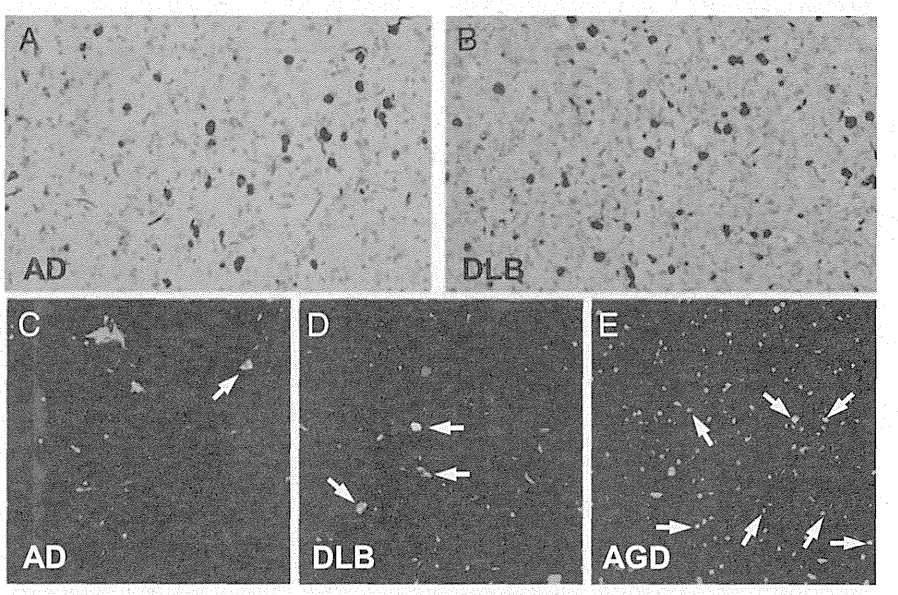

Fig. 3 Trans-activation response (TAR) DNA-binding protein of M_r 43 kDa (TDP-43)-positive structures in other neurodegenerative disorders. Neuronal cytoplasmic inclusions (NCIs) and short dystrophic neurites (DNs) stained with the pTDP-43-specific antibody (pS403/404) in the temporal cortex of the Alzheimer's disease (AD) case (A) and the dementia with Lewy bodies (DLB) case (B). (C–E) Double-label immunofluorescence histochemistry of the temporal cortex of AD (C) and DLB (D) and of the amygdala of argyrophilic grain disease (AGD) (E). The green fluorescence reveals the immunoreactivity for phosphorylated tau (AT8) in C and E, and that for phosphorylated alpha-synuclein in D, while the red fluorescence represents the immunopositivity for pS403/404 in C–E. Arrows indicate the colocalization of tau and pTDP-43 in C and E, and that of alpha-synuclein and pTDP-43 in D.

still unclear. A higher Braak NFT stage in the TDP-43 positive patients than in the TDP-43 negative ones was found in DLB+AD cases[91] and in our study of AD cases.[95] We also reported parallel distribution of TDP-43 positive structures and tau positive grains and higher AGD stages in cases with TDP-43 immunoreactivity than in those without TDP-43 immunoreactivity in AGD.[97] Double-label immunofluorescence microscopy reveals partial colocalization of tau and TDP-43 in AD, DLB, AGD, Guamanian PDC and CBD[83,84,86,87,90,91,97] or of α-synuclein and TDP-43 in DLB.[87,91,95] These findings suggest that there may be common factors or mechanisms that affect the conformation or modification of these proteins, leading to their intracellular accumulation.

Regarding the typing of TDP-43 pathology, neocortical TDP-43 pathology in AD and DLB corresponded to type A.[90,95,99] Immunoblot analyses of the sarkosyl insoluble fraction from AD and DLB cases with neocortical TDP-43 pathology also showed that the band pattern of these CTFs in AD and DLB corresponded to that of type A[58]. These results suggest that the morphological and biochemical features of TDP-43 pathology are common between AD or DLB and a specific subtype of FTLD-TDP. Since all FTLD-TDP cases with *GRN* mutations show type A pathology,[45] there may be genetic factors, such as mutations or genetic variants of *GRN* underlying the co-occurrence of abnormal deposition of TDP-43, tau and α-synuclein. Indeed, recently, *GRN* loss-of-function mutation has been confirmed in patients clinically diagnosed with AD[100–105] and Parkinson's disease.[106] The association between rs5848 variant in the 3′ untranslated region of *GRN* and risk of AD has been reported in a Taiwanese population,[107] suggesting that homozygous TT genotype accentuates the risk of AD. These findings suggest that PGRN reduction may induce both TDP-43 pathology and AD pathology.

Cell death and TDP-43 pathology

A report using a cell culture system showed that intracellular aggregate formation of TDP-43 induced cell death.[77] In brains of FTLD and ALS cases, basically, the occurrence of TDP-43-positive neuronal structures is related to degenerative changes.[36] However, the issue of the relation between the formation of TDP-43-positive inclusions and cell death may not be straightforward, since neuronal loss was not evident in the hippocampal granule cells[36] and the neostriatum[108] where TDP-43-positive structures were present in ALS cases. The reason for such a discrepancy between the results of the cell culture experiments and the findings of diseased brains should be discussed as a future issue.

CONCLUSION

The relevance of the pathological classification of TDP-43 proteinopathy is supported by clinical, biochemical and genetic correlations, although there is still highly significant heterogeneity in cases with type A pathology (Table 2). The results of the biochemical analyses of the diseased brains and the cellular models suggest that different strains of TDP-43 with different conformations may determine the clinicopathological phenotypes of TDP-43 proteinopathy, like prion disease. Detecting each TDP-43 strain in biological fluids may be useful for the differential diagnosis of TDP-43 proteinopathy. Furthermore, elucidating the mechanism of the conformational changes leading to the formation of multiple TDP-43 strains may be important for developing disease-modifying therapy for these diseases.

Table 2 Clinical, pathological and genetic associations of TDP-43 proteinopathy and other neurodegenerative disorders

Pathological subtype	Associated genes	Clinical phenotypes					Other diseases
		bvFTD	PNFA	SD		MND	
				Semantic aphasia	Prosopagnosia		
A	GRN, C9ORF72	+	+			PLS, ALS	AD, DLB
B	C9ORF72	+	+			ALS	
C				+			
					+	PLS	
D	VCP	+				ALS	

ALS, amyotrophic lateral sclerosis; AD, Alzheimer's disease; bvFTD, behavioral variant frontotemporal dementia; DLB, dementia with Lewy bodies; MND, motor neuron disease; PLS, primary lateral sclerosis; PNFA, progressive non-fluent aphasia; SD, semantic dementia.

