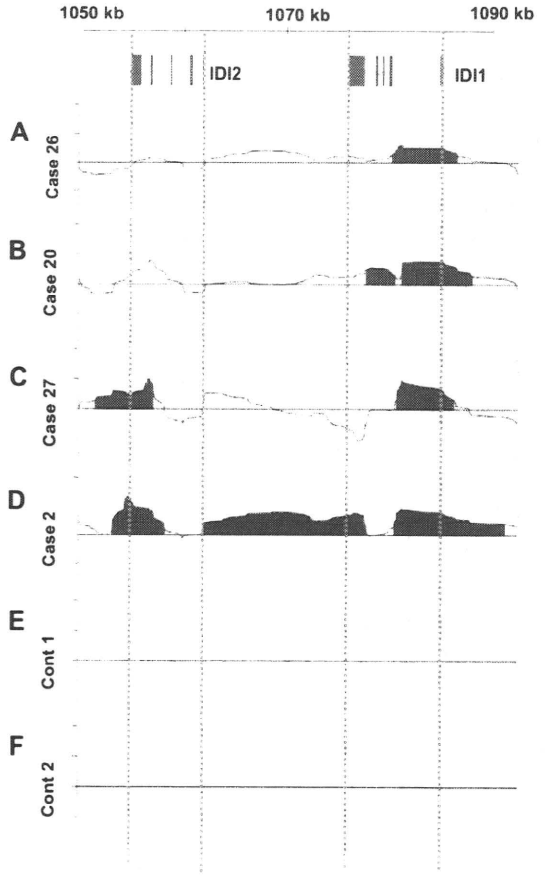


**Fig. 3.** Fine structure of copy-number gain in the IDI1/IDI2 gene region resolved by Agilent high-density custom tiling microarray. $Log_2$ ratio (*y*-axis) is plotted using moving average along the genome position (*x*-axis). Plots of four representative SALS patients are shown in (A)–(D) in parallel with plots of two control individuals (E and F). Copy-number gains are indicated as red areas. Red lines and blue lines represent copy-number plots of SALS patients and controls, respectively. Two grey lines indicate normal range of average $log_2$ ratios for probes among normal individuals.

losses and gains, in a complex multi-copy variable region by high-density tiling array were accessed by deviation of probe $log_2$ ratios that exceeded a given threshold of 1 SD from the mean probe ratio [21,22].

## 3. Results

### 3.1. Screening with whole-genome CNV beadchip

In search of a CNV associated with SALS, we conducted whole-genome screening by using the CNV beadchip for 11 SALS patients and 63 controls. Two CNV markers, one on chromosome 10p and the other on 2q, fulfilled our criteria: the association was statistically significant by Fisher's exact test and was accompanied by permutation or Bonferroni's correction, with a frequency of NA < 0.10. The former CNV marker, cnv1104p10, was located within the IDI1/IDI2 gene region on the 10p15.3 subtelomere. Among the measured samples, seven out of nine SALS patients displayed a copy-number gain at the CNV marker on the IDI1/IDI2 gene region, compared with 0 out of 59 control samples ($p = 3.70 \times 10^{-8}$ by Fisher's exact test, $p = 5.56 \times 10^{-6}$ by permutation test, $p < 0.05$ after Bonferroni's correction).

Fig. 1A shows the position of the CNV marker, cnv1104p10 (arrow), on the IDI1 gene. The region contains many complex repetitive structures surrounding the IDI1/IDI2 genes (Fig. 1B).

### 3.2. TaqMan qPCR

To validate the results of the CNV beadchip, qPCR analysis was done using larger numbers of SALS patients ($n = 83$) and healthy controls ($n = 100$). As shown in Fig. 2A, a copy-number gain at the site within the IDI1/IDI2 gene region was observed in 46 SALS patients, compared with 10 controls ($p = 4.86 \times 10^{-11}$, Odds Ratio 10.8, 95% Confidence Interval 4.75–26.64, Fisher's exact test) using Program Package R. The copy-number distribution was not significantly different between the two groups at a site 10 kb upstream to the region (Fig. 2B).

### 3.3. High-density tiling microarray analysis

We then analysed the extent of copy-number change along the 40-kb IDI1/IDI2 gene region with the high-density oligonucleotide tiling microarray in 32 SALS patients and 12 controls. A segmental copy-number gain was found in 19 of the SALS patients examined. Fig. 3 shows the detailed structure of segmental gains in four representative SALS patients and two controls along the 40-kb region (SALS, Fig. 3A–D; control, Fig. 3E and F). Fig. 4 shows the extent of the copy-number gain in the IDI1/IDI2 gene regions in all the 19 SALS patients with the gains. All the 19 SALS patients showed a segmental gain at the IDI1 gene, seven of whom at both the IDI1 and IDI2 genes.

## 4. Discussion

The previous genome-wide association studies of CNVs for SALS failed to detect common CNV locus associated with SALS [23–25]. The researchers used conventional genome-wide "SNP" genotyping platforms in which probes in repeat-rich regions were underrepresented: their HumanHap300 and HumanHap550 platforms covered only 25% and 40% of the CNVs, respectively, leading to the incapacity from capturing a large fraction of CNV [26]. Indeed, Cronin et al. noted the need to study CNVs located in regions not covered on the conventional SNP platforms [24]. We employed a "CNV-targeted platform" that places many CNV markers located in the CNV-rich regions, including the unstable regions, of the human genome. A segmental copy-number gain was found in many SALS patients in the IDI1/IDI2 gene region on 10p15.3 subtelomere. This region contains multiple low-copy repeats in a small region of the genome (Fig. 1B), which may account for the instability of this region and may trigger a segmental gain by an unequal crossing-over or end-joining event [27,28]. Such characteristics of the region may suggest possible involvement of the *de novo* occurrence of a segmental gain in SALS patients and have mechanical implications related to the sporadic nature of the disease.

IDI1 is an enzyme in the mevalonate pathway, which plays an essential role in the biosynthesis of cholesterol and many lipophilic molecules, including membrane-bound proteins [29]. In *Caenorhabditis elegans*, a homozygous mutation of the IDI1 gene resulted in paralysis during the first larval stage [30]. In mutant SOD1-transgenic rats, a model of ALS, cholesterol content in the myelin of the spinal cord decreased from the pre-symptomatic period [31]. Anderson et al. showed that dysfunctional cholesterol transport led to adult-onset motor neuron degeneration in male mice [32]. Zhai et al. suggested the significance of membrane-bound proteins in motor neuron degeneration in ALS [33]. Although the exact mechanism of alterations of lipid metabolism in ALS remains unknown, these findings suggest an involvement of cholesterol and lipid metabolisms in the pathogenesis of ALS.

In clinical settings, SALS patients with a high ratio of low-density to high-density lipoproteins survived longer than those

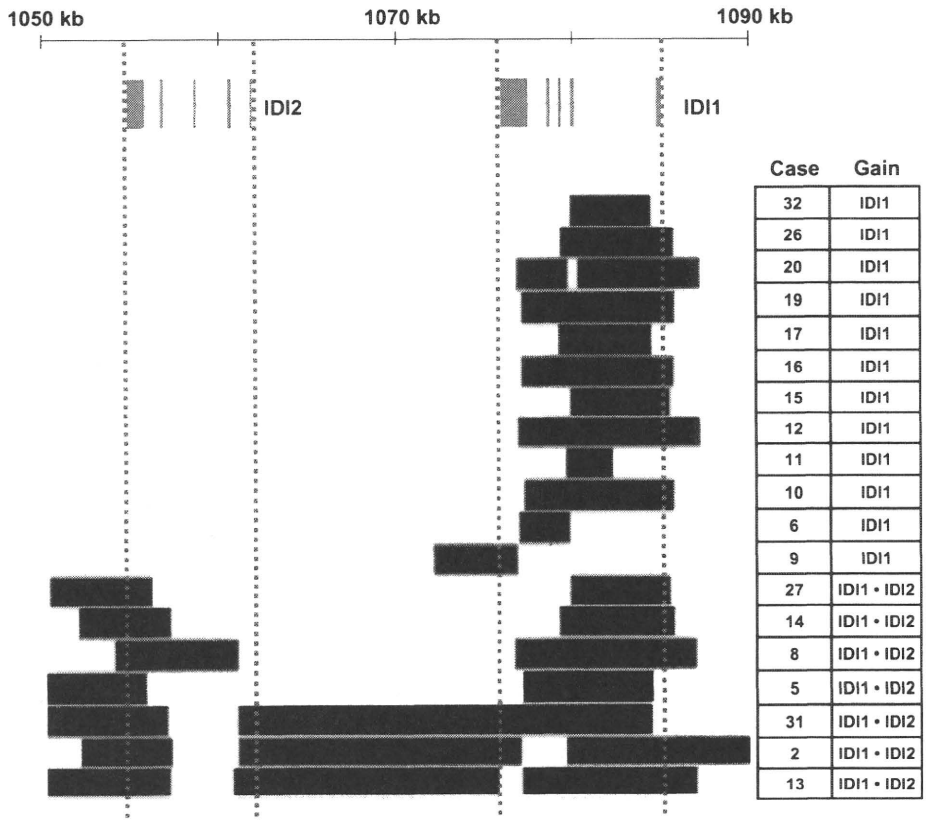


**Fig. 4.** The extent of copy-number gains (dark horizontal bars) in the IDI1/IDI2 gene region in 19 SALS patients (analysis by Agilent high-density custom tiling array). Case number and the presence of copy-number gain on IDI1 and/or IDI2 gene are indicated on right.

with a low ratio [34]. Moreover, statins, which inhibit HMG-CoA reductase, may cause SALS or SALS-like conditions [35]. HMG-CoA reductase is an enzyme in the mevalonate pathway, located upstream to IDI1. In a prospective study, SALS patients on statin medication showed an increase in the rate of neurological decline, as compared with those not on statin medication [36]. These data suggest that the mevalonate pathway may be involved in the pathogenesis of SALS. The present study showed a segmental copy-number gain in the IDI1/IDI2 gene region, which may cause a disruption of the genetic composition and result in a decreased expression of IDI1.

In conclusion, our study provides evidence for a segmental gain encompassing the IDI1/IDI2 gene region in many SALS patients. Further studies on the function of IDI1/IDI2 in motor neurons will contribute not only to the elucidation of the pathogenesis of SALS but also to the identification of a novel therapeutic target of the disease.

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## References

[1] C. Lagier-Tourenne, M. Polymenidou, D.W. Cleveland, TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration, Hum. Mol. Genet. 19 (2010) R46–R64.

[2] H. Maruyama, H. Morino, H. Ito, Y. Izumi, H. Kato, Y. Watanabe, Y. Kinoshita, M. Kamada, H. Nodera, H. Suzuki, O. Komure, S. Matsuur, K. Kobatake, N. Morimoto, K. Abe, N. Suzuki, M. Aoki, A. Kawata, T. Hirai, T. Kato, K. Ogasawara, A. Hirano, T. Takumi, H. Kusaka, K. Hagiwara, R. Kaji, H. Kawakami, Mutations of optineurin in amyotrophic lateral sclerosis, Nature 465 (2010) 223–226.

[3] A.J. Graham, A.M. Macdonald, C.H. Hawkes, British motor neuron disease twin study, J. Neurol. Neurosurg. Psychiatry 62 (1997) 562–569.

[4] M.A. van Es, P.W. van Vught, H.M. Blauw, L. Franke, C.G. Saris, P.M. Andersen, L. Van Den Bosch, S.W. de Jong, R. van't Slot, A. Birve, R. Lemmens, V. de Jong, F. Baas, H.J. Schelhaas, K. Sleegers, C. Van Broeckhoven, J.H. Wokke, C. Wijmenga, W. Robberecht, J.H. Veldink, R.A. Ophoff, L.H. van den Berg, ITPR2 as a susceptibility gene in sporadic amyotrophic lateral sclerosis: a genome-wide association study, Lancet Neurol. 6 (2007) 869–877.

[5] T. Dunckley, M.J. Huentelman, D.W. Craig, J.V. Pearson, S. Szelinger, K. Joshipura, R.F. Halperin, C. Stamper, K.R. Jensen, D. Letizia, S.E. Hesterlee, A. Pestronk, T. Levine, T. Bertorini, M.C. Graves, T. Mozaffar, C.E. Jackson, P. Bosch, A. McVe, A. Dick, R. Barohn, C. Lomen-Hoerth, J. Rosenfeld, D.T. O'connor, K. Zhang, R. Crook, H. Ryberg, M. Hutton, J. Katz, E.P. Simpson, H. Mitsumoto, R. Bowser, R.G. Miller, S.H. Appel, D.A. Stephan, Whole-genome analysis of sporadic amyo trophic lateral sclerosis, N. Engl. J. Med. 357 (2007) 775–788.

