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BRIEF REPORT

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Effects of histone modifications on increased expression of polyamine biosynthetic genes in suicide

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Abstract

Altered polyamine metabolism has been consistently observed as underlying the suicide process. We recently performed a global analysis of polyamine gene expression across the brains of suicide completers, and identified up-regulation of four genes, arginase II (*ARG2*), S-adenosylmethionine decarboxylase (*AMD1*), and antizymes 1 and 2 (*OAZ1* and *OAZ2*), which play essential roles in polyamine biosynthesis. To determine if a shared epigenetic mechanism is involved in their overexpression in the prefrontal cortex, we measured promoter levels of tri-methyl modified histone-3-lysine-4 (H3K4me3), a marker of open chromatin, and assessed its association with suicide and gene expression. We identified increased H3K4me3 in the promoter region of *OAZ1* in suicide, and found that H3K4me3 was correlated with the expression of *OAZ1* and *ARG2*. Overall, our findings indicate that the H3K4me3 modification plays an important role in the regulation of polyamine biosynthesis, and that this mechanism may be involved in the neurobiology of suicide.

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Introduction

Suicide accounts for one million deaths worldwide each year (Nock *et al.* 2008), and results from the interactions of a variety of clinical, social, genetical, and environmental factors. Although numerous studies over the last few decades have identified specific neurobiological alterations associated with suicide, we are far from reaching a comprehensive understanding of the underlying pathological processes. One biological pathway that has been investigated for its involvement in suicide is the polyamine system, a pathway which plays an important role in physiological and behavioural stress responses, and has been implicated in several psychiatric conditions (Fiori & Turecki, 2008). The levels of polyamines, which comprise agmatine, putrescine, spermidine, and spermine, are highly regulated through tight control of both their biosynthesis and catabolism, and it has thus been of great interest that the levels of both several polyamine metabolic enzymes, and the polyamines themselves, are altered in the brains of suicide completers (Chen *et al.* 2010; Guipponi *et al.* 2009; Sequeira *et al.* 2006).

To obtain a more comprehensive view of the involvement of dysregulated polyamine metabolism in suicide, we recently examined the expression of polyamine-related genes across 22 brain regions (Fiori *et al.* 2011), and identified altered expression of 14 genes in suicide completers. Three of these genes, spermidine/spermine N_1-acetyltransferase (*SAT1*), spermine oxidase (*SMOX*), and spermine synthase (*SMS*), had previously been found to demonstrate altered expression in suicide completers (Guipponi *et al.* 2009; Sequeira *et al.* 2006, 2007). As these genes are all involved at the higher stages of polyamine metabolism, these studies indicated that dysregulated expression of genes at this level was an important component of suicide pathology. However, in addition to these genes, our recent results also identified an up-regulation of several genes involved in lower levels of polyamine metabolism: arginase II (*ARG2*), S-adenosylmethionine (*SAM*) decarboxylase (*AMD1*), and antizymes 1 and 2 (*OAZ1* and *OAZ2*). These genes

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play essential roles in controlling polyamine biosynthesis, where *AMD1* is one of the main rate-limiting enzymes, and *OAZ1* and *OAZ2* are key regulators of the activity of ornithine decarboxylase (*ODC*), the second rate-limiting biosynthetic enzyme. As the involvement of these key polyamine biosynthetic genes represented novel findings, we were greatly interested in determining the underlying molecular mechanisms which may be involved in this differential expression in suicide.

