jects. The only difference between part A and part B is the subjects' rotation in part B just before they replace the cards. The most plausible explanation for their poor achievement in part B is that the patients were unable to use the information on changes in their body direction. In other words, they were defective in the processing of directional signals of the self, or unable to integrate information on the registered external spatial locations of objects with that on their body direction.

There are lines of evidence that the retrosplenial cortex is concerned with spatial navigation. For example, excision of the retrosplenial cortex of rats can impair spatial navigation [23, 24]. Recent neuroimaging studies in humans using PET or fMRI also revealed that the retrosplenial area was activated when subjects were involved in a large-scale navigation task, although the activation was usually bilateral [25–27]. Moreover, it is noteworthy that in rats, head direction cells – cells that are excited when rats are maintaining a certain heading or orientation within an environment – are found in the retrosplenial cortex in addition to several neural structures such as the anterior dorsal nucleus of the thalamus, lateral dorsal thalamus, lateral mammillary nuclei, striatum, and postsubiculum [28, 29]. These neural substrates may constitute a functional circuit dealing with directional signals of the self [28].

Several experiments in monkeys have shown that the retrosplenial cortex is reciprocally connected with the parahippocampal cortical areas, presubiculum, entorhinal cortex, mid-dorsolateral prefrontal cortex, superior temporal sulcus, and posterior parietal cortex [30–32]. Therefore, the retrosplenial cortex seems to be a pivotal constituent of both the medial temporal lobe memory system and head directional system as well as interacting with the lateral parietofrontal large-scale network, which contributes to the processing of information on the egocentric visuospatial locations of objects.

In conclusion, our 3 cases suggest that the right retrosplenial lesions were responsible for their HD. The CPT implied that HD patients cannot integrate information well on object locations in the space derived from an egocentric reference frame and that on changes in body direction. The right retrosplenial cortex may be a place where these different types of information necessary for navigation converge. The CPT is a very useful clinical tool for evaluating patients with HD.

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References

▶1 Aguirre GK, D'Esposito M: Topographical disorientation: a synthesis and taxonomy. Brain 1999;122:1613–1628.

▶2 Kase CS, Troncoso JF, Court JE, Tapia JF, Mohr JP: Global spatial disorientation. Clinico-pathologic correlations. J Neurol Sci 1977;34:267–278.

▶3 Levine DN, Warach J, Farah MJ: Two visual systems in mental imagery: dissociation of 'what' and 'where' in imagery disorders due to bilateral posterior cerebral lesions. Neurology 1985;35:1010–1018.

▶4 Stark M, Coslett HB, Saffran EM: Impairment of an egocentric map of locations: implications for perception and action. Cogn Neuropsychol 1996;13:481–523.

▶5 Wilson BA, Berry E, Gracey F, Harrison C, Stow I, Macniven J, Weatherley J, Young AW: Egocentric disorientation following bilateral parietal lobe damage. Cortex 2005;41:547–554.

▶6 Hécaen H, Tzortzis C, Rondot P: Loss of topographic memory with learning deficits. Cortex 1980;16:525–542.

▶7 McCarthy RA, Evans JJ, Hodges JR: Topographical amnesia: spatial memory disorder, perceptual dysfunction, or category specific semantic memory impairment? J Neurol Neurosurg Psychiatry 1996;60:318–325.

▶8 Landis T, Cummings JL, Benson DF, Palmer EP: Loss of topographic familiarity. An environmental agnosia. Arch Neurol 1986;43:132–136.

9 Takahashi N, Kawamura M, Hirayama K, Tagawa K: Non-verbal facial and topographic visual object agnosia – a problem of familiarity in prosopagnosia and topographic disorientation (in Japanese). No To Shinkei 1989;41:703–710.

▶10 Habib M, Sirigu A: Pure topographical disorientation: a definition and anatomical basis. Cortex 1987;23:73–85.

▶11 Takahashi N, Kawamura M, Shiota J, Kasahata N, Hirayama K: Pure topographical disorientation due to right retrosplenial lesion. Neurology 1997;49:464–469.

▶12 Bottini G, Cappa S, Geminiani G, Sterzi R: Topographic disorientation – a case report. Neuropsychologia 1990;28:309–312.

▶13 Obi T, Bando M, Takeda K, Sakuta M: A case of topographical disturbance following left medial parieto-occipital lobe infarction (in Japanese). Rinsho Shinkeigaku 1992;32:426–429.

▶14 Cammalleri R, Gangitano M, D'Amelio M, Raieli V, Raimondo D, Camarda R: Transient topographical amnesia and cingulated cortex damage: a case report. Neuropsychologia 1996;42:321–326.

▶15 Suzuki K, Yamadori A, Hayakawa Y, Fujii T: Pure topographical disorientation related to dysfunction of the viewpoint dependent visual system. Cortex 1998;34:589–599.

▶16 Katayama K, Takahashi N, Ogawara K, Hattori T: Pure topographical disorientation due to right posterior cingulated lesion. Cortex 1999;35:279–282.

▶17 Alsaadi T, Binder JR, Lazar RM, Doorani T, Mohr JP: Pure topographical disorientation: a distinctive syndrome with varied lesion location. Neurology 2000;54:1864–1866.

▶18 Valenstein E, Bowers D, Verfaellie M, Heilman KM, Day A, Watson RT: Retrosplenial amnesia. Brain 1987;110:1631–1646.

▶19 Takayama Y, Kamo H, Ohkawa Y, Akiguchi I, Kimura J: A case of retrosplenial amnesia (in Japanese). Rinsho Shinkeigaku 1991;31: 331–333.

▶20 Katai S, Maruyama T, Hashimoto T, Yanagisawa N: A case of cerebral infarction presenting as retrosplenial amnesia (in Japanese). Rinsho Shinkeigaku 1992;32:1281–1287.

▶21 Gainotti G, Aimonti S, Di Betta AM, Silveri MC: Retrograde amnesia in a patient with retrosplenial tumour. Neurocase 1998;4: 519–526.

▶22 Maguire EA: The retrosplenial contribution to human navigation: a review of lesion and neuroimaging findings. Scand J Psychol 2001;42:225–238.

▶23 Sutherland RJ, Whishaw IQ, Kolb B: Contributions of the cingulate cortex to two forms of spatial learning and memory. J Neurosci 1988;8:1863–1872.

▶24 Cooper BG, Mizumori SJY: Retrosplenial cortex inactivation selectively impairs navigation in darkness. Neuroreport 1999;10: 625–630.

▶25 Aguirre GK, D'Esposito M: Environmental knowledge is subserved by separable dorsal/ventral neural areas. J Neurosci 1997;17: 2512–2517.

▶26 Ghaem O, Mellet E, Crivello F, Tzourio N, Mazoyer B, Bethoz A, Denis M: Mental navigation along memorized routes activates the hippocampus, precuneus, and insula. Neuroreport 1997;8:739–744.

▶27 Maguire EA, Burgress N, Donnet JG, Frakowiak RSJ, Frith CD, O'Keefe J: Knowing where, and getting there: a human navigation network. Science 1998;280:921–924.

▶28 Taube JS: Head direction cells and the neurophysiological basis of a sense of direction. Prog Neurobiol 1998;55:225–256.

▶29 Chen LL, Lin LH, Green EJ, Barnes CA, MacNaughton BL: Head-direction cells in the rat posterior cortex. I. Anatomical distribution and behavioral modulation. Exp Brain Res 1994;101:8–23.

▶30 Kobayashi Y, Amaral D: Macaque monkey retrosplenial cortex. I. Three-dimensional and cytoarchitectonic organization. J Comp Neurol 2000;426:339–365.

▶31 Suzuki WA, Amaral D: Perirhinal and parahippocampal cortices of the macaque monkey: cortical afferents. J Comp Neurol 1994; 350:497–533.

▶32 Morris R, Pandya D, Petrides M: Fiber system linking the mid-dorsolateral frontal cortex with the retrosplenial/presubicular region in the rhesus monkey. J Comp Neurol 1999;407:183–192.

A Phase I Study of Aromatic L-Amino Acid Decarboxylase Gene Therapy for Parkinson's Disease

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Gene transfer of dopamine-synthesizing enzymes into the striatal neurons has led to behavioral recovery in animal models of Parkinson's disease (PD). We evaluated the safety, tolerability, and potential efficacy of adeno-associated virus (AAV) vector–mediated gene delivery of aromatic L-amino acid decarboxylase (AADC) into the putamen of PD patients. Six PD patients were evaluated at baseline and at 6 months, using multiple measures, including the Unified Parkinson's Disease Rating Scale (UPDRS), motor state diaries, and positron emission tomography (PET) with 6-[^{18}F]fluoro-L-*m*-tyrosine (FMT), a tracer for AADC. The short-duration response to levodopa was measured in three patients. The procedure was well tolerated. Six months after surgery, motor functions in the OFF-medication state improved an average of 46% based on the UPDRS scores, without apparent changes in the short-duration response to levodopa. PET revealed a 56% increase in FMT activity, which persisted up to 96 weeks. Our findings provide class IV evidence regarding the safety and efficacy of AADC gene therapy and warrant further evaluation in a randomized, controlled, phase 2 setting.

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INTRODUCTION

Dopamine replacement has been the standard pharmacotherapy for motor impairment in Parkinson's disease (PD). Although virtually all patients benefit from levodopa at an early stage of the disease, severe loss of nigrostriatal nerve terminals in advanced PD leads to profoundly decreased activities of dopamine-synthesizing enzymes, including aromatic L-amino acid decarboxylase (AADC), an essential enzyme that converts levodopa to dopamine. Failure to respond to levodopa therapy may result from a reduction in AADC activity, decreased dopamine storage capacity in synaptic vesicles, postsynaptic changes in striatal output neurons, and abnormalities of nondopaminergic neurotransmitter systems.[1,2] Systemic administration of high-dose levodopa enhances oscillations in motor performance and complications, including hallucinations, due to dopaminergic stimulation of the mesolimbic system.

One potential treatment for advanced PD is gene therapy to restore striatum-selective dopamine production. In addition to AADC, tyrosine hydroxylase, which converts L-tyrosine to levodopa, and guanosine triphosphate cyclohydrolase I, which catalyzes biosynthesis of the essential tyrosine hydroxylase cofactor, tetrahydrobiopterine, are necessary for efficient synthesis of dopamine.[2] Viral vector–mediated gene transfer of these dopamine-synthesizing enzymes has been shown to achieve behavioral recovery in animal PD models, with efficient transduction of striatal neurons that escape degeneration.[3–6] When tyrosine hydroxylase and guanosine triphosphate cyclohydrolase I are expressed in the striatum, levodopa can be synthesized continuously. This strategy would be useful for reducing motor fluctuations associated with intermittent levodopa intake. Gene transfer of AADC alone in combination with oral levodopa administration would be a safer strategy for initial clinical trials. In the latter approach, the patients still need to take levodopa to control motor symptoms, but excess production of dopamine could be avoided by reducing the dose of levodopa. We assessed the safety, tolerability, and the potential efficacy of intraputaminal infusion of recombinant adeno-associated virus (AAV) serotype 2 vector encoding human AADC (AAV-hAADC-2) in patients with mid- to late-stage PD. We also examined whether the short-duration response to levodopa, the antiparkinsonian response that parallels the plasma levodopa levels, would change after gene therapy.[7]

RESULTS

Patient disposition and baseline characteristics

Six patients (4 men, 2 women), mean age 60 (range, 51–68) years, were enrolled (**Table 1**). The mean disease duration was 10 (range, 5–18) years, and time on levodopa was 9.3 (range, 5–15) years. The average baseline daily levodopa and levodopa equivalent doses were 642 and 808 mg, respectively.

