the cellular effects of the mutation, and to 'package' abnormal tau molecules into aggregates, are able to function for longer. However, even in AD, accumulation of tau as PHF is not without detriment, with progressive loss of endoplasmic reticulum and ribosomes with accumulating PHF [57], indicating a decline in the nerve cell's synthetic machinery that eventually crosses a threshold to continued survival.

In sporadic FTLD with Pick bodies it is presumed that, as in FTDP-17, loss of microtubule function is likewise invoked through post-translational hyperphosphorylation changes in tau, although as yet the precise mechanism underpinning this is not known. Nonetheless, tau deposition is again about 10 times less than that in EOAD and the same mechanistic arguments (as in FTDP-17) for this smaller accumulation of tau may apply.

However, it could also be argued that the lower level of tau accumulated in FTLD compared with AD reflects the relative ease of degradation and clearance in each disorder with more pathological tau being removed from the brain during the course of the illness than is possible in AD. In AD, the hyperphosphorylated tau is assembled into highly insoluble PHF that become heavily modified through processes such as glycation [58]. Certainly, under the electron microscope, tau filaments in FTLD and AD have different ultrastructural appearances. In FTLD, the tau filaments are usually straight (with some twisted filaments) in those *MAPT* mutations in which Pick bodies are present [13], whereas the NFT-like structures in cases with exon 10 and splice mutations are composed of wide flat twisted ribbons [34], not PHF. These latter filamentous types may be more susceptible to degradation both internally and externally than PHF which even after cell death in AD remain largely intact as 'ghost tangles'. However, the lack of excessive tau in R406W mutation (compared with other *MAPT* mutations), where tau is present as PHF [6,21], would argue against this, although the long disease durations present in many patients with this particular mutation (in the three cases studied here disease duration was 7–29 years) suggests an unusually mild pathological phenotype with the low tau levels being a reflection of this. It would be interesting to compare tau loads using antibodies against glycation end-products or anti-tau antibodies like Alz50 and TG-3 which recognize tau only in specific conformations [59].

It might further be argued that even though the frontal cortex contains tau pathology for all three diseases under investigation, this might not be an appropriate or representative brain region to work on in a study of this kind, being affected relatively less than other regions (for example, temporal cortex) at end-stage disease at least as far as AD, and maybe also sporadic FTLD with Pick bodies [60] and FTDP-17 cases with N279K (in which a movement disorder is prominent) [20,23] are concerned. However, because the frontal cortex is not 'saturated' by tau pathology, even at end-stage disease, its study should better allow the relationship between tau deposition and *MAPT* mutation, or disease type, to be determined than would be the case were end-stage 'burnt out' regions like temporal cortex to be examined. The low levels of tau in frontal cortex in FTLD compared with those in AD could reflect declining disease in this region in FTLD, and that there could be regions of the brain in this latter disorder where the disease process is evolving more actively. However, histological examination of the brain in FTLD with respect to tau deposition refutes this where the level of tau deposition declines when moving in an anterior-posterior direction into better preserved regions of (frontal and parietal cortex) [13,34].

One potential weakness of the present study is that we have only employed phosphorylation-dependent tau antibodies and therefore the tau measurements we have obtained with any given antibody might reflect, at least partially, that proportion of tau molecules in which the epitope concerned is accordingly phosphorylated. As mentioned earlier in connection with AT100 immunostaining, different levels of phosphorylation at different epitopes on tau, or antibody accessibility, might explain the variations in mean tau load between antibodies, and between disease groups. In order to determine whether the antibodies we have used here have detected all, or only a proportion of, the insoluble tau pathology, it will be necessary to perform further analysis using a phospho-independent antibody, or to compare insoluble tau loads by biochemical techniques.

In conclusion, we suggest that the amount of insoluble tau deposited within the brain in FTLD does not depend in any systematic way upon the presence, or type, of *MAPT* mutation, or on what physiological change is generated. Furthermore, the brain tau load in FTLD would seem to be considerably less than that in EOAD, and this finding raises important questions relating to the role of aggregated tau in neurodegeneration – whether this represents an adaptive response aimed at promoting survival of neurones, or is indeed a detrimental change which brings about the demize of the affected cell.

Acknowledgements

This study was supported by a Wolfson Scholarship to AMS and Alzheimer's Research Trust Alzheimer's Disease Research Centre Grant to DMAM. The authors wish to thank the many other people who were involved in collecting and characterizing the FTLD cases with *MAPT* mutations, and the other FTLD and EOAD cases, and by doing so making possible this multicentre collaborative study.

References

1 Foster NL, Wilhelmsen K, Sima AA, Jones MZ, D'Amato CJ, Gilman S. Frontotemporal dementia and parkinsonism linked to chromosome 17: a consensus conference. *Ann Neurol* 1997; **41**: 706–15

2 Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon MJ, Nowotny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski JQ, Basun H, Lannfelt L, Neystat M, Fahn S, Dark F, Tannenberg T, Dodd PR, Hayward N, Kwok JBJ, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P. Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* 1998; **393**: 702–5

3 Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, Andreadis A, Wiederholt WC, Raskind M, Schellenberg GD. Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann Neurol* 1998; **43**: 815–25

4 Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc Natl Acad Sci USA* 1998; **95**: 7737–41

5 Houlden H, Baker M, Adamson J, Grover A, Waring S, Dickson D, Lynch T, Boeve B, Petersen RC, Pickering-Brown S, Neary D, Crauford D, Snowden JS, Mann D, Hutton M. Frequency of tau mutations in three series of non-Alzheimer's degenerative dementia. *Ann Neurol* 1999; **46**: 243–8

6 Rizzu P, Van Swieten JC, Joosse M, Hasegawa M, Stevens M, Tibben A, Niermeijer MF, Hillebrand M, Ravid R, Oostra BA, Goedert M, van Duijn CM, Heutink P. High prevalence of mutations in the microtubule-associated protein tau in a population study of frontotemporal dementia in the Netherlands. *Am J Hum Genet* 1999; **64**: 414–21

7 Rosso SM, Landweer EJ, Houterman M, Donker Kaat L, van Duijn CM, van Swieten JC. Medical and environmental risk factors for sporadic frontotemporal dementia: a retrospective case-control study. *J Neurol Neurosurg Psychiatry* 2003; **74**: 1574–6

8 Mann DM. The neuropathology and molecular genetics of frontotemporal dementia. In *Dementia*, edition 2nd edn. Eds J O'Brien, D Ames, A Burns. London: Arnold, 2000; 759–68

9 Reed LA, Wszolek ZK, Hutton M. Phenotypic correlations in FTDP-17. *Neurobiol Aging* 2001; **22**: 89–107

10 Murrell JR, Spillantini MG, Zolo P, Guazzelli M, Smith MJ, Hasegawa M, Redi F, Crowther RA, Pietrini P, Ghetti B, Goedert M. Tau gene mutation G389R causes a tauopathy with abundant pick body-like inclusions and axonal deposits. *J Neuropathol Exp Neurol* 1999; **58**: 1207–26

11 Ghetti B, Murrell JR, Zolo P, Spillantini MG, Goedert M. Progress in hereditary tauopathies: a mutation in the Tau gene (G389R) causes a Pick disease-like syndrome. *Ann N Y Acad Sci* 2000; **920**: 52–62

12 Lippa CF, Zhukareva V, Kawarai T, Uryu K, Shafiq M, Nee LE, Grafman J, Liang Y, St George-Hyslop PH, Trojanowski JQ, Lee VM. Frontotemporal dementia with novel tau pathology and a Glu342Val tau mutation. *Ann Neurol* 2000; **48**: 850–8

13 Pickering-Brown SM, Baker M, Yen S-H, Liu W-K, Hasegawa M, Cairns NJ, Lantos PL, Rossor M, Iwatsubo T, Davies Y, Allsop D, Furlong R, Owen F, Hardy J, Mann DMA, Hutton M. Pick's disease is associated with mutations in the *tau* gene. *Ann Neurol* 2000; **48**: 859–67

14 Rizzini C, Goedert M, Hodges JR, Smith MJ, Jakes R, Hills R, Xuereb JH, Crowther RA, Spillantini MG. Tau gene mutation K257T causes a tauopathy similar to Pick's disease. *J Neuropathol Exp Neurol* 2000; **59**: 990–1001

15 Neumann M, Schulz-Schaeffer W, Crowther RA, Smith MJ, Spillantini MG, Goedert M, Kretzschmar HA. Pick's disease associated with the novel Tau gene mutation K369I. *Ann Neurol* 2001; **50**: 503–13

16 Hayashi S, Toyoshima Y, Hasegawa M, Umeda Y, Wakabayashi K, Tokiguchi S, Iwatsubo T, Takahashi H. Late-onset frontotemporal dementia with a novel exon 1 (Arg5His) tau gene mutation. *Ann Neurol* 2002; **51**: 525–30

17 Rosso SM, van Herpen E, Deelen W, Kamphorst W, Severijnen LA, Willemsen R, Ravid R, Niermeijer MF, Dooijes D, Smith MJ, Goedert M, Heutink P, van Swieten JC. A novel tau mutation, S320F, causes a tauopathy with inclusions similar to those in Pick's disease. *Ann Neurol* 2002; **51**: 373–6

18 Hogg M, Grujic ZM, Baker M, Demirci S, Guillozet AL, Sweet AP, Herzog LL, Weintraub S, Mesulam MM, LaPointe NE, Gamblin TC, Berry RW, Binder LI, de Silva R, Lees A, Espinoza M, Davies P, Grover A, Sahara N, Ishizawa T, Dickson D, Yen SH, Hutton M, Bigio EH. The L266V tau mutation is associated with frontotemporal dementia and Pick-like 3R and 4R tauopathy. *Acta Neuropathol* 2003; **106**: 323–36

19 Pickering-Brown SM, Baker M, Nonaka T, Ikeda K, Sharma S, Mackenzie J, Simpson SA, Moore JW, Snowden JS, de Silva R, Revesz T, Hasegawa M, Hutton M, Mann DM. Frontotemporal dementia with Pick-type histology associated with Q336R mutation in the tau gene. *Brain* 2004; **127**: 1415–26
20 Wszolek Z, Pfeiffer RF, Bhatt MH, Schelper RL, Cordes M, Snow BJ, Rodnitsky RL, Wolters EC, Arwert F, Calne DB. Rapidly progressive autosomal dominant Parkinsonism and dementia with pallido-ponto-nigral degeneration. *Ann Neurol* 1992; **32**: 312–20
21 van Swieten JC, Stevens M, Rosso SM, Rizzu P, Joosse M, de Koning I, Kamphorst W, Ravid R, Spillantini MG, Niermeijer M, Heutink P. Phenotypic variation in hereditary frontotemporal dementia with tau mutations. *Ann Neurol* 1999; **46**: 617–26
22 Hasegawa M, Smith MJ, Iijima M, Tabira T, Goedert M. FTDP-17 mutations N279K and S305N in tau produce increased splicing of exon 10. *FEBS Lett* 1999; **443**: 93–6
23 Clark LN, Poorkaj P, Wszolek Z, Geschwind DH, Nasreddine ZS, Miller B, Li D, Payami H, Awert F, Markopoulou K, Andreadis A, D'Souza I, Lee VM, Reed L, Trojanowski JQ, Zhukareva V, Bird T, Schellenberg G, Wilhelmsen KC. Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17. *Proc Natl Acad Sci USA* 1998; **95**: 13103–7
24 Bugiani O, Murrell JR, Giaccone G, Hasegawa M, Ghigo G, Tabaton M, Morbin M, Primavera A, Carella F, Solaro C, Grisoli M, Savoiardo M, Spillantini MG, Tagliavini F, Goedert M, Ghetti B. Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in tau. *J Neuropathol Exp Neurol* 1999; **58**: 667–77
25 Delisle MB, Murrell JR, Richardson R, Trofatter JA, Rascol O, Soulages X, Mohr M, Calvas P, Ghetti B. A mutation at codon 279 (N279K) in exon 10 of the Tau gene causes a tauopathy with dementia and supranuclear palsy. *Acta Neuropathol* 1999; **98**: 62–77
26 D'Souza I, Poorkaj P, Hong M, Nochlin D, Lee VM, Bird TD, Schellenberg GD. Missense and silent tau gene mutations cause frontotemporal dementia with parkinsonism-chromosome 17 type, by affecting multiple alternative RNA splicing regulatory elements. *Proc Natl Acad Sci USA* 1999; **96**: 5598–603
27 Goedert M, Spillantini MG, Crowther RA, Chen SG, Parchi P, Tabaton M, Lanska DJ, Markesbery WR, Wilhelmsen KC, Dickson DW, Petersen PB, Gambetti P. Tau gene mutation in familial progressive subcortical gliosis. *Nat Med* 1999; **5**: 454–7
28 Mirra SS, Murrell JR, Gearing M, Spillantini MG, Goedert M, Crowther RA, Levey AI, Jones R, Green J, Shoffner JM, Wainer BH, Schmidt ML, Trojanowski JQ, Ghetti B. Tau pathology in a family with dementia and a P301L mutation in tau. *J Neuropathol Exp Neurol* 1998; **58**: 335–45
29 Nasreddine ZS, Loginov M, Clark LN, Lamarche J, Miller BL, Lamontagne A, Zhukareva V, Lee VM, Wilhelmsen KC, Geschwind DH. From genotype to phenotype. a clinical pathological, and biochemical investigation of frontotemporal dementia and parkinsonism (FTDP-17) caused by the P301L tau mutation. *Ann Neurol* 1999; **45**: 704–15
30 Arima K, Kowalska A, Hasegawa M, Mukoyama M, Watanabe R, Kawai M, Takahashi K, Iwatsubo T, Tabira T, Sunohara N. Two brothers with frontotemporal dementia and parkinsonism with an N279K mutation of the tau gene. *Neurology* 2000; **54**: 1787–95
31 Spillantini MG, Yoshida H, Rizzini C, Lantos PL, Khan N, Rossor MN, Goedert M, Brown J. A novel tau mutation (N296N) in familial dementia with swollen achromatic neurons and corticobasal inclusion bodies. *Ann Neurol* 2000; **48**: 939–43
32 Stanford PM, Halliday GM, Brooks WS, Kwok JB, Storey CE, Creasey H, Morris JG, Fulham MJ, Schofield PR. Progressive supranuclear palsy pathology caused by a novel silent mutation in exon 10 of the tau gene: expansion of the disease phenotype caused by tau gene mutations. *Brain* 2000; **123**: 880–93
33 Iseki E, Matsumura T, Marui W, Hino H, Odawara T, Sugiyama N, Suzuki K, Sawada H, Arai T, Kosaka K. Familial frontotemporal dementia and parkinsonism with a novel N296H mutation in exon 10 of the tau gene and a widespread tau accumulation in the glial cells. *Acta Neuropathol* 2001; **102**: 285–92
34 Pickering-Brown SM, Richardson AM, Snowden JS, McDonagh AM, Burns A, Braude W, Baker M, Liu WK, Yen SH, Hardy J, Hutton M, Davies Y, Allsop D, Craufurd D, Neary D, Mann DM. Inherited frontotemporal dementia in nine British families associated with intronic mutations in the tau gene. *Brain* 2002; **125**: 732–51
35 Grover A, DeTure M, Yen SH, Hutton M. Effects on splicing and protein function of three mutations in codon N296 of tau in vitro. *Neurosci Lett* 2002; **323**: 33–6
36 Halliday GM, Song YJC, Creasey H, Morris JG, Brooks WS, Kril JJ. Neuropathology in the S305S tau gene mutation. *Brain* 2006 (in press)
37 Taniguchi S, McDonagh AM, Pickering-Brown SM, Umeda Y, Iwatsubo T, Hasegawa M, Mann DM. The neuropathology of frontotemporal lobar degeneration with respect to the cytological and biochemical characteristics of tau protein. *Neuropathol Appl Neurobiol* 2004; **30**: 1–18
38 Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991; **41**: 479–86
39 Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* 1991; **82**: 239–59
40 Kertesz A, McGonagle P, Blair M, Davidson W, Munoz DG. The evolution and pathology of frontotemporal dementia. *Brain* 2005; **128**: 1996–2005

