Brain, Behavior, and Immunity 94 (2021) 308–317

Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

# Maternal autoimmunity and inflammation are associated with childhood tics and obsessive-compulsive disorder: Transcriptomic data show common enriched innate immune pathways

Hannah F. Jones [a,b,c], Velda X. Han [a,d], Shrujna Patel [a,c], Brian S. Gloss [e], Nicolette Soler [a,c,f], Alvin Ho [a,g], Suvasini Sharma [a,h], Kavitha Kothur [a,c], Margherita Nosadini [a,i], Louise Wienholt [c,j], Chris Hardwick [f], Elizabeth H. Barnes [k], Jacqueline R. Lim [c], Sarah Alshammery [a,c], Timothy C. Nielsen [l], Melanie Wong [m], Markus J. Hofer [n], Natasha Nassar [l], Wendy Gold [a,o,p], Fabienne Brilot [a,c,q], Shekeeb S. Mohammad [a,c], Russell C. Dale [a,c,q,*]

[a] Kids Neuroscience Centre, The Children's Hospital at Westmead, Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia
[b] Department of Neuroservices, Starship Children's Hospital, Auckland, New Zealand
[c] The Children's Hospital at Westmead Clinical School, Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia
[d] Khoo Teck Puat-National University Children's Medical Institute, National University Health System, Singapore
[e] Westmead Research Hub, Westmead Institute for Medical Research, Westmead, Sydney, New South Wales, Australia
[f] Department of Psychological Medicine, The Children's Hospital at Westmead, Sydney, New South Wales, Australia
[g] Department of Paediatrics and Adolescent Medicine, Queen Mary Hospital, Hong Kong
[h] Neurology Division, Department of Paediatrics, Lady Hardinge Medical College and Associated Kalawati Saran Children's Hospital, New Delhi, India
[i] Paediatric Neurology Unit, Department of Paediatrics, University of Padua, Italy
[j] Department of Clinical Immunology, Royal Prince Alfred Hospital, Sydney, New South Wales, Australia
[k] NHMRC Clinical Trials Centre, Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia
[l] Child Population and Translational Health Research, The Children's Hospital at Westmead Clinical School, Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia
[m] Department of Immunology, The Children's Hospital at Westmead, Sydney, New South Wales, Australia
[n] School of Life and Environmental Sciences and Charles Perkins Centre, Faculty of Science, University of Sydney, Sydney, New South Wales, Australia
[o] School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia
[p] The Children's Medical Research Institute, Westmead, Sydney, New South Wales, Australia
[q] The Brain and Mind Centre, University of Sydney, Sydney, New South Wales, Australia

ARTICLE INFO

*Keywords:*
Autoimmune disease
Inflammation
Innate immunity
Maternal immune activation
Tics
Tourette syndrome
Obsessive–compulsive disorder
Neurodevelopmental disorders
Transcriptome
Cytokines

ABSTRACT

Although genetic variation is a major risk factor of neurodevelopmental disorders, environmental factors during pregnancy and early life are also important in disease expression. Animal models demonstrate that maternal inflammation causes fetal neuroinflammation and neurodevelopmental deficits, and brain transcriptomics of neurodevelopmental disorders in humans show upregulated differentially expressed genes are enriched in immune pathways. We prospectively recruited 200 sequentially referred children with tic disorders/obsessive–compulsive disorder (OCD), 100 autoimmune neurological controls, and 100 age-matched healthy controls. A structured interview captured the maternal and family history of autoimmune disease and other pro-inflammatory states. Maternal blood and published Tourette brain transcriptomes were analysed for overlapping enriched pathways. Mothers of children with tics/OCD had a higher rate of autoimmune disease compared with mothers of children with autoimmune neurological conditions ($p = 0.054$), and mothers of healthy controls ($p = 0.0004$). Autoimmunity was similarly elevated in first- and second-degree maternal relatives of children with tics/OCD ($p < 0.0001$ and $p = 0.014$ respectively). Other pro-inflammatory states were also more common in mothers of children with tics/OCD than controls ($p < 0.0001$). Upregulated differentially expressed genes in maternal autoimmune disease and Tourette brain transcriptomes were commonly enriched in innate immune processes. Pro-inflammatory states, including autoimmune disease, are more common in the

* Corresponding author at: Kids Neuroscience Centre, Faculty of Medicine and Health, University of Sydney, Locked Bag 4001, Westmead, New South Wales 2145, Australia.
*E-mail address:* russell.dale@sydney.edu.au (R.C. Dale).

https://doi.org/10.1016/j.bbi.2020.12.035
Received 3 October 2020; Received in revised form 12 December 2020; Accepted 31 December 2020
Available online 8 January 2021

mothers and families of children with tics/OCD. Exploratory transcriptome analysis indicates innate immune signalling may link maternal inflammation and childhood tics/OCD. Targeting inflammation may represent preventative strategies in pregnancy and treatment opportunities for children with neurodevelopmental disorders.

## 1. Introduction

There is a substantial genomic contribution to most common neurodevelopmental disorders such as autism spectrum disorder (ASD), attention-deficit hyperactivity disorder (ADHD), tic disorders such as Tourette syndrome, and OCD (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2019). Furthermore, genomic research has uncovered shared genetic architecture across neurodevelopmental and neuropsychiatric disorders, with key overlapping loci involving synaptic plasticity and neurodevelopmental processes. (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2019; Schork et al., 2019) Brain transcriptomic analyses demonstrate downregulation of neuronal genes implicated in genome-wide association studies (GWAS), but upregulation of genes related to microglial function, suggesting inflammation may be an important environmental factor in the pathophysiology of neurodevelopmental disorders. (Lennington et al., 2016; Lisboa et al., 2019; Voineagu et al., 2011; Velmeshev et al., 2019) Microglia constitute 15% of brain cells and are important in synaptic pruning, neuronal differentiation, and neural circuit formation, (Paolicelli and Ferretti, 2017) and exposure to inflammation can affect microglial function in the perinatal and postnatal periods. (Estes and McAllister, 2016; Frick and Pittenger, 2016; Bilbo et al., 2018) Evidence for microglial activation and immune dysregulation in neurodevelopmental disorders aligns with an increasing understanding of the role of immune signalling in healthy neurodevelopment and disease. (Deverman and Patterson, 2009; Kipnis et al., 2012; Attwells et al., 2017; Martino et al., 2020)

Epidemiological studies associate immune dysregulation in pregnancy with adverse neurodevelopmental outcomes in children. (Estes and McAllister, 2016; Dalsgaard et al., 2015; Mataix-Cols et al., 2018) Animal models and early clinical studies demonstrate that cytokines induced by maternal immune activation correlate with altered brain morphology, neural circuits, microglial functioning, behaviour, and cognition in offspring. (Gumusoglu and Stevens, 2019; Rudolph et al., 2018)

Among the neurodevelopmental disorders, Tourette syndrome and OCD frequently co-exist, are closely genetically linked, and both involve cortico-striatal pathway dysfunction. (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2019; Yu et al., 2015; Hirschtritt et al., 2015) We designed a case-control study to investigate the frequency of inflammatory conditions in mothers and other relatives of children with tic disorders/OCD compared with children with autoimmune neurological conditions and healthy controls using a structured interview. Autoimmune disease was chosen as the primary exposure because the clinical features and biological biomarkers aid accurate reporting. Other pro-inflammatory states that were captured in mothers included asthma, eczema, smoking, anxiety/depression, pre-eclampsia and infection. (Bilbo et al., 2018; Furman et al., 2019) Moreover, we explored putative disease mechanisms by comparing maternal blood transcriptomes with published Tourette syndrome brain transcriptome data. (Lennington et al., 2016)

## 2. Methods

### 2.1. Participant selection

**Tic/OCD cohort:** We performed a prospective study of 200 children aged ≤ 16 years diagnosed with a tic disorder and/or OCD according to DSM-5 criteria and sequentially referred to a specialist clinic at the Children's Hospital at Westmead, Sydney, Australia, from February 2018 to June 2019 (Diagnostic and Statistical Manual of Mental Disorders, 2013).

**Controls:** We recruited a positive control group of 100 children with autoimmune neurological conditions treated at the Children's Hospital at Westmead, anticipating that they will have elevated familial autoimmunity due to the heritability of autoimmune disease. (Farh et al., 2015) The autoimmune neurological disorders included central nervous system (CNS) autoimmune demyelinating diseases (n = 54, 54%), autoimmune encephalitis (n = 14, 14%) and opsoclonus myoclonus ataxia syndrome (n = 10, 10%) and were diagnosed according to standard diagnostic or classification criteria (supplementary Fig. 1).

We also recruited a negative control group of 100 healthy age- and sex-matched children of hospital workers and their acquaintances. The inclusion criterion for healthy controls was absence of neurodevelopmental disorders in the child and siblings, but other childhood diseases, including autoimmune diseases, were acceptable in the proband and siblings.

