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Gene expression profiling of monocytes in recent-onset schizophrenia

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ABSTRACT

Immune–related mechanisms have been suggested to be involved in schizophrenia. Various studies have shown changes in monocytes isolated from the blood of schizophrenia patients, including changes in monocyte numbers, as well as altered protein and transcript levels of important markers. However, validation of these findings and understanding how these results are related to immune-related changes in the brain and schizophrenia genetic risk factors, is limited. The goal of this study was to better understand changes observed in monocytes of patients with early-onset schizophrenia. Using RNA sequencing, we analyzed gene expression profiles of monocytes isolated from twenty patients with early-onset schizophrenia and seventeen healthy controls. We validated expression changes of 7 out of 29 genes that were differentially expressed in previous studies including *TNFAIP3*, *DUSP2*, and *IL6*. At a transcriptome-wide level, we found 99 differentially expressed genes. Effect sizes of differentially expressed genes were moderately correlated with differential expression in brain tissue (Pearson's $r = 0.49$). Upregulated genes were enriched for genes in NF-κB and LPS signaling pathways. Downregulated genes were enriched for glucocorticoid response pathways. These pathways have been implicated in schizophrenia before and play a role in regulating the activation of myeloid cells. Interestingly, they are also involved in several non-inflammatory processes in the central nervous system, such as neurogenesis and neurotransmission. Future studies are needed to better understand how dysregulation of the NF-κB and glucocorticoid pathways affects inflammatory and non-inflammatory processes in schizophrenia. The fact that dysregulation of these pathways is also seen in brain tissue, provides potential possibilities for biomarker development.

1. Introduction

Schizophrenia is associated with an increased incidence of infections, immune-related disorders, and immune-related genetic associations (Benros et al., 2014; Khandaker et al., 2012a, 2012b; Ripke et al., 2014; Sekar et al., 2016). It is therefore hypothesized that the immune system contributes to schizophrenia pathogenesis. Which compartment of the immune system is changed and how this is related to the disease, is a subject of current studies. The immune system can be divided in the innate and adaptive immune system. Although associations between schizophrenia and the adaptive immune system have been found (Lynall et al., 2022; Miller et al., 2013; Sneeboer et al., 2020; van Mierlo et al., 2019), the role of the innate immune system has been investigated most extensively. Increased levels of innate immune system-related cytokines, chemokines, C-reactive protein (CRP) and the number of monocytes have been described in blood and cerebrospinal fluid (CSF) of schizophrenia patients (de Witte et al., 2014; Fernandes et al., 2015; Gallego et al., 2018; Goldsmith et al., 2016a; Mazza et al., 2019; Wang and Miller, 2018). A recent *meta*-analysis of *postmortem* brain transcriptome studies also showed an altered expression of innate immune networks, including NF-κB, interferon-signaling, and microglial networks (Gandal et al., 2018). Moreover, we have recently shown that around 10% of common variants associated with genetic risk to schizophrenia are associated with gene expression in monocytes (Lopes et al., 2022).

Monocytes are part of the peripheral innate immune system and involved in the first-line defense against invading pathogens and important for the initiation and control of inflammatory responses. During pathology, monocytes become activated, leave the bloodstream, and move into peripheral tissues to differentiate into tissue macrophages

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(Murphy and Weaver, 2017). The expression profile and function of monocytes partly overlap with myeloid cells present in the brain, including the perivascular macrophages and microglia. Studying monocytes in schizophrenia could therefore reveal specific monocyte-related disease mechanisms, as well as changes that are present in the entire myeloid cell population, including microglia and monocyte-derived brain macrophages.

Several studies have shown increased numbers of monocytes in the peripheral blood of patients with schizophrenia and first-episode psychosis (Mazza et al., 2020; Steiner et al., 2019), as well as CSF (Nikkilä et al., 1999). Meta-analyses have confirmed increased cytokine and inflammatory marker levels in blood and CSF, including IL-6, IL-8, and CRP. While IL-6 and IL-8 are produced primarily by innate immune cells, which includes monocytes, CRP is primarily produced in response to IL-6 by liver cells (Fernandes et al., 2015; Gallego et al., 2018; Goldsmith et al., 2016a,b; Marnell et al., 2005; Wang and Miller, 2018). Analyses of isolated monocytes using qPCR and flow cytometry have shown changes in the expression levels of inflammatory-related genes, such as *IL1B, IL6, TNF* and proteins, such as HLA-DR and TLR3, TLR4 (Summarized in Supplementary Table 1) (Chen et al., 2019; Drexhage et al., 2010; Kéri et al., 2017; Kowalski et al., 2001; Krause et al., 2012a; Krause et al., 2012b; Müller et al., 2012; Uranova et al., 2017; Weigelt et al., 2011). Functional analyses of monocytes showed an increased number of lysosomes (Uranova et al., 2017) and an altered response to inflammatory triggers (Chen et al., 2019; Kowalski et al., 2001; Krause et al., 2012a; Krause et al., 2012b; Müller et al., 2012; Uranova et al., 2017).

