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Spatial patterns of TDP-43 neuronal cytoplasmic inclusions (NCI) in fifteen cases of frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP)

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Abstract

Neuronal cytoplasmic inclusions (NCI) immunoreactive for transactive response DNA-binding protein (TDP-43) are the pathological hallmark of frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP). We studied the spatial patterns of the TDP-43 immunoreactive NCI in the frontal and temporal cortex of 15 cases of FTLD-TDP. The NCI were distributed parallel to the tissue boundary predominantly in regular clusters 50–400 μ m in diameter. In five cortical areas, the size of the clusters approximated to the cells of the cortico-cortical pathways. In most regions, cluster size was smaller than 400 μ m. There were no significant differences in spatial patterns between familial and sporadic cases. Cluster size of the NCI was not correlated with disease duration, brain weight, Braak stage, or disease subtype. The spatial pattern of the NCI was similar to that of neuronal inclusions in other neurodegenerative diseases and may reflect a common pattern of degeneration involving the cortico-cortical projections.

Keywords

Frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP); TAR DNA-binding protein of 43 kDa (TDP-43); Neuronal cytoplasmic inclusions (NCI); Spatial pattern

Introduction

The transactive response (TAR) DNA-binding protein of 43 kDa (TDP-43) is an RNA binding protein encoded by the highly conserved *TARDPB* gene on chromosome 1. TDP proteins have a glycine-rich domain and are believed to carry out essential cellular functions including the regulation of transcription, alternate splicing, and acting as a framework for nuclear bodies [1]. TDP-43 is a nuclear protein containing two RNA-recognition sequences as well as a glycine-rich C-terminal sequence and is widely expressed in body tissues [2]. In neurodegenerative disease, TDP-43 may be redistributed from the nucleus to the cytoplasm, is ubiquitinated, hyperphosphorylated, and then cleaved to generate C-terminal fragments [2]. These fragments then accumulate to form cellular inclusions which may cause cell death.

Recent studies suggest a significant role for TDP-43 in neurodegenerative disease. Hence, TDP-43 is a major pathological protein in frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP) previously called FTLN with ubiquitin immunoreactive inclusions (FTLD-U) [3], a disorder that accounts for approximately 10% of early-onset dementias. The spectrum of TDP proteinopathies includes most cases of sporadic and familial FTLD-TDP with or without associated motor neuron disease (MND). The majority of familial cases are linked to *progranulin* (*GRN*) mutations [4–6] and a smaller number to mutations of the *valosin-containing protein* (*VCP*) gene [7]. Accumulation of TDP-43 may also occur in Alzheimer's disease (AD) [8], dementia with Lewy bodies (DLB) [9], amyotrophic lateral sclerosis (ALS) [10], hippocampal sclerosis (HS) [11], and Guam-parkinsonism dementia complex (G-PDC) [12].

FTLD-TDP is characterized by a widespread atrophy largely affecting the frontal, temporal, and parietal lobes with neuronal loss, microvacuolation in the superficial cortical laminae, reactive atrophy, and TDP-43 immunoreactive lesions [13]. The latter include neuronal cytoplasmic inclusions (NCI), less frequent oligodendroglial inclusions (GI), neuronal intranuclear inclusions (NII), and dystrophic neurites (DN). Various subtypes of FTLD-TDP have been proposed based on the proportion and distribution of the TDP-43 immunoreactive lesions [14, 15].

NCI are a common feature of neurodegenerative disease [16], but the majority are characterized by abnormal aggregates of tau (tauopathies) or α -synuclein (synucleinopathies). Hence, FTLD-TDP proteinopathy, together with the NCI in neuronal intermediate filament inclusion disease (NIFID), are extensions to the molecular classification of neurodegenerative disease [17]. Abnormal neuronal intermediate filament (IF) aggregates in the form of NCI have been regarded as the pathological hallmark of NIFID [18]. Not all inclusions in NIFID, however, are immunolabeled by anti-neuronal IF antibodies and therefore, the primary molecular defect is uncertain [19]. Recently, 'fused in sarcoma' (FUS) protein (encoded by the *FUS* gene) has been shown to be a component of the inclusions of NIFID [20, 21].

