Neurofilament inclusion body disease: a new proteinopathy?

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Summary
We describe four cases of a new clinicopathological entity presenting with either a frontotemporal dementia or corticobasal degeneration syndrome with a mean age of onset of 45 years (range 41–50) characterized pathologically by deposition of neurofilament proteins. All four patients had a rapidly progressive course and have become mute and non-ambulatory, and three have died after mean illness duration of only 3 years (range 2–4). Both structural (MRI) and functional (PET and SPECT) imaging demonstrated frontal and temporal lobe and basal ganglia involvement. Gross neuropathological examination in the three deceased patients (the fourth patient, still alive, was diagnosed by brain biopsy) revealed changes affecting predominantly the frontal and temporal cortices, basal ganglia and brainstem. There was superficial linear spongiosis affecting the frontal lobes in all three autopsied patients, and severe caudate atrophy was noted in two of them and demonstrated on MRI in the living patient. On routine staining, there were numerous intracytoplasmic inclusions, which ranged from eosinophilic to basophilic. Some had a clearly defined basophilic margin, while others were granular with a hyaline core. With modified Bielschowsky silver technique, a small number of the inclusions were intensely stained. Inclusions were not labelled with other silver stains. Immunohistochemistry revealed that the inclusions were immunoreactive with antibodies to neurofilament heavy and light chain subunits and to ubiquitin, but not with antibodies to tau and α-synuclein. These neurofilament- and ubiquitin-positive inclusions were widespread, specific to neurons and occasionally intranuclear. The frequency and distribution of the inclusions and the silver and immunohistochemical profiles in these four cases is novel and has not been described in detail before. We propose the term neurofilament inclusion body disease for this entity.

Key words: neurofilament inclusion body disease; frontotemporal dementia; corticobasal degeneration; neurofilament; ubiquitin

Abbreviations: AD = Alzheimer’s disease; BIBD = basophilic inclusion body disease; CBD = corticobasal degeneration; CJD = Creutzfeldt–Jakob disease; DLB = dementia with Lewy bodies; DUI ± MND = dementia with ubiquitin-positive (tau- and α-synuclein-negative) inclusions with or without motor neuron disease; FTD = frontotemporal dementia; iPD = idiopathic Parkinson’s disease; MSA = multiple system atrophy; NIBD = neurofilament inclusion body disease; NF = neurofilament; PiD = Pick’s disease; PSP = progressive supranuclear palsy

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Introduction

A significant group of neurodegenerative diseases is characterized by intraneuronal inclusions, which may be cytoplasmic or intranuclear. An evolving molecular classification of such neurodegenerative disorders is based upon the biochemical nature of the protein deposits forming the inclusions. The microtubule-associated protein tau and the synaptic vesicle protein α-synuclein account for most of these neurodegenerative disorders (Goedert et al., 2001). Abnormal deposition of tau defines neurofibrillary tangles in the most common neurodegenerative disease, Alzheimer’s disease (AD). Furthermore, abnormalities of tau characterize corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) and frontotemporal dementia with parkinsonism linked to chromosome 17q (FTDP-17q), in which glial tau deposits are also frequently found (Lee et al., 2001). The morphologically distinct Pick bodies characteristic of Pick’s disease (PiD) are also composed of tau (Lowe, 1998; Lee et al., 2001; McKhann et al., 2001). The diseases associated with α-synuclein deposition are idiopathic Parkinson’s disease (iPD) and dementia with Lewy bodies (DLB), in which the pathological hallmark is the Lewy body (Spillantini et al., 1997). Lewy bodies are also commonly found in AD, especially in familial forms (Lippa et al., 1998). The glial cytoplasmic inclusions and intraneuronal inclusions of multiple system atrophy (MSA) also contain α-synuclein (Lippa et al., 1998; Spillantini et al., 1997). Many of the defining intracellular inclusions are also labelled by ubiquitin, which is an essential component of the proteasomal system, responsible for non-lysosomal degradation of intracellular proteins (Hershko and Ciechanover, 1998). The presence of ubiquitin in the inclusions is less specific, although it is the only identified component in the inclusions of dementia with ubiquitin-positive (tau- and α-synuclein-negative) inclusion bodies with or without motor neuron disease (DUI ± MND) (Rosser et al., 2000). The term DUI ± MND incorporates motor neuron disease–inclusion dementia (Jackson et al., 1996; Holton et al., 2002); dementia with inclusions tau- and synuclein-negative, ubiquitinized (Kertesz et al., 2000); and motor neuron disease-type dementia (Okamoto et al., 1991; Wightman et al., 1992).

The neuropathological differential diagnosis of the neurodegenerative disorders described above is complex and takes into account clinical information, the anatomical distribution of gross and microscopic changes, and the protein composition of the intracellular inclusions assessed by immunohistochemical and biochemical means.

We have identified four novel cases presenting with either the frontotemporal dementia (FTD) variant of frontotemporal lobar degeneration (Neary et al., 1998) or CBD syndrome, with microscopy demonstrating the presence of widespread intraneuronal inclusions. Unlike the pathological features described above, the inclusions were intensely immunoreactive to neurofilament (NF) antibodies, yet were not recognized by tau or α-synuclein antibodies. We have studied the clinical features, molecular composition, distribution and localization of the NF inclusions by performing a review of the medical records, semiquantitative immunohistochemical analysis and electron microscopy in these four cases. We propose the term NF inclusion body disease (NIBD) to describe these unique cases.