ACKNOWLEDGMENTS

I thank Drs M. Hasegawa, H. Akiyama, T. Nonaka, M. Hosokawa and T. Asada for advice and support. I also thank Ms H. Kondo, C. Haga and Y. Shimomura for their technical assistance. This work was supported by a Grant-in-Aid for Scientific Research (C) from Ministry of Education, Culture, Sports, Science and Technology of Japan, and grants from Ministry of Health, Labor and Welfare of Japan.

REFERENCES

1. Neumann M, Sampathu DM, Kwong LK *et al.* Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006; **314**: 130–133.
2. Arai T, Hasegawa M, Akiyama H *et al.* TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 2006; **351**: 602–611.
3. Davidson Y, Kelley T, Mackenzie IRA *et al.* Ubiquitinated pathological lesions in frontotemporal lobar degeneration contain the TAR DNA-binding protein, TDP-43. *Acta Neuropathol (Berl)* 2007; **113**: 521–533.
4. Neumann M, Kwong LK, Sampathu DM, Trojanowski JQ, Lee VM. TDP-43 proteinopathy in frontotemporal lobar degeneration and amyotrophic lateral sclerosis: protein misfolding diseases without amyloidosis. *Arch Neurol* 2007; **64**: 1388–1394.
5. Mackenzie IR, Bigio EH, Ince PG *et al.* Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. *Ann Neurol* 2007; **61**: 427–434.
6. Tan CF, Eguchi H, Tagawa A *et al.* TDP-43 immunoreactivity in neuronal inclusions in familial amyotrophic lateral sclerosis with or without SOD1 gene mutation. *Acta Neuropathol (Berl)* 2007; **113**: 535–542.
7. Mackenzie IR, Neumann M, Bigio EH *et al.* Nomenclature for neuropathologic subtypes of frontotemporal lobar degeneration: consensus recommendations. *Acta Neuropathol* 2009; **117**: 15–18.
8. Baker M, Mackenzie IR, Pickering-Brown SM *et al.* Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature* 2006; **442**: 916–919.
9. Cruts M, Gijselinck I, van der Zee J *et al.* Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature* 2006; **442**: 920–924.
10. Watts GDJ, Wymer J, Kovach MJ *et al.* Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nat Genet* 2004; **36**: 377–381.
11. Yokoseki A, Shiga A, Tan CF *et al.* TDP-43 mutation in familial amyotrophic lateral sclerosis. *Ann Neurol* 2008; **63**: 538–542.
12. Gitcho MA, Baloh RH, Chakraverty S *et al.* TDP-43 A315T mutation in familial motor neuron disease. *Ann Neurol* 2008; **63**: 535–538.
13. Sreedharan J, Blair IP, Tripathi VB *et al.* TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 2008; **319**: 1668–1672.
14. Kabashi E, Valdmanis PN, Dion P *et al.* TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat Genet* 2008; **40**: 572–574.
15. Van Deerlin VM, Leverenz JB, Bekris LM *et al.* TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. *Lancet Neurol* 2008; **7**: 409–416.
16. Renton AE, Majounie E, Waite A *et al.* A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 2011; **72**: 257–268.
17. DeJesus-Hernandez M, Mackenzie IR, Boeve BF *et al.* Expanded GGGGCC hexanucleotide repeat in

noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 2011; **72**: 245–256.
18. Ou SH, Wu F, Harrich D, Garcia-Martinez LF, Gaynor RB. Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. *J Virol* 1995; **69**: 3584–3596.
19. Wang H-Y, Wang I-F, Bose J, Shen C-KJ. Structural diversity and functional implications of the eukaryotic TDP gene family. *Genomics* 2004; **83**: 130–139.
20. Abhyankar MM, Urekar C, Reddi PP. A novel CpG-free vertebrate insulator silences the testis-specific SP-10 gene in somatic tissues: role for TDP-43 in insulator function. *J Biol Chem* 2007; **282**: 36143–36154.
21. Strong MJ, Volkening K, Hammond R *et al.* TDP43 is a human low molecular weight neurofilament (hNFL) mRNA-binding protein. *Mol Cell Neurosci* 2007; **35**: 320–327.
22. Ayala YM, Misteli T, Baralle FE. TDP-43 regulates retinoblastoma protein phosphorylation through the repression of cyclin-dependent kinase 6 expression. *Proc Natl Acad Sci U S A* 2008; **105**: 3785–3789.
23. Buratti E, Baralle FE. Characterization and functional implications of the RNA binding properties of nuclear factor TDP-43, a novel splicing regulator of CFTR exon 9. *J Biol Chem* 2001; **276**: 36337–36343.
24. Buratti E, Dork T, Zuccato E, Pagani F, Romano M, Baralle FE. Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. *EMBO J* 2001; **20**: 1774–1784.
25. Mercado PA, Ayala YM, Romano M, Buratti E, Baralle FE. Depletion of TDP 43 overrrides the need for exonic and intronic splicing enhancers in the human apoA-II gene. *Nucleic Acids Res* 2005; **33**: 6000–6010.
26. Bose JK, Wang IF, Hung L, Tarn WY, Shen CK. TDP-43 overexpression enhances exon 7 inclusion during the survival of motor neuron pre-mRNA splicing. *J Biol Chem* 2008; **283**: 28852–28859.
27. Buratti E, Brindisi A, Giombi M, Tisminetzky S, Ayala YM, Baralle FE. TDP-43 binds heterogeneous nuclear ribonucleoprotein A/B through its C-terminal tail. *J Biol Chem* 2005; **280**: 37572–37584.
28. Buratti E, Baralle FE. Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease. *Front Biosci* 2008; **13**: 867–878.
29. Mackenzie IRA, Baborie A, Pickering-Brown S *et al.* Heterogeneity of ubiquitin pathology in frontotemporal lobar degeneration: classification and relation to clinical phenotype. *Acta Neuropathol (Berl)* 2006; **112**: 539–549.
30. Mackenzie IRA, Baker M, Pickering-Brown S *et al.* The neuropathology of frontotemporal lobar degeneration caused by mutations in the progranulin gene. *Brain* 2006; **129**: 3081–3090.
31. Sampathu DM, Neumann M, Kwong LK *et al.* Pathological heterogeneity of frontotemporal lobar degeneration with ubiquitin-positive inclusions delineated by ubiquitin immunohistochemistry and novel monoclonal antibodies. *Am J Pathol* 2006; **169**: 1343–1352.
32. Leigh PN, Anderton BH, Dodson A, Gallo JM, Swash M, Power DM. Ubiquitin deposits in anterior horn cells in motor neurone disease. *Neurosci Lett* 1988; **93**: 197–203.
33. Mizusawa H. Hyaline and skein-like inclusions in amyotrophic lateral sclerosis. *Neuropathology* 1993; **13**: 201–208.
34. Piao YS, Wakabayashi K, Kakita A *et al.* Neuropathology with clinical correlations of sporadic amyotrophic lateral sclerosis: 102 autopsy cases examined between 1962 and 2000. *Brain Pathol* 2003; **13**: 10–22.
35. Geser F, Brandmeir NJ, Kwong LK *et al.* Evidence of multisystem disorder in whole-brain map of pathological TDP-43 in amyotrophic lateral sclerosis. *Arch Neurol* 2008; **65**: 636–641.
36. Nishihira Y, Tan CF, Hoshi Y *et al.* Sporadic amyotrophic lateral sclerosis of long duration is associated with relatively mild TDP-43 pathology. *Acta Neuropathol* 2009; **117**: 45–53.
37. Okamoto K, Hirai S, Yamazaki T, Sun X, Nakazato Y. New ubiquitin-positive intraneuronal inclusions in the extra-motor cortices in patients with amyotrophic lateral sclerosis. *Neurosci Lett* 1991; **129**: 233–236.
38. Wightman G, Anderson VER, Martin AJ *et al.* Hippocampal and neocortical ubiquitin-immunoreactive inclusions in amyotrophic lateral sclerosis with dementia. *Neurosci Lett* 1992; **139**: 269–274.
39. Okamoto K, Murakami N, Kusada H *et al.* Ubiquitin-positive intraneuronal inclusions in the extramotor cortices of presenile dementia patients with motor neuron disease. *J Neurol* 1992; **239**: 426–430.
40. Jackson M, Lennox G, Lowe J. Motor neuron disease-inclusion dementia. *Neurodegeneration* 1996; **5**: 339–350.
41. Iseki E, Li F, Odawara T *et al.* Ubiquitin-immunohistochemical investigation of atypical Pick's disease without Pick bodies. *J Neurol Sci* 1998; **159**: 194–201.
42. Bergmann M, Kuchelmeister K, Schmid KW, Kretzschmar HA, Schröder R. Different variants of frontotemporal dementia: a neuropathological and immunohistochemical study. *Acta Neuropathol (Berl)* 1996; **92**: 170–179.

