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# Ubiquitin Immunohistochemistry Suggests Classic Motor Neuron Disease, Motor Neuron Disease With Dementia, and Frontotemporal Dementia of the Motor Neuron Disease Type Represent a Clinicopathologic Spectrum

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**Abstract**

One of the characteristic pathologic changes in classic motor neuron disease (MND) is the presence of ubiquitin-immunoreactive (ub-ir) inclusions in the cytoplasm of lower motor neurons. In addition, cases of MND with dementia (MND-d) also have ub-ir neuronal cytoplasmic inclusions and dystrophic neurites in extramotor neocortex and hippocampus. Although this extramotor pathology is a highly sensitive marker for dementia in MND, similar changes are found in a subset of patients with frontotemporal dementia (FTD) with no motor symptoms (FTD-MND type). The purpose of this study is to more fully describe and compare the pattern of ub-ir pathology in these 3 conditions. We performed ubiquitin immunohistochemistry on postmortem tissue, representing a wide range of neuroanatomic structures, in cases of classic MND (n = 20), MND-d (n = 15), and FTD-MND type (n = 15). We found the variety of morphologies and the anatomic distribution of ub-ir pathology to be greater than previously documented. Moreover, the degree of overlap suggests that MND, MND-d, and FTD-MND type represent a spectrum of clinical disease with a common pathologic substrate. The only finding restricted to a specific subgroup of patients was the presence of ub-ir neuronal intranuclear inclusions in some cases of familial FTD.

**Key Words:** Amyotrophic lateral sclerosis, Frontotemporal dementia, Motor neuron disease, Neuronal inclusions, Ubiquitin.

## INTRODUCTION

The neuropathologic features of the classic amyotrophic lateral sclerosis (ALS) form of motor neuron disease (MND) include degeneration of upper and lower motor neurons (LMN) with corresponding degeneration of the corticospinal tracts and motor nerve roots (1). Ubiquitin-immunoreactive (ub-ir) cytoplasmic inclusions are often found in the surviving LMN and, less frequently, in upper motor neurons (2–4). The most characteristic types of inclusions are "filamentous skeins" and spherical "Lewy body-like" inclusions, which are felt to be highly sensitive and specific pathologic markers for MND (2–4). Dementia is an uncommon but well-recognized complication of MND (5). Dementia associated with MND is usually of the frontotemporal type, and the onset may precede, follow, or coincide with motor symptoms. In addition to pyramidal system degeneration, neuropathologic examination of patients with MND and dementia (MND-d) usually demonstrates ub-ir (tau-, synuclein-negative) dystrophic neurites and neuronal cytoplasmic inclusions in layer II neocortex and the dentate granule cells of the hippocampus (6). Although several studies have shown this extramotor pathology to be a highly sensitive marker of dementia in MND (6–10), similar cortical pathology is found in a subset of patients with frontotemporal dementia (FTD) without motor symptoms (11, 12). Such cases have been referred to as MND inclusion dementia (MNDID) or FTD-MND type. The relationship among classic MND, MND-d, and FTD-MND type is uncertain; however, some believe that they represent a clinicopathologic spectrum of disease (2, 4, 12). This concept is supported by clinical studies that have demonstrated subclinical pyramidal system involvement is a significant proportion of patients presenting with FTD (13) and mild cognitive deficits in many patients with MND (14–18). In addition, a number of families have been described in which different members may develop MND, MND-d, or FTD (12, 19).

It has long been recognized that a variety of subcortical regions, in addition to the pyramidal system, may show nonspecific degenerative changes in MND (5). Recently, a small number of reports have used ubiquitin immunohistochemistry to demonstrate more specific pathologic changes in these subcortical regions (8, 20–25). Most of these studies have focused on the pathology in a single anatomic region and a single disease. The purpose of the present study is to more fully catalogue the morphology and anatomic distribution of ub-ir pathology in classic MND, MND-d, and FTD-MND type and to compare the pathologic phenotypes of these 3 conditions.

## MATERIALS AND METHODS

### Study Subjects

We restricted our study to subjects who had been diagnosed with the most common, classic form of sporadic MND (that is, ALS) (1). We excluded patients with other clinical

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variants (such as primary lateral sclerosis, progressive muscular atrophy, and progressive bulbar palsy), familial cases with known gene mutations, and those with more unusual causes of MND (such as, Guamanian disease, postpolio syndrome, and spinal muscular atrophy).

We examined postmortem material from 20 patients with classic MND (without dementia), 15 patients with MND-d, 15 with FTD-MND type, and 10 neurologically normal, aged control subjects (Table 1). Clinical classification was based on retrospective chart review. All neurologic subjects had been evaluated in the Clinic for Alzheimer Disease and Related Disorders at Vancouver Coastal Health/University of British Columbia, the Vancouver General Hospital ALS clinic, or both.

Those classified as classic MND fulfilled current clinical diagnostic criteria for ALS (1). Although most had not undergone formal neuropsychologic testing, the examining neurologist had recorded the opinion that they were cognitively intact within the year before death. They included 11 men and 9 women, with a mean age at disease onset of 65 years (range, 42–78 years) and mean age at death of 67 years (range, 43–83 years). Two had a family history of MND, one with an autosomal-dominant inheritance pattern and one with only an affected daughter. No mutation of the superoxide dismutase gene had been found in either case.

