markedly in these ALS patients [Hayashi & Kato 1989, Mizutani et al. 1992]. Bunina bodies and ubiqutin-immunopositive inclusions have been observed in the neurons of the reticular formation of the medulla oblongata in patients with ALS [Bergmann 1993, Nakano et al. 1993]. This indicates that an ALS specific disease process exists in the neurons in the reticular formation.

It has been shown that propriospinal neurons at the cervical segment are located in laminae V-VII (cat and monkey [Molenaar and Kuypers 1978]) of Rexed [Rexed 1954], and that these neurons play roles in movement control and sensorimotor integration of the extremities [Martin 1996, Pierrot-Deseilligny 1996]. The reticulospinal and propriospinal tracts terminate in laminae V-VIII in the cervical cord (cat [Barilari & Kuypers 1969]). Thus, the reticulospinal neurons in the brain stem and propriospinal neurons in the spinal cord are considered to be closely linked to the degeneration of the ALF in classic ALS.

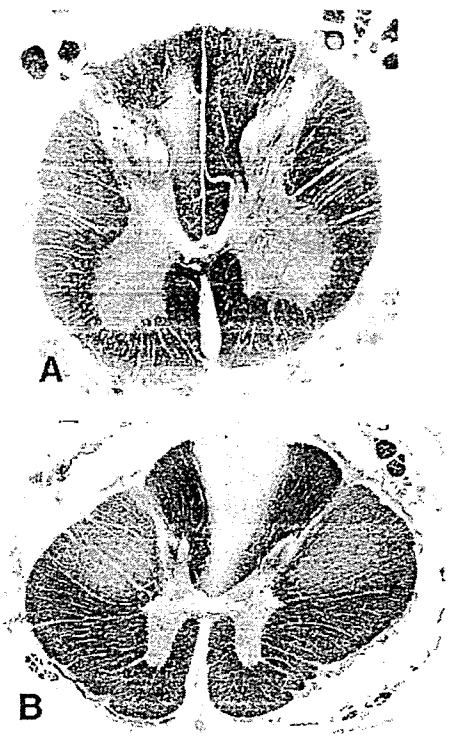

Figure 11. A familial ALS patient with SOD-1 gene mutation showing a degeneration of the left middle root zone (A) of the 12th thoracic segment. Mid-thoracic cord of a patient with SMON (B). Klüver-Barrera preparation.

Mechanism of the Middle Root Zone Degeneration in Familial ALS

Some patients of familial ALS with or without SOD-1 gene mutation show degeneration of the posterior funiculus, that is "middle root zone degeneration" (Fig. 11A) [Makifuchi and Ikuta 1977, Takahashi et al. 1994]. The pattern is quite different from that of the Wallerian

degeneration of the transverse myelopathy, or the subacute myelo-optico-neuropathy (SMON), an intoxication of clioquinol (hydoxyquinoline), which primarily involves the spinal ganglia and the axons [Tateishi J et al. 1972, 1973] (Fig, 11B). On the pathomechanism of this finding, Ikuta et al. (1982) reported a retrograde trans-synaptic degeneration of the afferent fibers to the degenerated Clarke's column, based on the findings of marked loss of neurons in the Clarke's column at the severely degenerated side of the posterior funiculus (Fig. 11A).

Acknowledgments

The authors are indebted to Emeritus Professor Fusahiro Ikuta, Niigata University, Niigata, Japan, and the Brain Research Center, Niigata Neurosurgical Hospital, Niigata, Japan, to proceed the research. This work was supported in part by grants from the Japanese Ministry of Education, Science, Sports and Culture (#14580735 to KO and # 14657376 to EK), and the Japanese Ministry of Health, Labor and Welfare (Research on Psychiatric and Neurological Diseases (H16-kokoro-017) to KO).

References

Altermark B, Lundberg A, Pinter M, Sasaki S (1987) Long C_3-C_5 propriospinal neurons in the cat. *Brain Res 404*: 382-388

Barilari MG, Kuypers HGJM (1969) Propriospinal fibers interconnecting the spinal enlargements in the cat. *Brain Res 14*: 321-330

Barnes S (1901) Degenerations in hemiplegia: with special reference to a ventrolateral pyramidal tract, the accessory fillet and Pick's bundle. *Brain 24*: 463-501

Bergmann M (1993) Motor neuron disease/amyotrophic lateral sclerosis-lesson from ubiquitin. *Path Res Pract 189*: 902-912

Charcot JM (1880) *Lecons sur les maladies du systeme nerveuz faites á la Salpetriere*. vol 2, 3rd edn. Delahaye, Paris, pp 213-226

Giok SP (1958) The fasciculus intermediolateralis of Loewenthal in man. Brain 81: 577-587

Hayashi H, Kato S (1989) Total manifestation of amyotrophic lateral sclerosis. ALS in the totally locked-in state. *J Neurol Sci 93*: 19-35

Holmes G (1909) The pathology of amyotrophic lateral sclerosis. *Rev Neurol Psychiatry 7*: 693-725

Ikuta F, Makifuchi T, Ichikawa T(1979) *Comparative studies of tract degeneration in ALS and other disorders*. In Tsubaki T, Toyokura Y (eds) Amyotrophic lateral sclerosis. University of Tokyo Press, Tokyo, pp 177-200

Ikuta F, Makifuchi T, Ohama E, Takeda S, Oyanagi K, Nakashima S, Motegi T (1982) Tract degeneration of the human spinal cord: Some observations on ALS and hemispherectomized humans. *Shinkei Kenkyu no Shinpo 26*: 710-736

Iwamoto Y, Sasaki S, Suzuki I (1990) Input-output organization of reticulospinal neurones, with special reference to connections with dorsal neck motoneurones in the cat. *Exp Brain Res 80*: 260-276

Kuru, M (1976) Sensory paths in the spinal cord and brain stem of man. Kimura Shoten, Tokyo

Lawyer, T Jr, Netsky MG (1953) Amyotrophic lateral sclerosis. A clinicoanatomic study of fifty-three cases. *Arch Neurol Psychiatry 69*: 171-192

Makifuchi T, & Ikuta F (1977) Degeneration of the accessory cuneate nucleus in familial ALS with the "middle root zone" involvement. *No to Shinkei 29*: 1332-1334

Martin GF, Humbertson AO Jr, Laxson LC, Panneton WM, Tschismadia I (1979) Spinal projections from the mesencephalic and pontine reticular formation in the North American opossum: A study using axonal transport techniques. *J Comp Neurol 187*: 373-400

Martin GF, Cabana T, Ditirro FJ, Ho RH, Humbertson AO Jr (1982) Raphespinal projections in the North American opossum: evidence for connectional heterogeneity. *J Comp Neurol 208*: 67-84

Martin JH (1996) Differential spinal projections from the forelimb areas of the rostral and caudal subregions of primary motor cortex in the cat. Exp Brain Res 108: 191-205

Mitsuyama Y, Takamatsu I (1971) An autopsy case of presenile dementia with motor neuron disease. *No to Shinkei 23*: 409-416

Mizutani T, Sakamaki S, Tsuchiya N, Kamei S, Kohzu H, Horiuchi R, Ida M, Shiozawa R, Takasu T (1992) Amyotrophic lateral sclerosis with ophthalmoplegia and multisystem degeneration in patients on long-term use of respirators. *Acta Neuropathol 84*: 372-377

Mochizuki Y, Mizutani T, Takasu T (1995) Amyotrophic lateral sclerosis with marked neurological asymmetry: clinicopathological study. *Acta Neuropathol 90*: 44-50

Molenaar I, Kuypers HGJM (1978) Cells of origin of propriospinal fibers and of fibers ascending to supraspinal levels. A HRP study in cat and rhesus monkey. *Brain Res 152*: 429-450

Nakano I, Iwatsubo T, Hashizume Y, Mizutani T, Mannen T (1992) Amyotrophic lateral sclerosis with dementia –lesions in the apical cortex and some deeper structures of the temporal lobes. *Neuropathol 12*: 69-77

Nakano I, Iwatsubo T, Hashizume Y, Mizutani T (1993) Bunina bodies in neurons of the medullary reticular formation in amyotrophic lateral sclerosis. *Acta Neuropathol 85*: 471-474

Nathan PW, Smith MC, Deacon P (1990) The corticospinal tracts in man. Course and location of fibres at different segmental levels. *Brain 113*: 303-324

Nathan PW, Smith M, Deacon P (1996) Vestibulospinal, reticulospinal and descending propriospinal nerve fibres in man. *Brain 119*: 1809-1833

Nyberg-Hansen R (1965) Sites and mode of termination of reticulo-spinal fibers in the cat. An experimental study with silver impregnation methods. *J Comp Neurol 124*: 71-100

Oyanagi K, Ikuta F, Horikawa Y (1989) Evidence for sequential degeneration of the neurons in the intermediate zone of the spinal cord in amyotrophic lateral sclerosis: a topographic and quantitative investigation. *Acta Neuropathol 77*: 343-349

Oyanagi K, Kawakami E, Morita T, Takahashi H (1999) Pursuit of the origin of the large myelinated fibers of the anterolateral funiculus in the spinal cord in humans in relation to the pathomechanism in amyotrophic lateral sclerosis. *Acta Neuropathol 98*: 635-640

Oyanagi K, Makifuchi T, Ikuta F (1983) A topographic and quantitative study of neurons in human spinal gray matter, with special reference to their changes in amyotrophic lateral sclerosis. *Biomed. Res 4*: 211-224

Oyanagi K, Makifuchi T, Ikuta F (1995) The anterolateral funiculus in the spinal cord in amyotrophic lateral sclerosis. *Acta Neuropathol 90*: 221-227

Parent A (1996) Carpenter's Human Neuroanatomy, 9th ed. Williams & Wilkins, Baltimore, pp 325-417

Pierrot-Deseilligny E (1996) Transmission of the cortical command for human voluntary movement through cervical propriospinal premotoneurons. *Prog Neurobiol 48*: 489-517

Reuter A (1931) Zur pathogenese der amyotrophischen Lateralsklerose. Deutz Nervenheilk 122: 237-254

Rexed B (1954) The cytoarchitectonic organization of the spinal cord in the cat. *J Comp Neurol 96*: 415-496