In the cerebral cortex of the tauopathies [22, 23], synucleinopathies [24, 25], and in NIFID [26], the various types of NCI often have a distinct spatial pattern, viz., they occur in distinct clusters which exhibit a regular periodicity parallel to the pia mater. This has led to the hypothesis that the NCI may develop in relation to clusters of cells associated with the modular structure of the cortex and specifically the cortico-cortical and cortico-hippocampal projections, and may result from the degeneration of these cells. The objective of the present study was to determine whether (1) the TDP-43 immunoreactive NCI in a group of fifteen FTLD-TDP cases exhibited a similar pattern of topographic distribution and (2) whether the spatial pattern of the NCI was consistent with degeneration of the cortico-cortical pathways.

Materials and methods

Cases

Fifteen cases of clinical and neuropathological cases of sporadic and familial FTLD-TDP were obtained from dementia centers in the USA and Canada (see Table 1). These cases formed part of a larger study with the aim of objectively quantifying the pathological changes in FTLD-TDP [27]. All cases exhibited FTLD with neuronal loss, microvacuolation in the superficial cortical laminae, and reactive atrophy consistent with the published consensus criteria for FTLD-TDP [14]. A variety of TDP-43-immunoreactive lesions was present in these cases including NCI, NII, DN, and GI consistent with a diagnosis of TDP-43 proteinopathy [15]. Of the 15 cases, five were identified as familial FTLD-TDP, and two of these cases were caused by the *GRN* gene [4–6]. The genetic defects in the remaining

familial cases have not been identified to date. None of the cases had coexisting MND (FTLD-MND) [28, 29] or HS [11]. Cases were assigned to subtype by a consultant neuropathologist based on the composite scheme of Cairns et al. [15], viz., type 1 cases were characterized by long DN in superficial cortical laminae with few or no NCI or NII, type 2 by numerous NCI in superficial and deep cortical laminae with infrequent DN and sparse or no NII, type 3 by pathology predominantly in superficial cortical laminae with numerous NCI, DN, and varying numbers of NII, and type 4 by numerous NII, and infrequent NCI and DN especially in neocortical areas.

Histological methods

After death, the consent of the next of kin was obtained for brain removal, following local Ethical Committee procedure and the 1995 Declaration of Helsinki (as modified Edinburgh, 2000). Tissue blocks were taken from the medial frontal lobe at the level of the genu of the corpus callosum to study the middle frontal gyrus (MFG) and the temporal lobe at the level of the lateral geniculate body to study the inferior temporal gyrus (ITG), parahippocampal gyrus (PHG), CA1/2 sectors of the hippocampus, and dentate gyrus (DG). Tissue was fixed in 10% phosphate-buffered formal-saline and embedded in paraffin wax. Immunohistochemistry (IHC) was performed on 4- to 10- μ m-sections with a rabbit antibody that recognizes TDP-43 epitopes (dilution 1:1,000; ProteinTech Inc., Chicago, IL, USA). Sections were counterstained with hematoxylin.

Morphometric methods

In areas of the cerebral cortex, the TDP-43 immunoreactive NCI were counted along a strip of tissue (1,600–3,200 μ m in length) located parallel to the pia mater, using 250 $\times$ 50 μ m sample fields arranged contiguously. The sample fields were located both in the upper (laminae II/III) and lower (laminae V/VI) laminae, the short edge of the sample field being orientated parallel to the pia mater and aligned with guidelines marked on the slide. In the hippocampus, the features were counted in the cornu ammonis (CA) in a region extending from the prosubiculum/CA boundary to the maximum point of curvature of the pyramidal layer before it extends to join the dentate fascia via CA3 and CA4. Hence, the region sampled encompassed approximately sectors CA1 and CA2, the short dimension of the contiguous sample field being aligned with the alveus. NCI have been commonly observed in the dentate gyrus fascia in FTLD-TDP [14, 30], and the sample field was therefore aligned with the upper edge of the granule cell layer. The majority of the NCI were similar to the C-type described by Yaguchi et al. [31] comprising large, intensely stained circular or crescent-shaped inclusions (Fig. 1).