Cases and controls were excluded if a sibling or relative was already recruited to the study, consent was declined, or the maternal history was unavailable (supplementary Fig. 2). The demographics of each group are summarised in Table 1.

### 2.2. Procedure

#### 2.2.1. Structured interview

A circa 50-question structured interview with over 400 data items was completed with the child's primary caregiver(s) either by phone or in person by a neurologist (HFJ) (supplementary text 1). The interview collected data regarding maternal immune conditions including autoimmune disease, asthma, atopy, and pre-eclampsia and infection during pregnancy with the proband, as well as autoimmunity and neuropsychiatric disorders in all first- and second-degree relatives. Postnatal immune factors in the proband were also captured such as recurrent infections, atopy, and autoimmune disease (see interview for definitions). For children with tics/OCD, data regarding tic/OCD onset, exacerbating and relieving factors, impact on function, and subjective efficacy of treatments were obtained. The clinician (RCD, SSM) recorded the child's comorbidities after a semi-structured clinical review, using DSM-5 criteria. (Diagnostic and Statistical Manual of Mental Disorders, 2013)

As autoimmune disease in the mothers was the primary exposure, diagnoses were classified as 'definite' or 'possible' depending on the level of supporting evidence for the diagnosis. 'Definite' diagnoses required specific treatment for the autoimmune disease, verifying documentation from a health practitioner, or a repeat blood test to confirm relevant autoantibodies (supplementary text 2). 'Possible' autoimmune disease diagnoses and inflammatory but not autoimmune conditions such as sarcoidosis, were only analysed with the 'definite' autoimmune disease group in a secondary analysis. For relatives other than the mothers, it was not feasible to obtain verifying documentation, and autoimmune diseases were reported *a priori*.

#### 2.2.2. Examination of the Pro-Inflammatory state in mothers with autoimmune disease: cytokine and transcriptome analyses

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 to investigate shared immune pathways. Blood samples were taken at the time of recruitment into the study. Cytokine analysis was performed for all mothers, and whole blood

**Table 1**
**Method of Interview and Demographics of Probands in the Tics/OCD and Control Groups.** Statistical comparisons using chi-squared, independent t-tests, and Mann Whitney U tests.

| | Tics/OCD (n = 200) | Autoimmune Neurological Controls (n = 100) | Healthy Controls (n = 100) | Tics/OCD vs Autoimmune Neurological Controls | Tics/OCD vs Healthy Controls |
|---|---|---|---|---|---|
| | | | | p-value | p-value |
| **Method of Interview** | | | | | |
| **Person interviewed** | | | | 0.27 | 0.097 |
| -Mother | 164 (82%) | 75 (75%) | 89 (89%) | | |
| -Father | 9 (4.5%) | 16 (16%) | 5 (5%) | | |
| -Mother and father | 27 (13.5%) | 7 (7%) | 6 (6%) | | |
| -Other | 0 (0%) | 2 (2%) | 0 (0%) | | |
| **Mode of interview** | | | | 0.44 | 0.0043 |
| -Phone | 136 (68%) | 63 (63%) | 52 (52%) | | |
| -In person | 64 (32%) | 37 (37%) | 45 (45%) | | |
| -Other | 0 | 0 | 3 (3%) | | |
| **Demographics** | | | | | |
| **Age of proband at interview (y)** | | | | | |
| -Mean (±SD) | 11.0 (±3.1) | 12.4 (±5.1) | 10.7 (±3.4) | 0.012 | 0.44 |
| **Sex of proband** | | | | | |
| -Male | 146 (73%) | 43 (43%) | 76 (76%) | <0.0001 | 0.58 |
| -Female | 54 (27%) | 57 (57%) | 24 (24%) | | |
| **Ethnicity**[a] | | | | | |
| -Caucasian | 167 (83.5%) | 56 (56%) | 79 (79%) | <0.0001 | 0.91 |
| -Middle Eastern/North African | 13 (6.5%) | 17 (17%) | 4 (4%) | 0.0043 | 0.44 |
| -Southern/Central Asian | 12 (6%) | 14 (14%) | 13 (13%) | 0.020 | 0.039 |
| -Aboriginal/Torres Strait | 8 (4%) | 1 (1%) | 2 (2%) | 0.28 | 0.50 |
| -Other | 21 (10.5%) | 20 (20%) | 14 (14%) | 0.024 | 0.37 |
| **Maternal age (y)** | | | | | |
| -At interview (mean (±SD)) | 42.2 (±5.8) | 42.0 (±7.4) | 41.6 (±5.9) | 0.80 | 0.43 |
| -At birth of proband (mean (±SD)) | 31.2 (±5.0) | 29.6 (±4.7) | 31.0 (±4.6) | 0.0088 | 0.66 |
| **Paternal age (y)** | | | | | |
| -At interview (mean (±SD)) | 44.7 (±6.8)[b] | 44.3 (±7.9) | 44.1 (±6.6) | 0.61 | 0.46 |
| -At birth of proband (mean (±SD)) | 33.7 (±6.4)[b] | 32.0 (±5.6) | 33.4 (±5.7) | 0.021 | 0.82 |
| **Mean number of relatives** | | | | | |
| −1st-degree relatives (±SD) | 3.2 (±0.9) | 3.6 (±1.4) | 3.4 (±0.8) | 0.030 | 0.021 |
| −2nd-degree relatives (±SD) | 8.0 (±2.7) | 8.9 (±3.2) | 8.1 (±1.9) | 0.020 | 0.19 |

Abbreviations: SD, standard deviation; y, years.
[a] Total response output i.e. if ≥ 2 ethnicities were reported, two ethnicities were recorded, and the individual was counted twice (Statistics New Zealand, 2005).
[b] n = 199.

RNA-sequencing analysis was performed in 14 samples from each group that passed RNA quality control testing (supplementary Table 1).

*2.2.2.1. Cytokine assays.* Serum was taken and stored at −80° Celsius until analysis. We used a panel that measured fifteen important pro- and anti-inflammatory 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) using a 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. The plate was read using the Luminex® 200™ platform (supplementary text 3).

*2.2.2.2. Transcriptome analysis.* Venous blood samples were collected in PAXgene™ blood RNA tubes (Qiagen, Hilden, Germany). The RNA transcriptomes were prepared by the Australian Genomics Research Foundation (AGRF), Adelaide, Australia, using Illumina's TruSeq stranded RNA sample preparation protocol. The RNA stranded samples were sequenced on the Illumina NovaSeq 6000 next generation sequencing platform 100 bp paired end run. The cleaned sequence reads were aligned against the *Homo sapiens* genome (Build version hg38), and the STAR aligner (v2.5.3a) was used to map reads to the genomic sequences.

### 2.3. Statistical analysis

We performed statistical analyses using SAS v9.4 and GraphPad Prism v8.2.0. We compared characteristics of the tic/OCD and control groups using independent t-tests, chi-squared tests, and Mann-Whitney U tests. We calculated odds ratios (OR) and 95% confidence intervals (CI) for associations between clinical features of children with tics/OCD and maternal autoimmunity without adjusting for multiple testing. We performed multivariate logistic regression to adjust for maternal neuropsychiatric disorders and perinatal factors associated with neurodevelopmental disorders which significantly differed between tic/OCD and control groups in univariate analyses. Any missing data were excluded from the analysis and noted in the tables. Cytokine profiles of cases were compared with healthy controls using the Mann-Whitney U test, and no adjustment was made for multiple comparisons.

Maternal transcriptome data were analysed by BSG and AGRF in the R statistical environment (R Core Team (2013)) with Tidyverse (2019). (Wickham, 2019) Differential gene expression (DGE) was determined using Empirical analysis of Digital Gene Expression data in R (edgeR) v3.26.8 using R 3.6.1. (Robinson et al., 2010) The top 1000 differentially expressed genes (DEGs) were tested for pathway enrichment in Gene Ontology (GO) terms, Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome pathways using the clusterProfiler and

ReactomePA v1.28.0 packages. (Young et al., 2010; Kanehisa and Goto, 2000; Yu and He, 2016; Yu et al., 2012) Gene Set Enrichment Analysis (GSEA) was also performed on the top 1000 DE genes and normalised enrichment scores were visualised using pheatmap v1.0.12. Published Tourette brain transcriptome Bam files mapped to hg19 were accessed with the authors' permission from www.synapse.org, (Lennington et al., 2016) and gene-level counts for UCSC refSeq genes were generated using HTSeq v0.11.2. Counts were background corrected (cpm > 1 in at least one sample), normalised, and case-control DGE was determined as above.

The primary statistical comparison of this study was the prevalence of maternal autoimmunity between the three study groups (at any time and at delivery of the proband), and all other comparisons should be considered as hypothesis generating only. Throughout the paper we present unadjusted p-values, and the reader should use their judgment in attributing significance for each comparison.

