

# Neuronal Intranuclear Inclusion Disease Presenting as Juvenile Parkinsonism

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**ABSTRACT: Background:** Diagnostic considerations for juvenile onset Parkinsonism (onset at <21 years of age) include juvenile Huntington disease, Wilson disease, dentatorubral-pallidolusian atrophy (DRPLA), storage diseases, and mitochondrial cytopathies. Neuronal Intranuclear Inclusion Disease (NIID) must also be considered. **Case Report:** We present a case of juvenile onset NIID with a predominantly Parkinsonian presentation, followed later by corticospinal, cerebellar, and lower motor neuron symptoms. **Conclusion:** Diagnosis of NIID can be made antemortem through rectal biopsy, however it was missed in this case. Rectal biopsy should be performed in all suspected cases, reviewed by an experienced neuropathologist and repeated if the suspicion for NIID is high. Pathologically, SUMO-1 immunohistochemistry appears to reliably label the neuronal inclusions and abnormal SUMOylation may play a part in the pathogenesis.

**RÉSUMÉ: Maladie des inclusions intranucléaires neuronales dont le tableau clinique est celui d'un parkinsonisme juvénile. Contexte :** La maladie de Huntington juvénile, la maladie de Wilson, l'atrophie dentato-rubro-pallido-lusienne, les maladies de surcharge et les cytopathies mitochondriales doivent faire partie du diagnostic différentiel du parkinsonisme juvénile (début avant l'âge de 21 ans). La maladie des inclusions intranucléaires neuronales (MIIN) doit également être considérée. **Observation :** Nous présentons une observation de MIIN à début juvénile dont le tableau clinique initial était surtout parkinsonien. Par la suite le patient a présenté des symptômes corticospinaux, cérébelleux et du neurone moteur inférieur. **Conclusion :** Le diagnostic de MIIN peut être posé par biopsie rectale avant le décès. Il a cependant été manqué chez ce patient. Une biopsie rectale devrait être faite chez tous les patients chez qui on soupçonne ce diagnostic et elle devrait être examinée par un neuropathologiste chevronné. On doit la répéter si le soupçon demeure. Au point de vue anatomopathologique, l'immunohistochimie à l'aide d'un anticorps anti-SUMO-1 semble marquer les inclusions neuronales de façon fiable et nous pensons qu'une SUMOylation anormale pourrait jouer un rôle dans la pathogenèse de la maladie.

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Juvenile onset Parkinsonism (onset at <21 years of age) is a rare condition. Diagnostic considerations include juvenile Huntington disease, Wilson disease, dentatorubral-pallidolusian atrophy (DRPLA), storage diseases, and mitochondrial cytopathies (see Table 1). Other genetic causes identified include mutation of PARK2 on chromosome 6q and the Contursi kindred of  $\alpha$ -synuclein mutations on chromosome 4q<sup>1</sup>. Among these, Neuronal Intranuclear Inclusion Disease (NIID), a slowly progressive multisystem degenerative disorder, is an important diagnostic consideration with an unknown underlying etiology.

We report a patient with juvenile Parkinsonism diagnosed pathologically with NIID whose diagnosis was missed during the patient's lifetime.

## CASE REPORT

A 10-year-old right handed boy presented with a two year history of gait difficulties, dysarthria, dysphagia, and sialorrhea. He gradually developed micrographia, and multiple episodes of oculogyric crises. On examination, he had a bilateral, high

frequency rest and postural tremor of the arms and head, and bradykinesia. Gait difficulties were typified by festination, decreased arm swing, and stooped posture (see video on-line).

Prenatal, birth and developmental history were normal. He had no history of viral illness, measles, or encephalopathy. Family history, including four siblings, was negative for similar problems. He was on no medications at the time and had never taken neuroleptics.

