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Distinct cortical immune-signaling profiles in autism spectrum disorder and severe mental illness

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Abstract

Background: Autism spectrum disorder (ASD) and severe mental illness (SMI), including schizophrenia and bipolar disorder, are clinically and developmentally distinct but share genetic and transcriptomic features involving synaptic and immune-related pathways, along with cellular evidence of altered neuroimmune states in the brain. This convergence raises the possibility of a shared inflammatory state, but direct measurements in human brain tissue are limited, leaving unclear whether these conditions share a cortical immune-signaling profile.

Methods: We measured 48 immune-related proteins by multiplex bead immunoassay in postmortem superior temporal gyrus (STG) from 72 human brain donors with ASD, severe mental illness (SMI), or unaffected controls. SMI included schizophrenia, schizoaffective disorder, bipolar disorder, or mood disorder with psychotic features. Thirty-two proteins met the detection threshold and were analyzed using permutation-based regression adjusted for age and postmortem interval and corrected for multiple comparisons, together with correlation analyses and partial least squares discriminant analysis.

Results: Rather than a common inflammatory signature, the findings revealed contrasting cortical immune-signaling profiles. ASD showed a selective pattern centered on IL-1–related signaling, with higher IL-1α, IL-18, and FLT-3L than both SMI and controls and higher M-CSF than controls, implicating both inflammatory and immune-cell support pathways. SMI displayed fewer individual protein differences, most prominently higher eotaxin. Multivariate analyses distinguished ASD, SMI, and controls, indicating differences in overall protein profiles than were apparent from individual proteins.

Limitations: ASD and SMI are heterogeneous diagnoses, and the groups include few female donors and varied medical histories. Postmortem tissue reflects the biological state at death, limiting interpretation of age-related change.

Conclusions: Substantial case overlap with our previous STG transcriptomic study provides cross-modal evidence that altered immune-related proteins in ASD occur alongside neuronal inflammatory and cellular-stress transcriptional changes. SMI is distinguished by elevated eotaxin and differences in overall immune-signaling profile. These cortical findings complement the peripheral literature and suggest neuroimmune profiles may differ between brain and peripheral systems. Together, these findings refine the neuroinflammatory hypothesis in ASD and SMI: both involve altered neuroimmune signaling, but through distinct cortical profiles rather than a shared immune-signaling state.

Background

Neurodevelopmental and psychiatric conditions encompass substantial heterogeneity in etiology, developmental course, and clinical presentation, yet may exhibit overlapping alterations in neural circuitry, immune-related biology, and neuropathological features [1–3]. Autism spectrum disorder (ASD), schizophrenia, and bipolar disorder exhibit disorder-specific molecular profiles while sharing transcriptomic alterations involving synaptic and immune-related processes [4]. Among the biological

features implicated across these conditions, altered immune signaling has been identified both peripherally and within the brain, raising the possibility of shared neuroinflammatory processes across neurodevelopmental and psychiatric conditions [5,6]. Neuroimmune signaling operates reciprocally, with cytokines and glia influencing neural development, connectivity, and synaptic function, while changes in neuronal activity and cellular state can in turn modify glial and immune signaling across the lifespan [7]. Together, these observations raise the possibility that altered neuroimmune biology represents a point of convergence across otherwise heterogeneous conditions, including ASD and severe mental illness, despite differences in etiology, developmental timing, and clinical phenotype.

Evidence for altered immune function in ASD comes from biofluid measures, including serum, plasma, and CSF as well as direct examination of brain tissue. Peripheral blood cytokine concentrations differ in ASD, with the IL-1 family of molecules among the more consistently supported findings [8–10]. However, peripheral cytokine measures do not necessarily reflect immune-related protein signaling within the brain [11]. Postmortem brain studies are limited, but have identified increased concentrations of several inflammatory mediators in brain tissue from ASD individuals [12,13]. Cellular studies similarly report alterations in microglial density, morphology, and phenotype across cortical regions, with some changes varying with age, alongside evidence of astrocyte involvement [12,14,15]. Transcriptomic studies identify altered immune- and glial-related cortical gene expression in ASD [16–18] and our prior transcriptomic analysis of superior temporal gyrus (STG) identified altered synaptic and neuroimmune signaling, including inflammatory signatures within neurons and gene-expression changes that varied with age [19]. Together, these findings provide converging evidence of altered neuroimmune function within the brain in ASD.

Neuroimmune alterations are also reported in conditions associated with severe mental illness, although findings within brain tissue are more heterogeneous [20,21]. Meta-analyses in schizophrenia-spectrum disorders identify persistent alterations in circulating inflammatory proteins across illness stages, with additional changes during acute illness [22] and peripheral cytokine and chemokine alterations are also reported in bipolar disorder [23]. Postmortem findings vary across brain regions: increased TNF-α and IL-6 and reduced IL-10 in prefrontal cortex in schizophrenia [24], whereas inflammatory mediators are largely unchanged in superior temporal cortex, with nominal differences that did not survive correction for multiple comparisons [25]. Transcriptomic studies further identify inflammatory subgroups within schizophrenia and bipolar disorder, indicating that immune-related alterations may characterize subsets of individuals rather than each diagnosis uniformly [26,27]. Cellular studies have reported increased microglial density in schizophrenia, with the strongest regional evidence observed in temporal cortex, although subsequent analyses have emphasized heterogeneity across regions and studies [20,21,28]. Together, these findings support neuroimmune involvement in conditions associated with psychosis, but do not establish a uniform cortical inflammatory profile.