Material and methods

Patients

Four patients with microscopic demonstration of neurodegeneration and unique features characterized by the presence of diffuse and intensely stained NF-positive inclusion bodies were recognized by three of the authors (T.R., J.L.H. and D.W.D.) over a 4 year period (1998–2002). Two of the patients resided in the United States (cases US1 and US2) and one each in Denmark (case D1) and the United Kingdom (case UK1). Three had complete post-mortem analysis, and one (D1) had a frontal lobe biopsy. As controls, we studied haematoxylin and eosin (H&E), NF and ubiquitin staining profiles in two normal control brains and pathological controls including three cases of DUI ± MND, two cases of AD and one case each of DLB and PiD. For each control case, we reviewed anterior frontal and temporal cortices and hippocampal sections.

Neuropathological procedure

In the post-mortem cases, consent to brain examination was obtained. Half of each brain was fixed in 10% formalin for a minimum of 4 weeks, while the other half was stored frozen. Each fixed half-brain hemisphere was sliced coronally and tissue blocks were taken from representative areas, including the frontal, temporal, parietal and occipital cortices, amygdala, hippocampus, basal ganglia, thalamus, midbrain,pons, medulla oblongata and cerebellum. Cervical cord was also available in cases US1 and US2. Following processing and paraffin wax embedding, 7 µm sections were cut and stained with routine methods including H&E, Nissl, periodic acid–Schiff/haematoxylin (PAS/H), Congo Red, thioflavin-S, luxol fast blue/cresyl violet, and silver impregnations (Bodian, modified Bielschowsky, and Gallyas methods).

In case D1, a biopsy of the right frontal lobe was performed, fixed in 10% formalin and processed for paraffin wax using standard procedures. Sections were cut and stained as described above.

Immunohistochemistry and antibodies

Immunohistochemistry was carried out with special emphasis on NF subunits. This involved the use of antibodies to phosphorylated NF (pNF) light, medium and heavy (L, M and H) subunits and antibody to non-phosphorylated NF heavy
subunit (non-pNF-H). An NF-cocktail antibody recognizing L and H subunits was also utilized.

Sections (7 μm) were cut from selected blocks. The sections were de-paraffinized in xylene and subsequently rehydrated in graded alcohol solutions. Endogenous peroxidase activity was neutralized in methanol/H2O2 solution for 10 min. Some sections were pretreated by pressure cooking (10 min) to unmask antigenic activity. Non-specific antigen binding was blocked in normal horse serum (Vectastain elite ABC kit; Vectorstain, Peterborough, UK). The primary antibodies made up in phosphate-buffered saline (PBS, 1%) to the dilutions stated below were applied to the sections and incubated for 1 h at room temperature. After several washes in PBS solution, the sections were incubated in prediluted biotinylated secondary antibody (Vectastain elite ABC kit; 30 min, room temperature). Following washes as described previously, the ABC reagent (Vectastain elite ABC kit) was applied to sections (30 min, room temperature). Visualization of immunolabelled structures was achieved by incubating sections in diaminobenzidine/H2O2 solution until coloration was visible (usually 3–4 min). Washing and counterstaining (Mayer’s haemotoxylin; WWR International, Poole, UK), clearing and mounting followed.

Primary antibody type (monoclonal unless stated), dilution, pretreatment and source were as follows: pNF-H (SMI 31, 1: 5000, pressure cooked) and non-pNF-H (SMI 32, 1: 500, pressure cooked; Sternberger Monoclonals, Lutherville, UK); pNF-M (BF10, 1: 400, pressure cooked; Affinity Research Products, Aurora, USA); pNF-L (Ab1, 1: 100, pressure cooked; Oncogene Research Products, Boston, USA); NF cocktail (1: 20, no pretreatment; ICN Pharmaceuticals, Aurora, USA); ubiquitin (1: 300 polyclonal, pressure cooked; DAKO, Ely, UK); tau (rabbit anti-tau Cat. No. A0024, reacts with both phosphorylated and non-phosphorylated tau, 1: 100, pressure cooked; DAKO, Ely, UK); B-crystallin (1: 1200, no pretreatment and source were as follows: pNF-H (SMI 31, 1: 5000, pressure cooked) and non-pNF-H (SMI 32, 1: 500, pressure cooked; Sternberger Monoclonals, Lutherville, UK); pNF-M (BF10, 1: 400, pressure cooked; Affinity Research Products, Aurora, USA); pNF-L (Ab1, 1: 100, pressure cooked; Oncogene Research Products, Boston, USA); NF cocktail (1: 20, no pretreatment; ICN Pharmaceuticals, Aurora, USA); ubiquitin (1: 300 polyclonal, pressure cooked; DAKO, Ely, UK); tau (rabbit anti-tau Cat. No. A0024, reacts with both phosphorylated and non-phosphorylated tau, 1: 100, pressure cooked; DAKO, Ely, UK); B-crystallin (1: 1200, no pretreatment and source were as follows: pNF-H (SMI 31, 1: 5000, pressure cooked) and non-pNF-H (SMI 32, 1: 500, pressure cooked; Sternberger Monoclonals, Lutherville, UK); pNF-M (BF10, 1: 400, pressure cooked; Affinity Research Products, Aurora, USA); pNF-L (Ab1, 1: 100, pressure cooked; Oncogene Research Products, Boston, USA); NF cocktail (1: 20, no pretreatment; ICN Pharmaceuticals, Aurora, USA); ubiquitin (1: 300 polyclonal, pressure cooked; DAKO, Ely, UK); tau (rabbit anti-tau Cat. No. A0024, reacts with both phosphorylated and non-phosphorylated tau, 1: 100, pressure cooked; DAKO, Ely, UK); B-crystallin (1: 1200, no pretreatment; Novocastra Laboratories, Newcastle Upon Tyne, UK); prion protein (i) KG9 (1: 50, formic acid and pressure cooked; obtained from Institute of Animal Health, Compton, UK) and (ii) 3F4 (1: 2000, formic acid and pressure cooked; obtained from DAKO).