Data analysis

To determine the spatial patterns of the NCI, the data were analyzed by spatial pattern analysis [32–34]. 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 $\times$ 250 μ m, 100 $\times$ 250 μ m, 200 $\times$ 250 μ m etc., up to a size limited by the length of the strip sampled. V/M was then plotted against field 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 while an increase in V/M to an asymptotic level suggests the presence of randomly distributed clusters. The statistical significance of a peak was tested using a t test [33]. The spatial patterns of the histological features in familial and sporadic cases were compared using a chi-square (χ^2) contingency table test. In addition, the degree of correlation between the cluster size of the NCI

(assuming a maximum size of 800 μm), with duration of disease, Braak stage [35], brain weight, and disease subtype was tested using Pearson's correlation coefficient (r).

Results

Typical examples of the spatial patterns shown by the TDP-43 immunoreactive NCI are shown in Fig. 2 and the data on which they are based listed in Table 2. In laminae II/III of the MFG, the V/M ratio was not significantly different to unity at any field size suggesting a random distribution of the NCI. By contrast, in the ITG, a V/M peak at field size 200 μm indicates the presence of regularly distributed clusters of NCI of average diameter 200 μm .

The spatial patterns exhibited by the NCI in each brain region of each case are shown in Table 3. In the cerebral cortex, 27/49 (55%) of brain regions analyzed revealed a regular distribution of clusters of NCI, a random distribution was present in 18/49 (37%) regions, a regular distribution of individual NCI in 1/49 (2%) regions, and larger non-regularly distributed clusters (>800 μm) in 3/49 (6%) regions. In sectors CA1/2 of the hippocampus, regularly distributed clusters of inclusions were present in 1/7 (1.4%) regions studied, a random distribution in 4/7 (57%) regions, a regular distribution in 1/7 (1.4%) regions, and large clusters were present in 2/7 (29%) regions. In the DG, regularly distributed clusters of NCI were present in 9/13 (69%) gyri and a random distribution in 4/13 (31%) gyri. In the data as a whole, six cortical areas showed evidence of clustering at two scales, i.e., smaller sized clusters of NCI were aggregated into larger clusters. In laminae II/III of the cortical areas exhibiting clustering, clusters were in the size range 400–800 μm in 3/18 (17%) gyri, smaller than 400 μm in 14/18 (78%) and larger than 800 μm in 1/18 (5.5%) gyri. In laminae V/VI, cluster sizes of inclusions were between 400 and 800 μm in 2/9 (22%) gyri, were smaller than 400 μm in 3/9 (33%) gyri, and larger than 400 μm in 2/9 (22%) gyri. Regularly distributed clusters of NCI were most common in the upper laminae of the cerebral cortex and in the DG.

The frequency of the different types of spatial pattern of the NCI were similar in familial and sporadic cases ($\chi^2 = 2.53$, 3DF, $P > 0.05$) and mean cluster size of the NCI was similar for both groups ($t = 0.27$ – 0.65 in different regions, $P > 0.05$). In addition, cluster size of the NCI was not significantly correlated with disease duration, Braak stage, brain weight, or disease subtype in the upper and lower cortical laminae, sector CA1/2 of the hippocampus, or in the DG.

Discussion

In the majority of regions, the TDP-43 immunoreactive NCI occurred in clusters that exhibited a regular periodicity parallel to the tissue boundary. This type of spatial pattern is similar to that reported for different types of neuronal inclusion characterized by tau [22, 23, 36], and α -synuclein [24, 25] immunoreactivity, and in NIFID characterized by α -internexin IHC [26]. To date, there have been no studies on the spatial patterns of FUS-immunoreactive NCI.

