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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,

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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 geometic mean of β-actin and GAPDH and was expressed relative to the control samples using $2^{-(\Delta\Delta Ct)}$ 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

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 Loats Image Analysis System (Westminister, 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, postmortem 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,-.37), 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 ($t = -.256$, $P = 1.0$, 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

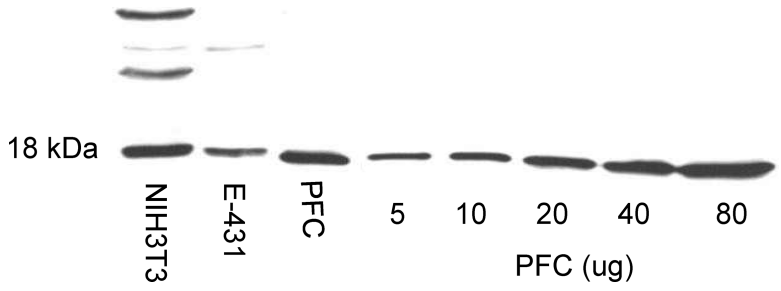

Figure 1. The specificity of spindle and kinetochore associated complex subunit 2 (SKA2) proteins was checked by running NIH3T3 cells and E431 immune lines along with membrane fraction of prefrontal cortex (PFC) from one control subject. Extracts from different cell lines as well as membrane fraction migrated to 18 kDa.

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[a]									
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[b]									
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, desipramine, nortriptyline, ethanol	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, ethanol	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, ethanol abuse
12.	SUICIDE	46	White	Female	16	6.8	Drug overdose / Nortryptyline 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, ethanol abuse, opioid abuse
18.	SUICIDE	74	White	Female	27	7.0	Venlafaxine overdose	Venlafaxine, ethanol	MDD, ethanol abuse
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

Table 2. *Continued*

	Group	Age (y)	Race	Gender	PMI (h)	Brain pH	Cause of Death	Psychotropic Drugs (at Time of Death)	Psychiatric Diagnosis
Schizophrenia Suicide Victims[c]									
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	Schizophrenia
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	Trifluoperazine	Schizophrenia
6.	SUICIDE	28	White	Male	13	7.1	Suicide, hanging	Thioridazine	Schizophrenia, ethanol abuse
7.	SUICIDE	35	White	Female	7	6.9	Suicide, multiple drugs intoxication	Amitriptyline, amoxapine, loxapine, nortriptyline	Schizophrenia
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, fluphenazine	Schizophrenia
16.	SUICIDE	54	White	Male	19	6.6	Suicide, bleeding	None	Schizophrenia
Other Suicide Victims[d]									
1.	SUICIDE	34	White	Male	16	6.4	GSW	Ethanol	Ethanol 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	Cocaine abuse
6.	SUICIDE	30	White	Male	32	6.5	Hanging	Cocaine, ethanol	Ethanol abuse, cocaine abuse, drug abuse
7.	SUICIDE	40	White	Male	26	6.7	GSW	Ethanol	Adjustment disorder
8.	SUICIDE	20	White	Male	32	6.9	Hanging	Ethanol	Ethanol 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	Adjustment disorder, conduct disorder
12.	SUICID	19	White	Male	15	6.9	GSW to chest	Fluoxetine	Dissociative disorder, substance abuse, PTSD
Depressed Nonsuicide Subjects[e]									
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	White	Male	24	7.1	ASCVD	Desmethylsertraline	MDD
12.	Nonsuicide depressed	47	White	Female	26	6.5	Diabetic ketoacidosis	Fluoxetine	MDD
Schizophrenia Nonsuicide Subjects[f]									
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.

[a] 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.

[b] 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.

[c] 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.

[d] 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.

[e] 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.

[f] Schizophrenia nonsuicide subjects (mean ± SD) age is 50.60 ± 16.17 years; PMI is 15.33 ± 3.62 hours; brain pH is 6.61 ± 0.29; 7 Black, 8 White; 10 Males, 5 Females.