Ethical Approval

Ethical approval was granted by the Sydney Children's Hospitals Network Human Research Ethics Committee (HREC/18/SCHN/227, HREC/12/SCHN/395).

## 3. Results

### 3.1. Description of cases with Tics/OCD

The diagnoses and comorbidities of the 200 children with tics/OCD are outlined in Table 2. One hundred and seventy-eight (89%) children had a tic disorder, 141 (70.5%) children had Tourette's syndrome, and 76 (38%) children had OCD. One hundred and fifty-four (77%) children had a diagnosis of at least one comorbidity, including OCD, and 109 (54.5%) children had two or more comorbidities. Forty-one (21.1%) children reported an abrupt onset (defined as reaching maximum severity within a week) to their initial tics or OCD symptoms, and 90 (45%) children identified a trigger at onset, most commonly an illness or stressful event. The clinical courses varied (supplementary text 1), but stress, tiredness, and infection were common exacerbating factors of tics or OCD symptoms (Table 2). Eighty-one (40.5%) children were reported to have a loss of learning ability, social skills, or other skills since the onset of their symptoms. A significant minority had a severe clinical course prompting presentation to a hospital (n = 46, 23%) or prolonged absence from school (n = 18, 9%). One hundred and forty-six (73%) children received psychological therapy, and 126 (63%) children were treated with at least one medication (Table 2).

**Table 2**
Diagnoses, Comorbidities and Clinical Features of the 200 Children with Tics/OCD (see interview in supplementary material for further details).

| Characteristic | Number of Cases |
|---|---|
| | n (%) |
| **Tic disorders** | 178 (89%) |
| Provisional tic disorder | 10 (5%) |
| Chronic motor tic disorder | 26 (13%) |
| Chronic vocal tic disorder | 1 (0.5%) |
| Tourette's syndrome | 141 (70.5%) |
| **Obsessive compulsive disorder** | 76 (38%) |
| Obsessive-compulsive disorder without tics | 22 (11%) |
| Obsessive-compulsive disorder and tic disorder | 54 (27%) |
| **Age at onset of tics** (mean (±SD))[a] | 6.1 (±2.9) years |
| **Age of onset of OCD** (mean (±SD))[b] | 8.1 (±3.2) years |
| **Comorbidities** | |
| Autism spectrum disorder | 57 (28.5%) |
| Significant learning disability[c] | 28 (14%) |
| Attention-deficit hyperactivity disorder | 67 (33.5%) |
| Oppositional defiant disorder/Conduct disorder | 26 (13%) |
| Any anxiety disorder(s) | 127 (63.5%) |
| Depression or mood disorder | 22 (11%) |
| Other neuropsychiatric comorbidities[d] | 7 (3.5%) |
| Neurological comorbidities[e] | 15 (7.5%) |
| **Onset of Tics/OCD** | |
| Onset to maximum intensity within 1 week | 41/194 (21.1%) |
| **Triggers at Onset** | |
| Reported trigger at onset of tics/OCD | 90 (45%) |
| -Infection/illness | 46 (23%) |
| -Stress/life event | 38 (19%) |
| -Medication | 4 (2%) |
| -Vaccination[f] | 10 (5%) |
| -Other | 3 (1.5%) |
| No reported trigger at onset | 109 (54.5%) |
| Unknown | 1 (0.5%) |
| **Clinical Course**[g] | |
| Monophasic | 5 (2.5%) |
| Chronic static | 3 (1.5%) |
| Mild fluctuations | 36 (18%) |
| Moderate fluctuations | 46 (23%) |
| Severe fluctuations | 20 (10%) |
| Relapsing remitting | 46 (23%) |
| Chronic progressive | 43 (21.5%) |
| Other | 1 (0.5%) |
| **Exacerbating factors of tics/OCD at any time in clinical course** | |
| Stress | 179/199 (89.9%) |
| Tiredness | 152/196 (77.6%) |
| Infection | 100/189 (52.9%) |
| Other | 51/200 (25.5%) |
| **Loss of skills in at least one domain at onset or with subsequent exacerbations in tics/OCD** | 81 (40.5%) |
| **Markers of Severity (at any time in clinical course)** | |
| Emergency department or hospital admission | 46 (23%) |
| Unable to attend school for ≥ 3 consecutive months | 18 (9%) |
| **Treatment for Tics/OCD or comorbidities** | |
| Psychoeducation only | 34 (17%) |
| Any psychology | 146 (73%) |
| Any pharmacological | 126 (63%) |
| -Any anti-depressant[h] | 78 (39%) |
| -Any alpha agonist | 70 (35%) |
| -Any neuroleptic | 57 (28.5%) |
| -Any stimulant/atomoxetine | 45 (22.5%) |
| **Improvement in symptoms (including comorbidities) reported with treatments**[i] | |
| Antibiotics[j] | 63/128 (49.2%) |
| Non-steroidal anti-inflammatory drugs[j] | 33/118 (28.0%) |
| Any alpha agonists | 44/70 (62.9%) |
| Any anti-depressant | 53/73 (72.6%) |
| Any neuroleptic | 44/55 (80%) |
| Any stimulant/atomoxetine | 32/43 (74.4%) |

[a] n = 176, unknown in 2 cases.
[b] n = 75, unknown in 1 case.
[c] Defined as needing special education i.e. special school placement or support unit.
[d] Eating disorder, post-traumatic stress disorder, bipolar disorder, psychosis.
[e] Includes epilepsy (all types), strabismus, migraine, and single cases of paroxysmal movement disorder, idiopathic intracranial hypertension and sensorineural hearing loss.
[f] Five caregivers reported infection and vaccination as a trigger.
[g] See supplementary material for graphs which were presented to caregivers during the interview.
[h] Selective serotonin reuptake inhibitors (SSRIs), serotonin-noradrenaline reuptake inhibitors (SNRIs) or tricyclic antidepressants (TCAs).
[i] Improvement with any treatment within the medication class is counted.
[j] Prescribed or administered for any indication.

### 3.2. Prevalence of definite autoimmune disease in mothers and reported autoimmunity in other first- and second-degree relatives

Sixty-one (30.5%) mothers of children with tics/OCD had definite autoimmune disease at any time, compared with 20 (20%) mothers of neurological autoimmune controls (p = 0.054), and 12 (12%) mothers of healthy controls (p = 0.0004, adjusted OR 2.70, 95%CI 1.28–5.68) (Fig. 1A, supplementary Tables 2 and 7). Thirty-eight (19%) mothers of children with tics/OCD had a definite autoimmune disease at the time of the delivery of the proband, compared with 13 (13%) mothers of neurological autoimmune controls (p = 0.19), and 9 (9%) mothers of healthy controls (p = 0.025) (Fig. 1B). The probands and the probands' siblings in the tic/OCD group had higher rates of autoimmune disease compared with healthy controls (p = 0.0003 and p = 0.018 respectively, Fig. 1D and 1E), and autoimmunity in any first-degree relative was more common in the tic/OCD group, compared with control groups (Fig. 1F). Among second-degree relatives, autoimmunity was more commonly reported in maternal grandmothers and maternal aunts of children with tics/OCD compared with healthy controls (p = 0.011 and p = 0.023 respectively, Fig. 1G), independent of reported autoimmune disease in the mother. Rates of autoimmunity in paternal second-degree relatives were similar between groups, except that paternal uncles of healthy controls had a higher proportion of autoimmune disease than paternal uncles in the tic/OCD group (p = 0.0009, Fig. 1H).

The autoimmune diseases of mothers of children with tics/OCD were heterogeneous, and only psoriasis was overrepresented compared with healthy controls (p = 0.0052) (supplementary Table 3 and supplementary Fig. 3). The increased odds of maternal autoimmunity in the tics/OCD group remained after adjusting for maternal neuropsychiatric disorders and perinatal factors associated with neurodevelopmental disorders (supplementary Tables 4–7). Only five (2.5%) mothers in the tics/OCD group had taken systemic immunomodulatory treatments during pregnancy, which was consistent with the management of the more frequent autoimmune diseases as well as clinical practice to minimise medications during pregnancy. Secondary analysis of the combined definite autoimmune diseases, possible autoimmune diseases, and inflammatory disorders revealed similar results to our primary analysis in maternal, first- and second-degree relatives (supplementary Table 8).

### 3.3. Other Pro-inflammatory factors in the mothers and children in the Tics/OCD cohort compared with controls

Mothers of children with tics/OCD had a greater number of pro-inflammatory conditions than mothers of neurological autoimmune and healthy controls (both p < 0.0001), independent of the sex of the child (Fig. 2a and 2b, supplementary Table 9a). Supplementary Table 9b shows the rates of pro-inflammatory diseases and conditions in mothers and probands in the tics/OCD and control groups. Anxiety/depression, asthma, infection in pregnancy, preeclampsia or pregnancy-induced hypertension, and smoking in pregnancy were more frequent in mothers of children with tics/OCD compared with mothers of healthy controls (p-values range from < 0.0001 for anxiety/depression to 0.022 for asthma). Asthma, recurrent infections, and hospitalisation for infection were also more frequent in the children with tics/OCD compared with healthy controls (p-values < 0.0001 to 0.0088, supplementary fig. 4 and supplementary Tables 9a and 9b).