Cortical immune signaling has largely been examined separately in ASD and severe mental illness, leaving unclear whether these conditions share a common profile or exhibit condition-specific patterns of neuroimmune alteration. Here, we measured a multiplex panel of cytokines, chemokines, and growth

factors in STG, a region structurally and functionally implicated in both ASD and schizophrenia [2,29–31]. The study included human brain donors with ASD, severe mental illness (SMI)—including schizophrenia, schizoaffective disorder, bipolar disorder, mood disorders with psychotic features—and unaffected controls. Substantial overlap of ASD and control donors with our prior STG transcriptomic study [19] allows the present protein findings to be considered alongside transcriptional changes in the same donors and cortical region. Given evidence for neuroimmune involvement across disorders, but differences in the specific molecular and cellular alterations reported, we hypothesized that ASD and SMI would have distinguishable cortical immune-signaling profiles, reflecting different perturbations of shared neuroimmune signaling rather than a common inflammatory state.

Materials and Methods

Cohort Demographics

Tissue and clinical data collection procedures were approved by the University of California, Davis Institutional Review Board (IRB) and Human and Anatomical Specimens Tissue Oversight Committee (HASTOC). Brain tissue was obtained from the University of Maryland Brain and Tissue Bank (NIH NeuroBioBank), the Harvard Brain Tissue Resource Center through the Autism Tissue Program, and the UC Davis Brain Endowment for Mental Health (BEMH) donation program. Informed consent was obtained from the next of kin at the time of donation. The study included 72 human brain donors: 29 unaffected controls (CTRL; 24M/5F), 17 with ASD (14M/3F), and 26 with severe mental illness (SMI; 19M/7F). Across all diagnostic groups, donors with documented bloodborne pathogens, including HIV and prion disease, a history of stroke, prolonged chemotherapy or radiation treatment, or a known serious neurological condition such as Parkinson's disease, Huntington's disease, Alzheimer's disease, or frontotemporal dementia were excluded prior to case selection. The unaffected control group (CTRL) was defined as individuals without a documented diagnosis of autism spectrum disorder, schizophrenia, bipolar disorder, or other psychotic disorder. Diagnoses for the ASD group were confirmed either through medical records or postmortem Autism Diagnostic Interview-Revised (ADI-R) interviews [32–34] conducted by clinicians from the Autism Tissue Program (now Autism BrainNet). Diagnoses for the SMI group were established through medical records or an adapted postmortem Structured Clinical Interview for DSM-5 (SCID-5) [35] completed with next of kin by trained mental health clinicians (TAN, SE, RG, DTZ, EL). The SMI group included individuals with schizophrenia, schizoaffective disorder, bipolar disorder with or without confirmed psychosis, major depressive disorder with psychotic features, and other psychotic disorders. For donors whose diagnosis could not be confirmed by SCID-5 because the interview could not be completed or did not yield a definitive diagnosis, assignment to the SMI group was based on available medical records and medical examiner reports. Further subject information, including age, sex, refined diagnosis, and postmortem interval (PMI), is provided in Supplementary Table 1.

Brain Tissue Collection

Sample processing details as well as partial data and diagnostic information from CTRL (n = 17) and ASD (n = 17) cases have previously been described [19]. Whole brain specimens collected through BEMH (n = 38) were bisected into separate hemispheres upon intake with alternating hemispheres across cases fixed or frozen. Hemispheres slated for freezing were coronally blocked into 1 cm thick slabs, snap frozen in liquid nitrogen vapor, and stored at -80C until use.

Homogenate Preparation

STG was sampled using 2 mm diameter tissue punches from fresh frozen tissue slabs (Fig. 1). Investigators were blind to the conditions of each case at the time of sampling and processing. All samples used in this study contained all available layers of STG for each case. Biopsy samples were homogenized using BioMasher II Micro Tissue Homogenizers (Kimble) and extraction medium (sterile PBS, Roche cOmplete protease inhibitor, 0.1% Triton X). The same investigator processed all samples to ensure consistency and minimize handling effects. Samples were processed uniformly on wet ice before centrifugation for 10 minutes at 1200 RPM ay 4 ℃ Aliquots of resultant supernatants were used for a multiplex bead immunoassay and protein normalization via bicinchoninic acid (BCA) assay (ThermoFisher Scientific).

Multiplex Bead Immunoassay

The immunoassay and protein normalization were run at the UC Davis MIND Institute Intellectual and Developmental Disabilities Research Center Biological and Molecular Analysis Core (IDDRC, University of California, Davis). Target analytes were measured using the MILLIPLEX Human Cytokine/ Chemokine/ Growth Factor Panel A Magnetic Bead Panel (MilliporeSigma, HCYTA-60K-PX48) on a Luminex 200 platform. All samples were processed according to manufacture guidelines. Briefly, 25 µL of standard or sample homogenate were incubated with capture antibody coated microbeads and incubated overnight. Subsequently, beads were incubated with the biotinylated reporter antibodies, the signal was then amplified using Streptavidin-phycoerythrin, and the plate was read on the Bioplex 200 platform (BioRad, Inc, Hercules, CA). Analyte protein concentration was determined using a 7-point standard curve based on the fluorescence intensity values from the standards following background subtraction. For normalization, a sample aliquot was used for a BCA assay to measure total protein concentration. Reported values represent the calculated protein concentrations normalized to total protein concentration and log-transformed using ln(value + 1).

