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ORIGINAL ARTICLE

Neuroinflammation and Not Tauopathy Is a Predominant Pathological Signature of Nodding Syndrome

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Abstract

Nodding syndrome (NS) is an epileptic disorder occurring in children in African onchocerciasis endemic regions. Here, we describe the pathological changes in 9 individuals from northern Uganda who died with NS (n = 5) or other forms of onchocerciasis-associated epilepsy (OAE) (n = 4). Postmortem examinations were performed and clinical information was obtained. Formalin-fixed brain samples were stained by hematoxylin and eosin and immunohistochemistry was used to stain astrocytes (GFAP), macrophages (CD68), ubiquitin, α-synuclein, p62, TDP-43, amyloid β, and tau (AT8). The cerebellum showed atrophy and loss of Purkinje cells with hyperplasia of the Bergmann glia. Gliosis and features of past ventriculitis and/or meningitis were observed in all but 1 participant. CD68-positive macrophage clusters were observed in all cases in various degrees. Immunohistochemistry for amyloid β, α-synuclein, or TDP-43 was negative. Mild to sparse AT8-positive neurofibrillary tangle-like structures and threads were observed in 4/5 NS and 2/4 OAE cases, preferentially in the frontal and parietal cortex, thalamic- and hypothalamic regions, mesencephalon and corpus callosum. Persons who died with NS and other forms of OAE presented similar pathological changes but no generalized tauopathy, suggesting that NS and other forms of OAE are different clinical presentations of a same disease with a common etiology.

Key Words: Epilepsy, Nodding syndrome, Onchocerciasis, Postmortem, Uganda.

INTRODUCTION

Nodding syndrome (NS) is an epileptic disorder characterized by repetitive forward dropping of the head and mental retardation. Sometimes nodding seizures are accompanied with stunted growth and/or underdeveloped secondary sexual characteristics (Nakalanga syndrome) (1, 2). Nodding seizures develop between the ages of 3 and 18 years in children previously developing normally, and often progress towards generalized convulsions at a later stage (3, 4). From around the year 2000 onwards, an epidemic of NS was observed in northern Uganda (1). There is a growing body of evidence suggesting that *Onchocerca volvulus* may be the cause of NS, in addition to other forms of epilepsy (onchocerciasis-associated epilepsy [OAE]) and Nakalanga syndrome (3). However, the pathophysiological mechanism causing NS remains to be determined.

A recent study suggested that NS is an autoimmune disease caused by cross-reactivity between *O. volvulus* antigens and the human protein leiomodin-1 (5). Leiomodin-1 antibodies were more abundant in sera from Ugandan patients with NS compared to unaffected village controls and leiomodin-1 antibodies were detected in the cerebrospinal fluid (CSF) from patients with NS (5). Leiomodin-1 was found to be expressed in mature and developing human neurons in vitro and localized in the murine CA3 region of the hippocampus, Purkinje cells in the cerebellum and cortical neurons, structures that also appear to be affected in patients with NS (5, 6). However, leiomodin-1 antibodies have not been detected in all NS cases, suggesting that there might be some additional factors responsible for the pathology, or that the etiology of NS may be heterogeneous.

Another hypothesis to explain NS pathophysiology is that either the complete *O. volvulus* microfilariae, parasite-derived products or the *Wolbachia* bacterial endosymbiont cross the blood-brain barrier and cause neuronal damage either

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by direct neurotoxicity or by a secondary inflammatory response (7–11). Before the introduction of ivermectin mass drug administration, microfilariae have been observed several times in CSF of *O. volvulus* infected persons: the first time by Hissette (7), and later by Mazzotti (9) during diethylcarbamazine therapy. In 1976, Duke et al (8) detected microfilaria in CSF from 5 persons heavily infected with *O. volvulus*. However, more recent studies were unable to detect microfilariae or *O. volvulus* DNA in CSF (12). Possible explanations could be that in the earlier studies, microfilariae observed in CSF originated from the skin or that in the more recent studies the microfilariae load of study participants was too low to detect microfilariae in CSF because of past ivermectin use.

Magnetic resonance imaging (MRI) brain scans performed in children with NS in northern Uganda showed generalized atrophy in the cerebral cortex as well as the cerebellum, although not in all patients (13). In addition, MRI scans of patients with NS and other forms of epilepsy in an onchocerciasis endemic area of Tanzania showed atrophy of the brain, changes in the hippocampal region, gliotic lesions, and subcortical signal abnormalities (12).

Between 2013 and 2017, several postmortem examinations were performed in Uganda on children who died with NS but not all histological findings were interpretable because of the way these postmortem samples were processed (14). When these samples were investigated for a second time in 2018, NS was suggested to be a novel neurodegenerative disorder associated with the deposition of tau-immunoreactive neurofibrillary tangles in the brain (6). It is, however, not clear whether the reported tau deposition is a cause of the disease or a consequence of another pathological event as tau pathology can be induced by seizure-associated phenomena (15) as a result from subtle repeated head injuries (16) or postinfection (17, 18).