A recent transcriptome study by Melbourne et al. compared monocytes from schizophrenia patients (N = 14) with an average disease duration of 30 years with controls (N = 14) and found 389 differentially expressed genes. The differentially expressed genes were enriched for interferon, glucocorticoid and LPS response signatures (Melbourne et al., 2021). These signatures were more strongly dysregulated with progression of illness duration. In the current study we set out to further analyze how the phenotype of monocytes is changed in early-onset schizophrenia (<3 years). We isolated monocytes from a cohort of twenty patients with a recent-onset psychotic disorder and compared these to twenty non-psychiatric controls matched on body-mass index (BMI), age, and sex. The monocytes were analyzed for transcriptome-wide changes in gene expression using RNA sequencing. The first aim of our study was to analyze whether we could replicate previous findings observed in monocytes (Chen et al., 2019; Drexhage et al., 2010; Kéri et al., 2017; Krause et al., 2012a; Krause et al., 2012b; Melbourne et al., 2021; Müller et al., 2012; Weigelt et al., 2011), as well as whether monocytes display similar immune changes as also found in brain tissue of schizophrenia patients (Gandal et al., 2018). Our second aim was to analyze potential other dysregulated pathways.

2. Methods

2.1. Sample collection

Patients with a recent-onset psychotic disorder and non-psychiatric controls were recruited into the tissue bank of the University Medical Center (UMC) Utrecht. Blood samples were collected between 2014 and 2018 as part of the CONTROLS study and as part of the baseline assessment of a randomized controlled trial consisting of placebo-simvastatin administration to study the treatment effect of simvastatin in schizophrenia (Begemann et al., 2015; Sommer et al., 2021). Inclusion criteria of the simvastatin study included: recent-onset of psychotic symptoms (<3 years), diagnosis with schizophrenia-spectrum disorder (DSM-IV 295.xx), and age between 18 and 50 years. Exclusion criteria were: chronic use of corticosteroids, statins, other lipid-lowering drugs or non-steroidal anti-inflammatory drugs (NSAIDS), pregnancy or breast-feeding, active liver, kidney, or muscle disease. Inclusion criterion for the CONTROLS study was an age between 18 and 70 years, exclusion criteria were a psychiatric illness as determined with the Comprehensive Assessment of Symptoms and History (CASH) (Andreasen et al., 1992), a family history of psychiatric illness, chronic use of corticosteroids, statins, other lipid-lowering drugs, or NSAIDs, pregnancy or breastfeeding, presence of diabetes mellitus or severe heart failure. All subjects provided written informed consent for participation.

Sample size for RNA-seq studies can be calculated using transcriptome-wide data of a similar study. Unfortunately, we did not have access to the complete transcriptome-wide dataset of the study by Melbourne et al. (Melbourne et al., 2021), required to perform the analysis. We therefore based our sample size on the size of this transcriptome study (n = 14 for each group). In addition, we used the qPCR data of the Drexhage et al. (2010) study and estimated a total number of 39 samples as the upper bound of the sample size to detect significant differences. Since the required data for a formal power analysis was not available the true sample size needed to detect differences may vary. For the current study, we matched 20 schizophrenia patients with 20 subjects from the CONTROLS study based on age, BMI, and sex. Demographics are summarized in Table 1, and further details are provided in Supplementary Table 2.

2.2. Monocyte isolation

The blood samples were collected in sodium-heparin tubes from which peripheral blood mononuclear cells (PBMCs) were enriched using a ficoll-Paque centrifugation protocol by the tissue bank within 24 h after blood withdrawal. The cells were subsequently frozen in 10% DMSO and stored in liquid nitrogen. Monocytes were positively selected from PBMCs by $CD14^{+}$ magnetic associated cell sorting (MACs) according to manufacturer's protocol (Miltenyi, Germany, 130–050-201) (Melief et al., 2016). The cells were subsequently lysed in Trizol reagent (Life Technologies, 15596018).

2.3. RNA isolation, library preparation, and sequencing

RNA trizol samples were processed with the miRNeasy mini kit (Qiagen, The Netherlands, 217004) using manufacturer's protocol including a DNAse (Qiagen, The Netherlands, 79254) step. The mRNA concentration was determined using a VarioSkan Flash microplate reader (Thermo Scientific, MA). RNA integrity number (RIN) was assessed using the RNA 6000 Pico kit (Agilent Technology, USA, 5067–1513) and Agilent 2100 bioanalyzer according to manufacturer's protocol. cDNA synthesis and library preparation were performed with the SMART-Seq® v4 Ultra® Low Input RNA Kit (Takara Bio, USA, Inc., R634891, R634898, R638509) and the SMARTer® ThruPLEX® DNA-seq Kit (Takara Bio, USA, Inc., R400407) and low Input Library Prep Kit v2 (Takara Bio, USA, Inc., 634899). Both according to manufacturer's protocol with an input of 5 ng RNA per sample. Shearing was performed using a Covaris AFA system. SPRI reagent (Beckman Coulter, USA) was subsequently used for size selection of fragmented cDNA (~500 bp per strand). Quantity and quality of the libraries were analyzed using the bioanalyzer. One monocyte sample (ID 22) was excluded for further analysis based on this QC step. Samples were divided over several batches based on the sample molarity and pooled for sequencing on an illumina platform (illumina, USA).

Table 1
Demographics of included donors. BMI = body mass index; RIN = RNA integrity number.