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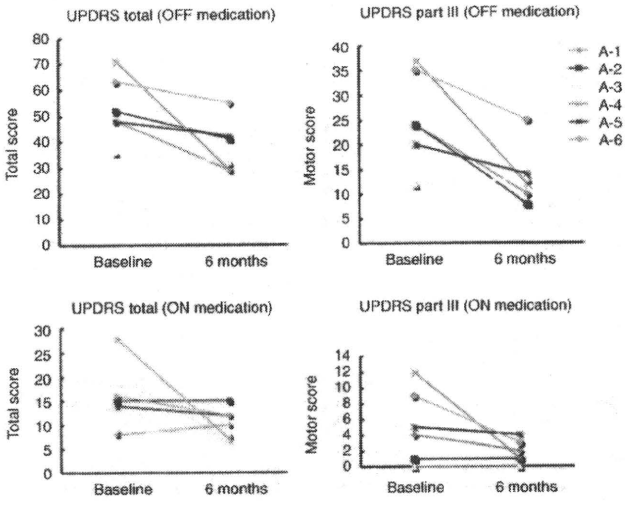

Figure 1 Changes in UPDRS scores. Absolute changes in scores from baseline to 6 months for individual patients. OFF, off-medication state; ON, on-medication state; UPDRS, Unified Parkinson's Disease Rating Scale.

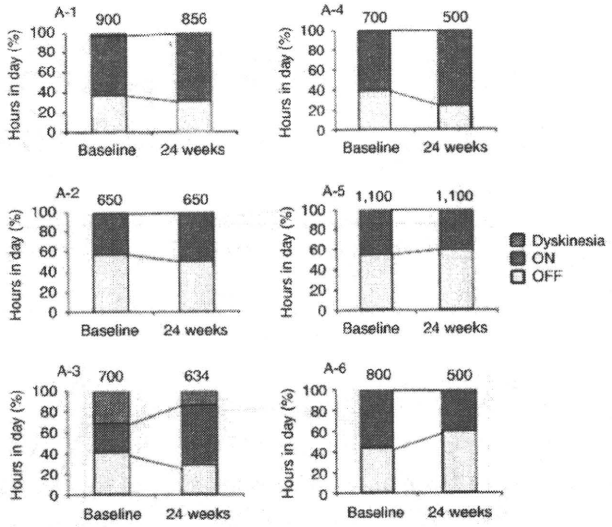

Figure 2 Evaluation of patients' diaries and daily doses of levodopa equivalents. For each 30-minute interval throughout the day, the patients recorded whether they were mobile (ON), immobile (OFF), or asleep. They also recorded the time with troublesome dyskinesias (Dyskinesia). The graph shows the percentage of hours in a day spent in each condition at baseline and at 6 months. The numbers on the bars indicate the mean daily doses of levodopa equivalents (mg). OFF, off-medication state; ON, on-medication state.

significant toxicity.[3,4,6,8,9] Recently, three phase I clinical trials of gene therapy for advanced PD demonstrated that AAV vector–mediated gene delivery into the subthalamic nucleus or putamen was safe and tolerable.[10–13] In this study, the safety of the AAV vectors for clinical use in the human brain was confirmed. Although one patient developed a venous hemorrhage in the subcortical white matter along the trajectory, it is well known that cerebral bleeding occasionally occurs in association with surgical procedures for deep brain stimulation in which electrodes are inserted into the basal ganglia through the frontal lobe white matter.[14,15] PET imaging in this patient showed that putaminal AADC expression was not affected by the subcortical venous hemorrhage and persisted up to 96 weeks. Thus, the venous hemorrhage was probably due to the surgical procedure and not gene transduction.

Although the present trial was a small, open-label study, and the nonblinded, uncontrolled analysis limits the interpretation, the initial efficacy outcomes are encouraging. Our patients showed improved motor performance in the OFF state. Levodopa has a relatively short plasma half-life (60–90 minutes), and antiparkinsonian effects observed after levodopa administration have generally been recognized as short- and long-duration responses. The short-duration response roughly parallels the plasma levodopa concentrations and is thought to be closely linked to dyskinesia, whereas the long-duration response builds up over weeks and improves trough (worst) motor performance in the OFF state.[7] Because the pattern of the short-duration response to levodopa did not change after gene therapy in our patients, the beneficial effect on the OFF state appears to be attributed to augmentation of the long-term response to levodopa.[16] In the preclinical studies with animal models of PD, AAV vectors mainly transduced medium spiny neurons that have dopamine receptors, and extracellular dopamine was increased in the striatum after administration of levodopa.[5,17] The mechanism underlying the long-duration response is not sufficiently understood, and future study is necessary to determine how nonphysiologic production of dopamine in the striatal neurons could enhance the response. It has been reported that the sustained long-duration response to levodopa is greater in patients treated with higher single doses of levodopa.[18] Thus, it is likely that increased dopamine in the putamen after gene transfer may enhance the stable long-duration response. Motor fluctuations in PD are associated with increased response to levodopa with a deeper trough in motor performance, rather than shortening of the response. Improving trough or OFF state motor function by augmenting the long-term response would likely reduce motor fluctuation.[16] Two of three patients in whom the short-duration response to levodopa was studied showed increased peak plasma levodopa concentrations after gene therapy. This finding may simply reflect variable absorbance of levodopa, and it remains to be elucidated whether changes in gastrointestinal absorption could be related to better motor performance in the OFF state.[19]

Activities and levels of AADC mRNA and protein are profoundly reduced in advanced PD,[2] but there are still several types of AADC-containing cells in the striatum, such as serotonin neurons, intrinsic dopamine neurons, AADC-containing "D" neurons, and glial cells.[20] These cells may act as a local source of dopamine. However, dopamine produced in nondopamine cells may not be taken up into dopamine cells and stored in synaptic vesicles, as dopamine transporter and vesicular monoamine transporter 2 are also reduced in advanced PD. The functional efficacy of dopamine produced from exogenous levodopa in these cells may be limited, at least in primates.[2,3] Striatal output neurons, main targets in AADC gene therapy, play a principal role in dopamine modulation of motor function in the basal ganglia. Dopamine synthesized in the striatal neurons themselves may more easily stimulate both synaptic and extrasynaptic receptors.

5 years of levodopa therapy; a minimum 8-point improvement in the UPDRS motor score after levodopa intake; and motor complications not satisfactorily controlled with medical therapy. The main exclusion criteria were atypical parkinsonism, dementia (MMSE score <20), and previous neurosurgical treatment for PD.

Vector and stereotaxic infusion. The vector used in this trial was a recombinant AAV2 with an expression cassette consisting of a human cytomegalovirus immediate-early promoter, followed by the human growth hormone first intron, complementary DNA of human AADC, and simian virus 40 polyadenylation signal sequence.[3–6] Clinical grade AAV-hAADC-2 was manufactured by Avigen (Alameda, CA) and provided by Genzyme (Boston, MA). The patients received AAV-hAADC-2 *via* bilateral intraputaminal infusions. Two target points were determined in the putamen that were sufficiently separated from each other in dorsolateral directions and identified on a magnetic resonance image. One burr hole was trepanned in each side of the cranial bone, through which the vector was injected into the two target points *via* the two-track insertion route. The vector-containing solution was prepared to a concentration of 1.5×10^{12} vector genome/ml, and 50 µl per point of the solution were injected at 1 µl/min; each patient received 3×10^{11} vector genome of AAV-hAADC-2.

Neutralizing antibody titers against AAV2 were determined by measuring β-galactosidase activities in HEK293 cells transduced with 5×10^3 vector genome/cell of AAV2 vectors expressing β-galactosidase in various dilutions of sera.[22]

PET. The AADC expression level in the putamen was assessed on PET imaging with FMT 6 days before surgery and 1 and 6 months after gene transfer. All patients stopped dopaminergic medications 18 hours before PET and took 2.5 mg/kg of carbidopa orally 1 hour before FMT injection. Subsequently, 0.12 mCi/kg of FMT in saline were infused into an antecubital vein, and a 90-minute dynamic acquisition sequence was obtained. The PET and magnetic resonance imaging data were co-registered with a fusion processing program (Syntegra; Philips, Amsterdam, The Netherlands) to produce the fusion images. Radioactivities within volumes of interest drawn in the putamen and occipital lobe were calculated between 80 and 90 minutes after tracer injection. A change in putaminal FMT uptake from baseline to 24 weeks was assessed using the putaminal–occipital ratio of radioactivities.

Statistical analysis. Values at baseline and 6 months after gene transfer were compared using Student's *t*-test (paired analyses). A two-sided *P* value <0.05 was taken to indicate significant differences. Two-way analysis of variance with Bonferroni correction of *P* values was used for the short-duration response to levodopa.

