Molecular pathology of suicide

A postmortem study

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CHAPTER 3

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.

Keywords

Bipolar disorder; dorsolateral prefrontal cortex; glia; suicide; sex differences; psychotic features

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 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). 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.

MATERIALS AND METHODS

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 grey 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 Table S2A-G). Demographic information and medical data were provided by SMRI. All analyses were performed by investigators unaware of the grouped information.

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.

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.

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.

RESULTS

Altered microglia mRNA expression in BD

An overview of mRNA expression in genes is presented in **Figure 1** and **Tables 2A** to **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.

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 **Figure 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.

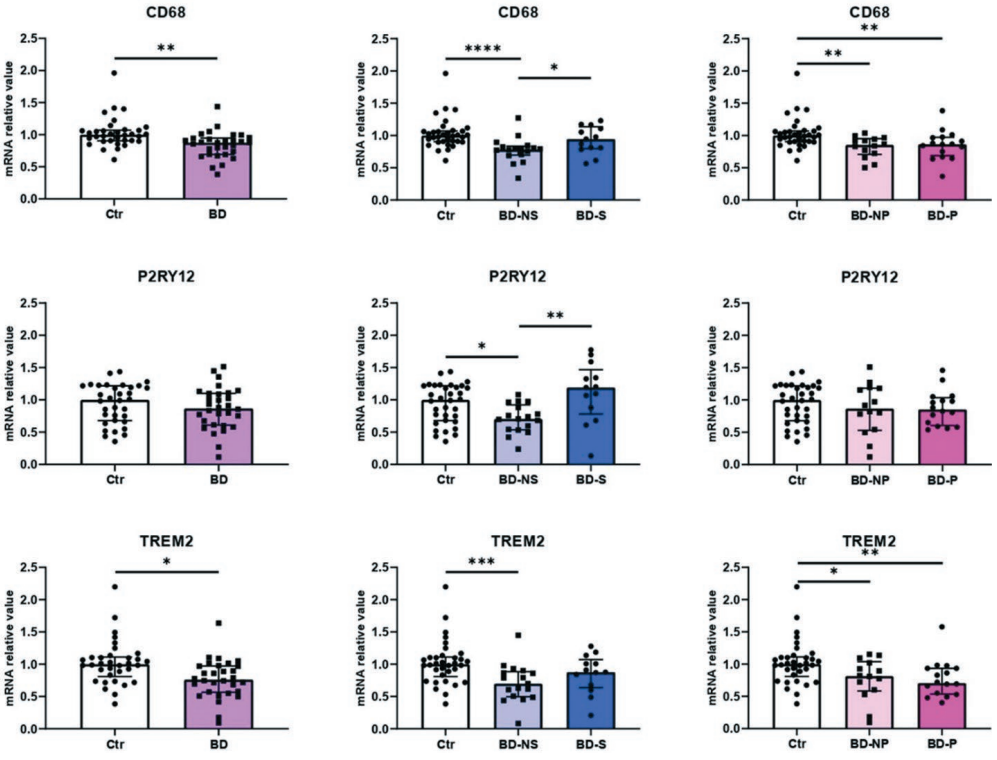

Figure 1. Transcript levels of microglia related genes (CD68, P2RY12 and TREM2) in the dorsolateral prefrontal cortex (DLPFC) in controls (Ctr, n = 34) and patients with bipolar disorder (BD, n = 30) that died of suicide (BD-S, n = 13) or causes other than suicide (BD-NS, n = 17), and with or without 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 $0.001 < P \leq 0.0001$.

