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Review

Is nodding syndrome an *Onchocerca volvulus*-induced neuroinflammatory disorder? Uganda's story of research in understanding the disease

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SUMMARY

Nodding syndrome is a devastating neurological disorder, mostly affecting children in eastern Africa. An estimated 10 000 children are affected. Uganda, one of the most affected countries, set out to systematically investigate the disease and develop interventions for it. On December 21, 2015, the Ministry of Health held a meeting with community leaders from the affected areas to disseminate the results of the investigations made to date. This article summarizes the presentation and shares the story of studies into this peculiar disease. It also shares the results of preliminary studies on its pathogenesis and puts into perspective an upcoming treatment intervention. Clinical and electrophysiological studies have demonstrated nodding syndrome to be a complex epilepsy disorder. A definitive aetiological agent has not been established, but in agreement with other affected countries, a consistent epidemiological association has been demonstrated with infection by *Onchocerca volvulus*. Preliminary studies of its pathogenesis suggest that nodding syndrome may be a neuroinflammatory disorder, possibly induced by antibodies to *O. volvulus* cross-reacting with neuron proteins. Histological examination of post-mortem brains has shown some yet to be characterized polarizable material in the majority of specimens. Studies to confirm these observations and a clinical trial are planned for 2016.
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1. Introduction

Nodding syndrome is a devastating neurological disorder. First reported in Tanzania in the 1960s,[1] subsequent reports have come from South Sudan[2–4] and Uganda.[5,6] An estimated 10 000 children are affected in these three countries, of whom 3320 are in Uganda. In 2012, the Government of Uganda put together a multisector programme to address the disease. The programme included research to better characterize the disease, develop interventions, and determine the cause. The Government also engaged the World Health Organization (WHO), the United States Centers for Disease Control and Prevention (US CDC), and the United Kingdom Department for International Development, and together with representatives from local and foreign universities, organized a scientific conference in 2012. At this conference, plans for the investigations were improved and resources shared, and these collaborations enabled input into the studies shared in this article . This article summarizes findings from the research, shares preliminary studies on the pathogenesis, and reports upcoming interventions. The work of local organizations involved in the rehabilitation of patients is also discussed.

2. Clinical features and complications of nodding syndrome

In affected persons, nodding syndrome is characterized by bouts of repetitive head nodding. Symptoms develop in children with previously normal development aged 3 to 18 years. Head nodding is the pathognomonic feature of nodding syndrome. The head nodding often occurs in association with feeding, a cold

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breeze, or cold weather.[4,7,8] Over time, this is complicated by tonic–clonic, focal motor, myoclonic, and atypical absence seizures, behavioural difficulties, declining cognitive and motor function, wasting, growth failure, and physical deformities, leading to severe disability and in some cases death. Psychiatric symptoms are also common, including depression and generalized anxiety, emotional symptoms, wandering, disorientation, and aggressive behaviour, and in some cases, disorganized behaviour with psychotic features.

The interictal electroencephalogram (EEG) is characterized by generalized slow wave activity, and multiple interictal epileptiform discharges are seen. Ictal activity consists of mostly generalized spike and spike and wave discharges.[6,7,9,10] The EEG and electromyogram (EMG) findings suggest that the head nodding may be atonic seizures.[6] In Tanzania, electrical patterns of atypical absences have also been observed.[11] Head nodding may also be induced by hyperventilation.[10] Available neuroimaging has mostly been obtained using low-resolution 0.2–0.5 T magnetic resonance imaging (MRI) and has shown generalized cerebral cortical and cerebellar atrophy. Non-specific gliotic changes and hippocampal atrophy have also been described in some children.[6,7,9,12]

It has been determined that the complications of nodding syndrome in untreated patients may develop through five clinical stages,[9] which provide potential opportunities for interventions to arrest progression (Figure 1).

3. Treatment and treatment outcomes

There is currently no specific treatment for nodding syndrome. With careful observations of small numbers of patients hospitalized for a few weeks, symptoms and signs amenable to symptomatic relief have been identified and a package of symptomatic therapies for care has been developed by the present researchers. A training manual has also been developed and this manual has been used to train health workers deployed to care for the patients.

