A., Lehky, T.J., Li, L., Ryder, J.E. *et al.* (2011) Efficacy and safety of dutasteride in patients with spinal and bulbar muscular atrophy: a randomised placebo-controlled trial. *Lancet Neurol.*, **10**, 140–147.
26. Parodi, S. and Pennuto, M. (2011) Neurotoxic effects of androgens in spinal and bulbar muscular atrophy. *Front. Neuroendocrinol.*, **32**, 416–425.
27. Hands, S.L. and Wyttenbach, A. (2010) Neurotoxic protein oligomerisation associated with polyglutamine diseases. *Acta Neuropathol.*, **120**, 419–437.
28. Naiki, H. and Nagai, Y. (2009) Molecular pathogenesis of protein misfolding diseases: pathological molecular environments versus quality control systems against misfolded proteins. *J. Biochem.*, **146**, 751–756.
29. Bauer, P.O. and Nukina, N. (2009) The pathogenic mechanisms of polyglutamine diseases and current therapeutic strategies. *J. Neurochem.*, **110**, 1737–1765.
30. Adachi, H., Katsuno, M., Minamiyama, M., Sang, C., Pagoulatos, G., Angelidis, C., Kusakabe, M., Yoshiki, A., Kobayashi, Y., Doyu, M. *et al.* (2003) Heat shock protein 70 chaperone overexpression ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model by reducing nuclear-localized mutant androgen receptor protein. *J. Neurosci.*, **23**, 2203–2211.
31. Katsuno, M., Adachi, H., Doyu, M., Minamiyama, M., Sang, C., Kobayashi, Y., Inukai, A. and Sobue, G. (2003) Leuprorelin rescues polyglutamine-dependent phenotypes in a transgenic mouse model of spinal and bulbar muscular atrophy. *Nat. Med.*, **9**, 768–773.

32. Waza, M., Adachi, H., Katsuno, M., Minamiyama, M., Sang, C., Tanaka, F., Inukai, A., Doyu, M. and Sobue, G. (2005) 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. *Nat. Med.*, **11**, 1088–1095.
33. Yang, Z., Chang, Y.J., Yu, I.C., Yeh, S., Wu, C.C., Miyamoto, H., Merry, D.E., Sobue, G., Chen, L.M., Chang, S.S. *et al.* (2007) ASC-J9 ameliorates spinal and bulbar muscular atrophy phenotype via degradation of androgen receptor. *Nat. Med.*, **13**, 348–353.
34. Miyazaki, Y., Adachi, H., Katsuno, M., Minamiyama, M., Jiang, Y.M., Huang, Z., Doi, H., Matsumoto, S., Kondo, N., Iida, M. *et al.* (2012) Viral delivery of miR-196a ameliorates the SBMA phenotype via the silencing of CELF2. *Nat. Med.*, **18**, 1136–1141.
35. Rinaldi, C., Bott, L.C., Chen, K.L., Harmison, G.G., Katsuno, M., Sobue, G., Pennuto, M. and Fischbeck, K.H. (2012) Insulinlike growth factor (IGF)-1 administration ameliorates disease manifestations in a mouse model of spinal and bulbar muscular atrophy. *Mol. Med.*, **18**, 1261–1268.
36. Katsuno, M., Adachi, H., Minamiyama, M., Waza, M., Doi, H., Kondo, N., Mizoguchi, H., Nitta, A., Yamada, K., Banno, H. *et al.* (2010) Disrupted transforming growth factor-beta signaling in spinal and bulbar muscular atrophy. *J. Neurosci.*, **30**, 5702–5712.
37. Yamanaka, T., Miyazaki, H., Oyama, F., Kurosawa, M., Washizu, C., Doi, H. and Nukina, N. (2008) Mutant Huntingtin reduces HSP70 expression through the sequestration of NF-Y transcription factor. *EMBO J.*, **27**, 827–839.
38. Huang, S., Ling, J.J., Yang, S., Li, X.J. and Li, S. (2011) Neuronal expression of TATA box-binding protein containing expanded polyglutamine in knock-in mice reduces chaperone protein response by impairing the function of nuclear factor-Y transcription factor. *Brain*, **134**, 1943–1958.
39. Wang, A.M., Miyata, Y., Klinedinst, S., Peng, H.M., Chua, J.P., Komiyama, T., Li, X., Morishima, Y., Merry, D.E., Pratt, W.B. *et al.* (2013) Activation of Hsp70 reduces neurotoxicity by promoting polyglutamine protein degradation. *Nat. Chem. Biol.*, **9**, 112–118.
40. Cummings, C.J., Sun, Y., Opal, P., Antalffy, B., Mestril, R., Orr, H.T., Dillmann, W.H. and Zoghbi, H.Y. (2001) Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. *Hum. Mol. Genet.*, **10**, 1511–1518.
41. Labbadia, J., Novoselov, S.S., Bett, J.S., Weiss, A., Paganetti, P., Bates, G.P. and Cheetham, M.E. (2012) Suppression of protein aggregation by chaperone modification of high molecular weight complexes. *Brain*, **135**, 1180–1196.
42. Malik, B., Nirmalananthan, N., Gray, A.L., La Spada, A.R., Hanna, M.G. and Greensmith, L. (2013) Co-induction of the heat shock response ameliorates disease progression in a mouse model of human spinal and bulbar muscular atrophy: implications for therapy. *Brain*, **136**, 926–943.
43. Popiel, H.A., Takeuchi, T., Fujita, H., Yamamoto, K., Ito, C., Yamane, H., Muramatsu, S., Toda, T., Wada, K. and Nagai, Y. (2012) Hsp40 gene therapy exerts therapeutic effects on polyglutamine disease mice via a non-cell autonomous mechanism. *PLoS ONE*, **7**, e51069.
44. Murata, S., Minami, Y., Minami, M., Chiba, T. and Tanaka, K. (2001) CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. *EMBO Rep.*, **2**, 1133–1138.
45. Miller, V.M., Nelson, R.F., Gouvion, C.M., Williams, A., Rodriguez-Lebron, E., Harper, S.Q., Davidson, B.L., Rebagliati, M.R. and Paulson, H.L. (2005) CHIP suppresses polyglutamine aggregation and toxicity in vitro and in vivo. *J. Neurosci.*, **25**, 9152–9161.
46. Adachi, H., Waza, M., Tokui, K., Katsuno, M., Minamiyama, M., Tanaka, F., Doyu, M. and Sobue, G. (2007) CHIP overexpression reduces mutant androgen receptor protein and ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model. *J. Neurosci.*, **27**, 5115–5126.
47. Montie, H.L., Cho, M.S., Holder, L., Liu, Y., Tsvetkov, A.S., Finkbeiner, S. and Merry, D.E. (2009) Cytoplasmic retention of polyglutamine-expanded androgen receptor ameliorates disease via autophagy in a mouse model of spinal and bulbar muscular atrophy. *Hum. Mol. Genet.*, **18**, 1937–1950.
48. Doi, H., Adachi, H., Katsuno, M., Minamiyama, M., Matsumoto, S., Kondo, N., Miyazaki, Y., Iida, M., Tohnai, G., Qiang, Q. *et al.* (2013) p62/SQSTM1 differentially removes thetoxic mutant androgen receptor via autophagy and inclusionformation in a spinal and bulbar muscular atrophy mouse model. *J. Neurosci.*, **33**, 7710–7727.
49. Song, W., Wang, F., Savini, M., Ake, A., di Ronza, A., Sardiello, M. and Segatori, L. (2013) TFEB regulates lysosomal proteostasis. *Hum. Mol. Genet.*, **22**, 1994–2009.
50. Medina, D.L., Fraldi, A., Bouche, V., Annunziata, F., Mansueto, G., Spampanato, C., Puri, C., Pignata, A., Martina, J.A., Sardiello, M. *et al.* (2011) Transcriptional activation of lysosomal exocytosis promotes cellular clearance. *Dev. Cell*, **21**, 421–430.
51. Parr, C., Carzaniga, R., Gentleman, S.M., Van Leuven, F., Walter, J. and Sastre, M. (2012) Glycogen synthase kinase 3 inhibition promotes lysosomal biogenesis and autophagic degradation of the amyloid-beta precursor protein. *Mol. Cell. Biol.*, **32**, 4410–4418.
52. Tsunemi, T., Ashe, T.D., Morrison, B.E., Soriano, K.R., Au, J., Roque, R.A., Lazarowski, E.R., Damian, V.A., Masliah, E. and La Spada, A.R. (2012) PGC-1alpha rescues Huntington's disease proteotoxicity by preventing oxidative stress and promoting TFEB function. *Sci. Transl. Med.*, **4**, 142ra197.
53. Chua, J.P., Reddy, S.L., Merry, D.E., Adachi, H., Katsuno, M., Sobue, G., Robins, D.M. and Lieberman, A.P. (2014) Transcriptional activation of TFEB/ZKSCAN3 target genes underlies enhanced autophagy in spinobulbar muscular atrophy. *Hum. Mol. Genet.*, **23**, 1376–1386.
54. Tanaka, K. and Matsuda, N. (2013) Proteostasis and neurodegeneration: the roles of proteasomal degradation and autophagy. *Biochim. Biophys. Acta*. doi: 10.1016/j.bbamcr.2013.03.012.
55. Adachi, H., Kume, A., Li, M., Nakagomi, Y., Niwa, H., Do, J., Sang, C., Kobayashi, Y., Doyu, M. and Sobue, G. (2001) Transgenic mice with an expanded CAG repeat controlled by the human AR promoter show polyglutamine nuclear inclusions and neuronal dysfunction without neuronal cell death. *Hum. Mol. Genet.*, **10**, 1039–1048.

