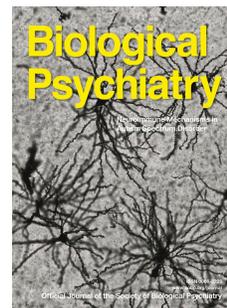# Journal Pre-proof

Systemic cell-adhesion molecules (CAM) in severe mental illness-potential role of intracellular CAM-1 in linking peripheral and neuro-inflammation.

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Systemic cell-adhesion molecules (CAM) in severe mental illness-potential role of intracellular CAM-1 in linking peripheral and neuro-inflammation.

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Running title: Intracellular CAM in severe mental illness

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ABSTRACT

Background: Cell adhesion molecules (CAM) orchestrate leukocyte trafficking and could link peripheral and neuro-inflammation in patients with severe mental illness (SMI), by promoting inflammatory and immune mediated responses and mediating signals across blood-brain-barrier (BBB). We hypothesized that CAMs would be dysregulated in SMI and evaluated if plasma levels of different vascular and neural CAMs. Dysregulated CAMs in plasma were further evaluated *in vivo* in leukocytes and brain tissue and *in vitro* in iPSC cells.

Methods: We compared plasma soluble (s) levels in circulating leukocytes of different vascular (VCAM-1, ICAM-1, P-SEL) and neural (JAM-A, N-CAD) CAMs in a large SMI sample of schizophrenia spectrum disorders (SCZ, n=895) and affective disorders (AFF, n=737) and heathy controls (HC, n=1070) controlling for age, sex, BMI, CRP and freezer storage time. We also evaluated mRNA expression of *ICAM1* and related genes encoding ICAM-1 receptors in leukocytes by microarray (n=842) and in available RNAseq from the CommonMind Consortium on post-mortem samples from the dorsolateral prefrontal cortex (n=474). The regulation of sICAM-1 in iPSC-derived neurons and astrocytes was assessed in SCZ patients and HC (n=8 of each).

Results: Our major findings: (1) increased sICAM-1 in SMI patients *vs.* HC; (2) increased *ITGB2* mRNA, encoding the beta chain of the ICAM-1 receptor, in circulating leukocytes from SMI patients and increased prefrontal cortex mRNA expression of *ICAM1* in SCZ; and (3) enhanced sICAM-1 release in iPSC-derived neurons from SCZ patients.

Conclusion: Our results support a systemic and cerebral dysregulation of sICAM-1 expression in SMI and especially in SCZ patients.

Introduction

Genetic and epidemiological studies support aberrant immune activation and inflammation as a pathogenic mechanism in severe mental illness (SMI) [1], including schizophrenia (SCZ) [2, 3] and affective disorders (AFF) [4]. Dysregulated activation of immune cells in the vasculature, including leukocyte subsets and endothelial cells and their bidirectional interactions, have been demonstrated as reflected by secreted activation markers preceding [5] and following diagnosis of SCZ and AFF [6-8]. These processes could directly affect the brain and potentially lead to progression of SMI involving both cell-mediated and soluble mediators [9]. Indeed, molecular neuroscience studies suggest that inflammation and immune activation can influence neuronal functioning and plasticity, and may predispose glial cells to a pro-inflammatory state associated with neurodegeneration.

These neuron-glia interactions include microglia, the primary immune cells of the central nervous system (CNS), and astroglia, both critical in maintaining neuro-homeostasis and involved in inflammatory dysregulation in neuropsychiatric disorders [10, 11]. Furthermore, these interactions may be neuro-developmentally dysregulated in SCZ [12]. Maternal infections may skew the immune phenotype of fetal microglia with potential negative long-term effects on microglia responsiveness during adulthood [13]. Furthermore, transplantation of iPSC derived glial progenitor cells from patients with childhood-onset SCZ into chimeric mice revealed impaired astroglial maturation and a behavioral phenotype consistent with SCZ [14]. Thus, early maternal immune activation may induce pathological interactions between neurons and glial cells, adversely affecting neurodevelopmental processes as outlined by Dietz et al. [15].

Although the blood-brain barrier (BBB) is not deteriorated in SMI as in neurological diseases and neurodegenerative disorders, increased permeability in SCZ has been demonstrated by post mortem transcriptomic studies showing dysregulated expression of tight junction-related genes in the prefrontal cortex [16, 17] and BBB transcripts enriched in brain endothelial cells such as *ICAM1* [18] as well as aberrant immunoreactivity of claudin-5 in brain sections from the parietal lobe [19]. While less evidence in AFF, MRI studies suggest subgroups of patients may have extensive and diffuse BBB dysfunction [20].

