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Sex difference in glia gene expression in the dorsolateral prefrontal cortex in bipolar disorder: Relation to psychotic features

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ABSTRACT

Background: Suicide, psychotic features and gender influence the epidemiology and clinical prognosis of bipolar disorder (BD). Differences in glial function between the genders might contribute to these clinical variables. Here we studied expression of glial genes in human post-mortem prefrontal cortex of BD and control subjects in relation to suicide, psychotic features and sex.

Methods: Real time PCR was used to detect transcriptional alterations of 16 glia-related genes in two brain areas, the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC), from 30 patients with BD subdivided by suicide and psychotic features, and from 34 well-matched control cases.

Results: We found no evidence of immune activation in BD. Instead, we found three microglial genes to be downregulated in the DLPFC of non-suicidal individuals with BD, i.e. CD68, triggering receptor expressed on myeloid cells 2 (TREM2) and purinergic receptor 12 (P2RY12). A remarkable sex difference was observed in the DLPFC of patients with BD: 14 glia-related genes were expressed at significantly higher levels in males, including all three glial cell types. A subset analysis showed that the sex differences were closely associated with the presence of psychotic features.

Conclusions: No evidence of immune activation was found in these two brain regions in BD. The sex-specific differences in glial gene expression in BD, found particularly in patients with psychotic features, may be associated with the potential co-existence of mania and psychotic features and could potentially contribute to the gender-biased characteristics in BD.

1. Introduction

Bipolar disorder (BD) is a multidimensional mood disorder with a strong genetic component in up to 85% of the patients (Goodwin and Jamison, 2007). Suicide rates in BD are approximately 20–30 fold higher, compared with the general population, and these cases occur mainly in a depressive episode (Gonda et al., 2012; Plans et al., 2018). As a heritable illness with neurodevelopmental impairments, this disorder often presents with diversified psychotic symptoms that are referred to approximately one-fifth of the lifetime suicidality in mood disorders and to more than 50% of the suicide ideations, independent of individual therapies or mood disorder classifications (Gesi et al., 2016; SanSegundo et al., 2018; Schaffer et al., 2015; Tsai et al., 2002). The descendants of people with psychosis and self-harm behaviors have a two to three times higher genetic risk for suicide than the general population (Andersen and Hynnekleiv, 2007). Suicide prevention thus has a high priority, but this requires a better understanding of the molecular mechanisms involved.

Sex difference may play an important role in the pathophysiology, cognitive and behavior phenotypes of BD. Clinical observations have revealed that, in females with BD, depressive episodes are a more frequent occurrence, and more often a previous family and personal history of suicidal behaviors is present (Nivoli et al., 2011). Male individuals with BD show higher risks of violent suicide attempts and psychotic features than females. Genetic data have further shown that elderly female individuals with BD without a family history of psychiatric disorders tended to have a later age of disease onset (Grigoroiu-Serbanescu et al., 2005) but a higher accumulation of cortical mitochondrial DNA (mtDNA) deletions than elderly men (Fuke et al., 2008). In particular, females with BD and psychotic features possessed

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Table 1
Demographic information.

	Ctr	BD	P
Age (year)[1]	45 (31–60)	44 (19–64)	0.66
Gender (M/F)	25/9	15/15	0.05
PMD (hour)[1]	28.5 (9–58)	33.5 (12–84)	0.13
Brain pH[1]	6.69 (6.00–7.03)	6.50 (5.92–6.97)	0.03
Brain weight (gram)[1]	1413 (1120–1900)	1420 (1170–1670)	0.49
Hemisphere	16 L/18 R	13 L/17 R	0.77
Age of onset (year)[1]	–	22.5 (14–48)	–
Duration of illness (year)[1]	–	18 (2–45)	–
Suicide	–	13	–
Psychotic features	–	16	–
Fluphenazine equivalents	–	19	–

Notes: Ctr, control; F, female; L, left; M, male; BD, bipolar disorder; L, left; PMD, postmortem delay; R, right.
[1] Data were shown as median with range.

more neuropsychological impairments than males suffering from the same conditions (Bräunig et al., 2009; Zanelli et al., 2013). Moreover, BD females treated with lithium tended to be more vulnerable to manic and depressive episodes than men (Queissner et al., 2018), but their cognitive performance showed more improvement after physical training (Fellendorf et al., 2017). However, the mechanisms behind the sex-differential vulnerability and prognosis remain unknown.

Regional differences in volume have been implicated in the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) of patients with BD. Information processing in the DLPFC, which has been related to the etiology of psychotic features, was preferentially reduced in psychotic BD, suggesting that psychosis might be accompanied by a disruption of the prefrontal control, which may result in the activation of the default network (Baker et al., 2014). Differences in the ACC, as measured by functional magnetic resonance imaging, have further been related to emotional processing, attentional and neurotransmitter impairments in pediatric and euthymic individuals with BD (Lee et al., 2018; Li et al., 2018; Soeiro-de-Souza et al., 2018). Patients in their first-episode mania with psychosis were further found to have a significant volume reduction of the entire ACC (Keramatian et al., 2016), whereas the right dorsal ACC volume was found to be increased in recent onset psychosis (de Azevedo-Marques Perico et al., 2011).

