Genetic contribution to microglial activation in schizophrenia

Jari Koistinaho (jari.koistinaho@helsinki.fi)
University of Helsinki https://orcid.org/0000-0001-6559-1153
Marja Koskuvi
Elina Pörsti
Ying Chieh Wu
Kalevi Trontti
University of Helsinki/Finland
Amanda McQuade
Ilkka Ojansuu
Tyrone Cannon
Yale University https://orcid.org/0000-0002-5632-3154
Jouko Lönnqvist
Sebastian Therman
Finnish Institute for Health and Welfare https://orcid.org/0000-0001-9407-4905
Jaana Suvisaari
https://orcid.org/0000-0001-7167-0990
Jaakko Kaprio
University of Helsinki https://orcid.org/0000-0002-3716-2455
Mathew Blurton-Jones
University of California, Irvine https://orcid.org/0000-0002-7770-7157
Iiris Hovatta
University of Helsinki/Finland https://orcid.org/0000-0002-5990-7892
Markku Lähteenvuo
Niuvanniemi hospital https://orcid.org/0000-0002-7244-145X
Taisia Rolova
Sarka Lehtonen
Univesity of Helsinki
Jari Tiihonen
Karolinska Institute https://orcid.org/0000-0002-0400-6798

Article

Keywords:

Posted Date: August 29th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1980131/v1

Title: Genetic contribution to microglial activation in schizophrenia

Running title: hiPSC-microglial activation in schizophrenia

Marja Koskuvi1,2, Elina Pörsti1, Ying-Chieh Wu1, Kalevi Trontti1,3,4, Amanda McQuade5,6,7, Ilkka Ojansuu8, Olli Vaurio8, Tyrone D. Cannon9, Jouko Lönnqvist10,11, Sebastian Therman10, Jaana Suvisaari10, Jaakko Kaprio12, Mathew Blurton-Jones5,6,7, Iiris Hovatta1,3,4, Markku Lähteenvuo8, Taisia Rolova1, Šárka Lehtonen1,13, Jari Tiihonen1,5,14, & Jari Koistinaho1,13

1 Neuroscience Center, University of Helsinki, Helsinki, Finland

2 Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

3 SleepWell Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland

4 Department of Psychology and Logopedics, University of Helsinki, Helsinki, Finland

5 Department of Neurobiology & Behavior, UC Irvine, Irvine, California, USA

6 Sue and Bill Gross Stem Cell Research Center, UC Irvine, Irvine, California, USA

7 Institute for Memory Impairments and Neurological Disorders, UC Irvine, Irvine, California, USA

8 Department of Forensic Psychiatry, University of Eastern Finland, Niuvanniemi Hospital, Kuopio, Finland

9 Department of Psychology and Psychiatry, Yale University, New Haven, Connecticut, USA

10 Mental Health Unit, Department of Public Health Solutions, National Institute for Health and Welfare, Helsinki, Finland

11 Department of Psychiatry, University of Helsinki, Helsinki, Finland

12 Institute for Molecular Medicine FIMM, University of Helsinki, Helsinki, Finland

13 A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland

14 Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Abstract

Several lines of evidence indicate the involvement of neuroinflammatory processes in the pathophysiology of schizophrenia (SCZ). Microglia are brain resident immune cells responding toward invading pathogens and injury-related products, and additionally, have a critical role in improving neurogenesis and synaptic functions. Hyperactivation of microglia in SCZ is one of the leading hypotheses for disease pathogenesis, but due to the lack of proper human cell models, the role of microglia in SCZ is not well studied. We used monozygotic twins discordant for SCZ and healthy individuals to generate human induced pluripotent stem cell-derived microglia to assess the transcriptional and functional differences in microglia between healthy controls, affected twins and unaffected twins. The microglia from affected twins had increased expression of several common inflammation-related genes, such as AIF1, HLA-DRA and IL1β compared to healthy individuals. Microglia from affected twins had also reduced response to IL1β treatment, but no significant differences in migration or phagocytotic activity. Ingenuity Pathway Analysis (IPA) showed abnormalities related to extracellular matrix signaling. RNA sequencing predicted downregulation of extracellular matrix structure constituent GO terms and hepatic fibrosis pathway activation that were shared by microglia of both affected and unaffected twins, but the upregulation of MHC class II receptors was observed only in affected twin microglia. Also, the microglia of affected twins had heterogeneous response to clozapine, minocycline, and sulforaphane treatments. Overall, despite the increased expression of inflammatory genes, we observed no clear functional signs of hyperactivation in microglia from patients with SCZ. We conclude that microglia of the patients with SCZ have genetic aberrations related to inflammation response and extracellular matrix without contributing to microglia hyperactivation.

Introduction

Neuroinflammation is a pathophysiological process involved in various neurological diseases. There is increasing evidence that dysregulation of neuroinflammation and immune response is also associated with neurodevelopmental disorders, including schizophrenia (SCZ) (1,2), autism spectrum disorder (ASD) (3) and attention deficit hyperactivity disorder (4), and with mood disorders such as bipolar disorder (BD) (5,6), and major depressive disorder (5,7).

Microglia are brain resident macrophages indispensable for innate immune system activation and inflammation as a response against invading pathogens or injury-induced products to protect neurons and other glial cells from damage. Although the exact etiology of SCZ remains largely unknown, it is a complex disorder that is thought to develop as the result of the interaction between genetic changes and environmental factors initiated already during embryonal development and continuing through childhood to adolescence and early adulthood. During embryonic development microglia support neurogenesis and modify synaptic network by synaptic pruning in early childhood and adulthood. Patients with SCZ have upregulation of various markers of neuroinflammation, which has led to the theory of prolonged microglia hyperactivation as a significant contributory factor in SCZ (8). Postmortem studies have revealed microglial activation in patients with SCZ, detected as increased microglial density based on ionized calcium-binding adapter molecule 1 (IBA1), major histocompatibility complex (MHC) class II, and cluster of differentiation 68 (CD68) immunoreactive cells (9–11). Consistently, there is evidence for elevated levels of pro-inflammatory cytokines interleukin 6 (IL6), tumor necrosis factor alpha (TNFα), interferon (IFN), and interleukin 1 beta (IL1β) in blood and brain of patients with SCZ (1,10,12).

The extended MHC locus has the strongest genetic association with SCZ known so far (13). The region contains polymorphic antigen-recognition human leukocyte antigen (*HLA*) genes, but also complement component 4 (*C4*) having the strongest individual genetic risk of common variants for SCZ (14). Due to high MHC locus polymorphism and lack of other microglia-specific genetic risk factors for SCZ, the availability of experimental models to study human microglial function in SCZ is limited, and the results have been inconclusive. Monocyte-derived microglia-like cells from patients with SCZ were shown to have increased synaptic pruning activity in neuronal co-cultures (15), while another study with a similar cell model failed to see gene expressional differences in the complement system or inflammatory pathways (16). In order to investigate microglia in SCZ without epigenetic impact, we chose to use human induced pluripotent stem cell (hiPSC) derived microglia. As the previous studies have suggested hyperactive phenotype in microglia in patients with SCZ, we focused especially on sensitivity to inflammatory stimuli and immune activation.

We also studied whether the antipsychotic drug clozapine and two other compounds, minocycline and sulforaphane, have the potential to modify the function of patient-derived microglia. Clozapine is the most common atypical antipsychotic drug used to treat treatment-resistant SCZ. Minocycline is an antibiotic drug, which has been shown to inhibit microglial activation and synaptic pruning, and thus holds potential for alternative treatment for SCZ (15,17,18). Sulforaphane is an antioxidant, which can modulate inflammation-related depression-like behavior in rodents (19,20). Clozapine and minocycline have multiple targets, which are not all well-known, while sulforaphane mainly induces NF-E2-related factor-2 (NRF2) activation. Even though it is not completely known how these compounds affect inflammation, previous studies have shown that all three of them can protect cells from LPS-induced immune activation potentially suppressing nuclear factor kappa B (NF-κB) pathway activation (19,21,22). NF-κB is a critical regulator of immune responses and controls the expression of various pro-inflammatory cytokines and acute phase proteins that are increased in the brain in people with SCZ.

In this study, we differentiated hiPSC-derived microglia-like cells (iMGLs) from our well-characterized monozygotic twins discordant for SCZ, i.e. cases with schizophrenia diagnosis and their healthy twins, and unrelated healthy controls to assess the genetic contribution to differences in gene expression and functions in microglia from affected and unaffected individuals. By using RNA sequencing we show dysregulation of commonly used pan-microglia genes and inflammation-related genes (*AIF1, HLA-DRA*, and *IL1β*) in affected twin microglia. While global gene expression profile was downregulated in both twins compared to healthy individuals, upregulation of 22.6% of the genes was seen only in clinical SCZ cases. Pathways related to extracellular matrix signaling were consistently aberrant in microglia of both affected and unaffected twin. Our test to rescue the expressional changes with applying clozapine, minocycline or sulforaphane gave heterogeneous response in affected twin microglia.

Materials and methods

Patient iPSC lines

hiPSCs were generated and characterized in previous study by Tiihonen, et al. (23). Supplementary tables 1 and 2 summarize the patient cohort and to which experiments they were included. Patient iPSCs were cultured in E8 medium (Gibco) on Matrigel (Corning) and splitted 1-2 times a week with 0.5 mM EDTA (Invitrogen) and tested negative for mycoplasma. Reagents are listed in supplementary table 3.

