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# Transcriptome analysis reveals disparate expression of inflammation-related miRNAs and their gene targets in iPSC-astrocytes from people with schizophrenia

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ABSTRACT

Despite the high heritability of schizophrenia (SCZ), details of its pathophysiology and etiology are still unknown. Recent findings suggest that aberrant inflammatory regulation and microRNAs (miRNAs) are involved. Here we performed a comparative analysis of the global miRNome of human induced pluripotent stem cell (iPSC)-astrocytes, derived from SCZ patients and healthy controls (CTRLs), at baseline and following inflammatory modulation using IL-1β. We identified four differentially expressed miRNAs (miR-337-3p, miR-127-5p, miR-206, miR-1185-1-3p) in SCZ astrocytes that exhibited significantly lower baseline expression relative to CTRLs. Group-specific differential expression (DE) analyses exploring possible distinctions in the modulatory capacity of IL-1β on miRNA expression in SCZ versus CTRL astroglia revealed trends toward altered miRNA expressions. In addition, we analyzed peripheral blood samples from a large cohort of SCZ patients (n = 484) and CTRLs (n = 496) screening for the expression of specific gene targets of the four DE miRNAs that were identified in our baseline astrocyte setup. Three of these genes, *LAMTOR4, IL23R*, and *ERBB3*, had a significantly lower expression in the blood of SCZ patients compared to CTRLs after multiple testing correction. We also found nominally significant differences for *ERBB2* and *IRAK1*, which similarly displayed lower expressions in SCZ versus CTRL. Furthermore, we found matching patterns between the expressions of identified miRNAs and their target genes when comparing our *in vitro* and *in vivo* results. The current results further our understanding of the pathobiological basis of SCZ.

## 1. Introduction

Schizophrenia (SCZ) is a severe mental disorder with a considerable disease burden and a lifetime prevalence of nearly 1% (McGrath et al., 2008; Correll et al., 2015). The main clinical features fall into three groups of symptoms: positive symptoms, negative symptoms, and cognitive impairments, with a substantial phenotypic heterogeneity (Gogtay et al., 2011). Although the currently available pharmacological interventions are associated with serious side effects (Patel, 2014) and about one-third of patients experience poor long-term outcome (Ijaz et al., 2018), they remain the primary treatment approach, especially during acute phases (Patel, 2014). Despite the high heritability of SCZ, details of its pathophysiology are still unknown. Identification of the fundamental disease mechanisms would be essential for the development of novel and more effective treatment modalities (Editorial, 2010; Rubinstein, 2010).

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Recent findings suggest that small non-coding RNAs, in particular microRNAs (miRNAs), are involved in the pathobiology and symptom development of SCZ (Beveridge and Cairns, 2012; Im and Kenny, 2012; Xu et al., 2013; Rajman and Schratt, 2017; Cao and Zhen, 2018). miRNAs are a class of short (~22 nt) non-coding RNAs that can functionally silence, and in some cases enhance, gene expression by complementary base-pairing with their relevant mRNA targets. This miRNA-based post-transcriptional control can potentially regulate the expression of multiple downstream target genes in almost all tissues and cell types (Winter et al., 2009). The functional importance of miRNA regulatory networks in neurodevelopment and brain physiology has been widely studied, and recent studies show that these networks play essential roles in SCZ, indicating that miRNAs could be utilized as potential biomarkers and therapeutic targets (Cao and Zhen, 2018).

Astrocytes are the most abundant glial cell type in the mammalian brain where they perform a wide spectrum of physiological activities, many of which are essential in normal brain functioning (Abbott et al., 2006), such as the immune surveillance of the CNS (Heneka et al., 2018). In recent years, they have emerged as potential mediators of inflammatory and immune-related abnormalities in SCZ (Khandaker et al., 2015). For example, studies on astroglia from human postmortem samples as well as preclinical animal models implicate a range of changes in astrocyte structure and function associated with neuropsychiatric disorders, including increased inflammation in SCZ (Kim et al., 2018). Neuroinflammation, as a pathophysiological phenomenon, can be the outcome of maladaptive or uncontrolled innate immune responses against various chemical, physical, or microbial danger signals in the brain. Neuroinflammation has been linked to dysregulated inflammasome activation, a critical component of innate immunity (Heneka et al., 2018). Activated inflammasomes induce the release of the inflammatory cytokines IL-1β and IL-18 by resident immunocompetent glial cells, such as microglia and astroglia (Heneka et al., 2018; Singhal et al., 2014; Swanson et al., 2019). These cytokines play a pivotal role in activating downstream inflammatory pathways during host defense, but have also been suggested to be involved in SCZ and other psychiatric disorders when secreted by glial cells in an uncontrolled manner (Khandaker et al., 2015). For instance, elevated blood and postmortem brain IL-1β levels have been associated with the severity and evolution of symptoms in SCZ (Zhu et al., 2018; Mohammadi et al., 2018). Aberrant inflammatory regulation in astrocytes have also been linked to brain volume reduction and cognitive impairment in SCZ (Kindler, et al., 2019). Furthermore, miRNAs have recently been suggested to be key players in astrocyte and microglia-mediated inflammation in SCZ (Mellios and Sur, 2012) and several other CNS pathologies (Karthikeyan et al., 2016).

To date, investigations into the glial biology and immunopathology of SCZ have been hindered by the inaccessibility of human brain tissue and by the lack of appropriate animal models. The latest methodological developments in human induced pluripotent stem cell (iPSC) biology have enabled studies of the cellular and molecular features of SCZ (Windrem et al., 2017; Falk et al., 2016). Direct reprogramming of fibroblasts to iPSCs and their subsequent differentiation into neurons (Brennand et al., 2011; Tran, et al., 2013) and astrocytes (Akkouh, et al., 2020) has been demonstrated to be a relevant and useful *in vitro* model in SCZ research. In the present study, we generated human iPSC-derived astroglia from SCZ patients and healthy controls, and used RNA sequencing to characterize the SCZ miRNome at baseline and following IL-1β-induced inflammatory activation. We identified four highly differentially expressed miRNAs which we studied further. In particular, we compared the expression of transcripts regulated by these miRNAs in the blood of SCZ patients and in astroglial cultures derived from SCZ patients and found concordant expression profiles, indicating a potential *in vivo* role for these miRNAs in SCZ.

## 2. Materials and methods

### 2.1. Recruitment of patients and collection of skin biopsies

This project is part of the ongoing Norwegian TOP (Thematically Organized Psychosis) study. Information about recruitment procedures, inclusion and exclusion criteria, and clinical assessments for the TOP study as a whole have been described in detail (Mørch et al., 2019; Simonsen et al., 2011) (additional details are discussed below). For iPSC reprogramming and astrocyte differentiation, fibroblast/skin biopsies were isolated from 3 CTRLs and 3 SCZ patients. For gene expression measurements of miRNA target genes in whole blood, a subset of the TOP sample consisting of 484 SCZ spectrum disorder patients (353 schizophrenia, 41 schizophreniform, and 90 schizoaffective) and 496 CTRL subjects was used. These participants were selected based on the availability of microarray gene expression data for the relevant markers. A detailed description of blood sampling procedures, microarray preprocessing, and quality control can be found in a previous report (Akkouh et al., 2018). Supplementary Tables S1 and S2 show further details about the study participants from whom skin biopsies and whole blood samples were obtained, respectively. All patients underwent a clinical examination that included diagnostic interviews based on Structured Clinical Interview in DSM-IV axis I Disorders (SCID-1) and structured assessments of clinical symptoms, use of psychotropic medication, smoking habits, alcohol consumption, and illicit substance use. Diagnostic evaluation was performed by trained clinical psychologists and psychiatrists. The main inclusion criteria for patients for both skin biopsy collection and for clinical sampling were confirmed diagnosis of SCZ according to the Diagnostic and Statistical manual of Mental Disorders (DSM)-IV, age between 18 and 65, and ability to give informed written consent. The main exclusion criteria were clinically significant brain injury, neurological disorder, ongoing infections, autoimmune disorders or any form of cancer. All participants have given written consent and the study was approved by the Norwegian Scientific Ethical Committees and the Norwegian Data Protection Agency. The project has been approved by the Regional Ethics Committee of the South-Eastern Norway Regional Health Authority (*REK#2012/2204*). The authors assert that all procedures contributing to this work comply with the ethical standards of relevant guidelines and regulations.