Interestingly, the volumetric alterations found in patients with mood disorders have been linked to stress-induced changes in glia (Czéh et al., 2007). Such structural glia alterations, indicative of hyper- or hypofunction of these cells, may possibly reflect measures of central inflammatory processes ongoing in the BD brain. In the periphery, elevated levels of inflammation-related markers have indeed been found in patients with BD (Jakobsson et al., 2015; Rolstad et al., 2015).

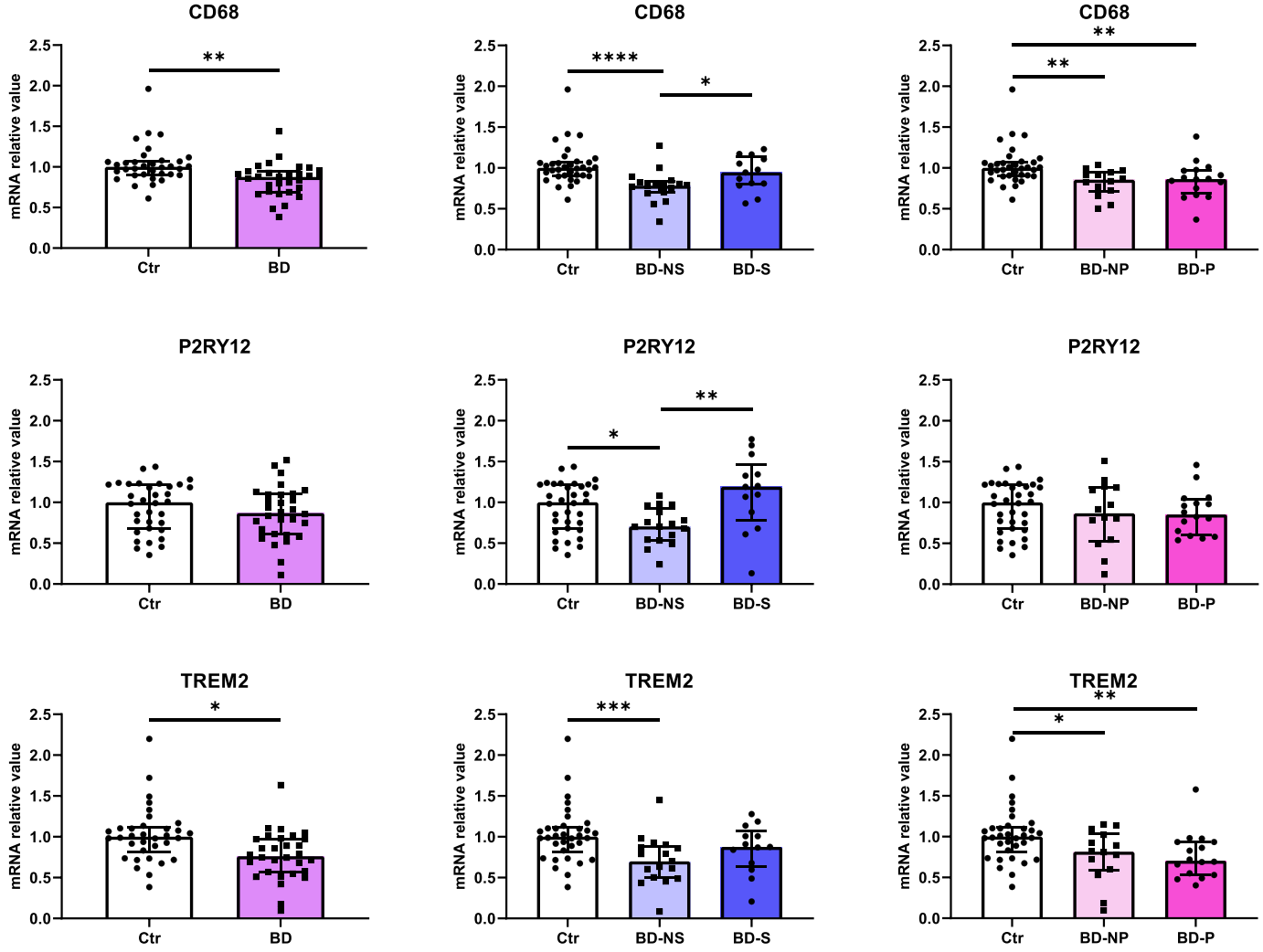

Fig. 1. Transcript levels of microglia related genes (CD68, P2RY12 and TREM2) in the dorsolateral prefrontal cortex (DLPFC) in controls (Ctr, n = 34) and patients with bipolar disorder (BD, n = 30) that died of suicide (BD-S, n = 13) or causes other than suicide (BD-NS, n = 17), and with or without psychotic features (BD-P, n = 16 or BD-NP, n = 14). Data shown in this graph were irrespective of gender and plotted as median with interquartile range. Note: * indicates $0.01 < P \leq 0.05$, ** indicates $0.001 < P \leq 0.01$, *** indicates $0.001 < P \leq 0.0001$, **** indicates $0.001 < P \leq 0.0001$.

Table 2A
Expression of glial genes in BD compared to their matched controls in the DLPFC and ACC.