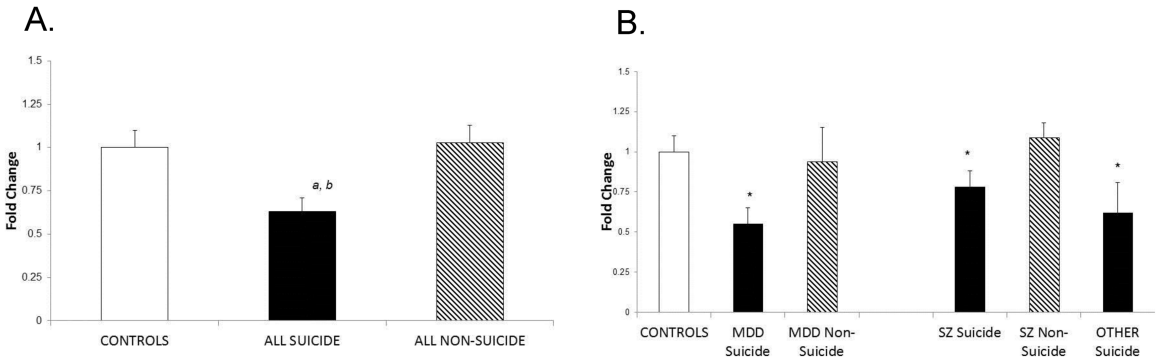

Figure 2. Mean mRNA expression levels of spindle and kinetochore associated complex subunit 2 (SKA2) in the prefrontal cortex (PFC) of normal controls, suicide victims, and nonsuicidal subjects from different diagnostic groups. The data are shown as fold change in mRNA levels and values are fold change ± 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) Mean mRNA expression levels of SKA2 in normal controls, depressed suicide victims, nonsuicidal depressed subjects, schizophrenic suicide victims, nonsuicidal schizophrenic subjects, and other (mainly substance abuse and/or conduct disorders) suicide victims. *$P < .05$.

Table 3. Statistical Summary for mRNA Expression of SKA2

Comparisons		T STAT	*P* value	Effect Size
Normal controls	Depressed suicide	-5.949763	<.0001	-.66
Normal controls	Other suicide	-4.52416	.0001	-.58
Normal controls	Schizophrenic suicide	-2.23897	0.3	-.37
Normal controls	Depressed nonsuicide	0.462002	1.0000	.07
Normal controls	Schizophrenic nonsuicide	0.760534	1.0000	.15
Depressed suicide	Other suicide	0.686473	1.0000	.104
Depressed suicide	Schizophrenic suicide	3.043836	.0196	.48
Other suicide	Schizophrenic suicide	2.111131	.2297	.37
Depressed nonsuicide	Schizophrenic nonsuicide	1.037718	.9148	.20

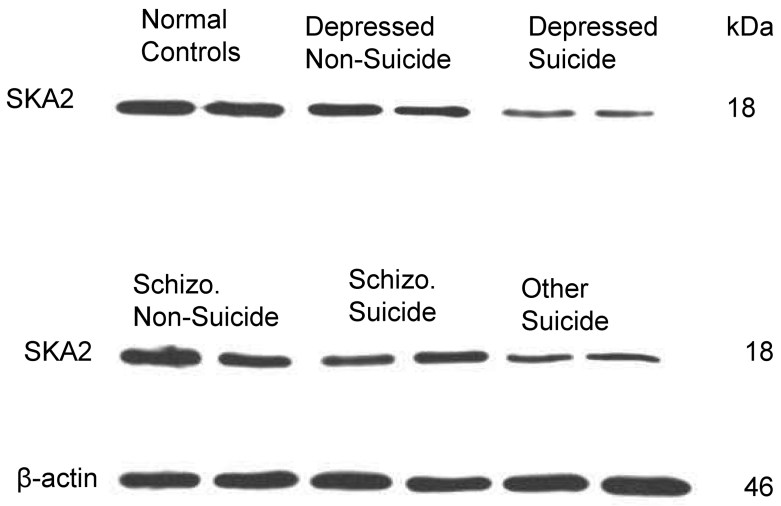

Figure 3. Representative Western blots showing the immunolabeling of spindle and kinetochore associated complex subunit 2 (SKA2) and beta-actin in the prefrontal cortex (PFC) membrane fraction of 2 subjects from the following groups: normal controls, depressed suicide, nonsuicidal depressed, schizophrenic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

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).

Protein Expression of SKA2 in Normal Controls, Suicide Victims, and Nonsuicidal Patients

Representative immunoblots of 2 subjects from the following groups—normal controls, depressed suicide, nonsuicidal depressed, schizophrenic suicide, nonsuicidal schizophrenic, and other suicide—are shown in the Figure 3. As can be seen, SKA2 protein expression appears to be decreased in suicide (ie, depressed suicide, schizophrenic suicide, and other suicide) victims, but not in nonsuicidal (ie, nonsuicidal depressed and nonsuicidal schizophrenic) subjects compared with normal control subjects.

The mean protein expression of SKA2 in suicide victims, nonsuicidal subjects, and normal controls are shown in Figure 4A. We examined if protein expression of SKA2, similar to its mRNA expression, was also altered in the PFC of suicide victims by comparing SKA2 protein expression between suicide victims, nonsuicidal subjects, and normal controls. The regression analysis showed differences between normal controls, total suicide victims, and total nonsuicidal subjects (F = 460.1, *P* <.0001). We then examined if there was a significant difference between the groups by posthoc *t* test with Bonferroni correction. We found that the protein expression of SKA2 was significantly decreased in suicide victims (t = 3.98, *P* =.0004, ES = .46) compared with normal control subjects and nonsuicidal patients (Figure 4A). 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.

