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Neuronal Intranuclear Inclusion Disease Without Polyglutamine Inclusions in a Child

Kathryn McFadden, MD, Ronald L. Hamilton, MD, Sam J. Insalaco, MD, Lawrence Lavine, DO, Majeed Al-Mateen, MD, Guoji Wang, MS, and Clayton A. Wiley, MD, PhD

From the Department of Pathology (KM, RLH, GW, CAW), University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, and Mary Bridge Children's Hospital (SJI, MA-M), Tacoma, Washington.

Abstract

Neuronal intranuclear inclusion disease (NIID) is a rare and heterogeneous group of slowly progressive neurodegenerative disorders characterized by the widespread presence of eosinophilic neuronal intranuclear inclusions (NII) accompanied by a more restricted pattern of neuronal loss. We report here the pathologic findings in a 13-year-old boy who died after a 6-year clinical history of progressive ataxia, extrapyramidal manifestations, and lower motor neuron abnormalities. Histological evaluation of the brain revealed widespread NII in most neurons. Marked loss of cerebellar Purkinje cells and neurons in the dentate nucleus, red nucleus, and spinal cord anterior horns was accompanied by a modest astrogliosis. Because of the abundance of NII and the absence of a relationship between NII and neuronal loss or microglial activation, we conclude that loss of cerebellar, brainstem, and spinal cord neurons reflects selective neuronal vulnerability. NII were immunoreactive for ubiquitin, glucocorticoid receptor, and SUMO-1, a small, ubiquitin-like protein purportedly involved in protein transport and gene transcription. NII were non-reactive for polyglutamine (1C2), TATA binding protein, promyelocytic leukemia protein, heat shock protein 90, tau, alpha-synuclein, neurofilament, and beta amyloid. The moderate ubiquitin and strong SUMO-1 staining of NII in juvenile cases is the reverse of the pattern noted in adult diseases, suggesting the two age groups are pathogenically distinct. We suggest that juvenile NIID is a spinocerebellar brainstem ataxic disease possibly related to an abnormality in SUMOylation.

Keywords

Intranuclear inclusions; Neurodegeneration

INTRODUCTION

Neuronal intranuclear inclusion disease (NIID) is a rare, slowly progressive and fatal neurodegenerative disorder characterized by the presence of eosinophilic neuronal intranuclear inclusions (NII) of the central, peripheral, and autonomic nervous systems with glial inclusions described in some cases. Neuronal loss and gliosis have been described in brainstem, cerebellar and spinal regions without relationship to NII. The initial disease descriptions (1–5) have been followed by approximately 30 case reports. Infantile, juvenile, and adult-onset cases have been described (6). The majority of cases are sporadic but several examples of familial occurrence have been reported (1,5,7). There is no obvious mode of inheritance although apparent

Send correspondence and reprint requests to: Dr. Kathryn A. McFadden, Presbyterian University Hospital, Neuropathology Division, 200 Lothrop Street A515, Pittsburgh, Pennsylvania; E-mail: mcfaddenka@msx.upmc.edu..

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autosomal dominant transmission has been found in some families (7,8). Given the variability in clinical and pathological findings it is unlikely that all these descriptions represent one disease.

Clinically, most cases of NIID present as a multisystem neurodegenerative process beginning in the second decade and progressing to death in 10 to 20 years. Neurological signs and symptoms vary widely, but usually include ataxia, extra-pyramidal signs such as tremor and oculogyral crises, lower motor neuron findings such as absent deep tendon reflexes, weakness, muscle wasting, foot deformities, and less apparent behavioral or cognitive difficulties (9). Patients eventually become incapacitated and die of repeated lower respiratory infections. Electrophysiological studies often show evidence of chronic denervation of muscle and reduced nerve conduction velocities (7). Imaging may show cerebral and/or cerebellar atrophy. There are no known specific laboratory findings besides histological analysis. Reported adult-onset cases are characterized by dementia and may represent a different pathological entity.

Macroscopic examination of the brain may be normal or show a mild reduction in cortical ribbon thickness with sparing of white matter volume. Mild to moderate cerebellar atrophy, more pronounced in the vermis, may also be evident. The substantia nigra is often nonpigmented. On light microscopy, the most striking histological feature is the widespread presence of well-demarcated, round, eosinophilic NII, 2 to 6 microns in diameter on average. On ultrastructural examination, NII are nonmembrane bound and consist of densely packed, randomly arranged filaments 8 to 15 nanometers in diameter with granular aggregates (10–12). Focal, severe neuronal loss is usually present in the substantia nigra, cerebellar Purkinje cell layer, dentate nucleus, red nucleus, and anterior horns. This is associated with mild to moderate gliosis. Investigators have noted that the distribution of neuronal loss, rather than the ubiquitous presence of intranuclear inclusions, correlates with clinical manifestations.

