

Brain Transcriptome of Autism Spectrum Disorders and Tourette Syndrome Defines Common Targetable Inflammatory Pathways Involving Cytokines, Complement, and Kinase Signalling

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Abstract

Background

Neurodevelopmental disorders (NDDs), including autism-spectrum disorders (ASD) and Tourette syndrome (TS) are common brain conditions which often co-exist, however current management focuses on symptom mitigation, with no approved treatments targeting disease mechanisms. There is accumulating literature implicating the immune system in NDDs, and transcriptomics of post-mortem brain tissue from individuals with NDDs has revealed an inflammatory signal.

Methods

We interrogated two RNA-sequencing datasets of ASD and TS (compared to age-matched controls) and identified the top 1000 differentially expressed genes, to explore commonly enriched pathways using an over-representation analysis through GO, KEGG and Reactome.

Results

In the ASD analysis, the top 1000 DEGs enriched 754 GO terms (all upregulated), 55 KEGG pathways (54 upregulated), and 109 Reactome pathways (all upregulated), involving inflammation, cytokines, complement, cell signalling and epigenetic regulation. In the TS analysis, the top 1000 DEGs enriched 419 GO terms (416 upregulated), 56 KEGG pathways (all upregulated), and 28 Reactome (all upregulated) pathways, including inflammation, cytokines, signal transduction and immune response to stimuli. Of the top 1000 DEGs from the ASD and TS analyses, 133 DEGs were shared. Interaction networks of the common protein-coding DEGs using STRING revealed 5 central up-regulated hub genes: *CSF2RB*, *HCK*, *HCLS1*, *LCP2* and *PLEK*, which are all kinases involved in cell signalling. Applying KEGG and Reactome analysis to these common DEGs identified pathways involving interleukins, complement activation, and cell signalling pathways.

Conclusions

These findings bring new evidence of shared inflammation in ASD and TS, and provide therapeutic opportunities targeting inflammation, epigenetic machinery, and cell signalling including kinases.

Background

Neurodevelopmental disorders (NDDs) such as autism-spectrum disorders (ASD) and tic disorders including Tourette syndrome (TS), are neurological conditions which commonly co-exist and have shared genetic contributions¹. ASD is characterised by social communication and language deficits, and repetitive stereotypical behaviour. Tics are repetitive stereotyped movements (motor tics) or vocalisations (vocal tics), and when present for more than 12 months, fulfill a diagnosis of TS. Tics are present in 11–22% of children with ASD, while ASD is present in 12% of children diagnosed with TS^{2–4}. Limited disease

specific treatments are currently available for NDDs, and management focuses on symptom mitigation and developmental support^{5,6}.

The genetic aetiology of neurodevelopmental disorders is thought to be due to variants in multiple genes that converge on common pathways^{7,8}. However genetic aetiologies in these disorders are unable to explain the wide phenotypic heterogeneity, instead the interaction between environmental and genetic factors are proposed to play an important role in pathogenesis of NDDs. In addition, immune dysregulation and inflammation have long been suggested to contribute to the pathophysiology, where early insults during gestation, such as maternal immune activation (MIA), can impact the development of the foetal brain⁹⁻¹⁶. MIA, encompassing maternal conditions such as infection, asthma, obesity, autoimmune disease, and psychosocial stress, are associated with increased incidence of NDDs in offspring, such as ASD and TS¹⁷⁻²⁰. MIA is thought to act as a disease primer, which in addition to genetic predisposition, results in increased expression of neurodevelopmental disorders²¹. Studies have also shown dysregulation in proinflammatory cytokines such as IL-12, TNF, monocyte chemoattractant protein 2 (MCP-2), and IL-2 in the brains and peripheral blood of individuals with ASD and TS²²⁻²⁵.

Transcriptomic analyses (RNA sequencing) of post-mortem brains from individuals with ASD have shown upregulated genes involved in inflammation and microglial dysregulation^{26,27}. Similarly, analysis of post-mortem brain striatum from individuals with TS identified up-regulated genes in immune and inflammatory pathways, and implicated microglial activation as a primary source of inflammation²⁸. In both the ASD and TS brain transcriptome studies, the downregulated genes were enriched in pathways involved in synaptic function and GABA neurotransmission, aligning with the genetic variation found in these disorders²⁶⁻²⁸. By contrast, the upregulated inflammatory findings were considered more likely to be due to environmental factors or secondary²⁶⁻²⁸.

Given the shared genetic heterogeneity and comorbidity of NDDs, there is an increasing need to examine common disease pathways. As inflammation has been reported in brain transcriptomics in both ASD and TS, we examined for shared gene expression in order to improve our understanding of the pathophysiology of NDDs and provide future potential therapeutic targets²⁶⁻²⁸.