Rose PK, Tourond JA, Donevan AH (1996) Morphology of single vestibulospinal collaterals in the upper cervical spinal cord of the cat: III Collaterals originating from axons in the ventral funiculus ipsilateral to their cells of origin. *J Comp Neurol 364*: 16-31

Shaw PJ (2001) Genetic inroads in familial ALS. *Nature Gen 29*: 103-102

Shinoda Y, Ohgaki T, Futami T (1986) The morphology of single lateral vestibulospinal tract axons in the lower cervical spinal cord of the cat. *J Comp Neurol 249*: 226-241

Smith MC (1951) The use of Marchi staining in the later stages of human tract degeneration. *J Neurol Neurosurg Psychiatry 14*: 222-225

Smith M (1957) Observations on the topography of the lateral column of the human cervical spinal cord. *Brain 80*: 263-272

Smith MC (1960) Nerve fibre degeneration in the brain in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry 23*: 269-282

Smith MC, Deacon P (1981) Helweg's triangular tract in man. *Brain 104*: 249-277

Swash M, Scholtz CL, Vowles G, Ingram DA (1988) Selective and asymmetric vulnerability of corticospinal and spinocerebellar tracts in motor neuron disease. *J Neurol Neurosurg Pshchiatr 51*: 785-789

Takahashi H, Makifuchi T, Nakano R, Sato S, Inuzuka T, Sakimura K, Mishina M, Honma Y, Tsuji S, Ikuta F (1994) Familial amyotrophic lateral sclerosis with a mutation in the Cu/Zn superoxide dismutase gene. *Acta Neuropathol 88*: 185-188

Takahashi H, Oyanagi K, Ikuta F (1993a) The intermediolateral nucleus in sporadic amyotrophic lateral sclerosis. *Acta Neuropathol 86*: 190-192

Takahashi H, Oyanagi K, Ikuta F, Tanaka M, Yuasa T, Miyatake T (1993b) Widespread multiple system degeneration in a patient with familial amyotrophic lateral sclerosis. *J Neurol Sci 120*: 15-21

Takahashi H, Oyanagi K, Ohama E, Ikuta F (1992) Clarke's column in sporadic amyotrophic lateral sclerosis. *Acta Neuropathol 84*: 465-470

Tateishi J, Ikeda H, Saito A, Kuroda S, Otsuki S (1972) Myelopathy in dogs induced by iodoxyquinoline. *Neurology 22*: 702-709

Tateishi J, Kuroda S, Saito A, Otsuki S (1973) Experimental myelo-optic neuropathy induced by Clioquinol. *Acta Neuropathol 24*: 304-320

Terao S, Li M, Hashizume Y, Osano Y, Mitsuma T, Sobue G (1997) Upper motor neuron lesion in stroke patients do not induce antegrade transneuronal degeneration in spinal anteior horn cells. *Stroke 28*: 2553-2556

Terao S, Sobue G, Hashizume Y, Mitsuma T, Takahashi A (1994) Disease-specific patterns of neuronal loss in the spinal ventral horn in amyotrophic lateral sclerosis, multiple system atrophy and X-linked recessive bulbospinal neuronopathy, with special reference to the loss of small neurons in the intermediate zone. *J Neurol 241*: 196-203

Ueki K (1966) *Hemispherectomy in the human with special reference to the preservation of function*. In Tokizane T, Shade JP (eds) Progress in brain research, 21B, Correlative neuroscience, Part B: Clinical studies. Elsevier, Amsterdam, pp 285-338

Yuasa R (1970) Amyotrophic lateral sclerosis with dementia. *Clin Neurol 10*: 569-577

For Expert 認知症性疾患のPET画像

脳血管性認知症

日本医科大学第2内科講師 山崎 峰雄
日本医科大学千葉北総病院脳神経センター 三品 雅洋
東京都老人総合研究所ポジトロン医学研究施設診療所長 石井 賢二
日本医科大学第2内科教授 片山 泰朗

脳血管性認知症(VaD)とは脳血管障害(CVD)病変によって引き起こされた認知症のことで，アルツハイマー病(AD)と併せて老年期認知症の大部分を構成する。しかし，臨床実地上，特に高齢者では両者の合併も多く，画像上脳梗塞を認める場合には診断を下すことが困難なことも多い。本稿ではNINDS-AIREN診断基準で典型的と考えられるVaD症例の画像を提示することで，VaDの特徴を明らかにする。

神経放射線学的所見をもとに多く行われている分類では，皮質を中心に梗塞巣が多発する多発梗塞性認知症(MID)，皮質下血管性認知症［これには白質病変主体のビンスワンガー型認知症と多発ラクナ(視床，脳弓，尾状核，淡蒼球，内包前脚など)が含まれる］と，海馬や視床など特定の領域が障害されることによって生じるstrategic infarct dementiaに分けられる。

多発梗塞性認知症(☞写真1参照)

皮質梗塞が多発することで生じるVaDは，皮質梗塞の度に局所症状を生じるとともに，知的機能が低下していく。MIDでは，ラクナ梗塞が基底核や深部白質に多発する皮質下血管性認知症と重複して認められることが多く，実際には両者を全く別物として取り扱うことは困難である。

多発性ラクナ梗塞による脳血管性認知症(☞写真2参照)

皮質病変で生じるMIDが病変部位の巣症状を呈するのに対して，多発ラクナによって生じるVaDは巣症状に乏しく，軽度の健忘，歩行障害，失禁，仮性球麻痺を呈することが多い。基本的にはビンスワンガー型認知症と同様にsmall arteryの障害で発症進行するVaDであり，病態はビンスワンガー型認知症とオーバーラップする点も多い。頭部MRI上は基底核や放線冠などにラクナが多発し，PETでは小梗塞部を超えた広範囲でびまん性に血流低下がみられることが多い。

ビンスワンガー型認知症(☞写真3参照)

ビンスワンガー型認知症では，頭部MRI T_2強調画像できわめて特徴的な，側脳室周囲の高信号域が観察される。1987年にHachinskiが側脳室周囲のT_2高信号域をleukoaraiosisと呼称することを提唱したが，このleukoaraiosisはVaDでのみ観察されるわけではなく，ADや認知症の明らかでない高齢者にも観察されることがあり，leukoaraiosisと表現される変化すべてが病的変化とは限らない点には注意を要する。一般に深部白質病変は①拡張したVirchow-Robin腔を示す古典的な神経病理学的な概念“état criblé”，②小梗塞巣とその周囲のグリオーシス，および③梗塞巣とは異なる「髄鞘染色での淡明化」の3種類の異なる病態からなると考えられるが，ビンスワンガー型認知症でみられる白質変化は，②および③の変化が主体で，前頭葉～放線冠～後頭葉にまで連続的に観察される深部白質病変である。PETを用いたビンスワンガー型認知症における酸素代謝の検討では，大脳皮質および白質で脳血流量(CBF)・酸素消費量($CMRO_2$)がともに低下しており，さらに酸素摂取率(OEF)の増加も認められず，虚血との因果関係ははっきりしない。しかし，神経学的に明らかな異常を認めないものの高度の深部白質病変部を示す症例や多発性脳梗塞に合併した白質病変例の検討では灰白質の脳血流は低下しているが，$CMRO_2$は保たれ，OEFは有意に増加している症例もあり，認知症が明らかではない段階での白

質病変部の成因に虚血性変化が深く関与している可能性が示されている。白質病変の脳循環代謝所見に関しては必ずしも一定の見解があるわけではないが，最近では虚血が病巣形成に一次的に関与することを示唆する報告が多い。

Strategic infarct dementia (☞写真4，5参照)

海馬，角回，帯状回前部などの皮質病変や視床，脳弓，尾状核，淡蒼球，内包前脚などの皮質下病変など，特定の部位に血管障害を生じて発症するstrategic infarct dementiaは，実際の臨床の場で遭遇することはMIDや皮質下血管性認知症のように多くはないが，臨床的エピソードと認知症の発症の因果関係が最も明瞭であり，診断も比較的容易である。PETでは梗塞巣より広範囲に脳血流，$CMRO_2$やブドウ糖代謝の低下を認めることが多く，神経線維連絡離断によるremote effectを端的に反映しており，診断上有意義である。

'07年04月18日(水) 15時22分 発信:042325[illegible]4
発信:三重大学医学部 図書館
R:264 P.02/03

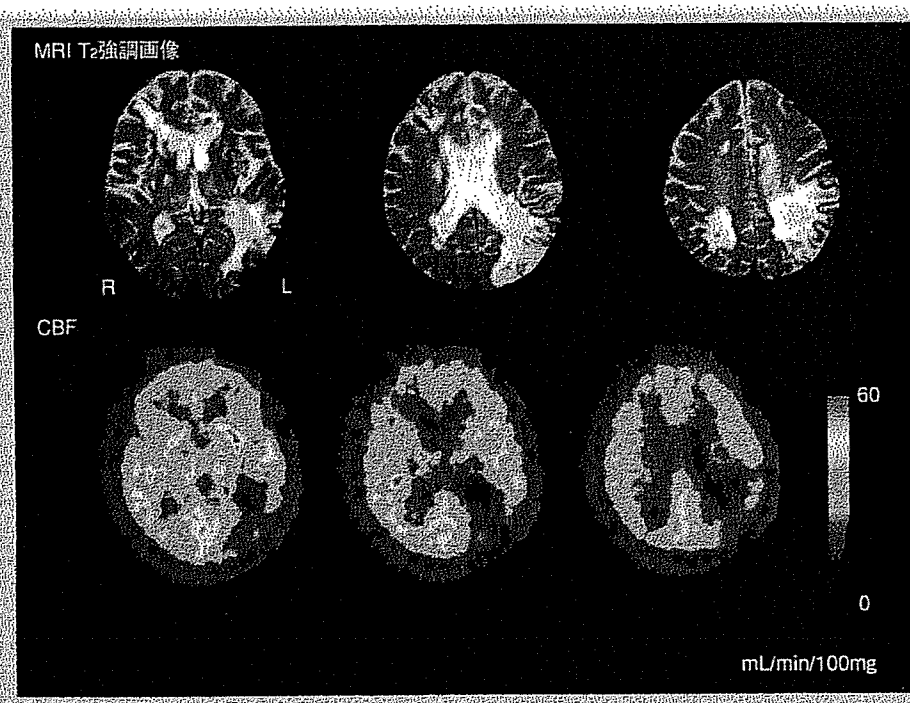

写真1

皮質に多発した脳梗塞に伴う認知症のMRI，PET画像

61歳男性。構音障害，歩行障害，麻痺，認知症が段階的に増悪した。MRI T_2強調画像では両側大脳基底核，放線冠，視床などにラクナ梗塞が多発している。[^{15}O]CO_2PETでは，前頭葉を中心に大脳皮質の脳血流の低下を認める。

