Atypical frontotemporal lobar degeneration with ubiquitin-positive, TDP-43-negative neuronal inclusions

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Frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) is the most common neuropathology associated with the clinical syndrome of frontotemporal dementia (FTD). Recently, TDP-43 was identified as the ubiquitinated pathological protein in both FTLD-U and sporadic amyotrophic lateral sclerosis. Although a number of studies have now confirmed that most sporadic and familial cases of FTLD-U are TDP-43 proteinopathies, there are exceptions. We describe six cases of early onset FTD with FTLD-U pathology that was negative for TDP-43, which we refer to as ‘atypical’ FTLD-U. All cases were sporadic and had very early onset FTD (mean age = 35 years), characterized by severe progressive psychobehavioural abnormalities in the absence of significant aphasia, cognitive-intellectual dysfunction or motor features. The neuropathological features were highly consistent, with small, round, neuronal cytoplasmic inclusions that were immunoreactive for ubiquitin (ub-ir), but negative for tau, α-synuclein, intermediate filaments and TDP-43. Cytoplasmic inclusions were most numerous in the neocortex, dentate granule cells and hippocampal pyramidal neurons. Ub-ir neuronal intra-nuclear inclusions were also present in neocortical and hippocampal neurons and had the unusual appearance of straight, curved or twisted filaments. We believe that these cases represent a new entity that is clinically and pathologically distinct from all currently recognized subtypes of FTLD. Moreover, the existence of such cases indicates that the designations of ‘FTLD-U’ and ‘TDP-43 proteinopathy’ should not be considered to be synonymous.

Keywords: frontotemporal lobar degeneration; frontotemporal dementia; FTLD-U; ubiquitin; TDP-43

Abbreviations: aFTLD-U = atypical FTLD-U; ALS = amyotrophic lateral sclerosis; BIBD = basophilic inclusion body disease; FTD = frontotemporal dementia; FTLD = frontotemporal lobar degeneration; IF = intermediate filament; MAPT = microtubule associated protein tau; MND = motoneuron disease; NCI = neuronal cytoplasmic inclusion; NF = neurofilament; NIFID = neuronal intermediate filament inclusion disease; NII = neuronal intra-nuclear inclusion; PGRN = progranulin; PNFA = primary non-fluent aphasia; PPA = primary progressive aphasia; SD = semantic dementia; TAR = transactive response; VCP = valosin containing protein


Introduction

The clinical syndrome of frontotemporal dementia (FTD) is characterized by progressive changes in behaviour, personality and/or language, with relative preservation of memory (The Lund and Manchester Groups, 1994; Neary et al., 1998; McKhann et al., 2001). Clinical subtypes include the frontal (behavioural) variant (fvFTD) and two forms of primary progressive aphasia (PPA); primary non-fluent aphasia (PNFA) and semantic dementia (SD). FTD is often associated with an extra-pyramidal movement disorder (Parkinsonism or corticobasal syndrome) or motoneuron disease (MND) (Lomen-Hoerth et al., 2002). FTD accounts for 5–15% of all dementia and is the second commonest cause in the presenile age group (Bird et al., 2003; Feldman et al., 2003). A family history of similar disease is present in 25–50%, indicating a significant genetic component (Stevens et al., 1998; Chow et al., 1999; Rosso et al., 2003). Autosomal dominant FTD may be associated with mutations...
in several genes, including those encoding the microtubule associated protein tau (MAPT), progranulin (PGRN), valosin containing protein (VCP) and charged multi-vesicular body protein 2B (CHMP2B), and several families with FTD and MND have shown genetic linkage to a locus on chromosome 9p (reviewed in Rademakers and Hutton, 2007).