NII are immunoreactive for ubiquitin and Thioflavin S and show variable autofluorescence. They are not immunoreactive for tau, neurofilament, amyloid precursor protein, glial fibrillary acidic protein (GFAP), or α - or β -tubulin. Occasional inclusions have been shown to be weakly positive for polyglutamine (10,13,14). Because of this, and clinical and morphologic similarities between NIID and certain CAG repeat polyglutamine diseases, it has been suggested NIID is caused by a CAG expansion in an unknown gene. However, NIID inclusions do not show the ubiquitous, strong immunopositivity for 1C2 (a marker for polyglutamine) seen in known CAG repeat disorders (14). NIID inclusions do consistently stain for ataxin-3. More recently Pountney et al (14) have found NII in familial, adult-onset cases to be consistently immunoreactive for SUMO-1 or small ubiquitin-like modifier-1. SUMOylation is a recently discovered pathway in nuclear protein processing, particularly of transcription factors (15). Whether this is true for the infantile/juvenile form is not known.

Clinical History

The subject of our study was born following an uncomplicated pregnancy and delivery. His early developmental milestones were normal and well documented on family videotapes. He had one episode of poststreptococcal glomerulonephritis at 4 years of age and recovered with no apparent sequelae. There were no subsequent hospitalizations, surgeries, or chronic medications. There was no family history of dementia, developmental disorder, movement disorder, motor handicaps, or seizures.

Tremors of his hands were initially noted at 7.5 years of age, approximately 3 weeks after his last mumps, measles, and rubella vaccine. One month later he exhibited difficulty walking and required assistance after falling. His tremors worsened. Subsequently, he complained of leg pain and weakness. His gait became stiff legged with the right leg rotated outwards. On examination at 8 years of age, he was found to have significant action and sustention tremor,

bilateral lower leg weakness (tibialis anterior), decreased deep tendon reflexes, and pes planus. He also exhibited impaired speech articulation.

Nerve conduction studies at this time showed slowing of motor and sensory conduction velocities in both the upper and lower extremities. Amplitudes of the compound muscle action potentials were at the lower limits of normal and sural sensory nerve action potential was unobtainable. Electromyography showed giant motor unit potentials suggestive of previous denervation followed by reinnervation without evidence of recent denervation. Sural nerve biopsy showed mild axonal degeneration and regeneration suggesting neuroaxonal dystrophy. A skin biopsy showed ultrastructural evidence of axonal and smooth muscle accumulation of electron lucent material reminiscent of glycoprotein or mucopolysaccharide. The changes were largely membrane-bound and not typical of the usual lysosomal storage disorders. No intranuclear inclusions were seen in any cell type.

Laboratory values for copper, ceruloplasmin, creatine kinase, lactate, erythrocyte sedimentation rate, anti-nuclear antibody, Lyme serology, thyroid panel, lipoprotein electrophoresis, lysosomal enzymes, and complete Charcot-Marie-Tooth disease DNA panel (Athena Diagnostics, Worcester, MA) were normal. Urine amino acids were normal. An EKG was normal.

Cerebrospinal fluid showed 60 leukocytes (99% mono-nuclear), 0 erythrocytes, protein 31 mg/dl, glucose 56, and a lactic acid of 1.0 (normal). Brain MRI at 8 years of age showed mild enlargement of the cerebellar sulci and mild vermian atrophy. These findings remained stable on follow-up 18 months later.

Between 8 and 9 years of age, he became confined to a wheelchair. He tired easily and napped frequently. Eventually speech production ceased and difficulty swallowing and excessive drooling were noted. Gastrostomy tube feeding was initiated. His tremor became more coarse and punctuated by prolonged episodes of shaking of the entire body. Extensor plantar reflexes were noted. He also developed oculogyric crises. His cognitive function appeared to remain intact and there was no discernible loss of vision or hearing. He received intravenous immunoglobulin for 2 years, which appeared to slow progression but showed no definite improvement. An initial trial of L-dopa caused vomiting and no noticeable improvement of symptoms.

At 11 years of age he weighed 50 pounds. He appeared alert but with “mask-like” facies and could not hold his head erect. Tremulous movements of the hands were constant without dystonic or choreoathetoid movements. Pupillary responses and extra ocular movements were normal as was fundoscopic examination. There was copious drooling and the tongue was atrophic with fasciculations. There was generalized muscular atrophy with contractures and areflexia throughout. Sensory examination was normal. Amantadine was initiated with improvement of symptoms. Three weeks later his facial expression was more animated and he was able to speak in hypophonic but comprehensible phrases. Tremors were reduced and he was able to maintain head control, lift his limbs against gravity and stand with assistance. This response, however, was temporary. A second trial of L-dopa caused nausea and exaggeration of tremors and was discontinued. A trial of pergolide mesylate produced similar results.

At 13 years of age, he developed an intercurrent respiratory infection and died of respiratory failure. An autopsy was performed. On autopsy, his emaciation, generalized muscle atrophy, and contractures were noted, as were bilateral pulmonary lobar consolidation and pericardial effusion.

