Published in final edited form as:
Acta Neuropathol. 2004 September ; 108(3): 213–223. doi:10.1007/s00401-004-0882-7.

α-Internexin aggregates are abundant in neuronal intermediate filament inclusion disease (NIFID) but rare in other neurodegenerative diseases

Nigel J. Cairns and **Kunihiro Uryu**
Center for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Maloney 3, HUP, 3600 Spruce Street, Philadelphia, PA 19104-4283, USA

Eileen H. Bigio
Department of Neuropathology, Northwestern University Medical School, Chicago, Illinois, USA

Ian R. A. Mackenzie
Department of Pathology and Laboratory Medicine, Vancouver General Hospital, Vancouver, British Columbia, Canada

Marla Gearing
Department of Pathology and Laboratory Medicine and Center for Neurodegenerative Disease, Emory University School of Medicine, Atlanta, Georgia, USA

Charles Duyckaerts
Laboratoire de Neuropathologie, Hôpital de la Salpêtrière, Paris, France

Hideaki Yokoo and **Yoichi Nakazato**
Department of Pathology, Gunma University School of Medicine, Maebashi, Japan

Evelyn Jaros and **Robert H. Perry**
Department of Neuropathology, Newcastle General Hospital, Newcastle-upon-Tyne, UK

Steven E. Arnold
Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

Virginia M.-Y. Lee and **John Q. Trojanowski**
Center for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Maloney 3, HUP, 3600 Spruce Street, Philadelphia, PA 19104-4283, USA

Abstract

Abnormal neuronal aggregates of α-internexin and the three neurofilament (NF) subunits, NF-L, NF-M, and NF-H have recently been identified as the pathological hallmarks of neuronal intermediate filament (IF) inclusion disease (NIFID), a novel neurological disease of early onset with a variable clinical phenotype including frontotemporal dementia, pyramidal and extrapyramidal signs. α-Internexin, a class IV IF protein, a major component of inclusions in NIFID, has not previously been identified as a component of the pathological protein aggregates of any other neurodegenerative disease. Therefore, to determine the specificity of this protein, α-internexin immunohistochemistry was undertaken on cases of NIFID, non-tau frontotemporal

Tel.: +1-215-6624708, Fax: +1-215-3495909, cairns@mail.med.upenn.edu.

dementias, motor neuron disease, α-synucleinopathies, tauopathies, and normal aged control brains. Our results indicate that class IV IF proteins are present within the pleomorphic inclusions of all cases of NIFID. Small subsets of abnormal neuronal inclusions in Alzheimer's disease, Lewy body diseases, and motor neuron disease also contain epitopes of α-internexin. Thus, α-internexin is a major component of the neuronal inclusions in NIFID and a relatively minor component of inclusions in other neurodegenerative diseases. The discovery of α-internexin in neuronal cytoplasmic inclusions implicates novel mechanisms of pathogenesis in NIFID and other neurological diseases with pathological filamentous neuronal inclusions.

Keywords

α-Internexin; Neurofilament; Intermediate filament; Neuronal intermediate filament inclusion disease; Frontotemporal dementia

Introduction

Many chronic progressive neurodegenerative disorders are characterized by the presence of abnormal protein aggregates in neurons and glia of the central nervous system [1, 16, 27, 37]. The identification of disease-specific abnormal protein inclusions has illuminated mechanisms of pathogenesis and has facilitated the molecular classification of the neurodegenerative diseases. Neuronal intermediate filament (IF) inclusion disease (NIFID) is a novel neurological disease with a clinically heterogeneous phenotype including progressive early-onset dementia, pyramidal and extrapyramidal signs. Grossly, there is focal atrophy of the frontal lobes, and to a lesser degree the temporal and parietal lobes, and, microscopically, there are intraneuronal, cytoplasmic, neuronal IF inclusions which contain neither tau nor α-synuclein [2, 4, 12, 15, 21, 38]. The inclusions are present in neocortex, where clusters of inclusions have been reported [3], subcortical nuclei and spinal cord.

Neurofilaments (NFs) are abundant IFs of the neuronal cytoskeleton and are composed of light (NF-L), medium (NF-M), and heavy (NF-H) subunits of approximately 68, 145, and 200 kDa, respectively [1, 24]. All three subunits are phosphorylated and most of the phosphorylation sites are located in the tail domain of NF-H [25, 36]. The use of phosphorylation-dependent and –independent antibodies to NF epitopes has enabled the immunohistochemical dissection of these proteins and has revealed that NFs within the perikaryon and proximal segments of axons and dendrites are normally hypophosphorylated, while NFs in axons are heavily phosphorylated. In neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), dementia with Lewy bodies (DLB) and motor neuron disease (MND), abnormal accumulations of phosphorylated NF proteins in the cell body have been reported [19, 26, 28, 29, 32, 33, 34, 35], although the significance of the phosphorylation of NF proteins within the cytoplasm is unclear. However, abnormal phosphorylation may impede axonal transport and contribute to neuronal dysfunction, while constitutive phosphorylation of NFs may protect them against proteolysis [17]. Mutations in NF-H and NF-L genes in MND have been associated with abnormal accumulations of NF proteins [1] and transgenic mice that overexpress NF proteins such as the NFH/LacZ mouse have selective degeneration of Purkinje cells with Lewy body-like inclusions [14].

In addition to the three NF triplet proteins, a fourth neuronal IF protein, α-internexin, has been classified as a type IV IF [8]. The gene for α-internexin is located on chromosome 10 and its transcript is a 499-amino acid protein with a molecular weight of 55.4 kDa and an apparent molecular mass of 66 kDa. The protein is expressed by most, if not all, neurons as they commence differentiation and its expression precedes that of the NF triplet proteins [22]. In the adult brain, α-internexin is expressed at relatively low levels in comparison to

the NF proteins, and there is selective anatomical expression with greater immunoreactivity being seen in the cerebellar granule cells, the source of thin-caliber parallel fibers [13], and in the neuron cell bodies and processes of cortical layer II neurons. α-Internexin also co-assembles with the NF triplet proteins [7]. A transgenic mouse model with overexpression of rat α-internexin has been shown to cause abnormal neurofilamentous accumulations and motor co-ordination deficits [9]. Recently, we described α-internexin as a major component of the pathological hallmark of NIFID [5]. Although previous studies have demonstrated the co-localization of NF epitopes in AD, PD, DLB, and MND [19, 25, 26, 28, 29, 32, 33, 34, 35], no study has demonstrated the presence of α-internexin as a component of the pathological inclusions of any neurodegenerative disease other than NIFID.

