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Prefrontal alterations in GABAergic and glutamatergic gene expression in relation to depression and suicide

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ABSTRACT

People that committed suicide were reported to have enhanced levels of gene transcripts for synaptic proteins in their prefrontal cortex (PFC). Given the close association of suicide with major depressive disorder (MDD), we here assessed whether these changes are related to suicide or rather to depression per se.

We used quantitative PCR to determine mRNA levels of 32 genes encoding for proteins directly involved in glutamatergic or GABAergic synaptic transmission in postmortem samples of the anterior cingulate cortex (ACC) and the dorsolateral PFC (DLPFC). Seventy-two brain samples from 3 groups of subjects were derived from the Stanley Medical Research Institute (SMRI): i) patients with MDD who committed suicide (MDD-S), ii) MDD patients who died of non-suicidal causes (MDD-NS) and iii) age-matched, non-psychiatric control subjects.

In the ACC, a significantly enhanced expression of genes related to glutamatergic or GABAergic synaptic transmission was found only in MDD-S patients, whereas in MDD-NS patients, decreased levels for these transcripts were found. Moreover, in the DLPFC, expression of these genes was decreased in MDD-S, relative to MDD-NS patients, whereas both groups showed increased expression compared to control subjects.

In conclusion, our findings indicate that MDD is associated with increases in GABA and glutamate related genes in the DLPFC (irrespective of suicide), while in the ACC, the increase in GABA and glutamate related genes may relate to suicide, rather than to MDD per se.

1. Introduction

Major depressive disorder (MDD) is a serious and disabling psychiatric illness that affects 121 million individuals worldwide, and is predicted to be the second most common cause of disability, after heart disease, by 2020 (Baune et al., 2007). MDD is also the most common psychiatric diagnosis associated with suicide. Up to 15% of those who are clinically depressed eventually commit suicide, and more than half of all people who commit suicide meet the criteria for depressive disorder (Alsalman and Alansari, 2016; Cavanagh et al., 2003). Several studies on a relationship between monoaminergic neurotransmission, depression and suicide have emerged in the past decades. However, both the limited efficacy and the delayed onset of the therapeutic effects of selective serotonin reuptake inhibitors (SSRIs), the most commonly prescribed antidepressant drugs, raise doubts as to whether the monoaminergic system is indeed in general a primary affected system in

depression (Lawrence et al., 2017; Thase et al., 2005; Trivedi et al., 2006).

In recent years, increasing evidence suggests that, in addition to hypothalamic neuroendocrine changes (Bao et al., 2008; Qi et al., 2013a; Swaab et al., 2005), an impaired balance between inhibition and excitation within the prefrontal cortex (PFC) and related limbic brain circuitry is involved in MDD, probably mediated by altered gamma-aminobutyric acid (GABA) and glutamate neurotransmission (Gao and Bao, 2011; Ghosal et al., 2017). Large-scale gene array studies in post-mortem tissue have provided strong support for alterations in GABAergic and glutamatergic neurotransmission in depression. For instance, Choudary and colleagues demonstrated significant down-regulation of SLC1A2 and SLC1A3, and up-regulation of several glutamate and GABA-A receptor subunits by using microarray analysis from MDD patients (Choudary et al., 2005).

Later, altered glutamate related genes, SLC1A2, SLC1A3 and GLUL

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were also found in the MDD patients by microarray and confirmed by quantitative real-time PCR (Q-PCR) and in situ hybridization (Bernard et al., 2011). Another study also demonstrates significant dysregulation of glutamate receptor subunit genes GRIA1 and GRIA3 in the subjects diagnosed with MDD by microarrays and a subgroup of identified genes was confirmed by Q-PCR (Duric et al., 2013). Moreover, morphometric studies have reported a reduced density and size of cortical neurons (Bernstein et al., 2016; Chana et al., 2003; Rajkowska et al., 1999), an effect that has been attributed to alterations in interneurons in some (Maciag et al., 2010; Rajkowska et al., 2007) but not all studies (Cotter et al., 2002; Lucassen et al., 2014). In particular the PFC has received considerable attention in recent years given its role in inhibitory control, emotions and mood, and prominent changes in depression and suicide (Fuster, 2008; Kekesi et al., 2012; Qi et al., 2013a, 2013b; Zhang et al., 2013).

As post-mortem studies have often been inconsistent, the exact molecular mechanisms underlying the aetiology and pathophysiology of depression and suicide, and factors that distinguish these, have so far remained obscure. One critical issue, however, for interpreting discrepancies between existing studies is the different case-control matching strategies. For instance, many studies that claimed to determine molecular alterations in relation to depression had selected depressed patient groups in which most patients had committed suicide, and compared these to non-psychiatric controls (Bernard et al., 2011; Deschwanden et al., 2011; Kimoto et al., 2015; Kunii et al., 2015; Martins-de-Souza et al., 2012; Matosin et al., 2014; Shelton et al., 2011). Without an adequate comparison to a disease control group, consisting of depressed patients who did not commit suicide, it is impossible to determine whether the reported alterations are due to depression or to suicide. On the other hand, studies that claimed to show changes in relation to suicide per se, often compared suicide cases to matched controls without any psychiatric disorder (Du et al., 2014; Kekesi et al., 2012; Lopez et al., 2014; Pandey et al., 2013; Poulter et al., 2008; Schiavone et al., 2016; Sequeira et al., 2012; Valdizan et al., 2010), thus disregarding the fact that the majority of suicide cases suffer from psychiatric disorders, including depression, anxiety, substance abuse, schizophrenia or personality disorders (Hawton and van Heeringen, 2009).

To investigate whether the aspect of suicide is relevant for the gene expression changes in depression reported before by others, we previously studied a group of well-documented depressed patients who did not commit suicide, and found that surprisingly few GABA and glutamate-related genes had changed in expression in the prefrontal cortex (PFC) (Zhao et al., 2012). This suggested that the alterations reported in

this brain area before, may be related to suicide rather than to depression per se. Furthermore, we recently found indications that depressed patients who committed suicide show different expression patterns of genes related to the glutamate-glutamine cycle (Zhao et al., 2016) and of stress related molecules, such as corticotropin-releasing hormone (CRH) and nitric oxide synthase (NOS), than depressed patients who died of causes other than suicide (Zhao et al., 2015).

Here, we follow up on these initial studies in a larger, well-characterized cohort from the Stanley Medical Research Institute (SMRI). We determined the expression patterns of genes involved in different aspects of both the GABA and glutamate pathways, comparing transcript levels from MDD patients who died of suicide, to MDD patients who died of other causes, and to matched controls without a neuropsychiatric disorder. Our results show that changes in the genes involved in glutamatergic and GABAergic neurotransmission may be related either to suicide or to depression per se, depending on the brain area studied.

2. Materials and methods

2.1. Material from the Stanley Medical Research Institute (SMRI)

Brain samples were collected from the SMRI (Bethesda, MD, USA). Permission to use the brain material was provided by the next of kin. Diagnoses were made according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV (American Psychiatric Association, 1994). All brains were examined microscopically to exclude cases with pathological signs of neurodegeneration or other lesions. Exclusion criteria included anyone over age 70, anyone with a history of seizures or other neurological disorders that might affect brain pathology and anyone with evidence of such conditions upon neuropathological examination. The cause of death for 17 of the 36 cases was suicide; the other cases, and all control subjects died from natural causes or accidents.

Post-mortem material of the anterior cingulate cortex (ACC, Brodmann area 24) and dorsolateral prefrontal cortex (DLPFC, Brodmann area 46) were obtained from MDD patients with accomplished suicide (MDD-S, N = 17), MDD patients who died of other causes (MDD-NS, N = 7), or matched controls (Ctr, N = 12) without a history of suicidal behaviour or any major psychiatric diagnosis. All groups were matched for age, gender, brain pH, brain weight, post-mortem delay (PMD), ethnicity, history of substance abuse, severity of substance abuse, and psychotic features (Table 1). All demographic information and medical data, including any lifetime use of

Table 1
Demographic information for SMRI subjects.

