Global gene expression profiling of the polyamine system in suicide completers

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Abstract

In recent years, gene expression, genetic association, and metabolic studies have implicated the polyamine system in psychiatric conditions, including suicide. Given the extensive regulation of genes involved in polyamine metabolism, as well as their interconnections with the metabolism of other amino acids, we were interested in further investigating the expression of polyamine-related genes across the brain in order to obtain a more comprehensive view of the dysregulation of this system in suicide. To this end, we examined the expression of genes related to polyamine metabolism across 22 brain regions in a sample of 29 mood-disordered suicide completers and 16 controls, and identified 14 genes displaying differential expression. Among these, altered expression of spermidine/spermine N\textsuperscript{1}-acetyltransferase, spermine oxidase, and spermine synthase, has previously been observed in brains of suicide completers, while the remainder of the genes represent novel findings. In addition to genes with direct involvement in polyamine metabolism, including \textit{S}-adenosylmethionine decarboxylase, ornithine decarboxylase antizymes 1 and 2, and arginase II, we identified altered expression of several more distally related genes, including aldehyde dehydrogenase 3 family, member A2, brain creatine kinase, mitochondrial creatine kinase 1, glycine amidinotransferase, glutamic-oxaloacetic transaminase 1, and arginyl-tRNA synthetase-like. Many of these genes displayed altered expression across several brain regions, strongly implying that dysregulated polyamine metabolism is a widespread phenomenon in the brains of suicide completers. This study provides a broader view of the nature and extent of the dysregulation of the polyamine system in suicide, and highlights the importance of this system in the neurobiology of suicide.

Introduction

Suicide is one of the leading causes of death worldwide (Nock \textit{et al.} 2008), and while efforts to identify the neurobiological mechanisms involved in suicidal behaviours have been ongoing for decades, we have yet to obtain a comprehensive understanding of the processes involved. Studies initially focused on examining the functioning of monoaminergic metabolism and neurotransmission; however, it has become exceedingly apparent that suicide comprises a far more extensive set of pathophysiological alterations. High-throughput technologies, such as gene expression microarrays, have provided considerable insight into these processes by highlighting molecular pathways which were not previously suspected to be involved in the neurobiology of suicide, including glutamatergic and \textgamma-aminobutyric acid (GABA) neurotransmission, as well as the polyamine system (Fiori & Turecki, 2010b).

The first microarray study to implicate the polyamine system in suicide completion identified decreased expression of spermidine/spermine \textit{N}\textsuperscript{1}-acetyltransferase (SAT1) across three brain regions in French-Canadian suicide completers (Sequeira \textit{et al.} 2006), a finding which has now been observed in additional brain regions and populations (Guipponi \textit{et al.}}
imidazoline receptors, believed to be a neurotransmitter, interacts with GABA receptors (Gilad more, polyamines have been shown to influence glutamatergic receptors (Williams, 1997). Additionally, polyamines also play essential roles in neurotransmission, in particular at cellular functions (Pegg, 2009), polyamines also play and spermine. In addition to their numerous intra-molecules, including agmatine, putrescine, spermidine and spermine. In addition to their numerous intracellular functions (Pegg, 2009), polyamines also play essential roles in neurotransmission, in particular at glutamatergic receptors (Williams, 1997). Furthermore, polyamines have been shown to influence GABA receptors (Gilad et al. 1992; Morgan & Stone, 1983) and dopaminergic pathways in the brain (Bo et al. 1990; Hirsch et al. 1987). Agmatine, which itself is believed to be a neurotransmitter, interacts with imidazoline receptors, α4 adrenoceptors, nicotinic acetylcholine receptors, and 5-HT3 receptors (Reis & Regunathan, 2000), and can influence nitric oxide (NO) transmission through effects on NO synthase (Galea et al. 1996). The levels of the polyamines are highly controlled through extensive regulation of factors involved in their metabolism, accumulation, and transport. The two rate-limiting enzymes involved in polyamine biosynthesis are ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AMDA1), while SAT1 is the rate-limiting enzyme in polyamine catabolism. The expression of each of these enzymes is influenced by numerous factors which affect their levels of transcription, translation, post-translational processing, and degradation (Pegg, 2009). Putrescine, spermidine and spermine possess a limited capacity to cross the blood–brain barrier (Shin et al. 1985), and as such, their levels in the CNS are largely due to endogenous synthesis. However, arginine, as well as both agmatine and ornithine, can be transported into the brain (Oldendorf & Szabo, 1976), and as such, factors affecting the metabolism of related amino acids can influence the polyamine system.

