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H3K4 tri-methylation in synapsin genes leads to different expression patterns in bipolar disorder and major depression

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Abstract

The synapsin family of neuronal phosphoproteins is composed of three genes (*SYN1*, *SYN2* and *SYN3*) with alternative splicing resulting in a number of variants with various levels of homology. These genes have been postulated to play significant roles in several neuropsychiatric disorders, including bipolar disorder, schizophrenia and epilepsy. Epigenetic regulatory mechanisms, such as histone modifications in gene regulatory regions, have also been proposed to play a role in a number of psychiatric disorders, including bipolar disorder and major depressive disorder. One of the best characterized histone modifications is histone 3 lysine 4 tri-methylation (H3K4me3), an epigenetic mark shown to be highly enriched at transcriptional start sites and associated with active transcription. In the present study we have quantified the expression of transcript variants of the three synapsin genes and investigated their relationship to H3K4me3 promoter enrichment in post-mortem brain samples. We found that histone modification marks were significantly increased in bipolar disorder and major depression and this effect was correlated with significant increases in gene expression. Our findings suggest that synapsin dysregulation in mood disorders is mediated in part by epigenetic regulatory mechanisms.

Keywords

Bipolar disorder; epigenetics; gene expression; H3K4me3; synapsin

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Supplementary material

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Statement of Interest

None.

Introduction

The synapsin family of neuronal phosphoproteins is composed of three genes (*SYN1, SYN2, SYN3*) with alternative splicing giving rise to 10 reported variants expressed at various developmental time-points and in various cell types (Porton *et al.* 1999; Sudhof *et al.* 1989). The genes are involved in synaptogenesis, synaptic transmission and synaptic plasticity (Corradi *et al.* 2008). Of the three synapsin genes, *SYN1* and *SYN2* are predominantly expressed by mature neurons, where they have been shown to associate with the cytoplasmic surface of synaptic vesicles and to represent >6% of their protein content (Cesca *et al.* 2010; Ferreira *et al.* 2000; Grafodatskaya *et al.* 2010; Huttner *et al.* 1983; Takamori *et al.* 2006). *SYN1* maps to chromosome Xp11.23 and has two known variants, *SYN1a* and *SYN1b* (Cesca *et al.* 2010; Kile *et al.* 2010) and *SYN2* maps to chromosome 3p25 and has two known variants, *SYN2a* and *SYN2b* (Cesca *et al.* 2010). Both *SYN1* and *SYN2* are differentially expressed in nerve terminals in the majority of the adult brain with demonstrated homology across numerous vertebrate and invertebrate organisms (Cesca *et al.* 2010; Grafodatskaya *et al.* 2010; Kile *et al.* 2010). *SYN3* maps to chromosome 22q12.3 and has been shown to produce up to six variants, although not all are expressed in the adult brain (Grafodatskaya *et al.* 2010; Porton *et al.* 1999). Its expression is much lower than that of *SYN1* or *SYN2* (Kao *et al.* 1998). The full-length *SYN1a* and *SYN1b* protein (isoform 3a) exhibits protein homology with the other two synapsins and consequently possible functional homology as well, while the other variants have been shown to have developmentally specific-expression and the majority to be limited to foetal neuron expression (Grafodatskaya *et al.* 2010; Porton *et al.* 1999). The only other *SYN3* variant that shows adult expression in the human brain is *SYN3g*. The function of the *SYN3* variants is not as well understood as that of *SYN1* or *SYN2*, but it has been suggested to be mainly localized to regions outside of the synapse in the adult brain and function in neurogenesis and synaptic plasticity (Corradi *et al.* 2008). The majority of brain regions jointly express synapsin variants at similar levels, suggesting that they are functionally complementary (Ullrich & Sudhof, 1995). However, deleting each of the three synapsin genes produces different phenotypes, indicating that the various gene products must differ in their function to some degree (Feng *et al.* 2002; Gitler *et al.* 2004; Li *et al.* 1995; Rosahl *et al.* 1995).

Synapsin genes have been proposed to play roles in several psychiatric disorders such as schizophrenia, bipolar disorder (BD) and epilepsy (Cesca *et al.* 2010; Fassio *et al.* 2011) in both genetic (Chen *et al.* 2004; Lee *et al.* 2005; Saviouk *et al.* 2007) and functional studies (Browning *et al.* 1993; Grebb & Greengard, 1990; Lopez de Lara *et al.* 2010; Mirnics *et al.* 2000; Nowakowski *et al.* 2002; Schroeder *et al.* 2010; Vawter *et al.* 2002). Given the evidence suggesting differential expression of synapsin genes in association with psychiatric phenotypes, it is interesting to study potential regulatory mechanisms that may underlie these changes. In this study, we set out to investigate epigenetic mechanisms, specifically the role of histone modifications, in explaining differential synapsin expression in BD.