	MDD-S	MDD-NS	Ctr	F or χ^2 ^b	P
Age (years, range)	40 (24–63)	46 (36–56)	47 (24–63)	1.40	0.26
Gender (M/F)	10/7	3/4	8/4	1.03	0.60
Race	16 W, 1H	7 W	11 W, 1H	0.53	0.74
PMD (hours, range)	29.6 (13–65)	29.9 (15–52)	25.3 (9–40)	0.54	0.59
Brain pH	6.67 (6.36–6.88)	6.60 (6.30–6.90)	6.64 (6.31–6.91)	0.57	0.57
Brain Weight (gram, range)	1480 (1170–1780)	1441 (1270–1590)	1444.83 (1200–1595)	0.30	0.75
Psychotic Feature ^a	9	3	–	0.10	0.75
Alcohol hx	11	5	6	1.03	0.60
Severity of Alcohol abuse ^b	2.18 (0–5)	1.29 (0–5)	2.08 (0–5)	0.51	0.61
Drug hx	5	3	4	0.40	0.82
Severity of Substance abuse ^b	0.88 (0–4)	1.43 (0–4)	0.75 (0–4)	0.47	0.63
Fluphenazine (lifetime) ^c	1041.18 (0–6500)	1314.29 (0–3000)	–	0.12	0.73
RIN	7.69 ± 0.66	7.49 ± 0.56	7.73 ± 0.95	1.21	0.55

Abbreviation: Ctr, control; F, female; hx, history; L, left; M, male; MDD-S, major depressed patients committed suicide; MDD-NS, major depressed patients died of causes other than suicide; PMD, postmortem delay; R, right; RIN, RNA integrity value.

^a Psychotic Feature tested without controls, by chi-square test.

^b Substance abuse and alcohol abuse was rated on a scale of 0–5.

^c Fluphenazine tested without controls.

Table 2
Clinico-pathological information of patients with MDD and control subjects.

	Patient code	Sex	Age (y)	PMD (h)	Brain pH	BW (g)	RIN	Brain Region	Medication	Psychotic feature	Cause of Death
MDD	1	F	48	24	6.36	1330	8.0	L DLPFC, L ACC	TCA	Yes	SUIC: OD
MDD	3	F	40	49	6.72	1450	8.8	L DLPFC, L ACC	SSRI	Yes	SUIC: HANGING
MDD	5	F	56	15	6.59	1370	8.4	L DLPFC, L ACC	antidepressant*	No	BURNS
MDD	7	M	28	26	6.7	1780	6.9	R DLPFC, R ACC	SSRI	Yes	SUIC: HANGING
MDD	9	M	35	19	6.6	1335	8.2	R DLPFC, R ACC	–	Yes	SUIC: HANGING
MDD	10	F	32	19	6.7	1280	8.9	L DLPFC, L ACC	–	Yes	SUIC: HANGING
MDD	12	F	32	19	6.8	1470	8	R DLPFC, R ACC	non-TCA, SSRI	No	SUIC: HANGING
MDD	13	M	63	31	6.6	1540	7.8	L DLPFC, L ACC	non-TCA	Yes	SUIC: HANGING
MDD	14	F	51	36	6.3	1440	7.3	L DLPFC, L ACC	MAOI, TCA	Yes	UNKNOWN
MDD	15	M	35	36	6.6	1710	6.3	L DLPFC, L ACC	–	Yes	SUIC: GSW
MDD	16	M	44	24	6.52	1550	7.9	R DLPFC, R ACC	TCA, SSRI	No	CARDIAC
MDD	17	M	56	38	6.59	1365	7.3	L DLPFC, L ACC	SNRI, anxiolytics	No	SUIC: OD
MDD	18	M	33	25	6.86	1640	7.6	R DLPFC, R ACC	–	No	SUIC: HANGING
MDD	20	M	34	24	6.79	1425	8.2	R DLPFC, R ACC	SSRI	No	SUIC: JUMPED
MDD	21	F	45	29	6.9	1350	7.4	L DLPFC, L ACC	SSRI	No	CARDIAC
MDD	22	M	53	21	6.64	1520	7.5	L DLPFC, L ACC	–	No	CARDIAC
MDD	24	M	62	65	6.57	1490	7.5	R DLPFC, R ACC	non-TCA	ND	SUIC: STABBED
MDD	25	F	36	32	6.74	1270	7.3	R DLPFC, R ACC	SARI, TCA	Yes	PULM EMBOL
MDD	27	M	40	52	6.48	1590	6.6	L DLPFC, L ACC	–	Yes	OD
MDD	29	F	28	40	6.68	1430	7.1	L DLPFC, L ACC	–	Yes	SUIC: OD
MDD	30	M	45	29	6.75	1514	7.9	L DLPFC, L ACC	SARI	No	SUIC: HANGING
MDD	31	M	24	21	6.61	1737	7.4	R DLPFC, R ACC	SNRI	No	SUIC: OD
MDD	32	F	45	13	6.58	1170	7.2	L DLPFC, L ACC	TCA	No	SUIC: OD
MDD	34	F	47	25	6.88	1495	7.7	L DLPFC, L ACC	TCA	No	SUIC: GSW
Ctrl	2	M	48	12	6.51	1410	8.6	R DLPFC, R ACC	–	No	CARDIAC
Ctrl	4	F	50	35	6.31	1520	7.9	L DLPFC, L ACC	anxiolytics	No	CARDIAC
Ctrl	6	M	50	11	6.5	1530	8.6	L DLPFC, L ACC	–	No	CARDIAC
Ctrl	8	M	63	37	6.5	1530	8	L DLPFC, L ACC	–	No	CARDIAC
Ctrl	11	M	24	17	6.6	1595	8.1	L DLPFC, L ACC	–	No	MVA
Ctrl	19	M	44	27	6.82	1410	7.2	R DLPFC, R ACC	–	No	ACUTE ALCOHOL POISONING
Ctrl	23	M	35	31	6.59	1520	8	R DLPFC, R ACC	–	No	MVA
Ctrl	26	M	63	40	6.91	1410	8.2	R DLPFC, R ACC	–	No	CARDIAC
Ctrl	28	M	34	9	6.56	1535	5.9	R DLPFC, R ACC	–	No	MVA
Ctrl	33	F	56	29	6.78	1278	8.9	L DLPFC, L ACC	–	No	CARDIAC
Ctrl	35	F	56	31	6.66	1400	6.1	L DLPFC, L ACC	–	No	OBESITY
Ctrl	36	F	39	24	6.88	1200	7.3	R DLPFC, R ACC	–	No	CARDIAC

* Type uncertain.

Abbreviations: ACC, anterior cingulate cortex; BZD, benzodiazepine; BW, brain weight; Ctrl, control; DLPFC, dorsolateral prefrontal cortex; F, female; GSW, gunshot wound; L, left; MAOI, MAO inhibitor; MDD, major disorder depression; M, male; MVA, motor vehicle accidents; OD, overdose; PMD, postmortem delay; R, right; RIN, RNA integrity number; SARI, Serotonin antagonist and reuptake inhibitors; SMRI, Stanley Medical Research Institute; SNRI, Serotonin-norepinephrine reuptake inhibitors; SSRI, selective serotonin reuptake inhibitor; SUIC, suicide; TCA, tricyclic antidepressant.