	Controls	SCZ patients
N	17	20
Sex (female/male)	3/14	4/16
Age (mean ± STDEV)	24.0 ± 3.9	23.1 ± 4.0
BMI (mean ± STDEV)	24.8 ± 5.3	24.9 ± 3.7
RIN (mean ± STDEV)	9.0 ± 0.2	8.7 ± 1.4
Antipsychotic use (N)	0	18

2.4. RNAseq analysis

RNAseq reads were aligned along the GRCh38/hg38 reference genome via STAR aligner v2.5 (Dobin et al., 2013) and genes were quantified using featureCounts(Liao et al., 2014). RNAseQC(Deluca et al., 2012) was used to calculate QC metrics using R (R v4.1.2) and included: exonic rate, intergenic rate, mapped reads, rRNA rate, genes detected, and mean per base coverage (Supplementary Table 3). Non-protein-coding genes and genes with a lower base mean than 1 were excluded from analyses. Raw gene counts were vst-normalized to shrink count-inflated gene-gene distances. We used principal component analysis (PCA) and inter-sample Pearson correlations to detect outliers and investigate confounders. Two samples (ID 36 and 37) were identified as outliers removed from further analyses (Supplementary Fig. 1A-B). The final sample size of our RNA-seq analysis was 20 cases and 17 controls.

A range of biological and technical variables have been shown to have an impact on gene expression profiles in monocytes. This includes demographic variables such as age, sex, smoking and BMI (Reynolds et al., 2015; So et al., 2021; Wright et al., 2012), but also technical factors related to the RNA-sequencing, including sequencing batch, base coverage, library size, % of exonic reads, number of detected genes, % rRNA (Navarro et al., 2021). To detect which of these factors are a major source of variation in our gene expression dataset, we used PCA and linear regression of these potential covariates on gene expression variance (Supplementary Fig. 2A). We then applied variancePartition (v1.24.0)(Hoffman and Schadt, 2016), which uses a linear mixed model to calculate the percentage of variation in expression due to selected covariates in each gene (Supplementary Fig. 2B). We selected covariates explaining a significant proportion of the variance (R^2 > 0.3 and visual inspection of variance partition plot) but were not correlated with status. Furthermore, we only selected one covariate if multiple covariates were highly correlated to account for collinearity (Supplementary Fig. 2C). The covariates that we selected based on these criteria for subsequent analyses were average base coverage, library size, % of exonic reads, number of detected genes, % rRNA, BMI, RIN, age, and batch.

Differential expression was calculated with DESeq2 (v1.34.0)(Love et al., 2014). We used a significance threshold of FDR < 0.1 as calculated with the Benjamini-Hochberg procedure. Gene ontology (GO) pathway analyses were performed on differentially expressed genes (DEGs) using the clusterProfiler package (v3.0.4)(Yu et al., 2012). GO molecular function, cellular component, and biological process pathways were included. To further annotate DEGs, gene set enrichment analysis (GSEA) on manually selected gene panels was performed. We used a microglial signature panel by Patir et al. (2019) (Patir et al., 2019) as well as NF-κB signaling and IFNy, IL4, and astrocyte immune response panels derived from gene expression networks in *postmortem* schizophrenia brain tissue from the latest *meta*-analysis by Gandal et al. (2018) (Gandal et al., 2018). We also added two panels comprising glucocorticoid and LPS response genes which were used in the paper by Melbourne et al. (2021) (Melbourne et al., 2021). The eight gene panels can be found in Supplementary Table 4. To test for the association between DEGs and genetic risk for schizophrenia, we extracted risk genes identified in the latest schizophrenia GWAS and TWAS studies (Gusev et al., 2018; Li et al., 2017; Pardiñas et al., 2018). Plots were generated using ComplexHeatmap (v1.10.2)(Gu et al., 2016), ggplot2 (v3.3.5)(Wickham, 2016), and RColorBrewer (v1.1.2)(Neuwirth and Brewer, 2014) packages.

2.5. Statistics

Correlation tests were performed in Pearson (nominal) with the basic R stats package (v3.6.2). A *p*-value < 0.05 was considered statistically significant. Principal components were calculated with the prcomp function and simple linear modeling was used to calculate the variance explained by covariates in the principal components using the lm function from the stats package. Differential gene statistical analyses were performed in R (v4.1.2) (CRAN:https://www.r-project.org). We calculated *q*-values using the Benjamini-Hochberg procedure to adjust *p*-values for multiple testing. The Benjamini-Hochberg procedure was also applied to correct for multiple testing in the statistical analyses of the gene panels. GSEA was performed by calculating odds ratios with Fisher's exact test. We considered a *q*-value of < 0.1 as statistically significant (referred to as FDR < 0.1).

We further analyzed whether our results are spurious and false-positive inflated by performing permutation testing on our DESeq2 analysis (n = 1000 permutations). For each permutation we randomly allocated group identity (i.e., SCZ/CTR) across samples and calculated group differences across genes modeled as the simplest DESeq2 expression (i.e., ~ 1 + status). For each analysis we then summarized the total number of DEGs discovered, as well as the q-value distribution for our original DEGs by calculating the respective means. Using the distribution of the mean values, we calculated a p-value for our original discovery using the cumulative probability discovery function.

3. Results

3.1. Demographics

The demographics of the patients and controls that were included in the final transcriptome analysis are summarized in Table 1 and more extensively described in Supplementary Table 2. The average age was 23 years for the patients and 24 years for the control groups. The groups were not significantly different in sex, age, and BMI. The RNA integrity (RIN) values of the RNA extracted from the monocytes, which is a quality measure for RNA, were good and not statistically different between cases and controls.