Epigenetic modifications have been investigated in various psychiatric phenotypes, including schizophrenia (Akbarian, 2010*a*, *b*), autism (Grafodatskaya *et al.* 2010), major depression (Schroeder *et al.* 2010) and suicide (Akbarian, 2010*b*). Interestingly, valproate, one of the most commonly used mood stabilizers in BD, is an inhibitor of histone deacetylases (Arent

et al. 2011; Kielland *et al.* 2006; Machado-Vieira *et al.* 2010; Tsankova *et al.* 2007), and thus it is possible that its stabilizing role in the disorder is mediated through inhibition of histone deacetylases. One of the best understood epigenetic mechanisms is histone methylation, particularly the tri-methylation of the fourth lysine tail on histone 3 (H3K4me3; Berger, 2007). This modification has been shown to be most abundant at transcriptional start sites (TSS) of genes and has been associated with increased transcription (Bernstein *et al.* 2005; Santos-Rosa *et al.* 2002; Schneider *et al.* 2004). H3K4me3 functions by opening up the chromatin and allowing transcriptional machinery to bind to the promoter region of genes, thus leading to the initiation of transcription. Enrichment of this mark typically leads to an increase in expression levels (Bannister & Kouzarides, 2011; Kouzarides, 2007; Santos-Rosa *et al.* 2002).

In this study, we analysed expression of *SYN1a*, *SYN1b*, *SYN2a*, *SYN2b*, *SYN3a*, and *SYN3g* (Supplementary Fig. 1) in post-mortem brains from BD patients, focusing on Brodmann Area 10 (BA 10) of the prefrontal cortex (PFC). Our choice to focus on the PFC was based on studies showing its importance in mood regulation as well as documented deficits in PFC-mediated working memory and executive function in BD patients (Blumberg *et al.* 2003; Quraishi & Frangou, 2002; Robinson *et al.* 2008). In addition, imaging studies have shown abnormalities in PFC biochemistry and function in BD patients during manic and depressive episodes, as well as during euthymia, suggesting the possibility of persistent neuropsychological deficits in BD (Malhi *et al.* 2007; Thompson *et al.* 2005). Furthermore, the mediofrontal cortex has been previously linked to mood regulation in BD. A study comparing BD patients with their at-risk but healthy siblings showed regional cerebral blood flow decreases in this region (BA 9/10) in patients but an increase in their siblings, suggesting that this brain region may be involved in BD (Kruger *et al.* 2006).

Since BD is characterized by alternating episodes of depression and mania, and the BD subjects investigated in this study died by suicide during a depressive episode, we included a comparison group of subjects with major depressive disorder (MDD) in order to control for possible effects that may be associated with depressive symptomatology. We compared both groups with a group of matched psychiatrically healthy controls.

Method and materials

Subjects

Post-mortem PFC brain tissue from BA 10 used in this study was obtained from the Quebec Suicide Brain Bank (www.douglasrecherche.qc.ca/suicide) as described elsewhere (Klempan *et al.* 2009; Lopez de Lara *et al.* 2010). Clinical information, toxicology and history of psychoactive prescription drugs were collected for both cases and controls. These data were found to have no influence on our results ; a detailed discussion is presented in Supplementary Methods, Supplementary Tables 1 and 2. All procedures in this study were approved by the ethics review board of our institution. Cases in this study were individuals who had a diagnosis of BD type I or type II (n=13) or MDD (n=18) and died by suicide. Controls were individuals who died suddenly and could not have undergone any resuscitation procedures or other type of medical intervention (n=14). Controls had neither current nor past psychiatric diagnoses. There were no significant group differences in

gender, age, post-mortem delay, pH and RNA integrity numbers (Table 1). We chose to focus on BA 10 as a representative PFC region and extracted total RNA from post-mortem brains.

Gene expression

Total messenger RNA (mRNA) was extracted from frozen brain tissue using the RNeasy Lipid Tissue Mini Kit (Qiagen, The Netherlands). For synthesis of complementary DNA, M-MLV reverse transcriptase (Gibco, USA) and oligo(dT)16 primers (Invitrogen, USA) were used.