	DLPFC			ACC		
	Fold change	P value	BHadj-p	Fold change	P value	BHadj-p
Astrocyte genes						
ALDH1L1	1.20	0.63		1.27	0.03	0.18
GFAP	−1.16	0.14		1.00	0.78	
GLT1	1.11	0.35		1.17	0.28	
GS	1.05	0.56		1.18	0.22	
S100b	−1.04	0.55		1.04	0.32	
Microglia genes						
CD68	**−1.15**	**0.000**	**0.006**	1.06	0.73	
CX3CR1	−1.21	0.05		−1.09	0.49	
HLA-DRA	−1.11	0.48		−1.21	0.41	
IBA1	−1.38	0.01	0.05	−1.42	0.02	0.13
P2RY12	−1.15	0.35		−1.09	0.55	
TREM2	**−1.31**	**0.002**	**0.013**	−1.24	0.008	0.12
TSPO	−1.09	0.47		1.10	0.11	
Oligodendrocyte genes						
MBP	−1.08	0.90		−1.02	0.35	
MOG	−1.05	0.35		1.10	0.09	
OLIG2	1.00	0.80		1.22	0.15	
PLP	−1.15	0.38		1.05	0.29	

Notes: ACC: anterior cingulate cortex; BD: bipolar disorder; BHadj-p: P value of Benjamini-Hochberg's adjustment; Ctr: control; DLPFC: dorsolateral prefrontal cortex.

For instance, the cytokine interleukin 10 was elevated in plasma in first-episode bipolar patients with psychotic features (Lesh et al., 2018), indicating potential monocyte/macrophage involvement in the psychotic etiology of BD. However, studies on postmortem frontal tissues have reported reductions in glial cell density in sublayers of both the DLPFC and ACC of patients with BD (Gittins and Harrison, 2011; Rajkowska et al., 2001). Pathologic changes in dendritic spines and reductions in oligodendrocyte specific proteins have been described in the isolated layers of the prefrontal cortex (PFC) in individuals with BD (Konopaske et al., 2014; Wesseling et al., 2014). Whether there is an immune process going on in the brain of patients with BD is thus still an open question.

Even though changes in peripheral immune markers were thought to point to an immune involvement in BD etiology, so far, the reported alterations in glia in the postmortem brain remain fragmentary and they vary per glial type, brain region, and the presence/absence of suicide, psychotic features and gender. The aim of our study was, therefore, to explore in patients with BD in a systematic way the transcriptional changes of markers of astrocytes, microglia and oligodendrocytes in BD, in relation to suicide, psychotic features and sex. Given their clear involvement in BD as outlined above, we focused on the DLPFC and ACC.

2. Materials and methods

2.1. Human brain samples from the Stanley Medical Research Institute (SMRI)

The Stanley Medical Research Institute (SMRI, Bethesda, MD, USA, Director: Dr. Maree J. Webster) provided 128 brain samples (25 male and 9 female controls and 15 male and 15 female patients with BD) for this study. The next of kin provided permission for the use of brain material. Diagnoses were made according to the Diagnostic and Statistical Manual of Mental Disorder IV (DSM-IV). The SMRI formulated exclusion criteria for all specimens, and all brain regions included were examined microscopically to exclude subjects with pathological signs of neurodegeneration or other lesions. The cause of death for 13 patients with BD was suicide (7 males and 6 females); the other cases and all control subjects died from natural causes or accidents. Sixteen patients with BD had psychotic features (7 males and 9 females) while 12 patients had no such features (for clinico-pathological details see Table 1).

The SMRI provided us with RNA from isolated gray matter of two brain areas: the DLPFC (Brodmann area 46) and ACC (Brodmann area 24) of each subject. The controls did not have suicidal behaviors or any major psychiatric diagnosis. Groups for comparison were well matched for sex, age, postmortem delay (PMD), month of death (MOD), and brain weight (BW) (see Table 1 and Tables S2A–G). Demographic information and medical data were provided by SMRI. All analyses were performed by investigators unaware of the grouped information.

2.2. Quantitative real-time PCR

cDNA synthesis was performed as described by us before (Wang et al., 2008). The RNA integrity value (RIN), an indicator of tissue RNA quality, did not show any significant difference between the diagnostic groups ($P = 0.76$). Our selection strategy towards the detected glial genes and their sequences for each primer pair are shown in Table S1. cDNA template (equivalent to 5 ng of total RNA) was amplified in a final volume of 10 µl using a 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. 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 of cDNA in multiple plates. Stable reference genes were selected to reduce the effect of sample variability (Vandesompele et al., 2002). The initial set of reference genes was: 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 ACC, ACTβ, TUBβ and GAPDH were selected. For DLPFC the selection of ACTβ, TUBα, TUBβ and UBC appeared to be the most appropriate.