In November 2017, we initiated a series of postmortem exams in persons with NS and other forms of epilepsy who died in northern Uganda in the same districts where the first postmortems were performed. The main reason for conducting a second postmortem series was the poor sample quality and limited clinical information available in the first series. In the second series of postmortem examinations, we improved the processing of the brain samples collected more detailed clinical and demographic information.

MATERIALS AND METHODS

Study Design

This postmortem study was conducted in the districts of Gulu, Kitgum, Pader, and Lamwo in northern Uganda, where an epidemic of NS appeared in late 1990s. Prior to the study, meetings with the local authorities, healthcare facilities, village health teams, and community members were organized in each district to inform them about the study, to facilitate recruitment and increase acceptance of the study. Individuals below the age of 30 years who died in the districts mentioned above and were suffering from NS or other forms of epilepsy were considered eligible. NS (13, 19) and OAE (3) cases were defined according to previously described criteria. Village health teams detected eligible candidates and informed the health centers. Both verbal and written information about the research and the autopsy procedure was provided in English and Acholi (the main local language) and permission was obtained from the next of kin. Demographic data and history of the illness were obtained from the medical records and interview with the next of kin. The question was asked whether the deceased had a history of febrile illness, severe malaria, measles, or another severe disease requiring hospitalization prior to the development of seizures, and whether there were known complications during the delivery. The premortem degree of autonomy was assessed using a modified Rankin scale (20). A complete postmortem exam was performed within 24 hours after death by an experienced pathologist (S.O. and F.O.). Postmortem results were communicated to the next of kin.

Gross Pathological Examination

Gross pathological changes and main characteristics were documented during external examination followed by removal of all major organs. These were examined for abnormalities, weighed and a sample was fixed in 10% neutral buffered formalin. The whole brain was removed, weighed, examined for macroscopic abnormalities and completely submerged in 10% neutral buffered formalin for at least 4 weeks for complete fixation. The formalin was replaced monthly.

Macroscopic Examination of the Brain

Formalin-fixed brains were first examined from the outside and special external features were noted. A first cut was made through the brainstem at the mesencephalon to separate the brain stem and cerebellum from the rest of the brain. Additionally, a cut in the sagittal plane through the corpus callosum was made and each hemisphere was cut in the frontal plane in slices of approximately 1 cm to allow close investigation. Both were sliced separately. The following samples were taken for paraffin embedding: frontal cortex, corpus callosum anterior and posterior, olfactory bulb, optic chiasm, basal ganglia, thalamus, hippocampus, lateral and parietal cortex, occipital cortex, cerebellar cortex, vermis, dentate nucleus, pons, and medulla oblongata. An additional sample was taken from all regions showing macroscopic abnormalities.

Histopathological Evaluation

Paraffin-embedded samples of the visceral organs (lungs, liver, kidney, and heart) and the whole brain were stained by hematoxylin and eosin. Peroxidase-based immunohistochemistry with hematoxylin counterstaining was performed to stain astrocytes (FLEX polyclonal rabbit antiglial fibrillary acidic protein), macrophages (FLEX monoclonal mouse antihuman CD68 KP1), ubiquitin, and phosphorylated protein tau (AT8). In a selection of slides, immunohistochemistry of P62 lck ligand, α-synuclein, N-terminal TDP-43 (TDP43), and amyloid-β residues 17–24 (4G8) was performed. Further details on the protocols for immunohistochemistry are shown in Supplementary Data Table S1. Neurofibrillary tangles were visualized by Gallyas silver

staining. A case of Alzheimer disease (AD) was used as a positive control for both AT8 and silver staining. The presence of tau-reactive neurofibrillary tangles was scored in 20 or more high-magnification fields (400×) of AT8-positive brain regions/areas, as "sparse" (when 1 or 2 tangles were present in any field), "mild" (3–10 tangles in any field), and "abundant" (more than 10 tangles present in any field).

PCR Detection of *O. volvulus* in Brain Samples

DNA was extracted from brain samples of cases 3–9 and the 2 controls in the following regions: cerebellum cortex, dentate nucleus, frontal cortex, hippocampus, olfactory nerve, pons, choroid plexus, thalamus, medulla elongate, optic nerve, and mammillary body. To detect potential invasion of the brain by *O. volvulus,* quantitative real-time PCR was performed for 3 different *O. volvulus* genes: the nematode *actin-2* gene (GenBank: M84916), the ND5 gene (GenBank: AY462885.1), and the O-150 repeat (GenBank: J04659.1) as described previously (21–23). More details on the primers and PCR protocols used are shown in the Supplementary Data.

Ethical Approval

Ethical approval was obtained from Lacor Hospital, the Uganda National Council for Science and Technology (UNCST), and the ethics committee from the University Hospital of Antwerp.

