Original paper

Folia Neuropathologica

Neuronal cytoplasmic inclusions in tau, TDP-43, and FUS molecular subtypes of frontotemporal lobar degeneration share similar spatial patterns

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Folia Neuropathol 2017; 55 (3): 185-192 DOI: https://doi.org/10.5114/fn.2017.70482

Abstract

The 'prion-like' transfer of pathogenic proteins may play a role in the pathogenesis of frontotemporal lobar degeneration (FTLD). Propagation of such proteins along anatomical pathways may give rise to specific spatial patterns of the 'signature' neuronal cytoplasmic inclusions (NCI) characteristic of these disorders. Hence, the spatial patterns of the NCI were compared in three molecular subtypes of FTLD: (1) two variants of FTLD-tau, viz. corticobasal degeneration (CBD) and Pick's disease (PiD), (2) FTLD with transactive response (TAR) DNA-binding protein 43 (TDP-43)-immunoreactive inclusions (FTLD-TDP), and (3) FTLD with 'fused in sarcoma' (FUS)-immunoreactive inclusions (FTLD-FUS). Regardless of molecular pathology, the NCI in the frontal and temporal cortex were most frequently aggregated into clusters, the clusters being regularly distributed parallel to the pia mater. In a significant proportion of regions, the regularly distributed clusters were in the size range 400–800 μm, approximating the dimension of cell columns associated with the cortico-cortical pathways. Clusters of NCI were significantly larger in FTLD-tau compared with FTLD-TDP and FTLD-FUS. The data suggest that cortical NCI in different molecular subtypes of FTLD all share a similar spatial pattern in the frontal and temporal cortex consistent with a 'prion-like' spread of pathological proteins along anatomical pathways. However, a more selective group of neurons appears to be affected in FTLD-TDP and FTLD-FUS than in FTLD-tau.

Key words: *frontotemporal lobar degeneration (FTLD), spatial patterns, neuronal cytoplasmic inclusions (NCI), 'prion-like' spread.*

Introduction

Most cases of frontotemporal lobar degeneration (FTLD) can be classified into three molecular subtypes: (1) FTLD with tau-immunoreactive inclusions (FTLD-tau), a heterogeneous group of disorders, examples of which include corticobasal degeneration (CBD), Pick's disease (PiD), and progressive supranuclear palsy (PSP), (2) FTLD with transactive response (TAR) DNA-binding protein 43 (TDP-43)-immunoreactive inclusions (FTLD-TDP), and (3) FTLD with 'fused in sarcoma' (FUS)-immunoreactive inclusions (FTLD-FUS) [42]. In all of these disorders, abnormally aggregated proteins result in the formation of phosphorylated 'signature' neuronal cytoplasmic inclusions (NCI), FUS

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protein in FTLD-FUS being additionally hypomethylated [48], most abundantly in the frontal and temporal cortex [15,29,52].

Many pathogenic proteins can exhibit 'prion-like' behaviour, i.e., they act as propagating entities or 'seeds' amplifying their pathogenic conformation similar to prion protein (PrP^{sc}) in prion diseases [35,37]. As a consequence, proteins may spread along anatomical pathways and the clinical phenotype of the disease could depend, in part, on variations in this spread and the specific pathways affected. If pathogenic proteins spread along anatomical connections, then the resulting NCI may exhibit a spatial distribution pattern in the cortex which reflects this spread [10]. In Creutzfeldt-Jakob disease (CJD), in which propagation of PrP^{sc} along anatomical pathways is well documented [19-21], PrP^{sc} deposits are clustered in the cerebral cortex, the clusters being regularly distributed parallel to the pia mater [11,13]. This pattern of clustering may therefore be a marker for the spread of PrP^{sc} [12,14] and other proteins among brain regions along anatomical pathways [10]. In addition, tau and TDP-43 but not FUS may share amyloid properties [23] and therefore inclusions in FTLD-FUS may have a different spatial pattern than in the other subtypes. Hence, the present study compared the spatial patterns of NCI in cases representing the three subtypes of FTLD: (1) two variants of FTLD-tau, viz. CBD and PiD, (2) FTLD-TDP, and (3) FTLD-FUS. Two main questions were addressed: (1) Are the spatial patterns of the NCI in the three disorders consistent with the propagation of pathological proteins? (2) Did molecular pathology influence the spatial patterns of the NCI?

