BRAIN
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The spectrum of disease and tau pathology of nodding syndrome in Uganda

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Nodding syndrome is an enigmatic recurrent epidemic neurologic disease that affects children in East Africa. The illness begins with vertical nodding of the head and can progress to grand mal seizures and death after several years. The most recent outbreak of nodding syndrome occurred in northern Uganda. We now describe the clinicopathologic spectrum of nodding syndrome in northern Uganda. The neuropathologic findings of 16 children or young adults with fatal nodding syndrome were correlated with the onset, duration and progression of their neurological illness. The affected individuals ranged in age from 14 to 25 years at the time of death with a duration of illness ranging from 6–15 years. All 16 cases had chronic seizures. In 10 cases, detailed clinical histories were available and showed that three individuals had a clinical course that was predominantly characterized by epilepsy, whereas the other seven individuals had progressive cognitive, behavioural and motor decline, in addition to epilepsy. The main neuropathologic findings included: tau pathology (16/16 cases), cerebellar degeneration (11/16 cases) and white matter degeneration (7/16 cases). The tau pathology was characterized by filamentous tau-positive deposits in the form of neurofibrillary tangles, pre-tangles and dot-like grains and threads in the neuropil. All cases showed some degree of tau pathology in the neocortex and in the locus coeruleus with frequent involvement of the substantia nigra and tegmental nuclei and lesser involvement of other grey matter sites, but there was a lack of glial tau pathology. The tau pathology in the neocortex showed a multifocal superficial laminar pattern. We conclude that nodding syndrome is a clinicopathological entity associated consistently with tau pathology, but our observations did not establish the cause of the disease, or an explanation for the tau pathology.

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Introduction

Nodding syndrome (NS) is a neurologic disease that affects children in East Africa.[1] The syndrome has recurred in three separate geographical isolates in the last 50 years, each occurring as an epidemic in a separate country. The first documented cases occurred in Tanzania in the1960s.[2] An outbreak emerged in Western Equatoria (present day South Sudan) in 1998,[3] and the presently described outbreak commenced in the early 2000s in northern Uganda.[4] The initial onset and progression of illness was the same in all isolates. Overt signs usually appear in childhood between 5 and 15 years of age with stereotypical head dropping movements (nodding) and grand mal seizures.[5] The mainstay of clinical management is antiepileptic medication and supportive care. NS may follow a progressive course of neurologic decline. This includes seizure progression and cognitive and psychiatric disability. In the late stages of the Tanzanian epidemic of NS, there was reportedly dementia and parkinsonism.[2] Multiple children in the same family are often affected by NS.

Environmental,[4] clinical,[5,6] serological[7] and neuropathological[8,9] investigations have been applied to the Ugandan outbreak, recently focusing on cases from the Kitgum district, in the northern part of the country. Despite ongoing research, the aetiology and pathogenesis of NS remains obscure. Clinical studies on NS have focused on the seizures, but relatively little is known about the other clinical neurologic manifestations of the disease or the case fatality rate. Initial neuropathological studies revealed tau pathology[8] and microglial activation (neuroinflammation).[9] In this study, we report the clinical and neuropathologic features of NS in 16 cases that arose during the current outbreak of the disease in Uganda.

Materials and methods

Subjects

Supplementary Table 1 lists the cases used for this study, including 16 cases of NS (Cases 1–16). Post-mortem examination and post-fixation examination of the brain were conducted based on consent from the families. The neuropathologic features of the NS cases were compared to cases of well-characterized tau proteinopathies, including progressive supranuclear palsy (three cases, Cases 31–33), corticobasal degeneration (three cases, Cases 34–36), Pick's disease (two cases, Cases 37 and 38) and globular glial tauopathy (three cases, Cases 39–41). In addition, 14 cases of sudden unexpected death in epilepsy or death related to seizures occurring in epilepsy were used for comparison. Three cases of epilepsy originated from Kitgum, Uganda (Cases 17–19) and the other epilepsy cases originated from Ontario (Cases 20–30). Cases from Ontario all represented deaths due to sudden unexpected death in epilepsy. The cases of globular glial tauopathy originated from Europe.[10] All other well-characterized tau proteinopathy cases originated from Canada. This research was carried out in accordance with the Declaration of Helsinki. This research was approved by the Research and Ethics Committee of Mulago Hospital, Infectious Disease Institute, Makerere University and the National Council of Science and Technology, Uganda.

Neuropathological examination

Histologic sections were obtained from formalin fixed brains that had been fixed for months to years. In some NS cases, the brains had already been sectioned and blocks had already been sampled, but some whole formalin fixed brains were available for sectioning. In all 16 cases, standardized blocks for paraffin embedding were obtained from the frontal, temporal, parietal and occipital cortex, hippocampus (level of the lateral geniculate body), basal ganglia, thalamic region, midbrain, pons, medulla and cerebellum. Some cases lacked selected blocks, and samples of spinal cord were only available in two cases. In addition, the frontal cortex at the level of the anterior corpus striatum was contiguously sampled in Cases 1–10. Sections from all blocks were prepared for routine staining with Luxol fast blue (LFB) and haematoxylin and eosin (H&E) staining and immunostaining with hyperphosphorylated tau (AT8). Selected sections were stained with a modified Bielschowky stain or the Gallyas stain. Additional immunohistochemical studies were conducted in Cases 1–10 using a panel of antibodies (Supplementary Table 2). Representative sections of the frontal cortex, basal ganglia, and pons and only the frontal cortex were immunostained with AT8 in the well-characterized tau proteinopathy and epilepsy cases, respectively. Brain tissue from a case of Alzheimer's disease and frontotemporal lobar degeneration with TDP-43 positive-inclusions were used as positive controls for immunoreactivity for the appropriate antibody. The primary antibody was omitted in the negative control. All immunohistochemical stains were prepared with a Leica ST4040 automated stainer. Primary antibody binding to tissue sections was visualized using the Leica BOND Polymer Refine Detection system. Assessment of tau pathology was made using a 5-point semiquantitative grading scale: 0, none; +, mild (isolated, sparse); ++, moderate; and +++, marked. The semiquantitative grading of tau pathology in Cases 2, 4, 6, 9 and 11 has already been reported.[8]

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary material.

Results

All 16 NS cases derived from communities in northern Uganda and the majority of cases originated from villages in the district of Kitgum. All patients had been refugees and resided in camps for internally displaced people (IDP) during the conflict between the Lord's Resistance Army and the military forces of the Ugandan government. During residency in the IDP camps, the diet variably consisted of maize, roots, tubers, pulses and groundnuts. Food was in short supply. Many children were malnourished, and diaorrhea and malaria were common.[11] Prior to and after residency in IDP camps, the families lived in villages. The villages had simple huts built on a clay soil base within large areas of cleared forest with small crop cultivars. Families were mostly composed of subsistence farmers and were multigenerational. The families mostly consumed a diet of grains (sesame and sorghum), plants (pumpkin, leafy green vegetables, corn and beans) and root vegetables. The staple diet includes posho (cooked cornmeal).

Onset, progression and clinical aspects of nodding syndrome

Basic clinical and demographic information was available for all 16 cases and a detailed clinical history was available in 10 cases. The 16 cases of NS ranged from 14–25 years of age at the time of death, nine males and seven females (Supplementary Table 1). Vertical head nodding commenced while living in IDP camps. All cases

had grand mal seizures that were treated with carbamazepine for the long-term control of seizures. However, the supply and distribution of antiepileptic medication was often inconsistent.