### 2.2. Generation of hiPSCs and differentiation of iPSC-astrocytes

The main aim of this study was to characterize and to compare the astrocyte-specific miRNome in patients with SCZ and in healthy controls. For this, we established a robust hiPSC-astrocyte monoculture model with high culture purity. The generation and characterization of human iPSCs were carried out as reported previously (Akkouh, et al., 2020). Astrocytes were differentiated from 3 SCZ and 3 CTRL iPSCs following two glial differentiation protocols with small modifications (Izrael et al., 2007; TCW et al., 2017) as published earlier (Akkouh, et al., 2020). Briefly, we reprogrammed fibroblasts isolated from patients and CTRLs using Sendai virus vector. On average, 3–5 clones were generated per donor iPSC lines and were cultured for at least 5–10 passages to allow time for the wash-out of virus before pluripotency testing. As shown in our previous publication (Akkouh, et al., 2020), intra-donor variability was not significant, and therefore one clone per donor was selected for experiments. iPSC colonies at passages 24–25 were transferred to 6-well tissue culture plates in Neural maintenance medium (NMM) consisting of 50% DMEM/F12 and 50% Neurobasal medium (both from Thermo Fisher) supplemented with 0.5% (v/v) N2, 1% (v/v) B27 (both from Invitrogen, Carlsbad, CA, USA), 5 μg/ml human insulin, 40 ng/ml triiodothyronine (T3), 10 μM β-mercaptoethanol, 1.5 mM L-glutamine, 100 μM NEAA, 100 U/ml penicillin and 100 μg/ml streptomycin (all from Sigma-Aldrich, St. Louis, MO, USA). 20 ng/ml EGF and 4 ng/ml bFGF (both from Peprotech, Rocky Hill, NJ, USA) were added to the cultures. Astroglia induction was done between

days 1–42 using the N2/B27 protocol as described in detail in (Akkouh, et al., 2020). For astrocyte maturation and enrichment, NMM medium was switched to ScienCell AM medium (ScienCell, Carlsbad, CA, USA) on day 42 and cells were further cultured for 60 additional days until day 102. Cultures were continuously biobanked from day 42 by freezing astrocytes in Sciencell AM medium + 10% DMSO at each passage, and storing them in liquid nitrogen. For the monitoring of astroglia differentiation, samples were continuously collected during the differentiation process at days 0 (iPSC), 7, 14, 21, 30, and 40. The mRNA expression of *PAX6*, *Nestin*, and the astrocyte-specific markers *GFAP*, *S100B*, *SLC1A2*, *SLC1A3*, *FABP7*, *AQP4*, *ALDH1L1*, and *ALDOC* was monitored using a custom-made TLDA gene array card (Thermo Fisher) on qPCR platform (Akkouh, et al., 2020). For additional quality control, cells were stained with anti-GFAP, anti-S100B and anti-AQP4 antibodies (all from Abcam, Cambridge, UK) and were analyzed using fluorescent microscopy on day 42 (GFAP) and day 102 (GFAP, S100B, AQP4). Finally, functional characterization of iPSC-astroglia was done using a glutamate uptake assay, and astrocytes were considered mature and were used for experiments on day 102 (Akkouh, et al., 2020). Each *in vitro* experiment was done in triplicates if not stated otherwise.

### 2.3. Inflammatory modulation of astrocytes

To mimic inflammasome activation *in vitro*, day 102 astrocytes were treated with 3 relevant working concentrations of IL-1β (0.1, 1, and 10 ng/ml; Peprotech) and the short-term (4 h) effect on early-response inflammatory cytokine genes were investigated using qPCR (Akkouh, et al., 2020). Since all applied concentrations strongly increased the expression of CXCL8/IL-8, IL6, and TNF genes in both SCZ and CTRL iPSC-astrocytes with no differences between patient and control lines, and since the most pronounced effect was seen at an IL-1β concentration of 10 ng/ml (Akkouh, et al., 2020), this concentration was therefore used in subsequent experiments. Cell pellets were collected after 4 h of incubation for miRNA extraction. Harvested samples were either immediately used for analyses or stored at −80 °C.

### 2.4. RNA extraction and miRNA sequencing

Total RNA was extracted from the mature astrocyte samples using the MagMax mirVana Total RNA Isolation Kit (Thermo Fisher) according to manufacturer's instructions. Approximately 1 million cells were used as input. RNA yield was quantified with a NanoDrop 8000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and RNA integrity was assessed with Bioanalyzer 2100 RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA). All samples had an RNA Integrity Number (RIN) > 8.5. Library preparation and single-end miRNA sequencing were carried out at the Norwegian High-Throughput Sequencing Centre (www.sequencing.uio.no). Briefly, libraries were prepared with the QIAseq miRNA Library Kit (QIAGEN), which integrates Unique Molecular Indices (UMIs) into the reverse-transcription process to eliminate PCR and sequencing bias. The prepared samples were then sequenced on an Illumina NextSeq 500 platform (Illumina) at an average depth of 22 million reads per sample using a read length of 75 base pairs single-end.

### 2.5. Data processing

Sequencing data were processed with the GeneGlobe Data Analysis Center (QIAGEN), which allowed us to make use of the integrated UMIs to identify individual miRNA species. First, Cutadapt (Martin, 2011) was used to trim reads for 3′ adapter sequences and low-quality bases, and then insert sequences and UMI sequences were identified. Reads with less than 16 bp insert sequences or less than 10 bp UMI sequences were discarded. Following trimming and cleaning, a sequential two-step alignment strategy was followed to map the reads to different databases. In the first step, reads were sequentially mapped to multiple references (miRbase mature, miRbase hairpin, piRNA, tRNA, mRNA and other RNA) allowing only perfect matches. In the second step, unmapped sequences from the first step were mapped to miRbase mature tolerating up to two mismatches. For miRNA mapping, miRbase v21 was used as reference, while piRNABank was used for piRNA mapping. For the remaining RNA species, the human GRCh38 reference was used. Mapping was performed with Bowtie (Langmead et al., 2009). Read counts were calculated for each RNA category from the mapping results.

### 2.6. Differential expression analysis

Differential expression (DE) analysis was carried out using only the read counts for mature miRNAs that were considered unique as identified by the UMIs. A pre-filtering step was applied in which only miRNAs with more than one count per million (CPM) in at least three samples (the least group size) were retained, leaving 1200 out of 1599 (75%) mature miRNA transcripts for downstream analyses. The statistical R package *DESeq2* (Love et al., 2014) was used for DE analyses applying default parameters. Four separate DE analyses were performed to examine the following: 1) baseline (untreated) differences between CTRL and SCZ astrocytes; 2) the effects of cytokine treatment (untreated vs. treated) in CTRL samples; 3) the effects cytokine treatment (untreated vs. treated) in SCZ samples; and 4) the effects of cytokine treatment across diagnostic groups (CTRL (untreated vs. treated) vs. SCZ (untreated vs. treated)). Sex and age differences were controlled for in 1), while in 2) and 3) the samples were paired and therefore perfectly matched on sex and age. In 4), the design formula was modified to take into account the fact that samples were nested within diagnostic groups, and this precluded the possibility of controlling for additional variables. An miRNA was defined as DE if the FDR was <0.1.

### 2.7. Target prediction and gene ontology enrichment analysis

Target prediction for significant miRNAs was performed with the R package *miRNAtap*, which integrates and aggregates ranked miRNA target predictions from five of the most commonly cited prediction algorithms: DIANA-microT (Maragkakis, et al., 2011), miRanda (Enright, et al., 2003), PicTar (Lall et al., 2006), TargetScan (Friedman et al., 2009), and miRDB (Wong and Wang, 2015). Default parameters were applied. The full set of ranked gene targets were then used as input to the R package *topGO* for Gene Ontology (GO) enrichment analysis. "Biological Process" was selected as the ontology and the Kolomonogorov Smirnov (KS) method was used for enrichment testing in order to make use of the rank information. A GO term was considered significantly enriched if the nominal p-value was <0.01, which is the default behavior of *topGO*.

### 2.8. ELISA assays

For sample preparation, $2.5 \times 10^5$ cells were cultured in 1 ml AM medium in 12-well plate. Culture supernatants were harvested 24 h after activation by 10 ng/ml IL-1β and the concentration of IL-6 and TNF-alpha cytokines were measured using Human IL-6 and TNF alpha ELISA kits (Thermo Fisher) following the manufacturer's recommendations. The precision of the ELISA kits were the following: Intra-Assay variation: CV < 10%; Inter-Assay variation: CV < 12% (CV% = SD/meanX100).