Cell adhesion molecules (CAMs) orchestrate leukocyte trafficking and their dysregulation could influence BBB permeability, promoting inflammatory and immune-mediated trafficking to the CNS, linking peripheral and neuro-inflammation in SMI [21-24]. Activated endothelial cells initially upregulate

selectins (e.g. p-selectin) to slow leukocytes on the endothelial surface before firm adhesion by integrins and Ig superfamily CAMs (e.g. intracellular CAM, ICAM; vascular CAM, VCAM). The expression of integrin recptors on leukocytes, that may bind CAMs and facillitate transmigration across the BBB, have been shown increased in SCZ [21, 25, 26]. Junctional adhesion molecules (JAM, e.g. JAM-A) and cadherins (CAD) maintain the structural integrity of cells and tissues with important roles during extravasation of leukocytes following firm adhesion [24, 27, 28]. Neural CAD (N-CAD) enables endothelial cell adhesion and communication with other cells, such as pericytes and astrocytes [29, 30]. These CAMs may shed from the cell surface by different mechanisms and their soluble (s) form released into the circulation. As shown in Supplemental Table 1, many previous studies have evaluated circulating CAMs showing quite consistent upregulation of proteins such as sICAM-1 in SMI [18, 31-37], while inconsistent regulation is found for sVCAM-1 [31-34] and sP-SEL [38-42], possibly due to differences in sample material and controlling for different demographics. To our knowledge, systemic levels of sJAM-A and sN-CAD, have not been evaluated in SMI. In addition,

Based on the role of CAMs in promoting inflammatory and immune responses and mediating signals across BBB, we hypothesized that evaluation of a range of circulating CAM reflecting leukocyte/vascular interactions such as sICAM-1, sVCAM-1 and sP-SEL, as well as neural CAMs such as sJAM-A and sN-CAD, could identify a novel pattern of dysregulation with clinical relevance for SMI. We focused on CAMs increased beyond the generalized systemic inflammation and metabolic distress in SMI as reflected by CRP and BMI, controlling for these factors. To test this hypothesis, we adopted a multi-step approach. Step 1: we evaluated plasma levels of different vascular and neural CAMs in patients with SMI (n=1632) and healthy controls (HC, n=1070) and associations with symptom severity. Step 2: among the observed regulated CAMs in step 1, we assessed microarray mRNA expression of the CAM and relevant receptors in leukocytes in a subset including 579 SMI patients and 263 HC. Step 3: we assessed dysregulated CAMs and corresponding receptors by estimating cell type-specific gene expression through computational deconvolution in leukocytes and RNA-seq data of dorsolateral prefrontal cortex (DLPFC) post mortem samples from the CommonMind Consortium (CMC, n=474). Step 4: we studied the regulation of dysregulated CAMs and their receptors in human induced pluripotent stem cell (iPSC)-derived neurons and astrocytes from SCZ and HC donors (n=8).

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Materials and methods

Setting and participants

The current study is part of the ongoing Thematically Organized Psychosis (TOP) Study including clinically stable patients 18-65 years, able to give a written informed consent, with a diagnosis of SCZ spectrum disorder (schizophrenia, schizoaffective disorder, schizophreniform disorder, delusional disorder, brief psychotic disorder and psychosis NOS) included in the diagnostic group "Schizophrenia" (SCZ), and affective disorder (bipolar I, bipolar II, bipolar NOS and major depressive disorder with psychotic features) included in the diagnostic group "Affective" (AFF) according to DSM-IV as described [4]. See the supplemental file for details. All participants gave written consent and the study was approved by the Regional Committees for Medical and Health Research Ethics (REC) in Norway and the Norwegian Data Protection Agency. Participants with C-reactive protein (CRP) >10 mg/L for any reason were excluded from the study.

Clinical assessments and medication

We evaluated patients with the Positive and Negative Syndrome Scale (PANSS)[43], Young Mania Rating scale (YMRS)[44] and the Global Assessment of Functioning Scale (GAF-S)[45]. All participants including HC were screened for medical history, physical examination and routine blood tests in addition to isolation of plasma for CAM analysis. Information regarding prescribed antipsychotics were obtained by clinical interviews and hospital records. We calculated "defined daily dose" (dddAP) according to the World Health Organization (WHO) principles, as described [46].