Normal investigations included: urine testing of organic acids, reducing substances, copper and porphyrins; serum testing

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of amino acids, proteins, immunoglobulins, pyruvate, ceruloplasmin, Vitamin E, creatine kinase, lactate, liver function tests; blood smear; hexosaminidase, sphingomyelinase,  $\beta$ -glucosidase,  $\beta$ -galactosidase, biotinidase and sialylation activity; genetic tests for autosomal dominant spinocerebellar ataxias, DRPLA, Friedreich ataxia and Huntington disease. Brain and spinal cord MRIs were repeatedly normal as was brain magnetic resonance spectroscopy and abdominal ultrasound. Skin and bone marrow biopsy revealed no signs of polyglucosan bodies or lysosomal storage disease. Electroencephalogram revealed brief generalized bursts of spike and wave activity. Electroretinogram was normal. Cerebrospinal fluid studies showed no abnormalities of tetrahydrobiopterin synthesis.

During the adolescent years, his Parkinsonism worsened with the tremor progressing to involve the legs; rigidity became more prominent. Oculogyric crises became more frequent lasting up to one hour. Dystonic postures were seen in his hands and feet. Poor postural reflexes lead to frequent falls and necessitated a wheelchair by his mid teens. Dysarthria and dysphagia rapidly progressed, and his lingual movements assumed an apraxic character. By the age of 16, he exhibited horizontal and vertical gaze evoked nystagmus. Hyperreflexia with extensor plantar

reflexes and spasticity were noted despite relatively preserved power. Cognition was relatively spared although emotional incontinence was present. Diffuse muscle fasciculations and atrophy of the interosseous muscles of the hands were seen and severe scoliosis of the spine had developed. Diffuse fibrillations and large motor unit potentials were found on EMG. Nerve conduction studies showed mild motor slowing in the lower limbs; sensory studies were normal. The differential diagnosis of NIID was raised and a rectal biopsy was performed at another institution and reported as normal (please see the pathology section below).

By the age of 18 he had become anarthric and reliant on percutaneous endoscopic gastrostomy tube feeding. Progressive contractures and scoliosis led to dyspnea and recurrent respiratory infections. He died at the age of 20 from pneumonia.

Throughout the course of the illness, he received multiple medication trials with levodopa which resulted in minimal transient improvement. Trials of trihexyphenidyl and amantadine were also unsuccessful.

#### PATHOLOGY

An unrestricted autopsy was performed. The fresh brain weight was 1,124g. General autopsy findings included diffuse cachexia (weight 28kg), generalized muscular atrophy and bilateral necrotizing bronchopneumonia. The brain showed severe depigmentation of the substantia nigra and general atrophy of the midbrain (Figure A). Significant atrophy was also seen in the thalamus and body of the caudate nucleus with concomitant dilatation of the third and lateral ventricles. The subthalamus and other nervous system tissues were grossly unremarkable.

**Table 1: Differential diagnosis in juvenile onset Parkinsonism, adapted from Paviour et al 2004<sup>1</sup>**

Hereditary Neurodegenerative disorders:	
	Parkin mutation related Parkinson's Disease
	NIID
	Huntington's Disease
	Neuroacanthocytosis
	PANK2 mutations (neurodegeneration with brain iron accumulation)
	Wilson's disease
	Spinocerebellar ataxia 2
	Spinocerebellar ataxia 3
	Dopa-responsive dystonia
	Tyrosine hydroxylase deficiency/mutation
	Rapid-onset dystonia-parkinsonism
	Niemann Pick type C
	Juvenile neuronal ceroid lipofuscinosis
	Dentatorubral-pallidoluyisan atrophy
	Mitochondrial cytopathies
Infectious:	
	Systemic lupus erythematosus
	Japanese encephalitis
	Subacute sclerosing panencephalitis
	Epstein-Barr virus encephalitis
	Post-encephalitic Parkinsonism
	Mycoplasma
Toxins/medications:	
	Cyanide, methanol, carbon monoxide, organophosphates, typical and atypical antipsychotics, metoclopramide, selective serotonin reuptake inhibitors, valproate
Autoimmune/inflammatory:	
	Postvaccine
	Extrapontine myelinolysis
	Bone marrow transplant

**Table 2: Common symptoms/signs seen throughout the course of NIID depending on age of onset, adapted from Takehashi et al, 2003<sup>5</sup>**

