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Changes in glial gene expression in the prefrontal cortex in relation to major depressive disorder, suicide and psychotic features

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ABSTRACT

Background: To establish whether major depressive disorder (MDD), suicidal behaviors and psychotic features contribute to glial alterations in the human prefrontal cortex.

Materials and methods: We compared mRNA expression using real-time qPCR of 17 glia related genes in the dorsolateral prefrontal cortex (DLPFC) and the anterior cingulate cortex (ACC) between 24 patients with MDD and 12 well-matched controls without psychiatric or neurological diseases. The MDD group was subdivided into i) MDD who died of suicide (MDD-S) or natural causes (MDD-NS) and ii) MDD with or without psychotic features (MDD-P and MDD-NP). The results were followed up with confounder factor analysis.

Results: Astrocyte gene aldehyde dehydrogenase-1 L1 (ALDH1L1) showed an increased expression in the DLPFC of MDD-NS and the ACC of MDD-NP. S100 calcium-binding protein B (S100B) was upregulated in the DLPFC of MDD compared to the controls. Microglial markers CD11B and purinergic receptor 12 (P2RY12) both showed decreased expression in the ACC of MDD-NS. CD68 was increased in the DLPFC of MDD in both, MDD-P and MDD-NP. The results were followed up with confounder factor analysis.

Results: Astrocite gene aldehyde dehydrogenase-1 L1 (ALDH1L1) showed an increased expression in the DLPFC of MDD-NS and the ACC of MDD-NP. S100 calcium-binding protein B (S100B) was upregulated in the DLPFC of MDD compared to the controls. Microglial markers CD11B and purinergic receptor 12 (P2RY12) both showed decreased expression in the ACC of MDD-NS. CD68 was increased in the DLPFC of MDD in both, MDD-S and MDD-P; compared to the controls. In addition, there was increased translocator protein (TSPO) expression in the DLPFC of MDD, especially MDD-NS. In the ACC, this gene had a lower expression in MDD-P than in MDD-NP. Myelin basic protein (MBP) mRNA in the DLPFC increased in MDD, in relation to psychotic features, but not to suicide.

Limitations: Sample volumes are relatively small.

Conclusions: Different glial functions in MDD were related to specific brain area, suicide or psychotic features.

1. Introduction

One-third of individuals with major depressive disorder (MDD) attempt suicide during their lifetime (Dong et al., 2019). Suicidal behavior is often associated with psychotic symptoms (Ma et al., 2019), while the presence of psychotic features in MDD predicts a double mortality rate, and a 2–5 times higher completion of suicide attempts during the acute episodes (Nelson et al., 2018; Suominen et al., 2009; Zalpuri and Rothschild, 2016). This elevated suicide mortality rate does not respond to antidepressants (Suominen et al., 2009), while tricyclic antidepressants have been reported to have a low impact but potentially exacerbate psychotic symptoms in patients with MDD (Glassman et al., 1975; Kantrowitz and Tampi, 2008). In addition, while family history of depression remains unclear among patients with MDD and psychotic features, family members of patients with psychotic features have a higher vulnerability to develop psychotic features (Nelson et al., 2018), which points to independence of psychotic features and MDD. Thus, the evidence above indicates that suicide and psychotic features can be considered as two heterogeneous conditions (Cardinal and Bullmore, 2011; Holma et al., 2010) that may be to a certain degree independent factors in MDD severity.

Based on previous work on neuronal abnormalities in both dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC), changes in neuron-glia interactions have been implicated as the neurobiological underpinning of MDD (Zhao et al., 2015, 2018, 2016). In our studies, glial-driven functional brain changes have been explored in...
MDD pathophysiology, and several suicide-/psychotic features-related specific glial alterations have been described in bipolar disorder and schizophrenia (SCZ) (Zhang et al., 2020a, 2020b). Within this framework, the pathological studies on the two regions that reported glial abnormalities required some follow up. Firstly, they mainly focused on the white, and not the grey matter, and are thus, intuitively, difficult to align to the neuronal changes in the cortex (Gittins and Harrison, 2011; Mosebach et al., 2013; Rajkowska et al., 2015; Schnieder et al., 2014; Schnieder et al., 2018; Torres-Platas et al., 2014; Webster et al., 2005).