MATERIALS AND METHODS

The brain was sectioned mid-sagittally with the right half frozen and the left half fixed in 10% buffered formalin. Multiple sections from cerebral cortex, basal ganglia, hippocampus, thalamus, midbrain, pons, medulla, cerebellum, and spinal cord were paraffin-embedded and examined by standard histologic techniques (hematoxylin and eosin [H&E]).

Immunohistochemical investigation was performed on selected 6- μ m-thick sections using a standard avidin-biotin-peroxidase technique. The following antibodies from DAKO (Carpinteria, CA) were used: ubiquitin (1:200), tau (1:200), neurofilament protein (NF, 1:50), Beta-amyloid (BA4, 1:500), glial fibrillary acidic protein (GFAP, 1:350), and CD68 (1:500). Additionally, alpha, B-crystalline (Vector, Burlingame, CA, 1:500), alpha-synuclein (Zymed, San Francisco, CA, 1:1000), glucocorticoid receptor (GR, 1:50), promyelocytic leukemia protein (PML, 1:50) and heat shock protein 90 (HSP 90, 1:50) (Santa Cruz Biotechnology, Santa Cruz, CA) were used. All antibodies were monoclonal except ubiquitin, tau, GFAP, GR, and HSP 90, which were rabbit polyclonal. Antigen retrieval for alpha-synuclein staining was performed by pretreatment with protease XXIV (Sigma, St. Louis, MO). Selected sections were also stained for Bielschowsky. Tissue samples from both cortical grey and white matter and basal ganglia were fixed in 3% glutaraldehyde and processed by standard methods for electron microscopy.

Antigen retrieval for immunofluorescence was performed by microwaving paraffin-embedded sections for 1 minute in Citra solution (BioGenex, San Ramon, CA). These were then incubated with 10% goat serum in PBS for 20 minutes followed by primary antibody overnight. Sections were then incubated with biotinylated secondary antibody and ABC. The primary antibodies used were monoclonal antibody to SUMO-1 (Zymed, 1:200) and anti-polyglutamine (1C2, Chemicon, Temecula, CA, 1:1000). Sections were counter-stained with hematoxylin and examined by transmission light microscopy. For fluorescence labeling, FITC, Cy3 and Cy5-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA, 1:200) were used. Fluorescence-labeled sections were examined using a ZEISS 510 laser confocal microscope and LSM 3.2 software package. Selected sections were doubly stained for SUMO-1/propidium iodide, SUMO-1/polyglutamine, SUMO-1/neurofilament, SUMO-1/GFAP, and triply stained for neurofilament/GFAP/polyglutamine and visualized by confocal laser scanning microscopy.

RESULTS

The fresh brain weighed 1,425 grams (consistent with age). The fixed left hemisected brain weighed 700 grams. The fixed spinal cord was available from the mid-cervical level to the cauda equina. Macroscopic examination of the fixed hemisected brain was unremarkable except for mild atrophy of the cerebellar hemisphere and vermis. The substantia nigra was not grossly pigmented (consistent with age). Mild atrophy of the ventral roots of the spinal cord was evident. Multiple cross sections of the spinal cord showed no other gross abnormalities.

Microscopic examination of H&E-stained sections revealed round, eosinophilic intranuclear inclusions in the majority of neurons in all neocortical areas sampled (Fig. 1A). The deep white matter was well myelinated. Glial inclusions were not seen. Neurons of the striatum and globus pallidus showed fewer NII. The thalamus exhibited numerous NII and mild gliosis particularly in the subthalamic nucleus. The hippocampus showed moderate to severe numbers of NII but no discernible neuronal loss within the CA1 region, subiculum, and entorhinal cortex. The lateral geniculate bodies and nuclei of the mid-brain, pons and medulla likewise exhibited numerous NII. The substantia nigra neurons showed severe neuronal loss with SUMO-1-positive NII in the majority of remaining neurons (Fig. 1B, C). The cerebellar cortex showed atrophy and severe depletion of Purkinje cells with modest Bergmann gliosis (Fig. 1D). NII

were seen in the majority of rare remaining Purkinje cells (Fig. 1E). The granular cell layer exhibited moderate neuronal loss but no discernible NII. The dentate nucleus showed moderate neuronal loss with numerous inclusions in remaining neurons. The spinal cord revealed abundant neuronal loss with frequent SUMO-1-positive intranuclear inclusions in the rare remaining anterior horn cells (Fig. 1E, G). Review of H&E-stained sections from the systemic autopsy demonstrated intranuclear inclusions in dorsal root ganglia neurons and autonomic neurons of Auerbach's plexus as well as adrenal medulla (Fig. 1H). The Table summarizes the distribution of inclusions and neuronal loss.