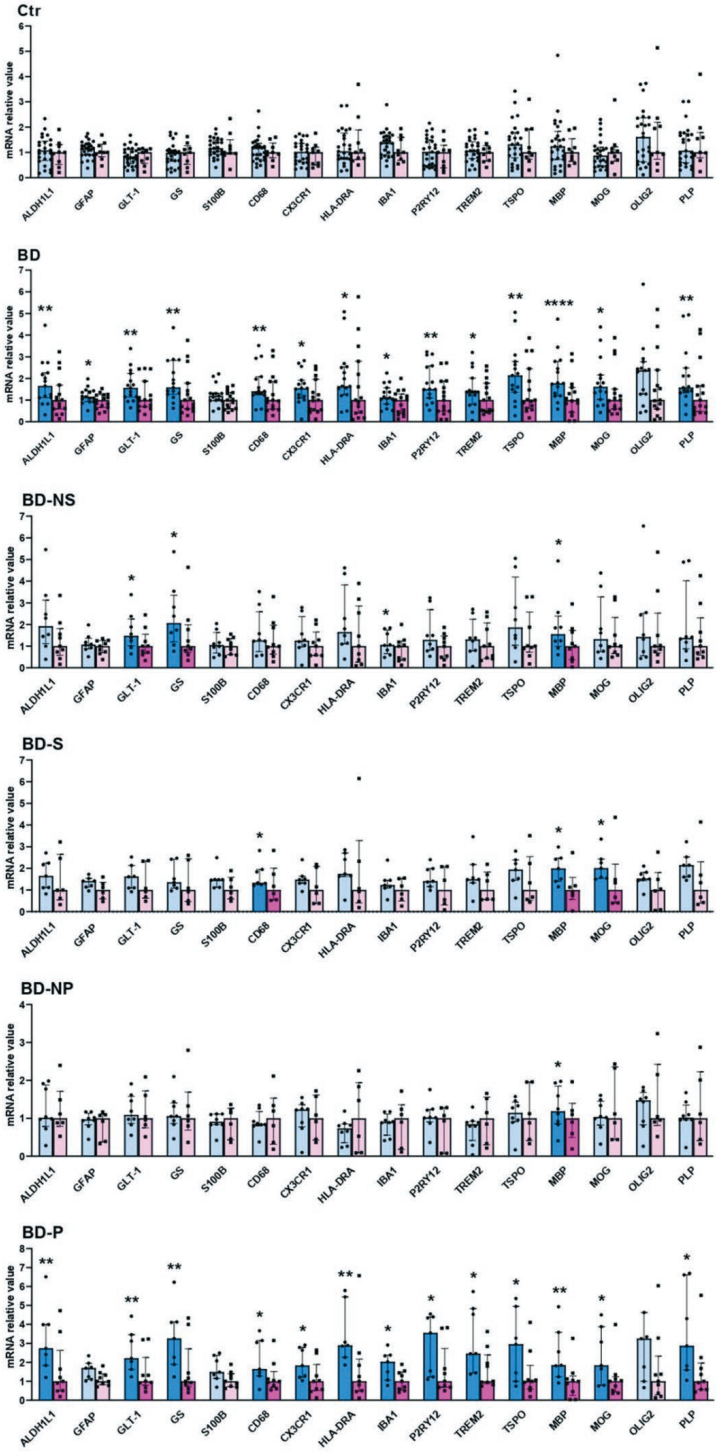
Ctr
BD
BD-NS
BD-S
BD-NP
BD-P
mRNA relative value
ALDH1L1
GFAP
GLT-1
GS
S100B
CD68
CX3CR1
HLA-DRA
IBA1
P2RY12
TREM2
TSPO
MBP
MOG
OLIG2
PLP

Figure 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 $0.001 < P \leq 0.0001$.

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.

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				

	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
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.

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				

	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
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.

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	BH-adj-p	Fold change	*P* value	BH-adj-p	Fold change	*P* value	BH-adj-p	Fold change	*P* value	BH-adj-p	Fold change	*P* value	BH-adj-p	Fold change	*P* value	BH-adj-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.

DISCUSSION

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

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.

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Contributors

Lin Zhang, Inge Huitinga and Dick F. Swaab designed the protocol. Lin Zhang undertook data collection. Ronald W. H. Verwer performed statistical analysis. Lin Zhang wrote the first draft. Dick F. Swaab and Paul J. Lucassen amended the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest Statement

None of the authors has anything to disclose.

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Table S1. Reference genes and target genes sequences.