Age of Onset	Most common symptoms and signs, other reported symptoms and signs.
<b>Infantile</b>	<b>Cerebellar (limb ataxia and/or dysarthria), involuntary movements (choreoathetosis and/or tremor), seizures, hyporeflexia, autonomic dysfunction.</b>
<b>Juvenile</b>	<b>Personality change or learning difficulty, pyramidal signs, involuntary movements (choreoathetosis and/or tremor), Parkinsonism, cerebellar signs, muscle atrophy, oculogyric crisis, facial weakness.</b>
<b>Adult</b>	<b>Dementia, Parkinsonism, hyporeflexia, autonomic dysfunction, cerebellar signs, muscle atrophy, involuntary movements.</b>

Routine H&E sections revealed ubiquitous neuronal intranuclear inclusions within the brain and spinal cord (Figure B). Inclusions varied in size and were most prominent in large neurons, solitary, neuronal and intranuclear. They exhibited strong and diffuse SUMO-1 (Figure C) and ubiquitin staining, while IC2 positivity was mild and rare; other immunohistochemical stains were negative. Ubiquitin staining revealed the intranuclear inclusions within smaller neurons, such as the granule neurons of the dentate gyrus (Figure D).

Prominent neuronal loss, gliosis and microglial activation were seen in the globus pallidus interna and substantia nigra (Figure E). Gliosis (Figure F) and microglial activation (Figure G) were elucidated by GFAP and CD68 immuno-histochemistry respectively. Residual neurons bore inclusions. Free melanin was seen in the substantia nigra, pars compacta, but Fe pigment deposition of the Hallervorden-Spatz type was not seen. Similar but less intense microscopic changes were seen in the thalamus. Several thalamic neurons uniquely displayed a vacuolated cytoplasm. Focal areas within the cerebellar cortex exhibited significant neuronal loss, gliosis and microglial activation. Bergmann type gliosis and 'torpedoes' (Figure H) were seen at high power; residual Purkinje cells bore inclusions. Mild neuronal loss and gliosis were detected elsewhere in the brainstem.

Sections from the spinal cord revealed prominent neuronal loss, gliosis and microglial activation within the anterior horns (Figure I) and Clarke's columns. Spino-cerebellar tracts were depleted of myelin bilaterally. No degenerative changes were detected in the corticospinal tracts. Sections from the quadriceps muscle revealed changes consistent with longstanding denervation atrophy and superimposed type II fiber atrophy.

The antemortem rectal biopsy performed at an outside institution was re-reviewed at our institution. The original rectal biopsy slides did not reveal any inclusions, but the sections were somewhat superficial and did not sample the submucous plexus satisfactorily. Therefore, additional deeper sections were requested from the archived paraffin blocks. Subsequent ubiquitin staining at our institution revealed occasional neurons (not seen in the original H&E sections), one of which displayed a large intranuclear inclusion (see Figure J).

## DISCUSSION

Neuronal Intranuclear Inclusion Disease is a neurodegenerative disorder which can present similar to other neurologic disorders including spinocerebellar ataxia<sup>2</sup>, Friedreich's ataxia<sup>3</sup>, and motor-sensory and autonomic neuropathy<sup>4</sup>. These conditions were ruled out using appropriate genetic testing in this patient. Clinical presentation of NIID varies depending on the age of onset (see Table 2)<sup>5</sup>. Parkinsonism is a relatively common clinical manifestation of juvenile onset NIID<sup>6-11</sup>. The gait displayed by this patient was distinct in that it had features of both Parkinsonism and spasticity. Characteristic gait impairment may be a useful clinical characteristic in the diagnosis of these patients.

Levodopa therapy has been reported to result in improvement for a limited duration in some cases<sup>7,8,12,13</sup>, although side effects such as nausea, worsening of tremors<sup>14</sup>, motor fluctuations<sup>12</sup>, and dyskinesias<sup>7</sup> can occur. Amantadine has also been reported to result in a temporary improvement of symptoms<sup>14</sup>. In our case

levodopa produced only a minimal transient benefit and its use was limited by subsequent worsening of symptoms and gastrointestinal side effects. Amantadine was not effective.

The majority of cases of NIID appear sporadic; however, cases have been reported in identical twins<sup>15,16</sup>, siblings<sup>17</sup>, and in successive generations in the same family<sup>18</sup>, suggesting a heritable component. Autosomal dominant transmission has been suggested in some cases<sup>16</sup>. It is unclear whether NIID is due to a single underlying genetic defect or represents multiple genetic and non-genetic etiologies.