[6] M.A. van Es, P.W. van Vught, H.M. Blauw, L. Franke, C.G. Saris, L. Van den Bosch, S.W. de Jong, V. de Jong, F. Baas, R. van't Slot, R. Lemmens, H.J. Schelhaas, A. Birve, K. Sleegers, C. Van Broeckhoven, J.C. Schymick, B.J. Traynor, J.H. Wokke, C. Wijmenga, W. Robberecht, P.M. Andersen, J.H. Veldink, R.A. Ophoff, L.H. van den Berg, Genetic variation in DPP6 is associated with susceptibility to amyotrophic lateral sclerosis, Nat. Genet. 40 (2008) 29–31.

[7] S. Cronin, S. Berger, J. Ding, J.C. Schymick, N. Washecka, D.G. Hernandez, M.J. Greenway, D.G. Bradley, B.J. Traynor, O. Hardiman, A genome-wide association study of sporadic ALS in a homogenous Irish population, Hum. Mol. Genet. 17 (2008) 768–774.

[8] M.A. van Es, J.H. Veldink, C.G. Saris, H.M. Blauw, P.W. van Vught, A. Birve, R. Lemmens, H.J. Schelhaas, E.J. Groen, M.H. Huisman, A.J. van der Kooi, M. de Visser, C. Dahlberg, K. Estrada, F. Rivadeneira, A. Hofman, M.J. Zwarts, P.T. van Doormaal, D. Rujescu, E. Strengman, I. Giegling, P. Muglia, B. Tomik, A. Slowik, A.G. Uitterlinden, C. Hendrich, S. Waibel, T. Meyer, A.C. Ludolph, J.D. Glass, S. Purcell, S. Cichon, M.M. Nöthen, H.E. Wichmann, S. Schreiber, S.H. Vermeulen, L.A. Kiemeney, J.H. Wokke, S. Cronin, R.L. McLaughlin, O. Hardiman, K. Fumoto, R.J. Pasterkamp, V. Meininger, J. Melki, P.N. Leigh, C.E. Shaw, J.E. Landers, A. Al-Chalabi, R.H. Brown Jr., W. Robberecht, P.M. Andersen, R.A. Ophoff, L.H. van den Berg, Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis, Nat. Genet. 41 (2009) -1087.

[9] J.C. Schymick, S.W. Scholz, H.C. Fung, A. Britton, S. Arepalli, J.R. Gibbs, F. Lombardo, M. Matarin, D. Kasperaviciute, D.G. Hernandez, C. Crews, L. Bruijn, J. Rothstein, G. Mora, G. Restagno, A. Chiò, A. Singleton, J. Hardy, B.J. Traynor, Genome-wide genotyping in amyotrophic lateral sclerosis and neurologically normal controls: first stage analysis and public release of data, Lancet Neurol. 6 (2007) 322–328.
[10] S. Cronin, B. Tomik, D.G. Bradley, A. Slowik, O. Hardiman, Screening for replication of genome-wide SNP associations in sporadic ALS, Eur. J. Hum. Genet. 17 (2009) 213–218.
[11] A. Chiò, J.C. Schymick, G. Restagno, S.W. Scholz, F. Lombardo, S.L. Lai, G. Mora, H.C. Fung, A. Britton, S. Arepalli, J.R. Gibbs, M. Nalls, S. Berger, L.C. Kwee, E.Z. Oddone, J. Ding, C. Crews, I. Rafferty, N. Washecka, D. Hernandez, L. Ferrucci, S. Bandinelli, J. Guralnik, F. Macciardi, F. Torri, S. Lupoli, S.J. Chanock, G. Thomas, D.J. Hunter, C. Gieger, H.E. Wichmann, A. Calvo, R. Mutani, S. Battistini, F. Giannini, C. Caponnetto, G.L. Mancardi, V. La Bella, F. Valentino, M.R. Monsurrò, G. Tedeschi, K. Marinou, M. Sabatelli, A. Conte, J. Mandrioli, P. Sola, F. Salvi, I. Bartolomei, G. Siciliano, C. Carlesi, R.W. Orrell, K. Talbot, Z. Simmons, J. Connor, E.P. Pioro, T. Dunkley, D.A. Stephan, D. Kasperaviciute, E.M. Fisher, S. Jabonka, M. Sendtner, M. Beck, L. Bruijn, J. Rothstein, S. Schmidt, A. Singleton, J. Hardy, B.J. Traynor, A two-stage genome-wide association study of sporadic amyotrophic lateral sclerosis, Hum. Mol. Genet. 18 (2009) 1524–1532.
[12] R. Fernández-Santiago, M. Sharma, D. Berg, T. Illig, J. Anneser, T. Meyer, A. Ludolph, T. Gasser, No evidence of association of FLJ10986 and ITPR2 with ALS in a large German cohort, Neurobiol. Aging (2009), May 21 (Epub ahead of print).
[13] R. Redon, S. Ishikawa, K.R. Fitch, L. Feuk, G.H. Perry, T.D. Andrews, H. Fiegler, M.H. Shapero, A.R. Carson, W. Chen, E.K. Cho, S. Dallaire, J.L. Freeman, J.R. González, M. Gratacòs, J. Huang, D. Kalaitzopoulos, D. Komura, J.R. MacDonald, C.R. Marshall, R. Mei, L. Montgomery, K. Nishimura, K. Okamura, F. Shen, M.J. Somerville, J. Tchinda, A. Valsesia, C. Woodwark, F. Yang, J. Zhang, T. Zerjal, J. Zhang, L. Armengol, D.F. Conrad, X. Estivill, C. Tyler-Smith, N.P. Carter, H. Aburatani, C. Lee, K.W. Jones, S.W. Scherer, M.E. Hurles, Global variation in copy number in the human genome, Nature 444 (2006) 444–454.
[14] J.R. Lupski, Genomic rearrangements and sporadic disease, Nat. Genet. 39 (2007) S43–S47.
[15] B.R. Brooks, El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis, J. Neurol. Sci. 124 (Suppl.) (1994) 96–107.
[16] H. Nagasawa, M. Wada, S. Arawaka, T. Kawanami, K. Kurita, M. Daimon, M. Adachi, T. Hosoya, M. Emi, M. Muramatsu, T. Kato, A polymorphism of the aldehyde dehydrogenase 2 gene is a risk factor for multiple lacunar infarcts in Japanese men: the Takahata Study, Eur. J. Neurol. 14 (2007) 428–434.
[17] C. Iseki, T. Kawanami, H. Nagasawa, M. Wada, S. Koyama, K. Kikuchi, S. Arawaka, K. Kurita, M. Daimon, E. Mori, T. Kato, Asymptomatic ventriculomegaly with features of idiopathic normal pressure hydrocephalus on MRI (AVIM) in the elderly: a prospective study in a Japanese population, J. Neurol. Sci. 277 (2009) 54–57.
[18] H. Stefansson, D. Rujescu, S. Cichon, O.P. Pietiläinen, A. Ingason, S. Steinberg, R. Fossdal, E. Sigurdsson, T. Sigmundsson, J.E. Buizer-Voskamp, T. Hansen, K.D. Jakobsen, P. Muglia, C. Francks, P.M. Matthews, A. Gylfason, B.V. Halldorsson, D. Gudbjartsson, T.E. Thorgeirsson, A. Sigurdsson, A. Jonasdottir, A. Jonasdottir, A. Bjornsson, S. Mattiasdottir, T. Blondal, M. Haraldsson, B.B. Magnusdottir, I. Giegling, H.J. Möller, A. Hartmann, K.V. Shianna, D. Ge, A.C. Need, C. Crombie, G. Fraser, N. Walker, J. Lonnqvist, J. Suvisaari, A. Tuulio-Henriksson, T. Paunio, T. Toulopoulou, E. Bramon, M. Di Forti, R. Murray, M. Ruggeri, E. Vassos, S. Tosato, M. Walshe, T. Li, C. Vasilescu, T.W. Mühleisen, A.G. Wang, H. Ullum, S. Djurovic, I. Melle, J. Olesen, L.A. Kiemeney, B. Franke, C. Sabatti, N.B. Freimer, J.R. Gulcher, U. Thorsteinsdottir, A. Kong, O.A. Andreassen, R.A. Ophoff, A. Georgi, M. Rietschel, T. Werge, H. Petursson, D.B. Goldstein, M.M. Nöthen, L. Peltonen, D.A. Collier, D. St Clair, K. Stefansson, Large recurrent microdeletions associated with schizophrenia, Nature 455 (2008) 232–236.
[19] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method, Methods 25 (2001) 402–408.
[20] A.J. de Smith, A. Tsalenko, N. Sampas, A. Scheffer, N.A. Yamada, P. Tsang, A. Ben-Dor, Z. Yakhini, R.J. Ellis, L. Bruhn, S. Laderman, P. Froguel, A.I. Blakemore, Array CGH analysis of copy number variation identifies 1284 new genes variant in healthy white males: implications for association studies of complex diseases, Hum. Mol. Genet. 16 (2007) 2783–2794.
[21] A.J. Sharp, S. Hansen, R.R. Selzer, Z. Cheng, R. Regan, J.A. Hurst, H. Stewart, S.M. Price, E. Blair, R.C. Hennekam, C.A. Fitzpatrick, R. Segraves, T.A. Richmond, C. Guiver, D.G. Albertson, D. Pinkel, P.S. Eis, S. Schwartz, S.J. Knight, E.E. Eichler, Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome, Nat. Genet. 38 (2006) 1038–1042.
[22] J.R. Lupski, Genomic disorders ten years on, Genome Med. 1 (2009) 42.
[23] H.M. Blauw, J.H. Veldink, M.A. van Es, P.W. van Vught, C.G. Saris, B. van der Zwaag, L. Franke, J.P. Burbach, J.H. Wokke, R.A. Ophoff, L.H. van den Berg, Copy-number variation in sporadic amyotrophic lateral sclerosis: a genome-wide screen, Lancet Neurol. 7 (2008) 319–326.
[24] S. Cronin, H.M. Blauw, J.H. Veldink, M.A. van Es, R.A. Ophoff, D.G. Bradley, L.H. van den Berg, O. Hardiman, Analysis of genome-wide copy number variation in Irish and Dutch ALS populations, Hum. Mol. Genet. 17 (2008) 3392–3398.
[25] L.V. Wain, I. Pedroso, J.E. Landers, G. Breen, C.E. Shaw, P.N. Leigh, R.H. Brown, M.D. Tobin, A. Al-Chalabi, The role of copy number variation in susceptibility to amyotrophic lateral sclerosis: genome-wide association study and comparison with published loci, PLoS ONE 4 (2009) e8175.
[26] G.M. Cooper, T. Zerr, J.M. Kidd, E.E. Eichler, D.A. Nickerson, Systematic assessment of copy number variant detection via genome-wide SNP genotyping, Nat. Genet. 40 (2008) 1199–1203.
[27] A. Itsara, G.M. Cooper, C. Baker, S. Girirajan, J. Li, D. Absher, R.M. Krauss, R.M. Myers, P.M. Ridker, D.I. Chasman, H. Mefford, P. Ying, D.A. Nickerson, E.E. Eichler, Population analysis of large copy number variants and hotspots of human genetic disease, Am. J. Hum. Genet. 84 (2009) 148–161.
[28] A.J. Sharp, D.P. Locke, S.D. McGrath, Z. Cheng, J.A. Bailey, R.U. Vallente, L.M. Pertz, R.A. Clark, S. Schwartz, R. Segraves, V.V. Oseroff, D.G. Albertson, D. Pinkel, E.E. Eichler, Segmental duplications and copy-number variation in the human genome, Am. J. Hum. Genet. 77 (2005) 78–88.
[29] A.C. Ramos-Valdivia, R. van der Heijden, R. Verpoorte, Isopentenyl diphosphate isomerase: a core enzyme in isoprenoid biosynthesis. A review of its biochemistry and function, Nat. Prod. Rep. 14 (1997) 591–603.
[30] J. Yochem, D.H. Hall, L.R. Bell, E.M. Hedgecock, R.K. Herman, Isopentenyl-diphosphate isomerase is essential for viability of *Caenorhabditis elegans*, Mol. Genet. Genomics 273 (2005) 158–166.
[31] I. Niebroj-Dobosz, J. Rafałowska, A. Fidziańska, R. Gadamski, P. Grieb, Myelin composition of spinal cord in a model of amyotrophic lateral sclerosis (ALS) in SOD1G93A transgenic rats, Folia Neuropathol. 45 (2007) 236–241.
[32] S. Anderson, N. Gustafsson, M. Warner, J.A. Gustafsson, Inactivation of liver X receptor beta leads to adult-onset motor neuron degeneration in male mice, Proc. Natl. Acad. Sci. USA 102 (2005) 3857–3862.
[33] J. Zhai, A.-L. Ström, R. Kilty, P. Venkatakrishnan, J. White, W.V. Everson, E.J. Smart, H. Zhu, Proteomic characterization of lipid raft proteins in amyotrophic lateral sclerosis mouse spinal cord, FEBS J. 276 (2009) 3308–3323.
[34] L. Dupuis, P. Corcia, A. Fergani, J.-L. Gonzalez De Aguilar, D. Bonnefont-Rousselot, R. Bittar, Dyslipidemia is a protective factor in amyotrophic lateral sclerosis, Neurology 70 (2008) 1004–1009.
[35] B.A. Golomb, E.K. Kwon, S. Koperski, M.A. Evans, Amyotrophic lateral sclerosis-like conditions in possible association with cholesterol-lowering drugs: an analysis of patient reports to the University of California, San Diego (UCSD) Statin Effects Study, Drug Saf. 32 (2009) 649–661.
[36] L. Zinman, R. Sadeghi, M. Gawel, D. Patton, A. Kiss, Are statin medications safe in patients with ALS? Amyotroph. Lateral Scler. 9 (2008) 223–228.