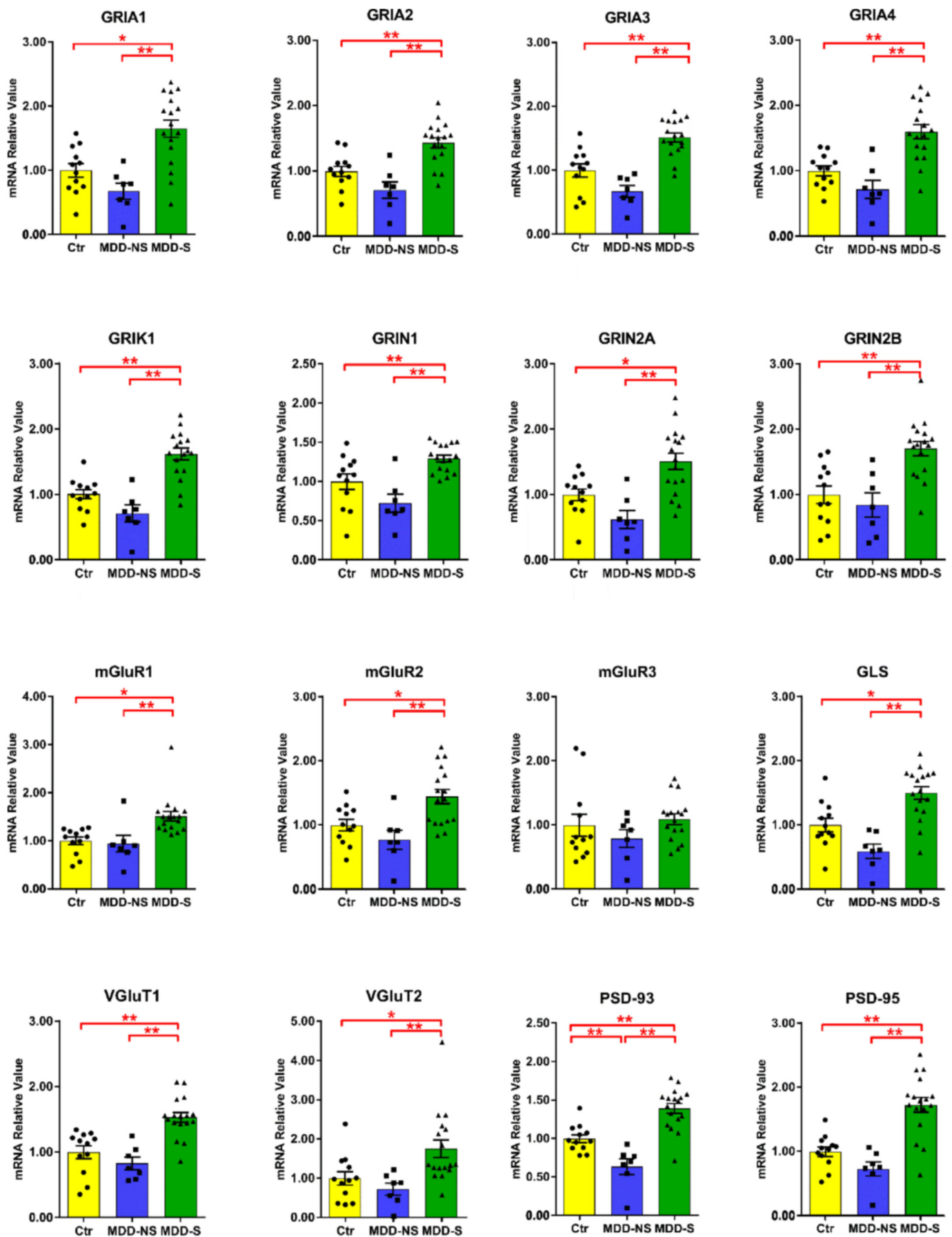
psychotropic medications or a history of substance abuse, were provided by the SMRI. General patient information is given in Table 1 and detailed information in Table 2. The SMRI provided us with RNA from isolated grey matter of the respective brain regions. All analyses were performed by investigators who were unaware of the diagnosis.

2.2. Quantitative real-time PCR

RNA isolation and cDNA synthesis were performed as described before (Wang et al., 2008; Zhao et al., 2012). 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 and the control group: 7.73 ± 0.95 , mean \pm SEM). For cDNA synthesis, an equal quantity of RNA (300 ng) for each sample was used for the synthesis of cDNA, mixed with 4.1 μ l mixture of oligo dT ($100 \mu\text{g ml}^{-1}$) and 10 x hexanucleotide (Roche, Basel, Switzerland) (40:1 for the oligodT and hexanucleotide mixture), heated to 80 °C for 10 min, after which the tubes were quickly transferred to ice. Then 1 μ l reverse transcriptase Superscript II RT (Invitrogen Life Technologies) was added together with a mixture of 5 μ l 5 first-strand buffer, 2.5 μ l 100 mM dithiothreitol, 1.5 μ l 10 mM dNTPs and 0.5 μ l RNase inhibitor. The synthesis 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 Supplementary

Information (SI Table 1 and our previous studies (Zhao et al., 2015, 2016). cDNA template (equivalent to 5 ng of total RNA) was amplified in a final volume of 20 μ l using a SYBR Green PCR master mix (Applied Biosystems, CA, USA) and a mixture of forward and reverse primers (each 2 pmol/ μ l) as described before (Wang et al., 2008; Zhao et al., 2012).

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 Q-PCR 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 on the proportion of explained variability regarding all target genes. A higher stability of a reference gene means that its variance is relatively lower compared with the other considered reference genes. Further, if the expression of a reference gene well reflects the variability of the samples it is expected that its application should reduce the overall variability of the target genes. Briefly: an initial selection of 6 possible reference genes was made based on the gene expression data and reference gene data from the literature. For the present study the most stable reference genes that also explained a considerable proportion of the target gene variability appeared to be actin-beta, tubulin-alpha, glyceraldehyde-3-phosphate



(caption on next page)

Fig. 1. Glutamate related genes in SMRI patients.

Fig. 1A is from the ACC of the SMRI patient cohort and Fig. 1B is from the DLPFC of the SMRI patient cohort. Transcript levels were expressed as fold-changes versus the control mean, and all P -values correspond to two-sided tests. Values of $P \leq 0.05$ were considered significant. Abbreviations: ACC, anterior cingulate cortex; Ctr, control; DLPFC, dorsolateral prefrontal cortex; MDD-S, major depressed patients that committed suicide; MDD-NS, major depressed patients that died from causes other than suicide. * indicates $P < 0.05$, ** indicates $P < 0.001$. For explanation of abbreviations of genes see [SI Table 1](#).

dehydrogenase.

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). Differences in clock time of death and month of death (circular parameters) between controls and patients with mood disorders were tested with the Mardia-Watson-Wheeler test. The gene expression values were 10 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 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. To avoid misinterpretation of our results because human postmortem data may not sufficiently conform to usually assumed theoretical distributions of test statistics, we applied a non-parametric resampling procedure (Zhao et al., 2016). Resampling was performed without replacement in two-group comparisons, thereby providing an alternative for the t -test. We generated 999 replicates consisting of randomly reallocated patients over the two groups in each comparison to obtain a permutation NULL distribution of the test statistic. The obtained NULL distribution mimics the hypothesis that the patients from either group actually belong to one and the same group (Davison and Hinkley, 1997). As test statistic, we used $T = (\text{mean}(\text{group}2) - \text{mean}(\text{group}1))/\text{se}$. 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 actually 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 999 permutation T -values together with the real observed T -value constitute a sample of 1000 “resampled observations”, that can be used to estimate how unusual the observed T -value is (Davison and Hinkley, 1997). Of course, the observed T -value may be on either side of the mean, therefore twice the minimal p -value was used to obtain a two-sided test (Zhao et al., 2016). P -values were corrected for multiple testing using the Benjamini-Hochberg criterion (Benjamini and Hochberg, 1995) and were considered statistically significant if their value was less than 0.05. 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).

3. Results

3.1. Transcription of genes encoding glutamatergic synapse markers

As an indication for changes in excitatory synaptic transmission in the PFC of MDD patients, we analysed Q-PCR values of 16 gene transcripts that encode proteins characteristic for glutamatergic synapses. The glutamatergic markers include genes encoding: AMPA-receptor subunits GRIA1, GRIA2, GRIA3, GRIA4; kainate-receptor subunit GRIK1; NMDA-receptor subunits GRIN1, GRIN2A, GRIN2B; metabotropic glutamate receptors GRM1 (mGluR1), GRM2 (mGluR2), GRM3 (mGluR3); glutaminase (GLS); vesicular glutamate transporters VGLUT1, VGLUT2; and membrane associated guanylate kinase (MAGUK) scaffolding proteins DLG2 (PSD-93) and DLG4 (PSD-95).

In the ACC of MDD-S patients, the gene expression of all of these

markers except mGluR3 was significantly increased (Fig. 1A, Table 3 and Table 4). In contrast, MDD-NS patients did not essentially differ from controls with respect to the genes studied: while there is a general trend for reduction in the MDD-NS group, which in most cases did not reach significance (except for PSD-93, $P = 0.008$). For all glutamatergic markers we tested (except mGluR3) expression levels were significantly higher in MDD-S versus MDD-NS patients (Fig. 1A, Tables 3 and 4).

For the DLPFC, a different picture emerged (Fig. 1B). Not only did we find a significant elevation in the levels of glutamatergic markers between the MDD-S patients and control subjects, but also between MDD-NS patients and control subjects. For all genes tested, the levels in MDD-S are either similar or smaller relative to MDD-NS patients (For more details, see Fig. 1, Tables 3 and 4). These findings indicate that the expression of genes involved in glutamatergic transmission is predominantly changed in the DLPFC of MDD patients irrespectively of whether or not they committed suicide, whereas in the ACC, the changes in gene expression critically depended on suicide, but not on MDD.

