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

Article *in* International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases · March 2016

DOI: 10.1016/j.ijid.2016.03.002

CITATIONS
66

READS
159

9 authors, including:

Richard Idro
Mulago hospital/Makerere University College of Health Sciences
196 PUBLICATIONS **4,820** CITATIONS
SEE PROFILE

Joseph Francis Wamala
World Health Organisation WHO, Juba, South Sudan
91 PUBLICATIONS **2,220** CITATIONS
SEE PROFILE

Catherine Abbo
Makerere University
72 PUBLICATIONS **1,008** CITATIONS
SEE PROFILE

Sylvester Onzivua
Makerere University
5 PUBLICATIONS **100** CITATIONS
SEE PROFILE

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

Author: Richard Idro Bernard Opar Joseph Wamala Catherine Abbo Sylvester Onzivua Deogratius Amos Mwaka Angelina Kakooza-Mwesige Anthony Mbonye Jane R Aceng

PII: S1201-9712(16)30988-2
DOI: http://dx.doi.org/doi:10.1016/j.ijid.2016.03.002
Reference: IJID 2555

To appear in: *International Journal of Infectious Diseases*

Received date: 14-1-2016
Revised date: 1-3-2016
Accepted date: 2-3-2016

Please cite this article as: Idro R, Opar B, Wamala J, Abbo C, Onzivua S, Mwaka DA, Kakooza-Mwesige A, Mbonye A, Aceng JR, Is nodding syndrome an *Onchocerca volvulus* induced neuro-inflammatory disorder? Uganda's story of research in understanding the disease, *International Journal of Infectious Diseases* (2016), http://dx.doi.org/10.1016/j.ijid.2016.03.002

Highlights

- Nodding syndrome is a complex epilepsy disorder characterised by atonic seizures.
- It is associated with multiple physical and functional disabilities and psychiatric manifestations.
- There is a consistent epidemiologic association with infection by the parasite *Onchocerca volvulus.*
- Preliminary studies suggest it is a neuro-inflammatory disorder induced by antibodies to *Onchocerca volvulus* cross reacting with neuron proteins.
- Studies to confirm these observations and a treatment trial are planned for 2016.

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

Authors

Richard Idro[a], Bernard Opar[b], Joseph Wamala[b], Catherine Abbo[a], Sylvester Onzivua[c], Deogratius Amos Mwaka[a], Angelina Kakooza-Mwesige[a], Anthony Mbonye[b], Jane R Aceng[b]

Institutions of affiliations

a. Makerere University College of Health Sciences, P.O Box 7072, Kampala, Uganda
b. Ministry of Health Headquarters, P.O Box 7272, Kampala, Uganda
c. Mulago hospital, P.O Box 7051, Kampala, Uganda

Corresponding author

Dr Richard Idro
Department of Paediatrics and Child Health
Makerere University College of Health Sciences
P.O Box 7072, Kampala, Uganda
Email: ridro1@gmail.com

Abstract

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

Keywords

Nodding syndrome, aetiology, *O. volvulus,* pathogenesis, treatment

1. Introduction

Nodding syndrome is a devastating neurological disorder. First reported in Tanzania in 1960's[1], subsequent reports have come from South Sudan[2-4] and Uganda[5 6]. **An estimated 10,000 children are affected in the three countries of whom, 3,320 are in Uganda.** In 2012, the Government of Uganda put together a multi-sectoral program to address the disease. The program included research to characterise the disease better, develop interventions and find the cause. **The Government also engaged the World Health Organization and the United States Centers for Disease Control, the United Kingdom Department for International Development and together with representatives of Local and foreign universities, organised a Scientific Conference in 2012 that improved plans for the investigations, shared resources and enabled collaborations that have input in to studies shared in the paper.** This paper summarises findings from these research, shares preliminary studies of pathogenesis and upcoming interventions. **The work of local organisations involved in the rehabilitation of patients is also mentioned.**