Secondly, age-dependent glial changes, that may indicate a compensatory response to, or underlying cause of, the neuronal dysfunction, have been found that complicate the pattern of glial pathology in MDD (Miguel-Hidalgo et al., 2000; Rajkowska and Stockmeier, 2013; Vostrikov and Uranova, 2011). Our earlier studies pointed out that MDD and suicide may differ in the alterations of key stress-related genes transcripts (Zhao et al., 2019). However, in studies on MDD so far, patients who died of suicide or non-suicidal causes were often lumped together (Ernst et al., 2011; Mosebach et al., 2013; Rajkowska et al., 2013). Additionally, suicide victims were compared to control subjects losing sight of the fact that the far majority of suicide cases have an underlying psychiatric illness (Klempnan et al., 2009a; Lutz et al., 2017; Nagy et al., 2015; Schnieder et al., 2018; Torres-Platas et al., 2014; Zhao et al., 2019). Moreover, none of the glia-related studies has so far analyzed psychotic features as a subgroup of patients with MDD. This may at least partly be an explanation for inconsistencies in the literature.

Therefore, we investigated here the expression levels of 17 glia-related genes in the prefrontal cortex (PFC) in MDD, taking into account suicide and psychotic features as separate subsets.

2. Materials and methods

2.1. Brain samples

RNA samples were obtained from the Stanley Medical Research Institute (SMRI, Bethesda, MD, USA). Permission for the use of brain material was provided by the next of kin. Diagnoses were based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). All brain regions were microscopically examined to exclude patients with pathological signs of neurodegeneration or other lesions. Exclusion criteria included subjects over age 70, subjects with a history of seizures or other neurological disorders that might affect brain pathology. The cause of death for 17 of 24 patients with MDD was suicide. Eleven patients with MDD had a history of psychotic features. The other cases and all control subjects died of natural causes or accidents.

The SMRI provided us with RNA from the isolated grey matter of the DLPFC (Brodmann area 46) and ACC (Brodmann area 24). They were obtained from patients with MDD (MDD, N = 24) and matched controls (Ctr, N = 12) without a history of suicidal behavior or any major psychiatric diagnosis. We distinguished the following subsets within the MDD group: 1) patients with MDD who died of suicide (MDD-S) or natural causes (MDD-NS) and 2) patients with or without psychotic features (MDD-P or MDD-NP). All groups were matched for age, brain pH, post-mortem interval, brain weight, history and severity of alcohol/ substance abuse (Tables 1 and S1). Table 1. Overall demographic information on the control and MDD subjects.

<table>
<thead>
<tr>
<th></th>
<th>Ctr</th>
<th>MDD</th>
<th>F or x²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, range)</td>
<td>47 (24-63)</td>
<td>42 (24-63)</td>
<td>1.23</td>
<td>0.64</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>8/4</td>
<td>13/11</td>
<td>0.51</td>
<td>0.47</td>
</tr>
<tr>
<td>Race</td>
<td>11W, 1H</td>
<td>23W, 1H</td>
<td>0.26</td>
<td>0.61</td>
</tr>
<tr>
<td>PMD (hours, range)</td>
<td>25.3 (9-40)</td>
<td>29.7 (13-65)</td>
<td>1.35</td>
<td>0.30</td>
</tr>
<tr>
<td>Brain pH</td>
<td>6.6 (6.31–6.91)</td>
<td>6.6 (6.3–6.9)</td>
<td>1.47</td>
<td>0.81</td>
</tr>
<tr>
<td>Brain Weight (gram, range)</td>
<td>1445</td>
<td>1469</td>
<td>1.69</td>
<td>0.63</td>
</tr>
<tr>
<td>Side of Brain Frozen (L/ R)</td>
<td>(1200–1595)</td>
<td>(1170–1780)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychotic Features</td>
<td>-</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol hx</td>
<td>6</td>
<td>16</td>
<td>0.94</td>
<td>0.33</td>
</tr>
<tr>
<td>Severity of Alcohol abuse</td>
<td>2.08 (0–5)</td>
<td>1.92 (0–5)</td>
<td>1.07</td>
<td>0.82</td>
</tr>
<tr>
<td>Drug hx</td>
<td>4</td>
<td>8</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Severity of Substance abuse</td>
<td>0.75 (0–4)</td>
<td>1.04 (0–4)</td>
<td>1.35</td>
<td>0.59</td>
</tr>
<tr>
<td>Fluphenazine (lifetime, range)</td>
<td>-</td>
<td>1121 (0–6500)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: Ctr, control; F, female; hx, history; L, left; M, male; MDD, major depressive disorder; PMD, postmortem delay; R, right. 1 Substance abuse and alcohol abuse was rated on a scale of 0-5.