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Original Article

Prevalence and Incidence of Amyotrophic Lateral Sclerosis in Japan

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ABSTRACT

Background: Previous studies have reported a high incidence of amyotrophic lateral sclerosis (ALS) in endemic foci in the Kii Peninsula, Japan. However, little is known about the ALS frequency in the whole country. Furthermore, the presence of ethnic variation in the incidence of ALS remains unknown.

Methods: We conducted a nationwide survey of ALS frequency in 2013 to estimate its annual prevalence and incidence. ALS was diagnosed based on the El Escorial Criteria. The study period was the 2009 fiscal year, from April 2009 to March 2010. To compare the incidence of ALS among prefectures, standardized incidence ratios (SIRs) and 95% confidence intervals (CIs) were calculated under the assumption of Poisson distribution.

Results: The annual crude prevalence and incidence rates per 100 000 people per year were 9.9 (95% CI 9.7–10.1) and 2.2 (95% CI 2.1–2.3), respectively. The age group with the highest prevalence as well as incidence was 70–79 years, and the male-female ratio was approximately 1.5. The annual incidence rate adjusted for age and sex using the 2000 U.S. standard population was 2.3 (95% CI 2.2–2.4) per 100 000 people. Some prefectures had significantly high SIRs: Okinawa, Nara and Wakayama in the Kii Peninsula, and Niigata for males; Kumamoto for females.

Conclusions: This is the first report on the annual prevalence and incidence of ALS in the representative population of Japan. We identified some prefectures with a high incidence of ALS. However, the incidence of ALS in the Japanese population was much lower than in the Caucasian populations of Europe and North America.

Key words: ALS; amyotrophic lateral sclerosis; epidemiology; incidence; Japan

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by the selective loss of upper and lower motor neurons. A total of 5%–10% of cases of ALS are familial, with the remainder believed to be sporadic.[1] The incidence of ALS is uniform across Caucasian populations,[2] but the presence of ethnic variation remains unknown.[3]

The Western Pacific form of ALS, termed ALS and parkinsonism-dementia complex (ALS/PDC), was identified in the 1950s in three distinct geographic isolates: Guam, Western New Guinea, and the Kii Peninsula of Japan.[4] The high prevalence and incidence of ALS/PDC has been reported in the Kii Peninsula (eg a prevalence of 47.7 per 100 000 people and an incidence of 9.54 per 100 000 person-years in the Kozagawa focus area of Wakayama prefecture).[5] However, little is known about the ALS frequency across Japan as a whole.

In this study, we estimate the prevalence and incidence of ALS in Japan. Furthermore, we compare the incidence between Japan and other countries, and examine geographic variation in the incidence of ALS among prefectures within the country.

METHODS

Specified disease treatment research program in Japan

Medical care is ensured for all people in Japan under the

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universal healthcare system, in which a 30% patient copayment is required for insurance-covered medical care. Some rare and intractable diseases, termed "specified diseases," remain difficult to treat, develop chronically, and constitute a great financial burden for patients and their family because of the high costs associated with long-term care and medicine. The specified disease treatment research program, which started in 1972, subsidizes the patient copayment for insured patients suffering from designated intractable diseases. A patient with ALS can receive that financial support, independent of disease severity.

Case ascertainment of ALS

When a patient is diagnosed with ALS by their doctor, they can apply to their prefectural government for assistance through the specified disease treatment research program, using the initial clinical application form for a new patient or the renewal form for an existing patient. Specialists in neurology serving on the committee on designated intractable diseases in each prefecture review the clinical application form, which is usually completed by a neurologist, based on the El Escorial Criteria (EEC).[6,7] As of 2009, there were 8555 neurologists in Japan.[8]

The criteria for ALS in this program are as follows[9]:

- adult onset, with a steady, progressive course;
- the presence of clinical or electrophysiological evidence of lower motor neuron (LMN) degeneration in at least two topographical anatomic regions (brainstem, cervical, thoracic or lumbosacral region), together with clinical evidence of upper motor neuron (UMN) degeneration in at least one region; and
- the absence of electrophysiological and pathological evidence of other disease processes that might explain the signs of LMN and/or UMN degeneration and neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs.

According to the criteria, patients with definite, probable, or possible ALS are included in this program, based on the revised EEC.[7,9]

Nationwide mail survey on amyotrophic lateral sclerosis

We sent a questionnaire on ALS to all 47 prefectural offices on February 11, 2013, requesting the following information:

- the number of patients with certified ALS who received financial aid for the treatment of a designated intractable disease in the 2009 fiscal year, classified by the categories (i) new or existing patients, (ii) sex, and (iii) age at which they were certified as a patient with ALS in the specified disease treatment research program (Note: patient's age at the time of diagnosis, application, and certification during the fiscal year is the same in most cases); and
- the total number of neurology specialists and the composition of the committee on designated intractable diseases.

The study period was the 2009 fiscal year, from April 2009 to March 2010, the latest fiscal year before occurrence of the Great East Japan Earthquake on March 11, 2011. We chose to examine events prior the earthquake because of the huge impact that it had on the population structure and quantity and quality of medical institutions and personnel in the Tohoku district, the consequences of which persist to date.

This study was approved by the Institutional Review Board of the National Institute of Public Health, Japan (NIPH-TRN#12009).

Statistical analysis

The annual prevalence rate was calculated from the observed number of all patients with ALS certified as eligible for financial aid for the treatment of a designated intractable disease during the 2009 fiscal year, divided by the population insured by national insurance. It was obtained by subtracting the population in receiving public assistance on July 31, 2009, from the total population on October 1, 2009. Overall, the insured population comprised approximately 99% of the total population. To calculate the annual incidence rate, the numerator was the observed number of patients with ALS newly certified as eligible for financial aid for the treatment of a designated intractable disease during the 2009 fiscal year. The rates were also calculated separately for age and sex.

To compare the incidence of ALS among different ethnic groups,[3] the rates were age- and sex-adjusted to the 2000 U.S. standard population using the 50- to 79-years age band by the direct method. We calculated 95% confidence intervals (CIs) under the assumption of Poisson distribution. In addition, geographic variation within the country was examined by calculating standardized incidence ratios (SIRs) and 95% CIs for all 47 prefectures using the indirect method under the assumption of Poisson distribution.[10,11] SIR with a 95% CI that did not include 1.0 was considered statistically significant.

RESULTS

All 47 prefectural offices returned their responses by June 2013. A special effort was made to achieve a response rate of 100% and confirm the data provided. Of 614 physicians comprising the committees on designated intractable diseases, the number of neurology specialists was 100, with at least 1 neurology specialist in the committee for each of 46 prefectures (unknown for Saga prefecture).

Table 1 shows the number of patients with ALS certified as eligible for financial aid for the treatment of a designated intractable disease during the 2009 fiscal year. Of a total of 10 237, including 6 patients younger than 20 years old, 2264 were patients with newly certified ALS.

Table 1. Number of patients with ALS certified as eligible for financial aid for the treatment of a designated intractable disease in Japan from April 2009 to March 2010

Age[a]	All patients			New patients		
(years)	Both	Male	Female	Both	Male	Female
0–4	0	0	0	0	0	0
5–9	1	1	0	0	0	0
10–14	2	0	2	0	0	0
15–19	3	1	2	1	1	0
20–24	10	8	2	2	2	0
25–29	19	14	5	2	1	1
30–34	50	23	27	8	4	4
35–39	95	58	37	13	7	6
40–44	226	120	106	41	17	24
45–49	319	180	139	55	26	29
50–54	558	326	232	99	54	45
55–59	1065	663	402	223	143	80
60–64	1590	955	635	337	217	120
65–69	1796	1103	693	394	234	160
70–74	1948	1111	837	450	269	181
75–59	1408	752	656	360	194	166
80–84	843	407	436	204	96	108
85+	304	120	184	75	37	38
Total	10237	5842	4395	2264	1302	962
Regrouped 20+	10231	5840	4391	2263	1301	962

[a]Age at which a patient was certified as eligible for financial aid for the treatment of a designated intractable disease from April 2009 to March 2010 (Note: Patient's age at the time of diagnosis, application and certification during the fiscal year is the same in most cases).

Table 2 shows the annual prevalence and incidence rates of patients with ALS aged 20 years or older. The annual crude prevalence was 9.9 (95% CI 9.7–10.1) per 100 000 people, with the highest age- and sex-specific rate of 27.1 (95% CI 26.2–28.0) per 100 000 people evident in the 70- to 79-years age group. The annual crude incidence was 2.2 (95% CI 2.1–2.3) per 100 000 people, with the highest age- and sex-specific rate of 6.5 (95% CI 6.1–7.0) per 100 000 people evident in the 70- to 79-years age group. Greater numbers of males were evident for both prevalence and incidence. To enhance the comparability of incidence rates among people of different ethnicities, incidence rates were standardized for the 50- to 79-years age band using the 2000 U.S. standard population (Table 2). The rate for both sexes combined was 2.3 (95% CI 2.2–2.4) per 100 000 people.

Table 3 shows the observed and expected numbers of patients with newly certified ALS, and SIRs with 95% CIs for each prefecture. Among males, SIRs were significantly high in Okinawa, Nara, Wakayama, and Niigata, and significantly low in Tokyo and Osaka. Among females, SIRs were significantly high in Kumamoto and significantly low in Miyazaki.