Material and methods

Cases

Demographic data and diagnostic criteria for the three molecular subtypes of FTLD are shown in Table I. CBD (n = 12) and PiD (n = 10) cases (FTLD-tau) were obtained from the Brain Bank, Department of Neuropathology, Institute of Psychiatry, King's College, London, UK. FTLD-TDP cases (n = 32) were obtained from Washington University, St Louis, MO., USA). Of the 32 FTLD-TDP cases, 20 were familial (at least one first degree relatives affected) and of these, 10 cases had *GRN* mutations [18,22,28,41,43], one had a *VCP* gene mutation, and one case was associated with the *C90RF72* gene [40,46]. The majority (n = 7) of the *GRN* cases were from a single hereditary dysphasic disinhibition dementia (HDDD) family (HDDD2) [43] and the remainder (n = 3) from a HDDD1 family [22]. No genetic defects have been identified to date in the remaining eight familial cases but none of these had a strong autosomal dominant pattern of inheritance. FTLD-FUS cases (n = 10) [16] were obtained from centres in Canada, Norway, Spain, Japan (one case from each), and from France, the UK, and the USA (two cases from each) [25].

Tissue preparation

After death, the consent of the next of kin was obtained for brain removal following local Ethical Committee procedures and the 1999 Declaration of Helsinki (as modified Edinburgh, 2000). Brain tissue was preserved in buffered 10% formalin or 4% paraformaldehyde. Tissue blocks were taken from frontal and temporal lobe, fixed in 10% phosphate buffered formal-saline, and embedded in paraffin wax. Immunohistochemistry (IHC) was performed on 6-8 µm sections using appropriate antibodies (Table I). Sections were counterstained with haematoxylin.

Morphometric methods

In the superior frontal gyrus (SFG), inferior temporal gyrus (ITG), and parahippocampal gyrus (PHG), NCI were counted along a strip of tissue (3200 to 6400 µm in length) located parallel to the pia mater, using 250 × 50 µm sample fields arranged contiguously [4]. The sample fields were located both in the upper (approximating to layers II/III) and lower (approximating to layers V/VI) cortex, the short edge of the sample field being orientated parallel with the pia mater and aligned with guidelines marked on the slide. The number of NCI present in each sample field was counted.

Data analysis

The data were analysed by spatial pattern analysis [2,4,6,7,9]. This method uses the variance-mean ratio (V/M) to determine whether the NCI were distributed randomly (V/M = 1), regularly (V/M < 1), or were clustered (V/M > 1) along a strip of tissue. Counts of NCI in adjacent sample fields were added together successively to provide data for increasing field sizes, e.g., 50 × 250 µm, 100 × 250 µm, 200 × 250 µm, etc., up to a size limited by the length of the strip sampled. V/M was plotted against field

Table I. Summary of demographic details, diagnostic criteria, and immunohistochemistry (IHC) of the frontotemporal lobar degeneration (FTLD) subtypes

Disorder	*n*	Age mean (SD)	M : F	IHC	Diagnostic criteria
FTLD-tau (CBD)	12	64.07 (9.07)	8 : 4	TP007 (tau)	NIH-ORD
FTLD-tau (PiD)	10	65.03 (11.3)	7 : 3	TP70 (tau)	Cairns *et al.* [26]
FTLD-TDP	32	71.03 (1.62)	16 : 16	pTDP-43	Cairns *et al.* [26]
FTLD-FUS	10	45.3 (12.1)	7 : 3	FUS	Cairns *et al.* [26]