Chromatin immunoprecipitation

DNA for chromatin immunoprecipitation (ChIP) was prepared from BA 10 of post-mortem brain tissues (regions adjacent to those selected for mRNA experiments) as described by Matevossian & Akbarian (2008). Briefly, 80 mg tissue was cleaved between adjacent nucleosomes with micrococcal nuclease (Sigma Aldrich, USA). A portion of selected intact nucleosomes was treated with anti-H3K4me3 antibody (Millipore, USA) and purified with protein G agarose beads (Millipore). The remainder was used as input control. Both input and bound fractions were digested with proteinase K before purifying DNA by phenol/chloroform extraction (Fiori & Turecki, 2011; Matevossian & Akbarian, 2008).

Quantitative real-time polymerase chain reaction

Samples were run on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, USA) in quadruplicate using standard quantitative real-time polymerase chain reaction (qRT-PCR) conditions and the TaqMan Fast Master Mix or the Power SYBR*1* Green PCR Master Mix (Applied Biosystems) as applicable. Relative expression for both mRNA and ChIP was calculated using the relative quantitation method (ΔΔ-Ct) with GAPDH as an endogenous control in the RQ Manager 1.2 software. TaqMan assays were used for gene expression (Applied Biosystems). Expression values are presented as relative quantification (RQ) values throughout and they represent $2^{-\Delta\Delta\text{-Ct}}$ metrics in reference to a pooled calibrator sample. For ChIP quantification, ratios of bound:input fractions were calculated for each sample by using custom SYBR Green primers designed (IDT, USA) in the promoter region ~500 bp upstream of the transcription start site. Primer sequences are available upon request.

Data analysis

Test coefficients and probability distributions were calculated using statistical software GraphPad Prism 5 and SPSS. Before any other statistical computation or graphical representation of results, outlier analyses were performed for each dataset. For this reason, select subjects may be missing from analyses on a case-by-case basis. For qRT–PCR experiments, relative quantitation was performed with GAPDH as an endogenous control in the RQ Manager 1.2 software (Applied Biosystems).

Results

SYN1 and *SYN2* have different expression profiles in BD and MDD

Demographic and post-mortem characteristics of the subjects included in the post-mortem expression study are reported in Table 1. As there were no significant differences between groups in these variables, we performed one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* tests to assess the differences in expression between subjects with BD and controls, as well as MDD and controls for the six synapsin variants (*SYN1a*, *SYN1b*, *SYN2a*, *SYN2b*, *SYN3a* and *SYN3g*) that are expressed in the adult human brain and that are structurally and functionally similar (Cesca *et al.* 2010). As shown in Fig. 1*a*, *b*, the *SYN1a* variant was differentially up-regulated in both BD and MDD (ANOVA p=0.0045), while the *SYN1b* variant was only significantly up-regulated in MDD (ANOVA p=0.0172). These results suggest distinct patterns between the two *SYN1* variants.

The result for *SYN2* showed opposing expression patterns for the two variants. As shown in Fig. 1*c*, *d*, *SYN2a* was significantly up-regulated in BD with no effect in MDD (ANOVA p=0.0001), while the converse was true for *SYN2b* (ANOVA p=0.0005). Considering that gene expression changes in the brain are usually subtle, we note that these significant results were accompanied by fairly high fold changes of 2.47 and 1.80 respectively. Furthermore, these differences between BD and MDD are highly significant when comparing the groups to one another. For *SYN2a*, the BD group has an average RQ expression value 2.81 times higher than the MDD group, while for *SYN2b* the MDD groups has an average RQ expression value 2.23 times higher than the BD group (see Table 2 for significance coefficients).

For *SYN3* we only detected *SYN3a* and *SYN3g* at quantifiable levels in our brain samples. The two variants have perfect homology in regard to their coding exons, although at the mRNA level *SYN3g* expresses an additional exon at the 5′ end. However, we did not detect differential expression in either the *SYN3a* variant (Fig. 2*e*) or the *SYN3g* variant (Fig. 2*f*) (ANOVA p=0.2121 and 0.1551 respectively).