DISCUSSION

This is the first report on the prevalence and incidence of ALS in a representative population of Japan. The annual crude prevalence and incidence rates per 100 000 people per year

Table 2. Annual prevalence and incidence rates of patients with ALS, aged 20 years or older, certified as eligible for financial aid for the treatment of a designated intractable disease in Japan from April 2009 to March 2010

	Both				Male				Female				M:F ratio
Age[a] (years)	Patients	Population[b]	Rate[c]	95% CI	Patients	Population[b]	Rate[c]	95% CI	Patients	Population[b]	Rate[c]	95% CI	
Prevalence													
20–29	29	14371936	0.2	0.1–0.3	22	7366821	0.3	0.2–0.5	7	7005115	0.1	0.0–0.0	3.0
30–39	145	18193826	0.8	0.7–0.9	81	9241101	0.9	0.7–1.1	64	8952725	0.7	0.6–0.9	1.2
40–49	545	16253995	3.4	3.1–3.6	300	8187477	3.7	3.3–4.1	245	8066518	3.0	2.7–3.4	1.2
50–59	1623	16631377	9.8	9.3–10.2	989	8235051	12.0	11.3–12.8	634	8396326	7.6	7.0–8.2	1.6
60–69	3386	17419338	19.4	18.8–20.1	2058	8393464	24.5	23.5–25.6	1328	9025874	14.7	13.9–15.5	1.7
70–79	3356	12393683	27.1	26.2–28.0	1863	5576087	33.4	31.9–35.0	1493	6816596	21.9	20.8–23.0	1.5
80+	1147	7736961	14.8	14.0–15.7	527	2627371	20.1	18.4–21.8	620	5109590	12.1	11.2–13.1	1.7
Crude	10231	103001116	9.9	9.7–10.1	5840	49627372	11.8	11.5–12.1	4391	53372744	8.2	8.0–8.5	1.4
Incidence[d]													
20–29	4	14371936	0.0	0.0–0.1	3	14371936	0.0	0.0–0.1	1	7005115	0.0	0.0–0.0	2.9
30–39	21	18193826	0.1	0.1–0.2	11	18193826	0.1	0.1–0.2	10	8952725	0.1	0.1–0.2	1.1
40–49	96	16253995	0.6	0.5–0.7	43	16253995	0.5	0.4–0.7	53	8066518	0.7	0.5–0.9	0.8
50–59	322	16631377	1.9	1.7–2.2	197	16631377	2.4	2.1–2.8	125	8396326	1.5	1.2–1.8	1.6
60–69	731	17419338	4.2	3.9–4.5	451	17419338	5.4	4.9–5.9	280	9025874	3.1	2.7–3.5	1.7
70–79	810	12393683	6.5	6.1–7.0	463	12393683	8.3	7.6–9.1	347	6816596	5.1	4.6–5.7	1.6
80+	279	7736961	3.6	3.2–4.1	133	7736961	5.1	4.2–6.0	146	5109590	2.9	2.4–3.4	1.8
Crude	2263	103001116	2.2	2.1–2.3	1301	103001116	2.6	2.5–2.8	962	53372744	1.8	1.7–1.9	1.5
Age-adjusted[e]			2.3	2.2–2.4			4.7	4.4–5.0			1.7	1.6–1.9	2.8

[a]Age at which a patient was certified as eligible for financial aid for the treatment of a designated intractable disease from April 2009 to March 2010 (Note: Patient's age at the time of diagnosis, application and certification during the fiscal year is the same in most cases).
[b]The population receiving public assistance on July 31, 2009, is subtracted from the total population on October 1, 2009.
[c]per 100 000 people per year.
[d]The numerator was the number of patients with ALS newly certified as eligible for financial aid for the treatment of a designated intractable disease during the study period.
[e]Age-adjusted incidence was standardized to the 2000 U.S. standard population using the 50- to 79-years age band for ethnic comparison.

Table 3. Observed and expected numbers of patients with ALS newly certified and the standardized incidence ratio by prefecture in Japan from April 2009 to March 2010

Prefecture	Male (n = 1301)			Female (n = 962)		
	O	E	SIR (95% CI)	O	E	SIR (95% CI)
Hokkaido	67	57.3	1.17 (0.91–1.48)	43	44.4	0.97 (0.70–1.30)
Aomori	16	14.3	1.12 (0.64–1.82)	14	11.8	1.19 (0.65–1.99)
Iwate	16	14.9	1.07 (0.61–1.74)	14	11.8	1.19 (0.65–1.99)
Miyagi	18	23.2	0.77 (0.46–1.22)	11	17.5	0.63 (0.31–1.13)
Akita	12	12.8	0.94 (0.48–1.64)	13	10.4	1.25 (0.66–2.13)
Yamagata	13	13.4	0.97 (0.52–1.66)	14	10.3	1.36 (0.74–2.28)
Fukushima	26	22.0	1.18 (0.77–1.73)	9	16.6	0.54 (0.25–1.03)
Ibaraki	41	31.0	1.32 (0.95–1.79)	21	21.7	0.97 (0.60–1.48)
Tochigi	16	20.7	0.77 (0.44–1.26)	9	14.7	0.61 (0.28–1.16)
Gunma	22	21.3	1.03 (0.65–1.56)	10	15.3	0.65 (0.31–1.20)
Saitama	70	71.9	0.97 (0.76–1.23)	46	48.3	0.95 (0.70–1.27)
Chiba	61	63.1	0.97 (0.74–1.24)	47	43.4	1.08 (0.80–1.44)
Tokyo	97	122.5	0.79 (0.64–0.97)	78	88.4	0.88 (0.70–1.10)
Kanagawa	87	86.1	1.01 (0.81–1.25)	70	59.9	1.17 (0.91–1.48)
Niigata	39	26.5	1.47 (1.04–2.01)	25	20.1	1.24 (0.80–1.83)
Toyama	13	12.1	1.07 (0.57–1.83)	12	9.4	1.28 (0.66–2.24)
Ishikawa	13	12.1	1.08 (0.57–1.84)	10	9.3	1.08 (0.52–1.99)
Fukui	10	8.7	1.16 (0.55–2.12)	4	6.6	0.61 (0.17–1.56)
Yamanashi	11	9.2	1.20 (0.60–2.14)	4	6.8	0.59 (0.16–1.51)
Nagano	24	24.1	0.99 (0.64–1.48)	20	17.8	1.12 (0.68–1.73)
Gifu	32	22.4	1.43 (0.98–2.02)	18	16.3	1.11 (0.66–1.75)
Shizuoka	39	40.5	0.96 (0.68–1.31)	30	29.3	1.03 (0.69–1.47)
Aichi	76	71.3	1.07 (0.84–1.33)	45	49.7	0.91 (0.66–1.21)
Mie	22	19.9	1.10 (0.69–1.67)	8	14.6	0.55 (0.24–1.08)
Shiga	19	13.4	1.42 (0.86–2.22)	8	9.7	0.83 (0.36–1.63)
Kyoto	19	26.4	0.72 (0.43–1.12)	14	19.9	0.70 (0.39–1.18)
Osaka	62	85.7	0.72 (0.55–0.93)	70	63.5	1.10 (0.86–1.39)
Hyogo	43	56.7	0.76 (0.55–1.02)	47	42.4	1.11 (0.81–1.47)
Nara	29	14.8	1.96 (1.31–2.81)	9	10.9	0.82 (0.38–1.56)
Wakayama	20	11.2	1.79 (1.10–2.77)	9	8.7	1.03 (0.47–1.96)
Tottori	7	6.4	1.09 (0.44–2.25)	3	5.1	0.59 (0.12–1.75)
Shimane	14	8.4	1.66 (0.91–2.78)	7	6.6	1.05 (0.42–2.17)
Okayama	24	20.8	1.15 (0.74–1.72)	14	15.7	0.89 (0.49–1.49)
Hiroshima	20	29.7	0.67 (0.41–1.04)	18	22.4	0.80 (0.48–1.27)
Yamaguchi	16	16.5	0.97 (0.55–1.57)	15	13.1	1.14 (0.64–1.88)
Tokushima	11	8.8	1.25 (0.62–2.24)	4	6.8	0.59 (0.16–1.50)
Kagawa	6	11.0	0.54 (0.20–1.19)	11	8.3	1.32 (0.66–2.36)
Ehime	10	15.7	0.64 (0.31–1.17)	15	12.4	1.21 (0.68–2.00)
Kochi	8	8.6	0.93 (0.40–1.84)	7	6.9	1.01 (0.41–2.09)
Fukuoka	43	47.5	0.90 (0.65–1.22)	46	37.9	1.21 (0.89–1.62)
Saga	13	8.7	1.50 (0.80–2.56)	11	7.0	1.57 (0.78–2.81)
Nagasaki	10	15.1	0.66 (0.31–1.22)	11	12.2	0.90 (0.45–1.61)
Kumamoto	21	19.2	1.09 (0.68–1.67)	24	15.3	1.57 (1.01–2.33)
Oita	11	13.1	0.84 (0.42–1.50)	14	10.3	1.35 (0.74–2.27)
Miyazaki	15	12.3	1.22 (0.68–2.02)	3	9.6	0.31 (0.06–0.92)
Kagoshima	16	18.3	0.87 (0.50–1.42)	16	14.6	1.09 (0.63–1.78)
Okinawa	23	11.4	2.02 (1.28–3.04)	11	8.3	1.32 (0.66–2.36)

were 9.9 (95% CI 9.7–10.1) and 2.2 (95% CI 2.1–2.3), respectively. The highest prevalence as well as incidence was evident in the 70- to 79-years age group, and the male-female ratio was approximately 1.5.