The clinical features in the children with tics/OCD that had the highest odds ratios with definite maternal autoimmunity were a chronic-progressive course and symptom improvement with non-steroidal anti-inflammatory drugs (NSAIDs), (OR 2.5, 95th CI 1.2–4.9, and OR 2.7, 95th CI 1.2–6.3 respectively, supplementary table 10). Infectious trigger at onset, comorbid diagnoses and pharmacological treatment were not associated with maternal autoimmune disease.

### 3.4. Cytokine measurements in mothers with autoimmune disease

Interferon-alpha and G-CSF were elevated in the mothers of children

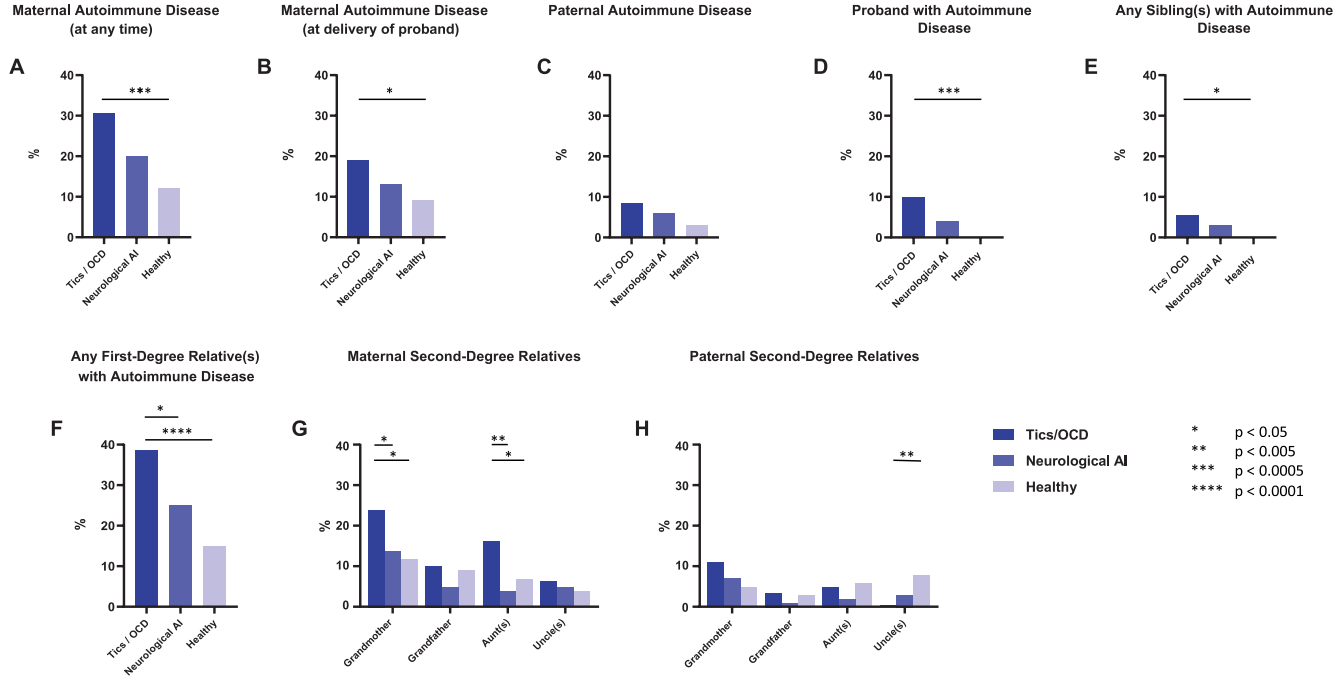


**Fig. 1.** Frequency of autoimmune disease in the mothers and other first- and second-degree relatives of children with tics/OCD compared with neurological autoimmune and healthy controls. The prevalence of reported autoimmune disease present in mothers at any time (A), mothers at delivery of proband (B), fathers (C), probands (D), any siblings (E), any first-degree relative(s) (F), and maternal and paternal second-degree relatives (G and H respectively) of children in each group. Elevated rates of autoimmune disease (noted with an asterisk(s)), were present in the tic/OCD cohort for mothers at any time, mothers at delivery of proband, probands, any siblings, maternal aunts, and maternal grandmothers. Paternal uncles of healthy controls had higher rates of autoimmune disease compared with the tics/OCD group. Abbreviations: AI, autoimmune.

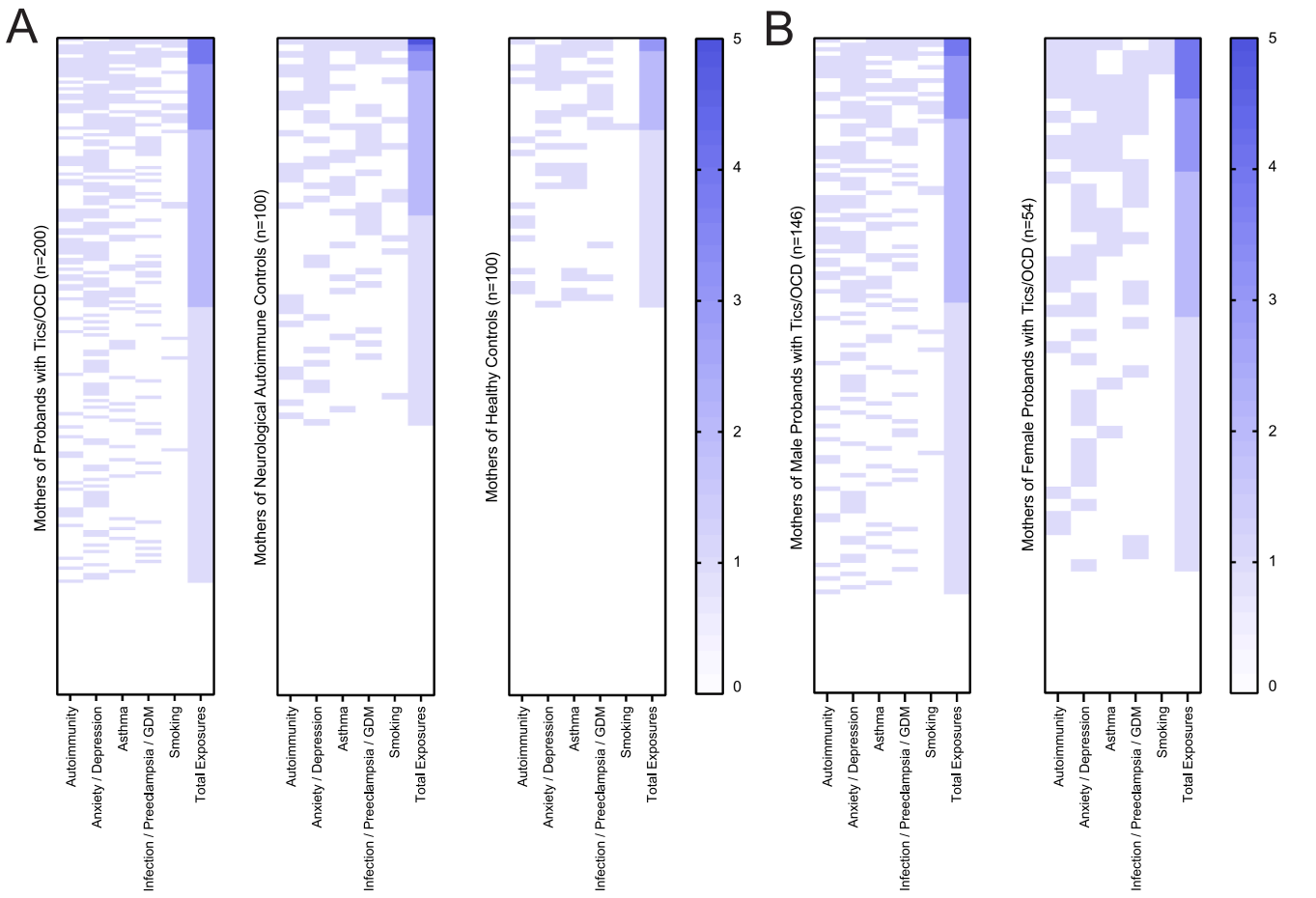


**Fig. 2.** Heat maps depicting the number of pro-inflammatory states in mothers of children with tics/OCD compared with neurological autoimmune and healthy controls. Data presented for definite autoimmune disease, anxiety/depression, asthma, pro-inflammatory pregnancy complications (infection, pre-eclampsia, gestational diabetes), smoking and total accumulative exposome. Each row represents one mother, and each column represents a pro-inflammatory state/pro-inflammatory states as labelled. The right column is shaded according to the accumulative number of exposures. **A.** Heat maps demonstrating a greater number of recognised pro-inflammatory states in mothers of children with tics/OCD compared with neurological autoimmune and healthy controls ($p < 0.0001$ for both). **B.** Heat maps showing that the number of pro-inflammatory factors in mothers of children with tics/OCD did not differ by sex of the proband ($p = 0.36$).

with tics/OCD and autoimmunity ($p = 0.033$ and $p = 0.015$ respectively), whereas interferon gamma-induced protein 10 (IP-10) and eotaxin were reduced compared to mothers of healthy controls ($p = 0.0095$ and $p = 0.017$ respectively, supplementary fig. 5).