The neuropathology associated with clinical FTD is also heterogeneous, with the common feature being relatively selective degeneration of the frontal and temporal lobes (frontotemporal lobar degeneration, FTLD) (Trojanowski and Dickson, 2001). Many cases of FTLD are characterized by the abnormal accumulation of hyperphosphorylated tau protein in neurons and glia. These tauopathies include cases with the pathological features of classical Pick’s disease, corticobasal degeneration, progressive supranuclear palsy and all familial cases caused by MAPT mutations. However, several recent studies have found that the most common pathology associated with clinical FTD is the presence of neuronal inclusions in the neocortex and hippocampal dentate granule cells that are immunoreactive for ubiquitin (ub-ir) but negative for tau and α-synuclein (Josephs et al., 2004; Johnson et al., 2005; Mackenzie et al., 2006c). This pattern of pathology was first recognized in patients with MND and dementia (Okamoto et al., 1992; Wightman et al., 1992) but was subsequently found to occur in a subset of FTD patients without motor features (Jackson et al., 1996). The term FTLD with ubiquitinated inclusions (FTLD-U) is now often used to describe this pathology, regardless of the associated clinical phenotype.

Until recently, it was uncertain whether FTLD-U represented a single disease process, with variable clinical expression, or if it included a number of discrete entities in which the ubiquitinated pathological protein was different. The recognition of several identifiable subtypes of FTLD-U pathology, each with relatively specific clinical correlation, was initially interpreted as suggesting that FTLD-U was a heterogeneous collection of diseases (Katsuse and Dickson, 2005; Mackenzie et al., 2006a; Sampathu et al., 2006). However, the recent identification of the transactive response (TAR) DNA-binding protein with M. 43 kD (TDP-43) as the major ubiquitinated pathological protein in all subtypes of FTLD-U and in sporadic amyotrophic lateral sclerosis (ALS) has provided strong support for the concept that FTD with FTLD-U pathology, MND-dementia and sporadic ALS are all part of a clinicopathological spectrum of disease (Arai et al., 2006; Neumann et al., 2006; Davidson et al., 2007).

TDP-43 is a nuclear protein expressed in a variety of tissues, with proposed functions that include transcription repression and activation of exon skipping (Buratti et al., 2001; Mercado et al., 2005). The physiological function of TDP-43 in the brain is currently unknown; however, it is normally localized to the nucleus of neurons and some glial cells (Neumann et al., 2006, 2007b). Initial reports provided several types of evidence that TDP-43 is the major pathological protein in FTLD-U and ALS, including: (i) co-localization of TDP-43 and ubiquitin in the pathological inclusions; (ii) specificity of TDP-43 immunoreactivity for the neuronal cytoplasmic inclusions (NCI) in these conditions; (iii) demonstration of abnormal C-terminal fragments of TDP-43 in post-mortem tissue; (iv) demonstration that the pathological forms of TDP-43 are ubiquitinated and hyperphosphorylated and (v) loss of the normal nuclear TDP-43 staining in cells harbouring NCI, suggesting loss of normal TDP-43 function (Arai et al., 2006; Neumann et al., 2006). The specific role of TDP-43 in neurodegeneration remains speculative, however, possibilities include the loss of some essential nuclear function and/or disruption of crucial intra-cellular processes by the accumulation of insoluble, mis-folded protein (Strong et al., 2007).

Although the initial reports suggested that TDP-43 was the pathological protein in all cases of FTLD-U (Arai et al., 2006; Neumann et al., 2006; Davidson et al., 2007), subsequent studies have identified some exceptions. A reassessment of the neuropathology of FTD linked to chromosome 3, found that the characteristic ub-ir NCI were not TDP-43 immunoreactivity (Cairns et al., 2007b; Holm et al., 2007). In addition, a multi-centred, international study designed to evaluate the frequency of TDP-43 proteinopathy, in a large and comprehensive collection of familial and sporadic FTLD-U, identified two sporadic cases with no TDP-43 reactivity (Cairns et al., 2007b). These findings indicate that there are small subsets of FTLD-U cases in which the pathological protein appears to be something other than TDP-43.

In this report, we describe the clinical and pathological features of six cases of sporadic FTD with FTLD-U pathology that is negative for TDP-43. The unusual and highly consistent clinical and pathological phenotypes suggest that these cases represent a specific disease entity, that is different from other cases of FTLD-U, and which we will refer to as ‘atypical’ FTLD-U (aFTLD-U).