Treatment aims at symptom relief and includes the use of sodium valproate for seizures and nutritional, behavioural, and physical therapy.[13] Patients now receive this care at 17 nodding syndrome treatment centres across the affected region (Figure 2). The outcomes of the intervention were audited in approximately 500 patients about a year after the initiation of treatment and significant improvements in seizure control, function, and quality of life were demonstrated.[14] With these improvements, about 40% had returned to school. Thus, it would appear that patients receiving appropriate antiepileptic treatment may not go through all five clinical stages of nodding syndrome and cognitive function may improve with seizure control. There have, however, been challenges with the supply of antiepileptic drugs, and the treatment centres have at times experienced stock-outs. This problem has decreased over time.

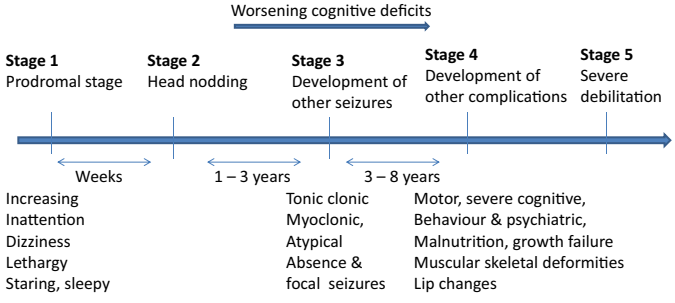

Figure 1. The natural history of nodding syndrome. Preliminary studies suggest that the symptoms and complications of nodding syndrome develop through five distinct but overlapping clinical stages over several years.

4. The aetiology of nodding syndrome

In trying to understand the aetiology of nodding syndrome, three questions were posed: Is nodding syndrome caused by a toxin or chemical? Is it a genetic disorder? Is it caused by an infection?

4.1. Is nodding syndrome caused by a toxin or chemical in the environment, or water or food eaten in the area? Is it related to chemicals used during the war that took place here?

The affected areas of northern Uganda and South Sudan have experienced long periods of warfare, exposure to war chemicals, and large internal population displacements. In the displaced people's camps, other than local food sources, food in the camps was supplied by the World Food Programme. However, the affected area in Tanzania experienced no such war or population displacement, raising doubts about the association with war or food relief. Moreover, although reports of nodding syndrome in northern Uganda started in 1997, cases increased rapidly from 2001, with peaks in 2003–2005 and 2008, 5–6 years after peaks in the number of wartime conflicts.[15] All the same, a series of studies of potential toxins was undertaken by investigators from the Ministry of Health, the US CDC, and local universities. Body fluids and tissue samples were obtained from cases and unaffected controls. None of the studies identified a specific toxin, however a vitamin B6 deficiency was present in the majority of cases (84%) and controls (75%).[16–18] Exposure to potential fungal contaminants in food could not, however, be excluded in the studies in South Sudan.[19]

4.2. Is nodding syndrome a genetic disorder?

The clustering of cases in selected areas and communities would suggest a geographically bounded exposure or genetic susceptibility. The case for a genetic cause became even stronger when it was observed that in over 60% of homes with cases, more than one child was affected (R. Idro, unpublished). However, it was clear from discussions with older members of the affected community that nodding syndrome was a relatively new disease within their community; a disease with similar presentation had not been observed in the Acholi land before the 1990s. With regard to the potential involvement of a recessive disorder, the affected communities do not practice consanguineous marriages . More specifically, investigators from the CDC performed exome sequencing on two children, one Ugandan and one South Sudanese, and found no association with known epilepsy genes.[18]

4.3. Is it caused by an infection?

Unlike Tanzania, the disease in Uganda and South Sudan has had the pattern of an epidemic, with very many children affected within a defined area and in short time. However, whatever the agent, the disease is unlikely infectious (i.e., unlikely to spread from one person to another), as nodding syndrome only develops in children. In a detailed epidemiological study of cases in Kitgum, Foltz et al. investigated the relationship between nodding syndrome and current or previous exposure to several infections and infestations, including cysticercosis, trypanosomiasis, malaria, and measles; no association was demonstrated with each of these.[16,18] Studies by the US CDC found no relationship with 19 different virus families.[18] The affected age group, the duration of symptoms, and the EEG