Microglia differentiation

We used the protocol adapted from McQuade, et al. (2018) (24) for hiPSC-microglia differentiation. Briefly, hiPSCs were first differentiated to hematopoietic stem cells (HPCs) with StemDiff Hematopoetic kit (StemCell Technologies) according the manufacturer's instructions. First, hiPSC colonies were de-attached with ReLeSR (StemCell Technologies) and seeded around 20-30 aggregates per Matrigel coated 6-well plate in E8 medium with 10 µM ROCK inhibitor (Y-27632, Sigma). The StemDiff medium was added onto the wells with well-distributed hiPSC-colonies on the next day. The medium was changed every other day until HPCs appeared after 11-13 days. Non-adherent HPCs were collected and 100-200,000 cells per well were seeded onto Matrigel-coated well on a 6-well plate in iMGL Base medium: DMEM/F12 (Gibco), 2 % ITS-G, 2 % B27, 2mM Glutamax, 1 % NEAA, 0.5 % N2, 0.5 % Penstrep (all from Invitrogen), 5 µg/ml insulin (Sigma) and 400 µM Monothioglycerol (Sigma) and added 100 ng/ml IL34 (PeproTech), 50 ng/ml TGFβ-1 (PeproTech), 25 ng/ml M-CSF (Peprotech) prior use (= iMGL differentiation medium). 1 ml of fresh medium was added every other day for 21-26 days. After the first 10-14 days of the differentiation, excessive media were collected from the wells leaving 1 ml on top of the cells. The media were centrifuged 300x g 4 min to collect the floating microglial precursors that were subsequently replated. To maturate the microglia, the cells were kept for three more days in iMGL maturation medium (iMGL differentiation medium + 100 ng/ml CX3CL1 (PeproTech) and 100 µg/ml CD200 (Biolegend)).

Drug treatments

Prepared hiPSC-microglia or hiPSC-macrophages were first re-plated depending on the application and matured two days. Then, the drugs were added for 24 hours in the same medium in the final concentration of 10 µM clozapine (Sigma), 10 µM minocycline (Sigma) or 5 µM D,L-Sulforaphane (Santa Cruz). In combined LPS and drug treatment, cells were pre-treated with clozapine, minocycline or sulforaphane for 1 hour before adding LPS for 24-hour (Sigma, 100 ng/ml) in iMGL maturation medium.

Statistics

As sample size was mostly four or five patients in a group, only non-parametric test were used. For comparing treatment effect paired tests were used: Friedman test and Wilcoxon matched-pairs signed rank test. For group comparison Kruskal-Wallis test and Mann-Whitney test were used. Exact p-values are given. Each data point represents separate patient line with pooled replicates. The data is presented as Mean + SD, except phagocytosis curves as Mean +/- SEM.

Data availability

Supplementary Data files contain raw RNA seq comparisons, IPA pathway analyses, and GO terms. The RNA sequencing data will be also available in GEO after manuscript acceptance.

Results

Human iMGLs were differentiated from pairs of monozygotic twins discordant for SCZ

We used a previously established protocol to generate human iMGLs in 40 days (24) (Figure 1a). The generated iMGLs had a ramified morphology and expressed pan-macrophage marker IBA1, and microglia-specific markers TREM2, P2RY12 and CX3CR1 (Figure 1b). iMGLs also upregulated macrophage- and microglia-related genes compared to their intermediate progenitors (hematopoietic progenitor cells, HPCs) and separately generated hiPSC-macrophages (iMϕ) (Figure 1c). iMGLs responded to 24-hour LPS treatment by significant reduction of *ITGAM, TREM2* and *P2RY12* gene expression (p= 0.029 in all, Mann-Whitney test). No significant batch differences were discovered (Suppl. figure 1b).

We generated iMGLs from a total of five monozygotic twin pairs, where one twin was diagnosed with SCZ (ST) and the other twin was healthy, but had thus a high genetic risk to develop SCZ (HT). Additionally, we generated iMGLs from five non-related healthy individuals (CTRL) (Figure 1d, Suppl. table 1). In order to identify disease-related expressional changes, we performed bulk RNA sequencing for four twin lines and four CTRL lines and compared group expressions to identify differentially expressed genes (DEGs). One of the twin pairs (pair 4) was excluded from RNA sequence due to poor sample quality (these lines produced consistently less iMGLs compared to the other lines and had abnormal activation sensitivity in culture). We first looked at the expression of common homeostatic microglial markers as their downregulation has been previously implicated in SCZ (25). None of analyzed markers showed within-pair differences, but the twins differed from CTRLs. *AIF1* (Iba1) was significantly upregulated in both ST and HT iMGLs compared to CTRLs by nominal p-value (ST p= $1.2x10^{-9}$, HT p= 0.032) and ST also by Bonferroni corrected p-value (adj.p= $1.3x10^{-6}$) (figure 1e). Also *PTPRC* (CD45) was upregulated in ST twins compared to CTRL iMGL (p= 0.0012, adj.p= 0.0081), while *TREM2* was downregulated in STs (p= 0.003) and *TMEM119* downregulated in HTs (p= 0.030) compared to CTRL. Other analyzed microglia-specific markers showed no significant group difference, e.g. *P2RY12, CX3CR1, ITGAM*, and *PROS1* (Suppl. figure 1c).

Twins with SCZ had aberrant expression of inflammatory genes and reduced IL6 response to IL1β

HLA-DR is one of the commonly used markers for microglial activation in postmortem studies (10,26,27). Expression of *HLA-DRA,* as well as many other HLA class II genes, was upregulated in our ST iMGLs vs. CTRL iMGLs (HLA-DRA p=$5.2x10^{-5}$, adj.p= 0.0049) (Figure 2a, Suppl. figure 2). The expression of pro-inflammatory cytokine *IL1β* was upregulated in ST vs. CTRL iMGLs (p= 0.021). On the other hand, *TSPO*, a controversial marker for inflammation used in PET imaging studies showed no significant alterations (28).

Microglia are extremely motile and survey their environment for injuries and pathogens. In response to ATP and ADP, molecules that are known to be secreted during neuronal damage, iMGLs showed more than five-fold increased migration (Figure 2b). ST iMGLs responded normally to both ATP and ADP, as the number of migrated iMGL cells did not differ between any of the groups (Kruskal-Wallis test). Next, we checked whether ST, HT and CTRL iMGLs respond differently to pro-inflammatory cytokines (Il1β, TNFα and IFNγ) measured as induced release of cytokines IL6, interleukin 8 (IL8) and TNFα and chemokine monocyte chemoattractant protein-1 (MCP1) 24 hours after exposure. In unstimulated conditions, release of IL6 and TNFα was undetectable. CTRL iMGLs significantly increased the release of IL6 (p= 0.030) and IL8 (p= 0.019) after all the cytokine treatments, while ST iMGLs significantly increased only MCP1 release (p= 0.0009, Friedman test). Between ST and CTRL

iMGLs, STs had significantly reduced IL6 response to IL1β treatment (p= 0.029, Mann-Whitney test). These data indicate that ST iMGLs are less responsive to central cell-to-cell pro-inflammatory signals.

Recognition of pathogens is the first step of innate immune activation and thus precedes pro-inflammatory cytokine release. Toll-like receptors (TLRs) detect various viral, bacterial and fungal structures. Interestingly, we observed that *TLR2* expression was significantly downregulated in ST iMGLs compared to CTRLs (p= 0.003), while *TLR4* receptor showed no difference (Figure 2e). To find out whether the decreased *TLR2* expression of ST iMGLs is reflected as an altered ability to detect pathogens and phagocytose them, we incubated iMGLs with pHrodo-labeled zymosan bioparticles (Figure 2f), which are recognized through TLR2, TLR6 and non-TLR receptor CLEC7A (29,30). A six-hour live imaging ST iMGL showed a slightly reduced phagocytosis of zymosan particles, but the reduction was not statistically significant (p=0.70 Mann-Whitney test) (Figure 2g-h).

Expression of neurotrophic factors and neurotransmitter receptors was reduced in SCZ iMGLs

In addition to neuroinflammation regulation, promotion of neurogenesis and synaptogenesis by neurotrophic factor secretion is another important function of microglia (31). Especially brain-derived neurotrophic factor (BDNF) released by microglia enhances learning-related synapse formation (32). BNDF is also one of the most considered biomarkers for SCZ, which has been found to be reduced in plasma samples in SCZ (33). Interestingly, *BDNF* was significantly less expressed in both ST and HT iMGLs compared to CTRL iMGLs (p= 0.005 and p= 0.008, respectively) (Suppl.figure 3a). iMGLs expressed also glial cell line-derived neurotrophic factor (*GDNF*), which has been reported to show *de novo* expression in microglia/macrophages upon neuroinflammation or injury (34), but its expression was similar in all three groups (Suppl.figure 3a). Another neurotrophic factor that showed altered expression in ST iMGLs was mesencephalic astrocyte-derived neurotrophic factor (*MANF*), which in turn was upregulated in ST iMGLs vs. CTRL (p= 0.006, adj.p= 0.008). MANF has been reported to induce anti-inflammatory microglia polarization after endoplasmic reticulum -associated stress (35).