Table 2. cDNA template (equivalent to 5 ng of total RNA) was amplified in a final volume of 20 µl using an SYBR Green PCR master mix (Applied Biosystems, CA, USA) and a mixture of forward and reverse primers (each 2 pmol/µl). Data were acquired and processed automatically by the Applied Biosystems 7300 Real-Time PCR System. The specificity of amplification was checked by melting curve analysis and electrophoresis of the products on an 8% polyacrylamide gel. Sterile water and RNA samples without the addition of reverse transcriptase during cDNA synthesis served as negative controls. The linearity of each qPCR assay was tested by preparing a series of dilutions of the same stock cDNA in multiple plates. To reduce the effect of sample variability, reference genes were selected based on expression stability measurement (Vandesompele et al., 2002) and the proportion of explained variability regarding all target genes. The higher stability of a reference gene means that its overall variability of the target genes. Briefly, we used the following reference genes: actin beta (ACTβ), glyceraldehyde-phosphate dehydrogenase (GAPDH), hypoxanthine phosphoribosyltransferase 1 (HPRT1), tubulin alpha (TUBα), tubulin beta (TUBβ) and ubiquitin C (UBC). For the comparisons in the DLPFC, the specific selection was ACTβ, TUBα, TUBβ and UBC; in the ACC, the corresponding selection was ACTβ, TUBβ and UBC.

2.2. Quantitative real-time PCR

RNA isolation and cDNA synthesis were performed as described before (Zhao et al., 2018), RNA integrity value (RIN), an indicator of human post-mortem tissue RNA quality (Stan et al., 2006), did not show any significant difference between the diagnostic groups in the SMRI material (RIN value of the ACC/DLPFC from MDD-S: 7.69 ± 0.66; MDD-NS: 7.49 ± 0.56; MDD-P: 7.56 ± 0.26; MDD-NP: 7.69 ± 0.10 and the control group: 7.73 ± 0.95, mean ± SEM). For cDNA synthesis, an equal amount of RNA (300 ng) for each sample was used for the synthesis of cdNA, mixed with a 4.1 ml mixture of oligo dT (100 mg ml-1) and 10 x hexanucleotide (Roche, Basel, Switzerland) (40:1 for the oligo-dT and hexanucleotide mixture), heated to 80°C for 10 min, after which the tubes were quickly transferred to ice. Then 1 ml reverse transcriptase Superscript II RT (Invitrogen Life Technologies) was added together with a mixture of 5 ml 5 first-strand buffer, 2.5 ml 100 mM dithiothreitol, 1.5 ml 10 mM dNTPs and 0.5 ml RNase inhibitor. The synthetic reaction was allowed to proceed for 1 h at 42°C, after which cdNA was stored at -20°C or used immediately. Additional information on all tested genes and the sequences for each primer pair is shown in Table S2. cDNA template (equivalent to 5 ng of total RNA) was amplified in a final volume of 20 µl using an SYBR Green PCR master mix (Applied Biosystems, CA, USA) and a mixture of forward and reverse primers (each 2 pmol/µl). Data were acquired and processed automatically by the Applied Biosystems 7300 Real-Time PCR System. The specificity of amplification was checked by melting curve analysis and electrophoresis of the products on an 8% polyacrylamide gel. Sterile water and RNA samples without the addition of reverse transcriptase during cDNA synthesis served as negative controls. The linearity of each qPCR assay was tested by preparing a series of dilutions of the same stock cDNA in multiple plates. To reduce the effect of sample variability, reference genes were selected based on expression stability measurement (Vandesompele et al., 2002) and the proportion of explained variability regarding all target genes. The higher stability of a reference gene means that its variance was relatively lower than the other considered reference genes.