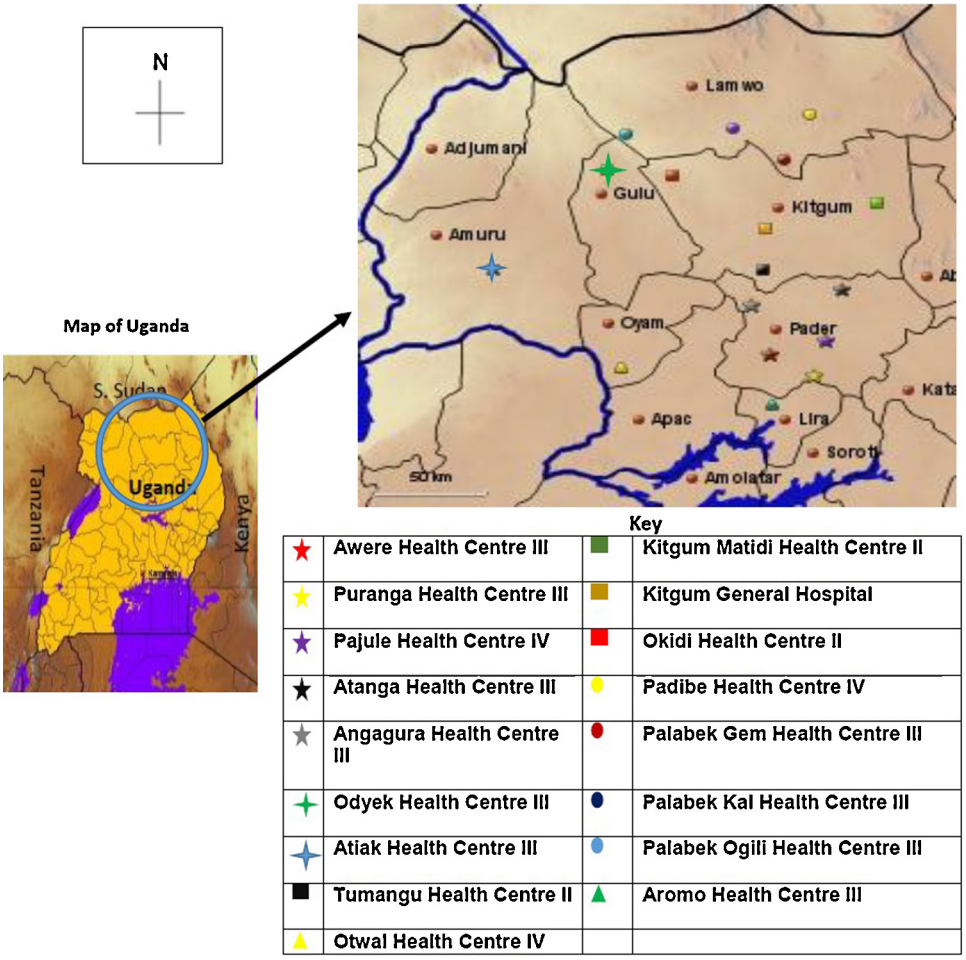

Figure 2. Map showing the locations of 17 specialized nodding syndrome treatment centres across northern Uganda. The map shows the locations of the 17 treatment centres in the seven districts of Oyam, Lira, Gulu, Amuru, Pader, Kitgum, and Lamwo.

recordings and brain MRI features indicate prion disease to be unlikely.[7,9] Indeed, no evidence of prion disease has been observed on histology. However, a strong epidemiological association has been documented between nodding syndrome and infection with *Onchocerca volvulus* (Table 1 [3,16,20]) (reviewed in Dowell et al.[18]).

O. volvulus has also been associated with other forms of epilepsy.[21] The nodding syndrome affected region in Uganda is crossed by the rivers Aswa and Pager and is endemic for *O. volvulus*. Global positioning systems (GPS) mapping of cases of nodding syndrome in the three most affected districts of Pader, Kitgum, and Lamwo shows dense clustering of nodding syndrome cases along the same rivers (Joseph Wamala et al., unpublished). However, the overlap with a similar mapping of other cases of epilepsy was incomplete (Figure 3). It is possible that some of the 'other forms of epilepsy' are cases of onchocerciasis-associated epilepsy and therefore located close to the Aswa and Pager rivers, while epilepsy cases further away from these rivers have another aetiology.

Despite the consistent association, it is unclear how *O. volvulus* may cause nodding syndrome and several questions have been asked. First, this parasite is endemic in many parts of Africa, Latin America, and Asia where it causes river blindness, yet nodding syndrome has only been reported in a few areas of Africa. Secondly, only children are affected. Third, it is unclear how the parasites can cause brain injury as there is hardly any evidence of breach of the blood–brain barrier and none has ever been demonstrated in brain tissue or in cerebrospinal fluid.[7] Alternative mechanisms other than direct parenchymal injury are likely.