Microglia express different neurotransmitter receptors for communicating with neurons and glia (36–38). We found that especially N-methyl-D-aspartate (NMDA)-receptor gene *GRIN2D* was highly expressed in iMGLs and showed downregulation in HT vs. CTRL (p= 0.015) (Suppl. figure 3b). Other neurotransmitter genes, such as kainate receptor *GRIK2* and GABA-receptor *GABRA2* were expressed only in low level, but were significantly downregulated in ST and HT compared to CTRL iMGLs (ST *GRIK2* p=0.0012; ST *GABRA2* p= 0.0035, and HT *GABRA2* p= 0.0045).

The C4 gene contributes to activation of complement system gene C3 to induce synaptic pruning (14). Even though no microglial complement genes (C1Q and C3) were expressed differently in ST or HT iMGLs (Suppl.figure 3c), the reduction in neurotrophic factors and neurotransmitter expression suggests reduced potential for synaptic remodeling in microglia from patients with SCZ.

Global downregulation of genes was associated with increased risk to develop SCZ and upregulation of MHC region to clinical manifestation of SCZ

We further examined comprehensively the DEGs between the study groups (Figure 3a). While the ST twins differed significantly from the HT twins only by 2 DEGs (adj.p<0.05, abs log2FC>1), we found more expressional changes related to clinical illness (ST vs. CTRL, 456 DEGs) than increased risk to develop SCZ (HT vs. CTRL, 123 DEGs) (Suppl. Data file 1-3). Interestingly, most of the SCZ-related gene expression changes were reductions, as 77.4 % of the DEGs in ST vs. CTRL and 99.2 % in HT vs. CTRL

were downregulations. These comparisons overlapped by 66 DEGs, which is 53.7 % of altered expression changes in the HT vs. CTRL comparison and 14.5 % in the ST vs. CTRL comparison (Figure 3b).

Next, we used Ingenuity Pathway Analysis (IPA) to identify canonical pathways related to DEGs in ST or HT vs. CTRL comparisons in iMGLs. Both twin comparisons to CTRL group shared the first four pathways, which together with Wound Healing Signaling Pathway and Pulmonary Fibrosis Idiopathic Signaling Pathway contained Hepatic Fibrosis / Hepatic Stellate Cell Activation (-log10(p-value)= 11.5 in ST vs. CTRL and 10.9 in HT vs. CTRL) and GP6 Signaling Pathway (-log10(p-value)= 5.1 in ST vs. CTRL and 4.5 in HT vs. CTRL). These pathways include genes encoding extracellular matrix molecules and have been identified in previous studies with the same cohort to be involved in hiPSC-neuron and hiPSC-astrocyte dysregulation in ST (39,40) (Figure 3 c-d, Suppl. Data files 4-5).

In order to study down- and upregulated DEGs separately, we used additionally Gene Ontology (GO) term analysis (Suppl. Data file 6-8). Downregulated GO Molecular function terms were similar between ST vs. CTRL and HT vs. CTRL (Figure 3e,g). The most significant functions were Extracellular matrix structural constituent (-log10(p-value)= 16.4 in ST vs. CTRL and 11.8 in HT vs. CTRL), Extracellular matrix structural constituent conferring tensile strength(-log10(p-value)= 11.1 in ST vs. CTRL and 7.3 in HT vs. CTRL), and Heparin binding (-log10(p-value)= 6.3 in ST vs. CTRL and 4.7 in HT vs. CTRL) terms. These pathways shared several different collagen genes.

On the other hand, the upregulated GO terms in STs compared to CTRLs were related to Protein heterodimerization (-log10(p-value)= 11.0) and MHC class II receptor activities (-log10(p-value)= 2.3) (Figure 3f). The first functional term contains several histone modification genes of which Clustered Histone genes *H3C12, H2BC17, H2AC13, H3C10*, and *H3C11* (5/18 DEGs) belong to a small cluster of histone genes in chromosome 6p22.1, which is also in close proximity of MHC region (Suppl. Data file 7) (41). Also nine out of 18 DEGs belonged to a large histone cluster in 6p22.2. MHC class II receptor activity term consists of HLA-genes (*HLA-DQA2* and *HLA-DQB2* genes in Suppl. figure 2). Thus, clinical manifestation of SCZ is associated with activation of chromosome 6 MHC region in iMGLs.

Heterogeneous response to different drug treatments in twins with SCZ

As STs showed specific dysregulations to several SCZ-associated genes, we tested if these transcriptomic changes can be rescued either with clozapine, an efficient atypical antipsychotic drug, minocycline, a tetracycline antibiotic proposed also as treatment of negative symptoms in SCZ due to its anti-inflammatory properties, and sulforaphane, an organosulfur isothiocyanate antioxidant with histone deacetylase inhibitory properties proposed for treatment of cognitive functions for patients with SCZ. We selected the drug concentration range from previously used cell culture studies with primary murine microglia or BV2 cell line microglia (15,21,42), and tested the suitable concentration by viability assay. Clozapine and sulforaphane had dose-dependent decreased effect on cell viability after 24 hours, while minocycline showed no clear toxicity up to 100 µM concentration (Figure 4a).

We tested the drug effect on phagocytosis, as minocycline has been previously shown to reduce phagocytosis of synaptic material dose-dependently in monocyte-derived microglia (15). Phagocytosis was significantly increased after 24-hour treatment with 10 µM sulforaphane, but no significant changes were discovered after clozapine or minocycline treatment (Suppl. figure 4a,c). We repeated the experiment with iMϕs, but again only sulforaphane significantly increased phagocytosis of zymosan, and the highest used concentration of clozapine and sulforaphane were toxic and caused significant reduction in phagocytosis (Suppl. figure 4b,d). As we could not detect any

response in phagocytosis of zymosan after minocycline treatment, this drug might inhibit only complement system mediated synapse phagocytosis as previously reported (15).

We continued using drug concentrations which did not affect viability (clozapine 10 µM, minocycline 10 µM and sulforaphane 5 µM), and performed RNA sequencing for drug treatments as well. All of the three drugs have been suggested to suppress NF-κB pro-inflammatory pathway activation as one beneficial mechanism. NF-κB downstream transcription factor *FOS* was significantly downregulated in CTRL iMGLs after 24-hour minocycline and sulforaphane treatments, but not after clozapine treatment (Figure 4b). However, the same downregulation was not seen in ST or HT twins untreated vs treated. None of the drugs had any significant effect on IL8 and MCP-1 release (suppl. figure 4e-g).

As clozapine inhibits NF-κB activation by inhibiting Akt pathway and sulforaphane promotes anti-inflammatory activation and microglia process elongation through Akt activation (21,43), we performed western plot for phosphorylated NF-κB and Akt for two male twin pairs. Clozapine reduced both Akt and NF-κB phosphorylation, and sulforaphane reduced NF-κB phosphorylation in HT (Suppl. figure 5a,b). In STs, we detected reduction of Akt phosphorylation after both clozapine and sulforaphane treatment, whereas the response of NF-κB phosphorylation varied. Sulforaphane especially induces NRF2 pathway, and this pathway's downstream targets *GCLM* and *HMOX1* were significantly upregulated on transcriptomic level in CTRL and HT untreated vs. sulforaphane as expected, but not in ST (Suppl. figure 5c).

ST microglia appeared non-responsive to drug treatments, so next we compared the significant DEGs between untreated and drug-treated in CTRL, ST or HT groups (p-value<0.05, abs log2FC >1). Interestingly, especially ST iMGLs had heterogeneous response to drug treatments (Figure 4 c clozapine, d minocycline, e sulforaphane, suppl. data file 12-17). While iMGLs of all CTRL lines responded similarly to drug treatments, iMGLs of ST1 patient had stronger response to clozapine, iMGLs of ST3 and ST5 patients a stronger response to minocycline and iMGLs of ST3 patient a stronger response to sulforaphane than iMGLs of other ST patients. Note that we had data from ST5 iMGL response only after minocycline treatment. The iMGLs of different HT lines showed consistent responses to both clozapine and minocycline treatments, with exception of HT5 iMGLs, which responded differently after clozapine and minocycline treatments.

Sulforaphane has been previously shown to upregulate BDNF in BV2 microglia cell line (20). While we did not detect upregulation of *BDNF* after sulforaphane treatment in iMGLs of any individual, we noticed a significant upregulation of *BDNF* expression after clozapine and minocycline treatments in ST iMGLs (Figure 4f). However, while minocycline has been shown to reduce expression of MHC class II genes, *TLR2* and *IL1β* in rodent microglia (17,18,44), minocycline treatment did not results in such gene expression changes in our iMGL cells of any individual. Instead, *HLA-DRA* expression was downregulated in CTRL but not ST or HT iMGL group after sulforaphane treatment. Similarly, *IL1β* expression was reduced only in CTRL iMGL group after clozapine and sulforaphane. None of the treatments resulted in altered *TLR2* expression in any iMGL group.