Given the importance of polyamine homeostasis, and the substantial evidence supporting a role for the dysregulation of the polyamine system in suicide and other psychiatric conditions, we were thus interested in obtaining a global view of the expression of genes directly and indirectly related to polyamine metabolism, across the brain of suicide completers. By examining the expression of these genes in a sample of mood-disordered suicide completers and controls, we were able to both identify differentially expressed genes, as well as observe the patterns of dysregulated expression across the brain, thus allowing us to develop a greater understanding of the genes and compensatory mechanisms mediating the effects of the polyamine system in the neurobiology of suicide.

Methods

Subjects

We analysed brain tissues from 29 male suicide completers with a history of mood disorders (major depressive disorder and bipolar disorder) and 16 male controls with no history of suicidal behaviour. This sample includes several of the subjects analysed in some of our previous studies using a different microarray platform, testing a limited number of brain regions and addressing different hypotheses (Sequeira et al. 2006). All subjects were characterized by the psychological autopsy method using structured clinical interviews eliciting Axis I diagnoses according to DSM-IV criteria, as detailed elsewhere (Kim et al. 2003). The average age, post-mortem interval (PMI), and pH of all subjects was 39.8 ± 14 yr, 27 ± 11 h, and 6.6 ± 0.3, respectively. There were no group differences in any of these variables as assessed using both Welch’s t test and the Wilcoxon rank sum test with continuity correction (Supplementary Table S1, online).

Brain samples were obtained from the Quebec Suicide Brain Bank (QSBB) (www.douglasrecherche.qc.ca/suicide), where tissues were processed and dissected at 4 °C, then snap-frozen in liquid nitrogen before storage at −80 °C, following standard procedures (Bird & Vonsattel, 1993). All subjects collected by the QSBB died suddenly without a prolonged agonal period. Brain tissues were dissected in accordance with standard neuroanatomical definitions (Nolte, 2002). We analysed a total of 22 brain regions: Brodmann areas (BA) 4, 6, 8/9, 10, 11, 20, 21, 24, 29, 38, 44, 45, 46, 47, as well as the amygdala, cerebellum, hippocampus,
hypothalamus, nucleus accumbens, anterior thalamus, dorsomedial thalamus, and lateral thalamus. This study was approved by our local institutional review board.

**Microarray analysis**

All RNA samples used in this study had minimum $A_{260}/A_{280} > 1.9$ and 28S/18S rRNA peak height ratios > 1.6, as assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies USA). Sample preparation and processing were performed as described in the Affymetrix GeneChip Expression Analysis Manual (Affymetrix, USA) in collaboration with Gene Logic Inc. (USA). Samples were analysed using the Human Genome U133 Plus 2.0 array.

GeneChip signal analysis was performed with Reference Robust Multiarray Average (refRMA) (Katz et al. 2006). Arrays were pre-filtered prior to statistical analysis by assessing several quality indicators, including  $\beta$-actin 5/3’ ratio, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) 5/3’ ratio, RawQ (noise), scale factor and percent of ‘present’ calls per array. Outlier subjects were excluded in regions where they did not pass quality standards, such that different numbers of subjects were included in the final analyses for each brain region (Supplementary Table S3).