Statistical Analysis

Data was evaluated for normality and variance using distribution graphs, Leven's test, and Shapiro-Wilk's test. We log-transformed the concentrations to reduce heteroscedasticity as is standard in the field of metabolomics, as cytokines typically follow a logarithmic scale due to their multiplicative nature. All

boxplots depict the median, interquartile range, and 1.5x IQR. For cohort demographics, Kruskal-Wallis with Dunn's correction was used for significance testing.

For protein analysis, only analytes with > 50% detection across samples were evaluated. Of the 48 proteins on the panel, 32 proteins met this threshold. To properly evaluate each analyte and control for PMI and age, a regression model using permutation-based F-tests (aovp, lmPerm package in R) was employed. For each test, 5,000 permutations were conducted with age and PMI as covariates. For each protein, group differences were evaluated using the model value ~ Dx + Age + PMI. Permutation values were floored at 1/ (# of permutations + 1). Significance testing was corrected using the Benjamini-Hochberg (BH) procedure. Additionally, age interaction terms were investigated using the model value ~ Dx + Age + PMI + Dx:Age. The Dx:Age interaction term was evaluated for significance and reported as an omnibus value. Pairwise comparisons were subsequently conducted by relative assessment of aging slopes across all three diagnostic groups.

Correlation matrices were developed to evaluate the relationships between every pair of protein between diagnoses. Pairwise complete Spearman correlation values were calculated and clustered using Ward's D2 method for the CTRL group. The matrices for ASD and SMI were then reorganized to match the structure of the CTRL group for effective comparison. R packages used for this analysis include Hmisc and pheatmap.

Partial least squares discriminant analysis (PLS-DA) was conducted in Metaboanalyst 6.0. Here the data was autoscaled. Missing values were imputed by 1/5 of the minimum positive value for left-censored data estimation. Each 2D scores plot displays the 95% confidence region of each cluster. Variable importance in projection (VIP) plots show VIP scores per analyte for the top 15 analytes. Models were verified using a 10-fold cross validation method measuring predictive accuracy, and significance testing was conducted using a permutation method (1000 permutations) evaluating the prediction accuracy during training.

Results

Main effects

There was no statistically significant difference in the age range between groups (range 10–73 years); however, the postmortem interval (PMI) was significantly greater in the severe mental illness group compared to others (Fig. 1) and was accounted for in the regression model during subsequent analyses.

Group-wise analysis of the main effect model (value ~ Dx + Age + PMI) revealed 8 statistically significant proteins after correction for multiple comparisons (Fig. 2). ASD exhibited the greatest number of differences. Proteins IL-1α (p_{adj} = 0.0024, p_{adj} = 0.0024; BH corrected), FLT-3L (p_{adj} = 0.0024, p_{adj} = 0.0024; BH corrected), and IL-18 (p_{adj} = 0.0024, p_{adj} = 0.0288; BH corrected) were all significantly elevated in ASD compared to both CTRL and SMI. Both IL-9 (p_{adj} = 0.00427; BH corrected) and M-CSF

(p_{adj} = 0.0416; BH corrected) were increased in ASD compared to CTRL but not compared to SMI. Only IL-4 (p_{adj} = 0.0367; BH corrected) was significantly decreased in ASD compared to CTRL. For SMI, two chemokines were significantly elevated. Eotaxin (p_{adj} = 0.0024, p_{adj} = 0.0024; BH corrected) was increased compared to both CTRL and ASD, and RANTES (p_{adj} = 0.0024; BH corrected) was increased compared to ASD.

Age interaction

An aging interaction term (Age:Dx) was evaluated to test differences in aging trajectories between groups using the model value ~ Dx + Age + PMI + Age:Dx. Overall, 19 proteins showed a significant interaction term after correction for multiple comparisons. However, only 3 proteins showed significant pairwise differences in aging trajectories (Fig. 3). FGF-2 exhibits altered aging within the CTRL group compared to ASD (p = 0.0254) and SMI (p = 0.0215). Eotaxin shows a similar trend for the CTRL group (p = 0.0376 for ASD; p = 0.0215 for SMI). Cytokine IL-12p70 shows an altered trajectory between ASD and SMI (p = 0.0283).

Network and profiling comparisons

Next, we explored the relationships between all observed proteins with each other by developing a clustered correlation matrix. Specifically, the CTRL group was hierarchically clustered, and the ASD and SMI groups were reorganized to match the structure (Fig. 4). These heatmaps demonstrate distinct modules that are relatively preserved across diagnostic groups, including a module containing strong relationships between MCP-1, G-CSF, IL-6, IL-8, GROα, and TNFα. However, another module consisting of M-CSF, FLT-3L, IL-18, IL-15, MIG/CXCL9, and IP-10 demonstrates a substantial loss of correlation in both ASD and SMI. Interestingly, proteins IL-5, TGFα, IFNα2, and IL-4 form a mostly negative correlation module for other proteins in CTRL. This relationship is attenuated in both ASD and SMI with generally more positive correlations across each matrix.