4. Expression analysis of genes of interest

We first analyzed expression changes in genes of interest that were selected based on monocyte analyses in previous studies (see Supplementary Table 1). From 29 genes/proteins that were differentially expressed in previous studies, we found significant differences in expression levels for *TNFAIP3*, *DUSP2*, and *IL6* ($\log_2$FC > 0.5 | $\log_2$FC < -0.5, FDR < 0.1) with the same direction of effect as reported before (Table 2, Fig. 1A). *IL1B*, *PDE4B*, *TNF*, and *F3* were significantly upregulated at a nominal *p*-value threshold of < 0.05. *CCL7*, *SERPINB2*, and *TLR4* were significantly downregulated at a nominal *p*-value threshold of < 0.05, which was the opposite effect direction previously described (Table 2, Supplementary Fig. 3A). We further compared fold changes between our current analysis and the monocyte study by Drexhage et al. (2010) (Drexhage et al., 2010) and found a high correlation between the effect sizes of both studies (Pearson's $r = 0.7$; Fig. 1B).

4.1. Transcriptome-wide differentially expressed genes.

Using PCA, we observed samples to separate by disease status after regressing out the confounders (Supplementary Figure 4A). We found 41 differentially expressed upregulated 106680540321500genes and 58 differentially expressed downregulated genes (FDR < 0.1) (Fig. 2A, Supplementary Table 5). 28 of these genes were differentially expressed with a $\log_2$ FC > 0.5 or < -0.5 (Supplementary Table 5). We permuted this analysis and found our results to be significantly at odds with random noise (p = 0.04 and p < 0.01 respectively) (Supplementary Figure 5A-B). These genes include several of the genes of interest (Table 2). Two previously fine-mapped schizophrenia GWAS loci containing genes, CSMD1 and FES, neared significance thresholds ($\log_2$ FC < -2 and FDR < 0.1, respectively). We then compared the effect sizes of our DEGs to those in *postmortem* brain tissues of schizophrenia patients derived by Gandal et al. (2018). Genes that were differentially expressed

Table 2
Expression levels of genes of interest. Raw expression (standard deviation) values shown for both control and schizophrenia donors. Differential expression effect sizes (third column) are shown as $\log_2$ of the fold change from control to schizophrenia as calculated with DESeq2. Nominal and FDR-adjusted *p*-values (*p*- and *q*-values respectively) are shown. sd = standard deviation. FDR = false discovery rate. Validated = Whether we replicated the respective gene's expression from previous studies. Validated expression legend: 1 = effect direction is replicated and significant at FDR threshold in our study. 2 = effect direction is replicated and significant at *p*-value threshold in our study. 3 = effect is not replicated in our study.

Gene symbol	CTR mean (sd)	SCZ mean (sd)	$\log_2$FC	*p*-value	FDR	Validated
IL6	44 (51)	276 (450)	1.97	< 0.01	0.06	1
TNFAIP3	10,025 (4727)	31,988 (25887)	1.36	< 0.01	0.02	1
TNF	615 (232)	1758 (1955)	1.1	0.01	0.28	2
F3	145 (199)	240 (288)	1.05	0.02	0.34	2
IL1B	10,148 (9161)	22,388 (23499)	0.93	< 0.01	0.21	2
DUSP2	8800 (8949)	14,991 (10006)	0.92	< 0.01	0.05	1
CCL20	127 (181)	247 (335)	0.87	0.1	0.61	3
PDE4B	6752 (5150)	10,362 (6715)	0.62	< 0.01	0.14	2
MAFF	818 (840)	1060 (786)	0.56	0.08	0.56	3
CXCL2	2921 (3452)	3754 (4000)	0.55	0.11	0.62	3
EREG	5982 (6968)	7326 (5949)	0.51	0.14	0.65	3
CXCL3	764 (975)	901 (1012)	0.48	0.23	0.74	3
PTGS2	17,831 (12860)	19,980 (9559)	0.27	0.3	0.78	3
ATF3	1629 (1401)	1630 (1085)	0.25	0.38	0.83	3
TLR2	7468 (1695)	7950 (1193)	0.14	0.08	0.56	3
PTX3	757 (586)	862 (676)	0.14	0.55	0.9	3
TLR5	1894 (551)	1928 (453)	0.12	0.09	0.57	3
EGR3	673 (770)	628 (416)	0.08	0.85	0.98	3
BCL2A1	5558 (3410)	5698 (3101)	0.05	0.76	0.96	3
TLR3	4 (7)	3 (4)	0.05	0.96	1	3
SPI1	14,275 (2423)	13,744 (2116)	0	0.93	0.99	3
CCL7	31 (59)	16 (51)	−1.71	0.04	1	3
CCL2	450 (835)	208 (215)	−0.94	0.07	0.54	3
SERPINB2	1737 (2562)	820 (450)	−0.67	0.02	0.37	3
TLR4	11,913 (3106)	9934 (2636)	−0.23	0.01	0.27	3
MXD1	12,013 (5920)	10,241 (3139)	−0.18	0.14	0.66	3
TREM1	8791 (3721)	7591 (1859)	−0.17	0.12	0.63	3
MAPK6	1791 (1155)	1596 (809)	−0.16	0.25	0.75	3
CDC42	11,152 (2413)	10,994 (2843)	−0.07	0.48	0.88	3

in our monocytes showed a moderate correlation in effect size with those of brain tissues (Pearson's $r = 0.49$, Fig. 2B).