NII in the cortex demonstrated rim-like immunoreactivity for ubiquitin (Fig. 1I). In all regions of the brain and spinal cord NII demonstrated intense immunostaining for SUMO-1 throughout the inclusion (Fig. 1J–L). Immunocytochemical stains for SUMO-1 were substantially more intense than those for ubiquitin. The immunocytochemical stain for glucocorticoid receptor was moderately positive in inclusions (not shown). The immunohistochemical stain for polyglutamine expansion (1C2) was negative in inclusions but showed strong cytoplasmic staining in neurons compared to an age matched control (not shown). The immunohistochemical stain for promyelocytic protein and heat shock protein 90 was negative. Immunofluorescence double labeling for SUMO-1/1C2 (Fig. 2A) showed cytoplasmic staining for 1C2 with no overlap with SUMO-1-positive inclusions. Triple labeling for microtubule associated protein-2 (MAP-2), GFAP, and 1C2 showed polyglutamine positivity in neuronal soma and nuclei (not in NII). Glial cells were negative (Fig. 2B). Double labeling for SUMO-1/GFAP (Fig. 2C) and SUMO-1/MAP-2 (Fig. 2D) showed NII to be confined to neurons. Double labeling for SUMO-1/propidium iodide showed the inclusions did not contain nucleic acids (Fig. 2E). Electron microscopy confirmed previous studies showing NII to be non-membrane bound collections of randomly oriented filaments and electron-dense granular material, distinct from the nucleolus (Fig. 3A–D).

Immunohistochemical stains for GFAP confirmed mild to moderate astrocytosis in areas of neuronal loss. The immunohistochemical stain for CD68 highlighted numerous, activated microglia in the grey and white matter, predominately in perivascular areas without any clear relationship to inclusion bearing neurons. CD3 immunohistochemical stains were essentially negative. The Table summarizes the distribution of astrocytosis and microgliosis.

The immunohistochemical stain for ubiquitin and SUMO-1 in sections from the systemic autopsy demonstrated inclusions in neurons of the adrenal medulla and Auerbach's plexus (Fig. 3E, F) and occasional inclusions in some cardiac cells. No inclusions were noted in other systemic organs.

DISCUSSION

NIID has been described in approximately 30 patients. It seems unlikely that these cases represent a single disease. Clinical features (age of onset, family history and pathology) suggest that the infantile/juvenile onset may be distinct from the adult-onset (6). The predominant neurological symptom in reported adult NIID cases is dementia (1,16,17), while infantile/juvenile cases show disproportionate ataxia and other movement symptoms. Both age groups show an excellent correlation between clinical symptoms and regions of neuronal damage and loss. Adult-onset disease displays neocortical pathology, while infantile/juvenile onset shows more prominent spino-cerebellar and brainstem neuronal damage and loss. The distribution and extent of inclusions also differs with age of onset suggesting different pathogenic mechanisms. Adult cases have shown a variable and smaller percentage of neurons (5%) with relatively indistinct central nervous system (CNS) nuclear inclusions as well as nuclear inclusions in up to 20% of glia (17). Some juvenile NIID reports have noted inclusions in non-neuronal CNS elements but the lineage of these rarely observed cells has not been confirmed

with double-label immunocytochemistry. Like previous infantile/juvenile cases, the subject's brain demonstrated massive numbers of intranuclear inclusions in the CNS that were entirely restricted to neurons. Outside of the CNS, most of the widely distributed peripheral nervous system neurons (e.g. myenteric plexus, adrenal medulla) had nuclear inclusions while all other cell types appeared spared with the exception of occasional cardiomyocytes (also reported by Oyer [18]).

NIID shows substantial clinical similarity to the spinocerebellar ataxias (SCAs). Most SCAs are the result of polyglutamine expansions and intranuclear inclusions have been reported in SCA1, 2, 3, 7, and 17. Strangely, the various proteins involved in these diseases show no homology except for CAG repeats exceeding a threshold of 35 to 40 repeats. Each disease is characterized by a stereotypical pattern of neurodegeneration of specific neurons presumed to be selectively vulnerable. Because deletion of these genes in humans is not associated with neurological disease, and homozygous mutations are not associated with earlier disease onset, CAG repeats are considered "toxic gain of function" mutations. Recent studies suggest that polyglutamine sequences resist eukaryotic proteasome digestion (19). Regardless of the disease, the longer the CAG repeat, the earlier the disease onset and the more abundant the NII.

The SCAs are variable in clinical presentation. In most cases there is limb and gait ataxia, dysarthria and hyporeflexia, although a tremor and/or positive Babinski sign are seen in some forms of SCA. Decline in cognitive function, seizure activity, sensory loss, and eye movement abnormalities are variably present. The subject's clinical presentation falls within this range of variation.