Methods

Data availability, and open-source bioinformatic analysis:

Human brain transcriptome data (RNA-seq) from two independent published studies were obtained with authors permission from synapse.org and analysed for differential gene expression and pathway enrichment analysis^{26,28}. Unlike TS, where only one study interrogating the brain transcriptome exists, there are a number of studies investigating ASD brain transcriptome²⁹⁻³¹. The current ASD dataset was chosen as it presented the largest cohort of samples^{26,27}. The ASD data were downloaded from synapse.org (ID: syn8234507) as count files, and RNA-seq metadata of 42 ASD cases were matched with

43 normal controls (NC)²⁶. The pre-frontal cortex (PFC) region was chosen for the ASD analysis given the large sample size with matched controls. The TS data was downloaded as BAM files from synapse.org (ID: syn3158906), which included putamen and the caudate nucleus regions from 9 TS cases and 9 normal controls²⁸. The bioinformatic workflow, including all utilised code and quality control figures can be found at <https://github.com/sarahalshammery/ASDTS>.

Demographic and clinical variables of cases and controls

Autism spectrum disorder

A total of 42 ASD cases and 43 normal control PFC samples were utilised in this analysis (Supplementary Table 1)²⁶. The ASD cohort selected (n = 42) consisted of nine female cases (21.43%) and 33 male cases (78.57%), with mean age of 26.38, median of 22.5 and range of 2–67 years. The normal control cohort selected (n = 43) comprised of nine females (20.93%) and 34 males (79.07%), with mean age of 28.63, median of 24, and range of 4–60 years. A Mann-Whitney test indicated no significant difference (U = 831, p-value = 0.5295) between the ages of the ASD cohort and normal controls.

Tourette syndrome

A total of 9 TS cases and 9 normal control caudate nucleus and putamen samples were included (Supplementary Table 1)²⁸. The TS cohort (n = 9) entailed four female cases (44.44%), and five male cases (55.56%) with mean age of 62.77, median of 52, and range of 29–84 years. The normal control (NC) cohort (n = 9) consisted of four (44.44%) females and five males (55.6%) with mean age of 58, median of 52, and range of 4–60 years. The full demographic data is in the supplementary material of the original study (See their Supplementary Table 2²⁸). There was no statistical differences in the age of the TS cases in comparison to normal controls²⁸.

Data quality control

The ASD dataset were prepared and sequenced as described (www.doi.org/10.7303/syn4587615), reads were mapped against the Genome Reference Consortium Human Build 37 (GRCh37, otherwise known as hg19). The TS dataset were mapped against GRCh37 (hg19), and gene level counts for REFSEQ genes were assessed using HTSeq-count²⁸. The raw counts for each dataset were converted to the counts per million (cpm) scale and filtered by requiring each gene to have >0.01 cpm of 50% within the samples. The data was normalised as per the EdgeR guide using Trimmed Mean of M-values (TMM) normalisation²⁸.

Differential gene expression analysis

The top 1000 genes following differential gene expression analysis of each dataset were considered differentially expressed genes (DEGs) in this investigation. The DEGs were identified by a quasi-likelihood (QL) negative binomial (NB) generalised log-linear model (glmQLF). Genes with a log fold change > 0

were considered to be up-regulated, and those below 0 were down-regulated. DEGs were visualised through a volcano plot using the ggplot 2 package³².

Pathway and Network enrichment analysis

Enrichments of the top 1000 DEGs were identified through an over-representation analysis using GO Biological Process, Reactome and the Kyoto Encyclopedia of Genes Genomes (KEGG), through the compareCluster function from the ClusterProfiler package (FDR < 0.05)^{33–39}. Given the perceived more significant mechanistic insights of the Reactome results, they are presented in the main text, whereas GO and KEGG are presented in the supplementary material.

The protein-coding DEGs which were common to both the ASD and the TS top 1000 DGE analyses, were visualised using a protein-protein interaction (PPI) network through the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; <https://string-db.org/>), with an interaction score > 0.4³⁹. The active interaction sources included experiments, co-expression, and gene fusion. The PPI network and functional enrichments and of GO, KEGG and Reactome from the DEGs common to both ASD and TS datasets were further imported into Cytoscape⁴⁰. CytoHubba, an app for Cytoscape was used to identify hub genes by ranking nodes by network features through the MCC method⁴¹. The expression of the hub genes in the disease cohorts compared to controls were visualised using the ggplot 2 package³².

Results

Transcriptional signatures

To identify relationships within the cases and their respective controls, we set out to explore differences based on transcriptome signatures. The ASD and TS cases were not observed to be transcriptionally distinct from their respective normal controls using hierarchal clustering analyses (Supplementary Figure 1 and Figure 2).

Differential gene expression analysis

Autism spectrum disorder: The top 1000 genes following DGE analysis within the PFC of ASD cases and normal controls were considered differentially expressed genes and used for further analysis. The differentially expressed genes consisted of 912 up-regulated genes and 88 down-regulated genes represented through a volcano plot (Figure 1A). Results of the DGE analysis can be accessed in supplemental material (Supplementary Table 2).