Gene	Full Name	Selection Strategy	Accession Code	Forward Primer	Reverse Primer
Reference Gene					
ACTβ	Actin beta		NM_001101	CCCAGCCATGTACGT TGCTA	TCACCGGAGTCCATCACGAT
GAPDH	Glyceraldehyde-phosphate dehydrogenase		NM_002046	CAAATTCCATGGCACCGTC	TCTCGCTCCTGGAAGATGGT
HPRT1	Hypoxanthine phosphori-bosyltransferase 1		NM_000194	GGACAGGACTGAACGT CTTGC	ATAGCCCCCCTTGAGCACAC
TUBα	Tubulin alpha		NM_006082	CTTTGAGCCAGCCAACC AGA	GTACAACAGGCAGCAAGCCAT
TUBβ	Tubulin beta		NM_006087	GGGCCAAGTTTTGGG AGGT	CACTGTCCCCATGGTATGTGC
UBC	Ubiquitin C		NM_021009	GCTGCTCATAAGACTCG GCC	GTCACCCAAGTCCCGTCCTA
Target Gene	**Full Name**	**Selection Strategy**	**Accession Code**	**Forward Primer**	**Reverse Primer**
Astrocyte					
ALDH1L1	Aldehyde dehydrogenase 1 family, member L1	Neuron-glia interaction (dopaminergic system)	NM_001270364	GAACACAGTGGTGATC AAGCC	GGAGGACGTTAACCACACCTT
GFAP	Glial fibrillary acidic protein	Astrocyte activation	NM_001363846	CCCACTCTGCTTTG ACTGAGC	CCTTCTTCGGCCTTAGAGGG
GLT1	Glutamate transporter 1	Neuron-glia interaction (glutamate-glutamine signaling)	NM_001195728	ATACCATTGACTCC CAGCATCG	GAGTTGCTTTCCCTGTG-GTTCTT

Target Gene	Full Name	Selection Strategy	Accession Code	Forward Primer	Reverse Primer
GS	Glutamine synthetase	Neuron-glia interaction (glutamate-glutamine signaling)	NM_001033056	TGTGTGGAAGAGTTG CCTGAG	TGGCAGCAGGCACGAGATAC
S100b	S100 calcium binding protein b	Blood-brain barrier (BBB) permeability	NM_006272	TGGAAAAAGCAACTC CATCAGAA	GAATCGCATGGGTCACGG
Microglia					
CD68	Cluster of differentiation 68	Microglia phagocytosis	NM_001040059	CTACCAGGCCCTCTGAGC	GGCGTGTCGAGGAAATAAGG
CX3CR1	Chemokine (C-X3-C motif) receptor 1	Microglia homeostasis	NM_001337	TTGGCCTGGTG GGAAATTTGT	AGGAGGTAAATGTCGGT GACACT
HLA-DRA	Human leukocyte antigen-DRA	Microglia activation and neuroinflammation	NM_019111	CCCAGGGAAGACCAC CTTT	CACCCTGCAGTCGTAAACGT
IBA1	Ionized calcium-binding adapter molecule 1	Microglia activation and neuroinflammation	NM_032955	AGGTGTCCAGTGGCT CCGGG	TGGCAGATCTCTTGCCCAGCA
P2RY12	Purinergic receptor 12	Microglia homeostasis, ATP signaling and coagulation state	NM_176876	TTCAAACCCTCCAGAA TCAACAG	GTGCACAGACTGGTGTTACC
TREM2	Triggering receptor expressed on myeloid cells 2	Microglial metabolism and anti-inflammatory activity	NM_018965	CCACCCACTTCCATCC TTCT	GTCCCTGGCTTCTGTCCAT
TSPO	Translocator protein	Neuron-glia interaction (GABAergic system)	NM_000714	TCTGGAAAGAGCTGG GAGG	TTGTCGGGCACCAAAGAAG