43. Woulfe J, Kertesz A, Munoz DG. Frontotemporal dementia with ubiquitinated cytoplasmic and intranuclear inclusions. *Acta Neuropathol (Berl)* 2001; **102**: 94–102.
44. Mackenzie IRA, Feldman H. Neuronal intranuclear inclusions distinguish familial FTD-MND type from sporadic cases. *Acta Neuropathol (Berl)* 2003; **105**: 543–548.
45. Cairns NJ, Neumann M, Bigio EH *et al.* TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. *Am J Pathol* 2007; **171**: 227–240.
46. Hatanpaa KJ, Bigio EH, Cairns NJ *et al.* TAR DNA-binding protein 43 immunohistochemistry reveals extensive neuritic pathology in FTLD-U: a midwest-southwest consortium for FTLD study. *J Neuropathol Exp Neurol* 2008; **67**: 271–279.
47. Mori F, Tanji K, Zhang HX *et al.* Maturation process of TDP-43-positive neuronal cytoplasmic inclusions in amyotrophic lateral sclerosis with and without dementia. *Acta Neuropathol* 2008; **116**: 193–203.
48. Arai T, Nonaka T, Hasegawa M *et al.* Neuronal and glial inclusions in frontotemporal dementia with or without motor neuron disease are immunopositive for p62. *Neurosci Lett* 2003; **342**: 41–44.
49. Arai T, Hasegawa M, Nonaka T *et al.* Phosphorylated and cleaved TDP-43 in ALS, FTLD and other neurodegenerative disorders and in cellular models of TDP-43 proteinopathy. *Neuropahology* 2010; **30**: 170–181.
50. Mackenzie IR, Neumann M, Baborie A *et al.* A harmonized classification system for FTLD-TDP pathology. *Acta Neuropathol* 2011; **122**: 111–113.
51. Josephs KA, Hodges JR, Snowden JS *et al.* Neuropathological background of phenotypical variability in frontotemporal dementia. *Acta Neuropathol* 2011; **122**: 137–153.
52. Kobayashi Z, Tsuchiya K, Arai T *et al.* Clinicopathological characteristics of FTLD-TDP showing corticospinal tract degeneration but lacking lower motor neuron loss. *J Neurol Sci* 2010; **298**: 70–77.
53. Ikeda M, Ishikawa T, Tanabe H. Epidemiology of frontotemporal lobar degeneration. *Dement Geriatr Cogn Disord* 2004; **17**: 265–268.
54. Ikeda K. Neuropathological discrepancy between Japanese Pick's disease without Pick bodies and frontal lobe degeneration type of frontotemporal dementia proposed by Lund and Manchester group. *Neuropathology* 2000; **20**: 76–82.
55. Sieben A, Van Langenhove T, Engelborghs S *et al.* The genetics and neuropathology of frontotemporal lobar degeneration. *Acta Neuropathol* 2012; **124**: 353–372.
56. Snowden J, Neary D, Mann D. Frontotemporal lobar degeneration: clinical and pathological relationships. *Acta Neuropathol (Berl)* 2007; **114**: 31–38.
57. Kwong LK, Uryu K, Trojanowski JQ, Lee VM. TDP-43 proteinopathies: neurodegenerative protein misfolding diseases without amyloidosis. *Neurosignals* 2008; **16**: 41–51.
58. Hasegawa M, Arai T, Nonaka T *et al.* Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol* 2008; **64**: 60–70.
59. Hasegawa M, Jakes R, Crowther RA, Lee VM, Ihara Y, Goedert M. Characterization of mAb AP422, a novel phosphorylation-dependent monoclonal antibody against tau protein. *FEBS Lett* 1996; **384**: 25–30.
60. Fujiwara H, Hasegawa M, Dohmae N *et al.* a-Synuclein is phosphorylated in synucleinopathy lesions. *Nat Cell Biol* 2002; **4**: 160–164.
61. Neumann M, Kwong LK, Lee EB *et al.* Phosphorylation of S409/410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies. *Acta Neuropathol* 2009; **117**: 137–149.
62. Kadokura A, Yamazaki T, Kakuda S *et al.* Phosphorylation-dependent TDP-43 antibody detects intraneuronal dot-like structures showing morphological characters of granulovacuolar degeneration. *Neurosci Lett* 2009; **463**: 87–92.
63. Arai T, Ikeda K, Akiyama H *et al.* Identification of amino-terminally cleaved tau fragments that distinguish progressive supranuclear palsy from corticobasal degeneration. *Ann Neurol* 2004; **55**: 72–79.
64. Clavaguera F, Bolmont T, Crowther RA *et al.* Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol* 2009; **11**: 909–913.
65. Desplats P, Lee HJ, Bae EJ *et al.* Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proc Natl Acad Sci U S A* 2009; **106**: 13010–13015.
66. Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem* 2009; **284**: 12845–12852.
67. Li JY, Englund E, Holton JL *et al.* Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med* 2008; **14**: 501–503.
68. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med* 2008; **14**: 504–506.
69. Nonaka T, Watanabe ST, Iwatsubo T, Hasegawa M. Seeded aggregation and toxicity of alpha-synuclein