Like SCA the relationship between NII and neuronal damage is unclear in NIID. Regions of chronic neuronal damage are so depleted of neurons that it can be difficult to determine whether identified residual neurons contain intranuclear inclusions. This may account for discrepancies in reports of whether affected neurons in NIID had inclusions. In the current case, inclusions were readily identified in residual neurons in regions of neurodegeneration. What is most striking about NIID is the abundance of inclusions in intact appearing neurons that clinically are assumed to be functional. In the case of our subject, despite having about 75% of cortical neurons containing inclusions, he retained subtle neurocognitive function such as sense of humor late into the disease per family and neurologist's report. It is possible to hypothesize that cortical NII occurred later than cerebellar NII, however, an animal model would be required to test this.

Despite reports of several familial cases of NIID (including a pair of identical twins), no causative gene has been identified. Cases of NIID have been specifically assessed for abnormal polyglutamine expansions in genes associated with SCA 1, 2, 3, 6, or 7 (8,10,13,14) without success. It is only natural to consider the possibility that a potentially new gene with polyglutamine expansion could underlie the pathogenesis of NIID. We and others (10,13,14, 20–22) have looked for histopathological evidence of such expansions, but only an occasional Nil weakly stained with an antibody (1C2) specific to abnormal polyglutamine. Nuclear inclusions in most polyglutamine disorders contain the specific aberrant expanded protein or polyglutamine track along with other proteins, but as in the current case, without nucleic acids. Ataxin-3 has been observed in up to 99% of NIID inclusions (23) while most other polyglutamine proteins are observed in less than 10% of inclusions.

What are possible mechanisms leading to formation of neuronal intranuclear inclusions in NIID? While an undefined protein with a polyglutamine expansion cannot be ruled out, other possibilities exist. A general defect in the cellular proteasome (24) might lead to the formation of protein aggregates. Certainly the circumferential staining with antibodies to ubiquitin would

be consistent with such a hypothesis. However, the presence of nuclear aggregates suggests a more specific proteolytic defect restricted to the nucleus. Intra-cytoplasmic and extracellular proteolysis are presumably not affected as once the affected neurons die, the inclusions are quickly degraded

Something unique about neuronal nuclear protein processing must account for the restriction of inclusions to these cells. SUMOylation is a newly discovered proteolytic pathway that occurs predominantly within the nucleus (25). The role of SUMOylation in NII and neurodegeneration was studied by Terashima et al in association with mutant atrophin-1 (26). These investigators show that inhibition of SUMOylation led to decreased atrophin-1 NII and decreased cell death. They hypothesized that SUMOylation of mutant atrophin-1 might trap important nuclear transcription factors. The role of SUMOylation specifically in familial NIID was recently addressed by Pountney et al (14). This autosomal dominant form of NIID is distinct from infantile/juvenile form in the absence of substantial Purkinje cell loss and stronger ubiquitin than SUMO-1 staining. These investigators showed inhibition of proteasome by lactacystin without transfection with expanded polyglutamine proteins led to formation of SUMO-1-positive intranuclear inclusions in 10% of cultured PC12 cells. Our finding of intense SUMOylation of NII in infantile/juvenile NIID is particularly intriguing as further study of the disease may disclose an entirely new and unique pathway by which neuronal nuclei process proteins like transcription factors.

To address the importance of SUMOylation in Huntington disease, Steffan et al transfected a variety of N-terminal Htt constructs into a neuronal cell line (27). They discovered that SUMOylation stabilizes Htt, decreases aggregate formation, increases repression of transcription, and increases cell degeneration. They hypothesize that SUMO masks cytoplasmic localization signals leading to increased nuclear localization and associated increased cytotoxicity. Since SUMO-1 and ubiquitin are conjugated to the same lysine residues, it would be expected that such conjugations could counteract each other. Decreased SUMOylation led to decreased cell death while decreased ubiquitination led to increased cell death. Degenerative diseases like NIID pose many unanswered questions but perhaps the most enigmatic one is: What confers selective neuronal vulnerability? The abundance of widely distributed NII in the face of restricted clinical and pathological disease supports the selective neuronal vulnerability theory. Many neurons have inclusions but some additional cell specific cofactor presumably accounts for the selective neuronal death. Is the cofactor the same in all of these diseases thus explaining their pathological similarities? Before designing therapies to disrupt NII it is crucial to know whether formation of NII is a protective response on the part of the afflicted neuron or, on the other hand, if NII formation is related to the pathogenesis of neuronal destruction. Lastly, the presence of NII in the absence of aberrant CAG repeats suggests that NIID may represent a new paradigm in neuronal nuclear protein processing critical for cell survival. Finding abundant SUMO-1 in NII in both familial and sporadic NED raises the possibility that the primary defect is one of aberrant SUMOylation leading to nuclear proteins resistant to degradation.