Analysis plan

We first assessed plasma CAMs in 1632 patients with SMI (SCZ=895, AFF=737) and 1070 HC, recruited between 2002 and 2017. As shown in Table 1, the groups were not well matched, as patients with AFF were older than HC and patients with SCZ, and BMI was higher in AFF and SCZ compared with HC. SCZ patients had more males compared to AFF and HC, while AFF had more females compared to HC. In CAMs dysregulated in plasma we proceeded with i) microarray mRNA analysis in leukocytes ii) cell type-specific gene expression through computational deconvolution of microarray blood expression data and RNA-seq data of dorsolateral prefrontal cortex (DLPFC) and iii)

regulation in human induced pluripotent stem cell (iPSC)-derived neurons and astrocytes. Methods are detailed below.

Protein measurements

Plasma levels of sICAM-1, sVCAM-1, sP-selectin (sP-SEL), sJAM-A and sN-CAD were measured in duplicate by enzyme immunoassays (EIA) using commercially available antibodies (R&D Systems, Minneapolis, MN, USA) as described in detail in the Supplemental file. Intra- and inter-assay coefficients of variation were <10% for all EIAs. For immunoassays, blood was taken using EDTA vials and the plasma was isolated within the next working day and stored at -80°C. Blood sampling was performed between 8 AM and 5 PM with some variation between patients and HC. A validation of the immune-assays with regard to performance, diurnal and post-prandial variation and effect bench/fridge time is presented in Supplemental Table 2.

RNA microarray analysis and gene expression imputation

Leukocyte mRNA expression was evaluated in 842: SCZ=338, AFF=241, HC=263, described in Supplemental Table 3. Blood samples were collected in Tempus Blood RNA Tubes (Life Technologies Corporation) and processed as described previously [47]. Briefly 200 ng of total RNA was biotin labelled and amplified using the Illumina TotalPrep-96 RNA Amplification Kit (Thermo Fisher, Waltham, MA, USA) and global gene expression quantification was performed with Illumina HumanHT-12 v4 Expression BeadChip (Illumina, Inc.). Multidimensional scaling and hierarchical clustering were used for regular quality control and removal of multiple batch effects (RNA extraction batch, RNA extraction method, DNase treatment batch, cRNA labelling batch, and chip hybridization). The "detectOutlier" function in the R package lumi identified 84 samples as outliers. Further details are in the Supplemental file.

The computational deconvolution tool CIBERSORTx [48] imputed cell type-specific gene expression for 10 major blood cell populations (see Figure 2A) as defined by the LM22 reference data set available through the web-based version of the tool (https://cibersortx.stanford.edu/). The "Group Mode" analysis type was used to impute cell expression, and differential expression (DE) analyses were carried out according to the guidelines recommended by the developers [49].

RNA-seq of brain samples from the CMC

RNA-seq data of DLPFC post-mortem brain samples was obtained from the CMC [50]. A subset of 474 samples (SCZ=214, AFF=45, HC=215) was used, including only donors with Caucasian ethnicity. Details on extraction procedures, yield and quality control are given in the Supplemental file as are details on the RNAseq. All cases had a read count of >25 million reads (mean: 39.2 million). A pre-filtering step excluded all lowly expressed and non-expressed genes (<1 count per million in > 50% of cases), retaining 16895 genes for analysis. DE analyses were carried out with the DESeq2 package [51], controlling for age, sex, post-mortem interval, and biobank repository (laboratory batch effects). Associations with FDR<0.05 were considered significant. For cell type-specific imputation of gene expression, CIBERSORTx was used [49], selecting the "High Resolution" data analysis mode. Gene expression reference profiles for human neurons, astrocytes, and endothelial cells were obtained from Zhang et al. [52] and used as input for imputation of cell type-specific expression.

Generation, differentiation and stimulation of astrocytes and neurons from donor iPSCs

For reprogramming and astrocyte and neuron iPSC differentiation, fibroblast/skin biopsies were isolated from 4 HC (mean age 45.5±13.9 years, 2 male) and 4 SCZ patients (mean age 25.0±3.6 years, 2 male). We differentiated and thoroughly characterized astrocytes and neurons from patient and control iPSCs following previously published glial and neuron differentiation and phenotyping protocols with small modifications (see Supplemental methods). Culture pellets and supernatants were collected after 0, 4, 7, 11 and 14 days of incubation for qPCR and EIA analysis as described above. Harvested samples were stored at −80 °C. Extraction procedure, RT-PCR reaction and qPCR analysis are detailed in the Supplemental file.