Tourette syndrome: The top 1000 differentially expressed genes within the striatum of individuals with TS and normal controls consisted of 711 up-regulated genes and 289 down-regulated genes, as shown in the volcano plot (Figure 1B). Results of the DGE analysis can be accessed in supplemental material (Supplementary Table 3).

Immune pathways are enriched in ASD and TS brain transcriptome

Autism spectrum disorder: To explore enriched terms and pathways in the top 1000 ASD DEGs, over-representation analyses were conducted through three databases (FDR <0.05). The GO analysis revealed 754 terms, all enriched by up-regulated DEGs, and involved many immune response and inflammatory signalling terms (Supplementary Table 2, Supplementary Figure 3). The top 3 GO terms were “response to lipopolysaccharide”, “response to molecule of bacterial origin” and “response to interferon-gamma”. Over-representation analysis using KEGG revealed 55 pathways, 54 of which were enriched by up-regulated genes (Supplementary Figure 4). The top 3 KEGG pathways (based on FDR) were “Systemic lupus erythematosus”, “Malaria” and “Neutrophil extracellular trap formation” (Supplementary Table 2). Enrichment of the DEGs using Reactome revealed 109 pathways, all enriched by up-regulated DEGs (Figure 2 A, C). Of the 109 pathways, the top 4 Reactome pathways (based on FDR) were “Interleukin-10 signalling”, “Signalling by interleukins”, “Interleukin-4 and interleukin-13 signalling” and “Interferon gamma signalling”. Overall, 25/109 Reactome pathways were involved in the immune response consisting of cytokine signalling, innate and adaptive immune response pathways, 16/109 pathways were involved in signal transduction including signalling by the NOTCH family, and 16/109 pathways belonged to gene expression including epigenetic regulation pathways. The full list of pathways from the three databases can be found in supplemental material (Supplementary Table 2).

Tourette syndrome: The top 1000 DEGs within the TS analysis enriched several terms and pathways from the three databases (FDR <0.05). GO over-representation analysis revealed 419 terms, 416 of which were enriched by up-regulated genes (Supplementary Table 3, Supplementary Figure 5). The top 3 enriched GO terms were “inflammatory response”, “cytokine-mediated signalling pathway” and “myeloid leukocyte activation”. Over-representation analysis using KEGG revealed 56 pathways, all enriched by up-regulated genes involved in the immune response and inflammatory signalling (Supplementary Table 3, Supplementary Figure 6). Of the 56 pathways, the top 3 KEGG pathways were “Staphylococcus aureus infection”, “Cytokine-cytokine receptor interaction” and “Viral protein interaction with cytokine and cytokine receptor”. Enrichment of the DEGs using Reactome revealed 28 pathways, all enriched by up-regulated DEGs (Figure 2 B, D). Of the 28 pathways, the top 3 Reactome pathways (sorted by FDR) were “Neutrophil degranulation”, “Interferon gamma signalling” and “Signalling by interleukins”. Overall, 18/28 Reactome pathways were involved in the immune response consisting of cytokine signalling, innate and adaptive immune response pathways, 5/28 pathways were involved in signal transduction, 3/28 pathways were involved in the homeostasis pathway, and 1/28 pathway was involved in response to stimuli. The full list of pathways from the three databases can be found in supplemental material (Supplementary Table 3).

Differentially expressed genes common to ASD and TS

Of the top 1000 DEGs from the ASD analysis, and the top 1000 DEGs from the TS analysis, 133 DEGs were found to be shared. The common protein-coding DEGs were mapped into a PPI network, and their expression in the ASD and TS cohorts was visualised (Figure 3A). From this network, we identified the top

five hub genes using Cytoscape and CytoHubba, which consisted of Colony Stimulating Factor 2 Receptor Subunit Beta (*CSF2RB*), Lymphocyte Cytosolic Protein 2 (*LCP2*), Hematopoietic cell kinase (*HCK*), Hematopoietic Cell-Specific Lyn Substrate 1 (*HCLST1*), and Pleckstrin (*PLEK*)^{40,41}. The raw data are presented in log scale for the five hub genes in cases compared to controls, shown for ASD (Figure 3B) and TS (Figure 3C). Inspection of the hub genes identified the first four as tyrosine kinases or associated with tyrosine kinase activity and cell signalling (Table 1). A full list of the common DEGs can be found in supplemental material (Supplementary Table 4).