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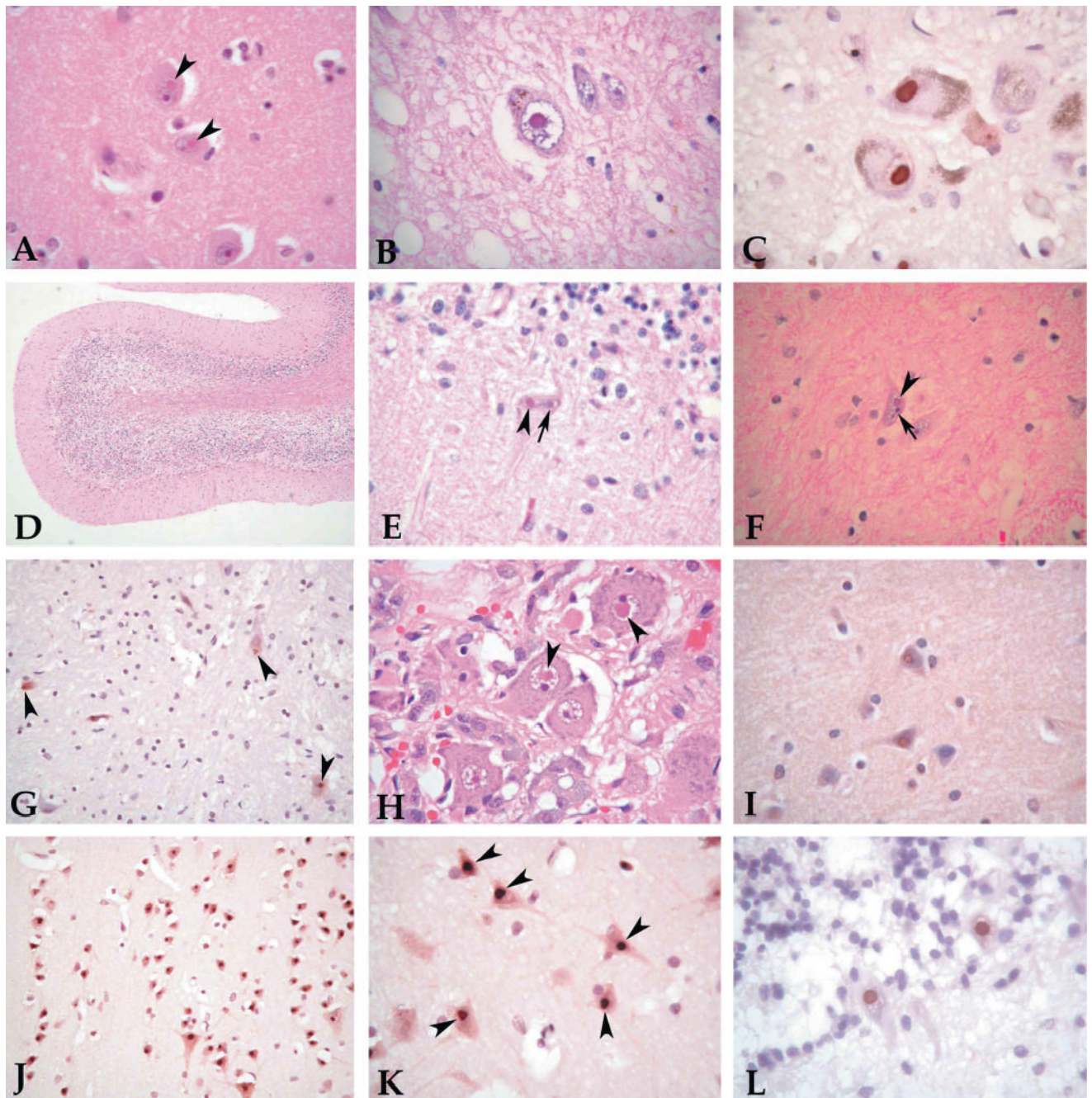
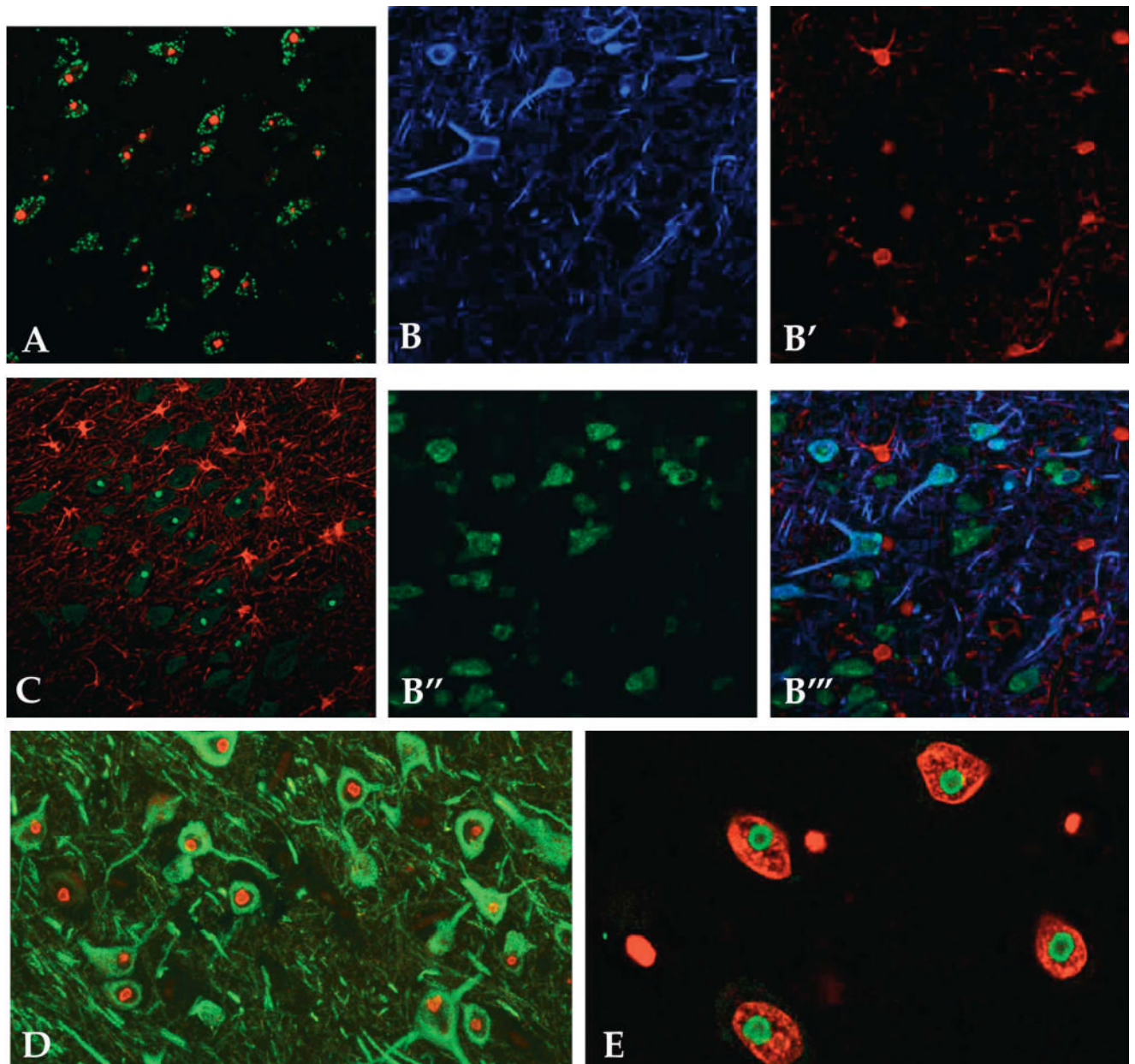