3.2. Transcription of genes encoding GABAergic synapse markers

To assess whether MDD and suicide also lead to gene expression changes of proteins characteristic for inhibitory synapses, we also tested the expression levels of a selection of 16 GABAergic markers. As GABAergic markers we used: GABA type A receptor subunits GABRA1, GABRA2, GABRA3, GABRA4, GABRA5, GABRB1, GABRB2, GABRB3, GABRD, GABRE, GABRG2, GABRQ; GABA type B receptor subunits GABBR1 and GABBR2; and GABA producing enzymes GAD65 and GAD67.

Similar to glutamate-related genes, several of these GABA-related genes were significantly increased in the ACC of MDD-S patients compared to MDD-NS patients, and/or to control subjects (Fig. 2A, and Tables 3 and 4). There was a general trend for a reduction in GABA-related genes in the MDD-NS group compared to controls, which in most of cases failed to reach significance (except for GABBR1, $P = 0.043$ and GAD65, $P = 0.038$). GABRA3 and GABRD were not altered in depression per se, but showed a markedly higher expression in MDD-S patients. (For more details, see Fig. 2A, Tables 3 and 4).

In the DLPFC, gene expression was increased in the MDD-S and MDD-NS group when compared to control subjects respectively, except for GABRA5 and GABRQ. It should also be noted that GABRA3, GABRA4, GABRD, GABRE were increased only in the MDD-NS patients compared to controls ($P = 0.007$, $P = 0.026$, $P = 0.007$ and $P = 0.007$, respectively) (For more details, see Fig. 2B, Tables 3 and 4). These findings show that the expression of genes that encode proteins involved in GABAergic transmission was predominantly increased in the ACC of MDD-S patients, as well as in the DLPFC of both MDD-S and MDD-NS patients.

3.3. Overall gene expression changes of glutamatergic and GABAergic synapse markers

Table 3 and Fig. 3 shows the general patterns that can be discerned in the expression of genes examined in this study. For MDD-S patients, both glutamatergic and GABAergic synaptic markers increased on average ~ 1.5 -fold in ACC as well as DLPFC. Notably, for MDD-NS patients a strikingly different pattern became evident. In comparison with control subjects, gene expression of both glutamatergic and GABAergic synaptic markers showed $\sim 30\%$ lower values in the ACC of MDD-NS patients instead of higher values as seen in MDD-S patients

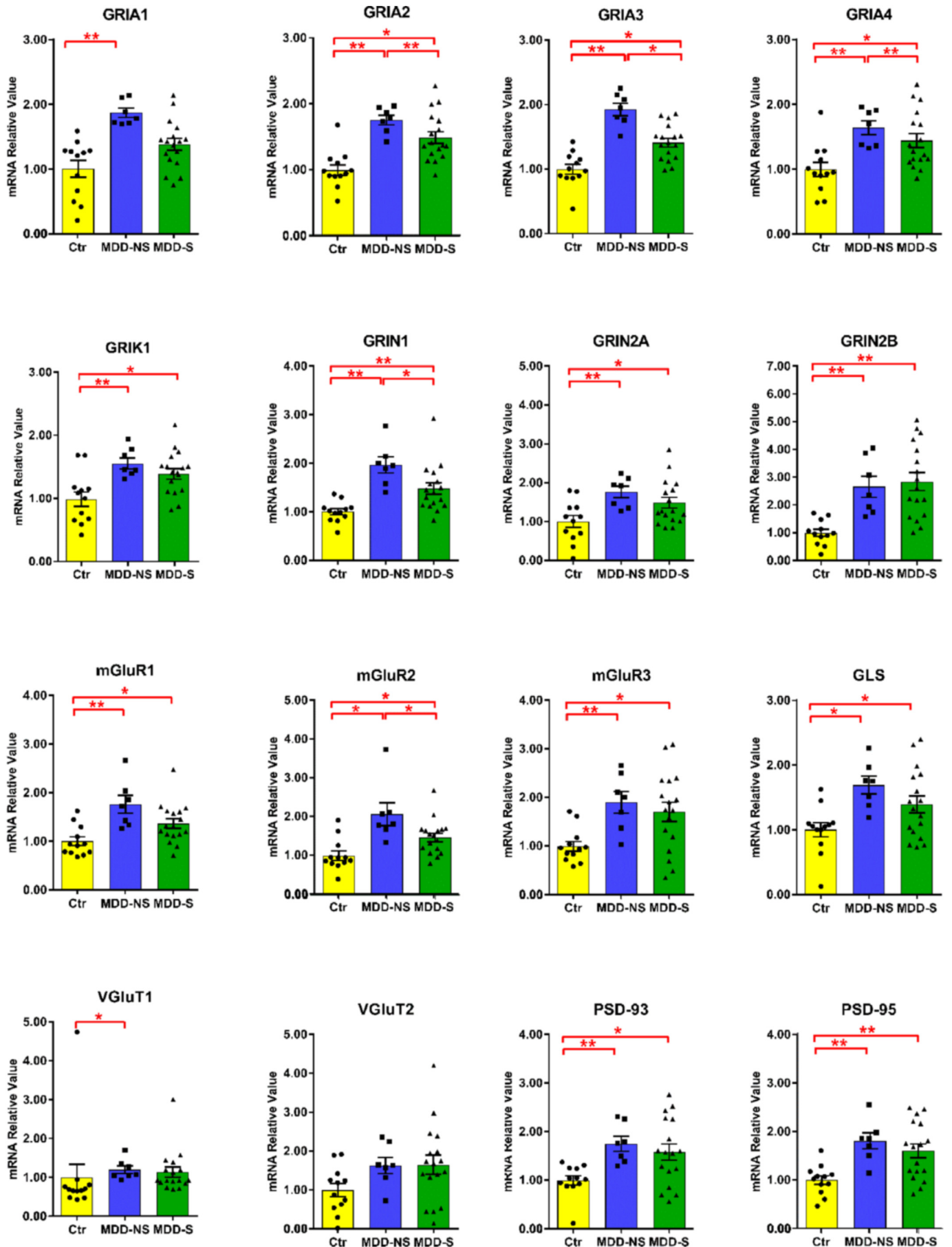


Fig. 1. (continued)

Table 3
Significant changes of target genes in SMRI subjects.

	ACC			DLPFC		
	MDD-S/ MDD-NS	MDD-S/ Ctr	MDD- NS/Ctr	MDD-S/ MDD-NS	MDD-S/ Ctr	MDD-NS/ Ctr
Glutamate related genes						
GRIA1	↑	↑	=	=	=	↑
GRIA2	↑	↑	=	↓	↑	↑
GRIA3	↑	↑	=	↓	↑	↑
GRIA4	↑	↑	=	↓	↑	↑
GRIK1	↑	↑	=	=	↑	↑
GRIN1	↑	↑	=	↓	↑	↑
GRIN2A	↑	↑	=	=	↑	↑
GRIN2B	↑	↑	=	=	↑	↑
mGluR1	↑	↑	=	=	↑	↑
mGluR2	↑	↑	=	↓	↑	↑
mGluR3	=	=	=	=	↑	↑
GLS	↑	↑	=	=	↑	↑
VGluT1	↑	↑	=	=	=	↑
VGluT2	↑	↑	=	=	=	=
PSD-93	↑	↑	↓	=	↑	↑
PSD-95	↑	↑	=	=	↑	↑
GABA related genes						
GABRA1	↑	↑	=	=	↑	↑
GABRA2	↑	↑	=	↓	↑	↑
GABRA3	↑	=	=	↓	=	↑
GABRA4	↑	↑	=	=	=	↑
GABRA5	↑	↑	=	=	=	=
GABRB1	↑	↑	=	↓	=	↑
GABRB2	↑	↑	=	=	↑	↑
GABRB3	↑	↑	=	↓	↑	↑
GABRD	↑	=	=	=	=	↑
GABRE	=	↑	=	=	=	↑
GABRG2	↑	↑	=	↓	↑	↑
GABRQ	↑	↑	=	=	=	=
GABRR1	↑	↑	↓	↓	↑	↑
GABRR2	↑	↑	=	↓	↑	↑
GAD65	↑	↑	↓	=	↑	↑
GAD67	↑	↑	=	=	↑	↑

The differences among MDD-S, MDD-NS and Ctr in the SMRI were tested with permutation tests respectively. The permutation *P*-values were corrected for multiple testing using the false discovery rate. *P* < 0.05 were considered significant. ↑ indicates significant increase; ↓ indicates significant decrease; = indicates no significant difference. For more detailed information on the result see Table 4.

Abbreviations: ACC, anterior cingulate cortex; Ctr, control; DLPFC, dorsolateral prefrontal cortex; MDD-S, major depressive disorder who committed suicide; MDD-NS, major depressive disorder who died of non-suicidal cause; For explanation of abbreviations of genes see SI Table 1.