Common differentially expressed genes in ASD and TS enrich immune pathways

As many of the enriched dysregulated pathways in ASD and TS overlapped, we set out to explore enriched pathways from the 133 DEGs common to both disorders, using overrepresentation analyses through KEGG and Reactome. The KEGG analysis revealed up-regulated genes enriched in 26 pathways in ASD and 25 pathways in TS, with the top three common pathways involving “Complement and coagulation cascades”, “TNF signaling pathway” and “Malaria” (Figure 4A). Using Reactome, the 133 DEGs common to ASD and TS involved up-regulated genes enriched in 14 pathways for ASD and 12 pathways for TS, with the top 3 common pathways implicating “Interleukin-4 and Interleukin-13 signaling”, “Interleukin-10 signaling” and “Signaling by Interleukins” (Figure 4B). The full list of pathways from the two databases can be found in the supplementary material (Supplementary Table 4).

Discussion

In this study we investigated enriched immune and inflammatory pathways in post-mortem brain tissue of individuals with ASD and TS, as well as pathways common to both disorders. Differential gene expression of the PFC region in ASD revealed that the majority (912 genes) of the top selected 1000 DEGs were up-regulated compared to normal controls. Analogous to this, in the striatum of TS, the majority (711 genes) of the identified top 1000 DEGs were also up-regulated compared to normal controls. This analysis validates the previous studies of up-regulated genes in post-mortem brains of individuals with ASD and TS^{28,42}.

The identified dominant signal of immune response and inflammation from the ASD GO enrichment analysis, aligns with studies investigating brain transcriptome and pathology of individuals with ASD, and supports the involvement of astrocytes and activated microglia^{26,42,43}. Of interest, the top GO term (by FDR) was response to lipopolysaccharide (LPS), a TLR4 agonist which stimulates an aberrant innate immune response in preclinical and clinical studies of NDDs^{44,45}. For example, studies have highlighted a differential innate immune response when monocytic cells from children with ASD are treated with LPS, characterised by dysregulated levels of cytokines including IL1B, vital in the neuro-immune crosstalk⁴⁶⁻⁴⁸.

The enriched pathways established by the KEGG and Reactome analyses in the ASD cases identified major cellular pathways with therapeutic potential. The differential expression of central immune genes

comprising cytokines, and CD cell markers (such as *IL1B*, *IL6*, *CD80*, *CD40*), support the reports of dysregulated cytokine levels in brains of individuals with ASD^{25,49}. Next, involvement of complement genes vital in phagocytosis (*C1QB*, *C1QC*, *C1R*, *C1S*), which play a central role in immunity, response to infection as well as synaptic pruning, further implicate the involvement of the immune system in ASD⁵⁰⁻⁵². In addition, the enrichment of histone subunits fundamental to gene expression and epigenetic regulation (*H3C13*, *H3C7*, *H2BC11*, *H2BC3*), supports the concept of potential association between epigenetic regulation and inflammation⁵³.

Analysis of the TS differentially expressed genes using GO identified numerous enriched immune response and inflammatory signalling terms. The enriched pathways highlighted by the KEGG and Reactome analyses in TS identified up-regulated DEGs involved in the immune response such as cytokine signalling (*IL1B*, *CXCL10*, *TNF*, *CCL2*)²⁴. In addition, pathways involving genes within major histocompatibility complexes II (i.e., *ICAM1*, *HLA-DRB1*, *HLA-DOA*) and the complement system (i.e., complement components *C3*, *C1*, and complement factor B) were enriched. These findings were similarly observed in the original analysis of these TS cases²⁸.

Given the substantial comorbidity and overlap between NDDs, we identified genes and pathways common to both ASD and TS. We identified 133 common DEGs, five of which were determined hub genes: *CSF2RB*, *HCK*, *HCLS1*, *LCP2*, and *PLEK*, which were all up-regulated in both disorders. Interestingly, the first four genes are either tyrosine kinases or associated with tyrosine kinase activity, and the fifth is a protein kinase substrate, which are key regulatory proteins involved in cell signalling, and are therapeutically targetable^{54,55}. The use of tyrosine kinase modulators in many oncologic diseases is well established, however these agents have also shown promise in preclinical models of non-oncologic neurological disorders such as Alzheimer's disease and Parkinson's disease, where inflammation and microglia are central to the pathogenesis⁵⁵⁻⁵⁷.

Our investigation has confirmed immune and inflammatory pathways are commonly enriched by up-regulated genes in ASD and TS. To further explore these intersecting findings, the 133 genes common to ASD and TS were analysed separately, which repeatedly identified enriched inflammatory pathways involving cytokine signalling and the complement system. These pathways involved immune genes (*IL1B*, *ICAM1*, and *JAK3*) and genes of the complement system (*C1QB*, *C1QC*, *C4B*), the latter of which is particularly relevant to NDDs, due to the importance of complement in neurodevelopmental processes such as neurogenesis and synaptic pruning⁵⁸. We utilised this approach as it allowed for comparison of the same genes within both disorders, while employing the distinct p values from each analysis, offering insight into the strength of each disorder's signal.