4.2. Gene set enrichment analysis

We subsequently performed a pathway analysis by selecting all genes with an FDR < 0.1 (n = 99) using GO pathway analysis. Genes upregulated in schizophrenia patients were significantly enriched for GO biological processes including inflammatory responses and regulation of hemopoiesis (Fig. 3A). For the downregulated genes we did not find significant enrichment of GO pathways. We complemented this step by performing GSEA on eight manually selected panels of microglia signature and schizophrenia risk genes, glucocorticoid, IL4, and LPS response genes as well as NF-κB, IFN, and astrocyte immune gene networks (Fig. 3B). We found significant enrichments for NF-κB signaling, LPS and glucocorticoid response gene sets further corroborating the GO pathway results (Fig. 3B). We next constructed principal components from the expression data of the LPS, NF-κB, and glucocorticoid response gene sets and tested for differential expression of the components that loaded strongest on status (Supplementary Figure 6A-D). Schizophrenia patients indeed showed a significantly higher expression of LPS (t = -4.23, p < 0.01, 95% CI = [1.68,4.81]) and NF-κB gene set-derived principal components (t = -3.18, p=<0.01, 95% CI = [-5.12,-1.12], Fig. 3C), validating our GSEA results on a transcriptome-wide level. Similarly, schizophrenia patients showed a significantly lower expression of principal components derived from the glucocorticoid response signature ($t = 4.50$, p=<0.01, 95% CI = [2.40,6.39], Fig. 3C).

5. Discussion

The goal of this study was to increase our understanding of changes in monocytes of patients with recent-onset schizophrenia. The first aim of our study was to analyze whether we could replicate findings observed in monocytes before (Chen et al., 2019; Drexhage et al., 2010; Kéri et al., 2017; Krause et al., 2012a; Krause et al., 2012b; Müller et al., 2012; Weigelt et al., 2011), as well as whether monocytes display similar immune changes as also found in brain tissue of schizophrenia patients (Gandal et al., 2018). Our second aim was to analyze potential other dysregulated pathways. To this extent, we isolated monocytes from recent-onset schizophrenia patients and controls and analyzed their transcriptome profiles.

We were able to validate earlier findings on monocytes in schizophrenia patients. Out of 29 genes and proteins, we replicated increased expression levels of *TNFAIP3*, *IL6*, and *DUSP2* at FDR < 0.1. *IL6* is a cytokine and *DUSP2* is a protein tyrosine phosphatase, which are both involved in pathogen defense and upregulated after immune activation. They are referred to as positive regulators of an inflammatory response (Jeffrey et al., 2006; Murphy and Weaver, 2017). Expression of both *IL6* and *TNFAIP3* is induced by NF-κB signaling (Lai et al., 2013; Melief et al., 2016; Zheng et al., 2016). *TNFAIP3* also plays a critical role in regulating inflammatory responses and mutations in this gene are associated with autoimmune diseases (Ma and Malynn, 2012). However, in contrast to *IL6*, *TNFAIP3* is known for its anti-inflammatory effects. More specifically, it has been shown to be a critical negative regulator of NF-κB activation (Catrysse et al., 2014). Our data, where we see an upregulation of both *TNFAIP3* expression and NF-κB pathways, may suggest a dysregulation of NF-κB-related regulatory mechanisms in schizophrenia, which would be interesting to study in functional follow-up studies on isolated monocytes.

In addition, we found a strong positive correlation of effect sizes between an earlier transcript level study of Drexhage et al. (2010) (Drexhage et al., 2010) and ours (Pearson's $r = 0.7$). At a pathway level our results in recent-onset patients are partly in line with the study of Melbourne et al. in patients with a medium and long-term illness duration. Both studies showed an association with NF-κB, LPS response- and glucocorticoid-related pathways. In Melbourne et al.'s study, the enrichment across these pathways increased with illness-duration which may explain the proportionally low number of differentially expressed genes we found. Contrary to the study by Melbourne et al., we did not find enrichment of interferon-related pathways.

Interestingly, we also found a moderate correlation (Pearson's r = 0.49) between the expression changes in monocytes, and the expression changes in *postmortem* brain tissue at a transcript and pathway level. An enrichment of NF-κB signatures was found for differential expressed genes in monocytes, as well as brain tissue. This suggests that monocytes partly capture gene expression alterations found in brain tissue. However, to test this hypothesis, a study with paired blood–brain samples is needed. Whether this association of transcriptional changes in