ST twin iMGLs did not activate differently after LPS-induced inflammation

Lipopolysaccharide (LPS) stimulation is the most common way to induce microglial inflammation. LPS activation occurs especially through TLR4-mediated NF-κB pathway activation. In order to see whether ST or HT iMGLs are more sensitive for LPS-induced activation, we treated iMGLs for 24 hours with LPS and measured the cytokine release and phagocytosis of zymosan bioparticles. However, no differences were observed in IL6, IL8 and MCP1 secretion after LPS treatment between the groups

(Figure 5a). Similarly, LPS had no significant effect on phagocytosis in any of the groups (p=0.49, CTRL; 0.54, ST; 0.63, HT; Friedman test) (Figure 5b). Clozapine, minocycline and sulforaphane have all been reported to suppress LPS-induced activations of mouse primary microglia or BV2 microglia (21,22,42,44,45). However, none of the drugs added one hour prior to LPS was able to reduce IL6, IL8, MCP1 or TNFα secretion.

Discussion

Microglia are brain residential immune cells which have arisen from the yolk sac and migrated to brain during development. Microglia are thus the first developed glial cell type in the brain. hiPSC differentiation allows us to generate a pure population of human microglia cell cultures (24,46). Human iMGLs are distinct from hematopoietic stem cells and monocytes and cluster closely with cultured human fetal and adult microglia by their RNA expression profile, as previously described (24). We also showed in this study that iMGL gene expression was different than in hiPSC-derived macrophages. All our hiPSC-lines included in the experiment were able to differentiate into iMGLs and by their morphology and marker expression resembled resting microglia. Even though pair 4 was excluded from RNA sequencing, we were able to include the lines to the other experiments after preparing additional iMGL batches and careful quality controls.

In this study, we showed that microglia from the patients with SCZ have a strong genetic component contributing to dysregulation of homeostatic and inflammatory gene expression. To our knowledge, this is the first time that hiPSC-microglia have been derived from patients with SCZ. So far, human microglia are mostly studied from postmortem tissue by their morphology, cell density, and the expression of different cellular markers in particular brain areas (26,27). Increase of microglial inflammation markers in postmortem SCZ brains is commonly detected when studying microglia density in different brain regions, although there are contradictive results as well (10,11,25,27). HLA-DRA cell density has been found increased in several studies, but gene expression studies have shown no difference in patients with SCZ (25,27,47). Similar variation has seen in cytokine and chemokine expression studies (27). However, analyses of postmortem samples represent only the late stage changes, and are affected by antipsychotic drugs while iMGLs recapitulate microglia phenotype that is only genotype-dependent.

Chronic hyperactivation leading to microglia priming has been proposed to be characterized by higher expression of inflammatory markers, higher sensitivity for activation, and exaggerated inflammatory response, when compared to unprimed microglia (48). Although iMGLs from patients with SCZ had increased transcriptomic expression of several inflammatory markers, they showed no hyperactivation in a sense of increased cytokine production or increased migration or phagocytosis. Moreover, LPS-induced inflammation had no additional effect on iMGL proinflammatory cytokine release or phagocytosis. The latest studies have pointed out that only a subgroup of patients with SCZ show signs of increased inflammation (10). Thus, it is possible that the patient iMGLs would not develop high inflammatory responses as hypothesized earlier.

The MHC locus is one of the earliest reported sites on the human genome associated with SCZ and the strongest association with SCZ in GWAS (13,49). Microglia are the main MHC class II -expressing antigen-presenting cell in the brain, which can attract antigen-specific CD4+ T lymphocytes to sites of inflammation (50,51). Our data suggest that upregulation of MHC class II genes is associated with clinical manifestation of SCZ. In fact, postmortem studies have found increased lymphocyte infiltration in a subset of patients with SCZ (52,53). Upregulation of MHC class II genes could be early pathophysiological change that leads to SCZ manifestation later in life. According to a new hypothesis

infiltrated regulatory T lymphocytes activate astrocytes, which in turn by increasing TGFβ secretion force microglia to sustain in non-inflammatory state and promote microglial phagocytosis and pruning in patients with SCZ (54,55). Further studies with co-cultures, cerebral organoids and xenotransplantation of microglia (46) are needed to assess the interaction between different cell types and their effect on microglia functions.

Downregulation of microglia transcriptome in SCZ have been shown by studies integrating RNA-sequence and genetic data (56). Downregulation of extracellular matrix constitution genes were detected from both twins compared to healthy individuals. Alterations in hepatic fibrosis and GP6 signaling pathways in microglia from patients with SCZ are shared with our previous studies in hiPSC-cortical neurons and hiPSC-astrocytes of the same individuals (39,40) indicating universal dysregulation of these pathways. The hepatic fibrosis pathways consists of mostly different collagen genes, which are not liver-specific despite the name of the pathway. Extracellular matrix molecules have multiple roles during development to support neuronal migration and synapse formation (57). Extracellular matrix components may induce or suppress microglial activation, their proliferation, migration and production of inflammatory cytokines as their ability to regulate synaptogenesis and neuronal transmission (57–59).

Neurogenesis is strongly influenced by microglia in the production, maturation, and integration of new neurons into circuitry by inducing neurotrophic factors and pruning weak synapses (31). Additionally, microglia-derived pro-inflammatory molecules have been shown to inhibit neurogenesis (60,61). We did not analyze whether our patient iMGLs had altered synaptic pruning, but expression of neurotrophic factors and transmitter receptors indicate microglia-regulated modulation of neural maturation and neural transmission in patients with SCZ independently from their possible role in synaptic pruning.

In summary, these data suggest that the increased risk to develop SCZ is associated with cell-autonomous downregulation of extracellular matrix and growth factor binding related transcriptional changes, and upregulation of inflammatory genes with clinical illness in microglia, without microglial hyperactivation. Microglia responses to clozapine, minocycline and sulforaphane differed from each other especially for individual patients in the group of SCZ. These findings demonstrate that drug candidates for treatment of SCZ and possibly for other mental disorders need to be tested in patient-derived models and not only in material derived from animals or healthy individuals.

Limitation of the study

Microglia adapt rapidly to environment changes and *ex vivo* microglia change their expression profile compared to *in vivo* (62). Thus, cultured microglia may not capture all expressional changes, but human microglia states are conserved across different *in vivo* and hiPSC-microglia experimental models although some key homeostatic genes have lower expression (63). The most common reason for a sample exclusion was spontaneous activation of microglia during differentiation, leading to cell apoptosis. This was more batch-specific than related to disease status. As some of the observed differences between the groups were high but not significant, availability of additional twin pairs discordant for SCZ could have increased the statistical power of our findings. Additional studies with large patient cohorts are needed to further validate our findings.

Acknowledgements

We thank technicians Anne Nyberg and Agnes Viherä for their help in cell culture.

Conflict of Interest

The authors declare no competing interests.

Funding

Jari Koistinaho has been supported by the Academy of Finland (grant 334525), Business Finland and Sigrid Juselius Foundation. Jaakko Kaprio has been supported by the Academy of Finland (grant 336823).

Author contributions

Jari Tiihonen and Jari Koistinaho conceived the study. Marja Koskuvi planned the experiments, differentiated microglia, prepared RNA and ICC samples, performed live cell experiments (phagocytosis, migration, MTT), run qRT-PCR, and assisted with CBA. Elina Pörsti assisted in microglia differentiation, performed ICC stainings, WB, and imaging, processed RNA sequencing data analysis with IPA and GO term analyses, and run CBA. Ying Chieh Wu established hiPSC-macrophage protocol and differentiated hiPSC-macrophages, assisted with CBA and WB. Kalevi Trontti, Iiris Hovatta performed RNA sequencing samples, supervised sequencing analyses and contributed to the interpretation of the results. Amanda McQuade and Mathew Blurton-Jones established the iMGL protocol and guided in iMGL differentiation. Tyrone D. Cannon, Jouko Lönnqvist, Sebastian Therman, Jaana Suvisaari, and Jaakko Kaprio gathered the data on twin pairs. Ilkka Ojansuu and Olli Vaurio performed skin biopsies and rating of symptoms. Jari Tiihonen and Markku Lähteenvuo coordinated patient data collection and contributed to the interpretation of the results. Šárka Lehtonen supervised microglia differentiation. Taisia Rolova supervised differentiation of microglia and macrophages, and experiments with phagocytosis, CBA and WB and contributed to the interpretation of the results. Marja Koskuvi wrote the first draft of the manuscript and prepared the figures and tables with the help from Jari Koistinaho, Jari Tiihonen and Elina Pörsti.

References

1. Fond G, Lançon C, Korchia T, Auquier P, Boyer L. The Role of Inflammation in the Treatment of Schizophrenia. Front Psychiatry. 2020 Mar 18;11:160.

2. Murphy CE, Walker AK, Weickert CS. Neuroinflammation in schizophrenia: the role of nuclear factor kappa B. Transl Psychiatry. 2021 Dec;11(1):528.

3. Siniscalco D, Schultz S, Brigida A, Antonucci N. Inflammation and Neuro-Immune Dysregulations in Autism Spectrum Disorders. Pharmaceuticals. 2018 Jun 4;11(2):56.

4. Dunn GA, Nigg JT, Sullivan EL. Neuroinflammation as a risk factor for attention deficit hyperactivity disorder. Pharmacol Biochem Behav. 2019 Jul;182:22–34.

5. Benedetti F, Aggio V, Pratesi ML, Greco G, Furlan R. Neuroinflammation in Bipolar Depression. Front Psychiatry. 2020 Feb 26;11:71.

6. Jones GH, Vecera CM, Pinjari OF, Machado-Vieira R. Inflammatory signaling mechanisms in bipolar disorder. J Biomed Sci. 2021 Dec;28(1):45.