Statistical Analyses

Statistical analyses were performed in R using the base statistical package. Associations between diagnosis (HC, SCZ, AFF, SCZ+AFF) and circulating levels CAM's were assessed using linear regression, controlled for freezer storage time, age, sex, BMI and circulating levels of CRP. Similarly, associations between diagnosis and bulk mRNA levels of ICAM-1 receptors were also assessed using linear regression with the same covariates. Release of sICAM-1 from in vitro cultures were assessed

by a univariate general linear model applying group (HC or SCZ) and time as fixed factors, ID number as random factor and the interaction time*group.

Results

Demographics and clinical characteristics

SCZ patients had more severe symptoms as reflected by PANSS and YMRS scores, and lower levels of functioning as reflected by GAF-S, compared to AFF. CRP levels were higher in AFF and SCZ compared to HC. As expected, use of AP was more frequent in SCZ compared to AFF, with a higher dddAP.

Circulating sCAMs in severe mental disorders

Differences in plasma sCAM levels between patients and HC and within diagnostic subgroups, controlled for age, sex, BMI, and CRP are shown in Figure 1, with coefficient estimates after shown in Supplemental Table 4. Patients with SMI were characterized by higher plasma sICAM-1 (p=3.0E-07), with the highest levels in SCZ (p=5.3E-08), although patients with AFF (p=6.7E-04) also had higher levels compared to HC. Patients with SMI as a whole also displayed higher levels of sJAM-A (2.4E-04) with a comparable increase in SCZ (2.4E-04) and AFF (p=0.009), while sVCAM-1, sP-SEL and sN-CAD showed no differences between diagnostic groups and HC.

Associations between sICAM-1, sJAM-A, symptoms scores and AP treatment

sICAM1 and sJAM-A levels were not associated with AP treatment (p=0.77 and p=0.33) or dddAP (p=0.59 and p=0.28), and there were no strong associations between plasma sICAM-1 and symptom scores as reflected by PANSS, YMRS and GAF-S (Supplemental Table 5), in SMI. A modest positive association was seen between PANSS negative scores (p=0.038) in the SMI group, mainly driven by an association in SCZ (p=0.063). In addition, sICAM-1 correlated with both BMI (r=0.14, p<0.001) and CRP (r=0.12, p<0.001), while no correlation was seen between sJAM-A and BMI or CRP (p>0.2).

Evaluation of ICAM1, F11R (JAM-A gene) and receptor genes in leukocytes

As shown in Figure 2A, *ICAM1* and *F11R* mRNA expression in circulating leukocytes was similar between all diagnostic groups and controls, and did not correlate with corresponding plasma levels of sICAM-1 (r=0.03, p=0.60) or sJAM-A (r=-0.01, p=0.86).

ICAM-1 and JAM-A mediated transmigration is facilitated by the β2-integrins, LFA-1 (CD11a/CD18 encoded by the *ITGAL/ITGB2* genes) and Mac-1 (CD11b/CD18 encoded by *ITGAM/ITGB2*). We evaluated mRNA expression of these integrins in circulating leukocytes. As shown in Table 2, *ITGB2* was increased in SMI as a whole and in SCZ and AFF subgroups, controlled for age, sex and BMI. Further controlling for CRP attenuated these associations, suggesting that enhanced systemic inflammation could contribute to elevated *ITGB2* expression in SMI. Imputation of cell type-specific gene expression of the receptors through computational deconvolution (Figure 2B) revealed that *ITGB2* expression was higher in neutrophils and monocytes in SZC and AFF, respectively, while it was decreased in CD4+ T cells from AFF (Figure 2C).

Evaluation of ICAM1, F11R and its receptors in the brain

We next assessed RNA-seq data in a large sample (n=474) of dorsolateral prefrontal cortex (DLPFC) post mortem tissues from the CommonMind Consortium (CMC) [50]. Differential expression analyses of the whole brain region (bulk RNA-seq) showed an elevated expression of *ICAM1 and ITGAL,* but not *F11R* in SCZ (Figure 3A) compared to HC, and for *ITGAL* also compared to AFF. ITGAM and ITGB2 were not regulated. To investigate whether this association was driven by a specific cell population, we imputed cell type-specific expression in neurons, astrocytes (the two most abundant cell types in the brain) and endothelial cells. While gene expression in neurons and endothelial cells could not be reliably imputed, likely due to inadequate statistical power for these cell types, we found that *ICAM1* expression was significantly increased in SCZ in the astrocyte subpopulation (Figure 3B) while ITGAL was not regulated in subpopulations.