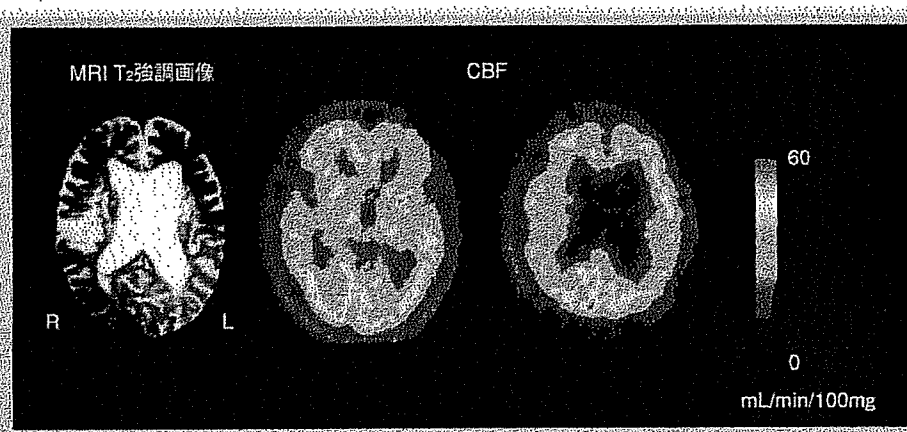

写真2

皮質下に多発した脳梗塞に伴う認知症のMRI，PET画像

75歳男性。構音障害，歩行障害，麻痺，認知症が段階的に増悪した。MRI T_2強調画像では両側大脳基底核，放線冠，視床などにラクナ梗塞が多発している。[^{15}O]CO_2PETでは，大脳基底核・視床と前頭葉を中心に大脳皮質の脳血流の低下を認める。

次回ページ数は 03 ページです。

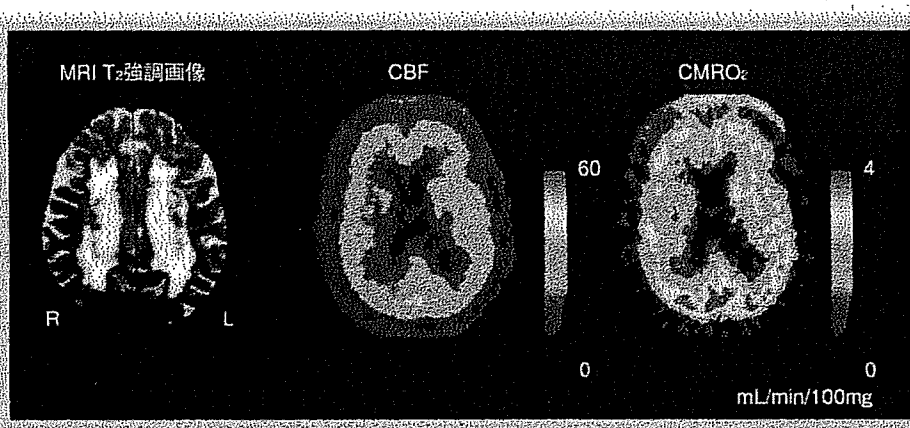

写真 3

ビンスワンガー型認知症の MRI，PET 画像

約 5 年の経過で健忘症状，計算力の低下などが徐々に進行した。MRI T_2強調画像では脳室周囲の白質にびまん性の高信号域を認める。[^{15}O]CO_2および[^{15}O]O_2PET では，前頭葉を中心に大脳皮質の脳血流と酸素代謝の低下を認める。

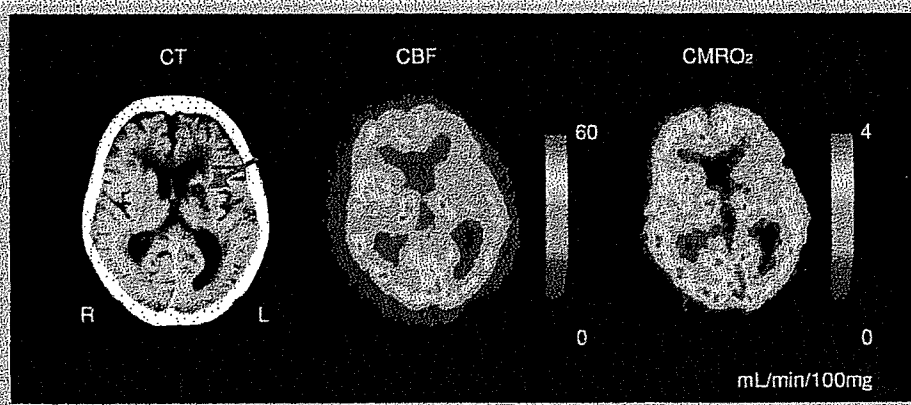

写真 4

左尾状核梗塞の CT，PET 画像

79歳女性，右利き。脳梗塞後に，発動性の低下と健忘症状を中心とする認知症を呈した。頭部 CT では，尾状核頭部，内包膝部，内包後脚に及ぶ脳梗塞を認める。[^{15}O]CO_2および[^{15}O]O_2PET では，左前頭葉と基底核部の脳血流と酸素代謝の低下を認める。

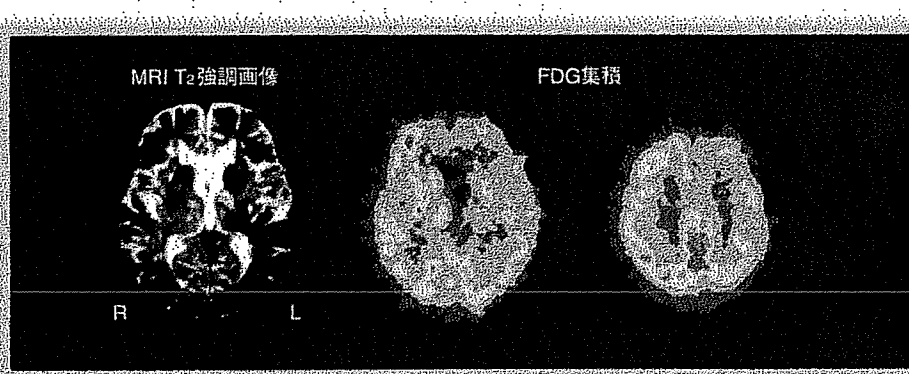

写真 5

左視床梗塞の MRI，PET 画像

82歳女性，右利き。脳梗塞発症後は傾眠傾向，慢性期には重度の健忘症を残した。MRI T_2強調画像で左視床前部の脳梗塞を認める。[^{18}F]FDG-PET では，左視床と左前頭葉～側頭葉～頭頂葉に広範なブドウ糖代謝の低下を認めた。

'07年04月18日(水) 15時22分 発信:042325c_J4
発信:三重大学医学部 図書館
R:264 P.03/03

Brain Advance Access published April 17, 2007

doi:10.1093/brain/awm065 *Brain* (2007) Page 1 of 9

TDP-43 is deposited in the Guam parkinsonism–dementia complex brains

Masato Hasegawa,[1,*] Tetsuaki Arai,[2,*] Haruhiko Akiyama,[2] Takashi Nonaka,[1] Hiroshi Mori,[3] Tomoyo Hashimoto,[4] Mineo Yamazaki[4] and Kiyomitsu Oyanagi[4]

[1]Departments of Molecular Neurobiology and [2]Psychogeriatrics, Tokyo Institute of Psychiatry, Tokyo Metropolitan Organization for Medical Research, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156-8585, [3]Department of Neuroscience, Osaka City University School of Medicine, 1-4-3 Asahimachi, Abenoku, Osaka 545-8585 and [4]Department of Neuropathology, Tokyo Metropolitan Institute for Neuroscience, Tokyo Metropolitan Organization for Medical Research, 2-6 Musashidai, Fuchu, Tokyo 183-8526, Japan

*These authors contributed equally to this work.

Correspondence to: Masato Hasegawa and Tetsuaki Arai, Tokyo Institute of Psychiatry, Tokyo Metropolitan Organization for Medical Research, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156-8585, Japan
E-mail: masato@prit.go.jp

TDP-43, a nuclear factor that functions in regulating transcription and alternative splicing, was recently identified as a component of the ubiquitin-positive, tau-negative inclusions specific for frontotemporal lobar degeneration (FTLD-U) and amyotrophic lateral sclerosis (ALS). In the present study, we carried out immunohistochemical and biochemical analyses of brains of Guamanians with the parkinsonism–dementia complex (G-PDC) using anti-TDP-43, anti-tau and anti-ubiquitin antibodies. Immunohistochemistry with anti-TDP-43 antibodies revealed various types of positive structures in the frontotemporal and hippocampal regions of G-PDC cases. Most of these structures were negative for tau. By immunoblot analysis with the TDP-43 antibody, an abnormal 45 kDa band, as well as a diffuse staining throughout the gel, was detected in the sarkosyl-insoluble fractions of G-PDC brains. Dephosphorylation has shown that abnormal phosphorylation takes place in the accumulated TDP-43 seen in FTLD-U and ALS. These results suggest that accumulation of TDP-43 is a common process in certain neurodegenerative disorders, including FTLD-U, ALS and G-PDC.