Gene expression is controlled by a variety of factors, and while genetic sequence variants have been functionally associated with the down-regulation of *SAT1* in suicide completers (Fiori & Turecki, 2010; Fiori *et al.* 2009; Sequeira *et al.* 2006), we theorized that the over-expression of four functionally related polyamine genes may be due to a shared mechanism. Epigenetic modifications, which influence gene expression without altering the basic genetic code, are an important source of non-coding functional variations, and it has been proposed that these modifications mediate the interaction between the genome and the environment, such that they may play an important role in conferring risk for neuropsychiatric disorders, including suicide (Zhang & Meaney, 2010). Epigenetic mechanisms include DNA methylation, microRNAs, and post-translational histone modifications, and several studies have now identified altered levels of epigenetic modifications in psychiatric disorders, including suicide (Akbarian *et al.* 2005; Ernst *et al.* 2009; McGowan *et al.* 2008). Of further interest, polyamine metabolism has been shown to influence epigenetic modifications (Ara *et al.* 2008; Schipper *et al.* 2007), and increased SAM, a biosynthetic precursor, has been implicated in the down-regulation of several genes in the prefrontal cortex of psychotic patients through altering their levels of promoter methylation (Guidotti *et al.* 2007). Furthermore, the expression of several polyamine-related genes has been shown to be influenced by epigenetic modifications, including both DNA methylation (Aleman *et al.* 2008; Ikeda *et al.* 2008), and histone methylation (Akbarian *et al.* 2005), indicating that epigenetic modifications are important modulators of the expression of polyamine genes.

Methods and Results

In order to assess the potential involvement of a shared epigenetic mechanism regulating the expression of *AMD1*, *ARG2*, *OAZ1*, and *OAZ2*, we focused on the tri-methyl modification of histone-3-lysine-4 (H3K4me3). This modification is a marker of the open, transcriptionally active chromatin state, and has been shown to be stable across a range of post-mortem intervals (PMIs) (Huang *et al.* 2006), thus making it an excellent candidate for our study. To determine the involvement of H3K4me3 in the up-regulation of these genes in suicide, we examined Brodmann area (BA) 44 (inferior frontal gyrus), a brain region which has been implicated in suicide and demonstrates altered expression of polyamine-related genes in suicide completers (Fiori *et al.* 2011; Klempan *et al.* 2009).

Our first objective was to identify a group of subjects in which the expression of each of our four genes of interest, *AMD1*, *ARG2*, *OAZ1*, and *OAZ2*, was significantly higher in suicide completers compared to non-suicide controls. We hypothesized that these subjects would represent a more homogenous group of suicide completers where H3K4me3 may be involved in the up-regulation of biosynthetic genes, thus allowing us to maximize the power of our epigenetic analyses. To this end, we first assessed the expression of *AMD1*, *ARG2*, *OAZ1*, and *OAZ2* in BA 44. The cause of death for each subject was assessed by the Quebec Coroner's office, and samples were obtained from the Quebec Suicide Brain Bank, where they were processed and dissected at 4 °C, then snap-frozen in liquid nitrogen before storage at −80 °C, following standard procedures. Psychiatric diagnoses were obtained using the psychological autopsy method with the Structured Clinical Interview for DSM-IV Axis I, as described elsewhere (Dumais *et al.* 2005). Written informed consent was obtained from next-of-kin for all subjects. This study was approved by our local institutional review board and performed in accordance with the Declaration of Helsinki. We focused on BA 44, a prefrontal cortex region believed to be involved in the neurobiology of suicide (Hercher *et al.* 2009), from which we extracted RNA, synthesized cDNA and assessed gene expression levels by quantitative real-time polymerase chain reaction (RT–PCR) using SYBR Green, using primers listed in Table 1.

We identified a group of 34 suicide completers for whom *AMD1*, *ARG2*, *OAZ1*, and *OAZ2* showed concomitant and significant ($p \leqslant 0.05$) up-regulation. This sample was comprised of male suicide completers, who were matched with an equal sample of male non-suicide controls for age, PMI, brain pH, and toxicological findings of medication (7 controls, 8 suicides). We next evaluated the involvement of H3K4me3 in the increased expression by analysing the levels of this modification in the promoter regions of each gene. To this end, we performed chromatin immunoprecipitation (IP) (Matevossian & Akbarian, 2008) in BA 44 tissue. Briefly, tissue samples were treated with