### 3.5. Transcriptomic analyses

Mothers with autoimmune disease who had children with tics/OCD ($n = 14$) had upregulated DEGs enriched in the neutrophil degranulation pathways and Toll-like Receptor (TLR) Cascades, compared with mothers of healthy controls (q-values = 2.0e-4 and 2.3e-2 respectively, ReactomePA, Fig. 3A). Gene ontology and KEGG pathway enrichment analysis showed an overrepresentation of DEGs associated with the innate immune and inflammatory responses (Fig. 3C and 3E).

The Tourette brain transcriptomes showed similar enrichment in innate immune pathways in Reactome, GO and KEGG analysis of DEGs (Fig. 3B, 3D and 3F). (Lennington et al., 2016) Additionally, GSEA demonstrated that the most enriched pathways common to both maternal blood and Tourette brain were neutrophil degranulation, innate immune system, and interleukin and cytokine signalling (Fig. 3G and supplementary fig. 6).

## 4. Discussion

Systemic chronic inflammation is a major contributor to the expression of common disease such as cardiovascular disease, cancer, and neurodegeneration, (Furman et al., 2019; Yuan et al., 2019) and transcriptome studies have provided new insight into a role for inflammation in neurodevelopment and related disorders. (Lennington et al., 2016; Lisboa et al., 2019; Voineagu et al., 2011; Velmeshev et al., 2019) We explored whether inflammation in mothers and other first- and second-degree relatives is associated with expression of tics/OCD in children, focusing on autoimmune disease. (Dalsgaard et al., 2015; Mataix-Cols et al., 2018; Murphy et al., 2010)

Autoimmune disease and other inflammatory states were more common in mothers of children with tics/OCD than controls, both at the time of delivery of the proband and at the time of interview. Although some mothers in our cohort were diagnosed with autoimmunity after the proband's delivery, autoimmune dysregulation typically begins years before the clinical manifestations of disease, (Arbuckle et al., 2003) and maternal autoimmunity has been associated with offspring neurodevelopmental disorders irrespective of time of onset. (Mataix-Cols et al., 2018, Nielsen et al., 2021)

The autoimmune diseases in our cohort were heterogeneous with variable innate, T-cell, and autoantibody mechanisms, and larger sample numbers are likely needed to identify a prevailing cytokine signature. Maternal psoriasis was the only specific condition that was elevated in mothers in the tics/OCD cohort. Psoriasis is an autoinflammatory disorder involving the Th17/IL-23 axis, and within this pathway, IL-17A has been strongly implicated in animal models of

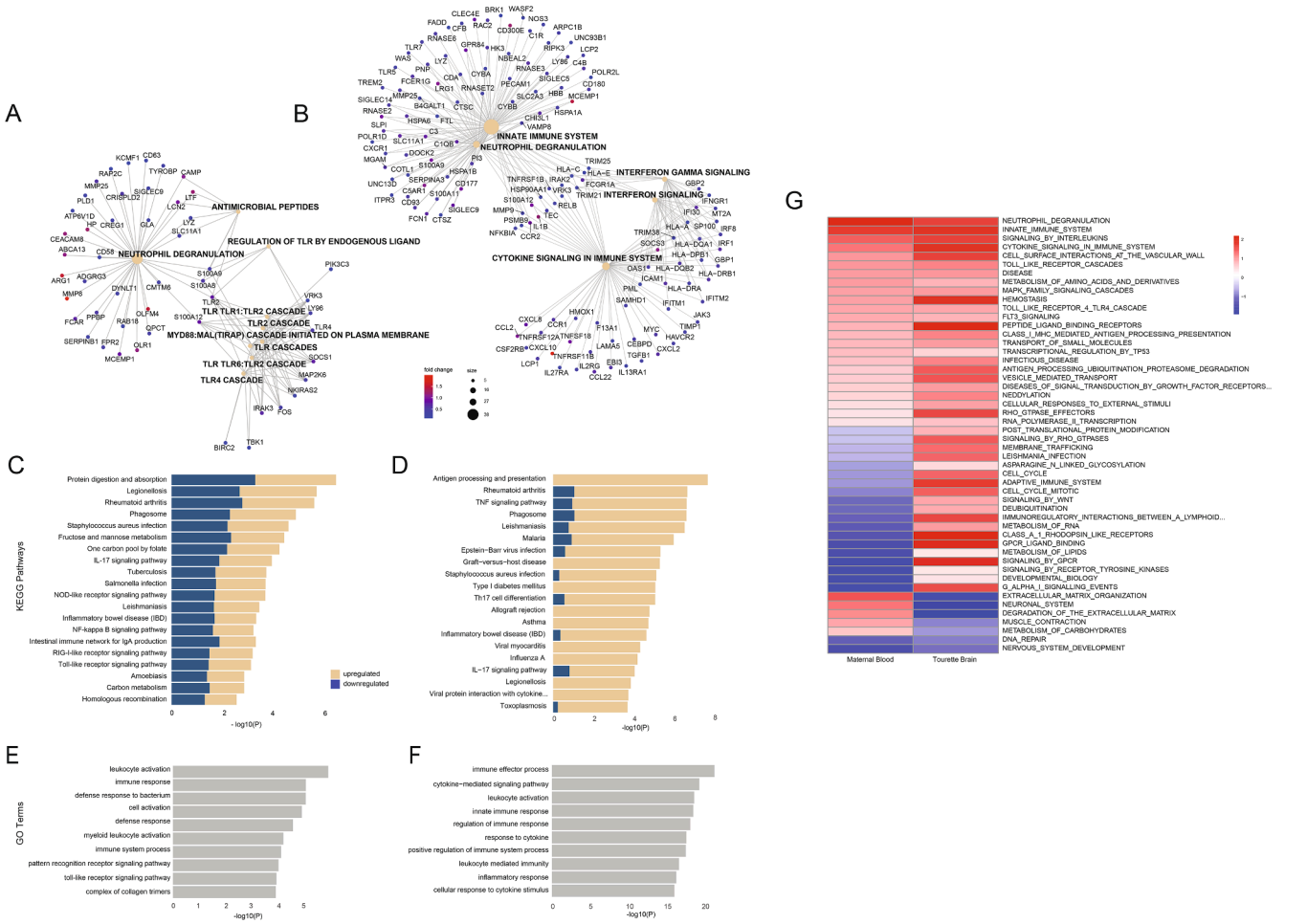


**Fig. 3.** Analysis of the top 1000 differentially expressed genes (DEGs) in the blood transcriptomes of mothers of children with tics/OCD with autoimmune disease compared with controls (A, C, E), and the top 1000 DEGs in brain transcriptomes of individuals with Tourette syndrome compared with controls (B, D, F). (Lennington et al., 2016) Panels A and B: Reactome Pathway Analysis of the upregulated genes within the top 1000 DEGs for maternal blood transcriptomes (A) and Tourette brain transcriptomes (B). Neutrophil degranulation pathways are enriched in both datasets (q-values: 2.0e-4 and 8.1e-8 respectively) and innate immune pathways are enriched in Tourette brain transcriptomes (q-value: 6.4e-13). Genes represented by points coloured according to logFC. Node sizes are binned according to the number of genes. Panels C and D: Middle row: The top 20 KEGG pathways enriched in DEGs for maternal blood transcriptomes (C) and Tourette brain transcriptomes (D) indicating common innate immune response pathways. Bars are coloured by the proportion of measured genes which are upregulated (tan) and downregulated (blue). The x-axis represents the unadjusted p-value. Panels E and F: The top 10 GO terms enriched in up- and down-regulated DEGs. Maternal blood transcriptome data is on the left (E) and Tourette brain transcriptome data is on the right (F). The x-axis represents the unadjusted P-value. (G) Heatmap of normalised enrichments scores (NES) of GSEA of ranked gene lists for both datasets trimmed to only demonstrate pathways with enrichment in both datasets. The full heatmap is provided in Supplementary Fig. 6C. GSEA demonstrates that the most upregulated pathways common to both maternal blood and Tourette brain were neutrophil degranulation, innate immune system, and interleukin and cytokine signalling. Abbreviations: GO, gene ontology; GSEA, gene set enrichment analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

maternal immune activation. (Reed et al., 2020) Psoriasis in mothers has also been associated with neurodevelopmental disorders in children in epidemiological cohorts and linked with ADHD in GWAS studies. (Croen et al., 2005; Tylee et al., 2018)