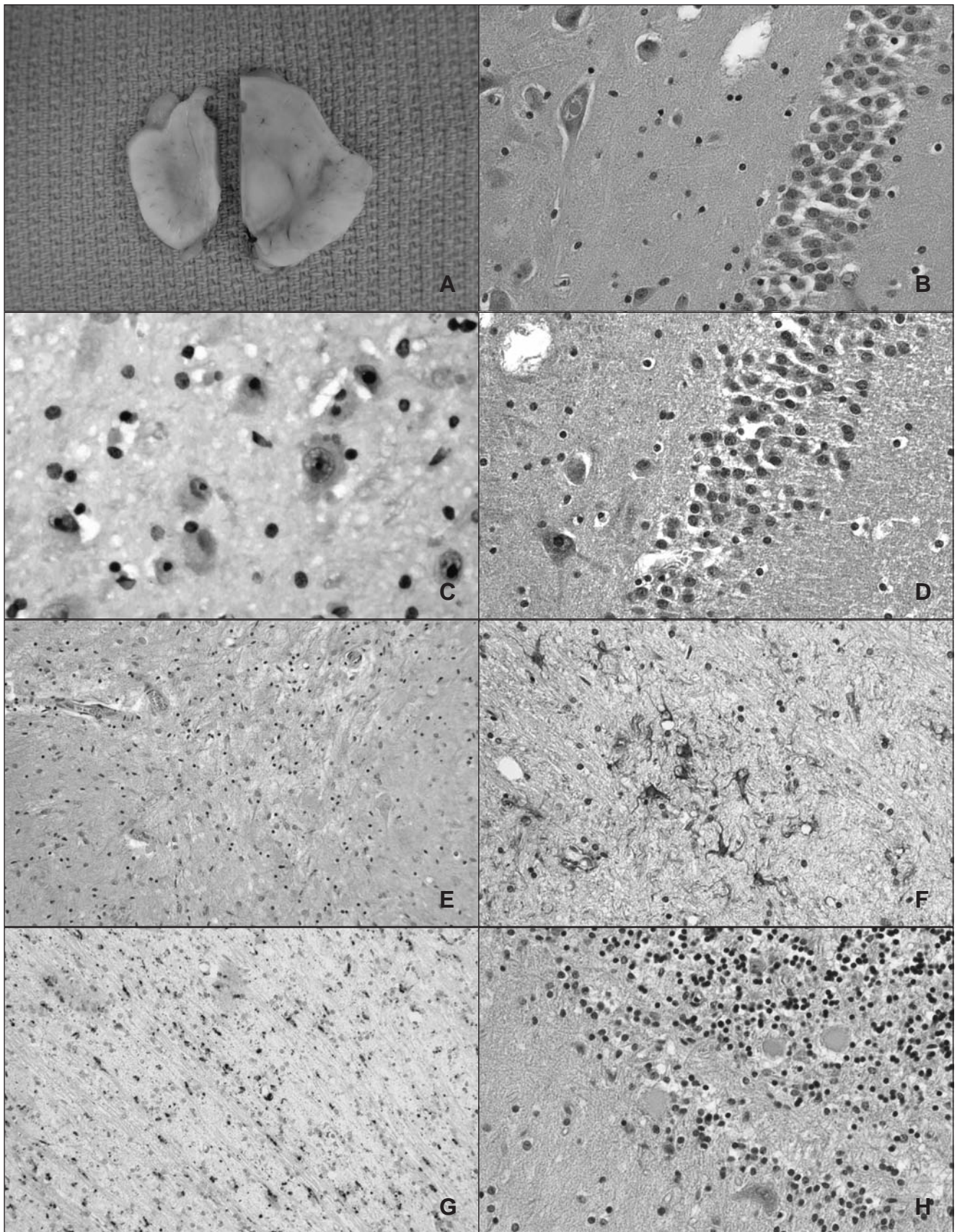
Intranuclear hyaline inclusions, the pathological hallmark and unifying feature of NIID, are present in many neurodegenerative disorders with known genetic etiologies including Spinocerebellar ataxia type 1, 2, 3, 7, and 17, Huntington's disease, Huntington's disease-like 2, spinobulbar muscular atrophy, and DRPLA<sup>19,20</sup>. Thus, NIID is a diagnosis of exclusion when genetic testing has ruled out these disorders.