RESULTS

Demographic and Clinical Information

Nine postmortem examinations were performed on individuals aged between 16 and 23 years at the time of death (median 18 years), 7 males and 2 females (Table 1). Seven had been living in the Kitgum district and 2 in the Pader district. Five presented with nodding seizures whereas 4 (cases 6, 7, 8, and 9) had a history of generalized tonic-clonic or absence seizures (case 8). The latter 4 cases met the criteria for OAE (3) and one of them (case 9) suffered from a gradual loss of sight and until he became completely blind by the age of 10 years. Participants without nodding seizures had a later onset of seizures (median 12 years) compared to NS cases (median 8 years); $p = 0.006$ and died at an older age (median 20 years) compared to those with NS (median 18 years); $p = 0.021$. However, all participants had suffered from the disease for a median duration of 9 years before death. All had received antiepileptic treatment but none was completely seizure-free and 4 died during seizures (Table 1). Adherence to antiepileptic drug treatment was reported to be poor and 6 were reported to have suffered from status epilepticus before. Six cases were not significantly disabled by their disease as they could carry out most routine household tasks. Case 2 was unable to perform routine household tasks or get dressed by himself but was able to walk without assistance (modified Rankin score 3). Two cases, 1 with nodding seizures (case 1) and 1 with generalized tonic-clonic seizures (case 6), were severely disabled prior to death (Modified Rankin scores of 4 and 5, respectively). All cases belonged to the Acholi tribe and received ivermectin treatment during the biannual mass distributions in the region. Only 2 of the cases had no siblings with epilepsy (Table 1). In the families of the other 7 cases, there were in total 14 siblings with other forms of epilepsy (9 with NS and 5 with another form of epilepsy) and in 5 families there was at least 1 person with NS and 1 person with another form of epilepsy.

Postmortem Examination

External postmortem examination showed that 5 cases had a wasted and unkempt appearance at the time of death, with dehydration and reduced subcutaneous fat and muscle bulk (Table 1). Three cases presented with Nakalanga features (3, 24) demonstrating stunted growth and underdeveloped secondary sexual characteristics, of which only 1 had a history of nodding seizures. The other 6 cases were fully developed according to their age. Two cases presented with burn scars acquired when falling in a fire while having seizures. Case 4 showed old therapeutic scars on the scapula from traditional healing practice.

Internal postmortem examination showed that most cases had abnormalities in the lungs: fibrinous adhesions to the chest wall, congestion, edema, or presence of gastric contents, either due to an underlying disease or aspiration of gastric contents during seizures. There was no common abnormality of the other major organs. Macroscopic examination of the brain and meninges appeared normal, with no visible lesions or scarring, no visible swelling, and normal blood vessels. One brain (case 6) was small (824 g), but did not show signs of atrophy. Two cases had slightly dilated ventricles (cases 2 and 7) and 3 showed autolysis (cases 2, 5, and 6). Individual case descriptions, as well as details on the weight of the organs, are provided in the Supplementary Data Table S2.

Neuropathology

Histological examination of the brains revealed general cortical neuronal loss in the cerebrum and atrophy and loss of Purkinje cells and granular cells in the cerebellum (Table 2). Neuronal loss in the cerebellum was more pronounced at the top of the folia and accompanied by hyperplasia of Bergmann glia (Fig. 1A, B). These pathological changes occurred in all cases in variable degrees, with the most severe pathology in cases 3 and 9 (Table 2). Gliotic lesions were observed in all cases, favoring the mesencephalon and cortical regions, including the molecular layer of the frontal cortex, periventricular space and around the aqueduct, indicating past ventriculitis or ependymitis granularis (Fig. 1C, D). The hippocampus was spared in most cases, with only 2 cases (cases 2 and 8) showing mild hippocampus sclerosis in the CA4 region. Foci of CD68-positive microglia were observed in all cases that were studied, mainly in the dentate nucleus, cerebellar vermis, medulla oblongata, hypothalamic regions, and frontal cortex (Fig. 1E, F; Table 2). Tau-immunoreactive neurofibrillary tangles and threads were observed in 4 of the 5 cases with a history of nodding seizures, and in 2 (cases 6 and 7) of the 4 cases with only tonic-clonic seizures (Tables 2 and 3). Tau depositions were mainly present in the cortical regions (superior gyrus of the

TABLE 1. Demographic and Clinical Information of the Cases in This Study

Characteristics	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
Age (years)	18	20	16	17	18	20	20	20	23
Gender	Male	Male	Female	Female	Male	Female	Male	Male	Male
District	Kitgum	Kitgum	Kitgum	Kitgum	Pader	Kitgum	Pader	Kitgum	Kitgum
Secondary sexual characteristics	Underdeveloped; stunted	Fully developed	Fully developed	Fully developed	Fully developed	Underdeveloped; stunted	Stunted	Fully developed	Fully developed
Clinical notes	Wasted	Well-nourished	Wasted	Well-nourished	Well-nourished	Wasted	Wasted	Well-nourished	Wasted
Cause of death	Septicemia secondary to lung infection	Asphyxia due to aspiration of gastric contents during seizures	Asphyxia due to aspirations of gastric content during seizures	Carbamazepine overdose	Asphyxia due to aspirations of gastric content during seizures	Septicemia	Severe dehydration following gastroenteritis	Suffocation during seizures	Liver failure, generalized metastatic Burkitt lymphoma
History of severe disease/ febrile illness	Measles	Severe malaria at 2 years	No	No	No	No	Severe malaria	Malnutrition as baby	Severe malaria as baby
Modified Rankin score	4	3	1	1	1	5	1	1	1
Nodding seizures	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Other seizures	Generalized tonic-clonic seizures	Generalized tonic-clonic seizures	Generalized tonic-clonic seizures	Generalized tonic-clonic seizures	Generalized tonic-clonic seizures	Generalized tonic-clonic seizures	Generalized tonic-clonic seizures	Absence and unclassified seizures	Generalized tonic-clonic seizures
Age at seizure onset	9	5	4	8	9	11	Childhood	12	13
Year of seizure onset	2006	2003	2003	2009	2010	2008	Unknown	2007	2008
Duration of disease (years)	9	15	12	9	9	9	Unknown	8	10
Antiepileptic drugs	Sodium Valproate	Carbamazepine and folic acid	Sodium valproate, carbamazepine and folic acid	Carbamazepine and folic acid	Sodium valproate and carbamazepine	Carbamazepine and folic acid	Sodium valproate	Carbamazepine and folic acid	Carbamazepine
Seizure frequency	Several/week	2–3/week	2/week	3/month	5/week	Several/week	1/month	Several/month	<1/month
History of status epilepticus	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes
Family member with NS/OFE	2 brothers with OFE	1 brother and 1 sister with NS	No	1 sister with NS; 2 sisters with OFE	2 brothers with NS	1 brother with NS; 1 brother with OFE	2 siblings with NS	No	1 brother with NS