### Table 1 Demographic features of four cases of neurofilament inclusion body disease

<table>
<thead>
<tr>
<th>Feature</th>
<th>UK1</th>
<th>US1</th>
<th>US2</th>
<th>D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country of residence</td>
<td>United Kingdom</td>
<td>United States</td>
<td>United States</td>
<td>Denmark</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>41</td>
<td>50</td>
<td>44</td>
<td>43</td>
</tr>
<tr>
<td>Age at death (years)</td>
<td>44</td>
<td>54</td>
<td>47</td>
<td>*</td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td>2.5</td>
<td>4</td>
<td>3.5</td>
<td>3</td>
</tr>
</tbody>
</table>

*Patient still alive, now terminal.

### Assessment of inclusion frequency

The frequency of the inclusions was assessed using H&E and both Ab1 and ubiquitin immunohistochemistry in a number of brain regions (see Table 3). A semiquantitative approach was used and a grading scale established, in which grade 0 was used for regions with no inclusions, + for a small number, ++ for a moderate number, +++ for numerous and ++++ for the most severely affected areas.

### Electron microscopy

Electron microscopy was performed in all three post-mortem cases. Formalin-fixed frontal and temporal cortices were post-fixed in buffered 1% osmium tetroxide, processed by conventional techniques and finally embedded in epoxy resin (Agar, Stanead, UK). Sections were cut on an LKB ultramicrotome, stained with uranyl acetate and lead citrate and viewed with a JEOL 1200 EX electron microscope (JEOL, Peabody, USA).

### Results

#### Clinical findings

The demographic data of all four cases are listed in Table 1. Clinical features of all four cases are outlined in Table 2, and individual case histories are reported in the Supplementary data (available at Brain Online). Two cases (UK1 and US2) presented with asymmetric cortical and basal ganglia symptoms, characterized by asymmetric loss of hand dexterity, apraxia and early L-dopa-unresponsive parkinsonism. The other two cases (US1 and D1), however, presented with frontal and temporal lobe features including poor organization and planning, loss of initiative, reduced hygiene, and personality change. Parkinsonism was also present in these cases but developed late and was asymmetric in one (US1).

Prominent features common to all cases included rapid progression of symptoms with early falling, mutism and loss of mobility. In some of the cases, headaches (UK1 and US1), swallowing difficulties (D1), dystonia (US1 and US2), supranuclear gaze palsy (UK1), obsessive–compulsive behaviours (US1 and US2), pathological laughter (UK1 and D1) and pathological crying (US2) were also noted to be present during the course of the illness.

Past medical history was significant for autoimmune disease (UK1, US1 and US2), a history of headaches (UK1,
US1 and D1) and a history of depression (UK1 and D1). One case (US2) had a positive family history of a neurodegenerative disorder (iPD followed by dementia in his father).

MRI of the brain in all four cases demonstrated mainly frontal and temporal lobe and caudate atrophy (Fig. 1). In case US1, in whom serial MRI scans 1 year apart were obtained, the most significant changes were noted in the posterior and lateral orbital gyri and limen insulae (Fig. 1). In addition, case D1 showed bilateral increased striatal signal (Fig. 2). [18F]fluorodeoxyglucose PET scan (UK1 and D1) and HMPAO single photon emission computer tomography (SPECT) scan (US1) demonstrated frontotemporal (UK1, US1 and D1) and basal ganglia (UK1 and D1) hypometabolism (Fig. 3).

Neuropsychometric testing (UK1, US1 and D1) demonstrated cognitive dysfunction with preserved performances on testing of visuospatial and visual perceptual function and impaired performance on tests sensitive to frontal and temporal lobe functions.

