Neuronal Intranuclear Inclusion Disease Presenting as Juvenile Parkinsonism

Katie M. Wiltshire, Christopher Dunham, Stuart Reid, Roland N. Auer, Oksana Suchowersky

ABSTRACT: Background: Diagnostic considerations for juvenile onset Parkinsonism (onset at <21 years of age) include juvenile Huntington disease, Wilson disease, dentatorubral-pallidoluysian 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-luysienne, les maladies de surcharge et les cytopathies mitochondriales doivent faire partie du diagnostic différencié 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 pathogénèse de la maladie.


Juvenile onset Parkinsonism (onset at <21 years of age) is a rare condition. Diagnostic considerations include juvenile Huntington disease, Wilson disease, dentatorubral-pallidoluysian 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 α-synuclein mutations on chromosome 4q. 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, reduced substances, copper and porphyrins; serum testing http://doi.org/10.1017/S031716710000994X
of amino acids, proteins, immunoglobulins, pyruvate, cerulo-
plasmin, Vitamin E, creatine kinase, lactate, liver function tests;
blood smear; hexosaminidase, sphingomyelinase, β-glucosidase,
β-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 degeneration 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\(^1\)

<table>
<thead>
<tr>
<th>Hereditary Neurodegenerative disorders:</th>
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<tr>
<td>Parkin mutation related Parkinson’s Disease</td>
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<td>NiID</td>
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<td>Huntington’s Disease</td>
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<td>Neuroacanthocytosis</td>
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<td>PANK2 mutations (neurodegeneration with brain iron accumulation)</td>
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<td>Wilson’s disease</td>
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<td>Spinocerebellar ataxia 2</td>
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<tr>
<td>Spinocerebellar ataxia 3</td>
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<tr>
<td>Dopa-responsive dystonia</td>
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<td>Tyrosine hydroxylase deficiency/mutation</td>
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<td>Rapid-onset dystonia-parkinsonism</td>
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<td>Niemann Pick type C</td>
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<tr>
<td>Juvenile neuronal ceroid lipofuscinosis</td>
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<tr>
<td>Dentatorubral-pallidolusyian atrophy</td>
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<td>Mitochondrial cytopathies</td>
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**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
- Extrapyramidal 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\(^5\)

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<tr>
<th>Age of Onset</th>
<th>Most common symptoms and signs, other reported symptoms and signs.</th>
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<tbody>
<tr>
<td><strong>Infantile</strong></td>
<td>Cerebellar (limb ataxia and/or dysarthria), involuntary movements (choreoathetosis and/or tremor), seizures, hyporeflexia, autonomic dysfunction.</td>
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<tr>
<td><strong>Juvenile</strong></td>
<td>Personality change or learning difficulty, pyramidal signs, involuntary movements (choreoathetosis and/or tremor), Parkinsonism, cerebellar signs, muscle atrophy, oculogyric crisis, facial weakness.</td>
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<tr>
<td><strong>Adult</strong></td>
<td>Dementia, Parkinsonism, hyporeflexia, autonomic dysfunction, cerebellar signs, muscle atrophy, involuntary movements.</td>
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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 ataxia2, Friedreich's ataxia3, and motor-sensory and autonomic neuropathy4. 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)5. Parkinsonism is a relatively common clinical manifestation of juvenile onset NIID6-11. 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 cases7,8,12,13, although side effects such as nausea, worsening of tremors14, motor fluctuations15, and dyskinesias2 can occur. Amantadine has also been reported to result in a temporary improvement of symptoms14. 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 twins15,16, siblings17, and in successive generations in the same family18, suggesting a hereditary component. Autosomal dominant transmission has been suggested in some cases19. 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 Spino-cerebellar ataxia type 1, 2, 3, 7, and 17, Huntington’s disease, Huntington’s disease-like 2, spinobulbar muscular atrophy, and DRPLA19,20. 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 al14 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-14. 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’14. 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 damage21. In particular, abnormal SUMOylation might lead to transcriptional dysregulation through nefarious effects on HDAC4 (histone deacetylase 4)22. 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 literature3,6,9,17,23 in our opinion a negative rectal biopsy should not be used to rule out the diagnosis. Reasons for this are several
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.

REFERENCES


Figure A-J: Tissues sampled for microscopy were routinely processed. Formalin fixed and paraffin embedded material for routine microscopy sectioned at 6 um, while sections for immunohistochemistry were cut at 3 um. 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); α-synuclein (Zymed, 1:100); Ubiquitin (Dakocytomation); CD43 (Novocastro, 1:100); CD68 (Ventana, predilution); Alzheimer precursor protein, APP (Chemicon, 1:4000); Ki67 (Dakocytomation, 1:100); Fastmyosin (Sigma, 1:1000); Slow-myosin (Sigma, 1:8000); 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×). 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×). E- Neuronal loss, gliosis and microglial activation in the pars compacta of the substantia nigra (H&E, 200×). F- Gliosis of the globus pallidus interna (GFAP, 400×). G- Microglial activation in the globus pallidus interna (CD68, 200×). H- High power microscopy of the focally atrophic cerebellar cortex illustrating Purkinje dropout, 'torpedoes' and Bergmann gliosis (H&E, 400×). I- Neuronal loss, gliosis and microglial activation in the anterior horn of the lumbar-sacral spinal cord (H&E, 400×). 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×).