# 孤発性ALSのゲノム解析

Genomic studies of sporadic ALS

加藤丈夫
Takeo KATO
山形大学医学部第三内科

◎筋萎縮性側索硬化症(ALS)全体の5～10%を占める家族性ALSの研究は近年，大きく進歩した．一方，ALSの90～95%を占める孤発性ALSの病因・病態は現在でも不明であるが，双生児研究により孤発性ALSの発症に遺伝的要因が関与していることが示された．そこで，孤発性ALSの発症に寄与する疾患感受性遺伝子(リスク遺伝子)を発見するため，種々の研究手法を用いて精力的に研究がなされた．しかし，研究間で結果が異なり，今後に大きな課題を残している．

Key word：一塩基多型，コピー数多型，染色体再構成，多因子疾患，ゲノムワイド解析

## 多因子疾患としての孤発性ALS

通常，病気は循環器疾患，呼吸器疾患，消化器疾患，神経疾患……など，臓器・系統別に分類されることが多い．一方，遺伝子の観点から分類すると“単一遺伝子病”と“多因子疾患(多因子遺伝病)”の2つに分類される．単一遺伝子病は1つの遺伝子の異常により発症し，Mendelの法則に則り遺伝する病気である．筋萎縮性側索硬化症(ALS)全体の5～10%を占める家族性ALSは単一遺伝子病であり，これまでいくつかの病因遺伝子(SOD1，alsin，TDP-43など)が知られており，最近，家族性ALSの新たな病因遺伝子としてoptineurinが同定された[1]．

一方，ALSの90～95%を占める孤発性ALSの病因・病態は現在でも不明であるが，双生児研究(British motor neuron disease twin study，下記)により，孤発性ALSの発症に遺伝的要因が関与していることが示唆された．したがって，孤発性ALSは多因子疾患と考えられ，その発症には“疾患感受性(病気のなりやすさ・なりにくさ)を高める複数の遺伝子(リスク遺伝子)”と“環境要因や生活習慣”が関与する可能性が指摘されている．

## 孤発性ALS発症に遺伝的要因が関与
### ――イギリスの双生児研究

Grahamら(1997)[2]は，孤発性ALSに罹患した77組の双生児(一卵性双生児26組，二卵性双生児51組)を調査・解析した．その結果，孤発性ALSの遺伝率(heritability)は0.38～0.85であると算出した(遺伝率が1とは100%遺伝子により規定されていることを意味し，ABO式血液型などがその一例である．一方，遺伝率が0とは遺伝子がまったく関与していないことを意味し，偶発的な事故による外傷などはその例である)．Grahamらの結果は，孤発性ALSであっても，その発症に遺伝的要因(遺伝子)が寄与していることを示している．さらに，彼らは環境因子についても検索し，工業用化学物質(industrial chemicals)，とくに石油化学物質(petrochemicals)や塗料(paints)の成分への曝露が孤発性ALSの有意な危険因子であると報告した．

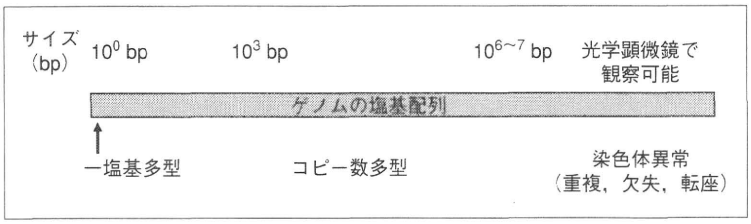


**図 1　ヒトゲノムの微小な変化と粗大な変化**

一塩基多型(SNP)は塩基配列の個人差の最小単位であり，特定の部位のひとつの塩基が個人間で異なる遺伝子多型である．一方，ゲノムのもっとも粗大な変化は染色体異常(重複，欠失，転座)であり，この変化は光学顕微鏡でみることができる．これらの中間に位置するのがコピー数多型(CNV)である．これは 1 kb 以上の塩基配列が欠失したり重複・挿入したりする変化であり，なかには数 Mb(数百万塩基)の塩基配列に及ぶものもある．

## ヒトゲノムの微小な変化と粗大な変化(図 1)

個人間でゲノムの塩基配列には約 0.1%の違いがあるとされている．この違いが形質(身長の大小や，顔の特徴の違いなど)や疾患感受性などの個体差を生む要因になっていると考えられている．したがって，医学・医療の分野でどのようなゲノムの違い(ゲノムの個体差)が個人の疾患感受性に影響するかを明らかにすることは，テーラーメイド医療(個別化医療)の確立やゲノム創薬にとって重要である．

ゲノムのもっとも粗大な変化には染色体の“重複”“欠失”“転座”の 3 種類があり，この変化は光学顕微鏡でみることができる．出生時より染色体が“重複”する変化としては 21 トリソミーの Down 症候群などがあり，染色体が“欠失”する例としては Williams 症候群などがある．“転座”する変化は染色体再構成(chromosomal rearrangements)ともよばれ，代表的な例として癌や主に後天的に肉腫や白血病で起こり，フィラデルフィア染色体がその代表的な例である．孤発性 ALS でも健常人に比べて高頻度に染色体再構成が起こっているとの報告があり，これについては後で述べる．

このようなゲノムの粗大な変化の対極に位置するのが一塩基多型(single nucleotide polymorphism：SNP)である．これは塩基配列の個人差の最小単位であり，特定の部位の 1 つの塩基が個人間で異なる遺伝子多型である．SNP はヒトゲノム中に 300 万～1,000 万存在すると見積もられており，もっとも多い遺伝子多型である．ほとんどの SNP はイントロンに存在し，遺伝子の機能に明らかな影響はないとされている．しかし，SNP が遺伝子のエクソン中に存在し，コードされる蛋白質のアミノ酸置換を伴うようであれば，蛋白質の機能に変化をもたらす可能性があり，さらには個体の形質や疾患感受性にも影響する可能性がある．エクソン以外の部位でも遺伝子の転写活性に影響するような部位に存在する SNP(たとえば，プロモーター領域の SNP や，イントロンの転写因子結合部位の SNP など)であれば，コードされる蛋白質の発現量に変化が起こる可能性があり，個体の形質や疾患感受性にも影響する可能性がある[3]．疾患感受性遺伝子を同定するため，これまで多くの疾患を対象にゲノムワイド SNP 解析がなされてきた．孤発性 ALS についてもゲノムワイド SNP 解析の報告がある(後述)．

上述の SNP(微小な変化)と染色体再構成(粗大な変化)の中間に位置するのがコピー数多型(copy number variation：CNV)である．これは 1 kb 以上の塩基配列が欠失したり重複・挿入したりする変化であり，なかには数 Mb(数百万塩基)の塩基配列に及ぶものもある．最近の研究によりヒトゲノム中には予想以上に CNV が多いことが明らかとなり，CNV 領域はゲノムの 1 割以上にもあたる 3 億 6,000 万塩基にも及ぶとの見積もある．CNV 領域に存在する遺伝子にはコピー数の変化が起こるので，コードされている蛋白質の発現量にも変化が起こる．その結果，個体の形質や疾患感受性に影響する可能性がある．CNV が孤発性疾患のリスクになる例として乾癬[4]，Crohn 病[5]，全身性エリテマトーデス[6]，ヒト免疫不全症候群[7]

表 1　孤発性 ALS のゲノムワイド SNP 関連解析

| Authors(year) | case | control | gene | OR | $p$-value | function of gene products/comments | ref. |
|---|---|---|---|---|---|---|---|
| Schymick et al.(2007) | 276 | 271 | — | — | — | nothing significant after Bonferroni correction | 11) |
| Dunckley et al.(2007) | 1,287 | 1,567 | FLJ10986 | 1.35 | $3.0\times10^{-4}$ | uncharacterized gene | 12) |
| van Es et al.(2007) | 1,337 | 1,356 | ITPR2 | 1.58 | $3.28\times10^{-6}$ | glutamate-mediated neurotransmission | 13) |
| van Es et al.(2008) | 1,767 | 1,916 | DPP6 | 1.3 | $5.04\times10^{-8}$ | type A neuronal transmembrane potassium channels | 16) |
| Cronin et al.(2008) | 958 | 932 | DPP6 | 1.37 | $2.53\times10^{-6}$ | type A neuronal transmembrane potassium channels | 17) |
| van Es et al.(2009) | 2,323 | 9,013 | UNC13A | 1.25 | $1.30\times10^{-9}$ | regulation of neurotransmitter release | 18) |
| Cronin et al.(2009) | 1,267 | 1,336 | — | — | — | nothing significant after Bonferroni correction | 19) |
| Chio et al.(2009) | 2,713 | 5,346 | — | — | — | nothing significant after Bonferroni correction | 15) |

などが報告されている．孤発性 ALS についてもゲノムワイド CNV 解析の報告がある(後述)．

## 孤発性ALSと染色体再構成(chromosomal rearrangements)

Kaneko ら(1995)[8]は日本人孤発性 ALS 患者 23 例の染色体検査を行い，1 例(4.3%)に染色体異常(転座)を認めた．Meyer ら(2003)[9]は 85 例の孤発性 ALS 患者の染色体検査を行い，5 例(5.9%)に染色体再構成を認めた．一般人口健常人での染色体再構成の頻度は 0.05〜0.1%であるので[10]，孤発性 ALS 患者での染色体再構成の頻度は有意に高い結果であった(OR：40.6，95%CI：16.5〜100.3，$p<0.0001$)．このように，孤発性 ALS 患者ではゲノムの再構成が起こりやすいことが示唆されたが，染色体再構成が起こっている部位(locus)は症例ごとに異なるため，この異常により孤発性 ALS の病因(リスク遺伝子)を一元的に説明するのは困難と思われる．

## ゲノムワイドSNP解析

孤発性 ALS の疾患感受性遺伝子を探索するため，多数の孤発性 ALS 患者と健常人の DNA を用いてゲノムワイド SNP 関連解析が行われた．最初の報告は Schymick ら(2007)[11]によるもので，孤発性 ALS 患者 276 例と健常対照者 271 例の DNA を用いてゲノムワイドに 555,352 SNPs の関連解析が行われた．しかし，多重解析の補正(Bonferroni 補正)を行うと孤発性 ALS に有意に関連する SNP は見出せなかった．

Dunckley ら(2007)[12]は 3 つの独立した case(孤発性 ALS)/control シリーズ〔Discovery series (case/control=386/542)→Replication series 1(同 766/750)→Replication series 2(同 135/275)〕を用いて，3 段階の SNP 関連解析を行った．Discovery series ではゲノムワイドに 766,955 SNPs について関連解析を行い，$p<0.05$ を呈した SNPs は Replication series 1 の DNA サンプルを用いて再度解析を行った．そして，3 つの case/control series のすべてで $p<0.05$ を呈した SNPs は 10 個見出され，そのうちの 1 つ，FLJ10986 遺伝子(機能不明)は Bonferroni 補正を行っても孤発性 ALS に有意に関連していた．

van Es ら(2007)[13]も多数の孤発性 ALS 患者(計 1,337 例)と対照例(計 1,356 例)の DNA を用いて，3 段階〔stage 1(case/control=461/450)→stage 2(同 563/603)→stage 3(同 313/303)〕の SNP 関連解析を行った．その結果，ITPR2(inositol 1,4,5-triphosphate receptor 2)遺伝子上の SNP が Bonferroni 補正を行っても孤発性 ALS に有意に関連していると報告した(Bonferroni 補正後の $p$ 値=0.012)．