2. Clinical features and complications of nodding syndrome

In affected persons, nodding syndrome is characterized by bouts of repetitive head nodding. Symptom develops in previously normally developing children ages three to 18 years. Head nodding is the pathognomonic feature of nodding syndrome. The head nods often occur in association with feeding, a cold breeze or cold weather [4 7 8]. Overtime, this is complicated by tonic-clonic, focal motor, myoclonic and atypical absence seizures, behaviour 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 generalised anxiety, emotional symptoms, wandering, disorientation, and aggressive behaviour and in some cases, disorganised behaviour with psychotic features. **The interictal electroencephalogram (EEG) is characterised by generalised slow wave activity and multiple interictal epileptiform discharges are seen. Ictal activity consists of mostly generalised spike and spike and wave discharges.[6 7 9 10] The EEG and electromyogram (EMG) suggests the head nods 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 neuro-imaging has mostly been obtained using low resolution 0.2-0.5T magnetic resonance imaging (MRI) and show generalised cerebral cortical and cerebellar atrophy. **Non-specific gliotic changes and hippocampal atrophy has also been described in some other children.[6 7 9 12]** We have determined that the complications of nodding syndrome

in **untreated patients may develop through five clinical stages**[9] which potentially provide opportunities for intervention 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 hospitalised for a few weeks, we identified symptoms and signs amenable to symptomatic relieve and developed a package of symptomatic therapies for care. We also developed a training manual and used this manual to train health workers who were deployed to care for the patients. Treatment aims at symptom relief and includes the use of sodium valproate for seizures, nutritional, behaviour and physical therapy.[13] Patients now receive this care in 17 nodding syndrome treatment centres across the affected region, **figure 2**. We audited the outcomes of the intervention in ~500 patients about a year after initiation of treatment and demonstrated significant improvements in seizure control, function and quality of life.[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 that cognitive function may improve with seizure control. There has however been challenges with the supply of antiepileptic drugs and the treatment centres have at times experienced stock outs. This problem has decreased overtime.**

4. The aetiology of nodding syndrome

In trying to understand the aetiology of nodding syndrome, we asked three questions; is nodding syndrome caused by a toxin or chemical? Is it a genetic disorder or is it caused by an infection?

4.1 Is nodding syndrome caused by a toxin or chemical in the environment, 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 had 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 Program. However, the affected area in Tanzania neither experienced such war nor 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 rapidly increased 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 Centers of Disease Control (CDC) and local universities. Body fluids and tissue samples were obtained from cases and unaffected controls. None of the studies identified a specific toxin but for vitamin B6 deficiency which 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 studies in South Sudan[19].**

4.2 Is nodding syndrome a genetic disorder?

Clustering of cases in selected areas and communities would suggest a geographically-bounded exposure or genetic susceptibility. The case for a genetic cause even became 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 is a relatively new disease within their community; a disease with similar presentation had not been observed in the Acholi land before the 1990's. The affected communities also do not practice consanguineous marriages to think about a recessive disorder. More specifically, investigators from the CDC performed **exome sequencing** on two children; one Ugandan and one South Sudanese child 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 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 epidemiologic study of cases in Kitgum, Foltz at el investigated the relationship between nodding syndrome and current or previous exposure to several infections and infestations including cysticercosis, trypanosomiasis, malaria, and measles and 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 recordings and brain MRI features meant prion disease was unlikely.[7 9] Indeed, no evidence of prion disease was observed on histology. However, a strong epidemiologic association has been documented between nodding syndrome and infection by O. *volvulus***, table 1** (reviewed in[18]).

Table 1; Infection by *Onchocerca volvulus* and nodding syndrome

Country and area of study	Test method	Cases, n/N (% positive)	Controls, n/N (% positive)	Odds ratio (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]

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 *Onchocerca volvulus.* Global Positioning Systems (GPS) mapping of cases of nodding syndrome in the three most affected districts of Pader, Kitgum and Lamwo show 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 closely 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 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 amino-butyric acid $(GABA)_{A,}$ $GABA_B$ and glycine receptors, are recognised causes of severe epileptic disorders[22]. Patients have seizures, psychiatric features and progressive encephalopathy. Preceding infections may play a role possibly, through molecular mimicry or indirectly by inducing cytokines and allowing pathogenic antibodies gain access to the brain (reviewed in[22 23]). *We hypothesise that nodding syndrome, which was previously unknown and is not universal in all O. volvulus endemic areas, is a neuro-inflammatory disorder caused by antibodies to either O. volvulus or its co-symbiotic bacteria, Wolbachia, cross reacting with host neuron surface proteins* **although evidence for neuro-inflammation has not been forth coming[6].**

.