2.3. Statistical analysis

S+ software (version 8.2, TIBCO, Seattle, WA, USA) was used for statistical analysis. The Chi-square test was used for analysis of categorical data (gender). For interval data, the Mann-Whitney test (2 samples) or the Kruskal-Wallis test with multiple comparisons (3 samples) was used (Conover, 1980). Before processing of gene expression data, the values were ^{10}log-transformed to enable simple reference gene correction and conventional statistical procedures. The reason for this transformation is that the observed Ct values used in order to quantify gene expression, appear as exponents of the PCR efficiency. Application of the log-transformation yields an additive statistical model and, after all statistical procedures have been finished, the data are back-transformed and presented as fold-changes. In multiple testing situations the Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) of *P*-values was applied. When the Kruskal-Wallis test was used in combination with the Benjamini-Hochberg correction, we proceeded in a 2-step way. As multiple comparisons in the Kruskal-Wallis test are only allowed if the global $P < 0.05$ (Conover, 1980), we first corrected the global *P*-values and then selected for further analysis only those genes for which this requirement was met. For each appropriate comparison, the corresponding *P*-values were pooled and corrected according to Benjamini-Hochberg. All tests were 2-sided.

3. Results

3.1. Altered microglia mRNA expression in BD

An overview of mRNA expression in genes is presented in Fig. 1 and

Table 2B
Expression of glial genes in BD with or without suicide compared to their matched controls in the DLPFC and ACC.

	Fold change			Global-p	BHadj-p	BHadj.p		
	BD-S/BD-NS	BD-S/Ctr	BD-NS/Ctr			BD-S/BD-NS	BD-S/Ctr	BD-NS/Ctr
DLPFC-astrocyte genes								
ALDH1L1	1.23	1.37	1.12	0.34				
GFAP	1.07	−1.07	−1.14	0.20				
GLT1	1.14	1.21	1.06	0.32				
GS	1.12	1.10	−1.02	0.33				
S100b	1.05	−1.01	−1.06	0.64				
DLPFC-microglia genes								
CD68	**1.20**	−1.05	**−1.27**	**0.000**	**0.003**	**0.01**	0.23	**0.000**
CX3CR1	1.35	−1.02	−1.34	0.02	0.06			
HLA-DRA	−1.11	−1.08	1.02	0.72				
IBA1	1.31	−1.18	−1.55	0.003	0.07			
P2RY12	**1.69**	1.19	**−1.41**	**0.005**	**0.03**	**0.004**	0.23	**0.01**
TREM2	1.25	−1.14	**−1.43**	**0.002**	**0.02**	0.09	0.23	**0.000**
TSPO	1.28	1.04	−1.22	0.47				
DLPFC-oligodendrocyte genes								
MBP	1.39	1.13	−1.23	0.04	0.06			
MOG	1.00	−1.13	−1.13	0.58				
OLIG2	1.36	1.18	−1.15	0.42				
PLP	1.11	−1.03	−1.14	0.43				
ACC-astrocyte genes								
ALDH1L1	−1.29	1.06	1.38	0.03	0.11			
GFAP	1.04	1.01	−1.03	0.85				
GLT1	−1.04	1.14	1.19	0.45				
GS	−1.08	1.16	1.25	0.33				
S100b	1.09	1.09	1.00	0.61				
ACC-microglia genes								
CD68	1.07	1.11	1.04	0.79				
CX3CR1	1.06	−1.07	−1.14	0.35				
HLA-DRA	1.04	−1.19	−1.24	0.69				
IBA1	1.22	−1.22	−1.48	0.02	0.11			
P2RY12	1.12	1.00	−1.12	0.84				
TREM2	1.24	−1.07	−1.32	0.01	0.11			
TSPO	−1.15	1.01	1.16	0.08				
ACC-oligodendrocyte genes								
MBP	1.00	−1.02	−1.01	0.64				
MOG	−1.28	−1.05	1.21	0.03	0.11			
OLIG2	−1.29	−1.03	1.24	0.09				
PLP	−1.31	−1.13	1.16	0.08				

Notes: ACC: anterior cingulate cortex; BD: bipolar disorder; BD-NS: bipolar disorder – non suicide; BD-S: bipolar disorder - suicide; BHadj-p: *P* value of Benjamini-Hochberg's adjustment; Ctr: control; DLPFC: dorsolateral prefrontal cortex.

Tables 2A–2C.

In the DLPFC, CD68 transcripts were downregulated in patients with BD compared to matched controls (Fold change = −1.15, $P = 0.006$). For the three group comparisons, we found that CD68 was only reduced in patients with BD who did not die by suicide (BD-NS vs. Ctr: $P < 0.0001$; BD-NS vs. BD-S: $P = 0.01$), whereas no difference was found between the controls and patients with BD that died from suicide ($P = 0.23$). Both BD with and without psychotic features revealed reduced CD68 gene expression compared to the controls (BD-NP vs. Ctr: $P = 0.002$; BD-P vs. Ctr: $P = 0.004$), while no difference was found for CD68 between BD with and without psychosis ($P = 0.70$).

In addition, the transcript level of TREM2 in the DLPFC was significantly reduced in patients with BD (Fold change = −1.31, $P = 0.01$). When compared to controls, a reduction of the expression of this gene was only found in patients with BD without completed suicide ($P = 0.0004$), while no difference was present between individuals with BD who died by suicide and controls ($P = 0.23$), nor between patients with BD with and without completed suicide ($P = 0.09$). TREM2 downregulation was present in patients with BD both with and without psychotic features, compared to the controls (BD-NP vs. Ctr: $P = 0.03$; BD-P vs. Ctr: $P = 0.002$). No significant changes were present between patients with and without psychosis ($P = 0.42$).