In the tauopathies and synucleinopathies, the spatial patterns of the NCI clusters within the cerebral cortex and hippocampus suggested that the inclusions were related to the cells of origin of specific cortico-cortical and cortico-hippocampal projections [37–39]. The cells of origin of the cortico-cortical projections are clustered and occur in bands that are more or less regularly distributed along the cortical strip. Individual bands of cells, approximately 500–800 μm in width depending on cortical region, traverse the cortical laminae in columns [38]. This pattern of cortical degeneration may also be present in FTLT-DTP. In approximately 58% of cortical regions studied, the clusters of NCI were regularly distributed

parallel to the pia mater consistent with their development in relation to these connections. However, in only five cortical areas did the estimated width of the NCI clusters approximate to the dimension of the cells of origin of the cortico-cortical projections. In the majority of cortical areas, the NCI developed in smaller clusters, usually between 50 and 200 μm in diameter; a size similar to the α -internexin positive NCI in NIFID [26]. Hence, the NCI affect only a subset of cells within a column. In five cortical areas, however, the smaller clusters of NCI were aggregated into larger 'superclusters' and in a further three areas, clusters larger than 800 μm in diameter were present. This observation suggests that in some regions smaller clusters of NCI may evolve into larger clusters as the disease develops [36]. There was no significant correlation between cluster size of the NCI and either disease duration or Braak stage, however, which argues against a simple relationship between cluster size and the developing disease.

NCI were more abundant in the upper than the lower cortical laminae in the majority of our cases. Hence, NCI affect the feedforward (FF) cortico-cortical connections more extensively than the feedback (FB) connections. In addition, there may be subcortical pathology in FTLN-TDP affecting the striatonigral system [10] and the serotonergic raphe nucleus which has ascending projections to the forebrain [40]. Hence, degeneration of these pathways could influence the spatial pattern of the NCI, especially in the lower laminae of the MFG. Significant densities of NCI were also a feature of the DG [14, 29, 30]. These NCI also exhibit a topographic distribution along the DG fascia, consistent with a degeneration of the afferent and/or efferent pathways involving the DG.

The TDP-43 immunoreactive NCI may be the direct cause or an indirect consequence of neural degeneration in FTLN-TDP [41]. TDP-43 is a nuclear protein and exhibits a granular pattern of staining in the nucleus of normal cells. During disease, TDP-43 is redistributed from the nucleus to the cytoplasm, is ubiquitinated, hyperphosphorylated, and then cleaved to generate C-terminal fragments [2] that accumulate to form insoluble NCI that may ultimately kill the cell. However, white matter pathology has also been reported in FTLN-TDP [38] and hyperphosphorylated and truncated TDP-43 has been detected in insoluble brain extracts from affected white matter regions. Hence, white matter degeneration could contribute to the neurodegenerative process in FTLN-TDP [42]. In other neurodegenerative disorders characterized by tau and α -synuclein-immunoreactive inclusions, synaptic disconnection may result in the aggregation of abnormal proteins and the formation of NCI [43]. A similar process may occur in FTLN-TDP with aggregates of TDP-43 accumulating in neuronal bodies as a consequence of synaptic disconnection [43]. Hence, accumulation of TDP-43 positive NCI in FTLN-TDP could be a consequence of the neuronal degeneration affecting the cortico-cortical pathways [43].

In conclusion, the spatial pattern of the TDP immunoreactive NCI in FTLN-TDP is similar to that reported for NCI characterized by tau [22, 23, 36], α -synuclein [24, 25], immunoreactivity, and in NIFID characterized by α -internexin IHC [26]. The similarity in the spatial pattern of inclusions in the cortex of different molecular pathologies supports the hypothesis that cortico-cortical breakdown is a common feature of these disorders. This degeneration results in the formation of NCI that differ in the molecular residues incorporated in the lesion. The data also suggest that the distribution of the NCI alone may not account for the degeneration of cortical columns in FTLN-TDP. The data further support the hypothesis that there are common pathological processes operating in a range of disorders that may be amenable to similar types of intervention.