Keywords: frontotemporal lobar degeneration; amyotrophic lateral sclerosis; ubiquitin; tau; inclusion

Abbreviations: ALS = amyotrophic lateral sclerosis; FTLD-U = frontotemporal lobar degeneration; G-PDC = Guam parkinsonism–dementia complex; NCI = neuronal cytoplasmic inclusions; NII = neuronal intranuclear inclusions

Received January 29, 2007. Revised March 7, 2007. Accepted March 8, 2007

Introduction

Ubiquitin-positive, tau-negative neuronal cytoplasmic inclusions (NCI) were first described in patients with amyotrophic lateral sclerosis (ALS) (Okamoto *et al.*, 1991). They were subsequently found in patients with frontotemporal lobar degeneration with motor neuron disease (FTLD-MND) (Okamoto *et al.*, 1992; Wightman *et al.*, 1992), and FTLD with MND-type inclusions but without MND (FTLD-MND-type) (Bergmann *et al.*, 1996; Jackson *et al.*, 1996; Iseki *et al.*, 1998). FTLD-MND and FTLD-MND-type are referred to as FTLD-U (Mackenzie *et al.*, 2006*a*). In some FTLD-U cases, neuronal intranuclear inclusions (NII) have been described (Woulfe *et al.*, 2001; Mackenzie and Feldman, 2003, 2005; Forman *et al.*, 2006), especially in those cases with autosomal dominant inheritance associated with mutations in progranulin gene (Baker *et al.*, 2006; Boeve *et al.*, 2006; Cruts *et al.*, 2006; Gass *et al.*, 2006; Huey *et al.*, 2006; Mackenzie *et al.*, 2006*b*; Masellis *et al.*, 2006; Mukherjee *et al.*, 2006; Pickering-Brown *et al.*, 2006; Snowden *et al.*, 2006) and in valosin-containing protein gene (Guyant-Marechal *et al.*, 2006). Recently, TDP-43, a ubiquitously expressed nuclear protein, was identified as the major component of the ubiquitin-positive inclusions in these disorders (Arai *et al.*, 2006; Neumann *et al.*, 2006, 2007; Davidson *et al.*, 2007). They include NCI, NII and dystrophic neurites in the hippocampus and frontotemporal cortex in cases of FTLD-U, and skein-like inclusions in the spinal cord in FTLD-MND and ALS cases.

© The Author (2007). Published by Oxford University Press on behalf of the Guarantors of Brain. All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org

The Guam parkinsonism–dementia complex (G-PDC) and amyotrophic lateral sclerosis (G-ALS) are neurodegenerative disorders of Chamorro residents of Guam. They are clinically characterized by either progressive cognitive impairment with extrapyramidal signs or motor neuron dysfunctions. G-PDC is characterized by severe neuronal loss and abundant neurofibrillary tangles (NFTs) in the temporal and frontal cortex, basal ganglia, thalamus and brainstem with a virtual absence of senile plaques (Hirano *et al.*, 1961; Nakano and Hirano, 1983; Oyanagi *et al.*, 1994*a*). Although environmental factors such as toxins in cycad seeds and minerals in the soils and drinking water have been implicated (Cox *et al.*, 2003; Hermosura *et al.*, 2005; Oyanagi *et al.*, 2006), the aetiology and the pathogenesis remain unknown. G-PDC exhibits similarities to FTLD-U in terms of the frontotemporal atrophy and the occurrence of ubiquitin positive inclusions in the dentate gyrus (Oyanagi *et al.*, 1994*b*; Ikemoto *et al.*, 1997). In the present study, we show that various types of tau-negative, TDP-43-positive structures are present in G-PDC brains. Immunoblot analysis revealed that hyperphosphorylated TDP-43 is deposited in the sarkosyl-insoluble fractions of G-PDC brains. These results suggest that a common pathogenic mechanism through conformational changes in TDP-43 may be associated with the neurodegeneration in FTLD-U, ALS and G-PDC.

Materials and methods

Materials

Brains from six cases of clinically and neuropathologically diagnosed G-PDC, two Japanese cases with Alzheimer's disease (AD) and two non-PDC non-ALS Guamanian controls were employed in this biochemical and immunohistochemical studies. Paraffin-embedded sections from three other G-PDC cases were also used for immunohistochemistry. The age, sex, brain weight, brain regions examined and diagnosis are given in Table 1.

Immunohistochemical analysis

Small blocks of frontal regions were dissected at autopsy or from fresh frozen brain samples and fixed overnight in 10% formalin neutral buffer solution (Wako). Blocks were cut on a vibratome at 50 μm thickness. The free-floating sections were treated with 3% H_2O_2/methanol for 30 min to block the internal peroxidase and incubated in 0.5% Triton X-100/PBS for 30 min. After blocking with 10% calf serum/PBS, sections were immunostained overnight with two well-characterized antibodies to TDP-43: a polyclonal (10782-1-AP, ProteinTech Group Inc., Chicago, IL; 1 : 3000) and a monoclonal (2E2-D3, Abnova Corporation, Taipei, Taiwan; 1 : 1000). Two monoclonal antibodies to phosphorylated tau (AT8; Innogenetics, Gent, Belgium, 1 : 1000 and PHF-1; generous gift from Dr P. Davies, 1 : 2000), a polyclonal and a monoclonal antibody to ubiquitin [Z0458; Dako, Denmark; 1 : 3000 and DF2 (Mori *et al.*, 1987); 1 : 200], and a monoclonal antibody to GFAP (6F2, DakoCytomation, Glostrup, Denmark; 1 : 100) were also used. For analysis of unfixed materials, Triton-X-insoluble pellets prepared from frozen brains (see below) were smeared on PLL-coated slide glasses and used. For immunostaining of the hippocampal region from G-PDC cases, 10% formalin-fixed and paraffin-embedded blocks were sectioned at 6 μm and stained with 10782-1-AP (1 : 300) and 2E2-D3 (1 : 100). Following treatment with the appropriate secondary antibody, labelling was detected using the avidin-biotinylated HRP complex (ABC) system coupled with a diaminobenzidine reaction to yield a brown precipitate. Pretreatment of tissues by autoclaving in 10 mM sodium citrate buffer for 10 min at 120°C was needed for staining with 2E2-D3 in paraffin-embedded sections.

Double-label immunofluorescence was performed using FITC and TRITC conjugated secondary antibodies. The sections were examined with a confocal laser microscope (LSM5 PASCAL; Carl Zeiss MicroImaging gmbh, Jena, Germany).

Immunoblot analysis

The sarkosyl-insoluble fractions were prepared as described (Arai *et al.*, 2006) with slight modifications. Frozen temporal or frontal cortex (0.5 g) from six cases of G-PDC, two cases with AD and two controls were homogenized in 10 volumes (5 ml) of buffer A (10 mM Tris–HCl, pH 7.5 containing 1 mM EGTA, 10% sucrose and 0.8 M NaCl). After addition of another 5 ml of buffer A containing 2% Triton-X100, the homogenate was incubated for 30 min at 37°C and spun at 100 000 × *g* for 30 min at 25°C. The pellet was homogenized in 10 volume of buffer A containing 1% Sarkosyl, incubated for 30 min at 37°C and spun at 100 000 × *g* for 30 min at 25°C. The sarkosyl-insoluble pellet was homogenized in 4 volumes of buffer A containing 1% CHAPS and spun at 100 000 × *g* for 20 min.

Table 1 Description of the subjects

Case no.	Age	Sex	Brain weight (g)	Regions	Clinical diagnosis	Neuropathological diagnosis
1(CON1)	68	F	990	Frontal	Diabetes, heart failure	Normal-aged brain
2(CON2)	43	F	1370	Frontal	Burn	Slight edematous brain
3(G-PDC1)	69	F	1050	Frontal	PDC	PDC
4(G-PDC2)	69	M	875		PDC	PDC
5(G-PDC3)	52	F	1025	Frontal	PDC, myocardiac infarct	PDC
6(G-PDC4)	52	M	1025	Frontal	PDC	PDC
7(G-PDC5)	56	M	1235	Frontal	PDC, pneumonia	PDC
8(G-PDC6)	56	F	875	Frontal	PDC	PDC
9(G-PDC7)	51	F	850	Hip, temp	PDC	PDC
10(G-PDC8)	64	M	1290	Hip, Temp	PDC	PDC
11(G-PDC9)	57	F	1150	Hip, Temp	PDC	PDC
12(AD1)	82	F	670	Temp	AD	AD
13(AD2)	75	F	730	Temp	AD	AD

