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Progress report for successful 2017 Brain Foundation Grant led by Dr Kavitha Kothur, Dr Hannah Jones, Dr Louise Wienholt and Professor Russell Dale

Scientific progress report: Identification of treatable immune mediated neurological and psychiatric disease using cytokines/chemokines as biomarkers of inflammation

Neuroinflammatory disorders represent a broad spectrum of diverse disorders including encephalitis, demyelinating disorders and other immune-mediated autoinflammatory central nervous system (CNS) disorders. Over the last decade, there has been a substantial improvement in the repertoire of diagnostic biomarkers of inflammatory or autoimmune brain disease in children. Despite that, many or most children with suspected inflammatory brain disease do not have a diagnostic biomarker to explain the symptoms and guide a therapeutic approach. Untreated, inflammatory and autoimmune disorders typically result in permanent neurological disability. The cytokines and chemokines play a complex pleiotropic role in inflammation including immune cell differentiation, proliferation and migration to the sites of inflammation and cellular injury. Cytokines and chemokines have been shown to be elevated in a number of inflammatory disorders of brain and can be potentially used as markers of inflammation.

Autism spectrum disorder (ASD), Tourette's disorder and obsessive compulsive disorder (OCD) are common neurodevelopmental and neuropsychiatric disorders caused by a complex interplay of genetic and environmental factors. Epidemiological studies, animal models, and case control studies indicate maternal immune activation may be an important factor involved in neurodevelopmental and psychiatric symptom expression. **A** sub group of children with Tics/OCD and Autism has unexplained sudden deterioration in response to infections causing severe functional impairment. These children improve on treatment with immune treatment.

These children usually have personal and family history of other autoimmune disorders and allergy supporting underlying genetic predisposition to immune dysregulation. Despite above studies, we unfortunately do not have biomarkers to identify inflammation and monitor disease response in these children with treatable immune conditions.

With the support from Brain foundation grant, our team collected the clinical data and therapeutic response of subgroup of children with neuropsychiatric and neurological disorders with fluctuating clinical course and Tics/OCD and Autism prospectively which was published in developmental medicine and child neurology. **(Jones HF, Ho ACC, Sharma S, Mohammad SS, Kothur K, Patel S, Brilot F, Guastella AJ, Dale RC; Immune-Neurodevelopment (Imm-Nd) Study Group.Maternal thyroid autoimmunity associated with acute-onset neuropsychiatric disorders and global regression in offspring.Dev Med Child Neurol. 2019 Aug;61(8):984-988. doi: 10.1111/dmcn.14167. Epub 2019 Feb 5.PMID: 30720202)**

In the above paper Dr Jones from our team reported eight children with neurodevelopmental/psychiatric disorders, all born to mothers with Hashimoto autoimmune thyroid disease. Seven of the eight families also reported autoimmunity in other first and second-degree family members. Of the eight index children, four were diagnosed with ASD. Rather than a static course, 7 of 8 children presented with an abrupt onset of neuropsychiatric symptoms (OCD n=6), tics (n=5) and/or psychosis (n=1) associated with an autistic or global regression at mean age 6.5 years (range 4-15). Most deteriorations appeared likely triggered by infection and followed a relapsing-remitting course. All children responded to immunomodulatory treatment as indicated by reductions in psychiatric symptoms. Seven children were also additionally successfully managed with conventional treatment.

Based on this data, we proposed that maternal autoimmunity might activate fetal microglia or alter transcription of neurodevelopmental vulnerability and/or immune genes that increase the expression and severity of neurodevelopmental problems, and susceptibility to deteriorations after infectious or stress. We selected 20 mothers with definite autoimmune disease from the tic/OCD cohort and 15 age-matched mothers from the healthy control cohort for

immunological studies. Cytokine analysis was performed in the 20 mothers from the tic/OCD group and 15 mothers of healthy controls. Serum was taken and stored at -80° Celsius until analysis. Fifteen cytokines (eotaxin, G-CSF, IFNα2, IFNɣ, GRO, IL-10, IL-12(p70), IL-13, IL-17A, IL-1ra, IL-6, IL-8, IP-10, MCP-1, TNFα) were assayed using the multiplex, bead-based immunoassay Milliplex® Map Human Cytokine/Chemokine Magnetic Bead Panel (Milliplex® Map system, Millipore Corporation, Missouri USA) according to the manufacturer's procedure and read using the Luminex® 200™ platform. Interferon-alpha and G-CSF were elevated in the mothers of children with tics/OCD and autoimmune disease, whereas interferon gamma-induced protein 10 (IP-10) and eotaxin were reduced compared to mothers of healthy controls (Fig below)