Table 2 Clinical features of four cases of neurofilament inclusion body disease

<table>
<thead>
<tr>
<th>Feature</th>
<th>UK1</th>
<th>US1</th>
<th>US2</th>
<th>D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presenting symptoms</td>
<td>Reduced left-hand dexterity</td>
<td>Poor organization and planning, reduced hygiene</td>
<td>Reduced right-hand dexterity, slow movements</td>
<td>Loss of memory and initiative, personality change</td>
</tr>
<tr>
<td>Parkinsonism [early]/[late]</td>
<td>[PI, akinetic-rigid gait, bradykinesia]</td>
<td>(Rigidity, hypophonia, hypomimia, bradykinesia)</td>
<td>[Hypomimia, rigidity, PI, reduced right-arm swing]</td>
<td>[Rigidity, PI, bradykinesia, slow gait]</td>
</tr>
<tr>
<td>Prominent clinical features during course of illness</td>
<td>Mute, falls, HA, NA, alien limb, supranuclear gaze palsy, l-dopa failure</td>
<td>Mute, falls, NA, torticollis, HA</td>
<td>Mute, falls, NA, spasticity, dystonia, l-dopa failure</td>
<td>Falls, voice change, mute disinhibited, swallowing difficulty, NA</td>
</tr>
<tr>
<td>Progression</td>
<td>Rapid</td>
<td>Rapid</td>
<td>Rapid</td>
<td>Rapid</td>
</tr>
<tr>
<td>Asymmetric signs</td>
<td>L &gt; R rigidity, apraxia, brisk reflexes</td>
<td>L upper limb rigidity</td>
<td>R upper limb rigidity, apraxia, brisk reflexes</td>
<td>NR</td>
</tr>
<tr>
<td>Other</td>
<td>Pathological laughter, increased sound sensitivity</td>
<td>OCB, 15 lbs weight gain</td>
<td>OCB, pathological crying, family history</td>
<td>Pathological laughter</td>
</tr>
<tr>
<td>MRI finding</td>
<td>FT and caudate atrophy</td>
<td>FT and caudate atrophy</td>
<td>FT atrophy</td>
<td>FT and caudate atrophy and signal changes in striatum</td>
</tr>
</tbody>
</table>

PI = postural instability; HA = headaches; NA = non-ambulatory; L = left; R = right; NR = not reported; OCB = obsessive–compulsive behaviour; FT = frontotemporal.

MRI of the brain in all four cases demonstrated mainly frontal and temporal lobe and caudate atrophy (Fig. 1). In case US1, in whom serial MRI scans 1 year apart were obtained, the most significant changes were noted in the posterior and lateral orbital gyri and limen insulae (Fig. 1). In addition, case D1 showed bilateral increased striatal signal (Fig. 2). [18F]fluorodeoxyglucose PET scan (UK1 and D1) and HMPAO single photon emission computer tomography (SPECT) scan (US1) demonstrated frontotemporal (UK1, US1 and D1) and basal ganglia (UK1 and D1) hypometabolism (Fig. 3).

Neuropsychometric testing (UK1, US1 and D1) demonstrated cognitive dysfunction with preserved performances on testing of visuospatial and visual perceptual function and impaired performance on tests sensitive to frontal and temporal lobe functions.

Table 3 Semiquantitation of inclusion frequency in neurofilament inclusion body disease in cases UK1 and US1

<table>
<thead>
<tr>
<th>Brain area</th>
<th>H&amp;E</th>
<th>Ab1</th>
<th>Ubiquitin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior frontal cortex</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Posterior frontal cortex</td>
<td>++++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Middle temporal gyrus</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Inferior temporal gyrus</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>Temporal pole</td>
<td>++++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Insular cortex</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Anterior cingulate gyrus</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Hippocampus CA1–CA4</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Dentate fascia</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Subiculum</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Amygdala</td>
<td>++++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Putamen</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thalamus</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Subthalamus</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pontine base</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Inferior olive</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Cerebellar Purkinje cells</td>
<td>+</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>Dentate nucleus</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

+++ = severely affected; +++ = numerous; ++ = moderate number; + = small number; 0 = no inclusions. *Staining is weak.

US1 and D1) and a history of depression (UK1 and D1). One case (US2) had a positive family history of a neurodegenerative disorder (iPD followed by dementia in his father).
Histological findings
The histological findings were similar for all cases and are therefore summarized with exceptions reported. Inclusions are depicted in Fig. 5.

Cerebral cortex. On routine microscopic examination, there was superficial linear spongiosis with thinning of the cortical ribbon affecting frontal, temporal and, to a lesser extent, the parietal lobes in all post-mortem cases. There was severe subpial and white matter gliosis and mild gliosis affecting cortical layers I–IV. H&E staining revealed intracytoplasmic inclusions ranging in size from 3 to 20 μm in all layers of the neocortex affecting all morphological types of neurons and often situated towards their apical dendrite (Fig. 5A). The appearance of the inclusions, irrespective of their localization, ranged from eosinophilic to basophilic, sometimes with a clearly defined basophilic margin (Fig. 5B). Inclusions appeared to be granular or mesh-like and often contained a hyaline, argyrophilic core resembling a ‘cherry’; therefore, we shall describe them as ‘cherry spots’. Such structures were found in several locations (Fig. 5C). The SMI 32 and Ab1 NF antibodies intensely stained most of what appeared to correspond to the cherry spots (Fig. 5K and L, respectively), while these were stained less frequently and less intensely by SMI 31 and rarely by BF10. The NF-cocktail antibody usually stained a larger part of the inclusions than the Ab1 and SMI 32 antibodies (Fig. 5M). Most of the inclusions were weakly immunoreactive for ubiquitin, although a proportion, often those situated in the superficial cortical laminae, were more strongly stained (Fig. 5J). Occasional inclusions were intranuclear, eosinophilic, up to 6 μm in diameter and frequently located in cortical layer II. Intranuclear inclusions (UK1 and US1 only) were strongly stained with both ubiquitin and Ab1 antibodies (Fig. 5O) and less strongly stained with SMI 32 and 1C2 antibodies (Fig. 5Q). Glial inclusions were not found.