### 2.9. Statistics

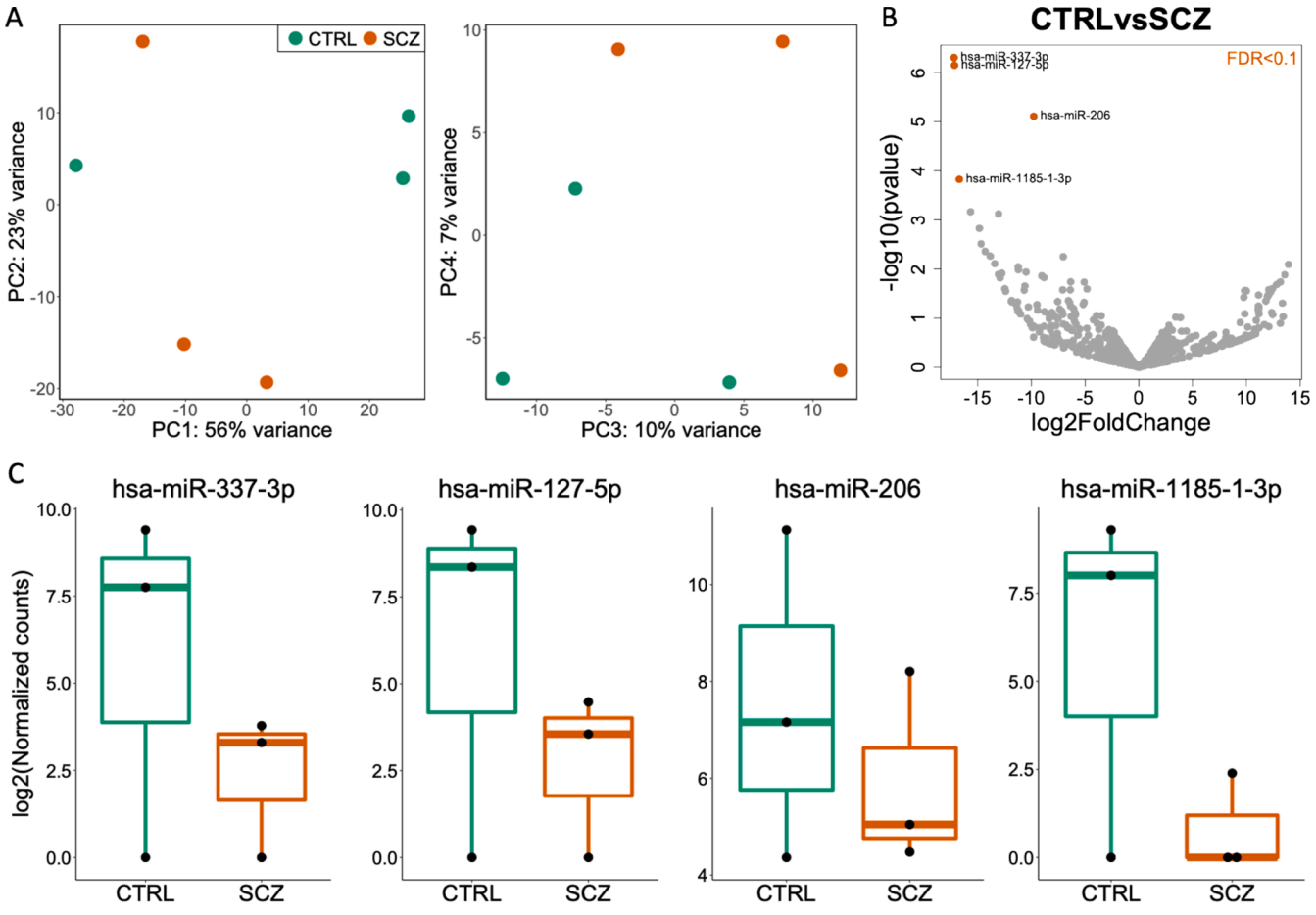
To test for significant differences in the blood gene expression levels of relevant miRNA target genes (*NFE2L2*, *FCGR1A*, *FCGR1B*, *FCGR1CP*, *IGF1*, *IRAK1*, *GSK3B*, *IL2*, *IL23*, *IL23R*, *LAMTOR4*, *MTOR*, *ERBB2*, *ERBB3*, *ERBB4*, *PIK3CA*) in SCZ patients compared to CTRLs, a multiple linear regression model with adjustment for age and sex differences was performed in R 3.4.1. Since some of the genes are represented by

multiple probes on the microarray chip, a total of 25 markers were tested (Suppl. Table S9). The selection of miRNA target genes was based on our target prediction results in combination with manual literature searches.

## 3. Results

### 3.1. Summary of miRNA sequencing output

An overview of the miRNA sequencing output can be found in Suppl. Fig. S1. Briefly, the average sequencing coverage was ~22 million reads per sample (Suppl. Fig. S1A), of which an average of ~25% (8–36%) mapped to miRNA genes (Suppl. Fig. S1B). When only mappable reads are considered, the miRNA fraction increases to 49%. We found no correlation between sequencing coverage and the percentage of miRNA reads. On average, ~84% of total miRNA reads were UMIs (Suppl. Fig. S1C). After filtering out lowly expressed miRNAs, 1200 genes were considered detected and thus tested in downstream analyses.




D

| hsa-miR-127-5p | | hsa-miR-206 | | hsa-miR-337-3p | |
|---|---|---|---|---|---|
| Gene | Rank product | Gene | Rank product | Gene | Rank product |
| HNRNPA2B1 | 1.658 | RPP21 | 2.174 | DDAH2 | 1.541 |
| RBP7 | 3.298 | LIPI | 4.115 | ELF1 | 2.856 |
| LXN | 3.354 | GLCCI1 | 4.476 | KRAS | 3.500 |
| CPSF6 | 3.517 | TMSB4X | 4.556 | ANP32E | 3.530 |
| LCLAT1 | 3.803 | NXT2 | 4.813 | PTEN | 3.566 |
| SHC3 | 4.147 | HACD3 | 5.241 | USP15 | 3.764 |
| RAB6A | 4.216 | ARCN1 | 5.519 | FAM104A | 4.314 |
| PEF1 | 4.690 | ZMAT3 | 6.331 | TNFAIP6 | 4.498 |
| TEX26 | 5.339 | PLPPR4 | 6.375 | ZFY | 4.899 |
| HNRNPR | 5.363 | EIF1AX | 6.381 | C5orf63 | 5.590 |

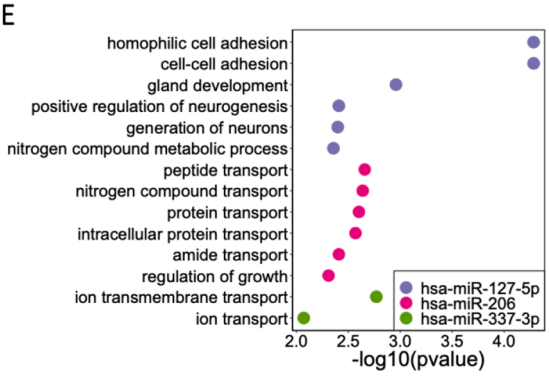



**Fig. 1.** Differential expression of miRNAs in SCZ iPSC-astroglia at baseline. A) PCA plots of baseline (untreated) astrocyte cultures. A clear separation on diagnosis is observed when clustering across PCs 3 and 4, explaining a relatively small proportion (17%) of the gene expression variance. All miRNA genes with detectable expression levels (n = 1200) were used as input. B) Volcano plot showing effect sizes (log fold changes) and raw p-values for the four miRNAs with differential expression in SCZ astrocytes compared to controls. C) Box plots of significant miRNAs. Black data points represent individual samples within each group. D) Top 10 genes predicted to be targets of the identified differentially expressed miRNAs. The target genes are ranked based on prediction results from five different sources as implemented in the *miRNAtap* tool. No target genes were identified for miR-1185–1-3p. E) GO enrichment analysis of the full set of predicted target genes for the indicated miRNAs. Only the top GO terms with lowest p-values are shown. For the full list, see Supplementary Table S4.

### 3.2. Differential expression of miRNAs in iPSC-astroglia at baseline

Recent advances suggest that certain miRNA classes are involved in the pathophysiology and symptom development of SCZ (Beveridge and Cairns, 2012; Im and Kenny, 2012; Xu et al., 2013; Rajman and Schratt, 2017; Cao and Zhen, 2018). To date however, little is known about exactly which miRNA species and their gene targets underlie putative cellular pathomechanisms. Therefore, we first performed an unbiased screening of the complete miRNome of human iPSC-astrocytes at baseline from both healthy CTRLs and SCZ patients (Fig. 1).

#### 3.2.1. Unsupervised clustering

We used principal component analysis (PCA) to cluster astrocyte samples based on all detected miRNAs (n = 1200). An incomplete separation on diagnosis was observed when clustering over the first two principal components (PCs), explaining 79% of the variation (Fig. 1A). The separation between CTRL and SCZ miRNomes became more pronounced when clustering over PC3 and PC4, but the explained total variation was only 17%, indicating that diagnostic status is not the major source of variation in miRNA gene expression (Fig. 1A).

#### 3.2.2. DE analyses between CTRL and SCZ groups

We idienified four miRNA genes with clear differential expression (FDR < 0.1) in SCZ astroglia, all of which exhibited substantially lower baseline expression compared to CTRL astroglia: miR-337-3p, miR-127-5p, miR-206, and miR-1185–1-3p (Fig. 1B-C; Suppl. Table S3). Strikingly, all of these significant DE miRNAs have previously been linked to the regulation of inflammation at both cellular and systems levels (Xia et al., 2018; Xie et al., 2012; Akehurst et al., 2015; Chu et al., 2019; Garcia-Lacarte et al., 2019). To identify the biological processes in which the four miRNAs are involved, we first searched for the potential target genes using multiple target prediction databases and then performed a GO enrichment analysis on the ranked targets (Fig. 1D). We found the strongest enrichment in target gene sets related to cell adhesion interactions and regulation of neurogenesis (miR-127-5p), protein transport and growth processes (miR-206), and regulation of transmembrane ion transport (miR-337-3p) (Fig. 1E; Suppl. Table S4). No target genes were identified for miR-1185–1-3p.

### 3.3. Effects of inflammatory modulation on the miRNome of iPSC-astrocytes

Glial cells, such as astrocytes, have recently emerged as potential candidates for brain inflammatory abnormalities in SCZ (Khandaker et al., 2015), and changes in human postmortem brain IL-1β levels have been identified as potential biomarkers in SCZ pathophysiology (Zhu et al., 2018; Mohammadi et al., 2018). These findings resonate with recent reports linking dysregulated inflammatory responses in astrocytes to cortical atrophy and cognitive impairment in SCZ (Kindler, et al., 2019). Although miRNAs have been proposed to play an important role in astroglia-mediated inflammation in CNS pathologies (Karthikeyan et al., 2016) including SCZ (Mellios and Sur, 2012), very little is known about their glia-specific expressions following inflammatory challenge. Therefore, we modelled an inflammatory environment by treating iPSC-astrocytes with IL-1β, a classical and prototypical pro-inflammatory cytokine, as described in our previous study (Akkouh, et al., 2020).