Detailed clinical history was available in 10 cases (Table 1) and showed that the course of illness began with nodding of the head in nine cases. The duration of the illness (defined as interval between symptom onset and death) ranged from 6–15 years. Old injuries sustained due to seizures occurred in four cases, including burns and fall-related trauma. Three of the 16 cases had a clinical course dominated by epilepsy with no progressive neurologic decline. The other seven cases had epilepsy with cognitive, behavioural and motor decline. Cognitive impairment was characterized by gradual reduction in language comprehension, inability to follow commands and illogical speech. There was progressive inability to participate in the activities of daily living and a decline in the attention to hygiene. Language deterioration to mutism occurred in some cases. Constant drooling often resulting in soaking of clothing in saliva was a prominent feature in most cases. The development of odd behaviour occurred in all cases and was the most prominent aspect of the clinical history. Bizarre behaviour included aggression, and hyperorality (four cases, including coprophagia in three cases). Two individuals slowly developed progressive weakness.

Neuropathologic and immunohistochemical findings in nodding syndrome

The most consistent finding on macroscopic examination was generalized cerebral atrophy (950–1154 g, in five cases for which brain weight was available). There was minimal atrophy of the cerebral cortex, that was only observed in the frontal cortex (Fig. 1A). We did not observe fenestration of the septum pellucidum, enlargement of the ventricles or prominent perivascular spaces. Routine staining revealed scattered neurofibrillary tangles in the upper cortical layers, locus coeruleus, tegmentum and substantia nigra. Ghost tangles were commonly observed in areas with concentrated tangle accumulation (Fig. 1B). The cerebellum showed varying degrees of diffuse atrophy of the cerebellar folia with Purkinje cells loss and Bergmann gliosis in 11 of 16 cases (Fig. 1C). There was white matter degeneration indicated by pallor of white matter staining in nine cases (Fig. 1D). In addition, variable myelin loss was found in the striatopallidal (pencil) fibres (Fig. 1E), transverse pontine fibres and to a lesser extent, commissural fibres.

The main immunohistochemical finding was heterogenous neuronal tau pathology in all 16 cases. The tau pathology was represented by neurofibrillary tangles, fine granular diffuse perikaryal staining (pre-tangles), neuropil threads and dot-like grains in the neuropil. The intensity and distribution of tau pathology varied greatly between cases. Tau pathology was observed in the cerebral cortex and sub-cortical grey matter, including the midbrain and brainstem (Table 2).

Cortical tau pathology was characterized by a spectrum of severity from minimal to marked and was found in all cases (Fig. 1). Cortical involvement had three basic histologic features: (i) tau pathology was largely multifocal in distribution and preferentially involved the gyral crests. In many cases, the crown of a single gyrus, or part of a gyrus, could be flanked by relatively uninvolved gyri. The multiple foci were often clearly delineated with sharp and well-demarcated borders; (ii) tau pathology was often only apparent in the upper cortical layers, most prominently in layers 2 and 3. This superficial laminar distribution was the most prominent pattern of cortical tau deposition; and (iii) the intensity of tau pathology ranged from a patchy superficial laminar pattern with dot-like grains, threads and pre-tangles to more extensive tau pathology throughout all cortical layers. The frontal lobes tended to be more extensively involved than other cortical regions. The overarching pattern of cortical tau pathology was multifocal involvement of the superficial cortex along the gyral crest, but not at the depths of sulci. The superficial laminar distribution of tau pathology did not include thorny astrocytes in the subpial zone. In addition, there was no preferential perivascular distribution of tau deposits at the depths of sulci, or in perivascular areas, except focally in Case 1, in a single section of the frontal cortex.

The tau pathology in subcortical grey matter (Fig. 2) was most consistently observed in the midbrain and pons but with heterogeneity been cases (Table 2) with relative sparing of cranial nerve nuclei, except for the oculomotor nuclear group. The presence of extra-cortical tau pathology was most consistently observed in the locus coeruleus, other tegmental nuclei, pontine nuclei and the substantia nigra. Tau pathology in the substantia nigra and locus coeruleus was not typically associated with marked neuronal loss or gliosis. Some intracellular tangles, mostly in the brainstem, were unstained by AT8 and most extracellular ghost tangles were also unstained by AT8; however, these tangles were stained using the Gallyas method. This appeared to correlate with the degree of tissue preservation. A peculiar observation was a tendency for a preferential distribution of tau pathology within the base of the pons. Specifically, the ventrolateral pontine nuclei (the outer shell) seemed to be preferentially involved by tau pathology, compared to other regions in the pontine base. Isolated tau pathology was also found the basal ganglia, thalamus and the medulla, including the inferior olivary nucleus (Fig. 3). Tau pathology was present to a lesser extent in the basal ganglia, cerebellum and dentate nucleus.

The hippocampus consistently lacked tau pathology in the dentate gyrus, the pyramidal cells and the neuropil. There was no evidence of significant glial tau pathology throughout the brain, or deposition of tau in the white matter. There was no hippocampal sclerosis, although one case did show focal neuronal loss and gliosis in Sommer's sector (Case 9).

A single case of NS in a 20-year old male (Case 13) with seizures and progressive neurologic decline had widespread cortical and subcortical tau pathology and cerebellar degeneration. There was extensive tau pathology in the substantia nigra and pons. The grey matter of the substantia nigra, pons and spinal cord showed lymphoplasmocytic cuffs (Fig. 4 A–D) with adjacent tau pathology including dots, threads and tangles (Fig. 4 E–H). In another case (Case 1), there was patchy cortical tau pathology observed as in the other cases of NS, but there were also localized perivascular foci of tau immunoreactivity at the depths of sulci in the frontal cortex. Additional clinicopathological description is provided in the Supplementary material.

Immunohistochemical studies with a range of antibodies revealed a variety of findings (Fig. 5). Some cases showed scattered swollen axons in white matter tracts, including the pontocerebellar fibres, identified by antibodies to β-amyloid precursor protein and phosphorylated neurofilament. Immunostaining with the antibody used to stain phosphorylated neurofilament (SMI31) that recognizes a shared epitope in the phosphorylated neurofilaments in axons and in hyperphosphorylated tau, labelled a subset of tangles. Immunostaining for synaptophysin, CD45 and CD68 was present but variable due to post-mortem loss of staining. However, microglial activation was highlighted by immunostaining with CD68 and was most prominent in the pons and basal ganglia. This was characterized by perivascular macrophages and clusters of CD68 positive cells in the corticospinal tracts, and pontocerebellar fibres.

Table 1 Clinical history of 10 cases of NS

Parameter	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Age/sex	19/M	14/F	16/F	18/M	18/F	14/M	15/M	20/F	13/F	18/M
Year of onset	2008	2010	2003	2005	2004	2008	2004	2008	2009	2002
Year of death	2014	2014	2015	2017	2017	2014	2012	2017	2014	2014
Duration	6 years	4 years	12 years	12 years	15 years	6 years	8 years	9 years	5 years	12 years
Nodding	+	+	+	+	+	+	+	−	+	+
Grand mal seizures	+	+	+	+	+	+	+	+	+	+
Drooling	−	−	−	+	+	+	+	−	+	+
Activities of daily living	Normal	Normal	Normal	Developed complete dependency	Developed complete dependency	Social isolation	Developed complete dependency	Social isolation	Developed complete dependency	Developed complete dependency
Hygiene	Normal	Normal	Normal	Progressive loss	Progressive loss	Progressive loss	Progressive loss	Smearing stool on chest	Progressive loss	Progressive loss
Language	Normal	Mutism at terminal phase	Normal	Nonsensical speech	Nonsensical speech with progression to mutism	Progressive reduction in speech	Nonsensical speech	Impaired in last 2 years of illness	Gradual decline to mutism	Nonsensical speech
Comprehension	Failure to progress in school	Normal	Normal	Gradual impairment	Gradual impairment	Unable to follow simple commands	Loss of recognition of people and places	Gradual impairment	Gradual impairment Unable to follow simple commands	Progressive inability to understand language and recognize objects but retained ability to recognize family
Behaviour	Normal	Normal	Normal	Odd behaviour with aggression, laughing crying, repeating songs, lack of empathy, walking without clothing	Gradual onset of odd behaviour, aggression, laughter	Bizarre behaviour including collecting stool, signing, aggression and agitation	Apathy, social isolation and would not wear clothing unless dressed by others	Aggression, removal of clothing in public, repetitive demands for food, defecation in public	Odd behaviour, social isolation and inappropriate laughter	Aggression, fire starting, social isolation, refusal to wear clothing, word repetition
Hyperorality	None	None	None	None	None	Coprophagia and placing objects in mouth	Coprophagia and placing objects in mouth	Coprophagia	Placing objects in mouth (rotten mangos)	None
Motor decline	None	None	None	Staggered gait	Forward bending/ leaning of torso	Unable to eat without assistance, difficulty swallowing	Unsteady gait	Unsteady, tremor, and unable to walk at end of illness	Progressive weakness, especially lower limbs	Progressive weakness, especially lower limbs