しかし，Fernández-Santiago ら(2009)[14]は，上記の FLJ10986 遺伝子と ITPR2 遺伝子上の SNPs が孤発性 ALS と関連しているかどうか，彼らの孤発性 ALS 患者と健常人の DNA を用いて検討したところ，いずれの SNP も統計学的に有意な関連は認められなかったと報告している．Chiò ら(2009)[15]はゲノムワイド SNP 関連解析(545,066 SNPs)を行い，これまでに報告のあった孤発性 ALS と有意に関連するとされる SNPs も含めて，いずれの SNPs も孤発性 ALS と有意な関連は認められなかったと報告している．

これまでに報告のあった孤発性 ALS のゲノムワイド SNP 関連解析のおもな研究を表 1 にまとめた[11-13,15-19]．この表からわかるように，これら

の研究間で結果が大きく異なっている．また，たとえ有意に関連するSNPが見出されてもオッズ比(OR)がきわめて低いことがあげられる．結果が不一致の理由としては，解析対象が異なること(人種・民族差など)や，false positiveの可能性などが考えられる．オッズ比が低いことに関しては，単独のSNPでは低いリスクであっても低リスクのSNPsが複数重なることで，より大きなリスクになる可能性などが考えられる．このように，孤発性ALSのゲノムワイドSNP関連解析は多くの関係者の努力と莫大な研究費を費やして行われてきたが，今日に至っても明確な結論は得られておらず，多くの課題と困難を将来に残している．

## ゲノムワイドCNV解析

上記のように，CNVは1kb以上の長さの塩基配列が欠失したり重複・挿入したりする変化である．このような，ゲノム上の大きな領域に変化が生じて発症する運動ニューロン疾患として若年発症脊髄性筋萎縮症(child-onset spinal muscular atrophy：SMA)が知られている．SMAは常染色体劣性の遺伝性疾患であり，第5染色体の5q13領域に存在する2つの似た遺伝子〔survival motor neuron(SMN)1遺伝子とSMN2遺伝子〕のうち，SMN1遺伝子の異常(多くは欠失・転座)が原因とされている．Veldinkら(2005)[20]は242例の孤発性ALS患者と175例の健常人のSMN1遺伝子のコピー数を検討し，SMN1遺伝子のコピー数の減少は孤発性ALSの有意なリスク(OR：4.1，95% CI：1.2～14.2，$p$=0.02)になると報告した．

孤発性ALSのゲノムワイドCNV関連解析については，Blauwら(2008)[21]，Croninら(2008)[22]およびWainら(2009)[23]が報告している．いずれの研究においても孤発性ALSの疾患感受性CNVの発見には至っていない．その理由のひとつとして，これら3つの研究ではSNP用DNAチップを用いてCNVを解析している点があげられる．一般に，SNPはゲノムの安定な領域に多く，CNVはゲノムの不安定な領域に多い．そして上記の論文の研究者も考察[22]しているように，彼らの用いたDNAチップはゲノムの不安定な領域に存在するCNVをカバーしていない．したがって今後，全ゲノムをカバーするようなCNVチップを用いて孤発性ALSのCNV関連解析を行う必要があると思われる．

## おわりに

近年の家族性ALS研究の著しい進歩に比べて孤発性ALSの疾患感受性遺伝子の研究はいまだスタートライン上にあるといっても過言ではない．この方面の今後の研究の発展を期待したい．

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### 文献

1) Maruyama, H. et al.：Mutations of optineurin in amyotrophic lateral sclerosis. *Nature*, **465**：223-227, 2010.
2) Graham, A. J. et al.：British motor neuron disease twin study. *J. Neurol. Neurosurg. Psychit.*, **62**：562-569, 1997.
3) Arawaka, S. et al.：The role of G-protein-coupled receptor kinase 5 in pathogenesis of sporadic Parkinson's disease. *J. Neurosci.*, **26**：9227-9238, 2006.
4) Hollox, E. J. et al.：Psoriasis is associated with increased beta-defensin genomic copy number. *Nat. Genet.*, **40**：23-25, 2008.
5) Fellermann, K. et al.：A chromosome 8 gene-cluster polymorphism with low human beta-defensin 2 gene copy number predisposes to Crohn disease of the colon. *Am. J. Hum. Genet.*, **79**：439-448, 2006.
6) Aitman, T. J. et al.：Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. *Nature*, **439**：851-855, 2006.
7) Gonzalez, E. et al.：The influence of CCL3L1 gene-containing segmental duplications on HIV-1 AIDS susceptibility. *Science*, **307**：1434-1440, 2005.
8) Kaneko, K. et al.：Cytogenetic analysis of 23 Japanese patients with amyotrophic lateral sclerosis. *Clin. Genet.*, **47**：158-160, 1995.
9) Meyer, T. et al.：High rate of constitutional chromosomal rearrangements in apparently sporadic ALS. *Neurology*, **60**：1348-1350, 2003.
10) Shaffer, L. G. and Lupski, J. R.：Molecular mechanisms for constitutional chromosomal rearrangements in humans. *Ann. Rev. Genet.*, **34**：297-329, 2000.
11) Schymick, J. C. et al.：Genome-wide genotyping in amyotrophic lateral sclerosis and neurologically normal controls：first stage analysis and public release of data. *Lancet Neurol.*, **6**：322-328, 2007.
12) Dunckley, T. et al.：Whole-genome analysis of sporadic amyotrophic lateral sclerosis. *N. Engl. J. Med.*, **357**：775-788, 2007.

13) van Es, M. A. et al. : ITPR2 as a susceptibility gene in sporadic amyotrophic lateral sclerosis : a genome-wide association study. *Lancet Neurol.*, **6** : 869-877, 2007.
14) Fernández-Santiago, R. et al. : No evidence of association of FLJ10986 and ITPR2 with ALS in a large German cohort. *Neurobiol. Aging*, 2009, May 21. [Epub ahead of print]
15) Chiò, A. et al. : A two-stage genome-wide association study of sporadic amyotrophic lateral sclerosis. *Hum. Mol. Genet.*, **18** : 1524-1532, 2009.
16) van Es, M. A. et al. : Genetic variation in DPP6 is associated with susceptibility to amyotrophic lateral sclerosis. *Nat. Genet.*, **40** : 29-31, 2008.
17) Cronin, S. et al. : A genome-wide association study of sporadic ALS in a homogenous Irish population. *Hum. Mol. Genet.*, **17** : 768-774, 2008.
18) van Es, M. A. et al. : Genome-wide association study identifies 19p13.3(UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nat. Genet.*, 2009, Sep.6. [Epub ahead of print]
19) Cronin, S. et al. : Screening for replication of genome-wide SNP associations in sporadic ALS. *Eur. J. Hum. Genet.*, **17** : 213-218, 2009.
20) Veldink, J. H. et al. : SMN genotypes producing less SMN protein increase susceptibility to and severity of sporadic ALS. *Neurology*, **65** : 820-825, 2005.
21) Blauw, H. M. et al. : Copy-number variation in sporadic amyotrophic lateral sclerosis : a genome-wide screen. *Lancet Neurol.*, **7** : 319-326, 2008.
22) Cronin, S. et al. : Analysis of genome-wide copy number variation in Irish and Dutch ALS populations. *Hum. Mol. Genet.*, **17** : 3392-3398, 2008.
23) Wain, L. V. et al. : The role of copy number variation in susceptibility to amyotrophic lateral sclerosis : genome-wide association study and comparison with published loci. *PLoS ONE*, **4** : e8175, 2009.

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ORIGINAL COMMUNICATION

# Progressive anterior operculum syndrome due to FTLD-TDP: a clinico-pathological investigation

**Mika Otsuki · Yoshitsugu Nakagawa · Fumiaki Mori · Hirotoshi Tobioka · Hideaki Yoshida · Yoshiharu Tatezawa · Toshio Tanigawa · Ikuko Takahashi · Ichiro Yabe · Hidenao Sasaki · Koichi Wakabayashi**

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**Abstract** Pathological investigation of progressive anterior operculum syndrome has rarely been reported. We describe clinico-pathological findings in a patient with progressive anterior operculum syndrome. A 74-year-old right-handed man had noticed speech and swallowing difficulties 1 year previously. Neurological examinations showed no abnormality other than a slight limitation of upward gaze and slow tongue movement without fibrillation. We investigated the patient using neuroimaging and neuropsychological examinations and observed him for 2 years until his death, at which point we obtained pathological findings. The patient's facial and masseteric muscles seemed hypotonic with drooling, but he could laugh and yawn normally, showing automatic voluntary dissociation. Palatal and pharyngeal reflexes were normal. Magnetic resonance imaging showed cortical atrophy in the temporal lobes bilaterally. $^{123}$IMP single photon emission computed tomography and positron emission tomography showed decreased blood flow and activity in the frontotemporal lobes, predominantly on the left side. Neuropsychological examinations showed no aphasia, dementia or other neuropsychological abnormality. Intubation fiberscopy, laryngoscopy and video fluorography showed no abnormality. After 6 months his anarthria and dysphagia became aggravated. He died of aspiration pneumonia 2 years after onset. Postmortem examination revealed neuronal degeneration with TDP-43-positive inclusions in the frontal, temporal and insular cortices,

M. Otsuki (✉)
School of Psychological Science,
Health Sciences University of Hokkaido, 1-2 Ainosato, Kita-ku,
Sapporo 002-8072, Japan
e-mail: miotsuki@hoku-iryo-u.ac.jp

Y. Nakagawa
School of Nursing and Social Services,
Health Sciences University of Hokkaido, 1757 Kanazawa,
Tobetsu Ishikari, Hokkaido 061-0293, Japan

F. Mori · K. Wakabayashi
Department of Neuropathology, Institute of Brain Science,
Hirosaki University, 5 Zaifu-cho, Hirosaki 036-8562, Japan

H. Tobioka
Department of Pathology, Otauru Kyokai Hospital,
1-6-15 Suminoe, Otaru 047-8510, Japan

H. Yoshida
Department of Surgery, Yoichi Kyokai Hospital,
85 Kurokawa-cho, Yoichi-cho, Yoichi-gun,
Hokkaido 046-0003, Japan

Y. Tatezawa
Department of Rehabilitation, Yoichi Kyokai Hospital,
85 Kurokawa-cho, Yoichi-cho, Yoichi-gun,
Hokkaido 046-0003, Japan

T. Tanigawa
Department of Internal Medicine, Yoichi Kyokai Hospital,
85 Kurokawa-cho, Yoichi-cho, Yoichi-gun,
Hokkaido 046-0003, Japan

I. Takahashi · I. Yabe · H. Sasaki
Department of Neurology, Hokkaido University,
N15, W7, Kita-ku, Sapporo 060-8638, Japan

consistent with frontotemporal lobar degeneration with TDP inclusions (FTLD-TDP). However, neuronal loss with gliosis was more prominent in the inferior part of the motor cortices, bilaterally. Progressive anterior operculum syndrome could be classified as a variant of FTLD-TDP.

**Keywords** Progressive anterior operculum syndrome · Foix–Chavany–Marie syndrome · FTLD · TDP-43

## Introduction

Anterior operculum syndrome or Foix–Chavany–Marie syndrome (FCMS), is characterized by anarthria and central voluntary paralysis of the lower cranial nerves with preserved automatic and emotional movement [7, 12]. Most of this syndrome is a result of cerebrovascular disease involving the anterior operculum, bilaterally or unilaterally [2, 3, 7, 11, 17, 19–21, 25, 26, 28, 29, 31]. However, it remains uncertain whether the causative lesion is the operculum proper or its vicinity, because other lesions are also suggested [13]. In addition to the reports of patients due to cerebrovascular disease, there are a few reports of patients with primary progressive pseudobulbar palsy showing both anarthria and dysphagia simultaneously [14, 22, 30], which can be referred to as anterior operculum syndrome or FCMS. Progressive pseudobulbar palsy has been reported as neurodegenerative disorders, including tauopathy, frontotemporal lobar degeneration (FTLD) with motor neuron disease, and dementia. However, there have been only a few detailed clinical descriptions or distinctive histologies [5, 6, 14, 15, 22, 23, 30, 32]. Histological information is necessary to clarify the causative lesion and etiology of primary progressive anterior operculum syndrome. Here, we can offer the information about a patient with primary progressive anterior operculum syndrome, in whom postmortem examination revealed marked neuronal loss with TDP-43-immunoreactive inclusions in the frontal lobes, particularly in the inferior part of the motor cortices, bilaterally. This is the first report of an autopsy-confirmed anterior operculum syndrome with FTLD and TDP-43-positive inclusions (FTLD-TDP) [18].