*CBD – corticobasal degeneration, FUS – 'fused in sarcoma', PiD – Pick's disease, TDP-43 – transactive response (TAR) DNA-binding protein 43, **n** – number of cases studied, M – male, F – female.*
Diagnostic criteria: National Institutes of Health – Office of Rare Diseases (NIH-ORD); diagnostic criteria for FTLD-TDP and FTLD-FUS according to Cairns et al. (2007)

size to determine whether the clusters of NCI were regularly or randomly distributed and to estimate the mean cluster size parallel to the tissue boundary. A V/M peak indicates the presence of regularly spaced clusters. The statistical significance of a peak was tested using the 't' distribution [4]. The effect of molecular pathology on the frequency of the different types of spatial pattern was tested using chi-square (X^2) contingency tables. In addition, mean cluster sizes of NCI were compared between FTLD subtypes using a one-way analysis of variance (ANOVA) (STATISTICA software, StatSoft Inc., 2300 East 14th St, Tulsa, Ok, 74104, USA) followed by Tukey's HSD *post-hoc* test.

Results

Examples of the NCI in the three FTLD subtypes are shown in Figures 1 and 2. Consistent morphological differences were apparent among disorders, spherical inclusions being predominant in PiD and FTLD-FUS, while NCI in CBD and FTLD-TDP were more variable, being spherical, spicular, or flame-shaped.

Examples of the spatial patterns of the NCI observed in a single brain region (ITG, layers II/III) in the three FTLD subtypes are shown in Figure 3. The V/M ratios of the NCI in CBD (FTLD-tau) increased with field size without reaching a peak, suggesting a large cluster of NCI at least 1600 mm in diameter. The V/M ratios of the NCI in FTLD-TDP and FTLD-FUS, however, reached peaks at field sizes of 200 mm and 100 mm respectively, suggesting clusters of inclusions, 200 mm and 100 mm in diameter, which were regularly distributed parallel to the pia mater.

A comparison of the spatial patterns exhibited by the NCI in FTLD-tau, FTLD-TDP, and FTLD-FUS in all cases and regions is shown in Table II. Most frequently, NCI were clustered and the clusters were regularly distributed parallel to the pia mater. This spatial pattern varied in frequency among subtypes