According to the *Report on Public Health Administration and Services 2009*,[12] the number of patients with ALS-awarded certificates of financial aid for the treatment of a designated intractable disease at the end of the 2009 fiscal year was 8492, which is 1745 fewer than the total of 10 237 identified in our study. The certificates must be returned to the governor of the patient's prefecture if they die. This difference almost corresponds to the number of deaths due to ALS between January 1 and December 31, 2009, recorded as 1797 in the country's vital statistics. We therefore believe that the published value in the report, given as 6.7 per 100 000 people, underestimates the annual crude prevalence of ALS, which has a high fatality rate.

How ethnicity influences an individual's risk of developing ALS is of great concern. A systematic review on the effect of ethnic variation on the incidence of ALS, using an incidence rate standardized to the 2000 U.S. standard population, provides some etiologic clues.[3] The age- and sex-adjusted incidence rate of our study, 2.3 per 100 000 people per year, was comparable to the rate of 2.0 per 100 000 person-years reported in Hokkaido, Japan.[3,13] These incidence rates are much lower than those reported in recent prospective studies: 6.4 in Ireland, 6.2 in Scotland, 5.5 in Italy (Northern), 5.3 in the U.S. (Washington), and 5.0 in Italy (Puglia); as well as in retrospective studies: 6.6 in the U.S. (Minnesota), 6.0 in Sweden, 5.6 in Canada, 5.3 in Norway, 4.6 in Libya, and 4.2 in Denmark.[3] In general, a low incidence is believed to be caused by the low occurrence of a disease, poor access to medical care, short life expectancy, and inter-disease competition.[3,14] These latter three conditions are unlikely to account for the low incidence of ALS in Japan, which provides ALS patients with subsidized public medicine and whose population has the longest life expectancy in the world. Therefore, if the difference is genuine, genetic or lifestyle factors that protect against ALS may confer a low risk of ALS in the Japanese population.

In addition, we found variation in the incidence of ALS among prefectures in Japan. Although the reason for that variation was unclear, the combined data of familial and sporadic ALS used in this study might reflect in part the geographic variation of genetic and/or environmental factors contributing to the occurrence of ALS. Among males, SIRs were high in Okinawa, in Nara and Wakayama on the Kii Peninsula, and in Niigata. Okinawa is where hereditary motor and sensory neuropathy with proximal dominancy (HMSN-P) was first reported in 1997.[15] The clinical features of HMSN-P include adult-onset ALS accompanied by mild sensory disturbance, progressing to bedridden incapacity.[16] Although HMSN-P is inherited as an autosomal dominant characteristic, some patients with HMSN-P may have been included in this study. According to recent reports, a high incidence of ALS persisted in males in Wakayama prefecture,[17,18] which is consistent with the findings of our study. Although it remains unclear why the SIR is high in Nara, one possible explanation is the immigration of residents with a family history of ALS from two major endemic foci in Wakayama and Mie prefectures to Nara prefecture in the Kii Peninsula. Future research is required to confirm the above hypothesis by comparing patients' place of birth with place of ALS diagnosis, using information obtained from the clinical application form.

Similarly, the reasons underlying the high SIR in Niigata among males are unclear. A previous mortality study reported that deaths among males due to ALS most often occurred

throughout Niigata, Gunma, Nagano, and Fukushima prefectures.[19] Considering these findings together, we may speculate that some unidentified foci with a high incidence of ALS exist in this region. Conversely, Tokyo and Osaka presented a slightly lower SIR in males, but the statistical significance was modest. For female patients, SIR was high in Kumamoto but low in Miyazaki; the statistical significance was modest. However, the reason for this discrepancy among prefectures was unclear.

The limitations of our present study should be taken into account. One is the case ascertainment of ALS. As described in the methods, however, this is not so serious, considering the following points: (1) ALS was usually diagnosed by neurologists; (2) the number of neurologists was large[8]; (3) neurologists have generally followed the clinical guidelines based on the EEC since the recommendation of its use by the research committee on ALS of Japan[9]; and (4) the agreement between judgments by the doctor who diagnosed and filled out the clinical application form and neurology specialists who reviewed it at the committee on designated intractable diseases was quite high.[20] The other is potential regional disparity affecting geographic variation in the incidence of ALS (eg number of neurologists and access to specialized medical care). Given the following point, however, the degree of regional disparity is likely to be small: prefectures with higher or lower concentrations of neurology clinics/departments[21] were not consistent with those in which the SIRs of ALS were high or low in our present study. Basically, all ALS patients have been guaranteed free medical access thanks to the provision of financial aid from universal medical insurance since 1961, countermeasures against intractable diseases including ALS since 1972, and nursing-care insurance since 2000.

In conclusion, we report here the annual prevalence and incidence of ALS in a representative population of Japan. We identified some prefectures with a high incidence of ALS. However, the incidence of ALS in the Japanese population was much lower than in Caucasian populations of Europe and North America.

ONLINE ONLY MATERIAL

Abstract in Japanese.

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REFERENCES

1. Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. N Engl J Med. 2001;344:1688–700.
2. Logroscino G, Traynor BJ, Hardiman O, Chiò A, Mitchell D, Swingler RJ, et al; EURALS. Incidence of amyotrophic lateral sclerosis in Europe. J Neurol Neurosurg Psychiatry. 2010;81:385–90.
3. Cronin S, Hardiman O, Traynor BJ. Ethnic variation in the incidence of ALS: a systematic review. Neurology. 2007;68:1002–7.
4. Kuzuhara S, Kokubo Y. Atypical parkinsonism of Japan: amyotrophic lateral sclerosis-parkinsonism-dementia complex of the Kii peninsula of Japan (Muro disease): an update. Mov Disord. 2005;20 Suppl 12:S108–13.
5. Yoshida S, Uebayashi Y, Kihira T, Kohmoto J, Wakayama I, Taguchi S, et al. Epidemiology of motor neuron disease in the Kii Peninsula of Japan, 1989–1993: active or disappearing focus? J Neurol Sci. 1998;155:146–55.
6. Brooks BR. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical limits of amyotrophic lateral sclerosis" workshop contributors. J Neurol Sci. 1994;124 Suppl:96–107.
7. Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2000;1:293–9.
8. Kuzuhara S. History of neurology and education on neurology in Japan. Rinsho Shinkeigaku. 2009;49:968–79.
9. Atsuta N, Watanabe H, Ito M, Tanaka F, Tamakoshi A, Nakano I, et al; Research Committee on the Neurodegenerative Diseases of Japan. Age at onset influences on wide-ranged clinical features of sporadic amyotrophic lateral sclerosis. J Neurol Sci. 2009;276:163–9.
10. Fay MP, Feuer EJ. Confidence intervals for directly standardized rates: a method based on the gamma distribution. Stat Med. 1997;16:791–801.
11. Schoenberg BS. Calculating confidence intervals for rates and ratios. Neuroepidemiology. 1983;2:257–65.
12. Ministry of Health, Labour and Welfare, Japan [homepage on the Internet]. [cited as 2014 January 29]. Available from http://www.mhlw.go.jp/toukei/saikin/hw/eisei/09/dl/kekka6.pdf (in Japanese).
13. Moriwaka F, Okumura H, Tashiro K, Hamada T, Matsumoto A, Matsumoto H, et al. Motor neuron disease and past poliomyelitis. Geographic study in Hokkaido, the northern-most island of Japan. J Neurol. 1993;240:13–6.
14. Neilson S, Robinson I, Alperovitch A. Rising amyotrophic lateral sclerosis mortality in France 1968–1990: increased life expectancy and inter-disease competition as an explanation. J Neurol. 1994;241:448–55.
15. Takashima H, Nakagawa M, Nakahara K, Suehara M, Matsuzaki T, Higuchi I, et al. A new type of hereditary motor and sensory neuropathy linked to chromosome 3. Ann Neurol. 1997;41:

771–80.
16. Nakagawa M. Wide spectrum of hereditary motor sensory neuropathy (HMSN). Rinsho Shinkeigaku. 2009;49:950–2 (in Japanese).
17. Kihira T, Yoshida S, Hironishi M, Miwa H, Okamato K, Kondo T. Changes in the incidence of amyotrophic lateral sclerosis in Wakayama, Japan. Amyotroph Lateral Scler Other Motor Neuron Disord. 2005;6:155–63.
18. Ishiura H, Takahashi Y, Mitsui J, Yoshida S, Kihira T, Kokubo Y, et al. C9ORF72 repeat expansion in amyotrophic lateral sclerosis in the Kii peninsula of Japan. Arch Neurol. 2012;69:1154–8.
19. Doi Y, Yokoyama T, Tango T, Takahashi K, Fujimoto K, Nakano I. Temporal trends and geographic clusters of mortality from amyotrophic lateral sclerosis in Japan, 1995–2004. J Neurol Sci. 2010;298:78–84.
20. Hioki A. Characteristics of receipt rate of financial aid for intractable disease patients in Gifu Prefecture, Japan. J Epidemiol. 2000;10:94–102.
21. Statistics and Information Department, the Ministry of Health, Labour and Welfare, Japan. Survey of Physicians, Dentists and Pharmacists (2008) [homepage on the Internet]. [cited as 2014 May 11]. Available from: http://www.e-stat.go.jp/SG1/estat/List.do?lid=000001060599 (in Japanese).