41 McKhann GM, Albert MS, Grossman M, Miller B, Dickson D, Trojanowski JQ. Workgroup on Frontotemporal dementia and Picks disease. Clinical and pathological diagnosis of frontotemporal dementia: report of the workgroup on Frontotemporal dementia and Picks disease. *Arch Neurol* 2001; **59**: 1203–4
42 Goedert M, Jakes R, Crowther RA, Six J, Lubke U, Vandermeeren M, Cras P, Trojanowski JQ, Lee VM. The abnormal phosphorylation of tau protein at Ser-202 in Alzheimer disease recapitulates phosphorylation during development. *Proc Natl Acad Sci USA* 1993; **90**: 5066–70
43 Mercken M, Vandermeeren M, Lubke U, Six J, Boons J, Van de Voorde A, Martin JJ, Gheuens J. Monoclonal antibodies with selective specificity for Alzheimer Tau are directed against phosphatase-sensitive epitopes. *Acta Neuropathol* 1992; **84**: 265–72
44 Goedert M, Jakes R, Crowther RA, Cohen P, Vanmechelen E, Vandermeeren M, Cras P. Epitope mapping of monoclonal antibodies to the paired helical filaments of Alzheimer's disease: identification of phosphorylation sites in tau protein. *Biochem J* 1994; **301**: 871–7
45 Mann DM, McDonagh AM, Pickering-Brown SM, Kowa H, Iwatsubo T. Amyloid beta protein deposition in patients with frontotemporal lobar degeneration: relationship to age and apolipoprotein E genotype. *Neurosci Lett* 2001; **304**: 161–4
46 Kondo J, Honda T, Mori H, Hamada Y, Miura R, Ogawara M, Ihara Y. The carboxyl third of tau is tightly bound to paired helical filaments. *Neuron* 1988; **1**: 827–34
47 Kosik KS, Orecchio LD, Binder L, Trojanowski JQ, Lee VM, Lee G. Epitopes that span the tau molecule are shared with paired helical filaments. *Neuron* 1988; **1**: 817–25
48 Zheng-Fischhofer Q, Biernat J, Mandelkow EM, Illenberger S, Godemann R, Mandelkow E. Sequential phosphorylation of Tau by glycogen synthase kinase-3β and protein kinase A at Thr212 and Ser214 generates the Alzheimer-specific epitope of antibody AT100 and requires a paired-helical-filament-like conformation. *Eur J Biochem* 1998; **252**: 542–52
49 Mailliot C, Sergeant N, Bussiere N, Caillet-Boudin ML, Delacourte A, Buee L. Phosphorylation of specific sets of tau isoforms reflects different neurofibrillary degeneration processes. *FEBS Lett* 1998; **433**: 201–4
50 Probst A, Tolnay. M, Langui D, Goedert M, Spillantini MG. Pick's disease: hyperphosphorylated tau protein segregates to the somatoaxonal compartment. *Acta Neuropathol* 1996; **92**: 588–96
51 Augustinack JC, Schneider A, Mandelkow EM, Hyman BT. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's Disease. *Acta Neuropathol* 2002; **103**: 26–35
52 Ferrer L, Barrachina M, Puig B. Anti-tau phosphospecific Ser^{262} antibody recognises a variety of abnormal hyperphosphorylated tau deposits in tauopathies including Pick bodies and argyrophilic grains. *Acta Neuropathol* 2002; **104**: 658–64
53 Goedert M, Spillantini MG, Cairns NJ, Crowther RA. Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron* 1992; **8**: 159–68
54 Alonso AC, Grundke-Iqbal I, Iqbal K. Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat Med* 1996; **2**: 783–7
55 Alonso AC, Mederlyova A, Novak M, Grundke-Iqbal I, Iqbal K. Promotion of hyperphosphorylation by frontotemporal dementia tau mutations. *J Biol Chem* 2004; **279**: 34873–81
56 Iqbal K, Alonso Adel C, Chen S, Chohan MO, El-Akkad E, Gong CX, Khatoon S, Li B, Liu F, Rahman A, Tanimukai H, Grundke-Iqbal I. Tau pathology in Alzheimer disease and other tauopathies. *Biochim Biophys Acta* 2005; **1739**: 198–210
57 Sumpter PQ, Mann DMA, Davies CA, Yates PO, Snowden JS, Neary D. An ultrastructural analysis of the effects of accumulation of neurofibrillary tangle in pyramidal cells of the cerebral cortex in Alzheimer's disease. *Neuropathol Appl Neurobiol* 1986; **12**: 305–19
58 Smith MA, Siedlak SL, Richey PL, Nagaraj RH, Elhammer A, Perry G. Quantitative solubilization and analysis of insoluble paired helical filaments from Alzheimer disease. *Brain Res* 1996; **717**: 99–108
59 Jicha GA, Lane E, Vincent I, Otvos L Jr, Hoffmann R, Davies P. A conformation- and phosphorylation-dependent antibody recognizing the paired helical filaments of Alzheimer's disease. *J Neurochem* 1997; **69**: 2087–95
60 Wechsler AF, Verity MA, Rosenschein S, Fried I, Scheibel AB. Pick's disease. A clinical, computed tomographic, and histologic study with golgi impregnation observations. *Arch Neurol* 1982; **39**: 287–90

Received 12 September 2005
Accepted after revision 24 January 2006

Acta Neuropathol (2006) 111: 329–340
DOI 10.1007/s00401-006-0048-x

ORIGINAL PAPER

Rohan de Silva · Tammaryn Lashley
Catherine Strand · Anna-Maria Shiarli
Jing Shi · Jinzhou Tian · Kathryn L. Bailey
Peter Davies · Eileen H. Bigio · Kunimasa Arima
Eizo Iseki · Shigeo Murayama · Hans Kretzschmar
Manuela Neumann · Carol Lippa · Glenda Halliday
James MacKenzie · Rivka Ravid · Dennis Dickson
Zbigniew Wszolek · Takeshi Iwatsubo
Stuart M. Pickering-Brown · Janice Holton
Andrew Lees · Tamas Revesz · David M. A. Mann

An immunohistochemical study of cases of sporadic and inherited frontotemporal lobar degeneration using 3R- and 4R-specific tau monoclonal antibodies

Received: 2 December 2005 / Revised: 20 January 2006 / Accepted: 21 January 2006 / Published online: 22 March 2006

Abstract The pathological distinctions between the various clinical and pathological manifestations of frontotemporal lobar degeneration (FTLD) remain unclear. Using monoclonal antibodies specific for 3- and 4-repeat isoforms of the microtubule associated protein, tau (3R- and 4R-tau), we have performed an

R. de Silva · A. Lees
Reta Lila Weston Institute of Neurological Studies,
University College London, Windeyer Building,
46 Cleveland St, W1T 4JF, London, UK

T. Lashley · C. Strand · J. Holton · T. Revesz
Department of Molecular Neuroscience,
Queen Square Brain Bank, Institute of Neurology,
University College London, Queen Square,
WC1N 3BG, London, UK

A.-M. Shiarli · J. Shi · J. Tian · K. L. Bailey
S. M. Pickering-Brown · D. M. A. Mann (✉)
Centre for Clinical Neurosciences, University of Manchester,
Greater Manchester Neurosciences Centre, Hope Hospital,
M6 8HD, Salford, UK
E-mail: david.mann@manchester.ac.uk
Tel.: +44-161-2062580
Fax: +44-161-2060388

J. Shi · J. Tian
Department of Care of the Elderly,
Dongzhimen Hospital,
100700, Beijing, P R China

P. Davies
Department of Pathology, Albert Einstein College of Medicine,
Bronx, NY, 10461, USA

E. H. Bigio
Northwestern University Alzheimer's Disease Center,
Chicago, IL, 60611, USA

K. Arima
National Centre of Neurology and Psychiatry,
Musashi Hospital, 187-8551, Tokyo, Japan

E. Iseki
Juntendo Tokyo Koto Geriatric Medical Center,
Juntendo University School of Medicine, 136-0075, Tokyo, Japan

S. Murayama
Department of Neuropathology, Tokyo Metropolitan
Institute of Gerontology, 173, Tokyo, Japan

H. Kretzschmar · M. Neumann
Reference Centre for Prion Diseases and Neurodegenerative
Diseases, Institute of Neuropathology, Marchioninistr. 17,
81377, Munchen, Germany

C. Lippa
Memory Disorders Centre, Drexel University College of Medicine,
Philadelphia, PA, 10129, USA

G. Halliday
Department of Neuropathology,
Prince of Wales Medical Research Institute,
University of New South Wales, NSW 2031, Randwick, Australia

J. MacKenzie
The Royal Infirmary, Foresterhill, AB25 2ZD, Aberdeen, Scotland

R. Ravid
Netherlands Brain Bank, Meibergdreef 33,
1105 AZ, The Netherlands

D. Dickson · Z. Wszolek · S. M. Pickering-Brown
Mayo Clinic, 4500 San Pablo Rd,
Jacksonville, FL, 32224, USA

T. Iwatsubo
Department of Neuropathology and Neuroscience,
University of Tokyo, 113-0033, Tokyo, Japan

immunohistochemical study of the tau pathology present in 14 cases of sporadic forms of FTLD, 12 cases with Pick bodies and two cases without and in 27 cases of familial FTLD associated with 12 different mutations in the tau gene (*MAPT*), five cases with Pick bodies and 22 cases without. In all 12 cases of sporadic FTLD where Pick bodies were present, these contained only 3R-tau isoforms. Clinically, ten of these cases had frontotemporal dementia and two had progressive apraxia. Only 3R-tau isoforms were present in Pick bodies in those patients with familial FTLD associated with L266V, Q336R, E342V, K369I or G389R *MAPT* mutations. Patients with familial FTLD associated with exon 10 N279K, N296H or +16 splice site mutations showed tau pathology characterised by neuronal neurofibrillary tangles (NFT) and glial cell tangles that contained only 4R-tau isoforms, as did the NFT in P301L *MAPT* mutation. With the R406W mutation, NFT contained both 3R- and 4R-tau isoforms. We also observed two patients with sporadic FTLD, but without Pick bodies, in whom the tau pathology comprised only of 4R-tau isoforms. We have therefore shown by immunohistochemistry that different specific tau isoform compositions underlie the various kinds of tau pathology present in sporadic and familial FTLD. The use of such tau isoform specific antibodies may refine pathological criteria underpinning FTLD.

Introduction

Frontotemporal lobar degeneration (FTLD) describes a clinically and pathologically heterogeneous group of forms of dementia that have onset of illness usually between ages of 35 and 75 years and affect males and females equally [41, 56]. A previous family history of a similar disorder occurs in about half of patients [41, 53, 56] and in many such familial cases a mutation in the gene encoding the microtubule associated protein, tau (*MAPT*) on chromosome 17 seems causal (see Ref. [15, 32] for recent reviews). To date, about 35 causal *MAPT* mutations in around 150 families have been identified and the term frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) was adopted [12] to accommodate the clinical and genetic features of such cases. At autopsy, patients with FTLD generally show atrophy of the frontal and temporal lobes of the brain associated with a degeneration and loss of large pyramidal neurons from such regions irrespective of clinical subtype or family history [41, 56]. However, under this umbrella of pathological change, histopathological differences occur [41, 55, 62] and over the years there have been several attempts at classification based upon microscopic appearances.