NA: not available; NS: nodding syndrome; OFE: other forms of epilepsy.

TABLE 2. Histopathological Changes in the Brains

Histological findings	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
Loss Purkinje cells	±	±	3+	+	−	±	+	±	3+
Hyperplasia Bergmann glia	+	±	+	±	±	±	±	±	3+
Activated microglia	NA	+	+	±	±	NA	±	±	±
Loss of neurons	+	+	+	+	−	−	+	+	+
Lymphocytes in meninges	NA	+	NA	NA	−	NA	NA	+	+
Cerebellum atrophy	+	+	+	+	+	−	+	+	+
Gliosis	−	+	2+	±	±	−	+	+	+
Neurofibrillary tangles	Present	Present	Present	Present	Absent	Present	Present	Absent	Absent
Past ventriculitis or meningitis	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

NA: not available.

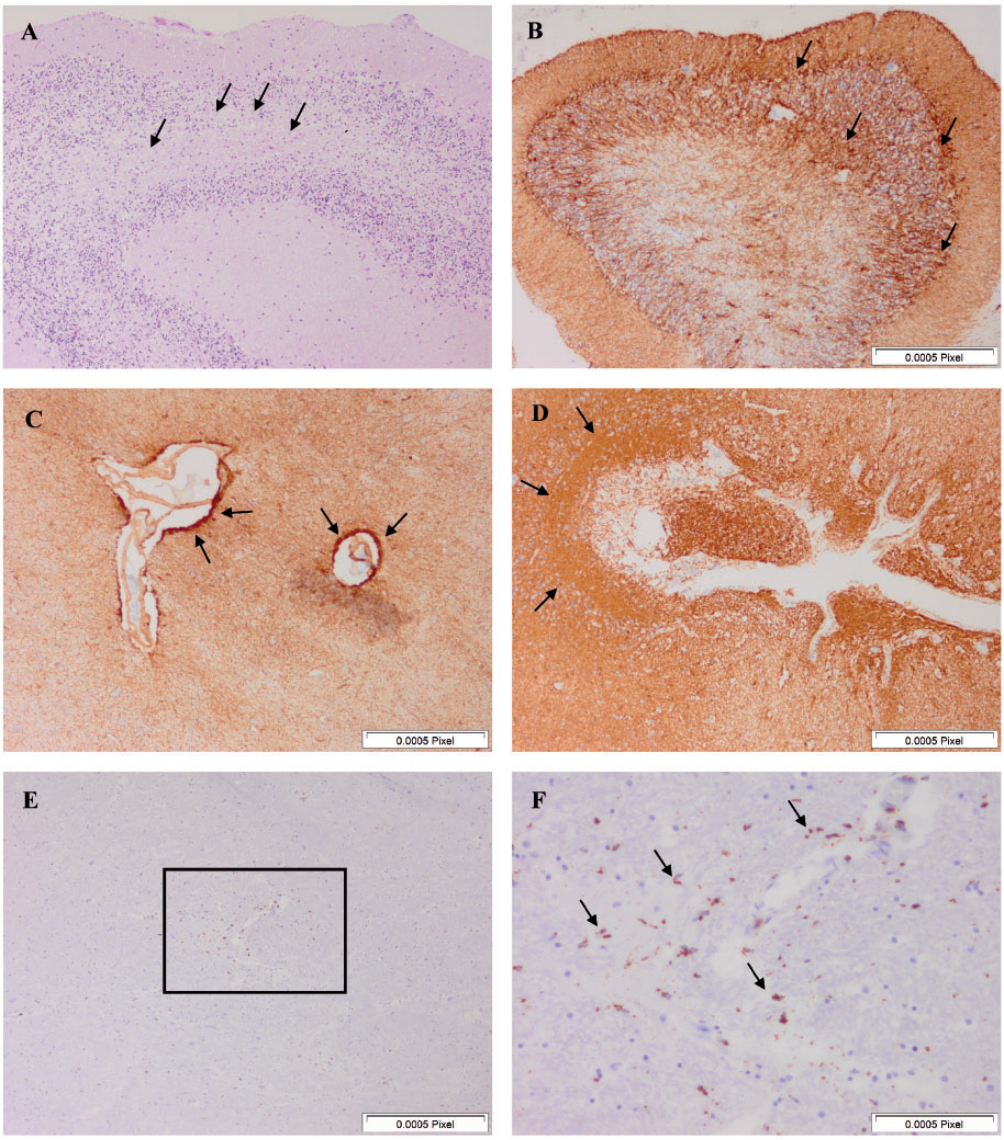

FIGURE 1. Main histological findings. **(A)** Loss of Purkinje cells at the top of the folia in the cerebellum (hematoxylin and eosin; arrows). **(B)** Hyperplasia of Bergmann glia in the cerebellum (GFAP; arrows). **(C, D)** Signs of past ependymitis granularis (GFAP; arrows). **(E, F)** Focus of activated microglia (CD68; black square and arrows).

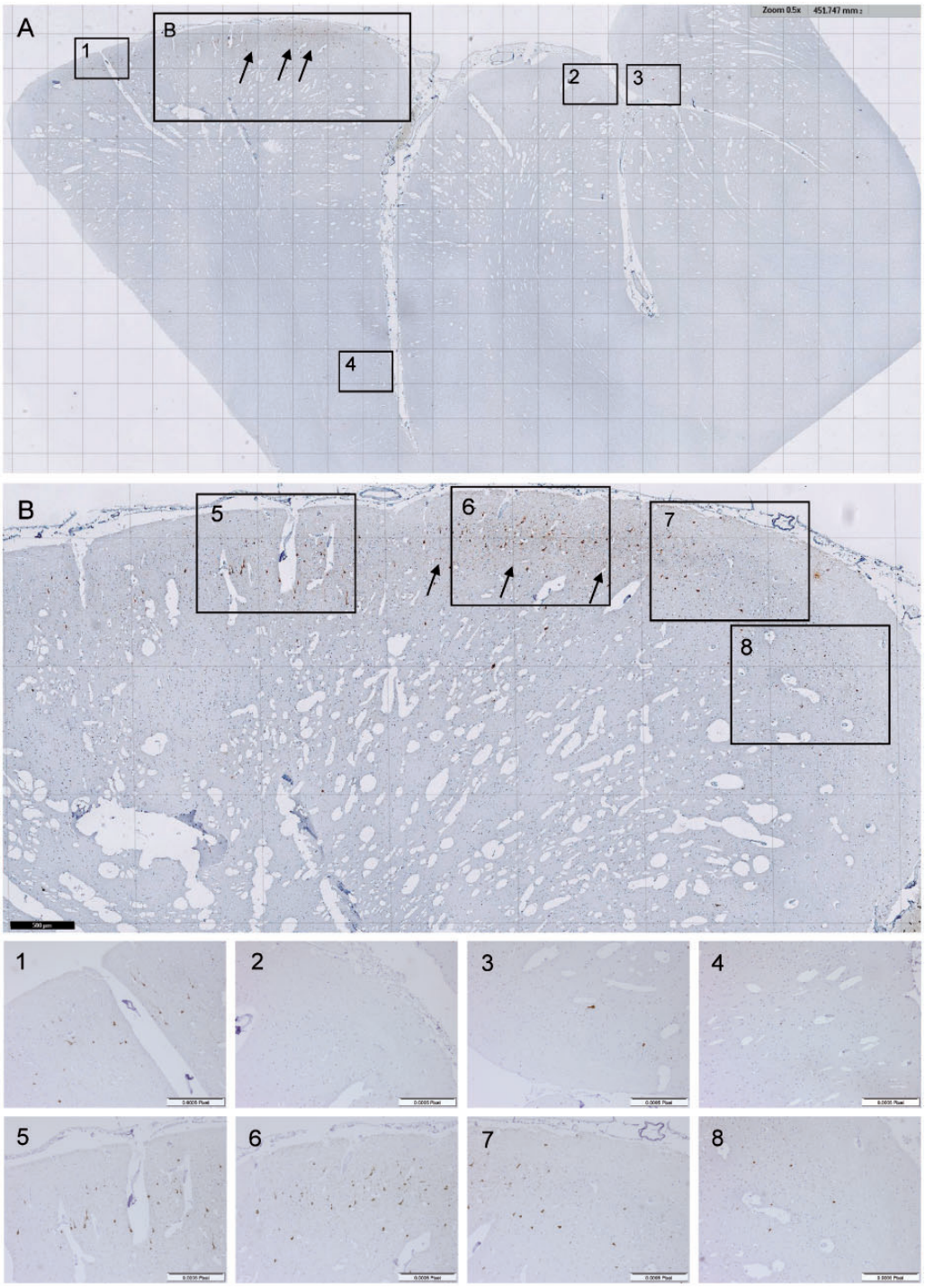

FIGURE 2. Focus of tau-immunoreactive neurofibrillary tangles (black arrows) in the parietal cortex of case 5. Gridlines: 1 × 1 mm. **(A)** Low-resolution scan (AT8) and **(B)** detail of **A**; 1–8: details of **A** and **B** (AT8).