Partial least squares discriminant analysis (PLS-DA) was used to evaluate the overall neuroimmune profiles of each diagnosis by clustering cases based on principal components (Fig. 5). Pairwise plotting shows that ASD is significantly different compared to CTRL (p = 0.003) and SMI (p = 0.008), and SMI is significantly different compared to CTRL (p = 0.025). Compared to CTRL, nine proteins had a VIP score greater than 1 and were primarily involved in distinguishing the ASD profile: IL-1α, FLT-3L, IL-18, M-CSF, IL-4, PDGF-AA, FGF-2, IL-5, and MCP-3. Compared to SMI, 12 proteins had a VIP score greater than 1 and included FLT-3L, eotaxin, IL-1α, RANTES, IL-18, IL-1RA, GROα, fractalkine, M-CSF, IFNα2, G-CSF, and IL-8. For CTRL and SMI profile separation, 11 proteins had a VIP score > 1: eotaxin, PDGF-AA, RANTES, IL-12p70, FGF-2, fractalkine, MCP-3, TNFα, EGF, VEGF-A, and MIG/CXCL9.

Discussion

ASD and a severe mental illness (SMI) group spanning schizophrenia, schizoaffective disorder, bipolar disorder, and mood disorders with psychotic features exhibit divergent cortical immune-signaling profiles within the superior temporal gyrus (STG). ASD exhibits higher IL-1α, IL-18, and FLT-3L than both controls and the SMI group, together with higher M-CSF and IL-9 and lower IL-4 than controls. The SMI group displays a more restricted pattern of individual protein differences, most clearly marked by higher eotaxin than both controls and ASD. Multivariate analysis further supports differences in the overall protein profiles among ASD, the SMI group, and controls, while eotaxin, FGF-2, and IL-12p70 vary in their relationships with age across groups. Overall, the findings support altered neuroimmune signaling in both clinical groups within the STG but indicate different patterns of cortical immune signaling rather than a common inflammatory state.

The ASD findings point to selective involvement of IL-1-related signaling and factors involved in immune-cell support and regulation, rather than a broad inflammatory response. In the multivariate analysis, IL-1α and FLT-3L contribute prominently to the separation of ASD from both comparison groups. In the correlation analysis, FLT-3L, IL-18, and M-CSF are strongly correlated in controls, whereas these relationships are attenuated in both clinical groups. Together, the concentration and correlation findings support selective alteration of specific immune-related processes rather than generalized inflammatory activation.

Our previous transcriptomic study of the same STG region in an extensively overlapping donor cohort provides an important point of comparison for the present protein findings. Zhang and colleagues [19] identified neuroinflammatory and neuronal-signaling alterations in STG, including increased AP-1 components such as FOS, JUN, and JUNB and NF-κB-related regulatory genes in laser-captured neurons. Additional transcriptional changes implicated heat-shock and unfolded-protein responses in bulk tissue and mitochondrial and bioenergetic processes in neurons. IL-1α and IL-18 signal through distinct receptors but converge on pathways that include MAPK/AP-1 and NF-κB-related signaling, providing a plausible pathway-level correspondence with part of the neuronal transcriptional profile. The protein measurements do not establish that IL-1α or IL-18 drive these neuronal transcriptional changes. Together, the two datasets provide cross-modal evidence that altered immune-related proteins in the STG of individuals with ASD occur alongside inflammatory and cellular-stress transcriptional changes within neurons in many of the same individuals. Cytokine and glial signaling can influence synaptic function, plasticity, and circuit refinement [5], providing a plausible biological link between these immune-related protein differences and the synaptic and neuronal transcriptional alterations reported across ASD and psychiatric conditions [4,19].

IL-1α provides a particularly informative example of how cortical protein findings differ from the peripheral inflammatory literature. IL-1α has received little direct study in brain tissue from individuals with ASD, and peripheral studies generally do not identify higher IL-1α in ASD [36,37]. A recent meta-analysis [10] likewise found no overall peripheral IL-1α difference, whereas IL-1β had the strongest evidence for elevation among the cytokines examined. In the present study, however, IL-1β was detectable in fewer than 50% of samples, precluding reliable group-level statistical analysis. IL-1α also

does not differ between the SMI group and controls, consistent with a previous report [25] of nominally lower concentrations in STG tissue from individuals with schizophrenia. IL-18 and IL-4 provide additional examples of divergence between cortical and peripheral findings: IL-18 is higher and IL-4 lower in the STG of individuals with ASD, whereas peripheral blood studies report decreased IL-18 and increased IL-4 in ASD [10,36,37]. Elevated IL-18 in the STG is also consistent with a previous report [38] of increased IL18 and IL18R expression in amygdala tissue from individuals with ASD, alongside increased IL18R without increased IL18 in dorsolateral prefrontal cortex. IL-9 is likewise higher in the STG of individuals with ASD in the present study, although relatively little is known about IL-9 in brain tissue from individuals with psychiatric conditions; both IL-9 and its receptor are expressed in the human central nervous system [39]. Together, these findings indicate that the inflammatory profile measured in blood does not directly mirror that observed within the brain.

The combination of IL-1-family cytokines with FLT-3L and M-CSF suggests involvement of both inflammatory signaling and pathways that support immune-cell development and maintenance, rather than generalized immune activation. FLT-3L promotes hematopoietic progenitor and dendritic-cell development, whereas M-CSF signaling through CSF1R supports microglial maintenance in the adult brain [40,41]. IL-1α adds another dimension because it is largely cell-associated, with intracellular and membrane-associated forms that can be released during cellular stress or injury [42]. Consequently, IL-1α measured in homogenized tissue may reflect intracellular, membrane-associated, and extracellular protein pools rather than extracellular cytokine alone [43,44]. IL-1 signaling occurs across endothelial cells, astrocytes, neurons, and microglia and is regulated in part by IL-1Ra and IL-1R2 [45]. IL-1RA also contributes to the multivariate separation of ASD and the SMI group, suggesting that differences in IL-1 pathway regulation may contribute to the broader group differences. Together, the protein pattern may reflect cellular stress, altered neural–glial communication, and compensatory signaling in addition to inflammatory activity [46].