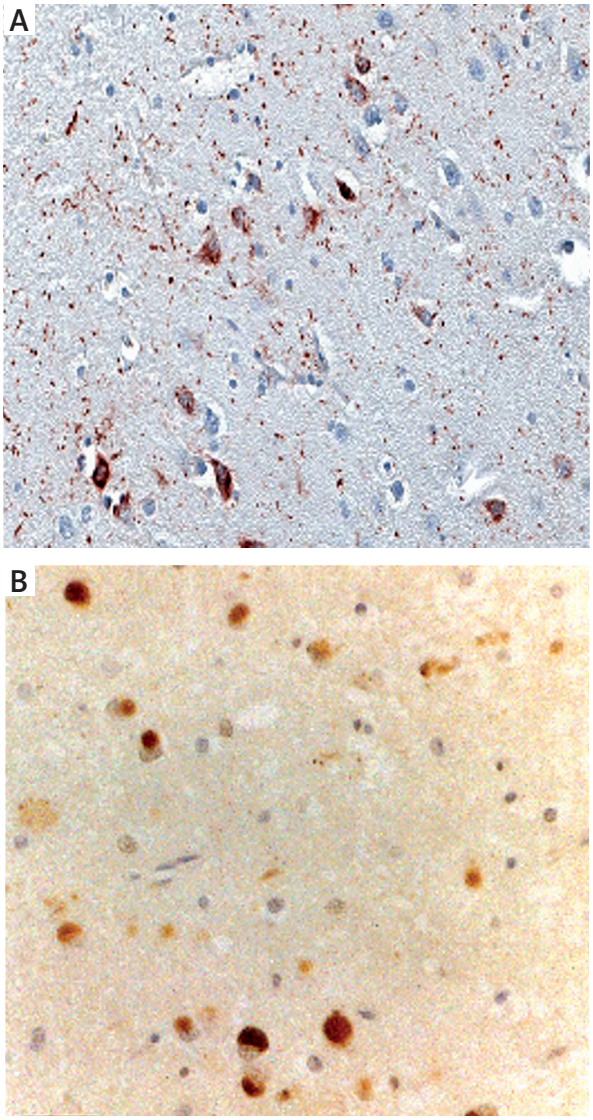

Fig. 1. Neuronal cytoplasmic inclusions (NCI) in frontotemporal lobar degeneration (FTLD) with tau-immunoreactive inclusions (FTLD-tau): **A)** corticobasal degeneration (CBD) (antibody TP007, bar = 100 μm) and **B)** Pick's disease (PiD) (Antibody tau TP70, bar = 50 μm).

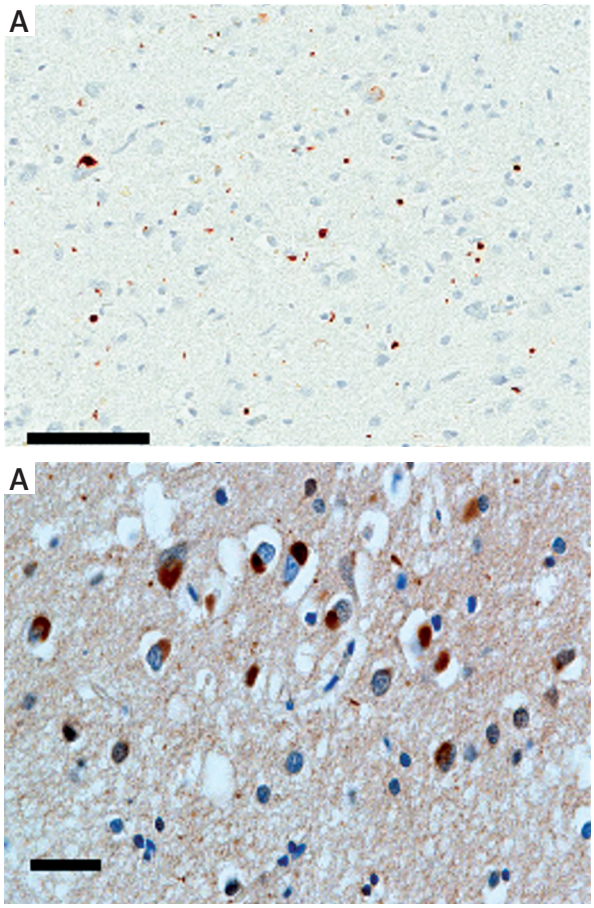

Fig. 2. Neuronal cytoplasmic inclusions (NCI) in frontotemporal lobar degeneration (FTLD): **A)** with TDP-43-immunoreactive inclusions (FTLD-TDP) (antibody pTDP-43, bar = 100 μm) and **B)** with FUS-immunoreactive inclusions (FTLD-FUS) (antibody FUS, bar = 100 μm).

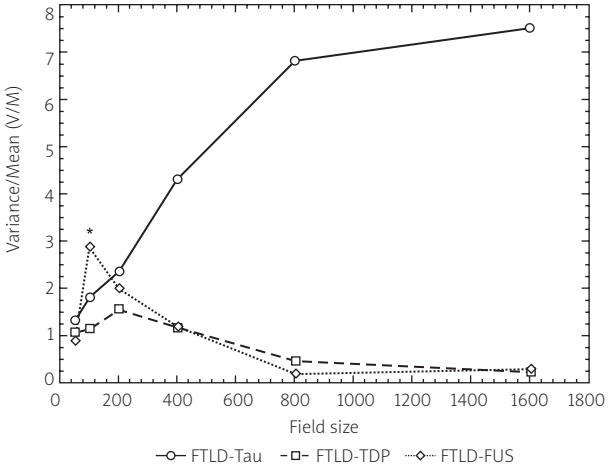

Fig. 3. Pattern analysis plots showing examples of the spatial patterns exhibited by neuronal cytoplasmic inclusions (NCI) in the inferior temporal gyrus (ITG): A comparison of three subtypes of frontotemporal lobar degeneration (FTLD), viz. FTLD-tau (CBD), FTLD-TDP, and FTLD-FUS (* indicates significant variance/mean peaks).