Table 4
Results of expression of target genes between the diagnostic groups and their matched control group.

	SMRI cohort								
	Fold changes			p.perm			Bhadj.p		
	MDD-S/MDD-NS	MDD-S/Ctr	MDD-NS/Ctr	MDD-S/MDD-NS	MDD-S/Ctr	MDD-NS/Ctr	MDD-S/MDD-NS	MDD-S/Ctr	MDD-NS/Ctr
ACC-Glutamate related genes									
GRIA1	2.7	1.7	0.6	0.002**	0.006**	0.096	0.005**	0.012*	0.120
GRIA2	2.2	1.5	0.7	0.002**	0.004**	0.072	0.005**	0.008**	0.095
GRIA3	2.4	1.6	0.7	0.002**	0.002**	0.068	0.005**	0.005**	0.091
GRIA4	2.4	1.6	0.7	0.002**	0.002**	0.060	0.005**	0.005**	0.082
GRIK1	2.6	1.6	0.6	0.002**	0.002**	0.044*	0.005**	0.005**	0.065
GRIN1	1.9	1.4	0.7	0.002**	0.004**	0.138	0.005**	0.008**	0.164
GRIN2A	2.8	1.5	0.6	0.004**	0.008**	0.044*	0.008**	0.015**	0.065
GRIN2B	2.3	1.9	0.8	0.002**	0.002**	0.466	0.005**	0.005**	0.486
mGluR1	1.7	1.5	0.9	0.002**	0.002**	0.556	0.005**	0.005**	0.568
mGluR2	2.1	1.5	0.7	0.004**	0.012*	0.186	0.008**	0.021*	0.210
mGluR3	1.6	1.2	0.8	0.054	0.308	0.414	0.077	0.336	0.437
GLS	2.9	1.5	0.5	0.002**	0.006**	0.034*	0.005**	0.012*	0.052

(continued on next page)

(Tables 3 and 4). As a result, the mutual difference between MDD-NS and MDD-S patients was 2.2-fold (*P* < 0.0001), i.e. larger than their separate differences with the controls. These results indicate that gene expression changes of synaptic proteins are in opposite direction in the ACC of MDD patients dependent on whether they had committed suicide or not. In the DLPFC (Figs. 1B and 2B, Tables 3 and 4), both MDD-NS and MDD-S patients showed on average increased gene expression as compared with controls. However, this increase was on average 20% higher in the DLPFC of MDD-NS patients compared with MDD-S patients (*P* < 0.0001; Fig. 3).

3.4. Potential confounders

We checked the matching of age, gender, brain pH, brain weight, PMD, ethnicity, history of substance abuse, severity of substance abuse or psychotic feature over our experimental groups (Table 1), and found that these factors will not have affected our conclusions. Although some of the group sizes were rather small, they were thus well-matched for possible confounders. However, a recent study concerning the DLPFC in relation with depression reported that gender may affect the expression of glutamate related genes (Gray et al., 2015). With respect to the comparison of MDD-S and MDD-NS Gray et al. observed that GRIN2B and GRM2 were higher expressed in the DLPFC of male MDD-S patients (Gray et al., 2015). We found that female MDD-S had a lower expression of GRIA3 as compared with female MDD-NS in the DLPFC, and a higher expression of mGluR1 in female MDD-S as compared with female MDD-NS in the ACC. The disparity between our results and those obtained by Gray et al. suggests that it would be very important to dedicate future gene expression studies to the separate analysis of females and males in the context of depression to shed more light on this matter. It was also brought to our attention that regardless of appropriate matching of PMD, brain pH and RIN, these factors may explain some of the remaining variability of expression of the studied genes. Supplementary Information section A shows that our conclusions are not affected by this, but it might be worthwhile to address their influence on gene expression in more detailed future investigations.

4. Discussion

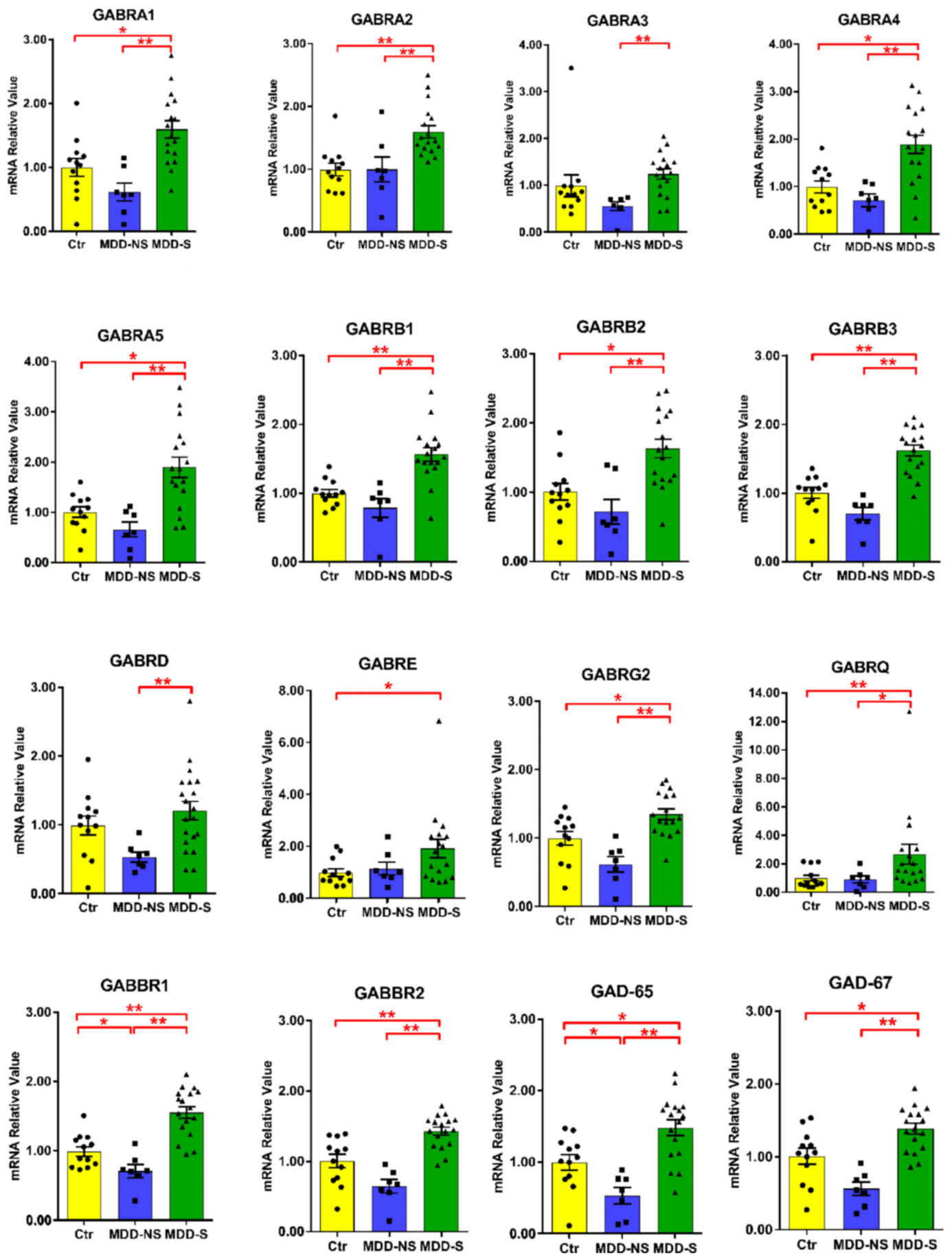
Several studies have suggested an increased expression of genes involved in synaptic transmission in the PFC of MDD patients (Choudary et al., 2005; Sequeira et al., 2007, 2009). However, since a large proportion of the MDD patients in these studies were suicide victims, it was unclear whether these gene changes were due to MDD or related to suicide. In brain material of the well-characterized SMRI

Table 4 (continued)