(continued)

Table 1 (continued)

Parameter	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Wandering	None	Present at terminal phase	Present at terminal phase	None	Aimless roaming	Aimless roaming	Aimless roaming	None	Aimless roaming prior to onset of weakness	Aimless roaming prior to onset of weakness
Old injuries	None	None	Burned in fire during seizure	None	None	Burned in fire during seizure	Injuries sustained in seizure-related falls	None	Multiple scars	None
Terminal Event Cause of death	Status epilepticus	Increasing seizures	Status epilepticus	Cachexia and empyema	Febrile illness (likely malaria)	Increased seizure frequency and heat stroke	Progressive neurologic decline	Progressive neurologic decline	Progressive neurologic decline	Progressive neurologic decline

Immunostaining for glial fibrillary acidic protein did not show marked diffuse gliosis in grey or white matter. Immunostaining for β-amyloid, phosphorylated TDP-43, phosphorylated α-synuclein, FUS and TAF-15 revealed no immunoreactive lesions in the cerebral cortex or hippocampus.

Comparison of nodding syndrome to primary tauopathies and epilepsy

The tau pathology in NS was compared to classical neurodegenerative tauopathies. One of the most conspicuous differences observed was the paucity of glial tau inclusions and lack of white matter involvement in NS. Despite the differences in glial tau deposition, there was some overlap in the distribution of neuronal tau pathology in NS in subcortical sites, compared to other tauopathies. This overlap involved tangles, pre-tangles, neuropil threads and dot-like grains, but not Pick bodies. All cases of NS, progressive supranuclear palsy, corticobasal degeneration, Pick's disease and globular glial tauopathy had neuronal tau pathology in the locus coeruleus (Fig. 6).

No tau pathology was found in the frontal cortex from 11 age-matched epilepsy cases from Canada.

Three cases of fatal epilepsy that occurred in the same villages as the NS cases were also examined (Cases 17, 18 and 19). None of these cases had a prodrome of nodding, although all of the affected individuals resided in the IDP camps over the same time-interval as the NS cases. In Case 17, an 18-year old male developed a grand mal seizure disorder within the first year of life with life-long debilitating seizures with progressive neurologic decline and death. Neuropathologic examination revealed severe right hemiatrophy of the entire right cerebral hemisphere (brain weight, 700 g) with complete sparing of the left cerebral hemisphere, brainstem and cerebellum. Microscopically, there was widespread and extensive neuronal loss and gliosis of the right cerebral cortex, hippocampus and subcortical nuclei. No tau pathology was present.

Case 18 was a 14-year-old male with a grand mal seizure disorder without neurological decline. He developed grand mal seizures shortly after leaving a IDP camp, at 8 years of age. He had normal growth and development. He often had aggressive episodes and postictal confusion. There was a remote history of a fall out of a tree while picking mangoes in early childhood. On the day of his death, he was agitated and died suddenly and unexpectedly while running. No seizure was observed. The dominant pattern was multifocal superficial cortical tau pathology in the frontal lobes. Rare foci of tau pathology chronic traumatic encephalopathy-neuropathologic change[12–14] were present in the frontal cortex (Supplementary Fig. 1). Otherwise, the neuropathological examination revealed tau pathology that was essentially indistinguishable from Case 1.

Case 19 was a 24 year old male who develop a grand mal seizure disorder without nodding or concomitant neurologic deterioration. Seizure onset occurred during residency in the IDP camp. Upon resettlement, the family kept pigs for slaughter. Death occurred suddenly and unexpectedly. Neuropathological examination revealed tau pathology largely limited to multifocal superficial cortical tauopathy in the frontal cortex. There was also tau pathology in the locus coeruleus and sparsely in the pontine nuclei. The brain also showed neurocysticercosis.

Discussion

The spectrum of disease in nodding syndrome

We studied 16 cases with fatal NS to establish a clinicopathological profile of the spectrum of disease. Our neuropathological