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REFERENCES

1. Fahn, S (2008). The history of dopamine and levodopa in the treatment of Parkinson's disease. *Mov Disord* **23** Suppl 3: S497–S508.
2. Nagatsu, T and Sawada, M (2007). Biochemistry of postmortem brains in Parkinson's disease: historical overview and future prospects. *J Neural Transm Suppl*: 113–120.
3. Bankiewicz, KS, Eberling, JL, Kohutnicka, M, Jagust, W, Pivirotto, P, Bringas, J *et al.* (2000). Convection-enhanced delivery of AAV vector in parkinsonian monkeys; *in vivo* detection of gene expression and restoration of dopaminergic function using pro-drug approach. *Exp Neurol* **164**: 2–14.
4. Bankiewicz, KS, Forsayeth, J, Eberling, JL, Sanchez-Pernaute, R, Pivirotto, P, Bringas, J *et al.* (2006). Long-term clinical improvement in MPTP-lesioned primates after gene therapy with AAV-hAADC. *Mol Ther* **14**: 564–570.
5. Muramatsu, S, Fujimoto, K, Ikeguchi, K, Shizuma, N, Kawasaki, K, Ono, F *et al.* (2002). Behavioral recovery in a primate model of Parkinson's disease by triple transduction of striatal cells with adeno-associated viral vectors expressing dopamine-synthesizing enzymes. *Hum Gene Ther* **13**: 345–354.
6. Shen, Y, Muramatsu, SI, Ikeguchi, K, Fujimoto, KI, Fan, DS, Ogawa, M *et al.* (2000). Triple transduction with adeno-associated virus vectors expressing tyrosine hydroxylase, aromatic-L-amino-acid decarboxylase, and GTP cyclohydrolase I for gene therapy of Parkinson's disease. *Hum Gene Ther* **11**: 1509–1519.
7. Nutt, JG (2008). Pharmacokinetics and pharmacodynamics of levodopa. *Mov Disord* **23** Suppl 3: S580–S584.
8. Leriche, L, Björklund, T, Breysse, N, Besret, L, Grégoire, MC, Carlsson, T *et al.* (2009). Positron emission tomography imaging demonstrates correlation between behavioral recovery and correction of dopamine neurotransmission after gene therapy. *J Neurosci* **29**: 1544–1553.
9. Muramatsu, S, Tsukada, H, Nakano, I and Ozawa, K (2005). Gene therapy for Parkinson's disease using recombinant adeno-associated viral vectors. *Expert Opin Biol Ther* **5**: 663–671.
10. Christine, CW, Starr, PA, Larson, PS, Eberling, JL, Jagust, WJ, Hawkins, RA *et al.* (2009). Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. *Neurology* **73**: 1662–1669.
11. Eberling, JL, Jagust, WJ, Christine, CW, Starr, P, Larson, P, Bankiewicz, KS *et al.* (2008). Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. *Neurology* **70**: 1980–1983.
12. Kaplitt, MG, Feigin, A, Tang, C, Fitzsimons, HL, Mattis, P, Lawlor, PA *et al.* (2007). Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet* **369**: 2097–2105.
13. Marks, WJ Jr, Ostrem, JL, Verhagen, L, Starr, PA, Larson, PS, Bakay, RA *et al.* (2008). Safety and tolerability of intraputaminal delivery of CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial. *Lancet Neurol* **7**: 400–408.
14. Ben-Haim, S, Asaad, WF, Gale, JT and Eskandar, EN (2009). Risk factors for hemorrhage during microelectrode-guided deep brain stimulation and the introduction of an improved microelectrode design. *Neurosurgery* **64**: 754–62; discussion 762.
15. Weaver, FM, Follett, K, Stern, M, Hur, K, Harris, C, Marks, WJ Jr *et al.*; CSP 468 Study Group. (2009). Bilateral deep brain stimulation vs best medical therapy for patients with advanced Parkinson disease: a randomized controlled trial. *JAMA* **301**: 63–73.
16. Nutt, JG, Carter, JH, Lea, ES and Sexton, GJ (2002). Evolution of the response to levodopa during the first 4 years of therapy. *Ann Neurol* **51**: 686–693.
17. Sánchez-Pernaute, R, Harvey-White, J, Cunningham, J and Bankiewicz, KS (2001). Functional effect of adeno-associated virus mediated gene transfer of aromatic L-amino acid decarboxylase into the striatum of 6-OHDA-lesioned rats. *Mol Ther* **4**: 324–330.
18. Zappia, M, Oliveri, RL, Bosco, D, Nicoletti, G, Branca, D, Caracciolo, M *et al.* (2000). The long-duration response to L-dopa in the treatment of early PD. *Neurology* **54**: 1910–1915.
19. Cersosimo, MG and Benarroch, EE (2008). Neural control of the gastrointestinal tract: implications for Parkinson disease. *Mov Disord* **23**: 1065–1075.
20. Kitahama, K, Geffard, M, Araneda, S, Arai, R, Ogawa, K, Nagatsu, I *et al.* (2007). Localization of L-DOPA uptake and decarboxylating neuronal structures in the cat brain using dopamine immunohistochemistry. *Brain Res* **1167**: 56–70.
21. Forsayeth, JR, Eberling, JL, Sanftner, LM, Zhen, Z, Pivirotto, P, Bringas, J *et al.* (2006). A dose-ranging study of AAV-hAADC therapy in Parkinsonian monkeys. *Mol Ther* **14**: 571–577.
22. Ito, T, Yamamoto, S, Hayashi, T, Kodera, M, Mizukami, H, Ozawa, K *et al.* (2009). A convenient enzyme-linked immunosorbent assay for rapid screening of anti-adeno-associated virus neutralizing antibodies. *Ann Clin Biochem* **46**: 508–510.

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Temporal trends and geographic clusters of mortality from amyotrophic lateral sclerosis in Japan, 1995–2004

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ABSTRACT

The present study examined temporal trends and geographic clustering of amyotrophic lateral sclerosis (ALS) mortality in Japan, during 1995–2004, using vital statistics based on death certificates. ALS was usually diagnosed by neurologists according to clinical guidelines that complied with the El Escorial Criteria. The underlying cause of death for ALS was coded as G12.2A. Regression analysis was used to examine temporal trends. Spatial scan statistic was used to detect any area of elevated risk as a cluster. A total of 12,173 (6864 male and 5309 female) ALS deaths were reported. Annual crude mortality rate per 100,000 population was 1.07 (1.26 for males and 0.89 for females) in 2004. Although the overall temporal trend was stable, the trend increased in the 70+ years age group (p for trend, <0.001 in males and <0.05 in females), while it declined in the under 70 years age group (p for trend, <0.01 for both sexes). Male preponderance and M/F ratio remained nearly constant over time. Three clusters were detected: two ($p<0.005$ in males and $p<0.05$ in females) in northeast and one ($p<0.05$ in males) in west-central Japan. Further research is needed to clarify contributing factors for the observed trends and clusters in ALS mortality.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is still a fatal neurodegenerative disease characterized by the selective loss of upper and lower motor neurons. Five to 10% of cases of ALS are familial; the others are believed to be sporadic [1]. While advances have been made in identifying disease-causing genes for familial ALS, very little is known about susceptibility genes or other risk factors for sporadic ALS. Many putative environmental risk factors (i.e., heavy metals, solvents, electrical and electromagnetic fields, poliovirus, mechanical trauma, heavy physical activity, cigarette smoking, and diet) have been previously reported; however, age and a family history are the only established risk factors for ALS [1–4].

Variation in mortality over time and by geographic location, sex and ethnicity can often be a source of etiological clues [5]. The mortality rate from motor neuron disease (MND), of which ALS accounts for 85% or more [1,5], was reported to have steadily increased from the 1950s to the 1990s in western countries [3,5–10]. In some European countries and the United States, a greater increase in ALS mortality was observed in females than in males in the past 30 years, causing a decrease in the male to female (M/F) ratio [5,8–10].

Contrary to the trends noted in many other countries, Japan has shown an unusual pattern of mortality from MND for decades. The age-adjusted MND mortality rate rose from the mid 1950s, peaking in the early 1960s, and declined in the early 1970s [11,12]. Thereafter, the rate slowly increased to that in the early 1950s for a period of 20 years between 1970 and 1990 [11]. A recent study reported that it decreased from 1995 through 2001, and the M/F ratio slightly increased [13].

The Western Pacific form of ALS, referred to as 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 [14,15]. Over the past four decades, the incidence of ALS/PDC has markedly declined in Guam [14]. On the other hand, a continuing high prevalence and incidence in Hohara in Mie prefecture [16] and Kozagawa in Wakayama prefecture [17] in the Kii peninsula are reported, although they temporarily declined in the 1980s.

In this study we examined the national ALS mortality data of Japan to reveal the recent temporal trends in ALS mortality and investigated whether or not any geographic clusters of ALS deaths exist in particular regions.

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2. Methods

The World Federation of Neurology Research Group on Neuromuscular Diseases published the El Escorial Criteria (EEC) in 1994 [18]. Based on these criteria, the research committee on ALS of Japan has updated the guidelines for diagnosis and treatments of ALS, and recommended it for clinicians and researchers [19]. ALS was usually diagnosed by neurologists using guidelines that complied with the El Escorial Criteria.

In 1995, the Statistics and Information Department of the Ministry of Health, Labour and Welfare, Japan, changed the coding for disease classification from that in the ninth version of International Classification of Diseases (ICD-9) to that in the tenth version (ICD-10). ICD-9 and ICD-10 designated MND with the respective four-digit codes of 3352 and G12.2. For the vital statistics of Japan after the year 1995, they added the capital letters A and B to G12.2 to differentiate ALS from other MND: G12.2A for ALS and G12.2B for primary lateral sclerosis, progressive bulbar palsy, spinal muscular atrophy, and other or unspecified MND. They followed the international rules for mortality statistics based on the underlying cause of death. The underlying cause of death was defined by the World Health Organization as the disease or injury that initiated the train of morbid events directly leading to death or the circumstances of the accident or violence that produced the fatal injury.

The national ALS mortality data from 1995 through 2004 were used for our present study. The data used were taken from the national mortality database of vital statistics based on death certificates, after obtaining permission for use from the Statistics and Information Department. The variables in the data file included the codes of the underlying cause of death for ALS along with age at death, sex, and place of residence where the deceased had lived. It did not contain any personal identifiable information (e.g., individuals' names or residential addresses).

The total number of deaths due to ALS from 1995 through 2004 was counted, and age-specific mortality rates were calculated according to 5-year age intervals. Annual crude mortality rates in 1995–2004 were calculated as the number of ALS deaths per million persons per year on the basis of the Japanese population for the respective year.

To examine temporal trends in deaths from ALS, annual age-adjusted mortality rates were calculated by the direct method using the total population of the 2005 census as a standard population. Linear regression analysis was used to examine temporal trends in mortality as well as M/F ratios in individual years as a continuous variable. Joinpoint regression analysis was used to provide annual percentage changes (APC) with 95% confidence intervals and p values for trends [20,21]. It was also performed in the groups of age at death, <70 and 70+ years. Statistical significance was determined as a p value for trend less than 0.05.

A cluster is defined as a geographically bounded group of occurrences of sufficient size and concentration to be unlikely to have occurred by chance without any assumptions about the shape or form of the cluster [22]. To detect clusters, the flexible spatial scan statistic [23–26] was used in our present study. It can detect clusters of any size and form located anywhere in the study region, whether or not they cross administrative borders. The most likely cluster can be detected as that with the maximum likelihood. P values were obtained using Monte Carlo hypothesis testing, comparing the test statistic from the observed data set with the test statistics from 999 random data sets generated under the null hypothesis of no clustering. Statistical significance was determined as a p value less than 0.05.

The flexible spatial scan statistic can be applied to geographically aggregated data. So, to eliminate the effects of age, we employed this statistic using the number of observed and expected deaths from ALS based on the vital statistics for each of the secondary medical care zones (SMCZ). At the time of this study, there were 359 SMCZs for medical care planning, each of which consisted of neighboring municipalities in all 47 prefectures of Japan, according to the Medical Service Law.

3. Results

A total of 12,173 (6864 male and 5309 female) ALS deaths were reported in Japan in the period between 1995 and 2004. Fig. 1 shows mortality rates due to ALS by 5-year age groups. The age-specific mortality rates rose steadily with age group up to the age of 75–79 years and then sharply declined for those aged 80 years and older.

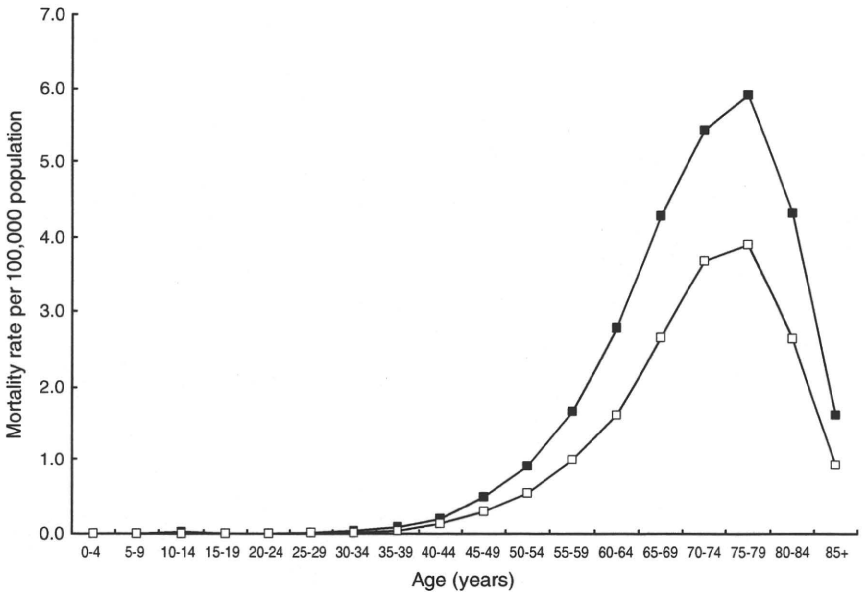

Fig. 1. Age-specific mortality rates from amyotrophic lateral sclerosis (ALS) by sex, Japan, 1995–2004 (■ = males and □ = females).