Table 1. Primers used to assess expression (RT–PCR) and H3K4me3 (ChIP). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an endogenous control

Gene	RT–PCR	ChIP
AMD1	GATGGAACTTATTGGACTATTCACATCAC	CAGCAGCTATAGGCCGTGG
	CTGTGCGACATTTAGAACTCTGATTAAC	GTGAGCACCGCCCTTATATATCC
ARG2	TTGCTGAGGAAATACACAATACAGG	CATCGAAGGCACGTCCCAGC
	GGTTAGCTGTAGTCTTCGCCTC	CCGCGCCTCCAACCAGC
OAZ1	GACAGCTTTGCAGTTCTCCTGG	TTTCACCAATCAGGCGCTGG
	TTCGGAGCAAGGCGGCTC	CCGCCTTTATAGATGCTGCG
OAZ2	GCTGATGGGAGCAAAGAAGG	CGGTGTCCTGGGAAACTGG
	AGCTGAAGGTCTTCAGGAGTG	GTCATGGCCTCCTGCACAGC
GAPDH	TTGTCAAGCTCATTTCCTGG	TACTAGCGGTTTTACGGGCG
	TGTGAGGAGGGGAGATTCAG	TCGAACAGGAGGAGCAGAGAGCGA

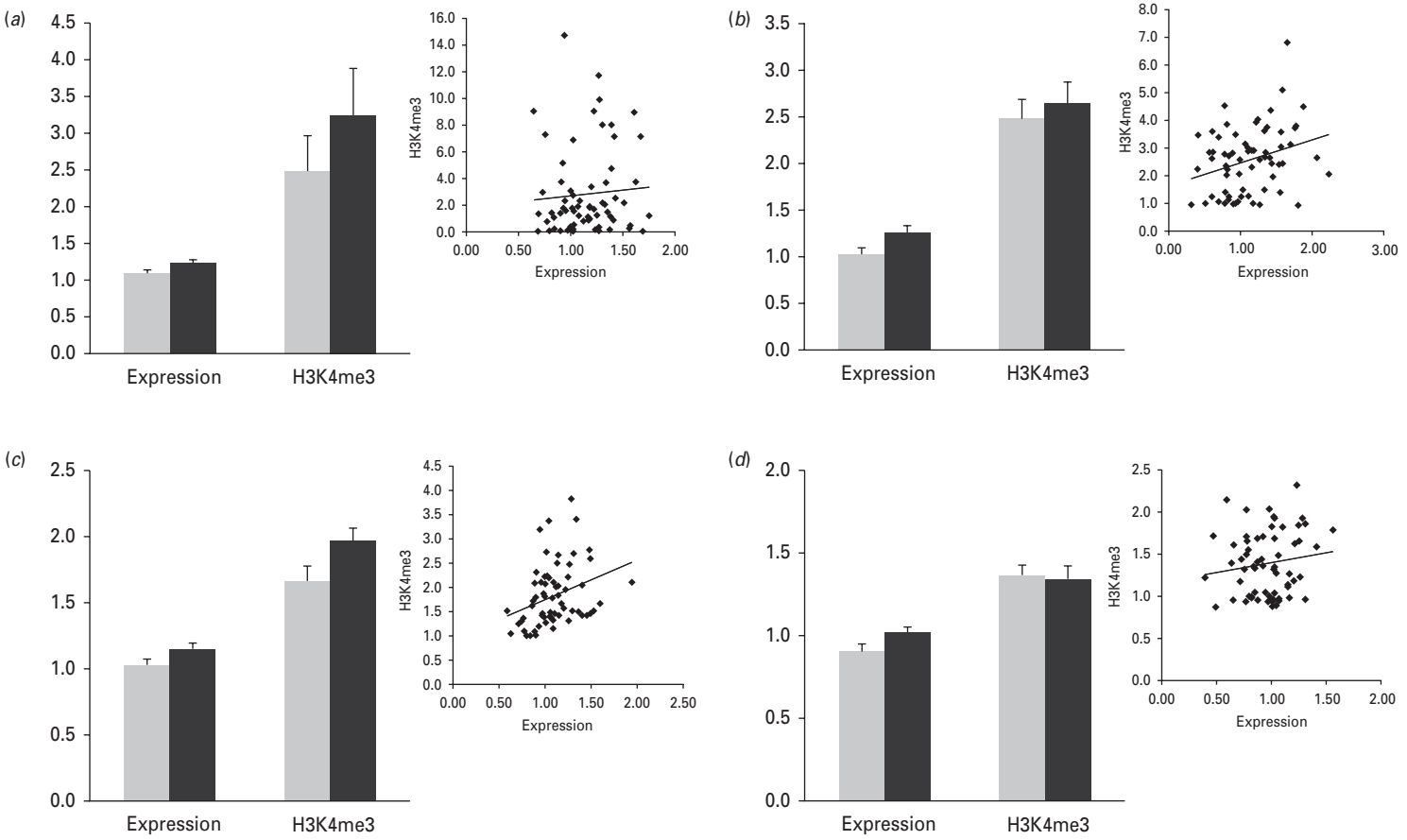
micrococcal nuclease, then intact nucleosomes were extracted, and a portion of sample was treated with anti-H3K4me3 antibody, while the remainder was used as an input control. The antibody-treated sample was purified using protein G agarose beads, and both input and bound (IP) fractions were digested with proteinase K before purifying DNA by phenol-chloroform extraction. Quantitative RT–PCR using SYBR Green, using primers listed in Table 1, was then used to measure DNA in each IP sample, using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as an endogenous control, and levels of H3K4me3 represented as IP/*GAPDH*. Similar findings were observed when IP was normalized to input (not shown). The results of these analyses are shown in Fig. 1. There were no significant differences between suicide completers and controls for *AMD1*, *ARG2*, or *OAZ2*; however, *OAZ1* displayed significantly elevated levels of H3K4me3 in the promoter region in suicide completers. H3K4me3 levels were not significantly different between individuals with positive and negative toxicology for psychotropic medication ($p = 0.41$), and when only individuals who had no evidence of psychotropic medication were included in the analyses, the differences between groups remained significantly different ($p = 0.013$).