The mean protein expression of SKA2 in each group is shown in Figure 4B. When we compared different groups against the normal controls, we found that the protein expression of SKA2 in depressed suicide (t = 3.67, *P* =.003, ES = .47), schizophrenic suicide (t = 2.27, *P* <.03, ES=.37), and other suicide groups (t = 3.14, *P* =.01, ES = .51) was significantly decreased compared with normal control subjects (Figure 4B; Table 4).There was no significant difference between any of the suicide groups compared against each other. When we compared the SKA2 protein expression in nonsuicidal subjects, consisting of 12 nonsuicidal

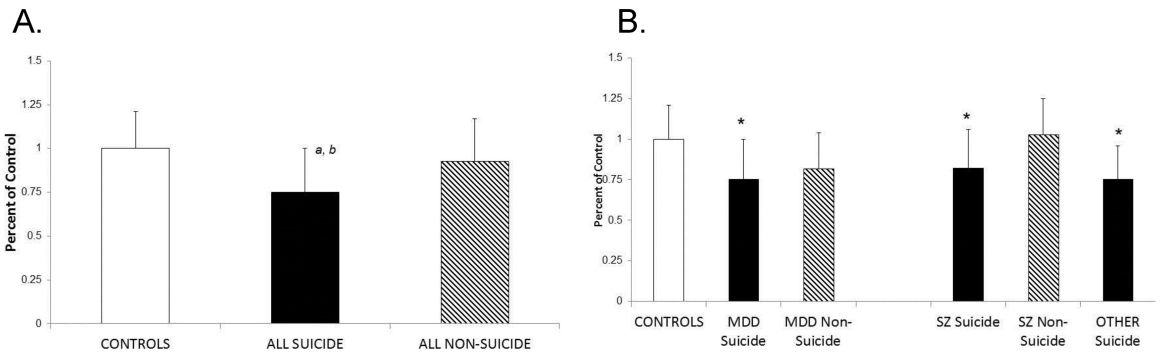

Figure 4. Protein expression levels of spindle and kinetochore associated complex subunit 2 (SKA2) in the prefrontal cortex (PFC) of normal controls, and suicide victims and nonsuicidal subjects from different diagnostic groups. The data are shown as percent of controls. Values are mean ± SD. (A) Mean protein expression levels of SKA2 in normal controls, depressed suicide victims, nonsuicidal depressed subjects, schizophrenic suicide victims, nonsuicidal schizophrenic subjects, and other (mainly substance abuse and/or conduct disorders) suicide victims. (a) Normal controls vs all suicides: $P < .001$. (b) All nonsuicidal vs all suicides: $P < .05$. (B) Mean protein expression levels of SKA2 in normal controls, depressed suicide victims, nonsuicidal depressed subjects, schizophrenic suicide victims, nonsuicidal schizophrenic subjects, and other (mainly substance abuse and/or conduct disorders) suicide victims. *$P < .05$.

Table 4. Statistical Summary for Protein Expression of SKA2

Comparisons		T STAT	P value	Effect Size
Normal controls	Depressed suicide	3.67543	.0028	.47
Normal controls	Other suicide	3.144044	.0148	.51
Normal controls	Schizophrenic suicide	2.275038	.3	.37
Normal controls	Depressed nonsuicide	-2.40996	.0603	-.39
Normal controls	Schizophrenic nonsuicide	-0.4643	1.0000	-.08
Depressed suicide	Other suicide	-0.00612	1.0000	-.001
Depressed suicide	Schizophrenic suicide	-0.86157	1.0000	-.14
Other suicide	Schizophrenic suicide	-0.76296	1.0000	-.15
Depressed nonsuicide	Schizophrenic nonsuicide	-2.48918	.0497	-.45

depressed (t = 2.41, $P = .06$, ES = .38) and 15 nonsuicidal schizophrenic subjects (t = -.46, $P = 1.0$, ES = .07), we found that the mean SKA2 protein expression in these subjects was not significantly different from that of the normal control subjects (Figure 4B; Table 4). These results therefore suggested that decreased SKA2 protein expression in suicide is independent of psychiatric diagnosis, since suicide victims from different diagnostic groups still showed a significant decrease in SKA2 protein expression, whereas nonsuicidal subjects did not show any significant difference compared with normal control subjects. These results also suggested that decreased SKA2 protein expression was specific to suicide.

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 knockdown 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 Staurosporine, 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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