7. Raison CL, Miller AH. Is Depression an Inflammatory Disorder? Curr Psychiatry Rep. 2011 Dec;13(6):467–75.

8. Monji A, Kato T, Kanba S. Cytokines and schizophrenia: Microglia hypothesis of schizophrenia: Cytokines and schizophrenia. Psychiatry Clin Neurosci. 2009 Jun;63(3):257–65.

9. Gober R, Ardalan M, Shiadeh SMJ, Duque L, Garamszegi SP, Ascona M, et al. Microglia activation in postmortem brains with schizophrenia demonstrates distinct morphological changes between brain regions. Brain Pathol [Internet]. 2022 Jan [cited 2022 Jun 8];32(1). Available from: https://onlinelibrary.wiley.com/doi/10.1111/bpa.13003

10. Fillman SG, Cloonan N, Catts VS, Miller LC, Wong J, McCrossin T, et al. Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia. Mol Psychiatry. 2013 Feb;18(2):206–14.

11. Steiner J, Mawrin C, Ziegeler A, Bielau H, Ullrich O, Bernstein HG, et al. Distribution of HLA-DR-positive microglia in schizophrenia reflects impaired cerebral lateralization. Acta Neuropathol (Berl). 2006 Sep;112(3):305–16.

12. Goldsmith DR, Rapaport MH, Miller BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. Mol Psychiatry. 2016 Dec;21(12):1696–709.

13. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014 Jul;511(7510):421–7.

14. Sekar A, Bialas AR, de Rivera H, Davis A, Hammond TR, Kamitaki N, et al. Schizophrenia risk from complex variation of complement component 4. Nature. 2016 Feb 11;530(7589):177–83.

15. Sellgren CM, Gracias J, Watmuff B, Biag JD, Thanos JM, Whittredge PB, et al. Increased synapse elimination by microglia in schizophrenia patient-derived models of synaptic pruning. Nat Neurosci. 2019 Mar;22(3):374–85.

16. Ormel PR, Böttcher C, Gigase FAJ, Missall RD, van Zuiden W, Fernández Zapata MC, et al. A characterization of the molecular phenotype and inflammatory response of schizophrenia patient-derived microglia-like cells. Brain Behav Immun. 2020 Nov;90:196–207.

17. Tikka T, Fiebich BL, Goldsteins G, Keinänen R, Koistinaho J. Minocycline, a Tetracycline Derivative, Is Neuroprotective against Excitotoxicity by Inhibiting Activation and Proliferation of Microglia. J Neurosci. 2001 Apr 15;21(8):2580–8.

18. Yrjänheikki J, Tikka T, Keinänen R, Goldsteins G, Chan PH, Koistinaho J. A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc Natl Acad Sci. 1999 Nov 9;96(23):13496–500.

19. Zhang J chun, Yao W, Dong C, Yang C, Ren Q, Ma M, et al. Prophylactic effects of sulforaphane on depression-like behavior and dendritic changes in mice after inflammation. J Nutr Biochem. 2017 Jan;39:134–44.

20. Tang R, Cao Q qian, Hu S wei, He L juan, Du P fei, Chen G, et al. Sulforaphane activates anti-inflammatory microglia, modulating stress resilience associated with BDNF transcription. Acta Pharmacol Sin. 2022 Apr;43(4):829–39.

21. Jeon S, Kim SH, Shin SY, Lee YH. Clozapine reduces Toll-like receptor 4/NF-κB-mediated inflammatory responses through inhibition of calcium/calmodulin-dependent Akt activation in microglia. Prog Neuropsychopharmacol Biol Psychiatry. 2018 Feb;81:477–87.

22. Kobayashi K, Imagama S, Ohgomori T, Hirano K, Uchimura K, Sakamoto K, et al. Minocycline selectively inhibits M1 polarization of microglia. Cell Death Dis. 2013 Mar;4(3):e525–e525.

23. Tiihonen J, Koskuvi M, Storvik M, Hyötyläinen I, Gao Y, Puttonen KA, et al. Sex-specific transcriptional and proteomic signatures in schizophrenia. Nat Commun. 2019 Dec;10(1):3933.

24. McQuade A, Coburn M, Tu CH, Hasselmann J, Davtyan H, Blurton-Jones M. Development and validation of a simplified method to generate human microglia from pluripotent stem cells. Mol Neurodegener. 2018 Dec;13(1):67.

25. Snijders GJLJ, Zuiden W, Sneeboer MAM, Berdenis van Berlekom A, Geest AT, Schnieder T, et al. A loss of mature microglial markers without immune activation in schizophrenia. Glia. 2021 May;69(5):1251–67.

26. Jurga AM, Paleczna M, Kuter KZ. Overview of General and Discriminating Markers of Differential Microglia Phenotypes. Front Cell Neurosci. 2020 Aug 6;14:198.

27. Trépanier MO, Hopperton KE, Mizrahi R, Mechawar N, Bazinet RP. Postmortem evidence of cerebral inflammation in schizophrenia: a systematic review. Mol Psychiatry. 2016 Aug;21(8):1009–26.

28. Plavén-Sigray P, Matheson GJ, Coughlin JM, Hafizi S, Laurikainen H, Ottoy J, et al. Meta-analysis of the Glial Marker TSPO in Psychosis Revisited: Reconciling Inconclusive Findings of Patient–Control Differences. Biol Psychiatry. 2021 Feb;89(3):e5–8.

29. Underhill DM. Macrophage recognition of zymosan particles. J Endotoxin Res. 2003 Jun 1;9(3):176–80.

30. Schorey J, Lawrence C. The Pattern Recognition Receptor Dectin-1: From Fungi to Mycobacteria. Curr Drug Targets. 2008 Feb 1;9(2):123–9.

31. Pérez-Rodríguez DR, Blanco-Luquin I, Mendioroz M. The Participation of Microglia in Neurogenesis: A Review. Brain Sci. 2021 May 18;11(5):658.

32. Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR, Lafaille JJ, et al. Microglia Promote Learning-Dependent Synapse Formation through Brain-Derived Neurotrophic Factor. Cell. 2013 Dec;155(7):1596–609.

33. Nieto RR, Carrasco A, Corral S, Castillo R, Gaspar PA, Bustamante ML, et al. BDNF as a Biomarker of Cognition in Schizophrenia/Psychosis: An Updated Review. Front Psychiatry. 2021 Jun 16;12:662407.

34. Duarte Azevedo M, Sander S, Tenenbaum L. GDNF, A Neuron-Derived Factor Upregulated in Glial Cells during Disease. J Clin Med. 2020 Feb 7;9(2):456.

35. Yang F, Li WB, Qu YW, Gao JX, Tang YS, Wang DJ, et al. Bone marrow mesenchymal stem cells induce M2 microglia polarization through PDGF-AA/MANF signaling. World J Stem Cells. 2020 Jul 26;12(7):633–58.

36. Liu H, Leak RK, Hu X. Neurotransmitter receptors on microglia. Stroke Vasc Neurol. 2016 Jun;1(2):52–8.

37. Domercq M, Vázquez-Villoldo N, Matute C. Neurotransmitter signaling in the pathophysiology of microglia. Front Cell Neurosci [Internet]. 2013 [cited 2022 Jul 29];7. Available from: http://journal.frontiersin.org/article/10.3389/fncel.2013.00049/abstract

38. Logiacco F, Xia P, Georgiev SV, Franconi C, Chang YJ, Ugursu B, et al. Microglia sense neuronal activity via GABA in the early postnatal hippocampus. Cell Rep. 2021 Dec;37(13):110128.

39. Tiihonen J, Koskuvi M, Lähteenvuo M, Trontti K, Ojansuu I, Vaurio O, et al. Molecular signaling pathways underlying schizophrenia. Schizophr Res. 2021 Jun;232:33–41.

40. Koskuvi M, Lehtonen Š, Trontti K, Keuters M, Wu Y, Koivisto H, et al. Contribution of astrocytes to familial risk and clinical manifestation of schizophrenia. Glia. 2022 Apr;70(4):650–60.

41. Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, Pe'er I, et al. Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature. 2009 Aug;460(7256):753–7.

42. Qin S, Yang C, Huang W, Du S, Mai H, Xiao J, et al. Sulforaphane attenuates microglia-mediated neuronal necroptosis through down-regulation of MAPK/NF-κB signaling pathways in LPS-activated BV-2 microglia. Pharmacol Res. 2018 Jul;133:218–35.

43. Wu Y, Gao M, Wu J, Hu P, Xu X, Zhang Y, et al. Sulforaphane triggers a functional elongation of microglial process via the Akt signal. J Nutr Biochem. 2019 May;67:51–62.

44. Henry CJ, Huang Y, Wynne A, Hanke M, Himler J, Bailey MT, et al. Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia. J Neuroinflammation. 2008 Dec;5(1):15.

45. Subedi L, Lee J, Yumnam S, Ji E, Kim S. Anti-Inflammatory Effect of Sulforaphane on LPS-Activated Microglia Potentially through JNK/AP-1/NF-κB Inhibition and Nrf2/HO-1 Activation. Cells. 2019 Feb 22;8(2):194.

46. Hasselmann J, Coburn MA, England W, Figueroa Velez DX, Kiani Shabestari S, Tu CH, et al. Development of a Chimeric Model to Study and Manipulate Human Microglia In Vivo. Neuron. 2019 Sep;103(6):1016-1033.e10.