Hippocampus. The hippocampal formation showed variable neuronal loss and spongiosis in the CA1 subregion with relative preservation of the CA2, CA3 and CA4 subregions. Many of the residual hippocampal neurons contained cytoplasmic inclusions that were often large and basophilic (Fig. 5B) and within which there was a well-defined eosinophilic hyaline cherry spot with a surrounding pale halo (Fig. 5C). The inclusions in the CA1–CA4 subregions had similar immunohistochemical staining characteristics to those in the neocortex. Some axons in CA1 showed fusiform dilations (Fig. 5N). In the granule cells of the dentate fascia, frequent neuronal cytoplasmic inclusions were noted (Fig. 5D), which appeared as single or occasionally multiple small and faintly eosinophilic. Rod-shaped intranuclear inclusions were also noted (US1 and UK1). The cytoplasmic inclusions of the granule cells were more frequently positively stained with the antibody to ubiquitin (Fig. 5E) than...
with the Ab1 or SMI 32 (Fig. 5F) antibodies, while the intranuclear inclusions were positive for ubiquitin but only rarely stained with SMI 32. There was variable neuronal loss and fibrillary gliosis in the subiculum, entorhinal and transentorhinal cortices as well as the amygdala. Frequent neuronal cytoplasmic inclusions were found within these four anatomical areas. In the subiculum, SMI 32 seems to have stained more inclusions than Ab1 (UK1 only).

Subcortical grey nuclei. The basal nucleus of Meynert had a normal neuronal population, and ubiquitin, SMI 32 and SMI 31 immunohistochemistry revealed very scanty cytoplasmic inclusions. The caudate nucleus exhibited severe atrophy with status spongiosis and gliosis in its medial aspect, most marked in cases UK1 and US1. The lateral aspect of the nucleus was less severely affected. Ubiquitin and SMI 32 stained rare inclusions within the ventrolateral caudate in cases UK1 and US1. There was moderate to severe gliosis in the globus pallidus and putamen, as well as a small number of axonal spheroids. The internal globus pallidus was affected more than the external globus pallidus, and there was an anteroposterior gradient of severity, with the anterior level most severely affected. A similar gradient was observed in the putamen, with the anterior and medial parts of the nucleus affected more severely than the posterior and lateral aspects. Few cytoplasmic inclusions were seen in the basal ganglia, and a proportion of these was positive with Ab1 antibody. The thalamus showed patchy rarefaction, with neuronal loss, gliosis and scattered intracytoplasmic inclusions most frequent in the medial thalamus. The subthalamic nucleus was well preserved with occasional intracytoplasmic inclusions.

Brainstem. The red nucleus contained large NF-positive spheroids and showed mild gliosis. The substantia nigra had mild neuronal loss with extracellular neuromelanin pigment and gliosis that was most marked in the ventral and lateral neuronal groups. A number of axonal spheroids were seen in the pars reticularis. The third nerve had a significant number of very thick axons. No inclusions were found in the third and fourth nerve nuclei. The raphe nuclei contained scanty inclusions in case US1 only. There was mild neuronal loss

Fig. 2 Case D1. An axial FLAIR (fluid attenuated inversion recovery) image demonstrates increased signal changes in striatum bilaterally.

Fig. 3 Case D1. An [18F]fluorodeoxyglucose PET scan reveals hypometabolism in the right frontotemporal region and right thalamus.
in the locus coeruleus and some of the neurons had NF-positive hyaline inclusions (US2 only). Rare Ab1-positive intraneuronal inclusions (UK1 only) and axonal spheroids were seen in the pontine base (UK1 and US1). In the inferior olive, there were large inclusions, some appearing filamentous (Fig. 5H), which were stained with both Ab1 immunohistochemistry and Bielschowsky’s silver impregnation but were negative for ubiquitin.

**Cerebellum.** In the cerebellar cortex, there was variable Purkinje cell loss and frequent torpedoes in two of the cases (UK1 and US1). These findings were not noted in the other post-mortem case (US2). In case UK1, the Purkinje cells often contained one or more ubiquitin-positive, but NF-negative, intranuclear inclusions (Fig. 5P), measuring 1–6 μm in diameter. No cytoplasmic inclusions were seen on routine stains or with immunohistochemistry.

**Spinal cord.** Where spinal cord sections were available (US1 and US2), no inclusions were seen in motor neurons. There was no degeneration of the corticospinal or other tracts.

A moderate number of inclusions were weakly stained with the modified Bielschowsky silver stain, while a small number, which mostly appeared to correspond in size to cherry spots, were intensely stained. Weakly stained inclusions could be demonstrated in the neocortex, hippocampus, basal ganglia and thalamus, while intensely stained inclusions were mainly found in the frontal cortex and hippocampus. The inclusions were not stained with Gallyas or Bodian silver impregnations.

The inclusions were not labelled by antibodies to either α-synuclein or tau or stained with PAS/H, Nissl, Congo Red or thioflavin-S methods. No cortical or brainstem Lewy bodies were found using routine stains or α-synuclein immunohistochemistry. α-Synuclein immunohistochemistry in the amygdala (UK1) was negative. None of the cases showed neurofibrillary pathology on tau immunohistochemistry. There were no glial inclusions identified on tau, α-synuclein or ubiquitin immunohistochemistry. No Aβ or prion protein deposition was observed. In the control cases stained with the same panel of anti-NF and ubiquitin antibodies, no inclusions with a similar staining profile were demonstrated.