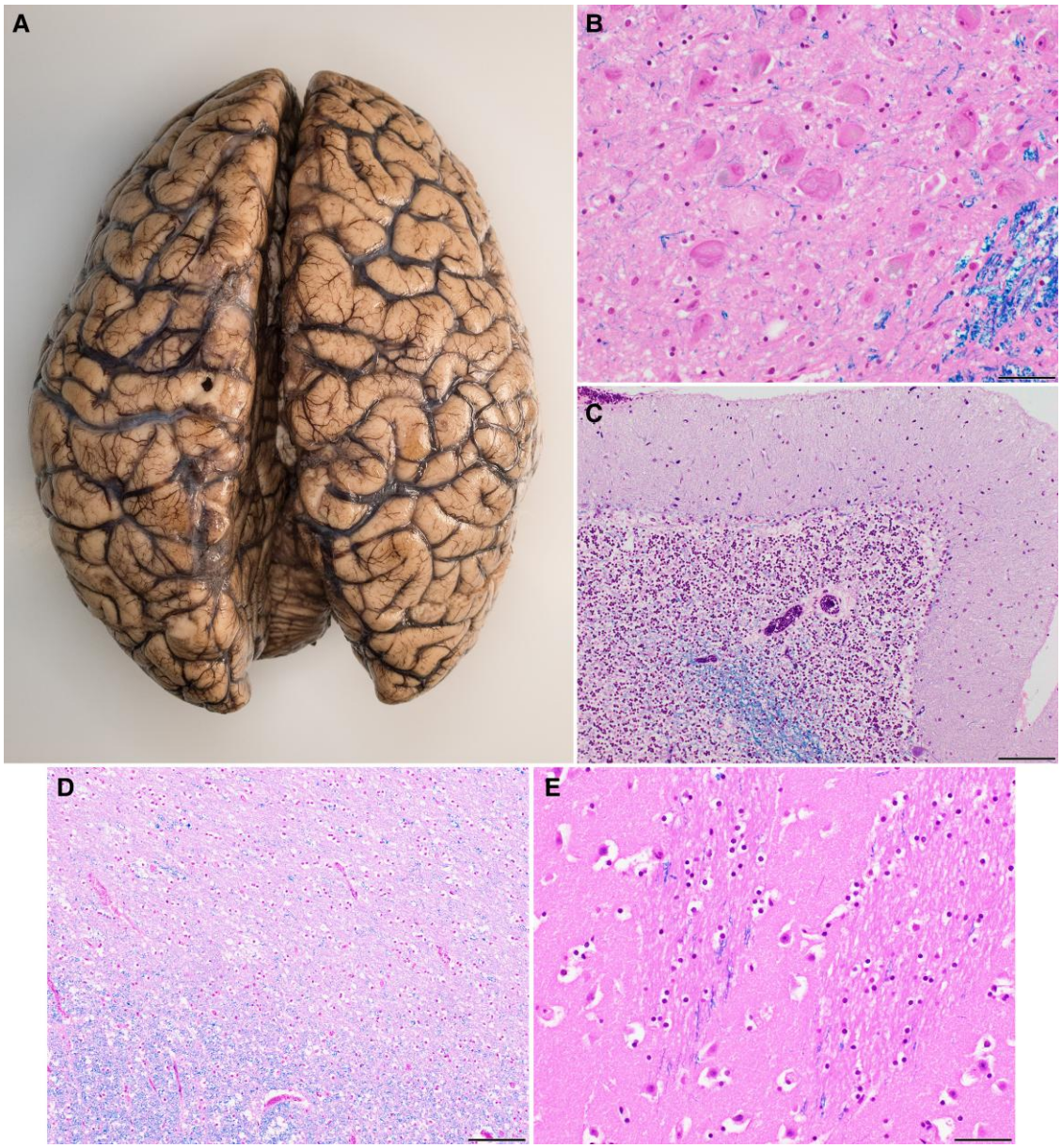

Figure 1 Neuropathological findings in NS. (**A**) View from the superior cerebral convexity showing minimal cortical atrophy. (**B**) Globose neurofibrillary tangles and ghost tangles in midbrain tegmentum (LFB/H&E, scale bar = 100 μm). (**C**) Cerebellar degeneration with loss of Purkinje cells and Bergmann gliosis (LFB/H&E, scale bar = 50 μm). (**D**) Degeneration with deep cerebral hemispheric white matter with relative preservation of myelin in optic radiation, in the lower portion of the image (LFB/H&E, scale bar = 50 μm). (**E**) Demyelination of the striatopallidal fibres (LFB/H&E, scale bar = 100 μm).

examinations indicate that NS is characterized by three hallmarks: tau pathology, cerebellar degeneration and white matter degeneration. The cerebellar and white matter pathology were not directly associated with tau-deposition in either the cerebellum or white matter. In addition, although NS was characterized by chronic seizures, hippocampal sclerosis or other manifestations of hippocampal pathology were not commonly observed in our cases.

The tau pathology in NS varied in intensity between the 16 cases, but all cases had tau pathology in the neocortex and in the locus coeruleus. In addition, tau pathology was frequently observed in tegmental nuclei and the substantia nigra. The deposition of tau in the neocortex and locus coeruleus suggests a primary role for both of these structures in NS, probably as the substrates of cortical epileptogenesis and noradrenergic neurotransmitter deficiency, respectively. The cortical tau pathology could also explain progressive deterioration in cognition and behavioural disinhibition. In some cases, the predilection for tau pathology in the tegmental nuclei could be the basis for other neurological disability such as gaze palsy. The basis for the cerebellar and white matter degeneration are unknown but likely represent secondary effects of neurodegeneration along tracts. For example, tau pathology was frequently observed in pontine nuclei in cases with cerebellar degeneration which could be explained by degeneration in the cerebropontocerebellar pathway.[15] However, the cerebellar degeneration in NS could also be explained as part of the NS pathological process, a secondary effect of recurrent seizures or anti-epileptic therapy. This is because both

Table 2 Distribution of tau pathology and cerebellar and white matter degeneration in cases of NS ($n = 16$[a])

Location	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16
Brain weight, g	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1154	1144	1075	990	950
Frontal cortex	+++	++	++	+++	+++	+++	+++	++	+++	+++	+++	++	++	++	++	++
Temporal cortex	++	+	+	++	++	++	+	+	+++	++	+++	+	+	–	++	++
Parietal cortex	+	++	++	++	+++	++	++	++	++	++	++	++	+	+	+	+++
Occipital cortex[b]	–	–	–	+	+	+	–	–	–	–	++	–	–	–	++	+
Amygdala	–	–	–	+	+	+	–	–	+	–	+	–	+	–	+	–
Hippocampus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Caudate/ putamen	–	–	–	+	–	–	–	–	+	+	+	–	–	–	–	–
Pallidum	–	–	–	+	–	–	–	–	–	–	++	–	–	–	–	–
Thalamus	+	+	–	+	++	–	+	–	+	+	++	–	+	+	+	+
Hypothalamus	–	+	–	NA	NA	+	NA	NA	+	NA	NA	NA	NA	+	+	–
Substantia nigra	–	+	–	+	+	+	+	–	++	+	NA	–	+	+	+	+
Locus coeruleus	+	NA	+	++	+	+	+	+	NA	+	++	+	+	+	+	+
Other tegmental nuclei	+	+	+	++	+	+	+	–	++	+	NA	+	+	–	+	–
Pontine nuclei	+	–	+	++	+	++	NA	–	NA	+	++	–	+	–	+	+
Inferior olivary nucleus	NA	–	–	NA	+	–	NA	NA	NA	NA	–	–	NA	–	+	–
Dentate nucleus	–	–	NA	–	NA	–	NA	–	+	+	–	–	NA	–	NA	–
Cerebellum	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–
Non-tau cerebellar degeneration[c]	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1	1
Non-tau white matter degeneration	0	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0

NA = not available.
[a]Cortical tau pathology was present in patchy foci and the grade represent the maximum intensity in cortical foci.
[b]Tau pathology in occipital lobe was limited to the extra-striate cortex.
[c]0 = absent; 1 = present.

epilepsy and carbamazepine are known to cause cerebellar degeneration.[16,17] In patients with epilepsy treated with carbamazepine, it may be impossible to determine if the cerebellar degeneration is due to one or the other. All NS cases in this study had recurrent seizures and were treated with carbamazepine. Thus, either or both could have contributed to the development of the cerebellar degeneration in our cases. Interestingly, tau pathology in the substantia nigra was not strongly correlated with marked loss of dopaminergic neurons and gliosis. This may be related to a latent and slow neurodegenerative process in NS. If this is true, then the aging cohort of individuals affected with NS in Uganda and South Sudan may slowly develop parkinsonism, as was documented in Tanzania.[2]

The clinicopathologic correlation in the 10 cases with detailed clinical information available revealed two groups of NS cases: three cases with an epilepsy-predominant clinical course and seven cases with chronic seizures and slow neurologic decline with cognitive and behavioural deterioration and motor impairment. The presence of increasing tau pathology, cerebellar degeneration and white matter degeneration was associated with the neurological deterioration. Otherwise, the age at death and duration of the disease was approximately the same for cases with epilepsy only, or epilepsy with neurological deterioration. This may indicate that NS has two clinical variants: a variant with seizures only, and a separate variant with a progressive neurodegenerative course. Therefore, inexorable neurologic deterioration may not be an invariable outcome in all cases of NS. This can only be resolved by a long-term prospective observational study of a group of NS cases with an epilepsy-predominant clinical picture at the commencement of clinical observation.