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CASE REPORT

Case of familial amyotrophic lateral sclerosis showing gadolinium-enhanced cranial nerves on magnetic resonance imaging associated with rapid progression of facial nerve palsy

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Key words
Cu/Zn superoxide dismutase, gadolinium enhancement, imaging, motor neuron disease, rapid progression.

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Abstract

The evaluation of cranial nerves in magnetic resonance imaging (MRI) at early developmental stage has not been established in amyotrophic lateral sclerosis (ALS). A 23-year-old man with familial ALS developed peripheral facial nerve palsy, and showed the striking gadolinium enhancement on MRI in those nerves at an early stage. His symptoms progressed rapidly and he died approximately 3 months after onset. We identified a missense mutation in exon 1 of the Cu/Zn superoxide dismutase gene (*SOD-1*), resulting in a Cys6Gly (C6G) amino acid substitution. Based on the rapid progression and neurotoxicity resulting from SOD-1 protein aggregation, the gadolinium enhancement of facial nerves might be caused by the rapid Wallerian degeneration and blood–brain barrier disruption at the early phase of progression.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of the motor system. A total of 10% of cases are familial,[1] and more than 10 genes have been identified as associated with familial ALS (FALS), including transactive response DNA binding protein 43 kDa (*TDP-43*), *FUS/TLS*, optineurin (*OPTN*) and Cu/Zn superoxide dismutase gene (*SOD-1*).[2] FALS patients with mutations of *SOD-1*, accounting for 20% of familial cases, have shown a variety of clinical courses depending on positions of gene mutation.[1,3]

Recently, a striking magnetic resonance imaging (MRI) finding was reported in one ALS case who showed gadolinium enhancement in the lumbar nerve roots associated with rapid development of weakness.[3] Gadolinium enhancement also occurs in cranial nerves undergoing Wallerian degeneration, as is often the case with axon injury.[4,5] So far, however, gadolinium enhancement to detect neurodegeneration of cranial nerves in ALS has not been reported. In the present report, we have been the first to report a FALS case with a C6G mutation in the *SOD-1* gene who showed gadolinium enhancement in bilateral facial nerves.

Case report

A 23-year-old man initially presented right facial nerve palsy and subsequently left facial nerve palsy after 2 weeks. On admission, neurological examination showed bilateral peripheral facial nerves palsy and weakness in soft palate elevation. All tendon reflexes were increased; however, neither fasciculation nor weakness was observed in the extremities. The patient's family pedigree included four other members with diagnoses of ALS (Fig. 1a). A nerve conduction study showed no response of the right facial nerve, and a response with reduced amplitude and normal distal latency from the left. A serological test showed no abnormalities. Serum lysozyme and angiotensin converting enzyme levels were normal. There was no evidence of systemic or paraneoplastic autoimmunity including anti-Hu and Yo antibody, or infection by human T-cell leukemia virus type-1 HIV. His cerebrospinal fluid (CSF) showed no remarkable findings in cells (1/μL), protein level (18 mg/dL) and myelin basic protein less than 40 pg/mL. No malignant cells were found in the CSF. Serological markers on CSF were all negative for cytomegalovirus, herpes simplex virus and varicella zoster virus. Of note, gadolinium considerably enhanced facial nerves on MRI (Fig. 1 b,c). Despite being treated with immunological therapy, the patient developed weakness in the upper extremity 7 weeks after onset, and was diagnosed with ALS by electromyography showing denervation in the tongue and abductor pollisis brevis. He died as a result of respiratory failure approximately 3 months after the onset.

Written informed consent for genetic analysis was obtained from the patient and his family, and the study was

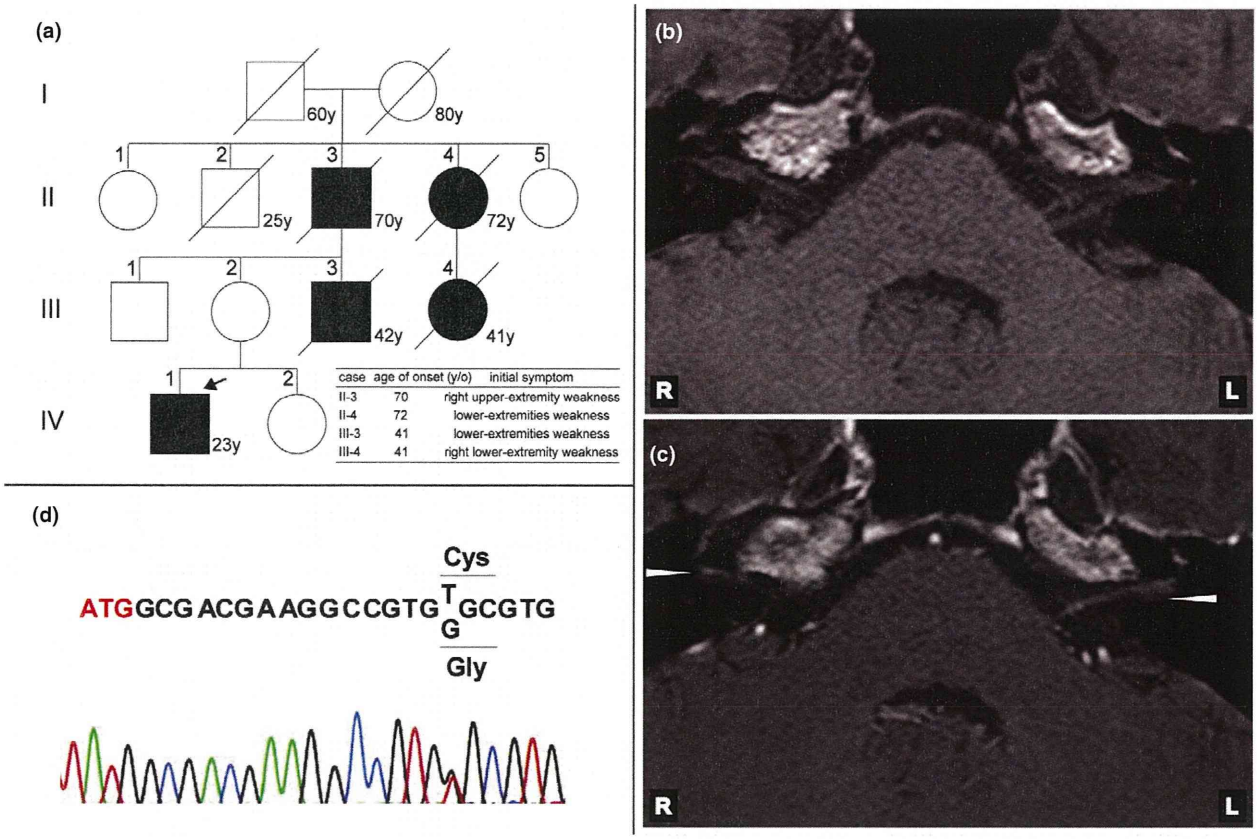

Figure 1 (a) Family pedigree of this patient. Shaded symbols show individuals diagnosed with amyotrophic lateral sclerosis (ALS). Arrow indicates the patient in the present report. Squares represent males and circles represent females, respectively. The table represents the age of onset and initial symptoms of other ALS cases in this family. (b) Axial images of T1-weighted, and (c) gadolinium-enhanced magnetic resonance imaging. Arrowheads indicate bilateral facial nerves enhanced by gadolinium. (d) A missense mutation in the *SOD-1* gene. Gene analysis showed a T to G heterozygous missense mutation in exon 1 of the *SOD-1* gene, resulting in the substitution of cysteine by glycine at amino acid 6.

carried out under approval by the ethical committees at Nagoya City University, RIKEN Brain Science Institute, Jichi Medical University. Genomic DNA prepared from the patient's lymphocytes was subjected to high resolution melting analysis, where a nucleotide substitution, changing Cys6 to Gly in SOD1, was identified (Fig. 1d). Other gene mutations reported in association with familial ALS, such as *FUS/TLS*, *TDP-43* and *OPTN*, were not identified.

Discussion

This patient showed the rapid progression of symptoms starting from facial nerve palsy and striking gadolinium-enhancement of those nerves on MRI. He showed an autosomal dominant pattern of inheritance, and we detected a missense mutation in the *SOD-1* gene.

The main cause of enhanced gadolinium uptake in cranial nerves is inflammatory or infectious neuropathy, sarcoidosis, trauma, neurolymphomatosis, meningeal carcinomatosis, demyelinating disorders and gliomatosis.[4,6] Those diseases were excluded in this case because of following evidences: normal CSF and serum findings, negative marker of infectious agents, and the lack of response to immunotherapy. In a previous study, gadolinium enhancement in the lumbar nerve root was described in association with Wallerian degeneration and related disruption of the blood–brain barrier at the developmental stage of symptoms.[3] Recently, in rodent model of ALS, the aberrant activation and infiltration of microglia related to the axonal degeneration could be linked to fine pathological changes of the endothelium.[7] Based on these, the present MRI results might be related to a disruption of the blood–brain barrier caused by an early stage of nerve degeneration and characteristic transition of endothelium, especially when disease progression is rapid.

