RESEARCH ARTICLE

The Expression of the Suicide-Associated Gene SKA2 Is Decreased in the Prefrontal Cortex of Suicide Victims but Not of Nonsuicidal Patients

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Abstract

Background: Recent study of genome-wide DNA methylation profiling in the postmortem brain of suicidal and nonsuicidal subjects found that gene expression of spindle and kinetochore associated complex subunit 2 (SKA2) is decreased in the postmortem brain of suicide victims compared with nonsuicidal, nonpsychiatric control subjects.

Methods: To determine if decreased SKA2 is specific to suicide and independent of diagnosis, we determined gene and protein expression of SKA2 in the prefrontal cortex obtained from suicide victims (n = 52), nonsuicidal psychiatric subjects (n = 27), and normal controls (n = 24). We determined gene expression by quantitative PCR technique and protein expression by Western blot. The postmortem brain samples were obtained from the Maryland Psychiatric Research Center.

Results: We found that protein and gene expression of SKA2 was significantly reduced in the prefrontal cortex of suicide victims compared with normal control subjects and nonsuicidal patients. We also found that SKA2 protein and gene expression in depressed suicide victims, schizophrenic suicide victims, and suicide victims with substance abuse and/or conduct disorders was significantly decreased compared with normal control subjects and also with nonsuicidal depressed or schizophrenic subjects.

Conclusions: This study shows that decreased gene and protein expression of SKA2 observed in the prefrontal cortex of suicide victims is specific to suicide, which was not observed in the brain of nonsuicidal patients. It also indicates reduced SKA2 expression in suicide is independent of psychiatric diagnosis, since it is observed in all diagnostic groups studied. Therefore, SKA2 may be a potential biomarker for suicide.

Keywords: SKA2, suicide, postmortem brain, schizophrenia, depression

Introduction

Suicide is a major public health concern. About 35,000 people die per year of suicide in the United States alone (Center for Disease Control, 2002; Goldsmith et al., 2002a, 2002b; CDC Prevention, 2007; CDC, 2009). Significant progress has been made in understanding the neurobiology of suicide, primarily based on studies of postmortem brain samples. These studies suggested several abnormalities in the brain of suicide victims (for review, see Turecki (2014) and Pandey (2013)). In a recent report, Guintivano et al. (2014) studied a genome-wide DNA methylation profiling in the postmortem brain from suicidal and nonsuicidal subjects and found that the DNA methylation scan identified an additive epigenetic and genetic association with suicide at rs720805 within the 3′ untranslated region of the spindle and kinetochore associated complex subunit 2 (SKA2) gene in 3 brain cohorts. This finding was also replicated in blood from live cohorts. Whereas the major focus of this study was on SKA2 DNA methylation,
they also studied the gene expression of SKA2 in suicide victims. They found that the gene expression of SKA2 in the postmortem brain of suicidal and nonsuicidal subjects was significantly reduced in suicidal subjects compared with controls. This study thus showed an abnormality of SKA2 gene in suicide pathogenesis. More recently, Niculescu et al. (2015) determined the expression of SKA2 in blood samples of suicidal patients and the postmortem brain of suicide victims. They observed decreased SKA2 gene expression in the blood of depressed patients and the brain of violent suicide completers. Studies of Guintivano et al. (2014) and Niculescu et al. (2015) thus strongly suggest dysregulation of SKA2 in suicide completers and its essential use as a blood marker for suicidality. It is not clear from their findings if decreased SKA2 gene expression in postmortem brain of suicide victims is independent of diagnosis (ie, whether suicide victims across all diagnostic groups have reduced SKA2 gene expression)—namely, if decreased SKA2 gene expression is specific for suicide and is not observed in the postmortem brain of nonsuicidal patients and if the protein expression of this gene is also altered in the postmortem brain of suicide victims.

To further extend the finding of decreased gene expression of SKA2 in suicide and to examine if this decrease is independent of psychiatric diagnosis and specific to suicide and is also associated with decreased protein expression, we have determined the mRNA and protein expression of SKA2 in the prefrontal cortex (PFC) obtained from suicide victims from different diagnostic groups, such as depression, schizophrenia, and other suicide groups (ie, substance abuse and/or conduct disorders) as well as from subjects from different diagnostic groups who died of natural causes (nonsuicidal patients) and from normal control subjects.

Methods

Subjects and Diagnoses

The study was performed in the PFC (Brodmann area 9) of 52 suicide victims (consisting of 24 depressed suicide victims, 16 schizophrenic suicide victims, and 12 other suicide victims with either substance abuse and/or conduct disorder), 27 nonsuicide patients (consisting of 12 depressed nonsuicide patients and 15 schizophrenic nonsuicidal patients), and 24 normal control subjects. Brain tissues were obtained from the Maryland Brain Collection at the Maryland Psychiatric Research Center, Baltimore, Maryland. All procedures were approved by the University of Maryland Institutional Review Board and by the University of Illinois Institutional Review Board.

Diagnostic Method

Subject diagnosis was based on the Structured Clinical Interview for DSM-IV (Spitzer et al., 1992). At least one family member and/or a friend, after giving written informed consent, underwent an interview. Diagnoses were made by a consensus of 2 psychiatrists from the data obtained in this interview, medical records from the case, and records obtained from the Medical Examiner’s office. Normal control subjects were verified as free from mental illnesses using these consensus diagnostic procedures.