Eotaxin most clearly distinguishes the SMI group from both ASD and controls. Its elevation is consistent with peripheral reports in schizophrenia and bipolar disorder [47,48]. The comparison with ASD is particularly informative because circulating eotaxin has also been reported to be elevated in ASD [9] whereas eotaxin concentrations in the STG do not differ between ASD and controls in the present study. Eotaxin undergoes bidirectional transport across the blood–brain barrier [49], so its higher cortical concentration may reflect both local and peripheral contributions. RANTES is also higher in the SMI group than in ASD, although neither clinical group differs from controls. The multiple cellular and vascular sources of RANTES further limit attribution of this tissue difference to a specific inflammatory process. Despite the relatively limited number of individual protein differences, multivariate analysis distinguishes the SMI group from both ASD and controls, indicating that differences in the overall protein profile extend beyond eotaxin alone. Together, these findings suggest that the neuroimmune alterations represented in this group differ in composition from those observed in ASD rather than reflecting a less pronounced form of the same pattern.

Relationships between immune-signaling proteins and age also vary across groups, most clearly for eotaxin, FGF-2, and IL-12p70. Eotaxin and FGF-2 have more positive relationships with age in controls than in either clinical group, whereas the relationship between IL-12p70 and age differs between ASD and SMI. Eotaxin has been linked to aging, reduced neurogenesis, and age-related cognitive decline [50,51]. In schizophrenia, serum eotaxin has also been reported to increase with age and illness duration [48] whereas in the present STG data its relationship with age is less positive in the SMI group than in controls. This cortical pattern does not support a simple model in which either clinical group represents an accelerated form of normal age-related inflammatory change. Instead, the relationship between immune signaling and age varies across the three groups. Because these data are cross-sectional, they reflect differences among individuals across the lifespan rather than within-person change and cannot establish when these age-related differences emerge.

Limitations

Postmortem tissue reflects the biological state present at death, warranting caution in interpreting developmental timing and causality. Postmortem interval and agonal state can affect protein preservation, with individual analytes differing in stability. Although PMI was longer in the SMI group, the primary protein differences remained after accounting for PMI. Heterogeneity across all three groups may contribute to within-group variation, and the SMI group spans multiple diagnostic categories with overlapping phenomenology and biology [52,53]. Co-occurring medical and neurological conditions and medication exposure may also influence protein concentrations [54]. Despite substantial individual variation, group-level differences remained after adjustment for age and PMI and correction for multiple comparisons, and multivariate analysis distinguished the three groups.

Conclusions

Overall, the results refine the broader inflammatory hypothesis across ASD and severe mental illnesses. Both clinical groups exhibit altered cortical immune-signaling profiles in STG, but the patterns differ in composition and organization. In ASD, IL-1-related signaling and immune-cell support factors recur across individual protein and multivariate findings and occur alongside inflammatory and cellular-stress transcriptional changes within neurons. The SMI group is distinguished most clearly by eotaxin, with additional differences apparent when proteins are considered together. Selected signaling proteins also relate differently to age across groups. Differences between cortical and peripheral findings further indicate that circulating immune profiles should not be assumed to represent signaling within brain tissue. Rather than supporting a single inflammatory process shared across diagnoses, these findings suggest that neuroimmune involvement may represent a point of biological convergence across neurodevelopmental and psychiatric conditions, while the specific patterns of cortical immune signaling remain condition-specific.

Declarations

Ethics Declarations

Tissue and clinical data collection procedures were approved by the University of California, Davis Institutional Review Board (IRB, ID: 2150714-1), in accordance with the Declaration of Helsinki, and Human and Anatomical Specimens Tissue Oversight Committee (HASTOC). Brain tissue was obtained from the University of Maryland Brain and Tissue Bank (NIH NeuroBioBank), the Harvard Brain Tissue Resource Center through the Autism Tissue Program, and the UC Davis Brain Endowment for Mental Health (BEMH) donation program. Informed consent was obtained from the next of kin at the time of donation.

Consent for Publication

Not applicable

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

TAN is founder and board member of Safari Health, Inc.

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Authors' Contributions

Study concept and design: ELC, CMS, TAN, JAV. Diagnostic information: AO, SE, DTL, RG, EL, and TAN. Acquisition of data: ELC, AO, and JS. Data analysis: ECH, KLH, and ELC. Drafting of the manuscript: ECH, ELC, KLH, and CMS. Preparation of figures: ECH and ELC. Critical revision of the manuscript: SE, TAN, KDM, KLH, and CMS. All authors have reviewed and approved the manuscript for submission.