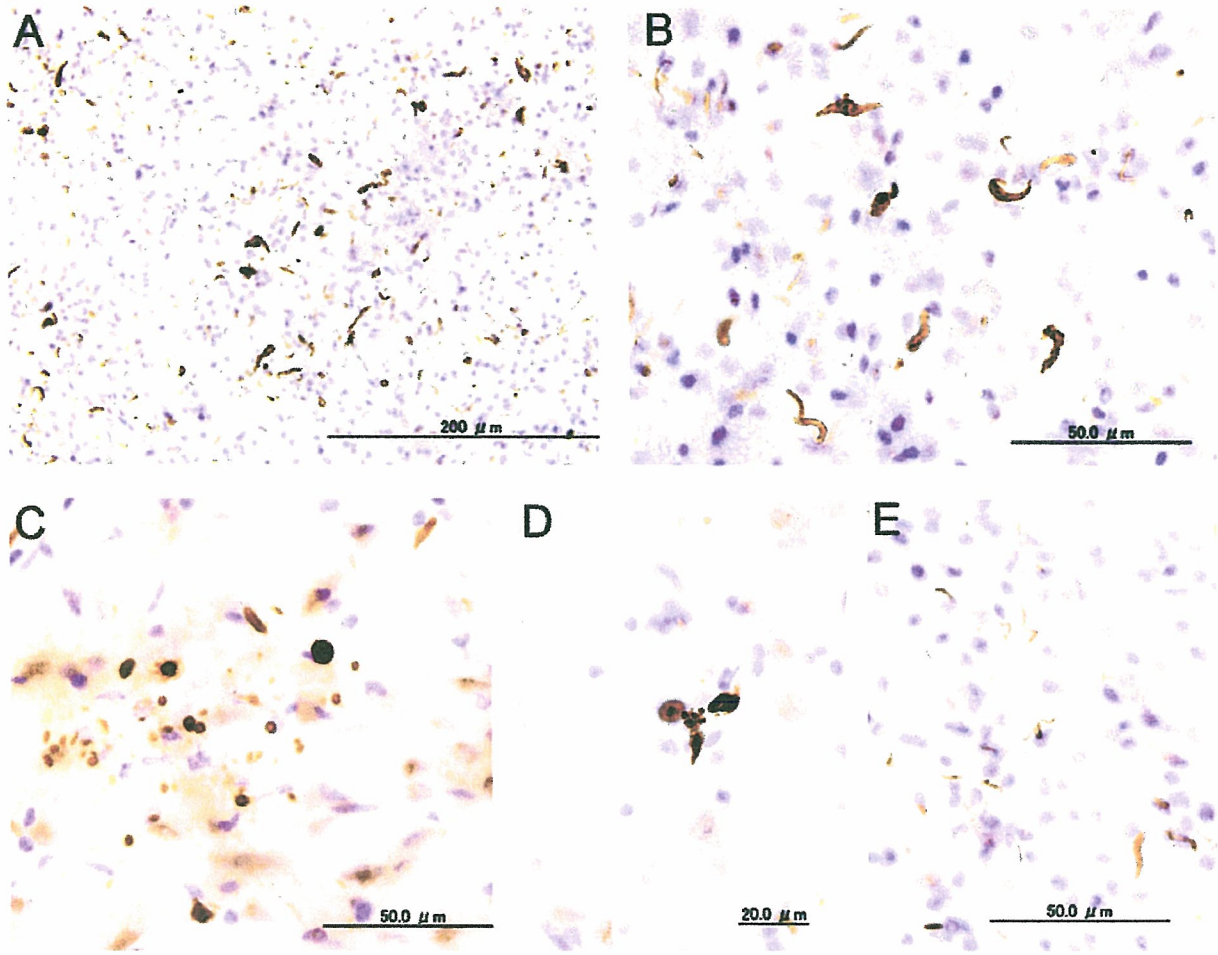

Fig. 1 Immunostainings of the frontal lobes of the G-PDC cases with the polyclonal antibody to TDP-43. **(A)** Numerous TDP-43 positive structures are observed in the white matter. **(B)** High magnification of coiled body-like or worm-like inclusions in the white matter. **(C)** Round-shape and dot-like TDP-43 positive structures in the grey matter. **(D)** Bud-shaped inclusions in the white matter. **(E)** Thin thread-like TDP-43 positive structures in the grey matter. The sections are counterstained with haematoxylin.

The pellet was sonicated in 0.8 volume of 7 M guanidine hydrochloride, dialysed against 30 mM Tris–HCl (pH 7.5), cleared by brief spin at 15 000 rpm and used for immunoblotting. For dephosphorylation, the sample was incubated with Lambda protein phosphatase as described (Arai *et al.*, 2006). For the analysis of proteins in white matter and grey matter, sarkosyl-insoluble proteins before and after dephosphorylation were prepared as described (Yamazaki *et al.*, 2005). Samples were run on SDS–PAGE using 10% polyacrylamide gel and the proteins were electrotransferred onto a polyvinylidene difluoride membrane, probed with the antibody to TDP-43, 10782-1-AP (1 : 3000) and the antibody to tau, HT7 (Innogenetics, Gent, Belgium; 1 : 3000), and detected as described (Arai *et al.*, 2006).

Results

Immunohistochemistry of G-PDC

Immunohistochemistry of G-PDC cases with the anti-TDP-43 antibodies revealed various types of inclusions. In vibratome sections of the frontal lobe, TDP-43 positive structures with various shapes (coiled-body-like, round-shape, dot-like, bud-shaped and thin thread-like) were present in both grey matter and white matter (Fig. 1A–E). These were similarly stained with both the polyclonal and monoclonal antibodies to TDP-43. The frequency of the inclusions varied from case to case, but they were detected in all six G-PDC cases examined. The number of TDP-43 positive structures in the white matter was much greater than that of ubiquitin positive ones in each G-PDC case. No such TDP-43-positive inclusions were observed on vibratome brain sections of the AD cases and the controls.

Various types of structures were also observed in paraffin sections of the hippocampal region of G-PDC cases (Fig. 2). In the granular cells of the dentate gyrus, numerous NCI (A, B) and a few NII (C) were positive for TDP-43. The nuclear staining for TDP-43 was reduced in neurons with cytoplasmic inclusions compared to that in non-affected

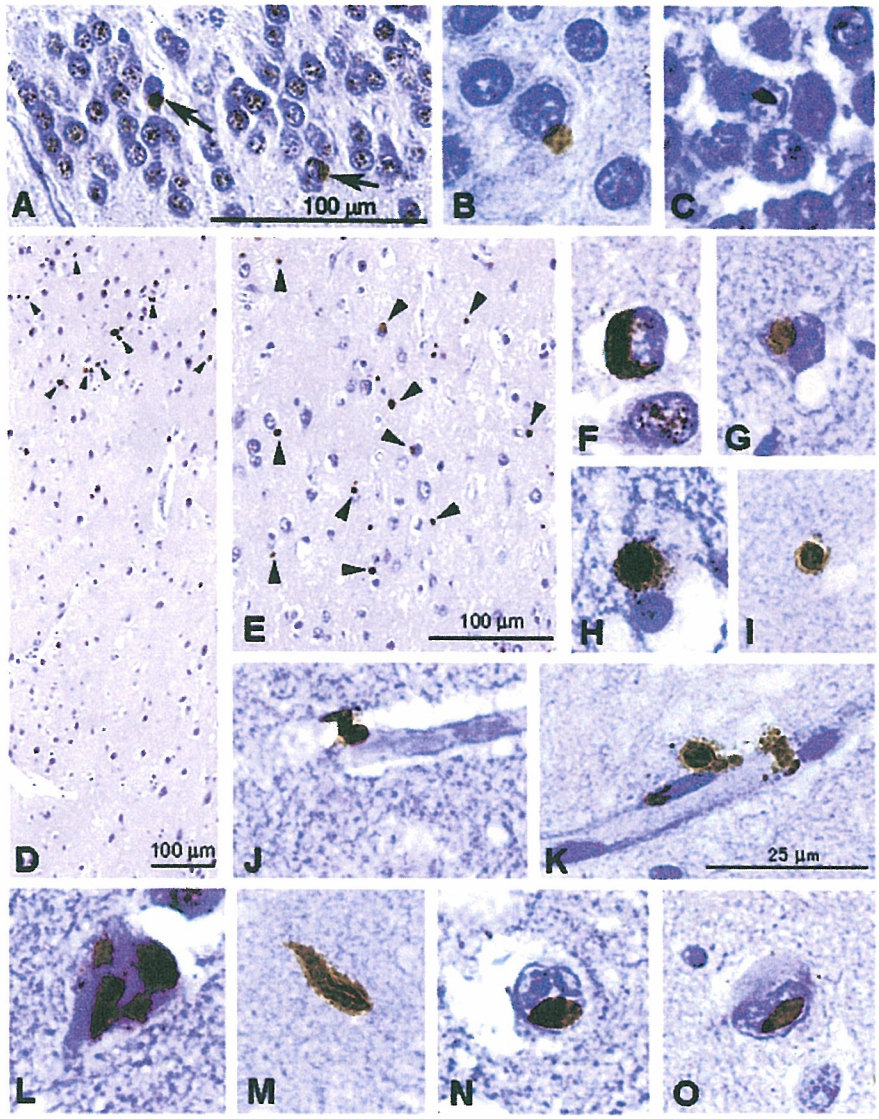

Fig. 2 Immunostainings of the hippocampus and the entorhinal cortex of the G-PDC cases. The polyclonal (**A**, **C**, **D**, **E**, **F**, **H**, **J**, **L** and **N**) and the monoclonal (**B**, **G**, **I**, **K**, **M** and **O**) antibodies to TDP-43 were used. In the granular cells of the dentate gyrus, frequent cytoplasmic inclusions with round or crescent shapes (arrows in **A**, **B**) and a few intranuclear inclusions (**C**) are observed. Note the absence of nuclear staining in neurons with cytoplasmic inclusions (arrows in **A**) compared to the granular staining of nuclei in nonaffected neurons. In the superficial layer of the entorhinal cortex, robust TDP-43 positive structures are seen (arrowheads in **D**, **E**). They include neuronal cytoplasmic inclusions (**F**, **G**), round structures (**H**, **I**), those associated with small blood vessels (**J**, **K**) or NFTs (**L**), the thread-like structure (**M**) and the intranuclear inclusions (**N**, **O**). The sections are counterstained with haematoxylin. Except for **A**, **D** and **E**, the same magnification is used as shown in **K**.

neurons as previously reported (Neumann *et al.*, 2006; Davidson *et al.*, 2007). In the parahippocampal cortex, TDP-43 positive structures tended to be more abundant in the superficial layer than in the deep layer (D, E). In addition to NCI (F, G), numerous round structures with similar size to glial nuclei were seen (H, I). There were also structures with round, dot-like or granular shape associated with small vessels (J, K). Occasional immunopositive structures associated with NFT were observed (L). Thread-like structures (M) and NII (N, O) were occasionally seen. All of these structures were positive for both the polyclonal and monoclonal antibodies to TDP-43, although the immunoreactivity was stronger with the polyclonal than with the monoclonal.