**Statistical analysis**

We performed a NetAffx query (www.affymetrix.com) for probesets related to arginine and proline metabolism, then expanded this list to include additional polyamine-related enzymes identified in the literature. We identified 69 genes, which were represented by a total of 147 probesets. Within each brain region, probesets were included in the analyses if they were considered present in at least 50% of all subjects.

In order to identify covariates for inclusion in statistical models, we performed Pearson correlations between the log$_2$ expression levels of all probesets across all regions (1703 tests) and each of the continuous variables for age, PMI, pH, and two RNA quality measures (5/3’ ratios of $\beta$-actin and GAPDH). The involvement of two dichotomous variables: toxicological findings of alcohol, or findings of other drugs, were assessed by first dichotomizing the covariates (independently for each covariate and also combining the two) and then performing $t$ tests comparing the transformed expression levels between the dichotomized groups. We then performed Shapiro-Wilk normality tests on the Pearson correlation values to determine if the distribution of correlations was normal and centred at zero. These results are shown in Supplementary Table S2. Variables which yielded $p$ values $\leq 0.05$ were considered to be correlated with expression and used to construct the six statistical models shown in Supplementary Table S4.

To identify probesets displaying differential expression in suicide completers, ANOVAs were performed using MAANOVA (Wu et al. 2003), a package for the R statistical environment, using the log$_2$-transformed expression values of each included probeset while controlling for the covariates included in each statistical model. Differential expression between the suicide and control groups was tested using the $F_S$ test (Cui et al. 2005). This is an $F$ test where the estimate of the variance in the denominator is obtained by shrinking the probeset-specific variance estimate towards the mean of the variance estimates for all 147 probesets using the James–Stein estimator. The amount of shrinkage depends on the degree of homogeneity of the variance estimates. When variance estimates are homogeneous, individual estimates are completely shrunk to the mean, effectively pooling the estimates. $p$ values for these tests were obtained using a permutation procedure.

The false discovery rates (FDRs) attached to each ANOVA $p$ value, for all comparisons across all brain regions, were estimated using two methods: Benjamini-Hochsberg (1995) and Efron (2004) implemented in R using the package FDRTOOL. A probeset was considered significantly differentially expressed if both FDR values were $\leq 0.1$ in at least one statistical model in any of the 22 brain regions.

The analysis provided an estimate of the log$_2$ fold change between the suicide and control groups for each probeset and each brain region. We then performed hierarchical clustering on the data matrix formed by these log$_2$ fold changes. This analysis must be considered exploratory, as the set of subjects included in the analysis varies between regions. We applied the DIANA (Kaufman & Rousseeuw, 1990) divisive clustering algorithm implemented in the R package using the Euclidean distance.

**Validation**

Total RNA for each brain region was extracted from adjacent tissue used for the microarray studies. cDNA synthesis was performed using oligo(dT)-priming (Invitrogen, USA). Real-time-polymerase chain reaction (RT–PCR) was performed in quadruplicate for each gene using SYBR Green on an ABI PRISM 7900HT Sequence Detection System (Applied
Biosystems, USA). β-actin, GAPDH, and β-2-microglobulin (B2M) were used as endogenous controls for relative quantitation. We used three different endogenous controls in order to account for differences in primer efficiencies, which can be an issue using SYBR Green chemistry (Liu & Saint, 2002). Primer sequences are given in Supplementary Table S6.

Results

Gene expression measurements are known to be influenced by specific post-mortem factors, many of which often cannot be avoided due to the difficulties inherent in recruitment of subjects from specific clinical populations. In this study, our statistical strategy first involved the identification of confounding variables which displayed a direct influence on the expression of polyamine-related genes, then controlled for these variables to identify gene expression changes specific to suicide. Using this methodology, we both avoided unnecessary statistical penalties, as well as identified genes whose expression differences would have otherwise been obscured.