Further, if the expression of a reference gene reflects the variability of the samples well, it is expected that its application should reduce the overall variability of the target genes. Briefly, we used the following reference genes: actin beta (ACTβ), glyceraldehyde-phosphate dehydrogenase (GAPDH), hypoxanthine phosphoribosyltransferase 1 (HPRT1), tubulin alpha (TUBα), tubulin beta (TUBβ) and ubiquitin C (UBC). For the comparisons in the DLPFC, the specific selection was ACTβ, TUBα, TUBβ and UBC; in the ACC, the corresponding selection was ACTβ, TUBβ and UBC.

2.3. Statistical analysis

Statistical analysis was conducted with IBM SPSS (version 20, SPSS) and TIBCO S+ software (version 8.2.0, TIBCO, Seattle, WA, USA). The
gene expression values were log-transformed before they were further processed for statistical analysis. The transformation of the essentially exponential expression data facilitates the application of reference gene correction and enables the application of conventional statistical methods. Thus, the residuals of the combination of selected reference genes can be simply subtracted from the original target gene values to obtain corrected gene expression values.

Given the higher variability in human postmortem data, that may not sufficiently conform to commonly assumed theoretical distributions of test statistics, we applied a non-parametric resampling procedure (Zhao et al., 2018). Resampling was performed without replacement in two-group comparisons, thereby providing an alternative for the t-test. We generated 9999 replicates consisting of randomly reallocated patients over the two groups in each comparison to obtaining a permutation NULL distribution of the test statistic. The obtained NULL distribution mimics the hypothesis that the patients from either group belong to one and the same group (Davison and Hinkley, 1997). As test statistic, we used \( T = (\text{mean (group2)} - \text{mean (group1)}) / \text{se}. \) In this formula, se is the standard error of the difference in means. If our data are normal with unknown, unequal variances in group2 and group1, while group1 and group2 observations belong to the same group, \( T \) should approximate the t-distribution with Welch modified degrees-of-freedom. In that case, the observed \( T \)-value would be sufficient to decide whether the two groups should be considered as one or not. If it is uncertain how appropriate the \( T \)-distribution is, the 9999 permutation \( T \)-values together with the real observed \( T \)-value constitute a sample of 10,000 “resampled observations”, that can be used to estimate how unusual the observed \( T \)-value is (Davison and Hinkley, 1997). Since the observed \( T \)-value can be on either side of the mean, testing was done two-sided (Zhao et al., 2018). P-values were corrected for multiple testing using the Benjamini-Hochberg criterion (Benjamini and Hochberg, 1995). After statistical analysis, group mean expression levels were back-transformed and expressed as fold-changes to compare their mutual differences (relative mRNA values in the figures). Figures were made with Graphpad Prism 8.1.2.

3. Results

3.1. Gene transcripts encoding astrocytic markers

As an indication for changes in astrocyte-related functions in the PFC of patients with MDD, we analyzed qPCR values of three gene transcripts: aldehyde dehydrogenase 1 family, member L1 (ALDH1L1), gliarial fibrillary acidic protein (GFAP), S100 calcium-binding protein B (S100B).