In the first case reports, made over a century ago by Arnold Pick, the characterising features of intraneuronal argyrophilic inclusions (Pick bodies) and swollen or ballooned neurons (Pick cells) were described and the eponym Pick's disease was coined to distinguish such cases from those of Alzheimer's disease where senile plaques and neurofibrillary tangles were the key pathology. However, recognizing that clinically similar forms of FTLD occurred without such Pick- or Alzheimer-type changes being present led to the scheme by Constantinidis [7] in which three variants of FTLD were identified: one with Pick bodies and Pick cells (type A), one with only Pick cells (type B) and one with neither (type C). Later immunohistochemical studies [33] indicated either a Pick-type of histology based on the presence of tau-positive Pick bodies (equivalent to Constantinidis type A) or a microvacuolar-type histology in which no tau intraneuronal inclusions (Pick bodies) were seen (equivalent to Constantinidis types B and C). Recent surveys [22, 31, 37, 41, 55, 62] indicate that about half of cases of FTLD show a histopathology based on the accumulation of insoluble aggregates of tau protein within neurons and glial cells of the cerebral cortex and hippocampus. In most other cases, termed FTLD-U, tau negative but ubiquitin positive inclusions usually occur within cerebral cortex and hippocampus and when clinical motor neuron disease (MND) is also present the term FTLD-MND can be ascribed. Sometimes, neither tau nor ubiquitin inclusions are seen; such cases have been labelled as dementia lacking distinctive histology. These observations are broadly in line with the recommendations of a consensus conference held in 2001 in an attempt to establish internationally accepted clinical and neuropathological criteria for FTLD [34]. Nonetheless, at the time such criteria were put forward it was recognized that they were likely to be interim and subject to review in the light of expanding knowledge of this disorder—a point reiterated in some most recent surveys [37, 55]

Tau-based pathology in FTLD can occur in either sporadic or familial cases. In sporadic cases of FTLD, tau pathology usually takes the form of Pick bodies and Pick cells, though in such cases some tau positive glial cells and dystrophic neurites can often be seen [66], though if cases of corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) or argyrophilic grain disease are included under the rubric of FTLD as has been the case in certain recent surveys [22, 31, 37] a neurofibrillary tangle-based tau pathology becomes more common.

In some familial cases (i.e., those with FTDP-17) with missense mutations within coding regions of exons 1, 9, 11, 12 and 13 of *MAPT* there are swollen nerve cells and rounded neuronal inclusions within large and small pyramidal neurones of the cerebral cortex and pyramidal and granule cells of the hippocampus reminiscent of the Pick bodies seen in sporadic disease [4, 14, 23, 29, 30, 39, 42, 45, 47, 50, 52]. Such mutations affect all six isoforms of tau, generating mutated tau molecules that (variably) lose their ability to interact with microtubules [20, 21, 23, 39, 42, 50, 51], increasing their propensity to self-aggregate into fibrils [20, 21, 23, 42, 47, 51]. Other *MAPT* mutations cluster around, or

lie within a predicted regulatory stem loop structure of a splice acceptor domain of *MAPT* pre-mRNA that determines the inclusion or exclusion of exon 10 by alternative splicing during gene transcription [2, 3, 5, 6, 9, 11, 17, 19, 20, 24, 25, 35, 40, 43, 46, 48, 52, 58–60], destabilizing the stem loop [24, 58] or strengthening [11, 18, 20] or destroying [11] splice enhancing, or splice silencing [11, 59] elements in the 5′ region of exon 10. Such cases show insoluble aggregated tau deposits as neurofibrillary tangle-like structures within large and smaller pyramidal cells of cortical layers III and V and prominently within glial cells in the deep white matter, globus pallidus and internal capsule [2, 17, 19, 35, 43, 46, 51, 58–60]. However, other exon 10 mutations do not affect the splicing of exon 10 [11, 24] but induce conformational changes in tau molecules containing exon 10 that interfere with microtubule function and lead to aggregation of the mutated tau into neurofibrillary tangles [18, 36, 59].

Although the brain tau isoform composition has been extensively analysed by western blotting both in cases of sporadic FTLD where Pick bodies [1, 8, 37, 53, 61, 65] or Pick-like bodies [37] are present and in many of the cases with FTDP-17 [2–6, 9, 14, 17, 19, 23, 25, 29, 30, 35, 36, 39, 42, 43, 45–47, 50, 52, 59, 60, 62, 65], certain ambiguities remain. For example, in cases of sporadic FTLD where Pick bodies are seen in histology, most western blotting studies have detected only tau isoforms with 3-repeat microtubule binding domains (3R-tau) [8, 37, 54], though other investigations have shown certain cases to show tau isoforms with both 3R-tau and 4-repeat microtubule binding domains (4R-tau) [1, 37, 62, 66] and in yet others only 4R-tau is seen [37, 62, 66]. Such disparities have led to controversies as to whether Pick bodies are composed of only 3R-tau, only 4R-tau or an admixture of 3R- and 4R-tau isoforms. Similarly, in some cases of FTDP-17 with Pick bodies (e.g., K257T *MAPT* mutation Ref. [50]) western blotting has likewise shown only 3R-tau to be present, whereas in other cases (e.g., L266V, G272V, G342V and G389R *MAPT* mutations) both 3R-tau and 4R-tau isoforms are seen [4, 14, 23, 31, 45]. One possible explanation for these apparent inconsistencies may lie in differing anatomical compartmentalizations of 3R- and 4R- tau isoforms between cases, a distinction that is lost upon the tissue homogenization required for western blotting. Specific immunohistological patterns associated with 3R- and 4R-tau isoforms cannot be distinguished using phospho-dependent and phospho-independent tau antibodies that recognize tau epitopes shared by all six tau isoforms.

In the present study, therefore, we have investigated in situ, using monoclonal antibodies specific for 3R- and 4R-tau isoform species, the isoform composition of the tau histopathological changes in 14 cases of sporadic FTLD and 27 cases of familial FTLD associated with 11 different mutations in *MAPT*, for which tau isoform patterns on western blotting have already been reported, in order to reconcile such inconsistencies and to provide further insight into disease classification by the use of tau antibodies not available when the last pathological criteria [34] were proposed.

Materials and methods

Brain tissues from 14 cases with sporadic FTLD (cases #1–14), in 12 of whom (cases #1–12) previous pathological investigations had shown Pick bodies to be present and five cases of familial FTLD (cases #30–34) with exon 10 +16 *MAPT* mutation were obtained from the Manchester Brain Bank. Seven of the sporadic FTLD cases (cases #1, 2, 4–7 and 13) had been included (as cases # 4–8, 10 and 12, respectively) in a previous study [66] investigating tau isoform composition in a series of 14 cases of FTLD. Eight of the sporadic FTLD cases (cases #1, 2, 4–7, 9 and 13) had been included (as cases #10, 11, 12, 13, 14, 15, 17 and 16, respectively) in a previous study of ours [62] also investigating tau isoform composition in FTLD. Tissues from the other 22 familial FTLD cases with *MAPT* mutations were kindly supplied in collaboration by colleagues from different centres across the world. Selected clinical and pathological details for all cases are given in Table 1. Full clinical and pathological descriptions for 25 of the 27 cases with *MAPT* mutations have been previously reported by the originating authors (see Table 1 for details of citation); the other two cases remain unreported to date.

Serial sections were cut at a thickness of 6 μm from formalin-fixed, wax-embedded blocks of frontal cortex (BA 8/9) from all 14 sporadic FTLD cases and 27 familial FTLD cases with *MAPT* mutations and from temporal cortex (BA 21/22) to include the hippocampus in the 14 sporadic FTLD cases alone and mounted onto APES-coated slides. One set of sections was immunostained for insoluble pathological tau proteins by a standard immunoperoxidase method using the phospho-dependent tau antibody AT8 (1:750) (Innogenetics, Belgium). AT8 antibody is raised against the phosphorylated Ser 202/Thr 205 epitope and immunoreacts with PHF-tau in AD [16]. It will detect all isoforms of tau in which this epitope is phosphorylated. Other sets of sections were stained with the 3R-tau specific monoclonal antibody RD3 [10] (1:3000; Upstate, Dundee, UK) and the 4R-tau specific monoclonal antibody ET3 (gift of P Davies, 1:100) as described [10]. Briefly, sections were deparaffinised in xylene and rehydrated in decreasing concentrations of alcohol. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol for 10 min. Sections were pressure cooked for 10 min in 0.01 M citrate buffer pH6.0. Sections were incubated in 10% non-fat milk to block non-specific staining, then with the primary antibodies RD3 and ET3 for 1 h at room temperature. This was followed by several washes in PBS and treatment with biotinylated

Table 1 Selected clinical and pathological details

Case	Pathology	*MAPT* mutation	Gender	Onset (years)	Death (years)	Duration (years)	APOE genotype	Brain weight (g)
1 [55, 62]	Pick bodies	None	F	53	60	7	3.3	960
2 [55, 62]	Pick bodies	None	M	46	56	10	3.3	1,150
3 [55, 62]	Pick bodies	None	M	54	63	9	n.a.	n.a.
4 [55, 62]	Pick bodies	None	F	52	62	10	3.4	928
5 [55, 62]	Pick bodies	None	F	76	84	8	3.3	1,235
6 [55, 62]	Pick bodies	None	F	50	58	8	2.2	1,065
7 [55, 62]	Pick bodies	None	M	63	74	11	2.3	990
8 [55, 62]	Pick bodies	None	M	73	77	4	3.3	n.a.
9 [55, 62]	Pick bodies	None	M	47	61	14	3.4	980
10 [55, 62]	Pick bodies	None	M	59	69	10	3.3	885
11 [55, 62]	Pick bodies	None	M	55	67	12	3.3	895
12 [55, 62]	Pick bodies	None	F	51	57	6	3.3	1,210
13 [55, 62]	NFT-like	None	F	57	64	7	3.3	1,000
14 [55, 62]	NFT-like	None	F	64	70	6	3.3	1,090
15 [23]	Pick bodies	L266V	M	32	36	3.5	n.a.	1,050
16 [2]	NFT, glial tangles	N279K	M	46	57	11	3.3	1,250
17 [2]	NFT, glial tangles	N279K	M	44	50	6	3.3	1,420
18 [6, 64]	NFT, glial tangles	N279K	M	44	50	6	3.4	1,290
19 [6, 64]	NFT, glial tangles	N279K	F	45	48	3	3.3	1,100
20 [6, 64]	NFT, glial tangles	N279K	M	56	58	2	2.3	1,400
21 [6, 64]	NFT, glial tangles	N279K	F	45	53	8	2.4	1,000
22 [6, 64]	NFT, glial tangles	N279K	M	57	63	6	3.4	1,100
23 [6, 64]	NFT, glial tangles	N279K	M	41	52	11	2.3	1,100
24 [25]	NFT, glial tangles	N296H	M	57	62	3	3.3	960
25 [51, 63]	NFT	P301L	M	~48	60	>12	3.3	1,331
26 [51, 63]	NFT	P301L	M	44	52	8	2.3	1,087
27 [51, 63]	NFT	P301L	F	54	76	22	2.2	1,006
28 [51, 63]	NFT	P301L	F	59	64	5	3.3	1,013
29 [19, 60]	NFT, glial tangles	S305S	F	48	51	3	n.a.	1,053
30 [46]	NFT, glial tangles	Exon 10 +16	M	50	61	11	3.4	1,016
31 [46]	NFT, glial tangles	Exon 10 +16	F	46	58	12	3.3	996
32 [46]	NFT, glial tangles	Exon 10 +16	M	43	55	12	3.4	1240
33 [46]	NFT, glial tangles	Exon 10 +16	F	52	65	13	2.3	1,040
34 [46]	NFT, glial tangles	Exon 10 +16	F	48	56	8	3.4	1,175
35[uc]	NFT, glial tangles	Exon 10 +16	M	57	63	6	3.3	1,440
36 [47]	Pick bodies	Q336R	M	58	68	10	3.3	1,102
37 [30]	Pick bodies	E342V	F	48	55	7	3.3	1,020
38 [42]	Pick bodies	K369I	F	52	61	9	n.a.	885
39[uc]	Pick bodies	G389R	M	45	49	4	n.a.	1,170
40 [51, 63]	NFT	R406W	M	63	70	7	3.3	1,121
41 [51, 63]	NFT	R406W	F	58	71	13	3.4	905

Superscript indicates case reference
[uc]Indicates unpublished case
NFT neurofibrillary tangle, *n.a.* APOE genotype or brain weight not available

anti-mouse (Dako 1:200) for 30 min and ABC (Dako) for 30 min. Peroxidase activity was developed with diaminobenzidine/ H_2O_2 solution [10].

The specificity of ET3 has been demonstrated previously in Western blots of recombinant 3R- and 4R-tau [27]. It has also been characterised in immunohistochemical studies of argyrophilic grain disease [13] and other tauopathies [23].

Results

Sporadic FTLD

Semi-quantitative rating data for AT8, ET3 and RD3 immunostaining in the 14 sporadic FTLD cases is given in Table 2.