To further investigate the potential association between ICAM-1 and SCZ in the brain, we finally assessed if iPSC-derived neurons and astrocytes generated from HC and patients with SCZ released sICAM-1 during long-term culturing. As shown in Figure 3C, neurons derived from patients with SCZ released increased levels of sICAM-1 over time compared to HC. Increasing sICAM-1 release was also observed in astrocytes during culture (Figure 3D), but with a similar temporal profile

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in SCZ and HC. We were not able to detect *ICAM1* mRNA expression in pellets from these cultures due to low expression (CT values >38).

Discussion

The present study evaluated previously investigated and novel sCAMs in a large sample of patients with SMI and HC. We confirm enhanced plasma sICAM-1 in patients with SMI, increased mRNA levels of *ITGB2*, encoding the beta chain of the ICAM-1 receptor, in circulating leukocytes from SMI patients and elevated DLPFC mRNA expression of *ICAM1* in SCZ. Novel findings of our study are the dysregulation of *ITGB2* in leukocytes in AFF, increased DLPFC mRNA expression of *ITGAL* in SCZ and enhanced release of sICAM-1 from neurons in SCZ. In addition, we report increased sJAM-A in SMI, but found no regulation in DLPFC samples.

Plasma and serum levels of sICAM-1 have previously been evaluated in SMI (reviewed in [53] and summarized in Supplemental Table 1). Our finding of increased sICAM-1 supports most studies reporting higher levels in SCZ [18, 32, 34, 35] and bipolar disorders [31, 33, 36, 37], while some studies report lower levels in SCZ [54, 55]. In these studies, higher sICAM-1 levels were related to stage of disease [31, 33, 34, 36, 37], inflammatory and cardio-metabolic burden [18, 35], while lower levels were associated with ICAM-1 SNPs [54] and AP treatment [31]. With some exceptions [34], and similar to our findings, these studies present only marginal or no correlation with symptom scores. Based on the widespread vascular expression of ICAM-1 [56] and role in atherosclerosis and metabolic disease [57, 58], increased levels driven by potential cardio-metabolic risk could obscure associations with symptom severity in SMI. Indeed, increased BMI and CRP correlated with sICAM-1 levels in our patients suggesting that enhanced subclinical inflammation could contribute to increased sICAM-1 in SMI, but in contrast to the above studies, we controlled for CRP and BMI in our analyses. Regardless of demographic or comorbid factors contributing to elevated systemic levels, sICAM-1 could link systemic inflammation and immune activation and neuro-inflammation in SMI. Regarding potential effects of AP treatment, we found no significant associations, but this may be best assessed by a temporal design. Taken together, in by far the largest study of SMI patients, we show that elevated sICAM-1 levels in both SCZ and AFF disorders, beyond the subclinical inflammation as assessed by CRP and metabolic risk as assessed by BMI.

Transmigration across the BBB is facilitated by the β2-integrins LFA-1 and Mac-1 [59], and our

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finding of increased *ITGB2* mRNA levels encoding the common beta chain protein for these integrins in SMI leukocytes, as shown previously for mRNA [25] and protein levels [26] in SCZ, further support that ICAM-1 mediated mechanisms could promote inflammatory cell trafficking across the BBB. Cai et al. demonstrated monocytes in the prefrontal cortex and brain tissue macrophages in close proximity to neurons in SCZ patients [18], and increased density of macrophages has been shown along the lateral ventricle [60, 61] and in the midbrain [62]. The imputed increased *ITGB2* in monocytes, support infiltration of these cells also in AFF as suggested by deconvolution of RNAseq data from post mortem samples of the dorsal striatum, estimating more monocytes in BD compared to HC [63]. The increased *ITGB2* on neutrophils in SCZ in our study, may suggest a role these cells in disruption of, or migration across, the BBB as seen in Alzheimer's disease [64]. Neutrophil counts correlate with reductions in grey matter volume and enlarged ventricles in SCZ [65]. Finally, the lower imputed *ITGB2* in CD4$^+$ T in AFF, suggest reduced migration of these cells following astrocyte-derived chemotaxis as shown in SCZ [66]. Since CD4$^+$ T$_{regs}$ are important anti-inflammatory regulators, their reduced ingress into the brain in SMI may contribute to a more inflammatory environment. Taken together, we hypothesize that an inflammatory systemic environment could increase β2-integrins in leukocytes in SMI, and exposure to elevated ICAM-1 could potentiate leukocyte arrest along the BBB vasculature and eventual migration into perivascular space, further promoting neuro-inflammation through secretion of inflammatory signals.