4.5 Preliminary studies show evidence of neuro-inflammation in nodding syndrome

The hypothesis has been supported by two separately conducted studies.

a) In the first study, and as part of a 2013 community survey of 215 nodding syndrome patients to describe the complications of nodding syndrome, we obtained serum samples 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). We also obtained serum samples from 11 nodding syndrome unaffected siblings and tested both sets of samples for the presence of antibodies against the neuron surface protein - VGKC complex protein - in the University of Oxford Neuro-Immunology laboratory. A positive test was defined as antibody levels greater than 150 pmol/L; 15/31 cases (48.3%) and 1/11 (9.1%) controls tested positive, p=0.03, (Idro R, 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 inclusion of only patients who had samples left over after several years of storage, multiple thaw and freeze cycles, and the use of commercial tests for specific VGKC proteins which do not have the same sensitivities. Our results so far do not yet indicate antibodies to a specific component of the VGKC-complex protein.

b) In the second study, investigators at the US Centers of Disease Control and the National Institutes of Health used protein array to profile auto-antibodies in 19 nodding syndrome cases and 19 unaffected controls. This approach detected >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 localisation of leiomodin-1 in mouse brain showed that it was 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 neuropathology in nodding syndrome may be caused by cross-reacting antibodies.

c) Although yet to be investigated, pathological host inflammatory responses in *O. volvulus* infected individuals may also be against *Wolbachia.* The Wolbachiae are intracytoplasmic symbiotic bacteria found in filarial worms. They are essential for the survival, reproduction and, probably, 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 pathogenicity and treatment with tetracyclines could eliminate the tissue injury.[27] Identification of any such variants could be crucial in elucidating other targets for intervention. Furthermore, *Wolbachia* exist in up to 11 sero-groups 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. Investigation of this hypothesis is part of the proposed studies from 2016-19, **figure 4**.

5. Post mortem studies

Between 2010 and 2015, postmortems studies were performed on children dying from Kitgum and Gulu districts. Grossly, the postmortem brains showed significant atrophy with copious cerebral spinal fluid. On histological examination, six of nine brain specimens examined in the USA had abnormalities in form of polarisable 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 apparently, they dissolved when the brains were stored in 70% alcohol. What they are, 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 provides 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, established a model rehabilitation system where patients receive comprehensive care and rehabilitation. Improvements in functional outcomes with this intervention has been impressive.

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

Earlier, Gulu University organised a follow up meeting to the 2012 conference in August 2015 at which results of studies 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 microfilaria, has little if any, effect on adult parasites. Instead, these continue to produce microfilaria 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'* germ-line and somatic cells of embryos[29] leading to marked reductions in host dermal microfilaria density, sterilisation and premature death of the adult parasite. Open-labelled trials have demonstrated that a six-week course of oral doxycycline, 100mg/day, results in >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 six-week treatment length is feasible in the community; in a trial in Cameroon, coverage of 73.8% was achieved with 97.5% compliance[32]. The tetracyclines however deposit in growing bone; may cause dental staining and hypoplasia thus, is contra-indicated in children <8years, pregnant and breast-feeding women. Rifampicin may be considered for younger children.[33 34]

Interaction with sodium valproate is limited. We therefore proposed a phase II trial of doxycycline 100mg daily for six weeks or placebo as treatment for children with nodding syndrome. Recruitment in to this trial will begin end of the first quarter of 2016. Concurrently, we will conduct a larger case control study to examine for evidence of neuro-inflammation or cross reacting antibodies in nodding syndrome and determine the effects of treatment with doxycycline on any such inflammatory responses. If we find evidence of neuro-inflammation and/or specific auto or cross reacting antibodies, immune-modulatory therapies may be considered. In addition, confirmation of a biologic association between nodding syndrome and infection by *O. volvulus* would allow 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 neuro-inflammatory disorder. We have made a lot of progress 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.

Role of funding agency

This work was supported by the Government of Uganda. Specific testing and assays were performed with support from the US Centers of Disease Control, the Waterloo Foundation (Grant No. 1025-1947) and University of Oxford. Dr Idro was also supported by the Wellcome Trust through the Directors Discretionary Research Fund to Prof Kevin Marsh. The funding agencies had no role in the design of the study, collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the manuscript for publication.