Cases with Pick bodies

Of the 14 cases with sporadic FTLD, 12 cases (cases #1–12) displayed Pick-type histology. Pick bodies were identified as defined by Kertesz et al. [28], as round or oval, compact intracytoplasmic neuronal inclusions, stained by Bielschowsky but not by Gallyas, tau-immunoreactive and located in dentate fascia, hippocampus and cerebral cortex. Clinically, nine cases (cases #1–7, 9 and 10) showed typical frontotemporal dementia, whereas case #8 had suffered from progressive aphasia and cases #11 and 12 from progressive apraxia. In 11 of these 12 cases (cases #1–4, 6–12), numerous Pick bodies were widespread within frontal and temporal cortex, chiefly in layers two and four and within dentate gyrus granule cells (Fig. 1a) and pyramidal cells of the

Table 2 Frequency of tau-immunoreactive pathological changes in frontal and temporal cortex and hippocampus, as detected in 14 patients with sporadic FTLD using AT8 (all tau isoforms), ET3 (4R-tau isoforms only) and RD3 (3R-tauisoforms only) antibodies

Case	Frontal			Temporal			Hippocampus			Tau biochemistry
	AT8	ET3	RD3	AT8	ET3	RD3	AT8	ET3	RD3	
1a	+ + + +	0	+ + +	+ + + +	0	+ + + +	+ + + +	0	+ + + +	n.d.
2a	+ + + +	0	+ + +	+ + +	0	+ + +	+ + +	0	+ + +	Mostly 3R, trace 4R [62, 66]
3[a]	+ + + +	0	+ + +	+ + + +	0	+ + + +	+ + + +	0	+ + +	n.d.
4[a]	+ + + +	0	+ + +	+ + + +	0	+ + + +	+ + +	0	+ + +	Mostly 3R, trace 4R [62, 66]
5[a,b]	+	0	0/+	+ + +	0	0/+	+ + + +	0	+ + +	Mainly 4R, some 3R [62, 66]
6[a]	+ + + +	0	+ + + +	+ + + +	0	+ + + +	+ + + +	0	+ + + +	Mostly 3R, trace 4R [62, 66]
7[a]	+ + +	0	+ +	+ + +	0	+	+ + + +	0	+ + +	n.d.
8[a]	+ + + +	0	+ + +	+ +	0	+	+ + + +	0	+ + +	n.d.
9[a]	+ + + +	0	+ + +	+ + + +	0	+ + + +	+ + + +	0	+ + + +	Mostly 3R, trace 4R [62, 66]
10[a]	+ + + +	0	+ + +	+ + + +	0	+ + + +	+ + + +	0	+ + + +	n.d.
11[a]	+ +	0	+	+ + +	0	+ + +	+ + + +	0	+ + + +	n.d.
12[a]	+ + +	0	+ +	+ + + +	0	+ + +	+ + + +	0	+ + + +	n.d.
13[b]	+ + +	+ +	0	+ + +	+	0	+	0/+	0	Mainly 4R [62, 66]
14[b]	+	0/+	0	+ +	+	0/+	+ + +	+	0	n.d.

Tau biochemical findings are shown (where known)
0 absent, 0/+ rare, + few, + + moderate number, + + + many, + + + + very many
[a]Pick bodies
[b]Neurofibrillary tangle-like structures, 3R 3-repeat tau, 4R 4-repeat tau, n.d. not done

hippocampus. However, in one elderly case (aged 83 years) (case #5) Pick bodies were widely present only in the granule cells of the dentate gyrus and pyramidal cells of the hippocampus, but less so within temporal neocortex. In this case occasional neurons showing diffuse cytoplasmic tau immunoreactivity (pretangles) and neurofibrillary tangle-like structures were seen in the frontal and temporal cortex (see also two cases reported by Mott et al. [37]. No other cases displayed such AD-type neurofibrillary changes. In cases #3, 4, 6 and 9, AT8-immunoreactive astrocytes were present within the cerebral cortex and white matter and a fine meshwork of dystrophic neurites (threads) was seen. These findings are typical of those seen using anti-tau antibodies such as AT8 which detect all hyperphosphorylated isoforms of tau and have been described on many occasions previously (e.g., Ref. [62, 66]).

In all 12 cases, the Pick bodies were strongly stained with RD3 antibody (Fig. 1b), usually to approximately the same extent as with AT8, but none were stained with

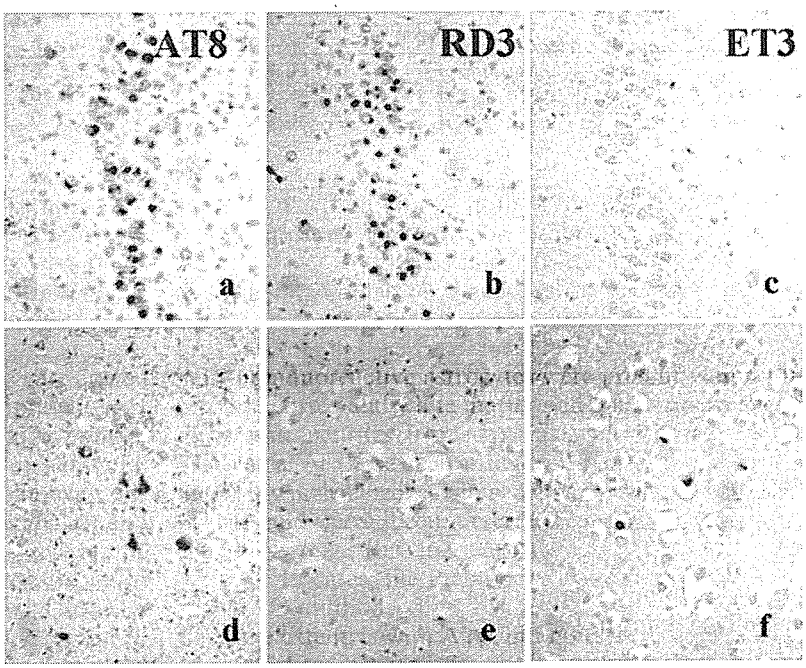

Fig. 1 Tau pathology in patients with sporadic FTLD. In patient #4 (**a–c**) Pick bodies within granule cells of the dentate gyrus of the hippocampus are immunoreactive with AT8 (**a**) and RD3 (**b**) antibodies, but not with ET3 (**c**) antibody. In patient #13 without Pick bodies (**d–f**) neurones with amorphous tau deposits or neurofibrillary tangle-like structures are immunoreactive with AT8 (**d**) and ET3 (**f**) antibodies, but not with RD3 antibody (**e**). Immunoperoxidase-haematoxylin, AT8 antibody (**a, d**), RD3 antibody (**b, e**), ET3 antibody (**c, f**). All 250 times microscope magnification

ET3 (Fig. 1c). In case #5 a minority of the Pick bodies within temporal neocortex were also stained with RD3, as were a few of the NFT-like structures in the frontal cortex, though neither were stained with ET3. In case #6 rare glial cells were diffusely stained with ET3.

Cases without Pick bodies

In the other two sporadic FTLD cases (cases #13 and 14) typical Pick bodies were absent from hippocampal dentate gyrus granule cells and pyramidal cells. In these, AT8 staining revealed numerous cells in both hippocampal regions containing fine, granular deposits of tau protein, which sometimes had NFT-like appearance. In both cases a few of the pyramidal cells, but none of the dentate gyrus granule cells, were stained with ET3, but not RD3, antibody. In frontal and temporal cortex fine amorphous deposits of tau protein which sometimes adopted a rounded, ring or crescent shape, other times a more NFT-like structure, were seen with AT8 staining (Fig. 1d). The amorphous tau deposits were sometimes similarly stained with RD3 and may be associated with ribosomes (Nissl bodies) (see also Papasozomenos Ref. [44]), whereas the rounded, ring or crescent shaped inclusions were only stained with ET3 (Fig. 1f) and not RD3 (Fig. 1e). In neither case were glial cell inclusions, or astrocytic plaques, of the type seen in CBD, present. These cases presented clinically with FTD and showed no features to distinguish them from the other 12 sporadic FTLD cases.

Familial FTLD

Semi-quantitative rating data for AT8, ET3 and RD3 immunostaining in the 27 familial FTLD cases is given in Table 3.

MAPT mutations in exons 9, 12 and 13

In cases #15 (exon 9, L266V), #36 (exon 12, Q336R), #37 (exon 12, E342V) and #38 (exon 12, K369I), many neurons containing diffuse deposits of tau protein were seen in frontal cortex in AT8 immunostained sections. In some cells, in each mutation, the insoluble tau was aggregated into rounded structures resembling the Pick bodies seen in the sporadic FTLD cases (Fig. 2a). These Pick-like bodies were likewise stained with RD3 anti-

Table 3 Frequency of tau-immunoreactive pathological changes in frontal cortex as detected in 27 patients with familial FTLD with *MAPT* mutations using AT8 (all tau isoforms), ET3 (4R-tau isoforms only) and RD3 (3R-tau isoforms only) antibodies

Case	*MAPT* mutation	AT8	ET3	RD3	Tau biochemistry
15[a,d]	L266V	++++[a,d]	+[d]	++++[a]	Mostly 3R[2]
16[c]	N279K	+++	+	0	4R only [2]
17[c]	N279K	+++	+++	0	4R only [2]
18[c]	N279K	+++	++	0	4R only [6]
19[c]	N279K	+++	+	0	4R only [6]
20[c]	N279K	+++	++	0	4R only [6]
21[c]	N279K	+++	++	0	4R only [6]
22[c]	N279K	++++	+++	0	4R only [6]
23[c]	N279K	++++	+++	0	4R only [6]
24[c]	N296H	++++	+++	0	4R only [25]
25[b]	P301L	++++	+	0	4R only [51, 63]
26[b]	P301L	+++	++	0	4R only [51, 63]
27[b,e]	P301L	++++	0	0/+	4R only [51, 63]
28[b,e]	P301L	++++	+++	+	4R only [51, 63]
29[c]	S305S	++	+++	0	4R only [19]
30[c]	Exon 10+16	+	0/+	0	n.d.
31[c]	Exon 10+16	+++	+	0	4R only [46]
32[c]	Exon 10+16	+++	++	0	4R only [46]
33[c]	Exon 10+16	++++	++++	0	n.d.
34[c]	Exon 10+16	++++	+++	0/+	n.d.
35[c]	Exon 10+16	+++	++	0	n.d.
36[a]	Q336R	++++	0/+	++++	n.d.
37[a,d]	E342V	+++[a,d]	0/+[d]	+[a]	Mostly 4R [30]
38[a]	K369I	++++	0	+++	3R and 4R [42]
39[a]	G389R	++	0	+	n.d.
40[b]	R406W	+++	+++	+++	3R and 4R [51, 63]
41[b]	R406W	+++	++	++	3R and 4R [51, 63]

Tau biochemical findings are shown (where known)
[a]Pick bodies present in neurones
[b]Neurofibrillary tangle-like structures in neurones
[c]Neurofibrillary tangle-like structures in neurons with glial cell tangles in white matter
[d]Glia in grey matter staining
[e]Some diffuse β-amyloid plaques, – absent, 0/+ rare, + few, ++ moderate number, +++ many, ++++ very many, 3R 3-repeat tau, 4R 4-repeat tau, n.d. not done

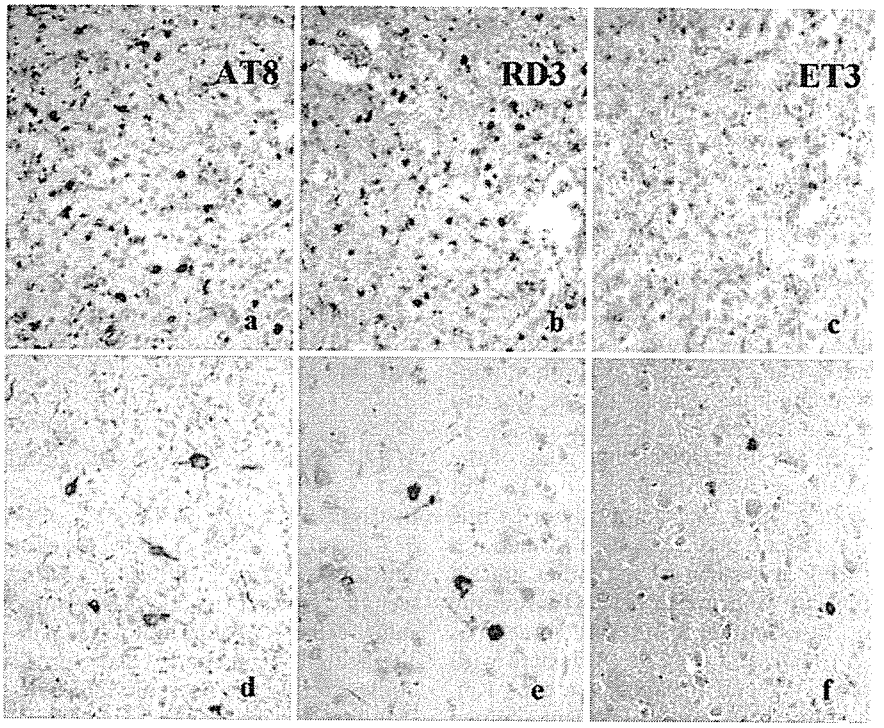

Fig. 2 Tau pathology in patients with familial FTLD. In patient #15 with *MAPT* L266V mutation (**a–c**), Pick bodies within pyramidal cells of the frontal cortex are immunoreactive with AT8 (**a**) and RD3 (**b**) antibodies, but not with ET3 (**c**) antibody. However, there are also numerous astrocytes within grey matter of frontal cortex that are immunoreactive with AT8 antibody (**a**), some of which are also reactive with ET3 antibody (**c**), but not with RD3 (**b**) antibody. In patient #40 with *MAPT* R406W mutation (**d–f**) neurones show amorphous tau deposits or neurofibrillary tangle-like structures that are immunoreactive with AT8 (**d**), RD3 (**e**) and ET3 (**f**) antibodies. In this patient numerous neuropil threads are seen but these are reactive only with AT8 antibody (**d**). Immunoperoxidase-haematoxylin, AT8 antibody (**a**, **d**), RD3 antibody (**b**, **e**), ET3 antibody (**c**, **f**). All 250 times microscope magnification

body (Fig. 2b) but not (or only very rarely so) with ET3 antibody (Fig. 2c). However, in L266V and E342V *MAPT* mutations numerous diffusely stained astrocytes were seen with AT8 antibody (Fig. 2a). These were also stained with ET3 (Fig. 2c), but not RD3 (Fig. 2b), antibody. In case #40 (exon 13, G389R), a few or a moderate number of pyramidal neurons with amorphous tau deposits were seen in AT8 stained sections, these sometimes having a rounded appearance resembling Pick bodies. Many thread-like structures were also seen within deeper layers of the frontal cortex. Occasionally, the Pick-like bodies were stained with RD3 antibody, but not with ET3.