**Inclusion frequency**

Semiquantitation of the inclusions found in cases UK1 and US1 revealed a few important patterns (Table 3). The inclusions were widely distributed, but they were most common in the anterior frontal and cingulate cortices and the hippocampal dentate fascia. Inclusions were also numerous, but less so, in the posterior frontal, temporal, parietal and insular cortices, subiculum, hippocampus proper and amygdala. They were scanty to moderate in the occipital cortex, basal ganglia and brainstem and were absent in the deep cerebellar nuclei. In the temporal cortex, there was a gradation whereby the superior temporal gyrus had fewer inclusions than the inferior temporal gyrus. Overall, the inclusions were most readily found on the H&E preparations, with the exception of the granule cells of the hippocampal dentate fascia and the inferior olive.

**Electron microscopy**

In all three post-mortem cases, cytoplasmic inclusions of the cortical neurons were noted to be composed of dense aggregates of filaments 11–16 nm in diameter and appeared consistent with NFs (Fig. 6A). These were surrounded by granule-coated fibrils and cellular organelles. In cell processes an occasional structure of ~3 μm diameter containing bundles of filamentous material, similar to those found in neurons, was seen (Fig. 6B).
Discussion

Clinical findings

The clinical presentation and progression of the cases we have described, for which we propose the term NIBD, differs from other neurodegenerative disorders. The presenting features of cases UK1 and US2 share many similarities to CBD syndrome, in that they both have asymmetric cortical features with parkinsonism, L-dopa resistance and apraxia. In a study of 14 patients with pathologically confirmed CBD, asymmetric hand clumsiness was the most common presenting symptom (Wenning et al., 1998). Similarly, the presenting symptoms and signs of cases US1 and D1 are in keeping with the features that help to define FTD, as both cases had insidious onset at age <65 years, prominent frontal features and prominent language dysfunction (Neary et al., 1998; see Supplementary data for histories). Parkinsonism develops in perhaps the majority of affected cases as FTD progresses (Knopman et al., 1990).

Most clinicopathological studies that have examined the progression of patients with CBD and FTD, as well as the duration of illness, have revealed a difference from our cases. The mean duration of illness until death for both CBD and FTD is generally >6 years (Boeve et al., 1999; Snowden et al., 1999).
of illness is ~6 months, although cases with a longer duration of illness have been reported (Parchi et al., 1999); MRI, EEG and CSF studies show basal ganglia and cortical changes, periodic complexes and increased 14-3-3 protein, respectively (Schroter et al., 2000; Zerr et al., 2000). These findings were not present in any of our cases except for D1, in whom MRI demonstrated striatal hyperintensity similar to that reported in CJD (Fig. 2). However, EEG findings and CSF results (see Supplementary data) were not consistent with a diagnosis of CJD. Furthermore, prion protein immunohistochemistry and western blotting (UK1, data not shown) were negative. DUI ± MND can have presenting features similar to our cases (Jackson et al., 1996; Rossor et al., 2000); however, in those reports, survival from first symptom was 8 and 9 years, respectively. Nevertheless, DUI can be rapidly progressive with duration of illness of ~3 years, but only in the setting of MND (i.e. when there is clinical and/or EMG evidence of motor neuron disease; Mitsuyama, 1993). None of our cases had any clinical or electrophysiological evidence for MND.

The other features that were prominent in our cases of NIBD were mutism, falls and terminal non-ambulation. It is difficult to determine the exact mechanism for the mutism, as the localization of mutism is diverse and has been described in many patients with bilateral cortical lesions (e.g. bilateral anterior cerebral infarcts; Bogousslavsky and Regli, 1990) and subcortical lesions (e.g. bilateral globus pallidus lesions; Ure et al., 1998). One clue, however, may have been the MRI findings in case US1, in which serial imaging 1 year apart demonstrates severe and more focal destruction of the inferior and posterior frontal lobes containing Broca’s area, out of proportion to other cortical areas and subcortical areas (Fig. 1). During this time, the patient had significantly reduced verbal output. A comparison with patients with FTD suggests that these findings may be significant, as mutism is common in FTD. Falls and terminal non-ambulation most likely result from progressive destruction of multiple pathways and structures that are required for ambulation. These include the cerebellar and frontal cortical–basal ganglia pathways. Pathological findings in NIBD revealed Purkinje cell loss with torpedoes and intranuclear inclusions within cerebellar Purkinje cells (UK1), while basal ganglia damage was present, sometimes severe (as in UK1 and US1, in whom the caudate nuclei were severely atrophic). Frontal cortical destruction with superficial linear spongiosis and numerous NF-positive intraneuronal inclusions were also a common feature.