Although autoimmunity was most evident in the mothers and maternal female relatives in our tic/OCD cohort, there were also higher observed rates of autoimmune disease in fathers of children with tics/OCD, as reported in epidemiological studies. (Mataix-Cols et al., 2018) Coinheritance of immune and neurodevelopmental disorders could be related to genes with dual immune and neurodevelopmental functions, as exemplified by some rare variants in OCD, (Cappi et al., 2016) transcription genes which regulate expression of immune and neuronal signalling pathways, (Autism Spectrum, 2017) or heritable epigenetic modifications. (Nardone and Elliott, 2016; Network, 2015)

Other disorders known to be pro-inflammatory such as asthma, pre-eclampsia, and depression, were more frequent in mothers of children with tics/OCD, concordant with epidemiological studies in neurodevelopmental disorders, and may be cumulative in their effects and risk. (Gong et al., 2019; Gumusoglu et al., 2020; Lee and Giuliani, 2019; Ayano et al., 2019) These disorders have innate immune activation in common, such as TLR signalling and activation. (Kumar et al., 2009) TLRs are pathogen- and damage-associated molecular pattern recognition receptors, which are highly expressed in microglia and neurons, and have been implicated in neurodevelopmental disorders, neuropsychiatric disorders, and stress-mediated neuroinflammation (Keri et al., 2017; Enstrom et al., 2010; De Miranda et al., 2010; Garate et al., 2013).

Brain transcriptomic studies of neurodevelopmental disorders have shown downregulation of synaptic and neuronal genes, but upregulation of inflammatory genes. (Lennington et al., 2016; Lisboa et al., 2019; Voineagu et al., 2011) The inflammatory signal reported in brain transcriptomic studies is generally not explained but presumed to be environmental or secondary. (Voineagu et al., 2011) Given our hypothesis

that maternal autoimmunity is involved in the expression of neurodevelopmental disorders in offspring, we performed transcriptomic analysis to determine if there were shared differentially expressed genes with brain transcriptomic studies. The transcriptomic data showed that common innate immune pathways were enriched in maternal autoimmunity and the neuroinflammation reported in Tourette brain samples including neutrophil degranulation, TLR cascades, and interleukin and cytokine signalling. Shared differential expression of genes in the TLR cascades highlights that these 'sensors' of the innate immune system could be important intermediaries in the immune-neurodevelopmental interface, an hypothesis which is supported by robust preclinical data. (Bilbo et al., 2018; De Miranda et al., 2010) Upregulation of inflammatory genes is similarly evident in brain transcriptomes in OCD and ASD, and suggest our findings may be extrapolated to other neurodevelopmental disorders. (Lisboa et al., 2019; Voineagu et al., 2011)

Maternal autoimmunity was not clearly associated with a distinct tics/OCD phenotype, although children of mothers with autoimmune disease were more likely to have a chronic progressive course and report improved symptoms with NSAIDs. Responsiveness to the cyclooxygenase-2 inhibitor, celecoxib, has been reported in both Tourette syndrome and OCD. (Müller, 2004; Sayyah et al., 2011) Infection provocation at onset and infection-associated symptom worsening were frequently reported irrespective of a maternal history of autoimmunity. (Singer et al., 2000) Abrupt, infection-provoked onset of tics or OCD is described in 'Paediatric Autoimmune Disease associated with Streptococcal Infection' (PANDAS), and 'Paediatric Acute-onset Neuropsychiatric Syndrome' (PANS), controversial entities proposed to be distinct disorders. However, our data suggest that infection-related exacerbations are common in tics/OCD in general and that PANDAS and PANS may represent a continuum with tic disorders and OCD, rather than separate disorders. The reporting of stress- and infection-related exacerbations suggests a role for immune-CNS crosstalk in the clinical course of tics/OCD. (Tian et al., 2012; Yirmiya and Goshen, 2011; Olvera Alvarez et al., 2018) Acute stress-induced neuronal activation triggers microglia and astrocytes to produce high levels of pro-inflammatory cytokines which stimulate the hypothalamus–pituitary-adrenal (HPA) axis and sympathetic nervous system, (Yirmiya and Goshen, 2011; Berens et al., 2017) and infection in the periphery provokes macrophages and immune cells to produce cytokines which provoke a pro-inflammatory response within the CNS. (Yirmiya and Goshen, 2011)

Our data also showed that children with tics/OCD had an excess of immune conditions, including autoimmune disease, many of whom did not have a parent with autoimmunity. Our findings indicate a role for immune dysregulation in the pathogenesis of tics/OCD postnatally, or shared pathogenic mechanisms between immune and neurodevelopmental disorders. (Wang et al., 2019; Perez-Vigil et al., 2016; Orlovska et al., 2017; Tsai et al., 2016) Previous studies have also shown higher rates of autoimmune disease in individuals with OCD and Tourette syndrome, (Perez-Vigil et al., 2016) and increased rates of OCD with specific autoimmune conditions. (Tylee et al., 2018; Wang et al., 2019)

As expected, there were high rates of anxiety, depression and other neuropsychiatric disorders in the mothers, fathers and second-degree relatives of children with tics/OCD. Some neuropsychiatric disorders are reported to be independently associated with autoimmune disease such as depression and psoriasis, (Kurd et al., 2010) but in our cohort the association between maternal autoimmune disease and tics/OCD in children remained after adjusting for maternal neuropsychiatric disorders. Anxiety and depression were the most common neuropsychiatric disorders in first-degree relatives of children with tics/OCD, including mothers, and could reflect environmental stressors not captured in our study such as financial strain and relationship conflict. The rates of tics and OCD in the parents of children with tics/OCD were lower than previously reported and may result from under-reporting or under-diagnosis. (Yu et al., 2015; Hirschtritt et al., 2015)

Important limitations to our study are latency in reporting and potential reporting bias. (O'Rourke et al., 2019) We sought to mitigate the effects of these limitations by verifying key diagnostic information about autoimmune disease and recruiting a positive control group of children with neurological autoimmune diseases whose parents were more likely to be aware of immune-related diseases in family members. In most instances, we interviewed the child's mother which may have resulted in more reporting of autoimmune disease and neuropsychiatric conditions in maternal second-degree relatives compared with paternal second-degree relatives, but this effect would have been consistent across patient and control groups. Similarly, blood samples used for analyses of cytokine profiles and transcriptomes were drawn years after delivery of the probands. However, given the chronic nature of autoimmune disease, we propose the immunological profiles detected reflect the maternal immune dysregulation present during pregnancy. Future studies should prospectively collect maternal and neonatal blood samples to further examine this association. A small proportion of mothers included in the biological investigations were taking immune therapy at the time of sample collection (4 of 20), which could have theoretically affected the findings.

There are important pro-inflammatory conditions, such as obesity, that were not investigated in our study and should be included in further research. A role for the microbiome in microglial maturation and functioning, gut-brain crosstalk, and neuropsychiatric disorders is also emerging, (Cryan et al., 2019; Rogers et al., 2016) and maternal and proband microbiomes should be included in future studies. (Martino et al., 2020) The high numbers of comorbidities and pharmacotherapies prescribed in our case cohort are consistent with children seen in a tertiary clinic and highlight that the generalisability of our findings to a community sample is uncertain.

Although we have emphasised the pro-inflammatory effects of the maternal diseases associated with tics/OCD in our study, the negative effects on fetal neurodevelopment are likely to be multi-factorial and include neuronal genetic factors (for depression) (Pearson et al., 2013) and thyroid hormone dysregulation (for autoimmune thyroid disease). (Brown et al., 2015) Other variables, including perinatal complications, were comparable between groups, (Williams et al., 2019) and medication use was adjusted for by logistic regression modelling.

Our findings demonstrate that maternal pro-inflammatory states, including autoimmune disease, are associated with tics/OCD in children, and support a possible role for maternal inflammation, in addition to immunogenetic and 'neurogenic' mechanisms in the aetiology of tic disorders and OCD. (Mataix-Cols et al., 2018) The breadth of immune conditions, including the heterogeneity of autoimmune diseases, and overlapping pathways in transcriptomic analysis of maternal blood and Tourette brain samples indicate that the innate immune response may be an important factor in disease expression. Inflammation is likely to be a more modifiable risk factor than susceptibility genes, and prospective studies which comprehensively assess pro-inflammatory states in mothers during pregnancy paired with detailed immunophenotyping, genomic and epigenomic testing, and careful evaluation of postnatal pro-inflammatory exposures in children, are needed to fully assess the role of inflammation as an environmental risk factor for neurodevelopmental disorders. Further understanding of the role of the immune system in neurodevelopment could unveil opportunities to mitigate risk to children by reducing exposure to inflammation and open new avenues for treatment.