Determination of mRNA Levels

RNA Extraction and Reverse Transcription

Total RNA was extracted from 100 mg of tissue using the TRIZOL reagent (Invitrogen) as per the manufacturer’s instructions and treated with DNase 1 (Invitrogen). The RNA yield was determined by absorbance at 260 nm using NanoDrop ND-1000 (NanoDrop Technologies, Montchanin, DE). RNA quality was assessed using Agilent Bioanalyzer 2100 (Agilent). All samples had 28S/18S ratios >1.2 and RNA integrity number >6.6. The mean RNA integrity number was 7.2 ± 0.6.

Expression levels of mRNA were determined using a 2-step real-time RT-PCR method. One microgram of total RNA was reverse transcribed using 50 ng random hexamers, 2 mM dNTP mix, 10 units ribonuclease inhibitor, and 200 units Moloney murine leukemia virus (MMLV)-reverse transcriptase enzyme in a final reaction volume of 20 µL.

Relative Real-Time PCR

Real-time PCR was performed using Pre-designed Taqman gene expression assays (Applied Biosystems, Foster City, CA) for all target and housekeeping genes on MX3005p sequence detection system (Agilent). The TaqMan assay IDs are in Table 1. To determine the stability and optimal number of housekeeping genes, we used geNORM version 3.4 (PrimerDesign) according to the manufacturer’s instructions (Vandesompele et al., 2002) and tested 12 commonly used reference genes of different functional classes in 10 samples from each test group. The average gene-stability measure (M) ranked β-actin and GAPDH as the most stable genes in our samples. PCR efficiency was tested over 5-log dilution series and confirmed that β-actin, GAPDH, and SKA2 had similar amplification efficiencies. For each primer/probe set, the PCR reaction was carried out using 10 µL of cDNA diluted 1:10-fold. Each quantitative PCR plate included a “no reverse transcriptase” and “no template” control to eliminate nonspecific amplification, one sample was run on a gel to confirm specificity, and samples were run in triplicates. Target gene quantitative PCR data was normalized to the geometric mean of β-actin and GAPDH and was expressed relative to the control samples using 2^(-ΔΔCT) method.

Determination of Protein Expression of SKA2 by Western Blot

Gel electrophoresis and immunolabeling of SKA2 proteins were performed by Western blot as previously reported (Dwivedi and Pandey, 2000). Equal amounts of protein samples (20 µg protein in each lane) obtained from membrane fraction were loaded onto 7.5% (w/v) acrylamide gel and subsequently transferred electrophoretically to enhanced chemiluminescent (ECL) nitrocellulose membranes (Amersham Pharmacia, Piscataway, NJ). The blots were incubated overnight at 4°C with primary antibody for SKA2 (Santa Cruz Biotechnology, Inc., catalog no. sc-136868) at a dilution of 1:3000 and with horseradish-peroxidase-linked secondary anti-rabbit antibody at a dilution of 1:3000 (Amersham Pharmacia) for 3 to 5 hours at room temperature. The signals were detected with the ECL Western-blot detection system (Amersham) followed by exposure to ECL-autoradiographic film (Amersham). The membranes were stripped using stripping solution (Chemicon International, Temecula, CA) and probed

Table 1. Taqman Primers/Probes Used for qPCR Analysis

| Taqman Accession | Probe Location (Exon Boundary) | Assay Function |
|------------------|-------------------------------|---------------|
| ACTB             | Hs99999903_m1 1-1             | Housekeeping (HK) |
| GAPDH            | Hs99999905_m1 3-3             | HK            |
| SKA2             | Hs00735057_m1 3-4             | Target gene   |

Guintivano et al. (2015)
with β-actin monoclonal primary (1:5000 for 2 hours; Sigma Chemical Co., St. Louis, MO) and anti-mouse secondary antibody (1:5000 for 2 hours). The bands on the autoradiograms were quantified using the Lofts Image Analysis System (Westminster, MD). A ratio of the optical density of SKA2 over the optical density of the corresponding β-actin band was calculated.

Before starting the experiment, immunolabeling of SKA2 proteins was characterized (Figure 1). The specificity of SKA2 proteins was checked by running NIH3T3 cells and E431 immune lines along with membrane fraction of PFC from one control subject. It was observed that extracts from different cell lines as well as membrane fraction migrated to 18 kDa (Figure 1). The appropriate concentration of proteins was selected by running 5 different concentrations (5–80 μg) of protein from membrane fraction of PFC. The optical density was linear between these concentrations of protein. We therefore used 20 μg of protein in subsequent experiments. Similarly, the antibody concentration and duration of exposure of the nitrocellulose membrane onto autoradiographic film were also characterized.

Statistical Analysis and Effect of Confounding Variables

The data analyses were performed using the SAS 9.2 statistical software package. Data normality was assessed by Shapiro-Wilk test, and all analyses were 2-tailed with a level of significance of P < .05. Linear regression was performed to compare the effects of 3 groups: normal control subjects, suicide victims, and nonsuicidal subjects for outcome measures of SKA2 on protein and gene expression by adjusting the effects of age, gender, post-mortem interval (PMI), and brain pH. For multiple comparisons, we used t tests with Bonferroni correction to adjust the type I error rates. We also performed posthoc t tests for pairwise comparisons.

To examine if the observed decrease in SKA2 expression in the PFC of suicide victims is related to the covariates, we examined the effects of age, gender, PMI, and brain pH on these parameters. There was no significant effect of age, gender, PMI, or brain pH on the protein or mRNA expression levels of SKA2 in any of the diagnostic groups.

Results

The demographic and clinical characteristics of normal controls, suicide victims, and nonsuicidal patients are shown in Table 2. Although the groups were well matched, there were some significant differences. There was a significant difference in age between normal controls with schizophrenic suicide (P = .05). There was a significant difference in PMI between normal controls and other suicide group (P < .01). There were significant differences in brain pH between normal controls vs schizophrenic suicide (P < .01), vs other suicide (P < .01), vs depressed nonsuicide (P < .01), and vs schizophrenic nonsuicide (P < .01) groups. However, these variables were used as covariates when analyzing the protein and mRNA expression of SKA2 in these groups.

mRNA Expression of SKA2 in Normal Control Subjects, Suicide Victims, and Nonsuicidal Patients

We determined the mRNA expression of SKA2 in the PFC (Brodmann area 9) of 24 normal control subjects, 52 suicide victims with different diagnoses, and 27 nonsuicidal subjects. The mean mRNA expression of SKA2 in these 3 groups is shown in Figure 2A. One-way ANOVA showed significant differences between the groups (F = 6447.06, P < .001), and when we compared the mRNA expression of SKA2 between these 3 groups, we found that the mRNA expression of SKA2 was significantly decreased (t = -5.40, P < .001, CI (-.99, -3.7), ES = .58) in suicide victims compared with normal control subjects (Figure 2A). The SKA2 expression in suicide victims was also significantly decreased (t = -5.70, P < .001, ES = .58) in the PFC of suicide victims compared with nonsuicidal patients (Figure 2A). When we compared the SKA2 expression between nonsuicidal patients and normal control subjects, we found that there was no significant difference (t = -2.56, P = 1.0, ES = .04) in the expression of SKA2 between normal control subjects and nonsuicidal patients (Figure 2A). These results suggested that decreased SKA2 expression was specific to suicide.

To examine the diagnostic specificity, that is, if decreased mRNA expression of SKA2 in suicide victims was independent of diagnosis, we divided the suicide victims into different diagnostic groups. The suicide group consisted of 24 depressed suicide victims, 16 schizophrenic suicide victims, and 12 suicide victims with other diagnoses, primarily substance abuse and/or conduct disorders. We found that mean mRNA expression of SKA2 in depressed suicide victims (t = 5.94, P < .001, ES = .66), schizophrenic suicide victims (t = 2.23, P < .03, ES = .37), and other suicide victims (t = 4.52, P < .001, ES = .58) was significantly different compared with normal control subjects, as shown in Figure 2B and Table 3. However, there was no significant difference between the 3 suicide groups compared against each other (Table 3).

On the other hand, the SKA2 mRNA expression was not significantly different in nonsuicidal depressed subjects and nonsuicidal schizophrenic subjects compared with normal control subjects (Figure 2B; Table 3), showing that decreased SKA2 mRNA expression was specific to suicide and independent of diagnosis.

We also compared the mRNA expression of SKA2 between depressed suicide victims and nonsuicidal depressed subjects.
Table 2. Demographic Characteristics of Subjects

| Group               | Age (y) | Race | Gender | PMI (h) | Brain pH | Cause of Death                          | Psychotropic Drugs (at Time of Death) | Psychiatric Diagnosis |
|---------------------|---------|------|--------|---------|----------|----------------------------------------|--------------------------------------|-----------------------|
| Normal Control Subjects |         |      |        |         |          |                                        |                                      |                       |
| 1. CONTROL          | 19      | Black| Male   | 11      | 6.9      | GSW                                    | None                                  | Normal                |
| 2. CONTROL          | 22      | Black| Male   | 19      | 6.9      | GSW                                    | None                                  | Normal                |
| 3. CONTROL          | 42      | White| Female | 23      | 7.2      | Pneumonia                              | None                                  | Normal                |
| 4. CONTROL          | 37      | Black| Male   | 5       | 7.1      | ASCVD                                  | None                                  | Normal                |
| 5. CONTROL          | 31      | Black| Male   | 8       | 7.2      | GSW                                    | None                                  | Normal                |
| 6. CONTROL          | 46      | Black| Male   | 9       | 7.1      | Multiple injuries                      | None                                  | Normal                |
| 7. CONTROL          | 33      | White| Male   | 15      | 7.0      | GSW                                    | None                                  | Normal                |
| 8. CONTROL          | 48      | White| Male   | 26      | 6.9      | ASCVD                                  | None                                  | Normal                |
| 9. CONTROL          | 40      | White| Female | 7       | 7.0      | ASCVD                                  | None                                  | Normal                |
| 10. CONTROL         | 23      | Black| Male   | 15      | 6.8      | GSW                                    | None                                  | Normal                |
| 11. CONTROL         | 83      | White| Male   | 20      | 7.1      | ASCVD                                  | None                                  | Normal                |
| 12. CONTROL         | 65      | Black| Female | 23      | 6.9      | ASCVD                                  | None                                  | Normal                |
| 13. CONTROL         | 35      | White| Male   | 24      | 6.9      | Crush injury to abdomen and chest      | None                                  | Normal                |
| 14. CONTROL         | 52      | White| Male   | 30      | 7.3      | ASCVD                                  | None                                  | Normal                |
| 15. CONTROL         | 37      | White| Male   | 24      | 7.0      | ASCVD                                  | None                                  | Normal                |
| 16. CONTROL         | 45      | White| Male   | 22      | 7.3      | ASCVD                                  | None                                  | Normal                |
| 17. CONTROL         | 26      | White| Male   | 12      | 6.9      | Arrhythmia                             | None                                  | Normal                |
| 18. CONTROL         | 47      | White| Male   | 10      | 7.0      | ASCVD                                  | None                                  | Normal                |
| 19. CONTROL         | 31      | White| Male   | 16      | 7.2      | MVA                                    | None                                  | Normal                |
| 20. CONTROL         | 60      | White| Male   | 15      | 7.1      | Accidental drowning                    | None                                  | Normal                |
| 21. CONTROL         | 28      | White| Male   | 13      | 6.8      | Electrocution                          | None                                  | Normal                |
| 22. CONTROL         | 45      | White| Female | 16      | 6.9      | Cardiac arrhythmia                     | None                                  | Normal                |
| 23. CONTROL         | 62      | White| Male   | 19      | 7.0      | Cardiac arrest                         | None                                  | Normal                |
| 24. CONTROL         | 53      | White| Male   | 15      | 6.9      | ASCVD                                  | None                                  | Normal                |
| Depressed Suicide Victims |       |      |        |         |          |                                        |                                      |                       |
| 1. SUICIDE          | 27      | White| Male   | 24      | 7.0      | GSW                                    | None                                  | MDD, ethanol abuse      |
| 2. SUICIDE          | 44      | White| Female | 11      | 7.2      | Drug overdose                          | Nortriptyline, MDD, ethanol abuse     |
| 3. SUICIDE          | 36      | White| Female | 10      | 7.1      | GSW                                    | None                                  | MDD                   |
| 4. SUICIDE          | 24      | White| Male   | 7       | 7.1      | GSW                                    | Ethanol, MDD                          |
| 5. SUICIDE          | 43      | White| Male   | 12      | 7.0      | Drug Overdose                          | None                                  | MDD, polysubstance abuse|
| 6. SUICIDE          | 53      | White| Male   | 23      | 6.9      | Jumped from height                     | None                                  | MDD                   |
| 7. SUICIDE          | 41      | White| Female | 27      | 7.1      | Drug Overdose                          | Amitriptyline, MDD, ethanol abuse     |
| 8. SUICIDE          | 22      | Black| Female | 16      | 7.3      | Drug overdose                          | None                                  | MDD                   |
| 9. SUICIDE          | 46      | White| Female | 21      | 6.9      | Drug overdose                          | Amitriptyline, desipramine, MDD       |
| 10. SUICIDE         | 36      | White| Female | 18      | 7.2      | GSW                                    | None                                  | MDD                   |
| 11. SUICIDE         | 38      | White| Male   | 24      | 7.0      | Drug overdose & Ethanol overdose       | Ethanol, MDD                          |
| 12. SUICIDE         | 46      | White| Female | 16      | 6.8      | Drug overdose / Nortriptyline Intoxication | Nortriptyline, MDD, panic disorder with agoraphobia |
| 13. SUICIDE         | 23      | White| Male   | 12      | 7.0      | Hanging                                | Paroxetine, MDD                       |
| 14. SUICIDE         | 18      | White| Male   | 17      | 6.3      | Hanging                                | None                                  | MDD                   |
| 15. SUICIDE         | 30      | White| Male   | 17      | 7.1      | Hanging                                | Venlafaxine, MDD                      |
| 16. SUICIDE         | 19      | White| Male   | 18      | 6.2      | CO intoxication                       | Ethanol, CO, MDD, ethanol abuse, polysubstance abuse |
| 17. SUICIDE         | 44      | White| Female | 30      | 7.2      | Drug overdose, Ethanol intoxication   | Fluoxetine, ethanol, MDD, opioid abuse |
| 18. SUICIDE         | 74      | White| Female | 27      | 7.0      | Venlafaxine overdose                  | Venlafaxine, ethanol, MDD             |
| 19. SUICIDE         | 25      | White| Male   | 14      | 6.8      | Hanging                                | Ethanol, MDD                          |
| 20. SUICIDE         | 23      | Black| Male   | 23      | 6.9      | Hanging                                | None                                  | MDD                   |
| 21. SUICIDE         | 63      | White| Male   | 19      | 6.9      | Drug overdose, Ethanol intoxication   | Ethanol, MDD                          |
| 22. SUICIDE         | 67      | White| Male   | 22      | 7.0      | GSW                                    | Fluoxetine, venlafaxine, MDD          |
| 23. SUICIDE         | 40      | White| Female | 20      | 7.0      | Drug overdose                          | Alprazolam, MDD                       |
| 24. SUICIDE         | 53      | White| Male   | 26      | 7.1      | Suicide by stab wound                  | Sertraline, MDD                       |
| Group                              | Age (y) | Race | Gender | PMI (h) | Brain pH | Cause of Death                          | Psychotropic Drugs (at Time of Death) | Psychiatric Diagnosis       |
|-----------------------------------|---------|------|--------|---------|----------|-----------------------------------------|---------------------------------------|----------------------------|
| Schizophrenia Suicide Victims³   |         |      |        |         |          |                                         |                                       |                            |
| 1. SUICIDE                        | 45      | Black| Male   | 10      | 6.8      | Suicide, stab wound                      | Haloperidol                           | Schizophrenia                 |
| 2. SUICIDE                        | 20      | White| Female | 11      | 6.5      | Jump from height, multiple injuries      | Haloperidol                           | Schizophrenia                 |
| 3. SUICIDE                        | 54      | White| Male   | 12      | 6.6      | Suicide, drowning                        | Haloperidol, ethanol abuse            | Schizophrenia, ethanol abuse |
| 4. SUICIDE                        | 20      | White| Male   | 23      | 6.4      | Drug overdose                            | Fluphenazine                          | Schizophrenia, ethanol abuse |
| 5. SUICIDE                        | 40      | White| Male   | 17      | 6.8      | Jump from height, multiple injuries      | Fluphenazine                          | Schizophrenia, ethanol abuse |
| 6. SUICIDE                        | 28      | White| Male   | 13      | 7.1      | Suicide, hanging                         | Thioridazine, amitriptyline, nortriptyline | Schizophrenia, ethanol abuse |
| 7. SUICIDE                        | 35      | White| Female | 7       | 6.9      | Suicide, multiple drugs intoxication     | Amitriptyline, loxapine, nortriptyline | Schizophrenia, ethanol abuse |
| 8. SUICIDE                        | 37      | White| Male   | 20      | 6.7      | Suicide, drowning                        | Haldol                                | Schizophrenia, ethanol abuse |
| 9. SUICIDE                        | 37      | White| Male   | 22      | 7.1      | Suicide, GSW to chest                   | None                                  | Schizophrenia, ethanol abuse |
| 10. SUICIDE                       | 51      | White| Female | 21      | 6.5      | Suicide, overdose                        | None                                  | Schizophrenia                 |
| 11. SUICIDE                       | 34      | White| Male   | 16      | 6.60     | Suicide, jumped from height, multiple injuries | Mesoridazine                          | Schizophrenia                 |
| 12. SUICIDE                       | 21      | White| Male   | 26      | 6.4      | Jumped from height, multiple injuries    | Fluphenazine                          | Schizophrenia                 |
| 13. SUICIDE                       | 23      | White| Male   | 20      | 6.6      | Jumped from height, multiple injuries    | None                                  | Schizophrenia, hallucinogen abuse |
| 14. SUICIDE                       | 45      | Black| Male   | 8       | 6.6      | Suicide, hanging                         | Olanzapine                            | Schizophrenia                 |
| 15. SUICIDE                       | 37      | White| Male   | 14      | 6.7      | Suicide, electrocution                   | Risperidone, thioridazine             | Schizophrenia                 |
| 16. SUICIDE                       | 54      | White| Male   | 19      | 6.6      | Suicide, bleeding                        | None                                  | Schizophrenia                 |
| Other Suicide Victims³⁴           |         |      |        |         |          |                                         |                                       |                            |
| 1. SUICIDE                        | 34      | White| Male   | 16      | 6.4      | GSW                                      | Ethanol                               | Alcohol abuse                 |
| 2. SUICIDE                        | 21      | White| Male   | 17      | 6.8      | GSW                                      | None                                  | Adjustment disorder, mixed    |
| 3. SUICIDE                        | 75      | White| Male   | 18      | 6.4      | GSW                                      | None                                  | Adjustment disorder, conduct disorder |
| 4. SUICIDE                        | 87      | White| Male   | 16      | 6.6      | GSW                                      | None                                  | Adjustment disorder, conduct disorder |
| 5. SUICIDE                        | 39      | White| Male   | 30      | 6.7      | Asphyxia                                 | Freon, cocaine, and metabolites       | Alcohol abuse, cocaine abuse, drug abuse |
| 6. SUICIDE                        | 30      | White| Male   | 32      | 6.5      | Hanging                                  | Cocaine, ethanol                      | Alcohol abuse, cocaine abuse, drug abuse |
| 7. SUICIDE                        | 40      | White| Male   | 26      | 6.7      | GSW                                      | Ethanol                               | Alcohol abuse                 |
| 8. SUICIDE                        | 20      | White| Male   | 32      | 6.9      | Hanging                                  | Ethanol                               | Alcohol abuse                 |
| 9. SUICIDE                        | 71      | White| Female | 24      | 6.5      | Drug overdose                            | None                                  | Adjustment disorder, mixed    |
| 10. SUICIDE                       | 24      | White| Male   | 22      | 6.8      | Hanging                                  | None                                  | Schizoaffective disorder      |
| 11. SUICIDE                       | 21      | White| Male   | 23      | 6.8      | Hanging                                  | None                                  | Adjustmant disorder, conduct disorder |
| 12. SUICIDE                       | 19      | White| Male   | 15      | 6.9      | GSW to chest                             | Fluoxetine                            | Dissociative disorder, substance abuse, PTSD |
| Depressed Nonsuicide Subjects¹    |         |      |        |         |          |                                         |                                       |                            |
| 1. Nonsuicide depressed           | 65      | White| Male   | 14      | 6.9      | ASCVD                                    | None                                  | MDD                         |
| 2. Nonsuicide depressed           | 55      | Black| Female | 8       | 6.4      | ASCVD                                    | Fluoxetine, ethanol                    | MDD, polysubstance abuse      |
| 3. Nonsuicide depressed           | 71      | White| Male   | 4       | 6.3      | ASCVD                                    | Bupropion                             | MDD                         |
| 4. Nonsuicide depressed           | 74      | Black| Female | 7       | 6.7      | ASCVD                                    | Paroxetine, thioridazine              | MDD                         |
| 5. Nonsuicide depressed           | 14      | White| Male   | 11      | 7.0      | MVA                                      | Sertraline                            | MDD, polysubstance abuse      |
| 6. Nonsuicide depressed           | 39      | White| Male   | 36      | 6.8      | Fatty Liver                              | Thioridazine                          | MDD                         |
| 7. Nonsuicide depressed           | 46      | Black| Male   | 20      | 7.1      | Seizure d/o                              | Fluoxetine, risperidone               | MDD                         |
| 8. Nonsuicide depressed           | 59      | White| Male   | 20      | 7.0      | ASCVD                                    | Sertraline                            | MDD, ethanol dependence      |
Table 2. Continued

| Group | Age (y) | Race | Gender | PMI (h) | Brain pH | Cause of Death | Psychotropic Drugs (at Time of Death) | Psychiatric Diagnosis |
|-------|---------|------|--------|---------|---------|----------------|--------------------------------------|---------------------|
| 9.    | Nonsuicide depressed | 46 | White | Female | 23 | 6.9 | Mixed Drug intoxication | Bupropion, lamotrigine | MDD, ethanol abuse, polysubstance abuse |
| 10.   | Nonsuicide depressed | 29 | White | Female | 22 | 6.9 | Obesity, Cardiomegaly | Fluoxetine, norfluoxetine | MDD |
| 11.   | Nonsuicide depressed | 49 | Male | 24 | 7.1 | ASCVD | Desmethylsertraline | MDD |
| 12.   | Nonsuicide depressed | 47 | Female | 26 | 6.5 | Diabetic ketoacidosis | Fluoxetine | MDD |
| Schizophrenia Nonsuicide Subjects | | | | | | | | |
| 1.    | Nonsuicide | 71 | White | Female | 12 | 6.8 | ASCVD | None | Schizophrenia |
| 2.    | Nonsuicide | 41 | Black | Female | 16 | 6.6 | Morbidly obese, dilated cardiomyopathy | Perphenazine | Schizophrenia |
| 3.    | Nonsuicide | 50 | Black | Female | 11 | 6.9 | Environmental hyperthermia, complication from schizophrenia | None | Schizophrenia |
| 4.    | Nonsuicide | 24 | Black | Male | 23 | 6.7 | ASCVD | Olanzapine | Schizophrenia |
| 5.    | Nonsuicide | 77 | White | Male | 17 | 6.8 | ASCVD | Risperidone | Schizophrenia |
| 6.    | Nonsuicide | 45 | Black | Female | 17 | 6.6 | Diabetic ketoacidosis | Haloperidol | Schizophrenia |
| 7.    | Nonsuicide | 47 | Black | Male | 20 | 7.0 | ASCVD | Fluphenazine | Schizophrenia, ethanol abuse, polysubstance abuse |
| 8.    | Nonsuicide | 55 | White | Male | 12 | 6.4 | ASCVD | Olanzapine | Schizophrenia, ethanol abuse |
| 9.    | Nonsuicide | 41 | Black | Male | 19 | 6.5 | ASCVD | Haloperidol | Schizophrenia, ethanol abuse |
| 10.   | Nonsuicide | 40 | White | Male | 14 | 6.6 | MVA | None | Schizophrenia, ethanol abuse, cannabis abuse, cocaine abuse |
| 11.   | Nonsuicide | 33 | Black | Male | 12 | 6.2 | Appendicitis/Peritonitis | Olanzapine | Schizophrenia |
| 12.   | Nonsuicide | 42 | White | Female | 14 | 6.8 | Liver Cirrhosis | None | Schizophrenia, cocaine abuse, polysubstance abuse |
| 13.   | Nonsuicide | 53 | White | Male | 14 | 6.1 | ASCVD | None | Schizophrenia |
| 14.   | Nonsuicide | 83 | White | Male | 18 | 7.0 | Electrocution, accidental | None | Schizophrenia |
| 15.   | Nonsuicide | 57 | White | Male | 11 | 6.2 | Allergic reaction | Haloperidol | Schizophrenia |

Abbreviations: ASCVD, atherosclerotic cardiovascular disease; CO, carbon monoxide; GSW, gunshot wound; MDD, major depressive disorder; MVA, motor vehicle accident.

* Normal controls (mean ± SD) age is 42.08 ± 15.35 years; PMI is 16.54 ± 6.56 hours; brain pH is 7.02 ± 0.15; 7 Black, 17 White; 20 Males, 4 Females.
* Depressed suicide victims (mean ± SD) age is 38.96 ± 15.40 years; PMI is 18.92 ± 6.02 hours; brain pH is 6.96 ± 0.25; 2 Black, 22 White; 14 Males, 10 Females.
* Schizophrenia suicide victims (mean ± SD) age is 36.31 ± 11.63 years; PMI is 16.19 ± 5.69 hours; brain pH is 6.68 ± 0.21; 2 Black, 14 White; 13 Males, 3 Females.
* Other suicide victims (mean ± SD) age is 40.08 ± 24.03 years; PMI is 22.58 ± 6.35 hours; brain pH is 6.67 ± 0.18; 12 White; 11 Males, 1 Females.
* Depressed nonsuicide subjects (mean ± SD) age is 49.50 ± 17.18 years; PMI is 17.92 ± 9.32 hours; brain pH is 6.80 ± 0.27; 3 Black, 9 White; 7 Males, 5 Females.
* Schizophrenia nonsuicide subjects (mean ± SD) age is 50.60 ± 16.7 years; PMI is 15.33 ± 6.62 hours; brain pH is 6.61 ± 0.29; 7 Black, 8 White; 10 Males, 5 Females.

Figure 2. Mean mRNA expression levels of spindle and kinetochore associated complex subunit 2 (SKA2) in the prefrontal cortex (PFC) of normal controls, suicide victims, and nonsuicidal subjects from different diagnostic groups. The data are shown as fold change in mRNA levels and values are fold change ± SEM. (A) Mean mRNA expression levels of SKA2 in normal controls, all suicide victims, and all nonsuicidal patients. (a) Normal controls vs all suicides: P < .0001. (b) All nonsuicidal vs all suicides: P < .0001. (b) All nonsuicidal vs all suicides: P < .0001. (b) All nonsuicidal vs all suicides: P < .0001. (b) All nonsuicidal vs all suicides: P < .0001.
and between schizophrenic suicide victims and nonsuicidal schizophrenic subjects (Table 3). We found that the mRNA expression of SKA2 was again significantly lower ($t = -3.68$, $P = .009$, ES$ = .56$) in depressed suicide victims compared with nonsuicidal depressed subjects, and it was also significantly lower in schizophrenic suicide victims compared with nonsuicidal schizophrenic subjects ($t = -3.61$, $P = .0012$, ES$ = .57$) (Figure 2B; Table 3). We also found that there was no significant difference ($t = 1.10$, $P = .82$, ES$ = .15$) in the protein expression levels of nonsuicidal patients compared with normal control subjects.

To examine if SKA2 protein expression was related to diagnosis or was independent of diagnosis and specific to suicide, we compared each diagnostic group separately with each other and also with normal control subjects as well as with each diagnostic group in the nonsuicidal patient group (Table 4). Suicide groups consisted of depressed suicide victims ($n = 24$), schizophrenic suicide victims ($n = 16$), and other suicide victims ($n = 12$), consisting primarily the subjects with substance abuse and/or conduct disorders.

### Table 3. Statistical Summary for mRNA Expression of SKA2

| Comparisons                         | T STAT | P value | Effect Size |
|-------------------------------------|--------|---------|-------------|
| Normal controls                     |        |         |             |
| Depressed suicide                   | -5.949 | <.0001  | -.66        |
| Other suicide                       | -4.524 | .0001   | -.58        |
| Schizophrenic suicide               | -2.238 | .03     | -.37        |
| Depressed nonsuicide                | 0.462  | 1.0000  | .07         |
| Schizophrenic nonsuicide            | 0.7605 | 1.0000  | .15         |
| Other suicide                       | 0.6864 | 1.0000  | .104        |
| Schizophrenic suicide               | 3.0438 | .0196   | .48         |
| Schizophrenic nonsuicide            | 2.1111 | .2297   | .37         |
| Other suicide                       | 1.0377 | .9148   | .20         |

**Figure 3.** Representative Western blots showing the immunolabeling of spindle and kinetochore associated complex subunit 2 (SKA2) and beta-actin in the prefrontal cortex (PFC) membrane fraction of 2 subjects from the following groups: normal controls, depressed suicide, nonsuicidal depressed, schizophrenic suicide, nonsuicidal schizophrenic, and other suicide (consisting mainly of substance abuse and/or conduct disorders).
**Discussion**

In this study, we found that the gene and protein expression of SKA2, a gene recently implicated in suicidal behavior (Guintivano et al., 2014), is significantly decreased in the PFC of suicide victims compared with normal controls. The protein and gene expression of SKA2 in the PFC of nonsuicidal patients was not significantly different from normal controls. Also, the gene and protein expression of SKA2 in the PFC was significantly lower in suicide victims compared with nonsuicidal patients. This observation suggests that decreased protein and mRNA expression of SKA2 is specific to suicide, as this decrease was not observed in nonsuicidal subjects.

We then examined if the decrease of SKA2 in the PFC of suicide victims is independent of diagnosis. We observed that SKA2 protein and gene expression was decreased in depressed suicide, schizophrenic suicide, and other suicide groups compared with normal controls and also compared with corresponding nonsuicidal groups, for example, depressed suicide vs depressed nonsuicide, suggesting that decreased SKA2 expression is not related to diagnosis (ie, it is independent of diagnosis, since the decrease was observed only in suicide victims).

In a recent study, Guintivano et al. (2014) reported altered DNA methylation of SKA2 in the blood of suicide patients and the brain of suicide victims. They also found decreased mRNA SKA2 expression in the PFC of suicide victims. However, it was not clear if the observed decrease in SKA2 mRNA expression was independent of diagnosis.

Our observation that mRNA expression of SKA2 is decreased in the PFC of suicide victims compared with controls is similar to that of Guintivano et al. (2014). Although the studies of Guintivano et al. (2014) focused more on DNA methylation in the brain and also in the blood, we focused on SKA2 abnormalities in suicide brain. In addition to mRNA expression, we have determined SKA2 protein expression in our cohort, and we also examined the diagnostic specificity of decreased SKA2 expression by determining SKA2 in suicide victims and nonsuicidal psychiatric subjects belonging to different diagnostic groups. We examined if decreased SKA2 expression was specific to suicide and not related to diagnosis. Our results suggest decreased gene and protein expression of SKA2 in suicide. The reasons, mechanisms, or functional consequences of this decrease are unclear, since the role of SKA2 in suicide and psychiatric disorders is not well known.

In a more recent study, Niculescu et al. (2015) found decreased SKA2 gene expression in violent suicide completers. We therefore also compared SKA2 expression between violent
and nonviolent suicide. Although the number of violent suicide completers was small, we did not find differences in SKA2 protein or mRNA expression between violent and nonviolent suicide completers.

SKA2 belongs to the SKA complex consisting of the proteins SKA1 and SKA2 (Hanisch et al., 2006). This complex was originally identified in a proteomic survey of the human mitotic spindle apparatus (Hanisch et al., 2006). A novel spindle and kinetochore associated (SKA) complex required for a timely anaphase onset consists of both SKA1 and SKA2. A third component of the SKA complex, known as SKA3, has also been identified (Raaijmakers et al., 2009), and the depletion of this gene (SKA3) from the complex results in mitotic arrest (Guimaraes and Deluca, 2009; Welburn et al., 2009). It is believed that this complex is required to generate stable kinetochore-microtubule attachments during mitosis in humans (Hanisch et al., 2006).

This SKA complex in general, and SKA2 in particular, may be associated with suicide/suicidal behavior through its interaction with glucocorticoid receptor (GR), which is involved in the feedback inhibition of hypothalamic-pituitary-adrenal (HPA) axis (Pariante and Lightman, 2008; Rice et al., 2008). Abnormal HPA axis function has been implicated in depression and completed suicide (Coryell and Schlesser, 2001). One of the tests by which HPA axis abnormalities have been assessed is the dexamethasone (DEX) suppression test (DST). DST was found to be abnormal in both depression and suicidal behavior (PLOTSKY ET AL., 1998; Coryell and Schlesser, 2001; Pariante and Lightman, 2008).

In addition, some studies suggest that abnormal DST may be a risk factor and even a predictor of completed suicide (Coryell and Schlesser, 2001; Yerevanian et al., 2004; Jokinen et al., 2007). For example, Yerevanian et al. (2004) found that DST nonsuppressors were significantly more likely to commit and complete suicide than DST suppressors. Other investigators have also found an association between DST nonsuppression and suicide (Coryell and Schlesser, 2001; Yerevanian et al., 2004; Jokinen et al., 2007; Le-Niculescu et al., 2013).

The DST nonsuppression (ie, abnormal DST) or the HPA axis hyperactivity in depressed and suicidal patients has been related to a deficiency in feedback mechanisms, primarily to altered GR in the brain (Nemeroff, 1996; Pariante and Lightman, 2008). Thus, GR is a key component regulating the HPA axis and it may be abnormal in suicide. Abnormality of this receptor in the postmortem brain has been implicated in suicide (Mcgowan et al., 2009; ALT ET AL., 2010; Pandey et al., 2013). For example, it has been found that the expression of GR and its target gene, GILZ, is decreased in the PFC and amygdala of suicide victims (Alt et al., 2010; Pandey et al., 2013). Abnormalities of DNA methylation of GR have also been reported in suicide subjects with early life trauma (Mcgowan et al., 2009). Thus, it is quite possible that abnormal DST suppression and GR expression observed in suicide (Raison and Miller, 2003; ALT ET AL., 2010; Pandey et al., 2013) may be related to a functional interaction between GR and SKA2, as suggested by some studies (Rice et al., 2008). Rice et al. (2008) studied in great detail the interaction of SKA2 with GR and found that SKA2 is colocalized with GR in the cytoplasm and moves to the nucleus in DEX-treated cells. They also found that in cells overexpressing a GR DNA construct, there was a partial translocation of SKA2 to the nucleus following glucocorticoid treatment. Since SKA2 has no nuclear localization, it may interact with GR in the cytoplasm to facilitate its movement to the nucleus. The main requirement for this process to occur is the overexpression of GR. Since there is some evidence to suggest that GR may be underexpressed in suicide brain (ALT ET AL., 2010; Pandey et al., 2013), this may lead to decreased SKA2 translocation to the nucleus. Similarly, underexpression of SKA2 in suicide may alter the translocation of GR to the nucleus (Rice et al., 2008).

Another possible mechanism of GR-SKA2 interaction may be the SKA2 effect on GR transactivation function. Rice et al. (2008) found that overexpression of SKA2 results in modest enhancement of GR transactivation, while knockdown of SKA2 markedly inhibits GR transactivation (Rice et al., 2008). Since we find decreased SKA2 expression in suicide, it may suggest decreased GR transactivation.

The strong interaction between GR and SKA2 suggests that abnormalities in HPA function in suicide may also be related to GR-SKA2 interaction. This assumption is further substantiated by the observation of Guintivano et al. (2014) that SKA2 genetic and epigenetic differences were associated with reduced suppression of salivary cortisol.

It has been shown that SKA2 may play a role in cell proliferation and apoptosis, as SKA2 knockout prevented cell proliferation (Rice et al., 2008). It was also found that DEX has a profound inhibitory effect on SKA2 expression, suggesting that SKA2 may play a role in antiproliferation or proapoptotic activity.

In addition to DEX, the levels of SKA2 are also regulated by Stauroporine, phorbol ester, and trichostatin A (Rice et al., 2008). All of these either induce apoptosis or inhibit cell proliferation. These findings may also suggest that abnormalities of SKA2 in suicide may either cause or be related to the observed abnormalities of protein kinase C (PKC) in suicide (Pandey et al., 2004). Since phorbol ester binds and activates PKC and since abnormalities of PKC enzymes have been reported in the suicide brain, it is possible that underexpression of SKA2 in suicide may be related to the reported abnormalities of PKC in suicide.

It therefore appears that the main factors regulating the expression of SKA2 are GR, PKC, and DEX. Abnormal expression of these factors—PKC and GR—has been reported in the suicide brain (Pandey et al., 2004, 2013).

What may be the consequences of SKA2 abnormalities in suicide? Decreased SKA2 expression may cause apoptosis and/or decreased cell proliferation through its interaction with GR and/or PKC. Reported volume and structural changes in the PFC of suicidal patients (Jollant et al., 2011) may be a consequence of SKA2 underexpression in the suicidal brain.

Identification of biomarkers with high sensitivity and specificity for suicide and/or suicidal behavior and genes that represent a risk factor for suicide may be important in the prevention and treatment of suicidal behavior. Recent studies have suggested that some genes and their DNA methylation such as SKA2 predict suicidal behavior with high specificity in patients with suicidal behavior. Also, Le-Niculescu et al. (2013) have identified SAT-1 gene to be specific to suicidal behavior. They also recently reported that SKA2 is indeed also a blood biomarker based on gene expression studies in blood samples of psychiatric patients and suicide completers (Niculescu et al., 2015). Gene and protein expression studies of SKA2 show promise as a biomarker for suicide, since it appears that it is not only specific to suicide but the SKA2 decrease observed in suicide victims is independent of diagnosis and not present in nonsuicidal psychiatric patients. Studies of this gene in blood of suicidal patients are needed to further validate the usefulness of this gene as a biomarker or vulnerability marker for suicide.

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Statement of Interest

None.

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