Both *ARG2* and *OAZ1* demonstrated significant positive correlations between expression and H3K4me3 levels, providing evidence that this modification plays an important role in regulating the expression of these genes, and which agrees well with H3K4me3 being a marker of transcriptionally active chromatin. Intriguingly, the results for both *ARG2* and *OAZ1* were primarily driven by strong correlations within the control group, suggesting that above a certain threshold level of H3K4me3 in the promoter, further increases have relatively little effect on expression. This effect should be further investigated. Neither *AMD1* nor *OAZ2* demonstrated significant correlations between expression and H3K4me3 in any group, suggesting that the levels of this modification in the promoter region have little influence on their expression in BA 44.

Discussion

Overall, the results of this study indicate that the up-regulation of *OAZ1* in suicide completers is associated with increased levels of H3K4me3 in the upstream promoter region, and that this modification is directly correlated with its expression in the brain. As mentioned above, *OAZ1* plays an important role in polyamine biosynthesis, through both its ability to bind and target *ODC* for degradation, as well as though inhibiting the uptake and inducing the secretion of polyamines from the cell (Pegg, 2006). Although previous studies have indicated that *OAZ1* activity is regulated primarily at the translational level through a unique frameshift mechanism (Pegg, 2006), our results have now demonstrated that transcriptional control also plays a role in regulating *OAZ1* levels. Furthermore, these results indicate that this form of epigenetic regulation is an important mechanism underlying the involvement of *OAZ1* in suicide.