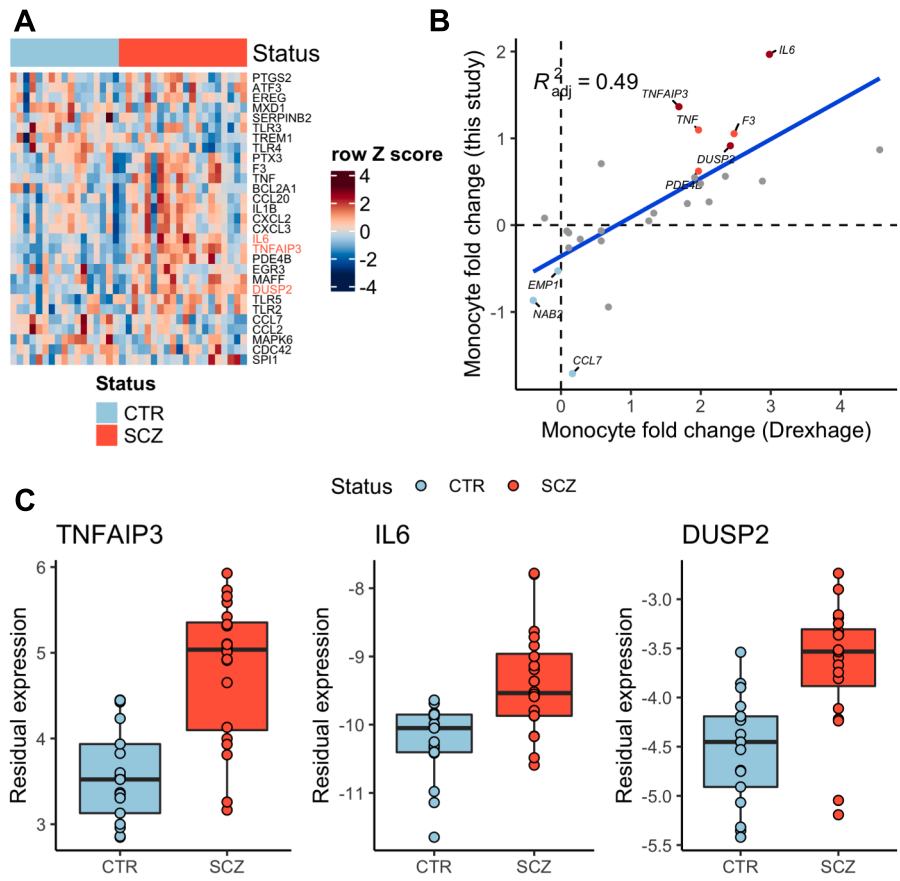

Fig. 1. Gene expression analysis of previously identified schizophrenia genes in monocytes. Monocytes were isolated and RNA sequencing was performed on 20 patients with recent-onset schizophrenia and 17 controls to analyze changes in gene expression. To account for multiple testing, we used Benjamini-Hochberg FDR-adjusted p-values (q-values) to determine significance (FDR < 0.1); A) Heatmap showing schizophrenia genes in monocytes previously found to be differentially expressed (upregulated). Genes in red were differentially expressed at $\log_2$FC > 0.5 or $\log_2$FC < -0.5 and FDR < 0.1. Values depicted are row Z-scores of residual gene expression. Rows (genes) have undergone unsupervised clustering (distances derived from Pearson correlations); B) Scatter plot showing correlation between effect sizes of differential expression in monocytes by Drexhage et al. (2010) (x-axis) and those of our study (y-axis). x- and y-axes are in $\log_2$FC. Blue line shows regression coefficient. Colored genes were differentially expressed at $\log_2$FC > 0.5 (red) or $\log_2$FC < -0.5 (blue) and nominal P-value < 0.05; C) Boxplots of *TNFAIP3*, *IL6*, and *DUSP2* residual gene expression between status groups (controls in blue, patients in red). CTR = control samples, SCZ = schizophrenia samples.

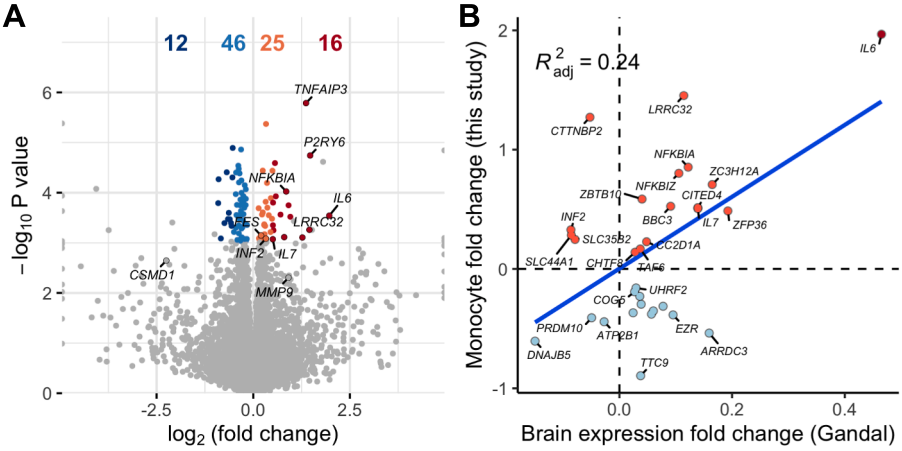

Fig. 2. Comparison of gene expression in monocytes versus brain tissues of schizophrenia patients. A) Volcano plot showing all differentially expressed genes at FDR < 0.1. Light-colored genes are differentially expressed at FDR < 0.1, dark-colored genes are differentially expressed at $\log_2$FC > 0.5 or $\log_2$FC < -0.5 and FDR < 0.1. Gene names are shown for i) genes that were previously found in either brain or monocyte samples of schizophrenia patients, and ii) genes that neared nominal significance threshold and were previously identified in a schizophrenia GWAS; B) Scatter plot showing correlation between effect sizes of differential expression of schizophrenia brain tissues (Gandal et al., 2018) (x-axis) and our monocytes (y-axis). x- and y-axes are in $\log_2$FC. Blue line shows regression coefficient.