and tau: cellular models of neurodegenerative diseases. *J Biol Chem* 2010; **285**: 34885–34898.
70. Luk KC, Song C, O'Brien P *et al.* Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. *Proc Natl Acad Sci U S A* 2009; **106**: 20051–20056.
71. Masuda-Suzukake M, Nonaka T, Hosokawa M *et al.* Prion-like spreading of pathological alpha-synuclein in brain. *Brain* 2013; **136**: 1128–1138.
72. Polymenidou M, Cleveland DW. The seeds of neurodegeneration: prion-like spreading in ALS. *Cell* 2011; **147**: 498–508.
73. Ren PH, Lauckner JE, Kachirskaia I, Heuser JE, Melki R, Kopito RR. Cytoplasmic penetration and persistent infection of mammalian cells by polyglutamine aggregates. *Nat Cell Biol* 2009; **11**: 219–225.
74. Collinge J, Sidle KC, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of "new variant" CJD. *Nature* 1996; **383**: 685–690.
75. Tsuji H, Arai T, Kametani F *et al.* Molecular analysis and biochemical classification of TDP-43 proteinopathy. *Brain* 2012; **135**: 3380–3391.
76. Hasegawa M, Nonaka T, Tsuji H *et al.* Molecular Dissection of TDP-43 Proteinopathies. *J Mol Neurosci* 2011; **45**: 480–485.
77. Nonaka T, Masuda-Suzukake M, Arai T *et al.* Prion-like properties of pathological TDP-43 aggregates from diseased brains. *Cell Rep* 2013; **4**: 124–134.
78. Josephs KA, Stroh A, Dugger B, Dickson DW. Evaluation of subcortical pathology and clinical correlations in FTLD-U subtypes. *Acta Neuropathol* 2009; **118**: 349–358.
79. Josephs KA, Dickson DW. Frontotemporal lobar degeneration with upper motor neuron disease/primary lateral sclerosis. *Neurology* 2007; **69**: 1800–1801.
80. Yokota O, Tsuchiya K, Arai T *et al.* Clinicopathological characterization of Pick's disease versus frontotemporal lobar degeneration with ubiquitin/TDP-43-positive inclusions. *Acta Neuropathol* 2009; **117**: 429–444.
81. Kosaka T, Fu YJ, Shiga A *et al.* Primary lateral sclerosis: upper-motor-predominant amyotrophic lateral sclerosis with frontotemporal lobar degeneration – immunohistochemical and biochemical analyses of TDP-43. *Neuropathology* 2012; **32**: 373–384.
82. Josephs KA, Whitwell JL, Murray ME *et al.* Corticospinal tract degeneration associated with TDP-43 type C pathology and semantic dementia. *Brain* 2013; **136**: 455–470.
83. Hasegawa M, Arai T, Akiyama H *et al.* TDP-43 is deposited in the Guam parkinsonism-dementia complex brains. *Brain* 2007; **130**: 1386–1394.
84. Geser F, Winton MJ, Kwong LK *et al.* Pathological TDP-43 in parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam. *Acta Neuropathol (Berl)* 2007; **115**: 133–145.
85. Miklossy J, Steele JC, Yu S *et al.* Enduring involvement of tau, beta-amyloid, alpha-synuclein, ubiquitin and TDP-43 pathology in the amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam (ALS/PDC). *Acta Neuropathol* 2008; **116**: 625–637.
86. Amador-Ortiz C, Lin WL, Ahmed Z *et al.* TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann Neurol* 2007; **61**: 435–445.
87. Higashi S, Iseki E, Yamamoto R *et al.* Concurrence of TDP-43, tau and alpha-synuclein pathology in brains of Alzheimer's disease and dementia with Lewy bodies. *Brain Res* 2007; **1184**: 284–294.
88. Hu WT, Josephs KA, Knopman DS *et al.* Temporal lobar predominance of TDP-43 neuronal cytoplasmic inclusions in Alzheimer disease. *Acta Neuropathol* 2008; **116**: 215–220.
89. Josephs KA, Whitwell JL, Knopman DS *et al.* Abnormal TDP-43 immunoreactivity in AD modifies clinicopathologic and radiologic phenotype. *Neurology* 2008; **70**: 1850–1857.
90. Uryu K, Nakashima-Yasuda H, Forman MS *et al.* Concomitant TAR-DNA-binding protein 43 pathology is present in Alzheimer disease and corticobasal degeneration but not in other tauopathies. *J Neuropathol Exp Neurol* 2008; **67**: 555–564.
91. Nakashima-Yasuda H, Uryu K, Robinson J *et al.* Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. *Acta Neuropathol (Berl)* 2007; **114**: 221–229.
92. Freeman SH, Spires-Jones T, Hyman BT, Growdon JH, Frosch MP. TAR-DNA binding protein 43 in Pick disease. *J Neuropathol Exp Neurol* 2008; **67**: 62–67.
93. Lin WL, Dickson DW. Ultrastructural localization of TDP-43 in filamentous neuronal inclusions in various neurodegenerative diseases. *Acta Neuropathol* 2008; **116**: 205–213.
94. Yokota O, Davidson Y, Bigio EH *et al.* Phosphorylated TDP-43 pathology and hippocampal sclerosis in progressive supranuclear palsy. *Acta Neuropathol* 2010; **120**: 55–66.
95. Arai T, Mackenzie IR, Hasegawa M *et al.* Phosphorylated TDP-43 in Alzheimer's disease and dementia with Lewy bodies. *Acta Neuropathol* 2009; **117**: 125–136.