from 45% of cortical gyri in FTLD-TDP to 74% in FTLD-FUS. Larger-scale clustering, in which the NCI occurred in clusters of at least 1600 mm in diameter, but without regular spacing, was also present in some regions. Within FTLD-tau, there were no significant differences between the spatial patterns of the NCI in CBD and PiD (X^2 = 2.37, 3DF, $p > 0.05$) and in FTLD-TDP between familial and sporadic cases (X^2 = 3.11, 6DF, $p > 0.05$). However, there were significant differences in the proportions of the different spatial patterns among FTLD subtypes (X^2 = 65.12, 9DF, $p < 0.001$), comparisons of the subtypes in pairs suggesting that FTLD-tau exhibited more frequent large-scale clustering than FTLD-TDP (X^2 = 51.15, 3DF, $p < 0.001$) and FTLD-FUS (X^2 = 21.47, 3DF, $p < 0.001$). In addition, the frequency of random distributions was higher in FTLD-TDP. In all subtypes, a small number of regions exhibited a more complex spatial pattern in which small, regularly distributed clusters were themselves aggregated into larger 'superclusters'.

A comparison of estimated cluster sizes of the NCI in the three subtypes is shown in Table III. There was a significant difference in mean cluster size among subtypes (F = 28.68, $p < 0.001$), *post-hoc* tests suggesting that cluster sizes were significantly larger in FTLD-tau than in FTLD-TDP and FTLD-FUS. In addition, the proportion of cortical regions in which regularly distributed clusters were within the size range 400-800 mm varied among subtypes (X^2 = 7.94, 2DF, $p < 0.05$), FTLD-tau variants exhibiting a greater proportion of regions within this size range (X^2 = 5.43, 1DF, $p < 0.05$) compared with FTLD-TDP and FTLD-FUS, which exhibited a similar range (X^2 = 1.42, 1DF, $p > 0.05$).

Discussion

The data suggest that in all three molecular subtypes of FTLD, the NCI were clustered in the frontal and temporal cortex and, in a significant proportion of gyri, the clusters were regularly distributed parallel to the pia mater [13]. The frequency of this spatial pattern varied among subtypes, being most frequent in FTLD-FUS (74%) and least frequent in FTLD-TDP

Table II. Frequency of the different types of spatial pattern (R – random, RG – regular) exhibited by neuronal cytoplasmic inclusions (NCI) in three subtypes of frontotemporal lobar degeneration (FTLD): FTLD-tau, viz. Pick's disease (PiD), and corticobasal degeneration (CBD), FTLD-TDP, and FTLD-FUS. Data in parentheses indicate the number of gyri in which regular clusters of inclusions were in the size range 400-800 μm

Disorder	Subtype	R	RG	Regular clusters (50-1600 μm)	Large clusters (≥ 1600 μm)
FTLD-tau	CBD	2	2	48 (24)	24
	PiD	1	0	27 (17)	20
FTLD-TDP	All cases	36	17	60 (28)	19
	GRN mutation	16	5	23 (11)	5
	Non-*GRN* familial cases	9	4	16 (7)	7
	Sporadic cases	11	8	21 (10)	7
FTLD-FUS	–	7	3	39 (11)	4

Chi-square (χ^2) contingency tests:
Between subtypes of FTLD-tau: $\chi^2 = 2.37$ (3DF, $p > 0.05$)
Between subtypes of FTLD-TDP: $\chi^2 = 3.11$ (6DF, $p > 0.05$)
All groups: $\chi^2 = 65.12$ (9DF, $p < 0.001$)
Comparing FTLD-tau and FTLD-TDP: $\chi^2 = 51.15$ (3DF, $p < 0.001$)
Comparing FTLD-tau and FTLD-FUS: $\chi^2 = 21.47$ (3DF, $p < 0.001$)
Comparing FTLD-TDP and FTLD-FUS: $\chi^2 = 12.05$ (3DF, $p < 0.01$)