In the DLPFC, S100B was the only gene that showed a significant increase (fold change = 1.25, \( P = 0.037 \)) in patients with MDD. In suicide- or psychotic features-related subset comparisons, however, no significance was found. A trend for an increase was found for ALDH1L1 (fold change = 1.32, \( P = 0.061 \)) in patients with MDD. The elevated transcript level of this gene was significant in the MDD-NS group (fold change = 1.60, \( P = 0.023 \)). Psychotic features did not significantly confound the detected change in astrocytic transcripts (see Table S2).

For the ACC, no changes were found between patients with MDD and controls, neither following subgrouping for suicide. However, in patients with MDD who did not have psychotic features, ALDH1L1 expression was higher than those with psychotic features (fold change = 1.38, \( P = 0.034 \)) and the control subjects (fold change = 1.43, \( P = 0.034 \)).

3.2. Gene transcripts encoding microglial markers

To assess whether MDD, suicide and psychotic features may be accompanied by gene expression changes of proteins characteristic for microglia, we tested the expression levels of the following 10 microglial markers. They are CD11B (CR3), CD45, CD68, chemokine (C-X3-C motif) receptor 1 (CX3CR1), human leukocyte antigen-DR alpha chain (HLA-DRA), ionized calcium-binding adapter molecule 1 (IBA1), purinergic receptor 12 (P2RY12), transmembrane protein 119 (TMEM119), triggering receptor expressed on myeloid cells 2 (TREM2) and translocator protein (TSPO).

Among these genes, both CD68 and TSPO revealed more than 20% increases in MDD in the DLPFC (CD68: fold change = 1.22, \( P = 0.037 \); TSPO: fold change = 1.25, \( P = 0.020 \)), and for CD68 in the suicide completers (MDD-S vs. Ctr: fold change = 1.23, \( P = 0.033 \). TSPO expression in MDD-NS (MDD-NS vs. Ctr: fold change = 1.40, \( P = 0.014 \)) significantly increased compared to the controls. For CD68, another elevation is present in patients with MDD and psychotic symptoms relative to the controls (fold change = 1.32, \( P = 0.015 \)).

In the ACC, no alterations were observed in the two-group comparisons. However, we found some genes differently expressed between MDD subgroups. For example, both CD11B and P2RY12 were approximately reduced by half in non-suicidal patients with MDD, but not in suicide completers compared to the controls (CD11B: MDD-NS vs. Ctr: fold change = -1.49, \( P = 0.031 \); MDD-S vs. MDD-NS: fold change = 1.59, \( P = 0.065 \); P2RY12: MDD-NS vs. Ctr: fold change = -1.47, \( P = 0.046 \); MDD-S vs. MDD-NS: fold change = 1.68; \( P = 0.065 \)). Additionally, TSPO showed opposite expressions between patients with and without psychotic features (MDD-P vs. MDD-NP: fold change = -1.27, \( P = 0.034 \)). These variable alterations per subgroup account for the unchanged transcript levels between MDD and controls.

3.3. Gene transcripts encoding oligodendrocytic markers

In addition, we investigated the following 4 oligodendrocyte genes: myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), oligodendrocyte transcription factor 2 (OLIG2) and myelin proteolipid protein (PLP).

In the DLPFC, MDDs showed increased MBP expression compared to the controls (fold change = 1.22, \( P = 0.003 \)), but the subgroup of suicide completers did not specially contribute to this elevation (MDD-S vs. Ctr: fold change = 1.20, \( P = 0.023 \); MDD-NS vs. Ctr: fold change = 1.26, \( P = 0.023 \); MDD-S vs. MDD-NS: \( P = 0.289 \)). Interestingly, the expression of this gene in patients with psychic features increased 30% compared to the controls (fold change = 1.30, \( P = 0.010 \)).

No changes were detected for oligodendrocyte markers from two- or three-group comparisons regarding MDD, suicide or psychotic features in the ACC.

An overview of significant changes is presented in Figs. 1–3. For more details, we refer to Tables 1 and 2A–C.

3.4. Sex differences

We did not find significant differences between the sexes, neither in the MDD nor in the matched control group.