Unlike *OAZ1*, we found no significant differences in the levels of H3K4me3 in the promoter regions of *AMD1*, *ARG2*, or *OAZ2*, nor was the expression of *AMD1* or *OAZ2* associated with this modification. As we investigated the levels of H3K4me3 at only one region of each gene, it is possible that this modification may still be involved in regulating the expression of these genes, but at genetic regions distinct from those examined in this study. Alternatively, the up-regulation of these genes may be due to additional



(e)

Gene	Expression *p* value	H3K4me3 *p* value*	Correlation *p* value	Correlation Pearson
AMD1	0.03	0.18	0.55	0.08
ARG2	0.03	0.31	0.02	0.30
OAZ1	0.04	0.03	0.09	0.32
OAZ2	0.04	0.37	0.34	0.12

Fig. 1. Expression and promoter levels of tri-methyl histone-3-lysine-4 (H3K4me3). Gene expression and levels of H3K4me3 (±S.E.M.) for 34 non-suicide controls (▒) and 34 suicide completers (■), as well as plots displaying the relationship between these measures for all subjects, are shown for (*a*) *AMD1*, (*b*) *ARG2*, (*c*) *OAZ1*, and (*d*) *OAZ2*. (*e*) Student's *t* test *p* values for the comparisons of expression and H3K4me3 between suicide completers and controls in 68 subjects, as well as Pearson correlations between expression and H3K4me3. * One-tailed.

epigenetic mechanisms such as DNA methylation and microRNAs, or other histone modifications. The polyamines themselves also represent potential candidates, as they not only interact strongly with nucleic acids, but are also well-known to regulate the expression and activity of polyamine metabolic enzymes. Given that we have observed altered polyamine profiles in the brains of suicide completers (Chen *et al.* 2010), it is possible that this mechanism is at least partially responsible for the differential expression of these genes. Interestingly, a recent study found that the overexpression of *OAZ* in cultured cells yielded a hypomethylation of genomic DNA and decreased levels of H3K9me2 (Yamamoto *et al.* 2010). It could thus be speculated that, through influencing the epigenetic mechanisms which control the expression of *AMD1*, *ARG2*, and *OAZ2*, the elevated levels of *OAZ1* may in fact be the cause of the coordinated expression. Finally, it is possible that distinct genetic or epigenetic mechanisms are separately involved in the elevated expression of each gene. However, given that the expression levels of the four genes were highly correlated, both in the initial sample as well as the smaller subgroup (Supplementary Table S1, online), the presence of a shared regulatory mechanism remains likely.

There are several limitations to this study. First, polyamines constitute a complex neurotransmitter system that, among other things, is involved in stress-response signalling and is tightly regulated by a number of different mechanisms (Fiori & Turecki, 2008). This study focused on only one histone modification in one region of each gene, and thus we cannot rule out the possibility that other epigenetic modifications are involved in their overexpression, or that H3K4me3 levels are altered in other regulatory regions of these genes. We are currently examining the role of additional epigenetic mechanisms in regulating the expression of these four genes, as well as expanding the genetic regions being examined. Second, although we selected our groups to be homogeneous in terms of age, pH, and PMI, it is possible that additional confounding post-mortem or clinical variables may have been present but which were not accounted for in this study. However, our sample size was large, making it relatively robust to the effects of potentially confounding variables. Finally, although the presence of psychiatric disorders in suicide completers can make it difficult to distinguish between the effects of suicide and comorbid diagnoses, our sample of suicide completers comprised individuals with a range of Axis I disorders, thereby allowing us to relate our findings specifically to suicide.

In conclusion, this study provides further evidence to support a role for the altered expression of polyamine biosynthetic genes in suicide, and identified the H3K4me3 modification as playing an important role in the up-regulation of *OAZ1* in the brains of suicide completers. Given the importance of the polyamine system in the pathophysiology of suicide, its regulation by epigenetic processes, which have the potential to be reversible, may provide a means through which the polyamine system could be targeted for therapeutic intervention.

Note

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

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Statement of Interest

None.

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