In cases #40 and 41 (exon 13, R406W) many nerve cells containing diffuse cytoplasmic tau deposits or well-formed NFT were seen in AT8 immunostained sections (Fig. 2d). These NFT were strongly stained with RD3 (Fig. 2e), but less so with ET3 (Fig. 2f).

MAPT mutations in exon 10

A similar tau pathology was seen in the 8 cases with N279K *MAPT* mutation in exon 10 (patients #16–23), albeit these cases were from two different kindreds and of different ethnic background (Japanese, cases #16 and 17 and North American/Caucasians, cases # 18–23). This was characterised in AT8 immunostained sections by the presence of pyramidal neurons in frontal cortex containing either amorphous cytoplasmic tau deposits or deposits resembling NFT (Fig. 3a), along with variable numbers of ballooned neurons and widespread glial cell tangles within grey matter and, especially within, white matter (Fig. 3d). These neuronal and glial cell tau deposits were strongly stained with ET3 (Fig. 3c, f) antibody, but not, or only rarely so in occasional cases, with RD3 antibody (Fig. 3b, e). The patient with *MAPT* N296H mutation (case #24) and those with *MAPT* exon 10 +16 splice site mutation (cases #30–35) showed similar changes to those with *MAPT* N279K mutation.

In the case with *MAPT* S305S mutation (case #29), a moderate number of small curvilinear, ring or crescent-shaped bodies were seen in neurons on AT8 staining. These were also stained with ET3 but not RD3 antibody. In cases with P301L mutation (cases #25–28), a moderate number of, or many, diffuse cytoplasmic and NFT-like tau aggregates were seen in pyramidal neurons on AT8 immunostaining, along with many neuropil threads (Fig. 3g). Many of the

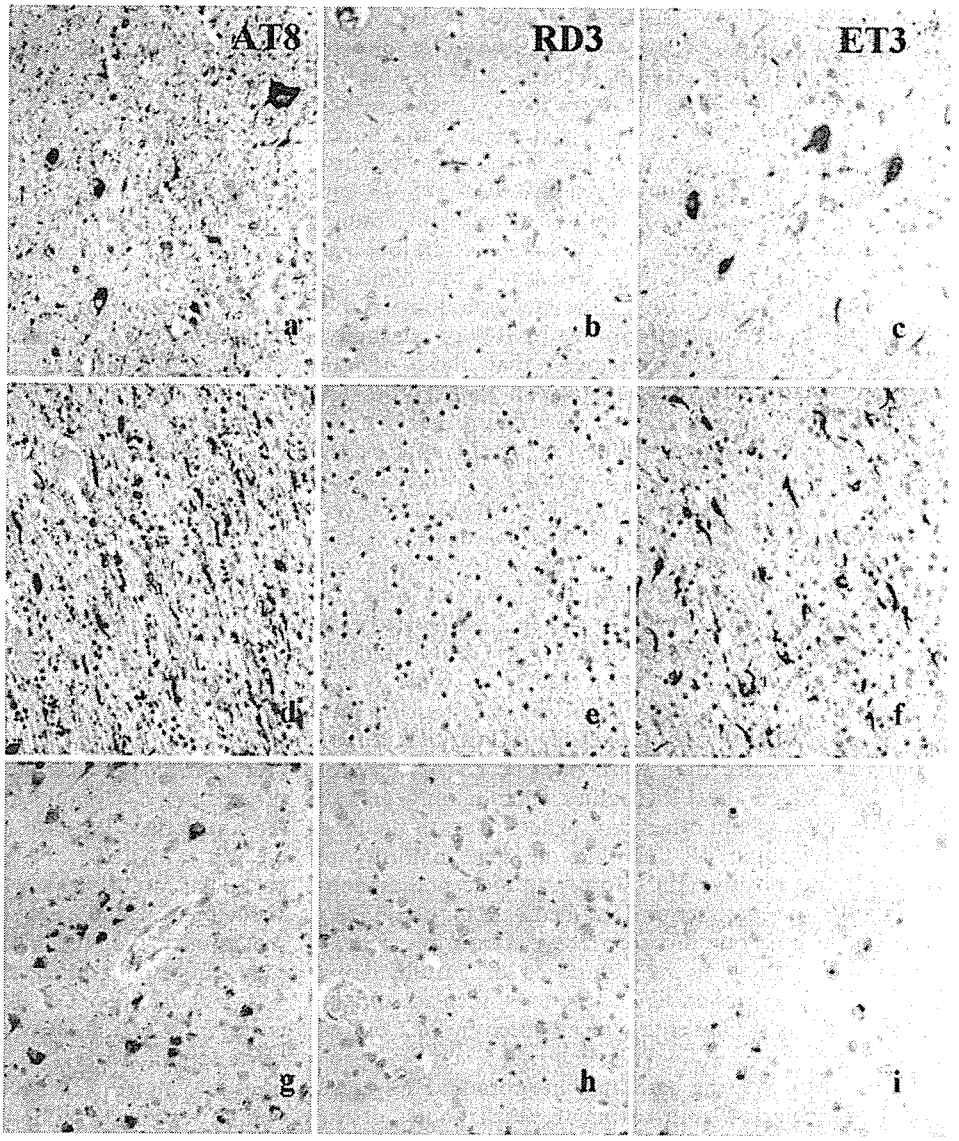

Fig. 3 Tau pathology in patients with familial FTLD. In patient #23 with *MAPT* N279K mutation (**a–f**), pyramidal cells of the frontal cortex are immunoreactive with AT8 (**a**) and ET3 (**c**) antibodies, but not with RD3 (**b**) antibody. Similarly, numerous glial cells within white matter of the frontal cortex contain tangles that are immunoreactive with AT8 antibody (**d**) and most of which are also reactive with ET3 antibody (**f**), but not with RD3 (**e**) antibody. In patient #25 with *MAPT* P301L mutation (**g–i**) neurones show amorphous tau deposits or neurofibrillary tangle-like structures that are immunoreactive with AT8 (**g**) and ET3 (**i**) antibodies, but not with RD3 antibody (**h**). In this patient numerous neuropil threads reactive only with AT8 antibody (**g**) are seen. Immunoperoxidase-haematoxylin, AT8 antibody (**a, d, g**), RD3 antibody (**b, e, h**), ET3 antibody (**c, f, i**). All 250 times microscope magnification

AT8-immunoreactive neurons were also stained with ET3 antibody, adopting a NFT-like appearance in some instances, but in others a perinuclear ring or crescent shaped structure was seen (Fig. 3i). In cases #25 and 26 none of the AT8-positive structures in nerve cells were stained with RD3 antibody (Fig. 3h), though in cases #27 and 28, respectively, rare or occasional nerve cells were RD3 reactive.

Discussion

In the present report, we have investigated by immunohistochemistry the tau isoform composition of the tau pathology present in 14 cases of sporadic FTLD and 27 cases of familial FTLD association with mutations in *MAPT* employing antibodies specific to 3R and 4R isoforms of tau.

Biochemical analysis of sarkosyl-insoluble tau extracted from brains of patients with sporadic FTLD where Pick bodies, or Pick body-like structures, are known to be present has variably shown only 3R-tau [8, 37, 54], a mixture of 3R- and 4R-tau [1, 37, 62, 66] or only 4R-tau [47, 62, 66] to be present. We find here that whenever Pick bodies are present in hippocampus and cerebral cortex in sporadic FTLD cases, these can only be detected using the antibody to 3R-tau. These findings build on an earlier report of ours [10] in which only a single case with Pick bodies was investigated and are in agreement with other immunohistochemical studies which have employed 4R-tau, but not 3R-tau, specific antibodies [1, 26]. Zhukareva and colleagues [66] investigated 14 cases of FTLD by immunohistochemistry using (different to the present study) 3R- and 4R-tau specific antibodies. In 12 of these patients and consistent with present findings, the Pick bodies were reactive only

with 3R-tau antibody. However, in seven of these cases 4R-tau species could be detected on western blot, though by immunohistochemistry this seemed to be localized in occasional cells bearing neurofibrillary tangles rather than Pick bodies and in neuropil threads [66]. Nonetheless, the other two cases in this latter study [66] displayed Pick bodies immunoreactive only to 4R-tau species and in these only 4R-tau was detected on western blot. Case #8 in the study by Zhukareva et al. [66] was case #13 in this present study. Consistent with Zhukareva et al. [66], we also found the tau pathology in this particular case reactive only with 4R-tau antibody. However, we are not convinced that these 4R-tau immunoreactive structures are indeed Pick bodies for the following reasons. First, they had a curvilinear, crescent or ring-shaped profile distinct in appearance from the rounded or oval, compact appearance associated with classic Pick bodies [28]. Second, no tau inclusions at all were present in either dentate gyrus granule cells or pyramidal cells of the hippocampus: the presence of Pick bodies in this region is pathognomic. In this present study, case #14 displayed a similar (to case #13) pattern of 4R-tau pathology. Therefore, in this present study, we find that in all cases of sporadic FTLD where unequivocal Pick bodies are present, these contain only 3R isoforms of tau. Nonetheless, it is acknowledged from the literature that there may be (rare) cases of FTLD in which the Pick bodies contain only 4R-tau isoforms [38, 66] and other cases where a few of the Pick bodies may contain 4R-tau while the great majority are only 3R-tau positive [1]. However, Pick bodies containing 4R-tau may better described as Pick-like bodies since, in at least one instance [38], electron microscopy has revealed these to be composed of parallel, long period twisted ribbon-like structures instead of the random, straight filaments typically seen in Pick bodies [28].

Consistent with these findings in sporadic FTLD, we could not detect 4R-tau within the Pick bodies of the frontal cortex of familial FTLD cases associated with *MAPT* mutations, L266V (case #15) (see also Ref. [23]), Q336R (case #36), E342V (case #37), K369I (case #38) and G389R (case #39), despite western blots showing 4R-tau species [23, 31, 43, 44] to be present in the brains of such patients (where frozen tissues were available for analysis). de Silva and co-workers reported similar findings in two other cases with *MAPT* G389R mutation [10]. Again this apparent discrepancy can be explained in terms of anatomical compartmentalization in the case of *MAPT* L266V and E342V mutations at least. In these mutations, tau-immunoreactive astrocytes are prominent and we have shown here that such cells are 4R-tau reactive and probably therefore responsible for the presence of 4R-tau isoforms on western blot. We have not been able to determine the anatomical correlates of 4R-tau isoforms seen on western blot in *MAPT* K369I and G389R mutations, though in a previous study of ours on one case with *MAPT* G389R mutation [45] 4R-tau was, on western blotting, a very minor constituent and therefore be undetectable by immunohistochemistry [10]. Similarly, Bronner et al. [4] have recently reported Pick bodies in *MAPT* G272V mutation to contain only 3R-tau isoforms on immunohistochemistry but because of rare 4R-tau containing NFT, western blotting showed a mix pattern of 3R- and 4R-tau isoforms.

Consistent with the many reports showing, by western blot, that patients with familial FTLD associated with exon 10 coding and splice site mutations contain only mutated 4R-tau isoforms [30] or selective increases in the proportion of wild-type 4R-tau [2, 6, 9, 19, 24, 25, 35, 40, 46, 60] in their brains, we found that the neuronal and glial tau pathology in *MAPT* N279K, N296H, P301L, S305S and the *MAPT* exon 10 +16 (see also Refs. [10] and [43] for exon 10 +3 mutation) mutations was exclusively associated with 4R-tau, except in two of the *MAPT* P301L mutation cases (cases #27 and 28) in whom rare 3R-tau immunoreactive cells were present and which might reflect the coincidental presence of minor Alzheimer-type pathology involving PHF, immunoreactive with both 3R- and 4R-tau antibodies because all six tau isoforms are equally represented. Some β-amyloid plaques were also present in these two *MAPT* P301L cases, again suggestive of additional Alzheimer-type pathology. Only in *MAPT* R406W mutation did we find the numerous NFT and neuropil threads [49, 63] immunoreactive with both 3R- and 4R-tau antibodies. Western blotting of the sarkosyl-insoluble tau extracted from the brains of patients with this particular mutation shows all six tau isoforms, similar to Alzheimer's disease, to occur [24, 63]. Indeed, ultrastructurally, the NFT in *MAPT* R406W mutation are in the form of paired helical filaments identical to those in AD [49]. However, it is not clear whether the same tangle in *MAPT* R406W mutation cases is both 3R- and 4R-tau immunoreactive, or whether separate populations of tangle bearing cells are involved, some containing exclusively 3R-tau, others 4R-tau, but the finding [10] in AD that NFT cells are doubly immunoreactive for 3R- and 4R-tau would make this unlikely. Notably, *MAPT* V337M mutation shares a very similar neurofibrillary pathology as *MAPT* R406Wmutation [57, 61] with all six tau isoforms being represented on western blot [57]. It might be inferred therefore that here too the tangles would be both 3R- and 4R-tau immunoreactive, though to our knowledge such investigations have not as yet been performed.

Although some studies [31, 37] have corralled cases of CBD and PSP, as tauopathies, under the umbrella of FTLD, as allowed under McKhann criteria [34], we did not include such cases within the present investigation. This was partly because we have already reported [10] that neurofibrillary tangles in PSP are stained only or mostly with antibody to 4R-tau, as is consistent with tau biochemical findings of mostly or only 4R-tau isoforms being present in sarkosyl insoluble fractions of tau [10, 37]. Furthermore, Mott et al. [37] have also shown that sarkosyl insoluble fractions of tau in CBD are likewise

composed mostly of 4R-tau and a similar pattern of 4R-tau immunoreactivity would therefore be anticipated. Indeed this was so in a single case of CBD we have so far studied (Unpublished data).