Methods

Study cases

All cases within our neuropathology database that had previously been given a pathological diagnosis of FTLD-U were re-evaluated (n = 83). These included cases with a clinical diagnosis of FTD (n = 40), MND-dementia (n = 20), MND without dementia but with cortical ubiquitin pathology (n = 10) and dementia, not otherwise specified (n = 13). In half of the MND-dementia cases (n = 10), the dementia fulfilled FTD clinical diagnostic criteria (Neary et al., 1998). There was a family history of similar disease in 39 cases (47%) and these included 14 with proven PGRN mutations and two members of a family with FTD and MND linked to chromosome 9p.

To determine the specificity of the type of intra-nuclear inclusions identified in the aFTLD-U cases, ubiquitin-stained sections from some other conditions were also examined; these included cases of inclusion body myopathy with Paget’s disease of bone and frontal dementia (IBMPFD) with VCP mutations (n = 5), familial FTD linked to chromosome 3 with CHMP2B mutations (FTD-3, n = 4), neuronal intermediate filament (IF) inclusion
Histochemistry, immunohistochemistry and immunofluorescence
Sections of formalin fixed, paraffin-embedded tissue from the frontal and temporal neocortex and hippocampus were examined in all cases. Histological stains included haematoxylin and eosin (HE), Luxol fast blue with cresyl violet (LFB/CV), Congo red and Bodian, Bielschowsky and Gallyas silver stains. All immunohistochemistry was performed using the Ventana BenchMark® XT automated staining system (Ventana, Tuscon, AZ, USA) and developed with aminoethylcarbazole (AEC). The primary antibodies employed recognized ubiquitin (DAKO anti-ubiquitin; 1:500, following microwave antigen retrieval), hyperphosphorylated tau (Innogenetics AT-8; 1:2000 following microwave antigen retrieval and Sigma TAU-2; 1:1000 with 3 h initial incubation at room temperature), α-synuclein (Zymed anti-α-synuclein; 1:10000, following microwave antigen retrieval), Aβ (DAKO anti-beta amyloid; 1:100 with initial incubation for 3 h at room temperature), α-internexin (Zymed anti-α-internexin; 1:500 with 3 h initial incubation at room temperature, following microwave antigen retrieval), non-phosphorylated neurofilament (NF) (DAKO anti-neurofilament protein; 1:2000, following protease digestion), phosphorylated neurofilament (pNF) (Sternberger SMI 31; 1:8000, following protease digestion), TDP-43 (ProteinTech Group anti-TDPBP; 1:100 following microwave antigen retrieval), p62 (BD Transduction Laboratories p62 Lck ligand; 1:500 following microwave antigen retrieval) and expanded polyglutamine repeat regions (Chemicon IC2; 1:1000, 24 h at room temperature following formic acid pre-treatment).

In cases that were found to have FTLD-U that was negative for TDP-43 (aFTLD-U), sections from additional anatomical regions (including basal ganglia, thalamus, brainstem, cerebellum and spinal cord) were also immunostained for ubiquitin. Double-labelling immunofluorescence studies were performed on 3µm sections of formalin fixed, paraffin embedded tissue representing frontal neocortex and hippocampus from cases of aFTLDU and a case of typical (TDP-43-positive) FTLD-U. Following microwave antigen retrieval, slides were incubated for 24 h at 4°C in a mixture of anti-TDP-43 (Protein Tech Group rabbit polyclonal TDP-43; 1:500) and anti-ubiquitin (Vector Laboratories mouse monoclonal anti-ubiquitin; 1:100). The sections were then rinsed and incubated for 45 min in a mixture of AlexaFluor 488 goat anti-mouse IgG (H+L) (Molecular Probes, emission peak 519 nm; 1:150) and AlexaFluor 594 donkey anti-rabbit IgG (H+L) (Molecular Probes, emission peak 613 nm; 1:150). The slides were covorsslapped using Vectashield Mounting Medium containing 1. 5 µg/ml 4′-diamidino-2-phenylindole (DAPI) as a nuclear counterstain and examined using a Zeiss Axiosplan II fluorescence microscope (Carl Zeiss Microscopy, Germany) with attached AxioCam digital camera using axiovision 4.1 software.