Materials and methods

Brain tissue collection, processing, and neuropathological assessment

Brain tissues from ten cases of NIFID were obtained from Canada, Norway, Spain, Japan (one case from each), and from France, the United Kingdom and the United States (two cases from each) and all displayed the pathological features previously described in NIFID [2, 4, 5, 12, 15, 38]. Five cases of MND, MND with dementia (three cases), frontotemporal lobar degeneration (FTLD) with MND-type inclusions (eight cases), FTLD (four cases), basophilic inclusion body disease (BIBD; two cases), AD (four cases) and two cases of Pick's disease, and one case each of corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP), four cases of PD, four cases of DLB, two cases of multiple system atrophy (MSA) and normal aged controls (four cases) were obtained from the Center for Neurodegenerative Disease Research, University of Pennsylvania School of Medicine (Table 1). After death, the consent of the next of kin was obtained for brain removal, following Local Ethics Committee procedures. Brain tissue was preserved in buffered 10% formalin.

Histology and immunohistochemistry

Tissue blocks were taken from representative areas including: the frontal, temporal, parietal, and occipital lobes, hippocampus, basal ganglia including the nucleus basalis of Meynert, thalamus, midbrain, pons, medulla oblongata, cerebellum, and spinal cord. Histological stains included: hematoxylin and eosin, Klüver-Barrera, and thioflavine S. Antigen retrieval was performed by heating sections in a solution of 0.5% ethylenediaminetetraacetic acid (EDTA) in 100 mmol/l TRIS, pH 7.6 at 100°C for 10 min. α-Synuclein immunohistochemistry (IHC) was enhanced by treatment in 80% formic acid for 5 min. IHC was undertaken on 6- to 10-μm-thick sections prepared from formalin- (cases NIFID1–7, 9, 10 and non-NIFID cases) or 4% paraformaldehyde- (case NIFID8) fixed, paraffin wax-embedded tissue blocks using the avidin-biotin complex detection system (Vector Laboratories, Burlingame, CA) and the chromogen 3,3′-diaminobenzidine (DAB), and sections were counterstained with hematoxylin as previously described [23].

Antibodies used included those that recognized epitopes of IF protein classes III, V and VI, and a panel of class IV neuronal IF proteins including: phosphorylation-dependent, non-phosphorylation-dependent, and phosphorylation-independent epitopes (Table 2). Anti-NF antibodies used in this study are well characterized and have been used previously to demonstrate epitopes of NF in neuronal inclusions in AD, Lewy body diseases, and MND [6, 19, 25, 26, 32, 33, 34, 35]. In addition, antibodies to α-synuclein [10], cytoskeletal, and other proteins associated with abnormal protein aggregates in neurodegenerative diseases were used in this study. For a complete list of antibodies used see Table 2.

Results

Pathological inclusions in NIFID contain α-internexin

Accumulations of α-internexin were observed in the cytoplasm of affected neurons and axons in the neocortex and underlying white matter of all lobes with the highest densities being found in the frontal and temporal lobes (Fig. 1) and only rare neuronal inclusions in the occipital lobe. Neuronal loss was present in layer II, and extended to the full thickness in the most severely affected parts of the frontal lobes in those cases with frontotemporal dementia (FTD; Table 1; NIFID1–6, 9 and10). The basal ganglia of these cases also showed marked atrophy and neuronal loss. Cases with clinical features of MND (Table 1; NIFID7, 8, and 10) displayed generally less involvement of the anterior frontal lobe, but marked neuronal loss and higher densities of inclusions in the precentral gyrus, corticospinal tract degeneration and inclusions in the spinal cord. Neuronal loss was not generally a prominent feature of the pyramidal neurons of the hippocampus nor the granule cells of the dentate gyrus, but neuronal cytoplasmic inclusions and axonal swellings were present in the granule neurons of the dentate gyrus and in affected gray and underlying white matter of all cases. Inclusions were also present in the basal ganglia, thalamus, and nuclei of the midbrain, pons, medulla oblongata, and, in two cases where the spinal cords were available, corticospinal tract degeneration was accompanied by neuronal inclusions in the central gray matter of the spinal cord; the motor neurons of the anterior horns were relatively well preserved. The most abundant aggregates of α-internexin were seen in areas that had only mild or no neuronal loss (Fig. 1). In areas of the most pronounced neuronal loss no or few IF inclusions were seen. These observations indicate that α-internexin aggregation may be an early event in the pathogenesis of the disease and that after neurons degenerate, the inclusions are released into the extracellular space where they are rapidly cleared, unlike the extracellular “ghost” tangles formed by tau filaments, which remain following degeneration of the neuron in which the tangle is located.

Neuronal α-internexin aggregates were present throughout the neuraxis and were pleomorphic; however, Pick body-like inclusions were the most abundant morphological type and could be seen in cortical laminae with the highest densities in layers V/VI. Swollen axons and axonal spheroids, similar to those found in amyotrophic lateral sclerosis and normal aging, but which are not specific to any neurodegenerative disease, were also seen in affected areas and in underlying white matter, corticospinal tracts and other white matter tracts.

Pathological inclusions in NIFID contain all class IV IF proteins

To determine the IF protein composition of the cytoplasmic inclusions, a panel of antibodies that recognize epitopes of all class IV IFs was employed. IHC demonstrated the presence within the inclusions of phosphorylation-dependent, non-phosphorylation-dependent, and phosphorylation-independent epitopes of NF-H, NF-M, NF-L and α-internexin (Fig. 2; Table 3). The most intense staining of the inclusions was obtained using anti-α-internexin and anti-phosphorylated NF-H (RMO 24) antibodies (Fig. 2c, d). The cytoplasmic inclusions were variably ubiquitinated as demonstrated by IHC (Fig. 2b). Pick body-like inclusions gave intense anti-ubiquitin staining (Fig. 2b, e), while other inclusions were pale or unstained in appearance (Fig. 2b). IHC of adjacent sections revealed these inclusions to contain neuronal IFs. There was variation in ubiquitin IHC between cases and this may reflect differences in methods of tissue preservation between centers because antigen retrieval enhanced staining in some cases. Rare intranuclear inclusions were observed in neurons containing cytoplasmic inclusions, and these were present in four out of ten cases. These intranuclear inclusions were compact, usually round, and contained ubiquitin epitopes but not IFs (data not shown).