Target Gene	Full Name	Selection Strategy	Accession Code	Forward Primer	Reverse Primer
Oligodendrocyte					
MBP	Myelin basic protein	Myelin stability and BBB permeability	NM_001025101	AAATGCCGAGAAGG CCAGTAC	CCCATTGTTCTGGTTCGCA
MOG	Myelin oligodendrocyte glycoprotein	Myelin sheath maintenance	NM_002433	TGAGAGGAAAACTTC GAGCAG	CGGCACAATTACAAACA GGG
OLIG2	Oligodendrocyte transcription factor 2	Neural repair	NM_005806	GTGTCCAGCGCCTCTCT	GGCAGCAGACGGA GACT
PLP1	Myelin proteolipid protein 1	Myelin formation and maintenance	NM_000533	CCTCACTGGCA CAGAAAAGCT	GGCCCCATAAA GGAAGAAGAA

Table S2A. Clinico-pathological information (Ctr-BD).

Group	Sex	Age (y)	PMD (hr)	MOD	CSF pH	BW (g)	RIN value	Hemisphere	Alcohol use	Drug use	Psychotic features	Fluphenazine equivalents (mg)	Cause of death
CTR1	F	44	28	5	6.59	1330	8.3	R DLPFC, R ACC	3	0	No	0	CARDIAC
CTR2	M	49	46	7	6.5	1605	8.3	R DLPFC, R ACC	0	0	No	0	CARDIAC
CTR3	M	53	9	12	6.4	1500	9.1	L DLPFC, L ACC	1	0	No	0	CARDIAC
CTR4	M	37	13	3	6.5	1600	8.3	L DLPFC, L ACC	0	0	No	0	CARDIAC
CTR5	M	51	31	4	6.7	1400	7.3	R DLPFC, R ACC	1	0	No	0	CARDIAC
CTR6	M	53	28	6	6	1340	8.4	L DLPFC, L ACC	0	0	No	0	CARDIAC
CTR7	F	38	33	7	6	1120	9.7	R DLPFC, R ACC	3	0	No	0	CARDIAC
CTR8	F	38	28	9	6.7	1350	8.8	R DLPFC, R ACC	0	0	No	0	CARDIAC
CTR9	M	60	47	10	6.8	1460	8.4	R DLPFC, R ACC	0	0	No	0	CARDIAC
CTR10	M	35	52	6	6.7	1700	8.7	R DLPFC, R ACC	0	0	No	0	MYOCARDITIS
CTR11	M	34	22	11	6.48	1480	8.2	R DLPFC, R ACC	0	0	No	0	CARDIAC
CTR12	M	47	21	1	6.81	1550	8.7	L DLPFC, L ACC	0	0	No	0	CARDIAC

Group	Sex	Age (y)	PMD (hr)	MOD	CSF pH	BW (g)	RIN value	Hemisphere	Alcohol use	Drug use	Psychotic features	Fluphenazine equivalents (mg)	Cause of death
CTR13	M	45	29	2	6.94	1405	8.5	R DLPFC, R ACC	1	0	No	0	CARDIAC
CTR14	F	34	24	2	6.87	1255	6.6	R DLPFC, R ACC	0	0	No	0	CARDIAC
CTR15	M	42	37	3	6.91	1340	7.8	L DLPFC, L ACC	4	3	No	0	CARDIAC
CTR16	F	44	10	3	6.2	1305	8.4	R DLPFC, R ACC	0	0	No	0	CARDIAC
CTR17	M	45	18	4	6.81	1585	8.0	L DLPFC, L ACC	0	0	No	0	CARDIAC
CTR18	M	49	23	6	6.93	1390	9.5	L DLPFC, L ACC	1	0	No	0	CARDIAC
CTR19	M	32	24	7	7.03	1415	8.3	L DLPFC, L ACC	2	0	No	0	CARDIAC
CTR20	M	55	31	8	6.7	1515	8.3	L DLPFC, L ACC	1	0	No	0	CARDIAC
CTR21	F	49	45	9	6.72	1435	8.6	L DLPFC, L ACC	0	0	No	0	CARDIAC
CTR22	F	33	29	2	6.52	1360	8.3	L DLPFC, L ACC	0	1	No	0	ASTHMA
CTR23	M	48	31	3	6.86	1580	7.0	R DLPFC, R ACC	0	0	No	0	CARDIAC
CTR24	M	50	49	3	6.75	1645	8.0	R DLPFC, R ACC	1	0	No	0	CARDIAC