monocytes and brain tissue is due to expression changes in microglia, or other cells such as astrocytes, is not yet clear. Recent *meta*-analyses of positron emission tomography studies with TSPO ligands (Plavén-Sigray et al., 2021), and studies on *postmortem* brain tissue (Snijders et al., 2021), suggest that microglia are not showing an immune-activation phenotype. High-resolution techniques, such as single nucleus RNA-seq or spatial transcriptomics studies, will be needed to shed light on which cell types contribute to the NF-κB signature observed in brain tissue. Furthermore, we did not find an enrichment for microglia core genes or astrocyte-specific immune network genes. Monocytes may therefore act as a peripheral proxy for inflammatory pathways shared between peripheral and glial cells, but not for the more specific glial genes, as expected.

A link between schizophrenia and the NF-κB pathway has been supported by several lines of evidence (Roussos et al., 2013; Volk et al., 2019). Notably, Volk et al. found dysregulation of a large range of NF-κB-related genes in *postmortem* brain tissue, but not in antipsychotic-exposed monkeys (Volk et al., 2019) suggesting that this is not an effect of antipsychotics. Nuclear factor NF-kappa-B (NF-κB) is a transcription factor that regulates the expression of various genes, including growth factors and cytokines (Liu et al., 2017). It plays a critical role in activation of the innate immune system, including monocytes and microglia. NF-κB genes are also widely expressed in non-immune cells of the developing and mature nervous system and play roles in several

