River blindness goes beyond the eye: autoimmune antibodies, cross-reactive with *Onchocerca volvulus* antigen, detected in brain of patients with Nodding syndrome

Christoph Kaiser[1], Sébastien D. S. Pion[2]

[1]Pediatrician, Pediatric Practice, Baden-Baden, Germany; [2]UMI 233, Institut de Recherche pour le Développement (IRD) and University of Montpellier 1, Montpellier, France

Correspondence to: Christoph Kaiser, MD, MSc. Pediatrician, Pediatric Practice, Balzenbergstrasse 73, 76530 Baden-Baden, Germany.
Email: dr.ch.kaiser@email.de.

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Nodding syndrome (NS) is a neurological disorder primarily characterized by paroxysmal head nodding seizures, affecting previously healthy children at the age between 3–18 years (1,2). Growing numbers of patients with NS were documented since the early 2000s in three distinct areas in the southern Sudan (3,4), southern Tanzania (1) and northern Uganda (5,6). In retrospect, NS was also found in western Uganda (7,8) and suspected cases were reported from Liberia (9) and Cameroon (10). In many NS patients, initial head seizure are followed by other seizures types, progressing physical and cognitive deterioration, and death (6,8). To date, the exact causation of NS is not clarified, but epidemiological and case-control studies have shown a consistent correlation between NS and infection with *Onchocerca volvulus (O. volvulus*), a parasite known as the cause of river blindness in large parts of tropical Africa (2,4,11,12). Because imaging studies and analysis of cerebrospinal fluid (CSF) failed to demonstrate the parasite in the central nervous system (CNS) of NS patients (1,4-6,13) it was suggested that an immunological process related to *O. volvulus* could play a role in the origination of NS (14,15).

Recently, Johnson *et al.* (16) presented a study on the possible involvement of auto-immune antibodies in NS pathogenesis in patients from two endemic areas of northern Uganda and South Sudan. In a broad search for potentially neurotoxic auto-antibodies in sera of 55 NS cases and matched unaffected village controls (UVC), four candidate proteins with more than 100-fold reactivity in NS sera above that of UVC sera were identified. The protein with highest comparative reactivity (33,000-fold increase) indicated auto-antibodies to leiomodin-1, a protein previously found expressed mainly in smooth muscle cells and other tissue outside the CNS. By ELISA testing, NS patient sera were found positive for leiomodin-1 antibodies significantly more frequently than those of UVC, and optical density was higher in NS patients. Leiomodin-1 antibodies were also detected in the CSF of eight out of 18 NS patients examined from northern Uganda. The protein with the second highest serum reactivity, antibodies to DJ-1 protein, could not be found in the CSF. Because no CSF samples from persons without NS from an African endemic area could be examined, CSF samples from eight North American patients with epilepsy were tested for comparison and found negative for leiomodin-1 antibodies.

In addition to the described case-control study, the authors carried out a number of *in vitro* experiments demonstrating: (I) that leiomodin-1 antibodies recognize leiomodin-1 protein in transfected human embryonic kidney cells; (II) that leiomodin-1 is detectable in cultured brain cells by immunostaining with anti-leiomodin antibodies from sera and CSF of NS patients; (III) that

leiomodin-1 is demonstrated in adult mouse brain sections in several distinct brain regions; (IV) that leiomodin-1 antibodies from sera of NS patients affect the functioning of an assay for human neuron viability, and this can be reversed by depletion of antibodies; (V) that the structure of leiomodin-1 protein to a large extent is homologous with the Tropomodulin protein found in *O. volvulus*; and (VI) that leiomodin-1 antibodies from NS patient CSF cross-react with *O. volvulus* whole-organism lysate. The authors speculate that infection with O. volvulus can lead to the formation of cross-reactive antibodies to leiomodin-1 and other yet unidentified proteins which can cross the blood-brain barrier (BBB) and elicit brain pathology in NS. Thus, NS may be an autoimmune reaction to *O. volvulus* infection.

Besides NS, two more clinical observations are lending support to the hypothesis that infection with O. volvulus can lead to brain disease. First, prior to the recognition of NS, an otherwise unexplained clustering of epilepsy was reported from several onchocerciasis endemic areas throughout West (17), Central and East Africa (18), and a strong positive correlation was demonstrated between the prevalence of onchocerciasis and that of epilepsy (19). A close association between onchocerciasis and epilepsy was also confirmed in numerous case-control studies (20). This phenomenon was denominated with the terms of "river epilepsy" (19), or onchocerciasis-associated epilepsy (OAE) (20). Second, as early as 1950, in south-eastern Uganda, Raper & Ladkin (21) described a condition characterized by growth retardation, physical deformities, retarded puberty, mental impairment, and epilepsy which they called "Nakalanga Syndrome", based on the term used by the local community. Nakalanga Syndrome was later reported from numerous other areas which all were endemic for onchocerciasis (22). Because of a considerable overlap of the symptoms and signs found in NS and Nakalanga Syndrome, it was suggested that both disorders may even be two manifestations of the same underlying disease (22).