In conclusion, we have shown here that whenever classic Pick bodies are present, be these in cases of sporadic FTLD or familial cases with *MAPT* mutations (L266V, Q336R, E342V, K369I), only 3R-tau isoforms are present in such structures. The presence of 4R-tau isoforms on western blot in *MAPT* L266V and E342V mutations relates to their presence in glial cells and not Pick bodies. We have therefore not been able to confirm previous reports of the presence of 4R-tau isoforms in Pick bodies [66] and given the structural differences between these and classic Pick bodies [38], it might be better for the moment to consider the latter as Pick body-like structures pending further investigation. Neurofibrillary and glial cell tangles in cases of familial FTLD with *MAPT* exon 10 mutations (i.e., N279K, N296H, P301L, S305S and exon 10 +16 mutations) were usually or exclusively composed of 4R-tau, consistent with biochemical findings of mostly or only 4R-tau isoforms within sarkosyl-insoluble fractions from brains of such cases. The use of such tau isoform-specific antibodies may help to further refine the pathological criteria underpinning FTLD.

Acknowledgements AMS was supported by a Wolfson Scholarship and Alzheimer's Research Trust Alzheimer's Disease Research Centre Grant to DMAM. TL is supported by the Parkinson's Disease Society. This work was supported by the Reta Lila Weston Trust for Medical Research (RdS, AL, TL) and the PSP (Europe) Association (KS), which also support the Queen Square Brain Bank. The authors thank the many other people who were involved in collecting and characterising the familial FTLD cases with *MAPT* mutations and the other sporadic FTLD cases and by doing so making this multicentre collaborative study possible. The authors would also like to thank the patients and their families, without whose generous support none of this research would have been possible.

References

1. Arai T, Ikeda K, Akiyama H, Shikamoto Y, Tsuchiya K, Yagishita S, Beach T, Rogers J, Schwab C, McGeer PL (2001) Distinct isoforms of tau aggregated in neurons and glial cells in brains of patients with Pick's disease, corticobasal degeneration and progressive supranuclear palsy. Acta Neuropathol 101:167–173
2. Arima K, Kowalska A, Hasegawa M, Mukoyama M, Watanabe R, Kawai M, Takahashi K, Iwatsubo T, Tabira T, Sunohara N (2000) Two brothers with frontotemporal dementia and parkinsonism with an N279K mutation of the tau gene. Neurology 54:1787–1795
3. Bird TD, Nochlin D, Poorkaj P, Cherrier M, Kaye J, Payami H, Peskind E, Lampe TH, Nemens E, Boyer P, Schellenberg GD (1999) A clinical pathological comparison of three families with frontotemporal dementia and identical mutations in the tau gene (P301L). Brain 122:741–756
4. Bronner IF, ter Meulen BC, Azmani A, Severijnen LA, Willemsen R, Kamphorst W, Ravid R, Heutink P, van Swieten JC (2005) Hereditary Pick's disease with the G272V tau mutation shows a predominant three-repeat tau pathology. Brain 128:2645–2653
5. Bugiani O, Murrell JR, Giaccone G, Hasegawa M, Ghigo G, Tabaton M, Morbin M, Primavera A, Carella F, Solaro C, Grisoli M, Savoiardo M, Spillantini MG, Tagliavini F, Goedert M, Ghetti B (1999) Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in tau. J Neuropathol Exp Neurol 58:667–677
6. Clark LN, Poorkaj P, Wszolek Z, Geschwind DH, Nasreddine ZS, Miller B, Li D, Payami H, Awert F, Markopoulou K, Andreadis A, D'Souza I, Lee VM, Reed L, Trojanowski JQ, Zhukareva V, Bird T, Schellenberg G, Wilhelmsen KC (1998) Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17. Proc Natl Acad Sci USA 95:13103–13107
7. Constantinidis RJ, Richard J, Tissot R (1974) Pick's disease: histological and clinical correlations. Eur Neurol 11:208–217
8. Delacourte A, Sergeant N, Wattez A, Gauvreau D, Robitaille Y (1998) Vulnerable neuronal subsets in Alzheimer's and Pick's disease are distinguished by their tau isoform distribution and phosphorylation. Ann Neurol 43:193–204
9. Delisle MB, Murrell JR, Richardson R, Trofatter JA, Rascol O, Soulages X, Mohr M, Calvas P, Ghetti B (1999) A mutation at codon 279 (N279K) in exon 10 of the tau gene causes a tauopathy with dementia and supranuclear palsy. Acta Neuropathol 98:62–77
10. de Silva R, Lashley T, Gibb G, Hope A, Reid A, Bandopadhyay R, Utton M, Strand C, Jowett T, Khan N, Anderton B, Wood N, Holton J, Revesz T, Lees A (2003) Pathological inclusion bodies in tauopathies contain distinct complements of tau with three or four microtubule-binding repeat domains as demonstrated by new specific monoclonal antibodies. Neuropathol Appl Neurobiol 29:288–302
11. D'Souza I, Poorkaj P, Hong M, Nochlin D, Lee VM, Bird TD, Schellenberg GD (1999) Missense and silent tau gene mutations cause frontotemporal dementia with parkinsonism-chromosome 17 type, by affecting multiple alternative RNA splicing regulatory elements. Proc Natl Acad Sci USA 96:5598–5603
12. Foster NL, Wilhelmsen K, Sima AA, Jones MZ, D'Amato CJ, Gilman S (1997) Frontotemporal dementia and parkinsonism linked to chromosome 17: a consensus conference. Ann Neurol 41:706–715
13. Fujino Y, Wang DS, Thomas N, Espinoza M, Davies P, Dickson DW (2005) Increased frequency of argyrophilic grain disease in Alzheimer disease with 4R tau-specific immunohistochemistry. J Neuropathol Exp Neurol 64:209–214
14. Ghetti B, Murrell JR, Zolo P, Spillantini MG, Goedert M (2000) Progress in hereditary tauopathies: a mutation in the tau gene (G389R) causes a Pick disease-like syndrome. Ann N Y Acad Sci 920:52–62
15. Goedert M (2005) Tau gene mutations and their effects. Mov Disord 20(Suppl 12):S45–S52
16. Goedert M, Jakes R, Crowther RA, Six J, Lubke U, Vandermeeren M, Cras P, Trojanowski JQ, Lee VM (1993) The abnormal phosphorylation of tau protein at Ser-202 in Alzheimer disease recapitulates phosphorylation during development. Proc Natl Acad Sci USA 90:5066–5070
17. Goedert M, Spillantini MG, Crowther RA, Chen SG, Parchi P, Tabaton M, Lanska DJ, Markesbery WR, Wilhelmsen KC, Dickson DW, Petersen PB, Gambetti P (1999). Tau gene mutation in familial progressive subcortical gliosis. Nat Med 5:454–457
18. Grover A, DeTure M, Yen SH, Hutton M (2002) Effects on splicing and protein function of three mutations in codon N296 of tau in vitro. Neurosci Lett 323:33–36
19. Halliday GM, Song YJC, Creasey H, Morris JG, Brooks WS, Kril JJ (2006) Neuropathology in the S305S tau gene mutation. Brain (in press)
20. Hasegawa M, Smith MJ, Iijima M, Tabira T, Goedert M (1999) FTDP-17 mutations N279K and S305N in tau produce increased splicing of exon 10. FEBS Lett 443:93–96
21. Hayashi S, Toyoshima Y, Hasegawa M, Umeda Y, Wakabayashi K, Tokiguchi S, Iwatsubo T, Takahashi H (2002)

Late-onset frontotemporal dementia with a novel exon 1 (Arg5His) tau gene mutation. Ann Neurol 51:525–530
22. Hodges JR, Davies RR, Xuereb JH, Casey B, Broe M, Bak TH, Kril JJ, Halliday GM (2004) Clinicopathological correlates in frontotemporal dementia. Ann Neurol 56:399–406
23. Hogg M, Grujic ZM, Baker M, Demirci S, Guillozet AL, Sweet AP, Herzog LL, Weintraub S, Mesulam MM, LaPointe NE, Gamblin TC, Berry RW, Binder LI, de Silva R, Lees A, Espinoza M, Davies P, Grover A, Sahara N, Ishizawa T, Dickson D, Yen SH, Hutton M, Bigio EH (2003) The L266V tau mutation is associated with frontotemporal dementia and Pick-like 3R and 4R tauopathy. Acta Neuropathol 106:323–336
24. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon MJ, Nowotny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski JQ, Basun H, Lannfelt L, Neystat M, Fahn S, Dark F, Tannenberg T, Dodd PR, Hayward N, Kwok JBJ, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P (1998) Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 393:702–705
25. Iseki E, Matsumura T, Marui W, Hino H, Odawara T, Sugiyama N, Suzuki K, Sawada H, Arai T, Kosaka K (2001) Familial frontotemporal dementia and parkinsonism with a novel N296H mutation in exon 10 of the tau gene and a widespread tau accumulation in the glial cells. Acta Neuropathol 102:285–292
26. Ishizawa K, Ksiezak-Reding H, Davies P, Delacourte A, Tiseo P, Yen S-H, Dickson DW (2000) A double-labelling immunohistochemical study of tau exon 10 in Alzheimer's disease, progressive supranuclear palsy and Pick's disease. Acta Neuropathol 100:235–244
27. Ishizawa T, Ko LW, Cookson N, Davies P, Espinoza M, Dickson DW (2002) Selective neurofibrillary degeneration of the hippocampal CA2 sector is associated with four repeat tauopathies. J Neuropathol Exp Neurol 61:1040–1047
28. Kertesz A, McGonagle P, Blair M, Davidson W, Munoz DG (2005) The evolution and pathology of frontotemporal dementia. Brain 128:1996–2005
29. Kobayashi T, Ota S, Tanaka K, Ito Y, Hasegawa M, Umeda Y, Motoi Y, Takanashi M, Yasuhara M, Anno M, et al (2003) A novel L266V mutation of the tau gene causes frontotemporal dementia with a unique tau pathology. Ann Neurol 53:133–137
30. Lippa CF, Zhukareva V, Kawarai T, Uryu K, Shafiq M, Nee LE, Grafman J, Liang Y, St George-Hyslop PH, Trojanowski JQ, Lee VM (2000) Frontotemporal dementia with novel tau pathology and a Glu342Val tau mutation. Ann Neurol 48:850–858
31. Lipton AM, White CL III, Bigio EH (2004) Frontotemporal lobar degeneration with motor neuron disease-type inclusions predominates in 76 cases of frontotemporal degeneration. Acta Neuropathol 108:379–385
32. Mann DMA (2005) The genetics and molecular pathology of frontotemporal lobar degeneration. In: Burns A, O'Brien J, Ames D, Arnold H (eds) Dementia, 3rd edn. , London, pp 689–701
33. Mann DMA, South PW, Snowden JS, Neary D (1993) Dementia of frontal lobe type: neuropathology and immunohistochemistry. J Neurol Neurosur Ps 56:605–614
34. McKhann GM, Albert MS, Grossman M, Miller B, Dickson D, Trojanowski JQ (2001) Workgroup on Frontotemporal dementia and Picks disease; Clinical and pathological diagnosis of frontotemporal dementia: report of the workgroup on Frontotemporal dementia and Picks disease. Arch Neurol 59:1203–1204
35. Mirra SS, Murrell JR, Gearing M, Spillantini MG, Goedert M, Crowther RA, Levey AI, Jones R, Green J, Shoffner JM, Wainer BH, Schmidt ML, Trojanowski JQ, Ghetti B (1999) Tau pathology in a family with dementia and a P301L mutation in tau. J Neuropathol Exp Neurol 58:335–345
36. Miyasaka T, Morishima-Kawashima M, Ravid R, Kamphorst W, Nagashima K, Ihara Y (2001) Selective deposition of mutant tau in the FTDP-17 brain affected by the P301L mutation. J Neuropathol Exp Neurol 60:872–884
37. Motoi Y, Iwamoto H, Itaya M, Kobayashi T, Hasegawa M, Yasuda M, Mizuno Y, Mori H (2005) Four-repeat tau positive Pick body-like inclusions are distinct from classic Pick bodies. Acta Neuropathol 110:431–433
38. Mott RT, Dickson DW, Trojanowski JQ, Zhukareva V, Lee VM, Forman M, van Deerlin V, Ervin JF, Wang DS, Schmechel DE, Hulette CM (2005) Neuropathologic, biochemical, and molecular characterization of the frontotemporal dementias. J Neuropathol Exp Neurol 64:420–428
39. Murrell JR, Spillantini MG, Zolo P, Guazzelli M, Smith MJ, Hasegawa M, Redi F, Crowther RA, Pietrini P, Ghetti B, Goedert M (1999) Tau gene mutation G389R causes a tauopathy with abundant pick body-like inclusions and axonal deposits. J Neuropathol Exp Neurol 58:1207–1226
40. Nasreddine ZS, Loginov M, Clark LN, Lamarche J, Miller BL, Lamontagne A, Zhukareva V, Lee VM, Wilhelmsen KC, Geschwind DH (1999) From genotype to phenotype: a clinical pathological, and biochemical investigation of frontotemporal dementia and parkinsonism (FTDP-17) caused by the P301L tau mutation. Ann Neurol 45:704–715
41. Neary D, Snowden JS, Mann DMA (2005) Frontotemporal dementia. Lancet Neurol 4:771–780
42. Neumann M, Schulz-Schaeffer W, Crowther RA, Smith MJ, Spillantini MG, Goedert M, Kretzschmar HA (2001) Pick's disease associated with the novel Tau gene mutation K369I. Ann Neurol 50:503–513
43. Neumann M, Mittelbronn M, Simon P, Vanmassenhove B, de Silva R, Lees A, Klapp J, Meyermann R, Kretzschmar HA (2005) A new family with frontotemporal dementia with intronic 10+3 splice site mutation in the tau gene: neuropathology and molecular effects. Neuropathol Appl Neurobiol 31:362–373
44. Papasozomenos SC (1989) Tau protein immunoreactivity in dementia of the Alzheimer type: II. Electron microscopy and pathogenetic implications. Effects of fixation on the morphology of the Alzheimer's abnormal filaments. Lab Invest 60:375–389
45. Pickering-Brown SM, Baker M, Yen S-H, Liu W-K, Hasegawa M, Cairns NJ, Lantos PL, Rossor M, Iwatsubo T, Davies Y, Allsop D, Furlong R, Owen F, Hardy J, Mann DMA, Hutton M (2000) Pick's disease is associated with mutations in the tau gene. Ann Neurol 48:859–867
46. Pickering-Brown SM, Richardson AM, Snowden JS, McDonagh AM, Burns A, Braude W, Baker M, Liu WK, Yen SH, Hardy J, Hutton M, Davies Y, Allsop D, Craufurd D, Neary D, Mann DMA (2002) Inherited frontotemporal dementia in nine British families associated with intronic mutations in the tau gene. Brain 125:732–751
47. Pickering-Brown SM, Baker M, Nonaka T, Ikeda K, Sharma S, MacKenzie J, Simpson SA, Moore JW, Snowden JS, de Silva R, Revesz T, Hasegawa M, Hutton M, Mann DM (2004) Frontotemporal dementia with Pick-type histology associated with Q336R mutation in the tau gene. Brain 127:1415–1426
48. Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, Wiederholt WC, Raskind M, Schellenberg GD (1998) Tau is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neurol 43:815–825
49. Reed LA, Grabowski TJ, Schmidt ML (1997) Autosomal dominant dementia with widespread neurofibrillary tangles. Ann Neurol 42:564–572