We first assessed the potential confounding effects of several variables commonly associated with post-mortem gene expression studies, including age, PMI, pH, and RNA quality (assessed by the 5′/3′ ratios of the housekeeping genes β-actin and GAPDH). To this end, we examined the correlation of each of these variables with the expression of all polyamine-related probesets, and considered a variable to have a significant effect on expression levels if the combination of all correlation values for that variable were not normally distributed. These results indicated that pH, age, and RNA quality may have confounding effects on gene expression levels (Supplementary Table S2). As the 5′/3′ ratio of GAPDH was highly correlated with that for β-actin, we retained β-actin 5′/3′ ratios as our RNA quality measure. We found no evidence to indicate that expression was influenced by PMI, or the presence of alcohol or drugs (not shown).

ANOVAwas performed to identify probesets which were significantly differentially expressed in the suicide completers compared to the controls, while controlling for each of the variables identified in the first step. The results for each probeset displaying significant differences are shown in Table 1 and Supplementary Table S5. In total, we identified 20 probesets, representing 14 genes, which were significantly differentially expressed in at least one brain region in at least one statistical model. Among these, seven significant findings were retained across all statistical models, including probesets representing ornithine decarboxylase antizyme 1 (OAZ1) and creatine kinase mitochondrial 1A (CKMT1A), as well as five representing SAT1.

Overall, 13 of the 22 brain regions contained at least one gene which displayed differential expression in our group. The primary motor cortex, BA 4, displayed the largest number of significant probesets, followed closely by the inferior frontal gyrus, BA 44. Among our statistical models, model 2 (age) yielded the most significant p values, while model 3 (RNA quality) generated the most significant probesets. However, the probesets yielding significant results were fairly consistent across models 1–3 (Supplementary Table S5), indicating that age and RNA quality have only minimal influence on the expression of our differentially expressed genes.

Altogether, the most significant findings were for SAT1, which showed decreased expression in the suicide completers in 10 of the 22 brain regions examined, which is in agreement with our previous findings (Klempman et al. 2009a, b; Sequeira et al. 2006, 2007). Among the SAT1 probesets, the most commonly differentially expressed was 230333_at. Although this probeset targets an intronic region between exons 3 and 4, preliminary studies found that this does represent a transcribed region of SAT1 (Supplementary Material and Supplementary Fig. S1). Two other genes, SMS and SMOX, have also previously been found to be differentially expressed in suicide completers (Klempman et al. 2009b; Sequeira et al. 2007), and these two genes displayed similar patterns of altered expression in our current study. Although the down-regulation of SAT1 was the most globally observed alteration in the suicide group, half of the differentially expressed genes displayed dysregulated expression in more than one brain region, with all but AMD1 showing a consistent direction across each associated brain region. In addition to demonstrating dysregulated expression across the greatest number of brain regions, calculations of fold changes (FC) between suicide completers and controls showed that SAT1 had the greatest decrease in expression, while ALDH3A2 demonstrated the largest increase in expression.

We performed RT–PCR to validate the most significant findings for each gene, with the exception of creatine kinase, brain (CKB) and CKMT1A, which we were unable to examine in the hypothalamus due to the small quantity of tissue available from this region. The results of this validation are shown in Supplementary Table S7. The majority of the genes showed significant correlations with microarray expression values or significant differences between groups, with the exception of CKMT1A/B and ODC.
antizyme 2 (OAZ2) in BA 4. However, there was a significant correlation between our RT–PCR results and the expression measured by the CKMT1A probe-set 235452_at. As probeset 202712_s_at measures both CKMT1A and CKMT1B, which are different isoforms encoded by the same gene (Friedman & Roberts, 1994), our results suggest that our significant findings in BA 4 may be due to altered expression of the CKMT1B isoform. The results for OAZ2 are less clear, as correlations between RT–PCR results and expression of two
other OAZ2 probesets gave both positive and negative correlations.