Group	Sex	Age (y)	PMD (hr)	MOD	CSF pH	BW (g)	RIN value	Hemisphere	Alcohol use	Drug use	Psychotic features	Fluphenazine equivalents (mg)	Cause of death
CTR25	M	32	13	8	6.57	1410	9.0	R DLPFC, R ACC	0	0	No	0	CARDIAC
CTR26	M	47	11	8	6.6	1495	8.3	L DLPFC, L ACC	0	1	No	0	CARDIAC
CTR27	M	46	31	9	6.67	1360	8.6	L DLPFC, L ACC	1	0	No	0	CARDIAC
CTR28	M	40	38	11	6.67	1498	8.7	L DLPFC, L ACC	1	0	No	0	CARDIAC
CTR29	M	51	22	11	6.71	1900	8.7	L DLPFC, L ACC	1	0	No	0	CARDIAC
CTR30	M	31	11	1	6.13	1335	8.4	R DLPFC, R ACC	1	1	No	0	PULM EMBOL
CTR31	M	48	24	1	6.91	1321	8.6	R DLPFC, R ACC	1	1	No	0	CARDIAC
CTR32	F	39	58	2	6.46	1260	8.4	R DLPFC, R ACC	4	0	No	0	CARDIAC
CTR33	M	47	36	6	6.57	1535	8.0	L DLPFC, L ACC	0	0	No	0	CARDIAC
CTR34	F	41	50	8	6.17	1290	7.3	R DLPFC, R ACC	0	0	No	0	CARDIAC
Median	–	45	28.5	–	6.69	1413	8.40	–	–	–	–	–	–
BD1	M	29	48	12	6.39	1570	9.2	R DLPFC, R ACC	3	3	Yes	9000	SUIC:JUMPED
BD2	M	29	60	1	6.7	1430	8.5	R DLPFC, R ACC	5	5	No	0	SUIC:CO

Group	Sex	Age (y)	PMD (hr)	MOD	CSF pH	BW (g)	RIN value	Hemisphere	Alcohol use	Drug use	Psychotic features	Fluphenazine equivalents (mg)	Cause of death
BD3	M	45	28	1	6.35	1480	9.1	L DLPFC, L ACC		5	Yes	10000	CARDIAC
BD4	M	41	70	1	6.71	1625	7.5	R DLPFC, R ACC	5	4	No	0	SUIC:OD
BD5	F	29	62	2	6.74	1330	7.8	R DLPFC, R ACC	3	5	Yes	0	OD
BD6	M	44	19	10	6.74	1660	9.3	L DLPFC, L ACC	2	1	No	0	SUIC:HANGING
BD7	F	48	18	5	6.5	1205	8.3	R DLPFC, R ACC	1	3	No	0	CARDIAC
BD8	M	42	32	6	6.65	1470	8.4	L DLPFC, L ACC	5	5	No	0	DROWNING
BD9	M	35	35	7	6.3	1490	7.8	R DLPFC, R ACC	1	2	Yes	30000	CARDIAC
BD10	F	59	53	7	6.2	1410	8.5	L DLPFC, L ACC	1	0	No	0	SUIC:OD
BD11	M	54	44	10	6.5	1510	9.2	R DLPFC, R ACC	2	2	No	0	SUIC:OD
BD12	F	35	17	11	6.1	1250	7.9	L DLPFC, L ACC	1	0	Yes	3000	SUIC:CO
BD13	F	42	49	2	6.65	1335	8.0	R DLPFC, R ACC	4	3	Yes	15000	OD
BD14	F	58	35	8	6.5	1440	9.5	R DLPFC, R ACC	1	1	Yes	12000	SUIC:GSW
BD15	M	64	16	6	6.1	1340	7.8	L DLPFC, L ACC	0	0	Yes	130000	PNEUMONIA