As expected, PCA of treated and untreated samples showed a

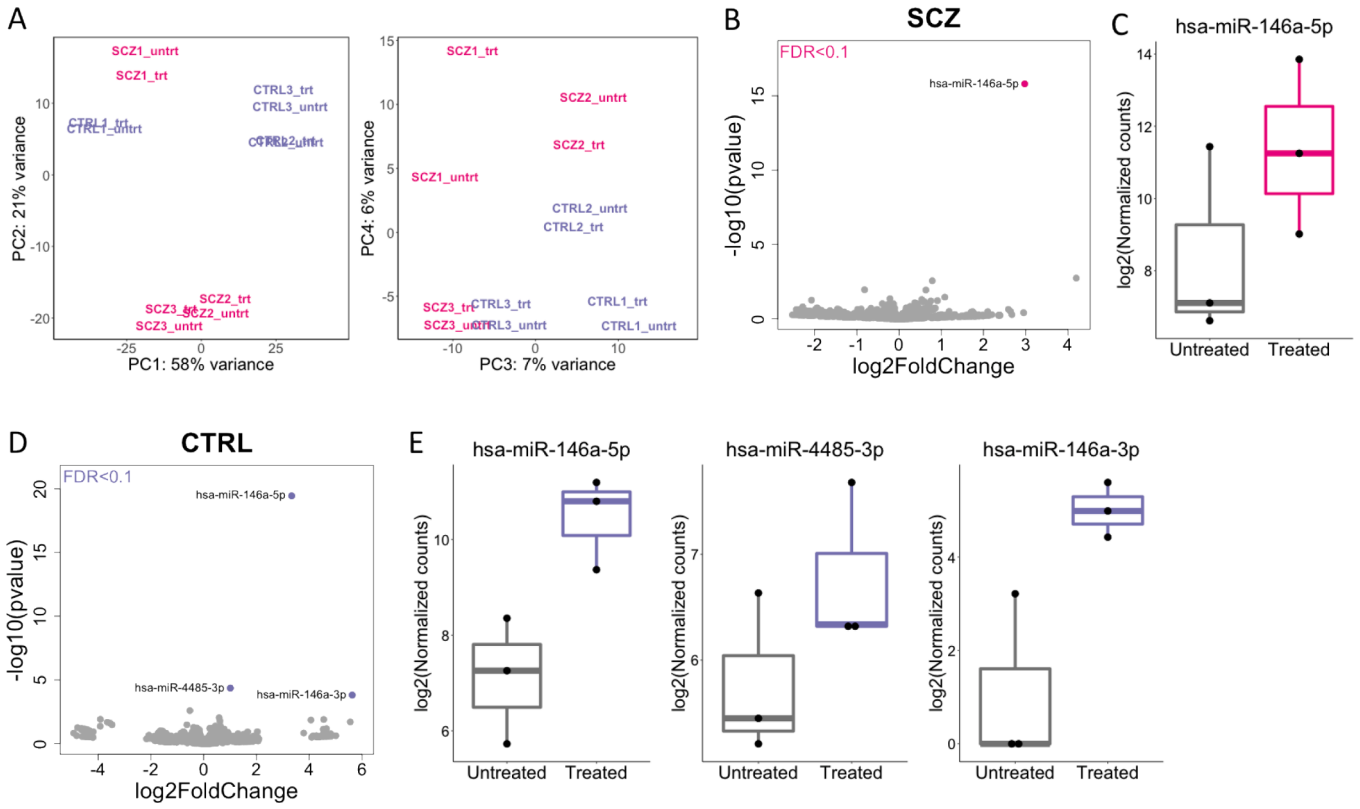



**Fig. 2.** Effects of cytokine treatment on miRNA expression in iPSC-astrocytes. A) PCA plots of all IL-1β-treated (trt) and untreated (untrt) samples. Clustering over the first two components show that donor effects are a major source of variance as samples from the same individual tend to cluster together. A clear separation on cytokine treatment and diagnosis is only seen when samples are clustered over PCs 3 and 4. B-C) Volcano and box plots showing the one miRNA (miR-146a-5p) that was significantly up-regulated by cytokine treatment in SCZ samples. D-E) Volcano and box plots showing three miRNA that were up-regulated by cytokine treatment in CTRL samples. The volcano plots were based on all miRNAs with detectable expression levels (n = 1200). The black data points in the box plots represent individual samples.

considerable separation on donors when clustering over the first two PCs, accounting for 79% of the total variation (Fig. 2A). Clustering over PC3 and PC4 revealed a clear albeit modest (11%) separation on IL-1β treatment and diagnostic status (Fig. 2A). These observations are in line with similar studies showing that donor effects are a greater source of variance than diagnostic status in iPCS-derived cellular models (Readhead et al., 2018).

### 3.3.1. DE analyses in CTRL and SCZ groups following inflammatory stimulation

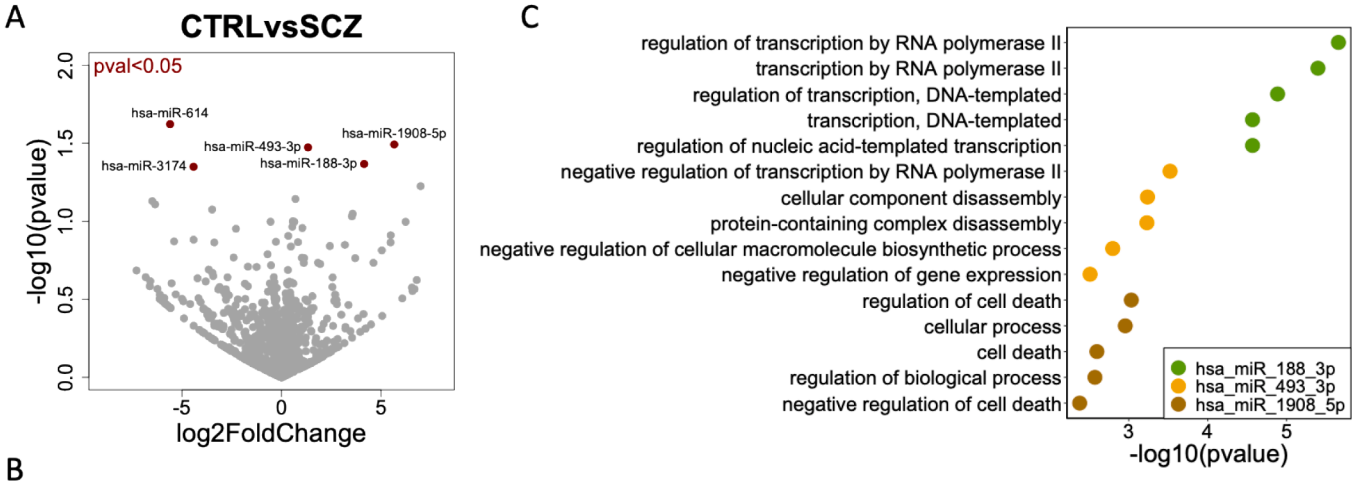
We next performed two separate DE analyses examining the miRNA expression-modulatory effects of IL-1β treatment in SCZ and CTRL samples, respectively. In the SCZ group, IL-1β significantly changed the expression level of miR-146a-5p (Fig. 2B-C; Suppl. Table S5). In the CTRL group, the treatment modulated the expression of miR-146a-3p, miR-146a-5p, and miR-4485-3p (Fig. 2D-E; Suppl. Table S6). GO enrichment analysis of predicted miR-146a-3p target genes (Suppl. Fig. S2A) revealed significant enrichment for gene sets related to classical biochemical components of the cellular stress reponse, such as regulation of several biosynthetic processes as well as organelle and actin cytoskeleton organization (Suppl. Fig S2B).

### 3.3.2. Group-specific effects of cytokine treatment on miRNA expression

To investigate whether SCZ iPSC-astrocytes respond differently to IL-1β treatment than CTRL astrocytes, we performed a group-specific DE analysis. Overall, five miRNAs were differentially modulated by cytokine treatment across diagnostic groups; however, these differences were only trending ($p > 0.05$) and did not survive correction for the number of tests performed (Fig. 3A; Suppl. Table S7). Two of the suggestive miRNAs, miR-188-3p and miR-1908-5p, showed similar expression trends in both CTRL and SCZ, where IL-1β treatment modestly decreased their levels in CTRLs and increased their expression in SCZ astrocytes (Suppl. Fig. S3). Secondly, miR-3174 and miR-614 also exhibited similar expression patterns, as IL-1β slightly increased their levels in CTRL, but not in SCZ astroglia (Suppl. Fig. S3). Finally, we did not observe any modulatory effect of the treatment on miR-493-3p levels, neither in CTRL nor in SCZ (Suppl. Fig. S3), neither did we observe significant differences in the IL-1β-induced IL-6 and TNF-alpha secretion of astrocytes between cases and controls (Suppl. Fig. S4). GO analysis of the predicted target genes (Fig. 3B) showed the strongest enrichment for biological processes involved in the control of cell proliferation (miR-188-3p) and in the negative regulation of transcription, translation and macromolecule biosynthesis (miR-493-3p) (Fig. 3C; Suppl. Tabl S8). Enrichments for biological activities pertaining to the regulation of apoptotic processes (miR-1908-5p) were also observed (Fig. 3C; Suppl. Tabl S8). The target genes for miR-3174 and miR-614 (Fig. 3B) were not significantly enriched for any GO terms.