50. Rizzini C, Goedert M, Hodges JR, Smith MJ, Jakes R, Hills R, Xuereb JH, Crowther RA, Spillantini MG (2000) Tau gene mutation K257T causes a tauopathy similar to Pick's disease. J Neuropathol Exp Neurol 59:990–1001
51. Rizzu P, van Swieten JC, Joosse M, Hasegawa M, Stevens M, Tibben A, Niermeijer MF, Hillebrand M, Ravid R, Oostra BA, Goedert M, van Duijn CM, Heutink P (1999) High prevalence of mutations in the microtubule-associated protein tau in a population study of frontotemporal dementia in the Netherlands. Am J Hum Genet 64:414–421
52. Rosso SM, van Herpen E, Deelen W, Kamphorst W, Severijnen LA, Willemsen R, Ravid R, Niermeijer MF, Dooijes D, Smith MJ, Goedert M, Heutink P, van Swieten JC (2002) A novel tau mutation, S320F, causes a tauopathy with inclusions similar to those in Pick's disease. Ann Neurol 51:373–376
53. Rosso SM, Landweer EJ, Houterman M, Donker Kaat L, van Duijn CM, van Swieten JC (2003) Medical and environmental risk factors for sporadic frontotemporal dementia: a retrospective case-control study. J Neurol Neurosur Ps 74:1574–1576
54. Sergeant N, Wattez A, Delacourte A (1999) Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: tau pathologies with exclusively exon 10 isoforms. J Neurochem 72:1243–1249
55. Shi J, Shaw CL, Du Plessis D, Richardson AMT, Bailey K, Julien C, Neary D, Snowden JS, Mann DMA (2005) Histopathological changes underlying frontotemporal lobar degeneration with clinicopathological correlation. Acta Neuropathol 110:501–512
56. Snowden JS, Neary D, Mann DMA (1996) Fronto-temporal lobar degeneration: Fronto-temporal dementia, progressive aphasia, semantic dementia. Churchill Livingstone, Edinburgh, pp 1–227
57. Spillantini MG, Crowther RA, Goedert M (1996) Comparison of the neurofibrillary pathology in Alzheimer's disease and familial presenile dementia with tangles. Acta Neuropathol 92:42–48
58. Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B (1998) Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. Proc Natl Acad Sci USA 95:7737–7741
59. Spillantini MG, Yoshida H, Rizzini C, Lantos PL, Khan N, Rossor MN, Goedert M, Brown J (2000) A novel tau mutation (N296N) in familial dementia with swollen achromatic neurons and corticobasal inclusion bodies. Ann Neurol 48:939–943
60. Stanford PM, Halliday GM, Brooks WS, Kwok JB, Storey CE, Creasey H, Morris JG, Fulham MJ, Schofield PR (2000) Progressive supranuclear palsy pathology caused by a novel silent mutation in exon 10 of the tau gene: expansion of the disease phenotype caused by tau gene mutations. Brain 123:880–893
61. Sumi SM, Bird TD, Nochlin D, Raskind MA (1992) Familial presenile dementia with psychosis associated with cortical neurofibrillary tangles and degeneration of the amygdala. Neurology 42:120–127
62. Taniguchi S, McDonagh AM, Pickering-Brown SM, Umeda Y, Iwatsubo T, Hasegawa M, Mann DM (2004) The neuropathology of frontotemporal lobar degeneration with respect to the cytological and biochemical characteristics of tau protein. Neuropathol Appl Neurobiol 30:1–18
63. van Swieten JC, Stevens M, Rosso SM, Rizzu P, Joosse M, de Koning I, Kamphorst W, Ravid R, Spillantini MG, Niermeijer M, Heutink P (1999) Phenotypic variation in hereditary frontotemporal dementia with tau mutations. Ann Neurol 46:617–626
64. Wszolek ZK, Pfeiffer RF, Bhatt MH, Schelper RL, Cordes M, Snow BJ Rodnitsky RL, Wolters EC, Arwert F, Calne DB (1992) Rapidly progressive autosomal dominant parkinsonism and dementia with pallido-ponto-nigral degeneration. Ann Neurol 32:312–320
65. Zarrantz JJ, Ferrer I, Lezcano E, Forcadas MI, Eizaguirre B, Atares B, Puig B, Gomez-Esteban JC, Fernandez-Maiztegui C, Rouco I, Perez-Concha T, Fernandez M, Rodruigez O, Rodriguez-Martinez AB, Martinez de Pancorbo M, Pastor P, Perez-Tur J (2005) A novel mutation (K317M) in the MAPT gene causes FTDP and motor neuron disease. Neurology 64:1578–1585
66. Zhukareva V, Mann DMA, Uryu K, Shuck T, Shah K, Grossman M, Miller BL, Hulette CM, Feinstein S, Trojanowski JQ, Lee VM-Y (2002) Sporadic Pick's disease: a tauopathy characterised by a spectrum of gray and white matter pathological tau isoforms. Ann Neurol 51:730–739

Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience Letters 400 (2006) 97–100
Neuroscience Letters
www.elsevier.com/locate/neulet

Autotaxin expression is enhanced in frontal cortex of Alzheimer-type dementia patients☆

Ken Umemura[a,1], Nobuyuki Yamashita[a,2], Xiaonian Yu[a], Kunimasa Arima[b], Takashi Asada[c], Takao Makifuchi[d], Shigeo Murayama[e], Yuko Saito[e], Kazutomi Kanamaru[e], Yuichi Goto[a], Shinichi Kohsaka[a], Ichiro Kanazawa[a,b], Hideo Kimura[a,*]

[a] *National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan*
[b] *National Center Hospital for Mental, Nervous, and Muscular Disorders, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan*
[c] *Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan*
[d] *National Saigata Hospital, Saigata, Japan*
[e] *Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan*

Received 11 January 2006; received in revised form 3 February 2006; accepted 6 February 2006

Abstract

We searched for genes differentially expressed in the frontal cortices of Alzheimer-type dementia (ATD) patients compared with those of non-ATD controls using DNA microarray and quantitative reverse transcription-polymerase chain reaction (RT-PCR) analyses. Here we show that the expression level of the autotaxin (also called lysophospholipase D or ecto-nucleotide pyrophosphatase/phosphodiesterase 2) gene was significantly greater in ATD cortices than in non-ATD cortices. In both ATD and non-ATD groups, the expression levels were greater in patients with the apoE ε3/ε4 genotype than in patients with the apoE ε3/ε3 genotype, although the differences were not statistically significant. These observations suggest that expression of the autotaxin gene and cell signaling by lysophosphatidic acid may be involved in the pathology of ATD, and that this cell signaling pathway may be a potential target of treatments for ATD.

Keywords: Alzheimer-type dementia; Microarray; Autotaxin; Brain

The frontal cortex has major roles in higher cognitive functions, and disintegration of cognition is a major behavioral symptom of Alzheimer-type dementia (ATD) as well as memory disturbance [3]. Severe atrophy is sometimes observed in the frontal cortex as well as other sites including the hippocampus and parahippocampal gyrus of ATD patients.

Genetic linkage studies of late-onset familial Alzheimer's disease (AD) showed that AD-susceptibility is linked to the apolipoprotein E (ApoE) gene located on chromosome 19 [7]. Amyloid deposits co-localize with Apo E [6] and amyloid β-protein binds to ApoE. The Apo E gene has three variants, ε2, ε3, and ε4, and ε3 is the most common allele. The frequency of the ε4 allele is higher in AD patients [8], and the copy number of this allele correlates with the age of onset of AD [1]. However, the sensitivity of ATD diagnosis using only this allele as a marker was no more than 65% [4]. The present study shows that autotaxin gene expression is greatly enhanced in the brains of ATD-patients compared with those of normal individuals. Expression of this gene tends to be greater in patients with apoE ε3/ε4 than in those with apoE ε3/ε3.

We tested postmortem brains listed in Table 1. Specimen sets A, B, and C were obtained from the National Center of Neurology and Psychiatry (NCNP; Kodaira, Tokyo, Japan) and National Saigata Hospital (Oogatamachi, Niigata, Japan). Specimen set D was from The Tokyo Metropolitan Institute of Gerontology (Itabashi, Tokyo, Japan). The patients in the non-ATD group of set D did not have amyotrophic lateral sclerosis or

☆ Supplementary material: A list of the age, gender, diagnosis for Alzheimer-type dementia, apo E genotype, and logarithmic value for the relative expression level of the autotoxin gene in each subject belonging to specimen sets C and D, normalized to that of the β-actin gene, determined by quantitative reverse transcription-polymerase chain reaction (RT-PCR) experiments (Excel document).

* Corresponding author. Tel.: +81 42 346 1725; fax: +81 42 346 1755.
E-mail address: kimura@ncnp.go.jp (H. Kimura).
[1] Present address: Gifu International Institute of Biotechnology Foundation, Kakamigahara, Gifu, Japan.
[2] Present address: Meiji Seika Kaisha Ltd., Yokohama, Kanagawa, Japan.

0304-3940/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2006.02.008

Table 1
Sets of specimens used

		Number of cases	Age (mean ± S.D.)
Set A[a]	ATD	7 (M: 4; F: 3)	79.6 ± 4.8
	Non-ATD (ALS)[b]	3 (M: 2; F: 1)	77.0 ± 8.9
Set B[a]	ATD	7 (M: 4; F: 3)	79.6 ± 4.8
	Non-ATD (ALS)[b]	5 (M: 4; F: 1)	72.0 ± 9.5
Set C[a]	ATD	9 (M: 4; F: 5)	77.6 ± 6.4
	Non-ATD (ALS)[b]	5 (M: 4; F: 1)	72.0 ± 9.5
Set D[c]	ATD	7 (M: 7; F: 0)	90.7 ± 6.2
	Non-ATD[d]	10 (M: 8; F: 2)	74.4 ± 7.9

[a] Set A is included in set B, and set B is included in set C.
[b] Patients had amyotrophic lateral sclerosis without dementia.
[c] Originated from an institution different from sets A–C.
[d] Patients did not have amyotrophic lateral sclerosis or dementia.

dementia, and no neuropathological symptoms of ATD were observed in their specimens. Four of them had cerebral or lacunar infarct, one cerebral hemorrhage, one small metastatic tumor, and the rest were diagnosed as unremarkable. The protocols used were approved by the local ethics committee of NCNP, National Saigata Hospital, and Tokyo Metropolitan Institute of Gerontology, respectively. Informed consent was obtained from the family of each patient. The frontal lobe was sliced into several pieces, and some were snap frozen in dry ice/ethanol and stored at −80 °C for DNA microarray and quantitative RT-PCR analyses.

Total RNA was prepared from frozen human cortical tissues using TRIzol reagent (Invitrogen), followed by purification using an RNeasy kit (Qiagen, Hilden, Germany), according to the protocols recommended by the manufacturers. To avoid potential biological hazards, homogenization of the tissues was carried out within a glove box, and all apparatus and waste that became in contact with the tissues were autoclaved at 134 °C for 1 h or immersed in disinfectant overnight. Sample preparation, hybridization, data acquisition and analysis of the DNA microarray experiment were performed according to the expression analysis technical manual for the GeneChip system (Affymetrix, Santa Clara, California). Briefly, 8–10 μg of each RNA sample was used for double-stranded cDNA synthesis, then, biotinylated antisense cRNA was synthesized. The cRNA was fragmented by heating in Mg^{2+}-containing buffer before being used for hybridization to microarrays. Human Genome U95Av2 arrays (Affymetrix) targeting about 12,000 genes were used. The hybridized arrays were stained with biotinylated anti-streptavidin antibody and streptavidin-R phycoerythrin conjugate, and the signals were measured with a confocal laser scanner (GeneArray Scanner, Affymetrix). The "signal" values representing the expression level of each gene were calculated with Microarray Suite ver 5.0 software (Affymetrix), and then statistical analyses and selection of genes of interest were performed using Data Mining Tool ver 3.0 (Affymetrix) and Genespring ver 4.1.2 (Silicon Genetics, Redwood City, California) bioinformatic algorithms. RNA prepared from each cortex was analyzed independently, and the DNA microarray experiment was repeated three times to confirm the reproducibility of results for specific genes.

cDNA was synthesized from total RNA samples using Multiple Reverse Transcriptase (Applied Biosystems, Foster City, California). Quantitative PCR by the TaqMan real-time quantitative PCR technique was performed using the PRISM 7700 Sequence Detection System (Applied Biosystems), as recommended in the instruction manuals distributed by Applied Biosystems. The forward primer, reverse primer, and TaqMan probe for each gene were designed using a sequence designing software (Primer Express version 1.5, Applied Biosystems). The nucleotide sequences for the autotoxin gene were as follows: 5′-TGCCGACAAGTGTGACGG-3′ for the forward primer, 5′-CCGGTGAGGCAGGATGAA-3′ for the reverse primer, and 5′-CCTCTCTCTGTGTCCTC-3′ for the Taqman probe. The initial amount of template in the PCR reaction was calculated based on the threshold cycle of the fluorescence curve. Relative amounts of transcripts for relevant genes and the β-actin gene in each sample were determined using an appropriate sample as a standard. The obtained values were converted to common logarithms, normalized to the means for all samples tested, and calibrated by deduction of the values for β-actin gene from those for the relevant genes of each sample.