The concept of a causal relationship between infection with *O. volvulus* and NS is still not universally accepted (23,24). Alternatively, it was proposed that NS could be a sequel of measles infection comparable to measles-induced subacute sclerosing panencephalitis (SSPE), and this could be triggered by malnutrition (24,25). In this view, the association between NS and onchocerciasis would be seen as a secondary phenomenon, possibly due to a greater susceptibility of patients with NS to *O. volvulus* infection. SSPE is a rare complication of measles caused by persisting infection of the virus in the CNS (26). Typically, after a time lapse of six years following measles infection, children present with initially subtle changes in cognition or behavior (stage 1), rapidly progressing to a condition with myoclonic jerks, epileptic seizures and dementia (stage 2), extrapyramidal symptoms and unresponsiveness (stage 3), to coma and autonomic failure, and a lethal outcome in 95% of cases (26). Although the clinical appearance and the relentless decline seen in a part of NS patients (6,7) may be reminiscent of SSPE, the sequence between disease onset and death of less than nine months seen in most patients with SSPE is much faster than what has been observed in NS patients (6,27). Myoclonus is reported as a frequent feature in SSPE and has been reported in connection with head nodding moves, but their description in SSPE did not fully resemble the head nodding seizures considered characteristic of NS (28). Probably, myoclonic movements in SSPE do not represent epileptic seizures in most cases (26,29). Whilst the diagnosis of SSPE would require the intrathecal demonstration of measles antibodies or antigen (26), a search for measles antigen in CSF was negative in samples of 16 NS patients from northern Uganda (12) and measles antibodies were so far not measured in case series obtaining CSF of patients with NS (1,4-6,13). With regard to the hypothesis that NS should be a form of SSPE, it would be unclear, why in many areas of tropical Africa where measles and childhood malnutrition are widespread but onchocerciasis is not endemic, SSPE is not found more frequently, and no cases of NS are found at all.

Numerous questions arise from the results presented by Johnson *et al.* (16): (I) Are leiomodin antibodies detectable in serum or CSF of other persons living in NS affected areas, with/without neurologic disease other than NS, and with/without *O. volvulus* infection status? Appropriate CSF samples could be obtained from patients undergoing diagnostic lumbar puncture because of neurologic disease other than NS or for spinal anesthesia. (II) Are leiomodin-1 antibodies also found in serum or CSF of patients with OAE or Nakalanga syndrome? (III) Can other brain reactive antibodies be detected in CSF of NS patients, as this is postulated by Johnson *et al.* (16)? (IV) Why are leiomodin-1 antibodies found in CSF and obviously are capable to cross the BBB, but the second abundant antibodies, DJ-1 antibodies, are not—and what are the factors facilitating or preventing BBB transfer (30,31)?

The most important question is probably that about

the clinical relevance of leomodin-1 antibodies in CSF of NS patients for neurologic pathology. Although Johnson *et al.* (16) provide evidence that leiomodin-1 antibodies are neurotoxic in vitro, this does not necessarily prove a pathogenic effect in the human brain. As leiomodin-1 antibodies are found in CSF of only eight of 16 NS patients, whilst all developed CNS symptoms, it may be conceived that they do not have an inevitable pathogenic effect *in vivo* and might be an epiphenomenon of *O. volvulus* infection (23-25,30). Some information on the clinical relevance of leiomodin-1 antibodies could possibly be gained in relating the data about antibody detection in CSF presented now (16) to the clinical data of the patients which were thoroughly assessed in the original survey of Sejvar *et al.* (5). It would be of great interest to analyze the presence or absence of CSF antibodies in relation to distinct clinical features of NS patients such as duration or frequency of head nodding seizures, or to associated symptoms and signs (other seizures, cognitive impairment, stunted growth, and others). The limited data base of 18 NS patients with CSF results in the study of Johnson *et al.* (16) could possibly be expanded by examination of CSF specimen from other well documented case series of NS patients for leiomodin-1 antibodies (1,4-6,13).

There is evidence that *O. volvulus* induced eye disease and overall mortality in onchocerciasis endemic areas are related to intensity of infection with the parasite (32,33), and this was also found for OAE (17,19). In correspondence, infection intensity with *O. volvulus* may also play a role in the origination of NS and explain some of the difference between NS patients and UVC now presented by Johnson *et al.* (16). For instance, the difference between NS and UVC for qualitative indicators of *O. volvulus* infection—microfilaridermia or *O. volvulus* serology—is strongly resembling that found in OAE (19). This also applies to the difference in detection of leiomodin-1 antibodies in sera of NS patients compared to UVC, which may in this regard be considered as an additional serological marker for *O. volvulus* infection. Optical densities of LMOD-1-Ab in the ELISA-test were higher in NS than UVC sera, indicating higher quantities of LMOD-1-Ab in NS sera. This could reflect a higher parasite load in NS patients. The hypothesis that NS is related to high infection intensity with *O. volvulus* could possibly be validated from the original data of the study of Johnson *et al.* (16) and the primary study of Foltz *et al.* (12) which in its methods section is mentioning that skin biopsies were examined for "presence and number of *O. volvulus* microfilaria" and also assessed ivermectin treatment status. The possible role of *O. volvulus* infection intensity in the etiology of NS—as well as OAE and Nakalanga syndrome—needs further investigation.

The study of Johnson *et al.* (16) is of significance, because for the first time it is presenting a plausible pathogenic agent that could establish the missing link between *O. volvulus* infection and cerebral pathology in NS. Additional investigations are needed for confirmation of the proposed concept of NS as an auto-immune brain disorder, and this could be helpful to develop more efficient therapeutic approaches. Besides opening new research perspectives, the results of Johnson *et al.* (16) are giving further evidence of the robust association between onchocerciasis and NS. Because onchocerciasis can be successfully controlled and possibly be eliminated (34), the decline of onchocerciasis prevalence in previously highly endemic areas should be followed be a reduction of the number of patients affected by NS in these areas, and also that of OAE and Nakalanga syndrome. If this expectation will prove to be true, the emerging phenomenon of onchocercal brain disease would possibly disappear even before its pathological mechanism could be fully elucidated. There would be no reason to regret this.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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