96. Schwab C, Arai T, Hasegawa M, Akiyama H, Yu S, McGeer PL. TDP-43 pathology in familial British dementia. *Acta Neuropathol* 2009; **118**: 303–311.
97. Fujishiro H, Uchikado H, Arai T *et al.* Accumulation of phosphorylated TDP-43 in brains of patients with argyrophilic grain disease. *Acta Neuropathol* 2009; **117**: 151–158.
98. Schwab C, Arai T, Hasegawa M, Yu S, McGeer PL. Colocalization of transactivation-responsive DNA-binding protein 43 and huntingtin in inclusions of Huntington disease. *J Neuropathol Exp Neurol* 2008; **67**: 1159–1165.
99. Josephs KA, Murray ME, Whitwell JL *et al.* Staging TDP-43 pathology in Alzheimer's disease. *Acta Neuropathol* 2013; **127**: 441–450.
100. Brouwers N, Nuytemans K, van der Zee J *et al.* Alzheimer and Parkinson diagnoses in progranulin null mutation carriers in an extended founder family. *Arch Neurol* 2007; **64**: 1436–1446.
101. Brouwers N, Sleegers K, Engelborghs S *et al.* Genetic variability in progranulin contributes to risk for clinically diagnosed Alzheimer disease. *Neurology* 2008; **71**: 656–664.
102. Le Ber I, Camuzat A, Hannequin D *et al.* Phenotype variability in progranulin mutation carriers: a clinical, neuropsychological, imaging and genetic study. *Brain* 2008; **131**: 732–746.
103. Kelley BJ, Haidar W, Boeve BF *et al.* Prominent phenotypic variability associated with mutations in Progranulin. *Neurobiol Aging* 2009; **30**: 739–751.
104. Rademakers R, Baker M, Gass J *et al.* Phenotypic variability associated with progranulin haploinsufficiency in patients with the common 1477C–>T (Arg493X) mutation: an international initiative. *Lancet Neurol* 2007; **6**: 857–868.
105. Perry DC, Lehmann M, Yokoyama JS *et al.* Progranulin mutations as risk factors for Alzheimer disease. *JAMA Neurol* 2013; **70**: 774–778.
106. Finch N, Baker M, Crook R *et al.* Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. *Brain* 2009; **132**: 583–591.
107. Lee MJ, Chen TF, Cheng TW, Chiu MJ. rs5848 variant of progranulin gene is a risk of Alzheimer's disease in the Taiwanese population. *Neurodegener Dis* 2011; **8**: 216–220.
108. Zhang H, Tan CF, Mori F *et al.* TDP-43-immunoreactive neuronal and glial inclusions in the neostriatum in amyotrophic lateral sclerosis with and without dementia. *Acta Neuropathol* 2008; **115**: 115–122.

Neuropathology and Applied Neurobiology (2014), **40**, 783–788 doi: 10.1111/nan.12157

Scientific correspondence

C9ORF72 repeat-associated non-ATG-translated polypeptides are distributed independently of TDP-43 in a Japanese patient with c9ALS

Hexanucleotide (GGGGCC) repeat expansion in a noncoding region of *C9ORF72* is the major genetic cause of frontotemporal dementia and amyotrophic lateral sclerosis (c9FTD/ALS) in the Caucasian population [1], but it is very rare in the Japanese population, possibly because of the difference in genetic background [2,3]. TDP-43 pathology indistinguishable from that of non-mutational ALS/FTLD-TDP has been observed in c9FTD/ALS [4]. In addition, the presence of p62-, ubiquitin- and ubiquilin-positive, and TDP-43-negative inclusions in the cerebellar cortex and hippocampus has been reported to be a unique and consistent feature in Caucasian patients with c9FTD/ALS [5,6]. Recently, it was demonstrated that these TDP-43-negative inclusions are derived from aggregated dipeptide repeat (DPR) proteins bidirectionally translated from the expanded repeat in *C9ORF72* by repeat associated non-ATG (RAN) translation, and that such DPR protein pathology is widely distributed in the central nervous system (CNS) [7,8]. However, it still remains unknown whether these distinct neuropathological features are also reproduced in Japanese patients with c9FTD/ALS. In the present study, we performed an immunohistochemical analysis focusing especially on DPR proteins in a Japanese patient with *C9ORF72* repeat expansion (c9ALS) [2], who to our knowledge represents the only autopsy case of this genetic disease to have been reported in the Japanese population so far.

The present study was conducted with approval from the Institutional Review Board of Niigata University. The clinical and pathological findings in this case have been reported previously (case 4 [9]). Briefly, the patient had a sibling who had also been diagnosed as having ALS. At the age of 61, he noticed hand clumsiness, and progressively developed bulbar palsy and limb weakness. He died 20 months after disease onset due to respiratory failure. He had no clinical features suggestive of dementia. The presence of *C9ORF72* repeat expansion was confirmed by repeat-primed PCR using the frozen cerebellar tissue [2]. The accurate hexanucleotide repeat length was unknown, because we could not perform Southern blot analysis due to lack of the amount of genomic DNA. Histologically, neuronal loss and gliosis were evident in the spinal anterior horns, brainstem motor nuclei and motor cortex, as well as degeneration in the anterior and lateral columns of the spinal cord. Bunina bodies and ubiquitin-positive skein-like inclusions were observed in the remaining lower motor neurones. Neuronal and glial (oligodendrocytic) cytoplasmic inclusions (NCIs and GCIs) recognized with anti-TDP-43 (polyclonal, Protein Tec Group, Chicago, IL, USA; 1:4000) were present in the lower motor nuclei and motor cortex, and much less frequently in the subcortical non-motor nuclei, such as the basal ganglia, whereas no such NCIs or GCIs were observed in the cerebellar cortex and hippocampus [9]. There were p62-positive and TDP-43-negative NCIs in the cerebellar granule cells and in the granule cells and pyramidal CA4-CA2 neurones of the hippocampus; at that time, we failed to show phosphorylated TDP-43 (pTDP-43)-positive NCIs in the lower motor nuclei and motor cortex [2].

