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Effects of promoter methylation on increased expression of polyamine biosynthetic genes in suicide

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ABSTRACT

Suicide is among the leading causes of death worldwide. The polyamine system has been increasingly implicated in the neurobiology of suicide. Previous research has indicated that epigenetic mechanisms play a role in explaining dysregulation of polyamine genes in suicide completers. Nevertheless, regulatory mechanisms explaining polyamine biosynthetic genes displaying dysregulated expression in suicide completers, including ornithine decarboxylase antizymes 1 and 2 (OAZ1 and OAZ2), S-adenosylmethionine decarboxylase (AMD1), and arginase 2 (ARG2), have yet to be elucidated. In this study, we investigated methylation patterns in the promoter region of OAZ1, OAZ2, AMD1, and ARG2 in Brodmann area 44 from a group of 33 suicide completers and 31 non-suicide controls. We found significant site-specific differences in methylation in the promoter of ARG2 and AMD1 that were also significantly negatively correlated with gene expression. These findings provide further support for a role for the involvement of epigenetic modifications in the regulation of genes associated with polyamine biosynthesis, and which may contribute to the complexity of suicidal behaviors.

1. Introduction

Suicide is an important cause of premature death around the world (Nock et al., 2008) and it is clear that biological factors play a role in underlying the suicide process. However, the exact molecular mechanisms at play remain largely unclear. Recently, the polyamine system has become an interesting target of research aimed toward understanding the neurobiological alterations associated with suicide (Fiori and Turecki, 2008).

Polyamines are ubiquitous aliphatic molecules, which include putrescine, spermine, spermidine, and agmatine, each of which is incorporated into a highly regulated metabolic pathway. This pathway includes 3 rate-limiting enzymes, one of which is S-adenosylmethionine decarboxylase (AMD1). The initial support for the involvement of the polyamine system in suicide came from observations that spermidine/spermine N^1-acetyltransferase (SAT1) gene expression was downregulated in numerous brain regions in suicide completers as compared to control subjects (Guipponi et al., 2009; Sequeira et al., 2006). Moreover, functionally characterized genetic variants in the promoter region of SAT1 were associated with suicide (Fiori et al., 2009; Sequeira et al., 2006). More recently, gene expression studies have identified altered expression of additional polyamine-related genes, including ornithine decarboxylase antizymes 1 and 2 (OAZ1 and OAZ2), arginase 2 (ARG2), and AMD1 in Brodmann area 44 (BA44) of suicide completers with a history of mood disorders (Fiori et al., 2011a).

Gene expression is controlled by a variety of factors including epigenetic modifications, which are of great interest due to their regulation by environmental factors (Nestler, 2009). Post-translational histone modifications and DNA methylation are examples of epigenetic mechanisms that modify gene expression without altering the DNA sequence (Nestler, 2009). To date, epigenetic modifications have been implicated in several psychiatric phenotypes, including schizophrenia (Akbarian et al., 2005), bipolar disorder (Hobara et al., 2010), and suicide (McGowan et al., 2008). Recently, our group found a significant increase in trimethylated histone 3-lysine 4 (H3K4me3), a marker of open chromatin, in suicide completers for OAZ1, and the levels of this modification were significantly correlated with expression of this gene (Fiori et al., 2011b). However, we found no evidence for elevated levels of H3K4me3 in the promoters of OAZ2, ARG2, or AMD1. To further investigate the effects of epigenetic factors on the altered expression levels of these four genes, we investigated the potential role of DNA methylation.

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Table 1
Subject information.

	Age	Brain pH	PMI	RIN	DNA quality	Sex	Ethnicity
Controls	43.6 ± 2.89	6.53 ± 0.0487	45.2 ± 4.72	6.4 ± 0.11	2.05 ± 0.0105	100% Males (34/34)	100% Caucasian (34/34)
Suicides	38.6 ± 2.25	6.61 ± 0.0464	33.9 ± 3.31	6.7 ± 0.11	2.06 ± 0.00616	100% Males (34/34)	100% Caucasian (34/34)

Detailed information on subjects used in both the gene expression and DNA methylation analyses.
Groups were controlled for age, brain pH, and PMI, as values did not differ significantly between groups ($p > 0.05$).
Values are expressed as either mean ± SEM or percentage.
RIN: RNA Integrity number; DNA Quality is expressed as the 260/280 ratio.