Contribution of authors

All authors participated in the design, conduct and write up of the individual studies that investigated the nodding syndrome in Uganda and that contributed the data for this manuscript. RI drafted the manuscript and all the other authors critically reviewed and revised the draft.

References

1. Jilek LA. [Mental Diseases and Epilepsy in Tropical Africa]. *Fortschritte der Neurologie, Psychiatrie, und ihrer Grenzgebiete* 1964;32:213-59.
2. Lacey M. Nodding disease: mystery of southern Sudan. *Lancet neurology* 2003;2(12):714.
3. Nodding syndrome - South Sudan, 2011. *MMWR. Morbidity and mortality weekly report* 2012;61(3):52-4.
4. Nyungura JL, Akim, T., Lako, A., Gordon, A., Lejeng, L., Gibson, W. Investigation into the Nodding syndrome in Witto Payam, Western Equatoria State, 2010. *Southern Sudan Medical Journal* 2011;4. (1):3-6.
5. Wasswa H. Ugandan authorities deal with a mysterious ailment that leaves people nodding continuously. *BMJ* 2012;344:e349.
6. Sejvar JJ, Kakooza AM, Foltz JL, Makumbi I, Atai-Omoruto AD, Malimbo M, et al. Clinical, neurological, and electrophysiological features of nodding syndrome in Kitgum, Uganda: an observational case series. *The Lancet. Neurology* 2013;12(2):166-74.
7. Winkler AS, Friedrich K, Konig R, Meindl M, Helbok R, Unterberger I, et al. The head nodding syndrome--clinical classification and possible causes. *Epilepsia* 2008;49(12):2008-15.
8. Winkler AS, Friedrich K, Meindl M, Kidunda A, Nassri A, Jilek-Aall L, et al. Clinical characteristics of people with head nodding in southern Tanzania. *Tropical doctor* 2010;40(3):173-5.
9. Idro R, Opoka RO, Aanyu HT, Kakooza-Mwesige A, Piloya-Were T, Namusoke H, et al. Nodding syndrome in Ugandan children--clinical features, brain imaging and complications: a case series. *BMJ open* 2013;3(5).
10. de Polo G, Romaniello R, Otim A, Benjamin K, Bonanni P, Borgatti R. Neurophysiological and clinical findings on Nodding Syndrome in 21 South Sudanese children and a review of the literature. *Seizure* 2015;31:64-71.
11. Spencer PS, Kitara, D.L., Gazda, S., Winkler, A. Nodding syndrome: 2015 international conference report and Gulu accord. *eNeurologicalSci* 2015.
12. Winkler AS, Friedrich K, Velicheti S, Dharsee J, Konig R, Nassri A, et al. MRI findings in people with epilepsy and nodding syndrome in an area endemic for onchocerciasis: an observational study. *African health sciences* 2013;13(2):529-40.
13. Idro R, Musubire KA, Byamah Mutamba B, Namusoke H, Muron J, Abbo C, et al. Proposed guidelines for the management of nodding syndrome. *African health sciences* 2013;13(2):219-32.
14. Idro R, Namusoke H, Abbo C, Mutamba BB, Kakooza-Mwesige A, Opoka RO, et al. Patients with nodding syndrome in Uganda improve with symptomatic treatment: a cross-sectional study. *BMJ open* 2014;4(11):e006476.
15. Landis JL, Palmer VS, Spencer PS. Nodding syndrome in Kitgum District, Uganda: association with conflict and internal displacement. *BMJ open* 2014;4(11):e006195.
16. Foltz JL, Makumbi I, Sejvar JJ, Malimbo M, Ndyomugyenyi R, Atai-Omoruto AD, et al. An Epidemiologic Investigation of Potential Risk Factors for Nodding Syndrome in Kitgum District, Uganda. *PloS one* 2013;8(6):e66419.
17. Korevaar DA, Visser BJ. Reviewing the evidence on nodding syndrome, a mysterious tropical disorder. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* 2013;17(3):e149-52.
18. Dowell SF, Sejvar JJ, Riek L, Vandemaele KA, Lamunu M, Kuesel AC, et al. Nodding syndrome. *Emerging infectious diseases* 2013;19(9):1374-84.