3.5. Confounder analysis

The \( P \)-values for matching for possibly confounding factors are given in Tables 1, S1B and S1C. There were no significant differences. Correlations between glia expression and non-matched factors, i.e. age of onset, disease duration and lifetime antipsychotic use, have been done and these factors did not alter our conclusions.

4. Discussion

We have found alterations in gene expression in astrocytes, microglia and oligodendrocytes in the DLPFC and ACC in relation to MDD, suicide and psychotic features. As discussed below, altered neuronal functions behind these changed glial transcripts may include dopamine (DA) and gamma-aminobutyric acid (GABA) signaling, blood-brain barrier (BBB) permeability and central nervous system (CNS) injury, microglia
phagocytosis, innate immune response and neuroinflammation, platelet coagulation and myelin stability.

In our previous study, we have found that patients with SCZ who died of non-suicidal causes presented elevated ALDH1L1 mRNA in the DLPFC (Zhang et al., 2020a). This present data show an increased expression of this gene in MDD, an effect that was not confounded by age (Barley et al., 2009). Although dopaminergic impairments may trigger psychotic symptoms (Pai et al., 2019), lower DA metabolites have been found in body fluids of suicide attempters (Roy et al., 1989, 1992), a change that was notably unrelated to the underlying psychiatric disorder (Pitchot et al., 2001). One possible explanation could be the stronger affinity of immunoglobulin G for DA found in the cerebral spinal fluid.

Fig. 1. Transcript levels of astrocyte related genes (ALDH1L1 and S100B) in the DLPFC and ACC in controls (Ctr) and patients with major depressive disorder (MDD) that died of suicide (MDD-S) or other causes than suicide (MDD-NS), and patients with (MDD-P) or without psychotic features (MDD-NP). Abbreviations: ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; * indicates 0.01 ≤ P < 0.05.
(CSF) of subjects with suicide attempts (Bergquist et al., 2002). However, although D2 dopaminergic dysfunction was reported to be related to DAD2 receptor (DAD2R) polymorphisms in suicide attempters (Suda et al., 2009), this possibility was not supported by other studies showing an unchanged binding in subcortical regions known to control DA synthesis, comparing suicide cases with controls (Allard and Norlen, 1997, 2001; Fitzgerald et al., 2017; Pare et al., 1969). One could thus assume that the binding of DA to DAD2R in the DLPFC of patients with suicide is not as strong as in non-suicides. In addition, in the ACC, we found an exclusive increase of ALDH1L1 expression in nonpsychotic

Fig. 2. Transcript levels of microglia related genes (Fig. 2A CD11B and CD68; Fig. 2B P2RY12 and TSPO) in the DLPFC and ACC in controls (Ctr) and patients with major depressive disorder (MDD) that died of suicide (MDD-S) or other causes than suicide (MDD-NS), and patients with (MDD-P) or without psychotic features (MDD-NP). Abbreviations: ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; * indicates $0.01 \leq P < 0.05$. 

Ctr MDD
0.0
0.5
1.0
1.5
2.0
CD11B-DLPFC
mRNA relative value
Ctr MDD-NS MDD-S
0.0
0.5
1.0
1.5
2.0
CD11B-DLPFC
mRNA relative value
Ctr MDD-NP MDD-P
0.0
0.5
1.0
1.5
2.0
CD11B-DLPFC
mRNA relative value

Ctr MDD
0.0
0.5
1.0
1.5
2.0
CD68-DLPFC
mRNA relative value
Ctr MDD-NS MDD-S
0.0
0.5
1.0
1.5
2.0
CD68-DLPFC
mRNA relative value
Ctr MDD-NP MDD-P
0.0
0.5
1.0
1.5
2.0
CD68-DLPFC
mRNA relative value