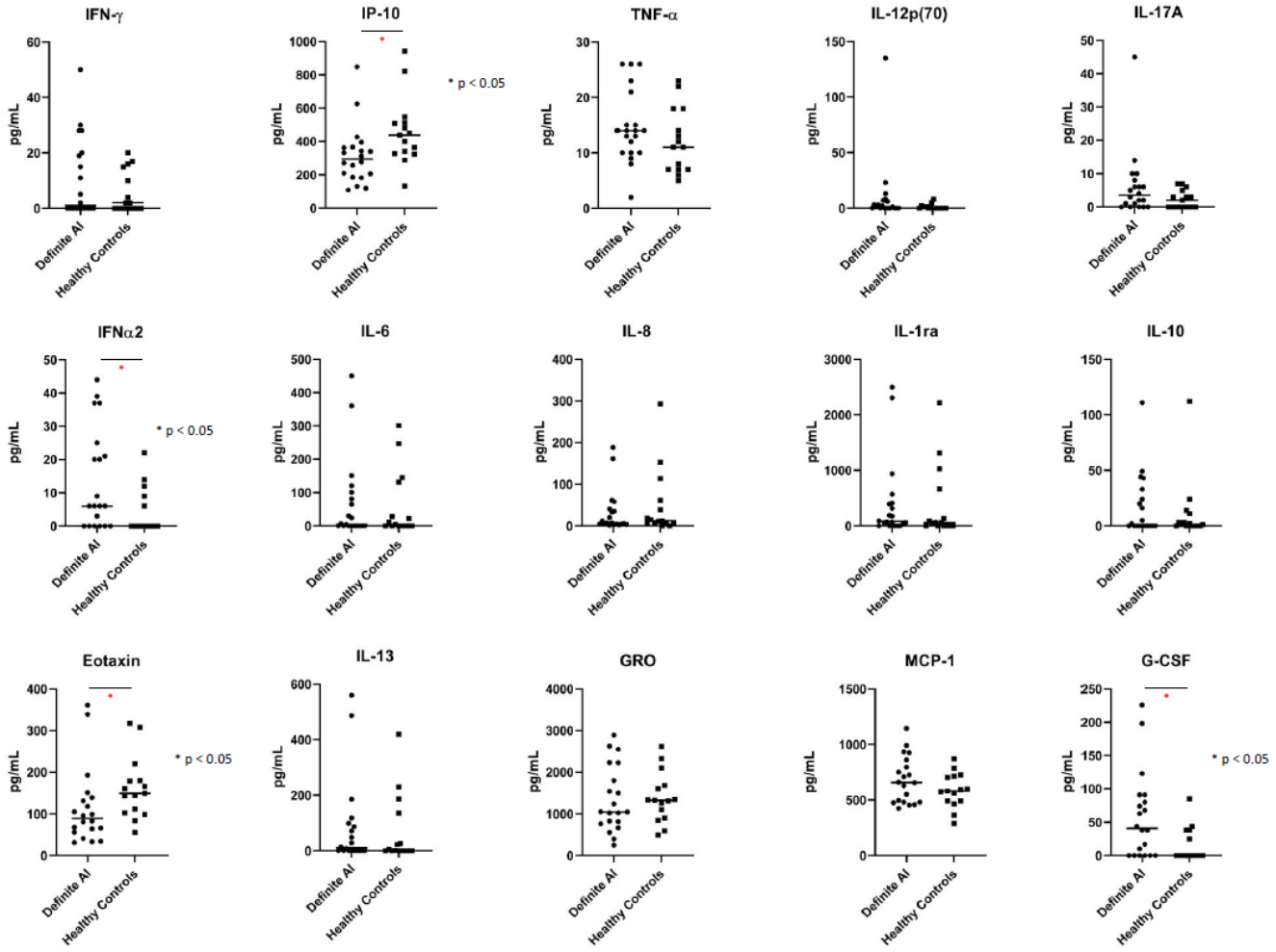

Fig 1: Cytokine profiles of Mothers of Children with Tics/OCD and Autoimmune Disease (n=20) vs Healthy controls (n=15). The median for each group is shown. AI = autoimmunity, G-CSF = Granulocyte Colony Stimulating Factor, GRO = Growth Stimulating Activity, IFN = interferon, IL = interleukin, IP-10 = Interferon gamma-induced protein 10, MCP-1 = monocyte chemoattractant protein-1. Statistically significant differences are marked with a bar and asterisk signifying $p<0.05$.

As described above, significant progress has been made with the Brain foundation funding to develop insights into identifying the maternal immune activation, and promising data has been obtained using cytokine/chemokine data in maternal serum to determine influence of maternal factors on fetal immune activation. This has been further extended to combine with transcriptome data which has been submitted for publication and is under peer review. Our data supports an association between maternal and familial autoimmune disease and other pro-inflammatory states and childhood tics and OCD. In the context of current evidence, our results highlight that targeting maternal inflammation in pregnancy may reduce vulnerability to neurodevelopmental disorders in offspring.