Moreover, we found a down-regulation in P2RY12 transcripts in patients with BD who died of natural causes relative to controls (Fold change = −1.41, $P = 0.01$) and between non-suicide patients with BD and suicide completers (Fold change = −1.69, $P = 0.004$). However, the differences between suicide completers and their controls, or between all BD subjects and their controls, were not significant (BD-S vs. Ctr: $P = 0.23$; BD vs. Ctr: $P = 0.35$).

None of the genes studied revealed any significant differences in the ACC.

3.2. Sex differences in BD in the DLPFC

A striking sex difference of the glia-related gene expression was found in individuals with BD in the DLPFC (see Fig. 2 and Table 3), with males having a higher expression of most detected genes. In a subset analysis, we found that these changes were more obvious in patients with psychotic features in their clinical history than in the other subsets. In addition, we noticed several other types of differences: GLT1, GS and P2RY12 showed similar significances in patients who had died of natural causes. Both CD68 and MOG revealed changes in the suicide subset. MBP exhibited this alteration in all subsets. No sex-specific change was found in the controls, and not in the BD group in the ACC for the two brain areas studied.

4. Discussion

To explore potential central immunological processes in BD and investigate whether they may be associated with suicide and/or

Table 2C
Expression of glial genes in BD with and without psychotic features and their matched controls in the DLPFC and ACC.

	Fold change			Global-p	BHadj-p	BHadj.p		
	BD-P/BD-NP	BD-P/Ctr	BD-NP/Ctr			BD-P/BD-NP	BD-P/Ctr	BD-NP/Ctr
DLPFC-astrocyte genes								
ALDH1L1	−1.04	1.16	1.20	0.80				
GFAP	−1.02	−1.17	−1.14	0.31				
GLT1	1.02	1.08	1.06	0.66				
GS	−1.17	−1.01	1.16	0.48				
S100b	−1.05	−1.08	−1.03	0.68				
DLPFC-microglia genes								
CD68	1.01	**−1.16**	**−1.17**	**0.002**	**0.03**	0.70	**0.004**	**0.002**
CX3CR1	−1.04	−1.22	−1.18	0.16				
HLA-DRA	1.37	1.13	−1.22	0.24				
IBA1	−1.07	−1.43	−1.34	0.03	0.16			
P2RY12	−1.02	−1.17	−1.15	0.62				
TREM2	−1.15	**−1.42**	**−1.23**	**0.005**	**0.04**	0.42	**0.002**	**0.03**
TSPO	−1.13	−1.15	−1.02	0.55				
DLPFC-oligodendrocyte genes								
MBP	−1.14	−1.16	−1.02	0.24				
MOG	1.07	−1.04	−1.11	0.59				
OLIG2	−1.32	−1.22	1.08	0.09				
PLP	−1.01	−1.14	−1.13	0.63				
ACC-astrocyte genes								
ALDH1L1	1.48	1.52	1.03	0.007	0.09			
GFAP	1.14	1.06	−1.07	0.41				
GLT1	1.38	1.28	−1.08	0.03	0.09			
GS	1.35	1.44	1.06	0.02	0.09			
S100b	1.33	1.21	−1.10	0.19				
ACC-microglia genes								
CD68	1.16	1.14	−1.02	0.22				
CX3CR1	1.28	1.02	−1.25	0.15				
HLA-DRA	−1.15	−1.23	−1.06	0.76				
IBA1	−1.17	−1.40	−1.19	0.03	0.09			
P2RY12	1.12	−1.02	−1.14	0.41				
TREM2	−1.14	−1.31	−1.15	0.04	0.09			
TSPO	−1.04	1.07	1.12	0.05				
ACC-oligodendrocyte genes								
MBP	1.15	1.05	−1.09	0.32				
MOG	1.35	1.27	−1.06	0.04	0.09			
OLIG2	1.21	1.31	1.09	0.04	0.09			
PLP	1.43	1.26	−1.14	0.05				

Notes: ACC: anterior cingulate cortex; BD: bipolar disorder; BD-NP: bipolar disorder – nonpsychotic features; BD-P: bipolar disorder – psychotic features; BHadj-p: *P* value of Benjamini-Hochberg's adjustment; Ctr: control; DLPFC: dorsolateral prefrontal cortex.

psychotic features, we analyzed mRNA expression in the DLPFC and ACC from individuals with BD and matched controls. We also paid special attention to possible gender differences. Some alterations in microglia-related gene expression in the DLPFC were only present in patients who did not attempt suicide (i.e. a decreased expression of the microglial genes CD68, P2RY12 and TREM2). We did not find psychotic features confound our data. A striking finding was the prominent sex difference in glial gene expression in the same region. The clear sex differences in glia-related genes in the DLPFC were only observed in this brain region and only in BD, especially in patients with psychotic features.