Nuclear TDP-43 staining varied much from case to case even in controls as previously reported (Davidson *et al.*, 2007). Furthermore, within cases showing TDP-43 nuclear staining, this was not evenly present in all nuclei, namely, a

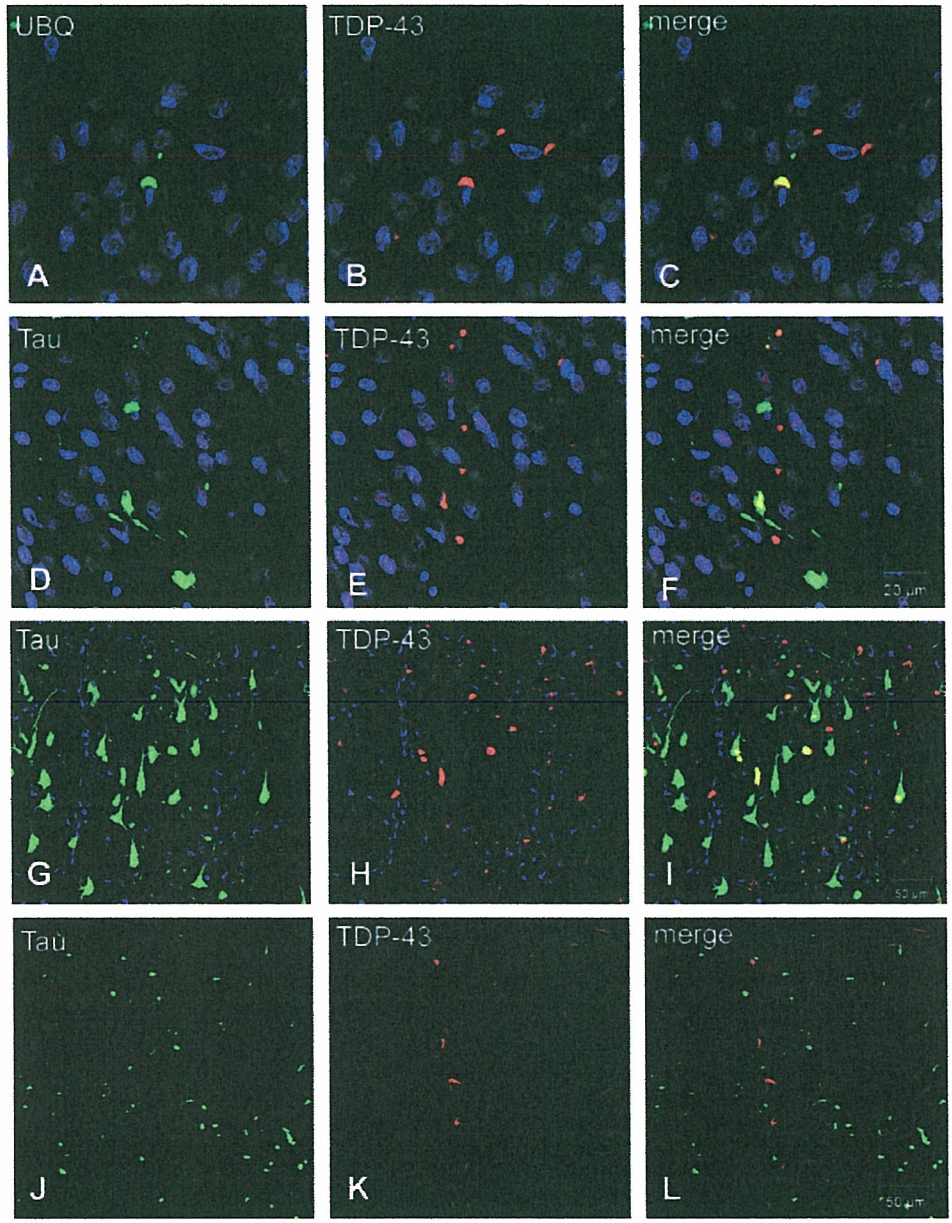

Fig. 3 Double-label immunofluorescence of the G-PDC cases with anti-ubiquitin (DF2 in **A**) or anti-tau (PHF-1 in **D** and **G**; AT8 in **J**) and anti-TDP-43 (**B**, **E**, **H** and **K**). Merged images are shown in **C**, **F**, **I** and **L**. In the granular cells in the dentate gyrus, a crescent inclusion shows colocalization (yellow fluorescence in **C**) of ubiquitin (green fluorescence in **A**) and TDP-43 (red fluorescence in **B**). Structures which are ubiquitin positive and TDP-43 negative (green in **C**) or ubiquitin negative and TDP-43 positive (red in **C**) are also observed. In the same region, colocalization of tau and TDP-43 are seen in some structures (yellow in **F**), but many TDP-43 positive structures are negative for tau (red in **F**). In the temporal cortex (**G**–**I**), most of the structures stained for tau (**G**) and those stained for TDP-43 (**H**) are independent, although colocalization of tau and TDP-43 is observed in part of NFTs (**I**). In the white matter of the frontal lobe, the distribution of structures positive for tau (**J**) and of those positive for TDP-43 (**K**) are virtually independent (**L**). In **A**–**I**, the cell nuclei are stained with TO-PRO-3 (Invitrogen, Tokyo, Japan), producing a blue colour. Scale bars are shown in **C**, **F**, **I** and **L**.

mix of TDP-43 positive and negative nuclei was seen (data not shown).

Figure 3 shows double immunofluorescence staining with anti-ubiquitin (A) or anti-tau (D, G and J) and anti-TDP-43 antibodies (B, E, H and K). Some NCI in the dentate gyrus were positive for both ubiquitin and TDP-43 (C). Most TDP-43 positive structures in the dentate gyrus and the temporal cortex were negative for tau, although

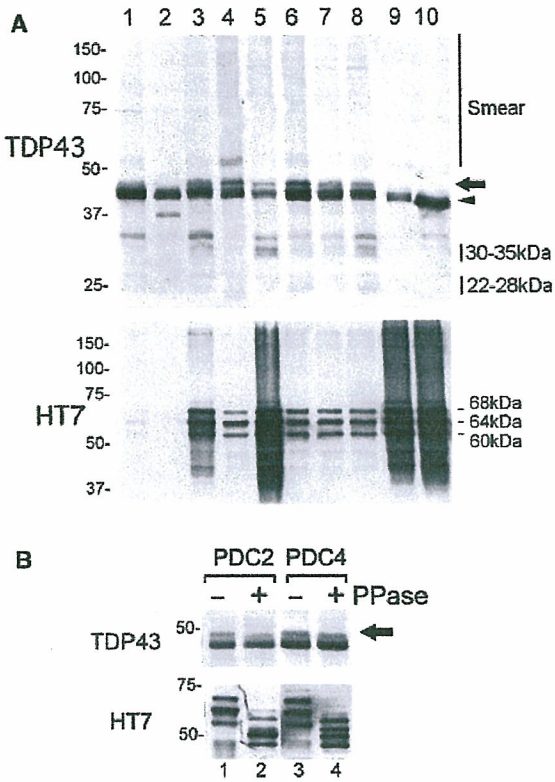

Fig. 4 Immunoblotting of the sarkosyl-insoluble fraction with anti-TDP-43 antibody (upper panel) and HT7 (lower panel). **(A)** Lanes 1 and 2, control (cases 1 and 2); lanes 3–8, G-PDC (cases 3–8); lanes 9 and 10, AD (cases 12 and 13). A positive band of 43 kDa (arrow head) is commonly seen in all cases, while an additional band of 45 kDa (arrow) as well as diffuse smear stainings are observed only in G-PDC brains (lanes 3–8). Several bands with 30–35 or 22–28 kDa are evident in five of six G-PDC cases (lanes 3, 5–8), although weak bands at 30–35 kDa are visible in a control case (lane 1) and in an AD case (lane 10). The triplet bands of 68, 64 and 60 kDa, typical for hyperphosphorylated tau in AD, and additional smear immunoreactivities are detected in all G-PDC cases examined. However, the intensities are much weaker than those found in two typical AD cases. **(B)** Immunoblotting of sarkosyl-insoluble fractions from the G-PDC cases before and after dephosphorylation. Partial mobility shift of the 45 kDa TDP-43-positive band (arrow) is observed after dephosphorylation of samples with lambda protein phosphatase. Mobility shifts in the PHF-tau bands are also detected with the HT7 antibody.

parts of NFTs were positive for TDP-43 (F, I). Virtually, no colocalization of tau and TDP-43 was observed in the white matter of the frontal lobe (L).

Immunoblot analysis of G-PDC

Figure 4 illustrates the results of immunoblotting of sarkosyl-insoluble fractions from two controls, six G-PDC cases and two AD cases with an anti-TDP-43 antibody or a phosphorylation independent anti-tau antibody HT7. By immunoblotting with HT7, the three major abnormal tau bands of 60, 64 and 68 kDa were detected in all G-PDC cases (Fig. 4A, lower panel). Although the pattern was indistinguishable to that seen in AD brains, the intensities of these tau bands in G-PDC cases were apparently weaker than those in two AD cases. By immunoblotting with the anti-TDP-43 antibody, a major band of 43 kDa corresponding to full-length TDP-43 was seen in all samples examined. In addition to the 43 kDa band, an abnormal 45 kDa band was observed in all G-PDC cases examined (lanes 3–8) which was not seen in the two controls (lanes 1 and 2) and two AD cases (lanes 9 and 10) (Fig. 4A, upper panel). Moreover, a diffuse smear staining was more prominent in G-PDC cases than in controls and AD cases (Fig. 4A, upper panel). Several positive bands of 30–35 or 22–26 kDa were evident in five of six G-PDC cases (lanes 3, 5–8), although faint bands at 30–35 kDa were visible in a control case (lane 1) and in an AD case (lane 10). After dephosphorylation of the samples with lambda protein phosphatase, a partial shift of the 45 kDa band was observed (Fig. 4B), suggesting that phosphorylation takes place in the full-length TDP-43. Similar results were obtained in the experiments with alkaline phosphatase at 37°C for 2 h (data not shown).

In order to confirm the deposition of TDP-43 in the white matter biochemically, the grey and white matters of two G-PDC cases were separated from each other macroscopically and the sarkosyl-insoluble fractions were immunoblotted with the anti-TDP-43 antibody. The abnormal 45 kDa band and smear stainings were detected in both the grey matter and the white matter (Fig. 5) and the intensities were slightly stronger in the sarkosyl-insoluble fractions from the white matter than in those from the grey matter in both cases (Fig. 5, upper panel). In contrast, immunoreactivities of tau bands detected with HT7 were much stronger in the grey matter than in the white matter in case PDC1 and similar deposits were detected in case PDC5 (Fig. 5, lower panel).

Discussion

TDP-43 is thought to function in transcriptional repression and exon skipping (Buratti *et al.*, 2001; Wang *et al.*, 2002; Ayala *et al.*, 2005; Buratti *et al.*, 2005). It was first identified as a protein capable of binding to a TAR DNA of the human immunodeficiency virus 1 (HIV-1) long terminal repeat region (Ou *et al.*, 1995). TDP-43 interacts with several nuclear ribonucleoproteins including heterogeneous nuclear RNP A/B and survival motor neuron protein, inhibiting alternative splicing (Wang *et al.*, 2002; Buratti *et al.*, 2005). The physiological function of TDP-43 in brain cells has not yet been determined. The present study showed numerous TDP-43 positive, tau-negative structures with various types of morphologies in white and grey matters of G-PDC brains.