SMRI cohort									
Fold changes			p.perm			Bhadj.p			
	MDD-S/MDD-NS	MDD-S/Ctr	MDD-NS/Ctr	MDD-S/MDD-NS	MDD-S/Ctr	MDD-NS/Ctr	MDD-S/MDD-NS	MDD-S/Ctr	MDD-NS/Ctr
VGluT1	1.9	1.6	0.9	0.002**	0.002**	0.316	0.005**	0.005**	0.341
VGluT2	3.2	1.9	0.6	0.002**	0.010*	0.328	0.005**	0.018*	0.350
PSD93	2.5	1.4	0.6	0.002**	0.002**	0.004**	0.005**	0.005**	0.008**
PSD95	2.6	1.7	0.7	0.002**	0.002**	0.074	0.005**	0.005**	0.096
ACC-GABA related genes									
GABRA1	3.0	1.8	0.6	0.002**	0.012*	0.150	0.005**	0.021*	0.173
GABRA2	1.8	1.6	0.9	0.002**	0.002**	0.680	0.005**	0.005**	0.680
GABRA3	2.8	1.4	0.5	0.004**		0.100	0.008**		0.123
GABRA4	3.2	1.9	0.6	0.002**	0.006**	0.208	0.005**	0.012*	0.232
GABRA5	3.4	1.9	0.5	0.002**	0.006**	0.090	0.005**	0.012*	0.114
GABRB1	2.4	1.5	0.6	0.002**	0.002**	0.124	0.005**	0.005**	0.149
GABRB2	2.7	1.7	0.6	0.004**	0.010*	0.154	0.008**	0.018*	0.176
GABRB3	2.4	1.7	0.7	0.002**	0.002**	0.066	0.005**	0.005**	0.089
GABRD	2.3	1.4	0.6	0.004**	0.214	0.078	0.008**	0.236	0.100
GABRE	1.5	1.7	1.1	0.124	0.014*	0.624	0.149	0.023*	0.631
GABRG2	2.5	1.4	0.6	0.002**	0.014*	0.054	0.005**	0.023*	0.077
GABRQ	3.2	2.4	0.7	0.014*	0.004**	0.544	0.023*	0.008**	0.562
GABBR1	2.3	1.6	0.7	0.002**	0.002**	0.028*	0.005**	0.005**	0.043*
GABBR2	2.5	1.5	0.6	0.002**	0.004**	0.056	0.005**	0.008**	0.079
GAD65	2.7	1.5	0.6	0.002**	0.012*	0.024*	0.005**	0.021*	0.038*
GAD67	3.3	1.6	0.5	0.002**	0.016*	0.058	0.005**	0.026*	0.081
DLPFC-Glutamate related genes									
GRIA1	0.7	1.5	2.1	0.094	0.052	0.002**	0.125	0.075	0.007**
GRIA2	0.8	1.5	1.8	0.002**	0.016*	0.002**	0.007**	0.028*	0.007**
GRIA3	0.7	1.5	2.0	0.012*	0.004**	0.002**	0.023*	0.010*	0.007**
GRIA4	0.9	1.5	1.7	0.002**	0.004**	0.002**	0.007**	0.010*	0.007**
GRIK1	0.9	1.5	1.7	0.154	0.006**	0.002**	0.187	0.013*	0.007**
GRIN1	0.7	1.4	2.0	0.020*	0.002**	0.002**	0.034*	0.007**	0.007**
GRIN2A	0.8	1.8	2.2	0.106	0.012*	0.002**	0.139	0.023*	0.007**
GRIN2B	1.0	2.9	2.8	0.924	0.002**	0.002**	0.934	0.007**	0.007**
mGluR1	0.8	1.4	1.8	0.058	0.016*	0.004**	0.082	0.028*	0.010*
mGluR2	0.7	1.5	2.1	0.026*	0.006**	0.004**	0.042*	0.013*	0.010*
mGluR3	0.8	1.6	1.9	0.422	0.032*	0.002**	0.450	0.049*	0.007**
GLS	0.8	1.5	1.9	0.174	0.004**	0.004**	0.209	0.010*	0.010*
VGluT1	0.9	1.4	1.6	0.320	0.140	0.028*	0.361	0.177	0.043*
VGluT2	0.8	1.9	2.3	0.598	0.150	0.040*	0.624	0.185	0.059
PSD93	0.8	1.6	1.9	0.316	0.028*	0.002**	0.361	0.043*	0.007**
PSD95	0.9	1.6	1.8	0.296	0.002**	0.002**	0.342	0.007**	0.007**
DLPFC-GABA related genes									
GABRA1	0.9	1.6	1.7	0.678	0.010*	0.004**	0.692	0.021*	0.010*
GABRA2	0.7	1.4	2.2	0.004**	0.018*	0.006**	0.010*	0.031*	0.013*
GABRA3	0.7	1.5	2.1	0.026*	0.046*	0.002**	0.042*	0.067	0.007**
GABRA4	0.7	1.5	2.1	0.146	0.224	0.014*	0.182	0.265	0.026*
GABRA5	0.8	1.6	1.9	0.384	0.110	0.040*	0.419	0.143	0.059
GABRB1	0.7	1.5	2.3	0.012*	0.066	0.004**	0.023*	0.091	0.010*
GABRB2	0.8	1.8	2.1	0.468	0.006**	0.002**	0.494	0.013*	0.007**
GABRB3	0.8	1.4	1.7	0.022*	0.002**	0.002**	0.036*	0.007**	0.007**
GABRD	0.7	1.4	2.0	0.126	0.074	0.002**	0.161	0.100	0.007**
GABRE	0.8	1.7	2.1	0.396	0.062	0.018*	0.427	0.086	0.031*
GABRG2	0.8	1.4	1.9	0.006**	0.006**	0.002**	0.013*	0.013*	0.007**
GABRQ	1.0	1.3	1.3	0.964	0.380	0.268	0.964	0.419	0.314
GABBR1	0.8	1.4	1.8	0.004**	0.002**	0.002**	0.010*	0.007**	0.007**
GABBR2	0.7	1.4	1.9	0.002**	0.012*	0.002**	0.007**	0.023*	0.007**
GAD65	0.9	1.5	1.6	0.340	0.004**	0.006**	0.380	0.010*	0.013*
GAD67	0.9	1.5	1.6	0.608	0.012*	0.006**	0.628	0.023*	0.013*

The differences among MDD-S, MDD-NS and Ctr in the SMRI were tested with permutation tests respectively. The permutation P-values were corrected for multiple testing using the false discovery rate. P < 0.05 were considered significant and marked as bold Italics, * indicates P < 0.05, ** indicates P < 0.001. Fold changes were calculated using the mean gene expression values.

Abbreviations: ACC, anterior cingulate cortex; Bhadj.p, Benjamini-Hochberg adjusted p-values; Ctr, control; DLPFC, dorsolateral prefrontal cortex; MDD-S, major depressive disorder who committed suicide; MDD-NS, major depressive disorder who died of non-suicidal cause; p.perm, p-values obtained using permutation tests; For explanation of abbreviations of genes see SI Table 1.



(caption on next page)

Fig. 2. GABA related genes in SMRI patients.

Fig. 2A is from the ACC of the SMRI patient cohort and Fig. 2B is from the DLPFC of the SMRI patient cohort. Transcript levels were expressed as fold-changes versus the control mean, and all *P*-values correspond to two-sided tests. Values of $P \leq 0.05$ were considered significant. Abbreviations: ACC, anterior cingulate cortex; Ctr, control; DLPFC, dorsolateral prefrontal cortex; MDD-S, major depressed patients that committed suicide; MDD-NS, major depressed patients that died from causes other than suicide. * indicates $P < 0.05$, ** indicates $P < 0.001$. For explanation of abbreviations of genes see [SI Table 1](#).

cohort, we found the expression of both glutamate- and GABA-related genes to be increased in the ACC of MDD-S patients, as compared to MDD-NS patients or to non-psychiatric control subjects. In contrast, both GABA and glutamate-related genes were lower in the MDD-NS patients as compared to control subjects, confirming our earlier study (Zhao et al., 2012). Moreover, in the DLPFC, we found the expression of both glutamate- and GABA-related genes to be decreased in the MDD-S patients compared to MDD-NS patients. There was an increased expression in GABA and glutamate related genes when MDD-S patients and MDD-NS were compared to non-psychiatric control subjects. Together, our findings indicate that increases in glutamatergic and GABAergic neurotransmission in the ACC is not related to MDD, but rather to suicide, and MDD is associated with increases in GABA and glutamate related genes in the DLPFC, irrespective of suicide.

By using the HG-U133AB chipset on 17 cortical and subcortical brain regions, earlier studies also suggested that in particular glutamatergic and GABAergic gene expression patterns may be associated with suicidality (Sequeira et al., 2007, 2009, 2012; Turecki, 2014). The highest number of suicide-specific alterations were found in several PFC subareas and the hippocampus (Sequeira et al., 2009). Our current Q-PCR data on the Stanley Foundation cohort confirms that the GABA and glutamate system are altered in the PFC after suicide, and further suggest that these gene expression changes may have a major impact on ACC functioning in MDD-S patients specifically.