temporal lobe, frontal and parietal cortex, and sulcus calcarinus), where foci of neurofibrillary tangles and threads were located in the cortical layers 1–3, at the top of the gyri and in the mesencephalon, thalamic and hypothalamic regions and basal nucleus (Table 3; Fig. 2; Supplementary Data). Cases 2, 3, and 4 showed the most abundant tau deposition, however, there was no association between tau pathology and any clinical or demographical characteristic, including age at the time of death, gender, or duration of disease but all 3 presented with nodding seizures. Figures 2 and 3 represent the samples with the most dense tau depositions observed in this case series. In the 3 most severely affected cases, the superior gyrus of the

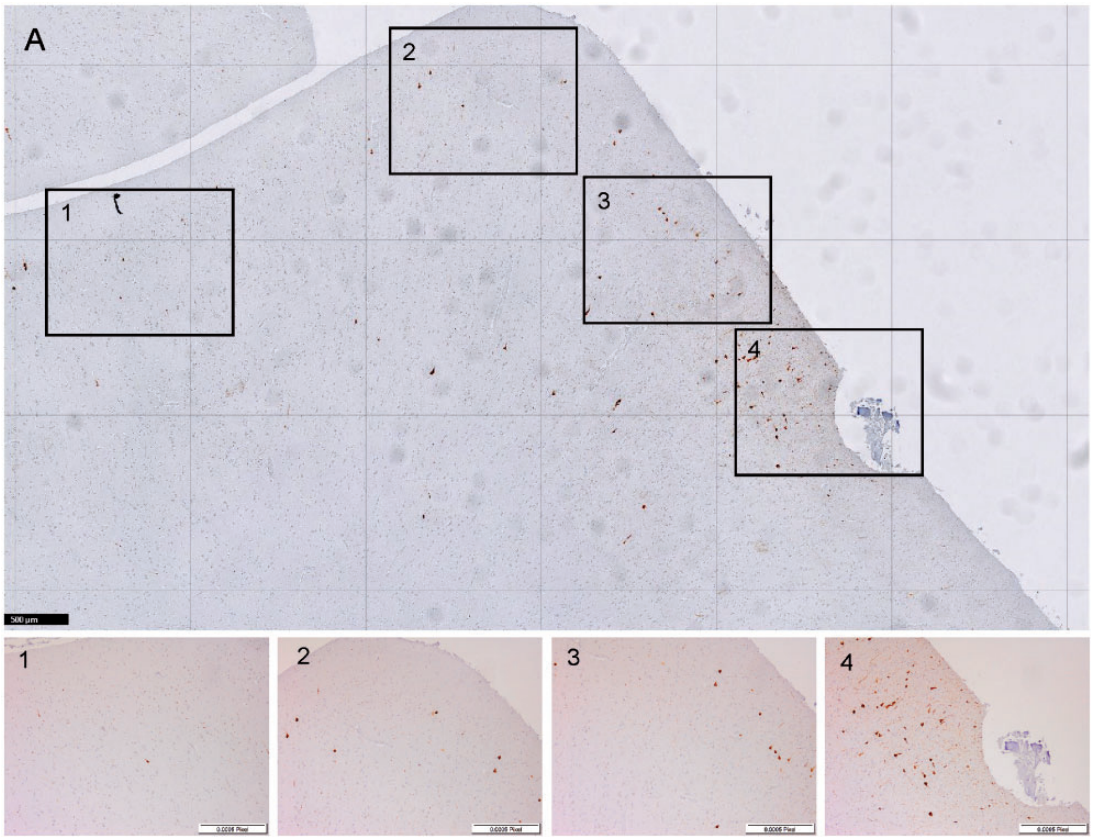

FIGURE 3. Focus of tau-immunoreactive neurofibrillary tangles in the superior gyrus of the temporal lobe of case 8. Gridlines: 1 × 1 mm. **(A)** Low-resolution scan (AT8) and 1–4: details of **A** (AT8).

temporal lobe contained foci of abundant tau deposition (Table 3). Sparse tau-immunoreactive neurofibrillary tangles were found in the pyramidal cells in the dentate gyrus of the hippocampus of one of the 2 controls. No astrocytic tau deposition was observed anywhere. Staining for Ubiquitin and P62 protein confirmed the presence of neurofibrillary tangles and threads but did not show any evidence of Lewy bodies. Staining for amyloid β, phosphorylated tau (TDP43) and α-synuclein were all negative. PCR results for the 3 genes of *O. volvulus* tested here were negative in all samples.

DISCUSSION

The brains of individuals who died with NS or another form of OAE were characterized by neuronal loss, especially in the cerebellum at the top of the folia, with associated hyperplasia of Bergmann glia and foci of gliosis throughout the brain, confirming previous MRI findings (12). Contrary to previous MRI findings in people with NS (12) and postmortem investigation of people who died with epilepsy (25), the hippocampus appeared normal in most cases, with very mild sclerosis in the CA4 region in only 2 cases. Fibrosis and sclerosis at the ventricles, aqueduct, and meninges indicate past ependymitis granularis, ventriculitis, and meningitis. There was no difference in both macroscopic and neuropathologic findings between the NS cases and the other epilepsy cases without a history of head nodding.

Neurofibrillary tangles and threads were found in 6 individuals, favoring the superficial layers of the cortical regions, the mesencephalon, thalamic and hypothalamic regions, and basal nucleus. Tau deposition was highly focal, with a gradient from the core of the focus to the direct surrounding regions to no deposition in the deep cortical layers. There were no signs of generalized tauopathy. Tau deposits were observed in 4 of the 5 NS cases and in 2 of the 4 cases with other forms of OAE, with the most abundant depositions in the NS cases. This may explain why in the previous postmortem study, only done in persons with NS, tau deposits were observed in all cases. This is also in accordance with the fact that NS is a more severe form of OAE associated with high disability, as was recently shown in a study in South Sudan (26). The highest amount of tau deposition was present in the temporal lobe followed by the frontal and parietal cortex, suggesting these regions experienced most stress. This is in accordance with the clinical presentation of NS and other forms of OAE which are characterized by severe cognitive impairment, convulsive seizures, hallucinations and behavioral changes (4). Differences in disease presentation and severity can also explain the differences between localization and amount of tau deposits.

Seven of the 9 cases had siblings with NS and/or other forms of epilepsy. All cases with nodding seizures also had

TABLE 3. Distribution and Intensity of Neurofibrillary Tangles in the Brains

Brain region	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
Medulla oblongata (tegmentum)	–	Threads	–	–	–	Threads	±	–	–
Medulla oblongata (oliva inferior)	–	NA	Threads	NA	NA	±	NA	–	NA
Pons	–	NA	NA	NA	–	±	±	–	–
Mesencephalon	–	±	±	–	–	±	±	–	–
Cerebellum vermis	–	NA	–	–	–	NA	–	–	–
Dentate nucleus	NA	NA	–	–	–	–	±	–	–
Frontal cortex	±	+	2+	+	–	+	+	–	–
Parietal cortex	NA	2+	+	+	–	NA	±	–	NA
Basal cortex	–	NA	±	±	±	NA	±	–	–
Sulcus calcarinus	NA	–	2+	2+	NA	±	NA	–	–
Cingulate gyrus	–	NA	±	±	–	+	NA	–	–
Temporal lobe (superior gyrus)	±	2+	2+	2+	NA	NA	±	–	–
Hippocampus (dentate gyrus)	–	+	±	±	–	–	–	–	–
Corpus callosum	–	NA	±	±	–	+	NA	–	–
Thalamus	–	±	NA	±	–	NA	±	–	–
Hypothalamus (anterior part)	NA	±	NA	NA	NA	NA	±	NA	–
Hypothalamus (central part)	–	±	NA	NA	–	NA	±	NA	–
Mammillary body	NA	±	±	NA	NA	NA	NA	NA	–
Caudate nucleus	NA	NA	±	NA	–	NA	–	–	–
Putamen and pallidum	±	±	+	NA	–	+	±	NA	–
Basal nucleus	NA	±	2+	NA	NA	NA	NA	–	–

±: sparse; +: mild; 2+: abundant; NA: not available.

generalized tonic-clonic seizures. Two of the cases without nodding seizures presented Nakalanga features. Our study, therefore, adds evidence that NS and other forms of epilepsy meeting the OAE criteria are different phenotypic presentations of 1 disease with a common etiology, but a wide clinical spectrum.

In the period 2013–2017, 12 postmortems on patients with NS were performed, 5 of which were described recently (6). In all of them, deposition of tau-reactive neurofibrillary tangles was reported, present in the same brain regions but markedly more abundant compared to our study series (6). Seven cases of this first cohort have not yet been described and it would be interesting to know which degree of tauopathy these presented.

The differences in tau detection between the 2 postmortem studies are likely not due to genetic differences in study populations, as all individuals belonged to the Acholi tribe living either in the Kitgum or Pader district. All individuals developed their first seizures while living in internal displaced person camps between 2005 and 2010. However, individuals included in our series were older when they died compared to those in the study reported by Pollanen et al. Median ages of death were 20 and 14 years ($p = 0.0062$), respectively. While ageing is a risk factor for development of tau pathology (27), it is unclear why we observed less and milder tauopathy. While individuals in both studies had been treated with antiepileptic drugs, based on the longer survival time, it is likely that several individuals in the more recent postmortem series had received better treatment and care. However, most cases in our series still presented weekly or monthly seizures and 6 had experienced status epilepticus.

Deposition of insoluble phosphorylated tau (p-tau) is observed in many other neurological disorders, with a distinction between diseases where protein tau dysfunction is the primary pathogenic event, such as AD (28, 29), and disorders where deposition of p-tau is secondary to another pathological phenomenon, such as chronic traumatic encephalopathy (CTE), temporal lobe epilepsy (TLE), and several postinfectious disorders (18, 29–31). NS shows some similarities and differences with these so-called secondary tauopathies. For example, CTE develops as a consequence of repeated traumatic brain injury, like head impacts or epileptic seizures (29). Like in NS, tau-deposition in CTE occurs in the form of threadlike neurites and large grain-like and dot-like structures preferentially affecting superficial cortical layers, subcortical nuclei including the hypothalamic nuclei, amygdala, nucleus accumbens, thalamus, midbrain, tegmentum, and isodendritic core (29, 30, 32). However, CTE is also characterized by the presence of tau-positive depositions in the depth of the sulcus and perivascular, and neurofibrillary tangles are present in the hippocampus and astrocytes at the glia limitans, which is not the case in NS (29, 30). Furthermore, a postmortem study investigating brains of people who died with epilepsy describes p-tau immunereactive neurofibrillary tangles with a similar distribution to NS, except that the hippocampus is affected in TLE (29, 32). In CTE, TLE as well as NS, deposition of p-tau is sharp and localized, preferentially in the gray matter and more intense around the sulci and pia mater, with the highest density of p-tau-positive neurons in the superficial cortical layer (29, 32). Moreover, in people with epilepsy, the severity of seizures was also not correlating to p-tau deposition (29).

The neuropathology in NS also shows similarities to p-tau depositions in postencephalitic Parkinsonism (PEP) (31) and subacute sclerosing panencephalitis (SSPE), both postinfectious disorders (18, 33). An epidemic of PEP was observed after the first world war following a disease called encephalitis lethargica, of which the etiology has never been identified (34). PEP is linked to autoimmunity against deep gray matter with severe degeneration of substantia nigra, neuronal loss and gliosis, and tau accumulation was observed in the microglia and other neurons, preferentially in the brainstem and subthalamic nuclei (31, 34, 35). Unlike NS, in PEP p-tau is also frequently observed in the hippocampus but only occasionally in the frontal and temporal cortices (35). SSPE sometimes occurs after measles infection and was shown to be associated with depositions of p-tau-reactive neurofibrillary tangles (18, 33). Similar to NS, large individual differences were found in tau depositions of SSPE cases and they were only observed in approximately 20% of cases (18). The main difference between tau deposition in NS and SSPE is the involvement of the hippocampus and pyramidal cells in SSPE and the more diffuse distribution of tau deposits in all layers of the brain (18), whereas in our study and that of Pollanen et al (6), the hippocampus was clear and the tau deposits were very localized. It has been suggested that deposition of p-tau neurofibrillary tangles is a secondary event to SSPE and this is likely also the case in NS (17).

The histopathology of NS is different from primary tauopathies like AD, fronto-temporal dementia (FTD), frontobasal degeneration, or argyrophiric grain disease (28, 34). The main difference between NS and AD and FTD is the location and abundance of the tau deposition, which is more diffuse and abundant in AD and FTD, with the hippocampus being the first region affected (27). Furthermore, many primary tauopathies are associated with the deposition of other insoluble proteins, such as β amyloid or the formation of Lewy bodies, which were not observed in NS (34).

All cases presented with some degree of cerebellar atrophy. Detailed reports of neurological exams were not available we do not know how many of these cases presented with cerebellar signs. However, walking abnormalities have been observed in persons with advanced NS disease. In a study in an onchocerciasis-endemic region in Maridi County in South Sudan, walking abnormalities were reported in 7.5% of persons with nodding seizures (26).

The strength of our study is that, in comparison of the first published postmortem study, we obtained relatively detailed clinical and demographic data and that we included cases with other forms of OAE besides NS. Most cases had been treated at the Kitgum General Hospital and information about the antiepileptic treatment regimen and seizure frequency was available. Families of the deceased were visited after the postmortem examination to provide information on the study progress and to cross-verify the demographical data and disease history.

A weakness of our study is the small sample size, with only 5 cases of NS and 4 cases with other forms of OAE. Performing postmortem exams in northern Uganda was challenging for logistical and sociological reasons. Furthermore, due to difficult transportation of the deceased from the village to the mortuary and the high temperatures, the brain of 3 cases showed signs of autolysis and therefore some samples had to be excluded from analysis.

In conclusion, our postmortem exams of 9 persons with OAE showed that only 6 of them presented tau-reactive neurofibrillary tangles. The amount and the distribution of the tau deposition are markedly different from primary tauopathies like AD but has some resemblances with several so-called secondary tauopathies such as TLE and SSPE. We, therefore, conclude that NS is not a primary tauopathy. Based on our postmortem findings, it is difficult to speculate about the pathophysiological mechanism of OAE. No histopathological evidence of a parasitic infection was found and also PCR testing for *O. volvulus* DNA was negative in all individuals (data not shown). However, all individuals presented their first seizures at least 7 years before they died. Therefore, we cannot exclude that at the moment of the first seizures microfilariae may have been able to enter the brain and caused permanent damage leading to recurrent seizures. Given the strong epidemiological evidence that *O. volvulus* infection is the trigger of the seizures, NS could be an autoimmune reaction following infection or could be triggered by parasite- or *Wolbachia*-derived factors crossing the blood-brain barrier. Signs of past ventriculitis and meningitis and gliotic lesions point in the direction of a postinfection encephalopathy or postinfection autoimmune reaction. To identify such a process, additional studies need to be performed on CSF samples, preferably of individuals early after the onset of seizures to identify potential neurotoxic antibodies or compounds.

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