In addition to above, as part of identifying treatable neurological disorders in children, we investigated intrathecal inflammation using cerebrospinal fluid (CSF) cytokines and chemokines in paediatric epilepsy subgroup patients with frequent daily seizures. The prominent elevation of CSF cytokines and chemokines in Febrile infection-related epilepsy syndrome (FIRES) and to a lesser extent febrile status epilepticus group(FSE) (Fig 2) highlights that the cytokine/chemokine elevation is significantly associated with the etiology of the underlying process, targeted timely treatment of which may help reduce inflammation and improve patient outcomes.

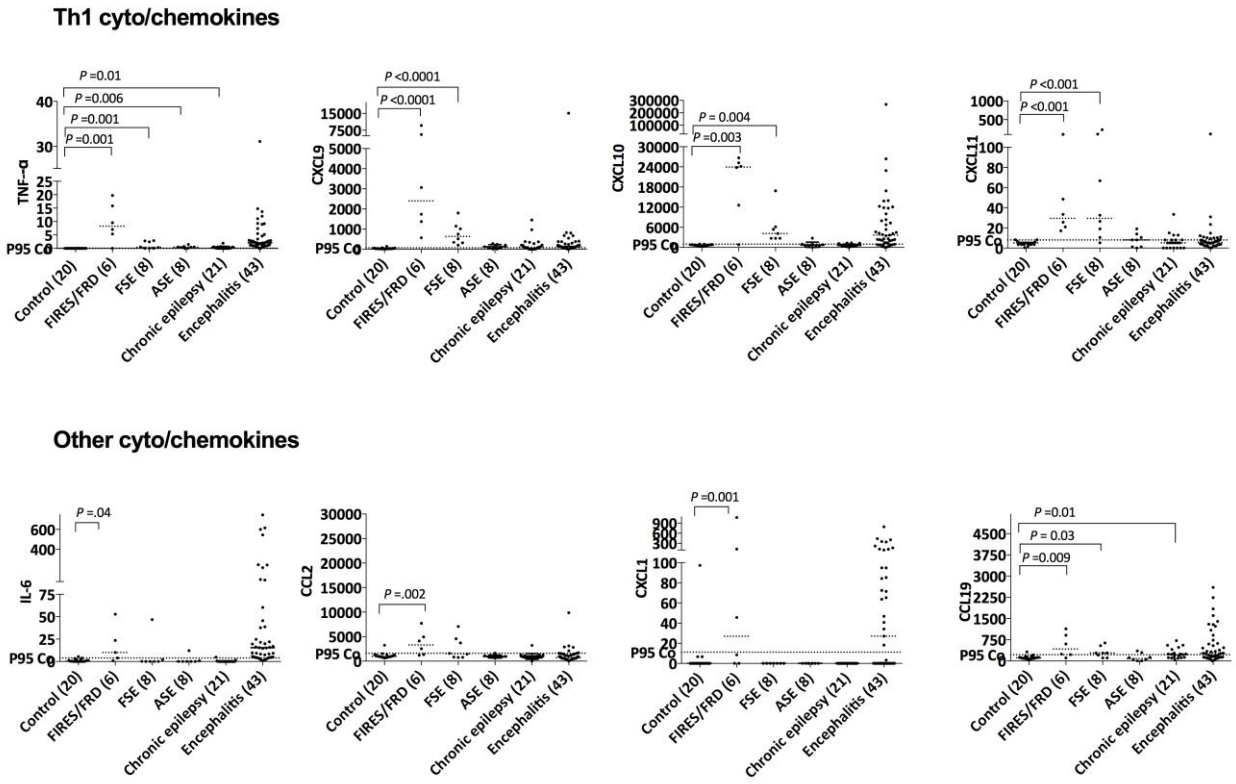

Fig 2: The cytokine/chemokines that were significantly elevated in epilepsy subgroups compared to non inflammatory neurological controls (n=20). Compared to controls, Th1 associated cytokine/chemokines (TNF-α, CXCL9, CXCL10, CXCL11), CSF IL-6, CCL2, CXCL1 and CCL19 were elevated in FIRES/FRD. Similarly in FSE, TNF-α, CXCL9, CXCL10, CXCL11 and CCL19 were elevated compared to controls. In chronic epilepsy, TNF-α and CCL19 were mildly elevated. The encephalitis group has been presented for comparison (n=43).

Dotted lines represent medians. The 95th centile of the 20 non-inflammatory controls is presented (P95 Co).

This work has been published in ILAE home journal "Epilepsia" (impact factor 5.5). **(Kothur K, Bandokar S, Gill D, Dale RC et al. Etiology is the key determinant of neuroinflammation in epilepsy: Elevation of cerebrospinal fluid cytokines and**

chemokines in febrile infection-related epilepsy syndrome and febrile status epilepticus. Epilepsia 2019 Aug;60(8):1678-1688)

We hope that some of the discoveries presented above and the publications emanating from this work will be instrumental in research towards the identification of inflammation and their mechanisms in other neurological disorders.

We greatly appreciate the support from Brain Foundation Australia.

Your Sincerely
Kavitha Kothur, Hannah Jones, Dr Louise Wienholt and Professor Russell C Dale