Our findings show that expression of almost all glutamate receptor subunits was increased in MDD-S patients. This is in line with a microarray study that observed a global up-regulation of AMPA-receptors in suicide victims who suffered from MDD as compared to non-psychiatric control subjects or suicide victims without a history of MDD, although other psychiatric diagnoses were present in the latter group of patients (Sequeira et al., 2009). This notion on AMPA-receptors is further supported by our observation that suicide victims have an increased gene expression of PSD-93 and PSD-95. These membrane associate guanylate kinases (MAGUK) scaffolding proteins are required for synapse stabilization and their expression levels directly influence the average synapse strength or synapse quantity (Keith and El-Husseini, 2008). Because the AMPA-, NMDA-, kainate-receptor, mGluR subunits and MAGUKs were all significantly increased in the ACC of depressed patients who committed suicide, it is possible that, on average, ACC neurons in suicide victims have stronger or more excitatory synapses. Alternatively, ACC neurons of MDD-S patients suffer from a loss of glutamatergic synapses, similar as DLPFC neurons (Kang et al., 2012), for which these neurons try to compensate by increasing the production of glutamate receptor subunits and MAGUKs. Recently, Gray and colleagues (Gray et al., 2015) have detected differences in the expression of glutamate receptor genes in DLPFC of a mixed group of MDD subjects (suicide victims and non-suicide victims), and similarly as we, found that the most of the genes showed higher levels of expression in MDD subjects as compared with non-psychiatric control subjects. Given the evidence that AMPA receptor potentiators possess antidepressant-like properties (Neis et al., 2016; Nishimura et al., 2005), our findings of abnormal AMPA receptor expression regulation in MDD suggests that this may reflect a compensatory mechanism.

The altered gene expression in suicide may also influence behavioural functioning of the patients. For example, if the PFC of a depressed patient is activated while mood is still low, this could diminish the inhibition of impulsive behaviour and lead to an increased risk to commit suicide. Increased activation together with low mood may also be the basis of the increased risk of suicidal behaviour reported among

children and adolescents who start with SSRI treatment (Barbui et al., 2009), and in studies that show a seasonal suicide peak when daylight starts to increase in spring (Woo et al., 2012). In agreement with this possibility a recent fMRI study showed altered frontal-based brain function (e.g., decreased ACC functional connectivity with ventrolateral PFC/DLPFC) that directly relates to past suicidal behaviour in schizophrenia (Minzenberg et al., 2016).

Earlier studies have shown alterations in molecular components of the GABAergic system in relation to possible mechanisms that may underlie suicide (Choudary et al., 2005; Klempan et al., 2009; Lee et al., 2009; Merali et al., 2004; Sequeira et al., 2009). In support, we also found an overall increase in the expression of genes encoding GABAergic proteins in both ACC and DLPFC of suicide victims. Merali and others showed in their studies a dysregulation of GABA-A receptor subunits, and the GABA-A receptor promoter was found to be hypermethylated in the frontal cortex of suicide victims compared to control subjects (Merali et al., 2004; Poulter et al., 2008, 2010). It has been shown that several GABA-A receptor subunits were shown to be increased in depressed individuals after accomplished suicide. For heteromeric proteins, like GABA-A receptors, which have highly variable functions, it may be particularly important to have the correct balance of receptor subunits (Nishimura et al., 2005). In that respect, the increased GABRA4 mRNA level that we found, is interesting as it might play a central role in tonic inhibition as opposed to phasic inhibition, the altered ratio between GABRA4 and rest of GABA subunits suggesting that the balance between these two modes of inhibition may be altered.

We found that mRNA expression of glutamatergic and GABAergic proteins are similarly altered, leaving it unclear whether the overall balance between excitation and inhibition might be disturbed in the suicide patients. Possibly these alterations have started with variations in excitation in cortical regions and were followed by proportional changes in inhibition (Atallah and Scanziani, 2009; Haider et al., 2006; Okun and Lampl, 2008). Our findings support a scenario where suicidal activity is characterized by an overall change in synaptic activity in ACC. Functional magnetic resonance imaging has shown that neuronal activity of the ACC was elevated in depressed patients who did a suicide attempt (Vannoy et al., 2007), while suicide ideation was also associated with an increased error related activation in the ACC (Barbui et al., 2009; Matthews et al., 2012).

In the DLPFC, reductions in GAD65/GAD67 immuno-positive neuropil (Gos et al., 2009) and decreased GAD67 protein level with western blotting (Karolewicz et al., 2010) were reported in subjects with MDD. It should be noted, however, that both studies concerned a mixed group of suicide and non-suicide patients. Neuroimaging studies also reported consistent reductions in GABA in MDD (Sanacora et al., 1999, 2004; Sanacora and Saricicek, 2007; Schur et al., 2016), or in relation to stress (Houtepen et al., 2017). In the present study, we observed altered expression level of the majority of GABA receptor subunits and enzymes in MDD patients in both ACC and DLPFC independent on whether they did or did not commit suicide. It is tempting to speculate that this disrupted GABAergic gene expression will have significantly contributed to the GABA deficits observed by neuroimaging studies in MDD. Notably, gene microarray analysis also observed an upregulation of GABRA1 and GABRB3 in the DLPFC of subjects with depressive symptoms who committed suicide, which is in line with our findings and idea (Choudary et al., 2005; Sequeira et al., 2007).

Medication to treat depression is a potential confounder that is difficult to analyse because some medication may have remained

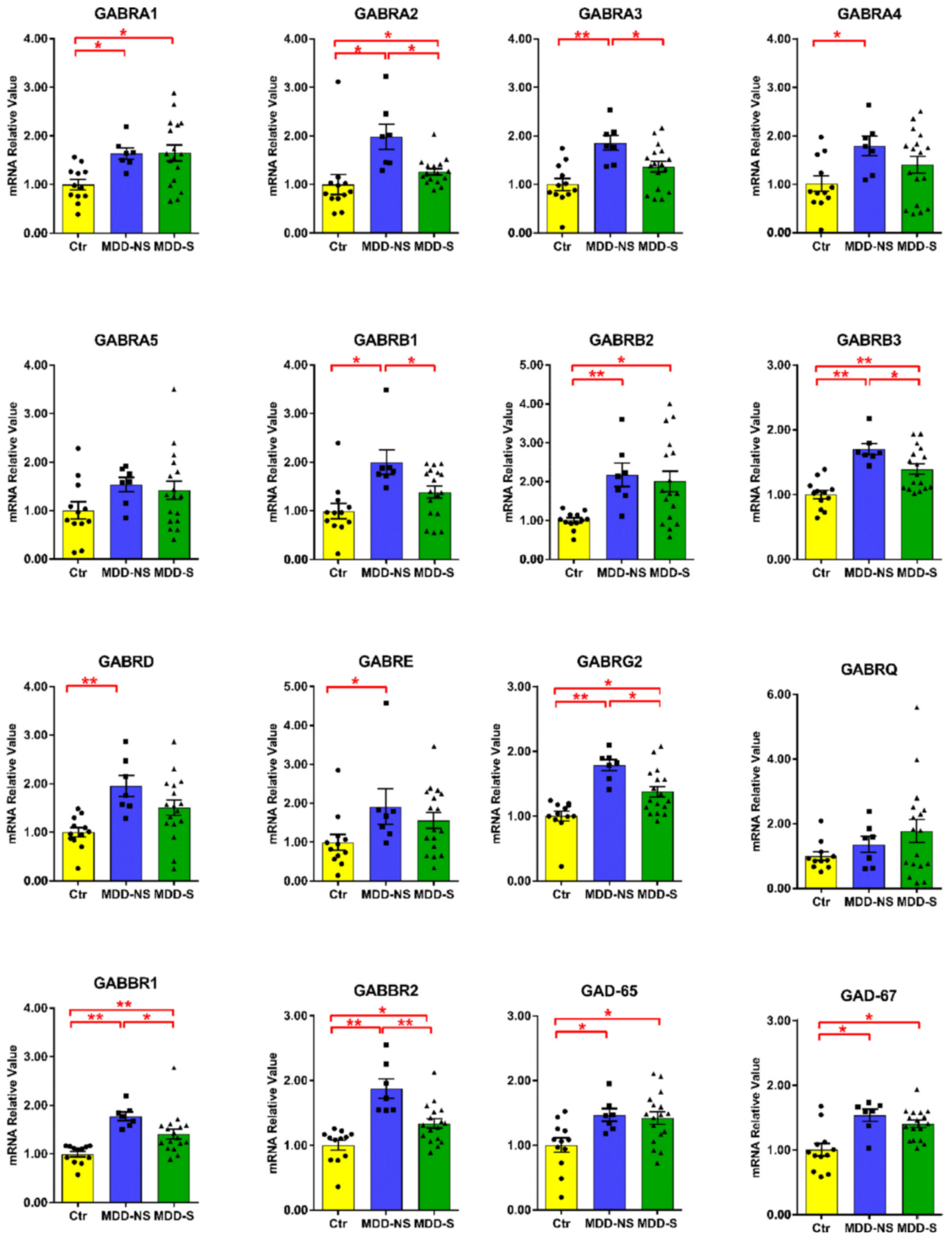


Fig. 2. (continued)