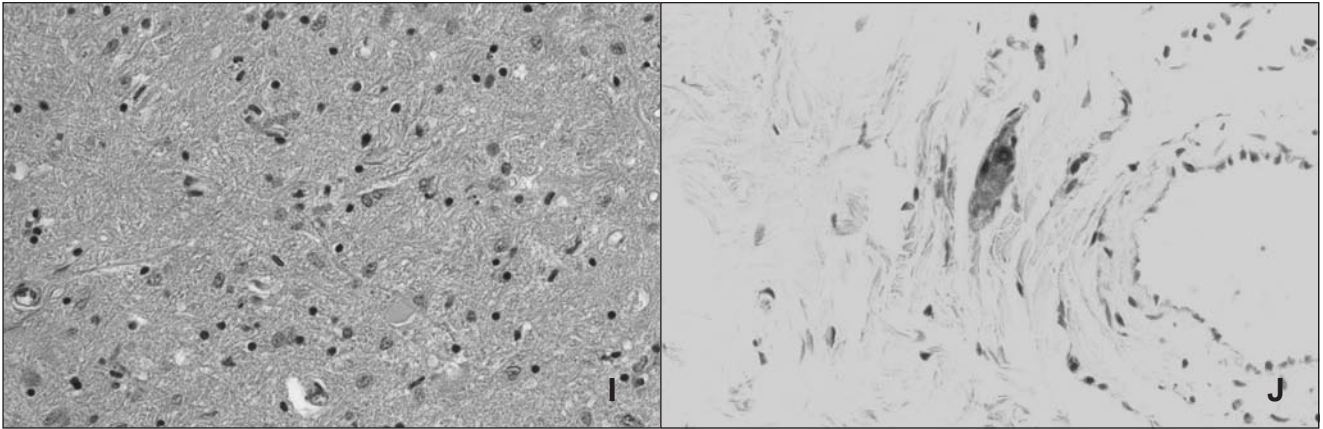
Histology in NIID reveals ubiquitous neuronal intranuclear inclusions but more discrete neuronal loss, gliosis and microglial activation in the deep gray matter, brainstem, cerebellum and spinal cord. It is these later pathologic changes which likely underlie the clinical symptomatology of NIID. The pathology of our case generally conforms to those previously reported in the literature, although some differences are notable. First, in our case, the globus pallidus interna and thalamus both displayed neuronal loss, gliosis and microglial activation. These changes have not been well described previously. Second, although the case reported by McFadden et al<sup>14</sup> described nigral neuronal loss, no gliosis and only minimal microglial activation were seen. In contrast, our case prominently exhibited both of the later features in the substantia nigra. Finally, cases describe prominent pathology in the dentate nucleus; in contrast, with the exception of neuronal inclusions, no other pathologic changes were identified here.

Immunohistochemical workup of NIID cases generally reveal strong and diffuse inclusion staining with ubiquitin and SUMO-1<sup>14</sup>. Meanwhile, IC2 positivity, marking cellular foci of polyglutamine deposition, is mild and sparse at best. Our findings are similar to previously reported cases and suggest that NIID is not a polyglutamine expansion disorder, but possibly a disorder of 'SUMOylation'<sup>14</sup>. SUMO-1's numerous molecular actions seem concentrated within the nucleus, including roles in protein trafficking, gene transcription, chromosome separation during mitosis and repairing DNA damage<sup>21</sup>. In particular, abnormal SUMOylation might lead to transcriptional dysregulation through nefarious effects on HDAC4 (histone deacetylase 4)<sup>22</sup>. Therefore, although discrete foci of neuronal loss, gliosis, and microglial activation within the nervous system appear to best correlate with clinical symptomatology, the ubiquitous SUMO-1 positive neuronal inclusions may still play an active role in the pathogenesis of NIID.