Our current study identified commonly enriched inflammatory pathways, however, several questions regarding the involvement of the immune response in ASD and TS remain unanswered. The cause of the identified inflammatory signals is still ambiguous, in addition to its nature. Research investigating the source of inflammation in NDDs has suggested it is an environmental or secondary component, rather

than genetic^{28, 42}. In particular, the influence of MIA, which could create a neuroinflammatory environment in offspring, may alter immune signalling pathways and epigenetic control of cell function during the critical periods of development¹⁶. In addition, the identified inflammatory signal might be casual and pathogenic, or alternatively reactive or protective in origin, which cannot be deduced from the current investigation. Further functional and mechanistic explorations of tissue from individuals with NDDs might elucidate the nature of this inflammation.

Despite our findings, this study has a number of caveats. Firstly, our analysis involved different brain regions from the two disorders, prefrontal cortex for ASD, and caudate and putamen for TS, as corresponding brain region data was not available for the two disorders at the time of analysis.

Secondly, the majority of the samples within the two datasets were not children, as cohorts of paediatric post-mortem brain samples are scarce. Therefore, our analysis represents late-stage disease, and it is unclear if the findings will be reflected in younger cohorts. It is not known whether the inflammatory signal seen in ASD and TS accumulates over the course of life, or is present in childhood.

Finally, the approach taken to identify differentially expressed genes differs from other statistical analysis often used in DGE analysis. Most cases of ASD and TS, along with other NDDs, are understood to involve the accumulation of many common risk variants in converging biological pathways, therefore we analysed the DGE of the top 1000 genes rather than employing a stringent statistical cut-off in order to unravel a wide range of genes. Although this approach may identify false positives where individual genes are not statistically dysregulated, it improves the cumulative power of pathway analysis, enabling many genes with small changes to be included.

Conclusions

The results from this study confirm the presence of inflammation and involvement of a dysregulated immune response within post-mortem brain of individuals with ASD and TS. Our findings bring new evidence of shared dysregulation of immune response and inflammatory pathways in NDDs and indicates a role for inflammation as an important environmental factor in the expression of these disorders. Findings from this work, when considered within the broad literature, provide a rationale to treat inflammation. The implicated pathways involved in inflammation, cell signalling including tyrosine kinases, and epigenetic machinery, may open doors for treatment through drug repurposing of monoclonal antibodies, kinase inhibitors and epigenetic modulators such as HDAC inhibitors, respectively.

List Of Abbreviations

ASD Autism spectrum disorders

BAM Binary Alignment Map

Cpm counts per million

DEG Differentially expressed genes

DGE Differential gene expression

FDR False discovery rate

GO Gene ontology

GRCh37 Genome Reference Consortium Human Build 37

IL-12 Interleukin 12

IL-2 Interleukin 12

KEGG Kyoto encyclopedia of genes and genomes

LPS Lipopolysaccharide

MCP2 monocyte chemoattractant protein 2

MIA Maternal immune activation

NC normal control

NDDs Neurodevelopmental disorders

PFC Prefrontal cortex

PPI Protein-protein interaction

REFSEQ Reference sequences

STRING Search tool for the retrieval of interacting genes/proteins

TMM Trimmed mean of M-values

TNF Tumour necrosis factor

TS Tourette syndrome

Declarations

Ethics approval and consent to participate

Approval to obtain the RNA-sequencing datasets of autism spectrum disorders and Tourette syndrome were obtained with author's permission, data was accessed from synapse.org.

Consent for publication

Not applicable.

Availability of data and materials

The autism spectrum disorders dataset was downloaded from synapse.org (ID: syn8234507). The Tourette syndrome dataset was downloaded from synapse.org (ID: syn3158906). The code used to analyse these datasets can be found at <https://github.com/sarahalshammery/ASDTS>.

Competing interests

The authors declare that they have no competing interest.

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Authors' contributions

SA analysed, interpreted, and wrote the results of this investigation. SP, HFJ, VXH, WAG and RCD assisted in the interpretation and writing of the results. BSG assisted in the analysis and interpretation of the results. All authors read and approved the final manuscript.

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Tables

Table 1 Up-regulated hub genes in ASD and TS

Hub genes shared in autism spectrum disorders (ASD) and Tourette syndrome (TS) following differential expression analyses. The common differentially expressed genes (top 1000 genes each) from each disorder's analysis were imported into STRING and Cytoscape to identify hub genes. The top 5 hub genes were selected.