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Figures

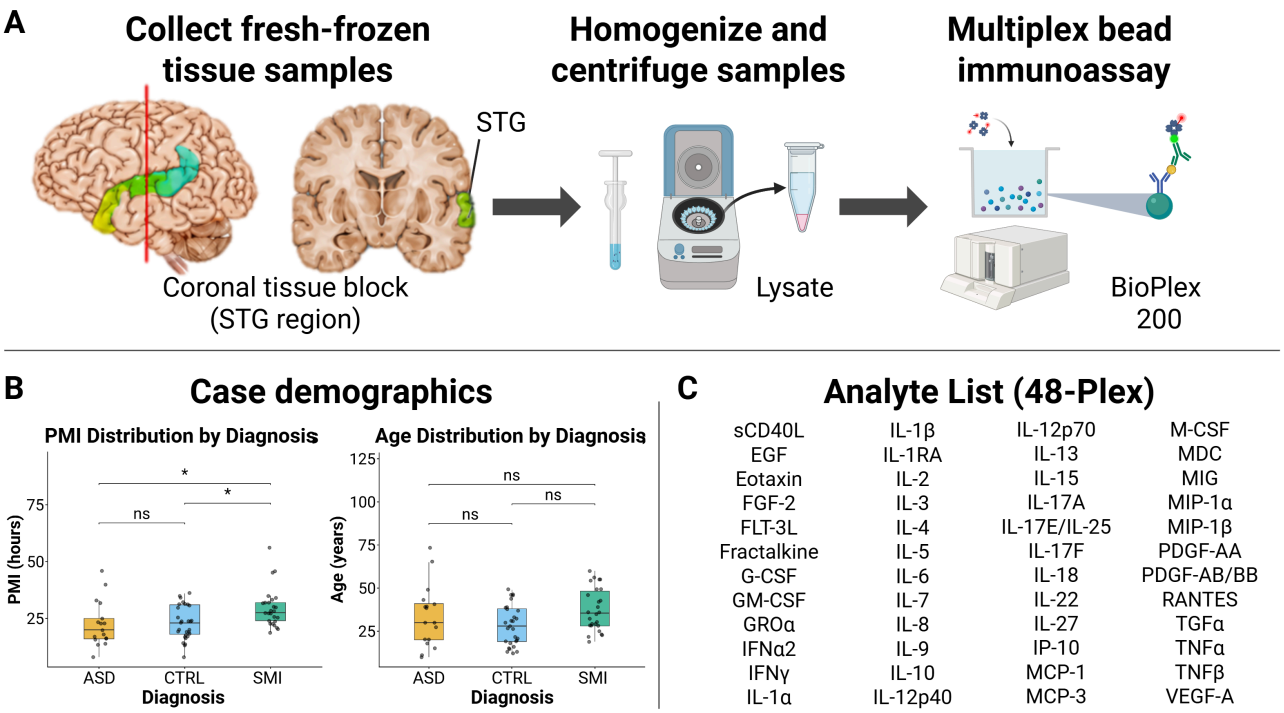


Figure 1

Experiment Overview. Project overview **A)** STG sample processing workflow. **B)** Pairwise comparison of age and PMI across major diagnostic groups (* p<0.05). **C)** List of 48 cytokines, chemokines, and growth factors measured in this study.