Table 1
Annual mortality rate per 100,000 population from amyotrophic lateral sclerosis (ALS), Japan, 1995–2004.

Year	Number of deaths			Crude mortality rate			Age-adjusted mortality rate[a]		
	Total	Male	Female	Total	Male	Female	Male	Female	M/F ratio
1995	1031	579	452	0.82	0.94	0.71	1.27	0.81	1.56
1996	1166	658	508	0.93	1.07	0.79	1.43	0.90	1.59
1997	1144	634	510	0.91	1.03	0.79	1.34	0.88	1.52
1998	1179	666	513	0.93	1.08	0.79	1.38	0.86	1.60
1999	1181	673	508	0.93	1.09	0.78	1.36	0.83	1.64
2000	1202	666	536	0.95	1.07	0.83	1.33	0.86	1.55
2001	1225	692	533	0.96	1.11	0.82	1.34	0.85	1.57
2002	1337	761	576	1.05	1.22	0.88	1.43	0.88	1.64
2003	1338	749	589	1.05	1.20	0.90	1.38	0.87	1.58
2004	1370	786	584	1.07	1.26	0.89	1.41	0.85	1.67

[a] Direct age-adjusted to the year 2005 census population. *P* values for trends are 0.200 for males, 0.899 for females and 0.122 for M/F ratios.

Annual crude mortality rates per 100,000 population increased from 0.94 to 1.26 for males and from 0.71 to 0.89 for females. After adjustment by age, ALS mortality rates have been stable during this period in both males and females. Mortality rates were higher for males than for females over the entire period. The M/F ratios remained almost constant (Table 1).

After stratification by age, age-adjusted mortality rates showed completely different temporal trends in the age groups of <70 years and 70+years (Figs. 2 and 3). Age-adjusted mortality rates have significantly increased in the older age group (p for trend, <0.001 in males and <0.05 in females), while they have significantly decreased in the younger age group (p for trend, <0.01 for both sexes).

The most likely, second, and third clusters are shown in Table 2, and each cluster is located on maps in Figs. 4 and 5. For males, two statistically significant clusters were identified, but both clusters were detected in different regions from the Kii Peninsula. In a part of the Kii Peninsula including Hohara and Kozagawa villages, which have been well known hyperendemic ALS foci for many years, a third cluster containing 236 observed deaths was detected, but it was not statistically significant. For females, one statistically significant cluster was identified. It was adjacent to the most likely cluster found in males, and some areas of these two clusters overlapped.

4. Discussion

Previous studies have reported a rise and fall in mortality from MND or ALS in Japan over the past five decades [11–13]. Our present study demonstrated that the recent temporal trend in age-adjusted mortality rate due to ALS was stable during the 10-year period between 1995 and 2004, since the introduction of ECC and ICD-10. When stratified by age into <70 and 70+years at death, we found opposite directions in age-adjusted mortality rates for the two groups: a clear upward trend in the older age group and a downward trend in the younger age group. This pattern is unique compared to those reported from previous studies [2,3,5,6,8–13]. In the United States, an increasing trend is seen for all ages except age 45 to 54 years [5]. In the United Kingdom and Norway, the trend is upward in the 60+ or 65+years age group but it is static in the under 60 years age group [8,10].

The increased ALS mortality in the older age group is common to all studies. It is noteworthy that in our present study there was a decreasing trend in the under 70 years age group. One of the hypotheses explaining this phenomenon is that age at onset may be chronologically delayed with time. According to a 10-year prospective population-based study conducted in Italy, mean onset age (SD) has become slightly higher: 64.2(11.2)years in 1995–1999 and 65.4 (11.1)years in 2000–2004 [27]. Although the data are cross-sectional in Japan, there is a substantial difference in age at onset between 61.8 (12.2)years in 1989–1999 [17] and 65.4(10.7)years in 2003–2006 [28]. As another hypothetical explanation, it may be that survival duration has been prolonged by the improvement of treatment such as pharmacotherapy (e.g., rilusole) combined with nutritional and respiratory support [1]. Remarkable progress in home-visit nursing care has been made in Japan, with the universal availability of

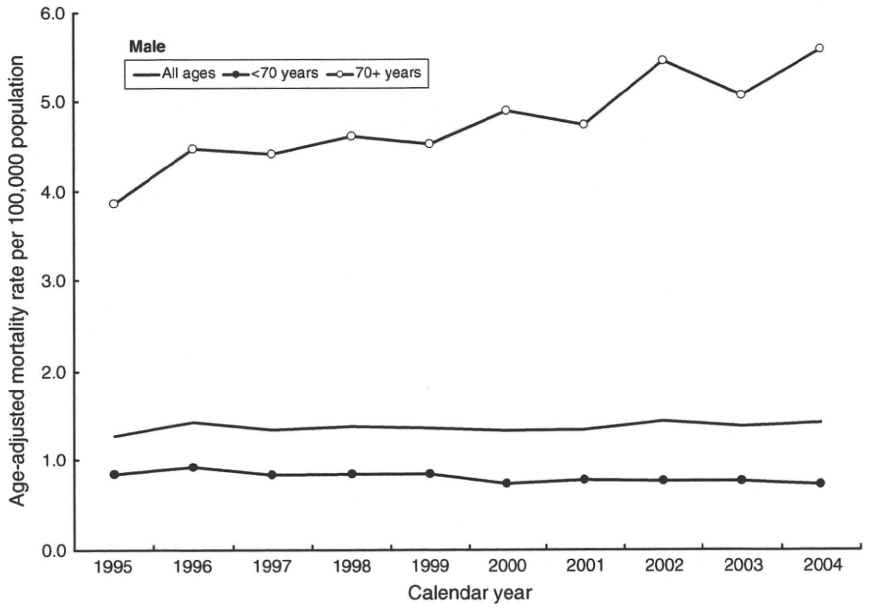

Fig. 2. Age-adjusted mortality rates from amyotrophic lateral sclerosis (ALS) in males, Japan, 1995–2004. The annual percentage changes (95% confidence intervals) were 0.5(−0.3, 1.4), −2.1(−3.2, −1.0) and +3.3(2.1, 4.5) for all age groups, age group of <70 years and age group of 70+years, respectively.

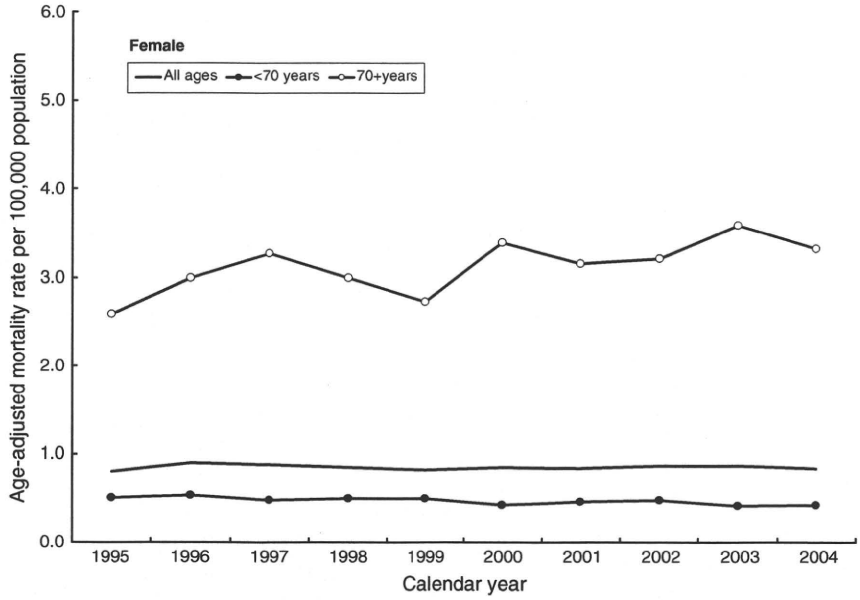

Fig. 3. Age-adjusted mortality rates from amyotrophic lateral sclerosis (ALS) in females, Japan, 1995–2004. The annual percentage changes (95% confidence intervals) were 0.0 (−0.7, 0.8), −2.3(−3.6, −0.9) and +2.3(0.3, 4.1) for all age groups, age group of <70 years and age group of 70+ years, respectively.

mechanical ventilatory support for ALS patients. A very high proportion of Japanese ALS patients (29.3%) received tracheotomy positive pressure ventilation (TPPV) compared to those in North America and Europe (2.1–5.4%) [28–33]. Based on the large-scale registered data on patients with ALS in Japan, about 40% of those under TPPV received care at home and almost 30% survived 9 years or more after the introduction of TPPV [28]. In our clinical experience, younger patients and their families are more likely to choose TPPV than older ones (unpublished). As a result, younger patients could survive longer. This may lead to prolongation of the age at death, particularly for younger patients, which may cause mortality to decrease in younger patients and consequently increase in older patients. To determine the duration of survival, both age at symptom-onset and age at death are required, but our present study lacked the former. Further research is needed to justify the aforementioned hypotheses.

Regarding the M/F ratio of ALS mortality over time, we found male preponderance that remained stable in our present study. This finding is inconsistent with the reported narrowing of the male to female gap in mortality [4,8–10] and incidence [2–4,34]. As some authors pointed out, the reduced M/F ratio may be partly caused by the changing lifestyle (e.g., smoking) of women, which has become more similar to that of men, in western countries [3,4,34]. According to OECD health data, for example, the prevalence of smoking has become similar for both sexes in western countries, but it is still markedly different between sexes in Japan (58.8% for males and 15.2% for females in 1995; 46.9% for males and 13.2% for females in 2004) [35]. In addition, we have to take into account gender difference in socioeconomic status that might affect patients' choice of treatment and long-term care. But we could not examine this factor because of the limited data in our current study. Further investigation is required to verify potential factors explaining the M/F ratio of ALS mortality over time.

The present study identified three statistically significant clusters of ALS mortality. Two (A1 in Fig. 4 and B1 in Fig. 5), which partially overlapped, were located in the northeastern part of the mainland of Japan. As shown in Fig. 4, one more cluster (A2) was geographically separated from the other (A1), at a short distance from the Kii Peninsula. In the Kii Peninsula, a cluster (A3) was observed but it did not reach statistical significance at the SMCZ level. Recent epidemiological surveys reported a continuing high incidence and prevalence in the subfoci of Hohara and Kozagawa villages of the Kii Peninsula [16,17,36]. Our cluster analysis may not be able to detect clustering in the two villages, which are much smaller units of area than SMCZ. These four clusters (A1–A3 and B1) are promising candidate areas for further analytical studies on ALS.