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Fig. 1. Neuronal cytoplasmic inclusions (NCI) (*arrow*) in the upper laminae of the frontal lobe in frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP) (TDP-43 immunohistochemistry, hematoxylin, *bar* 30 μ m)

Fig. 2. Examples of the spatial patterns exhibited by TDP-43 positive neuronal cytoplasmic inclusions (NCI) in frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP) (significant V/M peak)**

Table 1
Demographic features and gross brain weight of the frontotemporal lobar degeneration with frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP) cases studied

Case	Center	Sex	Onset	Death	Duration	BW	F/S	Braak	ST
A	H	M	55	59	4	1,440	–	0	2
B	H	M	73	79	6	1,180	–	2	3
C	V	F	–	82	–	940	S	0	1
D	V	M	69	72	3	–	S	0	2
E	V	M	68	71	3	1,400	S	1	2
F	V	F	63	71	8	1,050	GRN	0	1
G	V	F	55	70	15	–	S	3	1
H	V	M	66	71	5	1,230	F	2	3
I	P	M	61	65	4	1,100	S	2	2
J	P	M	69	75	6	1,000	F	3	2
K	P	M	61	64	3	1,330	S	1	2
L	CD	F	57	73	16	834	S	0	2
M	W	F	65	77	12	810	GRN	4	1
N	W	M	57	65	8	960	S	0	2
O	W	M	43	50	7	1,060	F	–	3

HHarvard Brain Tissue Resource Center, VVancouver General Hospital, PDepartment of Pathology, University of Pittsburgh, CDUniversity of California, Davis, WWashington University School of Medicine, Mmale, Ffemale, Onset, death, and duration are given in years, BWBrain weight (gm), F/Sfamilial or sporadic, GRN = *progranulin* mutation, Braak Braak stage based on distribution of NFT, ST disease subtype according to Cairns et al. [14], (–) data not available

Table 2

Number of neuronal cytoplasmic inclusions (NCI) (per $50 \times 250 \mu\text{m}$ contiguous field) in laminae II/III of the middle frontal gyrus (MFG) and the inferior temporal gyrus (ITG) in a case of frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP)

MFG	ITG			
	Field	N	Field	N
1	0	17	1	2
2	0	18	0	0
3	0	19	0	3
4	0	20	0	4
5	0	21	0	5
6	1	22	0	6
7	0	23	0	7
8	2	24	0	8
9	0	25	0	9
10	0	26	1	10
11.	0	27	0	11
12	1	28	2	12
13	0	29	0	13
14	0	30	0	14
15	0	31	1	15
16	2	32	1	16

The first column of each set of data indicates the number of the field in the contiguous sequence (32 plots in total) and N the number of NCI recorded within the plot

Table 3

Spatial pattern of the TDP-43 immunoreactive neuronal cytoplasmic inclusions (NCI) (*MTG* middle temporal gyrus, *ITG* inferior temporal gyrus, *PHG* parahippocampal gyrus, *HC* hippocampus, *DG* dentate gyrus) in areas of the frontal and temporal lobe in 15 cases of frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP)

Case	MTG		ITG		PHG		HC	DG
	II/III	V/VI	II/III	V/VI	II/III	V/VI		
A	R	R	200	100	R	R	>400	R
B	R	50	-	-	R	-	>400	100
C	200	-	100	R	-	-	R	200
D	50	-	400	-	-	-	50	-
E	-	-	50	>800	-	-	R	100
F	50	-	50	-	100	R	-	-
G	-	-	-	R	-	-	-	100
H	-	-	R	R	R	RG	-	100
I	-	-	R	-	50,200	R	R	R
J	50,200	>400	-	-	>800	-	-	200
K	-	-	-	R	50,400	-	R	100
L	50	200	50,400	100,400	50	-	-	R
M	50	50,400	50	-	100	100	-	200
N	200	-	R	100	-	-	-	400
O	50	R	R	-	-	-	-	R

Data represent the dimensions (μm) measured parallel to the pia mater, alveus or dentate gyrus granule cell layer of regularly distributed clusters of NCI with the following exceptions: *R* random distribution, *RG* regular distribution of individual aggregates. Data preceded by > indicate large scale clustering without regular spacing. Where two figures are present, clustering occurs at two scales, i.e., smaller clusters are aggregated into larger 'superclusters'. (-) indicates either tissue section unavailable or density of inclusions was too low to determine spatial pattern