Pathogenesis of nodding syndrome; Preliminary findings

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ICNC 2022
17th INTERNATIONAL CHILD NEUROLOGY CONGRESS
ANTALYA, TURKEY | OCTOBER 3-7, 2022

INTRODUCTION

Nodding syndrome (NS) is an epileptic encephalopathy characterized by head-nodding. The pathogenesis is poorly understood but epidemiological studies have demonstrated a consistent association with *Onchocerca volvulus*. We hypothesized that NS is a neuro-inflammatory disorder with antibodies to *O.volvulus* or its symbiont bacteria, *Wolbachia,* cross-reacting with neuronal proteins.

Figure 1: Images of children affected with nodding syndrome

METHODS

We conducted a case-control study of 154 children with long-standing NS and 154 age-matched unaffected community children (CC) in Uganda. *O.volvulus* seroreactivity was assessed by anti-Ov16 IgG ELISA. We tested plasma samples for hu-Leiomodin and also performed a preliminary study to assess the presence of any neuronal or glial antibodies in 50 CC sera and 50 NS sera/paired CSFs by immunohistochemistry (IHC) on rat brain sections and immunofluorescence on live/fixed cultured rat hippocampal neurons. The presence of antibodies to specific extracellular (LGI1, CASPR2, GABAaR, GABAbR) or intracellular (leiomodin-1) antigens was assessed by cell-based assays. Also, we conducted MRIs of children with nodding syndrome and conducted an exploratory proteomic analysis of CSF proteins.

RESULTS

In plasma, *Ov-16* IgG were detected in 95% NS vs 55% CC while leiomodin-1 autoantibodies were obsevered in 27% of NS cases compared to 23% of controls.. Preliminary evidence for neuronal-reactive antibodies found 18/50 (36%) NS sera bound to brain sections, principally to the Purkinje cells and molecular layer of the cerebellum, compared with 3/50(6%) CC sera. NS sera also bound to live neurons (13/50; 26%; CC binding 18%) and to fixed/ permeabilised neurons, 14/50(28%, CC binding 18%) consistent with an intracellular target. Antibodies to specific antigens in NS sera included 8/50(16%) to GABAbR and 7(14%) to GABAaR. In addition, 14/50 (28%) NS sera (CC 18%) and 5/50(10%) NS CSF bound to leiomodin-1 in fixed/permeabilised cells. MRI studies demonstrated significant atrophy of the brain. Preliminary proteomic analsysis show differential expression of CSF preteome between children with NS compared to Lukemia controls.

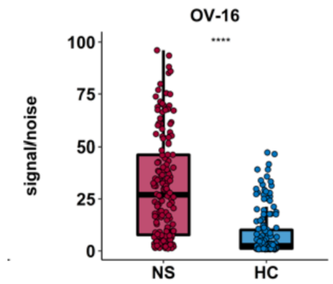

Figure 2: Image showing the level of plasma anti-Ov-16 antibody levels in children with Nodding syndrome compared to healthy community controls.

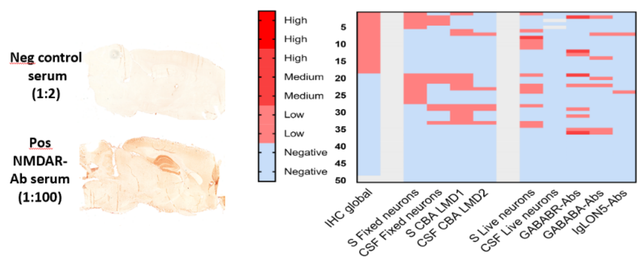

Figure 3 : **Summary of immunohistochemistry findings**. *left*, Image of sample mouse brain sections tested with CSF from NS participants, Right, summary of all mouse brain binding experiments from 50 samples. red shows strong binding, blue shows negative samples

RESULTS

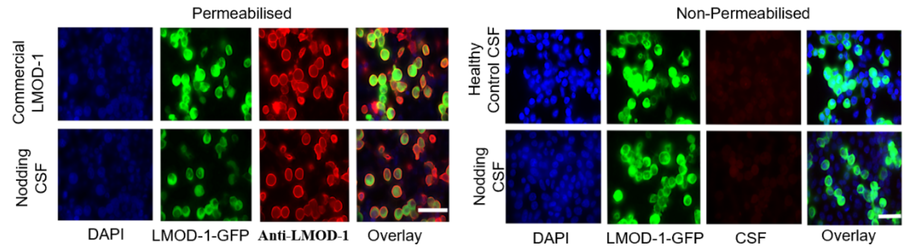

Figure 4: Establishment of a cell-based assay to test LMOD-1 antibody binding using HEK293 cells

None of the 100 CSFs bound to the surface of these cells.The commercial antibody bound strongly ONLY when the cells were permeabilised to expose the intracellular antigens

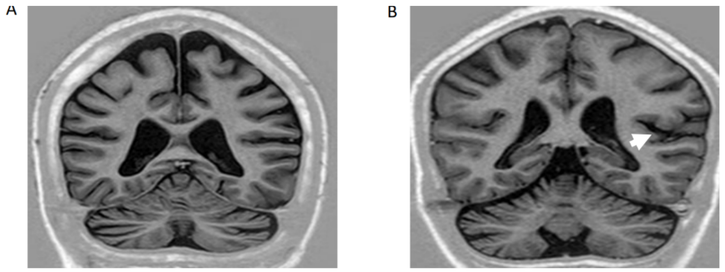

Figure 5: MRI of participants with NS and OAE :
A) 16 yo girl with NS with global cerebral and cerebellar atrophy (T1- weighted inversionrecovery).B) 15 yo boy with NS with left temporal lobe atrophy and bilateral cerebellar atrophy (white arrow showing the temporal lobe atrophy) (T1-weighted inversion recovery) .

CONCLUSION

These preliminary studies confirm that antibodies to *O.volvulus*-specific antigens are more common in NS than in controls. The presence of increased IgG reactivity of NS sera with brain tissue, particularly the cerebellum, which show atrophy on MRI in NS, is consistent with our hypothesis of *O.volvulus*-induced neuroreactive antibodies in NS. Further analysis of proteomic data is ongoing.

ACKNOWLEDGEMENTS

This study was jointly funded by the Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement through an African Research Leadership Award to RI and KM, grant number MR/M025489/1. The award is also part of the EDCTP2 programme supported by the European Union. contact: ridro1@gmail.com