47. Sinkus ML, Adams CE, Logel J, Freedman R, Leonard S. Expression of immune genes on chromosome 6p21.3–22.1 in schizophrenia. Brain Behav Immun. 2013 Aug;32:51–62.

48. Norden DM, Muccigrosso MM, Godbout JP. Microglial priming and enhanced reactivity to secondary insult in aging, and traumatic CNS injury, and neurodegenerative disease. Neuropharmacology. 2015 Sep;96:29–41.

49. Inta D, Lang UE, Borgwardt S, Meyer-Lindenberg A, Gass P. Microglia Activation and Schizophrenia: Lessons From the Effects of Minocycline on Postnatal Neurogenesis, Neuronal Survival and Synaptic Pruning. Schizophr Bull. 2016 Jun 27;sbw088.

50. Schetters STT, Gomez-Nicola D, Garcia-Vallejo JJ, Van Kooyk Y. Neuroinflammation: Microglia and T Cells Get Ready to Tango. Front Immunol. 2018 Jan 25;8:1905.

51. Roche PA, Furuta K. The ins and outs of MHC class II-mediated antigen processing and presentation. Nat Rev Immunol. 2015 Apr;15(4):203–16.

52. Schlaaff K, Dobrowolny H, Frodl T, Mawrin C, Gos T, Steiner J, et al. Increased densities of T and B lymphocytes indicate neuroinflammation in subgroups of schizophrenia and mood disorder patients. Brain Behav Immun. 2020 Aug;88:497–506.

53. Busse S, Busse M, Schiltz K, Bielau H, Gos T, Brisch R, et al. Different distribution patterns of lymphocytes and microglia in the hippocampus of patients with residual versus paranoid schizophrenia: Further evidence for disease course-related immune alterations? Brain Behav Immun. 2012 Nov;26(8):1273–9.

54. Murphy CE, Weickert CS. A new suspect in the unsolved case of neuroinflammation in schizophrenia. Mol Psychiatry. 2021 Dec;26(12):7105–6.

55. Corsi-Zuelli F, Deakin B. Impaired regulatory T cell control of astroglial overdrive and microglial pruning in schizophrenia. Neurosci Biobehav Rev. 2021 Jun;125:637–53.

56. Gandal MJ, Zhang P, Hadjimichael E, Walker RL, Chen C, Liu S, et al. Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. Science. 2018 Dec 14;362(6420):eaat8127.

57. Crapser JD, Arreola MA, Tsourmas KI, Green KN. Microglia as hackers of the matrix: sculpting synapses and the extracellular space. Cell Mol Immunol. 2021 Nov;18(11):2472–88.

58. Strackeljan L, Baczynska E, Cangalaya C, Baidoe-Ansah D, Wlodarczyk J, Kaushik R, et al. Microglia Depletion-Induced Remodeling of Extracellular Matrix and Excitatory Synapses in the Hippocampus of Adult Mice. Cells. 2021 Jul 22;10(8):1862.

59. Haw RTY, Tong CK, Yew A, Lee HC, Phillips JB, Vidyadaran S. A three-dimensional collagen construct to model lipopolysaccharide-induced activation of BV2 microglia. J Neuroinflammation. 2014 Dec;11(1):134.

60. Zhang J, Rong P, Zhang L, He H, Zhou T, Fan Y, et al. IL4-driven microglia modulate stress resilience through BDNF-dependent neurogenesis. Sci Adv. 2021 Mar 19;7(12):eabb9888.

61. Sato K. Effects of Microglia on Neurogenesis. Glia. 2015 Aug;63(8):1394–405.

62. Gosselin D, Skola D, Coufal NG, Holtman IR, Schlachetzki JCM, Sajti E, et al. An environment-dependent transcriptional network specifies human microglia identity. Science. 2017 Jun 23;356(6344):eaal3222.

63. Popova G, Soliman SS, Kim CN, Keefe MG, Hennick KM, Jain S, et al. Human microglia states are conserved across experimental models and regulate neural stem cell responses in chimeric organoids. Cell Stem Cell. 2021 Dec;28(12):2153-2166.e6.

Figure legends

Figure 1. Characterization of human iMGLs derived from monozygotic twins discordant for SCZ. a) Timeline for iMGL differentiation. Generated iMGLs expressed **b)** on protein level pan-macrophage marker IBA1, and microglia-specific markers P2Y12, TREM2 and CX3CR1 (nuclei on blue), and **c)** on transcriptomic level *AIF1* (IBA1), *ITGAM* (CD11b), *P2RY12* and *TREM2*. Kruskal-Wallis test, Mann-Whitney test, n=3-4 lines. **d)** Illustration of patient cohort created with BioRender. **e)** RNA expression of *AIF1*, *TREM2*, *PTPRC* (CD45) and *TMEM119* based on bulk RNA sequencing. Scale bar 50 µm. CTRL= healthy individuals; ST= affected twin; HT= healthy twin. HPC= hematopoietic stem cell, iMϕ= hiPSC-macrophage, iMGL= hiPSC-microglia like cell, iMGL+LPS = LPS-treated iMGL.

Figure 2. Inflammation, cytokine release and phagocytosis in iMGLs. a) Gene expression of inflammatory related *HLA-DRA*, *TSPO* and *IL1β* based on RNA sequencing. **b)** Representative image after 4-hour migration assay. Cells were masked with magenta for analysis. Scale bar 800 µm. **c)** The number of migrated cells normalised to unstimulated condition. Performed Kruskal-Wallis test (shown) and Mann-Whitney test (non-significant, not shown). **d)** Release of IL6, IL8 and MCP1 cytokines after 24 hours treatment with either 20 ng/ml IL1β, 20 ng/ml TNFα or 20 ng/ml IFNγ. Friedman test and Mann-Whitney test. **e)** Gene expression of *TLR2* and *TLR4*. **f)** Representative image from phagocytosis after 6 hours (Scale bar 100 µm) and **g)** phagocytosis of pHrodo-zymosan bioparticles. **h)** Phagosytosis between the groups after 6 hours. Mann-Whitney test. n= 4-5 cell lines.

Figure 3. iMGL expression profile and pathway analysis. a) Overview of differentially expressed genes (DEGs) in different comparisons. **b)** Venn diagram of shared DEGs between HT vs. CTRL and ST vs. CTRL. Ingenuity Pathway Analysis (IPA) canonical pathways **c)** from ST vs. CTRL and **d)** HT vs. CTRL comparisons. Cutt-offs: Fold change > abs 2, adjusted p. < 0.05. **e)** Downregulated and **f)** upregulated Gene Ontology (GO) Molecular function terms from ST vs. CTRL comparison and **g)** downregulated terms from HT vs. CTRL comparison. RNAseq n=4 lines in each group.

Figure 4. iMGL responses to different drug treatments. a) Viability of iMGLs after 24-hour treatment with clozapine, minocycline and sulforaphane. Tested with 4 independent experiments with two control, two HT and two ST lines (n=6 lines) (Wilcoxon test). **b)** NFκB pathway downstream gene *FOS*. Heatmaps of CTRL, ST and HT line gene expression changes after **c)** 10 µM clozapine **d)** 10 µM minocycline and **e)** 5 µM sulforaphane treatments. No data from ST5 patient after clozapine and sulforaphane. **f)** Gene expression differences after drug treatments in *BDNF, HLA-DRA, TLR2* and *IL1β*. RNAseq n=3-4 lines in each group.

Figure 5. Drug treatment protection from LPS-induced inflammation in iMGLs. a) IL6, IL8 and MCP1 secretion after 24-hour 100 ng/ml LPS treatment between the groups. Mann-Whitney test. **b)** LPS effect on pHrodo-zymosan phagocytosis. **c)** IL6, IL8, MCP1 and TNFa secretion after 30 min pre-treatment with 10 µM clozapine, 10 µM minocycline or 5 µM sulforaphane and 24-hour 100 ng/ml LPS treatment. Friedman test and Wilcoxon matched-pairs signed rank test.

Figure 1.