In the present study, we confirmed that NCIs and GCIs recognized with a 'polyclonal' antibody against pTDP-43 (pS409/410; Cosmo Bio, Tokyo, Japan; 1: 1000) were present with the same distribution pattern as that of TDP-43 mentioned above (Figure 1**a**,**c**). Immunostaining with an antibody against another RNA-binding protein, RNA-binding motif 45 (RBM45) (polyclonal, Sigma-Aldrich, St. Louis, MO, USA; 1:50), which has been known to accumulate in inclusions in ALS [10], also revealed a distribution of positive inclusions strikingly similar to that of TDP-43-positive inclusions, although such NCIs and GCIs were comparatively small in number. The characteristic morphologies of NCIs and GCIs were shared by both anti-TDP-43/anti-pTDP-43 and anti-RBM45 (Figure 1**a**–**d**).

We generated polyclonal antibodies against putative DPR proteins from the GGGGCC repeat by RAN translation [11]. Although immunostaining with three antibodies against different polypeptides, poly Gly-Ala (GA), poly Gly-Pro and poly Gly-Arg, arising from RAN translation revealed similar DPR protein pathology, abundant positive

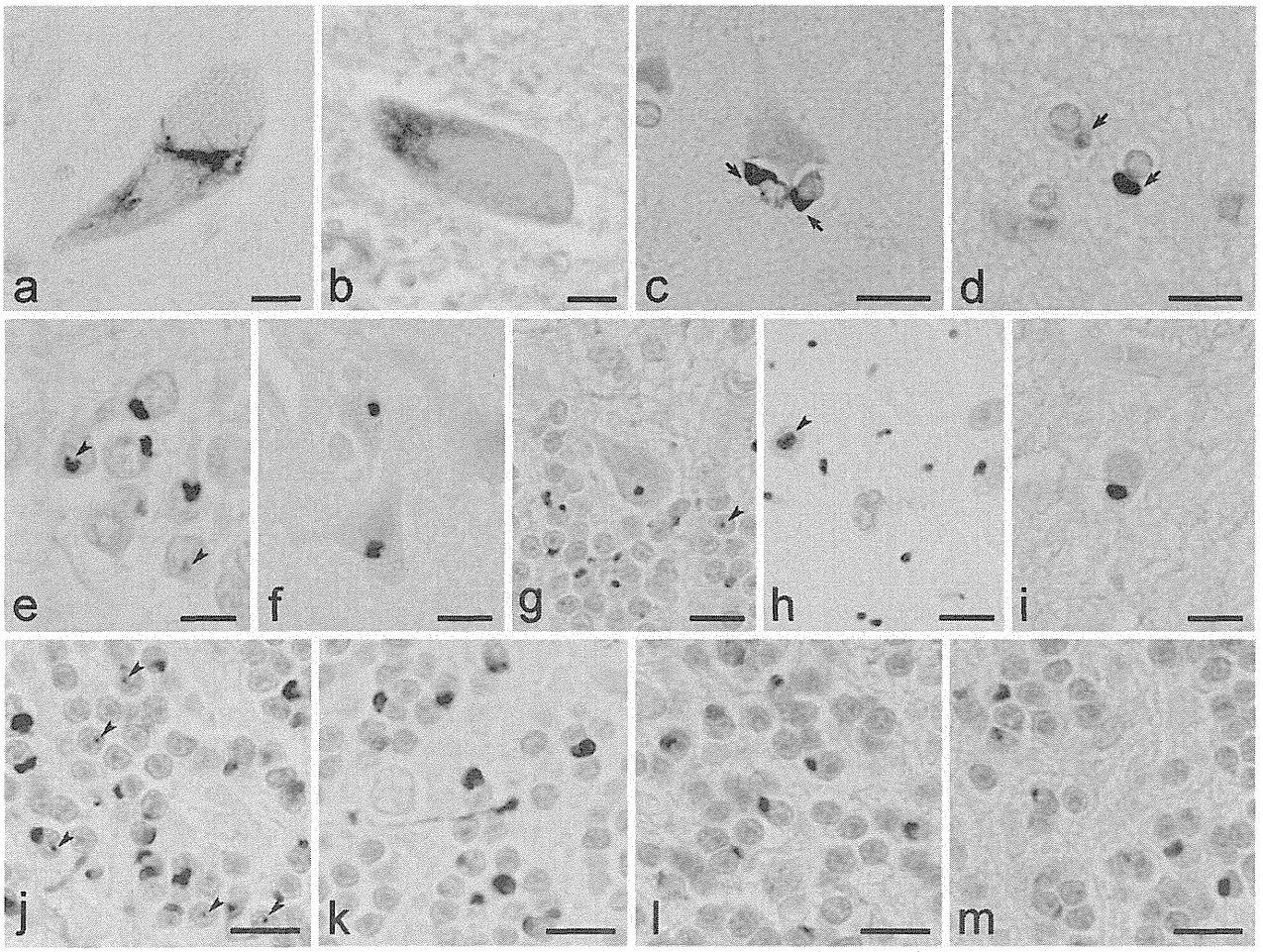

Figure 1. Immunohistochemical study. (**a–d**) Skein-like cytoplasmic inclusions positive for phosphorylated TDP-43 (pTDP-43) (**a**) and RBM45 (**b**) in the cervical anterior horn cells. C-like or fibrillary tangle-like glial cytoplasmic inclusions positive for pTDP-43 (**c**) and RBM45 (**d**) in the motor cortex. Note glial cytoplasmic inclusions indicated by arrows (**c**,**d**). (**e–h**) Numerous poly Gly-Ala (GA)-positive neuronal cytoplasmic and intranuclear inclusions (arrowheads) are observed widely beyond the regions showing TDP-43-positive inclusions [(**e**) hippocampal dentate gyrus, (**f**) temporal cortex, (**g**) cerebellar granular-Purkinje layer, (**h**) cerebellar molecular layer; also see Table 1]. A Purkinje cell also contains a positive cytoplasmic inclusion (**g**). Punctate or linear inclusions in the neuropil of the cerebellar molecular layer are also evident (**h**). (**i**) Here, an oligodendroglial cytoplasmic inclusion is shown in the precentral subcortical white matter. (**j–m**) In the cerebellar granular layer, it is evident that GA-positive neuronal cytoplasmic inclusions (**j**) are also positive for ubiquilin (**k**), ubiquitin (**l**) and p62 (**m**). Note GA-positive intranuclear inclusions indicated by arrowheads (**j**). Bars: 10 μm (**a–m**).