Ctr MDD
0.0
0.5
1.0
1.5
2.0
CD68-ACC
mRNA relative value
Ctr MDD-NS MDD-S
0.0
0.5
1.0
1.5
2.0
CD68-ACC
mRNA relative value
Ctr MDD-NP MDD-P
0.0
0.5
1.0
1.5
2.0
CD68-ACC
mRNA relative value
MDDs, suggesting that region-specific changes in DAD2R could be involved in MDD pathogenesis (Larisch et al., 1997). On basis of these observations, we presume that the ACC, but not the DLPFC, may serve as a specific DA binding site that presents different binding abilities when comparing MDD and psychotic features per se Suhara et al. (2002), Takahashi et al. (2006). The elevated S100B expression we observed in MDD brain samples may contribute to the increased S100B levels reported in peripheral blood (Gos et al., 2013; Hamidi et al., 2004; Schroeter et al., 2013). However, CSF S100B was reported to remain unchanged in MDD, likely reflecting different alterations among different brain areas (Schmidt et al., 2015). An alternative possibility, i.e. that a compromised BBB permeability could affect the DLPFC of depressed patients, awaits further study (Loftis et al., 2018). Klempan et al. (2009b) reported

Fig. 2. (continued).
Reduced levels of S100B transcripts in the DLPFC of MDD-S relative to controls, while in our comparison, we did not find any significant alteration. However, a previous rodent study showed that drug overdose increased S100B concentration in CSF (Vicente et al., 2007) and there are five suicide victims in our collection who died of a drug overdose, this may have contributed to a higher S100B expression in the MDD-S abolishing significance.

CD11B expression decreased in MDD, confirming observations in rodent models. In both depression models, neonatal (early life stress) and adult (social defeat stress), CD11B expression was found to be increased in the PFC (Ambrée et al., 2018; Giridharan et al., 2019). Our data indicate that there is no increased neuro-inflammation in non-suicidal individuals with MDD. Given the unchanged CD68 expression found after our three-group comparison, we deduce that reduced innate inflammatory state and synapse pruning may be present in the ACC in patients with MDD who died naturally (Michailidou et al., 2015).

In human postmortem studies, complement C3b was found to be deposited in peripheral capillaries of suicide completers (Thorell, 1989; Varis et al., 1993). Our current data showed a trend for an increase in CD11b, a C3 receptor, in MDD-S compared to the rest of the patients, which supports this finding. Additionally, a reduced hypo-thrombotic state has been found in peripheral plasma of patients with MDD relative to suicide attempters and healthy controls and independent of suicidality. It should be noted that this condition went together with a higher pro-coagulant activity, solely in MDDs who had presented suicidal behaviors. In our ACC data, P2RY12, a key regulator in platelet aggregation (Dorsam and Kunapuli, 2004), showed a similar crosstalk suggesting that, in suicide, an increased platelet aggregation may be induced by capillary injury that activated complement system (Yang et al., 2016).

We found an increase of CD68 mRNA in the DLPFC in suicide completers. This is an extension of the finding that some types of microglia in the prefrontal white matter were associated with suicide (Schmieder et al., 2014). These observations also agree with previous studies reporting a peripheral and cerebral increase of monokines, that is mainly produced by monocytes and macrophages, and are indicative of microglia activation in suicide. For instance, plasma tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) were both increased in MD patients with suicidality (Li et al., 2013; Monfrim et al., 2014), while TNF-α levels in patients who attempted suicide seem to be even higher compared to those who only displayed suicidal ideation (Janelidze et al., 2011). In addition, in the PFC of suicidal patients, an increased expression of cytokines, that promote monocyte-macrophage activation (IL-4 and IL-13), or are centrally secreted by macrophages...
(IL-1β) and TNF-α, supports a CD68-driven microglial activation in suicide completers with MDD (Pandey et al., 2012; Tonelli et al., 2008). Of note, Steiner et al. reported an increased microgliosis in the DLPFC of patients who accomplished suicide and included both patients with MDD and SCZ (Steiner et al., 2008). Since we previously found an unchanged CD68 expression in the PFC of SCZ patients that committed suicide, their observation may be influenced by a large number of MDD-S cases. In addition, enhanced microglial phagocytosis has been assumed to participate in the pathophysiology of psychotic diseases that could be inhibited by antipsychotics (Chen et al., 2013; Rakic et al., 2016). We here provide additional molecular proof showing that CD68 was increased in the DLPFC of patients with MDD and psychotic features.