SYN2 expression is modulated by H3K4me3 enrichment at the promoter region distinctly for BD and MDD

Given that expression of synapsin variants was increased in BD cases, we chose to investigate whether these changes were epigenetically regulated. We investigated levels of tri-methylation of the fourth lysine tail of histone 3 (H3K4me3) using ChIP and designed primers for each independent promoter in the first 500 bp upstream of the TSS, since H3K4me3 has been shown to be enriched in this region. The *SYN1a* and *SYN1b* variants share a promoter (Supplementary Fig. 1). As shown in Fig. 2*a*, this promoter was highly enriched in the MDD group with no change in the BD group (ANOVA p=0.005). There was also a significant difference when comparing the BD and MDD groups to one another, with a fold change of 3.22 (see Table 3 for significance coefficients). However, when following up this analysis with a Pearson's correlation between expression and H3K4me3 enrichment RQ values (Fig. 3*a*, *b*), we found no significant effect. For simplicity, all three diagnostic groups were included in this analysis since the expression patterns were very similar for the

SYN1a and *SYN1b* variants. However, we found that separate analyses by diagnostic status (as for *SYN2* below) yield the same non-significant correlation results (data not shown).

The *SYN2a* and the *SYN2b* variants also share a promoter (Supplementary Fig. 1), which was significantly highly enriched in the H3K4me3 modification (ANOVA p=0.0187), as shown in Fig. 2*b*. Only the BD group, however, was significantly different from controls in Tukey's *post-hoc* test. Given the divergent expression of variants in the two disorders, Pearson's correlations were computed on the groups that had significantly different gene expression effects – BD–control (Con) for *SYN2a* and MDD–Con for *SYN2b* – and these correlations were highly significant (Fig. 3*c*, *d*).

Discussion

In this study we investigated expression patterns of synapsin variants and possible epigenetic regulatory mechanisms in the PFC (BA 10) of post-mortem brains from patients with BD, as well as MDD and controls with no psychiatric history. We focused on the PFC because of its involvement in mood regulation, working memory and executive function (Blumberg *et al.* 2003; Quraishi & Frangou, 2002; Robinson *et al.* 2008). Overall, we found that *SYN1a* and *SYN2a* were upregulated in the BD brain samples. The most striking gene expression finding was for *SYN2*, where the gene was over-expressed in BD compared to controls and this effect was accounted for by the longer variant, *SYN2a*. The converse was found in post-mortem brains from patients with MDD, where we saw a significant up-regulation of the *SYN2b* variant, but no change for *SYN2a*. This expression difference between the two disorders may not be aetiologically relevant, considering evidence that synapsin variants have overlapping function in the brain (Cesca *et al.* 2010). However, when looking to identify functional individualities in various synapsin isoforms, Gitler *et al.* found a unique role for *SYN2a* during synaptic activity at glutamatergic synapses (Gitler *et al.* 2008). This is of potential relevance, as alterations in glutamatergic transmission and plasticity have been indicated in BD (Chen *et al.* 2010; Eastwood & Harrison, 2010; Ongur *et al.* 2008; Sanacora *et al.* 2008). Furthermore, in a separate investigation of the effect of lithium treatment on synapsin expression in neuronal cell lines we found that this mood stabilizer classically used in BD treatment affected *SYN2a* but not *SYN2b* expression (Cruceanu *et al.* 2012). Based on this evidence, our findings could reflect a subtle but distinct mechanism of regulation of the *SYN2* gene in the brains of patients with different mood disorders.

The second part of our study was to determine whether the observed up-regulation in gene expression was mediated through epigenetic modifications. To our knowledge, no previous studies have tried to identify histone modifications in the synapsin genes in relation to mood disorders, so we quantified H3K4me3 levels in the promoter regions of synapsin variants. Overall, we showed an increase in H3K4me3 levels at synapsin promoters in mood disorders, with patterns that are disease-specific. For the *SYN1* variants, there was no significant correlation between mRNA expression and H3K4me3 enrichment. Although both the gene expression and the epigenetic findings for *SYN1a* and *SYN1b* are interesting, the two appear to be independent phenomena or part of a much more complex mechanism.