The limitations of our present study should be taken into account. One is case ascertainment for ALS based on the underlying cause of

Table 2
Geographic clusters of ALS mortality detected using flexible spatial statistics, Japan, 1995–2004.

Cluster	Approximate cluster location[a]	Number of SMCZs[b]	Expected cases	Observed cases	Relative risk	*p* value
Males (N=6864)						
Most likely cluster	A1: a part of Niigata–Gunma–Nagano–Fukushima	12	115.70	181	1.56	0
Second cluster	A2: a part of Hyogo–Kyoto–Osaka	7	182.16	254	1.39	0.03
Third cluster	A3: a part of Kii Peninsula (Wakayama–Mie–Nara–Osaka)	11	178.34	236	1.32	0.36
Females (N=5309)						
Most likely cluster	B1: a part of Gunma–Tochigi–Saitama	12	118.67	178	1.50	0.02
Second cluster	B2: a part of Hokkaido Island	11	172.54	231	1.34	0.26
Third cluster	B3: a part of Aichi–Gifu–Shizuoka–Shiga	12	276.71	384	1.26	0.3

[a] The clusters of A1–A3 and B1--B3 correspond to those on maps in Figs. 4 and 5, respectively.
[b] Abbreviated secondary medical care zones.

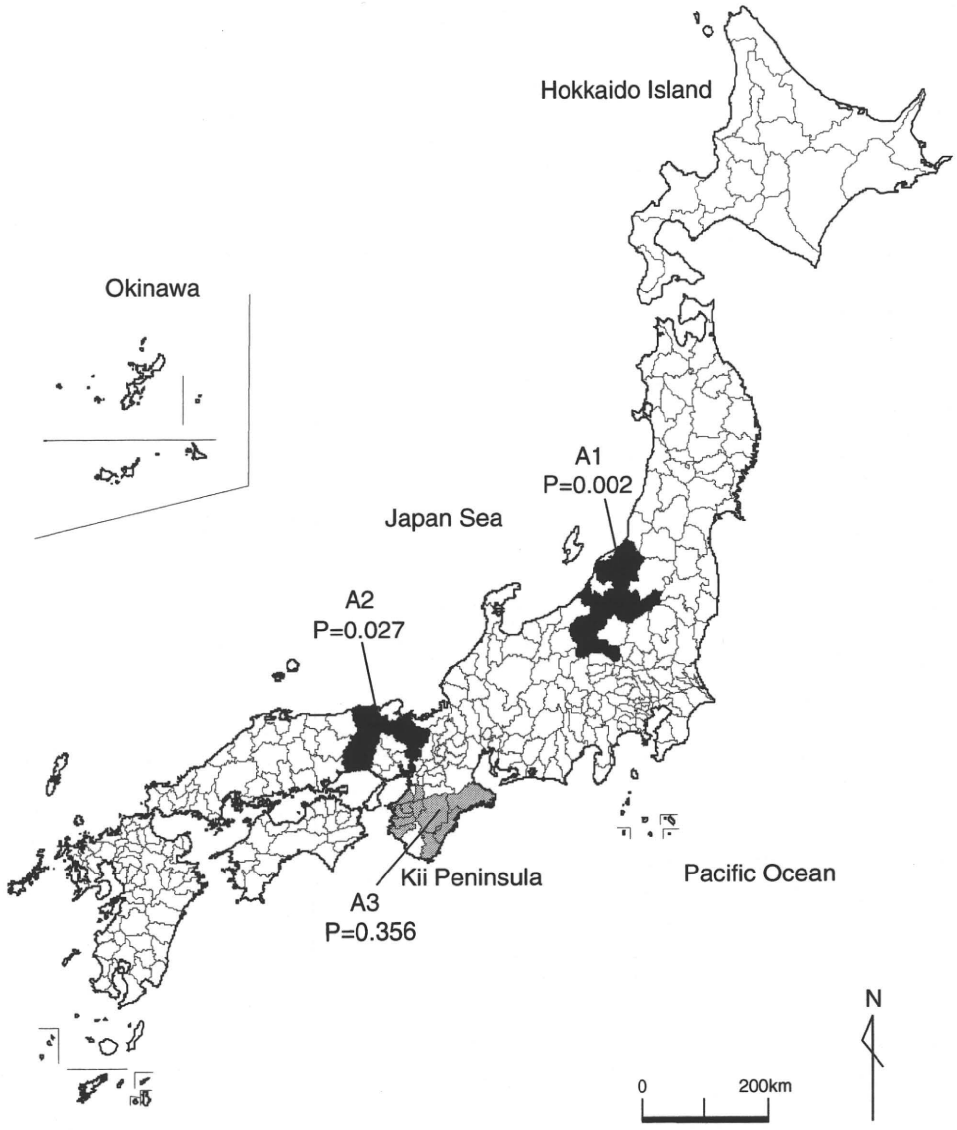

Fig. 4. Geographic clusters of age-adjusted deaths from amyotrophic lateral sclerosis (ALS) for males, Japan, 1995–2004. A1, A2 and A3 are the most likely, second and third geographic clusters, respectively (black (A1 and A2) and gray (A3) clusters with and without statistical significance, respectively).

death recorded on death certificates. This is not so serious, however, considering the following points: (1) ALS was usually diagnosed by neurologists, (2) the number of neurologists was large (e.g., 8555 as of March 31, 2009), and (3) neurologists have generally followed the clinical guidelines based on ECC since the recommendation of its use by the research committee on ALS of Japan [18,19]. The other is regional disparity affecting geographic clustering (e.g., number of neurologists and access to specialized medical care). Taking the following points together, it seems that the degree of regional disparity is small. The average number of neurology clinics/departments was 4.75 per 100,000 population [37]. The areas with higher or lower concentrations of neurology clinics/departments were not consistent with the locations in which the clusters of ALS mortality were detected in our present study. Basically, all ALS patients have been guaranteed free medical access by 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 have provided new evidence of ALS mortality in Japan during the 10-year period between 1995 and 2004: 1) The overall temporal trend in age-adjusted ALS mortality is stable. 2) The trend is going up in the 70+ years age group while it is going down in the under 70 years age group. 3) Male preponderance and M/F ratio remain nearly constant. 4) Some geographic clusters are detected. Current thinking on complex diseases like ALS is that multiple genetic and environmental factors contribute to disease liability [38]. Further research is needed to clarify contributing factors for the observed trends and clusters in ALS mortality.

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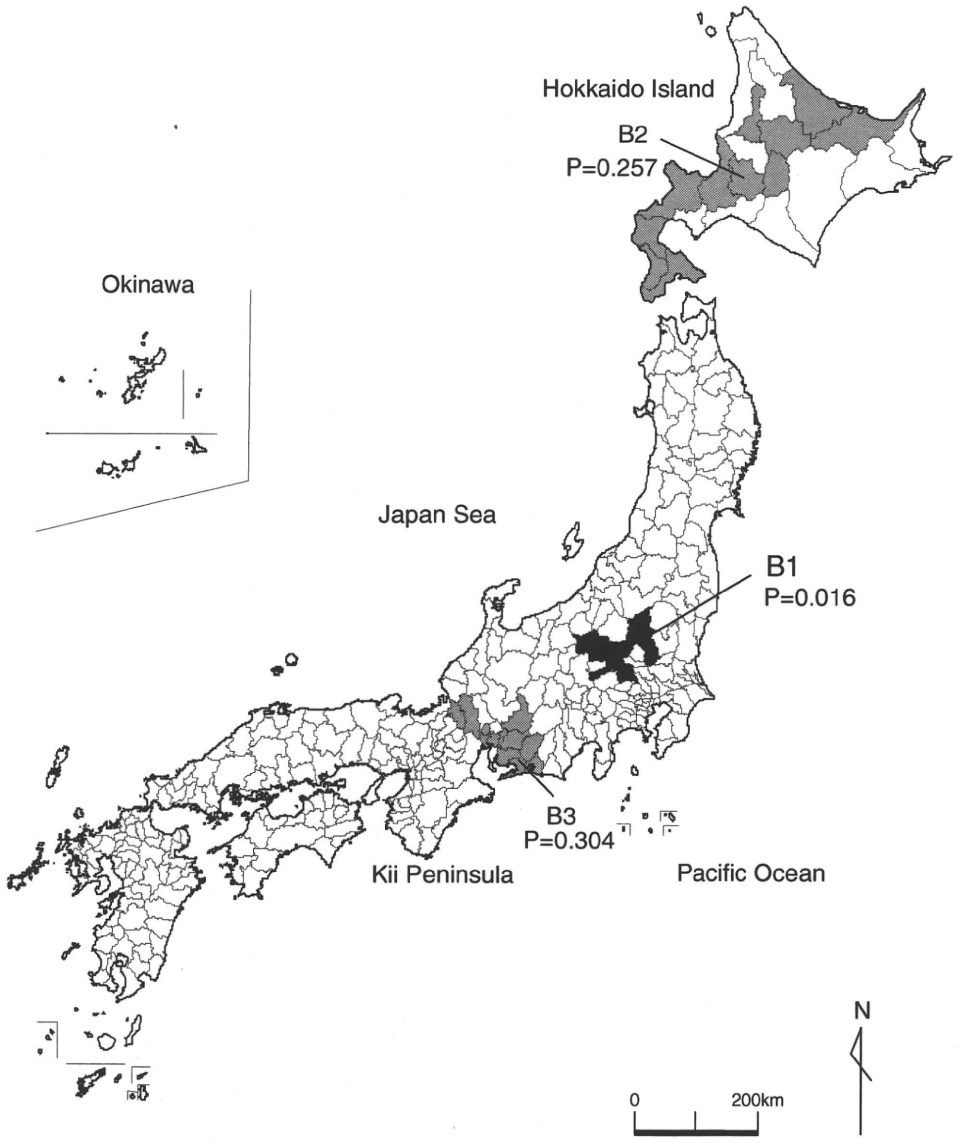

Fig. 5. Geographic clusters of age-adjusted deaths from amyotrophic lateral sclerosis (ALS) for females, Japan, 1995–2004. B1, B2 and B3 are the most likely, second and third geographic clusters, respectively (black (B1) and gray (B2 and B3) clusters with and without statistical significance, respectively).

References

[1] Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. N Engl J Med 2001;344: 1688–700.

[2] Armon C. An evidence-based medicine approach to the evaluation of the role of exogenous risk factors in sporadic amyotrophic lateral sclerosis. Neuroepidemiology 2003;22:217–28.

[3] Beghi E, Logroscino G, Chiò A, Hardiman O, Mitchell D, Swingler R, et al, EURALS Consortium. The epidemiology of ALS and the role of population-based registries. Biochim Biophys Acta 2006;1762:1150–7.

[4] McGuire V, Nelson LM. Epidemiology of ALS. In: Mitsumoto H, Przedbroski S, Gordon P, editors. Amyotrophic lateral sclerosis. New York: Taylor & Francis; 2006. p. 17–41.

[5] Noonan CW, White MC, Thurman D, Wong LY. Temporal and geographic variation in United States motor neuron disease mortality, 1969–1998. Neurology 2005;64: 1215–21.