4.4. Cross-reacting antibodies, complex epilepsy, and the pathogenesis of nodding syndrome

Antibodies against neuron surface proteins such as the voltage-gated potassium channel complex (VGKC), the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), gamma-aminobutyric acid $(GABA)_A$, $GABA_B$,

Table 1
Infection by *Onchocerca volvulus* and nodding syndrome

Country and area of study	Test method	Cases, *n*/*N* (% positive)	Controls, *n*/*N* (% positive)	OR (95% CI)	Reference
South Sudan, Amadi, 2001	Skin snip	29/30 (96.7%)	17/34 (50.0%)	29 (3.5, 238)	Tumwine et al.[20]
South Sudan, Lui, 2001	Skin snip	35/39 (89.7%)	15/31 (48.4%)	9.3 (2.6, 32.6)	Tumwine et al.[20]
South Sudan, Lui, 2002	Skin snip	12/13 (92.3%)	7/16 (43.8%)	15.4 (1.6, 149)	Tumwine et al.[20]
Uganda, Kitgum, 2009	ELISA	37/39 (94.9%)	20/41 (48.8%)	14.4 (2.7, 78)	Foltz et al.[16]
South Sudan, Witto and Maridi, 2010	Skin snip	29/38 (76.3%)	18/38 (47.4%)	3.2 (1.2, 8.7)	CDC[3]

OR, odds ratio; CI, confidence interval; CDC, US Centers for Disease Control and Prevention.

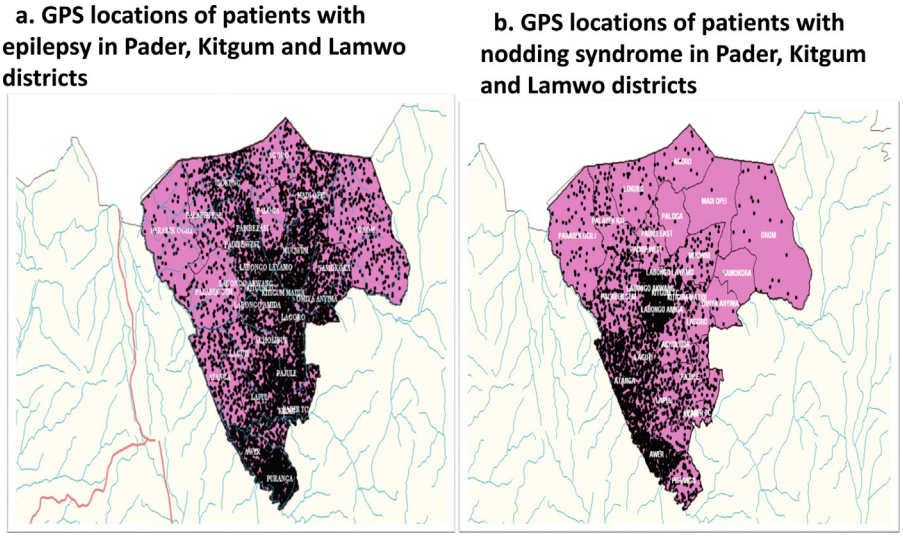

Figure 3. GPS locations of individual patients with nodding syndrome or other forms of epilepsy in the three most affected districts of Kitgum, Pader, and Lamwo in northern Uganda. The maps are similar, but the overlap is incomplete. (a) Cases of nodding syndrome are highly clustered along the Aswa and Pager river banks. (b) Patients with other forms of epilepsy are also clustered, but the overlap in the distribution with cases of nodding syndrome is incomplete.

and glycine receptors are recognized causes of severe epileptic disorders.[22] Patients have seizures, psychiatric features, and progressive encephalopathy. Preceding infections may possibly play a role through molecular mimicry or indirectly by inducing cytokines and allowing pathogenic antibodies to gain access to the brain (reviewed by Vincent et al.[22,23]). It is hypothesized that nodding syndrome, which was previously unknown and is not universal in all *O. volvulus* endemic areas, is a neuroinflammatory disorder caused by antibodies to either *O. volvulus* or its co-symbiotic bacteria, Wolbachia, cross-reacting with host neuron surface proteins, although evidence for neuroinflammation has not been forthcoming.[6]

4.5. Preliminary studies show evidence of neuroinflammation in nodding syndrome

The hypothesis of neuroinflammation in nodding syndrome has been supported by two separately conducted studies .