inclusions were recognized most clearly with anti-poly GA (1:1000). The distribution pattern of DPR protein-positive NCIs was apparently different from that of TDP-43-positive NCIs, the latter being clearly associated with neuronal loss in the lower and upper motor neurones systems. DPR protein-positive NCIs were widely distributed in the brain (Figure 1**e–h**,**j**), with the highest frequency in the hippocampal dentate gyrus (Figure 1**e**) and cerebellar granular layer (Figure 1**g**,**j**), and were distributed almost evenly in the cerebral neocortex examined (Figure 1**f**). DPR protein-positive GCIs were comparatively rare (Figure 1**i**), although TDP-43- and RBM45-positive GCIs were observed frequently (Figure 1**c**,**d**). Neuronal intranuclear inclusions were frequently associated with anti-DPR proteins (Figure 1**e**,**g**,**h**,**j**), but not with anti-TDP-43. DPR protein-positive punctate or filamentous structures were also encountered in the neuropil of the cerebellar molecular layer (Figure 1**h**), which were never recognized with anti-TDP-43, and only rarely with anti-ubiquilin (monoclonal, clone 5F5, Abnova, Walnut, CA, USA; 1:10 000). DPR protein-positive NCIs appeared as irregular dot-like, granular or star-like inclusions

Table 1. Summary of regional immunohistochemical findings

	*Lower motor neurones**	*Motor cortex*	*Non-motor cortex†*	*Dentate gyrus*	*Hippocampal CA4*	*Cerebellar granule cells*
Neuronal loss	++	+	–	–	–	–
TDP-43	+	+	–	–	–	–
pTDP-43	+	+	–	–	–	–
RBM45	+	+	–	–	–	–
p62	+	++	++	++	++	++
Ubiquitin	+	+	+	+	+	++
Ubiquilin	–	+	+	++	+	++
DPR‡	–	++	++	+++	++	+++

Severity of neuronal loss are represented as: – = not noted, + = mild, ++ = moderate-severe.
Neurones containing each antibody-positive inclusions were counted per 100 neurones in high-power fields, and ratio is represented as: – = none, + = ~10%, ++ = 10~30%, +++ = >30%.
*Cervical anterior horn cells and hypoglossal nucleus.
†Frontal and temporal cortex were examined.
‡poly Gly-Ala.

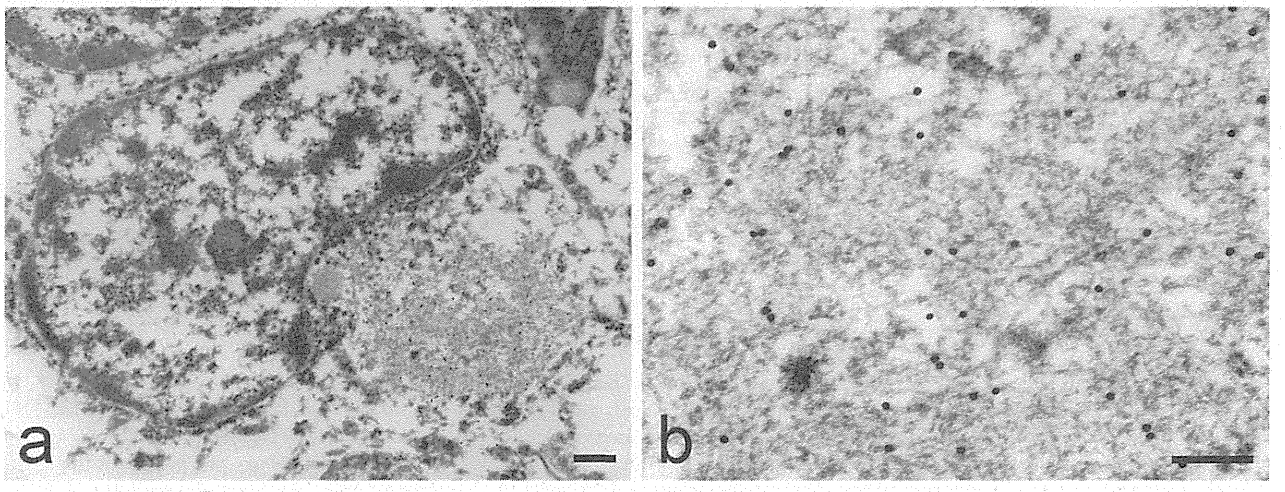

Figure 2. Immunoelectron microscopy for the presence of ubiquilin. (**a**) A cerebellar granule cell is seen to have a round filamentous cytoplasmic aggregate. (**b**) Higher-magnification view of the aggregate, showing immunogold particles on the randomly arranged, approximately 10-nm-wide filamentous structures. Bars: (**a**) 500 nm, (**b**) 200 nm.

(Figure 1**e–h,j**) that generally differed in morphology from TDP-43- and RBM45-positive NCIs (Figure 1**a–d**). Such inclusions were frequently labelled with antibodies against ubiquilin, ubiquitin (polyclonal, Dako, Glostrup, Denmark; 1:800) and p62 (monoclonal, BD biosciences, San Jose, CA, USA; 1:500) (Figure 1**j–m**). The immunohistochemical findings are summarized in Table 1.

Under immunogold-labelling electron microscopy for ubiquilin in the cerebellar granular layer tissue, the labelled NCIs were shown to be composed of randomly arranged filamentous structures (Figure 2**a,b**); their morphological features were very similar to those of ubiquitin-positive and TDP-43-negative NCIs in the entorhinal cortex in a case of c9FTD/ALS [12]. Double-labelling immunofluorescence revealed that co-localization of TDP-43 (monoclonal, clone 2E2-D3, Abnova, Taipei, Taiwan; 1:250) and RBM45 (Figure 3**a–c**), but not TDP-43 (2E2-D3) and DPR proteins (Figure 3**d–f**), was a feature in the cytoplasmic inclusions. The majority of p62-positive NCIs were positive for DPR proteins, whereas p62-positive GCIs were negative for DPR proteins (Figure 3**g–i**).