## Case presentation

A 74-year-old right-handed man consulted our hospital with a complaint of insidious onset of speech and swallowing impairment over the previous 9 months. There was no evidence of memory impairment from the history, because he could continue his drapery job without any difficulty. At the first consultation, his facial and masseteric muscles seemed hypotonic and he held his mouth half open with a tendency to drool. His voice was hypotonic and he could manifest only laborious, syllabic phonemes or short phrases with dysprosody, whose articulation was distorted and varied depending on the situation, demonstrating anarthria. He was unable to imitate oro-bucco-facio-linguo-pharyngeal movements, such as pretending to cough, blow out a match, stick out his tongue, and click his tongue voluntarily. The most striking characteristic was the persistent dissociation between automatic (spontaneous) and voluntary movements of facial expression. For example, although he could not move his facial muscles, mouth or tongue on command, he sometimes laughed clearly at jokes or smiled at acquaintances, and he sometimes swallowed his saliva. He could occasionally lick his lips, keep candy in his mouth and sometimes drink water.

### Neurological examination

He was alert, fully oriented and cooperative. He could not speak but could adequately answer every question in writing, with no difficulty in finding words or comprehending language. His cranial nerves were normal except for a slightly limited upward gaze, but he could spontaneously move his eyes in other natural situations. Motor and sensory systems were normal with normal muscle tones without muscle wasting or fasciculation and no pathological reflexes were elicited. Palatal reflex was attenuated and gag reflex was normal. His coordination, posture and gait were normal.

### Neuropsychological examination

The results of neuropsychological examination are summarized in Table 1. The western aphasia battery (WAB) showed a perfect score in comprehension and in confrontation naming which was expressed by writing, because of anarthria. Reading showed a somewhat low score, because of impaired reading aloud; however, there was no difficulty in reading comprehension. Writing was not flawless, because he showed morphological impairment and a tendency toward micrographia. He could draw simple geometrical figures such as triangles and cubes, and he also demonstrated a good score (SS score 11) in the block design task from WAIS-R, which showed that he did not have constructional impairment. All of the neuropsychological examinations indicated that he preserved general intelligence and language ability without aphasia, except for speech output.

### Magnetic resonance imaging of the brain

Magnetic resonance imaging (MRI) demonstrated marked cortical atrophy with multiple ischemic lesions in the

**Table 1** Neuropsychological examinations

| | 9 M | 15 M |
|---|---|---|
| Western aphasia battery | | |
| Comprehension | 10/10 | 10/10 |
| Confrontation naming | 20/20[a] | [e] |
| Reading | 8/10[b] | – |
| Writing | 8.4/10[c] | – |
| Raven's colored matrices | 29/36[d] | 2 /36 |
| The Wechsler adult intelligence scale-revised | | |
| Verbal IQ | 83[a] | 8.[a] |
| Performance IQ | 98 | – |
| Full IQ | 89 | – |

*M* months after onset, – not administered

[a] by writing due to severe anarthria

[b] lower scores due to inability of reading aloud, but reading comprehension was perfect

[c] writing showed morphological impairment and a tendency toward micrographia

[d] this is within the age-matched normal range

[e] it was difficult for him because of his deteriorated writing ability

corona radiate, medial temporal lobe and brainstem, bilaterally. The scattered ischemic lesions seemed not to directly affect his severe impairment of speech and swallowing (Fig. 1a).

## Further examinations and evolution

During the 6 months after the first consultation (15 months after onset), the patient's symptoms deteriorated and he was admitted for further examinations. His facial expression seemed hypotonic, with sustained drooling, and his writing was impaired with phonological and verbal paragraphia. His penmanship was distorted and showed micrographia; however, he was courteous and well oriented as ever.

Neurological examination revealed almost the same condition as seen 6 months previously, except for additional findings of exaggerated jaw jerk (masseteric reflex). Palmomental, bilateral orbicularis oculi, orbicularis oris and snout reflexes were also easily elicited, and there was rigidity in the upper and lower extremities.

## Laryngo-pharynx investigation

Intubation fiberscopy showed no abnormality. Laryngoscopy showed no vocal cord paralysis. Video fluorography revealed that the patient was unable to initiate swallowing, but reflex swallowing was preserved; therefore, once food was placed into the posterior pharynx, he could swallow it.

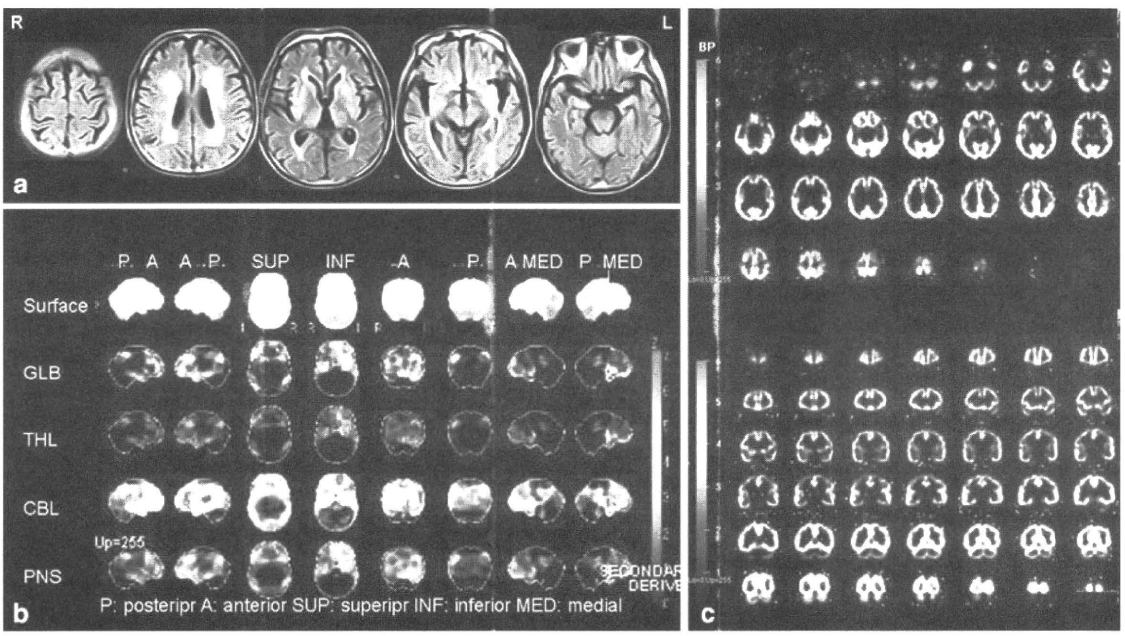


**Fig. 1** Neuroimaging. **a** Magnetic resonance imaging of the patient using the fluid-attenuated inversion recovery method showed marked cortical atrophy accompanied with multiple ischemic lesions in the bilateral corona radiate, medial temporal lobe and brainstem. **b** $^{123}$IMP SPECT, three-dimensional stereotaxic surface projection images. GLB (the *second row*) shows *Z* score images normalized by the whole brain count demonstrated the decrease of $^{123}$IMP uptake in the frontotemporal regions bilaterally but predominantly on the left side. **c** PET Dynamic C-11-flumazenil brain positron emission computed tomography demonstrated the diffuse decrease of activities in the frontal and temporal lobes bilaterally by binding potential images

Functional neuroimaging examinations

The quantitative measurement of regional cerebral blood flow using [N-isopropyl-(iodine-123)-p-iodoamphetamine] ($^{123}$IMP) single photon emission computed tomography (SPECT) demonstrated that the uptake of $^{123}$IMP was decreased in the frontotemporal regions bilaterally but predominantly on the left side (Fig. 1b). Dynamic C-11-flumazenil brain positron emission computed tomography (PET) demonstrated the diffuse decrease of activities in the frontal and temporal lobes bilaterally (Fig. 1c).

Neurophysiological examinations

Electromyography (EMG) of his lingual muscle, left sternomastoid muscle and left first dorsal interosseous muscle demonstrated that he could not spontaneously contract his tongue but resting lingual EMG was normal. EMG of sternomastoid and first dorsal interosseous muscles showed no abnormality.

Hematological and biochemical investigations

All hematological and biochemical investigations were normal, including thyroid function. Cerebrospinal fluid was acellular with normal total protein but there was an increase of tau protein (548 pg/ml, normal <150 pg/ml).

After these examinations, he returned home. He was re-admitted to our hospital a month later, because of dehydration. In the following 2 months, he suffered aspiration pneumonia and died 20 months after the onset of the first symptom. An autopsy was performed and neuropathological examination was limited to the brain.

Neuropathological investigation

His slightly atrophic brain weighed 1,210 g after fixation. The cerebral hemispheres were sectioned coronally and multiple samples were taken bilaterally. Histologically, microvacuolation was found in layer II of the frontal, temporal and insular cortex (Fig. 2a, b). Neuronal loss with gliosis was more prominent in the inferior part of the motor cortices, bilaterally, which was more severe on the left than the right side (Figs. 2c, d, Fig. 3). Moderate loss of neurons with gliosis was found in the amygdaloid nucleus. Gliosis without obvious neuronal loss was evident in the ventral putamen and anteromedial regions of the thalamus. In the brainstem, moderate loss of neurons with gliosis was found in the hypoglossal nucleus. The pyramidal tracts showed slight myelin pallor. TDP-43 immunohistochemistry revealed numerous neuronal cytoplasmic inclusions in the frontal and temporal cortex, amygdaloid nucleus, dentate gyrus, claustrum, neostriatum, and thalamus, and a few in the substantia nigra, facial nucleus, hypoglossal nucleus and inferior olivary nucleus (Fig. 2e–g). TDP-43-immunoreactive glial inclusions were also found in the frontal white matter and globus pallidus (Fig. 2h). Cystatin C immunohistochemistry confirmed the absence of Bunina

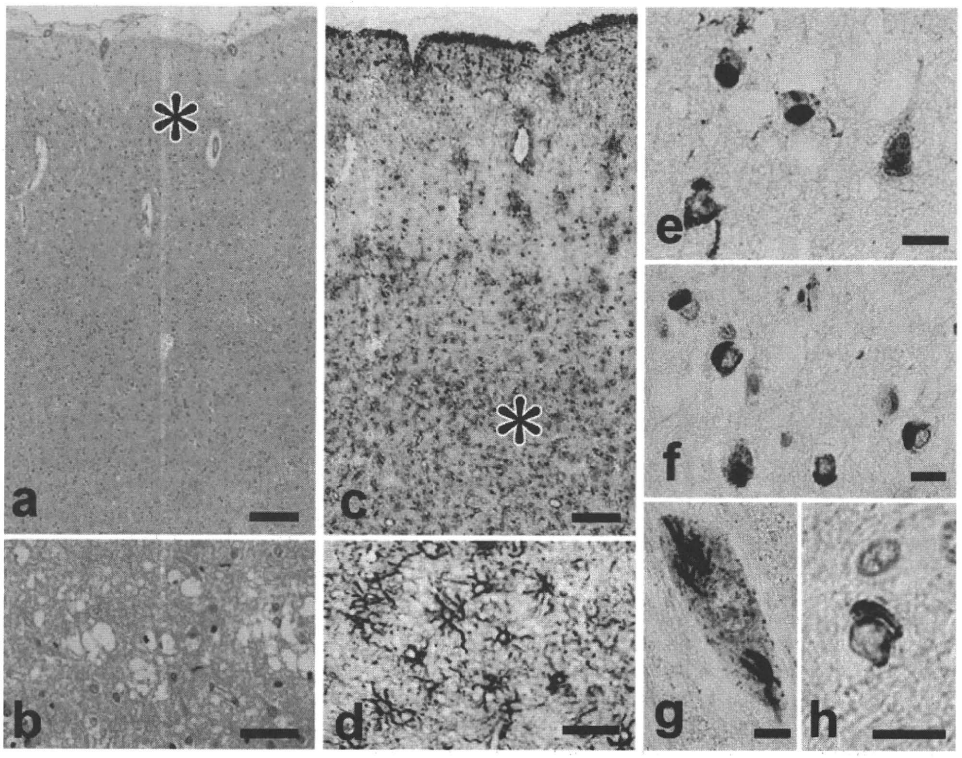


**Fig. 2** **a–d** Serial sections of the inferior part of the left motor cortex. **a** Moderate to marked loss of neurons with superficial spongiosis. **b** Higher magnification view of the area indicated by *asterisk* in **a** showing marked vacuolation in the neuropils. **c** Marked gliosis in all cortical layers. **d** Higher magnification view of the area indicated by *asterisk* in **c** showing reactive astrocytes with thick processes. TDP-43-positive neuronal cytoplasmic inclusions and neurites in the frontal cortex (**e**) and amygdaloid nucleus (**f**). **g** TDP-43-positive skein-like inclusions in the facial nucleus. **h** TDP-43-positive glial inclusions in the frontal white matter. *Bars* 200 μm in **a**, **c**, 50 μm in **b**, **d** and 10 μm in **e–h**. **a**, **b** H&E; **c**, **d** GFAP immunostain; **e–h** TDP-43 immunostain

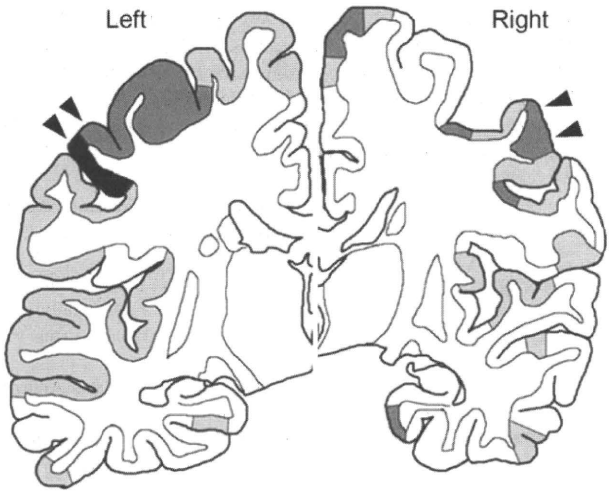


**Fig. 3** Schematic representation of the distribution of neuronal loss in the patient. Shading intensity corresponds to the severity of involvement. *Arrowheads* indicate the motor cortex

bodies in the lower motor neurons. Based on the above findings, our case was diagnosed as having type 2 of FTLD-U according to Sampathu's classification [24] or FTLD-TDP using a newly established nomenclature [18]. Moreover, many neurofibrillary tangles were found in the hippocampal formation, and some in the frontal and temporal neocortex (Braak stage IV). Many senile plaques were also seen in the cerebral neocortex (Braak stage C). No Lewy bodies were found in our case.