We next used a hierarchical clustering algorithm in order to gain a better understanding of the relationship amongst the alterations in polyamine-related gene expression across each brain region. Figure 1 depicts a heat map of the log₂ fold changes from statistical model 5 (β-actin and pH) between the suicide and control groups for 19 of the 20 probesets with a significant expression difference in at least one region, as well as the dendogram obtained from the hierarchical clustering of the regions and probesets. We initially carried out these analyses using all 20 significant probesets, however the SAT1 probeset 230333_at was removed as its very strong and extensive down-regulation obscured our ability to detect relationships between all other components (not shown). Model 5 was chosen for this analysis as it resulted in the greatest range of log₂ fold changes. The clustering clearly separated two groups of genes, with SAT1 (probesets 210592_s_at and 213988_s_at), SMOX and GATM being mostly down-regulated across the brain. In addition, probeset 202053_s_at in ALDH3A2 was distinguished from all other included probesets by its extensive overexpression across multiple regions. These expression differences were particularly strong in the cluster of regions formed by BA 4, BA 21, BA 38, BA 44, and all regions of the thalamus. Finally, these results demonstrate a distinct pattern of dysregulated expression in the hypothalamus.

<table>
<thead>
<tr>
<th>Probe Set</th>
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<tr>
<td>OAZ1_215952_s_at</td>
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<td>OAZ2_201364_s_at</td>
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**Fig. 1.** Heat map generated using the DIANA algorithm with the log₂ fold changes from 19 probesets between suicide completers and controls in model 5 (β-actin and pH). AMY, Amygdala; BA, Brodmann area; HC, hippocampus; HT, hypothalamus; Thal-A, anterior thalamus; Thal-D, dorsomedial thalamus; Thal-L, lateral thalamus.

Discussion

We investigated the expression of 69 polyamine-, arginine-, and proline-related genes, across 22 brain regions, in a sample of controls and mood-disordered suicide completers, and identified 14 genes displaying altered levels of gene expression, as well as several
clusters of genes and brain regions which appear to be particularly important for the involvement of these systems in suicide.

As with our previous gene expression studies in suicide completers, SAT1, the main rate-limiting enzyme in polyamine catabolism and interconversion, demonstrated the most widespread and consistent alterations in expression. Previous studies by our group have also identified up-regulated expression of SMS and decreased expression of SMOX in suicide completers (Klempen et al. 2009b; Sequeira et al. 2007), and these two genes displayed a similar pattern of dysregulated expression in the current study. The consistency of the findings for these three genes, with our use of an expanded sample set and different microarray platform, reinforces the validity of our previous findings, and highlights their importance in suicide.

In this study, our focused approach and expanded sample allowed us to identify additional polyamine-related genes which show differential expression in suicide completers. Whereas our original studies highlighted genes involved in controlling the levels of the higher polyamines, the current study also revealed the importance of enzymes involved in the biosynthesis of the lower polyamines. AMD1 is one of the rate-limiting biosynthetic enzymes, and acts on S-adenosylmethionine (SAM) to produce the amino-propyl donor required for the conversion of putrescine to spermidine, and spermidine to spermine. AMD1 levels are regulated at multiple levels, including transcription, translation, pro-enzyme processing, enzymatic activity, and protein degradation, and several of these processes are known to be influenced by the polyamines (Pegg, 2009). In addition to its essential role in polyamine biosynthesis, its effects on the levels of SAM and decarboxylated SAM influences the synthesis of several neurotransmitters, as well as levels of DNA methylation and histone modifications (Ara et al. 2008; Duranton et al. 1998; Frostesjo et al. 1997; Guidotti et al. 2007; Mischoulon & Fava, 2002; Schipper et al. 2007), each of which may play a role in the antidepressant effects of SAM in humans (Williams et al. 2005). Post-mortem studies have found elevated levels of SAM in BA 9 of psychotic, but not depressed patients (Guidotti et al. 2007), whereas depressed individuals display a down-regulation of AMD1 in BA 21 (Aston et al. 2005). Interestingly, given the important role of putrescine on AMD1 activity, AMD1 is down-regulated in BA 4, a region demonstrating elevated putrescine levels in suicide completers (Chen et al. 2010). Although ODC is the second major rate-limiting enzyme in polyamine biosynthesis, and plays the leading role in the polyamine stress response (Gilad & Gilad, 2003), post-mortem studies in humans have not detected altered expression of this gene in suicide or other psychiatric conditions. However, as this gene is extensively regulated at numerous levels, the apparent lack of alterations in mRNA levels does not necessarily rule out its involvement in suicide. Thus it is of great interest that we identified increased expression of two genes, the antizymes OAZ1 and OAZ2, which directly influence ODC activity and protein levels. The ODC antizymes are a class of three related isoforms which bind ODC and target it for degradation, as well as inhibit the uptake and induce the secretion of polyamines from the cell (Mangold, 2005). Interestingly, translation of each antizyme is controlled by a unique frameshift which is induced by elevated polyamine levels (Mangold, 2005). Taking into account the presence of increased polyamine levels (Chen et al. 2010) in conjunction with elevated antizyme mRNA levels, it seems likely that protein levels of antizyme are also increased, with the potential effect of decreasing ODC activity in these brain regions. A recent study found OAZ1 to be down-regulated in BA 10 of schizophrenia patients (Maycox et al. 2009), thus emphasizing the importance of antizymes in psychiatry.