### 3.4. Expression of target genes in circulating immune cells matches the expression patterns of their targeting miRNAs in in vitro iPSC-astrocyte cultures

Astrocytes secrete a diverse range of miRNAs contained in exosomes (Jovicic and Gitler, 2017), which can pass the blood–brain barrier, and this mechanism has been hypothesized as a form of remote target gene regulation between the CNS and circulation, as well as a possible way for brain-immune bidirectional communication (Matsumoto et al., 2018; Saint-Pol et al., 2020). Furthermore, the diagnostic value of circulating miRNAs isolated from total peripheral blood samples has recently been proposed in SCZ (Liu et al., 2017). We thus examined peripheral blood




B

| hsa-miR-188-3p | | hsa-miR-493-3p | | hsa-miR-1908-5p | | hsa-miR-3174 | | hsa-miR-614 | |
|---|---|---|---|---|---|---|---|---|---|
| Gene | Rank product | Gene | Rank product | Gene | Rank product | Gene | Rank product | Gene | Rank product |
| ZNF799 | 1.871 | FOXP2 | 2.240 | ESPN | 1.225 | CHCHD3 | 1.000 | HOOK2 | 0.693 |
| ZNF546 | 2.500 | YTHDF2 | 3.062 | GNG7 | 3.240 | CHAC2 | 1.587 | NLRP8 | 0.874 |
| ZNF559-ZNF177 | 3.659 | SRSF11 | 3.362 | DPP9 | 4.272 | PSMB5 | 1.732 | FOXD1 | 1.140 |
| ZNF594 | 4.330 | FAM102B | 3.932 | ZNF784 | 6.423 | TIPRL | 2.000 | TEX101 | 1.414 |
| ZNF624 | 4.486 | MRVI1 | 4.738 | ISLR2 | 6.595 | GIMAP2 | 3.037 | HCAR3 | 1.803 |
| TMEM178B | 4.583 | C17orf49 | 4.827 | UBXN6 | 8.216 | NAT2 | 3.969 | EPB41L5 | 2.345 |
| ZNF559 | 4.871 | DKK1 | 5.209 | PRRT1 | 8.441 | ZNF572 | 4.822 | GTPBP6 | 2.449 |
| PITPNM1 | 4.950 | SOSTDC1 | 5.441 | NTN5 | 8.544 | ZFYVE21 | 5.016 | STT3A | 2.739 |
| ZNF441 | 5.037 | DPY30 | 5.831 | LSP1 | 8.573 | NDUFA6 | 5.524 | EXOSC7 | 3.317 |
| SURF4 | 5.992 | WDR87 | 6.801 | FKBP8 | 9.539 | PMP22 | 5.916 | NTM | 3.482 |

**Fig. 3.** Group-specific effects of cytokine treatment on miRNA expression. A) Five miRNAs displayed a suggestive ($p < 0.05$) disease-specific association with cytokine treatment, i.e. IL-1β modulated the expression of these miRNAs differently in SCZ astrocytes than in CTRL astrocytes. B) The top 10 target genes of the five identified miRNAs as predicted by aggregating results from multiple prediction algorithms. C) GO enrichment test using the full set of predicted target genes for each suggestive miRNA. Only the most significantly enriched GO terms are shown. No GO term was significantly enriched by the predicted target genes of miR-3174 and miR-614.

samples from a large cohort of SCZ patients (n = 484) and healthy CTRLs (n = 496) and tested whether a selected panel of target genes (see Materials and Methods) for the four DE miRNAs identified in our baseline astrocyte cultures (Fig. 1A-C) were differentially expressed between diagnostic groups. We found that three genes, *LAMTOR4, IL23R*, and *ERBB3*, had a significantly lower expression in the blood of SCZ patients relative to CTRLs after multiple testing correction (FDR < 0.1; Fig. 4; Suppl. Table S9). In addition, trending differences (p < 0.05) were found for two other genes, *ERBB2* and *IRAK1*, both of which had lower expression levels in SCZ versus CTLR (Fig. 4; Suppl. Table S9).

Notably, when comparing our *in vivo* and *in vitro* results (Figs. 1 and 4), we found matching patterns between the expression of miRNAs and their target mRNAs. The blood expression levels of the selected targets of all four miRNAs, which exhibited lower expression in SCZ iPSC-astroglia versus CTRL at baseline, were also lower in SCZ blood samples relative to healthy control samples: miR-337-3p and *LAMTOR4,* miR-1185-1-3p and *IL23R*, miR-127-5p and *ERBB2/ERBB3*, and miR-206 and *IRAK1* (Fig. 1C and Fig. 4) (Akehurst et al., 2015; Chu et al., 2019; Wang et al., 2014). These findings indicate a possible SCZ-specific alteration in top-down brain-immune system regulation of genes involved in innate immune responses (*IL23R*; *IRAK1*) and in cellular proliferation and metabolism (*ERBB3*, *ERBB2*, *LAMTOR4*).

## 4. Discussion

A mounting body of evidence suggests that certain miRNA classes are involved in the pathophysiology and symptom development of SCZ (Beveridge and Cairns, 2012; Im and Kenny, 2012; Xu et al., 2013; Rajman and Schratt, 2017; Cao and Zhen, 2018). A good example is miR-137, which has an important role in modulating neuronal maturation, neurotransmission, and synaptic plasticity, and for which dysregulation has been linked to SCZ, bipolar disorder, and to the cognitive symptoms of various psychiatric disorders (Thomas et al., 2018). Dysregulation in the expression of certain miRNA species and of exosomal miRNA-cargo in astrocytes and microglia has been linked to neurotoxic inflammation in neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS) (Varcianna et al., 2019; Rostalski et al., 2019). Furthermore, recent reports exposed several miRNAs as potential culprits in astrocyte and microglia-mediated neuroinflammation in SCZ (Mellios and Sur, 2012) and other brain pathologies (Karthikeyan et al., 2016). However, our knowledge is still scarce about the exact miRNA species and their gene targets that mediate glial functions and glia-neuron crosstalk in SCZ.

In the present study, our goal was to obtain the complete miRNome of human iPSC-astrocytes at baseline and following inflammatory stimulation. We identified four miRNAs, miR-127-5p, miR-206, miR-337-3p and miR-1185–1-3p, which displayed lower expression in SCZ astroglia expressions relative to CTRL astroglia. All four of these miRNAs are involved in the regulation of inflammation in various systemic and tissue microenvironment-specific contexts (Xia et al., 2018; Xie et al., 2012; Akehurst et al., 2015; Chu et al., 2019; Garcia-Lacarte et al., 2019). We then ran gene enrichment analyses to gain insight into the biological processes the four miRNAs may regulate. We found that the strongest enrichments were associated with neurogenesis and cell-to-cell adhesion processes (miR-127-5p), protein transport and growth modulation (miR-206), and control of transmembrane ion transport (miR-337-3p). These biological processes can be linked to the astroglial regulation of neuroplasticity (Haydon and Nedergaard, 2015)