A positron emission tomography (PET) study showed that TSPO was increased in the frontal cortex in patients with MDD who did not present with severe suicidality (Li et al., 2018). This is in line with our results in the DLPFC, but not the ACC. TSPO, as a benzodiazepine receptor, was assumed to participate in the pathophysiology of psychotic diseases that are commonly found in patients with first-episode psychosis compared to healthy controls (Ota et al., 2017). Data from blood samples report higher MBP transcripts in patients with psychotic features-related alterations in TSPO expression, which is consistent with an imaging study on patients with a high risk for psychotic features (Hafizi et al., 2018a, 2018b).

Our data together with the findings of others indicate that changes in oligodendrocyte related transcripts in MDD may be Brodmann area-specific and differ between the grey and white matter (Mosebach et al., 2013; Fantazzato et al., 2017; Rajkowska et al., 2015). We did not find evidence to support the possibility that suicide is an independent factor in oligodendrocyte expression. This is in agreement with two Canadian studies indicating that suicide does not contribute to altered oligodendrocytic activities in the PFC (Lutz et al., 2017; Tanti et al., 2017). Data from blood samples report higher MBP transcripts in patients with first-episode psychosis compared to healthy controls (Ota et al., 2015; Santoro et al., 2015). However, postmortem studies in the human brain did not reveal elevated oligodendrocyte-related gene expression in relation to psychosis (Aston et al., 2005; Barley et al., 2009; Chandley et al., 2013; Matthews et al., 2012). Thus, the increased

### Table 2B

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold change</th>
<th>P-perm</th>
<th>BHadj.p</th>
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<tr>
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<td>MDD-S/MDD-NS</td>
<td>MDD-S/Ctr</td>
<td>MDD-NS/Ctr</td>
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</tr>
<tr>
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<td>0.030</td>
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<tr>
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<td>0.112</td>
</tr>
<tr>
<td>IBA1</td>
<td>1.33</td>
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<td>0.113</td>
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</tr>
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<td>0.264</td>
</tr>
<tr>
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<td>PLP</td>
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</tr>
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</table>

Note: ACC: anterior cingulate cortex; BHadj.p: P value of Benjamini-Hochberg’s adjustment; Ctr: control; DLPFC: dorsolateral prefrontal cortex; MDD: major depressive disorder; MDD-NS: MDD patients died of non-suicidal reasons; MDD-S: MDD patients died of suicide; P-perm: P value of permutation test.
MBP expression we report in the DLPFC may be the first possible cortical region from where the increased plasma MBP levels are derived. This suggests stronger demyelination in patients with MDD and psychotic features, especially when MBP increases BBB permeability by inducing cerebral inflammation (D’Aversa et al., 2013).

In conclusion, differential changes were observed in the expression of several glial transcripts, in relation to MDD, suicide and psychotic features that were region-specific. Overall, MDD shows glia-related changes that may be relevant for BBB integrity, microglial phagocytosis, DA and GABA signaling, and myelination. While both suicide and psychotic features confound microglial phagocytosis, only psychotic features impact myelin stability.

5. Limitations

Inherent to postmortem studies is the sample volumes in this study relatively small. However, subjects of the collection studied have been well matched to reduce the output variabilities. Correlation analysis between glia expression and fluphenazine lifetime dosage has been performed but we did not find significance.

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CRediT authorship contribution statement

Lin Zhang: Visualization, Data curation, Writing – original draft. Ronald W.H. Verwer: Formal analysis. Juan Zhao: Writing – review & editing. Inge Huitinga: Visualization, Writing – review & editing. Paul J. Lucassen: Writing – review & editing. Dick F. Swaab: Visualization, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

None to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jad.2021.08.098.
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