α-Internexin in other neurodegenerative diseases and normal aging

Epitopes of NF subunits have previously been identified as components of subsets of neurofibrillary tangles (NFTs) and dystrophic neurites in AD, Lewy bodies in PD and DLB, and in swollen axons and spheroids in MND and normal aging. For the first time, we demonstrate that α-internexin is a component of a small subset (~5%) of NFTs, dystrophic neurites of neuritic plaques (~5%), and occasional swollen achromatic neurons in AD (Table 4; Fig. 4a–c). No α-internexin immunoreactivity was seen in the hallmark neuronal or glial fibrillary inclusions of the tauopathies: Pick's disease, CBD, and PSP, although occasional swollen α-internexin-immunostained achromatic neurons (Pick cells) were seen in Pick's disease (Fig. 4f). α-Internexin staining was observed in a small fraction (~5%) of Lewy bodies in PD and DLB (Table 4). The most intense staining was observed in the core region of Lewy bodies with the corona being relatively unstained (Fig. 4d, e). α-Synuclein IHC on adjacent sections confirmed the presence of α-synuclein in these inclusions (data not shown). Pale bodies in PD were unstained by α-internexin IHC (Fig. 4e). In another α-synucleinopathy, MSA, the glial cytoplasmic inclusions were unstained. No ubiquitin-positive neuronal skein inclusions or Lewy body-like inclusions in cases of MND were labeled by anti-α-internexin antibodies. Rarely, epitopes of α-internexin were seen in swollen axons and spheroids in cases of FTLD, FTLD-MND, MND, MND-D, BIBD, (~1% of ubiquitin-positive inclusions; Fig. 4h), in neuronal inclusions within the granule cells of the dentate fascia in FTLD-MND (Fig. 3), and in normal aged control subjects (Fig. 4g; Table 4). The α-internexin containing swollen axons and spheroids were present in neo- and archicortex, underlying white matter, subcortical nuclei, and adjacent fiber tracts, but were not seen in the large diameter corticospinal tracts of the spinal cord. Rare, swollen achromatic α-internexin-positive neurons were occasionally seen in superficial and deep cortical laminae, subcortical and brainstem nuclei in a variety of neurodegenerative diseases (Fig. 4i) and some normal aged subjects (Fig. 4g).

Discussion

NIFID is a recently described neurological disorder [2, 3, 4, 12, 15], characterized by neuronal loss and pathological neuronal aggregates of all class IV IF proteins (α-internexin and the triplet NF proteins). This study using a panel of anti-IF proteins has confirmed and extended our earlier observations that all class IV IF proteins are present within the inclusions of NIFID [5], while epitopes of classes III, V, and VI IFs are absent from the inclusions of NIFID. Previously we have shown that epitopes of NF proteins are present within the pathological inclusions of several neurodegenerative disorders including AD, PD, DLB, and MND [19, 26, 32, 33, 34, 35]. Here, we report for the first time that α-internexin is also present in small subsets of the pathological neuronal inclusions of AD, PD, DLB, FTLD-MND, and rarely in the swollen axons and spheroids of MND, non-tau FTDs, and normal aged brain.

The observation that α-internexin is a major component of the neuronal IF inclusions of NIFID is surprising because α-internexin is expressed at relatively low levels in adult brain in comparison to the NF triplet proteins [22]. α-Internexin is normally differentially expressed in discrete neuronal populations: higher levels of expression have been reported in neurons with small caliber axons such as layer II neurons, cerebellar granule and basket cells [13, 22]. The widespread distribution of inclusions throughout the neocortex, subcortical nuclei, brainstem and spinal cord, in NIFID does not coincide with the populations of neurons that have generally higher levels of expression of this protein. The only correspondence we observed between neurons expressing higher levels of α-internexin and the density of cytoplasmic inclusions was in neocortical layer II neurons in some, but not all, cases. Neuronal loss and microvacuolation in the superficial layers, especially layer II, were seen to varying degrees in all cases of NIFID. As α-internexin is a neurodevelopmentally

regulated protein and precedes the NF triplet proteins in axon growth [13, 22], increased expression of α-internexin in vulnerable neurons may be a regenerative response. Increased expression or failure of axonal transport of α-internexin in vulnerable and degenerating neurons may contribute to the formation of pathological intracytoplasmic inclusions in this disease [9].

NF accumulations have been reported in several human neurological diseases including MND, PD, DLB, PSP, Charcot-Marie-Tooth (CMT) disease, diabetic neuropathy, and giant axonal neuropathy [1]. In this study, we report the presence of another IF, α-internexin, in rare swollen axons and spheroids of MND and non-tau FTDs. Unlike the NF-rich and peripherin-positive swollen axons and spheroids routinely seen in the corticospinal tracts of MND [18, 30], and to a lesser extent in normal aging, α-internexin-positive, peripherin-negative spheroids were only seen in cortex, underlying white matter and subcortical nuclei. α-Internexin-positive inclusions were not seen in the anterior horn motor neurons, but were occasionally seen in the interneurons of the central gray matter of the spinal cord in cases of NIFID and reported previously [5]. The restricted sites of expression of α-internexin spheroids in MND may reflect the relatively less frequent involvement of neurons with small axons in the MNDs. Although mutations in the copper/zinc superoxide dismutase (SOD) gene have been found in 1–2% of cases of ALS, and mutations in NF genes have been associated with CMT disease, PD, and MND, no case of NIFID had a family history of neurological or psychiatric disease. On the other hand, the early age at onset might signify that NIFID is a recessive genetic disorder; this idea remains to be examined in more detail.

IHC studies of NFs in MND and MND with dementia have demonstrated a somatodendritic distribution of NF phosphorylation [32]. Neurofilamentous aggregates including cytoplasmic inclusions in the anterior horn cells, swollen axons and spheroids show a pattern of NF phosphorylation that reflects a centrifugal gradient of phosphorylation, such that NF subunits in the perikaryon are in a primitive state of phosphorylation and become increasingly phosphorylated as they migrate to the synapse. Thus, normal dendrites and axons show the strongest NF immunoreactivity with pNF antibodies and neuronal perikarya express predominantly hypophosphorylated epitopes of all NF subunits. In NIFID the neuronal cytoplasmic pleomorphic inclusions contained predominantly α-internexin and phosphorylated NFs, indicating abnormal cellular localization of these proteins. Thus, the cytoplasmic location of pNF aggregates in NIFID contrasts with the constitutive axonal distribution of these proteins and their presence in axonal swellings and spheroids in MND and normal aging.

AD is characterized pathologically by β-amyloid deposits, NFTs, neuritic plaques and neuropil threads, which contain abnormal filamentous aggregates of hyperphosphorylated tau protein [27]. Comparative epitope analyses of the cytoskeletal proteins in NFTs, neuritic plaque neurites and neuropil threads have shown that epitopes of NF subunits are present within these structures [33, 34]. This study extends these observations by demonstrating that another IF protein is a component of a small subset of the NFTs and neuritic plaque neurites of AD. The co-localization of α-internexin in these inclusions may reflect the population of vulnerable neurons with small caliber axons, aberrant transport, proteolysis, cross-linking, or a regenerative response. The relatively small subset of tau- and ubiquitin-positive structures that contain α-internexin indicates that this protein has only a minor role in the pathogenesis of the neuronal inclusions in AD.