## Discussion

Our patient showed progressive anarthria and dysphagia coincidentally, with conspicuous automatico-voluntary dissociation with selective impairment of volitional facial, pharyngeal, lingual, masticatory and ocular movements but without automatic movements and reflexes, all of which are symptoms consistent with anterior operculum syndrome or FCMS [7]. The patient manifested exaggerated jaw jerk accompanied by snout, orbicularis oculi and oris, and palmomental reflexes in the presence of the gag reflex. Mao et al. [19] advocated that decreased gag reflex is a sign that can distinguish anterior operculum syndrome from other pseudobulbar palsies; however, retaining and decreasing the gag reflex does not always seem a decisive finding of significance for a diagnosis of anterior operculum syndrome, because Davies et al. [9] reported that 37% of 140 healthy people lacked a gag reflex.

Several investigators have reported progressive loss of speech output in anterior operculum syndrome [5, 6, 8, 10, 14, 15, 22, 23, 27, 30, 32]. Although progressive anarthria was common in these cases, the other clinical symptoms and the prognosis seemed to be heterogenous. Among these reports, some patients showed anarthria accompanied with pseudobulbar palsy, whereas the other patients showed advanced speech impairment for several years [6, 22, 23, 32] or more than 10 years [5, 15] before the onset of dysphagia. Our patient's speech impairment was consistent with so-called pure anarthria [16] from the viewpoint of speech and language terminology, but simultaneously accompanied with dysphasia, without time lag. Our patient must be differentiated from primary progressive anarthria [6]. Thus, we designate this syndrome as primary progressive anterior operculum syndrome. Aggravation of the symptoms in our patient was rapid as compared with other primary progressive anarthria with late-onset FCMS [5, 15, 22, 23, 32].

To our knowledge, there have been only three patients with primary pesudobulbar palsy demonstrating both anarthria and dysphagia simultaneously [14, 22, 30]. Lampl et al. [14] reported a case of primary progressive pseudobulbar palsy, in whom postmortem examination was not performed. Patient 3 reported by Tyrell et al. [30] and Patient 2 reported by Nakajima et al. [22] finally showed muscle wasting and fasciculation, indicating evidence of motor neuron disease. Neuropathological examination in Patient 2 of Nakajima et al. [22] revealed frontotemporal degeneration with superficial spongiosis, tau accumulation in the medial temporal lobe, pyramidal tract degeneration, and moderate neuronal loss in the spinal anterior horn. In our case, microvacuolation was found in the superficial layers of the frontal, temporal and insular cortex. In addition, TDP-43-immunoreactive neuronal inclusions were abundant in these areas, consistent with histopathological features of FTLD-TDP [18]. It is noteworthy that neuronal loss with gliosis was more prominent in the inferior part of the motor cortices, bilaterally. Moreover, moderate neuronal loss was found in the hypoglossal nucleus and TDP-43-positive inclusions were seen in the hypoglossal and facial nuclei, suggesting that our patient could be diagnosed as FTLD-TDP with motor neuron disease. In our patient, moderate Alzheimer pathology was also evident. Recent studies have shown that TDP-43 is also deposited in the brain of patients with neurodegenerative tauopathies, including Alzheimer's disease [1]. However, the distribution and extent of TDP-43 pathology in FTLD-TDP with or without motor neuron disease is much more severe and widespread than in Alzheimer's disease [4]. We feel that the causative lesion of anterior operculum syndrome in our patient was in the frontal lobes, especially in the inferior part of the motor cortex.

Clinico-pathological investigation of this patient will provide diagnostic and nosologic criteria for FTLD-TDP. We concluded that there is a peculiar clinical syndrome

which shows progressive anterior operculum syndrome as a variant of FTLD-TDP. Evaluation of patients with progressive speech output and dysphagia should include FTLD-TDP in the differential diagnosis.

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## References

1. Amador-Ortiz C, Lin W-L, Ahmed Z, Personett D, Davies P, Duara R, Graff-Radford NR, Hutton ML, Dickson DW (2008) TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. Ann Neurol 61:435–445
2. Besson G, Bogousslavsky J, Regli F, Maeder P (1991) Acute pseudobulbar or suprabulbal palsy. Arch Neurol 48:501–507
3. Biller J, Asconape J, Challa VR, Tool JF, McLean WT (1981) A case for cerebral thromboangiitis obliterans. Stroke 12:686–689
4. Brandmeir NJ, Geser F, Kwong LK, Zimmerman E, Qian J, Lee VM, Trojanowski JQ (2008) Severe subcortical TDP-43 pathology in sporadic frontotemporal lobar degeneration with motor neuron disease. Acta Neuropathol 115:123–131
5. Broussolle E, Tommasi M, Mauguiere F, Chazot G (1992) Progressive anarthria with secondary Parkinsonism: a clinico-pathological case report. J Neurol Neurosurg Psychiatry 55:577–580
6. Broussolle E, Backchine S, Tommasi M, Laurent B, Bazin B, Cinotti L et al (1996) Slowly progressive anarthria with late onset anterior operculum syndrome: a variant form of frontal cortical atrophy syndromes. J Neurol Sci 144:44–58
7. Bruyn GW, Gathier JC (1969) The operculum syndrome. In: Vinken PJ, Bruyn GW, Biemond A (eds) Localization in clinical neurology. Handbook of clinical neurology, vol 2. North Holland, Amsterdam, pp 776–783
8. Cohen J, Benoit N, Van Eeckhout P, Ducarne B, Brunet P (1993) Pure progressive aphemia. J Neurol Neurosurg Psychiatry 56:2923–2924
9. Davies AE, Kidd D, Stone SP, MacMahon J (1995) Pharyngeal sensation and gag reflexin healthy subjects. Lancet 345:487–488
10. Didic M, Ceccaldi M, Poncet M (1998) Progressive loss of speech: a neuropsychological profile of oremotor dysfunction. Eur Neurol 39:90–96
11. Ferrari G, Boninsegna C, Beltramello A (1979) Foix–Chavaney–Marie syndrome: CT study and clinical report of three cases. Neuroradiology 18:41–42
12. Foix C, Chavany JA, Marie J (1926) Diplegie facio-linguo-masticatrice d' origine cortico sous-corticale sans paralysie des membres. Rev Neurol (Paris) 33:214–219
13. Gordon A (2007) The opercular syndrome or, the Foix–Chavaney–Marie syndrome. J R Coll Physicians Edinb 37:103–106
14. Lampl Y, Steinmetz A, Gilad R, Eshel Y, Chamocitz D, Sarova P (1997) Anterior operculum syndrome localized by SPECT. J Nucl Med 38:1122–1124
15. Lang C, Reichwein J, Iro H, Treig T (1989) Foix–Chavany–Marie-syndrome-neurologica: neuropsychological, CT, MRI and SPECT findings in a case of progressive for more than 10 years. Eur Arch Psychiatry Neurol Sci 239:188–193
16. Lecours AR, Lhermitte F (1976) The ''pure form'' of the phonetic disintegration syndrome (pure anarthria): anatomical-clinical report of a historical case. Brain Lang 3:88–113
17. Levine DN, Mohr JP (1979) Language after bilateral cerebral infarctions: role of the minor hemisphere in speech. Neurology 29:927–938
18. Mackenzie IR, Manuela Neumann, Bigio EH, Cairns NJ, Alafuzoff I, Krill J et al (2009) Nomenclature for neruopathologic subtypes of frontotemporal lobar degeneration: consensus recommendations. Acta Neuropathol 117:15–18
19. Mao CC, Coull BM, Golper LAC, Rau MT (1989) Anterior operculum syndrome. Neurology 39:1169–1172
20. Marchiori GC, Trincia G, Cusumano S (1991) Labio-glosso-pharyngo-laryngeal paralysis caused by two brain lesions: cortical and subcortical. Ital J Neurol Sci 12:419–422
21. Mariani C, Spinnler H, Sterzi R, Valler G (1980) Bilateral perisylvian softenings: bilateral anterior operculum syndrome (Foix–Chavaney–Marie syndrome). J Neurol 223:269–284
22. Nakajima M, Iwabuchi S, Fukutake T, Hirayama K (1994) Progressive anterior operculum syndrome in cousins. Adv Neurol Sci 38:581–587 (in Japanese with English abstract)
23. Sakurai Y, Maruyama S, Fukusako Y, Bando M, Iwata M, Inoue K (1998) Progressive aphemia in a patient with Pick's disease: a neuropsychological and anatomic study. Neurol Sci 159:156–161
24. Sampathu DM, Neumann M, Kwong LK, Chou TT, Micsenyi M, Truax A et al (2006) Pathological heterogeneity of frontotemporal lobar degeneration with ubiquitin-positive inclusions delineated by ubiquitin immunohistochemistry and novel monoclonal antibodies. Am J Pathol 169:1343–1352
25. Sandyk R (1983) The operculum syndrome. A case report. S Afr Med J 63:578–579
26. Sandyk R, Brennnan MJW (1983) The operculum syndrome. J Comput Assist Tomogr 7:130–131
27. Silveri MC, Cappa A, Salvigni BL (1993) Speech and language in primary progressive anarthria. Neurocase 9:213–220
28. Starkstein SE, Berthier M, Leiguarda R (1988) Bilateral operculum syndrome and crossed aphemia due to a right insular lesion: a clinicopathological study. Brain Lang 34:253–261
29. Toghi H, Sano M, Takahashi S, Chiba K, Matsuoka S (1988) Bilateral anterior iperculum syndrome: localizing value of SPECT and MRI. Neuroradiology 30:589–591
30. Tyrrel PJ, Kartsounis LD, Frackoiwiak RSJ (1991) Progressive loss of speech output and orofacial dyspraxia associated with forntal lobe hypomnetabolism. J Neurol Neurosurg Psychiatry 54:351–357
31. Villa G, Catagirone C (1984) Speech suppression withput aphasia after bilateral perisylvian softenings (bilateral rplandicoperculum damage). Ital J Neurol Sci 5:77–83
32. Weller M, Poremba M, Dichgans J (1990) Operculum syndrome without opercular lesions: Foix–Chavaney–Marie syndrome in progressive supranuclear motor system degeneration. Eur Arch Psychiatry Neurol Sci 239:370–372

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ORIGINAL COMMUNICATION

# Writing errors as a result of frontal dysfunction in Japanese patients with amyotrophic lateral sclerosis

**Sachiko Tsuji-Akimoto · Shinsuke Hamada · Ichiro Yabe · Itaru Tamura · Mika Otsuki · Syoji Kobashi · Hidenao Sasaki**

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**Abstract** Loss of communication is a critical problem for advanced amyotrophic lateral sclerosis (ALS) patients. This loss of communication is mainly caused by severe dysarthria and disability of the dominant hand. However, reports show that about 50% of ALS patients have mild cognitive dysfunction, and there are a considerable number of case reports on Japanese ALS patients with agraphia. To clarify writing disabilities in non-demented ALS patients, eighteen non-demented ALS patients and 16 controls without neurological disorders were examined for frontal cognitive function and writing ability. To assess writing errors statistically, we scored them on their composition ability with the original writing error index (WEI). The ALS and control groups did not differ significantly with regard to age, years of education, or general cognitive level. Two patients could not write a letter because of disability of the dominant hand. The WEI and results of picture arrangement tests indicated significant impairment in the ALS patients. Auditory comprehension (Western Aphasia Battery; WAB IIC) and *kanji* dictation also showed mild impairment. Patients' writing errors consisted of both syntactic and letter-writing mistakes. Omission, substitution, displacement, and inappropriate placement of the phonic marks of *kana* were observed; these features have often been reported in Japanese patients with agraphia resulted from a frontal lobe lesion. The most frequent type of error was an omission of kana, the next most common was a missing subject. Writing errors might be a specific deficit for some non-demented ALS patients.