[6] Chancellor AM, Warlow CP. Adult onset motor neuron disease: worldwide mortality, incidence and distribution since 1950. J Neurol Neurosurg Psychiatry 1992;55:1106–15.

[7] Román GC. Neuroepidemiology of amyotrophic lateral sclerosis: clues to aetiology and pathogenesis. J Neurol Neurosurg Psychiatry 1996;61:131–7.

[8] Seljeseth YM, Vollset SE, Tysnes OB. Increasing mortality from amyotrophic lateral sclerosis in Norway? Neurology 2000;55:1262–6.

[9] Worms PM. The epidemiology of motor neuron diseases: a review of recent studies. J Neurol Sci 2001;191:3–9.

[10] Day TG, Scott M, Perring R, Doyle P. Motor neuron disease mortality in Great Britain continues to rise: examination of mortality rates 1975–2004. Amyotroph Lateral Scler 2007;8:337–42.

[11] Kondo K, Tsubaki T. Changing mortality patterns of motor neuron disease in Japan. J Neurol Sci 1977;32:411–24.

[12] Neilson S, Robinson I, Kondo K. A new analysis of mortality from motor neurone disease in Japan, 1950–1990: rise and fall in the postwar years. J Neurol Sci 1993;117:46–53.

[13] Okamoto K, Kobashi G, Washio M, Sasaki S, Yokoyama T, Miyake Y, et al. Descriptive epidemiology of amyotrophic lateral sclerosis in Japan, 1995–2001. J Epidemiol 2005;15:20–3.

[14] Kusui K. Epidemiological study on amyotrophic lateral sclerosis (ALS) and other neighboring motor neuron diseases in Kii Peninsula. Psychiatr Neurol Jpn 1962;64:85–99 (in Japanese).

[15] Wiederholt WC. Neuroepidemiologic research initiatives on Guam: past and present. Neuroepidemiology 1999;18:279–91.

[16] 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.

[17] 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.

[18] 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.
[19] Research Committee on Neurodegenerative Diseases of Intractable Diseases, the Ministry of Health, Labour and Welfare, Japan. Amyotrophic lateral sclerosis. In: Disease Prevention Study Group, editor. Guidelines of diagnoses and treatments for intractable diseases. 3rd ed. Tokyo: Tokyo Roppo Publishing; 2005. p. 80–9. (in Japanese).
[20] Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. Stat Med 2000;19:335–51.
[21] National Cancer Institute, U. S. National Institutes of Health. Statistical Research and Applications: Joinpoint Regression Program Version 3.4.2. Available from: http://srab.cancer.gov/joinpoint/.
[22] Lawson AB. Statistical methods in spatial epidemiology. 2nd ed. West Sussex: John Wiley & Sons; 2006.
[23] Tango T, Takahashi K. A flexibly shaped spatial scan statistic for detecting clusters. Int J Health Geogr 2005;4:11 Available from: http://www.ij-healthgeographics.com/content/4/1/11.
[24] Doi Y, Yokoyama T, Sakai M, Nakamura Y, Tango T, Takahashi K. Spatial clusters of Creutzfeldt–Jakob disease mortality in Japan between 1995 and 2004. Neuroepidemiology 2008;30:222–8.
[25] Tango T. Statistical methods for disease clustering. In: Gail M, Krickeberg, Samet JM, Tsiatis A, Wong W, editors. New York: Springer; 2010.
[26] Department of Technology Assessment and Biostatistics, National Institute of Public Health, Japan. The Flexible Scan Statistics Version 3.0.5. Available from: http://www.niph.go.jp/soshiki/gijutsu/download/flexscan/index.html.
[27] Chiò A, Mora G, Calvo A, Mazzini L, Bottacchi E. PARALS. Epidemiology of ALS in Italy: a 10-year prospective population-based study. Neurology 2009;72:725–31.
[28] 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.
[29] Smyth A, Riedl M, Kimura R, Olick R, Siegler M. End of life decisions in amyotrophic lateral sclerosis: a cross-cultural perspective. J Neurol Sci 1997;152(Suppl 1):S93–6.
[30] Hayashi H. Ventilatory support: Japanese experience. J Neurol Sci 1997;152(Suppl 1):S97–S100.
[31] Borasio GD, Gelinas DF, Yanagisawa N. Mechanical ventilation in amyotrophic lateral sclerosis: a cross-cultural perspective. J Neurol 1998;245(Suppl 2):S7–S12.
[32] Sorenson EJ, Stalker AP, Kurland LT, Windebank AJ. Amyotrophic lateral sclerosis in Olmsted County, Minnesota, 1925 to 1998. Neurology 2002;59:280–2.
[33] Forbes RB, Colville S, Cran GW, Scottish Motor Neurone Disease Register. Unexpected decline in survival from amyotrophic lateral sclerosis/motor neuron disease. J Neurol Neurosurg Psychiatry 2004;75:1753–5.
[34] Logroscino G, Traynor BJ, Hardiman O, Chio' A, Couratier P, Mitchell JD, et al, EURALS. Descriptive epidemiology of amyotrophic lateral sclerosis: new evidence and unsolved issues. J Neurol Neurosurg Psychiatry 2008;79:6–11.
[35] OECD. OECD Health Data 2010; June 2010. Available from: http://www.oecd.org/document/16/0,3343,en_2649_34631_2085200_1_1_1_37407,00.html.
[36] 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 Mot Neuron Disord 2005;6:155–63.
[37] Statistics and Information Department, the Ministry of Health, Labour and Welfare, Japan. Survey of physicians, dentists and pharmacists; 2004. Available from: http://www.mhlw.go.jp/toukei/saikin/hw/ishi/04/index.html. (in Japanese).
[38] Simpson CL, Al-Chalabi A. Amyotrophic lateral sclerosis as a complex genetic disease. Biochim Biophys Acta 2006;1762:973–85.

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Is lesion of Exner's area linked to progressive agraphia in amyotrophic lateral sclerosis with dementia? An autopsy case report

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Abstract. Agraphia, as a neuropsychological symptom of ALS, especially ALS with dementia (ALS-D), has recently attracted more attention. However, the brain lesion responsible has not been identified. Here we present an autopsy case of ALS-D of a patient with obvious agraphia, without aphasia, that also presented cerebrospinal degeneration with TDP-43-pathology compatible with ALS-D. Of the pre-motor frontal lobe cortices, degeneration and immuno-histochemical pathology were most obvious in the caudal area of the left middle frontal gyrus, or Exner's area. Assuring this area plays a pivotal role in the kanji and kana formation used in writing the Japanese language, this case of ALS-D showed both agraphia and Exner's area stressed pathological lesions. It may thus be the first case to indicate an intimate relationship between the neuropsychological symptoms and an associated lesion for ALS-D.

Keywords: Amyotrophic lateral sclerosis with dementia (ALS-D), fronto-temporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP), progressive agraphia, Exner's area

1. Introduction

Amyotrophic lateral sclerosis with dementia (ALS-D) is a nosological condition presenting motor neuron disease (MND) and dementia. The clinical features of dementia in ALS-D are of the frontal lobe type, and ALS-D is located within a framework of fronto-temporal lobar degeneration (FTLD) [1]. Although analysis of language function in ALS-D is considered difficult to carry out, due to severe bulbar palsy, we previously reported that writing disorder may exist in the early stage [2]. Another report, in Japanese, also describes writing disorder in a patient with ALS-D [3]. Here we describe an autopsied case of ALS-D, presenting progressive agraphia without aphasia, and discuss its clinicopathological relationship.

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2. Case history

A 73 year-old Japanese woman, with a history of breast cancer, was admitted to our hospital with speech disorder and personality change. Her family history provided no clues as to onset of these changes. Six months before admission, she became disoriented about dates and resistant to correction, and after three months, while playing cards with her family, she began missing her turn and became argumentative about continuing. At about the same time, her speech began to be slurred and became difficult, and her activities were slow down.

On admission, she showed no abnormality except for evidence of the surgery for breast cancer. She was cooperative during the neurological examination and

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cognizant of time and place. Her answers to questions were cogent. She could name objects and repeat sentences correctly, although her speech was slurred, slow-pitched, monotonous, and difficult. The tongue showed no atrophy nor fasciculation. Her facial expressions were fairly normal. Although there was no amyotrophy in the extremities, muscle tone was spastic, especially in the lower extremities. Jaw jerk and tendon reflex were exaggerated. Pathological grasping reflex, Hoffman reflex and Trömner reflex were induced bilaterally. Planter reflex was flexor bilaterally. No cerebellar ataxia was observed. As is mentioned later, her writing errors were, however, of great interest. She was not conscious of her illness and scored 7/18 on the frontal assessment battery [4]. Hasegawa's dementia rating scale (revised) was 23/30, suggesting mild cognitive impairment (normal range is above 21/30) [5]. Routine laboratory data were all within normal limits including those for syphilis, anti-human T cell leukemia virus 1 antibody, thyroid function, vitamin B_{12} and folic acid. The cerebrospinal fluid examination was unremarkable. Needle electromyography performed on the tongue, diaphragm, and anterior tibial muscle revealed mild chronic denervation. Magnetic resonance images (MRI) of the head revealed atrophy of the anterior part of the temporal and frontal lobes, slightly predominant on the right. Single photon emission computed tomography (SPECT) images disclosed decreased uptake of tracer, bilaterally, in the frontal and temporal lobes. After discharge she was observed as an outpatient, but her mental condition and motor symptoms gradually deteriorated. About five months after discharge, gastrostomy was performed for severe dysphagia. At which time, she had tetraparesis and could not respond to the examiner's instructions. She was transferred to the nursing hospital. Tracheostomy was not performed, and artificial ventilation not administered at the request of her family. The total duration of her illness was 18 months and she died of sudden respiratory failure.

3. Analysis of language function

Japanese language uses two distinct writing systems: kana characters, composed of simple phonograms with unambiguous phonetic readings, and kanji characters, a system of several thousand morphograms or ideograms.

On admission, her speech showed severe dysarthria and little intonation, and sounds and syllables were inconsistent. She occasionally had telegraphic speech and paraphrasia during spontaneous speech. The results of the WAB, examined during the first two weeks of admission, revealed spontaneous speech was non-fluent due to severe dysarthria. Repetition was mildly impaired. Although she could repeat nine words (each word was composed of two to ten characters) and two short sentences (each sentence was composed of eight and nine characters) without an error, she made two errors in repeating a long sentence composed of 20 characters, i.e. "新しい甘酒を五本のひょうたんに入れなさい" (Put fresh sweat alcohol into five gourds, in English). Object naming was mildly impaired (18/20), however, she could name all objects given phonemic cues. Sentence completion and responses in conversation were slightly impaired (8/10 and 8/10, respectively). As shown in Yes/No questions (60/60) and auditory word recognition (59/60), comprehension was not impaired.