This study is the first to report the presence of α-internexin in a subset of Lewy bodies in PD and DLB. α-Synuclein is the major component of the Lewy bodies and dystrophic neurites in PD and DLB and glial and neuronal inclusions in MSA [16]. Epitopes of NF subunits have been previously reported in spatially separate domains of the Lewy body [19,

35], but not in the glial cytoplasmic inclusion of MSA. The pale bodies in PD and DLB were unstained by α-internexin IHC, indicating that either these structures constitute a distinct population of α-synuclein inclusions, or that they represent an early stage in the formation of classical Lewy bodies, which have an eosinophilic core and pale corona. Only a small subset (~5%) of Lewy bodies was labeled by α-internexin IHC. Like the small fraction of NFTs and dystrophic neurites in AD that was stained, the co-localization of α-internexin in Lewy bodies may reflect a population of vulnerable neurons, aberrant transport, proteolysis, cross-linking, or a regenerative response.

Rare, swollen achromatic neurons that contained moderate to intense α-internexin immunoreactivity were occasionally seen in NIFID, non-tau FTDs, MND, tauopathies, and α-synucleinopathies, but not in the normal aged control subjects. The presence of this pattern of staining may be explained by disrupted transport or a regenerative response in degenerating neurons in a spectrum of neurodegenerative diseases.

The mechanisms leading to IF aggregation in the cytoplasm and proximal axons of NIFID are unknown. This study shows that α-internexin is a major component, along with NF proteins, of the pathological inclusions in NIFID, but an uncommon finding in other neurodegenerative diseases. Although NFs aggregate in neurodegenerative diseases, the role of α-internexin in this process is currently unknown. It is possible that a failure of axonal transport may contribute to the abnormal cytoplasmic accumulation because transgenic mouse models have demonstrated abnormal NF accumulation and subsequent aggregation of NF [14]. Also, NF subunit proteins have been shown to act as pathological chaperones that facilitate the formation of intraneuronal filamentous tau aggregates that cause neurodegeneration in transgenic mice (T44), which overexpress the shortest human brain tau isoform [20]. Toxic insults, including elevated levels of the neurotransmitter glutamate may also contribute to decreased axonal transport and NF accumulation [1, 11]. The role of α-internexin in the pathogenesis of NIFID and other neurodegenerative disorders characterized by pathological aggregates of IF remains to be elucidated.

Acknowledgments

We thank Dr. D. Munoz for tissue samples and the staff of the Center for Neurodegenerative Disease Research of the University of Pennsylvania for technical support, and we thank the families of patients whose generosity made this research possible. Support for this work was provided by grants from the National Institute on Aging of the National Institutes of Health (AG-09215, AG-10124, AG-17586 to V.M.-Y.L. and J.Q.T., and AG-10130 and ES12068 to M.G.), and from the Wellcome Trust (GR066166AIA) to N.J.C. V.M.-Y.L. is the John H. Ware, 3rd Professor of Alzheimer's Research and J.Q.T. is the William Maul Measey-Truman G. Schnabel, Jr. M.D., Professor of Geriatric Medicine and Gerontology.

References

1. Al-Chalabi A, Miller CCJ. Neurofilaments and neurological disease. BioEssays. 2003; 25:346–365. [PubMed: 12655642]
2. Bigio EH, Lipton AM, White CL 3rd, Dickson DW, Hirano A. Frontotemporal dementia and motor neurone degeneration with neurofilament inclusion bodies: additional evidence for overlap between FTD and ALS. Neuropathol Appl Neurobiol. 2003; 29:239–253. [PubMed: 12787321]
3. Cairns NJ, Armstrong RA. Clustering of neurofilament inclusions in the temporal lobe in dementia with neurofilament inclusions. Acta Neuropathol. 2003; 106:125–128. [PubMed: 12732935]
4. Cairns NJ, Perry RH, Jaros E, Burn D, McKeith IG, Lowe JS, Holton J, Rossor MN, Skullerud K, Duyckaerts C, Cruz-Sanchez FF, Lantos PL. Patients with a novel neurofilamentopathy: dementia with neurofilament inclusions. Neurosci Lett. 2003; 341:177–180. [PubMed: 12697277]
5. Cairns NJ, Zhukareva V, Uryu K, Zhang B, Bigio E, Mackenzie IRA, Gearing M, Duyckaerts C, Yokoo H, Nakazato Y, Jaros E, Perry RH, Lee VM-Y, Trojanowski JQ. α-Internexin is present in

the pathological inclusions of neuronal intermediate filament inclusion disease. Am J Pathol. 2004 in press.