In the first study, and as part of a 2013 community survey of 215 nodding syndrome patients to describe the complications of nodding syndrome, serum samples were obtained from 31 nodding syndrome patients with *O. volvulus* infection on microscopic examination of skin snips (microfilaria parasite density of 1–20 parasites/μl in saline in which the skin snips had been incubated overnight). Serum samples were also obtained from 11 nodding syndrome unaffected siblings. Both sets of samples were tested for the presence of antibodies against the neuron surface protein – VGKC complex protein – at the University of Oxford Neuro-Immunology Laboratory. A positive test was defined as an antibody level greater than 150 pmol/l. Fifteen of the 31 cases (48.3%) and one of the 11 controls (9.1%) tested positive ($p = 0.03$) (R. Idro et al., unpublished). No patient or control subject had antibodies to the intracellular glutamic acid decarboxylase, which is also associated with complex epilepsy.

An earlier pilot study of Tanzanian patients did not find antibodies to the VGKC complex proteins.[24] There were, however, concerns with the Tanzanian study, particularly the inclusion of only patients who had samples left over after several years of storage and multiple thaw and freeze cycles, and the use of commercial tests for specific VGKC proteins, which do not have the same sensitivities. The results so far do not yet indicate antibodies to a specific component of the VGKC complex protein.

In the second study, investigators at the US CDC and the National Institutes of Health used a protein array to profile autoantibodies in 19 nodding syndrome cases and 19 unaffected controls. This approach detected a >1.5-fold increase in antibodies to 167 probes representing 137 individual proteins in pooled patient sera compared to controls. Specifically, antibodies to leiomodin 1 were increased in 11/19 (58%) cases vs. 5/19 (26%) controls.[25] Leiomodin is better known as a muscle protein, but the localization of leiomodin 1 in mouse brain has shown it to be focally expressed in cortical neurons, in Purkinje cells in the cerebellum, and in the CA3 region of the hippocampus. All three areas have been shown to suffer atrophy on brain MRI in earlier studies.[7,9,12] Antibodies to leiomodin 1, which shares 83% sequence similarity with a conserved region of *O. volvulus* tropomyosin, were neurotoxic in vitro to mouse brain and showed cross-reactivity to *O. volvulus* tropomyosin, supporting the hypothesis that a neuropathology in nodding syndrome may be caused by cross-reacting antibodies.

Although yet to be investigated, pathological host inflammatory responses in *O. volvulus*-infected individuals may also be against Wolbachia. Wolbachia are intracytoplasmic symbiotic bacteria found in filarial worms. They are essential for the survival, reproduction, and probably for the pathogenesis of *O. volvulus*. *O. volvulus* extracts depleted of Wolbachia with doxycycline do not induce the inflammation seen in *O. volvulus*-associated corneal keratitis.[26] Variant species may increase the pathogenicity, and treatment with tetracyclines could eliminate the tissue injury.[27] The identification of any such variants could be crucial in elucidating other targets for intervention. Furthermore, Wolbachia exist in up to 11 serogroups or super-groups (A–K). Super-groups A, B, E, H, I, and K are commonly found in arthropods, while groups C, D, and J are limited to filariae. It is unknown whether unique super-groups exist in regions with nodding syndrome and in patients with nodding syndrome, or whether new and virulent super-groups have evolved. An investigation of this hypothesis is part of the proposed studies for 2016 to 2019 (Figure 4).

5. Post-mortem studies

Between 2010 and 2015, post-mortem studies were performed on children who had died in the districts of Kitgum and Gulu. Grossly, the post-mortem brains showed significant atrophy with copious cerebrospinal fluid. On histological examination, six of