Despite the documented antemortem suspicion of NIID, subsequent rectal biopsy initially failed to identify the neuronal intranuclear inclusions. Upon re-review at our institution, which included ubiquitin immunohistochemistry, a single neuron exhibited an inclusion. Although the antemortem diagnosis of NIID using rectal biopsy has been reported in the literature<sup>3,6,9,17,23</sup> in our opinion a negative rectal biopsy should not be used to rule out the diagnosis. Reasons for this are several







**Figure A-J:** Tissues sampled for microscopy were routinely processed. Formalin fixed and paraffin embedded material for routine microscopy sectioned at 6  $\mu$ m, while sections for immunohistochemistry were cut at 3  $\mu$ m. Standard avidin-biotin-peroxidase immunohistochemical staining techniques were employed. Primary antibodies utilized, including source and dilution (in brackets), were as follows: Glial fibrillary acidic protein, GFAP (Dako, 1:4000); Tau (Pierce Biotechnology, 1:100);  $\alpha$ -synuclein (Zymed, 1:100); Ubiquitin (Dakocytomation); CD43 (Novocastro, 1:100); CD68 (Ventana, predilution); Alzheimer precursor protein, APP (Chemicon, 1:4000); Ki67 (Dakocytomation, 1:100); Fast-myosin (Sigma, 1:1000); Slow-myosin (Sigma, 1:800); Neurofilament protein, NFP (Biogenex, 1:500); Small ubiquitin-like modifier 1, SUMO-1 (Zymed, 1:200); IC2 (Chemicon, 1:1000). A- Gross axial hemisections of the midbrain depict marked generalized atrophy and severe depigmentation of the substantia nigra in our patient (left) versus control (68 year old; right). B- End folium and dentate granule layer of the hippocampus. A large eosinophilic neuronal intranuclear inclusion is easily detectable in an end folial neuron (H&E, 400 $\times$ ). C- SUMO-1 immunohistochemistry highlights the inclusions. D- End folium and dentate granule layer of the hippocampus. Ubiquitin immunohistochemistry highlights both the large (CA4 hilar) and small (dentate granule neurons) inclusions (400 $\times$ ). E- Neuronal loss, gliosis and microglial activation in the pars compacta of the substantia nigra (H&E, 200 $\times$ ). F- Gliosis of the globus pallidus interna (GFAP, 400 $\times$ ). G- Microglial activation in the globus pallidus interna (CD68, 200 $\times$ ). H- High power microscopy of the focally atrophic cerebellar cortex illustrating Purkinje dropout, 'torpedoes' and Bergmann gliosis (H&E, 400 $\times$ ). I- Neuronal loss, gliosis and microglial activation in the anterior horn of the lumbosacral spinal cord (H&E, 400 $\times$ ). J- Ubiquitin immunohistochemistry of the antemortem rectal biopsy. On deeper sectioning into the paraffin block, the submucous plexus is better seen and a large inclusion is identified (400 $\times$ ).

fold: 1) not all neurons contain inclusions, suggesting the possibility of sampling error; 2) small neurons tend to have small inclusions which can be difficult to see, even with appropriate immunohistochemical stains; 3) enteric neurons can be somewhat scarce in a rectal biopsy, especially when sampling and/or sectioning of the paraffin block is superficial. In that light, postmortem evaluation remains the best means of obtaining the proper diagnosis and repeat biopsies should be considered if the suspicion for NIID is high.

## CONCLUSION

This case typifies adolescent type NIID with a predominantly Parkinsonian presentation, followed later by corticospinal, cerebellar, and lower motor neuron symptoms. These symptoms appear to reflect focal neuronal loss, gliosis and microglial activation in the globus pallidus, thalamus, brainstem, cerebellum and spinal cord. Pathologically, SUMO-1 immunohistochemistry appears to reliably label the neuronal inclusions in this disorder, and abnormal SUMOylation may play a part in the pathogenesis. Clinical suspicion should be raised for NIID in juveniles presenting with features of Parkinsonism when other more common causes have been ruled out. Neuronal Intranuclear Inclusion Disease broadens the differential of juvenile Parkinsonism, and heightened awareness of this unique clinicopathologic entity may facilitate an antemortem diagnosis.

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