Finally, there were other clinical findings including saccadic eye movement abnormality (UK1), increased sound sensitivity (UK1), obsessive–compulsive behaviours (US1 and US2), pathological laughter or crying (UK1, US2 and D1) and past medical history of headache (UK1, US1 and D1), depression (UK1 and D1) and autoimmune disorders (UK1, US1 and UK2). These findings were limited to one or a few cases and at present are of uncertain significance.
Table 4 Pathological features of NIBD compared with BIBD, DUI and PiD-UNI

<table>
<thead>
<tr>
<th>Feature</th>
<th>NIBD*</th>
<th>BIBD†</th>
<th>DUI*‡</th>
<th>PiD-UNI§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Age range of onset (years)</td>
<td>41–50</td>
<td>29–60</td>
<td>43–59</td>
<td>48</td>
</tr>
<tr>
<td>Mean duration of illness (years)</td>
<td>3</td>
<td>6</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Clinical diagnosis</td>
<td>FTLD/CBD</td>
<td>FTLD</td>
<td>FTLD</td>
<td>FTLD</td>
</tr>
<tr>
<td>Lobar atrophy</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Caudate atrophy</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Superficial cortical spongiosis</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Nigral pallor</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Nigral cell loss</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Neuronal intracytoplasmic inclusions</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Neuronal intranuclear inclusions</td>
<td>Yes</td>
<td>No</td>
<td>Yes†</td>
<td>No</td>
</tr>
<tr>
<td>Argyrophilia of inclusions</td>
<td>Yes (O)</td>
<td>Yes (O)</td>
<td>No</td>
<td>Yes (F)</td>
</tr>
<tr>
<td>Immunohistochemical profile</td>
<td>UB, NF (I)</td>
<td>NF (W)</td>
<td>UB</td>
<td>NF (I)</td>
</tr>
<tr>
<td>Main distribution of inclusions</td>
<td>FT, HP, DG</td>
<td>BS, BG</td>
<td>FT, DG</td>
<td>FT, HP</td>
</tr>
</tbody>
</table>

NIBD = neurofilament inclusion body disease; BIBD = basophilic inclusion body disease; DUI = dementia with ubiquitin-positive inclusions; PiD-UNI = Pick’s disease with unusual neuronal inclusions; FTLD = frontotemporal lobar degeneration; CBD = corticobasal degeneration; O = occasional; F = frequent; UB = ubiquitin; NF = neurofilament; I = intense staining; W = weak or negative staining; FT = frontotemporal; HP = hippocampus CA1–CA4; DG = dentate granule cells; BS = brainstem; BG = basal ganglia.

*Analysis of our data; †adapted from Munoz-Garcia and Ludwin (1984); ‡adapted from Holton et al. (2002); §adapted from Yokoo et al. (1994); Woulfe et al. (2001).

Only one case (US2) had a positive family history of an early onset neurodegenerative disease (see Supplementary data). The patient’s father had developed signs and symptoms of iPD in his early 50s, followed a few years later by dementia. Unfortunately, post-mortem examination was not performed. The development of iPD in his early 50s followed by dementia is very intriguing. The differential diagnosis of parkinsonism and dementia is wide. A diagnosis of iPD, however, is very unlikely. More likely would be a diagnosis of DLB (McKeith et al., 1996). We know his father was treated with L-dopa therapy, but we do not know whether he responded to the treatment. With the young age of onset and short duration of illness (<5 years), NIBD is clearly a diagnostic possibility.

MRI studies in all four cases revealed prominent frontal and temporal lobe atrophy and increased signal in the striatum in one case (D1). The distribution of atrophy is in keeping with the clinical presentation. The increased signal in the striatum in case D1 is unusual, as this is uncommon in CBD and FTLD syndromes (Hauser et al., 1996; Larsson et al., 2000), although it is more common in CJD (as discussed above).

The findings on functional neuroimaging (PET and SPECT) and neuropsychological tests are also in keeping with the clinical presentation and can be useful ancillary studies.

Pathological findings

Pathological study of all four cases showed similar findings of NF-containing neuronal cytoplasmic inclusions in several brain areas, including the neocortex and limbic structures, with moderate numbers in deep grey and brainstem nuclei. In at least two of the cases (UK1 and US1), we were able to demonstrate neocortical ubiquitin-positive intranuclear inclusions, which were also frequent in the cerebellar Purkinje cells in one case (UK1).