<i>Gene</i>	<i>Gene Name</i>	<i>Type of protein</i>	<i>Protein Function</i>	<i>Reference</i>
<i>CSF2RB</i>	Colony Stimulating Factor 2 Receptor Subunit Beta	High affinity common beta chain for IL-3, IL-5 and colony stimulating factor (CSF).	Involved in signal transduction, and interaction with protein kinases.	59
<i>HCK</i>	Hematopoietic cell kinase	Non-receptor tyrosine-protein kinase and member of the <i>Src</i> family.	Involved in phagocytosis, regulating of the actin skeleton, and FcγRI activation.	60-63
<i>HCLS1</i>	Hematopoietic Cell-Specific Lyn Substrate 1	Actin-binding protein of Lyn, and member of the <i>Src</i> family of protein tyrosine kinases.	Involved in signal transduction, antigen-receptor signalling, apoptosis, and regulation of F-actin filaments.	64-66
<i>LCP2</i>	Lymphocyte cytosolic protein 2 or SLP-76 Tyrosine Phosphoprotein	Signal transducing adaptor protein.	Involved in T cell antigen receptor-activated protein tyrosine kinase pathway, phosphorylation of <i>Src</i> family kinases, and actin reorganization.	67-71
<i>PLEK</i>	Pleckstrin or p47	Substrate of protein kinase C.	Largely unclear – reported to mediate protein kinase C family, plays a role in phagocytosis and cytoskeletal organisation.	72, 73

Figures

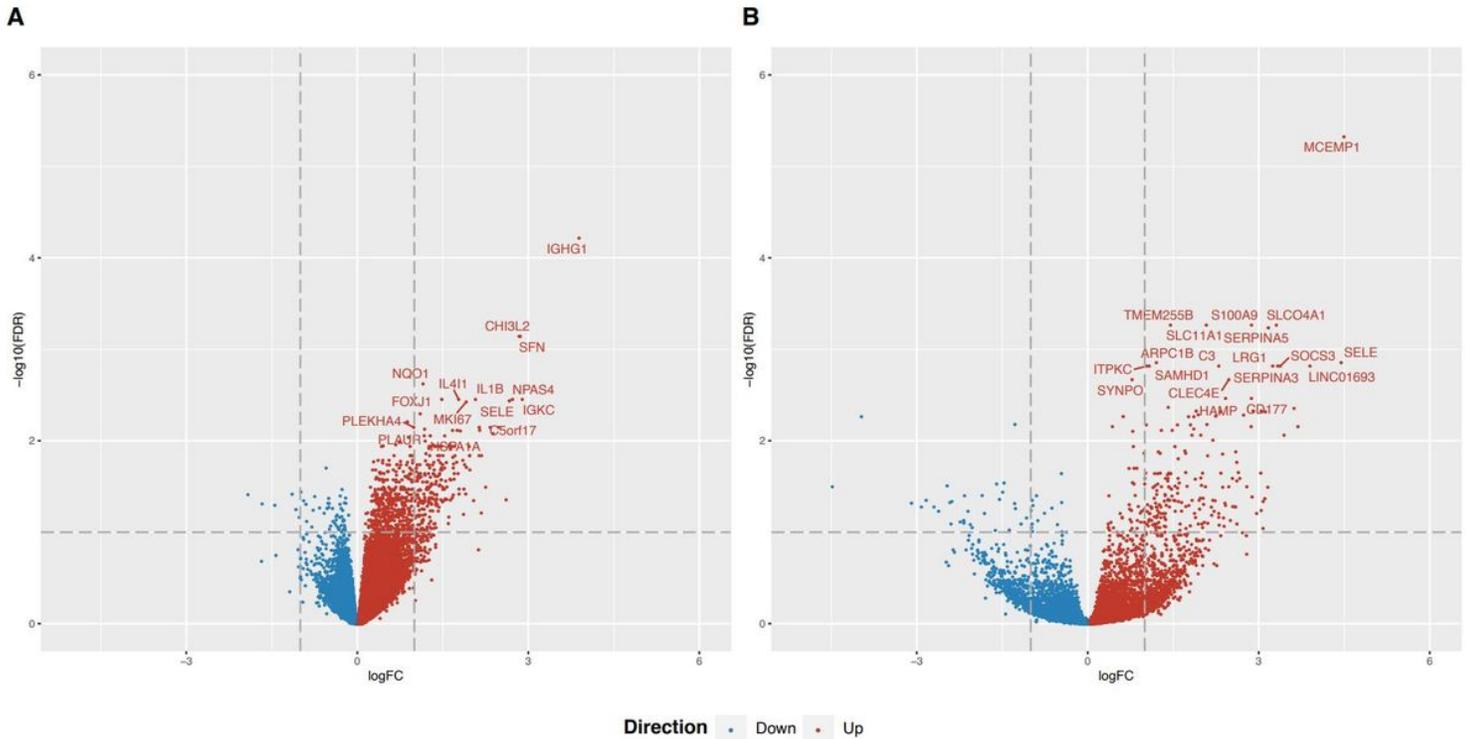


Figure 1

Volcano plot of differentially expressed genes in ASD and TS. Differential gene expression analysis was performed on the transcripts of A) autism spectrum disorders (ASD) cases and normal controls and B) Tourette syndrome (TS) cases and normal control samples. The y-axis represents statistical significance ($-\log_{10}(\text{FDR})$) and the x-axis represents gene expression log fold change ($\log_{2}(\text{FC})$). The dashed vertical lines ($\pm 1 \log_{2}(\text{FC})$) represent log fold change of gene expression between cases and normal controls, where up-regulated genes (red) are positive on the x-axis, and down-regulated genes (blue) are negative. The dashed horizontal line ($>1 -\log_{10}(\text{FDR})$) represents the statistical significance of gene expression, increasing towards the top. The top 20 differentially expressed genes were labelled for ease of viewing, all of which had an up-regulated expression in both the ASD and TS datasets.