Another Japanese case of FALS with the same C6G mutation was previously reported.[8] In that report, the patient was a 46-year-old woman who initially presented facial weakness and subsequently weakness of her upper extremities after 2 weeks. She died as a result of respiratory failure after 2 months of the onset. Her clinical course was similar to the present patient, except for the point that the present patient was relatively young compared with the previous patient. Although there was no description of MRI findings in the previous patient, a post-mortem pathological study showed severe loss of anterior horn and Betz cells in her motor systems. Her younger sister also presented

progressive weakness accompanied with upper and lower extremities, and died of respiratory failure 3 months after the clinical onset. Thus, both FALS pedigrees were similar in the point of the rapid progression of symptoms; however, initial symptoms and experimental findings were various, even among different members of the same family.

One proposed mechanism of rapid progression in the present patient is as a result of the position of the mutation in *SOD-1*. As Cys6 resides close to the dimer interface of the SOD-1 homodimer, its mutation likely reduces protein stability of SOD-1 and induces protein misfolding.[8,9] This aberrant protein aggregation might cause rapid toxicity in the motor neuron, including facial nerves. Thus, the present FALS case with C6G mutation in *SOD-1* showed gadolinium enhancement, reflecting rapid motor nerve degeneration.

References

1 Ticozzi N, Tiloca C, Morellic C *et al.* Genetics of familial Amyotrophic lateral sclerosis. *Arch. Ital. Biol.* 2011; **149**: 65–82.
2 Al-Chalabi A. ALSoD (ALS online genetics database). Cited in 11 October 2013. Available from URL: http://alsod.iop.kcl.ac.uk/
3 Young NP, Laughlin RS, Sorenson EJ. Gadolinium enhancement of the lumber roots in a case of ALS. *Amyotroph. Lateral Scler.* 2010; **11**: 207–9.
4 Saremi F, Helmy M, Farzin S, Zee CS, Go JL. MRI of cranial nerve enhancement. *Am. J. Roentgenol.* 2005; **185**: 1487–97.
5 Nakao Y, Sakihama N, Kumagami H. Vascular permeability changes associated with experimentally induced facial nerve lesions in the rabbit. *Eur. Arch. Otorhinolaryngol.* 1995; **252**: 255–7.
6 Hosoya T, Adachi M, Yamaguchi K, Yamaguchi K, Kato T, Sugai, Adachi M Abducens nerve enhancement demonstrated by multiplanar reconstruction of contrast-enhanced three-dimensional MRI. *Neuroradiology* 2001; **43**: 295–301.
7 Zhong Z, Deane R, Ali Z *et al.* ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration. *Nat. Neurosci.* 2008; **11**: 420–2.
8 Kohno S, Takahashi Y, Miyajima H, Serizawa M, Mizoguchi K. A novel mutation (Cys6Gly) in the Cu/Zn superoxide dismutase gene associated with rapidly progressive familial amyotrophic lateral sclerosis. *Neurosci. Lett.* 1999; **276**: 135–7.
9 Brotherton T, Polak M, Kelly C *et al.* A novel ALS SOD1 C6S mutation with implications for aggregation related toxicity and genetic counseling. *Amyotroph. Lateral Scler.* 2011; **12**: 215–9.

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ORIGINAL ARTICLE

Relationship between dementia severity and behavioural and psychological symptoms in early-onset Alzheimer's disease

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Key words: *Alzheimer's disease, behavioural and psychological symptoms (BPSD), early-onset, Neuropsychiatric Inventory (NPI), severity of dementia.*

Abstract

Background: The features of behavioural and psychological symptoms of dementia (BPSD) are influenced by dementia stage. In early-onset Alzheimer's disease (EOAD), the association between BPSD and dementia stage remains unclear because of the difficulty of recruiting subjects with a wide range of disease severity. We used a combination of community-based and hospital-based approaches to investigate the relationship between dementia severity and BPSD in EOAD patients.

Methods: Sixty-three consecutive EOAD outpatients and 29 EOAD patients from a community-based survey were divided into three dementia severity groups according to the Clinical Dementia Rating scale (CDR): mild (CDR 0.5–1, $n = 55$), moderate (CDR 2, $n = 17$), and severe (CDR 3, $n = 20$). BPSD were rated using the Neuropsychiatric Inventory.

Results: Scores of the Neuropsychiatric Inventory subscales agitation, euphoria, apathy, disinhibition, irritability, and aberrant motor behaviour increased significantly with increased dementia severity. Hallucinations were greater in the moderate group than in the mild group. For delusions, depression, and anxiety, no significant differences were observed among the three severity groups.

Conclusions: The pattern of apathy, agitation, disinhibition, irritability, and aberrant motor behaviour worsening with severity progression in EOAD is similar to the pattern in late-onset Alzheimer's disease. In contrast, hallucinations, depression, and anxiety showed different patterns in EOAD.

INTRODUCTION

Alzheimer's disease (AD) is the most frequent diagnosis in dementia. AD is divided into early-onset (EOAD) and late-onset (LOAD) according to whether the onset is before or after 65 years of age. Previous reports based on large clinicopathologic studies have shown that the pathologies of EOAD and LOAD are not qualitatively different.[1,2] However, several studies have revealed distinct neurocognitive and neuroimaging features in EOAD patients. For example, language problems and visuospatial dysfunction are common in EOAD,[3] and in a neuroimaging study, EOAD patients exhibited a more severe reduction in grey matter in the bilateral parietal and posterior cingulate cortices and precuneus region.[4] These findings suggest it may be necessary to consider EOAD and LOAD as separate diseases clinically.

'Behavioural and psychological symptoms of dementia' (BPSD) is a descriptive term that encompasses a heterogeneous group of non-cognitive symptoms and behaviours occurring in patients with dementia.[5] The development of BPSD is associated with more rapid cognitive decline,[6] greater impairment in activities of daily living,[7] lower quality of life for patients and caregivers,[8] and earlier institutionalization.[9] Different types of dementia present with characteristic behavioural profiles that reflect specific brain regions affected and neurotransmitter

abnormalities. Recent studies have suggested the features of BPSD are influenced by the dementia stage.[10–19] Although a relationship between dementia severity and BPSD has been reported in LOAD patients,[10,11,13,14,19] there has been no similar study in EOAD. We think that EOAD patients may have a characteristic pattern of BPSD as dementia severity progresses, and comprehending the pattern is important to direct treatment and to plan care and support for patients and their caregivers. It is hard to recruit a sufficient number of EOAD patients for valid statistical analysis. Additionally, because many patients seen in the memory clinic have mild dementia, it is difficult to recruit a wide range of dementia severity in hospital-based studies. In the present study, we solved these problems by combining a hospital-based study with a community-based study, and we investigated the relationship between dementia severity and BPSD in EOAD patients.

METHODS

Subjects

All procedures followed the Clinical Study Guidelines of the Ethics Committee of Kumamoto University Hospital and were approved by the internal review board. After receiving a complete description of all procedures for the present study, all patients or their caregivers, if surrogate consent was needed, provided written informed consent.

Sixty-three patients with EOAD who consecutively visited Kumamoto University Hospital between April 2007 and May 2013 comprised the hospital outpatients in our study. Patients underwent general physical, neurological, and neuropsychological examinations, including the Mini-Mental State Examination (MMSE).[20] All underwent structural neuroimaging with magnetic resonance imaging of the brain and routine laboratory tests. In addition to these 63 hospital outpatients, we included 29 EOAD patients from our community-based survey on the prevalence of early onset dementia.[21] This study was conducted in the same catchment area as our university hospital in 2008. We visited these EOAD patients at home, in the hospital, or in the nursing home and examined them using the same procedure as used on the outpatients. We referred to blood work and structural neuroimaging with computed tomography or magnetic resonance imaging of the brain that had been performed by their regular doctors. The purpose of combining these two patient groups was to investigate BPSD of EOAD over a range of disease severity.

Patients who satisfied the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association diagnostic criteria for probable AD were put into the EOAD group if they were under 65 years old at examination.[22] We decided to classify subjects by age at first assessment to avoid the possibility that inaccurate medical histories would confound the age of onset.[23] Patients were excluded from the study if they had developmental abnormalities, serious psychiatric disease, a history of substance abuse, or significant neurologic antecedents, such as brain trauma, brain tumour, epilepsy, and inflammatory disease. Patients without a reliable informant were also excluded.

Assessments and measures

Neuropsychiatrists who examined patients rated the severity of dementia using the Clinical Dementia Rating (CDR).[24] We classified the patients into three groups according to the CDR: mild dementia (CDR 0.5–1, $n = 55$), moderate dementia (CDR 2, $n = 17$), and severe dementia (CDR 3, $n = 20$).

We assessed patients' comprehensive BPSD through a structured caregiver interview that employed a Japanese version of the Neuropsychiatric Inventory (NPI).[25,26] The NPI was used to rate 10 symptoms on the basis of a patient's condition during the month before the interview: the symptoms were delusions, hallucinations, agitation, depression, anxiety, euphoria, apathy, disinhibition, irritability, and aberrant motor behaviour (AMB). According to the criteria-based rating scheme, the severity of each manifestation was classified into four grades (from 1 to 3; 0 if absent), and the frequency of each manifestation was also classified into five grades (from 1 to 4; 0 if absent). The NPI score (severity × frequency) was calculated for each manifestation (range of possible scores: 0–12). The presence of a symptom was expressed as an NPI subset score >0. The maximum total score on the NPI for the 10 manifestations is 120.

The CDR and NPI assessments were performed within 1 month of the first visit or on the day we visited patients in their home.