Cai et al. demonstrated a 68% higher *ICAM1* mRNA expression in DLPFC in 37 SCZ patients, in particular in a subgroup with high inflammation, with enrichment in the brain endothelium [18]. Herein we extend these findings showing increased *ICAM1* mRNA expression in SCZ in a large DLPFC post mortem sample, with imputed expression primarily in astrocytes. Inflammatory challenge may induce *ICAM1* mRNA and cell surface expression on cultured astrocytes [67-69], and the higher levels in post mortem samples could reflect a higher inflammatory environment *in vivo* as found by Cai et al. We were not able to detect any *ICAM1* mRNA using qPCR from our iPSC derived cells suggesting low levels. Still, spontaneous sICAM1 correlates strongly with membrane bound expression on astrocytes [70], and is detected in supernatants of resting cells [70, 71]. Thus, the lack of difference between SCZ and HC in unstimulated cells support that increased astrocyte derived ICAM-1 in SCZ is due to a higher inflammatory environment. In contrast, the higher secretion of sICAM-1 from neurons in SCZ, without an inflammatory trigger, could point to some genotypic

differences between SCZ patients and HC. Interestingly, enhanced oxidative stress is a feature of iPSC-derived neurons in SCZ [72], and it is tempting to hypothesize that ICAM-1 may promote oxidative-stress induced neuronal loss [73]. Astrocytes express low levels of LFA-1, which are enhanced by IFNγ [74], and we speculate that the increased *ITGAV* imputed in SCZ astrocytes could promote inflammatory signaling involving glial cells, neurons and infiltrating leukocytes. sICAM-1 may induce MIP-2 release in both mouse astrocytes and brain microvascular endothelial cells [70], which could advance recruitment of leukocytes and inflammation in the brain [75]. In addition to regulation of vascular permeability, ICAM-1-dependent adhesion to astrocytes induces TNF secretion, suggesting a direct role in inflammatory signal transduction [76]. Similar effects of sICAM-1 have shown in endothelial cells and macrophages, invoking a range of inflammatory responses [77, 78]. Thus, ICAM-1-related mechanisms in SMI may not be restricted to transmigration of leukocytes across the BBB, but could both enhance and be enhanced by inflammation within the CNS.

A novel finding in our study was the increased sJAM-A levels in SMI. JAM-A is expressed on endothelial cells, epithelial tight junctions and leukocytes [79, 80] and mediate transmigration of leukocytes across the BBB in a LFA-1 dependent manner [81]. However, we observed no regulation of F11R in the DLPFC samples. A study evaluating sJAM-A as a BBB breakdown marker in human brain microvascular cells found no effects of inflammatory or hypoxic challenge and detected no upregulation in patients with clinically active multiple sclerosis [82]. As JAM-A is also expressed in atherosclerotic plaques [83] and increased soluble levels have been demonstrated in patients with atherosclerotic disease [84], comorbid conditions could contribute to increased sJAM-A in SMI.

A strength of our study was the large number of patients and controls. While previous studies on sICAM-1 have been relatively consistent, not requiring this statistical power, our study brings confidence to markers such as sP-SEL and sVCAM-1, where previous studies are highly inconsistent. P-SEL is abundantly expressed in platelets [85] and serum would therefore not reflect *in vivo* circulating levels but rather *ex vivo* release during platelet degranulation, which may be less relevant in SMI. Nonetheless, while we did not detect systemic dysregulation of P-selectin, VCAM-1 and NCAD, this does not necessarily imply that these proteins have no role in the progression of SMI, but merely that this was not reflected by circulating levels in our study.

Limitations to our study include the blood sampling protocol, which was not optimal with differences between patients and HC in time of day when samples were obtained and with isolation of

plasma the next day. However, our validation experiments found no systematic effects on the measured CAMs. Samples were collected and stored at -80°C over a period of 15 years, however controlling for this had no major impact on the results. The lack of smoking status in our control population is a limitation of our study. RIN was only determined in a subsample of our leukocyte mRNA samples, and quality control to remove samples with lower numbers of detected transcripts and low signal-noise ratio was performed with an R algorithm, resulting in removal of 84 samples. We were not able to impute gene expression for neurons or endothelial cell in the DLPFC post mortem samples, most probably due to low expression of the ICAM-related genes and low number of the cells compared to astrocytes in the cortex. Also, to ensure a more homogeneous study population, we a set of RNAseq data from the CMC collection due to technical differences with regard to strand specificity as described by the authors [50], as well as non-Caucasian samples. While these strict criteria negatively impacted the sample size, our sample is still much larger than the sample size used in most similar studies. Patients were chronic with stable disease and results could be different in acute patients.