## CRediT authorship contribution statement

**Hannah F. Jones:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, review and editing. **Velda X. Han:** Conceptualization, data analysis, writing – review and editing. **Shrujna Patel:** Conceptualization, data analysis, writing – review and editing. **Brian S. Gloss:** Conceptualization, data analysis, writing – review and editing. **Nicolette Soler:** Data curation and analysis, writing – review and editing. **Alvin Ho:** Conceptualization, data

curation and analysis, writing – review and editing. **Suvasini Sharma:** Conceptualization, data curation and analysis, writing – review and editing. **Kavitha Kothur:** Conceptualization, data curation and analysis, writing – review and editing. **Margherita Nosadini:** Conceptualization, data curation and analysis, writing – review and editing. **Louise Wienholt:** Data curation and analysis, writing – review and editing. **Chris Hardwick:** Conceptualization, data curation and analysis, writing – review and editing. **Elizabeth H. Barnes:** Conceptualization, data analysis, writing – review and editing. **Jacqueline R. Lim:** Data analysis, writing – review and editing. **Sarah Alshammery:** Data analysis, writing – review and editing. **Timothy C. Nielsen:** Data analysis, writing – review and editing. **Melanie Wong:** Conceptualization, data analysis, writing – review and editing. **Markus J. Hofer:** Conceptualization, writing – review and editing. **Natasha Nassar:** Data analysis, writing – review and editing. **Wendy Gold:** Data analysis, writing – review and editing. **Fabienne Brilot:** Conceptualization, Data curation, Formal analysis, Resources, Writing – review and editing. **Shekeeb S. Mohammad:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing –review and editing. **Russell C. Dale:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, review and editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

*Acknowledgements*

We gratefully acknowledge the neurologists at the Children's Hospital at Westmead, Dr Sushil Bandodkar, Dr Andrew Williams and the hundreds of study participants who contributed to this research. We also gratefully acknowledge the use of the PsychENCODE Consortium data published as, 'Transcriptome Analysis of the Human Striatum in Tourette Syndrome.' Data were generated as part of the PsychENCODE Consortium supported by: U01MH103339, U01MH103365, U01MH103392, U01MH103340, U01MH103346, R01MH105472, R01MH094714, R01MH105898, R21MH102791, R21MH105881, R21MH103877, and P50MH106934 awarded to: Schahram Akbarian (Icahn School of Medicine at Mount Sinai), Gregory Crawford (Duke), Stella Dracheva (Icahn School of Medicine at Mount Sinai), Peggy Farnham (USC), Mark Gerstein (Yale), Daniel Geschwind (UCLA), Thomas M. Hyde (LIBD), Andrew Jaffe (LIBD), James A. Knowles (USC), Chunyu Liu (UIC), Dalila Pinto (Icahn School of Medicine at Mount Sinai), Nenad Sestan (Yale), Pamela Sklar (Icahn School of Medicine at Mount Sinai), Matthew State (UCSF), Patrick Sullivan (UNC), Flora Vaccarino (Yale), Sherman Weissman (Yale), Kevin White (UChicago) and Peter Zandi (JHU).

Financial support for the study was granted by the Petre Foundation, Brain Foundation, NHMRC Investigator Grant (APP1193648), and Cerebral Palsy Alliance. There was no role of the study sponsors in the study design, collection, analysis or interpretation of data, report writing, or decision to submit for publication.

Author contributions: All named authors have made a substantial contribution to the study conception or design; or the acquisition, analysis, or interpretation of data; drafted the manuscript or revised it critically for important intellectual content; approved the final version of the manuscript for publication; and agreed to be accountable for all aspects of the work.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2020.12.035.

## References

Arbuckle, M.R., McClain, M.T., Rubertone, M.V., et al., 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N. Engl. J. Med. 349, 1526–1533.

Attwells, S., Setiawan, E., Wilson, A.A., et al., 2017. Inflammation in the Neurocircuitry of Obsessive-Compulsive Disorder. JAMA Psychiatry 74, 833–840.

Autism Spectrum Disorders Working Group of The Psychiatric Genomics C. (2017). Meta-analysis of GWAS of over 16,000 individuals with autism spectrum disorder highlights a novel locus at 10q24.32 and a significant overlap with schizophrenia. Mol. Autism,8, 21.

Ayano, G., Maravilla, J.C., Alati, R., 2019. Risk of autistic spectrum disorder in offspring with parental mood disorders: A systematic review and meta-analysis. J. Affect. Disord. 248, 185–197.

Berens, A.E., Jensen, S.K.G., Nelson 3rd., C.A., 2017. Biological embedding of childhood adversity: from physiological mechanisms to clinical implications. BMC Med. 15, 135.

Bilbo, S.D., Block, C.L., Bolton, J.L., Hanamsagar, R., Tran, P.K., 2018. Beyond infection - Maternal immune activation by environmental factors, microglial development, and relevance for autism spectrum disorders. Exp. Neurol. 299, 241–251.

Brown, A.S., Surcel, H.M., Hinkka-Yli-Salomaki, S., Cheslack-Postava, K., Bao, Y., Sourander, A., 2015. Maternal thyroid autoantibody and elevated risk of autism in a national birth cohort. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 57, 86–92.

Cappi, C., Brentani, H., Lima, L., et al., 2016. Whole-exome sequencing in obsessive-compulsive disorder identifies rare mutations in immunological and neurodevelopmental pathways. Transl. Psychiatry 6, e764.

Croen, L.A., Grether, J.K., Yoshida, C.K., Odouli, R., Van de Water, J., 2005. Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. Arch. Pediatr. Adolesc. Med. 159, 151–157.

Cross-Disorder Group of the Psychiatric Genomics Consortium, 2019. Genomic Relationships, Novel Loci, and Pleiotropic Mechanisms across Eight Psychiatric Disorders. Cell 179, 1469–1482.

Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., et al., 2019. The Microbiota-Gut-Brain Axis. Physiol. Rev. 99, 1877–2013.

Dalsgaard, S., Waltoft, B.L., Leckman, J.F., Mortensen, P.B., 2015. Maternal history of autoimmune disease and later development of Tourette syndrome in offspring. J. Am. Acad. Child Adolesc. Psychiatry 54, 495–501.

De Miranda J, Yaddanapudi K, Hornig M, Villar G, Serge R, Lipkin WI (2010). Induction of Toll-like receptor 3-mediated immunity during gestation inhibits cortical neurogenesis and causes behavioral disturbances. mBio, 1.

Deverman, B.E., Patterson, P.H., 2009. Cytokines and CNS development. Neuron 64, 61–78.

Diagnostic and Statistical Manual of Mental Disorders: DSM-5. (2013) Fifth ed. Arlington, VA: American Psychiatric Association.

Enstrom, A.M., Onore, C.E., Van de Water, J.A., Ashwood, P., 2010. Differential monocyte responses to TLR ligands in children with autism spectrum disorders. Brain Behav. Immun. 24, 64–71.

Estes, M.L., McAllister, A.K., 2016. Maternal immune activation: Implications for neuropsychiatric disorders. Science 353, 772–777.

Farh, K.K., Marson, A., Zhu, J., et al., 2015. Genetic and epigenetic fine mapping of causal autoimmune disease variants. Nature 518, 337–343.

Frick L, Pittenger C. Microglial (2016) Dysregulation in OCD, Tourette Syndrome, and PANDAS. J. Immunol. Res. 2016, doi.10.1155/2016/8606057..

Furman, D., Campisi, J., Verdin, E., et al., 2019. Chronic inflammation in the etiology of disease across the life span. Nat. Med. 25, 1822–1832.

Garate, I., Garcia-Bueno, B., Madrigal, J.L., et al., 2013. Stress-induced neuroinflammation: role of the Toll-like receptor-4 pathway. Biol. Psychiatry 73, 32–43.

Gong, T., Lundholm, C., Rejno, G., et al., 2019. Parental asthma and risk of autism spectrum disorder in offspring: A population and family-based case-control study. Clin. Exp. Allergy 49, 883–891.

Gumusoglu, S.B., Chilukuri, A.S.S., Santillan, D.A., et al., 2020. Neurodevelopmental Outcomes of Prenatal Preeclampsia Exposure. Trends Neurosi 43, 253–268.

Gumusoglu, S.B., Stevens, H.E., 2019. Maternal Inflammation and Neurodevelopmental Programming: A Review of Preclinical Outcomes and Implications for Translational Psychiatry. Biol. Psychiatry 85, 107–121.