Microglia plays an important role in central immune responses. Upon activation, microglia can aggravate neural inflammation by releasing specific pro-inflammatory cytokines. Functional abnormalities of microglia have been implicated in the onset of some mood disorders and have therefore been proposed as novel therapeutic targets. Even though pro-inflammatory cytokines are elevated in the peripheral circulation in BD, there are only limited studies that have analyzed microglia in the BD brain. Our data on unaltered microglial transcripts in BD agree with those from Sneeboer et al. (2019). In addition, we found a down-regulation of CD68–, P2RY12- and TREM2-mRNA expression in the DLPFC in individuals with BD who did not attempt suicide. De Baumont et al. have reported changes in CD68 and TREM2 expression in BD compared to schizophrenia (SCZ) (de Baumont et al., 2015). As an M2 microglia marker, this reduced TREM2 expression reminded us of a reduced anti-inflammatory activity in non-suicidal BD cases as reported before (Zhang et al., 2018). Interestingly, TREM2 has a more significant reduction in psychotic victims, indicating that a decrease in TREM2-mRNA expression may be related more to the psychotic features than to BD *per se*. Indeed, psychotic symptoms might be associated with increases in microglia indoleamine 2,3 dioxygenase expression following TREM2 deficiency, as was suggested based on TREM2 knock down studies (Penberthy, 2007); this was thought to result from increases in pro-inflammatory cytokine release in the periphery in BD (Anderson et al., 2016; Takahashi et al., 2005).

CD68 and P2RY12 both showed an elevated expression in suicide completers with BD relative to those non-suicidal patients and had levels that met those of the control subjects, suggesting that microglia are activated during suicidal behaviors. In our previous study, we found an increase in P2RY12 expression in suicide completers with SCZ (Zhang et al., 2020), which is consistent with a disturbed purine metabolism in the PFC in suicide (Pantazatos et al., 2017). In major depressive disorder (MDD), we also found an upregulation of CD68 transcript in this cohort, but not in patients who died of non-suicidal causes, as compared to controls (unpublished data), suggesting an enhanced microglial phagocytosis in suicide completers among individuals who are suffering from depression-related psychiatric diseases, and that this increase might be related to the ATP signaling activation.

Human postmortem brain studies on the relationship between glial expression and sex differences in BD are of general interest because of

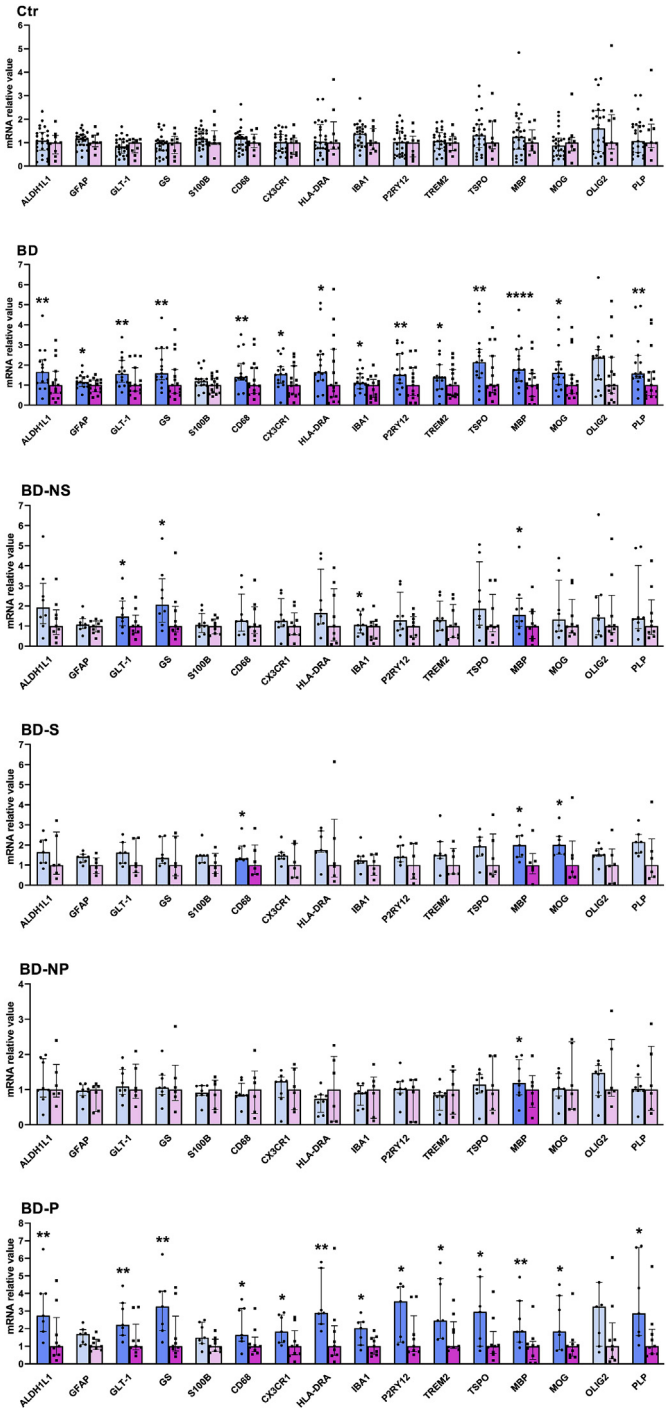

Fig. 2. Transcript levels of glial genes in the DLPFC in controls and patients with BD and their subsets between males and females. Sample volumes: Ctr-male: n = 25, Ctr-female: n = 9; BD-male: n = 15, BD-female: n = 15; BD-NS-male: n = 8, BD-NS-female: n = 9; BD-S-male: n = 7, BD-S-female: n = 6; BD-NP-male: n = 8, BD-NP-female: n = 6; BD-P-male: n = 7, BD-P-female: n = 9. Data were plotted as median with interquartile range. Note: * indicates $0.01 < P \leq 0.05$, ** indicates $0.001 < P \leq 0.01$, *** indicates $0.001 < P \leq 0.0001$, **** indicates $0.001 < P \leq 0.0001$.