Ubiquitin-positive inclusions have already been described in the granular cells of the hippocampal dentate gyrus in G-PDC cases. Most of these ubiquitin-positive inclusions

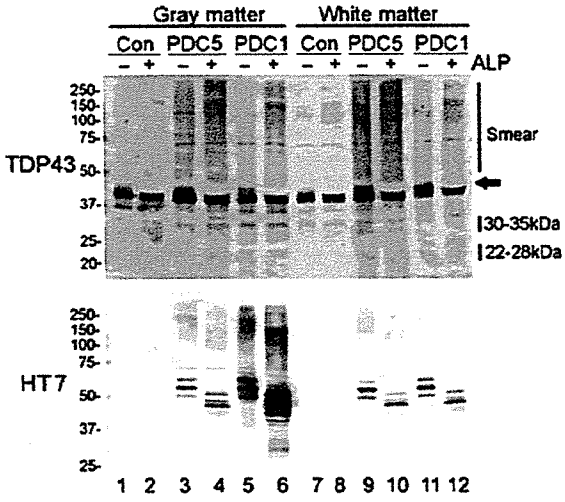

Fig. 5 Immunoblotting of the sarkosyl-insoluble fractions from grey or white matters of G-PDC cases with anti-TDP-43 antibody and HT7 before and after dephosphorylation. Lanes 1, 2, 7 and 8, control (case 2); lanes 3, 4, 9 and 10, G-PDC5 (case 7); lanes 5, 6, 11 and 12, G-PDC1 (case 3). Sarkosyl-insoluble fractions from grey matter (lanes 1–6) and white matter (lanes 7–12) before (lanes 1, 3, 5, 7, 9 and 11) and after (lanes 2, 4, 6, 8, 10 and 12) treatment with alkaline phosphatase (ALP) are immunoblotted with the TDP-43 antibody (upper panel) or HT7 (lower panel). The 45 kDa TDP-43 band (arrow) and the smeared stainings, which are not seen in control cases, are detected in both grey and white matter of the G-PDC cases. In both G-PDC cases, the intensities of the immunoreactivities for TDP-43 are stronger in the white matter than in the grey matter. In contrast, abundant tau is detected in the grey matter of the G-PDC1 case but less tau is observed in the white matter of G-PDC1 and in grey and white matter of G-PDC5 cases.

were reported to be tau-positive (Ikemoto *et al.*, 1997). In the present study, however, we showed that most of the TDP-43 positive inclusions in the granular cells in the hippocampus of G-PDC cases were negative for tau. These findings suggest that the occurrence of TDP-43 positive and tau negative neuronal inclusions in the hippocampus is common to FTLD-U, ALS and G-PDC. Some of the TDP-43-positive inclusions were also immunoreactive for ubiquitin, suggesting that partial ubiquitination may occur on the deposited TDP-43, as seen on tau in AD or α-synuclein in DLB.

On the other hand, the morphology and the distribution of some TDP-43 positive structures in the G-PDC cases seem to be different from those reported in FTLD-U cases (Arai *et al.*, 2006; Neumann *et al.*, 2006; Davidson *et al.*, 2007). For instances, in the frontal region, TDP-43 positive structures with various shapes were more pronounced in the white matter than in the grey matter in G-PDC cases, whereas NCI and dystrophic neurites were prominent in the superficial cortical layer in FTLD-U cases. The TDP-43 positive round structures (Fig. 2H and I) and those associated with small vessels (Fig. 2J and K) found in the parahippocampal and temporal cortices of G-PDC cases in this study have not so far been described in FTLD-U cases. These structures might not be considered corpora amylacea, based on the following points. First, the double immunofluorescence staining with antibodies to GFAP and TDP-43 showed that these TDP-43 positive structures were negative for GFAP (data not shown). Second, the laminar distribution of those was different from that of corpora amylacea, which is reported to be common in the surface glial feltwork in the outer part of layer I covering the cortex (Cavanagh, 1999). Finally, pretreatment of the section with 1 N KOH, which is reported to reduce the staining of corpora amylacea (Cavanagh, 1999), did not affect the staining of these structures with anti-TDP-43 antibodies (data not shown). It also seems unlikely that these TDP-43 positive round structures are normal nuclei since these are negative for haematoxylin (Fig. 2H–K) and for TO-PRO-3 (Fig. 3H). We speculate the possibility that these are degenerating nuclei or swollen processes like spheroids, but the nature of those should be further investigated.

The present biochemical studies demonstrate that hyperphosphorylated TDP-43 with a molecular weight of 45 kDa, fragments or splicing isoforms with lower molecular weight and the smearing substances with diffuse staining, similar to those found in FTLD-U and ALS, were deposited in the sarkosyl-insoluble fractions of G-PDC brains. The recovery of normal full-length TDP-43 in the sarkosyl-insoluble fraction might be due to its presence in the nucleus. These results suggest that accumulation of TDP-43 is a common process in certain neurodegenerative disorders, including ALS, FTLD-U and G-PDC, and similar biochemical alterations and conformational changes in TDP-43 may occur in these diseases.

It is unclear whether there are any relationships between the deposition of hyperphosphorylated tau and the accumulation of TDP-43. The occasional occurrence of TDP-43-positive structures associated with NFTs in the hippocampal and temporal regions of G-PDC cases may indicate some association between tau and TDP-43. However, it has to be noted that in a case of G-PDC (PDC3), the western blot of the sarkosyl insoluble fraction showed the most abundant tau (Fig. 4A, lower panel, lane 5) but the least amount of TDP-43 (Fig. 4A, upper panel, lane 5) among the all PDC cases examined. Although some unique tau positive structures, such as the granular hazy inclusions in astrocytes and the fine granules in white matter, have been previously reported in G-PDC (Oyanagi *et al.*, 1997; Yamazaki *et al.*, 2005), the association between these structures and TDP-43 was not examined in this study. Further studies will be needed to elucidate the role of the association between tau and TDP-43 in the pathogenesis of G-PDC.

There has been a long history of debate for the nosology of G-PDC. It is distinguished from AD by the laminar distribution of NFT (Hof *et al.*, 1991), the prominent glial pathology (Oyanagi *et al.*, 1997) and the relative absence of

amyloid plaques (Gentleman *et al.*, 1991; Schmidt *et al.*, 1998). The nature of α-synuclein pathology is also different between Parkinson's disease (PD) and G-PDC, i.e. the frequency of Lewy bodies in the substantia nigra is lower in G-PDC than in PD (Hirano *et al.*, 1966; Oyanagi and Wada, 1999), while the density of α-synuclein positive structures in the cerebellum is higher in G-PDC than in PD (Sebeo *et al.*, 2004). As for TDP-43, the predominance of white matter TDP-43 profiles is very unlike FTLD-U variants so far described. These findings suggest that G-PDC represents combined neurodegenerative disorders, in which tau, α-synuclein and TDP-43 are simultaneously involved, but does not represent mere co-existence of multiple common degenerative diseases, including AD, PD and FTLD-U.

In conclusion, the results of the present study suggest that a common pathogenic mechanism through the process of biochemical and structural changes in the TDP-43 molecule in neurons and/or glial cells may be related to the neurodegeneration in ALS, FTLD-U and G-PDC. The deposition of TDP-43 in brains of G-ALS patients should be analysed as well. It might also be important to investigate the relationship between environmental or genetic factors and dysfunction or deposition of TDP-43 in these disorders.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas—Research on Pathomechanisms of Brain Disorders (to M.H.) and a Grant-in-Aid for Scientific Research (B) (to M.H.), both from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2006; 351: 602–11.

Ayala Y, Pantano S, D'Ambrogio A, Buratti E, Brindisi A, Marchetti C, et al. Human, drosophila, and C. elegans TDP43: nucleic acid binding properties and splicing regulatory function. J Mol Biol 2005; 348: 575–88.

Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 2006; 442: 916–9.

Bergmann M, Kuchelmeister K, Schmid KW, Kretzschmar HA, Schröder R. Different variants of frontotemporal dementia: a neuropathological and immunohistochemical study. Acta Neuropathol (Berl) 1996; 92: 170–9.

Boeve BF, Baker M, Dickson DW, Parisi JE, Giannini C, Josephs KA, et al. Frontotemporal dementia and parkinsonism associated with the IVS1 + 1G→A mutation in progranulin: a clinicopathological study. Brain 2006; 129: 3103–14.

Buratti E, Brindisi A, Giombi M, Tisminetzky S, Ayala YM, Baralle FE. TDP-43 binds heterogeneous nuclear ribonucleoprotein A/B through its C-terminal tail. J Biol Chem 2005; 280: 37572–84.

Buratti E, Dork T, Zuccato E, Pagani F, Romano M, Baralle FE. Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. EMBO J 2001; 20: 1774–84.

Cavanagh JB. Corpora-amylacea and the family of polyglucosan diseases. Brain Res Rev 1999; 29: 265–95.

Cox PA, Banack SA, Murch SJ. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proc Natl Acad Sci USA 2003; 100: 13380–3.

Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 2006; 442: 920–4.

Davidson Y, Kelley T, Mackenzie IRA, Pickering-Brown S, Du Plessis D, Neary D, et al. Ubiquitinated pathological lesions in frontotemporal lobar degeneration contain the TAR DNA-binding protein, TDP-43. Acta Neuropathol (Berl) 2007; doi: 10.1007/s00401-006-0189-y.

Forman MS, Mackenzie IR, Cairns NJ, Swanson E, Boyer PJ, Drachman DA, et al. Novel ubiquitin neuropathology in frontotemporal dementia with valosin-containing protein gene mutations. J Neuropathol Exp Neurol 2006; 65: 571–81.

Gass J, Cannon A, Mackenzie IR, Boeve B, Baker M, Adamson J, et al. Mutations in progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. Hum Mol Genet 2006; 15: 2988–3001.

Gentleman SM, Perl D, Allsop D, Clinton J, Royston MC, Roberts GW. Beta (A4)-amyloid protein and parkinsonian dementia complex of Guam. Lancet 1991; 337: 55–6.

Guyant-Marechal L, Laquerriere A, Duyckaerts C, Dumanchin C, Bou J, Dugny F, et al. Valosin-containing protein gene mutations. Clinical and neuropathological features. Neurology 2006; 67: 644–51.