Hirschtritt, M.E., Lee, P.C., Pauls, D.L., et al., 2015. Lifetime prevalence, age of risk, and genetic relationships of comorbid psychiatric disorders in Tourette syndrome. JAMA Psychiatry 72, 325–333.

Kanehisa, M., Goto, S., 2000. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 28, 27–30.

Keri, S., Szabo, C., Kelemen, O., 2017. Uniting the neurodevelopmental and immunological hypotheses: Neuregulin 1 receptor ErbB and Toll-like receptor activation in first-episode schizophrenia. Sci. Rep. 7, 4147.

Kipnis, J., Gadani, S., Derecki, N.C., 2012. Pro-cognitive properties of T cells. Nat. Rev. Immunol. 12, 663–669.

Kumar, H., Kawai, T., Akira, S., 2009. Toll-like receptors and innate immunity. Biochem. Biophys. Res. Commun. 388, 621–625.

Kurd, S.K., Troxel, A.B., Crits-Christoph, P., Gelfand, J.M., 2010. The risk of depression, anxiety, and suicidality in patients with psoriasis: a population-based cohort study. Arch. Dermatol. 146, 891–895.

Lee, C.H., Giuliani, F., 2019. The Role of Inflammation in Depression and Fatigue. Front. Immunol. 10, 1696.

Lennington, J.B., Coppola, G., Kataoka-Sasaki, Y., et al., 2016. Transcriptome Analysis of the Human Striatum in Tourette Syndrome. Biol. Psychiatry 79, 372–382.
Lisboa, B.C.G., Oliveira, K.C., Tahira, A.C., et al., 2019. Initial findings of striatum tripartite model in OCD brain samples based on transcriptome analysis. Sci. Rep. 9, 3086.
Martino, D., Johnson, I., Leckman, J.F., 2020. What Does Immunology Have to Do With Normal Brain Development and the Pathophysiology Underlying Tourette Syndrome and Related Neuropsychiatric Disorders? Front. Neurol. 11, 567407 https://doi.org/10.3389/fneur.2020.567407.
Mataix-Cols, D., Frans, E., Perez-Vigil, A., et al., 2018. A total-population multigenerational family clustering study of autoimmune diseases in obsessive-compulsive disorder and Tourette's/chronic tic disorders. Mol. Psychiatry 23, 1652–1658.
Müller, N., 2004. Anti-inflammatory therapy with a COX-2 inhibitor in Tourette's syndrome. Inflammopharmacology 12, 271–275.
Murphy, T.K., Storch, E.A., Turner, A., Reid, J.M., Tan, J., Lewin, A.B., 2010. Maternal history of autoimmune disease in children presenting with tics and/or obsessive-compulsive disorder. J. Neuroimmunol. 229, 243–247.
Nardone, S., Elliott, E., 2016. The Interaction between the Immune System and Epigenetics in the Etiology of Autism Spectrum Disorders. Front. Neurosci. 10, 329.
Network, Pathway Analysis Subgroup of Psychiatric Genomics C. Psychiatric genome-wide association study analyses implicate neuronal, immune and histone pathways. Nat. Neurosci., 8, 199–209.
Nielsen, T.C., Nassar, N., Shand, A.W., Jones, H., Guastella, A.J., Dale, R.C., Lain, S.J., 2021. Association of maternal autoimmune disease with attention-deficit/hyperactivity disorder in children. JAMA Pediatr. e205487.
Olvera Alvarez, H.A., Kubzansky, L.D., Campen, M.J., Slavich, G.M., 2018. Early life stress, air pollution, inflammation, and disease: An integrative review and immunologic model of social-environmental adversity and lifespan health. Neurosci. Biobehav. Rev. 92, 226–242.
Orlovska, S., Vestergaard, C.H., Bech, B.H., Nordentoft, M., Vestergaard, M., Benros, M. E., 2017. Association of Streptococcal Throat Infection With Mental Disorders: Testing Key Aspects of the PANDAS Hypothesis in a Nationwide Study. JAMA Psychiatry 74, 740–746.
O'Rourke, J.A., Ravichandran, C., Howe, Y.J., et al., 2019. Accuracy of self-reported history of autoimmune disease: A pilot study. PLoS ONE 14, e0216526.
Paolicelli, R.C., Ferretti, M.T., 2017. Function and Dysfunction of Microglia during Brain Development: Consequences for Synapses and Neural Circuits. Front. Synaptic Neurosci. 9, 9.
Pearson, R.M., Evans, J., Kounali, D., et al., 2013. Maternal depression during pregnancy and the postnatal period: risks and possible mechanisms for offspring depression at age 18 years. JAMA Psychiatry 70, 1312–1319.
Perez-Vigil, A., Fernandez de la Cruz, L., Brander, G., Isomura, K., Gromark, C., Mataix-Cols, D., 2016. The link between autoimmune diseases and obsessive-compulsive and tic disorders: A systematic review. Neurosci. Biobehav. Rev. 71, 542–562.
Reed, M.D., Yim, Y.S., Wimmer, R.D., et al., 2020. IL-17a promotes sociability in mouse models of neurodevelopmental disorders. Nature 577, 249–253.
Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140.
Rogers, G.B., Keating, D.J., Young, R.L., Wong, M.-L., Licinio, J., Wesselingh, S., 2016. From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. Mol Psych 21, 738–748.
Rudolph, M.D., Graham, A.M., Feczko, E., et al., 2018. Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring. Nat. Neurosci. 21, 765–772.
Sayyah, M., Boostani, H., Pakseresht, S., Malayeri, A., 2011. A preliminary randomized double-blind clinical trial on the efficacy of celecoxib as an adjunct in the treatment of obsessive-compulsive disorder. Psychiatry Res. 189, 403–406.
Schork, A.J., Won, H., Appadurai, V., et al., 2019. A genome-wide association study of shared risk across psychiatric disorders implicates gene regulation during fetal neurodevelopment. Nat. Neurosci. 22, 353–361.
Singer, H.S., Giuliano, J.D., Zimmerman, A.M., Walkup, J.T., 2000. Infection: a stimulus for tic disorders. Pediatr. Neurol. 22, 380–383.
Statistics New Zealand. Understanding and Working with Ethnicity Data. A technical paper. Wellington 2005 [Available from: http://archive.stats.govt.nz/browse_for_stats/population/census_counts/review-measurement-of-ethnicity/papers.aspx#gsc.tab=0].
Tian, L., Ma, L., Kaarela, T., Li, Z., 2012. Neuroimmune crosstalk in the central nervous system and its significance for neurological diseases. J. Neuroinflammation 9, 155.
Tsai, C.S., Yang, Y.H., Huang, K.Y., Lee, Y., McIntyre, R.S., Chen, V.C., 2016. Association of Tic Disorders and Enterovirus Infection: A Nationwide Population-Based Study. Medicine (Baltimore) 95, e3347.
Tylee, D.S., Sun, J., Hess, J.L., et al., 2018. Genetic correlations among psychiatric and immune-related phenotypes based on genome-wide association data. Am. J. Med. Genet. B Neuropsychiatr. Genet. 177, 641–657.
Velmeshev, D., Schirmer, L., Jung, D., et al., 2019. Single-cell genomics identifies cell type-specific molecular changes in autism. Science 364, 685–689.
Voineagu, I., Wang, X., Johnston, P., et al., 2011. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature 474, 380–384.
Wang, L.Y., Chen, S.F., Chiang, J.H., Hsu, C.Y., Shen, Y.C., 2019. Systemic autoimmune diseases are associated with an increased risk of obsessive-compulsive disorder: a nationwide population-based cohort study. Soc. Psychiatry Psychiatr. Epidemiol. 54, 507–516.
Wickham (2019). Welcome to the tidyverse Journal of Open Source Software, 4, 1686.
Williams, A., Grantz, K., Seeni, I., et al., 2019. Obstetric and neonatal complications among women with autoimmune disease. J. Autoimmun. 103, 102287.
Yirmiya, R., Goshen, I., 2011. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav. Immun. 25, 181–213.
Young, M.D., Wakefield, M.J., Smyth, G.K., Oshlack, A., 2010. Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol. 11, R14.
Yu, G., He, Q.Y., 2016. ReactomePA: an R/Bioconductor package for reactome pathway analysis and visualization. Mol. BioSyst. 12, 477–479.
Yu, D., Mathews, C.A., Scharf, J.M., et al., 2015. Cross-disorder genome-wide analyses suggest a complex genetic relationship between Tourette's syndrome and OCD. Am. J. Psychiatry 172, 82–93.
Yu, G., Wang, L.-G., Han, Y., He, Q.-Y., 2012. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS: A Journal of*. Integr. Biol. 16, 284–287.
Yuan, N., Chen, Y., Xia, Y., Dai, J., Liu, C., 2019. Inflammation-related biomarkers in major psychiatric disorders: a cross-disorder assessment of reproducibility and specificity in 43 meta-analyses. Transl. Psychiatry 9, 233.