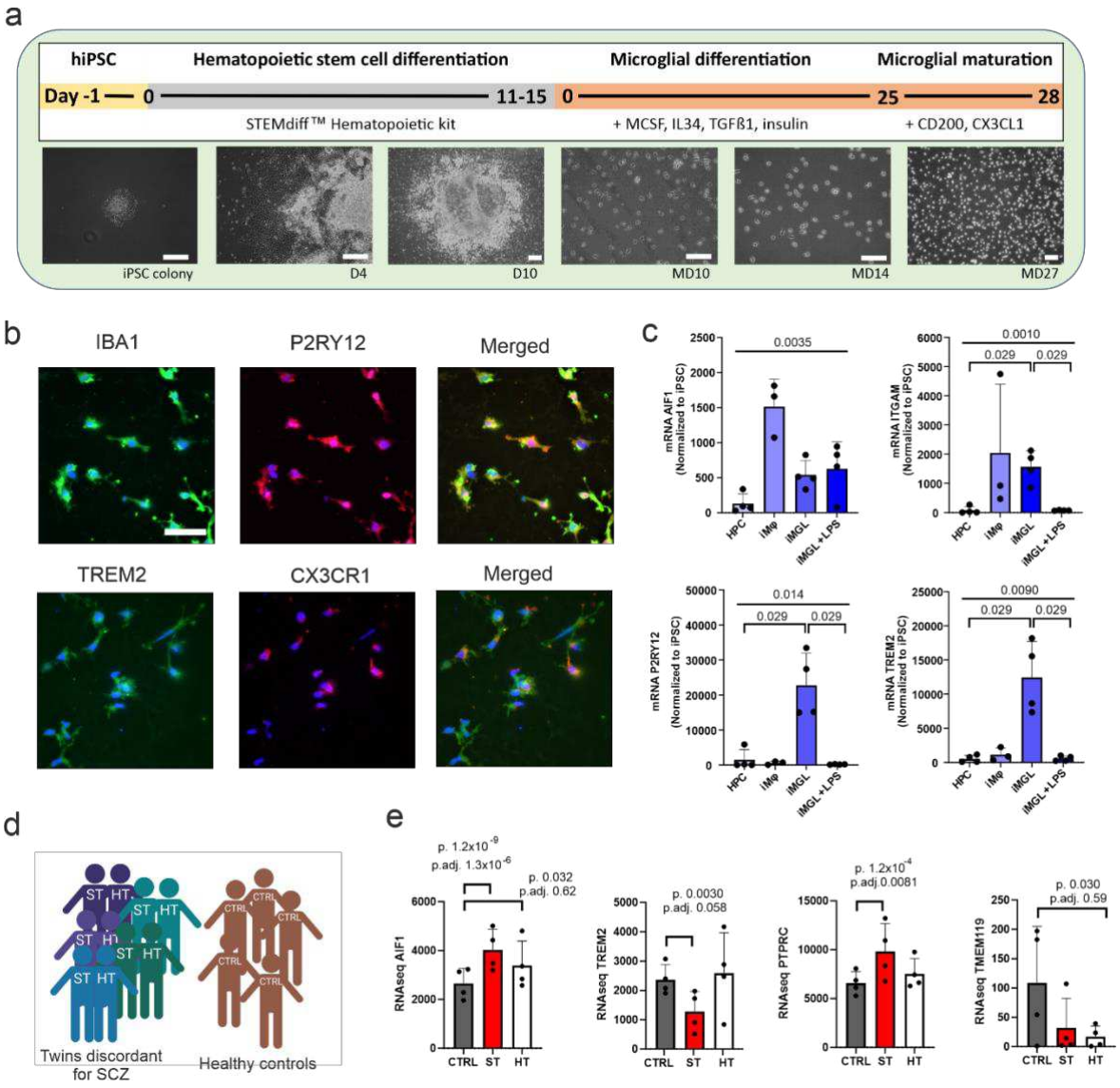

a
hiPSC
Hematopoietic stem cell differentiation
Microglial differentiation
Microglial maturation
Day -1
0
11-15
0
25
28
STEMdiff™ Hematopoietic kit
+ MCSF, IL34, TGFß1, insulin
+ CD200, CX3CL1
iPSC colony
D4
D10
MD10
MD14
MD27
b
IBA1
P2RY12
Merged
TREM2
CX3CR1
Merged
c
mRNA AIF1 (Normalized to iPSC)
mRNA ITGAM (Normalized to iPSC)
mRNA P2RY12 (Normalized to iPSC)
mRNA TREM2 (Normalized to iPSC)
HPC
iMφ
iMGL
iMGL+LPS
d
Twins discordant for SCZ
Healthy controls
e
RNAseq AIF1
RNAseq TREM2
RNAseq PTPRC
RNAseq TMEM119
CTRL
ST
HT

Figure 2.

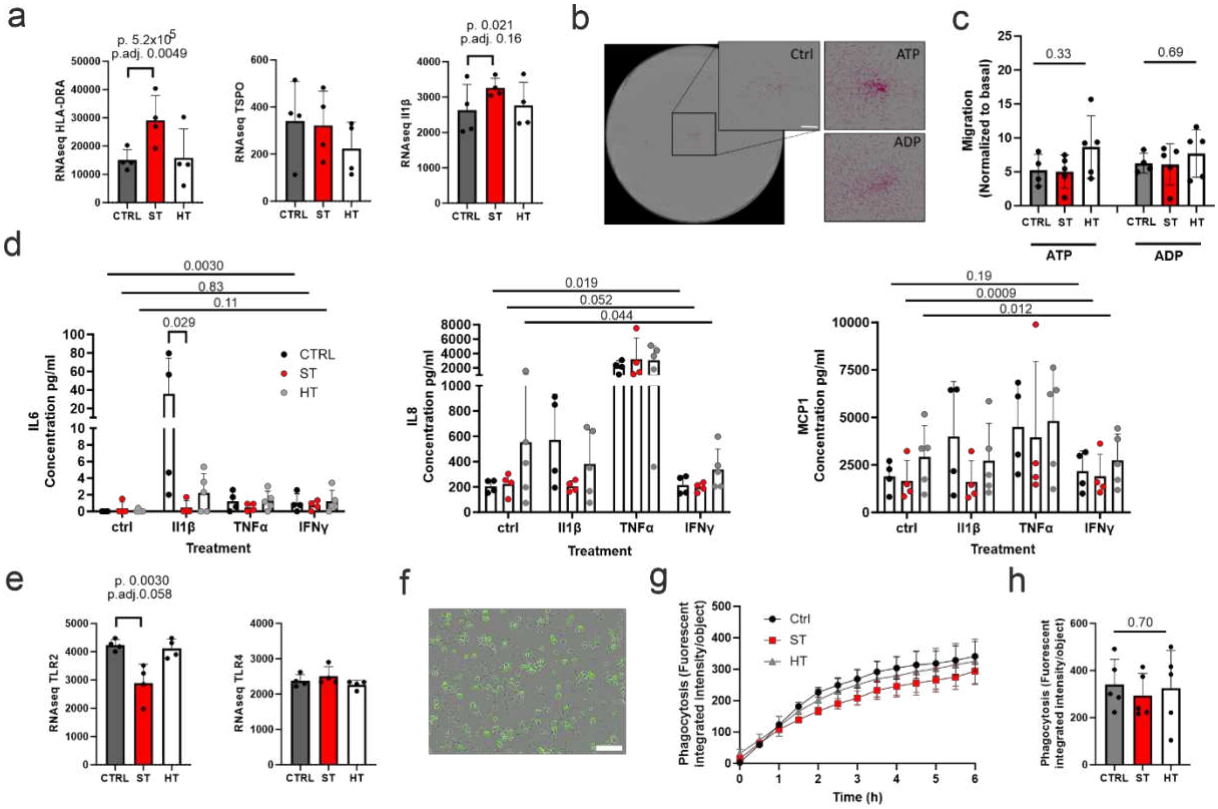


Figure 3.

a

Comparison	p<0.05 log2FC>|1|	adj.p <0.05, log2FC>|1|		
		Total	Down	Up
ST vs. HT	357	2	2	0
ST vs. CTRL	1586	456	353	103
HT vs. CTRL	1011	123	122	1

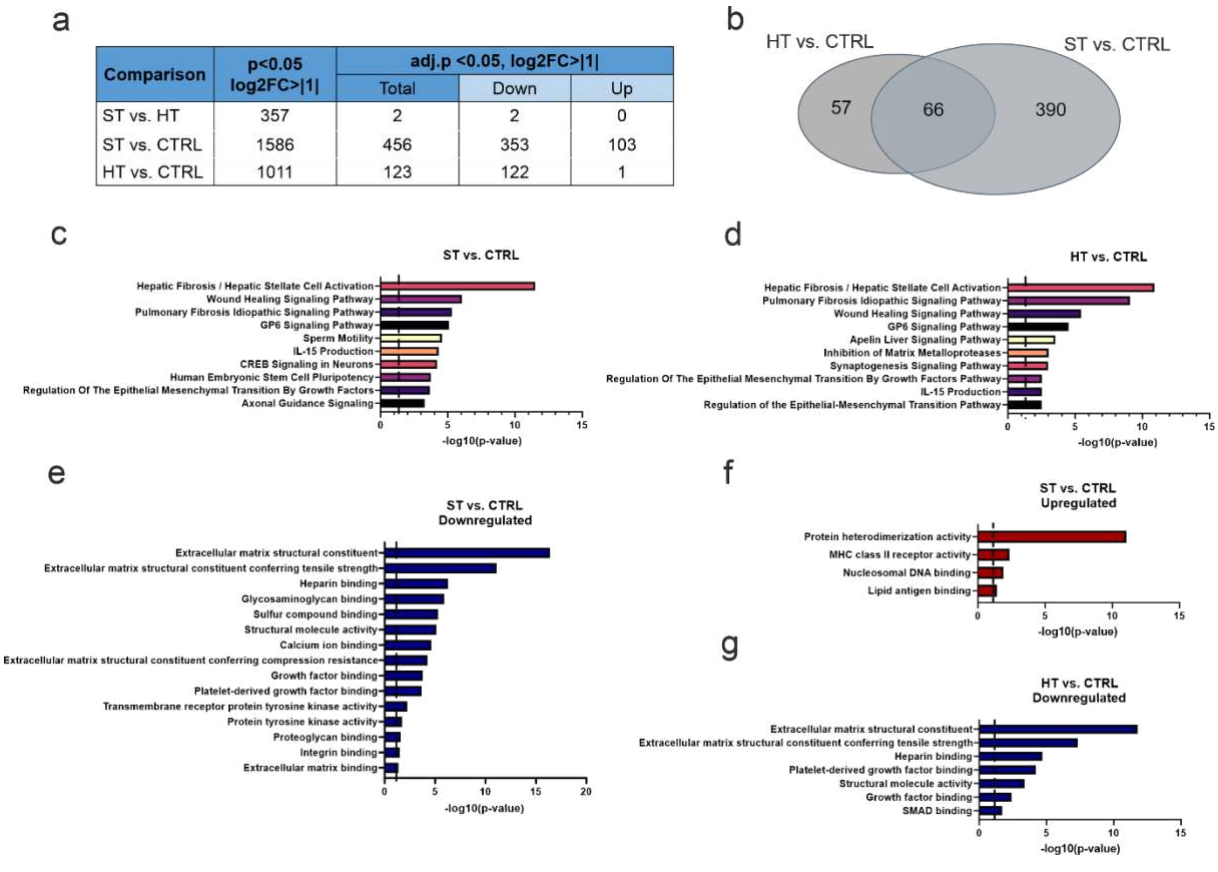


Figure 4.