The production of ornithine by arginases is also an important site for the control of polyamine metabolism as well as other arginine-related pathways. Arginase II (ARG2) is the mitochondrial variant and is found at high levels in the brain where it plays important roles in both polyamine and NO metabolism (Vockley et al. 1996). Arginase induction can result in decreased NO synthesis as well as increased polyamine biosynthesis through the reduction of arginine and elevation in ornithine levels (Morris, 2009). As such, the up-regulation of ARG2 is particularly interesting given the relationship of NO with suicidal behaviours (Lee et al. 2006; Reif et al. 2009). Furthermore, altered arginase activity has been observed in plasma of patients with major depressive disorder, schizophrenia, and bipolar disorder (Elgun & Kumbasar, 2000; Yanik et al. 2003, 2004).

Our previous findings of decreased expression of SAT1 and SMOX, combined with the elevated expression of SMS, had been theorized to result in increased levels of the higher polyamines with a concomitant decrease of putrescine and agmatine. However, the interpretation is less clear when combined with the current gene expression results in which elevated expression of AMD1 and ARG2 should be expected to increase polyamine biosynthesis whereas elevated expression of the ODC antizymes, OAZ1 and...
OAZ2, should have decreased conversion of ornithine to putrescine. Our group has also recently found increased levels of putrescine and spermidine in the brains of suicide completers (Chen et al. 2010), indicating that the picture is even more complicated. Although measurements of spermine and ornithine will be required to gain a more complete profile, it seems clear that while some of the alterations in gene expression are involved in the aetiology of suicide, others likely represent compensatory mechanisms.

In addition to enzymes directly involved in polyamine metabolism, we identified several genes with more distal relationships which may influence the availability of substrates for polyamine biosynthesis. These include aldehyde dehydrogenase 3 family, member A2 (ALDH3A2), CKB, CKMT1A/B, glycine amidinotransferase (GATM), glutamic-oxaloacetic transaminase 1, soluble (GOT1), and arginyl-tRNA synthetase-like (RARSL). Interestingly, three of these enzymes, CKB, CKMT1A/B, and GATM are involved in creatine metabolism, which plays an essential role in controlling levels of adenosine 5'-triphosphate (ATP) in the cell (Hemmer & Wallimann, 1993). CKB, CKMT1A, and CKMT1B represent different isoforms of creatine kinase, and are involved in the regeneration of cellular ATP (Friedman & Roberts, 1994). Both CKB and CKMT1A were up-regulated in the hypothalamus, which may indicate a higher energy use in this structure, or could reflect a compensatory mechanism to increase low ATP levels. Intriguingly, the hypothalamus displayed a pattern of dysregulated expression which was distinct from those of other brain regions, which is of great interest given the strong support for the involvement of the hypothalamic–pituitary–adrenal (HPA) axis system in suicide (Ernst et al. 2009). In BA 4, we observed a down-regulation of GATM, the rate-limiting enzyme in creatine biosynthesis (Wyss & Kaddurah-Daouk, 2000), as well as increased expression of CKMT1A and/or CKMT1B, which is in agreement with our previous findings of significant alterations in ATP production in the prefrontal cortex of suicide completers (Klempan et al. 2009b). Finally, alterations in CKB have previously been observed in several brain regions of patients with schizophrenia (Martins-de-Souza et al. 2009a, b), and mutations in GATM appear to have behavioural and cognitive effects (Schulze, 2003), emphasizing the important neurobiological consequences resulting from dysregulated creatine metabolism.