Table III. Estimated mean cluster sizes (SE – standard error) of the neuronal cytoplasmic inclusions (NCI) in the cortex of three subtypes of frontotemporal lobar degeneration (FTLD), viz. FTLD-tau, FTLD-TDP, and FTLD-FUS

Subtype	Mean cluster size (μm)	Range (μm)	SE	Percentage of gyri in range 400-800 (μm)
FTLD-tau (CBD)	1990	100-1600	243.9	32
FTLD-tau (PiD)	3229	200-1600	385.9	51
FTLD-TDP	302	50-1600	97.2	10
FTLD-FUS	474	50-1600	134.4	21

Comparison of cluster sizes (1-way ANOVA with 'Tukey HSD' post-hoc test): Between disorders $F = 23.52$ ($p < 0.001$), post-hoc FTLD-tau > FTLD-TDP = FTLD-FUS
Comparison of proportions of regions with cluster sizes in the range 400-800 mm:
All groups $\chi^2 = 7.94$ (2DF, $p < 0.05$)
Comparing FTLD-tau and. FTLD-TDP + FTLD-FUS $\chi^2 = 5.43$ (1DF, $p > 0.05$)
Comparing FTLD-TDP and FTLD-FUS $\chi^2 = 1.42$ (1DF, $p > 0.05$)

(45%). Similar spatial patterns have been observed in Alzheimer's disease (AD) [3,8], in various synucleinopathies such as Parkinson's disease dementia (PDD) [10] and in CJD [11,13]. Hence, although FUS may not share the amyloid-like properties of tau and TDP-43 [23], the similar spatial patterns exhibited suggest that similar pathological processes may be present in diseases characterised by different molecular pathologies [34].

The regular distribution of clusters of NCI is consistent with their development in association with the cells of origin of specific cortical pathways [3,14]. In cortical regions, these cells are clustered and occur in bands which are regularly distributed along the cortex. Individual bands of cells traverse the cortical layers and, in the primate brain, vary in width from 400-500 mm up to 800-1000 μm depending on the region [30,36]. In a proportion of gyri, the width of the NCI clusters and their distribution along the cortex suggest an association with these pathways with two exceptions. First, in some gyri, NCI occurred in larger clusters greater than 800-1600 mm, especially in FTLD-tau, and in some regions, small clusters of NCI were aggregated into larger 'superclusters'. These results suggest that the smaller, regularly distributed clusters of inclusions could 'coalesce' to form larger clusters as the disease develops [3]. Second, NCI were randomly distributed in some gyri, especially in FTLD-TDP, which may be the result of the low density of pTDP-43-immunoreactive inclusions observed in some cases [15].

Differences in cluster sizes of NCI among subtypes suggest variation in the degree to which specific cortical columns may be affected by the different molecular pathologies. Hence, significantly larger clusters of NCI were observed in FTLD-tau than in FTLD-

TDP and FTLD-FUS, and in the latter two disorders the clusters were usually smaller than the estimated diameter of the cell columns of the cortico-cortical projections. Hence, a more localised pattern of cortical degeneration appears to be present in FTLD-TDP and FTLD-FUS. Compared with tau, which is widespread in neurons and important in the assembly and stabilisation of microtubules [27,51], both TDP-43 and FUS have more specific roles, TDP-43 in mRNA function, DNA repair, and in non-coding RNA metabolism [45] while FUS is important in regulating gene expression including transcription, splicing, and RNA transport [31]. In both TDP-43 and FUS, nuclear clearance results in the immediate aggregation in the cytoplasm of cells [33]. In addition, TDP-43 can also repress non-conserved cryptic axons, many of which are cell type specific, and therefore loss of TDP-43 function could result in the degeneration of specific groups of cells [38,39].