Finally, the study had a cross-sectional design, making the causality described suggestive. We do not suggest sICAM-1 represents a clinically useful biomarker in these patients, as there was a large overlap in the sICAM-1 distributions of SMI patients and controls.

In conclusion, our results support a systemic and cerebral dysregulation of sICAM-1 signaling in SMI and especially in SCZ, potentially contributing to CNS pathology in this patient group.

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Figure Legends

Figure 1. Circulating sCAMs in severe mental disorders Evaluation of sCAM levels between SMI patients (n=1632) and HC (n=1070) and within SCZ (n=895) and AFF (n=737) subgroups, controlled for age, sex, BMI, and CRP. **p<0.01, ***p<0.001 *vs.* HC

Figure 2. Expression of leukocyte ICAM-1, JAM-A and integrin genes in SMI. A) Microarray mRNA expression of *ICAM1* and *F11R* (encoding JAM-A) in circulating leukocytes. **B)** data were deconvoluted with CIBERSORTx. Heatmap showing imputed cell expression of genes encoding β2-integrins, LFA-1 (CD11a/CD18 encoded by the *ITGAL/ITGB2* genes) and Mac-1 (CD11b/CD18 encoded by *ITGAM/ITGB2*) according to cell type. **C)** Imputed mRNA expression of *ITGB2* across diagnostic groups. *p<0.05, **p<0.001 in FDR adjusted t-tests.

Figure 3. Regulation of ICAM-1, JAM-A and integrins in brain tissue and brain cells in SMI. A)
Expression of *ICAM1*, *F11R* (encoding JAM-A) and *ITGAL* in bulk RNA-seq data from 474 DLPFC
donors (SCZ=214, AFF=45, HC=215). *FDR<0.05, **FDR<0.001 *vs.* HC; †FDR<0.05 *vs.* AFF. **B)**
Imputed expression of *ICAM1* in astrocytes. *p<0.05. Release of sICAM-1 at different time-points from
iPSC-derived **C)** neurons and **D)** astrocytes from SCZ and HC (n=8 in each group). The insert shows
estimated marginal means and 95% CIs from the mixed model regression with p-value reflecting the
diagnostic group effect.

Table 1. Demographics and clinical information in patients with severe mental illness and healthy
control participants

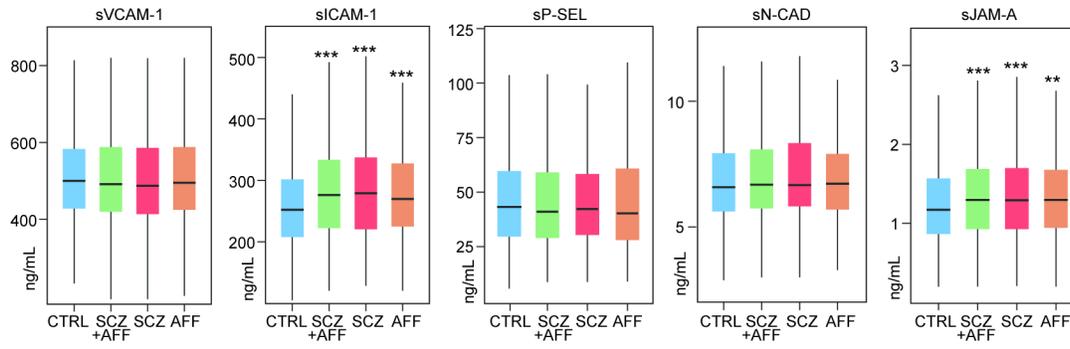|	n	SCZ mean±SD or %	n	AFF mean±SD or %	n	HC mean±SD or %	ANOVA p	SCZ vs HC p	AFF vs HC p	SCZ vs AFF p
Age, yrs	895	32.8±13.3	737	38.6±13.7	1070	32.5±10	<2e-16	0.92	<2e-16	<2e-16
Sex (Male)	425	57.7 %	363	40.6 %	559	52.2 %	7.0E-12	0.03	3.0E-07	8.3E-12
BMI, kg/m^2	455	26±5.3	779	25.9±4.8	849	24.7±3.9	1.1E-08	5.1E-06	3.0E-07	0.98
PANSS total	555	63.6±16.6	421	45.4±10.2	-	-	-	-	-	<2e-16
PANSS neg	561	15.8±6.2	421	10.0±3.4	-	-	-	-	-	<2e-16
PANSS pos	559	15.3±5.4	423	9.8±3.4	-	-	-	-	-	<2e-16
PANSS gen	558	32.5±8.4	422	25.6±5.9	-	-	-	-	-	<2e-16
YMRS	472	5.2±5.1	416	3.1±4.4	-	-	-	-	-	1.6E-10
GAF-S	602	42.7±11.7	437	58.1±11.6	-	-	-	-	-	<2e-16
CRP, mg/L	509	3.5±3.6	376	3.2±3.4	968	2.3±2.6	1.3E-14	<2e-16	5.3E-06	0.14
AP use	525	71.2 %	223	24.9 %	0	0.0 %	-	-	-	<2e-16
ddd AP	525	1.18±0.82	223	0.87±0.64	-	-	-	-	-	9.1E-07