Nodding syndrome compared to similar diseases

Supplementary Table 3 compares the similarities and differences between NS and other diseases with tau deposits. There was an overlap in neuronal tau pathology in subcortical grey matter sites between NS, progressive supranuclear palsy, Parkinsonism-dementia complex of Guam[18,19] and post-encephalitic parkinsonism.[20] Interestingly, these diseases are not only unified by similar subcortical tau pathology, but three of these diseases share a combined three repeat and four repeat tau profile.[21] In addition, the common involvement of the locus coeruleus is observed across the tau proteinopathies. The main differences between NS and the other tauopathies was the lack of hippocampal tau pathology, the lack of diffuse glial tau pathology, and a novel pattern of cortical tau pathology in

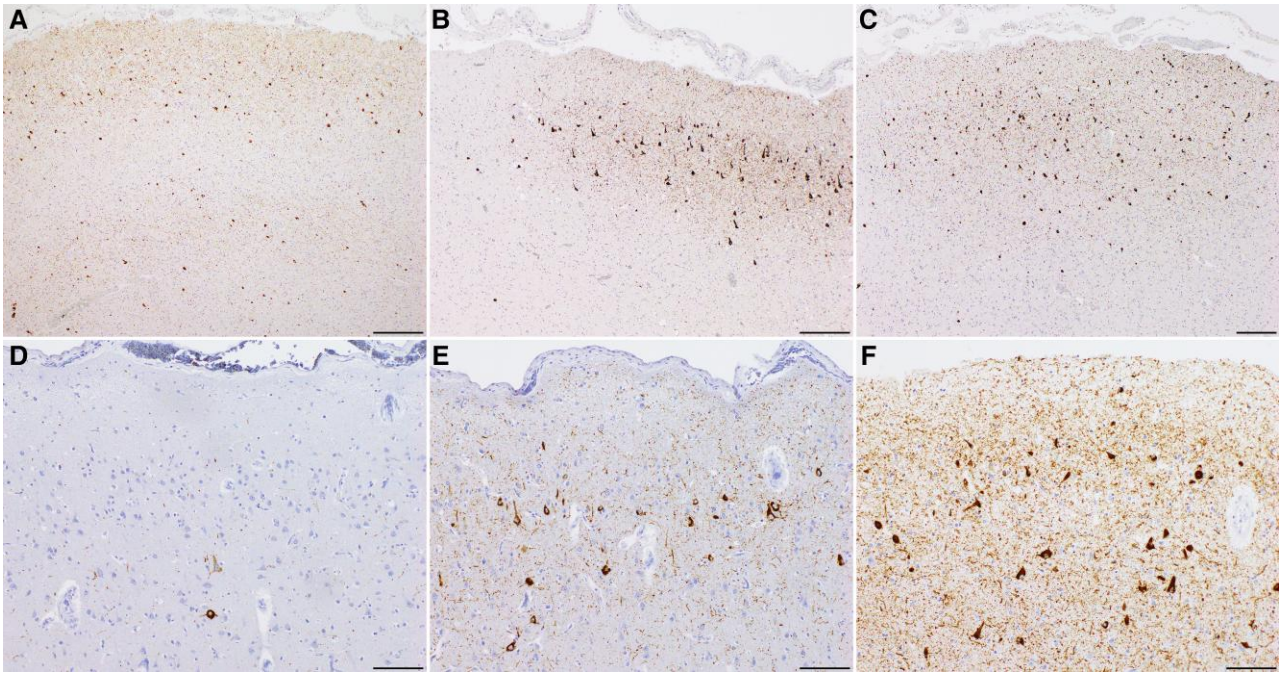

Figure 2 Cortical tau pathology in NS. (**A** and **C**) Cortical tau pathology at crest of gyri. (**B**) Well demarcated border of the multifocal superficial cortical tau pathology pattern. (**D**) Mild cortical tau pathology with dots and pre-tangles. (**E**) Moderate cortical tau pathology with a full spectrum of tau pathology. (**F**) Marked cortical tau pathology. (AT8, scale bar = 100 μm).

NS. The patchy nature of the cortical tau pathology in NS is somewhat reminiscent of chronic traumatic encephalopathy[22]; however, it must be emphasized that the tau pathology contrasted with chronic traumatic encephalopathy due to the opposite distribution of the tau deposition across the width of gyri and the lack of preferential accumulation in the depths of sulci. There was a relative absence of characteristic glial tau pathology, which seems to be an important hallmark of many well-characterized tau proteinopathies.

We also compared the neuropathologic findings and the clinicopathologic correlation between NS and epilepsy. Two cases of epilepsy in the same geographical catchment area as NS had tau pathology that was indistinguishable from the epilepsy-predominant form of NS. However, cases of epilepsy from Canada and a Ugandan case of epilepsy associated with cerebral hemiatrophy had no tau pathology. This suggests that the spectrum of disease in NS, in the affected communities in northern Uganda included: epilepsy alone, epilepsy with a prodrome of nodding, and a form of NS with a progressive neurodegenerative decline. The spectrum is unified by the presence of tau pathology. This implies that the clinical entity of NS has a broader set of clinical presentations than previously recognized. However, it is also important to mention that not all forms of epilepsy occurring in the study area had tau pathology, i.e. tau pathology was not a non-specific finding in all forms of epilepsy in the geographic area of the study. This is exemplified by the 20-year-old male with fatal epilepsy with cerebral hemiatrophy without any tau pathology (Case 17). Furthermore, there is anecdotal evidence that the appearance of NS in Uganda was also associated with an increased prevalence of epilepsy in individuals of similar age. Thus, the common denominator of tau pathology in these cases is likely explained by a unifying cause for epilepsy and NS in northern Uganda.

It has been suggested that recurrent seizures could secondarily cause accumulation of filamentous tau in NS.[9] On this basis, it has been suggested that tau pathology could simply reflect a nonspecific reaction to repeated neuronal excitation, as a consequence of ongoing seizures. The presence of tau pathology in surgically resected temporal lobes from cases of temporal lobe epilepsy could support this view.[23] However, tau pathology has not been reported as a universal finding in other types of epilepsy and tau pathology was not detected in Canadian cases of fatal epilepsy in our study. Furthermore, the non-random distribution of tau pathology in the NS brain, and particularly the additional and consistent involvement of subcortical structures, is more reminiscent of a neurodegenerative process. Indeed, the natural history of epilepsy has been studied extensively for decades and does not include universally progressive neuropsychiatric and motor decline (such as parkinsonism), nor the formation of neurofibrillary tangles and other manifestations of tau proteinopathy in the cerebral cortex, brainstem, and cerebellum. The clinicopathologic correlation and the involvement of multiple brain regions with both tau pathology and other degenerative changes indicates that epilepsy is more likely a symptom of the underlying brain injury rather than the primary process that causes the neuropathological changes in NS.

There is circumstantial evidence that NS may be related to the aftermath of an outbreak of an infectious disease. It is well known that subacute sclerosing panencephalitis due to infection with the measles virus includes tau pathology in the form of neurofibrillary tangles.[24,25] But, the necrotizing/sclerosing process of subacute sclerosing panencephalitis is not mirrored in NS, and intranuclear Cowdry type A inclusion bodies were not seen in NS brains. Tau pathology has also been described in the aftermath of West Nile virus encephalitis.[26] Specifically, infection with the West Nile virus can produce neurofibrillary tangles in the substantia nigra, in the absence of severe loss of dopaminergic neurons. This correlates well with our findings of tau pathology with relative neuronal preservation in the substantia nigra in cases of NS.