Results
In 77 of 83 (93%) of our cases with FTLD-U pathology, the uh-ir neuronal inclusions were also immunoreactive for TDP-43. Most of these also showed some TDP-43-positive glial inclusions, of the type previously described (Neumann et al., 2007b). However, six cases were found to have pathology that was consistent with FTLD-U but with no abnormal TDP-43 immunostaining (aFTLD-U).

Clinical features of aFTLD-U
Cases with aFTLD-U had a mean age of onset of 35.3±4.1 years with a mean duration of disease of 8.3±2.2 years (Table 1). In this small cohort, there was a striking female predominance (5:1). None of the subjects had a family history of similar disease although one had several cousins described as having mental retardation and epilepsy. All subjects fulfilled clinical diagnostic criteria for fvFTD (Table 2) (Neary et al., 1998). The typical presentation was that of gradually progressive psychobehavioural decompensation in the absence of significant cognitive-intellectual difficulties, other than impaired attention. Poor performance on visuospatial testing and calculation by one patient (Case 4) was felt to be secondary to inattention. All patients demonstrated early decline in personal standards of hygiene and grooming. Disinhibition affected the domains of interpersonal conduct (i.e. social intrusiveness, rudeness, inappropriate singing and making animal noises), affect regulation (i.e. inappropriate laughter) and sexual propriety (i.e. inappropriate touching, kissing and grabbing, getting into bed with other patients, public masturbation) (Table 3). Abnormal feeding behaviour, usually overeating, was common and two patients demonstrated classical hyperorality. Several subjects exhibited aggressive, violent, anti-social behaviour that included cruelty to animals and physically assaulting co-workers, relatives and co-patients. One subject served two jail terms (one for theft and another for assaulting a co-worker), while another subject was involved in three hit-and-run accidents and lost her job for fighting with her supervisor. Three patients had psychosis, two with paranoid delusions and one with auditory hallucinations. Two patients with psychosis also had a thought disorder, manifest by disorganized, incoherent verbal output in the absence of aphasia. Language dysfunction was limited to aspontaneity, stereotypy, perseveration, echolalia and repetitive speech. In all cases, speech was fluent early in the disease and only one subject demonstrated mild early anomia that did not progress. None of the subjects had significant motor dysfunction or movement disorder with the exception of one patient who demonstrated choreiform movements of the hands.

The relatively young age of onset, together with the predominant psychobehavioural dysfunction, resulted in an initial psychiatric diagnosis. In some patients, the presence of depression, psychosis and thought disorder reinforced the impression of a primary psychiatric illness. All patients required institutionalization in a chronic psychiatric facility, usually within the first 2 years of their illness. The late phase of disease was characterized by neurological deterioration and psychobehavioural regression. Patients became mute, incontinent, demonstrated fecal
smearing, primitive reflexes, paratonic rigidity and required total care.

**Neuropathology of aFTLD-U**

The average brain weight was 981 ± 126 g (Table 4). All cases showed symmetric atrophy of the frontal and temporal lobes and the striatum. Gross pallor of the substantia nigra was recorded in two cases. Microscopic examination of the cerebral cortex showed chronic non-specific degenerative changes of neuronal loss and gliosis with superficial spongiosis. The frontal cortex was severely affected in all cases while involvement of the temporal cortex was more variable, with the inferior and middle gyri more affected than the superior temporal gyrus. There was minimal involvement of the parietal cortex and the occipital lobes were spared. All cases showed near complete loss of pyramidal neurons from the subiculum and CA1 sector of the hippocampus (hippocampal sclerosis). Severe degenerative changes were also consistently present in the striatum and zona compacta of the substantia nigra. The globus pallidus, periaqueductal grey and thalamus were always affected but to a variable degree. There was no involvement of the pons, medulla, spinal cord or cerebellum.

No pathological changes were identified with special histochemical stains or immunohistochemistry for Aβ, tau, α-synuclein, NF, pNF or α-internexin proteins. Specifically, there were no senile plaques, neurofibrillary tangles, Pick bodies, Lewy bodies, Lewy neurites, glial inclusions or hyaline conglomerate inclusions.