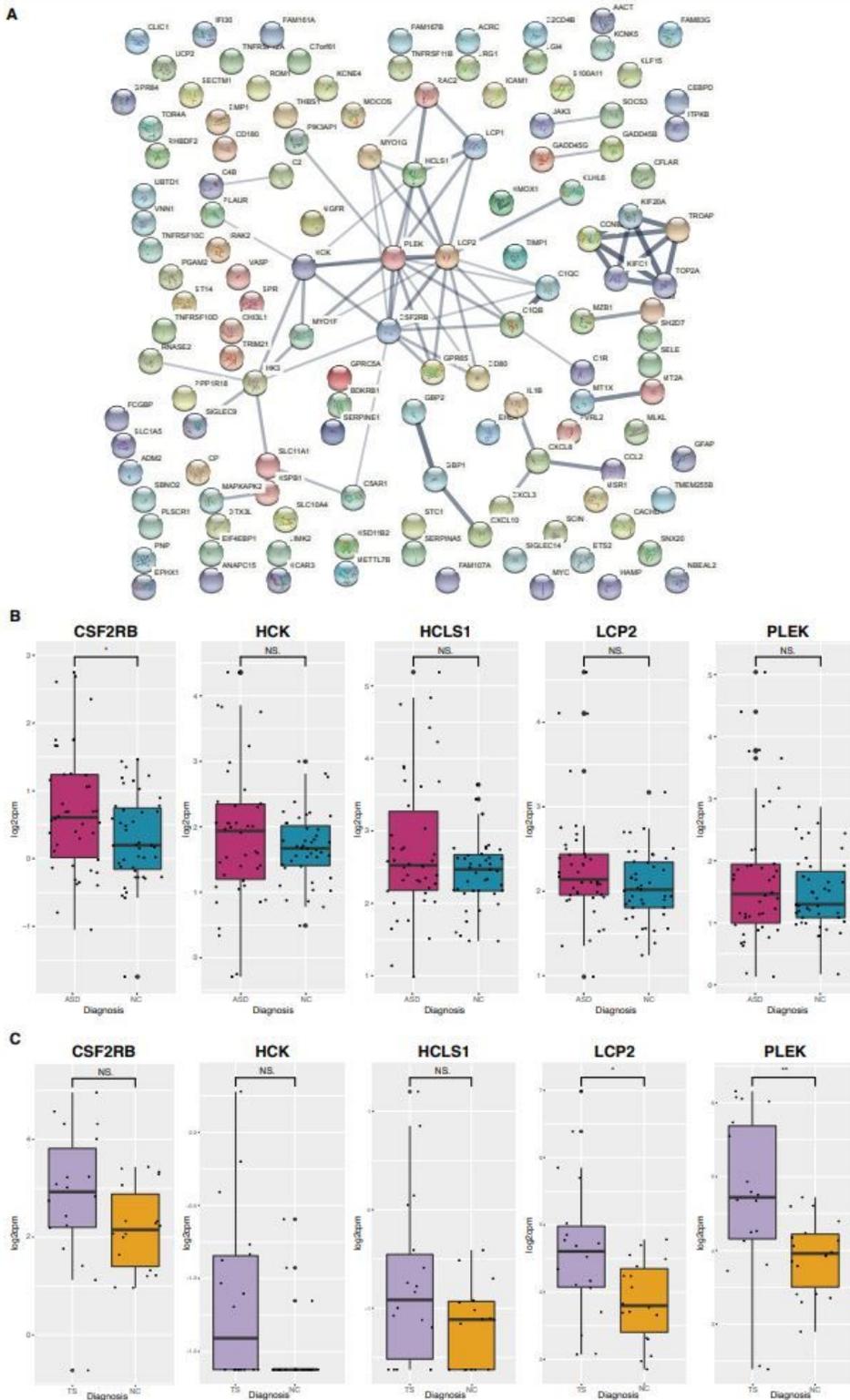


Figure 3

Protein network of common differentially expressed genes and expression of hub genes in ASD and Tourette syndrome. A) Protein-protein-interaction (PPI) network of genes found to be commonly differentially expressed in autism spectrum disorders (ASD) and Tourette syndrome (TS). The network consists of nodes (circles) and edges (lines) representative of predicted functional associations of the common protein-coding genes. Edge thickness is indicative of the strength of predicted evidence. Panels