the clear sex-specific differences in the vulnerability to develop psychiatric disorders and in this case in particular because of the sex differences in BD symptoms. To our best knowledge, and for the first time, we here report higher levels of gene expression of the three types of glia in the DLPFC of male individuals relative to females with BD, in particular in patients with BD and psychotic features. Interestingly, also in autism spectrum disorder (ASD) in the postmortem frontal cortex, astrocyte and microglia gene expression levels were significantly higher in adult males compared to females (Werling et al., 2016). A higher glial cell density in male patients with BD has further been proposed to sustain an increased gray matter volume by anti-apoptotic or proliferative/supportive effects of glia (Keshavarz, 2017). Further, a seasonal pattern of manic episodes has been mentioned in relation to psychotic BD in males (Hochman et al., 2016). One may presume that this seasonal pattern may also be, as shown in animal studies, glia-mediated and possibly sex hormone determined (Forlano and Bass, 2005a, b). We performed gender analysis of our transcriptional glia data in the DLPFC and ACC in both MDD and SCZ, but no sex related changes were found (Zhang et al., 2020). We thus deduced that these glia differences in relation to sex are more specific when mania is accompanied by psychotic features.

In our study, sex-based differences appeared mainly in mature astrocytes expressing GS and GLT1. These are both biochemical parameters relevant for glutamate signaling. Sex differences in the glutamate pathway were recently reported to contribute to psychiatric diseases such as MDD, SCZ, ASD and attention deficit hyperactivity disorder (Wickens et al., 2018). Interestingly, we found that P2RY12 shared a similar sexually dimorphic expression pattern in non-suicidal individuals with BD, indicating that glial-dependent purinergic signaling may be disturbed by stress-related diseases and involved in the regulation of glutamate transmission. On the other hand, the immature astrocytic marker S100b did not reveal any significant differences between the sexes, suggesting that astrocytes in the DLPFC are sexually differentiated to the same extent in patients with BD as in controls.

Microglia-related sex differences seem to be brain region and species specific. The enhanced microglia density and soma size that were found before in the somatosensory cortex, hippocampus and amygdala of male adult mice (Guneykaya et al., 2018) were not recapitulated in our human prefrontal data in controls. Following a restrained stress exposure, adult rodents showed different states of microglial activity and proliferation, i.e. the proportion of ramified microglia was increased more in females than in males (Bollinger et al., 2016). However, when males were exposed to social defeat stress they displayed higher phagocytic activity and proliferation of microglia than females (Lehmann et al., 2016). Importantly, there was a shift from an activated state to an anti-inflammatory state in female prefrontal cortical microglia following stress exposure (Bollinger et al., 2016). The strongly lowered microglia mRNA expression in such female mice may predict a weaker innate immune function than in males. When lithium is prescribed to BD females, the total glial cell numbers increased (Keshavarz, 2017) and more manic and depressive episodes were reported than in males (Queissner et al., 2018).

In MDD, opposite changes in gene expression were reported for both sexes in the DLPFC: male patients showed an increase, and females a decrease in oligodendrocyte expression relative to controls (Seney et al., 2018). Notably, OLIG2, a gene determining the differentiation and proliferation of oligodendrocytes, was not significantly different between the sexes in our data. But in our study, MBP and PLP1, two genes closely interacting with each other, showed an, approximately, double increase in BD males compared to females, suggesting the presence of sex differences in the process of myelination, which could include myelin sheath compaction, stabilization, maintenance and/or neuronal survival. Our data provide evidence for a clear sex difference in immature oligodendrocyte expression in BD. In addition, the sex difference in expression of CD68 and MOG supported the possibility of increases in microglial activation/phagocytosis that may occur in response to myelin injury and regeneration in the DLPFC of suicide cases.

A few limitations in our study should be mentioned. Firstly, the relatively small sample size in the subsets (BDNS and BDS, BDNP and BDP) precluded a further exploration of sex differences in patients with BD with and without suicide and psychotic features. Secondly, antipsychotic medication may, at least in theory, have affected P2RY12

Table 3
Sex differences in glial gene expression (male/female) in Ctr and BD (and its subgroups) in the DLPFC.