**Table 1** Demographics and presenting clinical features of patients with atypical FTLD-U

| Case | Gender | Onset (years) | Death (years) | Duration (years) | Family history | Behaviour | Language | Psychosis | Thought disorder | Cognitive-intellectual function | Motor/movement
|------|--------|---------------|---------------|-----------------|----------------|-----------|----------|-----------|-----------------|-----------------------------|-----------------
| 1    | Female | 38            | 29            | 1               | +++           | +++       | Mild anoma | —         | —               | —                           | —               |
| 2    | Female | 37            | 42            | 1               | —             | +++       | Aspontaneity | —         | —               | —                           | —               |
| 3    | Female | 40            | 36            | 8               | —             | +++       | Aspontaneity | —         | —               | —                           | —               |
| 4    | Female | 32            | 39            | 1               | —             | +++       | Aspontaneity | —         | —               | —                           | —               |
| 5    | Male   | 29            | 36            | 1               | —             | +++       | Repetitive | —         | —               | —                           | —               |
| 6    | Female | 36            | 42            | 7               | —             | +++       | Echolalia | —         | —               | —                           | —               |
|      |        |               |               | 6               | —             | —         | —         |           | —               | —                           | —               |

— = No abnormality; + = mild abnormality; ++ = moderate abnormality; +++ = severe abnormality; NA = not available.

**Table 2** Clinical diagnostic criteria for FTD (Neary et al. 1998) in patients with atypical FTLD-U

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— = Present; — = Absent.

Ubiquitin immunohistochemistry demonstrated a highly consistent and unique pattern of pathology in all cases. In the neocortex, moderate numbers of ub-ir NCI were present in both large and small neurons, primarily within middle and
deeper cortical layers (Fig. 1). Most inclusions were well defined, round, oval or crescent-shaped and approximately the size of the nucleus. Rarely, one neuron contained multiple small round NCI (Fig. 2). Staining intensity was variable but most were strongly immunoreactive. Some additional neurons showed diffuse granular cytoplasmic immunoreactivity. The NCI were only rarely visible on HE stain where they appeared faintly eosinophilic or basophilic. They were not seen with silver stains, Congo red or Nissl stain. Small numbers of short, tortuous ub-ir neurites were also present in the same cortical regions but these were never numerous. In addition to NCI, occasional ub-ir neuronal intra-nuclear inclusions (NII) were present (see description below) (Fig. 3). Ub-ir pathology was most abundant in the frontal and temporal neocortex with minimal if any involvement of the parietal cortex and complete sparing of the occipital region.

In the hippocampus, small oval or crescentic NCI tended to be numerous in the dentate granule cell layer with fewer in CA4 pyramidal neurons (Fig. 2). NII were more numerous than in the neocortex, being present in ~1–2% of dentate granule cells and CA4 pyramidal neurons (Fig. 3). This meant that in standard 3 μm thick coronal sections, taken at the level of the lateral geniculate body, between 15 and 30 NII were identified in the dentate fascia, in each case. These NII had a highly unusual morphology, appearing as thick rods or wiry filaments (Fig. 3). Short examples that partially spanned the nucleus were straight, slightly curved or had multiple bends, producing a worm-like appearance. Many were much longer, being C-shaped, forming complete rings or with numerous bends and twists, resulting in an irregular 3D configuration (appreciated by focusing up and down on the section). Neurons with an NII sometimes also contained an NCI (Fig. 3).

The striatum consistently showed small numbers of NCI and neurites but no NII. Some NCI were also usually present in the nucleus basalis, hypothalamus, periaqueductal grey matter and third cranial nerve nucleus. Despite consistently showing moderate to severe degenerative changes, the globus pallidus and substantia nigra rarely contained any ub-ir pathology. NCI were not present in the pons, medulla, spinal cord or cerebellum.

Surprisingly, immunohistochemistry for TDP-43 did not label any NCI, NII or neurites in any anatomical regions in these cases. Neurons showed the normal diffuse nuclear staining pattern, confirming that the technique had worked with adequate sensitivity (Fig. 2). The findings on immunohistochemistry were confirmed with double-labelling immunofluorescence, which showed absence of TDP-43 co-localization with the ub-ir NCI and NII (Fig. 4).