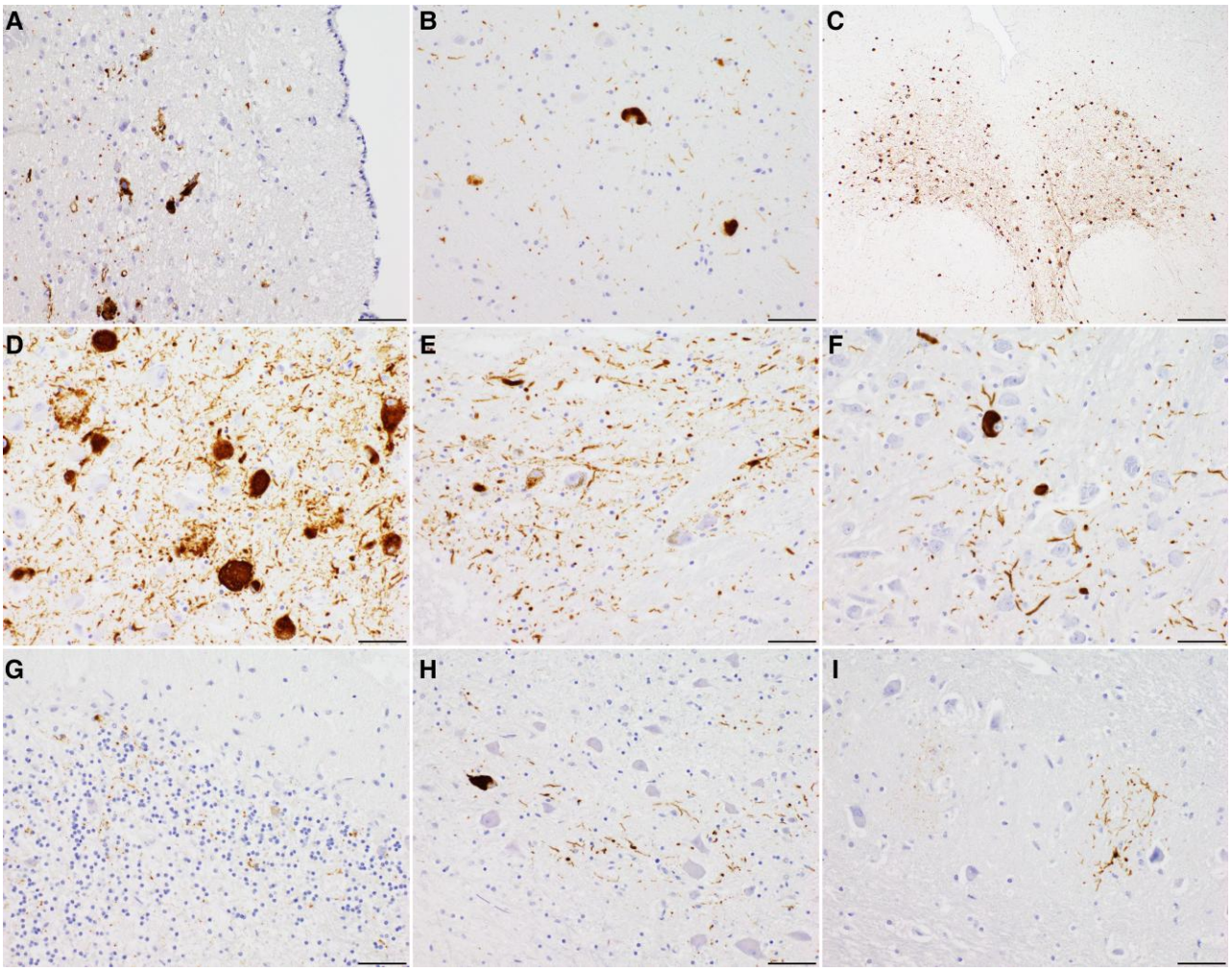

Figure 3 Representative images of tau pathology in subcortical grey matter in NS. (**A**) Hypothalamus. (**B**) Thalamus. (**C**) Oculomotor nuclear group. (**D**) Oculomotor nuclear group. (**E**) Substantia nigra. (**F**) Basal pontine nuclei. (**G**) Cerebellum. (**H**) Dentate nucleus. (**I**) Inferior olivary nucleus. (AT8, scale bar = 100 μm).

During the pandemic of encephalitis lethargica, many of the survivors of the acute illness subsequently developed parkinsonism.[27] Many of these individuals then died after decades of severe disability characterized by akinesia and rigidity. Neuropathologic examination revealed neurofibrillary degeneration often with involvement of the midbrain and brainstem.[20,28,29] Interestingly, although many cases of post-encephalitic parkinsonism were clearly associated with a clinical history of encephalitis lethargica, an acute encephalitic illness was not always recorded.[20] Although the virus responsible for encephalitis lethargica was never isolated, post-encephalitic parkinsonism is generally accepted to be the consequence of virus-mediated neurodegeneration. Although a possible candidate for the cause of encephalitic lethargica is the influenza virus, this has not been confirmed.[30,31] The distribution of tau pathology in NS partially overlaps with that described in post-encephalitic parkinsonism with shared involvement of the tegmental nuclei, locus coeruleus and substantia nigra. One NS case had tau pathology that coincided with perivascular lymphocytic infiltrates and microglial nodules. This single case could also represent an outlier with the coincidental occurrence of encephalitis and NS. Therefore, further neuropathologic studies are needed in NS cases to determine if other 'overlap cases' occur.

Tau pathology in nodding syndrome compared to chronic traumatic encephalopathy

Although the patchy nature of cortical tau pathology in NS could be interpretated as reminiscent of chronic traumatic encephalopathy,[12] the pathognomonic features of chronic traumatic encephalopathy, such as the patchy distribution of perivascular tau deposition at the depths of sulci, were clearly not seen in NS. Therefore, our NS cases did not fulfill the neuropathologic criteria for chronic traumatic encephalopathy neuropathologic change.[32] Rare perivascular foci were only found in Cases 1 and 18. The dominant pattern in NS was superficial cortical tau pathology along the crests of gyri, in contrast to the pathognomonic lesion in chronic traumatic encephalopathy. In addition, the other consistently observed features of chronic traumatic encephalopathy[12] were not found in our NS cases: (i) none of the macroscopic observations of the NS cases had fenestration of the septum pellucidum, enlargement of the frontal and temporal horns and prominent perivascular spaces; (ii) the hippocampus is not involved by tau pathology in