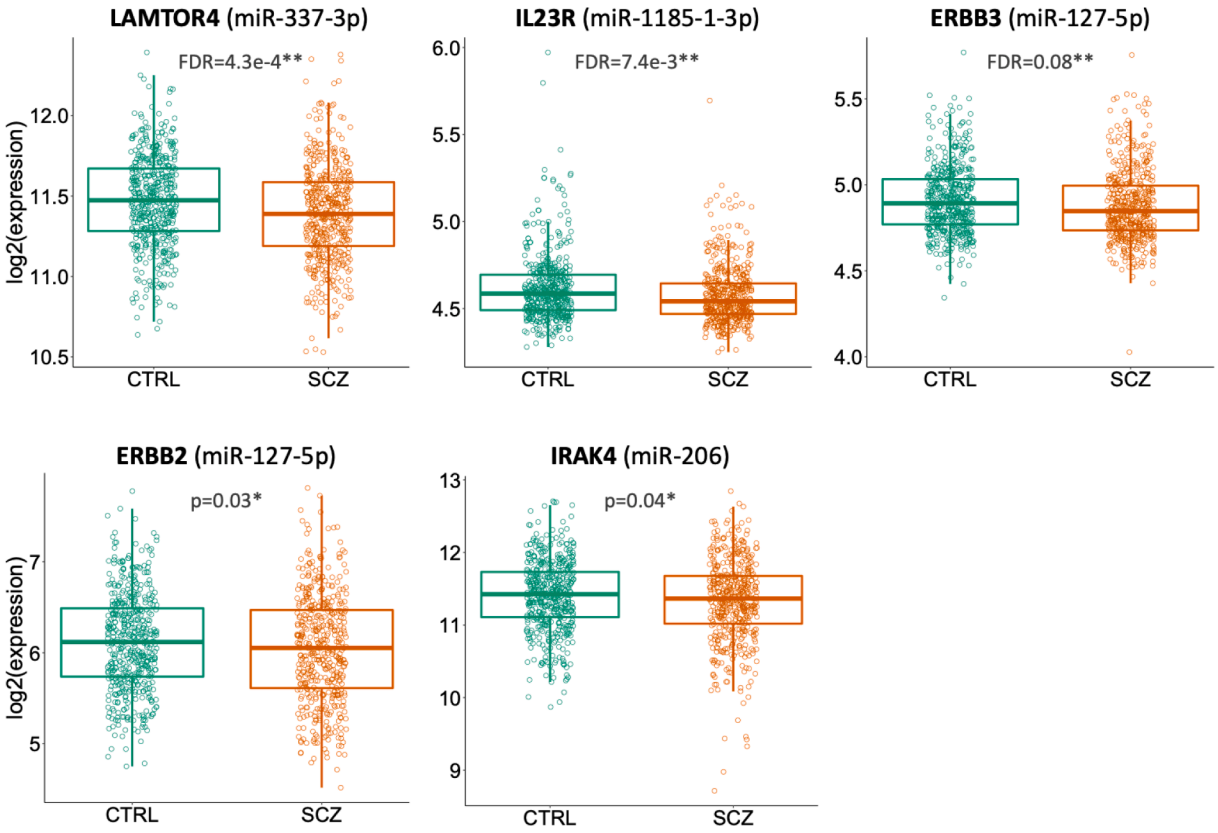



**Fig. 4.** Differential expression analysis of selected target genes in circulating immune cells. A selected panel of predicted targets of the four miRNAs with differential expression in baseline SCZ astrocytes were examined in whole blood samples from SCZ patients (n = 484) and healthy CTRLs (n = 496) using microarrays. A total of 25 probes representing 16 individual genes were examined. Three genes, *LAMTOR4*, *IL23R*, and *ERBB2*, were significantly (FDR < 0.1) down-regulated in SCZ cases, while *ERBB2* and *IRAK1* displayed a nominally significant (p < 0.05) down-regulation in SCZ. **FDR < 0.1. *p-value < 0.05. The gene names in brackets represent gene-targeting miRNAs based on *in silico* predicition algorithms and available literature.

suggesting that decreased expression of the four miRNAs may be involved in the aberrant formation and sculpting of neural tissue as well as the impaired neuroplasticity observed in SCZ (Bhandari et al., 2016). In line with our findings, down-regulation of two miR-127 analogs, miR-127-3p and 5p, has been demonstrated in frontotemporal dementia (Piscopo et al., 2018) and in the prefrontal cortex of Parkinson's Disease patients (Hoss et al., 2016), respectively, suggesting a pathobiological role for these miRNA species in the cognitive symptoms of different neuropsychiatric disorders. It has also been reported that expression of miR-206 in the brain shows significant allelic association to SCZ (Hansen et al., 2007; Hauberg et al., 2016). Furthermore, down-regulation or miR-337-3p has been shown to play a role in synaptic abnormalities in a mouse model of SCZ by potentially influencing the onset of age-related cognitive decline observed during the symptom evolution of the disorder (Earls et al., 2012). miR-337 is also one of the clozapine-associated miRNAs previously shown to influence ligand-receptor interactions at 5-HT2A serotonin receptor sites (Santarelli et al., 2013). While we found no target genes for miR-1185-1-3p in our enrichment analysis, down-regulation of this miRNA has been seen in depression (Zhang et al., 2016) and has been suggested to be involved in the negative symptoms of SCZ (van den Berg et al., 2020). Our results corroborate these findings by showing a decreased baseline expression of miR-127-5p, miR-206, miR-337-3p, and miR-1185-1-3p in SCZ-astrocytes, suggesting a cell type-specific role of these miRNA species in SCZ pathogenesis.

The gliopathy hypothesis of SCZ is an emerging paradigm in psychiatry research that implicates glia-related inflammatory abnormalities, such as abnormal brain IL-1β levels, in disease pathophysiology (Khandaker et al., 2015; Zhu et al., 2018; Mohammadi et al., 2018). Recent findings associate dysregulated inflammatory responses in astrocytes with cortical atrophy and cognitive impairment in SCZ (Kindler, et al., 2019). miRNAs have been causally linked to astroglia-mediated inflammation in CNS pathologies (Karthikeyan et al., 2016) including SCZ (Mellios and Sur, 2012), but very little is known about their astrocyte-specific expressions following inflammatory modulation. Therefore, we next treated iPSC-astrocytes with IL-1β, and performed two separate DE analyses examining the miRNA expression-modulatory effects of the treatment in SCZ and CTRL samples. We found that IL-1β significantly changed the expression level of only one miRNA species, miR-146a-5p, in the SCZ group. This is in good agreement with recent studies reporting miR-146a as an IL-1β-responsive element (Nahid et al., 2015), and an inflammatory biomarker miRNA involved in the regulation of a plethora of innate immune responses (Nahid et al., 2011; Saba et al., 2014). In the CTRL group, by contrast, IL-1β significantly changed the expression levels of three miRNA species: miR-146a-3p, miR-146a-5p, and miR-4485-3p, all of which have also been associated with inflammation and related cellular stress responses in previous *in vitro* and *in vivo* studies (Nahid et al., 2015, 2011; Saba et al., 2014). miR-4485 has also been associated with inflammatory responses following different environmental insults, such as heat-shock (Rong and Liu, 2020) and UV irradiation (Hwang et al., 2020). Notably, SCZ astrocytes exhibited a much lower degree of IL-1β-provoked upregulation of miR-146a-5p than CTRL astrocytes, suggesting a decreased ability to respond to inflammatory stress. In line with these findings, our GO enrichment analysis of predicted miR-146a-3p target genes showed significant enrichments for classical biochemical components of cellular stress reponse, such as regulation of several biosynthetic processes as well as organelle and actin cytoskeleton reorganization. The observed disparate expression of miRNAs following IL-1β modulation in SCZ and CTRL astrocytes suggests an impaired ability of SCZ-astroglia to respond to inflammatory challenge, and an overall decrease in stress resilience compared to CTRLs.

To further investigate if there is a difference in the response to IL-1β treatment in SCZ versus CTRL astrocytes, we performed a group-specific DE analysis. We found only trending, but not significant differences in the expression of five miRNA species, which were differentially modulated by the inflammatory treatment across diagnostic groups, and no differences in the IL-1β-induced inflammatory cytokine secretion of astrocytes. It is important to note that this latter phenomenon may be an inssue of statistical power due to low donor numbers (n = 3) between SCZ and CTRLs. Two of the five miRNAs, miR-188-3p and miR-1908-5p, displayed moderate trends in which both were downregulated by IL-1β treatment in CTRLs, but upregulated in SCZ astrocytes. Interestingly, abnormal upregulations of both miR-188 and miR-1908 have been associated with neurodegenerative disorders (Jovicic and Gitler, 2017; Wang et al., 2018). miR-188 has recently been shown to be enriched in astroglia in a mouse model of ALS, a neurodegenerative disorder in which astrocytes play a crucial role in disease pathogenesis (Jovicic and Gitler, 2017). In addition, upregulation of miR-1908 has been shown to impair amyloid clearance by targeting ApoE, and elevated levels of this miRNA has been found in peripheral blood cells of Alzheimer's disease patients (Wang et al., 2018, 2019). This is suggestive of a dysregulated astrocytic response to infections or chronic inflammation in SCZ, where abnormally high levels of miR-188 and miR-1908 may predispose patients to neurodegeneration. Two other miRNAs, miR-3174 and miR-614, exhibited increased expression levels in CTRL, but not SCZ astrocytes following inflammatory modulation. miR-3174 and miR-614 have been shown to drive (Li et al., 2017) and inhibit (Zhang et al., 2018) apoptosis and autophagy in cancer cells, respectively. Both processes are also well known during and after inflammatory stress responses in the human brain (Corps et al., 2015), and are central to the survival and post-stress functions of astrocytes following brain injuries, inflammation and oxidative stress (Karve et al., 2016). Our results indicate an inability of SCZ astrocytes to regulate the expression of these cell survival-related miRNAs under inflammatory conditions. It is important to note that the lack of statistical significance in our DE analyses may be due to small sample sizes, and therefore warrants further investigation into the modulatory effects of IL-1β on the levels of the identified miRNAs in human astrocytes.