6. Carden MJ, Trojanowski JQ, Schlaepfer WW, Lee VM-Y. Two-stage expression of neurofilament polypeptides during rat neurogenesis with early establishment of adult phosphorylation patterns. J Neurosci. 1987; 7:3499–3504.
7. Ching GY, Liem RKH. Roles of head and tail domains in α-internexin's self-assembly and coassembly with the neurofilament triplet proteins. J Cell Sci. 1998; 111:321–333. [PubMed: 9427681]
8. Ching GY, Liem RKH. Structure of the gene for the neuronal intermediate filament protein α-internexin and functional analysis of its promoter. J Biol Chem. 1991; 29:19459–19468. [PubMed: 1717465]
9. Ching GY, Chien C-L, Flores R, Liem RKH. Overexpression of α-internexin causes abnormal neurofilamentous accumulations and motor coordination deficits in transgenic mice. J Neurosci. 1999; 19:2974–2986. [PubMed: 10191315]
10. Duda JE, Giasson BI, Mabon ME, Lee VM, Trojanowski JQ. Novel antibodies to synuclein show abundant striatal pathology in Lewy body diseases. Ann Neurol. 2002; 52:205–210. [PubMed: 12210791]
11. Durham HD. Aggregation of intermediate filaments by 2,5-hexanedione: comparison of effects on neurofilaments, GFAP filaments and vimentin filaments in dissociated cultures of mouse spinal cord dorsal root ganglia. J Neuropathol Exp Neurol. 1988; 47:432–442. [PubMed: 3385438]
12. Duyckaerts C, Mokhtari K, Fontaine B, Hauw J-J. Maladie de Pick généralisée: une démence mal nommée caractérisée par des inclusions neurofilamentaires. Rev Neurol (Paris). 2003; 159:224–224.
13. Fliegner KH, Kaplan MP, Wood TL, Pintar JE, Liem RKH. Expression of the gene for the neuronal intermediate filament protein α-internexin coincides with the onset of neuronal differentiation in the developing rat nervous system. J Comp Neurol. 1994; 342:161–173. [PubMed: 8201029]
14. Galvin JE, Nakamura M, McIntosh TK, Saatman KE, Sampathu D, Raghupathi R, Lee VM-Y, Trojanowski JQ. Neuro-filament-rich intraneuronal inclusions exacerbate neurodegenerative sequelae of brain trauma in NFH/LacZ transgenic mice. Exp Neurol. 2000; 165:77–89. [PubMed: 10964487]
15. Gearing M, Castellano AA, Hunter SB, Brat DJ, Glass JD. Unusual neuropathologic findings in a case of primary lateral sclerosis. J Neuropathol Exp Neurol. 2003; 62:555–555.
16. Goedert M, Spillantini MG, Serpell LC, Berriman J, Smith MJ, Jakes R, Crowther RA. From genetics to pathology: tau and alpha-synuclein assemblies in neurodegenerative diseases. Philos Trans R Soc Lond B Biol Sci. 2001; 356:213–227. [PubMed: 11260802]
17. Goldstein E, Sternberger NM, Sternberger LA. Phosphorylation protects neurofilaments against proteolysis. J Neuroimmunol. 1987; 14:149–160. [PubMed: 3029175]
18. He CZ, Hays AP. Expression of peripherin in ubiquitinated inclusions of amyotrophic lateral sclerosis. J Neurol Sci. 2004; 217:47–54. [PubMed: 14675609]
19. Hill WD, Lee VM-Y, Hurtig HI, Murray JM, Trojanowski JQ. Epitopes located in spatially separate domains of each neurofilament subunit are present in Parkinson's disease Lewy bodies. J Comp Neurol. 1991; 309:150–160. [PubMed: 1716646]
20. Ishihara T, Higuchi M, Zhang B, Yoshiyama Y, Hong M, Trojanowski JQ, Lee VM-Y. Attenuated neurodegenerative disease phenotype in tau transgenic mouse lacking neurofilaments. J Neurosci. 2001; 15:6026–6035. [PubMed: 11487626]
21. Josephs KA, Holton JL, Rossor MN, Braendgaard H, Ozawa T, Fox NC, Petersen RC, Pearl GS, Ganguly M, Rosa P, Laursen H, Parisi JE, Waldemar G, Quinn NP, Dickson DW, Revesz T. Neurofilament inclusion body disease: a new proteinopathy? Brain. 2003; 126:2291–2303. [PubMed: 12876145]
22. Kaplan MP, Chin SSM, Fliegner KH, Liem RKH. α-Internexin, a novel neuronal intermediate filament protein, precedes the low molecular weight neurofilament protein (NF-L) in the developing brain. J Neurosci. 1990; 10:2735–2748. [PubMed: 2201753]

23. Lantos PL, Cairns NJ, Khan MN, King A, Revesz T, Janssen JC, Morris H, Rossor MN. Neuropathologic variation in frontotemporal dementia due to the intronic tau 10(+16) mutation. Neurology. 2002; 58:1169–1175. [PubMed: 11971082]
24. Lee K, Cleveland DW. Neuronal intermediate filaments. Annu Rev Neurosci. 1996; 19:187–217. [PubMed: 8833441]
25. Lee VM-Y, Carden MJ, Schlaepfer WW, Trojanowski JQ. Monoclonal antibodies distinguish several differentially phosphorylated states of the two largest rat neurofilament subunits (NF-H and NF-M) and demonstrate their existence in the normal nervous system of adult rats. J Neurosci. 1987; 7:3478–3488.
26. Lee VM-Y, Otvos L Jr, Schmidt ML, Trojanowski JQ. Alzheimer disease tangles share immunological similarities with multiphosphorylation repeats in the two large neurofilament proteins. Proc Natl Acad Sci USA. 1988; 85:7384–7388. [PubMed: 2459703]
27. Lee VM-Y, Goedert M, Trojanowski JQ. Neurodegenerative tauopathies. Annu Rev Neurosci. 2001; 24:1121–1159. [PubMed: 11520930]
28. Leigh PN, Dodson A, Swash M, Brion J-P, Anderton BH. Cytoskeletal abnormalities in motor neuron disease: an immunohistochemical study. Brain. 1989; 112:521–535. [PubMed: 2706443]
29. Manetto V, Sternberger NH, Perry G, Sternberger LA, Gambetti P. Phosphorylation of neurofilaments is altered in amyotrophic lateral sclerosis. J Neuropathol Exp Neurol. 1988; 47:642–653. [PubMed: 2459315]
30. Migheli A, Pezzulo T, Attanasio A, Schiffer D. Peripherin immunoreactive structures in amyotrophic lateral sclerosis. Lab Invest. 1993; 68:185–191. [PubMed: 8441252]
31. Pawlyk AC, Giasson BI, Sampathu DM, Perez FA, Lim KL, Dawson VL, Dawson TM, Palmiter RD, Trojanowski JQ, Lee VM. Novel monoclonal antibodies demonstrate biochemical variation of brain parkin with age. J Biol Chem. 2003; 278:48120–48128. [PubMed: 12972409]
32. Schmidt ML, Carden MJ, Lee VM-Y, Trojanowski JQ. Phosphate dependent and independent neurofilament epitopes in the axonal swellings of patients with motor neuron disease and controls. Lab Invest. 1987; 56:282–294. [PubMed: 2434727]
33. Schmidt ML, Lee VM-Y, Trojanowski JQ. Relative abundance of tau and neurofilament epitopes in hippocampal neurofibrillary tangles. Am J Pathol. 1990; 136:1069–1075. [PubMed: 1693468]
34. Schmidt ML, Lee VM-Y, Trojanowski JQ. Comparative epitope analysis of neuronal cytoskeletal proteins in Alzheimer's disease senile plaque neurites and neuropil threads. Lab Invest. 1991; 64:352–357. [PubMed: 1706004]
35. Schmidt ML, Murray J, Lee VM-Y, Hill WD, Wertkin A, Trojanowski JQ. Epitope map of neurofilament protein domains in cortical and peripheral nervous system Lewy bodies. Am J Pathol. 1991; 139:53–65. [PubMed: 1713021]
36. Sternberger LA, Sternberger NH. Monoclonal antibodies distinguish phosphorylated and non-phosphorylated forms of neurofilaments. Proc Natl Acad Sci USA. 1983; 80:6126–6130. [PubMed: 6577472]
37. Trojanowski JQ, Dickson D. Update on the neuropathological diagnosis of frontotemporal dementia. J Neuropathol Exp Neurol. 2001; 60:1123–1126. [PubMed: 11764085]
38. Yokoo H, Oyama T, Hirato J, Sasaki A, Nakazato Y. A case of Pick's disease with unusual neuronal inclusions. Acta Neuropathol. 1994; 88:267–272. [PubMed: 7528965]