Of particular interest is whether the observed spatial patterns could reflect the 'prion-like' behaviour of pathogenic proteins [32,35,37,47]. Several observations are consistent with this hypothesis. First, tau may exit host cells, transfer between cells, gain access to new cells, and create pathology within these cells [47]. Second, by analogy with the scrapie form of prion protein (PrP^{sc}), nucleation or seeding activity of tau may result in a core of an NCI of transferred tau surrounded by additional layers of cytoplasmic tau contributed by the host cell. Third, the spatial patterns of NCI in all three subtypes exhibited a similar spatial pattern to PrP^{sc} deposits in CJD [11,13]. Fourth, TDP-43 is a dimeric nuclear protein in which the C-terminal region exhibits 'prion-like' behaviour [49], the majority of gene mutations associated with frontotemporal dementia (FTD) and motor neuron disease (MND) being located in this region [49]. Moreover, tandem repeats of the 'prion-like' Q/N region of TDP-43, when fused to additional TDP-43, can cause aggregate formation in neuronal and non-neuronal cell lines [24]. Fifth, aggregates of phosphorylated TDP-43 (pTDP-43) are frequently present in axons of hypoglossal and facial nerves and in spinal cord anterior cells in MND, consistent with propagation of the protein [44], while FUS activity is found in granules in gray matter of the brain stem and spinal cord, which co-localise with synaptophysin [1], consistent with transport of the protein and synaptic disconnection. Sixth, stress granules are foci of cytoplasmic RNA formed in response to stress and, among many other proteins, also exhibit TDP-43 and FUS immunoreactivity [17]. Hence, the domains involved in the phase separation of liquid droplets such as stress granules may be a precursor to aggregation and propagation of proteins. Hence, cell-to-cell transfer of pathological proteins along anatomical pathways may be a common mechanism determining cortical degeneration in a variety of FTLD molecular subtypes.

In conclusion, FTLD characterised by NCI expressing different molecular pathologies exhibit similar spatial patterns in the cerebral cortex, consistent with an association with specific anatomical pathways. The data provide some support for the hypothesis that a 'prion-like' cell-to-cell transfer of pathogenic proteins occurs across different subtypes of FTLD. Different FTLD subtypes therefore may be amenable to similar interventions; e.g., immunotherapy which targets extracellular pathogenic proteins could lead to their removal, thus preventing or slowing cell-to-cell propagation [50].

Acknowledgments

I would like to thank the families of patients, whose generosity made this research possible. In addition, the following are thanked for making tissue sections available for this study: Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, MO, USA, Brain Bank, Institute of Psychiatry, King's College London, London, UK, William Ellis (Department of Pathology, University of California, Davis, Sacramento, CA, USA), Ronald L. Hamilton (Department of Pathology, University of Pittsburgh, Pittsburgh, PA, USA), Ian R. A. Mackenzie (Department of Pathology, Vancouver General Hospital, Vancouver, Canada), E. Tessa Hedley-Whyte (Massachusetts General Hospital and Harvard Brain Tissue Resource Center, Belmont, MA, USA), Marla Gearing (Center for Neurodegenerative Disease, Emory University, Atlanta, GA, USA), Robert Perry Department of Neuropathology, Newcastle General Hospital, Newcastle-upon-Tyne, NE4 6BE, UK), Charles Duyckaerts, Laboratoire de Neuropathologie, Hôpital de la Salpêtrière, AP-HP, 75651, Paris, France), Felix Cruz-Sanchez (Institute of Neurological and Gerontological Sciences, International University of Catalonia, Barcelona, Spain), Kari Skullerud (Department of Pathology, Rikshospitalet, N-0027, Oslo, Norway), Eileen Bigio (Depart-

ment of Pathology, Northwestern University Medical School, Chicago, Illinois, USA), and Hideaki Yokoo (Department of Pathology, Gunma University School of Medicine, Maebashi, Japan).

Disclosure

Author reports no conflict of interest.

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