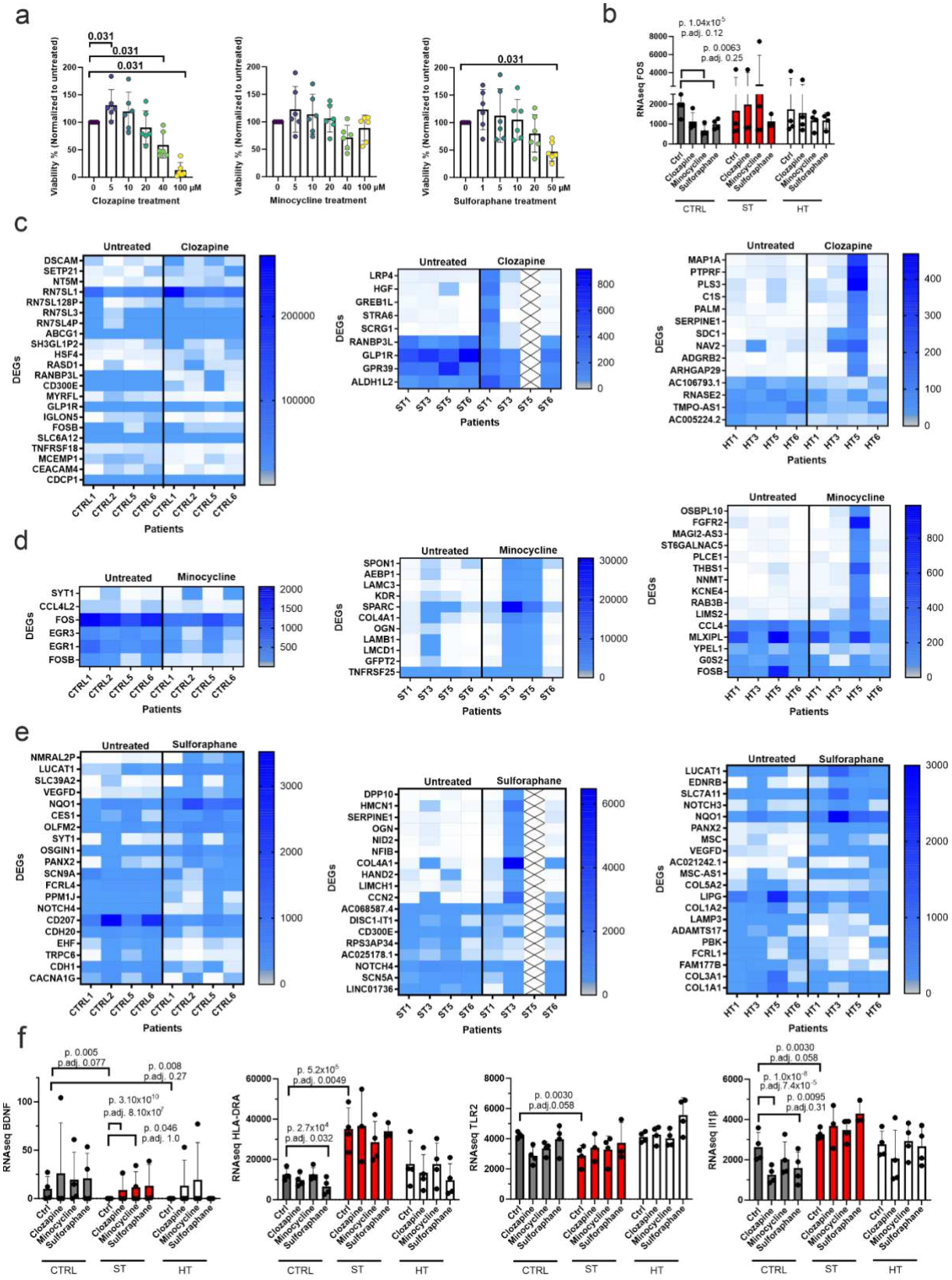
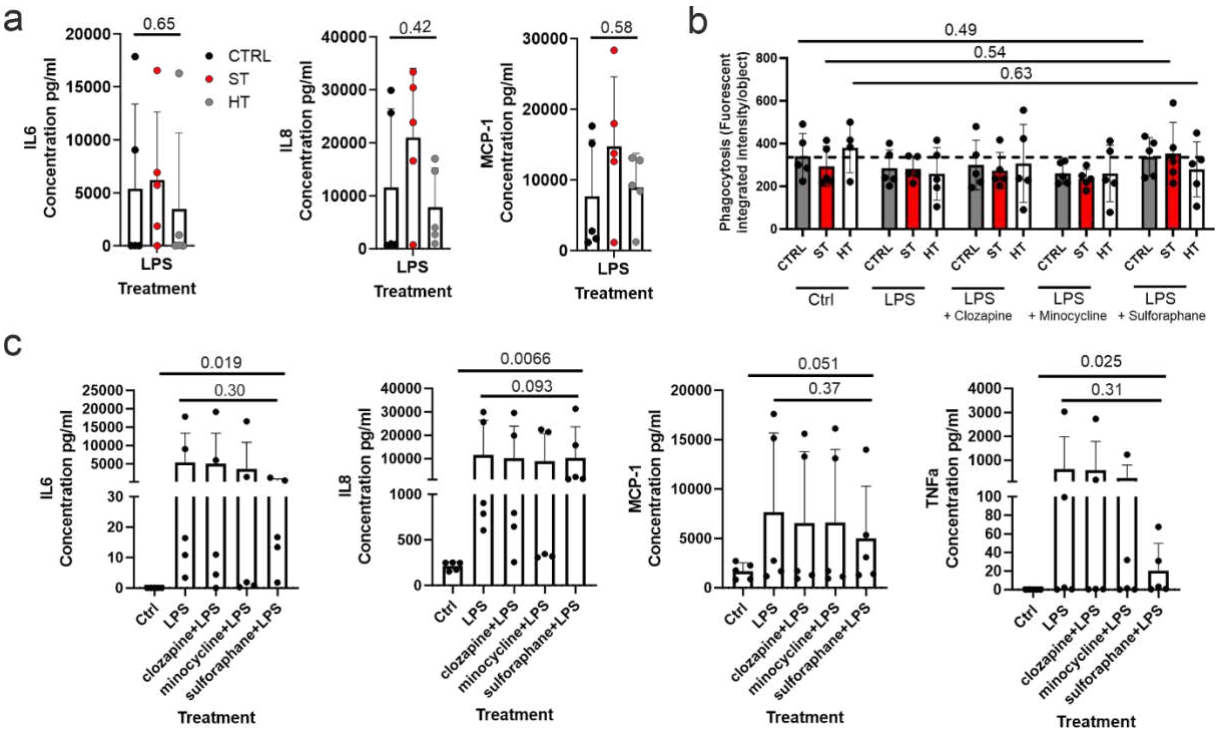

a
Viability % (Normalized to untreated)
Clozapine treatment
Minocycline treatment
Sulforaphane treatment
b
RNAseq FOS
CTRL
ST
HT
c
Untreated
Clozapine
DEGs
Patients
d
Untreated
Minocycline
e
Untreated
Sulforaphane
f
RNAseq BDNF
RNAseq HLA-DRA
RNAseq TLR2
RNAseq Il1β

Figure 5.


a
0.65
0.42
0.58
CTRL
ST
HT
IL6 Concentration pg/ml
IL8 Concentration pg/ml
MCP-1 Concentration pg/ml
LPS
Treatment
b
0.49
0.54
0.63
Phagocytosis (Fuorescent Integrated Intensity/object)
CTRL ST HT
Ctrl
LPS
LPS + Clozapine
LPS + Minocycline
LPS + Sulforaphane
c
0.019
0.30
0.0066
0.093
0.051
0.37
0.025
0.31
IL6 Concentration pg/ml
IL8 Concentration pg/ml
MCP-1 Concentration pg/ml
TNFa Concentration pg/ml
Ctrl
LPS
clozapine+LPS
minocycline+LPS
sulforaphane+LPS
Treatment

Supplementary Files

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

- MPSCZiMGLSupplementaryinformation.pdf
- MPSCZiMGLsuppldatafiles.xlsx