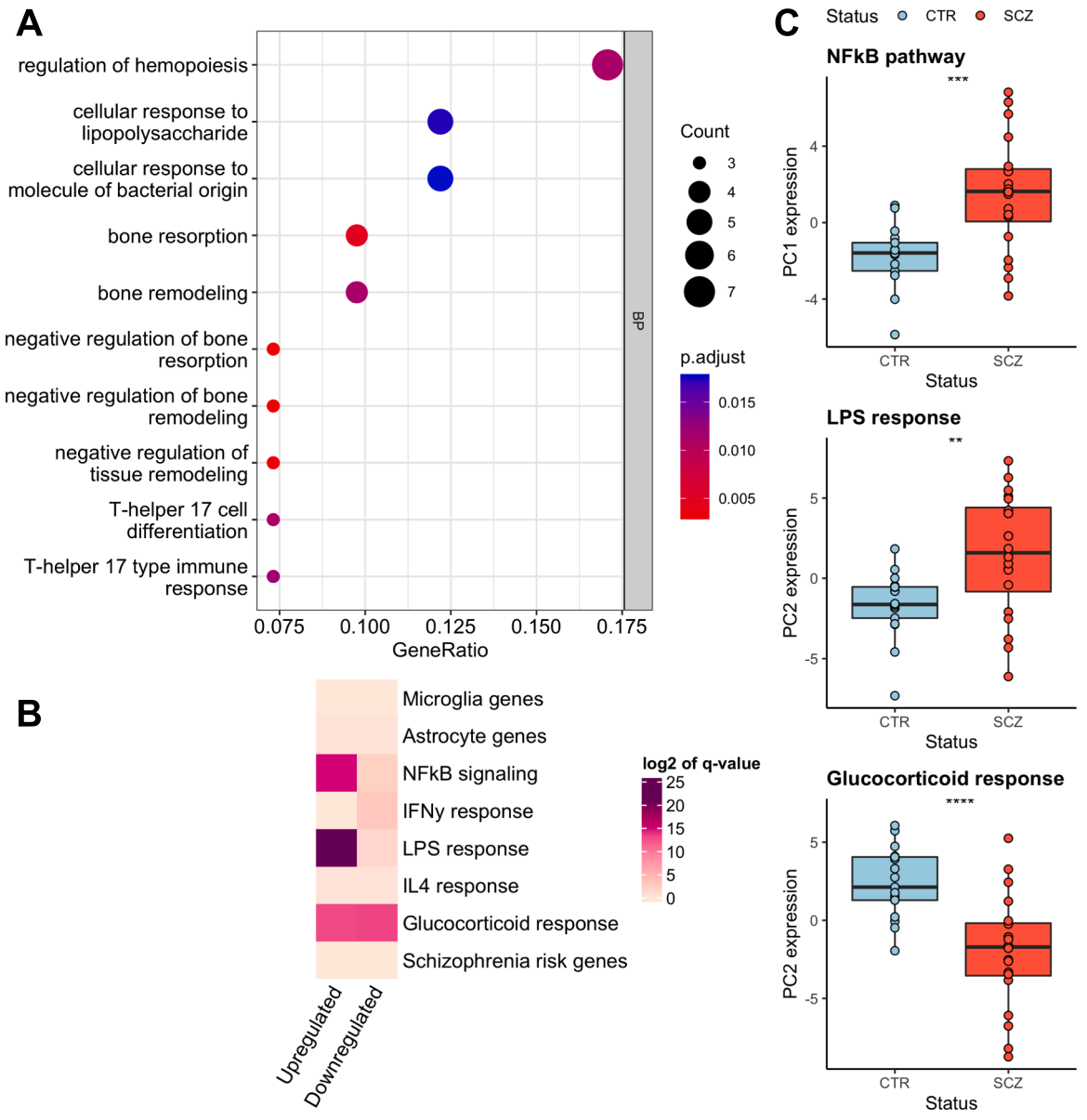

Fig. 3. Explorative transcriptome analyses in monocytes of patients versus controls. Unbiased RNA sequencing analysis of monocytes isolated from twenty schizophrenia patients and seventeen controls. Annotation of 99 differentially expressed genes (DEGs) found at FDR < 01. A) Dotplot showing gene-ontology pathway analysis of our 99 DEGs. y-axis shows pathway names, x-axis shows ratio of genes landing in the respective pathway. p.adjust shows Benjamini-Hochberg corrected p-values (FDR). Counts show number of hits in the respective pathway; B) Heatmap of $\log_2$ q-values (FDR) from the GSEA between our 99 DEGs and hand-selected gene sets separated by whether the genes were down- or upregulated; C) Boxplots of principal component expression by status group (controls in blue, patients in red). Boxplots are shown for the three significantly enriched pathways (NF-κB, LPS response, and glucocorticoid pathways). Principal components were constructed based on expression of genes in each of the three gene lists. Each dot represents the principal component expression per sample. *= p <=0.05; **= p <=0.01; ***= p <=0.001; ****= p <=0.0001.

neurodevelopmental processes, such as neurogenesis, axon guidance, and synaptic density (Gutierrez and Davies, 2011).

We also found enrichment of glucocorticoid response genes among the differentially expressed genes in monocytes. Changes in the hypothalamic–pituitaryadrenal (HPA) axis have been associated with schizophrenia (Cherian et al., 2019), with signs of overactivity of the HPA axis in neuroendocrine studies. We found a downregulation of glucocorticoid pathways in monocytes, which might reflect a feedback loop on the immune system. We did not find enrichment for schizophrenia risk genes, suggesting that GWAS-associated gene loci for schizophrenia map to celltypes other than monocytes. Of note, the gene set used in our paper is based on a TWAS analysis using gene expression data in brain tissue. Our data do not exclude the possibility of causal SNPs influencing the expression of myeloid cell-type specific genes. An example of this is the increased expression of *FES* which was previously associated with a schizophrenia risk SNP (Lopes et al., 2022).

Among the differentially expressed genes, we found two genes that have also been prioritized in genetic studies of schizophrenia. At a nominal level we found downregulated expression of *CSMD1*, which was previously shown to contain schizophrenia risk loci (Ripke et al., 2014). Whether the risk alleles increase or decrease *CSMD1* expression has, however, not yet been reported. *FES*, on the other hand, was first identified as a schizophrenia GWAS risk gene (Ripke et al., 2014) and later fine-mapped by Lopes Paiva et al. (2022) (Lopes et al., 2022) to

contain both expression and splicing quantitative trait loci (QTL) in monocytes and microglia respectively. QTL effect size analysis suggests that the monocyte-specific SNPs in the *FES* gene region are associated with an increased expression of this gene. In line with these findings, we found an upregulated expression of *FES* in monocytes of schizophrenia patients. Its function, especially in monocytes of schizophrenia patients, remains to be elucidated. *FES* encodes a tyrosine kinase which plays a role and myeloid cell development and cytokine signaling (Hackenmiller et al., 2000; van der Wel et al., 2020).

The strengths of this study are the analysis of cells from early-stage schizophrenia patients and well-matched controls; the in-depth profiling of these cells; and the combination of a hypothesis-driven as well as unbiased analysis of the data. The current study also has limitations. First, a larger cohort would have provided more power to detect genes with smaller effect sizes or changes in subgroups of patients and would decrease the chance of false positive findings. In addition, although we matched the groups and regressed out effects caused by sex, BMI, and technical covariates, we were not able to control for every potential confounder, such as smoking and use of medication. Antipsychotics and antidepressants have been shown to have an effect on the immune system (Dinesh et al., 2020; Szałach et al., 2019). In our dataset, we found that the use of anxiolytics and antidepressants did not show an effect on gene expression, whereas antipsychotic use was explaining a low proportion of the variance. As 18 of the 20 patients were using antipsychotics, we were not able to include this as a covariate. To address this issue, follow-up studies should include individuals without and with medication. Smoking is known to be more prevalent in schizophrenia patients across disease (Sagud et al., 2019) as well as to increase NF-κB pathway activation stages (Rom et al., 2013). In our study, 14 out of 20 patients are smoking, and 3 out of 17 controls. During our quality control analysis, we found no effect of this covariate on gene expression (Supplementary Fig. 2). However, to test whether there would be an interaction effect, we need a considerably larger sample size.

In conclusion, we replicated an upregulated expression of *IL6, TNFAIP3*, and *DUSP2* in monocytes of schizophrenia patients, as well as an association with genes related to the NF-κB/LPS response pathways. These findings are in line with previous studies on *postmortem* brain tissue and blood. These markers/pathways have important functions in regulation of the innate immune system but also play non-inflammatory roles in the central nervous system. These findings could therefore reflect potential interesting changes for monocytes, as well as more general changes in these pathways in the central nervous system. Follow-up studies are needed to further understand the role of NF-κB signaling in schizophrenia pathogenesis. The fact that we can detect these changes in blood provide possibilities for future biomarker development.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2023.04.019.

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