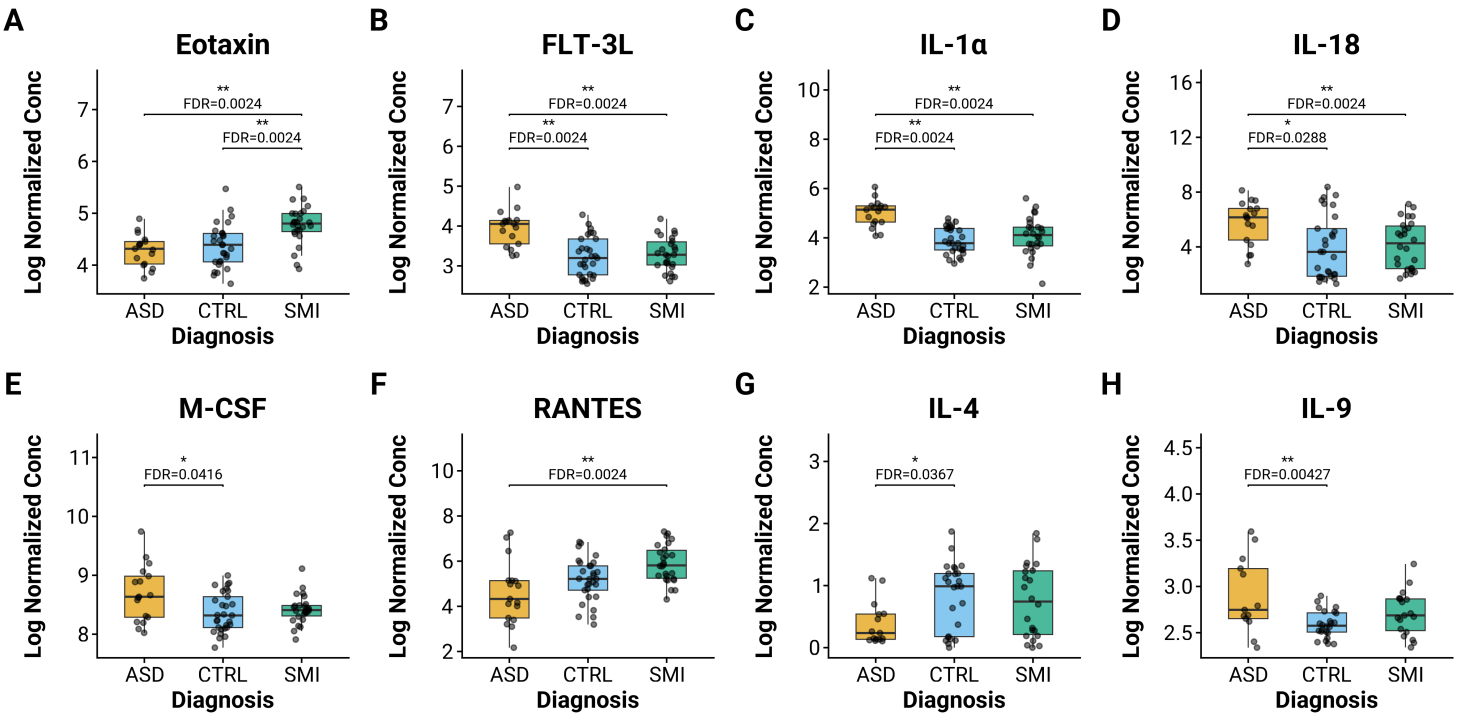


Figure 2

Cytokines and Chemokines Show Altered Expression Across Diagnostic Groups. Statistically significant neuroimmune results. **A-H)** Log-transformed normalized protein concentrations were plotted between diagnoses. Significance testing was conducted using a permutation-based linear regression model and corrected for multiple comparisons (Benjamini-Hochberg; BH). * p<0.05; ** p<0.01.

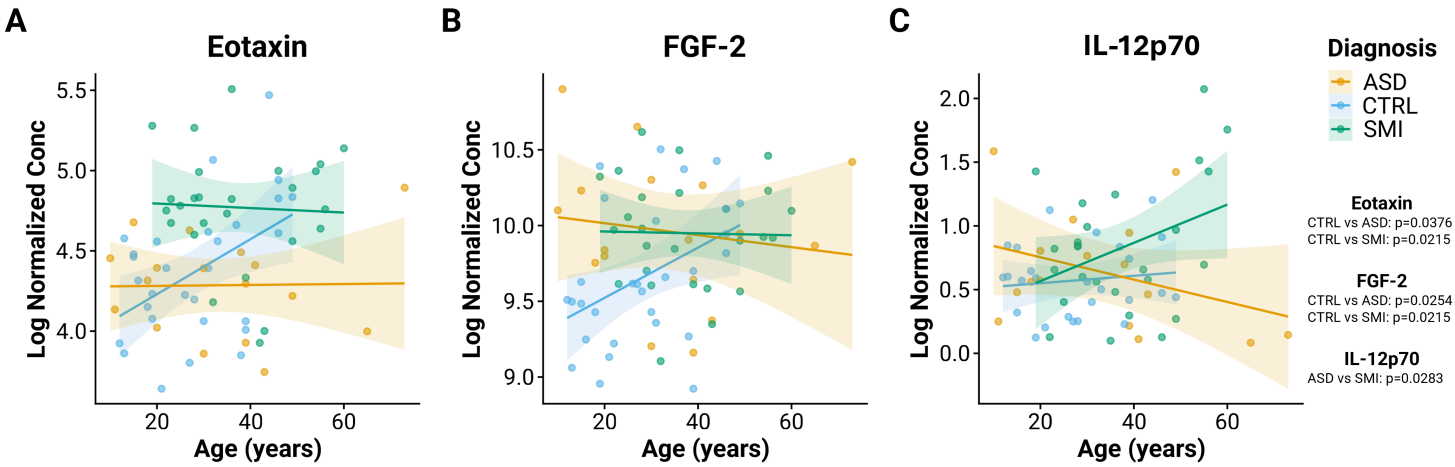


Figure 3

Immune Signaling Proteins Exhibit Altered Aging Trajectories. Significantly different age-related trajectories (pairwise comparisons of slopes) across diagnostic cohorts. **A, B)** Eotaxin and FGF-2 showed significantly different aging trajectories between CTRL and ASD and between CTRL and SMI. **C)**IL-12p70 had significantly different trajectories between ASD and SMI. Confidence bands represent 95% confidence intervals for each regression line.