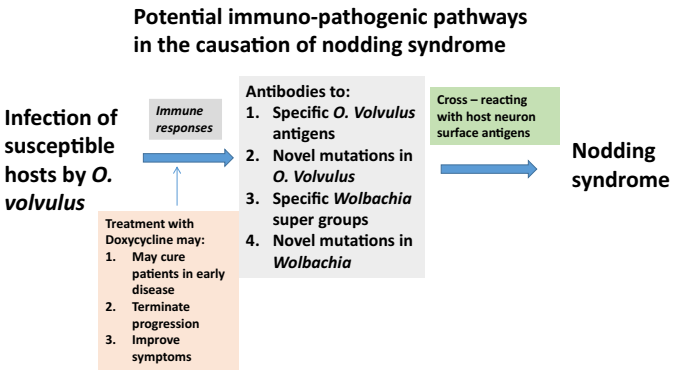

Figure 4. Potential immune-pathogenic pathways in the causation of nodding syndrome.

nine brain specimens examined in the USA had abnormalities in the form of polarizable crystal-like materials of different sizes, mainly in the brainstem, but also in the white matter. There was no inflammatory reaction associated with these materials, but they apparently dissolved when the brains were stored in 70% alcohol. What they are and their nature and significance are yet to be established (Dr Sylvester Onzivua, personal communication).

6. Rehabilitation services

A major challenge has been the provision of rehabilitation services for patients. Although the current guidelines for the management of patients clearly provide for this, the provision of this care is still inadequate. Most of the treatment centres do not have the human resource capacity to provide this service. However, a local non-governmental organization, Hope for Humans, has established a model rehabilitation system where patients receive comprehensive care and rehabilitation. Improvements in functional outcomes with this intervention have been impressive.

7. Planned studies on the pathogenesis and treatment of nodding syndrome

Gulu University organized a follow-up meeting to the 2012 conference in August 2015, at which the results of studies performed at different laboratories were shared. The conference concluded that reliable neuropathological data and biological markers for nodding syndrome are urgently needed, especially if these are present throughout the clinical evolution of nodding syndrome.[11]

Currently, there is no specific treatment for nodding syndrome. In addition, there is no routine treatment for the adult Onchocerca. Ivermectin, which kills microfilariae, has little if any effect on adult parasites. Instead, these continue to produce microfilariae for a lifespan lasting 5–15 years.[28] Killing the adult worms could potentially alter the course of nodding syndrome. This may be achieved by targeting Wolbachia. Antibiotic depletion of Wolbachia results in extensive apoptosis in the adult *O. volvulus* germline and somatic cells of embryos,[29] leading to marked reductions in host dermal microfilaria density, sterilization, and premature death of the adult parasite. Open-label trials have demonstrated that a 6-week course of oral doxycycline, 100 mg/day, results in a >90% reduction in Wolbachia levels in filarial tissue and in dermal microfilaria density over a period of 6–11 months,[30,31] and early death of the adult parasite. The 6-week treatment length is feasible in the community. In a trial in Cameroon, coverage of 73.8% was achieved with 97.5% compliance.[32] However, the tetracyclines deposit in growing bone and may cause dental staining and hypoplasia, and thus are contraindicated in children aged <8 years and in pregnant and breast-feeding women. Rifampicin may be considered for younger children.[33,34] Interaction with sodium valproate is limited.

The authors have therefore proposed a phase II trial of doxycycline 100 mg daily for 6 weeks or placebo as treatment for children with nodding syndrome. Recruitment into this trial will begin at the end of the first quarter of 2016. Concurrently, a larger case–control study will be conducted to seek evidence of neuroinflammation or cross-reacting antibodies in nodding syndrome and to determine the effects of doxycycline treatment on any such inflammatory responses. If evidence of neuroinflammation and/or specific autoantibodies or cross-reacting antibodies is found, immune-modulatory therapies may be considered. In addition, confirmation of a biological association between nodding syndrome and infection with *O. volvulus* would allow an escalation of treatments and prevention. Indeed, if pilot studies indicate a potential role for doxycycline, this would offer a cheap intervention beneficial to the older child. It would also be a proof of principal that treatment is possible and that similar strategies can be explored for younger children.

8. Conclusions

Nodding syndrome is a devastating disorder that affects developing brains and manifests with epilepsy and is complicated by multiple physical and functional disabilities and psychiatric manifestations. It is associated with infection with *O. volvulus* and may be a neuroinflammatory disorder. Much progress has been made in the care of children in Uganda; symptoms and function improve with symptomatic treatments. This progress has been supported by detailed research, and further research is on the way.

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Author contributions: All authors participated in the design, conduct, and write-up of the individual studies investigating nodding syndrome in Uganda and contributing data for this manuscript. RI drafted the manuscript and all the other authors critically reviewed and revised the draft.

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