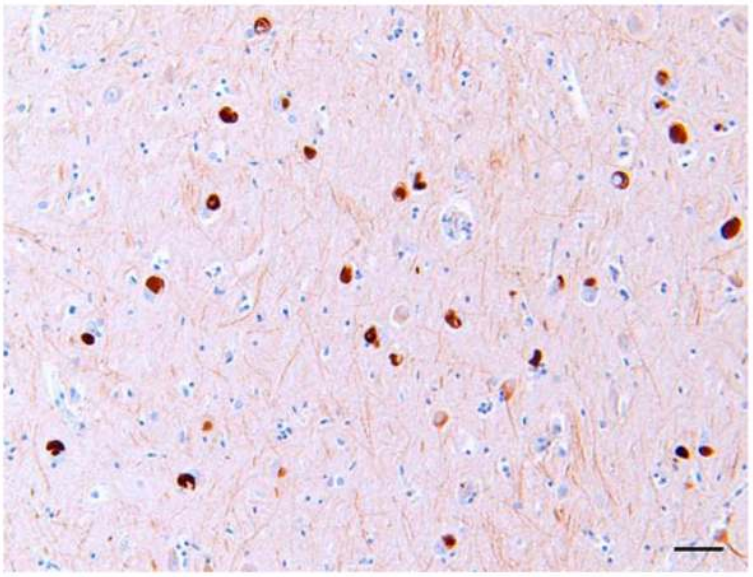

Fig. 1.

Low-power photomicrograph showing neuronal intracytoplasmic inclusions in the subiculum of the temporal lobe of a case of neuronal intermediate filament inclusion disease (NIFID). The inclusions are compact, pleomorphic, and intensely stained. α-Internexin immunohistochemistry; *bar* 100 μm

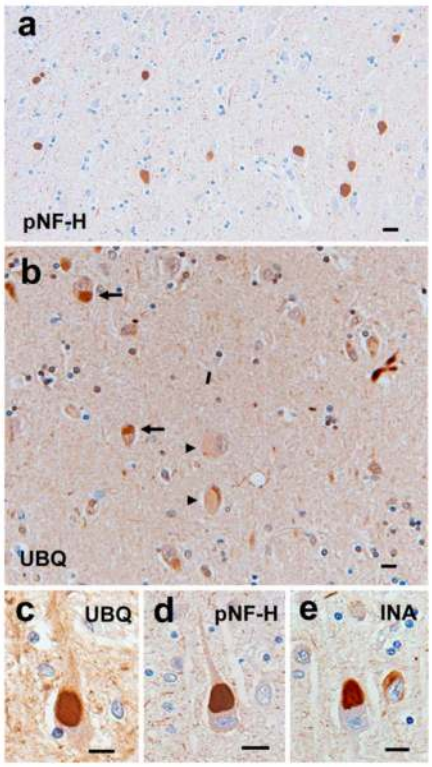

Fig. 2.

Neuronal cytoplasmic inclusions in NIFID contain epitopes of class IV IF proteins. Intracytoplasmic inclusions are labeled by: (**a**), anti-pNF-H (RMO 24) and anti-ubiquitin (*UBQ*) antibodies (**b**) in the subiculum of case NIFID9. Intracytoplasmic inclusions are variably ubiquitinated (**b**; *arrows* Pick body-like inclusions, *arrowheads* pale or unstained inclusions) Ubiquitin immunohistochemistry. **c**–**e** Consecutive sections of a compact neuronal inclusion labeled by ubiquitin (**c**), pNF-H (RMO 24) (**d**), and α-internexin (**e**, *INA*) antibodies (*IF* intermediate filament, *pNF* phosphorylated neurofilament). *Bars* **a** 20 μm; **b**–**e** 10 μm

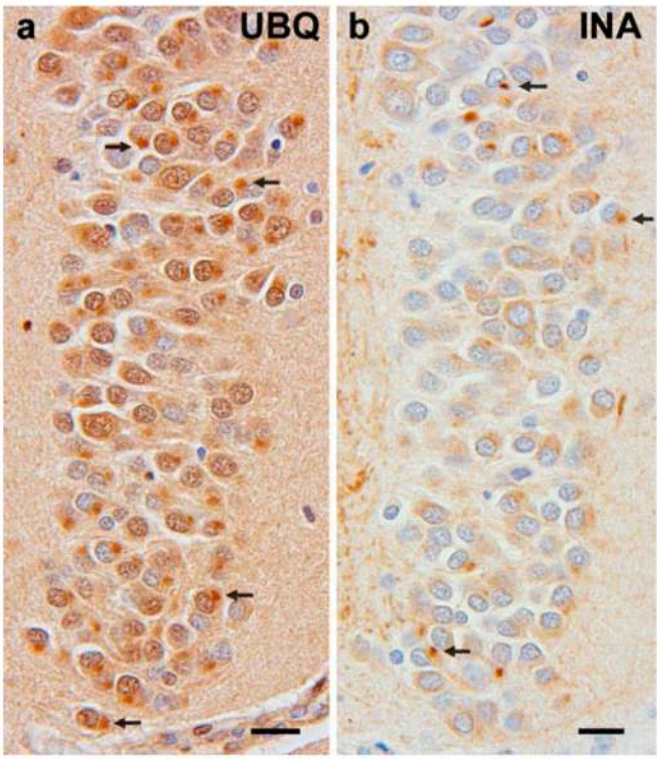

Fig. 3.

a Many neurons in the dentate gyrus contain cytoplasmic ubiquitinated inclusions (*arrows*) in a case of FTLD-MND. **b** In an adjacent section, only a small subset of neuronal inclusions in **a** contains aggregates of α-internexin (*INA*; *arrows*). The α-internexin aggregate (*arrow*) occupies a small fraction of the total volume of the inclusion. Immunohistochemistry for **a** ubiquitin, **b** α-Internexin. *Bars* 10 μm

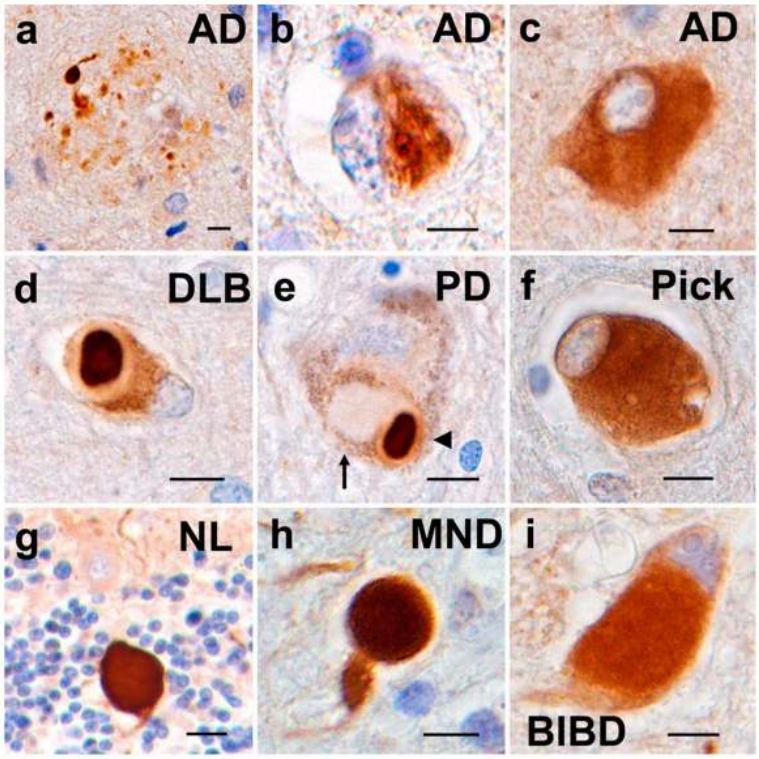

Fig. 4.