A number of conditions can be considered in the differential diagnosis of NIBD (Table 4). The absence of α-synuclein and tau pathology in all our cases excludes the diagnosis of a number of neurodegenerative conditions with neuronal inclusions, including PD, DLB, MSA, PiD, CBD, PSP or AD (Lowe, 1998). The presence of ubiquitin- and NF-positive inclusions excludes the diagnosis of dementia lacking distinctive histology (Knoopman et al., 1990; Mann, 1998). The immunohistochemical profile of NIBD is also different from that seen in basophilic inclusion body disease (BIBD) (Munoz-Garcia and Ludwin, 1984; Munoz, 1998; Holton et al. 2002), as in this condition the basophilic inclusions are negative or weakly positive for NFs (Munoz-Garcia and Ludwin, 1984; Tsuchiya et al., 2001). The distribution of the basophilic inclusions is also different from that of the NIBD inclusions, as they are found mainly in the basal ganglia and brainstem (Munoz-Garcia and Ludwin, 1984), sparse in the cortex and absent in the hippocampus. As the inclusions in NIBD are variably positive for ubiquitin, a comparison with DUI ± MND is also necessary. In DUI ± MND, the inclusions are limited largely to the cortex and hippocampus, in which only dentate granule cells are involved (Okamoto et al., 1991; Jackson et al., 1996; Rossor et al., 2000; Holton et al., 2002). In contrast, inclusions are frequently found not only in the dentate granule cells, but also throughout the hippocampal formation, including subiculum in NIBD. Furthermore, the inclusions in NIBD are more prominent than those in DUI ± MND and can be easily seen in H&E-stained sections. Most importantly, the
inclusions in DUI ± MND are negative for NFs, which is also confirmed by the current study. A necessary consideration of our NF inclusions is their possible relationship with NF-positive inclusions within motor neurons in MND (Sobue et al., 1990) or the eosinophilic and basophilic inclusions, labelled with NF and ubiquitin antibodies and described in a single case reported by Arima et al. (1998). There were no MND-type inclusions with ubiquitin and NF immunohistochemistry within motor neurons of either the cranial nerve nuclei, which were available for our studies in all three post-mortem cases (UK1, US1 and US2), or the cervical cord studied in two of the cases (US1 and US2). Furthermore, neuronal inclusions, similar to those seen in NIBD, are not seen in the cerebral cortices and deep grey nuclei in MND (Lowe and Leigh, 2002). It could be of interest that, in the case of Arima et al. (1998) diagnosed clinically and confirmed by post-mortem as MND, the intracytoplasmic ubiquitin-positive inclusions were found in cortical neurons and hippocampal dentate granule cells. However, these were reported to be only weakly positive or negative for NF using the SMI 31 antibody recognizing pNF-H. The entity described by Yokoo et al. (1994) as ‘Pick’s disease with unusual inclusions’ (PiD-UNI) in 1994 is, with a few differences, the most similar to our four cases. In their case, the inclusions were NF-positive with an immunohistochemical profile and distribution similar to ours. However, in PiD-UNI, ubiquitin studies were reported as negative and neither intranuclear inclusions nor axonal spheroids were reported. A further difference is that, clinically, unlike our cases, their case had a long disease duration of 13 years, even though the early disease course was rapidly progressive. Pietrini et al. (1993) described a case of the panencephalitic type of CJD with neuropathological features similar to PiD. In their case, there were numerous NF-positive swollen neurons and a small number of Pick body-like argyrophilic intraneuronal inclusions. However, there is no reference to the presence of NF-positive intraneuronal inclusions of the kind we observed in our cases. The other only report of significant interest was a recent report of five patients with a novel neurofilamentopathy (Cairns et al., 2003). Similar to our cases, their cases had an early-onset dementia and intraneuronal, cytoplasmic, NF-positive inclusions. Little clinical information was given, but their patients seem all to have had an FTD. No mention of a CBD syndrome was made. Unlike in our cases, NF-L staining was less impressive than NF-H staining. Also, no mention was made of intranuclear inclusions, inclusions within the inferior olive, axonal swellings outside the cerebellum or the presence of filamentous structures in cell processes, which we were able to demonstrate with electron microscopy.

One of the striking features noted in our cases was the presence of neuronal ubiquitin-positive intraneuronal inclusions. Although such intranuclear inclusions were only rarely found in most areas, they were numerous in the hippocampal dentate granule cells and the cerebellar Purkinje cells (UK1). Intranuclear inclusions are rather rare in cases with dementia, although neuronal ubiquitinated intranuclear inclusions have been described in three cases of DUI ± MND (Woulfe et al., 2001) and a single case report of a demented woman (Weidenheim and Dickson, 1995). Intranuclear neuronal inclusions are a common feature of polyglutamine disorders (Davies et al., 1997; Hayashi et al., 1998; Holmberg et al., 1998; Li et al., 1998; Pang et al., 2002) and neuronal intranuclear hyaline inclusion disease (Munoz-Garcia and Ludwin, 1986), which may also be a polyglutamine disorder (Lieberman et al., 1998; Takahashi et al., 2000). Case D1 underwent genetic testing for Huntington’s disease and dentatorubral-pallidolusian atrophy and tested negative (see Supplementary data). The relationship of these disorders to our four cases of NIBD is not clear at present and requires further investigation, especially since the antibody 1C2, which identifies polyglutamine sequences, occasionally labelled cortical intranuclear inclusions in our NIBD cases.

Two of the four cases (UK1 and US2) of NIBD were treated with dopamine therapy and both failed to improve symptomatically. This is not surprising, as pathology was not limited to the substantia nigra, but was also extensive and severe in the basal ganglia. Replacement anticholinesterase therapy is also unlikely to be beneficial in NIBD, as the nucleus basalis of Meynert is preserved. This is not to say that trials with either category of medication are not worth pursuing.

The mechanism for NF deposition in NIBD is currently unknown, but there are a few theoretical possibilities based on results from mouse genetic and human NF studies (Nixon, 1993; Julien, 1999). These include ‘sporadic’ NF gene mutations, post-translational modifications including abnormal phosphorylation of the subunit components of NF (NF-L, NF-M and NF-H) and failure of axonal NF transport.

In summary, NIBD is a unique neurodegenerative disorder with overlapping clinical features of frontotemporal lobar degeneration and CBD syndrome. A diagnosis of NIBD should be considered in patients who present with either a CBD or FTD syndrome, with rapid progression without clinical or EMG evidence of MND, with early falls, mutism, akinesia and terminal immobility. MRI evidence of frontal and temporal lobe atrophy would support the diagnosis. The main morphological features that support the pathological diagnosis of NIBD is α-synuclein- and tau-negative neuronal intracytoplasmic inclusions, many of which contain NFs and variable amounts of ubiquitin, and some of which are argyrophilic with modified Bielschowsky. Such inclusions are strikingly numerous in neocortices and limbic structures. Further characterization of NIBD is needed to clarify the significance and mechanism of the NF deposition in this disorder.

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