Statistical analysis

Demographic and clinical variables, NPI total score, and the score of each individual NPI domain were evaluated between groups with χ^2 analysis, one-way ANOVA, and the post-hoc Tukey test. For all analyses, a *P*-value <0.05 was regarded as statistically significant. Statistical operations were performed with SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The overall sample's mean age ± SD at examination was 59.0 ± 3.8 years, and the mean MMSE score ± SD was 13.4 ± 8.8. The 63 participants in the hospital-based study included 50 mild cases, 12 moderate cases, and 1 severe case. The 29 participants in the population-based study included 5 mild cases, 5 moderate cases, and 19 severe cases. The majority of participants in this study received in-home care (*n* = 81). Seven subjects received hospital treatment, and four received nursing home care.

No significant differences were found in age, sex ratio, and educational level among the three severity groups; these demographic variables are shown in Table 1. Dementia severity was significantly associated with disease duration. The MMSE score showed a significant decrease with dementia severity.

Table 2 shows the prevalence of neuropsychiatric symptoms according to dementia severity group. Apathy was the most common behaviour, which was exhibited by 82.6% of all patients, followed by depression (39.1%), anxiety (35.9%), and irritability (33.7%). Disinhibition was the least common, developed by only 5% of all EOAD patients.

Figure 1 shows the mean NPI total scores in the three groups. The total NPI score increased across each group with significant differences between the mild and moderate dementia groups ($P < 0.05$) and between the mild and severe groups ($P < 0.01$).

The NPI subscale scores of agitation, euphoria, apathy, disinhibition, irritability, and AMB increased with dementia severity (Table 3). Significant differences were found between the severe group and both the mild and moderate groups in terms of agitation ($P < 0.01$), euphoria ($P < 0.05$), disinhibition ($P < 0.05$), and irritability ($P < 0.05$); these subscales did not differ between the mild and moderate groups. Severity progression increased apathy ($P < 0.01$), with significant differences between all groups. The severe group had significantly greater AMB ($P < 0.01$) than the mild group. There were more hallucinations in the moderate group than in the mild group ($P < 0.05$), with no further increase in the severe group. Three NPI

Table 1 Patient demographics and clinical characteristics

	Mild (*n* = 55)	Moderate (*n* = 17)	Severe (*n* = 20)	*P*-value
Age at examination, mean ± SD (year)	58.8 ± 4.1	58.8 ± 3.7	59.7 ± 3.1	0.69†
Sex (men/women)	19/36	6/11	6/14	0.95‡
Education, mean ± SD (year)	12.5 ± 2.3	11.3 ± 2.9	11.7 ± 2.3	0.16†
Duration, mean ± SD (year)	2.7 ± 1.7	4.7 ± 2.1	9.4 ± 4.4	<0.001†
MMSE, mean ± SD	19.2 ± 4.5	9.4 ± 5.6	0.8 ± 1.9	<0.001†

†One-way ANOVA. ‡χ^2 test. MMSE, Mini-Mental State Examination.

Table 2 Prevalence of neuropsychiatric symptoms in each stage of dementia

NPI item	Total (%) (*n* = 92)	Mild (%) (*n* = 55)	Moderate (%) (*n* = 17)	Severe (%) (*n* = 20)
Delusions	14.1	12.7	17.6	15.0
Hallucinations	8.7	1.8	23.5	15.0
Agitation	26.1	20.0	23.5	45.0
Depression	39.1	41.8	47.1	25.0
Anxiety	35.9	40.0	41.2	20.0
Euphoria	8.7	5.5	0	25.0
Apathy	82.6	76.4	82.4	100
Disinhibition	5.4	1.8	0	20.0
Irritability	33.7	32.7	29.4	40.0
AMB	26.1	16.4	41.2	40.0

AMB, aberrant motor behaviour; NPI, Neuropsychiatric Inventory.

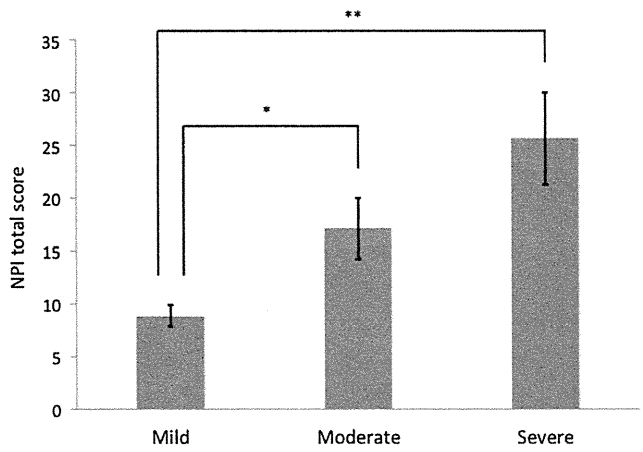

Figure 1 Mean Neuropsychiatric Inventory (NPI) total score by dementia group. Error bars represent standard error of the mean. *$P < 0.05$, **$P < 0.01$ by a multiple comparison using Tukey test.

Table 3 Mean scores for each NPI subscale by dementia severity group

NPI item	Dementia severity				
	Mild (n = 55)	Moderate (n = 17)	Severe (n = 20)	P-value	Pairwise comparisons†
Delusions	0.33 ± 1.04	1.59 ± 3.99	0.90 ± 2.79	0.127	
Hallucinations	0.02 ± 0.14	1.47 ± 3.39	0.90 ± 2.77	0.017	Mild < moderate
Agitation	0.67 ± 1.51	0.76 ± 1.68	3.60 ± 4.94	<0.001	Mild, moderate < severe
Depression	1.18 ± 1.88	1.47 ± 3.00	0.55 ± 1.10	0.34	
Anxiety	1.40 ± 2.31	2.00 ± 3.39	0.95 ± 2.14	0.45	
Euphoria	0.13 ± 0.61	0	1.15 ± 2.83	0.013	Mild, moderate < severe
Apathy	3.36 ± 3.19	6.29 ± 4.15	10.20 ± 2.75	<0.001	Mild < moderate < severe
Disinhibition	0.02 ± 0.14	0	1.45 ± 3.33	0.002	Mild, moderate < severe
Irritability	1.00 ± 1.81	0.65 ± 1.22	2.70 ± 4.05	0.014	Mild, moderate < severe
AMB	0.75 ± 2.08	2.88 ± 4.00	3.65 ± 4.89	0.001	Mild < severe

Data are shown as mean ± SD. †Post-hoc Tukey test. AMB, aberrant motor behaviour; NPI, Neuropsychiatric Inventory.

subscale scores (delusions, depression, and anxiety) showed no significant difference in the three severity groups.

DISCUSSION

To our knowledge, this is the first study attempting to clarify the relationship between dementia severity and neuropsychiatric symptoms in EOAD patients. It is difficult to recruit many outpatients in the severe stage of dementia for a cross-sectional study for any degenerative disease, including EOAD. Longitudinal studies require a time commitment from patients and their caregivers, and longitudinal studies for degenerative diseases often suffer from attrition as participants drop out. To address these issues, we performed a cross-sectional study but combined patients who were recruited from a community-based study with outpatients recruited from a memory clinic. Therefore, we were able to compare EOAD patients with a wide range of dementia severity. We think this method is very useful for cross-sectional studies researching uncommon and slowly progressive diseases if the same diagnostic and assessment methods are available in both hospital-based and community-based studies.

There have been two previous hospital-based studies on the prevalence of neuropsychiatric symptoms in EOAD patients that used NPI, and one of them used the same method of assessing NPI symptom criteria (an NPI subset score >0) as our study. [23] Our results were very similar to their findings except for the symptoms of apathy (82.6% vs 56.5%) and irritability (33.7% vs 19.6%). These differences may be due to our inclusion of more severe patients (mean MMSE score: 13.4 vs 17.4).

In the present study, agitation, apathy, disinhibition, irritability, and AMB became worse with increased dementia severity. This pattern was consistently observed in previous studies of LOAD patients.[10,13,19] In AD, agitation and AMB have been associated with a greater neurofibrillary tangle burden in the orbitalfrontal cortex, and apathy has been associated with the anterior cingulate area.[27] Aggravation of these symptoms might be associated directly with advancing brain damage around these areas, and therefore, this pattern might be common in both EOAD and LOAD patients.

Hallucinations were higher in the moderate group than in either the mild or severe groups in the current study. Several studies of LOAD patients have reported that hallucinations become worse with dementia severity.[13,14,19] This may reflect a difference in the pattern of cortical dysfunction. Neuroimaging investigations revealed that patients with EOAD exhibited a more severe reduction in grey matter in the bilateral parietal and posterior cingulate cortices and precuneus region than those with LOAD.[4] Therefore, hallucinations in EOAD might appear in a milder stage than in LOAD, reflecting a difference in the pattern of brain damage.

Depression and anxiety had a relatively high frequency and showed no significant difference among the three groups. These two symptoms have been grouped together by several factor analysis studies on AD and also showed a similar pattern in our study.[28–30] Both symptoms appeared in the mild or moderate stage and decreased in the severe stage. In AD patients, mainly LOAD, depression has been reported to be more severe in the moderate stage.[12,16] We think

this discrepancy might be due to a high occurrence of depression in the mild group of EOAD patients. There are many EOAD patients who have a role in society or family at the time of disease onset, potentially creating a greater risk of poor social adjustment from an early stage. Depression and anxiety are likely influenced by both psychosocial factors and advancing brain damage in EOAD.