	Ctr			BD			BD-NS			BD-S			BD-NP			BD-P		
	Fold change	*P* value	BHadj-p	Fold change	*P* value	BHadj-p	Fold change	*P* value	BHadj-p	Fold change	*P* value	BHadj-p	Fold change	*P* value	BHadj-p	Fold change	*P* value	BHadj-p
Astrocytic genes																		
ALDH1L1	1.08	0.55		**1.66**	**0.004**	**0.008**	1.91	0.02	0.06	1.65	0.05		1.01	0.90		**2.73**	**0.001**	**0.008**
GFAP	1.16	0.71		**1.15**	**0.02**	**0.03**	1.07	0.25		1.41	0.02	0.05	−1.03	0.25		1.69	0.04	0.070
GLT1	0.83	0.68		**1.56**	**0.001**	**0.005**	**1.47**	**0.009**	**0.046**	1.61	0.06		1.09	0.70		**2.21**	**0.001**	**0.008**
GS	0.98	0.92		**1.58**	**0.0006**	**0.003**	**2.06**	**0.003**	**0.02**	1.35	0.12		1.05	0.80		**3.25**	**0.001**	**0.008**
S100B	1.17	0.63		1.20	0.05		1.05	0.70		1.48	0.02	0.06	−1.10	0.44		1.48	0.03	0.06
Microglial genes																		
CD68	1.19	0.63		**1.41**	**0.002**	**0.006**	1.27	0.05		**1.33**	**0.01**	**0.046**	−1.18	0.25		**1.64**	**0.01**	**0.03**
CX3CR1	1.02	0.20		**1.54**	**0.02**	**0.02**	1.25	0.34		1.47	0.05		1.23	1.00		**1.83**	**0.003**	**0.01**
HLA-DRA	1.04	0.80		**1.64**	**0.01**	**0.02**	1.64	0.02	0.06	1.75	0.20		−1.36	0.90		**2.89**	**0.001**	**0.008**
IBA1	1.37	0.38		**1.10**	**0.008**	**0.01**	1.05	0.04	0.07	1.22	0.09		−1.11	0.37		**2.03**	**0.004**	**0.01**
P2RY12	1.02	0.27		**1.51**	**0.0003**	**0.002**	**1.29**	**0.002**	**0.02**	1.41	0.03	0.07	1.02	0.03	0.06	**3.54**	**0.005**	**0.01**
TREM2	1.07	0.34		**1.37**	**0.02**	**0.03**	1.30	0.34		1.49	0.05		−1.19	0.90		**2.46**	**0.004**	**0.01**
TSPO	1.31	0.60		**2.14**	**0.003**	**0.007**	1.86	0.04	0.07	1.93	0.03	0.07	1.14	0.52		**2.95**	**0.01**	**0.02**
Oligodendrocytic genes																		
MBP	1.24	0.47		**1.77**	**0.0000**	**0.000**	**1.55**	**0.001**	**0.02**	**1.99**	**0.003**	**0.02**	**1.18**	**0.007**	**0.02**	**1.85**	**0.001**	**0.008**
MOG	0.87	0.80		**1.61**	**0.03**	**0.03**	1.32	0.29		**2.00**	**0.01**	**0.046**	1.03	0.80		**1.84**	**0.007**	**0.02**
OLIG2	1.61	0.65		2.37	0.09		1.42	0.70		1.52	0.02	0.05	1.47	0.61		3.25	0.04	0.07
PLP	1.06	0.83		**1.57**	**0.004**	**0.008**	1.38	0.15		2.15	0.02	0.05	1.02	0.70		**2.86**	**0.003**	**0.01**

Notes: BD: bipolar disorder; BD-NP: BD without psychotic features; BD-NS: patients with BD died of non-suicidal causes; BD-P: BD with psychotic features; BD-S: patients with BD died of suicide; BHadj-p: *P* value of Benjamini-Hochberg's adjustment; Ctr: controls.

mRNA expression in both the DLPFC and ACC. Information on such medication was provided by SMRI as a lifetime dosage in fluphenazine equivalents for 19 individuals with BD. A positive correlation was present between this medication equivalent and P2RY12 expression in both the DLPFC (rho = −0.49, $P = 0.041$) and ACC (rho = −0.54, $P = 0.026$). However, since there was no significant alteration in the DLPFC ($P = 1.00$) or ACC ($P = 0.26$) among the three groups (control and subjects with BD with and without an intake of fluphenazine), it is unlikely that antipsychotics have influenced our main conclusions.

5. Conclusions

Different expression patterns are present in BD when comparing suicide as a confounder. In our data, different genes (decreased CD68, P2RY12 and TREM2) were decreased in patients with BD who did not die by suicide, while individuals with BD who died from suicide showed significantly higher expression of CD68 and P2RY12 mRNA than patients who died of other causes. These findings indicate that in individuals with BD, a stronger prefrontal microglial phagocytosis activity may be present, together with an ATP signaling activation, and a stronger purinergic metabolism in suicide cases. A remarkable sex difference was present in the expression of these three types of glia in BD, especially in those with psychotic features. These changes were observed in particular in the DLPFC. No such changes were found in our SCZ and MDD studies.

CRediT authorship contribution statement

Lin Zhang: Data curation, Writing - original draft. **Ronald W.H. Verwer:** Formal analysis. **Paul J. Lucassen:** Writing - review & editing. **Inge Huitinga:** Methodology, Conceptualization. **Dick F. Swaab:** Supervision, Funding acquisition, Conceptualization, Writing - review & editing.

Declaration of competing interest

None of the authors has anything to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2020.03.003.

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