The most interesting epigenetic finding was the enrichment of H3K4me3 at the *SYN2* promoter. Unlike the *SYN1* data, the H3K4me3 enrichment in the *SYN2* promoter correlated with the expression up-regulation shown for the individual variants on disease-specific lines. This finding suggests that gene expression of *SYN2a* in BD, and *SYN2b* in MDD, are regulated, at least in part, by changes in H3K4me3 levels at the *SYN2* promoter. H3K4me3 is a marker for open chromatin and subsequent enhanced expression, so once the chromatin has been opened, transcription levels are dependent on transcription factors binding. The promoter region where we detected H3K4me3 enrichment is between 176 bp and 395 bp upstream of the transcription start site. Our attempt to design primers in regions closer to the TSS did not yield quantifiable H3K4me3 levels. Interestingly, within this region there are two binding sites for the transcription factor adaptor-related protein complex 2, α-1 subunit (*AP-2α*). These sites were first identified by Petersohn *et al.* through DNA-protein binding assays *in vitro* (Petersohn *et al.* 1995) and the direct role of *AP-2α* in regulating *SYN2* expression was validated through knock-down experiments in primary mid-brain embryonic mouse neurons by Skoblenick *et al.* (2010). The latter showed an increase in neuronal *SYN2* expression mediated through *AP-2α* following dopamine D_1 receptor stimulation or dopamine D_2 receptor inhibition (Skoblenick *et al.* 2010). As dopamine dysfunction has been well characterized in both BD and MDD (Hashimoto *et al.* 2007), *AP-2α* is a likely candidate for mediating the role of *SYN2* in these disorders. Furthermore, *AP-2α* has been shown to be regulated by lithium and carbamazepine (Akbarian, 2010*a*; Rao *et al.* 2007), two common mood stabilizer treatments used for BD, as well as by antidepressants such as citalopram and imipramine (Damberg *et al.* 2000).

Although the H3K4me3 findings are of interest, considering that the two *SYN2* variants share a promoter, the disease-specific expression cannot alone be explained by this epigenetic mechanism. Since the *SYN2* variants are only dissimilar at the 3′ end, other regulatory mechanisms could explain the differential expression of these two *SYN2* transcripts in BD and MDD. One such mechanism could be microRNA regulation, a class of regulatory molecules that frequently act at 3′ sites and have been shown to be dysregulated in BD post-mortem brains (Kim *et al.* 2010; Miller & Wahlestedt, 2010; Moreau *et al.* 2011).

As with all post-mortem brain studies, there are technical limitations to take into account, such as the relatively small sample size and the possible confounders associated with using frozen tissue for expression studies. To account for this, we ensured that the three diagnostic groups had no significant differences in brain pH, post-mortem delay, as well as RNA integrity for expression studies (Table 1). Furthermore, as explained in greater detail in Supplementary Methods, we performed thorough post-mortem investigations on all subjects in an attempt to gather all the relevant medical history information as well as toxicology analyses at time of death. No significant effect of these potential covariates was identified in this study in regard to gene expression or epigenetic modifications (Supplementary Methods, Supplementary Tables 1 and 2).

Another limitation of this study is that we only investigated one epigenetic modification to try to explain our gene expression findings. It has been noted in the literature that epigenetic mechanisms seem to work in concert (Kouzarides, 2007) and, accordingly, it is entirely possible that H3K4me3 enrichment is only one piece of the puzzle, particularly concerning

the results for *SYN1* variants. The present study serves to demonstrate the involvement of epigenetic mechanisms in synapsin gene regulation in mood disorders, but it would be interesting to follow up our findings with a more in-depth look at various levels of epigenetic regulation, not just in terms of histone modifications but also DNA methylation.

The main findings of this study are two-fold. First, we showed distinct synapsin profiles for BD and MDD post-mortem brain mRNA expression. These findings are interesting because they potentially indicate a molecular marker for distinguishing the two clinically similar disorders. Second, we showed that for *SYN2* the changes in expression are correlated with enrichment of H3K4me3, an epigenetic mark associated with transcriptional activation. To our knowledge, this is the first study to identify an epigenetic mechanism to be involved in the regulation of this gene. As with any molecular studies of disease, independent replication in additional post-mortem sample sets is extremely important to validate that the findings are truly relevant for the disorder and do not merely characterize the studied population. Future studies are warranted to understand the extent of epigenetic regulation of the *SYN2* gene in BD, as well as the processes by which the *SYN2a* and *SYN2b* variants are distinctly expressed in the PFC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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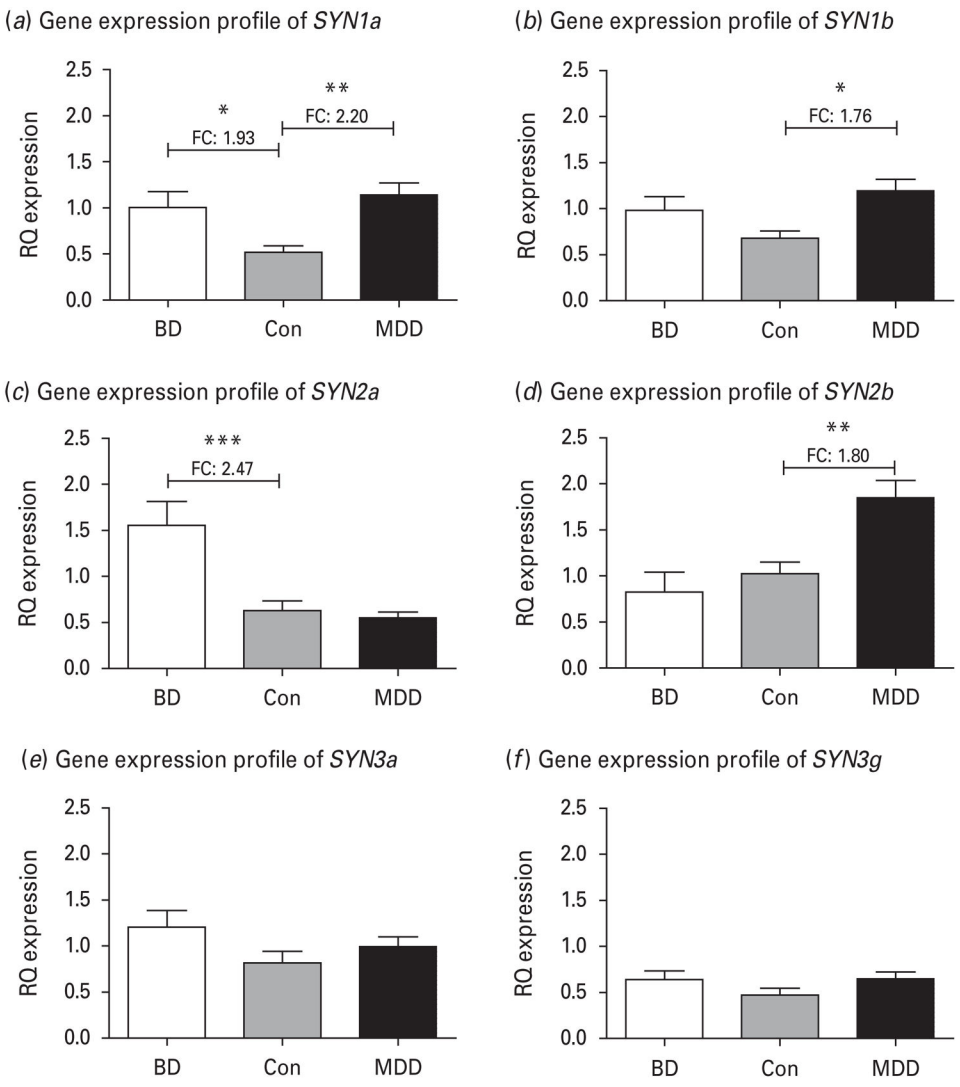

Fig. 1.

Brain expression relative quantification (RQ) values from quantitative real-time polymerase chain reaction relative to GAPDH as an endogenous control. Data are presented as RQ expression values, which represent $2^{-\Delta\Delta\text{-Ct}}$ metrics. The groups compared are bipolar disorder (BD), major depressive disorder (MDD) and non-psychiatric controls (Con) using analysis of variance (ANOVA) analyses followed by Tukey's post tests. (*a*) Relative quantitative expression for variant *SYN1a* (ANOVA p=0.0045 ; after outlier analysis BD n=13, MDD n=15, Con n=11). (*b*) Relative quantitative expression for variant *SYN1b* (ANOVA p=0.0172 ; after outlier analysis BD n=13, MDD n=15, Con n=11). (*c*) Relative quantitative expression for variant *SYN2a* (ANOVA p=0.0001 ; after outlier analysis BD n=13, MDD n=12, Con n=13). (*d*) Relative quantitative expression for variant *SYN2b* (ANOVA p=0.0005 ; after outlier analysis BD n=13, MDD n=15, Con n=11). (*e*) Relative quantitative expression for variant *SYN3a* (ANOVA p=0.2121 ; after outlier analysis BD n=12, MDD n=12, Con n=12). (*f*) Relative quantitative expression for variant *SYN3g* (ANOVA p=0.1551 ; after outlier analysis BD n=12, MDD n=14, Con n=12).

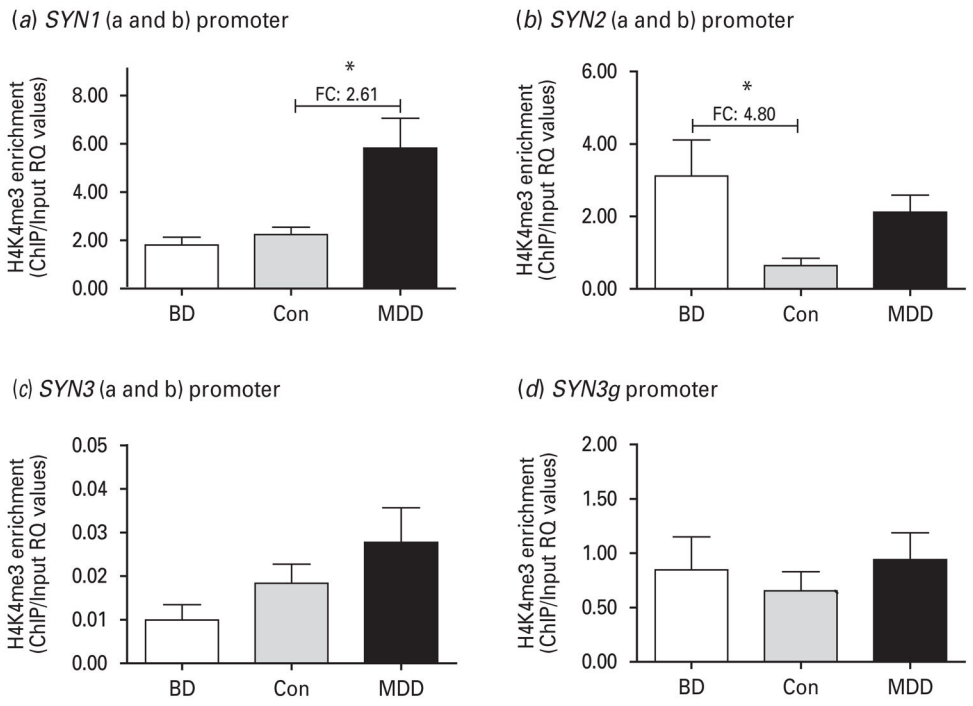

Fig. 2.

Histone 3 lysine 4 tri-methylation (H3K4me3) enrichment results for four different promoter regions representing the specific synapsin variants. Data are presented as relative quantification (RQ) expression values, which represent $2^{-\Delta\Delta\text{-Ct}}$ metrics. The groups compared are bipolar disorder (BD), major depressive disorder (MDD) and non-psychiatric controls (Con) using analyses of variance (ANOVA) followed by Tukey's post tests. (*a*) H3K4me3 enrichment for the shared promoter of variants *SYN1a* and *SYN1b* (ANOVA p=0.005 ; after outlier analysis BD n=12, MDD n=15, Con n=12). (*b*) H3K4me3 enrichment for the shared promoter of variants *SYN2a* and *SYN2b* (ANOVA p=0.0187 ; after outlier analysis BD n=9, MDD n=8, Con n=10).

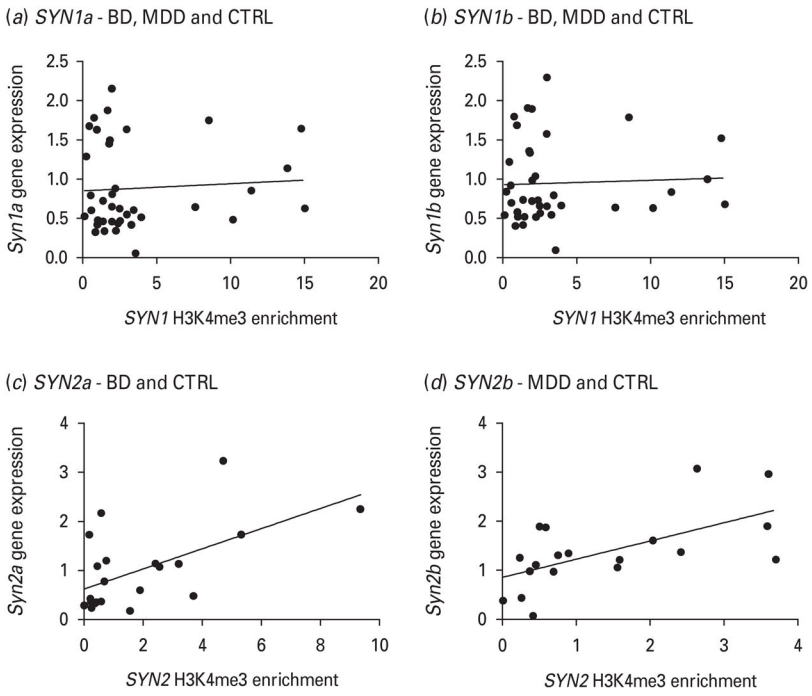

Fig. 3.

Pearson's correlations of gene expression relative quantification (RQ) values *vs.* histone 3 lysine 4 tri-methylation (H3K4me3) enrichment RQ values at the promoter region of the various synapsin variants. (*a*) For the correlation between the *SYN1a* variant expression and the *SYN1* promoter H3K4me3 enrichment, the two-tailed *p* value is 0.6833 (not significant). (*b*) For the *SYN1b* variant the same correlation is also not significant, with a *p* value of 0.7825. For the *SYN2* variants, since gene expression was so discrepant across diagnostic groups, with each variant showing an effect in a different disorder, correlations were computed accordingly. (*c*) For the *SYN2a* variant, the correlation for the bipolar disorder and control (Con) groups had a two-tailed *p* value of 0.0052. (*d*) For the *SYN2b* variant the correlation for the major depressive disorder and Con groups had a two-tailed *p* value of 0.0054.

Table 1

Brain sample group demographics (presented as mean±S.E.M.) for BD, MDD and controls

Status	Gender Male/female	Age	Brain pH	Post-mortem delay	RNA integrity no.
BD	9/4	44.00±4.05	6.63±0.07	30.38±6.31	6.63±0.30
MDD	11/7	52.00±3.81	6.72±0.06	20.28±4.32	6.34±0.21
Con	12/3	41.73±6.04	6.56±0.05	24.03±4.62	6.48±0.18
Group differences	n.s.	0.076	0.161	0.458	0.563

BD, Bipolar disorder; MDD, major depressive disorder; Con, controls.

Group differences were computed using one-way analysis of variance.

Table 2

Gene expression results

	BD *vs*. Con	MDD *vs*. Con	BD *vs*. MDD
SYN1a			
ANOVA *p* value	0.0045**		
Tukey's multiple comparison test (*q*)	3.575	4.844	1.089
Significance	*	**	n.s.
SYN1b			
ANOVA *p* value	0.0172*		
Tukey's multiple comparison test (*q*)	2.356	4.244	1.736
Significance	n.s.	*	n.s.
SYN2a			
ANOVA *p* value	0.0001***		
Tukey's multiple comparison test (*q*)	5.661	0.487	6.037
Significance	***	n.s.	***
SYN2b			
ANOVA *p* value	0.0005***		
Tukey's multiple comparison test (*q*)	1.049	4.624	5.626
Significance	n.s.	**	***
SYN3a			
ANOVA *p* value	0.2121		
Tukey's multiple comparison test (*q*)	2.098	2.278	0.054
Significance	n.s.	n.s.	n.s.
SYN3g			
ANOVA *p* value	0.1551		
Tukey's multiple comparison test (*q*)	2.798	1.329	1.567
Significance	n.s.	n.s.	n.s.

For each of the six synapsin variants, gene expression was quantified using quantitative real-time polymerase chain reaction. Data are presented as relative quantification expression values, which represent $2^{-\Delta\Delta\text{-Ct}}$ metrics one-way analyses of variance (ANOVA) and Tukey's *post-hoc* tests were computed for the three diagnostic groups: bipolar disorder (BD), control (Con) and major depressive disorder (MDD).

p Values are presented along with significance levels (* $p \leq 0.05$; ** $p \leq 0.001$; ** $p \leq 0.0001$).

Table 3

Histone 3 lysine 4 tri-methylation (H3K4me3) enrichment results

	BD *vs*. Con	MDD *vs*. Con	BD *vs*. MDD
SYN1 a+b			
ANOVA *p* value	0.005**		
Tukey's multiple comparison test (*q*)	0.4217	4.046	4.211
Significance	n.s.	*	*
SYN2 a+b			
ANOVA *p* value	0.0187*		
Tukey's multiple comparison test (*q*)	4.267	2.366	1.501
Significance	*	n.s.	n.s.

For each of the four different synapsin promoter regions, chromatin immunoprecipitation (ChIP) or input enrichment was quantified using quantitative real-time polymerase chain reaction.

We report ChIP: input ratios of relative quantification expression values, which represent $2^{-\Delta\Delta\text{-Ct}}$ metrics.

One-way analyses of variance (ANOVA) and Tukey's *post-hoc* tests were computed for the three diagnostic groups: bipolar disorder (BD), control (Con) and major depressive disorder (MDD).

p Values are presented along with significance levels (* $p \leq 0.05$; ** $p \leq 0.001$; ** $p \leq 0.0001$).