The only other antibody that demonstrated the ub-ir inclusions was against the ubiquitin-binding protein p62. This method labelled the NCI and neurites but did not stain the NII. The NII also failed to stain with the 1C2 antibody that recognizes expanded polyglutamines.

The unusual type of NII, present in all cases of aFTLD-U, was not found in any case of TDP-43-positive FTLD-U. All cases with proven PGRN or VCP mutations had lentiform NII, characteristic of these genetic subtypes (Forman et al., 2006; Mackenzie et al., 2006b), however these were easy to distinguish based on their different morphology, anatomical distribution and TDP-43-immunoreactivity. No NII were seen in any of the cases of FTD with CHMP2B mutations, Alzheimer’s disease or non-demented controls. The only cases that were found to have NII of similar appearance, distribution and immunophenotype (ub-ir, IF-negative) were the two examples of NIFID.

**Discussion**

The recent identification of TDP-43 as the disease protein in FTLD-U and ALS has been a major breakthrough in our understanding of the pathogenesis and inter-relationship of these conditions and has defined a new class of neurodegenerative disorders (TDP-43 proteinopathies) (Forman et al., 2007; Kwong, et al., 2007; Neumann et al., 2007a). Initial reports suggested that pathological TDP-43 is both a specific and sensitive marker of all subtypes of FTLD-U and ALS (Arai et al., 2006; Neumann et al., 2006; Davidson et al., 2007). Although subsequent studies have confirmed the importance of TDP-43 proteinopathy within this group of disorders, some important exceptions have emerged. A recent multi-centred, international study evaluated TDP-43 in a large collection of cases of FTLD-U (∼193), selected to represent all major genetic, clinical and pathological subtypes.
(Cairns et al., 2007b). TDP-43 pathology was a consistent finding in all familial cases caused by mutations in PGRN or VCP and those linked to chromosome 9p. However, familial cases from the Danish family with FTD linked to chromosome 3 (FTD-3), caused by a mutation in CHMP2B, were an exception, having ub-ir inclusions in the hippocampal dentate granule cells that were not reactive for TDP-43 (a finding that was described in more detail in a separate report by Holm et al., 2007). In addition, although the vast majority (97%) of sporadic FTLD-U cases in that study were found to be TDP-43-positive, there were two cases with no TDP-43 immunoreactivity (discussed below) (Cairns et al., 2007b). Another study evaluated a large series of ALS (n = 111) and found that all sporadic cases and all familial cases without SOD1 mutations had TDP-43-positive inclusions in both lower motor neurons and glial cells, however, cases with SOD1 mutations had no immunohistochemical or biochemical evidence of pathological TDP-43 (Mackenzie et al., 2007). More recently, the central role of TDP-43 in sporadic ALS has even been challenged, by evidence that the NCI contain other ubiquitinated proteins (Sanelli et al., 2007). A number of recent studies have also raised questions about the disease specificity of TDP-43 by demonstrating some degree of TDP-43 pathology in a variety of conditions, outside the usual spectrum of FTD and ALS; these include hippocampal sclerosis dementia, (Amador-Ortiz et al., 2007; Cairns et al., 2007b; Probst et al., 2007), ALS–Parkinsonism–dementia complex of Guam (Geser et al., 2008), classical Pick’s disease (Freeman et al., 2008) and a significant proportion of cases of Alzheimer’s disease, Parkinson’s disease and dementia with Lewy bodies (Amador-Ortiz et al., 2007; Higashi et al., 2007; Nakashima-Yasuda et al., 2007). Recent review articles have tended to stress the central role of TDP-43 in FTLD-U and ALS and, although they
**Fig. 1** Ubiquitin-immunoreactive neuronal cytoplasmic inclusions in deep layers of neocortex (A and B). Inclusions varied in shape and included round (C), oval and crescentic (D) forms. Ubiquitin immunohistochemistry. Scale bar: A, 75 μm; B, 38 μm; C and D, 8 μm.