The exosome-mediated astroglial secretion of miRNAs and their transportation via the blood–brain barrier is a putative mechanism that may allow the glial compartment of the brain to communicate with and regulate gene expression in peripheral immune cells (Jovicic and Gitler, 2017; Matsumoto et al., 2018; Saint-Pol et al., 2020). This hypothesis is gaining ground in recent psychoneuroimmunological research, emphasizing the potential diagnostic value of circulating miRNAs in SCZ (Liu et al., 2017). To investigate a possible astrocyte-peripheral immune regulatory axis in SCZ, we examined peripheral blood samples from a large cohort of SCZ patients (n = 484) and healthy CTRLs (n = 496). Screening for a selected panel of genes that are targets of the four differentially expressed miRNAs previously identified in our iPSC-astrocyte baseline setup, we found that three of these genes, *LAMTOR4, IL23R*, and *ERBB3*, displayed significantly lower expressions in the blood of SCZ patients relative to CTRLs after multiple testing correction. We also found a trend for lower expression of *ERBB2* and *IRAK1* in SCZ patients versus CTLRs. Thus, our clinical *in vivo* and iPSC *in vitro* results showed matching expression patterns between the four miRNAs and their target genes, supporting the notion of a top-down brain-immune system regulatory mechanism with disease-specific alterations in SCZ. Interestingly, the modulated target genes in the clinical sample were factors critically involved in innate immune responses (*IL23R*, *IRAK1*) (Mogensen, 2009; Szabo and Rajnavolgyi, 2013), cellular metabolism and cell cycle regulation, but also in the immune control of pathogenic infections (*ERBB3*, *ERBB2*, *LAMTOR4*) (Ho et al., 2017). The observed lower expression of these genes in SCZ patients may represent *miRNA-based transcriptional gene activation* where miRNAs bind nascent RNA transcripts, gene promoter regions, or enhancer regions and positively regulate gene expression, thus decreased trends in miRNA expression are expected to be associated with decreased expression of their gene targets (Orang, 2014; O'Brien et al., 2018). It may also involve abnormal secretion of exosomes and/or disparate amounts of miRNA cargo-load in SCZ-astroglia secreted exosomes (Jovicic and Gitler, 2017). The phenomenon may also be the result of

dysregulated peripheral immunity, but can also be the consequence of a compensatory mechanism seen in long-term, chronic inflammatory conditions (Fekete et al., 2012). For instance, chronic inflammatory stimulation of dendritic cells, a myeloid cell type that plays a critical role in bridging innate and adaptive immunity, has been linked to sustained IRAK1 suppression (Hedl et al., 2007). The same phenomenon was observed in monocytes of septic patients (Escoll et al., 2003). Furthermore, bacterial LPS-induced miRNAs have been shown to act via down-regulation of the innate immune Toll-like receptor signaling component of IRAK1 through miR-146a (Taganov, 2006). This represents an evolutionally conserved immune tolerance mechanism that may be involved in the detected SCZ-specific expression patterns of miRNAs and gene targets, and may also support the inflammatory gliopathy hypothesis of SCZ, where chronic, low-grade inflammation may play a causative role in the anomaly (Khandaker et al., 2015).

In summary, we performed a comparative analysis of the global miRNome of human astrocytes differentiated from iPS cells derived from SCZ patients and healthy controls, at both baseline and following inflammatory modulation. We identified four differentially expressed miRNAs in SCZ astrocytes that exhibited significantly lower baseline expression relative to CTRLs, and indications of differential inflammatory modulation. We also analyzed peripheral blood samples from a large cohort of SCZ patients and healthy controls and found patterns of expression of these four miRNAs and their target genes that matched our *in vitro* results. These data have implications for the role of astroglia and a hypothesized astroglia-peripheral immune regulatory axis in the pathogenesis of SCZ.

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## Appendix A. Supplementary data

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## References

Abbott, N.J., et al., 2006. Astrocyte-endothelial interactions at the blood-brain barrier. Nat. Rev. Neurosci. 7 (1), 41–53.
Akehurst, C., et al., 2015. Differential expression of microRNA-206 and its target genes in preeclampsia. J. Hypertens. 33 (10), 2068–2074.
Akkouh, I.A., et al., 2018. Expression of TCN1 in blood is negatively associated with verbal declarative memory performance. Sci. Rep. 8 (1) https://doi.org/10.1038/s41598-018-30898-5.
Akkouh, I.A., et al., 2020. Decreased IL-1β-induced CCL20 response in human iPSC-astrocytes in schizophrenia: potential attenuating effects on recruitment of regulatory T cells. Brain Behav. Immun. 87, 634–644.
Beveridge, N.J., Cairns, M.J., 2012. MicroRNA dysregulation in schizophrenia. Neurobiol. Dis. 46 (2), 263–271.
Bhandari, A., et al., 2016. A review of impaired neuroplasticity in schizophrenia investigated with non-invasive brain stimulation. Front. Psychiatry 7. https://doi.org/10.3389/fpsyt.2016.00045.
Brennand, K.J., et al., 2011. Modelling schizophrenia using human induced pluripotent stem cells. Nature 473 (7346), 221–225.
Cao, T., Zhen, X., 2018. Dysregulation of miRNA and its potential therapeutic application in schizophrenia. CNS Neurosci. Ther. 24, 586–597.
Chu, H., et al., 2019. MicroRNA-206 promotes lipopolysaccharide-induced inflammation injury via regulation of IRAK1 in MRC-5 cells. Int. Immunopharmacol. 73, 590–598.
Corps, K.N., et al., 2015. Inflammation and neuroprotection in traumatic brain injury. JAMA Neurol. 72 (3), 355. https://doi.org/10.1001/jamaneurol.2014.3558.
Correll, C.U., et al., 2015. Effects of antipsychotics, antidepressants and mood stabilizers on risk for physical diseases in people with schizophrenia, depression and bipolar disorder. World Psychiatry 14 (2), 119–136.
Earls, L.R., Fricke, R.G., Yu, J., Berry, R.B., Baldwin, L.T., Zakharenko, S.S., 2012. Age-dependent microRNA control of synaptic plasticity in 22q11 deletion syndrome and schizophrenia. J. Neurosci. 32 (41), 14132–14144.
Editorial, 2010. A decade for psychiatric disorders. Nature 463, 9.
Enright, A.J., et al., 2003. MicroRNA targets in Drosophila. Genome Biol 5:R1.
Escoll, P., et al., 2003. Rapid up-regulation of IRAK-M expression following a second endotoxin challenge in human monocytes and in monocytes isolated from septic patients. Biochem. Biophys. Res. Commun. 311 (2), 465–472.
Falk, A, et al., 2016. Modeling psychiatric disorders: from genomic findings to cellular phenotypes. Mol. Psychiatry 21 (9), 1167–1179.
Fekete, T., et al., 2012. Constraints for monocyte-derived dendritic cell functions under inflammatory conditions. Eur. J. Immunol. 42 (2), 458–469.
Friedman, R.C., et al., 2009. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 19 (1), 92–105.
Garcia-Lacarte, M., et al., 2019. miR-1185-1 and miR-548q are biomarkers of response to weight loss and regulate the expression of GSK3B. Cells 8 (12), 1548. https://doi.org/10.3390/cells8121548.
Gogtay, N., et al., 2011. Age of onset of schizophrenia: perspectives from structural neuroimaging studies. Schizophr. Bull. 37 (3), 504–513.
Hansen, T., et al., 2007. Brain expressed microRNAs implicated in schizophrenia etiology. PLoS ONE 2 (9), e873.
Hauberg, M.E., et al., 2016. Schizophrenia risk variants affecting microRNA function and site-specific regulation of NT5C2 by miR-206. Eur. Neuropsychopharmacol. 26 (9), 1522–1526.
Haydon, P.G., Nedergaard, M., 2015. How do astrocytes participate in neural plasticity? Cold Spring Harb. Perspect. Biol. 7 (3), a020438. https://doi.org/10.1101/cshperspect.a020438.
Hedl, M., et al., 2007. Chronic stimulation of Nod2 mediates tolerance to bacterial products. Proc. Natl. Acad. Sci. U.S.A. 104 (49), 19440–19445.
Heneka, M.T., et al., 2018. Inflammasome signalling in brain function and neurodegenerative disease. Nat. Rev. Neurosci. 19 (10), 610–621.
Ho, J., et al., 2017. The Role of ErbB Receptors in Infection. Trends Microbiol. 25 (11), 942–952.
Hoss, A.G., et al., 2016. microRNA profiles in Parkinson's disease prefrontal cortex. Front. Aging Neurosci. 8 https://doi.org/10.3389/fnagi.2016.00036.
Hwang, J., et al., 2020. Photoprotective activity of topsentin, a bis(indole) alkaloid from the marine sponge spongosorites genitrix, by regulation of COX-2 and Mir-4485 expression in UVB-irradiated human keratinocyte cells. Mar. Drugs 18 (2), 87. https://doi.org/10.3390/md18020087.
Ijaz, S., et al., 2018. Antipsychotic polypharmacy and metabolic syndrome in schizophrenia: a review of systematic reviews. BMC Psychiatry 18 (1). https://doi.org/10.1186/s12888-018-1848-y.
Im, H.I., Kenny, P.J., 2012. MicroRNAs in neuronal function and dysfunction. Trends Neurosci. 35 (5), 325–334.
Izrael, M., et al., 2007. Human oligodendrocytes derived from embryonic stem cells: effect of noggin on phenotypic differentiation in vitro and on myelination in vivo. Mol. Cell. Neurosci. 34 (3), 310–323.
Jovicic, A., Gitler, A.D., 2017. Distinct repertoires of microRNAs present in mouse astrocytes compared to astrocyte-secreted exosomes. PLoS ONE 12, e0171418.
Karthikeyan, A., et al., 2016. MicroRNAs: key players in microglia and astrocyte mediated inflammation in CNS pathologies. Curr. Med. Chem. 23 (30), 3528–3546.
Karve, I.P., et al., 2016. The contribution of astrocytes and microglia to traumatic brain injury. Br. J. Pharmacol. 173 (4), 692–702.
Khandaker, G.M., et al., 2015. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. Lancet Psychiatry 2 (3), 258–270.
Kim, R., et al., 2018. Astroglial correlates of neuropsychiatric disease: from astrocytopathy to astrogliosis. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 87, 126–146.
Kindler, J., et al., 2019. Dysregulation of kynurenine metabolism is related to proinflammatory cytokines, attention, and prefrontal cortex volume in schizophrenia. Mol. Psychiatry 2019. https://doi.org/10.1038/s41380-019-0401-9 [Epub ahead of print].
Lall, S., et al., 2006. A genome-wide map ofconserved microRNA targets in C. elegans. Curr. Biol. 16 (5), 460–471.
Langmead, B., et al., 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 10 (3), R25. https://doi.org/10.1186/gb-2009-10-3-r25.
Li, B., et al., 2017. miR-3174 contributes to apoptosis and autophagic cell death defects in gastric cancer cells by targeting ARHGAP10. Mol. Ther. Nucleic Acids 9, 294–311.
Liu, S., et al., 2017. Diagnostic value of blood-derived micrornas for schizophrenia: results of a meta-analysis and validation. Sci. Rep. 7 (1) https://doi.org/10.1038/s41598-017-15751-5.
Love, M.I., et al., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15 (12) https://doi.org/10.1186/s13059-014-0550-8.