HC, healthy control participants; SCZ, Schizophrenia; AFF, affective disorders; BMI, body mass index;
PANSS, Positive and Negative Syndrome Scale; YMRS, Young Mania Rating Scale; GAF-S, Global
Assessment of Functioning Scale; CRP, C-reactive protein; AP, antipsychotics; ddd, defined daily
dose. Categorical data are given as percent while continuous data are given as mean±SD

**Table 2. Associations between mRNA expression of ICAM-1 receptor genes in leukocytes and
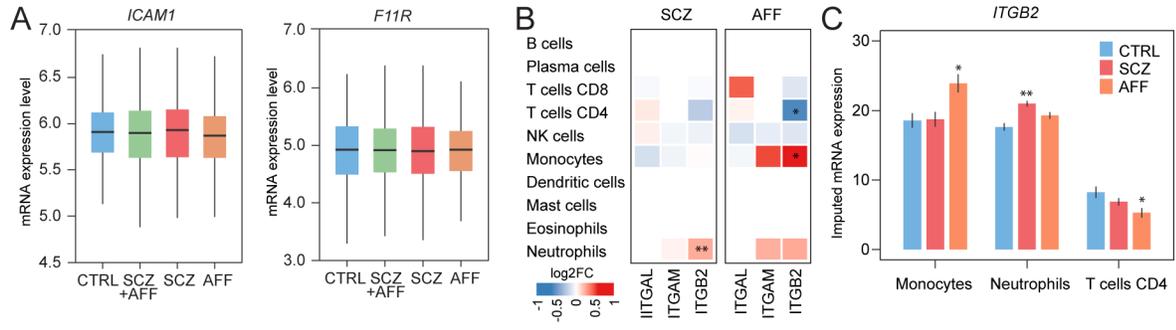diagnosis, controlled for age, sex, BMI, CRP and clo**

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Model	Outcome	SCZ+AFF	p	SCZ	p	AFF	p
Age	*ITGAM*	0.042±0.028	0.134	0.056±0.031	0.070	0.014±0.036	0.698
	ITGB2	0.048±0.012	6.4E-05	0.046±0.013	0.001	0.044±0.015	8.3E-04
	ITGAL	-0.013±0.001	0.402	-0.011±0.022	0.605	-0.013±0.025	0.617
Age+SEX +BMI	*ITGAM*	-0.005±0.027	0.865	0.028±0.030	0.349	-0.058±0.033	0.075
	ITGB2	0.050±0.013	1.1E-04	0.049±0.015	0.001	0.052±0.017	0.002
	ITGAL	0.022±0.020	0.277	0.013±0.022	0.573	0.041±0.026	0.119
Age+sex+ BMI+CRP	*ITGAM*	-0.011±0.028	0.706	0.013±0.031	0.677	-0.06±0.034	0.078
	ITGB2	0.038±0.013	0.005	0.031±0.016	0.052	0.047±0.018	0.009
	ITGAL	0.022±0.021	0.307	0.002±0.023	0.940	0.059±0.028	0.037

The table shows coefficients±SD compared to healthy controls. BMI, body mass index; CRP, C-reactive protein; *ITGAM*, Integrin alpha M; *ITGB2*, Integrin beta 2.