Subjects with MND-d also fulfilled diagnostic criteria for ALS. In addition, they were documented to have cognitive deficits of sufficient scope and severity to warrant a diagnosis of dementia. In most cases, the dementia was said to have prominent frontal features; however, a detailed description and the results of neuropsychologic testing were not available in all cases. There were 9 male and 6 female subjects in this group. The mean age at which neurologic symptoms onset was 59 years (range, 39–77 years) and mean age at death was 63 years (range, 41–81 years). Seven subjects presented with dementia, 5 presented with motor symptoms, and 3 developed both simultaneously. Eight patients with MND-d had a family history. For 2 patients, multiple family members in each generation were affected, suggesting an autosomal-dominant inheritance pattern; however, the clinical phenotype varied and included MND-d, MND only, or dementia only. Six other subjects with MND-d had a family history in which the pattern of inheritance was less clear, with at least one other family member having MND-d (2 cases), MND or dementia (one case), MND only (one case), or dementia only (2 cases).

All of those classified as FTD-MND type had received a clinical diagnosis of dementia without any record of pyramidal system abnormality. Most of the more recent cases fulfilled current diagnostic criteria for FTD (26). Cases that had been evaluated before the recognition of FTD as a clinical syndrome were often noted to have evidence of prominent frontal lobe dysfunction. Neuropathologic examination demonstrated the characteristic changes of MND type ub-ir inclusions in extramotor cortex (6, 27). Pathologic changes, characteristic of other causes of FTD (28), were excluded. This group included 8 male and 7 female subjects. The mean age at disease onset was 60 years (range, 50–70 years) and the mean age at death was 68 years (range, 58–82 years). Nine had a family history of FTD-MND with an inheritance pattern usually, suggesting an autosomal-dominant trait with high penetrance. Three of the patients were cousins within the same family and, although each of them had FTD without motor symptoms, some other affected family members had MND-d. Two other subjects within the FTD-MND type group were brothers. Each of the other 4 subjects with familial FTD-MND type was from different families. Most of the families with an autosomal-dominant pattern of MND-d or FTD-MND type had been studied for mutations in the tau gene with none identified.

The subjects in the control group had no documented neurologic illness and had a mean age at death of 69 years (range, 60–80 years).

## Histochemistry and Immunohistochemistry

Formalin fixed, paraffin-embedded tissue sections were stained using hematoxylin and eosin (H&E), Gallyas silver, and modified Bielschowsky silver methods. All immunohistochemistry, with the exception of the 1C2 antibody, was performed using the Ventana ES automated staining system (Ventana, Tucson, AZ). The primary antibodies used recognized the following proteins: ubiquitin (anti-ubiquitin; DAKO, Glostrup, Denmark; 1:500, after microwave antigen retrieval), nonphosphorylated neurofilament (NF) (antineurofilament protein; DAKO; 1:2000, after protease digestion), phosphorylated neurofilament (pNF) (SMI 31; Sternberger, Lutherville, MD; 1:8000, after protease digestion), hyperphosphorylated tau (AT-8; Innogenetics, Gent, Belgium; 1:2000 after microwave antigen retrieval and TAU-2; Sigma, Oakville, Canada; 1:1000 with 3 hours initial incubation at room temperature), α-synuclein (anti-α-synuclein; Zymed, San Francisco, CA; 1:10,000 after microwave antigen retrieval), Aβ protein (anti-beta amyloid; DAKO; 1:100 with formic acid pretreatment and initial incubation for 3 hours at room temperature) and polyglutamine containing proteins (1C2; Chemicon, Temecula, CA; 1:1000, 24 hours at room temperature, after formic acid pretreatment).

All available archival tissue blocks from each case were immunostained for ubiquitin. Silver stains and immunohistochemistry using other primary antibodies were performed on sections from selected regions. Ub-ir pathology was assessed in each of the following anatomic regions when available: frontal neocortex, temporal neocortex, parietal neocortex, occipital neocortex, cingulum, insula, hippocampus, caudate nucleus, putamen, globus pallidus, nucleus basalis, thalamus, subthalamus, hypothalamus, substantia nigra, red nucleus, periaqueductal grey matter, superior colliculus, occulomotor nucleus, locus ceruleus, trigeminal motor nucleus, nuclei of the basis pontis, inferior olivary nucleus, dorsal motor nucleus of the vagus, hypoglossal nucleus, cerebellar cortex, dentate nucleus, and anterior grey matter of the spinal cord.

## Quantitation

We decided to use a semiquantitative grading method to rate the severity of the pathologic changes. Although an unbiased stereologic method of counting inclusions would have resulted in data that could be subjected to formal statistical analysis, this was felt to be inappropriate for several reasons. Because many of the cases were obtained retrospectively, we were limited to using the archival material available. This meant that we could not be certain that tissue blocks representing

**TABLE 1.** Clinical Summary of Study Subjects

| Case No. | Sex | MND Onset (years) | Dementia Onset (years) | Death (years) | Family History |
|---|---|---|---|---|---|
| 1 | M | 46 | No | 47 | Autosomal-dominant MND |
| 2 | F | 63 | No | 64 | MND (daughter) |
| 3 | F | NA | No | 72 | No |
| 4 | F | 72 | No | 73 | No |
| 5 | F | 64 | No | 65 | No |
| 6 | M | NA | No | 65 | No |
| 7 | M | 71 | No | 73 | No |
| 8 | M | 78 | No | 79 | No |
| 9 | M | 72 | No | 72 | No |
| 10 | F | 42 | No | 43 | No |
| 11 | M | 75 | No | 75 | No |
| 12 | M | 53 | No | 53 | No |
| 13 | F | 65 | No | 66 | No |
| 14 | M | 57 | No | 57 | No |
| 15 | M | NA | No | 65 | No |
| 16 | M | 53 | No | 56 | No |
| 17 | F | 72 | No | 72 | No |
| 18 | F | 58 | No | 83 | No |
| 19 | F | 73 | No | 82 | No |
| 20 | M | 71 | No | 73 | No |
| Mean | 11 M:9 F | ~65 | No | 67 | |
| 21 | F | 56 | 54 | 58 | Autosomal-dominant MND-d, MND, or dementia |
| 22 | F | 39 | 39 | 41 | Autosomal-dominant MND-d, MND, or dementia |
| 23 | M | 79 | 77 | 81 | MND-d (twin brother, sister) |
| 24 | M | 73 | 63 | 74 | MND-d (2 brothers) |
| 25 | M | 60 | 59 | 62 | MND (brother), dementia (mother) |
| 26 | M | 55 | 56 | 56 | MND (father, 2 aunts) |
| 27 | F | 60 | 56 | 64 | Dementia (father, sister, aunt) |
| 28 | F | 65 | 66 | 68 | Dementia (mother) |
| 29 | M | 59 | 56 | 60 | No |
| 30 | M | 48 | 48 | 50 | Adopted |
| 31 | M | 53 | 53 | 54 | No |
| 32 | F | 48 | 49 | 50 | No |
| 33 | M | 72 | 73 | 76 | No |
| 34 | M | 72 | 69 | 77 | No |
| 35 | F | 70 | 71 | 76 | No |
| Mean | 9 M:6 F | 60 | 59 | 63 | |
| 36 | M | No | 55 | 69 | Autosomal-dominant FTD-MND type or MND-d* |
| 37 | M | No | 61 | 66 | Autosomal-dominant FTD-MND type or MND-d* |
| 38 | F | No | 55 | 60 | Autosomal-dominant FTD-MND type or MND-d* |
| 39 | F | No | 56 | 61 | Autosomal-dominant FTD-MND type |

*(continued on next page)*

**TABLE 1.** *(continued)* Clinical Summary of Study Subjects

| Case No. | Sex | MND Onset (years) | Dementia Onset (years) | Death (years) | Family History |
|---|---|---|---|---|---|
| 40 | F | No | 50 | 58 | Autosomal-dominant FTD-MND type |
| 41 | M | No | 55 | 63 | Autosomal-dominant FTD-MND type |
| 42 | F | No | <66 | 71 | Autosomal-dominant FTD-MND type |
| 43 | M | No | 66 | 71 | FTD-MND type (brother, cousin, nephew)† |
| 44 | M | No | 58 | 66 | FTD-MND type (brother, cousin, nephew)† |
| 45 | F | No | 55 | 70 | No |
| 46 | F | No | NA | 82 | No |
| 47 | M | No | NA | 71 | No |
| 48 | M | No | 70 | 71 | No |
| 49 | M | No | 69 | 72 | No |
| 50 | F | No | 65 | 70 | No |
| Mean | 8 M:7 F | No | ~60 | 68 | |

*, Cousins within same family.
†, Brothers in the same family.
MND, motor neuron disease; MND-d, motor neuron disease with dementia; FTD-MND type, frontotemporal dementia with MND type inclusions; NA, information not available.

a particular anatomic region were taken from the exact same site or in the same orientation in all cases. Furthermore, many of the regions we assessed were relatively small structures, containing few neurons per sections, making accurate counts difficult (such as, cranial nerve nuclei). Finally, in addition to neuronal inclusions, we wanted to evaluate dystrophic neurite pathology, a change that is not amenable to numeric counting. Recognizing the limitations these factors placed on the accuracy of the data we would collect, we decided that a semiquantitative method was most appropriate. A similar approach has been used in many of the previous studies in this field (4, 6, 8–10, 12, 20, 24, 27).

Ub-ir pathologic changes were graded using the following semiquantitative system: −, none; +, rare (pathologic lesions were only found in a small proportion of 20× microscopic fields examined); ++, mild (a small number of pathologic structures were present in most 20× fields); +++, moderate (moderate numbers of pathologic structures were present in virtually every 20× field examined); and ++++, severe (large numbers of pathologic structures were present in virtually every field 20× examined). The reliability and reproducibility of this grading system has been demonstrated previously (29). The final score was an estimated average for the entire anatomic region being assessed.

## RESULTS

Silver stains and immunohistochemistry for tau, α-synuclein, NF, pNF, and Aβ protein demonstrated pathologic changes in some study subjects of each group, including some controls. The number and type of lesions and their anatomic distribution were similar to what has previously been reported for neurologically normal individuals in this age group (30–32). Diffuse and neuritic senile plaques, neurofibrillary tangles, neuropil threads, dystrophic axons, axonal spheroids, and Lewy bodies (in one control case) were present in some individuals (not illustrated); however, the numeric frequency and anatomic distribution was never sufficient to fulfill any current disease-specific neuropathologic diagnostic criteria. In addition to these lesions, ubiquitin immunohistochemistry demonstrated some other patterns of staining that were seen in all groups of study subjects and control cases with equal frequency. These included 1) delicate, diffuse cytoplasmic granules in some neurons, especially in the thalamus, hypothalamus, and substantia nigra; 2) coarse, chunky cytoplasmic granules in neurons of the inferior olivary nucleus; 3) thin, straight cytoplasmic filaments in large neurons of the caudate and putamen; 4) small, dot-like structures in white matter; and 5) small numbers of dystrophic neurites in the upper layers of neocortex (not illustrated). These changes have been described previously in neurologically normal, aged individuals and were not considered to be related to the disease processes being studied unless their number or frequency was markedly increased (31, 33).

In each of the 3 groups of study subjects, ubiquitin immunohistochemistry demonstrated pathologic changes in excess of what was seen in controls, involving a wide range of anatomic sites. These neuronal inclusions were of several different morphologic types, each with a characteristic anatomic distribution. No glial inclusions were identified. The proportion of cases showing a particular pathologic change and the severity of the change differed between the groups (Tables 2–6).

### Patterns of ub-ir Pathology

#### Dense Neuronal Cytoplasmic Inclusions and Dystrophic Neurites in Extramotor Cerebral Cortex

Cytoplasmic inclusions were most common in small neurons of layer II neocortex (Fig. 1A, B) and dentate granule



 

**TABLE 2.** Extramotor ub-ir Cerebral Cortical Pathology

| | Neocortical Neurites | Neocortical Inclusions | Hippocampal Inclusions |
|---|---|---|---|
| FTD-MND type (15/15) | +++ | +++ | +++ |
| MND-d (15/15) | ++ | ++ | +++ |
| MND (9/20) | + | ++ | ++ |

The score is the mean for all affected cases in the group.

cells of the hippocampus (Fig. 1C, D). They tended to be positioned immediately adjacent to the nucleus and varied in shape to include oval, crescentic, and annular forms. Short, twisted dystrophic neurites were also present in layer II neocortex, in numbers far exceeding the rare examples seen in control subjects (Fig. 1A, B). The neocortical pathology tended to show an anterior-to-posterior gradient (frontal and temporal > insula and cingulum > parietal > occipital).

This cerebral cortical pathology was present in all cases of FTD-MND type (by definition), and all MND-d. FTD-MND type cases tended to have more severe neocortical involvement than MND-d, whereas the hippocampal involvement was similar between the 2 groups (Table 2). Nine of the 20 (45%) cases of classic MND had similar cortical pathology; however, it tended to be slightly less severe than in either of the other 2 groups.

## Dense Neuronal Cytoplasmic Inclusions and Dystrophic Neurites in Subcortical Grey Matter

Dystrophic neurites and dense neuronal cytoplasmic inclusions similar to those seen in the cerebral cortex were also found in many subcortical grey matter structures. The striatum was involved in all cases of FTD-MND type and MND-d, with the caudate tending to be more severely affected than the putamen (Table 3; Fig. 2A–C). Cases of FTD-MND type tended to have more severe pathology than MND-d. Eight cases of classic MND (40%) had similar pathology but usually to a milder degree. Seven of the MND cases with striatal pathology also had cerebral cortical involvement (see previously).

Small numbers of dense cytoplasmic inclusions were also found in a variety of other subcortical regions in all 3 patient groups (Table 4). Cases of FTD-MND type had the highest frequency of involvement, with the dorsomedial and anterior nuclei of the thalamus being affected in slightly more than half the cases and the globus pallidus, periaqueductal grey, and superior colliculus each involved in 25% to 30% of cases. The same areas were affected in a smaller proportion of cases of MND-d and MND. The nucleus basalis and red nucleus were rarely affected, and there were many areas examined that did not show any of this type of inclusion in any patients (subthalamic nucleus, hypothalamus, locus ceruleus, cranial nerve nuclei, pontine nuclei, inferior olive, cerebellar cortex, and cerebellar dentate nucleus).

**TABLE 3.** Striatal ub-ir Pathology

| | Caudate Neurites | Caudate Inclusions | Putamen Neurites | Putamen Inclusions |
|---|---|---|---|---|
| FTD-MND type (15/15) | +++ | ++ | ++ | ++ |
| MND-d (15/15) | ++ | ++ | + | + |
| MND (8/20) | + | + | + | + |

The score is the mean for all affected cases in the group.

## Filamentous Skeins and Lewy Body-Like Cytoplasmic Inclusions in Lower Pyramidal Motor Neurons

In cases of classic MND, ub-ir cytoplasmic inclusions were present in LMN of the spinal cord in 18 of 20 (90%) cases and in the brainstem (hypoglossal and/or trigeminal motor nucleus) in 10 of 17 (59%) (Table 5; Fig. 2D–F). Cases of MND-d had a similar frequency with spinal cord involvement in 10 of 11 (91%) and brainstem involvement in 10 of 13 (77%). Filamentous skeins (Fig. 2D, E) were more frequent than Lewy body-like inclusions (Fig. 2F). In some cases of MND and MND-d, these inclusions were numerous and affected most of the remaining lower motor neurons. Unfortunately, only 3 cases of FTD-MND type had spinal cord tissue available for examination. One of these had small numbers of both filamentous skeins and Lewy body-like inclusions affecting a normal complement of anterior horn motor neurons. Brainstem motor nuclei were involved in 4 of 14 (27%) cases of FTD-MND type. Filamentous skeins were more numerous and present in all affected cases of FTD-MND type, whereas Lewy body-like inclusions were only present in 2 cases.

## Filamentous Skeins and Lewy Body-Like Cytoplasmic Inclusions in Substantia Nigra

Filamentous skeins and Lewy body-like inclusions were also present in the cytoplasm of pigmented neurons of the substantia nigra (Fig. 2G, H). Skeins were more common and present in all affected cases (Table 6). Lewy body-like inclusions were most common in cases with MND-d, being present in 7 of 15 (47%) affected cases compared with 3 of 18 (17%) affected classic MND and only 1 of 14 (7%) affected FTD-MND type. Small numbers of filamentous skeins were occasionally found in other anatomic regions, including the thalamus, red nucleus, and periaqueductal grey matter.

## Tangled Filaments in Inferior Olivary Neurons

Tangled masses of ub-ir coarse filaments were present in the cytoplasm of neurons in the inferior olivary nucleus in 7 of 18 (39%) cases with classic MND, 3 of 14 (21%) with MND-d, and 4 of 15 (27%) with FTD-MND type (Fig. 2I). These had a distinct morphology and were easily distinguished from the more chunky, crystalline granules that were commonly seen in both neurologic subjects and controls (33). The filamentous inclusions tended to be more numerous in affected cases of FTD-MND type (mean score = ++) compared with MND and MND-d (mean score = +, each).

## Neuronal Intranuclear Inclusions

Ub-ir inclusions were present in the nuclei of small neurons in 7 of 15 cases of FTD-MND type and one case of

**TABLE 4.** Dense ub-ir Cytoplasmic Inclusions in Subcortical Structures

| | Caud | Put | Thal | Gp | Pag | Sup Coll | Nuc Bas | Red Nuc |
|---|---|---|---|---|---|---|---|---|
| FTD-MND type | 12/12++ | 15/15++ | 6/11+ | 3/12+ | 3/12+ | 3/11+ | 1/8++ | 1/11+ |
| MND-d | 13/13++ | 14/15+ | 5/14+ | 3/12+ | 1/12+ | 2/12+ | 1/8++ | 2/14+ |
| MND | 5/18+ | 5/18+ | 3/15+ | 1/11+ | 3/18+ | 3/16+ | 1/7++ | 0/18 |

The score is the mean for all affected cases in the group.

caud, caudate; put, putamen; thal, thalamus; gp, globus pallidus; pag, periaqueductal grey; sup coll, superior colliculus; nuc bas, nucleus basalis; red nuc, red nucleus.

MND-d (Fig. 3). All affected cases had a strong familial history of neurologic disease, usually suggesting an autosomal-dominant trait with high penetrance. No neuronal intranuclear inclusions (NII) were seen in any cases of familial or sporadic classic MND. NII were most often lentiform in shape and frequently distorted the nuclear membrane (Fig. 3, inset). Less commonly, they appeared as straight or curved rods. Occasional round examples were interpreted as one of the aforementioned types seen in cross-section. NII showed no immunoreactivity for polyglutamines.

In all affected cases of FTD-MND type, NII were frequent in small neurons of the superficial layers of cerebral cortex and the striatum. In a few cases, they were also present in smaller numbers in the globus pallidus, thalamus, periaqueductal grey matter, and dentate granule cells of the hippocampus. One case of MND-d had rare NII, restricted to the dentate granule cells of the hippocampus.

## DISCUSSION

The use of ubiquitin immunohistochemistry has greatly enhanced our understanding of the neuropathology of MND and MND-d (2–4) and has allowed for the recognition of FTD-MND type as a specific subtype of FTD (12). In addition to showing changes that are felt to be characteristic of each condition, a number of recent studies have used this technique to identify previously unrecognized patterns of pathology. The sensitivity, specificity, and clinical correlation of these newly described changes have yet to be established. The purposes of this study were 1) to more fully define the morphology and anatomic distribution of ub-ir neuropathologic changes in MND, MND-d, and FTD-MND type; and 2) to compare the patterns of pathology between these conditions to clarify whether they are distinct entities or represent a clinicopathologic spectrum of disease.

In classic MND, ub-ir cytoplasmic inclusions (filamentous skeins and Lewy body-like inclusions) are found in LMN of the brainstem and spinal cord and are felt to be a highly sensitive and specific marker for this disease (2–4). In agreement with this, we found inclusions in the vast majority of our cases of classic MND (90%) and MND-d (91%), with the spinal cord being affected more frequently than the brainstem. There is little published information on LMN involvement in FTD-MND type, and the reported frequency varies widely, from "rare" to present in 100% of cases (2, 11, 12). One of the problems in establishing the true frequency is the fact that the spinal cord is often not examined in autopsies on cases without a history of motor symptoms. Although the assessment of LMN in our FTD-MND type cases was usually restricted to the brainstem, we did identify cytoplasmic inclusions in 27% of cases. It seems likely that this is an underestimate of the true frequency of LMN involvement and suggests that a significant proportion of patients with FTD-MND type have inclusions of a type previously felt to be specific for MND. The clinical relevance of this finding is supported by a recent study that found that 50% of unselected patients with FTD, without a prior diagnosis of ALS, either met clinical diagnostic criteria for ALS (14%) or had milder pyramidal motor symptoms (36%) (13).

It is well recognized that some patients with otherwise typical MND also develop dementia (MND-d), often with prominent features of frontotemporal lobe dysfunction (FTD) (5). Although the exact incidence is uncertain, dementia is generally thought to be an uncommon complication, occurring in approximately 5% of MND cases (5). Okamoto et al were first to describe a unique pattern of neuropathology in such cases, consisting of ub-ir (tau-, synuclein-negative) neurites and neuronal cytoplasmic inclusions in layer II neocortex and dentate granule layer of the hippocampus (27). Subsequent studies confirmed that these extramotor ub-ir inclusions are a consistent finding in MND-d (6–10). The sensitivity of this

**TABLE 5.** Ub-ir Cytoplasmic Inclusions in Lower Pyramidal Motor Neurons

| | Spinal cord | cn XII | cn V |
|---|---|---|---|
| MND | 18/20++ | 9/16++ | 5/12+++ |
| MND-d | 10/11++ | 7/13++ | 8/12+ |
| FTD-MND type | 1/3+ | 4/14++ | 1/5+++ |

The score is the mean for all affected cases in the group and includes both filamentous skeins and Lewy body-like inclusions.

cn XII, hypoglossal nucleus; cn V, motor nucleus of the trigeminal nerve.

**TABLE 6.** Ub-ir Cytoplasmic Inclusions in Pigmented Neurons of Substantia Nigra

| | Filamentous Skeins | Lewy Body-Like Inclusions | Inclusions (Either Type) |
|---|---|---|---|
| MND | 7/18++ | 3/18+ | 9/18++ |
| MND-d | 11/15++ | 7/15++ | 13/15++ |
| FTD-MND type | 12/14+ | 1/14++ | 12/14+ |

The score is the mean for all affected cases in the group.

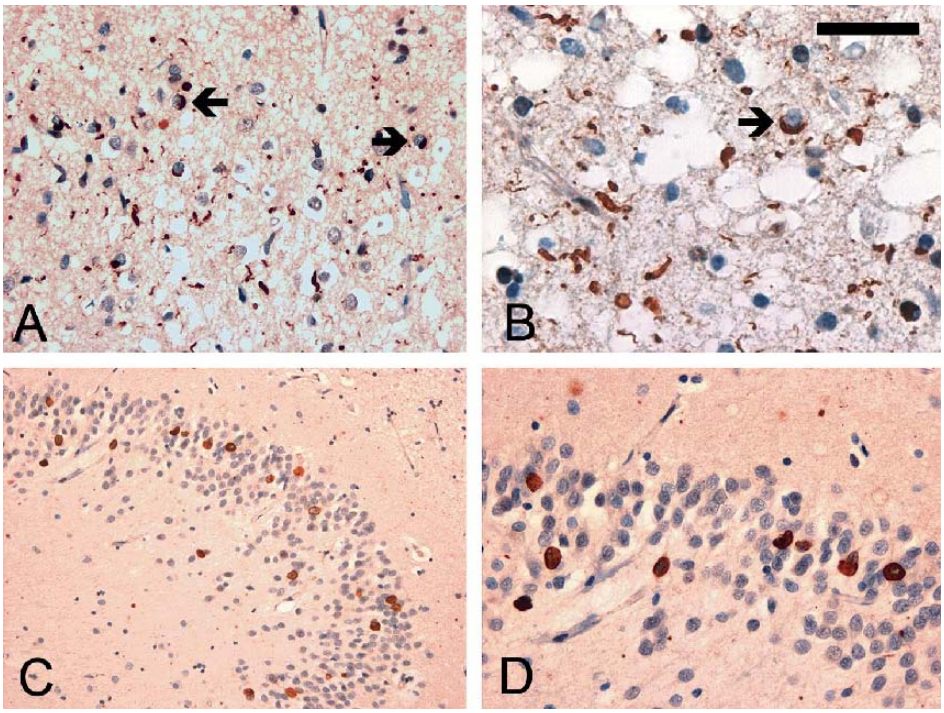


**FIGURE 1.** Ubiquitin-immunoreactive pathologic changes in the cerebral cortex in cases of MND, MND with dementia, and FTD-MND type. **(A, B)** Extramotor frontal neocortex with numerous neurites and neuronal cytoplasmic inclusions (arrows) in layer II. **(C, D)** Neuronal cytoplasmic inclusions in hippocampal dentate granule cells. Scale bar = **(A)** 200 μm, **(B)** 100 μm, **(C)** 400 μm, **(D)** 100 μm.

association and the recognition of identical changes in some patients with pure FTD (lacking motor symptoms, FTD-MND type) (11, 12) suggests that this pattern of pathology is closely related to the pathogenesis of dementia in MND. The specificity of this finding is less certain, however, because several studies have reported similar changes in a significant proportion of nondemented patients with MND (7–10, 27). In the present study, we found ub-ir inclusions and neurites in the extramotor neocortex and hippocampus in all cases of FTD-MND type (by definition) and confirmed that this pathology is a consistent finding in patients with MND-d. The fact that this pathology was slightly more severe in the FTD-MND type group than in patients with MND-d may reflect differences in the average disease duration. In addition, we found similar ub-ir pathology in almost half of our patients with MND in whom there was no documented history of dementia. Although the morphology and anatomic distribution of the inclusions was similar, the amount of this pathology tended to be less in the nondemented patients with MND. Several previous studies have reported similar findings, of extramotor, ub-ir pathology in 20% to 60% of nondemented patients with MND, with the extent of pathology tending to be less severe than in MND-d (7–10, 27). This suggests there may be a preclinical stage of cognitive dysfunction in which extramotor pathology is accumulating in many patients with MND but has not yet reached sufficient levels to cause overt dementia. This hypothesis is supported by several clinical studies in which neuropsychologic testing has demonstrated mild degrees of cognitive dysfunction in 25% to 50% of patients with MND (14–18). In addition, neuroimaging studies have shown abnormal blood flow and metabolism in frontal and temporal regions in many patients with MND who are not clinically demented (15, 17, 34).

In addition to the pyramidal system and cerebral cortex, a wide variety of subcortical regions may show evidence of chronic degeneration in MND (5). The most commonly affected areas include the basal ganglia, thalamus, and substantia nigra. Recently, ubiquitin immunohistochemistry has been used to demonstrate unique pathologic changes in these regions, which are more specific than just neuronal loss and gliosis. Dense round or crescentic cytoplasmic inclusions similar to those seen in extramotor neocortex and hippocampus have been described in small striatal neurons in some patients with classic MND and MND-dementia (8, 23, 24). Separate reports have identified similar inclusions and dystrophic neurites in a few cases of familial FTD-MND type (21, 25). In this study, we found ub-ir cytoplasmic inclusions and dystrophic neurites in the striatum in all cases of MND-d and FTD-MND type, regardless of family history. Of the patients with MND without dementia, just less than half were found to have ub-ir striatal pathology that was generally less severe than that seen in the demented patients. Similar pathology was also found in a wide range of other subcortical structures, particularly in the FTD-MND group. This pathology did have some anatomic specificity, however, because many regions never showed involvement (for example, basis pontis and cerebellum).

Pigmented neurons of the substantia nigra have been reported to contain ub-ir cytoplasmic inclusions in some cases of MND and MND-d, with morphology resembling those found in LMN (filamentous skeins and Lewy body-like inclusions) (20, 22). We found nigral inclusions, of these types, to be more common in cases of MND-d than MND without dementia (87% vs. 50%, respectively). Although not previously described, we also found small numbers of filamentous skeins in most of our cases of FTD-MND type. Moreover,

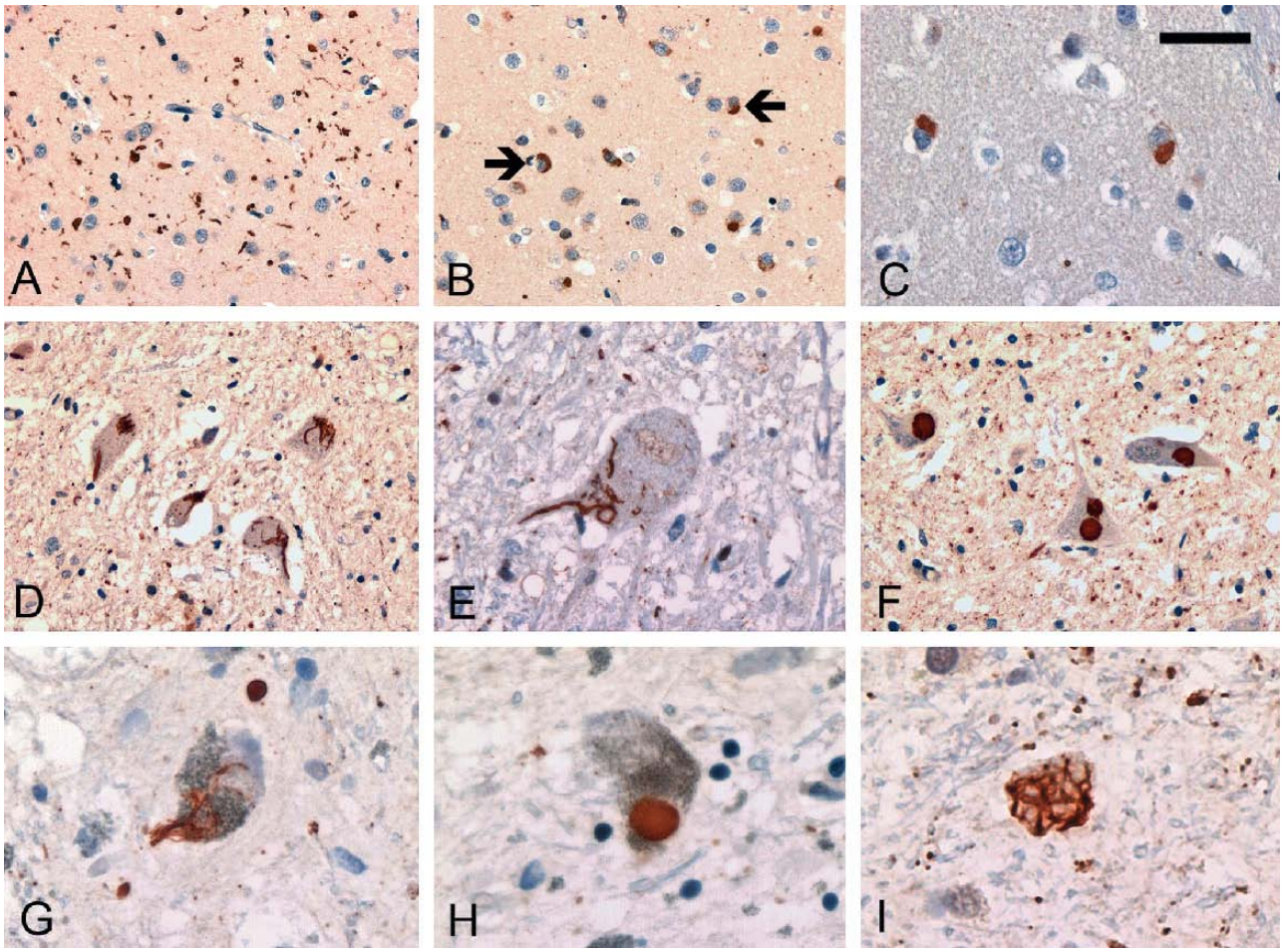


**FIGURE 2.** Ubiquitin-immunoreactive pathologic changes in subcortical regions in cases of MND, MND with dementia, and FTD-MND type. **(A–C)** Striatum containing numerous neurites **(A)** and dense neuronal cytoplasmic inclusions ([**B, C**]; arrows). **(D–F)** Lower motor neurons in the brainstem (**D**, hypoglossal nucleus) and spinal cord **(E, F)** containing filamentous skein-like cytoplasmic inclusions **(D, E)** and Lewy body-like inclusions **(F)**. **(G, H)** Pigmented neurons of substantia nigra containing filamentous skein-like cytoplasmic inclusion **(G)** and Lewy body-like inclusion **(H)**. **(I)** Neuron in the inferior olivary nucleus with cytoplasmic inclusion composed of thick, coiled filaments. Scale bar = **(A)** 200 μm, **(B)** 100 μm, **(C)** 50 μm, **(D)** 200 μm, **(E)** 75 μm, **(F)** 200 μm, **(G)** 75 μm, **(H)** 75 μm, **(I)** 50 μm.

filamentous skeins were occasionally found in anatomic regions not previously reported, including the thalamus, red nucleus, and periaqueductal grey matter.

We are not aware of any previous reports describing involvement of the inferior olivary nucleus in MND, MND-d, or FTD-MND type. However, we found filamentous cytoplasmic inclusions in olivary neurons in some subjects from all 3 neurologic groups, but not in controls. These inclusions had a slightly different appearance than the delicate skeins found in LMN and the substantia nigra, with the filaments seeming to be slightly thicker and often coiled into a spherical arrangement. Their filamentous nature also distinguished these from the granular inclusions that are often found in the olivary nucleus in normal aging (33).

Clinical correlation for the various patterns of subcortical pathology has not been studied in detail. Patients with MND (with or without dementia) may develop a variety of nonpyramidal features (including parkinsonism, cerebellar signs, autonomic dysfunction, and sensory abnormalities) that likely reflect degeneration of specific subcortical structures or systems (1, 5). One study in which ub-ir inclusions were identified in the substantia nigra of 4 patients with MND-d found no record of extrapyramidal clinical features on retrospective review of the medical records (20). However, in a recent semiquantitative study, we compared material from patients with MND-d with and without a history of parkinsonism and found the degree of ub-ir pathology in the striatonigral system to be greater in those that had extrapyramidal features (29).

The one type of pathology that was restricted to a specific subgroup of our patients was lentiform ub-ir NII. Similar to the results of a previous study of ours (which included many of the same cases), we found NII only in patients with familial FTD (19). This unique type of inclusion was first described in detail by Woulfe et al in 3 patients with FTD-MND type (25).

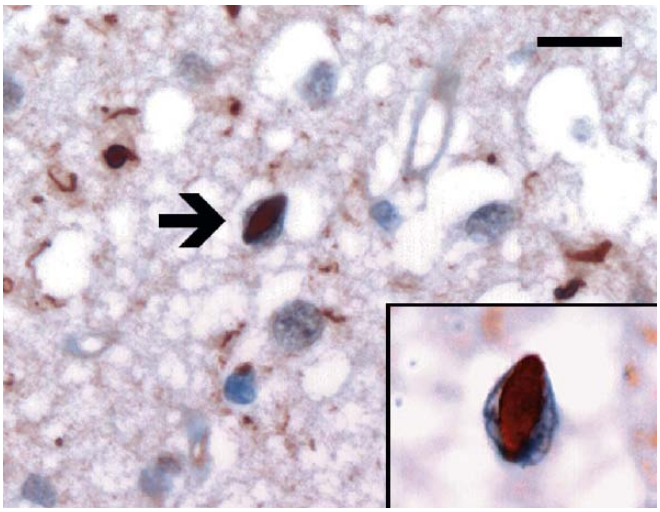

**FIGURE 3.** Lentiform neuronal intranuclear inclusions in a case of familial FTD-MND type. Scale bar = 50 μm.

The patients in their study all had at least one relative affected with dementia; however, the significance of this family history was not further evaluated. Two additional reports of families with autosomal-dominant FTD-MND type each made brief mention of similar NII in affected family members who underwent postmortem examination (35, 36). A recently published study confirmed that NII are highly specific for familial FTD, but also found similar inclusions in at least one case that appeared to be sporadic (37). More recent personal experience (unpublished) and communication with several other neuropathologists suggests that although the presence of numerous lentiform ub-ir NII is highly specific for familial FTD, small numbers of similar inclusions may be found in a more restricted anatomic distribution in some cases of sporadic FTD. Genetic analysis of 2 of our families combined with published data on other families has linked autosomal-dominant FTD-MND type with NII to chromosome 17q21, with the region of interest between markers D17S1787 and D17S931 (35, 36, 38).

The consistency with which specific anatomic sites are affected in each of the clinical groups (that is, LMN involvement in patients with MND and extramotor cortical involvement in patients with dementia) (Table 7) suggests that the formation of ub-ir pathology plays a direct role in the pathogenesis of clinical symptoms. However, we have demonstrated that a milder degree of similar ub-ir pathology is found in the same anatomic regions in a significant proportion of patients who do not yet demonstrate corresponding clinical features. This suggests there is an early stage in the disease process during which the pathology is developing but has not yet reached sufficient severity to cause overt clinical expression. The existence of this preclinical phase is supported by a number of recent clinical studies (13–18). Taken together, these clinical and pathologic findings are most consistent with the concept that MND, MND-d, and FTD-MND type each represent part of a clinicopathologic spectrum of disease rather than discrete entities.

**TABLE 7.** Overlap in Anatomic Distribution of ub-ir Pathology

| | Lower Motor Neurons | Striatonigral System | Extramotor Cerebral Cortex |
|---|---|---|---|
| MND | >90% | 60% | 45% |
| MND-d | >90% | 100% | 100% |
| FTD-MND type | >25% | 100% | 100% |

It should be noted that in this study, we included cases of MND that represent only the most common, classic type (ALS) (1). As a result of insufficient numbers, we did not include examples of less common clinical variants of MND (such as primary lateral sclerosis, progressive muscular atrophy, and progressive bulbar palsy) and, therefore, cannot comment as to whether these fit into the disease spectrum under consideration. We also excluded familial cases know to have mutations in the Cu/Zn superoxide dismutase (SOD 1), some of which have pathologic changes that are quite different from sporadic MND, likely reflecting a distinct pathogenesis (39). Finally, there are several other disease entities that fall under the broad heading of MND that we did not consider because they appear to have a unique pathogenesis and molecular pathology (such as, Guamanian ALS/parkinsonism–dementia complex, neuronal intermediate filament inclusion body disease) (40, 41). Therefore, the findings and interpretations of the present study extend to include only the most common classic subtype of MND.

In summary, we have demonstrated the use of ubiquitin immunohistochemistry in defining the pathologic changes that characterize MND, MND-d, and FTD-MND type. We have found the variety of morphologies and the anatomic distribution of ub-ir pathology in each of these conditions to be greater than previously documented. Moreover, the degree of overlap in the neuropathology suggests that MND, MND-dementia, and FTD-MND type may represent a spectrum of clinical disease with a common pathologic substrate. Additional studies are needed to more fully evaluate the various clinical–anatomic–pathologic correlations.

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