Group	Sex	Age (y)	PMD (hr)	MOD	CSF pH	BW (g)	RIN value	Hemisphere	Alcohol use	Drug use	Psychotic features	Fluphenazine equivalents (mg)	Cause of death
BD16	M	59	84	7	6.65	1300	7.3	L DLPFC, L ACC	1	0	No	500	SLEEP APNEA
BD17	M	51	23	3	6.67	1590	9.5	L DLPFC, L ACC	3	0	Yes	1200	CARDIAC
BD18	F	63	32	4	6.97	1290	8.9	R DLPFC, R ACC	0	0	No	0	CARDIAC
BD19	F	44	37	5	6.37	1200	7.3	L DLPFC, L ACC	0	0	Yes	30000	MYOCARDITIS
BD20	F	56	26	5	6.58	1170	8.2	R DLPFC, R ACC	5	4	No	25000	DROWNING
BD21	F	43	39	5	6.74	1505	9.3	R DLPFC, R ACC	2	1	Yes	4500	SUIC:OD
BD22	M	35	22	6	6.58	1390	8.5	L DLPFC, L ACC	2	2	Yes	2000	DROWNING
BD23	F	50	62	8	6.51	1400	8.3	R DLPFC, R ACC	1	2	Yes	15000	SUIC:OD
BD24	F	49	38	9	6.39	1190	8.5	L DLPFC, L ACC	5	1	Yes	0	OD
BD25	F	33	24	11	6.51	1450	8.6	R DLPFC, R ACC	5	2	No	3000	SUIC:HANGING
BD26	F	41	28	2	6.44	1360	8.7	R DLPFC, R ACC	4	4	No	3000	CARDIAC
BD27	F	43	57	10	5.92	1340	6.3	R DLPFC, R ACC	4	4	Yes	10000	OD
BD28	M	56	23	2	6.07	1670	9.3	L DLPFC, L ACC	4	0	Yes	10000	SUIC:OD

Group	Sex	Age (y)	PMD (hr)	MOD	CSF pH	BW (g)	RIN value	Hemisphere	Alcohol use	Drug use	Psychotic features	Fluphenazine equivalents (mg)	Cause of death
BD29	M	48	23	3	6.9	1466	8.4	R DLPFC, R ACC	5	2	No	0	SUIC:HANGING
BD30	M	19	12	5	5.97	1484	7.9	L DLPFC, L ACC	2	5	No	2000	OD
Median	–	44	33.5	–	6.50	1420	8.45	–	–	–	–	–	–
P value	0.05	0.66	0.13	NS	**0.03**	0.49	0.76	0.77	–	–	–	–	–

Notes: ACC, anterior cingulate cortex; BD, bipolar disorder; BW, brain weight; CSF, cerebrospinal fluid; CTR, control; DLPFC, dorsolateral prefrontal cortex; F, female; L, left; M, male; MOD, month of death; N.A., not mention; NS, not significant; OD, overdose drugs; PMD, postmortem delay; R, right; RIN, RNA integrity number; SUIC, suicide. Scale of alcohol and drug use: 0, little or none; 1, social; 2, moderate past; 3, moderate present; 4, heavy past; 5, heavy present.

Table S2B. Demographic information (Ctr-BD-NS-BD-S).

	Ctr	BD-NS	BD-S	*P*
Age (year, range)	45 (31–60)	44 (19–64)	44 (29–59)	0.90
Gender (M/F)	25/9	8/9	7/6	0.14
PMD (hour, range)	28.5 (9–58)	32 (16–84)	39 (17–70)	0.23
Brain pH	6.69 (6.00–7.03)	6.50 (6.10–6.97)	6.51 (6.1–6.74)	0.07
Brain weight (gram, range)	1413 (1120–1900)	1340 (1170–1590)	1466 (1250–1670)	0.01
Hemisphere	16 L/18 R	9 L/8 R	4 L/9 R	0.46
Fluphenazine equivalents	–	12	7	–

Notes: BD-NS, patients with bipolar disorder who died of non-suicidal reasons; BD-S, patients with bipolar disorder who died of suicides; Ctr, control; F, female; L, left; M, male; PMD, postmortem delay; R, right.