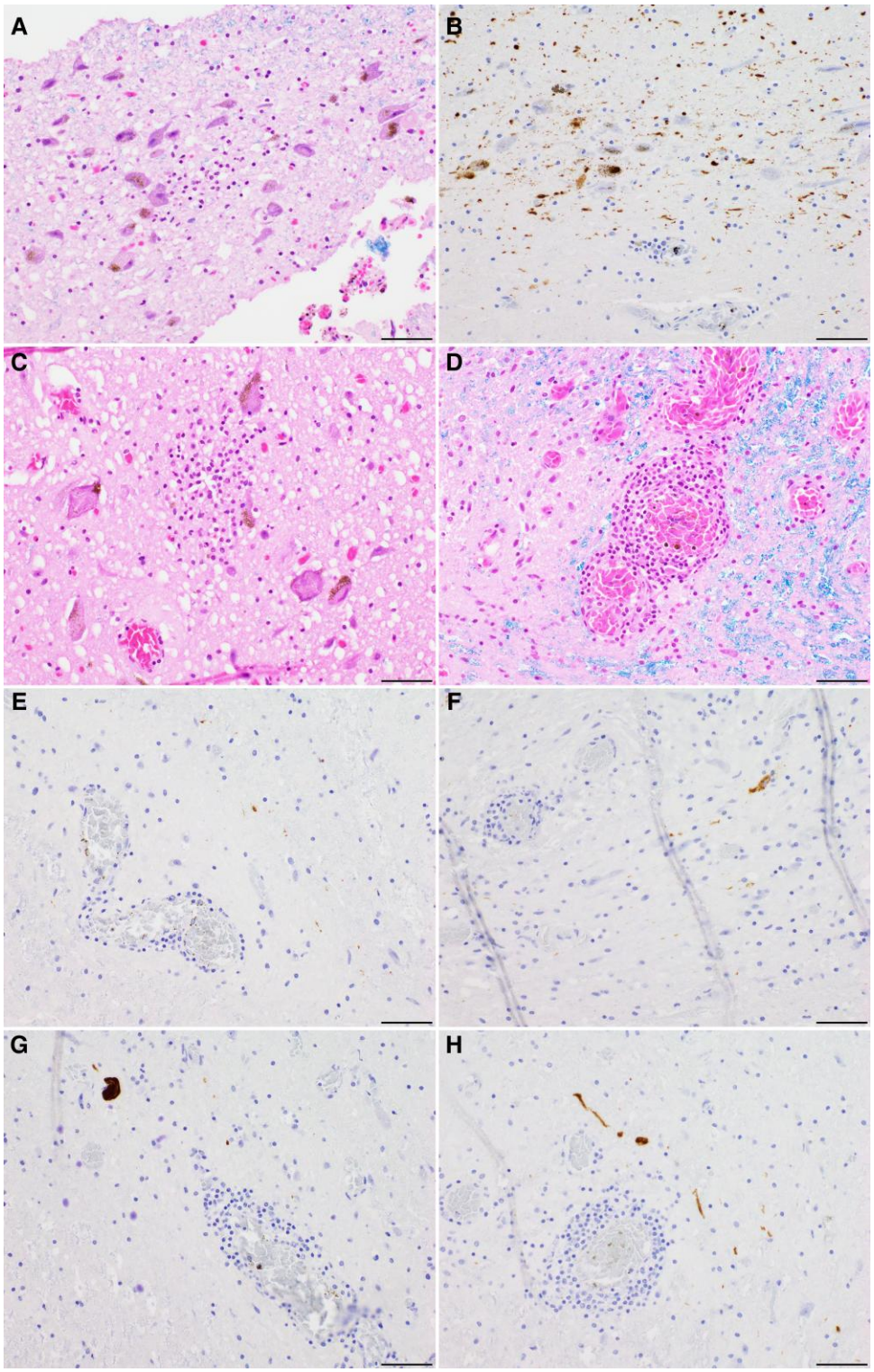

Figure 4 Neuropathological findings in a case of NS with overlapping encephalomyelitis and tau pathology. (**A**) Substantia nigra showing neurofibrillary tangles and microglial nodule (LFB/H&E). (**B**) Tau pathology in substantia nigra (AT8). (**C** and **D**) Perivascular lymphocytic cuffs in spinal cord grey matter (LFB/H&E). (**E–H**) Tau pathology in spinal cord grey matter adjacent to perivascular cuffs, ranging from dot-like grains, threads and a tangle (AT8). (AT8, scale bar = 100 μm).

NS; (iii) the consistent presence of TDP-43 in chronic traumatic encephalopathy is absent in NS; (iv) astrocytic tau pathology were not present in NS; (v) there was no specific history of head injury, although two cases each had a history a burns or falls; and (vi) the demographic profile and natural history of NS is unlike chronic traumatic encephalopathy. Chronic traumatic encephalopathy and other neurodegenerative diseases are frequently associated with limbic pathology. Therefore, it will be important to determine

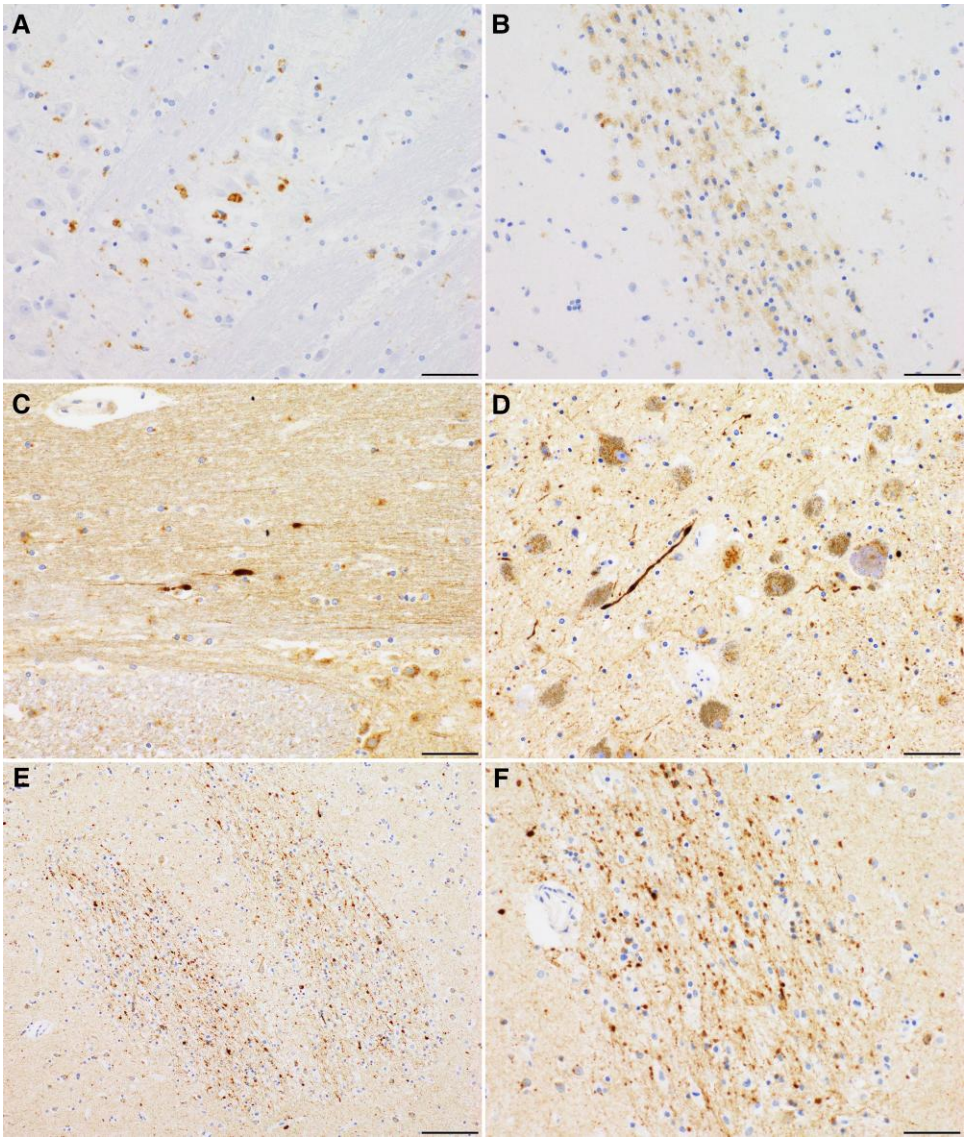

Figure 5 Immunohistochemical findings in NS. (**A**) CD68-positive macrophages in the pons. (**B**) CD68-positive macrophages in the striatopallidal fibres. (**C**) β-amyloid precursor protein immunoreactive axonal varicosities involving the transverse pontine fibres. (**D**) Axonal varicosities in the locus coeruleus with immunoreactive for phosphorylated neurofilament. (**E**) β-amyloid precursor protein immunoreactive axonal varicosities involving the striatopallidal bundles (Scale bar = 100 μm). (**F**) Higher magnification of **E** (Scale bar = 200 μm).

if the ageing cohort of NS patients in Uganda develop increasing limbic tau pathology. Further longitudinal neuropathologic studies are needed to understand the whole spectrum of tau pathologies in NS, particularly with ageing of the patients.

Nodding syndrome and autoimmunity

It is well recognized that autoimmune mechanisms can cause disorders of the CNS. Specifically, an infection could be the substrate for eliciting an autoimmune reaction. There are at least four lines of evidence to support an autoimmune mechanism in NS. First, microglial activation is present in the NS brain and may represent a neuroinflammatory manifestation of an autoimmune process.[9] Second, autoantibodies to glutamate receptors have been demonstrated in some cases of NS.[33] Third, the recently discovered anti-IgLON5-related tauopathy is the first tauopathy that is likely caused by an autoimmune attack of a neuronal antigen.[34] IgLON5 is a neuronal adhesion molecule that is ubiquitously distributed in the brain, although it is unclear how the autoantibody could mediate tau aggregation. Fourth, like anti-IgLON5 tauopathy, there is over-representation of HLA haplotypes in NS.[33] Overall, anti-IgLON5-related tauopathy may represent the cardinal example of an autoimmune neurodegenerative tauopathy, and a similar mechanism could be operative in NS.