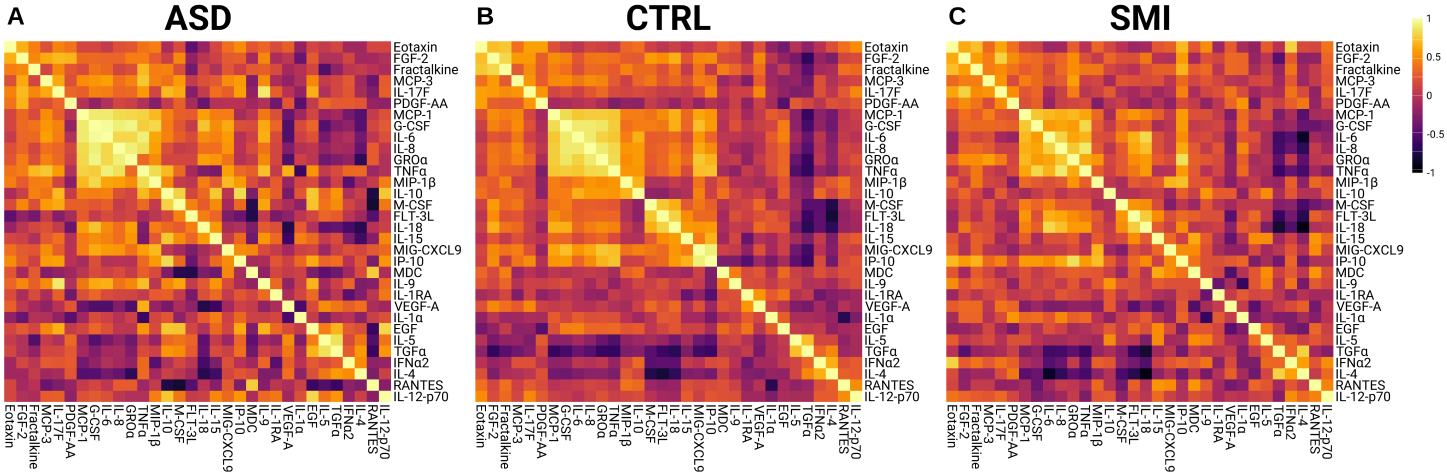


Figure 4

Relationships Between Immune Proteins are Impacted in ASD and SMI. Pairwise complete Spearman correlations are hierarchically clustered for the CTRL group, with the ASD and SMI groups matching

structure. Red indicates a positive correlation; blue represents a negative correlation.

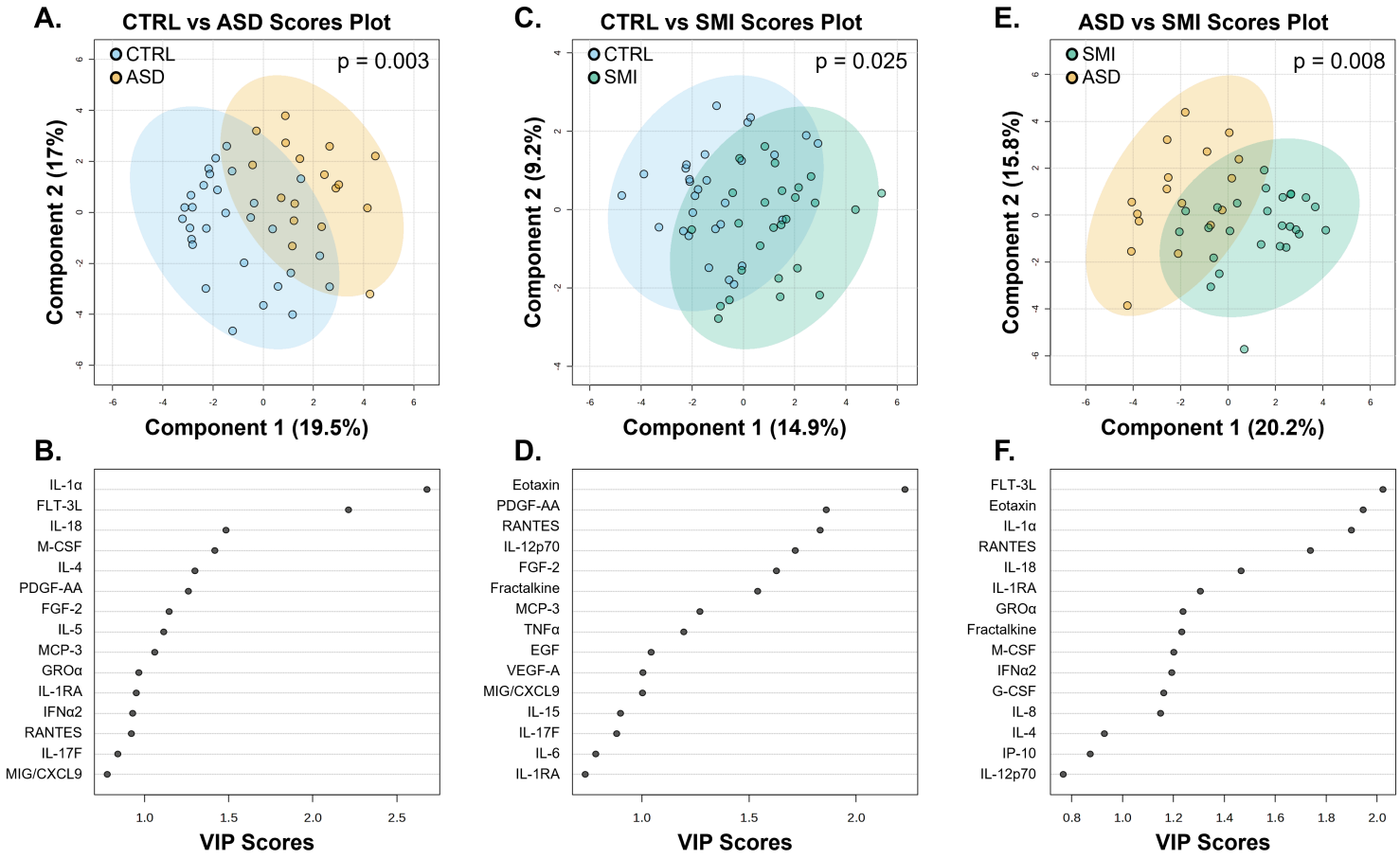


Figure 5

ASD Exhibits a Distinct Neuroimmune Profile Compared to CTRL and SMI. Neuroimmune profiles distinguish all diagnostic cohorts. **A, C, E)** Partial least squares discriminant analysis (PLS-DA) across groups on a pairwise basis. 2D scores plots represent individual cases and 95% confidence region. **B, D, F)** The top 15 analytes contributing to cluster separation are ranked by variable importance in projection (VIP) scores.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx