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Isoform-level brain expression profiling of the spermidine/spermine N1-Acetyltransferase1 (*SAT1*) gene in major depression and suicide

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Abstract

Low brain expression of the spermidine/spermine N-1 acetyltransferase (*SAT1*) gene, the rate-limiting enzyme involved in catabolism of polyamines that mediate the polyamine stress response (PSR), has been reported in depressed suicides. However, it is unknown whether this effect is associated with depression or with suicide and whether all or only specific isoforms expressed by SAT1, such as the primary 171 amino acid protein-encoding transcript (SSAT), or an alternative splice variant (SSATX) that is involved in SAT1 regulated unproductive splicing and transcription (RUST), are involved. We applied next generation sequencing (RNA-seq) to assess gene-level, isoform-level, and exon-level SAT1 expression differences between healthy controls (HC, N = 29), DSM-IV major depressive disorder suicides (MDD-S, N = 21) and MDD non-suicides (MDD, N = 9) in the dorsal lateral prefrontal cortex (Brodmann Area 9, BA9) of medication-free individuals postmortem. Using small RNA-seq, we also examined miRNA species putatively involved in SAT1 post-transcriptional regulation. A DSM-IV diagnosis was made by structured interview. Toxicology and history ruled out recent psychotropic medication. At the gene-level, we found low SAT1 expression in both MDD-S (vs. HC, $p = 0.002$) and MDD (vs. HC, $p = 0.002$). At the isoform-level, reductions in MDD-S (vs. HC) were most pronounced in four transcripts including SSAT and SSATX, while reductions in MDD (vs. HC) were pronounced in three transcripts, one of which was reduced in MDD relative to MDD-S (all $p < 0.1$ FDR corrected). We did not observe evidence for differential exon-usage (i.e. splicing) nor differences in miRNA expression. Results replicate the finding of low SAT1 brain expression in depressed suicides in an independent sample and implicate low SAT1 brain expression in MDD independent of suicide. Low expressions of both SSAT and SATX isoforms suggest that shared transcriptional mechanisms involved in RUST may account for low SAT1 brain expression in depressed suicides. Future studies are required to

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Conflict of Interest

The authors have no relevant conflicts of interest to report. Dr Mann receives royalties for commercial use of the C-SSRS from the Research Foundation for Mental Hygiene and has stock options in Qualitas Health, a start-up company working on a PUFA nutritional supplement.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nbd.2015.04.014.

understand the functions and regulation of SAT1 isoforms, and how they relate to the pathogenesis of MDD and suicide.

Keywords

Transcriptome; Psychiatric genomics; Major depressive disorder; RNAseq; ncRNA; Suicidal behavior

Introduction

Involvement of the polyamine system in suicide was first suggested by a reported deficit in spermidine/spermine N-1 acetyltransferase (SAT1), the rate-limiting enzyme in the catabolism of polyamines spermidine and spermine in the polyamine stress response (PSR), in multiple brain regions of suicides (Sequeira et al., 2006). In this study, an exploratory whole-genome approach (Affymetrix HG-U133A cDNA microarray) identified low SAT1 mRNA levels in suicides, and low mRNA and protein levels were confirmed by semiquantitative reverse transcription–polymerase chain reaction (RT–PCR), immunohistochemistry and Western blot analyses (Sequeira et al., 2006). Low SAT1 expression in the brain has been confirmed by other groups in both depressed and non-depressed suicides (Sequeira et al., 2007; Guipponi et al., 2009; Klempan et al., 2009a,b). Conversely, higher SAT1 has been found in the blood of suicidal attempters, non-attempters who are suicide ideators and suicides with bipolar disorder or psychosis (Le-Niculescu et al., 2013), providing further evidence for a role of SAT1 dysregulation in suicidal behavior. What is not clear is whether this abnormality is also present in major depressive disorder independently of suicidal behavior.

Microarray studies of the brains of suicides have identified more potentially responsible genes and molecular pathways, including SAT1 and the polyamine system (Fiori and Turecki, 2010a). However, hybridization-based methods have several limitations, which include reliance upon existing knowledge about genome sequence, high background levels due to cross-hybridization (Okoniewski and Miller, 2006; Royce et al., 2007), and a limited dynamic range of detection due to both background and saturation of signals (Wang et al., 2009). Critically, hybridization-based cDNA microarrays and protein assays are limited to detecting particular (protein coding) mRNA transcripts and protein outputs of a gene, yet it is now evident that the vast majority of multi-exon genes undergo alternative splicing, which greatly increases the functional diversity of RNA and protein species (Li et al., 2014).

According to the recent human genome assembly (GRCh38), there are 10 annotated isoforms of the SAT1 gene, 5 of which are known protein-coding sequences (CDS). One isoform (SSAT, Ensemble name SAT1-001) is the mRNA transcript that codes for the 171 amino acid (aa) functional form of the SAT1 enzyme and was the target of microarray based and RT–PCR studies above (Sequeira et al., 2006; Klempan et al., 2009a,b; Le-Niculescu et al., 2013). A second isoform is an alternative splice variant (SSATX, ensemble name SAT1-002) that is targeted for nonsense mediated decay and plays a role in the regulation of SSAT mRNA levels and SAT1 protein levels via regulated unproductive splicing and transcription (RUST) (Pegg, 2008).

It is unknown whether this alternative splice variant (SSATX) is also altered in suicide and depression. Given that this isoform plays a role in SSAT expression via regulated unproductive splicing and translation (RUST), this could explain the low expression of SAT1 in suicide and depression. Furthermore, it is unknown whether other SAT1 isoforms (albeit with yet to be determined biological functions) are differentially expressed in suicide and depression. Heretofore such questions have been difficult to answer because traditional gene-level psychiatric genomic studies have not examined the expression of transcript isoforms: isoform-specific antibodies are generally not available, and experiments such as Western blot and chromatin immunoprecipitation combined with microarray technology (ChIP-chip) do not routinely provide resolution at the isoform level (Li et al., 2014). Although previous microarray-based SAT1 studies in depressed suicides included probesets targeting more than one SAT1 isoform (Sequeira et al., 2006) (for instance probeset 230333_at in (4) appears to target exon4 in the SSATX isoform, but was not included in their primary analysis), these previous studies did not aim to profile relative expressions of these distinct SAT1 isoforms. In the current study, RNA-seq was used to reconstruct all known SAT1 isoforms, as well as predict new ones, and profile their relative expression in suicide and depression. This approach allows for a more comprehensive evaluation of SAT1 expression at isoform resolution.

Next generation sequence (NGS) technologies allow deep sequencing of all RNA types, and hence provide information on the transcriptome at isoform-level resolution (Trapnell et al., 2012). Unlike hybridization-based approaches, RNA-Seq is not limited to detecting previously identified transcripts, but also novel transcripts including many tissue specific non-protein-coding RNAs. A third key advantage is that RNA-Seq has very low, if any, background signal because the cDNA sequences can be unambiguously mapped to unique regions of the genome, and it avoids technical issues inherent to microarray probe performance such as cross-hybridization, non-specific hybridization and limited detection range of individual probes (Zhao et al., 2014). RNA-seq has been shown to be highly accurate for quantifying expression levels, as determined using quantitative PCR (qPCR) (Nagalakshmi et al., 2008) and spike-in RNA controls of known concentration (Mortazavi et al., 2008).

SAT1 is arguably one of the most consistently implicated genes in depressed suicides based on cDNA microarrays (Sequeira et al., 2006; Fiori et al., 2009, 2010; Guipponi et al., 2009; Klempan et al., 2009b; Fiori and Turecki, 2010a,b, 2011; Le-Niculescu et al., 2013; Lopez et al., 2014), but it is unknown whether this gene is involved in MDD independently of suicide, which isoforms in particular are dysregulated, and whether the gene undergoes differential splicing in suicide and depression. In the current study, we applied NGS whole-transcriptome profiling (RNA-Seq) and examined SAT1 gene-level, isoform-level and exon-level expression in major depressive disorder (MDD) with and without suicide. We tested levels of the SSAT and SSATX isoforms as well as all other isoforms for which we found evidence in the transcriptome data. Here we use a hypothesis-driven approach and focus only on the SAT1 gene in order to reduce risk of false negatives due to overly conservative multiple comparisons correction. An exploratory (whole-exome) analysis of this sample is reported elsewhere (manuscript in preparation).

Posttranscriptional mechanisms have been proposed to account for low SAT1 expression in suicide or MDD via microRNA (miRNA) post-transcriptional regulation (Lopez et al., 2014). Therefore we also examined group differences in specific miRNA expression in a subset of RNA samples which were enriched for miRNA, and sought potential correlations and anticorrelations with SAT1 transcript expression. Furthermore, whereas previous studies examined non-depressed suicides in addition to depressed suicides (Sequeira et al., 2006, 2007; Klempan et al., 2009a,b), here we examined MDD-suicides (MDD-S) as well as MDD non-suicides (MDD). This third group allowed us to test the effects of major depressive disorder (MDD) alone (and not suicide) on SAT1 expression by comparing MDD-S vs. HC, as well as the effects of suicidal behavior in the context of MDD by comparing MDD-S vs. MDD. Diagnosis was by SCID I and all subjects were free of neuropathology and had negative toxicological screens for psychotropic medication and illicit drugs.

Materials and methods

Samples

Fifty-nine clinical samples were obtained from the brain collection of The Division of Molecular Imaging and Neuropathology, at the New York State Psychiatric Institute and Columbia University. All procedures for brain collection and psychological autopsy were approved by the applicable Institutional Review Boards. Psychiatric diagnosis in the suicides and absence of diagnoses in the controls were determined by the Structured Clinical Interview for DSM IV (SCID-I and II) as part of a psychological autopsy described elsewhere (Kelly and Mann, 1996). All subjects were selected because they died suddenly to avoid metabolic complications related to agonal issues. All brains were free of gross neuropathology and had negative brain toxicology for psychotropic, illicit psychoactive drugs and neurotoxic drugs. There were no diagnoses of Alcohol or Drug Use Disorders. Antemortem medication history for three months ruled out recent exposure to psychotropic medication and confirmed results of comprehensive peripheral toxicology. Brain samples were dissected from Brodmann Area 9 as previously reported (Sibille at al., 2004).

We applied gene-level (DESeq2 (Love et al., 2014), Cufflinks/Cuffnorm (Trapnell et al., 2013) workflow #1, Supplementary Fig. 1), isoform-level (Cufflinks/Cuffnorm workflow #1) and exon-centric (DEXSeq (Anders et al., 2012)) analyses to examine differential expression between 21 MDD-S (subjects with major depressive disorder and suicide), 9 MDD (subjects with MDD and no suicide), and 29 sudden death healthy control (HC) subjects with no MDD and no suicide (59 samples total). Small RNA analysis (miRNA differential expression) uses an age and sex matched subset which included 9 MDD-S, 9 MDD and 9 HC. Note that the main results reported throughout the text derive from the full dataset of 59 samples (DESeq2 and DEXSeq analyses, Cufflinks/Cuffnorm Workflow #1, Supplementary Fig. 1, left pathway), which all adjust for age and gender (and RIN scores in select additional analyses in a subset of 57 samples). The only exceptions are the reported results based on Cufflinks/Cuffdiff (Trapnell et al., 2013) Workflow #2, (Supplementary Fig. 1, left pathway), in which a subset of 17 MDD-S and 17 HCs were used in age and gender matched analyses using Cuffdiff2 (Cuffdiff2 does not yet allow adjustment for nuisance covariates such as age and gender). These results are reported for further corroboration of

the results using an algorithm specifically developed to address statistical challenges and measurement error related to RNA-seq data in estimating abundance and differential expression at isoform-level resolution (Trapnell et al., 2013).

RNA sample preparation and sequencing

RNA was extracted from the dorsal prefrontal cortex (BA9) using previously established protocols (Sibille et al., 2004). Total RNA extractions were conducted according to the guidelines recommended by the NIH Roadmap Epigenomics Mapping Consortium (REMC). We used the Ambion's mirVana miRNA isolation kit (#. AM1560, Life Technologies, Carlsbad, CA, USA) to isolate total RNA from postmortem brain tissue (Brodmann Area 9). The coding RNA library was generated using the TruSeq Stranded Total RNA Sample Prep kit (Illumina, San Diego, CA, USA) which includes rRNA depletion and chemical fragmentation. The miRNA libraries were generated using the TruSeq Small RNA Sample Prep Kit (Illumina, San Diego, CA, USA) following the manufacturer's recommendations (no rRNA depletion or chemical fragmentation was performed).

Paired-end, strand specific sequencing for total RNA was performed on Illumina HiSeq 2500 with 100 bp read lengths, while single-end sequencing was performed for microRNA on Illumina MiSeq with 50 bp read lengths. For each clinical sample, raw RNA-seq reads were aligned and mapped to the Ensembl GRCh37 human reference genome and assembled using Tophat v2.0.9. resulting in BAM files for each of 59 samples. Between 17,000,000 and 57,000,000 reads were obtained for each sample, and ~75–90% of reads were successfully mapped to the genome for each sample. Of these reads, ~10–15% aligned to multiple genomic loci. Read statistics for each sample (subject) are listed in Supplementary Table 1.

Gene-level and isoform-level differential expression analyses

Cufflinks/Cuffnorm—Preprocessing and differential expression analyses used Cufflinks (v.2.2.0) software (Trapnell et al., 2012). These assemblies (BAM files from tophat2 alignment) were merged together to provide a uniform basis for calculating gene and transcript expression in each sample using Cufflinks v2.2.0. Cuffquant v2.2.1 (4237) was then used to quantify gene and isoform-level expression values, and the output CXB files were then input to Cuffnorm v2.2.1 (4237) which produced normalized expression values for each sample (Supplementary Fig. 1, workflow #1). Default settings for Cufflinks v2.2.0 were used (i.e. cufflinks –p 8 –o ${1}_clout ${1}_thout/accepted_hits.bam, ${1} refers to sample name). We note that cuffcompare predicted only one novel transcript (code j) near the SAT1 gene locus, however across the whole genome it predicted 147,016 novel transcripts not included in the human GRCh37 assembly (i.e. number of transcripts with code “j”). Cuffnorm produced normalized expression values (normalized FPKM) which were subsequently analyzed using SPSS v20.0.0 (see below). The reads and the merged assembly were also used to calculate expression levels and the effect size and statistical significance of observed differences using Cuffdiff v.2.2.1 (4237), (Supplementary Fig. 1, workflow #2).

Normalized FPKM (Fragments Per Kilobase Of Exon Per Million Fragments Mapped) expression values (from Cuffnorm) were submitted to linear mixed effects models (LMMs)

in order to assess and adjust for the effects of age and sex, to account for correlations in isoform values from each sample, and to test for group * isoform interaction effects (Supplementary Fig. 1, workflow #1). This workflow was applied as it was more computationally efficient than the traditional Cuffdiff analysis (workflow #2) and allowed for the comparison of all three groups in a single model while also including additional covariates such as age and sex. Although workflow #2 only used an age and sex-matched subset of our data, we report results from this workflow #2 in order to confirm that the traditional Cuffdiff analysis, that calculates p-values very differently compared with workflow #1 (i.e. based on explicit sampling from the beta negative binomial distribution (13)), yields results consistent with workflow #1. Age and sex effects were included in all LMMs as nuisance covariates consistent with previous related studies (Klempan et al., 2009a,b). For gene-level model (no correlated, repeated measures), an LMM effectively reduces to a general linear model, with fixed effect group and age and gender as covariates. For isoform, TSS and CDS level LMM, isoform ID (TSS/CDS) was included as an additional fixed factor, while a random subject factor was also included.

Although LMMs are generally robust to deviations from normality in the independent variable, for posthoc pair-wise comparisons we applied non-parametric Mann–Whitney U to test for difference in median expression values between each group. Relative to the two-sample t-test, this test is more robust to the presence of outliers and is considerably more efficient when assumptions of normality do not hold. For sample sizes greater than approximately 20–25, the Mann–Whitney U follows a distribution, and U scores can be converted to z-scores (done by default in SPSS). Therefore in the text we report z-scores and p-values, and in Tables 2 and 3 and we additionally report the U statistics and medians in each group. All reported p-values are two-tailed. We applied Benjamini–Hochberg False Discovery Rate (FDR) (Benjamini et al., 2001) correction to control the FDR among 30 p-values resulting from group pair-wise isoform-level comparisons (Table 4). There was no evidence of sex/age by isoform interactions, Akaike Information Criteria, (AIC) increased when these terms were included in the model, and they did not explain significant variance in the overall model ($F = 0.61/1.46$, $p = 0.79/0.17$, data not shown). Therefore we did not adjust for these covariates prior to the primary pair-wise non-parametric group comparisons for each isoform. The case was similar for the LMMs which included TSS groups and protein coding (CDS) isoforms. However results for select comparisons are reported in the main text after adjusting for age and sex. Additional variables that could potentially confound transcript expression results, such as PMI, pH and RNA integrity (RIN), were not different among the groups (see Table 1). However, additional analyses which covaried for RIN score were conducted in order to rule out RNA quality as a potential confound.

At the time of writing of this manuscript, the most recent assembly which is compatible with Cufflinks was the GRCh37 reference annotation (http://cole-trapnell-lab.github.io/cufflinks//igenome_table/index.html), and the GRCh38 annotated genome for Tophat/Cufflinks was not yet available. However, the assembled SAT1 transcripts were manually compared to the updated GRCh38 genome assembly at useast.ensemble.org/Homo_sapiens and only one additional transcript is included in GRCh38 that is not included in GRCh37 (SAT-201 in Table 2). Therefore the current Ensembl Genome Assembly (GRCh38) contains only one additional protein coding transcript (SAT1-201) that is not included in the previous version

(GRCh37). Isoforms IDs corresponding to transcripts in the GRCh38 assembly are shown in Supplementary Fig. 2.

DESeq2—In addition to Cufflinks/Cuffnorm, we used DESeq2 v.1.6.3 (Love et al., 2014) to assess differential expression between groups. In contrast to Cufflinks/Cuffnorm, DESeq2 ignores ambiguously mapped reads, and applies a Generalized Linear Model (GLM) to count data assuming a negative binomial distribution. This approach allows for adjustment of nuisance effects (here age, gender and RIN score) and uses shrinkage estimation for dispersion and fold changes (pooling information both across samples and across all genes) to improve stability and interpretation of estimates. However, a disadvantage is that DESeq2 does not attempt to calculate relative expression values for isoforms. Therefore, primary gene-level results are based on DESeq2, while isoform-level results are based on Cufflinks/Cuffnorm. We also report gene-level results based on Cufflinks/Cuffnorm to facilitate comparison with isoform-level results using FPKM (as opposed to normalized counts as with DESeq2).

For each BAM file resulting from Tophat2, read counts per gene were summarized using summarizeOverlaps function in the GenomicAlignments R library (Lawrence et al., 2013) and a transcript database derived from the GRCh37 human genome assembly. For each gene, read counts were fit to a GLM with log link function (~age + sex + condition), where condition was the factor of interest with three levels: MDD-S, MDD and CON. A likelihood ratio test (LRT) was performed, whereby the likelihood of the above model given the data was compared to a reduced model which excluded condition factor. The LRT was performed in order to test all three levels at once (equivalent to an omnibus F-test which avoids multiple tests when comparing each group to the other). In order to estimate log2 fold changes (LFC, or log2 FC) for each pairwise group comparison, contrasts of individual coefficients and associated p-values were generated. For a comparison of group 1 vs. group 2, fold change (FC) is defined as the ratio of group 2 vs. group 1, i.e. if the value in group 1 is A and the value in group 2 is B, the FC is B/A. *DESeq2* reports the standard error for each shrunken LFC estimate, obtained from the curvature of the coefficient's posterior at its maximum. For significance testing in group comparisons, *DESeq2* uses a Wald test: the shrunken estimate of LFC is divided by its standard error, resulting in a z-statistic, which is compared to a standard normal distribution. For significant testing in the LRT, an analysis-of-deviance between the full model (with the condition factor) and the reduced model is computed by means of a χ^2 likelihood-ratio test (Anders et al., 2012).

Exon-level differential expression analyses

We applied DEXSeq v1.12.2 (Anders et al., 2012), an “exon-centric” analysis which explicitly tests for differential exon usage by comparing a fully specified and reduced GLM using a likelihood ratio test (LRT, see methods for DESeq2 analysis). For each gene, the read counts that map to unique splicing events (i.e. exon deletions, insertions or alternate start/end sites, here referred to as “exon”) are modeled as ~ sample + exon + exon:condition + age:exon + sex:exon in the full model, and ~ sample + exon + age:exon + sex:exon in the reduced model. Note that the “sample” term models the overall gene expression value for each sample (i.e. read counts mapping to the whole gene), while “exon” models the

deviation about this mean for each exon (i.e. unique splicing event) in the gene. Age:exon and sex:exon co-vary for interaction effects of age and gender with “exon”. Note that age and sex or not included as main effects since overall gene expression is modeled separately for each sample, thus absorbing any variance attributable to inter-individual differences. Thus this approach explicitly tests for evidence of differential exon usage between groups (here modeled using the exon:condition interaction term) after accounting for group differences in overall gene expression.

Small RNA-seq analysis

For microRNA, the resulting FastQ files (adaptor sequences already trimmed) were first converted to FASTA format and processed according to the Small RNA Analysis pipeline in CLC Genomics Workbench version 7.5 (http://www.clcbio.com/products/clc-genomics-workbench/) using default settings (i.e. mature length variants were allowed 2 additional or missing upstream and downstream base-pairs and 2 mismatched base-pairs, and alignment was strand-specific) Unique reads were extracted and counted and subsequently merged and annotated according to known miRNA species included in miRBase Release 21. Total counts for each miRNA were divided by the total library size to arrive at an expression value for each sample. These expression values were then submitted to one-way Kruskal–Wallis analysis of variance, a non-parametric version of 1-way ANOVA, with three levels: MDD-S, MDD and HC. Spearman rank correlation was also applied to examine potential relationships with SAT1 isoform 5 and 6 expression levels.

Isoform co-expression matrices

Pairwise correlations (Spearman's rank coefficient) across all 59 samples were computed among the 10 SAT1 isoforms. In addition, potential differences in these correlations among groups (HC vs. MDD-S) were tested for by constructing a separate matrix for HC (N = 29) and one for MDD-S (N = 21), and then computing the difference matrix (HC–MDD-S). Significant differences were inferred non-parametrically by comparison against a null (noise) distribution in which difference matrices were computed while shuffling group labels (10,000 iterations). Set-level p-values were determined by comparing the number of absolute differences above a particular threshold (here 0.6, 0.7 and 0.8) in the real dataset (using actual HC and MDD-S labels) vs. the number of supra-threshold absolute differences obtained in the null dataset.

Results

Demographics

A total of 29 healthy controls (HC), 21 MDD-S and 9 MDD samples were included in the analysis. Mean age and gender ratios were not different between groups (see Table 1). In addition, effects of age and sex on SAT1 expression were assessed and included as nuisance covariates (see below).

Transcriptome annotation of the SAT1 gene

Reads from each sample were first assembled with Cufflinks (to enable discovery of new transcripts) and subsequently mapped to the GRCh37 reference genome. Cufflinks identified

10 transcripts (iso-forms) from the SAT1 gene at X:23791108–238045309. Cuffcompare was used to compare the assembled transcript fragments with the GRCh37 reference annotation and revealed that 9 isoforms were exact matches with known variants (Table 2, class codes "="), and one was identified as a potentially new splice variant (Table 2, class code "j").

SAT1 gene-level expression in MDD and suicide

With DESeq2, we observed significant gene-level differences in SAT1 expression (Table 3, Fig. 1). In particular, SAT1 expression was low in both MDD-S and MDD groups relative to CON (MDD-S vs. CON, log2 FC = −0.41, p = 0.002; MDD vs. CON, log2 FC = −0.47, p = 0.002), while there was no evidence for suicide-specific effects (MDD-S vs. MDD, log2 FC = 0.06, p = 0.70). The DESeq2 likelihood ratio test (LRT, testing for overall effect of group status) remained significant after covarying for RIN score (stat = 10.34, p = 0.0056). Although DESeq2 applies a scaling factor which normalizes counts by library size, we conducted an additional analysis which excluded the upper quartile of samples with the highest library sizes. Results remained significant in this downsampled analysis (22 CON, 16 MDD-S, 6 MDD, χ^2 = 11.5, p = 0.003), confirming that differences in library size do not account for the observed effects.

Gene-level expression differences as assessed through Cufflinks/Cuffnorm were consistent with results obtained with DESeq2 above. There was an effect of group on SAT1 gene-level expression ($F_{2,54}$ = 3.3, p = 0.04) and no evidence of age or sex effects (age: $F_{1,54}$ = 0.19, p = 0.67, sex: $F_{1,54}$ = 0.20, p = 0.7, Supplementary Table 2). RNA quality (as measured through RIN) did not account for these differences, since SAT1 group differences remained after covarying for RIN, age and sex (new $F_{2,51}$ = 3.04, p = 0.056, note this analysis included 57 samples since one MDD-S and one HC were missing RIN scores.) Non-parametric (Mann–Whitney U) pair-wise comparisons between each group indicated greater SAT1 expression in HC relative to both MDD-S (z = 2.64, p = 0.008) and MDD (z = 2.31, p = 0.02, Table 4).

SAT1 isoform-level expression in MDD and suicide

Normalized FPKM expression values (see Materials and methods) for the ten SAT1 isoforms were entered into a repeated measures linear mixed-effects model with fixed factors group, isoform ID and group × isoform interaction, a random subject factor, and age and gender as nuisance covariates. There was a main effect of isoform ID ($F_{9,97}$ = 77.9, p < 0.001) and group ($F_{2,76}$ = 10.5, p < 0.001), as well as a significant group * isoform interaction ($F_{18,97}$ = 2.6, p = 0.001), indicating that isoforms were differentially expressed between the groups (Table 4, Supplementary Table 2).

Isoform 5 exhibited the highest overall expression (Fig. 2A, #5, black dots) and was higher in HCs relative to MDD-S (z = 2.31, p = 0.02, Table 4), showed a trend towards being higher in HCs relative to MDD (z = 1.6, p = 0.11), and did not differ between MDD-S and MDD groups (z = −0.34, p = 0.76). These differences remained after adjusting for sex and age for HCs vs. MDD-S (z = 1.98, p = 0.048), but not for HCs vs. MDD (z = 0.98, p = 0.33), with similar results after adjusting for RIN score (HCs vs. MDD-S, z = 1.9, p = 0.052 and HCs

vs. MDD , z = 1.12, p = 0.24). This isoform (isoform 5, Fig. 2B, black) corresponds to ensemble SAT1-001 (henceforth referred to as SSAT) and is the mRNA transcript responsible for coding the functional form of the 171 amino acid SAT1 protein having the Uniprot ID P21673 (Casero and Pegg, 1993). Although the Mann–Whitney U test is robust in the presence of outliers, we nevertheless verified that HC vs. MDD-S differences for this isoform remained statistically significant even after removing outliers (seven asterisks and circles in Fig. 2C, new z = −2.15, p = 0.032). For additional confirmation, we ran HC vs. MDD-S comparison using the traditional Cuffdiff v.2.20 differential expression pipeline (workflow #2, Supplementary Fig. 1, left side) on an age and gender matched subset of the full sample (N = 17 each group, HC mean FPKM = 29.9, MDD-S mean FPKM = 22.3, log2 (fold-change) = −0.42, one-tailed p = 0.044).

The transcripts with the second and third highest overall expression (Isoforms 3 and 9) exhibited ~ 5 fold lower expression relative to iso-form 5. Isoform 3 did not differ between MDD-S and HC groups (z = −0.46, p = 0.65), but was lower in MDD relative to both MDD-S (z = −3.42, p < 0.001) and HC groups (z = −3.42, p < 0.001). This is a known protein-coding transcript (Table 2), however it's functions are largely unknown and it does not have a Uniprot ID. Isoform 9 (non-protein coding transcript) was higher in HC relative to both MDD-S (z = 2.49, p = 0.012) and MDD (z = 2.28, p = 0.021).

Isoform 6 (SAT1-002, also known as SSATX) corresponds to a well-characterized splice-variant of the SAT1 gene involved in regulated un-productive transcript and translation (RUST) and which is targeted for non-sense mediated decay (Nikiforova et al., 2002; Hyvönen et al., 2012). Overall, expression of this isoform was ~25 fold lower than iso-form 5. Nevertheless, group differences were detected in directions similar to isoform 5 (HC vs. MDD-S: z = 2.31, p = 0.02, HC vs. MDD: z = 1.36, p = 0.18, MDD-S vs. MDD: z = −0.48, p = 0.66, Fig. 2D). The HC vs. MDD-S differences remained after adjusting for sex and age (z = 2.3, p = 0.02), with similar results after adjusting for RIN score (z = 2.6, p = 0.008). An additional HC vs. MDD-S comparison using the traditional Cuffdiff v.2.20 differential expression pipeline (workflow #2, Supplementary Fig. 1, left side) on an age and gender matched subset of the full sample was consistent with this result (N = 17 each group, HC mean FPKM = 1.42, MDD-S mean FPKM = 0.42, log2 (fold_change) = −1.73, p = 0.054).

SAT1 transcription start site (TSS) and protein coding (CDS) differences in depression and suicide

The Cufflinks software groups together isoforms of a gene that have the same TSS; these TSS groups represent isoforms that are all derived from the same pre-mRNA. Therefore group differences in relative abundances of particular TSS groups would suggest that regulation at the transcription level could (at least partially) account for the observed lower expression of SAT1 in MDD-S. Cufflinks identified 6 different TSS groups for the SAT1 gene (Table 2, Supplementary Fig. 3, right panel). One TSS group (TSS 2) contained five isoforms that included isoforms 5 and 6 mentioned above, while the other five isoforms were grouped into separate TSS groups. Note that in the case of SAT1 the only difference of the TSS analysis with the previous, isoform-level analysis is that expression levels for isoforms 2, 3, 4, 5 and 6 are pooled together into a single TSS, in order to more directly assess

differences in expression according to TSS. Applying a similar mixed-effects model as above (with the exception that isoforms expression levels were grouped according to their 6 TSS) revealed a group * TSS interaction ($F_{10,98.3} = 2.55$, $p = 0.009$, Table 4, Supplementary Table 2). As expected, TSS 2 (which represent expression levels for five isoforms) was the most abundant and exhibited greatest overall group differences relative to the other TSS groups (i.e. isoforms): HC vs. MDD-S ($z = 2.6$, $p = 0.009$) and HC vs. MDD ($z = 2.28$, $p = 0.021$, Supplementary Table 3, Supplementary Fig. 3).

Similarly, Cufflinks identifies transcripts with annotated coding sequences (CDS) and allows for analysis restricted to differential output of protein-coding sequences. The SAT1 gene contains four known CDS (Table 2, Supplementary Fig. 3, right panel). Applying a similar mixed-effects model as above (with the exception that only the four isoforms with known CDS were included in the model) revealed a significant group * CDS interaction ($F_{6,87.4} = 3.98$, $p = 0.001$, Table 4, Supplementary Table 2). As already stated above, CDS 1 (equivalent to isoform 5, the primary SAT1 protein coding transcript) was most abundantly expressed overall and was lower in MDD-S relative to HC ($z = -2.31$, $p = 0.02$). In addition, CDS 4 (equivalent to isoform 2) was also low in MDD-S relative to HC ($z = -2.4$, $p = 0.015$). In contrast, CDS 3 (equivalent to isoform 3) was low in MDD relative to both HC ($z = -3.4$, $p < 0.001$) and DS ($z = -3.4$, $p < 0.001$).

Isoform co-expression

Correlation of gene (isoform) expression may be indicative of shared functions and/or regulatory mechanisms (Lee et al., 2004). Thus, we determined pair-wise Spearman rank correlations between each isoform across all 59 samples. Eight of 45 unique isoform pairs exhibited positive correlations that survived Bonferroni correction for multiple comparisons (Supplementary Fig. 4, red). Isoform 5–isoform 9 (a non-coding transcript) exhibited the strongest correlation ($R = 0.63$, $p < 0.0001$). Isoform 5 was also significantly correlated with isoform 1 ($R = 0.582$, $p < 0.0001$) and isoform 2 ($R = 0.427$, $p = 0.001$).

We also sought differences in co-expression (correlations) between groups, as this could potentially indicate an altered functional relationship in MDD or MDD-S. However, no significant group differences in pair-wise isoform correlations were observed (all set level p-values were 0.5 or greater, see Materials and methods).

Differential exon-used in suicide and depression

For exon-level analysis, we applied DEXSeq (Anders et al., 2012) (see Materials and methods) in order to test for differential exon usage (i.e. differential splicing) of SAT1 in MDD with or without suicide. After adjusting for overall differences in SAT1 gene expression, none of the 24 exons were significant after FDR correction (Fig. 3, all adjusted p-values > 0.8, Supplementary Table 4). Only one bin (E011, which uniquely corresponds to isoform 3) exhibited some evidence for a trend-level effect for differential exon usage after adjusting for overall differences in gene-level expression ($p = 0.053$ uncorrected, Fig. 3, Supplementary Table 4).

MicroRNA expression in suicide and depression

Previous work has suggested reduced SAT1 expression in MDD-S is influenced by particular species of miRNA (small, non-coding RNA molecules that play an important role in the post-transcriptional regulation of mRNA) (Lopez et al., 2014). Four miRNA species (mir139, mir-34c, mir-195 and mir-320c) were hypothesized to be potential regulators of SAT1 mRNA based on target prediction analysis, differential (higher) expression in depressed suicides, and correlation with SAT1 mRNA. Therefore, we sought group differences in expression of these four miRNA species and correlation with isoform 5 and 6 expression values. Library sizes and total counts of each miRNA species are listed in Supplementary Table 5. Group differences in expression values of these four miRNAs were not detected (3-way Kruskal–Wallis: mir-139, $p = 0.7$; mir-34c $p = 0.67$; mir-320c $p = 0.56$; mir-195 $p = 0.94$, Table 5, left half). We also did not detect any correlations between these miRNAs with SAT1 isoform 5 and 6 expression levels (all p-values > 0.18) (Table 6), except for a trend-level positive correlation between mir-195 and isoform 5 ($R = 0.38$, $p = 0.06$) and negative correlation between mir-195 and isoform 6 ($R = -0.37$, $p = 0.07$).

Discussion

Low brain SAT1 expression in MDD

We found low SAT1 in MDD vs. HC but no differences in gene-level SAT1 expression in MDD-S vs. MDD. These findings indicate a relationship with MDD and not suicide and are consistent with a previous study (Klempan et al., 2009b) that also did not observe differences in SAT1 brain expression between non-depressed suicides and controls, and a study that found lower SAT1 expression in BA46 in depressed suicides vs. non-depressed suicides (Klempan et al., 2009a). Both studies suggest that low brain SAT1 expression may be specifically involved in the path-ophysiology of major depression, and not suicide. A recent study found *elevated* SAT1 expression in the blood of bipolar suicidal ideators and suicides vs. non-suicidal patients (Le-Niculescu et al., 2013). These findings are in the opposite direction of our results and previous reports and may be due to this study examining a different psychiatric population (bipolar disorder vs. MDD) and RNA from a different source (blood vs. brain).

We found a significant group × isoform interaction when restricting analyses to protein coding (CDS) mRNA. Because all four protein-coding (CDS) mRNAs have the same TSS (and hence derive from the same premRNA), differential CDS expression among the groups suggests that additional post-transcriptional processes (alternative splicing, microRNA regulation, RNA editing etc.) contribute to low SAT1 expression in MDD-S. In addition, we found that isoform 3 (SAT1-003, a transcript known to code for a truncated, 120 aa protein) was lower in MDD vs. both HC and MDD-S. That it was not low in MDD-S suggests a potential interaction between suicide and MDD regarding expression of this isoform. Further studies are needed to understand the biological function of this SAT1 protein isoform and its potential role in major depression.

Low SSAT and SSATX isoform expression in major depression and suicide

The expression of rate-limiting enzymes of polyamine metabolism, including SAT1, is regulated by multiple mechanisms at transcriptional, post-transcriptional, translational and post-translational levels (Pegg, 2008; Hyvönen et al., 2012). The SAT1 enzyme was discovered during studies of an increase in putrescine and a loss of spermidine and spermine in rodent liver due to carbon tetrachloride (Hölttä et al., 1973; Matsui and Pegg, 1980), and the cDNA was first cloned in human lung carcinoma cells which exhibited increased SSAT expression in response to polyamine analogs (Casero et al., 1991). SAT1 transcription is induced by polyamines via binding of polyamine-modulated factor 1 and Nrf-2 to the SAT1 promoter region (Wang et al., 1999, 2001). In addition, SSAT mRNA is stabilized and its translation is accelerated by polyamines (Butcher et al., 2007; Pegg, 2008), and SAT1 enzyme protein is protected from degradation by polyamine-induced conformational change (Coleman et al., 1995). In addition to these mechanisms, SAT1 expression is regulated by RUST (Hyvönen et al., 2006) in which production of isoform 6 (SSATX), that contains a premature stop codon in the additional exon 4, targeting the mRNA for rapid degradation via nonsense-mediated mRNA decay (NMD) (Wagner and Lykke-Andersen, 2002). In the case of SAT1, RUST appears to be mediated by intracellular levels of polyamines: high polyamine levels promote production of SSAT mRNA, low polyamine levels promote the production of SSATX mRNA (Hyvönen et al., 2006). Mutagenesis and knockdown experiments demonstrate that silencing SSATX via small interfering RNA increases SAT1 activity, suggesting RUST contributes to the regulation of SAT1 activity (Hyvönen et al., 2012). It appears SSATX is present at low level background and its level is dynamically regulated by polyamine levels. Further studies are required to understand the exact mechanisms whereby polyamines affect SSATX production (Hyvönen et al., 2012).

A posttranscriptional mechanism has been proposed to account for low SAT1 expression in suicide or MDD via micro RNA (miRNA) post-transcriptional regulation via four miRNA species (Lopez et al., 2014). However, we did not observe differential expression in these miRNAs between MDD-S, MDD and HC, but there was a trend-level correlation/anticorrelation between mir-195 and isoform 5/6 across all samples. It is possible that differences in methodology (RNA-seq vs. real-time PCR), unaccounted-for variables in our population group, and/or limited sample size influenced our ability to detect differences in miRNA expression previously reported (Lopez et al., 2014).

We observed a 2–3 fold decrease in SSATX expression in the MDD-S relative to HC. At low polyamine levels, SSATX is normally produced, and when polyamine levels are too high, polyamines or analogs inhibit the inclusion of exon 4 (the exon containing the stop codons targeting the RNA for NMD) (Pegg, 2008), thus producing the 171 amino acid protein-coding SSAT mRNA. That SSATX, which shares the same TSS, is under shared control (by polyamine levels) with SSAT, and is constitutively lower in MDD-S, all suggest that regulatory mechanisms at the transcription-level, or further upstream, may also account for low SAT1 expression and activity in MDD and suicide.

Stressful live events are risk factors for suicidal behavior and MDD (Bao et al., 2008; Klempan et al., 2009b). The synthesis of polyamines and their inter-conversion form the basis for the polyamine stress response (PSR), a stress reaction pathway involving increases

in polyamine metabolism initiated by exposure to acute or chronic stressors (Casero and Pegg, 1993; Gilad et al., 1995; Klempan et al., 2009b). Changes in polyamine synthesis and catabolism may be transient or long-lived, with effects on apoptosis, neuroprotection, transcription and translation, and regulation of andenylate cyclase and ion channels (NMDA, voltage-gated Ca^{2+}) (Gilad and Gilad, 2003; Jänne et al., 2004; Klempan et al., 2009b). The PSR is a dynamic process that varies with the type, intensity, and duration of stressors, and indicating that the PSR is an adaptive mechanism in reaction to stressful events (Gilad and Gilad, 2003). Under persistent stressful conditions, however, the PSR may be maladaptive as may be reflected by polyamine accumulation in the brain (Gilad and Gilad, 2003). If so, chronically higher levels of polyamines spermine and spermidine could potentially explain lower SSATX expression in MDD-S, since this transcript is produced when intracellular polyamine levels are low (Pegg, 2008). Alternatively, lower SSATX could reflect a compensatory mechanism to produce more SSAT (via reduced RUST) in response to lower SSAT output (via post-transcriptional mechanisms such as in Lopez et al., 2014).

Differential SAT1 splicing in MDD

The lack of strong evidence for differential splicing is consistent with the observation that over half of SAT1 isoforms (including SSAT and SSATX) were in the direction of lower expression in MDD-S and MDD groups relative to HC (through to varying degrees of effect size and statistical significance), with the exception being isoform 3, which was significantly lower in MDD relative to both HC and MDD-S groups (and not different between MDD-S and HC groups, Table 5, Supplementary Fig. 3). Interestingly, isoform 3 was the only source of reads (from SAT1 gene) that contributed to count levels in bin E011 in the DEXSeq analysis (Fig. 3) which is the bin showing trend-level evidence for differential exon usage ($p = 0.053$ uncorrected, Supplementary Table 4). Taken together, the above isoform and exon-level analyses both suggest preliminary evidence for differential SAT1 splicing involving isoform 3 in MDD, but not suicide. Replication of this result in a larger MDD sample size would be required to confirm this hypothesis.

Differences in non-coding SAT1 RNA isoforms

We observed less expression of isoform 9 (a non-coding transcript) in MDD-S vs. HCs. Unlike proteins or microRNAs, non-coding RNA (ncRNA) functions cannot currently be inferred from sequence or structure (Mercer et al., 2009). Potential functions of ncRNA include chromatin remodeling, transcriptional regulation and post-transcriptional regulation such as splicing, editing, transport, translation and degradation, all of which implicate ncRNA in disease etiology (Mercer et al., 2009; Taft et al., 2010). Interestingly, this isoform exhibited the highest correlation with isoform 5 (SSAT) expression across the whole sample ($R = 0.63$, $p < 0.001$, Supplementary Fig. 4). That this SAT1 ncRNA is highly correlated with SSAT, and is also low in MDD-S and MDD, suggests that it may be involved in transcriptional regulatory control of SAT1 and could in part explain lower SAT1 levels in MDD.

Similarly, we observed lower expression of isoform 2 (mRNA coding a 150 aa protein product with unknown function) in MDD-S and MDD. This isoform was also correlated with isoform 5 across the whole sample ($R = 0.43$, $p = 0.001$). It was previously

hypothesized that translation of the SSATX (isoform 6) mRNA could generate a truncated SSAT protein of only 71 aa (described in Kee et al., 2004) which might theoretically inactivate SSAT by forming heterodimers, providing another means by which SSAT activity would be low in cells with low polyamine levels (Pegg, 2008). In other genes, it was recently demonstrated that co-expression of the human cannabinoid receptor coding region splice variants (hCB) affects the function of hCB receptor complexes (Bagher et al., 2013). It is thus possible that this isoform, and/or either of the other two known SAT1 protein-coding isoforms, could provide an additional layer of control of SSAT activity at the post-translational level, via interactions with the SSAT enzyme protein. Further studies are required to explore the above hypotheses.

Conclusion

We used RNA-seq to quantify gene expression and resolve isoform-level differences in SAT1 gene expression in major depression with and without suicide. Gene-level analyses indicate that SAT1 is lower in both MDD-S and MDD, suggesting a role for low brain SAT1 expression in MDD independent of any deficit in relation to suicidal behavior (Table 7). Isoform-level analyses show that differential expression is most pronounced for specific isoforms including SSAT (transcript coding for the 171 aa functional SAT1 enzyme) and SSATX, an alternative splice variant involved in SAT1 RUST, which are both lower in MDD-S. We did not observe evidence for differential exon usage (splicing) of SAT1 in depression and suicide after adjusting for overall differences in gene expression, nor evidence for differential expression of putative miRNA post-transcriptional regulators of SAT1 (Lopez et al., 2014). Taken together, these results are consistent with a model in which transcriptional regulation (such as those involved in SAT1 RUST) is a primary cause for low SAT1 expression. Additional coding and non-coding transcripts are low in MDD-S and/or MDD, and are also correlated with SSAT expression, suggesting their involvement in additional regulatory mechanisms of SSAT activity that may explain its low expression.

Limitations

SAT1 down-regulation in the cortex of MDD cases who died by suicide (Klempan et al., 2009b), does not confirm a causal relationship between low SAT1 expression and MDD or suicidal behavior. Our knowledge of ncRNA and isoform-level protein function is limited, but progress is being facilitated by emerging computational and bioinformatics approaches (Li et al., 2014). Future studies will be required to better understand the biological function of SAT1 isoforms and their role in major depression and suicidal behavior.

Although RNA-seq has been shown to be highly accurate and has been well validated by others (Mortazavi et al., 2008; Nagalakshmi et al., 2008; Zhao et al., 2014), isoform-level findings reported here should be regarded as exploratory and provisionary until they can be replicated in independent samples. Due to the complexity, labor and time involved in designing isoform-specific primers, quantitative PCR (qPCR) validation of the isoform-level findings is outside the scope of the current study. In addition, qPCR uses an exponential amplification system that introduces different types of biases and variability that are not observed with direct RNAseq, and it is arguable that RNAseq should be the gold standard

for differential expression microarray and qPCR analyses (Costa et al., 2013; SEQC/MAQC-III Consortium, 2014).

We examined putative SAT1 small RNA (miRNA) regulators identi-fied by Lopez et al. (2014). This study performed a comprehensive search of five miRNA target prediction databases (microRNA.org, TargetScan, miRWalk, RNA Hybrid and RNA22) which yielded over 200 miRNAs predicted to target SAT1. This list was narrowed down to 10 by including only miRNAs that were expressed in the human brain, that were predicted by all 5 databases, and that also targeted the SMOX gene. Our lack of positive findings in attempting to replicate miRNA expression results from Lopez et al. may reflect limited sensitivity to detect differences owing to hidden variability in our sample and methods (small RNA-seq vs. RT-PCR). It is possible there are other miRNA species involved in SAT1 regulation that were not detected in Lopez et al. Only two of the five databases used in Lopez et. al. have been updated since 2012. Although other miRNA databases are being continuously updated, given the number of total miRNA prediction databases (over ten according to http://en.wikipedia.org/wiki/MicroRNA_and_microRNA_target_database), and given the relatively high number of miRNA that would be predicted to bind SAT1 based on Lopez et al. (over 200), exploring additional miRNA that may target SAT1 was considered outside the scope of the current study.

Evidence suggests that SSATX can be up-regulated by hypoxia (Kim et al., 2005). Since we observed *reduced* expression of this iso-form in MDD-S relative to HC, hypoxia during suicide is unlikely to have played a role in these findings. Hypoxia is also unlikely to account for increase expression in the HC, since only sudden death controls were included.

Despite accumulating evidence implicating SAT1 in depression and/or suicide (Sequeira et al., 2006; Fiori et al., 2009, 2010; Guipponi et al., 2009; Klempan et al., 2009b; Fiori and Turecki, 2010a,b, 2011; Le-Niculescu et al., 2013; Lopez et al., 2014) including the current report, the mechanistic relationship between SAT1 expression, polyamine contents with depression and suicide remains unclear. Constitutively lower brain SAT1 expression in MDD could either be a result of, or directly contribute to, maladaptive polyamine stress response (PSR) during chronic stress (Gilad and Gilad, 2003). Transgenic mice that either under-express or over-express SAT1 mice have been generated and proposed as models for type 2 diabetes (Alhonen et al., 1999, 2009; Niiranen et al., 2006). Future behavioral studies could examine depression-related endophenotypes (i.e. anhedonia, behavioral despair, neuroendocrine disruption, and anxiety-related behavior) in these transgenic mice in order to test whether SAT1 expression may play a causal role in MDD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Alhonen L, Pietilä M, Halmekytö M, Kramer DL, Jänne J, Porter CW. Transgenic mice with activated polyamine catabolism due to overexpression of spermidine/spermine N1-acetyltransferase show enhanced sensitivity to the polyamine analog, N1, N11-diethylnorspermine. Mol. Pharmacol. 1999; 55:693–698. [PubMed: 10101027]

Alhonen L, Uimari A, Pietilä M, Hyvönen MT, Pirinen E, Keinänen TA. Transgenic animals modelling polyamine metabolism-related diseases. Essays Biochem. 2009; 46:125–144. [PubMed: 20095974]

Anders S, Reyes A, Huber W. Detecting differential usage of exons from RNA-seq data. Genome Res. 2012; 22:2008–2017. [PubMed: 22722343]

Bagher AM, Laprairie RB, Kelly ME, Denovan-Wright EM. Co-expression of the human cannabinoid receptor coding region splice variants (hCB1) affects the function of hCB1 receptor complexes. Eur. J. Pharmacol. 2013; 721:341–354. [PubMed: 24091169]

Bao AM, Meynen G, Swaab DF. The stress system in depression and neurode-generation: focus on the human hypothalamus. Brain Res. Rev. 2008; 57:531–553. [PubMed: 17524488]

Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav. Brain Res. 2001; 125:279–284. [PubMed: 11682119]

Butcher NJ, Broadhurst GM, Minchin RF. Polyamine-dependent regulation of spermidine-spermine N1-acetyltransferase mRNA translation. J. Biol. Chem. 2007; 282:28530–28539. [PubMed: 17690107]

Casero RA, Pegg AE. Spermidine/spermine N1-acetyltransferase—the turning point in polyamine metabolism. FASEB J. 1993; 7:653–661. [PubMed: 8500690]

Casero RA, Celano P, Ervin SJ, Applegren NB, Wiest L, Pegg AE. Isolation and characterization of a cDNA clone that codes for human spermidine/spermine N1-acetyltransferase. J. Biol. Chem. 1991; 266:810–814. [PubMed: 1985966]

Coleman CS, Huang H, Pegg AE. Role of the carboxyl terminal MATEE sequence of spermidine/spermine N1-acetyltransferase in the activity and stabilization by the polyamine analog N1, N12-bis(ethyl)spermine. Biochemistry. 1995; 34:13423–13430. [PubMed: 7577929]

Costa C, Giménez-Capitán A, Karachaliou N, Rosell R. Comprehensive molecular screening: from the RT-PCR to the RNA-seq. Transl. Lung Cancer Res. 2013; 2:87–91. [PubMed: 25806219]

Fiori LM, Turecki G. Gene expression profiling of suicide completers. Eur. Psychiatry. 2010a; 25:287–290. [PubMed: 20392610]

Fiori LM, Turecki G. Association of the SAT1 in/del polymorphism with suicide completion. Am. J. Med. Genet. B Neuropsychiatr. Genet. 2010b; 153B:825–829. [PubMed: 19851986]

Fiori LM, Turecki G. Epigenetic regulation of spermidine/spermine N1-acetyltransferase (SAT1) in suicide. J. Psychiatr. Res. 2011; 45:1229–1235. [PubMed: 21501848]

Fiori LM, Mechawar N, Turecki G. Identification and characterization of spermidine/spermine N1-acetyltransferase promoter variants in suicide completers. Biol. Psychiatry. 2009; 66:460–467. [PubMed: 19446796]

Fiori LM, Wanner B, Jomphe V, Croteau J, Vitaro F, Tremblay RE, et al. Association of polyaminergic loci with anxiety, mood disorders, and attempted suicide. PLoS ONE. 2010; 5:e15146. [PubMed: 21152090]

Gilad GM, Gilad VH. Overview of the brain polyamine-stress-response: regulation, development, and modulation by lithium and role in cell survival. Cell. Mol. Neurobiol. 2003; 23:637–649. [PubMed: 14514021]

Gilad GM, Gilad VH, Casanova MF, Casero RA. Polyamines and their metabolizing enzymes in human frontal cortex and hippocampus: preliminary measurements in affective disorders. Biol. Psychiatry. 1995; 38:227–234. [PubMed: 8547444]

Guipponi M, Deutsch S, Kohler K, Perroud N, Le Gal F, Vessaz M, et al. Genetic and epigenetic analysis of SSAT gene dysregulation in suicidal behavior. Am. J. Med. Genet. B Neuropsychiatr. Genet. 2009; 150B:799–807. [PubMed: 19051286]

Hölttä E, Sinervirta R, Jänne J. Synthesis and accumulation of polyamines in rat liver regenerating after treatment with carbon tetrachloride. Biochem. Biophys. Res. Commun. 1973; 54:350–357. [PubMed: 4741572]

Hyvönen MT, Uimari A, Keinänen TA, Heikkinen S, Pellinen R, Wahlfors T, et al. Polyamine-regulated unproductive splicing and translation of spermidine/ spermine N1-acetyltransferase. RNA. 2006; 12:1569–1582. [PubMed: 16809818]

Hyvönen MT, Uimari A, Vepsäläinen J, Khomutov AR, Keinänen TA, Alhonen L. Tissue-specific alternative splicing of spermidine/spermine N1-acetyltransferase. Amino Acids. 2012; 42:485–493. [PubMed: 21809078]

Jänne J, Alhonen L, Pietilä M, Keinänen TA. Genetic approaches to the cellular functions of polyamines in mammals. Eur. J. Biochem. 2004; 271:877–894. [PubMed: 15009201]

Kee K, Foster BA, Merali S, Kramer DL, Hensen ML, Diegelman P, et al. Activated polyamine catabolism depletes acetyl-CoA pools and suppresses prostate tumor growth in TRAMP mice. J. Biol. Chem. 2004; 279:40076–40083. [PubMed: 15252047]

Kelly TM, Mann JJ. Validity of DSM-III-R diagnosis by psychological autopsy: a comparison with clinician ante-mortem diagnosis. Acta Psychiatr. Scand. 1996; 94:337–343. [PubMed: 9124080]

Kim K, Ryu JH, Park JW, Kim MS, Chun YS. Induction of a SSAT isoform in response to hypoxia or iron deficiency and its protective effects on cell death. Biochem. Biophys. Res. Commun. 2005; 331:78–85. [PubMed: 15845361]

Klempan TA, Sequeira A, Canetti L, Lalovic A, Ernst C, ffrench-Mullen J, et al. Altered expression of genes involved in ATP biosynthesis and GABAergic neurotrans-mission in the ventral prefrontal cortex of suicides with and without major depression. Mol. Psychiatry. 2009a; 14:175–189. [PubMed: 17938633]

Klempan TA, Rujescu D, Mérette C, Himmelman C, Sequeira A, Canetti L, et al. Profiling brain expression of the spermidine/spermine N1-acetyltransferase 1 (SAT1) gene in suicide. Am. J. Med. Genet. B Neuropsychiatr. Genet. 2009b; 150B:934–943. [PubMed: 19152344]

Lawrence M, Huber W, Pagès H, Aboyoun P, Carlson M, Gentleman R, et al. Software for computing and annotating genomic ranges. PLoS Comput. Biol. 2013; 9:e1003118. [PubMed: 23950696]

Lee HK, Hsu AK, Sajdak J, Qin J, Pavlidis P. Coexpression analysis of human genes across many microarray data sets. Genome Res. 2004; 14:1085–1094. [PubMed: 15173114]

Le-Niculescu H, Levey DF, Ayalew M, Palmer L, Gavrin LM, Jain N, et al. Discovery and validation of blood biomarkers for suicidality. Mol. Psychiatry. 2013; 18:1249–1264. [PubMed: 23958961]

Li HD, Menon R, Omenn GS, Guan Y. The emerging era of genomic data integration for analyzing splice isoform function. Trends Genet. 2014; 30:340–347. [PubMed: 24951248]

Lopez JP, Fiori LM, Gross JA, Labonte B, Yerko V, Mechawar N, et al. Regulatory role of miRNAs in polyamine gene expression in the prefrontal cortex of depressed suicide completers. Int. J. Neuropsychopharmacol. 2014; 17:23–32. [PubMed: 24025154]

Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014; 15:550. [PubMed: 25516281]

Matsui I, Pegg AE. Effect of thioacetamide, growth hormone or partial hepatectomy on spermidine acetylase activity of rat liver cytosol. Biochim. Biophys. Acta. 1980; 633:87–94. [PubMed: 7448208]

Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat. Rev. Genet. 2009; 10:155–159. [PubMed: 19188922]

Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat. Methods. 2008; 5:621–628. [PubMed: 18516045]

Nagalakshmi U, Wang Z, Waern K, Shou C, Raha D, Gerstein M, et al. The transcriptional landscape of the yeast genome defined by RNA sequencing. Science. 2008; 320:1344–1349. [PubMed: 18451266]

Niiranen K, Keinänen TA, Pirinen E, Heikkinen S, Tusa M, Fatrai S, et al. Mice with targeted disruption of spermidine/spermine N1-acetyltransferase gene maintain nearly normal tissue polyamine homeostasis but show signs of insulin resistance upon aging. J. Cell. Mol. Med. 2006; 10:933–945. [PubMed: 17125596]

Nikiforova NN, Velikodvorskaja TV, Kachko AV, Nikolaev LG, Monastyrskaya GS, Lukyanov SA, et al. Induction of alternatively spliced spermidine/spermine N1-acetyltransferase mRNA in the human kidney cells infected by venezuelan equine encephalitis and tick-borne encephalitis viruses. Virology. 2002; 297:163–171. [PubMed: 12083816]

Okoniewski MJ, Miller CJ. Hybridization interactions between probesets in short oligo microarrays lead to spurious correlations. BMC Bioinforma. 2006; 7:276.

Pegg AE. Spermidine/spermine-N(1)-acetyltransferase: a key metabolic regulator. Am. J. Physiol. Endocrinol. Metab. 2008; 294:E995–E1010. [PubMed: 18349109]

Royce TE, Rozowsky JS, Gerstein MB. Toward a universal microarray: prediction of gene expression through nearest-neighbor probe sequence identification. Nucleic Acids Res. 2007; 35:e99. [PubMed: 17686789]

SEQC/MAQC-III Consortium. A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequencing Quality Control Consortium. Nat. Biotechnol. 2014; 32:903–914. [PubMed: 25150838]

Sequeira A, Gwadry FG, Ffrench-Mullen JM, Canetti L, Gingras Y, Casero RA, et al. Implication of SSAT by gene expression and genetic variation in suicide and major depression. Arch. Gen. Psychiatry. 2006; 63:35–48. [PubMed: 16389195]

Sequeira A, Klempan T, Canetti L, ffrench-Mullen J, Benkelfat C, Rouleau GA, et al. Patterns of gene expression in the limbic system of suicides with and without major depression. Mol. Psychiatry. 2007; 12:640–655. [PubMed: 17353912]

Sibille E, Arango V, Galfalvy HC, Pavlidis P, Erraji-Benchekroun L, Ellis SP, et al. Gene expression profiling of depression and suicide in human prefrontal cortex. Neuropsychopharmacology. 2004; 29:351–361. [PubMed: 14603265]

Taft RJ, Pang KC, Mercer TR, Dinger M, Mattick JS. Non-coding RNAs: regulators of disease. J. Pathol. 2010; 220:126–139. [PubMed: 19882673]

Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. 2012; 7:562–578. [PubMed: 22383036]

Trapnell C, Hendrickson DG, Sauvageau M, Goff L, Rinn JL, Pachter L. Differential analysis of gene regulation at transcript resolution with RNA-seq. Nat. Biotechnol. 2013; 31:46–53. [PubMed: 23222703]

Wagner E, Lykke-Andersen J. mRNA surveillance: the perfect persist. J. Cell Sci. 2002; 115:3033–3038. [PubMed: 12118059]

Wang Y, Devereux W, Stewart TM, Casero RA. Cloning and characterization of human polyamine-modulated factor-1, a transcriptional cofactor that regulates the transcription of the spermidine/spermine N(1)-acetyltransferase gene. J. Biol. Chem. 1999; 274:22095–22101. [PubMed: 10419538]

Wang Y, Devereux W, Stewart TM, Casero RA. Characterization of the interaction between the transcription factors human polyamine modulated factor (PMF-1) and NF-E2-related factor 2 (Nrf-2) in the transcriptional regulation of the spermidine/spermine N1-acetyltransferase (SSAT) gene. Biochem. J. 2001; 355:45–49. [PubMed: 11256947]

Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. 2009; 10:57–63. [PubMed: 19015660]

Zhao S, Fung-Leung WP, Bittner A, Ngo K, Liu X. Comparison of RNA-Seq and microarray in transcriptome profiling of activated T cells. PLoS ONE. 2014; 9:e78644. [PubMed: 24454679]

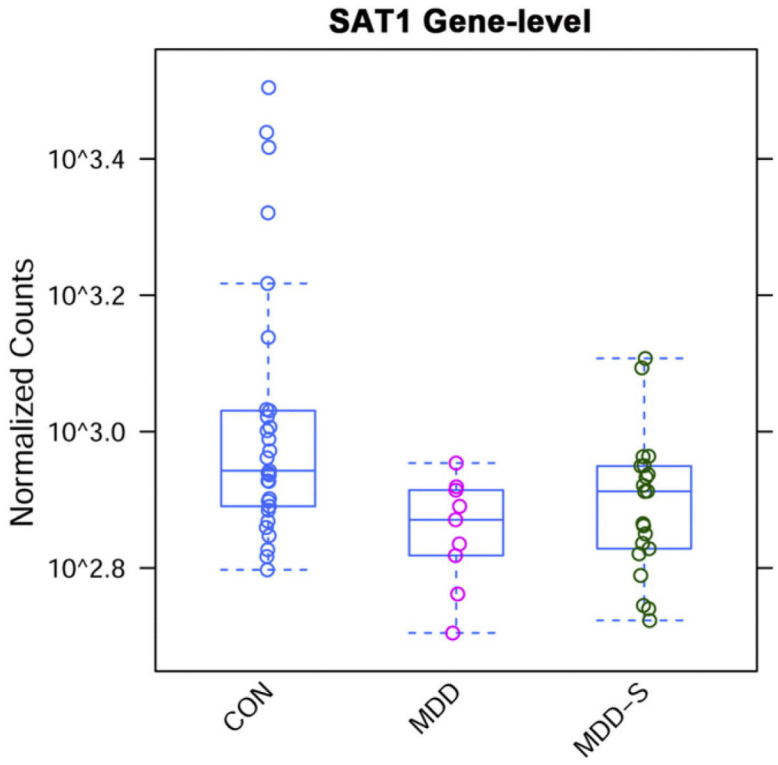

Fig. 1.

Gene-level SAT1 expression in depression with and without suicide. Boxplots for normalized counts in CON, MDD and MDD-S obtained from DESeq2 (see Materials and methods). Expression in both MDD and MDD-S was low relative to CON ($\log_2$ fold changes (FC) and p-values are reported in Table 1). The top of each box represents the 75th percentile, the bottom of the box represents the 25th percentile, and the line in the middle represents the 50th percentile. The whiskers (the lines that extend out the top and bottom of the box) represent the highest and lowest values that are not outliers or extreme values. Outliers (values that are between 1.5 and 3 times the interquartile range) and extreme values (values that are more than 3 times the interquartile range) are those points beyond the whiskers.

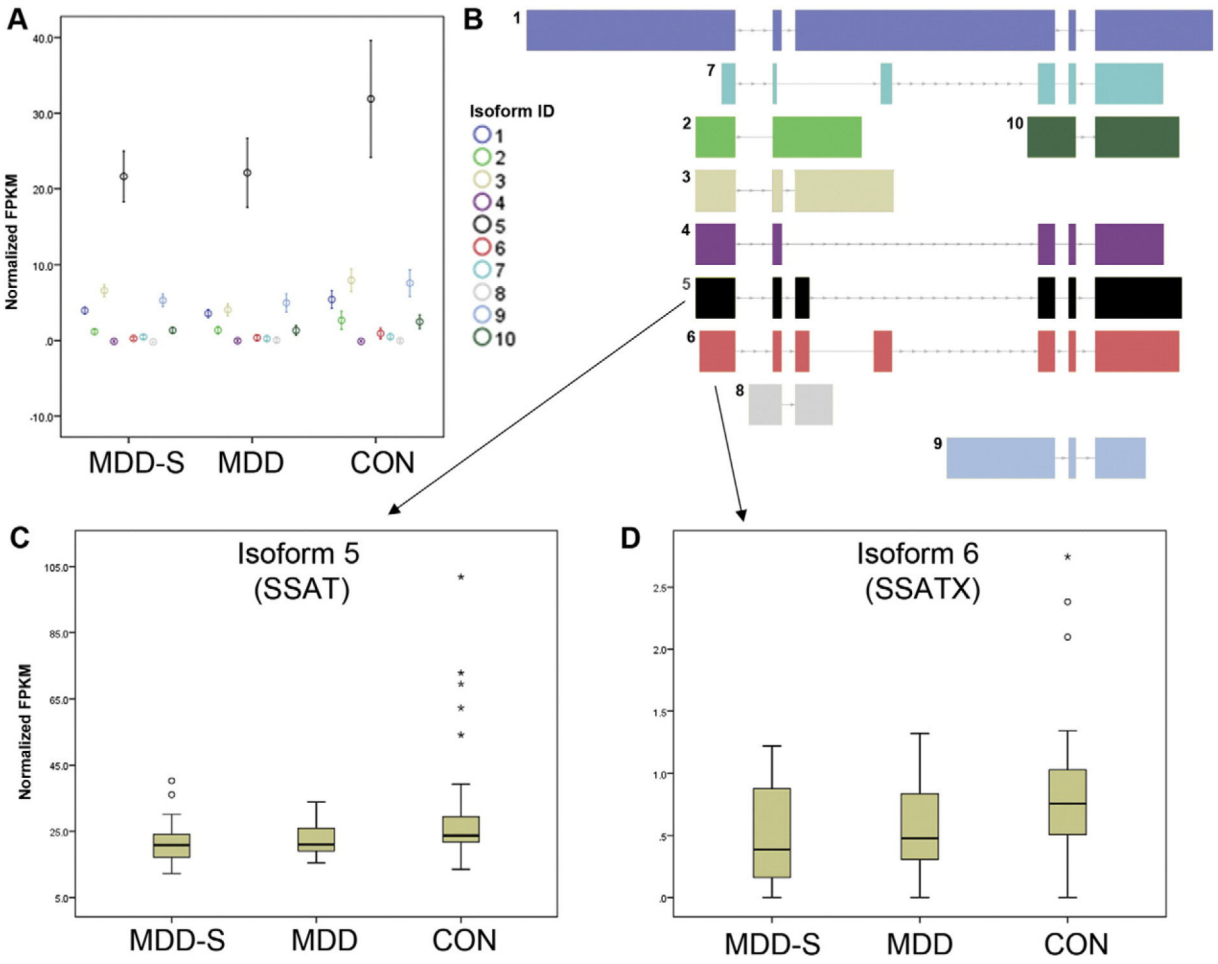

Fig. 2.

Isoform-level SAT1 expression in depression with and without suicide. (A) Average normalized FPKM values for each isoform are plotted for each group (MDD-S, HC and MD), after adjusting for age and gender. Isoform 5 (SAT1-001, or SSAT, the primary protein encoding transcript, black) shows the greatest overall expression in all groups, and is also elevated in HC relative to MDD-S and MD. Isoform 6 (SAT1-002, or SSATX, the alternate splice variant to SAT1-001 that regulates SAT1 expression via RUST, green) shows low expression relative to other isoforms (~50 fold less than isoform 5), and is also elevated in HC relative to MDD-S and MDD (see Table 2). Error bars represent 95% CI. (B) Color-coded track plots for 10 SAT1 isoforms. (C) Separate box-plots for isoform 5. The top of each box represents the 75th percentile, the bottom of the box represents the 25th percentile, and the line in the middle represents the 50th percentile. The whiskers (the lines that extend out the top and bottom of the box) represent the highest and lowest values that are not outliers or extreme values. Outliers (values that are between 1.5 and 3 times the interquartile range) and extreme values (values that are more than 3 times the interquartile range) are represented by circles and asterisks respectively beyond the whiskers. (D) Separate box-plot for isoform 6. Note that one outlier (FPKM ~ 10) in the HC group is not visible since the y-axis was rescaled for display purposes.

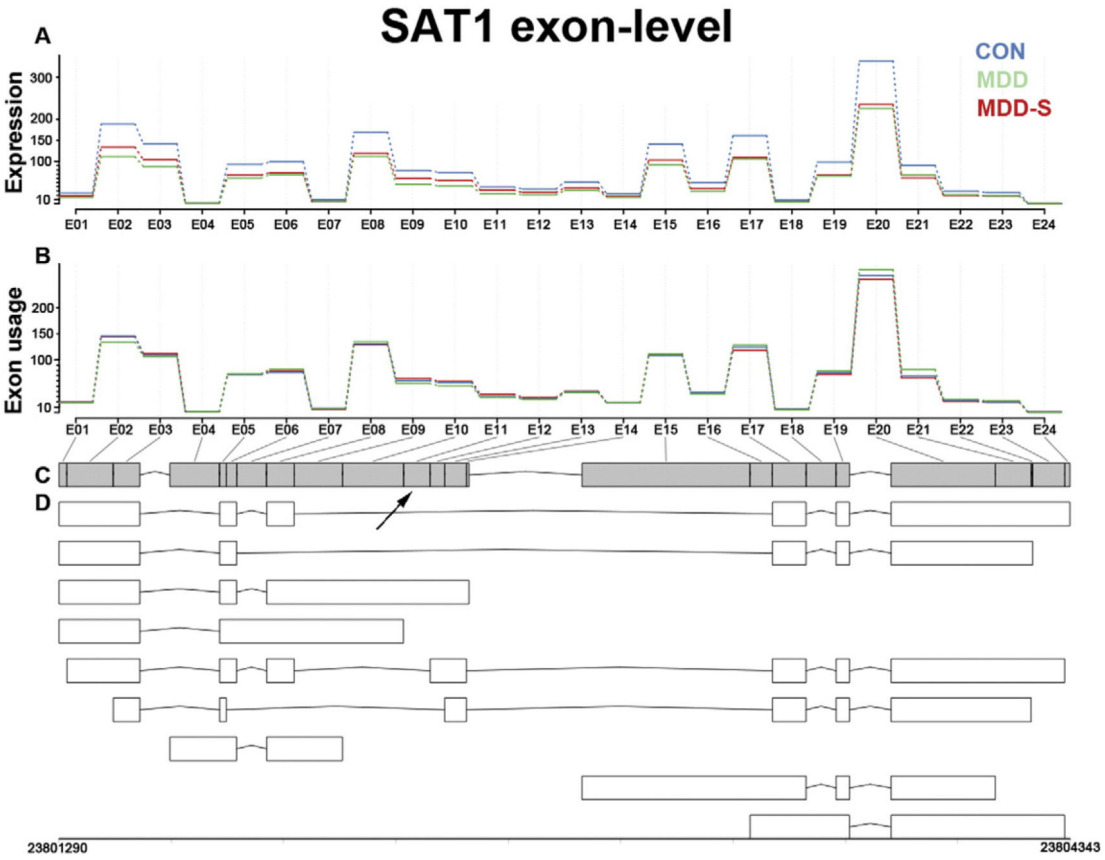

Fig. 3.

SAT1 exon-level expression before and after adjusting for overall gene-level group differences. A) Top row indicates mean normalized counts for each of 24 splicing events (i.e. bins, or “exons”, see Materials and methods) in the SAT1 gene across for the three groups CON, MDD and MDD-S. B) There are no significant differences in mean normalized counts in any exon after removing overall effect of gene expression and correcting for multiple comparisons. There was a trend-level effect for E011 (arrow, $p = 0.053$ uncorrected, Supplementary Table 4). C) The 24 unique splicing events (here referred to as “exon”) along the SAT1 gene. D) For reference, the nine known SAT1 transcripts according to Human genome assembly GRCh37 are shown.

Table 1

Demographics.

Total RNA	**MDD-S**	**HC**	**MDD**	
Sample size	N = 21	N = 29	N = 9	Statistic
Age (mean years, sd)	52.0 (21.7)	43.5 (21.3)	57.7 (15.5)	F(2,56) = 2.0, p = 0.14
Sex (number, % Female)	8 (38%)	6 (21%)	3 (33%)	ChiSq = 1.9, p = 0.39
RIN (mean, sd)	6.6 (1.5)	7.1 (1.3)	7.4 (1.1)	F(2,53) = 1.2, p = 0.32
PMI (mean, sd)	16.1 (7.0)	13.2 (4.7)	15.1 (4.4)	F(2,54) = 1.6, p = 0.22
Brain pH (mean, sd)	6.4 (0.3)	6.5 (0.3)	6.4 (0.3)	F(2,55) = 0.8, p = 0.46
Micro RNA				
Sample size	N = 9	N=9	N=9	
Age (mean years, sd)	59.2 (14.2)	56.7 (16.0)	57.7 (15.5)	F(2,24) = 0.06, p = 0.94
Gender (number, % Female)	3 (33%)	3 (33%)	3 (33%)	ChiSq = 0, p = 1
RIN (mean, sd)	7.7 (0.9)	7.4 (1.3)	7.4 (1.1)	F(2,23) = 0.3, p = 0.77
PMI (mean, sd)	13.3 (6.0)	16.7 (3.8)	15.1 (4.4)	F(2,24) = 1.1, p = 0.36
Brain pH (mean, sd)	6.3 (0.3)	6.4 (0.4)	6.4 (0.3)	F(2,24) = 0.2, p = 0.82

Table 2

Assembly and mapping of transcripts to GRCh37 Ensemble Reference Genome. The right half (Ensemble Name through Flags) is taken from Ensemble transcript table for SAT1, while the left half lists ID's for transcripts, TSS and CDS assembled and identified in the current dataset Cuffcmp Class codes "=" indicate exact matches with known transcripts, while "j" indicates potentially novel splicing variant.

Cuffcmp class Code	Iso ID	TSS ID	CDS ID	Ensemble name	Ensemble ID	Length	Protein	Biotype	RefSeq	Flags
=	5 (aka SSAT)	2	1	SAT1-001	ENST00000379270	1065 bp	171 aa	Protein coding	NM_002970 NP_002961	GENCODE basic
Not *detected in the assembled dataset*				SAT1-201 *	ENST00000612412	1074 bp	199 aa	Protein coding	-	GENCODE basic
=	3	2	3	SAT1-003	ENST00000379253	909 bp	120 aa	Protein coding	-	GENCODE basic
=	4	2	2	SAT1-005	ENST00000379254	868 bp	143 aa	Protein coding	-	GENCODE basic
=	2	2	4	SAT1-004	ENST00000379251	801 bp	150 aa	Protein coding	-	GENCODE basic
=	6 (SSATX)	2	NA	SAT1-002	ENST00000489394	1135 bp	No protein product	Processed transcript	NR_027783	
=	9	5	NA	SAT1-006	ENST00000474223	1033 bp	No protein product	Processed transcript	-	
=	10	6	NA	SAT1-007	ENST00000462639	826 bp	No protein product	Processed transcript	-	
=	7	3	NA	SAT1-009	ENST00000463236	735 bp	No protein product	Processed transcript	-	
=	8	4	NA	SAT1-008	ENST00000487713	432 bp	No protein product	Processed transcript	-	
j	1	1	*This transcript does not occur in the GRCh37 assembly to which transcripts were aligned (nor the current GRCh38 assembly), and may potentially represent a novel transcript or pre-mRNA ?*							

*This transcript appears in GRCh38, but not GRCh37. Isoform 5 encodes the SAT1 enzyme protein and is also referred to in the literature as SSAT, while Isoform 6 is an alternate splice variant targeted to non-sense mediated decay (referred to in the literature as SSATX).

Table 3

Gene-level group differences in SAT1 expression using normalized counts in DESeq2. lfcSE is log fold change standard error.

DESeq2 Results	baseMean	log2FoldChange	lfcSE	Stat	p value
Likelikhood ratio test (~age + sex + condition vs. ~age + sex)				$chi^2 = 12.097$	0.002
MDD-S vs. CON	957.409	–0.407	0.131	Z = –3.114	0.002
MDD-S vs. MDD	957.409	0.060	0.157	Z = 0.384	0.701
MDD vs. CON	957.409	–0.467	0.154	Z =–3.035	0.002

Table 4

Linear mixed model results for effects of interest at gene, isoform, TSS and CDS levels using normalized FPKM values from Cufflinks/Cuffnorm.

Source	Numerator df	Denominator df	F	Sig.
Dependent variable: SAT1 gene level expression				
Group	2	54	3.315	0.044
Dependent variable: TCONS isoform, 10 levels				
Group	2	75.872	10.552	0
Isoform ID	9	97.197	77.893	0
Group * Isoform ID	18	97.197	2.598	0.001
Dependent variable: TSS group, 6 levels				
Group	2	61.977	6.56	0.003
TSS ID	5	98.307	92.773	0
Group * TSS ID	10	98.307	2.55	0.009
Dependent variable: CDS group, 4 levels				
Group	2	63.3	5.627	0.006
CDS ID	3	87.391	117.885	0
Group * CDS ID	6	87.391	3.975	0.001

Table 5

Pair-wise group comparisons at SAT1 gene and isoform levels. Shaded rows indicate isoforms with a FDR < 0.1.

	ID	Group1 Median	Group 2 Median	Mann-Whitney U	Z	Exact sig. (2-tailed)	Adjusted p-value (FDR)
MDD-S_vs_HC							
Gene level		41.267	47.087	170	−2.644	0.008	
Isoform Level	1	3.94	4.87	217	−1.71	0.088	0.285
	2	1.29	1.74	182	−2.408	0.015	0.078
	3	6.76	6.94	281	−0.462	0.654	0.77
	4	1.07E-05	9.62E-06	282	0.442	0.668	0.77
	5	20.88	23.70	187	−2.31	0.02	0.078
	6	0.39	0.76	187	−2.31	0.02	0.078
	7	0.60	0.36	290	0.285	0.785	0.81
	8	1.00E-10	0.00E+00	262	0.835	0.412	0.71
	9	5.34	6.60	178	−2.486	0.012	0.078
	10	1.57	1.99	219	−1.681	0.095	0.285
MDD_vs_HC							
Gene level		41.26	47.087	63	−2.318	0.019	
Isoform level	1	3.87	4.87	63	−2.318	0.019	0.078
	2	1.79	1.74	93	1.288	0.206	0.412
	3	4.02	6.94	31	−3.416	0	0
	4	2.41E-05	9.62E-06	120	0.361	0.736	0.81
	5	21.01	23.70	84	−1.597	0.115	0.313
	6	0.48	0.76	91	−1.356	0.184	0.41
	7	0.40	0.36	117	0.464	0.661	0.77
	8	3.05E-08	0.00E+00	109	0.738	0.478	0.717
	9	5.30	6.60	64	−2.283	0.021	0.078
	10	1.31	1.99	92	−1.322	0.196	0.412
MDD-S_vs_MDD							
Gene level		41.267	41.26	85	0.43	0.689	
Isoform level	1	3.94	3.87	77	0.792	0.449	0.71
	2	1.29	1.79	78	−0.747	0.476	0.71
	3	6.76	4.02	19	3.417	0	0
	4	1.07E-05	2.41E-05	81	−0.611	0.563	0.77
	5	20.88	21.01	87	−0.339	0.756	0.81
	6	0.39	0.48	84	−0.475	0.657	0.77
	7	0.60	0.40	75	0.882	0.397	0.717
	8	1.00E-10	3.05E-08	60	−1.561	0.125	0.313
	9	5.34	5.30	83	0.52	0.625	0.77
	10	1.57	1.31	92	0.113	0.929	0.92

Table 6

miRNA (identified in (21)) expression in depression and suicide and correlation with SAT1 isoforms 5 and 6.

	MDD-S	MDD	HC	p-value	Iso5 R	p-value	Iso6 R	p-value
mir-139	3.60E–04	4.00E–04	3.30E–04	0.7	0.23	0.26	–0.28	0.18
mir-34c	4.40E–05	4.60E–05	4.10E–05	0.67	–0.022	0.92	–0.21	0.32
mir-320c	0.00E + 00	0.00E + 00	0.00E + 00	0.56	0.24	0.26	–0.004	0.98
mir-195	2.20E–04	2.70E–04	2.40E–04	0.94	0.38	0.06	–0.37	0.07

Table 7

Summary of significant results.

Analysis	Transcript	Ensembl ID	Comparison	Group median FPKM (HC; MDD; MDD-S)	Z-score	Pval	CorrP
Gene level	SAT1	ENSG00000130066	HC > MDD-S	47.09; 41.26; 41.27	3.1	0.002	0.003
			HC > MDD		3.0	0.002	0.003
Isoform-level	Isoform 2	ENST00000379251	HC > MDD-S	1.74; 1.79; 1.29	2.4	0.015	0.078
	Isoform 5 (SSAT)	ENST00000379270	HC > MDD-S	23.70; 21.01; 20.88	2.3	0.02	0.078
	Isoform 6 (SSATX)	ENST00000489394	HC > MDD-S	0.76; 0.48; 0.39	2.3	0.02	0.078
	Isoform 9	ENST00000474223	HC > MDD-S	6.60; 5.30; 5.34	2.5	0.012	0.078
			HC > MDD		2.3	0.021	0.078
	Isoform 1	NA	HC > MDD	4.87; 3.87; 3.94	2.3	0.019	0.078
	Isoform 3	ENST00000379253	HC > MDD	6.94; 4.02; 6.76	3.4	0	0
			MDD-S > MDD		3.4	0	0