In contrast to the results described above, her writhing was severely impaired. Although writing speed was slow, her formation of written characters showed no distortion. She made five errors in writing the same sentence as used in repetition: "新しい甘酒を五本のひょうたんに入れなさい" (she made two errors in repetition). She made four errors during writing six kanji words (each word was composed of two kanji characters), two errors in six kana words (each word was composed of three or four kana characters), and thirteen errors in four sentences (each sentence was composed of nine or ten characters). Most of the errors were omission of kana characters and adjuncts, such as postpositional particles. For example: "山上１本立て居す" instead of "山の上に木が１本立って居ます". The sentence is written, as normal, using a mixture of kanji and kana, and an English translation is: "There is a tree on the mountain". "の" and "に" are both particles, written in kana letters. And "木が" is the subject of the sentence, composed of "木"("tree") and "が"(a postpositional particle). "ま" is one of the so-called "okurigana", or kana characters added after kanji to indicate inflection. Other types of errors were substitution or addition of letters. Her ability to copy both letters and sentences was unimpaired. She could copy the sentence "新しい甘酒を五本のひょうたんに入れなさい" without an error.

After about three months, during follow up at the outpatient clinic, perseverative errors and paragraphia emerged. Moreover, morphology of her written characters became slightly distorted.

When tube feeding was necessary, she was no longer able to hold writing implements.

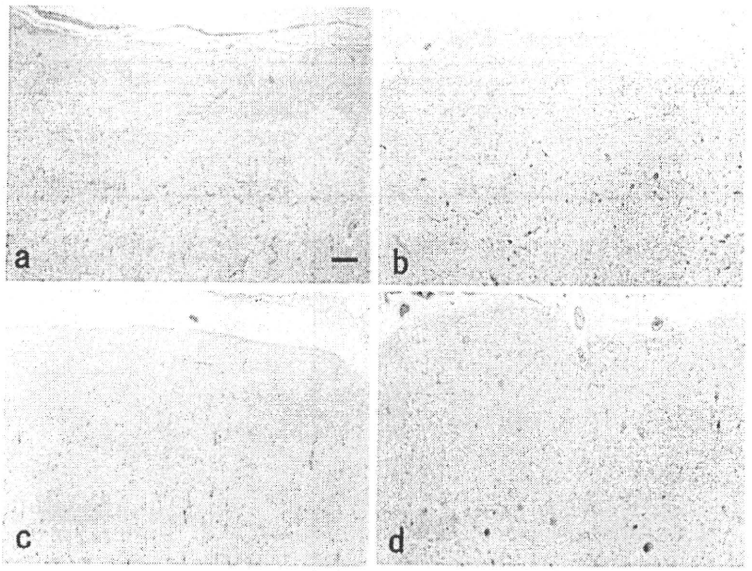

Fig. 1. Superficial spongiosis of the left frontal lobe cortices. a: superior frontal gyrus, b: middle frontal gyrus, c: inferior frontal gyrus, d: precentral gyrus. Severe spongiosis in the middle frontal (b) and precentral (d) gyri are shown. HE stain. Scale bar = 400 μm. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/BEN-2010-0276)

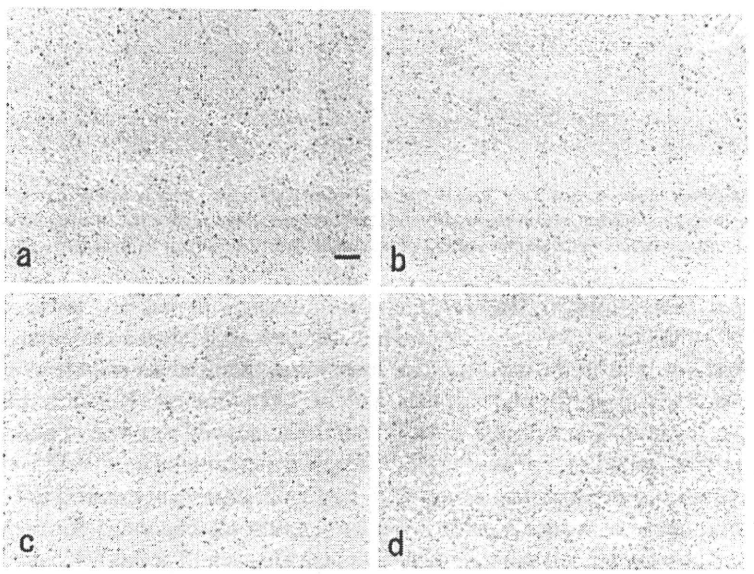

Fig. 2. Subcortical white matter of the left frontal lobe. a: superior frontal gyrus, b: middle frontal gyrus, c: inferior frontal gyrus, d: precentral gyrus. Numerous macrophages are observed in the middle frontal (b) and precentral (d) gyri. HE stain. Scale bar = 200 μm. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/BEN-2010-0276)

4. Neuropathological findings

General pathological examination revealed severe thinning of the diaphragma.

The brain weighed 1060 g prior to fixation. Macroscopic examination revealed circumscribed atrophy of the anterior temporal and frontal lobes bilaterally. Both lateral ventricles were slightly dilated. The substantia nigra was significantly depigmented.

Sections of paraffin-embedded tissue were stained with hematoxylin and eosin (H & E), Klüver-Barrera, and Bodian stains. In addition, immunohistochemistry for selected areas was performed using anti-ubiquitin (Dako; Japan; 1:100), anti-tau (AT8; Innunogenetics;

Table 1
Summary of lesion distribution

lesion (gyrus)	neuronal loss	superficial spongiosis	macrophage invasion	TDP pathology
superior frontal	mild	moderate	none	mild
middle frontal	mild	severe	severe	moderate
inferior frontal	mild	mild	none	mild
precentral	severe	severe	severe	moderate

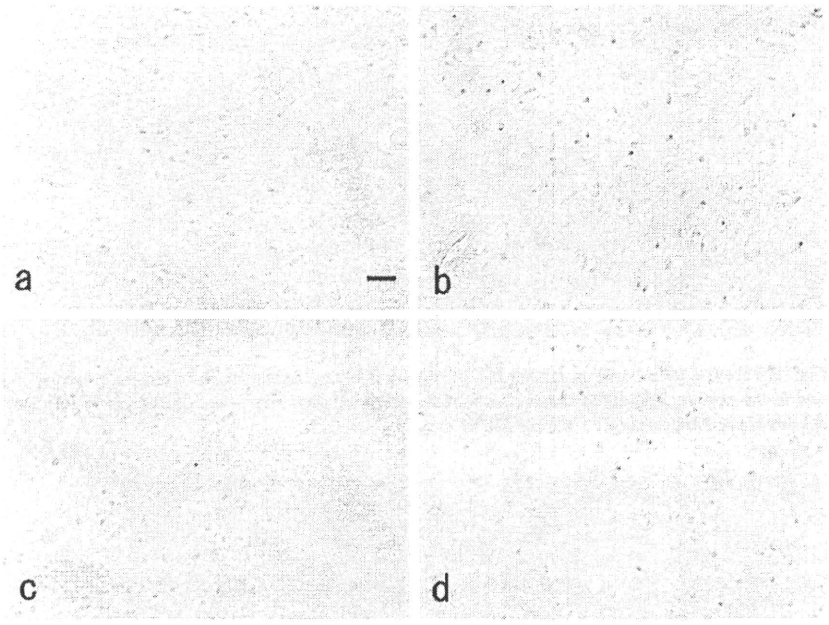

Fig. 3. Neuronal intra-cytoplasmic inclusions in the cortical neurons. a: superior frontal gyrus, b: middle frontal gyrus, c: inferior frontal gyrus, d: precentral gyrus. Numerous inclusions can be seen in the middle frontal (b) and precentral (d) gyri. Anti-phosphorylated-TDP-43-immunostain. Scale bar = 80 μm. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/BEN-2010-0276)

1:1000), and anti-phosphorylated-TDP-43 (p403/404, Cosmo Bio; California; 1:1000) antibodies.

Superficial spongiosis, neuronal loss, gliosis and rarefaction of the neuropil were observed in the frontal and temporal lobe cortices, especially in the precentral gyrus and posterior part of the middle frontal gyrus bilaterally (Fig. 1). Superficial spongiosis was also observed in the other frontal cortex, but to a lesser degree. Plenty of macrophages were observed in the subcortical white matter of these areas (Fig. 2). There were several clusters of macrophages in the deeper cortical layer of the precentral gyrus, indicating loss of Betz cells. Moderate neuronal loss was observed in the transitional area between CA1 and subiculum. Only a few neurofibrillary tangles were observed in the parahippocampal gyrus. No pathological change was observed in the left parietal and occipital lobes.

The pyramidal tracts showed almost complete axonal loss in the bilateral cerebral peduncles and pyramids. Neurons in the hypoglossal and ambiguous nuclei were preserved in number, however a few Bunina bodies were observed in the remaining neurons. The substantia nigra showed severe neuronal loss and gliosis with no Lewy bodies. The locus ceruleus showed mild neuronal loss and two Lewy bodies. The cerebellum was unremarkable. There were no neurofibrillary tangles in the brainstem or cerebellum.

In the spinal cord, moderate to severe loss of anterior horn cells and several Bunina bodies in the remaining neurons were observed. The pyramidal tracts showed almost complete axonal loss in the lateral columns. The posterior columns were unremarkable. Neurons in Onuf's nucleus were preserved in number.

Immuno-histochemistry showed ubiqutin and TDP-43-positive and tau-negative neuronal intracytoplasmic inclusions in the hipocampal dentate gyrus and frontal lobe cortex (Fig. 3). We detected these inclusions in the middle frontal and precentral gyri much more than in other frontal lobe cortices. Only a small number of TDP-43 positive dystrophic neurites were observed.

TDP-43 positive neuronal intranuclear inclusions and glial cells were not observed.

Summary of the pathological changes including immunohistochemistry are shown in Table 1.

5. Discussion

The presented case features a combination of progressive pseudobulbar palsy, frontal lobe type dementia, pyramidal tract signs and progressive amyotrophy. MRI revealed atrophy of the frontal and temporal lobes. Decreased blood flow in the frontal and temporal lobes was demonstrated by SPECT images. Pathological features of the case are: degeneration of both upper and lower motor neurons, degeneration of frontal and temporal lobes, Bunina bodies of the remaining lower motor neurons and TDP-43 pathology. These features are fully compatible with the diagnosis of ALS-D.

In progressive nonfluent aphasia, agraphia with effortful writing containing spelling error and agrammatism may be observed [6]. Agrammatism refers to omission or incorrect use of grammatical terms, including articles, prepositions, auxiliary verbs, inflexions, and derivations. From an initial stage, the presented patient showed progressive speech output disorder due to pseudobulbar palsy. Although telegraphic speech and occasional paraphasia were observed, frequency of such errors was quite less than that of writing errors. As shown in the WAB result, comprehension, naming and repetition were almost preserved when obvious agraphia was observed. Therefore, we believe the agraphia observed in our case did not derive from aphasia. Although perseverative errors were observed at a late stage, this can be attributed to frontal lobe dysfunction.

Omission of kana letters in Japanese and related syntactic errors in English have been reported in ALS with or without dementia [2,3,7,8]. The writing errors observed in our case concur well with previous descriptions. Although the lesion associated with pure agraphia is thought to be in the left frontal or parietal lobe [9], detailed clinicopathological analysis of ALS is unreported. A single autopsied case from the United States suggests that the left frontal or parietal lobe may be linked with the lesion responsible for syntactic errors in writing [7]. However, pathological findings related to agraphia were not described.