Epitopes of α-internexin are present in subsets of neuronal inclusions of neurodegenerative diseases and normal aging. Dystrophic neurites of a neuritic plaque (**a**), a neurofibrillary tangle in the hippocampus (**b**), and a swollen achromatic neuron in the frontal lobe of a case of AD (**c**) contain epitopes of α-internexin. The core regions of a subset of Lewy bodies in DLB (**d**) and PD (**e**) contain intense α-internexin immunoreactivity. A neuromelanin-containing neuron contains a classical Lewy body with an intensely stained core and lightly stained ring (*arrowhead*), and a pale body is unstained (*arrow*; **e**) within the same cell. A swollen achromatic neuron or "Pick cell" in the frontal lobe of a case of Pick's disease (**f**) is immunostained. A swollen axon is present in the granule cell layer of the cerebellum of a normal (*NL*) aged subject (**g**), while in the same field a Purkinje cell is unstained. A swollen axon and axonal spheroid in the medulla of a case of MND contain α-internexin epitopes (**h**). A swollen achromatic neuron in the medulla of a case of BIBD contains diffuse α-internexin staining (**i**) (*AD* Alzheimer's disease, *DLB* dementia with Lewy bodies, *PD* Parkinson's disease, *MND* motor neuron disease, *BIBD* basophilic inclusion body disease). α-Internexin immunohistochemistry; *bars* 10 μm

Table 1

Summary of demographic information of cases (*NL* neuropathologically normal, *NIFID* neuronal intermediate filament inclusion disease, *MND* motor neuron disease, *MNDD* MND with dementia, *FTLD* frontotemporal lobar degeneration, *FTLD+MND* FTLD with MND-type inclusions, *BIBD* basophilic inclusion body disease, *AD* Alzheimer's disease, *PICK* Pick's disease, *CBD* corticobasal degeneration, *PSP* progressive supranuclear palsy, *PD* Parkinson's disease, *DLB* dementia with Lewy bodies, *MSA* multiple system atrophy, *PMI* post-mortem interval, *n/a* not available)

Case	Sex	Age at onset (years)	Duration (years)	Age at death (years)	Brain weight (g)	PMI (hours)	Ref.
NIFID-1	F	52	2.7	54	813	24	[4]
NIFID-2	F	38	3	41	904	15	[4]
NIFID-3	F	23	5	28	860	n/a	[4]
NIFID-4	M	47	3	50	1,200	n/a	[4]
NIFID-5	M	39	3.5	43	950	n/a	[12]
NIFID-6	M	32	3	35	n/a	n/a	[12]
NIFID-7	M	56	4	60	1,250	24	[2]
NIFID-8	M	48	4	52	1,310	24	[15]
NIFID-9	M	48	13	61	850	2	[38]
NIFID-10	F	25	4	29	710	n/a	
Mean		40.8	4.5	45.3	983	17.8	
(range)		(23–56)	(2.7–13)	(28–61)	(710–1,310)	(2–24)	
NL-1	M			43	1,545	31	
NL-2	M			49	1,300	5	
NL-3	M			62	1,360	5	
NL-4	M			65	1,346	26	
Mean				57	1,388	17	
(range)				(43–65)	(1,300–1,545)	(5–31)	
MND-1	F	85	2	87	1,120	31	
MND-2	M	n/a	n/a	38	1,406	5	
MND-3	F	44	10	54	1,359	12	
MND-4	F	66	7	73	1,520	17	
MND-5	F	62	2	64	1,166	4	
MNDD-1	F	70	3	73	1,120	12	
MNDD-2	F	55	1	56	1,190	8	

Case	Sex	Age at onset (years)	Duration (years)	Age at death (years)	Brain weight (g)	PMI (hours)	Ref.
MNDD-3	M	44	2	46	1,496	10	
FTLD+MND-1	M	52	2	54	1,536	4	
FTLD+MND-2	M	47	7	54	985	5	
FTLD+MND-3	F	52	11	63	990	6	
FTLD+MND-4	F	n/a	n/a	54	813	12	
FTLD+MND-5	F	45	4	49	1,182	6	
FTLD+MND-6	M	63	8	71	1,390	17	
FTLD+MND-7	M	55	2	57	1,200	9	
FTLD+MND-8	F	68	8	76	934	14	
FTLD-1	F	56	6	62	1,010	17	
FTLD-2	M	89	3	92	1,341	13	
FTLD-3	F	n/a	n/a	68	1,022	6	
FTLD-4	M	46	10	56	1,200	22	
BIBD-1	M	29	10	39	1,070	n/a	
BIBD-2	M	54	6	60	1,040	4	
Tauopathies							
AD-1	F	62	11	73	947	11	
AD-2	M	43	9	52	1,219	20	
AD-3	F	53	10	63	1,119	14	
AD-4	F	50	7	57	1,314	7	
PICK-1	M	71	3	74	1,240	15	
PICK–2	M	69	7	76	1,199	8	
CBD	F	64	7	71	1,041	7	
PSP	F	68	11	79	1,064	17	
α-Synucleinopathies							
PD-1	F	49	26	75	1,187	6	
PD-2	F	60	12	72	1,236	6	
PD-3	M	76	8	84	1,543	6	
PD-4	M	54	21	75	1,258	6	
DLB-1	M	72	9	81	1,212	8	
DLB-2	F	57	15	72	1,118	5	

Acta Neuropathol. Author manuscript; available in PMC 2012 December 07.