Maragkakis, M., et al., 2011. DIANA-microT Web server upgrade supports Fly and Worm miRNA targetprediction and bibliographic miRNA to disease association. Nucleic Acids Res 39(Web Server issue):W145-148.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 17 (1), 10. https://doi.org/10.14806/ej.17.110.14806/ej.17.1.200.
Matsumoto, J., et al., 2018. The transport mechanism of extracellular vesicles at the blood-brain barrier. Curr. Pharm. Des. 23 (40), 6206–6214.
McGrath, J., et al., 2008. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol. Rev. 30 (1), 67–76.
Mellios, N., Sur, M., 2012. The emerging role of microRNAs in Schizophrenia and autism spectrum disorders. Front. Psychiatry 3, 39.
Mogensen, T.H., 2009. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin. Microbiol. Rev. 22 (2), 240–273.
Mohammadi, A., et al., 2018. Brain, blood, cerebrospinal fluid, and serum biomarkers in schizophrenia. Psychiatry Res. 265, 25–38.
Mørch, R.H., et al., 2019. Inflammatory markers are altered in severe mental disorders independent of comorbid cardiometabolic disease risk factors – inflammatory markers and immune activation in severe mental disorders. Psychol. Med. 49 (10), 1749–1757.
Nahid, M.A., et al., 2011. MicroRNA in TLR signaling and endotoxin tolerance. Cell. Mol. Immunol. 8 (5), 388–403.
Nahid, M.A., et al., 2015. Interleukin 1β-responsive microRNA-146a is critical for the cytokine-induced tolerance and cross-tolerance to toll-like receptor ligands. J. Innate Immun. 7 (4), 428–440.
O'Brien, J., et al., 2018. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front. Endocrinol. 9 https://doi.org/10.3389/fendo.2018.00402.
Orang, A.V., et al., 2014. Mechanisms of miRNA-mediated gene regulation from common downregulation to mRNA-specific upregulation. Int. J. Genomics 2014, 970607.
Patel, K.R., et al., 2014. Schizophrenia: overview and treatment options. P T 39, 638–645.
Piscopo, P., et al., 2018. Circulating miR-127-3p as a potential biomarker for differential diagnosis in frontotemporal dementia. J. Alzheimers Dis. 65 (2), 455–464.
Rajman, M., et al., 2017. MicroRNAs in neural development: from master regulators to fine-tuners. Development 144 (13), 2310–2322.
Readhead, B., et al., 2018. Expression-based drug screening of neural progenitor cells from individuals with schizophrenia. Nat. Commun. 9 (1) https://doi.org/10.1038/s41467-018-06515-4.
Rong, H.T., Liu, D.W., 2020. Identification of differentially expressed miRNAs associated with thermal injury in epidermal stem cells based on RNA-sequencing. Exp. Ther. Med. 19, 2218–2228.
Rostalski, H., et al., 2019. Astrocytes and microglia as potential contributors to the pathogenesis of C9orf72 repeat expansion-associated FTLD and ALS. Front. Neurosci. 13 https://doi.org/10.3389/fnins.2019.00486.
Rubinstein, E., 2010. Psychiatry: medicine benefits from cultural and personal insights. Nature 463, 424.
Saba, R., et al., 2014. MicroRNA-146a: a dominant, negative regulator of the innate immune response. Front. Immunol. 5 https://doi.org/10.3389/fimmu.2014.00578.
Saint-Pol, J., et al., 2020. Targeting and crossing the blood-brain barrier with extracellular vesicles. Cells 9 (4), 851. https://doi.org/10.3390/cells9040851.
Santarelli, D.M., et al., 2013. Gene-microRNA interactions associated with antipsychotic mechanisms and the metabolic side effects of olanzapine. Psychopharmacology 227 (1), 67–78.
Simonsen, C., et al., 2011. Neurocognitive dysfunction in bipolar and schizophrenia spectrum disorders depends on history of psychosis rather than diagnostic group. Schizophr. Bull. 37 (1), 73–83.
Singhal, G., et al., 2014. Inflammasomes in neuroinflammation and changes in brain function: a focused review. Front. Neurosci. 8 https://doi.org/10.3389/fnins.2014.00315.
Swanson, K.V., et al., 2019. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat. Rev. Immunol. 19 (8), 477–489.
Szabo, A., Rajnavolgyi, E., 2013. Collaboration of Toll-like and RIG-I-like receptors in human dendritic cells: tRIGgering antiviral innate immune responses. Am. J. Clin. Exp. Immunol. 2, 195–207.
Taganov, K.D., et al., 2006. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc. Natl. Acad. Sci. U.S.A. 103, 12481–12486.
Tcw, J., et al., 2017. An efficient platform for astrocyte differentiation from human induced pluripotent stem cells. Stem Cell Rep. 9 (2), 600–614.
Thomas, K.T., et al., 2018. microRNAs sculpt neuronal communication in a tight balance that is lost in neurological disease. Front. Mol. Neurosci. 11 https://doi.org/10.3389/fnmol.2018.00455.
Tran, N.N., et al., 2013. Modeling schizophrenia using induced pluripotent stem cell-derived and fibroblast-induced neurons. Schizophr. Bull. 39, 4–10.
van den Berg, M.M.J., et al., 2020. Circulating microRNAs as potential biomarkers for psychiatric and neurodegenerative disorders. Prog. Neurobiol. 185, 101732. https://doi.org/10.1016/j.pneurobio.2019.101732.
Varcianna, A., et al., 2019. Micro-RNAs secreted through astrocyte-derived extracellular vesicles cause neuronal network degeneration in C9orf72 ALS. EBioMedicine 40, 626–635.
Wang, S., et al., 2014. Prognostic and biological significance of microRNA-127 expression in human breast cancer. Dis. Markers 2014, 1–12. https://doi.org/10.1155/2014/401986.
Wang, M., et al., 2019. MicroRNAs in Alzheimer's disease. Front. Genet. 10 https://doi.org/10.3389/fgene.2019.00153.
Wang, Z., Qin, W., Wei, C.B., Tang, Y., Zhao, L.N., Jin, H.M., Li, Y., Wang, Q., Luan, X.Q., He, J.C., Jia, J., 2018. The microRNA-1908 up-regulation in the peripheral blood cells impairs amyloid clearance by targeting ApoE. Int. J. Geriatr. Psychiatry 33 (7), 980–986.
Windrem, M.S., et al., 2017. Human iPSC glial mouse chimeras reveal glial contributions to schizophrenia. Cell Stem Cell 21 (2), 195–208.e6.
Winter, J., et al., 2009. Many roads to maturity: microRNA biogenesis pathways and their regulation. Nat. Cell Biol. 11 (3), 228–234.
Wong, N., Wang, X., 2015. miRDB: an online resource for microRNAtarget prediction and functional annotations. Nucl. Acids Res. 43 (D1), D146–D152.
Xia, P., et al., 2018. Mulberrin (Mul) reduces spinal cord injury (SCI)-induced apoptosis, inflammation and oxidative stress in rats via miroRNA-337 by targeting Nrf-2. Biomed. Pharmacother. 107, 1480–1487.
Xie, T., et al., 2012. MicroRNA-127 inhibits lung inflammation by targeting IgG Fcγ receptor I. J. Immunol. 188 (5), 2437–2444.
Xu, B., et al., 2013. Derepression of a neuronal inhibitor due to miRNA dysregulation in a schizophrenia-related microdeletion. Cell 152 (1-2), 262–275.
Zhang, Y., et al., 2016. Clinical predictor and circulating microRNA profile expression in patients with early onset post-stroke depression. J. Affect. Disord. 193, 51–58.
Zhang, J., et al., 2018. Upregulation of miR-614 promotes proliferation and inhibits apoptosis in ovarian cancer by suppressing PPP2R2A expression. Mol. Med. Rep. https://doi.org/10.3892/mmr10.3892/mmr.2018.8714.
Zhu, F., et al., 2018. Altered Serum Tumor Necrosis Factor and Interleukin-1β in First-Episode Drug-Naive and Chronic Schizophrenia. Front. Neurosci. 12 https://doi.org/10.3389/fnins.2018.00296.