**Fig. 2** Ubiquitin-immunoreactive neuronal cytoplasmic inclusions (NCI) in dentate granule cells of hippocampus (A). Immunostaining for TDP-43 showed the normal diffuse nuclear pattern but did not label any inclusions (B). NCI were round, oval or crescentic (C). Rarely, one neuron contained multiple small NCI (D). Ubiquitin (A, C and D) and TDP-43 (B) immunohistochemistry. Scale bar; A and B, 38 μm; C and D, 8 μm.
acknowledge familial cases with CHMP2B and SOD1 mutations as important exceptions, they do not mention the possible existence of sporadic TDP-43-negative cases (Forman et al., 2007; Kwong, et al., 2007; Neumann et al., 2007a). Even the most recent neuropathological diagnostic and nosologic criteria for FTLD, written specifically to incorporate recent molecular pathological advances, do not include a category for sporadic TDP-43-negative FTLD-U (Cairns et al., 2007a).

In the present study, we found an absence of TDP-43 immunoreactivity in 6 of 83 (7%) of our cases with FTLD-U pathology and in 12% of FTLD-U with an FTD clinical phenotype. Due to the manner in which these cases were obtained, it is not possible for us to estimate the frequency of aFTLD-U in the FTD population overall, but while it would seem that such cases are probably not common, they are also not rare.

Our aFTLD-U cases showed an unusual and highly consistent clinical phenotype and neuropathology, suggesting that they represent a specific disease entity. All cases appeared to be sporadic. The clinical presentation was characterized by very early onset severe psychobehavioural abnormalities, usually in the absence of significant cognitive or motor features. Speech and language abnormalities were typical of frontal lobe dysfunction and there was no associated PPA. Behavioural abnormalities were sufficiently severe to require early institutionalization. The neuropathology fulfilled criteria for FTLD-U by having ub-ir neuronal inclusions that were negative for tau, α-synuclein and IFs. There was a predominance of NCI with very little neuritic pathology. The NCI were well defined, homogenous and had the appearance of small Pick bodies and were distributed throughout the neocortex, hippocampus and in a variety of subcortical structures. The unusual filamentous NII were less numerous and had a more restricted anatomical distribution, but were a consistent finding and perhaps the most unique feature.

Comparison of our aFTLD-U cases with TDP-43-positive FTLD-U shows a number of significant differences. The clinical phenotype of aFTLD-U is much more homogenous and does not include the high frequency of a positive family history, PPA or the range of extra-pyramidal and pyramidal system dysfunction commonly seen in other cases of FTLD-U. In addition, the age of onset in aFTLD-U is significantly younger (35.3 versus 60.5 years for sporadic FTLD-U) (Cairns et al., 2007b), with all cases on-setting by age 40 years. The pattern of ub-ir pathology in aFTLD-U most closely resembles the subtype of FTLD-U referred to as type 3a by Mackenzie et al. (2006a) and type 2 by Sampathu et al. (2006), having a pancortical involvement with a predominance of NCI and relatively few neurites. However, the morphology of the NCI in aFTLD-U is different, having a well-defined and smooth outer contour as opposed to the more irregular NCI typical of the corresponding FTLD-U subtype. The finding of NCI in hippocampal pyramidal neurons is also unusual for FTLD-U and none of the cases we examined had the filamentous type of NII found in all of our aFTLD-U.

Our cases of aFTLD-U also show significant differences when compared with other types of FTLD characterized by ub-ir, tau-negative, TDP-43-negative neuronal inclusions, including FTD-3, basophilic inclusion body disease (BIBD) and NIFID (Cairns et al., 2007a).
FTD-3 has only been reported in a single large Danish family (Gydesen et al., 2002). A small number of additional cases of FTD and of ALS have been reported with CHMP2B variations of questionable pathogenicity (Parkinson et al., 2006). The age of onset in FTD-3 is older than in our aFTLD-U cases, ranging from 46 to 65 years (mean = 57 years) (Gydesen et al., 2002). Although the initial presentation is that of a frontal lobe syndrome, most patients later develop florid motor features with Parkinsonism, dystonia, pyramidal signs and myoclonus. The neuropathology of FTD-3 is also different, with NCI largely restricted to the dentate granule cells and minimal involvement of the neocortex (Holm et al., 2007). Furthermore, we did not find any NII in the FTD-3 cases examined.