The *P*-values for the differences in paired comparisons (Fig. 1a and b) were determined by the two-tailed Mann–Whitney *U*-test. For more detailed data analyses taking influences of multiple parameters of the subjects into account, multiple linear regression analysis was employed. The *P*-values for regression coefficients for the regressors were determined by the two-tailed one sample *t*-test.

To determine genes specifically expressed in AD brains, total RNA was prepared from seven frozen cortices of ATD cases and three cortices of ALS cases without dementia ("specimen set A" in Table 1), and DNA microarray experiments (GeneChip system; Affymetrix) targeting about 12,000 known human genes were performed. In ALS without dementia, the neuropathological symptoms appear in motor neurons but not in the central nervous system. Therefore, ALS cases were used as the control for ATD in specimen set A. The experiment was repeated three times for each RNA sample to exclude experimental artifacts. Genes showing higher (76 genes) and lower (36 genes) expressions in the ATD group were selected. The resultant genes were further examined by quantitative reverse transcription-polymerase chain reaction (RT-PCR) with TaqMan chemistry using specimen set B, which includes set A (Table 1). Fourteen genes showed higher and three genes lower expressions in ATD. Since the difference in expression of these genes could reflect the difference between ATD and ALS, the resultant genes were re-evaluated using an additional specimen set, set D, along with set C, which is an expansion of sets A and B. Specimen set D was provided by an institution different from sets A–C, and the control subjects were not ALS cases. Although the differential expressions of most of these genes were not observed in set D, it was confirmed for the autotaxin gene in both set C and set D: greater expression in ATD (Fig. 1a).

Specimen set D differs from set C in: (i) ALS morbidity, (ii) considerably older average age of the ATD group than non-ATD group, and (iii) a steeper gender ratio. Therefore, correlation between age or gender, in addition to the diagnosis (ATD or non-

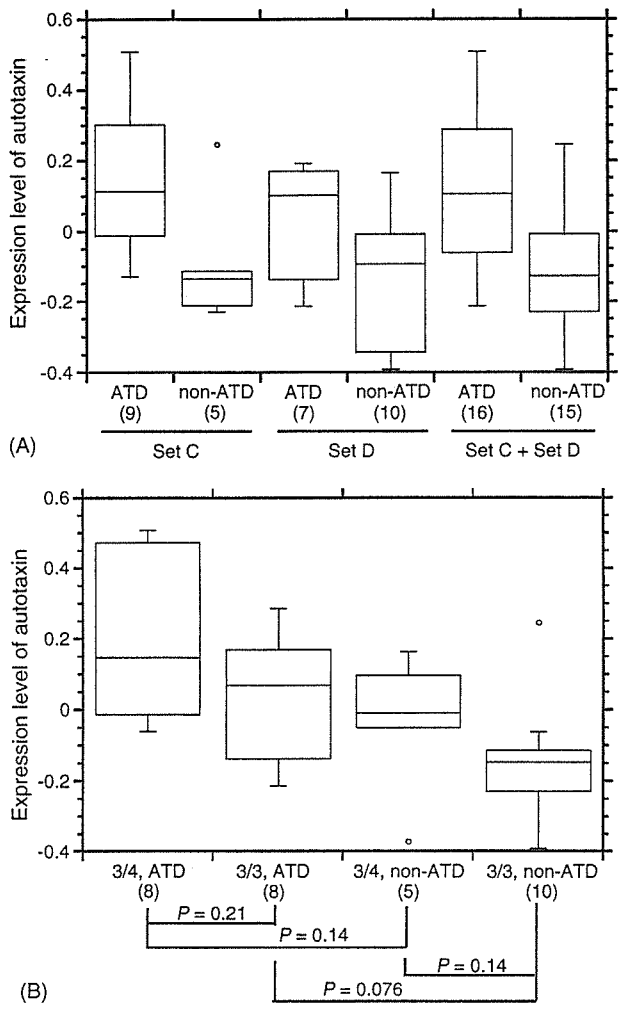

Fig. 1. Box-whisker plot representing the distribution of the expression level of the autotaxin gene. Using total RNA prepared from frozen cortices as the template, two-step quantitative RT-PCR was performed. The values were converted to common logarithms, normalized to the means for all of set C and set D samples. The normalized values for the β-actin gene were deducted from those for the autotaxin gene, and the distribution of the margin is represented. In parentheses are the numbers of samples belonging to each group. (a) Comparisons between ATD groups and non-ATD groups in specimen set C, set D, or set C plus set D. The *P*-value for each pair of ATD and non-ATD groups by the Mann–Whitney *U*-test is 0.028 for set C, 0.079 for set D, and 0.0064 for set C plus set D. (b) Two-way comparisons among ATD groups and non-ATD groups with the apoE genotype of ε3/ε4 (3/4) or ε3/ε3 (3/3) in specimen set C plus set D. The *P*-value for each comparison by the Mann–Whitney *U*-test is indicated.

ATD), and the expression of the autotaxin gene was statistically examined. When the expression data (using both specimen sets) were subjected to multiple linear regression analysis with the diagnosis, age, and gender as indicator variables, the *P*-value for the regression coefficient for each regressor, i.e. the diagnosis, age, and gender, in the regression model was 0.00070, 0.041, and 0.90, respectively. This indicates that diagnosis and age are probable factors linked to expression of the autotaxin gene, but gender is not. Next, the diagnosis, age, and specimen set (whether sets C or D) were introduced as indicator variables. The *P*-value for the regression coefficient for each of diagnosis, age, and specimen set, was 0.0025, 0.091, and 0.77, respectively. This suggests that the specimen set is not linked to expression of the autotaxin gene after the data are normalized for diagnosis and age. In other words, when the expression of the autotoxin gene is to be analyzed, specimen set C and set D may be merged as long as the diagnosis and the age are dealt with as affecting parameters. Age was slightly negatively correlated with autotaxin expression (see below). Because the average age of the ATD group was considerably older than the non-ATD group in specimen set D, for the purpose of examining the greater expression of the autotoxin gene in the ATD group, the merging of specimen set D into set C would not lead to a false positive result if age is not dealt with as an affecting parameter, though a false negative result is possible. When specimen set D was merged with set C, the significance of the differential expression of the autotoxin gene between ATD and non-ATD groups was shown more clearly even without consideration of the age ($P = 0.0064$; Fig. 1a).

For each ATD and non-ATD group in all specimens, the expression level of the autotaxin gene also appeared to be dependent on apoE genotype: a greater level in the apoE ε3/ε4 group than in the ε3/ε3 group (Fig. 1b). The differences were, however, not significant ($P = 0.21$ for the ATD group and $P = 0.14$ for the non-ATD group). At the same time, for each of the apoE ε3/ε4 and ε3/ε3 groups in all specimens, the expression level of the autotaxin gene appeared to be greater in the ATD group than in the non-ATD group ($P = 0.14$ for the apoE ε3/ε4 group and $P = 0.076$ for the ε3/ε3 group; Fig. 1b). In order to take age into account, stepwise multiple linear regression analysis was performed, with diagnosis, age, and apoE genotype being introduced as indicator variables. The *P*-value for the regression coefficient for each regressor, i.e. diagnosis, age, and apoE genotype, in the final regression model was 0.0013, 0.059, and 0.086, respectively. When these factors were introduced as the regressors in this order, the adjusted R^2 value at each of the three steps, which indicates the proportion of the variance of the data explicable by the regression with compensation for the number of regressors introduced, was 0.222, 0.312, and 0.362, respectively. A step-by-step increase of the adjusted R^2 value and relatively small *P*-values indicate that both age and apoE genotype were effective regressors. In the final regression model, the R^2 value (without adjustment for the degree of freedom) was 0.425, and the partial regression coefficients for diagnosis, age, and apoE genotype, were 0.275, −0.0077, and 0.120, respectively. They correspond to: a 1.88-fold increase in ATD subjects compared to a non-ATD subjects, a 0.84-fold decrease per 10 years of additional age, and a 1.32-fold increase in apoE ε3/ε4 subjects compared to ε3/ε3 subjects.

Autotaxin was originally isolated as an autocrine motility factor secreted by melanoma cells which promotes cell motility and metastasis [10], and was recently found to be identical to the lysophosphatidic acid (LPA)-producing enzyme, lysophospholipase D [11,12] and to NPP2, which belongs to the ectonucleotide pyrophosphatase/phosphodiesterase family [5]. LPA induces hyperphosphorylation of Tau protein [9], growth cone collapse and neurite retraction [2]. In addition, tyrosine phosphorylation of focal adhesion kinase (FAK), which is increased by amyloid β peptide [13], is modulated by LPA. These observa-

tions suggest that autotaxin may be involved in ATD pathology. Since autotaxin is a secreted enzyme, the observation that its expression level was greater in the ATD group than the non-ATD group of both apoE ε3/ε4 and ε3/ε3 in all specimens suggests the possibility that the amount of autotaxin protein or LPA in cerebro-spinal fluid can be used as a risk factor marker of ATD with any apo E genotype.

We believe that this is the first demonstration that the autotaxin gene is more strongly expressed in the brain of ATD patients. The present study suggests the possibility that the inhibition of the activity of autotaxin or signal transduction cascade for LPA in the brain might serve as a treatment or a prevention against ATD.

Acknowledgement

This work was supported by a grant from the Organization for Pharmaceutical Safety and Research.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2006.02.008.

References

[1] E.H. Corder, A.M. Saunders, W.J. Strittmatter, D.E. Schmechel, P.C. Gaskell, G.W. Small, A.D. Roses, J.L. Haines, M.A. Pericak-Vance, Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families, Science 261 (1993) 921–923.

[2] N. Fukushima, J.A. Weiner, J. Chun, Lysophosphatidic acid (LPA) is a novel extracellular regulator of cortical neuroblast morphology, Dev. Biol. 228 (2000) 6–18.

[3] M.D. Kopelman, Frontal dysfunction and memory deficits in the alcoholic Korsakoff syndrome and Alzheimer-type dementia, Brain 114 (1991) 117–137.

[4] R. Mayeux, A.M. Saunders, S. Shea, S. Mirra, D. Evans, A.D. Roses, B.T. Hyman, B. Crain, M.X. Tang, C.H. Phelps, Utility of the apolipoprotein E genotype in the diagnosis of Alzheimer's disease. Alzheimer's disease centers consortium on apolipoprotein E and Alzheimer's disease, N. Engl. J. Med. 338 (1998) 506–511.

[5] J. Murata, H.Y. Lee, T. Clair, H.C. Krutzsch, A.A. Arestad, M.E. Sobel, L.A. Liotta, M.L. Stracke, cDNA cloning of the human tumor motility-stimulating protein, autotaxin, reveals a homology with phosphodiesterases, J. Biol. Chem. 269 (1994) 30479–30484.

[6] Y. Namba, M. Tomonaga, H. Kawasaki, E. Otomo, K. Ikeda, Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease, Brain Res. 541 (1991) 163–166.

[7] M.A. Pericak-Vance, J.L. Bebout, P.C. Gaskell Jr., L.H. Yamaoka, W.Y. Hung, M.J. Alberts, A.P. Walker, R.J. Bartlett, C.A. Haynes, K.A. Welsh, et al., Linkage studies in familial Alzheimer's disease: Evidence for chromosome 19 linkage, Am. J. Hum. Genet. 48 (1991) 1034–1050.

[8] A.M. Saunders, W.J. Strittmatter, D. Schmechel, P.H. George-Hyslop, M.A. Pericak-Vance, S.H. Joo, B.L. Rosi, J.F. Gusella, D.R. Crapper-MacLachlan, M.J. Alberts, Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease, Neurology 43 (1993) 1467–1472.

[9] C.L. Sayas, M.T. Moreno-Flores, J. Avila, F. Wandosell, The neurite retraction induced by lysophosphatidic acid increases Alzheimer's disease-like *Tau* phosphorylation, J. Biol. Chem. 274 (1999) 37046–37052.

[10] M.L. Stracke, H.C. Krutzsch, E.J. Unsworth, A. Arestad, V. Cioce, E. Schiffmann, L.A. Liotta, Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein, J. Biol. Chem. 267 (1992) 2524–2529.

[11] A. Tokumura, E. Majima, Y. Kariya, K. Tominaga, K. Kogure, K. Yasuda, K. Fukuzawa, Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase, J. Biol. Chem. 277 (2002) 39436–39442.

[12] M. Umezu-Goto, Y. Kishi, A. Taira, K. Hama, N. Dohmae, K. Takio, T. Yamori, G.B. Mills, K. Inoue, J. Aoki, H. Arai, Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production, J. Cell Biol. 158 (2002) 227–233.

[13] C. Zhang, H.E. Qiu, G.A. Krafft, W.L. Klein, Protein kinase C and F-actin are essential for stimulation of neuronal FAK tyrosine phosphorylation by G-proteins and amyloid beta protein, FEBS Lett. 386 (1996) 185–188.