Table S2C. Demographic information (Ctr-BD-NP-BD-P).

	Ctr	BD-NP	BD-P	*P*
Age (year, range)	45 (31–60)	46 (19–63)	43.5 (29–64)	0.83
Gender (M/F)	25/9	8/6	7/9	0.11
PMD (hour, range)	28.5 (9–58)	30 (12–84)	36 (16–62)	0.29
Brain pH	6.69 (6.00–7.03)	6.62 (5.97–6.97)	6.39 (5.92–6.74)	0.02
Brain weight (gram, range)	1413 (1120–1900)	1440 (1170–1660)	1395 (1190–1670)	0.76
Hemisphere	16 L/18 R	5 L/9 R	8 L/8 R	0.70
Fluphenazine equivalents	–	5	14	–

Notes: BD-NP, patients with bipolar disorder but without psychotic features; BD-P, patients with bipolar disorder and psychotic features; Ctr, control; F, female; L, left; M, male; PMD, postmortem delay; R, right.

Table S2D. Demographic information (Ctr-M vs. Ctr-F).

	Ctr-M	Ctr-F	*P*
Age (year, range)	47 (31–60)	39 (33–49)	0.06
Gender (M/F)	25	9	–
PMD (hour, range)	28 (9–52)	29 (10–58)	0.32
Brain pH	6.70 (6.00–6.94)	6.52 (6.00–6.87)	0.09
Brain weight (gram, range)	1495 (1321–1900)	1305 (1120–1435)	0.0003
Hemisphere	11 L/14 R	7 L/2 R	0.08

Notes: Ctr-F, control female; Ctr-M, control male; L, left; PMD, postmortem delay; R, right.

Table S2E. Demographic information (BD-M vs. BD-F).

	BD-M	BD-F	*P*
Age (year, range)	44 (19–64)	44 (29–63)	0.62
Gender (M/F)	15	15	–
PMD (hour, range)	28 (12–84)	37 (17–62)	0.35
Brain pH	6.58 (5.97–6.74)	6.50 (5.92–6.97)	0.85
Brain weight (gram, range)	1484 (1300–1670)	1335 (1170–1505)	0.0005
Hemisphere	9 L/6 R	4 L/11 R	0.07
Psychotic features	7	9	–
Suicide	7	6	–
Fluphenazine equivalents (lifetime dosage, mg)	1200 (0–130000)	3000 (0–30000)	0.41

Notes: BD-F, female patients with bipolar disorder; BD-M, male patients with bipolar disorder; L, left; PMD, postmortem delay; R, right.

Table S2F. Demographic information (Ctr-M vs. BD-M).

	Ctr-M	BD-M	*P*
Age (year, range)	47 (31–60)	44 (19–64)	0.46
PMD (hour, range)	28 (9–52)	28 (12–84)	0.40
Brain pH	6.70 (6.00–6.94)	6.58 (5.97–6.74)	0.03
Brain weight (gram, range)	1495 (1321–1900)	1484 (1300–1670)	0.95
Hemisphere	11 L/14 R	9 L/6 R	0.33
Psychotic features	–	7	–
Suicide	–	7	–
Fluphenazine equivalents	–	9	–

Notes: BD-M, male patients with bipolar disorder; Ctr-M, male control subjects; L, left; PMD, postmortem delay; R, right.

Table S2G. Demographic information (Ctr-F vs. BD-F).

	Ctr-F	BD-F	*P*
Age (year, range)	39 (33–49)	44 (29–63)	0.11
PMD (hour, range)	29 (10–58)	37 (17–62)	0.53
Brain pH	6.52 (6.00–6.87)	6.50 (5.92–6.97)	0.88
Brain weight (gram, range)	1305 (1120–1435)	1335 (1170–1505)	0.61
Hemisphere	7 L/2 R	4 L/11 R	0.02
Psychotic features	–	9	–
Suicide	–	6	–
Fluphenazine equivalents	–	10	–

Notes: BD-F, female patients with bipolar disorder; Ctr-F, female control subjects; L, left; PMD, postmortem delay; R, right.