**Keywords** Assessment of cognitive disorders/dementia · Amyotrophic lateral sclerosis · Agraphia · Frontal lobe dysfunction

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S. Tsuji-Akimoto (✉) · S. Hamada · I. Yabe · H. Sasaki
Department of Neurology, Hokkaido University Graduate School of Medicine, Sapporo, Japan
e-mail: tujitti@jb3.so-net.ne.jp

S. Hamada
e-mail: shamada@med.hokudai.ac.jp

I. Yabe
e-mail: yabe@med.hokudai.ac.jp

H. Sasaki
e-mail: h-isasak@med.hokudai.ac.jp

I. Tamura · M. Otsuki
School of Psychological Science, Health Sciences University of Hokkaido, Sapporo, Japan

I. Tamura
e-mail: tamurait@hoku-iryo-u.ac.jp

M. Otsuki
e-mail: miotsuki@hoku-iryo-u.ac.jp

S. Kobashi
Graduate School of Engineering, University of Hyogo, Himeji, Japan
e-mail: kobashi@eng.u-hyogo.ac.jp

## Introduction

Recently, considerable evidence has been gathered for cognitive impairment in amyotrophic lateral sclerosis (ALS). After the consensus criteria for frontotemporal lobar dementia (FTLD) were established, the frequency of dementia reported in ALS patients increased from 6 to 23%, and as many as 50% showed cognitive change if mild frontal dysfunction was present [17, 20, 21, 26]. One of the

most intensively investigated cognitive problems was verbal fluency [17]. Though slowness and inarticulation of speech caused by oropharyngeal motor dysfunction was present, subjects' verbal fluency for the first letter was also impaired. Functional magnetic resonance imaging revealed reduced activation of the middle and inferior frontal gyrus and anterior cingulated gyrus [1]. Many other methods of examination have been reported to effectively identify mild cognitive impairment, however, the patterns of abnormality vary so much that it is unclear which examination is most suitable for screening [32].

Some patients who seem mentally normal are unable to write well when they cannot articulate. Loss of communication is a critical problem for both patients and caregivers. In a retrospective study of Japanese ALS patients having dementia, writing errors were prominent; agraphia and anosognosia were specifically noted in the case of ALS with dementia [13]. The study also found a high prevalence of agraphia among non-demented bulbar-onset ALS patients [15]. Several cases of ALS patients with excessive spelling mistakes were reported [8, 10, 16, 21, 28]; however, the mechanism of agraphia is not well understood. Some single photon emission computed tomography studies have speculated that the cause of agraphia may be reduction in cerebral blood flow caused by a lesion in the frontal lobe, but the number of patients was too small to draw a definite conclusion.

In this study, we clarified that writing errors were frequently noted among non-demented ALS patients. Writing errors may be indicated in the early diagnosis of mild cognitive impairment. Analysis of error patterns is helpful for improving communication in patients at advanced stages of ALS.

## Patients and methods

### Patients

Of all the sporadic ALS patients admitted to the neurological department of Hokkaido University Hospital and Hokuyukai Hospital from May to November 2008, 18 patients agreed to participate in the study. ALS was clinically diagnosed according to the revised El Escorial Criteria (rEEC) [7]. Patients who met Neary's FTLD criteria or scored under mean – 2SD on Raven's colored progressive matrices (RCPM) were excluded [21]. Patients with respiratory symptoms [% forced vital capacity, (FVC) $<70\%$], and a history of neurological or psychiatric illness were also excluded. Among the patients examined, one was left-handed. Sixteen volunteers who did not have any neurological disease were examined as controls; they were adjusted to the patients for age and educational period. The general cognitive status was assessed by RCPM, and all 16 controls scored above the mean − 2SD. Patients and controls gave written informed consent according to the Helsinki II Declaration and the study was approved by the Ethics Committee of Hokkaido University Hospital.

### Neuropsychological examination

The following neuropsychological examinations were performed: RCPM, forward and backward digit span (adopted Wechsler Adult Intelligent Scale; WAIS-R), trail making test (TMT)-A and -B, frontal assessment battery (FAB)-4 and 5, paced auditory serial addition task (PASAT)-2 and PASAT-3, imitating three kinds of hand patterns and a copy of a 2-D cube, three kinds of picture arrangements (PA) (Japanese version of WAIS-R IV 3-5), naming and pointing to 12 objects (6 pictures and 6 real ordinary objects), auditory comprehension (Western Aphasia Battery: WAB-IIC), repetition (WAB-III), reading and dictation of both kanji and kana, and composition. Reading and dictation tests were performed using 47 kanji characters and 47 pairs of kana. The kanji characters used for reading and dictation were selected from the Japanese educational kanji for the third grade in elementary schools. The kana pairs were selected such that all 50 letters were included.

### Investigation of writing ability

For investigation of writing ability, the subjects were asked to give a written description of the stories for the Japanese version of WAIS-R IV (iii)–(v) and WAB-IV (a picture of a picnic). To quantify the writing error for statistic analysis, we designed the writing error index (WEI). WEI was calculated as the number of errors divided by the total number of words. The errors included not only easy mistakes such as omission, substitution, and displacement of a letter, but also complex mistakes such as writing an incomprehensible sentence and a grammatical mistake, were also checked as one error. The detail of the calculating rule is described in supplementary file 1 and 2. WEI was calculated by two examiners (one was experimentally blinded), and the mean values of their assessments were used in the analysis.

### Statistical analysis

Comparative analysis between ALS patients and controls was performed by Student's $t$ test or Wilcoxon-Mann–Whitney's test in accordance with variance, using KaleidaGraph 4.0. Correlations among batteries were calculated with Pearson's formula using StatCell 2 software, an Excel ad-on.

**Table 1** The Profile of ALS patients and controls

| | Patients | Control | *p* value |
|---|---|---|---|
| Number (*n*) | 18 | 16 | |
| Age (mean, ±SD) | 65.4 ± 11.5 | 64.5 ± 8.4 | 0.8007 |
| Female:male | 9:9 | 11:5 | |
| Disease duration (months, mean, ±SD) | 19.4 ± 7.0 | – | |
| Educational period (years, mean, ±SD) | 10.8 ± 2.5 | 12.0 ± 4.1 | 0.1279 |
| RPCM (mean, ±SD) | 28.8 ± 5.5 | 31.1 ± 4.1 | 0.1741 |
| WER exam (*n*) | 16 | 16 | |
| ALS-FRS-R (total) (mean ± SD) | 34.7 ± 7.5 | | |
| rEEC at examination | | | |
| Definite | 4 | | |
| Probable | 8 | | |
| Laboratory-supported probable | 1 | | |
| Possible | 3 | | |
| Suspect | 2 | | |
| Onset | | | |
| Brain stem | 10 | | |
| Cervical | 6 | | |
| Thoracic | 0 | | |
| Lumbosacral | 1 | | |
| Multiple | 1 | | |

*n* Number of patients, *SD* standard deviation, *RCPM* Raven's colored progressive matrices, *WEI* writing error index, *ALSFRS-R* revised ALS functional rating scale, *rEEC* revised El Escorial Criteria

## Results

The profile of the patients is shown in Table 1. Patients categorized under "suspected or possible ALS" at the time of examination deteriorated to "possible or probable ALS" by August 2009. One patient who had not been diagnosed with dementia at the time of examination was suspected of behavior change 10 months later. Two patients could not speak clearly because of severe dysarthria. One patient could perform neither dictation nor composition because of disability of the dominant hand, and another patient refused to write all four compositions for the WEI because her education had been erratic and incomplete in her school-going days.

The results of the neuropsychological examination are shown in Table 2. The results of PA and WEI showed that the patients in the ALS group had significant impairment compared to the controls. Forward and reverse digit span, 2 seconds-version of PASAT (PASAT-2), auditory comprehension (WAB-IIC), and kanji dictation also showed statistically significant impairment. However, we did not label these subjects as cognitively impaired. The individual score of digit span was within normal limits. The results of the 3 seconds-version of PASAT means the PASAT-2 was influenced by motor impairment.

WEI was significantly higher in ALS patients than control groups, and a high WEI index was also found in some patients with good dictation performance. Among 16 patients examined for dictation, three patients apparently showed agraphia both in kana and kanji dictations. Four patients had low scores only in the kanji dictation. The pathological implication of an isolated kanji dictation was unclear because a kanji dictation is easily influenced by education. In the kanji-limited impaired group, one patient refused to write the compositions (as mentioned before). The WEI of the other three patients were 14.4, 12.6, and 12.9, respectively, which were much higher than the average WEI of controls. Furthermore, among nine patients who scored well on the dictation, three patients showed WEI indexes higher than 10.

On composition, writing errors were of two types—syntactic and non-syntactic. Non-syntactic errors included both kana and kanji, and the number of errors of kanji showed a smaller difference between patients and controls (Fig. 1a). Kana errors included omissions, substitutions, displacements, incorrect phonetic marks (a pair of dots that convert an unvoiced consonant to a voiced consonant when it is added in the right upper corner); and imperfect characters (Fig. 2). Among 122 total kana errors, 60 errors were omissions. Omission of a kana-letter was a characteristic of ALS patients, but it was sometimes observed in the controls, too; however, most omissions were a single letter in controls. On the other hand, the patients had a higher number of total omissions. Sometimes, sequences of letters were deleted within a word or extended to the next word. Postpositional particles, represented by a single kana letter and corresponding to the preposition in English, were frequently omitted. Other frequent errors in the patients were phonetic marks and phonologically plausible substitutions of kana letters at postpositions. Syntactic errors were classified into four major

**Table 2** Results of neuropsychological examinations

| | Number of patients | Full score | ALS (mean ±SD) | Control (mean ±SD) | *p* value |
|---|---|---|---|---|---|
| Digit span (forward)* | 18 | 12 | 6.9 ± 2.2 | 8.9 ± 2.5 | 0.018 |
| Digit span (reverse)* | 18 | 12 | 5.6 ± 2.9 | 7.7 ± 1.6 | 0.015 |
| TMT B/A | 16 | | 2.9 ± 1.3 | 3.0 ± 2.2 | 0.885 |
| TMT B-A | 16 | | 110.3 ± 69.8 | 80.3 ± 77.5 | 0.258 |
| RCPM | 18 | 37 | 28.8 ± 5.5 | 31.1 ± 4.1 | 0.174 |
| FAB-4 | 18 | 3 | 2.4 ± 1.1 | 2.9 ± 0.3 | 0.062 |
| FAB-5 | 18 | 3 | 1.7 ± 1.3 | 1.9 ± 1.3 | 0.635 |
| PASAT-3 | 16 | 60 | 29.5 ± 15.8 | 38.6 ± 16.1 | 0.133 |
| PASAT-2* | 16 | 60 | 22.5 ± 10.9 | 32.2 ± 11.2 | 0.026 |
| Picture arrangements* | 18 | 3 | 1.8 ± 1.2 | 2.9 ± 0.4 | 0.009 |
| Parietal lobe function | 15 | 4 | 3.7 ± 0.6 | 3.8 ± 0.5 | 0.645 |
| Naming | 18 | 12 | 11.9 ± 0.3 | 12 ± 0.0 | 0.333 |
| Pointing | 18 | 12 | 12 | 12 | – |
| Audiotory comprehension* (WABII-C) | 18 | 80 | 72.0 ± 8.0 | 77.6 ± 3.6 | 0.016 |
| Repetition (WABIII) | 16 | 100 | 91.5 ± 22.1 | 99.5 ± 1.2 | 0.169 |
| Dictation (kana) | 16 | 47 | 44.5 ± 5.7 | 46.8 ± 0.8 | 0.140 |
| Dictation (kanji)* | 16 | 47 | 36.5 ± 15.6 | 46.5 ± 0.6 | 0.022 |
| Writing error index (WEI)* | 16 | | 12.7 ± 8.2 | 2.9 ± 3.0 | <0.001 |
| Non-syntactic error (index)* | 16 | | 9.6 ± 8.6 | 2.3 ± 2.3 | 0.004 |
| Syntactic error (index)* | 16 | | 2.4 ± 2.8 | 0.5 ± 0.9 | 0.028 |

*SD* standard deviation, *n* number of patients, *TMT* trail making test, *RCPM* Raven's colored progressive matrices, *FAB* frontal assessment batteries, *PASAT* paced auditory serial addition task, *WAB* Western aphasia battery, *WEI* writing error index

patterns: (1) missing subject, (2) unfinished sentence (loss of one or more phrases including verb), (3) mismatch between a subject and its verb, and (4) inappropriate use of conjunctions (Fig. 1b). Missing a subject is a common feature of spoken Japanese, and some controls omitted subjects when writing Japanese. Therefore, it was difficult to determine whether this was an actual mistake. However, as shown in Fig. 1b, the total number of missing subjects in ALS cases was much higher than that in the control cases. When two or more people were present in a story, subjectless sentences were often unclear if the reader was not shown the pictures.