The relationship between the other differentially expressed genes and the polyamine system is not straightforward. ALDH3A2 is a microsomal enzyme involved in the metabolism of aldehydes, including those produced during putrescine catabolism. Deficiency of this gene is associated with Sjögren–Larsson syndrome, which is characterized by physical and neurological defects resulting from the accumulation of fatty alcohols (Rizzo & Craft, 1991). Up-regulation may reflect increased polyamine catabolism, or may be a consequence its involvement in lipid metabolism, which has also been implicated in suicidal behaviours (Ernst et al. 2009). Indeed, our multivariate analyses demonstrated a distinct pattern of dysregulated expression for this gene, which may indicate that its involvement in suicide is not related to polyamine metabolism. Along the same line, GOT1 catalyses the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate, which is an essential reaction in several metabolic pathways, thereby making it difficult to determine if its up-regulation is directly related to polyamine metabolism. Finally, RARSL uses arginine to produce arginyl-tRNA, which is essential for protein synthesis, and plays a role in protein degradation (Morris, 2009).

There are several limitations to this study. As with all studies of this nature, the effects of post-mortem factors as well as other confounding clinical variables may influence the interpretation of results. However, we used statistical methods to identify and control for confounding variables, and by statistically assessing the effects of these and other variables on overall expression, we were able to identify the most relevant factors in terms of quality (β-actin 5′/3′ ratio), post-mortem (pH), and clinical (age) characteristics. Given that our results were fairly consistent across the models, our expression results appear relatively robust to the effects of these variables. Nonetheless, we cannot rule out the possibility that additional confounding factors may have been present which were not controlled for. By expanding our sample to include individuals with depression and bipolar disorder, we may have obscured our ability to detect suicide-specific alterations by introducing disease-specific effects. Additionally, as we did not have a suicide group without mood disorders, we are unable to completely dissociate the processes related to suicide from those associated with mood disorders. Nonetheless, in doing so we may have generated results which can be better generalized to other populations. Post-mortem toxicological assessments found that the majority of our subjects were not taking medication at the time of death, and we found no indication that the expression of polyamine probesets was related to the presence of alcohol or drugs; however, we cannot exclude the possibility that medication use prior to this period...
could have accounted for some of our differential gene expression. Finally, it is possible that additional quality or post-mortem variables were present which were not accounted for in our statistical models.

Microarray studies generate large amounts of data, potentially leading to false-positive findings when inadequate statistical methods are used to control for multiple testing. Conversely, the use of overly stringent correction methods can prevent the identification of biologically relevant alterations in expression. By pre-selecting probesets to include only those which may be relevant for polyamine metabolism, we have greatly decreased the amount of statistical correction required for multiple testing, while at the same time, we have limited false positive results by the use of two separate FDR methods.

In conclusion, we have performed a global analysis of the expression of polyamine-related genes across many brain regions of suicide completers, and have identified several genes displaying significantly altered expression. This study has allowed us to obtain a broader view of the polyamine-related processes associated with suicide, and has highlighted the importance of the polyamine system in the psychopathology of suicide.

Note
Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

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Statement of Interest
None.

References