FIGURE 1.

Distribution and immunoreactivity of neuronal intranuclear inclusions. (A) H&E, cerebral cortex neurons with large inclusions (arrowheads). (B) H&E, substantia nigral neuron. (C) SUMO-1 in 2 pigmented neurons of the substantia nigra. (D-E) H&E, cerebellar cortex, low power (D). Residual Purkinje cell with an intranuclear inclusion (arrowhead) and nucleolus (arrow) (E). (F) H&E, residual cervical spinal anterior horn cell neuron with inclusion (arrowhead) adjacent to nucleolus (arrow). (G) SUMO-1, residual cervical cord anterior horn cells with intranuclear inclusions (arrowheads). (H) H&E, inclusions in medullary neurons of adrenal gland (arrowheads). (I) Ubiquitin, cerebral cortex pyramidal neurons with rim staining

of inclusions. (**J, K**) SUMO-1, cerebral cortex neurons with somal and nuclear staining (**J**) and intranuclear inclusions (arrowheads) (**K**). (**L**) SUMO-1, Purkinje neurons.

**FIGURE 2.**

Immunofluorescent confocal microscopy of cerebral cortex. **(A)** Double-label immunocytochemical staining for SUMO-1 (red) and polyglutamine antibody (1C2, green). **(B)** Triple label immunostaining for microtubule-associated protein-2 (MAP-2, **[B]**, blue) glial fibrillary acidic protein (GFAP, **[B']**, red), 1C2 (**[B'']**, green) and the merged image (**[B''']**). **(C)** Double-label. Immunofluorescent staining for GFAP (red) and SUMO-1 (green). **(D)** Double-label immunofluorescent staining of the cerebral cortex for MAP-2 (green) and SUMO-1 (red). **(E)** Immunofluorescent staining of the cerebral cortex for SUMO-1 (green) counterstained with propidium iodide (red).