Hermosura MC, Nayakanti H, Dorovkov MV, Calderon FR, Ryazanov AG, Haymer DS, et al. A TRPM7 variant shows altered sensitivity to magnesium that may contribute to the pathogenesis of two Guamanian neurodegenerative disorders. Proc Natl Acad Sci USA 2005; 102: 11510–5.

Hirano A, Kurland LT, Krooth RS, Lessell S. Parkinsonism-dementia complex, an endemic disease on the island of Guam. I. Clinical features. Brain 1961; 84: 642–61.

Hirano A, Malamud N, Elizan TS, Krurland LT. Amyotrophic lateral sclerosis and Parkinsonism-dementia complex on Guam. Further pathologic studies. Arch Neurol 1966; 15: 35–51.

Hof PR, Perl DP, Loerzel AJ, Morrison JH. Neurofibrillary tangle distribution in the cerebral cortex of parkinsonism-dementia cases from Guam: differences with Alzheimer's disease. Brain Res 1991; 564: 306–13.

Huey ED, Grafman J, Wassermann EW, Pietrini P, Tierney MC, Ghetti B, et al. Characteristics of frontotemporal dementia patients with a progranulin mutation. Ann Neurol 2006; 60: 374–80.

Ikemoto A, Hirano A, Akiguchi I, Kimura J. Comparative study of ubiquitin immunoreactivity of hippocampal granular cells in amyotrophic lateral sclerosis with dementia, Guamanian amyotrophic lateral sclerosis and Guamanian parkinsonism-dementia complex. Acta Neuropathol (Berl) 1997; 93: 265–70.

Iseki E, Li F, Odawara T, Hino H, Suzuki K, Kosaka K, et al. Ubiquitin-immunohistochemical investigation of atypical Pick's disease without Pick bodies. J Neurol Sci 1998; 159: 194–201.

Jackson M, Lennox G, Lowe J. Motor neuron disease-inclusion dementia. Neurodegeneration 1996; 5: 339–50.

Mackenzie IRA, Baborie A, Pickering-Brown S, Du Plessis D, Jaros E, Perry RH, et al. Heterogeneity of ubiquitin pathology in frontotemporal lobar degeneration: classification and relation to clinical phenotype. Acta Neuropathol (Berl) 2006*a*; 112: 539–49.

Mackenzie IRA, Baker M, Pickering-Brown S, Hsiung GYR, Lindholm C, Dwosh E, et al. The neuropathology of frontotemporal lobar degeneration caused by mutations in the progranulin gene. Brain 2006*b*; 129: 3081–90.

Mackenzie IRA, Feldman H. Neuronal intranuclear inclusions distinguish familial FTD-MND type from sporadic cases. Acta Neuropathol (Berl) 2003; 105: 543–8.

Mackenzie IRA, Feldman HH. Ubiquitin immunohistochemistry suggests classic motor neuron disease, motor neuron disease with dementia, and frontotemporal dementia of the motor neuron disease type represent a clinicopathological spectrum. J Neuropathol Exp Neurol 2005; 64: 730–9.

Masellis M, Momeni P, Meschino W, Heffner R Jr, Elder J, Sato C, et al. Novel splicing mutation in the progranulin gene causing familial corticobasal syndrome. Brain 2006; 129: 3115–23.

Mori H, Kondo J, Ihara Y. Ubiquitin is a component of paired helical filaments in Alzheimer's disease. Science 1987; 235: 1641–4.

Mukherjee O, Pastor P, Cairns NJ, Chakraverty S, Kauwe JSK, Shears S, et al. HDDD2 is a familial frontotemporal lobar degeneration with ubiquitin-positive tau-negative inclusions caused by a missense mutation in the signal peptide of progranulin. Ann Neurol 2006; 60: 314–22.

Nakano I, Hirano A. Neuron loss in the nucleus basalis of Meynert in parkinsonism-dementia complex of Guam. Ann Neurol 1983; 13: 87–91.

Neumann M, Mackenzie IR, Cairns NJ, Boyer PJ, Markesbery WR, Smith CD, et al. TDP-43 in the ubiquitin pathology of frontotemporal dementia with VCP gene mutations. J Neuropathol Exp Neurol 2007; 66: 152–7.

Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006; 314: 130–3.

Okamoto K, Hirai S, Yamazaki T, Sun X, Nakazato Y. New ubiquitin-positive intraneuronal inclusions in the extra-motor cortices in patients with amyotrophic lateral sclerosis. Neurosci Lett 1991; 129: 233–6.

Okamoto K, Murakami N, Kusada H, Yoshida M, Hashizume Y, Nakazato Y, et al. Ubiquitin-positive intraneuronal inclusions in the extramotor cortices of presenile dementia patients with motor neuron disease. J Neurol 1992; 239: 426–30.

Ou SH, Wu F, Harrich D, Garcia-Martinez LF, Gaynor RB. Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. J Virol 1995; 69: 3584–96.

Oyanagi K, Kawakami E, Kikuchi-Horie K, Ohara K, Ogata K, Takahama S, et al. Magnesium deficiency over generations in rats with special references to the pathogenesis of the Parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam. Neuropathology 2006; 26: 115–28.

Oyanagi K, Makifuchi T, Ohtoh T, Chen KM, Gajdusek DC, Chase TN. Distinct pathological features of the gallyas- and tau-positive glia in the Parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam. J Neuropathol Exp Neurol 1997; 56: 308–16.

Oyanagi K, Makifuchi T, Ohtoh T, Chen KM, van der Schaaf T, Gajdusek DC, et al. Amyotrophic lateral sclerosis of Guam: the nature of the neuropathological findings. Acta Neuropathol (Berl) 1994*a*; 88: 405–12.

Oyanagi K, Makifuchi T, Ohtoh T, Ikuta F, Chen KM, Chase TN, et al. Topographic investigation of brain atrophy in parkinsonism-dementia complex of Guam: a comparison with Alzheimer's disease and progressive supranuclear palsy. Neurodegeneration 1994*b*; 3: 301–4.

Oyanagi K, Wada M. Neuropathology of parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam: an update. J Neurol 1999; 246 ((Suppl 2:II): 19–27.

Pickering-Brown SM, Baker M, Gass J, Boeve BF, Loy CT, Brooks WS, et al. Mutations in progranulin explain atypical phenotypes with variants in MAPT. Brain 2006; 129: 3124–6.

Schmidt ML, Lee VMY, Saido T, Perl D, Schuck T, Iwatsubo T, et al. Amyloid plaques in Guam amyotrophic lateral sclerosis/parkinsonism-dementia complex contain species of A beta similar to those found in the amyloid plaques of Alzheimer's disease and pathological aging. Acta Neuropathol (Berl) 1998; 96: 487–94.

Sebeo J, Hof PR, Perl DP. Occurrence of α-synuclein pathology in the cerebellum of Guamanian patients with parkinsonism-dementia complex. Acta Neuropathol (Berl) 2004; 107: 497–503.

Snowden JS, Pickering-Brown SM, Mackenzie IR, Richardson AMT, Varma A, Neary D, et al. Progranulin gene mutations associated with frontotemporal dementia and progressive non-fluent aphasia. Brain 2006; 129: 3115–23.

Wang I-F, Reddy NM, Shen C-KJ. Higher order arrangement of the eukaryotic nuclear bodies. Proc Natl Acad Sci USA 2002; 99: 13583–8.

Wightman G, Anderson VER, Martin AJ, Swash M, Anderson BH, Neary D, et al. Hippocampal and neocortical ubiquitin-immunoreactive inclusions in amyotrophic lateral sclerosis with dementia. Neurosci Lett 1992; 139: 269–74.

Woulfe J, Kertesz A, Munoz DG. Frontotemporal dementia with ubiquitinated cytoplasmic and intranuclear inclusions. Acta Neuropathol (Berl) 2001; 102: 94–102.

Yamazaki M, Hasegawa M, Mori O, Murayama S, Tsuchiya K, Ikeda K, et al. Tau-positive fine granules in the cerebral white matter: a novel finding among the tauopathies exclusive to parkinsonism-dementia complex of Guam. J Neuropathol Exp Neurol 2005; 64: 839–46.

Neuropathology, in press.

Amyotrophic Lateral Sclerosis-Dementia with Severe Degeneration of the Substantia Nigra and Clinical Parkinsonism: Report of an Autopsy Case

Masayuki Shintaku[1)], Kiyomitsu Oyanagi[2)], and Daita Kaneda[3)]

1) Department of Pathology, Osaka Red Cross Hospital

2) Department of Neuropathology, Tokyo Metropolitan Institute of Neuroscience

3) Department of Neurology, Osaka Red Cross Hospital

Running Title: ALS-dementia with Parkinsonism

Address correspondence to: Masayuki Shintaku, M.D., Department of Pathology, Osaka Red Cross Hospital, Tennoji, Osaka 543-8555, Japan.

(Tel.) 06-6774-5111 (Fax.) 06-6774-5131

(E-mail) masa-s@sings.jp

Abstract

An autopsy case of amyotrophic lateral sclerosis-dementia (ALS-D) with clinically overt parkinsonism and severe degeneration of the substantia nigra is reported. The patient is a 78-year-old man, who died after a clinical course of two years characterized by parkinsonism which was responsive to L-DOPA treatment. Motor neuron symptoms and dementia were not apparent antemortem. Autopsy revealed severe degeneration of the substantia nigra without α-synucleinopathy-related changes. Finely granular mineralization of necrotic neurons was a unique finding in the substantia nigra. Mild loss of spinal anterior horn cells with the appearance of several Bunina bodies and degeneration of the hippocampal subiculum and temporal cortex were also noted. A small number of ubiquitinated intracytoplasmic inclusions were found in neurons of the dentate fascia of the hippocampus and the temporal and frontal cortices. Although degeneration of the substantia nigra is a common finding in ALS-D, patients seldom develop clinically overt parkinsonism. This case indicates that patients with ALS-D ~~rarely present with~~ predominantly parkinsonian clinical features and these symptoms and signs can be improved by L-DOPA treatment.

Key words: amyotrophic lateral sclerosis-dementia, degeneration, mineralization, parkinsonism, substantia nigra