Our case reveals a left frontal lobe lesion with emphasis on the caudal part of the middle frontal gyrus, combined with immuno-histochemistry and other ALS-related indicators. Frontal and temporal lobe lesions including the hippocampal dentate gyrus have already been associated with dementia symptom in ALS-D [1]. A detailed description of each neuropsychological symptom and lesion remains to be elucidated, however.

The posterior part of the left middle frontal gyrus (Exner's area) is thought to play a pivotal role in writing letters and lesions there to cause agraphia [10,11]. Only a small number of cases of pure agraphia due to circumscribed lesions in Exner's area have been reported thus far [12–15]. Reports from Japan reveal phonological errors such as omission and paragraphia of kana letters associated with Exner's area lesion in cerebrovascular disease [15–17]. These errors concur well with the writing disorders observed in our case.

In the case described here, lesion distribution in the left frontal lobe was concentrated in Exner's area and no pathological change was observed in the left parietal lobe, which is another lesion associated with agraphia. We believe this is the first report that links clinicopathology in progressive agraphia without aphasia and detailed pathological examination including immunohistochemistry. Of course, further accumulation of clinicopathological examination focusing ALS-D and agraphia would be required to clarify our hypothesis.

Acknowledgements

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References

[1] I.R.A. Mackenzie, The neuropathology of FTD associated with ALS, *Alzheimer Dis Assoc Disord* 4 (2007), S44–S49.

[2] H. Ichikawa, S. Koyama, H. Ohno, K. Ishihara, K. Nagumo and M. Kawamura, Writing errors and anosognosia in amyotrophic lateral sclerosis with dementia, *Behav Neurol* **19** (2008), 107–116.

[3] M. Kanzaki, M. Sato, G. Ogawa, N. Miyamoto, K. Motoyoshi, K. Kamakura and K. Takeda, A case of dementia with motor neuron disease associated with agraphia – the omission of kana letters–, *Clin Neurol* **44** (2004), 673–676 (in Japanese with English abstract).

[4] B. Dubois, A. Slachevsky, I. Litvan and B. Pillon, The FAB: a Frontal Assessment Battery at bedside, *Neurology* **55** (2000), 1621–1626.

[5] T. Hosokawa, Y. Yamada, A. Isagoda and R. Nakamura, Psychometric equivalence of the Hasegawa Dementia Scale-Revised with the Mini-Mental State Examination in stroke patients, *Perceptual and Motor Skills* **79** (1994), 664–666.

[6] D. Neary, J.S. Snowden, L. Gustafson, U. Passant, D. Stuss, S. Black, M. Freedman, A. Kertesz, P.H. Robert, M. Albert, K. Boone, B.L. Miller, J. Cummings and D.F. Benson, Frontotemporal lobar degeneration. A consensus on clinical diagnostic criteria, *Neuroloy* **51** (1998), 1546–1554.

[7] J.H. Ferguson and F. Boller, A different form of agraphia: Syntactic writing errors in patients with motor speech and movement disorders, *Brain Lang* **4** (1977), 382–389.

[8] M. Satoh, K. Takeda and S. Kuzuhara, Agraphia in intellectually normal Japanese patients with ALS: omission of kana letters, *J Neurol* **256** (2009), 1455–1460.

[9] P. Marcie and H. Hécan, Agraphia: writing disorders associated with unilateral cortical lesions, in: *Clinical Neuropsychology*, K.N. Heilman and E. Valenstine, eds, Oxford University Press, New York and Oxford, 1979, pp. 92–127.

[10] S. Exner, *Unterschingen über die Localisation der Functionen in der Grösshirnrinde des Menschen*, W. Braumuller, Wien, 1881.

[11] J.M. Nielsen, *Agnosia, Apraxia, Aphasia. Their value in cerebral localization*, P.B. Hoeber, New York, 1946.

[12] H.C. Gordinier, A case of brain tumor at the base of the second left frontal convolution, *Am J Med Sci* **117** (1899), 526–535.

[13] G. Aimard, M. Devic, M. Lebel, P. Trouillas and D. Boisson, *Agraphie pure (dynamique?) d'origine frontale. A propos d'un observation*, *Rev Neurol* **131** (1975), 505–512.

[14] S.W. Anderson, A.R. Damasio and H. Damasio, Troubled letters but not numbers. Domain specific cognitive impairment following focal damage in frontal cortex, *Brain* **113** (1990), 749–766.

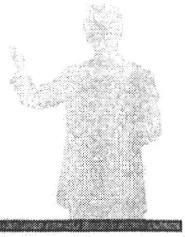
[15] K. Abe, R. Yokoyama, S. Yorifuji and T. Yanagihara, A jargon agraphia and selective agraphia of Kana resulting from an infarct in the left middle frontal gyrus, *Jpn J Neuropsychol* **9** (1993), 196–201 (in Japanese with English abstract).

[16] H. Tohgi, K. Saitoh, S. Takahashi, H. Takahashi, K. Utsugisawa, H. Yonezawa, K. Hatano and T. Sasaki, Agraphia and acalculia after a left prefrontal (F1, F2) infarction, *J Neurol Neurosurg Psychiatry* **58** (1995), 629–632.

[17] Y. Sakurai, K. Matsumura, T. Iwatsubo and T. Momose, Frontal pure agraphia for kanji or kana: Dissociation between morphology and phonology, *Neurology* **49** (1997), 946–952.

パーキンソン病の遺伝子治療—実用化に向けて

〔65頁グラフ欄参照〕



内科懇話会

◆key word
パーキンソン病
遺伝子治療
AAVベクター
臨床研究

自治医科大学神経内科学教授
中野今治◉なかの いまはる

本日はパーキンソン病の遺伝子治療について、単純化してお話を進めたいと思います。まず、遺伝子を入れるためにウイルスベクターを使いますので、アデノ随伴ウイルス(adeno-associated virus：AAV)から簡単に話します。

次に遺伝子治療を理解するためのパーキンソン病の病態について触れ、遺伝子治療、前臨床研究—主にサルの話—それから臨床研究、最後に実際に我々がヒトで行った臨床研究第Ⅰ、Ⅱ相についてお話しします。

治療用ウイルスベクター

治療用ウイルスベクターは、概念的には**図1**(以下、図はグラフ欄参照)に示すように、ウイルス遺伝子をタンクの中の荷物に譬えますと、被っているカプシドを外してウイルス遺伝子を取り出すと、荷台が空になったトラックができます。つまり、ウイルスのDNAだけが取り外されて、本来の遺伝子はなくなってしまいます。

その代わり治療遺伝子を組み込み、このタンクローリーの荷物だけが入れ替わったDNAを作り、最後にまたカプシドを被せます。そうすると、核酸の入れ替わったウイルスができる、そういうものをウイルスベクターと呼んでいます。

AAVベクターには長所と短所があり、最大の利点は非病原性です。それから、ターゲットとしている神経は非分裂細胞ですが、それに遺伝子導入が可能です。また、いったん入れると薄まることがなく、長期間その効果が続きます。さらに重複感染が可能であり、複数の遺伝子を別々のベクターに搭載して導入することができるという利点があります。

欠点は、ベクターの大量作製法の開発が遅れていることで、実用化が足踏みしている状況です。また、挿入できる遺伝子が小さいため、治療遺伝子が限られてくることです。

パーキンソン病の治療で使うAAVベクター(**図2**)は、両端のITRの間にサイトメガロウイルスのプロモーターを組み込み、チロシン水酸化酵素(TH)、GTPシクロヒドラーゼ1(GCH)、実際にヒトに入れる芳香族L—アミ

ノ酸脱炭酸酵素（AADC）という酵素の三つの遺伝子をそれぞれ組み込んだ構造になっています。

パーキンソン病の病態

パーキンソン病には、振戦（震え）、筋強剛（筋肉が硬くなる）、寡動、立ち直り反射の障害の4大症候があります。進行すると、運動症状が薬の影響で変動するようになってきます。例えば薬が効いている間（オンステート）は非常によく動ける、ところが薬が切れてきます（オフステート）と、ほとんど動けなくなります。我々が目指しているのは、薬が切れて動けない状態の患者に対して、この遺伝子治療を行いたいということです。

パーキンソン病は黒質線条体系のドパミンニューロンが変性・脱落する疾患で、中脳に黒質があり、そこから黒質線条体系の尾状核、被殻にドパミン系の投射が行われています。黒質で作られたドパミンが線条体に投射して、ここでドパミンが放出されることで運動がスムーズに行われるという機構になっています。

ドパミンの合成系にはTH、GCH、AADCの三つの酵素が関与しています（図3）。本来我々が持っているTHの補酵素がテトラヒドロビオプテリンで、それを作るための律速酵素がGCHです。この二つでチロシンからL-DOPAができます。L-DOPAから脱炭酸でドパミンができて、放出されるという機構になっています。

一方、線条体固有のニューロンがあります。パーキンソン病では、黒質のニューロンが脱落しますので、ドパミンの放出が行われなくなります（図4）。線条体固有のニューロンは障害されない、健在であるということが、我々が考えているパーキンソン病の遺伝子治療のキーポイントです。

黒質線条体系と錐体路の関係

これからちょっと話が複雑になります。我々の運動は、最終的には錐体路系を通り手足を動かしていますが、一つは小脳からの投射系が一次運動野に達して調整をする。もう一つが黒質線条体系の錐体外路系という機構で運動を調整しています（図5）。

一次運動野の投射系皮質脊髄路に、まず視床からの興奮性の刺激が伝達されることが分かっています。そして、淡蒼球内節から視床の興奮性のニューロンを抑制する経路があり、さらにこれを抑制する経路、そして黒質からの投射系は直接路が一つあるのですが、直接路は興奮性の線維がD1レセプターを持っている抑制性のニューロンを興奮させます。これは抑制系ですので、それによって淡蒼球内節の興奮は抑えられ、視床のニューロンの抑制が外れて興奮し、正常な人ではこのように脊髄路が興奮し、動くことができるわけです。

もう一つの経路は間接路ですが、なかなか理解しにくいです。

抑制系の興奮経路はD2というレセプターを持ち、被殻のニューロンを抑制します。次の淡蒼球外節のニューロンが興奮し、視床下核というニューロンを抑制します。このニューロンは淡蒼球内節ニューロンを興奮させるのですけれども、視床下核ニューロンが抑制されますので淡蒼球内節ニューロンが興奮できなくて、やはり視床のニューロンは興奮した状態になって、運動が伝わるというメカニズムになっています。

ですから、直接路であっても間接路であっても、最終的にはBetz巨細胞、あるいは運動皮質のニューロンを興奮させて、我々の手足を動かしているという状態になっています。

パーキンソン病では黒質のニューロンが落ちますので、興奮系の投射も、抑制系の投射も落ちてきて（図6）、直接路系で説明しますと、これが興奮しなくなります。そうすると視床下核に抑制がかからなくなり、淡蒼球の抑制性のニューロンが興奮し、視床のニューロンを抑える。そのために運動野のニューロンが興奮できなくなって、動きが悪くなります。

間接路はなかなか複雑ですが、結局は、この視床下核は興奮しすぎて、淡蒼球内節の抑制系の神経を興奮させます。最終的には視床のニューロンが抑制されて、興奮性の刺激が運動野に伝わらなくなって動きが悪くなります。