To investigate the implication of disease duration, severity, and general cognitive status in writing errors, we calculated Pearson's correlation between WEI and the parameters. As shown in Table 3, WEI was not associated with the total, bulbar, and upper limb parts of the revised ALS functional rating scale (ALSFRS-R), while it was mildly associated with disease duration and the RCPM score [9]. Indeed, the patient having the worst ALSFRS-R with moderate bulbar symptoms performed best both in writing and other cognitive examinations.

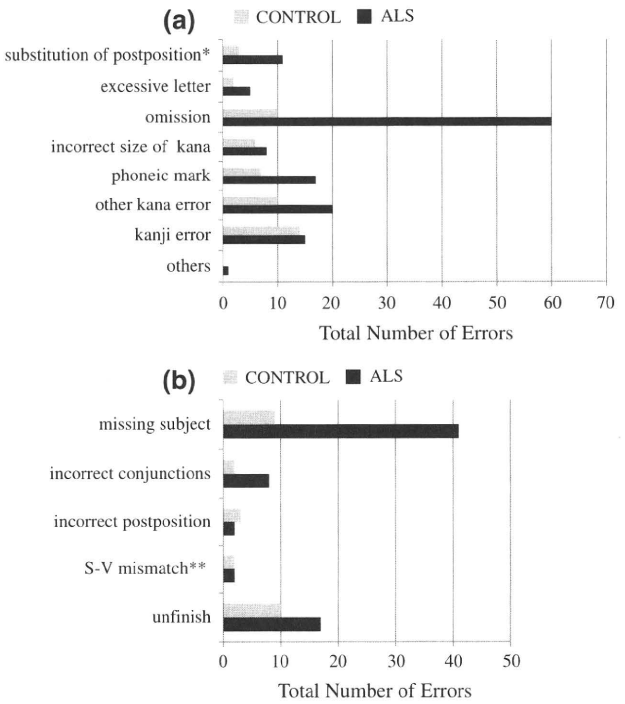


**Fig. 1** Comparing error patterns between ALS and controls. **a** Classification of non-syntactic errors. Increase of omission was prominent. *Substitution of postposition means a phonologically plausible error of postposition. **b** Missing subject was **S–V mismatch means mismatch between subject and verb

## Discussion

We demonstrated that ALS patients had mild disability with regard to spontaneous writing and PA. Spontaneous

writing, loss of subject, letter omissions, substitutions, and displacements were frequently exhibited by ALS patients. Some patients experienced difficulties in articulation and writing because of motor complications. However, none of the patients in this study had any difficulty in both naming and pointing, and none exhibited paraphasia. Thus, we concluded that our subjects did not have aphasia. None of them had dementia, aphasia, or lexical problems at the time of the examination. Results of auditory comprehension, syntactic error at composition and frequent omission of postpositions might show agrammatism. However, to understand a sentence, several elements are necessary in addition to grammatical ability, such as concentration, short-term memory, and working memory. Hence, agrammatism should be carefully determined.

Subjects also did not show constructional apraxia, limb apraxia, ideomotor apraxia, or visuospatial agnosia. The patients who performed writing tasks could hold and manage a pen. Hence, we concluded that they had agraphia. Agraphia is classified into several types—pure agraphia, aphasic agraphia, agraphia with alexia, apraxic agraphia, and spatial agraphia [11]. Our patients were classified as having pure agraphia. Especially in ALS patients, agraphia should not be diagnosed solely on the basis of the dictation scores because even patients who get a 100% score on a dictation sometimes have difficulty in writing sentences or a composition. Therefore, we think that performance in the dictation of short words is not a proper indicator of mild agraphia in non-demented ALS.

Pure agraphia can result from several lesions. The posterior middle frontal gyrus of the dominant side (including Exner's area) is classically considered as the writing center, as proven by the patients with stroke of the region [3, 27, 29, 33]. Pure agraphia is often characterized by substitution, displacement, addition, repetition, and omission. Written Japanese has two types of symbols—kana (phonogram) and kanji (morphogram). A kana alone does not mean anything; it represents only a syllable. The dictation of kana is similar to that of regular words in European languages. Tohgi reported that a patient who had difficulty in kana writing had lesion involvement of the middle frontal gyrus and the upper part of the inferior frontal gyrus [33]. He had greater disability for spontaneous writing than for dictation. He frequently made mistakes in phonic marks, contracted sounds (e.g., kya and sho), and postpositional letters. In particular, presence of the lesions created problems in the selection and placement of letters. The features reported were similar to those of our ALS patients. The presence of lesions in the left superior parietal lobule is also known to indicate pure agraphia. Basso et al. [5] described two pure agraphic patients who had these lesions, but according to several case reports, the characteristics were different from those of pure agraphia of the frontal

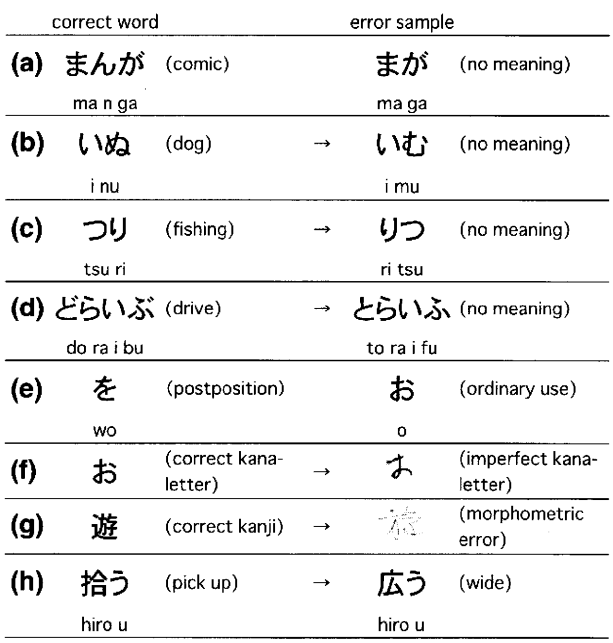


**Fig. 2** Samples of non-syntactic errors. **a**–**f** were samples of kana error, and g, h were kanji. **a** Omission of kana letter. **b** Substitution of a kana letter. **c** Displacement of kana letters. **d** Phonetic marks were omitted in the case of 2 kana letters. Marks consisted of a pair of dots written in the right upper corner of a kana letter when it is read as b, d, g, z. **e** Phonologically plausible substitution of kana. All postpositional particles with the pronunciation "wo" must be written as "を" instead of "お". The letter "お" is for ordinary use and not used as a postposition. The pronunciation of these letters is not distinguishable in spoken Japanese. **f** An imperfect kana letter. **g** Morphometric error of kanji. **h** Phonologically plausible substitution of kanji. Two kanji are pronounced in the same way but differ in their meanings

**Table 3** Correlation between WEI and patient-profiles

| | Pearson's correlation |
|---|---|
| Disease duration | 0.573 |
| ALSFRS-R (total) | −0.193 |
| ALSFRS-R1-3 (bulbar) | −0.170 |
| ALSFRS-R4-5 (upper limbs) | −0.326 |
| RCPM | −0.418 |

*WEI* writing error index, *ALSFRS-R* ALS functional rating score revised, *RCPM* Raven's colored progressive matrices

lesions. The main problem associated with this lesion is clumsiness in forming the shape of the letter; this is called apraxic (or ideational) agraphia [6, 24]. It is attributed to neither apraxia nor movement disorder of the hand. People who have this lesion experience difficulty in writing letters in the correct sequence and forget the order of strokes [24]. In the present study, some patients wrote ill-shaped letters, but we did not conclude that our patients had apraxic agraphia because they showed motor disability of the dominant hand, and written symbols other than letters were

also ill-shaped. The left posterior inferior temporal gyrus is known to be associated with Japanese pure agraphia, limited to kanji recall [19, 31]. In our study, performance in kanji dictation was significantly impaired in the patients compared to the controls. However, we think the lesion was not responsible because errors in kana were more frequently noted in the composition. The patients showed equal impairment in both kana and kanji recall.

Satoh [28] reported that 3 of 16 ALS patients without dementia had a tendency to omit kana in dictation. The subjective dictation words consisted of 2–5 kana letters each, and if a word contained three or more kana letters, the likelihood of the patient omitting letters was greater. They also described some patients who showed kanji agraphia. Ichikawa et al. [15] recently reported that bulbar-onset ALS patients showed high prevalence of agraphia characterized by omission, substitution of kana, and misuse of postposition. They reported the details of patients in a series of two Japanese reports. While they emphasized kana-error represented by omission, they also illustrated patients having kanji-dominant writing error [12, 14]. Errors of kana and kanji were independent of each other in many patients and they speculated that writing errors of ALS patients might be classified into two types arising from different lesions, as FTLD has two types of aphasia, progressive aphasia and semantic dementia.

Agraphia was also reported in many cases of primary lateral sclerosis (PLS) [25, 34]. Similar to our study, the pattern of errors varied from agrammatism to misspelling. In a study on PLS, it was found that words containing less than five letters were spelt correctly; thus, the length of a word affects misspelling. On the basis of the strong association between misspelling and severity of dysarthria (bulbar part of ALSFRS-R), the researchers proposed that it was because of a deficit in rehearsal for spelling when subjects could not speak. Ichikawa, et al. [15] pointed out that agraphia was frequently noted in patients with bulbar-onset ALS. They did not mention limb-onset ALS. In our study, however, WEI and the bulbar part of the ALSFRS-R were not correlated; thus, the results did not support the rehearsal theory. Because only a few patients of the limb-onset group could perform writing tests in this study, it is unclear whether agraphia is specific to bulbar-onset patients. We think that agraphia was independent of motor disability.

Difficulty in PA was another characteristic of ALS. PA in WAIS-III reflected performance IQ, but because it is a complex task, PA was not classified into specific secondary indices. Further, PA was excluded from WAIS-IV. Only a few reports have described what poor performance in PA implies. In psychiatric disorders, PA and explanation of the story have been considered to be associated with social cognition [2, 30]. However, PA requires more complex factors than social cognition. To understand each picture, social cognition represented by speculation of relationships, social experiences, knowledge of behavior, and insight into others' emotions is required. Moreover, for PA, speculation of time sequence and working memory is necessary. Both defects in social cognition and working memory are critical features of frontotemporal dementia. In the present study, PA was scored from 0 to 3 points, and the patients were clearly divided into two groups: those who could perform the task easily (3 or 2 points) and those who could not (0 or 1 points, sometimes 2 points but with long consideration). PA might be useful for screening of mild cognitive disturbance associated with ALS. Moreover, PA and WEI were highly correlated. Confusion of a story line as determined from the uncorrected picture sequence could affect the WEI, but the result that the subject of the picnic picture (WAB IV; composition) had the same tendency of error showed that the mistakes were not because of the uncorrected sequences.

Impairment in PA and agraphia might be the most sensitive symptoms of mild cognitive impairment. According to the consensus criteria for cognition in ALS patients, tests should be completed within 20 min [32]. PA involving the arrangement of one set of pictures is easy to perform within 3 min.

Recent findings on the accumulation of TAR-DNA binding protein 43 kDa (TDP-43) both in ALS and FTD patients further support the notion of a common pathway for both these diseases [4, 18, 22]. On the other hand, in the case of patients who undergo long-term mechanical ventilation and show a modest extra-motor lesion, extension of the extra-motor pathology is not affected by either severity of the motor symptom or disease duration [23]. In the present study, some patients were cognitively intact, and the patient with the best WEI had the worst ALSFRS-R. The disagreement between motor function and agraphia also supports this view, and the writing examination might be a sensitive indicator of the future possibility of cognitive involvement. Therefore, patients with agraphia need to be carefully observed with regard to progression of their condition to dementia.

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**Conflict of interest** The authors report no conflicts of interest.

## References

1. Abrahams S, Goldstein LH, Simmons A, Brammer M, Williams SC, Giampietro V, Leigh PN (2004) Word retrieval in amyotrophic lateral sclerosis: a functional magnetic resonance imaging study. Brain 127:1507–1517