Case	Sex	Age at onset (years)	Duration (years)	Age at death (years)	Brain weight (g)	PMI (hours)	Ref.
DLB-3	M	73	8	81	1,380	20	
DLB-4	M	72	5	77	1,307	14	
MSA-1	M	45	9	54	1,491	15	
MSA-2	M	53	2	55	1,400	7	

Table 2

Primary antibodies used for immunohistochemical analysis (*NF* neurofilament, *Pind* phosphate independent epitope in NF-M or NFH, *P–* non-phosphorylated, *P+*/++/+ + + mildly/moderately/highly phosphorylated, *MPR* multiphosphorylation repeat in NF-M or NF-H, *NA* not applicable, *1–36/1–39* number of amino acids in peptides that model sequences in the MPRs of NF-H and NF-M [27])

Antibody	Dilution	Epitope	Reference/source
Type III intermediate filament proteins			
Vimentin	×1,000	NA	Sigma, St Louis, MO
Desmin	×1,000	NA	Boehringer Mannheim, Indianapolis, IN
Glial fibrillary acidic protein	×1,000	NA	DAKO, Carpinteria, CA
Peripherin	×100	C terminus	Chemicon, Temecula, CA
Type IV intermediate filament proteins			
RMO 24	×1	NF-H, P+++/tail within MPR	[32]
RMO 34	×100	NF-H, P++/ tail within MPR	[19]
RMO 217	×1	NF-H, P++/tail within MPR	[32]
TA51	×1,000	NF-H, P+/tail within MPR	[32]
HO21	×1	NF-H, P+/1–36 within MPR	[27]
RMd 09	×1	NF-H, P–/tail within MPR	[32]
RMO 14	×1	NF-H, Pind	[35]
RMd 020	×1	NF-H, P–	[35]
RMO 304	×1	NF-H, P–	[35]
SMI 31	×1,000	NF-H and NF-M, P++	Sternberger, Lutherville, MA
SMI 32	×1,000	NF-H, P–	Sternberger
RMO 55	×1	NF-M, P++	[35]
RMO 281	×1	NF-M, P++	[35]
RMO 100	×1	NF-M, P+/1–39 within MPR	[27]
RMO 189	×1	NF-M, Pind/core	[35]
RMd 020	×1	NF-M, Pind	[27]
NFL	×1,000	NF-L, Pind/tail	[6]
NR4	×1,000	NF-L	Sigma
α-Internexin	×400	NA	Zymed, San Francisco, CA
Type V intermediate filament protein			
Lamin A/C	×100	NA	Santa Cruz Biotechnology, Santa Cruz, CA
Type VI intermediate filament protein			
Nestin	×100	NA	(gift from Dr. C. Messam)
Other proteins			
Actin	×100	NA	Sigma
α-Tubulin	×100	NA	Sigma
β-Tubulin	×100	NA	Serotec, Raleigh, NC
PHF1	×500	Tau, P++	(gift from Dr. P. Davies)
Syn 303	×1,000	α-Synuclein	[10]
PRK8	×100	Parkin/second ring domain	[31]
PRK28	×100	Parkin/second ring domain	[31]

Antibody	Dilution	Epitope	Reference/source
KAM-SA100	×100	DJ-1	Stressgen, Victoria, BC, Canada
1C2	×1,000	Polyglutamine	Chemicon
SD-G6	×100	Superoxide dismutase	Sigma
Ubiquitin	×1,000	NA	DAKO

Table 3

Summary immuno-histochemical profile of neuronal cytoplasmic inclusions in NIFID (+++ Inclusions intensely immunoreactive, ++ moderately,+ lightly stained, *0* unstained)

Antibody	Epitope	Neuronal inclusion
Type III intermediate filament proteins		
Vimentin	NA	0
Desmin	NA	0
Glial fibrillary acidic protein	NA	0
Peripherin	C terminus	0
Type IV intermediate filament proteins		
RMO 24	NF-H, P+++/tail within MPR	+++
RMO 34	NF-H, P++/tail within MPR	++
RMO 217	NF-H, P++/tail within MPR	++
TA51	NF-H, P+/tail within MPR	++
HO21	NF-H, P+/1–36 within MPR	++
RMd 09	NF-H, P–/tail within MPR	++
RMO 14	NF-H, Pind	+
RMd 020	NF-H, P–	++
RMO 304	NF-H, P–	+
SMI 31	NF-H and NF-M, P++	+++
SMI 32	NF-H, P–	++
RMO 55	NF-M, P++	+
RMO 281	NF-M, P++	+
RMO 100	NF-M, P+/1–39 within MPR	+
RMO 189	NF-M, Pind/rod	++
RMd 020	NF-M, Pind	++
NFL	NF-L, Pind/tail	+
NR4	NF-L	++
α-Internexin	NA	+++
Type V intermediate filament protein		
Lamin A/C	NA	0
Type VI intermediate filament protein		
Nestin	NA	0
Other proteins		
Actin	NA	
α-Tubulin	NA	0
β-Tubulin	NA	0
PHF1	Tau, P++	0
Syn 303	α-Synuclein	0
PRK8	Parkin/second ring domain	0
PRK28	Parkin/second ring domain	0
KAM-SA100	DJ-1	0

Antibody	Epitope	Neuronal inclusion
1C2	Polyglutamine	0
SD-G6	Superoxide dismutase	0
Ubiquitin	NA	0 to +++

Table 4

Specificity of α-internexin immunohistochemistry in neurodegenerative diseases

Diagnosis	Neuronal cytoplasmic inclusions	Dystrophic neurites, axonal swellings or spheroids	Glial inclu-sions
Neuronal intermediate filamentopathy			
NIFID	10/10	10/10	0/10
Non-Tau FTD/MND			
MND	0/5	3/5[a]	0/5
MNDD	0/3	2/3[a]	0/3
FTLD+MND	2/8 (~5%)[b]	5/8[a]	0/8
FTLD	0/4	3/4[a]	0/4
BIBD	0/2	2/2[a]	0/2
Tauopathies			
AD	2/4 (~5%)[c]	4/4 (~5%)[c]	0/4
PICK	0/2	0/2	0/1
CBD	0/1	1/1	0/1
PSP	0/1	0/1	0/1
α-Synucleinopathies			
PD	4/4(~5%)[d]	1/4	0/4
DLB	4/4(~5%)[d]	0/4	0/4
MSA	0/2	0/2	0/2
Normal aged controls	0/5	1/5	0/4

[a] In cases of non-tau frontotemporal dementias (FTDs) and MND, α-internexin-positive structures within neuronal processes were rare and there was a small subset (~1%) of ubiquitinated dystrophic neurites, swollen axons and spheroids

[b] A subset of inclusions in the granule neurons of the dentate gyrus in FTLD-MND

[c] A subset (~5%) of tau- and ubiquitin-positive neurofibrillary tangles and the dystrophic neurites of a subset of neuritic plaques (~5%) was labeled by anti-α-internexin antibodies

[d] In PD and DLB α-internexin-positive Lewy bodies were seen in all cases but these represented a small fraction (~5%) of all Lewy bodies