BIBD is a term that has been used for a small number of clinically and pathologically heterogeneous cases, in which the common finding is NCI that are basophilic with HE stain. The clinical phenotypes include sporadic and familial ALS, ALS with dementia and pure FTD (Munoz-Garcia and Ludwin, 1984; Kusaka et al., 1993; Hamada et al., 1995; Tsuchiya et al., 2001; Ishihara et al., 2006). The morphology and anatomical distribution of the NCI vary among cases and many reports antedate the widespread use of immunohistochemistry. One of the earliest descriptions included two
patients with FTD on-setting around age 30 years (Munoz-Garcia and Ludwin, 1984). Recent review of the pathology of one of these cases found the inclusions to be ub-ir but negative for TDP-43 (Cairns et al., 2007b). However, unlike our cases of aFTLD-U, the NCI were clearly visible on HE stain, weakly argyrophilic and stained for Nissl substance (Munoz-Garcia and Ludwin, 1984). The anatomical distribution of NCI was also different in those cases of BIBD compared with aFTLD-U, being scarce in the neocortex and absent in the hippocampus. A more recent study included four cases of BIBD, most with an older age of onset (mean = 47 ± 12 years) (Yokota et al., 2007). Three were diagnosed with FTD while the other had MND and dementia. NCI were again more common in subcortical structures and these authors found the basophilic inclusions to be negative for both ubiquitin as well as TDP-43.

Like aFTLD-U, NIFID is also a sporadic, early onset FTD; however, most cases also demonstrate significant cognitive impairment, language dysfunction and motor features (Cairns et al., 2004; Mackenzie and Feldman, 2004). By definition, our aFTLD-U cases are not NIFID since they do not have inclusions that are immunoreactive for class IV IF (light, medium and heavy neurofilament subunits and α-internexin). Also, the inclusions in NIFID are more pleomorphic and tend to be visible with HE. However, it is interesting that the most common type of NCI, in most cases of NIFID, is small Pick body-like inclusions that resemble those of aFTLD-U in size, shape and anatomical distribution and that do not always immunostain for IF (Bigio et al., 2004; Mackenzie and Feldman, 2004). In addition, the two cases of NIFID we examined were the only other condition in which we found filamentous NII, similar to those in our aFTLD-U cases. NII have previously been reported in cases of NIFID, although as an inconsistent feature (Josephs et al., 2003; Cairns et al. 2004; Mackenzie and Feldman, 2004).

The only previous report of TDP-43-negative FTLD-U was in the study by Cairns et al. (2007b). Although detailed clinical and pathological information was not provided, one of their two cases was similar to our examples of aFTLD-U, having a relatively early onset at age 46 (personal communication, Dr. Nigel Cairns) and pathology that was classified as type 2 according to the system of Sampathu et al. (2006).

In summary, we have described six cases of early-onset sporadic FTD that we believe represent a newly recognized clinicopathological entity. The pathology is consistent with FTLD-U but differs from the majority of cases by the absence of pathological TDP-43. Recognition of these cases raises a fundamental question regarding terminology. The term FTLD-U was originally developed for pathology characterized by inclusions that are immunoreactive for ubiquitin but not for other neurodegenerative proteins, which at the time meant negative for tau and α-synuclein. The assumption was that the ubiquitinated protein (or proteins) in these cases would eventually be identified and this would allow more specific nomenclature and reclassification. This is precisely what happened when a small subset of cases were discovered to have NCI immunoreactive for IF; these cases were given a new designation (NIFID) and removed from the FTLD-U category. If this convention is maintained, then most cases should now be renamed (possibly ‘FTLD-TDP’) and the term FTLD-U restricted to cases where the ubiquitinated protein is still unknown, such as the ones we have described and FTD-3. However, such a decision will require consensus agreement by FTLD neuropathologists.

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References