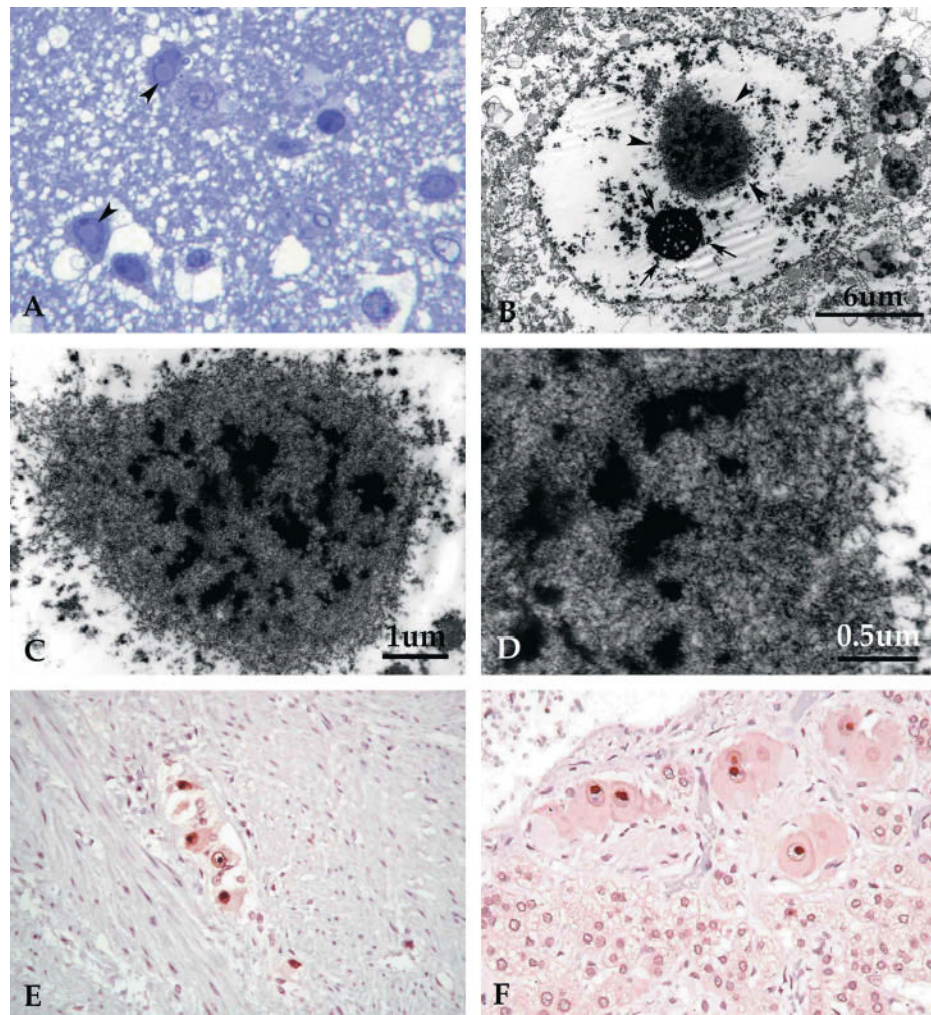


FIGURE 3.

(A) Plastic embedded cerebral cortex counterstained with toluidine blue demonstrates moderately vacuolated cerebral cortex with several neuronal nuclei containing pale staining nuclear inclusions with perinuclear halos (arrowheads). (B) Electron micrograph of nucleus with inclusion (arrowheads) and a more densely osmophilic nucleolus (arrows). (C) Higher power electron micrograph demonstrating fibrillar and dense granular structure of nuclear inclusion. (D) Higher power electron micrograph demonstrating transition from fibrillar to granular material within the inclusion. (E) SUMO-1, Auerbach's plexus ganglion cells. (F) SUMO-1, adrenal gland ganglion cells.

TABLE

Pathologic Findings

Area	Neuronal Loss	Inclusions (remaining nuclei)	Gliosis (GFAP)	Microgliosis (CD68)
Hippocampus (CA1, 2,3-4 and Dentate)	0	+++	+	+
Amygdala	0	+++	+	+
Lateral geniculate nucleus	0	+++	+	+
Caudate/Putamen	0	+	+	NE
Globus Pallidus	0	+	++	NE
Nucleus Basalis	0	+++	NE	NE
Thalamus	0	+++	+	NE
Subthalamic nucleus	+	+++	0	NE
Substantia nigra	+++	+++	0	+
Red nucleus	++	++	+	NE
Pontine tegmentum	NE	+++	0	NE
Basis pontis	0	+++	0	NE
Inferior olive	0	+++	++	NE
Purkinje cells	+++	+++	+++	+
Granular cells	++	0	0	+
Dentate nucleus	++	+++	++	++
Anterior horn cells	+++	+++	++	++
Dorsal root ganglia	0	++	NA	0

Values for neuronal loss, gliosis, and microgliosis are based on a 3+ scale (0, none; +, mild, ++, moderate; +++ = severe). Values for inclusions are based on frequency counts in remaining neurons over 10 high-power fields (0, 0%; +, <25%; ++, 25–50%; +++, >50%). NA, not applicable, NE, not evaluated.