B and C: Hub genes central to the network were identified using Cytoscape, and the expression (log2cpm; y-axis) of the five hub genes in cases and normal control (NC) cohorts. Expression of the five hub genes in B) ASD cases (n=42; pink) against normal controls (n= 43; blue) and C) TS cases (n =9; purple) and normal controls (n =9; yellow). The median is shown as the black line in each group's box, with the small black dots representing each sample. The whiskers on either side of the boxes represent the minimum (Q1-1.5*IQR) and maximum (Q3+1.5*IQR) log2cpm excluding outliers. The big black dots represent potential outliers (** = $p < 0.01$, * = $p < 0.05$, NS = non-significant, Mann-Whitney-Wilcoxon Test). Network generated using STRING.

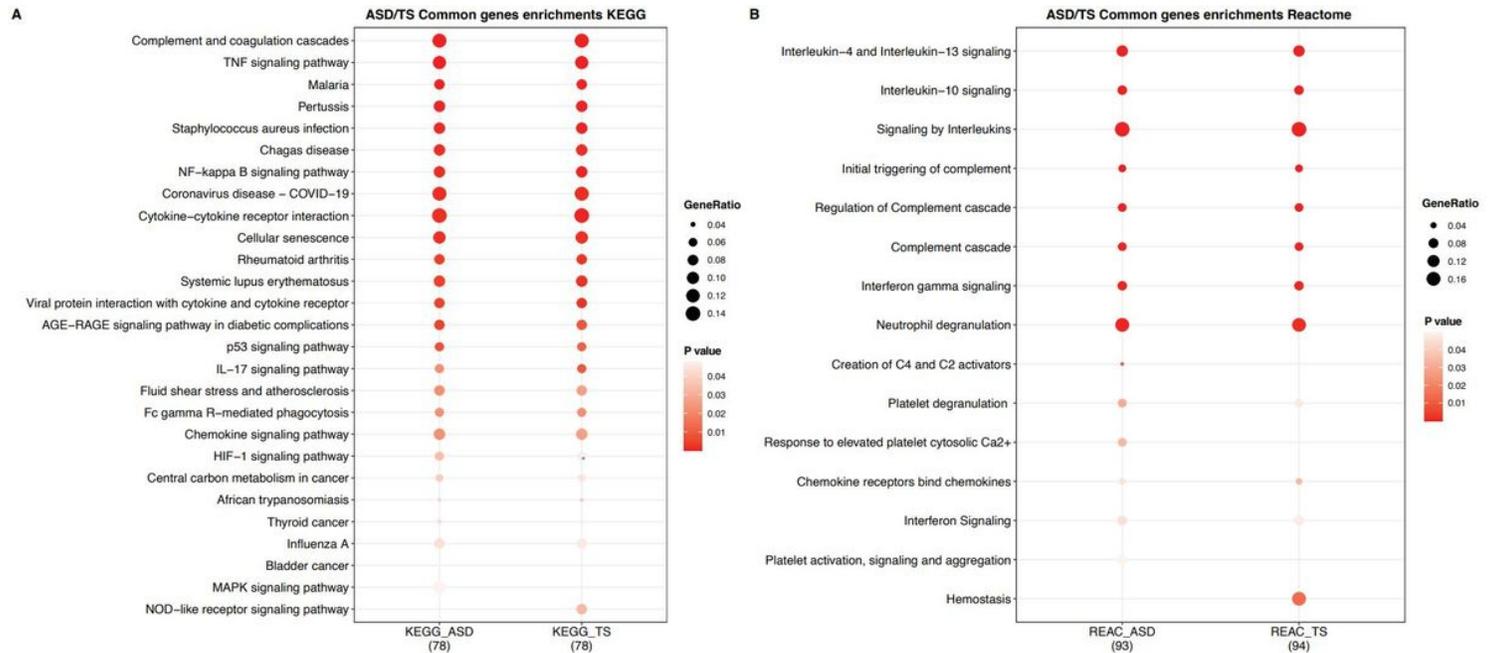


Figure 4

Overrepresented KEGG and Reactome pathways common to ASD and Tourette syndrome. Pathway enrichment analyses (FDR < 0.05) of the up-regulated differentially expressed genes (133 genes) common to autism spectrum disorders (ASD) and Tourette syndrome (TS) using A) KEGG B) Reactome. Of the common 133 genes, 78 genes enriched pathways in ASD and TS using KEGG (A). Of the common 133 genes, 93 genes enriched pathways in ASD and 94 genes enriched pathways in TS using Reactome (B). Larger dots represent a bigger gene ratio of the differentially expressed genes in the database to that of the total annotated genes, while red dots represent statistically significant pathways.

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