19. Spencer PS, Vandemaele K, Richer M, Palmer VS, Chungong S, Anker M, et al. Nodding syndrome in Mundri county, South Sudan: environmental, nutritional and infectious factors. *African health sciences* 2013;13(2):183-204.
20. Tumwine JK, Vandemaele K, Chungong S, Richer M, Anker M, Ayana Y, et al. Clinical and epidemiologic characteristics of nodding syndrome in Mundri County, southern Sudan. *African health sciences* 2012;12(3):242-8.
21. Kaiser C, Asaba G, Leichsenring M, Kabagambe G. High incidence of epilepsy related to onchocerciasis in West Uganda. *Epilepsy research* 1998;30(3):247-51.
22. Vincent A, Bien CG, Irani SR, Waters P. Autoantibodies associated with diseases of the CNS: new developments and future challenges. *Lancet neurology* 2011;10(8):759-72.
23. Vincent A, Irani SR, Lang B. Potentially pathogenic autoantibodies associated with epilepsy and encephalitis in children and adults. *Epilepsia* 2011;52 Suppl 8:8-11.
24. Dietmann A, Wallner B, Konig R, Friedrich K, Pfausler B, Deisenhammer F, et al. Nodding syndrome in Tanzania may not be associated with circulating anti-NMDA-and anti-VGKC receptor antibodies or decreased pyridoxal phosphate serum levels-a pilot study. *African health sciences* 2014;14(2):434-8.
25. Johnson T, Tyagi R, Lee PR, Lee M-h, Johnson KR, Kowalak J, et al. Detection of auto-antibodies to leiomodin-1 in patients with nodding syndrome. *Journal of Neuroimmunology*;275(1):103.
26. Kluxen G, Horauf A. [Ocular onchocerciasis: a key role for Wolbachia]. *Der Ophthalmologe : Zeitschrift der Deutschen Ophthalmologischen Gesellschaft* 2007;104(10):860-5.
27. Min KT, Benzer S. Wolbachia, normally a symbiont of Drosophila, can be virulent, causing degeneration and early death. *Proceedings of the National Academy of Sciences of the United States of America* 1997;94(20):10792-6.
28. Udall DN. Recent updates on onchocerciasis: diagnosis and treatment. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007;44(1):53-60.
29. Landmann F, Voronin D, Sullivan W, Taylor MJ. Anti-filarial activity of antibiotic therapy is due to extensive apoptosis after Wolbachia depletion from filarial nematodes. *PLoS pathogens* 2011;7(11):e1002351.
30. Hoerauf A, Volkmann L, Hamelmann C, Adjei O, Autenrieth IB, Fleischer B, et al. Endosymbiotic bacteria in worms as targets for a novel chemotherapy in filariasis. *Lancet* 2000;355(9211):1242-3.
31. Hoerauf A, Mand S, Volkmann L, Buttner M, Marfo-Debrekyei Y, Taylor M, et al. Doxycycline in the treatment of human onchocerciasis: Kinetics of Wolbachia endobacteria reduction and of inhibition of embryogenesis in female Onchocerca worms. *Microbes and infection / Institut Pasteur* 2003;5(4):261-73.
32. Tamarozzi F, Tendongfor N, Enyong PA, Esum M, Faragher B, Wanji S, et al. Long term impact of large scale community-directed delivery of doxycycline for the treatment of onchocerciasis. *Parasites & vectors* 2012;5:53.
33. Townson S, Hutton D, Siemienska J, Hollick L, Scanlon T, Tagboto SK, et al. Antibiotics and Wolbachia in filarial nematodes: antifilarial activity of rifampicin, oxytetracycline and chloramphenicol against Onchocerca gutturosa, Onchocerca lienalis and Brugia pahangi. *Annals of tropical medicine and parasitology* 2000;94(8):801-16.
34. Specht S, Mand S, Marfo-Debrekyei Y, Debrah AY, Konadu P, Adjei O, et al. Efficacy of 2- and 4-week rifampicin treatment on the Wolbachia of Onchocerca volvulus. *Parasitology research* 2008;103(6):1303-9.

Figure legends

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.

Figure 2: A 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.

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.

3a; Cases of nodding syndrome are highly clustered along the Aswa and Pager river banks.

3b; Patients with other forms of epilepsy are also clustered but the overlap in the distribution with cases of nodding syndrome is incomplete.

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

Figure 4