The strength of the present study is that we examined BPSD among EOAD patients with a wide range of dementia severity. However, several methodological issues limit the interpretation of the results of this study. First, the homogeneity of this cohort is open to question given the combination of a hospital-based study with a community-based study, although all subjects underwent the same diagnostic and assessment procedures. Second, we did not investigate medication use. Taking anti-dementia and/or psychotropic agents could affect the results of the study. Third, the cross-sectional study design may not have been optimal because behavioural disturbances can fluctuate and may not be present at every examination.[31] A longitudinal study may offer additional information on the course of BPSD in EOAD patients. Despite these limitations, we believe that our findings may be useful for the management and care of EOAD patients in their home or in institutions.

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REFERENCES

1 Newton RD. The identity of Alzheimer's disease and senile dementia and their relationship to senility. *J Ment Sci* 1948; **94**: 225–249.
2 Neumann MA, Cohn R. Incidence of Alzheimer's disease in large mental hospital; relation to senile psychosis and psychosis with cerebral arteriosclerosis. *AMA Arch Neurol Psychiatry* 1953; **69**: 615–636.
3 Mendez MF. The accurate diagnosis of early-onset dementia. *Int J Psychiatry Med* 2006; **36**: 401–412.
4 Ishii K, Kawachi T, Sasaki H *et al*. Voxel-based morphometric comparison between early- and late-onset mild Alzheimer's disease and assessment of diagnostic performance of z score images. *AJNR Am J Neuroradiol* 2005; **26**: 333–340.
5 Finkel SI, Costa e Silva J, Cohen G, Miller S, Sartorius N. Behavioral and psychological signs and symptoms of dementia: a consensus statement on current knowledge and implications for research and treatment. *Int Psychogeriatr* 1996; **8** (Suppl 3): 497–500.
6 Stern Y, Tang MX, Albert MS *et al*. Predicting time to nursing home care and death in individuals with Alzheimer disease. *JAMA* 1997; **277**: 806–812.
7 Lyketsos CG, Steele C, Baker L *et al*. Major and minor depression in Alzheimer's disease: prevalence and impact. *J Neuropsychiatry Clin Neurosci* 1997; **9**: 556–561.
8 González-Salvador T, Lyketsos CG, Baker A *et al*. Quality of life in dementia patients in long-term care. *Int J Geriatr Psychiatry* 2000; **15**: 181–189.
9 Steele C, Rovner B, Chase GA, Folstein M. Psychiatric symptoms and nursing home placement of patients with Alzheimer's disease. *Am J Psychiatry* 1990; **147**: 1049–1051.
10 Mega MS, Cummings JL, Fiorello T, Gornbein J. The spectrum of behavioral changes in Alzheimer's disease. *Neurology* 1996; **46**: 130–135.
11 Harwood DG, Barker WW, Ownby RL, Duara R. Relationship of behavioral and psychological symptoms to cognitive impairment and functional status in Alzheimer's disease. *Int J Geriatr Psychiatry* 2000; **15**: 393–400.
12 Lyketsos CG, Steinberg M, Tschanz JT, Norton MC, Steffens DC, Breitner JC. Mental and behavioral disturbances in dementia: findings from the Cache County Study on Memory in Aging. *Am J Psychiatry* 2000; **157**: 708–714.
13 Lopez OL, Becker JT, Sweet RA *et al*. Psychiatric symptoms vary with the severity of dementia in probable Alzheimer's disease. *J Neuropsychiatry Clin Neurosci* 2003; **15**: 346–353.
14 Piccininni M, Di Carlo A, Baldereschi M, Zaccara G, Inzitari D. Behavioral and psychological symptoms in Alzheimer's disease: frequency and relationship with duration and severity of the disease. *Dement Geriatr Cogn Disord* 2005; **19**: 276–281.
15 Srikanth S, Nagaraja AV, Ratnavalli E. Neuropsychiatric symptoms in dementia-frequency, relationship to dementia severity and comparison in Alzheimer's disease, vascular dementia and frontotemporal dementia. *J Neurol Sci* 2005; **236**: 43–48.
16 Caputo M, Monastero R, Mariani E *et al*. Neuropsychiatric symptoms in 921 elderly subjects with dementia: a comparison between vascular and neurodegenerative types. *Acta Psychiatr Scand* 2008; **117**: 455–464.
17 Kashibayashi T, Ikeda M, Komori K *et al*. Transition of distinctive symptoms of semantic dementia during longitudinal clinical observation. *Dement Geriatr Cogn Disord* 2010; **29**: 224–232.
18 Thompson C, Brodaty H, Trollor J, Sachdev P. Behavioral and psychological symptoms associated with dementia subtype and severity. *Int Psychogeriatr* 2010; **22**: 300–305.
19 Canevelli M, Adali N, Cantet C *et al*. Impact of behavioral subsyndromes on cognitive decline in Alzheimer's disease: data from the ICTUS study. *J Neurol* 2013; **260**: 1859–1865.
20 Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189–198.

21 Ikejima C, Ikeda M, Hashimoto M *et al*. Multicenter population-based study on the prevalence of early onset dementia in Japan: vascular dementia as its prominent cause. *Psychiatry Clin Neurosci* 2014; **68**: 216–224.
22 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; **34**: 939–944.
23 Toyota Y, Ikeda M, Shinagawa S *et al*. Comparison of behavioral and psychological symptoms in early-onset and late-onset Alzheimer's disease. *Int J Geriatr Psychiatry* 2007; **22**: 896–901.
24 Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. *Br J Psychiatry* 1982; **140**: 566–572.
25 Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. *Neurology* 1994; **44**: 2308–2314.
26 Hirono N, Mori E, Ikejiri Y *et al*. [Japanese version of the Neuropsychiatric Inventory—a scoring system for neuropsychiatric disturbance in dementia patients]. *No To Shinkei* 1997; **49**: 266–271.
27 Tekin S, Mega MS, Masterman DM *et al*. Orbitofrontal and anterior cingulate cortex neurofibrillary tangle burden is associated with agitation in Alzheimer disease. *Ann Neurol* 2001; **49**: 355–361.
28 Frisoni GB, Rozzini L, Gozzetti A *et al*. Behavioral syndromes in Alzheimer's disease: description and correlates. *Dement Geriatr Cogn Disord* 1999; **10**: 130–138.
29 Aalten P, Verhey FR, Boziki M *et al*. Neuropsychiatric syndromes in dementia. Results from the European Alzheimer Disease Consortium: part I. *Dement Geriatr Cogn Disord* 2007; **24**: 457–463.
30 Spalletta G, Musicco M, Padovani A *et al*. Neuropsychiatric symptoms and syndromes in a large cohort of newly diagnosed, untreated patients with Alzheimer disease. *Am J Geriatr Psychiatry* 2010; **18**: 1026–1035.
31 Aalten P, de Vugt ME, Jaspers N, Jolles J, Verhey FR. The course of neuropsychiatric symptoms in dementia. Part II: relationships among behavioural sub-syndromes and the influence of clinical variables. *Int J Geriatr Psychiatry* 2005; **20**: 531–536.

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Family history of frontotemporal lobar degeneration in Asia – an international multi-center research

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ABSTRACT

Background: Previous studies in western countries have shown that about 30%–50% of patients with frontotemporal lobar degeneration (FTLD) have a positive family history, whereas the few epidemiological studies on FTLD done in Asia reported much lower frequencies. It is not clear the reason why the frequencies of FTLD with positive family history were lower in Asia. Furthermore, these findings were not from studies focused on family history. Therefore, it is necessary to conduct further studies on the family history of FTLD in Asia. This international multi-center research aims to investigate the family histories in patients with FTLD and related neurodegenerative diseases such as progressive supranuclear palsy (PSP), corticobasal syndrome (CBS), and motor neuron diseases in a larger Asian cohort.

Methods: Participants were collected from five countries: India, Indonesia, Japan, Taiwan, and Philippines. All patients were diagnosed with behavioral variant frontotemporal dementia (bvFTD), semantic dementia (SD), progressive non-fluent aphasia (PA), frontotemporal dementia with motor neuron disease (FTD/MND), PSP, and corticobasal degeneration (CBD) according to international consensus criteria. Family histories of FTLD and related neurodegenerative diseases were investigated in each patient.

Results: Ninety-one patients were included in this study. Forty-two patients were diagnosed to have bvFTD, two patients had FTD/MND, 22 had SD, 15 had PA, one had PA/CBS, five had CBS and four patients had PSP. Family history of any FTLD spectrum disorder was reported in 9.5% in bvFTD patients but in none of the SD or PA.

Conclusion: In contrast to patients of the western countries, few Asian FTLD patients have positive family histories of dementia.

Key words: Asia, epidemiology, family history, bvFTD, FTLD

Introduction

Frontotemporal lobar degeneration (FTLD) is the second most common cause of early-onset dementia after Alzheimer's disease (AD; Neary, 1999). FTLD includes three clinical subtypes: frontotemporal dementia (bvFTD or behavioral variant FTD) characterized by a progressive deterioration of behavior and personality, as well as semantic dementia (SD) and progressive non-fluent aphasia variants of progressive aphasia (PA; Neary *et al.*, 1998). The broader FTLD spectrum also includes FTD with motor neuron disease (FTD/MND) and parkinsonian syndromes such as progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD). While CBD requires a pathological diagnosis, the purely clinical entity is termed corticobasal syndrome (CBS).

Researches in western countries frequently report a strong family history in FTLD patients. In a community-based study by the Cambridge group, almost one-third of the participants (29%) with

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