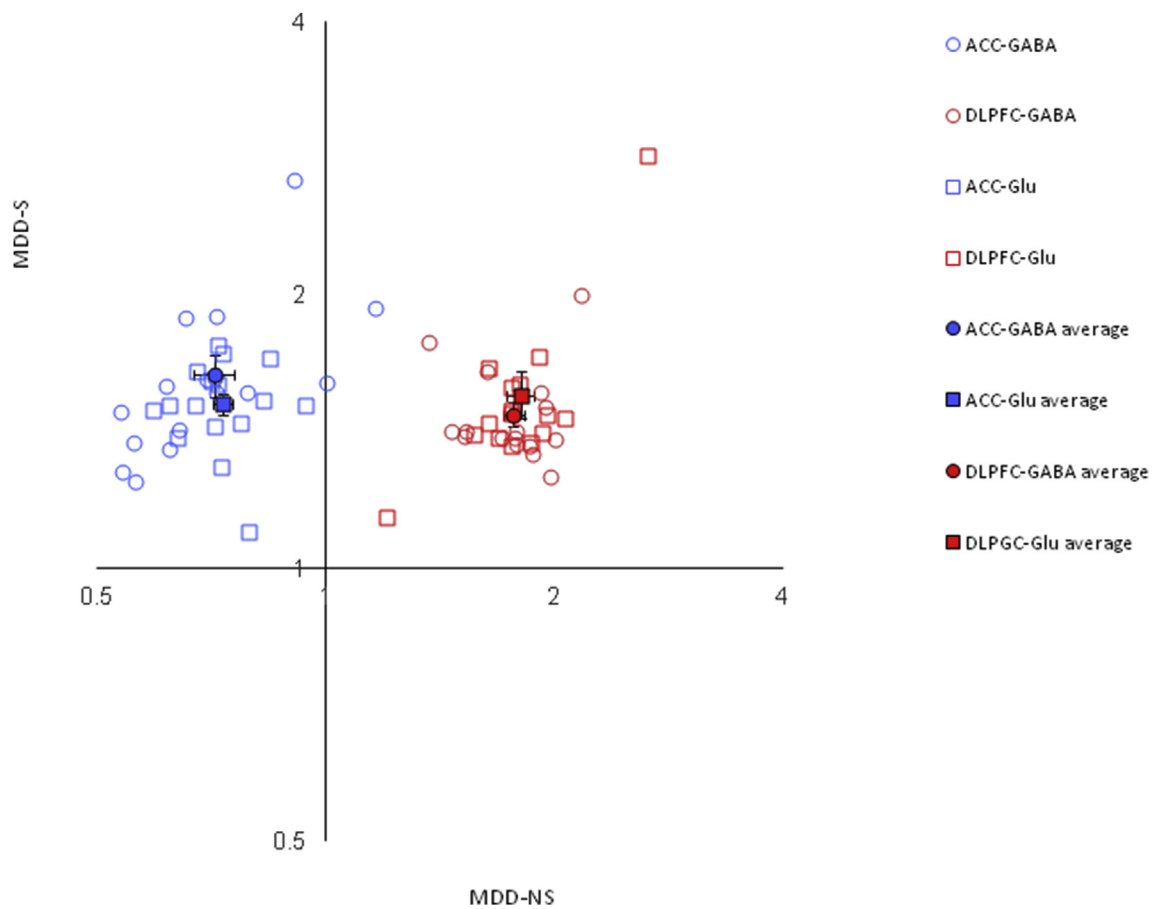


Fig. 3. Plot for different gene expression profiles in ACC and DLPFC MDD-S vs MDD-NS.

Each circle stands for a Glutamate related gene while GABA related genes are represented in squares. Each dot (circle or square) represent a gene with the average expression in MDD-S group (X axis) and MDD-NS (Y axis). ACC is in blue and DLPFC is in red. Note that the gene expression changes of synaptic proteins are in opposite direction in the ACC of MDD patients dependent on whether they had committed suicide or not.

Abbreviations: ACC, anterior cingulate cortex; Ctr, control; DLPFC, dorsolateral prefrontal cortex; MDD-S, major depressed patients that committed suicide; MDD-NS, major depressed patients that died from causes other than suicide.

unreported. Below, we briefly address the medication data in relation to our results. If we ignore possibly combined medications, we found that tricyclic antidepressants (TCA, N = 6) were associated with significantly decreased expression of 28 genes in the ACC but not in the DLPFC. Selective serotonin reuptake inhibitors (SSRI, N = 7) showed no association with gene expression in either ACC or DLPFC. Interestingly, a recent animal study also did not find changes of GRIA1 and GRIA3 expression in the cortex of rats treated with the SSRI fluoxetine (Duric et al., 2013). In our cohort, there were only two patients (Number 17 and 31) who had been taking serotonin and norepinephrine reuptake inhibitors (SNRI), therefore, we did not have enough observations to report on such compounds. This also held for serotonin antagonist and reuptake inhibitors (SARI, N = 5). Considering the possible implications for the interpretation of gene expression involved in depression and suicide it will be important to study a very large group of patients whose medication has been well documented.

The suicide-related gene expression changes that trended towards an elevation (Figs. 1A and 2A) appeared to be specific for the ACC; the changes towards a reduction (Figs. 1B and 2B) were mainly observed in the DLPFC. The ACC is a cortical area that, among other things, has been linked to the emotional reaction to pain rather than to the perception of pain itself (Price, 2000). Hence, the intense psychological pain prior to the act of suicide may relate to hyperactivity at ACC synapses. This possibility is supported by a coordinate-based meta-analysis of functional MRI studies (van Heeringen et al., 2014). Further

studies are needed to establish whether selectively suppressing ACC activity by targeting GABA or glutamatergic synapses locally, could be used as a therapeutic approach towards the prevention of suicide. The emergence of intravenous ketamine therapy has been celebrated as perhaps the “the most important break-through in antidepressant treatment in decade” (Insel T, 2014). Studies showed that the antidepressant responses to ketamine started at 3–4 h post-infusion (Aan Het Rot et al., 2012), and this rapid antidepressant effect is also true for patients with treatment-resistant depression (Fekadu et al., 2009; Nemeroff, 2007). Furthermore, ketamine decreased suicidal ideation with treatment-resistant depression within 40 min, and these decreases remained significant through the first 4 h post-infusion which appears that one of the earliest effects of the drug is a profound reduction in suicidal thoughts (DiazGranados et al., 2010). The mechanism through which ketamine infusion produces antidepressant effect remains obscure. A common hypothesis states that it acts by binding NMDA-receptors, thereby preventing signaling processes triggered by the receptor (Adams and Moghaddam, 2001; Moghaddam et al., 1997; Moghaddam and Adams, 1998). A recent study proposed that it is a metabolite of ketamine called hydroxynorketamine (HNK) that has antidepressant activity by increasing the levels of AMPA-receptors at synapses, thereby enhancing neural activity (Zanos et al., 2016). The necessity of AMPA activation implies that ketamine/HNK induces synaptogenesis by increasing glutamate signaling rather than by protecting neurons from glutamate excitotoxicity (Newport et al., 2015). The linkage between NMDA-receptor antagonism and AMPA-receptor

activation is crucial as it might help clarify how ketamine/HNK can be neuroprotective in some contexts (Brunson et al., 2001; Yan and Jiang, 2014) but potentially neurotoxic in others (Yan and Jiang, 2014; Yan et al., 2014; Zuo et al., 2014). This dual potential of ketamine must be considered when contemplating its therapy in the clinical setting.

Conflict of interest

None to declare.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jpsychires.2018.04.020>.

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