In the present Japanese case of c9ALS, the histological and molecular pathology of sporadic ALS was observed: especially, the occurrence of Bunina bodies and TDP-43-

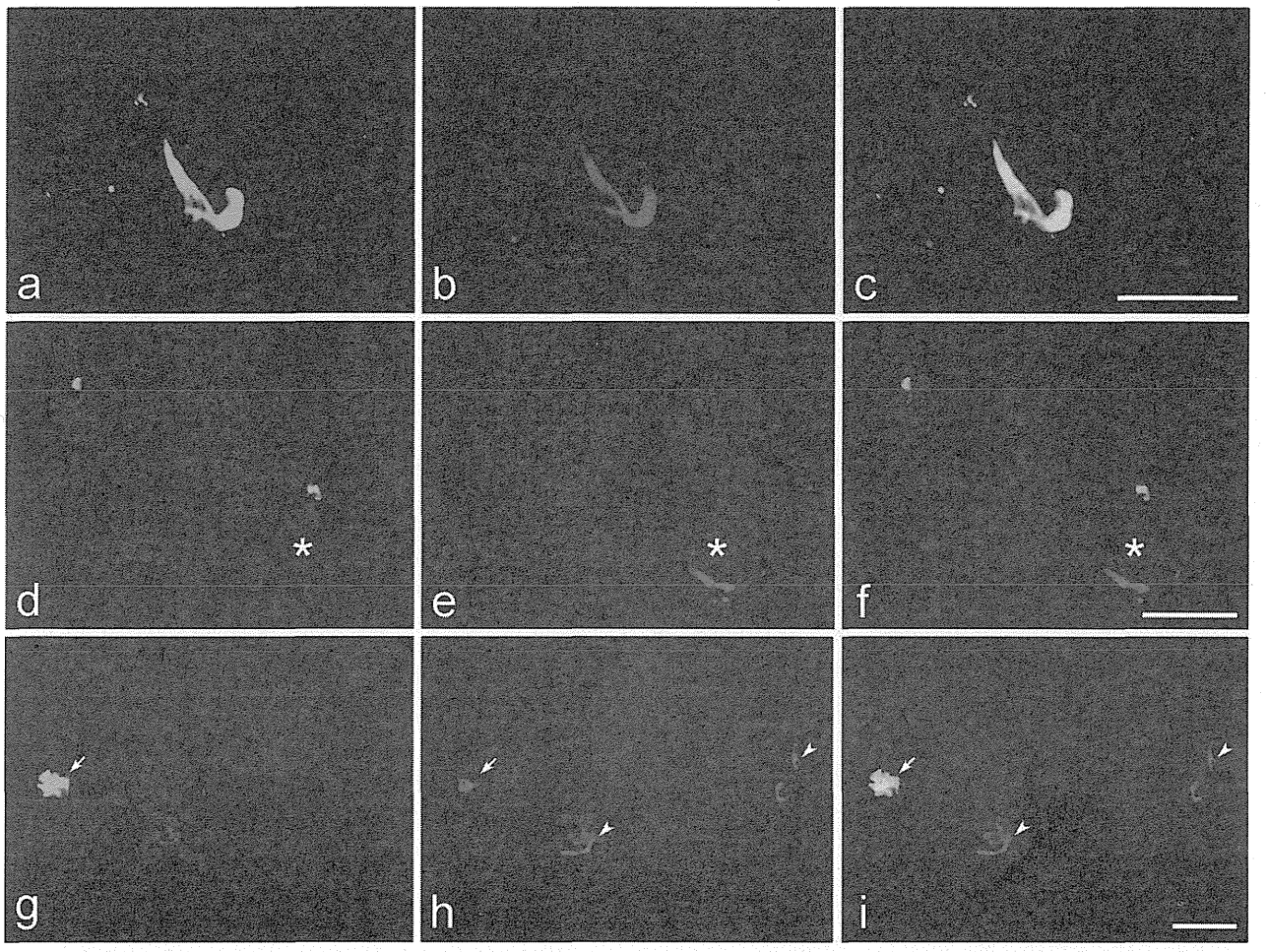

Figure 3. Double-labelling immunofluorescence for the presence of TDP-43, RBM45, DPR proteins and p62. (**a–i**) Inclusions observed in the motor cortex cells. (**a–c**) Colocalization of RBM45 (**a**, green) and TDP-43 (**b**, red) is evident in the cytoplasmic inclusions in an oligodendrocytic cell (**c**, merge). (**d–f**) Poly Gly-Ala (GA) (**d**, green) and TDP-43 (**e**, red) never co-localize in the cytoplasmic inclusions (**f**, merge), although co-existence of poly GA- and TDP-43-positive inclusions are rarely observed in the same cells, mostly in the neuronal cells (asterisks) (**d–f**). (**g–i**) Co-localization of poly GA (**g**, green, arrow) and p62 (**h**, red, arrow) is evident in cytoplasmic inclusions in a neuronal cell (**i**, merge, arrow). Poly GA-negative (**g**, green) and p62-positive inclusions (**h**, red, arrowheads) are also evident in two oligodendrocytic cells (**i**, merge, arrowheads). Bars: 10 μm (**a–i**).

positive NCIs was confirmed. It is important to note that the TDP-43 pathology and neuronal loss were correlated with each other; this was also the case for another RNA-binding protein, RBM45, which has been reported to be increased in the cerebrospinal fluid of ALS patients, and also colocalized with TDP-43 in inclusions of patients with ALS and FTLD-TDP [10]. In addition, the occurrence of p62-positive, and TDP-43-negative granular or star-shaped NCIs in the cerebellar cortex and hippocampus was a feature. Importantly, such p62-positive and TDP-43-negative NCIs were also labelled with antibodies against DPR proteins we generated. The DPR protein pathology was distributed independently of TDP-43 pathology: co-localization of DPR proteins and TDP-43 was extremely rare, if present, in the same inclusion, indicating that unlike TDP-43 and RBM45, there was no correlation between DPR protein pathology and neuronal loss (Table 1), or between DPR protein pathology and clinical symptoms, as pointed out previously in Caucasian cases of c9FTD/ALS [13].

It is noteworthy that most recently, the DPR proteins have been reported to be translated from not only a sense direction of the repeats but also an antisense direction (CCCCGG); in fact, two additional DPR proteins (poly