Other possible aetiologies of nodding syndrome

Infection with the nematode *Onchocerca volvulus* (onchocerciasis) has been epidemiologically linked to NS.[35–37] However, there is no evidence that the nematode enters the CNS. It has been proposed that an antibody to an epitope shared between a nematode-derived protein and human brain leiomodin-1 may result in an autoimmune reaction as a basis for how the nematode could cause NS.[7] However, a subsequent study did not support the pathogenic role for leiomodin-1 autoantibodies in NS.[38] Our neuropathologic findings do not indicate

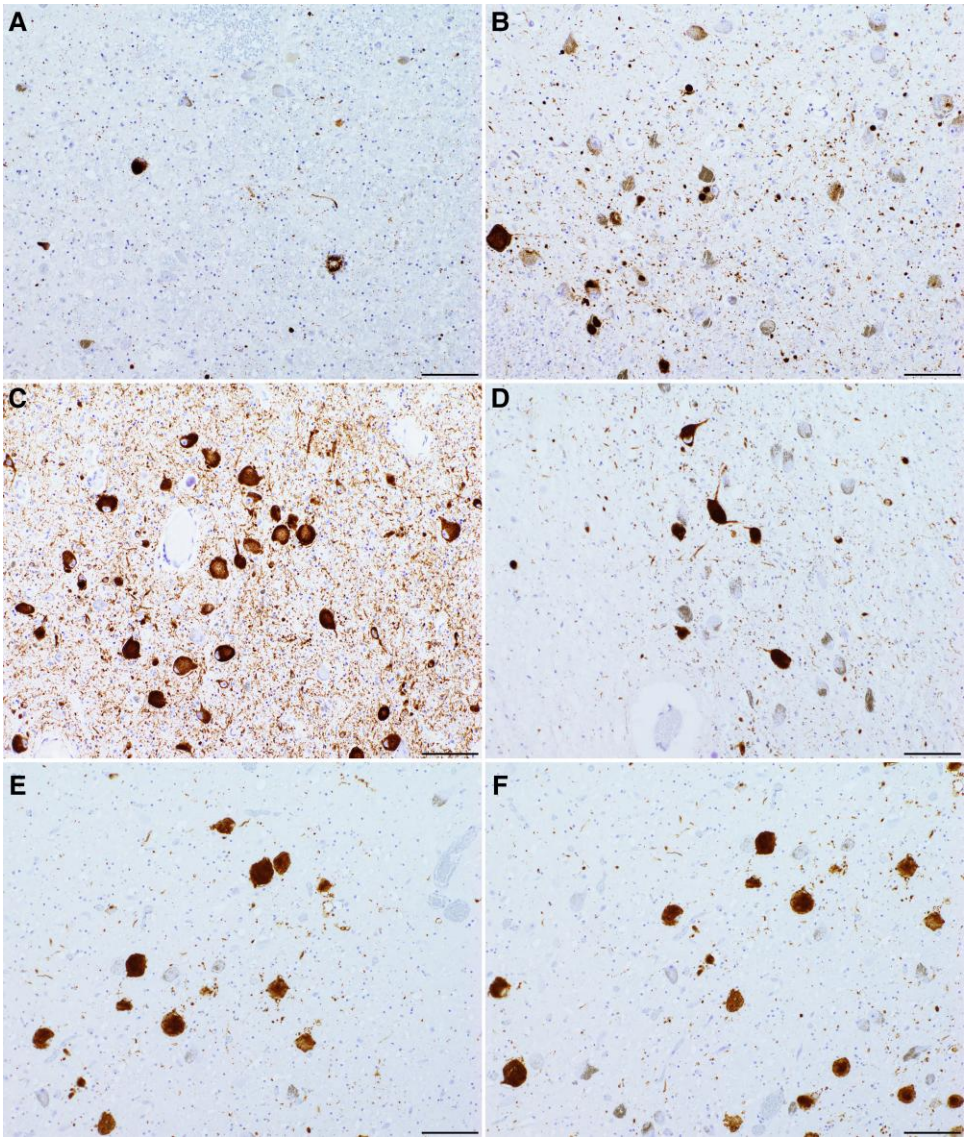

Figure 6 Tau pathology in the locus coeruleus in NS and other tauopathies. (**A**) Globular glial tauopathy. (**B**) Pick's disease. (**C**) Corticobasal degeneration. (**D**) Progressive supranuclear palsy. (**E** and **F**) NS. (AT8, scale bar = 100 μm).

how onchocerciasis could cause NS. But, serological studies of antibodies to *Onchocerca volvulus* were not available for our cases.

Other considerations for possible cofactors in NS pathogenesis could include neurotoxins. Dietary neurotoxins can cause disease in malnourished individuals at the time of famine and are well-known to cause neurological disorders in Africa. The paradigm example is konzo, an endemic irreversible spastic paraparesis caused by ingestion of cyanogenic cassava.[39] Another classical example is neurolathyrism, another spastic paraparesis caused by the ingestion of grass peas which contain a neurotoxic amino acid.[40] On this basis, neurotoxin(s) cannot be excluded as a cause or contributing factor in NS, although common mycotoxins have been excluded as a factor.[41] Although the mystery of the Parkinsonism-dementia complex of Guam has never been solved, there is some indication that an acquired environmental factor contributes to the disease. This may represent a neurotoxin, although this is a matter of debate that has not been settled. Despite this, the similarities between NS and Parkinsonism-dementia complex of Guam (e.g. progressive neurologic disease in a geographical isolate, clusters of affected individuals within the same family and tau pathology) could indicate a similar environmental factor, such as a neurotoxin that initiates or promotes tau pathology.

NS is associated with multiple cases occurring in siblings,[42] and there is some evidence of HLA preponderance in cases of NS.[33] These clusters may represent a common environmental/infection exposure or a genetic predisposition.

Limits of this study

The main limitation of this study is that we cannot know if our post-mortem cases are fully representative of all clinical cases. Specifically, the post-mortem cases were skewed to NS patients with a progressive course rather than epilepsy only. The high prevalence of a progressive course in our study of fatal cases, may not reflect the most common clinical course of NS. This simply may be related to overrepresentation of the more lethal form of the disease in a post-mortem study.

Another limitation of our study is that the immunohistochemical analysis of NS brain tissue was hampered by post-mortem changes. The logistics of conducting post-mortem examinations in rural Africa often result in a prolonged post-mortem interval. The cytoskeletal proteins are resistant to proteolysis, despite a prolonged post-mortem interval, but other epitopes such as markers for lymphocytes are not as resistant.

The observation of a single case of NS (Case 13) with overlapping encephalomyelitis and tau pathology could imply a key causal role for viral infection. However, this single case could also represent an outlier with the coincidental occurrence of encephalitis and NS. Therefore, further neuropathological studies are needed in NS cases to determine if other 'overlap cases' occur. It will also be important to determine if there is a quantitative relationship between cerebral tau burden and neurological progression with increased disability. The similarities and differences between the tau pathology in NS and other diseases with tau deposition must also be further explored.

Conclusion

We conclude that NS is a nosologically distinct entity characterized by periodic outbreaks of the disease in Africa. The clinicopathological spectrum is unified by epilepsy, tau pathology and neurological decline, in most fatal cases. NS shares features with other tau proteinopathies, however, peculiar features including a pattern of superficial cortical tau pathology in NS, largely involving gyral crowns, allows distinction from the main tauopathies. The cause of the tau pathology in NS has not yet been established.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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