2. Materials and methods

2.1. Subjects

Post-mortem brain tissue from BA44, which has been previously shown to demonstrate altered levels of polyamine genes in suicides (Fiori et al., 2011a), was obtained from the Quebec Suicide Brain Bank. Tissue was dissected at 4 °C, snap-frozen in liquid nitrogen, and stored at −80 °C following standard procedures. The Quebec Coroner's office assessed the cause of death for each subject, cases and controls alike, and psychiatric diagnoses were obtained using psychological autopsy following the Structured Clinical Interviews for DSM-IV Axis 1 (Dumais et al., 2005). Control subjects deceased from either natural ($n = 18$) or accidental ($n = 16$) causes. Methods of suicide included: hanging ($n = 25$), overdose ($n = 4$), jumping ($n = 2$), cutting ($n = 1$), drowning ($n = 1$), and shooting ($n = 1$). Of the 34 subjects in the suicide group, 28 were known to have an axis I disorder, which included schizophrenia ($n = 3$), major depressive disorder ($n = 12$), depressive disorder not otherwise specified ($n = 3$), bipolar disorder ($n = 7$), and substance abuse ($n = 3$). Of those, 9 subjects had prescriptions for psychiatric medications. Eight control subjects had prescriptions for non-psychiatric medications. All subjects, including controls, were French-Canadian males and were matched for age, post-mortem interval (PMI), and brain pH. Detailed information on subjects is found in Table 1. Written informed consent was obtained from next-of-kin for all subjects, and our local institutional review board approved this study.

2.2. Gene expression

RNA extracted from BA44 of 34 non-suicide controls and 34 suicide completers using QIAGEN's RNeasy Mini kit was used to synthesize cDNA. All subjects had RNA Integrity Number (RIN) values greater than 5.0, as determined using the Agilent 2100 Bioanalyzer and Agilent RNA 6000 Nano Assay protocol (Table 1). Gene expression of all four genes was measured using quantitative real-time polymerase chain reaction (qRT-PCR) on Applied Biosystems' *7900HT* Fast Real-Time PCR System. SYBR green primers for each gene are listed in Table 2, where glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. The 9600 emulation thermal cycle protocol was: 50 °C for 2 min, 95 °C for 10 min, and 40 repetitions of 95 °C for 15 s and 60 °C for 1 min. The data was extracted by relative quantification using Applied Biosystems' SDS 2.4 and RQ Manager 1.2.1 software.

2.3. Methylation analysis

Genomic DNA was extracted from BA44 of 31 non-suicide controls and 33 suicide completers using QIAGEN's QIAamp DNA Mini Kit. Concentrations of genomic DNA were assessed using the Thermo Scientific Nanodrop 1000 spectrophotometer and each sample had a 260/280 ratio greater than 1.8 (Table 1). Three non-suicide controls and one suicide completer were excluded from this analysis due to poor DNA quality. Genomic DNA from each sample was bisulfite-treated using QIAGEN's Epitect Bisulfite Kit following the manufacturer's guidelines. At the Génome Québec Innovation Centre, Sequenom's EpiTYPER was used to characterize differentially methylated CpGs in the promoter region of each gene. Promoter regions were defined as approximately 500 base pairs (bp) upstream of the transcription start site (TSS) (Fig. 1). In order to meet the mass threshold necessary for accurate measurement by Sequenom's EpiTYPER, CpGs within the promoter region were clustered together by the EpiTYPER software. As such, certain analyzed fragments contained more than one CpG. Levels of methylation were assessed for each cluster of CpGs.

2.4. Statistics

Statistical analyses were performed on SPSS version 18 and GraphPad Prism 5. Differences in methylation between groups and across all CpGs were assessed using a 2-way, mixed model ANOVA followed by LSD post-hoc analyses. In all cases, p-values were considered statistically significant for $p \leq 0.05$, and all p-values were corrected for age, PMI, and psychiatric medication.

3. Results

In this study, we first examined the level of gene expression of OAZ1, OAZ2, AMD1, and ARG2 (Fig. 2). For all of these genes, we found increased expression levels in suicides as compared to the non-suicide controls ($p = 0.035$, 0.041, 0.025, and 0.027, respectively). We then examined the methylation levels in a region of the promoter, upstream of the TSS, for each gene. Due to the repressive function of methylation in gene promoters (Maunakea et al., 2010), we expected generally low levels in these functionally active promoters. Indeed, mean methylation levels across the promoter of the 4 genes were between 3% and 8%, with OAZ1 being the only gene to show overall methylation group differences ($p = 0.029$). OAZ2 also showed an overall group difference, although it was a trend toward significance ($p = 0.057$) Each of the four genes,

Table 2
Gene expression primers.

Gene	Forward primer	Reverse primer
GAPDH	TTGTCAAGCTCATTTCCTGG	TGTGAGGAGGGGAGATTCAG
AMD1	GATGGAACTTATTGGACTATTCACATCAC	CTGTGCGACATTTAGAACTCTGATTAAC
ARG2	TTGCTGAGGAAATACACAATACAGG	GGTTAGCTGTAGTCTTCGCCTC
OAZ1	GACAGCTTTGCAGTTCTCCTGG	TTCGGAGCAAGGCGGCTC
OAZ2	GCTGATGGGAGCAAAGAAGG	AGCTGAAGGTCTTCAGGAGTG

Primers used to assess gene expression by qRT-PCR. Glyceraldehyde-3-phosphate (GAPDH) was used as the endogenous control. All primers are listed from 5′ → 3′.